Yeast perfume factory: metabolic engineering of Saccharomyces cerevisiae for plant isoprenoid biosynthesis

Gionata, S.; Knuf, C.; Siavash, P.; Chen, Y.; Maury, Jerome; Schalk, M.; Daviet, L.; Nielsen, Jens; Siewers, V.

Published in:
F E B S Journal

Link to article, DOI:
10.1111/j.1742-4658.2010.07680.x

Publication date:
2010

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):
A1 – Ageing

A1.01
Abstract withdrawn

A1.02
BDNF Mediated Angiogenesis Potential is Decreased Associated with Aging
D. Cai, L. Cao, S. Chen, D. Li, X. Shen, X Zheng and X. Liu
Ji Nan University, Key Laboratory for Regenerative Medicine,
Ministry of Education, Guangzhou, China

The mechanism of age-related decrease of angiogenic potential in myocardium is still unclear. Cardiac microvascular endothelial cells (CMECs) play a key role in cardiac angiogenesis. In this study, using the CMECs which are isolated from young and old hearts, we found that the migration and proliferation capacity of old CMECs were diminished. BDNF was able to increase the migration and proliferation of CMECs no matter in young and old CMECs, however, the effects in old CMECs was less potent compared with the young CMECs. In vivo study showed that delivery of BDNF in young heart in ischemic situation was able to increase the vessel numbers in both infarct and border zone significantly, but not in young heart in non-ischemic situation. The microarray results showed that 84 genes were up-regulated, while 81 genes were down-regulated upon BDNF treatment. The functional annotations of genes are cell migration, blood vessel morphogenesis, angiogenesis, regulation of proliferation, cell cycle regulation, etc, which have been shown the strong potential effects in migration, proliferation and angiogenesis. The results of present study revealed that BDNF-TrkB pathway play an important role in angiogenesis of myocardium. Although CMECs express BDNF consistently, however, BDNF might not initiate the angiogenesis in heart individually in vivo. BDNF-mediated angiogenic potential might depend on the cross talk with focal micro-environment. Importantly, senescence of CMECs was able to impair the BDNF-mediated migration and proliferation capacity. It might contribute to age-related decrease of angiogenic potential in myocardium and poor regenerative capacity seen in aged heart.

A1.03
Memory enhancing effects of saffron in adult & aged mice are correlated with the antioxidant protection: In vitro and in vivo studies
M. Papandreou1, M. Tsachaki2, S. Efthimiopoulos1, P. Cordopatis1, F. Lamari1 and M. Margaritis1
1University of Patras, Biology, Lab. Human & Animal Physiology, Patras, Greece, 2University of Athens, Biology, Lab. Animal & Human Physiology, Athens, Greece

Oxidative stress is implicated in senescence and age-related pathologies, with memory deficits as the commonest manifestations. Herbal ingredients are sought to forestall/reverse those deficits as dietary components/supplements. The effect on cognitive function of a 7-day, intraperitoneal administration of saffron was examined in healthy adult and aged mice by step through test. Results showed that saffron-treated mice exhibited significant improvement in learning and memory. Experiments in whole brain homogenates revealed that saffron administration resulted in significantly lower brain lipid peroxidation (malondialdehyde, 44–63%) and higher antioxidant parameters (glutathione, ascorbic acid, total antioxidant power). Salt- and detergent-soluble AChE activity was significantly decreased only in adult mice. Thus, the significant cognitive enhancement conferred by saffron administration in adult and aged mice, is closely related to the antioxidant reinforcement; AChE inhibition (in adult mice) plays also a minor role. Studying further the antioxidant potential, the effect(s) of saffron and crocetin (main crocin metabolite), were examined against H2O2-induced toxicity in SH-SY5Y and HEK293 cells. Cell viability and scavenging of free radicals after co-treatment with H2O2 (250–750 μM) and the tested compounds (1–250 μg/ml saffron, 1–125 μM crocetin) were determined with MTT and DCF assays. Results showed that saffron and crocetin provide strong protection in rescuing cell viability and repressing ROS production in the SH-SY5Y cells; moderate effects in HEK293 cells. Considering, thus, earlier metabolic studies, crocetin appears to be responsible for the in vivo effects.

A1.04
Protective effects of triphlorethol-A against formaldehyde-induced oxidative damage and apoptosis: role of mitochondria-mediated caspase-dependent pathway
R. Zhang1, K. A. Kang1, M. J. Piao1, K. C. Kim1, J. Y. Choi2, J. Choi3, J. Park4 and J. W. Hyun1
1School of Medicine, Jeju National University, Jeju Island, Republic of Korea, 2Department of Pharmacology, School of Medicine, Ewha Womans University, Seoul, Republic of Korea, 3Faculty of Environmental Engineering, University of Seoul, Seoul, Republic of Korea, 4Division of Hematology and Oncology, Department of Internal Medicine, Gachon University of Medical Science, Gil Hospital, Incheon, Republic of Korea

The toxicity of formaldehyde (HCHO) has been attributed to its ability to form adducts with DNA and proteins. Triphlorethol-A, derived from Ecklonia cava, was reported to exert a cytoprotective effect against oxidative stress damage via an antioxidant mechanism. The aim of this study was to examine the mechanisms underlying triphlorethol-A ability to protect Chinese hamster lung fibroblast (V79-4) cells against HCHO-induced damage. Triphlorethol-A significantly decreased the HCHO-induced intracellular reactive oxygen species (ROS) production. Triphlorethol-A prevented increased cell damage induced by HCHO via inhibition of mitochondria-mediated caspase-dependent apoptosis pathway. Triphlorethol-A diminished HCHO-induced mitochondrial dysfunction including loss of mitochondrial membrane action potential (Ψ) and adenosine triphosphate (ATP) depletion. Furthermore, the anti-apoptotic effect of triphlorethol-A was exerted through inhibition of c-Jun NH2-terminal kinase (JNK) which was enhanced by HCHO. Our data indicate that triphlorethol-A exerts a cytoprotective effect in V79-4 cells against HCHO-induced oxidative stress by inhibiting the mitochondria-mediated caspase-dependent apoptotic pathway.
Antioxidant effect of Jeju water containing vanadium component
K. A. Kang1, R. Zhang1, M. J. Piao1, K. C. Kim1, C. M. Lim1, A. D. Kim2 and J. W. Hyun1
1School of Medicine, Jeju National University, Jejusi, Republic of Korea, 2Department of Marine Life Science, Jeju National University, Jejusi, Republic of Korea

The aim of this study was to examine the antioxidant effect of Jeju water containing vanadium component (20–25 ppb). Cells were incubated for 10 passages in media containing deionized distilled water (DW group) and Jeju water (JW group). DW and JW groups did not show to scavenge 1,1-diphenyl-2-picrylhydrazyl radical. Electron spin resonance spectrometer data showed that JW group significantly scavenged superoxide radicals induced by Fenton reaction (H2O2 + FeSO4), and hydroxyl radicals induced by xanthine/xanthin oxidase system as compared to DW group. Furthermore, JW group significantly scavenged intracellular reactive oxygen species in human Chang liver cells as compared to DW group, which are measured by using fluorospectrometer, flow cytometer, and confocal microscope after staining 2,7’-dichlorodihydrofluorescein diacetate. These results suggest that Jeju water containing vanadium component showed antioxidant effect via scavenging radicals.

Effect of aging and oxidative stress on elongation factor-2 in hypothalamus and hypophysis
S. Argüelles Castilla1, M. Cano2, A. Machado de la Quintana1 and A. Ayala1
1University of Seville, Biochemistry and Molecular Biology, Seville, Spain, 2University of Seville, Animal Physiology and Biology, Seville, Spain

The hypothalamic-hypophysis system (HHS) is a major part of the neuroendocrine system. The output of this unit regulates several body functions. One common feature of hormones secreted by this system is that they are peptides whose size range from 9–36 amino acids. As the organisms age, a considerable diminution of the protein synthesis takes place in several tissues. Among the possible causes of the decline of translation in old animals are the modifications of elongation factor-2 (eEF-2). We studied whether the level of this protein was affected in the HHS in old animals. The effects of aging are compared to those of an oxidant compound (cumene hydroperoxide) administrated to young rats. To test this, eEF-2 levels, adduct formation with both malondialdehyde (MDA) and 4-hydroxynonenal (HNE), and two oxidative stress markers were compared in old rats versus young rats treated with cumene hydroperoxide (CH), a compound that has been used in experimental models to induce lipid peroxidation. The results indicate that oxidative stress could be involved in the alterations of eEF-2, which forms adducts with MDA and HNE. The alterations of eEF-2 levels, secondary to lipid peroxidation and adduct formation with these aldehydes could contribute to the suboptimal hormone production from these tissues during aging. Besides eEF-2, proteomic analysis shows that several other proteins are affected.
the cells and can cause replicative senescence especially in the mother cells where the agedness and retention of old and damaged materials are the main concern. The importance of protein segregation is also demonstrated to be a beneficial mechanism to decrease clonal senescence.

A1.10 Down-regulation of protein kinase CKII induces the p53-p21Cip1/WAF1-pathway-dependent senescence in human colon cancer cells

J. J. Kim, J.-Y. Kang and Y.-S. Bae
Kyungpook National University, Daegu, Republic of Korea

Protein kinase CKII plays a critical role in cell growth and proliferation. The expression level of CKII is greatly enhanced in a variety of tumor or leukemic cells. We have previously shown that the down-regulation of protein kinase CKII activity is tightly associated with cellular senescence of human fibroblast IMR-90 cells. Here, we examined the roles of p53 and p21Cip1/WAF1 in senescence development induced by CKII inhibition using wild-type, isogenic p53−/− and isogenic p21−/− HCT116 human colon cancer cell lines. A senescent marker appeared after staining for senescence-associated β-galactosidase activity in wild-type HCT116 cells treated with CKII inhibitor or CKIIα siRNA, but this response was almost abolished in p53− or p21Cip1/WAF1-null cells. Increased cellular levels of p53 and p21Cip1/WAF1 protein occurred with the inhibition of CKII. CKII inhibition upregulated p53 and p21Cip1/WAF1 expression at post-transcriptional and transcriptional level, respectively. Rb phosphorylation significantly decreased in cells treated with CKII inhibitor. Taken together, this study shows that the activation of the p53-p21Cip1/WAF1-Rb pathway acts as a major mediator of cellular senescence induced by CKII inhibition.

A1.11 Abstract withdrawn

A1.12 Differences in ageing and stress resistance in clonal relative to sexual populations of the fissiparous starfish Coscinasterias muricata

C. Oweson1, H. Nilsson Sköld2, B. Carney Almroth3, M. Sköld3 and Steve Wing4
1University of Gothenburg/Marine Ecology-Kristineberg, Fiskebäckskil, Sweden, 2University of Gothenburg/Zoology, Gothenburg, Sweden, 3The Board of Fisheries, Lysekil, Sweden, 4Otago University, Dunedin, New Zealand

In organisms that propagate by asexual cloning the parental body is the reproductive unit, why such species have despite experimental evidence been considered potentially immortal due to presumed relocation of energy investment into body maintenance, rather than into gonad production. We have used the starfish Coscinasterias muricata, which can reproduce either fissionarily or sexually, to analyse if clonal animals are more stress-resistant than their sexually reproducing counterparts. To use C. muricata as a study organism is of high relevance, since the species naturally use both reproduction strategies, the starfish can be easily maintained in the lab and their size makes the sampling simple. We have studied the animals on whole animal level, cellular and protein level. Since telomere length has previously been related to health and fitness in a variety of species, analysis of the relative telomere length is of high interest. To complement the telomere study, we have also studied differences in telomerase activity between these two groups. To verify if these two groups differ in ability to respond to stressors we have analysé different parameters of oxidative stress and used a robustness assay to measure their sustainability to physical exhaustion. In conclusion, we present experimental evidence for increased stress resistance in a clonal species. The results support the theoretical assumption long telomeres may be a potential mechanism for this.

A1.13 Abstract withdrawn

A1.14 Aging and oxidative stress in two populations of Atlantic cod fish: Effects of commercial fishing

B. Carney Almroth1, M. Sköld1, J. Hjelm3, L. Förlin1 and H. Nilsson Sköld1
1University of Gothenburg, Zoology, Göteborg, Sweden, 2Swedish Board of Fisheries, Institute of Marine Research, Lysekil, Sweden, 3University of Gothenburg, Marine Ecology, Fiskebäckskil, Sweden

Sexual reproduction and ageing are closely related and regarded as opposite regulators of each other due to the costs of reproduction. Gender also plays a role in ageing. We have addressed these relationships in wild cod fish populations where extensive fishing has caused genetic shifts in populations, resulting in sexual maturation at younger ages and smaller sizes. We have measured a number of oxidative stress parameters known to vary with age, in both adult fish tissue as well in eggs. Samples were analysed from genetically similar cod populations collected at two sites, Kattegat, a trawled region, and Öresund, a protected area. Fish ranged in age from 2 to 8 years. Our results indicate that male cod have significantly higher catalase activities in liver tissue than females, and that neither sex displays changes in CAT with age. Decreases in glutathione content (total and oxidized) correlates strongly with aging in males from both sites, but not females. GSH is also not affected in eggs. Fish from Kattegat had significantly lower levels of GSSG and CAT activity, indicating lower oxidative stress in these fish with early maturation. Protein carbonyls and lipid peroxides in liver tissue do not correlate with age, nor do these variables differ between genders. We do see a trend towards increasing protein carbonyls in eggs with female age (p = 0, 083), indicating a possible negative maternal affect with age. In conclusion, we observed gender differences in oxidative stress and potential negative maternal effects with age in wild Atlantic cod, and differences in oxidative stress between populations.

A1.15 Genetic association study between length of telomeres and healthy aging

R. Ranka1, L. Pliss1, A. Kruminia2 and V. Baumanis1
1Latvian Biomedical Research and Study Centre, Riga, Latvia, 2Riga Stradiuss University, Riga, Latvia

Introduction: Telomeres are repetitive DNA sequences at the ends of linear chromosomes that consist of 5–15 kb pairs of multiple copies of TTAGGG sequences. Telomeres shorten with each cell division by 50–200 bp owing to the so-called “end replication problem”. Although telomere length is known to play a critical role in cellular senescence, the relationship of telomere length to aging and longevity in humans is not well understood. Human population studies have correlated decreased telomere length in
Materials and methods: Three age groups were studied: individuals with age 18–40 years, individuals with age 65–75 years, and the centenarian group (with age above 90 years). DNA was isolated from leukocytes samples and telomere length was determined by telomere restriction fragment analysis.

Results: The mean length of telomeres in younger age group was 10.8 kb. The mean length of telomeres in 65–75 age group was 6.7 kb. Significant correlation between telomere length and age was observed in groups 18–40 and 65–75 years. Surprisingly, the mean telomere length in the centenarian group was slightly longer (7.6 kb) than in 65–75 age group.

Conclusion: The preliminary results of the present study in different age groups including centenarians found a positive link between telomere length and longevity.

A1.16 QPCR as standard test for determine programmed cell death and the response to different stresses in Saccharomyces cerevisiae E. Mera and D. Petranovic

Chalmers University of Technology, Chemical and Biological engineering, Göteborg, Sweden

The finding of the apoptotic marker YAC1 in baker’s yeast Saccharomyces cerevisiae a decade ago opened the possibility of study apoptosis in yeast as a model to understand the programmed cell death (PCD) in higher organisms. However, apoptosis is not the only cellular death routine in eukaryotes; necrosis and autophagy are too. All these routines have different characteristics and several assays are routinely used to differentiate these pathways, such as co-staining of annexin-V (AnnV) and propidium iodide (PI) to discriminate between early apoptosis, primary necrosis and late apoptosis, TUNEL test for DNA fragmentation, ROS determination with dihydroethidium (DHE) and nuclear fragmentation and chromatin condensation observed with DAPI staining. These tests can assign the type of PCD of the cell but most of the time these tests are qualitative. The use of quantitative PCR (QPCR) for establishing the changes in expression levels during different stimuli and conditions could describe the differences between the PCD routines in a quantitative manner.

In this work we test groups of genes whose transcription changes during unfolded protein response (UPR), apoptosis, necrosis, autophagy and general stress response in the baker’s yeast Saccharomyces cerevisiae with the aim to establish a standard test for quantitative determination of activated death pathways.


1Pukyong National University, Chemistry, Busan, Republic of Korea, 2Food and Safety Research Center, Busan, Republic of Korea, 3Dong-Eui University, Chemistry, Busan, Republic of Korea

Eiseniabicyclis (Kjellman.E.bicyclis) Setchellisaperennial brown alga, belonging to the family Laminariaceae. Fucofuroeckol A (FF) an deckol (EK) were isolated from E. bicyclis, and their antioxidative and matrixmetalloproteinase (MMP)-2 inhibitory effects were investigated. EK and FF showed significant antioxidant activities in several antioxidant assays, such as DPPH, hydroxyl, superoxide anion and peroxyinitrate radicals scavenging activities using the electron spin resonance spectrometry technique and intracellular reactive oxygen species by DCFH-DA method. In MMP-2 inhibitory assay, FF and EK showed strong direct inhibition on MMP-2 dose-dependently. FF and EK also inhibited protein expression of MMP-2 in human fibrosarcoma cells. Therefore, these results suggested that FF and EK have remarkable antioxidant activities and strong potential as valuable natural MMP-2 inhibitor to develop cosmeceuticals for anti-wrinkle formation.

A1.18 Antiglycation activity of pyridoxal 5’-phosphate R. Mironova1, I. Ivanov2, N. Stambolieva3, I. Ivanov1 and T. Niwa4

1Department of Gene Regulations, Institute of Molecular Biology, Sofia, Bulgaria, 2Sofia University “St. Kl. Ohridsky”, Faculty of Biology, Sofia, Bulgaria, 3Institute of Organic Chemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria, 4Department of Clinical Preventive Medicine, Nagoya University School of Medicine, Nagoya, Japan

Glycation is a spontaneous chemical reaction, first discovered about a century ago by the French chemist Maillard. After him the reaction was called the Maillard reaction. In the last decades it has been recognized that the Maillard reaction is implicated in physiological processes such as senescence and ageing. In the glycation reaction carbonyl compounds such as reducing sugars interact with NH2-biomolecules including proteins, DNA and amino lipids, and thus impair their physiological function. The deleterious consequences of the Maillard reaction have prompted the active search for compounds capable to counteract the deleterious consequences of the Maillard reaction in vivo. In the present study we provide evidence that the vitamin B6 vitamer pyridoxal 5’-phosphate (PLP) exhibits carbonyl trapping activity. Under physiological conditions in vitro (37°C, pH 7) PLP interacts with the highly toxic dycarbonyl compounds 3-deoxyglucosone and methylglyoxal, the reaction reaching thermodynamic equilibrium after approximately 96 hours. Under the same conditions glycerol was also found to react with PLP while the model reaction of pyridoxal 5’-deoxyglucosone failed to give any detectable products. Based on electrospray ionization tandem mass spectrometry coupled to liquid chromatography and NMR spectroscopy we propose a structure for the reaction product of PLP with 3-deoxyglucosone. This product was detected also in the urine of rats with streptozotocin-induced diabetes. Data we provide in this study point to a novel physiological function of PLP. In addition to its cofactor activity PLP seems to play in vivo a role in detoxification of highly reactive dycarbonyl compounds.

A1.19 Boolean model of yeast apoptosis I. Kazemzadeh, M. Cvijovic and D. Petranovic Nielsen

Chalmers University of Technology, Chemical and Biological Engineering, Gothenburg, Sweden

Programmed cell death (apoptosis) is mediated through different pathways based on different stimuli and like most biological processes it is the result of sequential activation /inhibition signals acting as input to downstream components. In the simplest possible way this input/output feature of any cellular process like apoptosis can be represented by a discrete model called Boolean...
Phenolic compounds in the turkish table olive cultivars

E. Savas1, S. Beyaztas2 and O. Arslan2

1Balıkesir University, Sarsuluk Vocational School, Balıkesir, Turkey, 2Balıkesir University, Science and Art Faculty, Balıkesir, Turkey

Olive oil is the fat of choice in the Mediterranean area, where the diet has been associated with a lower incidence of coronary heart disease and certain cancers. Phenols in extra virgin olive oil are responsible for its peculiar pungent taste and for its high stability. Recent findings demonstrate that olive oil phenolics inhibit oxidation of low-density lipoproteins (the most atherogenic ones) and possess other potent biological activities that if demonstrated in vivo, could partially account for the observed healthful effects of diets that include high-quality olive oil and other foods rich in flavonoids and phenols. There is increasing interest in olive phenolic compounds because of their biological properties as well as their contribution to the colour, taste and shelf life of olive products. In the Spanish and Californian procedures, olives are treated with a diluted aqueous NaOH solution, that brings about a lye-treatment, followed by palmitic (16.85%), linoleic (16.01%), stearic (2.82%) and linolenic (0.61%) of the Domat variety. In all processed olive samples, mineral and fatty acid compositions have been determined separated by high-performance liquid chromatography (HPLC).

The changes of the chemical composition during processing three Turkish table olive cultivars (Olea europea L.)

E. Savas

Balıkesir University, Sarsuluk Vocational School, Balıkesir, Turkey

Different olive varieties subjected to the same processing method react differently, depending on their varietal, chemical and physical characteristics. In the Californian method the olives are processed by successive treatments using 1 to 2% (w/v) concentrations of sodium hydroxide solutions (lye) that penetrate the fruit to the pit. At the end of each lye treatment the olives are washed with water and aerated. This aerobic alkali treatment tends to cause dramatic changes in the texture of the flesh. This leads to a softening which makes the end product less marketable. The objective of this study was determine fatty acids and mineral content to monitor changes in the composition of table olives after the lye treatment. Domat, Edremit and Gemlik varieties crude and processed table olive samples were considered for their fatty acid and mineral compositions. The mineral contents of three olive varieties were determined by ICP and found to be excellent. Olives were found to be rich in Ca, Fe, K, Mg, Na and P minerals. Also, K, Na and P contents of the Gemlik variety were found higher than those of other varieties. Fatty acids methyl esters (FAME) analysis of olive samples were determined by GC. Oleic acid (% 73.63) was present in the highest concentration, followed by palmitic (16.8%), linoleic (16.01%), stearic (2.82%) and linolenic (0.61%) of the Domat variety. In all processed olive samples, mineral and fatty acid compositions have been affected by alkaline treatment, negatively.

Anti-proliferative effect of papaverine in HepG2 cells

S. Kazemi Noureini1 and M. Wink2

1Tarbiat Moallem University of Sabzevar, Biology, Sabzevar, Islamic Republic of Iran, 2Institute of Pharmacy and Molecular Biotechnology, Department of Biology, Heidelberg, Germany

Plants of genus Papaveraceae with many valuable secondary metabolites have been used for different purposes in traditional medicine. This study is focused on papaverine effects on growth rate of HepG2 cells as a model for hepatocarcinoma. LD50 concentration of papaverine in this cell line was measured equal to 130 μM using neutral red uptake and MTT cytotoxicity methods. Growth rate and population doubling time of the cells under long-exposure to papaverine at two different concentrations corresponding to LD10 (defined as the concentration of papaverine which causes 10% reduction of cell viability) and 10 fold lower equal to 5 and 0.5 μM respectively, for 48 hours in successive passages were evaluated by using cell counting after trypan blue staining. TRAP (Telomerase Repeat Amplification Protocol) assay was used to compare immortality of the 48 hours treated cells to untreated controls. Data collected showed reduced cell growth in HepG2 cells exposed to 5 μM papaverine for 48 hours per passage over 41 days. The number of doublings in control cells over this period was 23.2, while the papaverine-treated cells passed only 15.7 doublings. Doubling time was increased to 62.58 hours (47% longer) comparing to 42.39 hours for untreated control. TRAP assay indicated a 55% reduction of telomerase activity in treated cells at LD50. Real time RT-PCR showed diminished hTERT expression in the treated cells to 65% of untreated cells. In conclusion papaverine shows strong growth limiting effect in HepG2 cell line and probably is a valuable compound against cancer.

Cell senescence induction by Chelidonine in MCF7 cells

S. Kazemi Noureini1 and M. Wink2

1Tarbiat Moallem University of Sabzevar, Biology, Sabzevar, Islamic Republic of Iran, 2Institute of Pharmacy and Molecular Biotechnology, Biology, Heidelberg, Germany

Chelidonine, a tertiary hexahydro-benzophenanthridine alkaloid of Chelidonium majus and one of the alkaloids of Ukraine, has been shown to induce apoptosis in cell culture. Although Ukrain is known as an anticancer drug, the mechanism of action of the components still remained to be well understood. This study has...
focused on immortality and growth of MCF7 cells as a model for breast cancer after treatment with chelidonine. LD50 of chelidonine in MCF7 cells after 48 hour treatment was measured 37 μM by neutral red uptake and MTT cytotoxicity tests. Growth rate of treated cells under long exposure to sub-apoptotic concentrations of chelidonine was estimated by cell counting after trypan-blue staining. In every passage the cells were treated only for 48 hour, and followed by normal medium. Treated cells exhibit strong growth inhibition after four times treatment with 0.2 μM chelidonine, so that the cell growth curve reached plateau and the treated cells failed in re-plating. At this time, growth of the treated cells shows almost 60% decline comparing to controls while cell viability was not affected. The number of cell doublings in treated cells was eight, while untreated controls passed 18 doublings. The treated cells morphologically appear to be aged with a large cell volume and high cytoplasmic to nuclear ratio. Induction of senescence in long-time treated cells was shown by β-galactosidase activity, a commonly used biomarker for cell senescence. Expression level of some genes related to cell senescence is under estimation.

A1.24
Lipid peroxidation damage of retinal pigment epithelium contributes to the pathogenesis of age-related macular degeneration

J. Kopitz1, T. Krohne2 and F. Holz2
1University of Heidelberg, Pathology, Heidelberg, Germany,
2University Hospital Bonn, Eye Hospital, Bonn, Germany

Age-related macular degeneration (AMD) is the leading cause of legal blindness in developed countries, and prevalence will increase rapidly due to demographic changes. Progressive dysfunction of the retinal pigment epithelium (RPE) is considered central to the pathogenesis of AMD. In particular, lysosomal dysfunction induced by lipid peroxidation products, like malondialdehyde (MDA) or 4-hydroxynonenal (HNE), seems to play a pivotal role. We found that lipid peroxidation-related protein modifications on photoreceptor outer segment (POS) proteins inhibit their lysosomal degradation in RPE cells. Lipid peroxidation products exerted striking inhibitory effects on lysosomal cysteine proteases. Feeding of RPE cells with HNE- or MDA-modified POS resulted in an 8-fold increase in cellular autofluorescence, indicating lipofuscinogenesis. In polarized RPE cells we observed apical-to-basolateral transcytosis of undegraded HNE- or MDA-modified POS, which, in vivo, may contribute to sub-RPE deposit formation and drusen biogenesis, a hallmark of AMD. Autophagy activity, measured as 3-methyladenine-sensitive turnover of radiolabeled endogenous proteins, was reduced by pretreating the cells with lipid peroxidation-modified POS by 40%. In conclusion, lipid peroxidation products generated in the outer retina due to its unique physiological characteristics, such as high tissue oxygen concentration, intense light exposure and high abundance of polyunsaturated fatty acids, severely affect RPE lysosomal function, resulting in lipofuscinogenesis, extracellular deposition of undegraded material and reduced autophagy, finally leading to senescence and degeneration of the RPE, as seen in AMD.
A2 – Molecular Immunology

A2.01
Isolation of a novel nanobody against HER-2/neu using phage display technology
F. Sheikholeslamii1, M. J. Rasaei2, M. A. Shokrgozar3 and M. Mokhtari Dizaji4
1Institute Pasteur of Iran, Research & Development Department, Tehran, Islamic Republic of Iran, 2Tarbiat Modares University-Medical Sciences Faculty, Clinical Biochemistry Dept., Tehran, Islamic Republic of Iran, 3Institute Pasteur of Iran, 4Institute Pasteur of Iran, Cell Bank Dept., Tehran, Islamic Republic of Iran, 5Tarbiat Modares University-Medical Sciences Faculty, Medical Physics, Tehran, Islamic Republic of Iran

Camelid sera contain functional antibodies without light chains. The various domains of an antibody are named as VHH. They have some biological, medical and biotechnological advantages over conventional antibodies. Nanobodies are well expressed in microorganisms (Escherichia coli, fungi and yeast) with high stability, good solubility and easy production in large quantities. In this study, we identified a nanobody that recognizes extra cellular domain of human epidermal growth factor receptor 2 (HER-2/neu) that over expressed in a number of various solid tumors are associated with over expression of erbB-2. Our nanobody (SR-87) has been isolated from immune phage nanobody repertoire. The soluble antibody was purified following immobilized metal affinity chromatography (IMAC) and characterized by SDS-PAGE, Western blotting and ELISA methods. SR-87 was characterized and showed good affinity (10-10 M-1) and specificity towards HER-2 in comparison to murine monoclonal antibodies. This single domain antibody (14 KD) may be useful for targeting HER-2 marker on the surface of tumor cells. SR-87 was conjugated to gold – silica nanoshells and applied them to SK-Br-3 cell line which over expressed HER2 and HelaS3 cell line which didn’t have any HER2 receptors. The cells were irradiated with NIR laser and evaluated for nanoshell binding and viability. The photothermal therapy was generated enough heat to destroy SK-Br-3 cells while controls with no nanoshells or the nonspecific antibody binding, show no therapy.

A2.02
Blood serum levels of IL-1â, IL-6 and TNF-â in patients on maintenance hemodialysis
A. Sotoodeh Jahromi1, M. Shojaei2 and A Madani3
1Jahrom University of Medical Science, Immunology, Jahrom, Islamic Republic of Iran, 2Jahrom University of Medical Science, Internal Medicine, Jahrom, Islamic Republic of Iran, 3Hormozgan University of Medical Science, Hygiene, Bandar Abbas, Islamic Republic of Iran

Introduction: Dialysis provides effective and safe treatment of ESRD, but patients who are maintained on chronic dialysis are at risk for cardiovascular disease. One major risk factor for cardiovascular disease in adult patients with ESRD is chronic inflammation. Cytokines are essential mediators of immune response and inflammatory reactions. During a hemodialysis (HD), cytokines are released mainly by monocytes activated by endotoxin-type compounds in dialyzer fluid. Complement factors and direct contact with dialyzer membrane. Aim of this study was to examine effects of the duration of HD therapy upon systemic profile of the pro-inflammatory cytokines (IL-1 â, TNF-â and IL-6) in patients on regular maintenance HD.

Methods: The study included 43 CRF patients, aged 59.32 ± 14.43 years, on regular HD maintenance therapy for mean 26.44 ± 41.29 months and 43 age and sex matched healthy controls. It was designed to assess serum levels of inflammatory cytokines: IL-1â, IL-6 and TNF-â in CRF patients on regular maintenance HD.

Results: The serum IL-1â, IL-6 and TNF-â level were statistically significantly higher in patients than in the controls. There were statistically significant positive correlations between the duration of HD therapy and serum levels of the inflammatory cytokines.

Conclusions: Elevated serum IL-1â, IL-6 and TNF-â levels in our CRF patients on regular maintenance HD indirectly confirm importance of HD in amplification of the chronic inflammation substantially depend on the duration of dialysis treatment.

A2.03
Anticardiolipin antibody in acute myocardial infarction
A. Sotoodeh Jahromi, M. Shojaei and N. Akbari
Jahrom University of Medical Science, Jahrom, Islamic Republic of Iran

Background: Antiphospholipid (aPL) antibodies – both the lupus anticoagulant and anticardiolipin antibodies – are closely associated with arterial and venous thrombosis. The purpose of the present study was to determine whether the presence of aPL antibodies, namely, anti-cardiolipin (aCL) antibodies, are a risk factor for acute myocardial infarction (MI).

Methods: This case control study was carried out on 45 patients with acute myocardial infarction and 45 age, sex and MI risk factors matched healthy persons (control group) referring to peymeh hospital of Jahrom between 2006 March to 2007 February. Using commercial enzyme-linked immunosorbent assay (ELISA) kit, the presence of anti-cardiolipin (aCL) IgG in the patients’ and the controls’ sera was determined.

Results: The prevalence of aCL IgG in the patient 62.29 ± 13.245 years (including 68.89% men and 31.10% women) and in the control group 61.71 ± 12.297 years (including 53.30% men and 47.70% women), was 18.60% and 11.60% respectively (p = 0.366).

Conclusion: This study shows no significant association between presence of aCL IgG and acute myocardial infarction. Future larger studies may be required to determine the precise role of aCL IgG in the pathogenesis of different subtypes of ischaemic heart diseases and Myocardial infarction.

A2.04
The interleukins IL-13 and IL-18 in the primary breast cancer tumor tissue
N. Srahovic1, Z. Muminovic2, J. Muanovic-Mustedanagic2, Z. Muminovic2, L. Begic1 and A. Softic1
1Department of Biochemistry, University of Tuzla, Tuzla, Bosnia and Herzegovina, 2University Clinical Center Tuzla, Tuzla, Bosnia and Herzegovina

Some recent literature data suggest possible role of interleukins in the pathogenesis of breast cancer. The aim of this study was to investigate the presence and the expression levels of the IL-13
and IL-18 in the primary breast cancer tumor in relation to the unchanged breast tissue and pathohistological factors (lymph node status, tumor size, histological grade), estrogen and progesterone receptor status. The expression levels of IL-13 and IL-18 in the primary tumor tissue and unchanged surrounding tissue in 50 breast cancer patients and in breast tissue in 20 patients with benign breast diseases were determined using three-step immunohistochemical staining, as well as the hormones receptor status. IL-13 and IL-18 were present in breast cancer tumor, surrounding tissue and breast tissue in patients with benign breast disease. The expression of these interleukins was significantly higher in breast cancer tumor compared with surrounding tissue (p < 0.05). In addition, the IL-13 expression was significantly higher in breast cancer tumor compared with breast tissue in patients with benign breast diseases (p < 0.01), whereas IL-18 expression was not. No significant differences between IL-13 and IL-18 expressions were noticed considering the lymph node status. In relation to pathohistological factors no significant correlations in both interleukins expression were found, excluding significant correlation between IL-13 expression and tumor size in patients with lymph node-negative breast cancer (p = 0.05). However, expression level of analyzed interleukins in tumors in lymph node-negative patients was inversely correlated to hormone receptors, but not statistically significant.

A2.06
Autoactivation of MASP-2: Role of exosite interactions

1 Eötvös Loránd University, Laboratory of Structural Chemistry and Biology, HAS-ELTE Protein Modelling Group, Budapest, Hungary, 2Institute of Enzymology Hungarian Academy of Sciences, Budapest, Hungary, 3Eötvös Loránd University, Laboratory of Structural Chemistry and Biology, Institute of Chemistry, Budapest, Hungary

The complement system is a key element of innate immunity in vertebrates. A cascade of enzyme reactions, triggered by a recognition protein complex, results in opsonization and destruction of the pathogen cell. The recognition complexes of the classical and lectin pathways of complement consist of structurally related proteins and act analogously. MASP-2, a modular serine protease of the recognition complex of the lectin pathway is responsible for the first proteolytic event of the cascade: its autoactivation. Our aim is to explore the structural background of the narrow substrate-specificity as well as autoactivation of MASP-2 and other related enzymes in atomic details. We report the structure of the active form of the catalytic fragment of MASP-2 crystallized in a new crystal form. The structure was refined to 2.5 Ångstrom resolution. In the structure there is enzyme-product relationship between two symmetry-related molecules. In addition to the contacts corresponding to a canonical serine protease-peptide interaction there are extended exosite interactions as well between the two MASP-2 molecules. Exploring these exosite regions should help us to understand the high selectivities and high autoactivation rates of MASP-2 and C1r, two related activation-initiating enzymes of the lectin and the classical pathways, respectively. Support from EMBL and Hungarian Scientific Research Fund (OTKA) grants F67937 and K68408 is acknowledged.

A2.08
Electrostatic allostery – A novel mechanism for neutralization of protein antigens by antibodies

J. Dmitrov1, L. Roumenina1, J.-L. Plantier2, B. Atanasov3, S. Kaveri1 and S. Lacroix-Desmazes4
1INSERM U872, Centre de Recherche des Cordeliers, Paris, France, 2Faculté de Médecine RTH Laennec, Université de Lyon, Lyon, France, 3Institute of Organic Chemistry, Sofia, Bulgaria

The binding of antibodies usually causes steric hindrance of functionally important sites on their target molecules. In the present study by using theoretical and experimental approaches, we demonstrate a unique role for protein electrostatics in neutralization of the coagulation factor VIII (FVIII) by a human pathogenic antibody – BO2C11. Kinetic and thermodynamic analyses of BO2C11 binding to FVIII indicated that this interaction is characterized by an ionic strength dependency that is uncommon for other protein-protein interactions. By using continuum electrostatic calculations, we further demonstrated that BO2C11 binding to FVIII induces long-distance perturbations in the electrostatic potential and in the local electrostatic parameters (degree of ionization, proton affinity and electrostatic energy) of charged residues in the C2 domain of FVIII. The effects were not consecutive of structural alternations in C2. The distant changes in the electrostatic parameters were not delocalized, but affected predominantly the residues that constitute a binding site for von Willebrand factor (VWF) – a protein essential for FVIII stability and half-life in the circulation. Replacement of the in silico predicted electrostatic hotspots by alanine by site directed mutagenesis of FVIII resulted in considerable decrease in the binding to VWF. Thus, the allosteric perturbation of surface electrostatics at a VWF binding site on C2 could explain the pathogenic effect of the BO2C11 in preventing FVIII binding to VWF. Our findings suggest that some antibodies modify their targets by alteration of protein surface electrostatics at a long-distance from the binding site.

A2.09
Different molecular mechanisms of alternative complement pathway dysregulation result in common glomerular endothelial damage and contribute to the pathogenesis of the atypical hemolytic uremic syndrome

1Centre de Recherche des Cordeliers, INSERM UMR 872, Paris, France, 2INSERM U45, Hôpital Necker, Paris, France, 3Academic Renal Unit, University of Bristol, Southmead Hospital, Bristol, UK, 4Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges-Pompidou, Service d’Immunologie Biologique, Paris, France

Complement is a major innate immune defense against pathogens, tightly regulated to prevent host tissue damage. The atypical hemolytic uremic syndrome (aHUS) is characterized by endothelial damage leading to renal failure and is highly associated with abnormal alternative pathway regulation. We characterized the functional consequences of 4 aHUS-associated mutations in Factor B (FB) and C3 (forming the alternative
A2.10
Abstract withdrawn

A2.11
Expression of endothelial selectin ligands on leukocytes following repeated dives in SCUBA divers

Y. Cikes Culic1, A. Markotić1, M. Ljubkovic2, T. Bresković2, J. Marinovic Ljubkovic2 and Z. Dujic2
1Department of Medical Chemistry and Biochemistry, University of Split School of Medicine, Split, Croatia 2Department of Physiology, University of Split School of Medicine, Split, Croatia

Leukocyte cell surface adhesion molecule CD11b, decorated with CD15s, plays a critical role in the regulation of β2 integrin function during neutrophil endothelial transmigration. Hyperbaric oxygenation reduces neutrophil-endothelial cell adhesion, which is mediated by Mac-1 (CD11b/CD18) β2-integrin. This study investigated the expression of CD15 and CD15s, on leukocytes following repeated trimix (a mixture of oxygen, helium and nitrogen) dives in two series: in the first series seven divers performed six consecutive dives from 55–80 m, while in the second series seven divers performed three consecutive dives from 63–65 m. Five divers took part in each of the two series. CD15s and CD15s were determined before and after the 1st and the last dive. Leukocyte subpopulations were not elevated after either the first or last dives in series I. Only CD15+ CD15s+ granulocytes were significantly decreased after the 1st dive (p = 0.006). In the second series the monocyt proportion was increased (p = 0.014) and lymphocytes decreased (p = 0.020) within the total leukocyte population, while CD15s+ monocytes and CD14+ CD15s+ granulocytes were elevated (p = 0.019, and p = 0.018, respectively) after the 1st dive. CD15+ CD14+ granulocytes were decreased after the last dive in the second series (p = 0.048 and 0.017, respectively), while CD15s+ granulocytes were decreased only after the last dive in the second series (p = 0.006). The current findings of decreased endothelial selectin ligand CD15s expression on CD15+ granulocytes after certain dives point to the role of this subpopulation in the endothelial damage prevention.

A2.12
ER aminopeptidase 1 single Nucleotide Polymorphisms can influence antigenic peptide processing

I. Enoumachiou1, R. Kemal1, I. York2, Y. Goto3, M. Tsujimoto3, A. Hatorii4 and Efstratios Stratatsi-Stratatsi5,6
1National Centre for Scientific Research “Demokritos”, IRRP, Protein Chemistry laboratory, Agia Paraskevi, Greece, 2Department of Microbiology and Molecular Genetics, Biomedical Physical Sciences, Michigan State University, East Lansing, MI, US, 3RIKEN Wako, Laboratory of Cellular Biochemistry, Saitama, Japan, 4Department of System Chemotherapy and Molecular Sciences, Graduate School of Pharmaceutical Sciences, Kyoto University, Saky, Kyoto, Japan

ERAP1 is an ER aminopeptidase that plays crucial roles in the generation and destruction of MHC class I-restricted antigenic peptides. Recently, large population studies have linked coding ERAP1 single nucleotide polymorphisms (SNPs) with predisposition to autoimmune diseases and virally induced cancer. We hypothesized that this link is due to ERAP1’s role in antigen peptide processing, through the aberrant generation or destruction of key antigenic epitopes that initiate or sustain autoimmunity or elicit anti-viral responses. To test this hypothesis we overexpressed and purified allelic versions of ERAP1 and tested their ability to generate antigenic peptides in vitro. We found that, for several but not for all of the epitopes tested, major antigenic peptide generation rates were dependent on the ERAP1 allele used and in patterns that were also epitope dependent. Furthermore, the generation rate of specific antigenic peptides suspected to be linked with autoimmunity was highly dependent on the presence of the specific ERAP1 SNPs also linked with autoimmune disease. Our results suggest that ERAP1 SNPs may impose specificity changes in the enzyme. Furthermore, our findings provide support to the concept that antigen peptide processing is the biochemical mechanism behind the link of ERAP1 SNPs and autoimmune disease predisposition.

A2.13
PolyCTLDesigner: A program for designing cytotoxic T-cell polyepitope immunogens

D. V. Antonets, A. Z. Maksyutov and S. I. Bazhan
State Research Center of Virology and Biotechnology “Vector”, Theoretical, Novosibirsk region, Koltsovo, Russian Federation

T-cell epitopes are important tools for diagnosis and treatment of infectious, autoimmune or cancer diseases as well as for the development of novel polyepitope vaccines. Although immunogenicity of the peptide is known to be crucially determined by its MHC-binding affinity it was also shown to be dependent on amino acid residues which flank the epitope and affect efficiency of its proteasomal release and TAP-dependent transporting into endoplasmic reticulum. Here we present a program that tries to take these considerations into account when designing primary structure of cytotoxic T-cell immunogen. The PolyCTLDesigner software constructs polyepitope CTL immunogen selecting superior spacers for every pair of selected epitopes, choosing appropriate epitope matchings and selecting optimal arrangement of epitopes within designed construction using graph theory approach, thus increasing efficiency of polyepitope processing and favoring presentation of target epitopes. It also tries to minimize the number of “non-target” epitopes within desired polyepitope immunogen and is able to assist in collecting the set of peptides covering selected HLA repertoire with desired rate of redundancy using known genotypic HLA allele frequencies data together with either known
or predicted specificity of selected peptides towards different allo-
types of HLA class I molecules. PolyCTLDesigner is integrated with
previously created T-cell epitope prediction software named
TEpredict. Both programs were written in Python programming
language. They could be freely downloaded from TEpredict pro-

**A2.14**

Studies of structural and functional properties of orthopoxviral CrmB proteins

T. V. Tregubchak and S. N. Shchelkunov

State Research Center of Virology and Biotechnology “Vector”,
Novosibirsk region, Koltsovo, Russian Federation

CrmB proteins of variola (VARV), monkeypox (MPXV) and
cowpox (CPXV) viruses were produced in baculovirus expression
system. Despite sharing high sequence identity, CrmB proteins of
VARV, MPXV and CPXV differed in their efficiencies of inhibiting
cytotoxic effect of human, mouse and rabbit TNFs in L929
mouse fibroblast cells. Of these CrmBs only VARV-CrmB was
shown to have pronounced protective effect in the experimental
model of LPS-induced shock in SPF BALB/c mice. Gel-filtration of
the lysates of S21 insect cells infected with recombinant bacu-
lovirus containing the gene coding for either MPXV- or CPXV-
CrmB revealed that TNF-neutralizing activity was mainly associ-
ated with fractions whose molecular weight was about 90 kDa,
corresponding to homodimers of CrmB proteins. Whereas gel-fil-
trations of similar preparations containing recombinant VARV-
CrmB protein revealed that TNF-neutralizing activity was pre-
dominantly associated with the fraction of high molecular weight
(> 500 kDa), corresponding to large multimeric complexes of
VARV-CrmB. CrmB proteins consist of N-terminal TNF-binding
domain and C-terminal chemokine binding one. To study influ-
ences of these domains and their species-specific distinctions on
biological activity and some physicochemical characteristics of
VARV and CPXV-CrmB, we modelled spatial structures of these
proteins and developed several mutant and truncated forms of
these CrmBs. Designed mutant forms of VARV- and CPXV-
CrmB were also produced in baculoviral expression system. And
now properties of these recombinant proteins are being compara-
tively studied. The work was supported by Russian Foundation
for Basic Research (grant 090400055a).

**A2.15**

Biochemical evidence for specific pairwise interactions of mouse NKR-P1B/D/Clr-b
receptors engaged in lectin – lectin interactions

P. Haas1, K. Kotynkova1, O. Vanek1, P. Pompach2, P. Novak2,
M. Holubova2, Petra Cedalova1 and K. Bezouska1

1Charles University, Faculty of Science, Prague, Czech Republic,
2Academy of Science of Czech Republic, Institute of Microbiology
v.v.i., Prague, Czech Republic

Mouse NKR-P1B/D/Clr receptor pair represents a recently dis-
covered example of lectin – lectin interactions. In order to study
this interaction by biochemical techniques, we have amplified the
individual cDNA clones for the receptors by RT-PCR from B6/
BL mice spleens and transfected DNA fragments coding for the
extracellular ligand binding domains into pET-30 bacterial expres-
sion vectors. During expression proteins precipitated into inclu-
sion bodies, from which they could be refolded in vitro. Using ion
cyclotron resonance mass spectrometry, we have confirmed the
quality of the refolding for Clrb checking the disulfide bonding.

In order for the NKR-P1B to fold properly, the third cysteine
which does not fit into the pattern usual for this family of recep-
tors was substituted for serine. The resulting C118S NKR-P1B,
just as the Clrb, was shown to be monomeric in solution. More-
over, we produced uniformly 15N-labeled variants of these pro-
teins, and measured 1H/15N-HSQC spectra providing additional
evidence for proper folding of these proteins. Using gel filtration and
analytical ultracentrifugation we were unable to prove the interac-
tion between Clrb and NKR-P1B in these monomeric forms. Using
SPR technique a specific weak interaction was shown to occur
only at pH = 4 while at physiological pH no interaction was
observed. Further efforts to prepare the receptors in dimeric
forms in which they appear on the membrane, and experiments to
see if and under which conditions these forms interact will follow.
Supported by grants from Ministry of Education of Czech
Republic (MSM_21620808 and 1M0505), and from The Grant

**A2.16**

Association of Fcγ receptor IIa (CD32a) with lipid rafts regulates ligand binding activity

S. Bournazos1, S. Hart2, L. Chamberlain3, M. Glennie4 and
I. Dransfield1

1University of Edinburgh, MRC Centre for Inflammation
Research, Edinburgh, UK, 2Hall York Medical School/University
of Hull, Cottingham, UK, 3University of Edinburgh, Edinburgh,
UK, 4University of Southampton, Southampton, UK

Binding of immunoglobulins to myeloid cells via Fc receptors is a
key event in the control of innate and acquired immunity. Fcγ
receptor IIa (CD32a) is a receptor for multivalent IgG expressed
by myeloid cells and its association with microdomains rich in
cholesterol and sphingolipids, termed as lipid rafts has been
reported to be essential for efficient signalling. However, for many
myeloid cell types, ligand binding to CD32a is suppressed by as
yet undefined mechanisms. In this study, we have examined the
role of CD32a-lipid raft interactions in the regulation of IgG
binding to CD32a. CD32-mediated IgG binding was measured by
flow cytometry using fluorescent-labelled IgG complexes in several
cell types. We have introduced point mutations in the transmem-
brane and juxtamembrane region of CD32 and assessed the
association of these mutants with lipid rafts by confocal immuno-
fluorescence and extraction and analysis of detergent-resistant
domains. Disruption of lipid raft structure following depletion or
sequestration of membrane cholesterol greatly inhibited CD32a-
mediated IgG binding. Furthermore, specific CD32a mutants,
which show reduced association with lipid rafts (A224S and
C241A) displayed decreased levels of IgG binding compared with
wild type CD32a. In contrast, constitutively lipid raft-associated
C241A receptor IIa (CD32a) is a receptor for multivalent IgG expressed
by myeloid cells and its association with microdomains rich in
cholesterol and sphingolipids, termed as lipid rafts has been
reported to be essential for efficient signalling. However, for many
myeloid cell types, ligand binding to CD32a is suppressed by as
yet undefined mechanisms. In this study, we have examined the
role of CD32a-lipid raft interactions in the regulation of IgG
binding to CD32a. CD32-mediated IgG binding was measured by
flow cytometry using fluorescent-labelled IgG complexes in several

**A2.17**

Searching for new interaction partners and substrates of tissue transglutaminase in
differentiated NB4 cells

I. Nemet, K. Csomós, E. Csösz, L. Fész and Z. Balajthy

University of Debrecen, Department of Biochemistry and
Molecular Biology, Debrecen, Hungary
Tissue transglutaminase (TG2) is a member of the transglutaminase family of enzymes that covalently cross-link proteins in a Ca²⁺ dependent manner. TG2 also has a guanosine triphosphatase (GTPase) activity, protein disulﬁde isomerase activity and protein kinase activity. TG2 is present in various cellular compartments including cytoplasm, nucleus, and extracellular matrix and involved in the terminal differentiation and immune cells. The NB4 is an acute promyelocytic leukemia cell line which could be differentiated into neutrophil granulocytes with all trans retinoic acid treatment. We have published that during differentiation process of NB4 cells the expression of TG2 was highly increased and contributed to the expression of GP91phox. Our working hypothesis is that TG2 could perform these distinct functions through its cross linking activity and/or mediate it by protein-protein interaction. Our aim is to identify new substrates and interaction partners in differentiating NB4 cells. For identiﬁcation of substrates of TG2, differentiated cells were permeabili-zed and an artiﬁcial TG2 substrate (biotinilated pentylamine) was added to it. After cell lysis, the biotinilated proteins were puriﬁed using streptavidin beads, separated with 2D-PAGE and then identiﬁed with HPLC-MS/MS. With this method we could identify several new possible substrates of TG2. For identiﬁcation of interacting partner proteins of TG2, after lysis of differentiated cells immunoprecipitation were carried out standard procedures. The precipitated proteins were separated with SDS-PAGE or with 2D-PAGE. Gels were stained with Sypro Ruby and protein band/spots were identiﬁed by HPLC-MS/MS analysis.

A2.18 Carboxylated calixarenes bind strongly to CD69 and protect CD69⁺ killer cells from apoptosis induced by tumor cell surface ligands

D. Adamek, A. Kádečk, R. Snajdrová, K. Krenk, M. Vancurová, P. Lhoták, V. Kren, K. Bezouska
1Faculty of Science Charles University, Prague, Czech Republic, 2Institute of Molecular Genetics, Academy of Sciences of Czech Republic, Prague, Czech Republic, 3Institute of Chemical Technology, Prague, Czech Republic, 4Institute of Microbiology, Academy of Sciences of Czech Republic, Prague, Czech Republic

CD69 is expressed at cell surface as homodimeric receptor belonging to C-type family. We have recently identiﬁed carboxylated calixarenes as a new class of noncarbohydrate ligands for CD69 receptor. Binding activities of synthesized carboxylated calixarenes were tested using plate binding, plate inhibition, and plate precipitation assays using recombinant human CD69 protein. In direct binding assays we have employed the principle of ﬂuorescence quenching. Human N-PBMC were isolated on ficoll-lixarene had the highest afﬁnity in the direct binding assays, and proved to be the most speciﬁc inhibitor identiﬁed so far in receptor precipitations and cellular activation experiments. Moreover these compounds also proved effective at protection of CD69-high lymphocytes from apoptosis triggered by a multivalent ligand SiaTnTRI2 or antibody crosslinking. Carbohydrated calixarenes investigated here set a new paradigm for noncarbohydrate ligands for CD69 making them attractive candidates for protection of killer cells in combine animal tumor therapies. This work was supported by Ministry of Education of Czech Republic (MSM 0021620808 and 1M0505), and by the Grant Agency of Czech Republic.

A2.19 Clearance of dying autophagic cells induces the inﬂammosomal pathway in human and primed mouse macrophages

G. Ayna, G. Petrovski and L. Fesus
University of Debrecen, Biochemistry and Molecular Biology, Debrecen, Hungary

Autophagy is now recognized as possible inducer of a distinct cell death mechanism happening under various circumstances (Mizushima N., Nature Reviews, 2008). Clearance of dying autophagic (AU) MCF7 cells but not living or apoptotic ones can lead to pro-inﬂammatory response in human macrophages (Petrovski et al., Autophagy, 2007). These dying cells could induce activation of caspase-1 (IL-1β converting enzyme) as early as 1 hour after being co-incubated with human blood-bom macrophages. Upon observations in human system, we decided to establish a mouse model and used the mouse Ba/F3 cell line (IL-3 dependent BM derived pro-B cells) as a possible AU cell clearance model. Ba/F3 cells have been shown to undergo death under IL-3 depletion with signs of autophagy (Wirawan and Vandenabeele et al., 15th Euroconference, ECDO, 2007). Our recent results show that AU Ba/F3 cells but not living cells can induce the IL-1 release from LPS primed mouse peritoneal macrophages. According to our results, Ba/F3 cells are partially dying after IL-3 depletion. Furthermore, rapamycin (m-TOR inhibitor) treatment trigger more cell death during IL-3 depletion compared to non-treated cells. On the other hand, LC3H levels are elevated upon IL-3 depletion with/without rapamycin treatment compared to living cells. These observations may indicate the importance of autophagy in cell death. Mechanisms behind these observations will be clariﬁed by using knock out mice system to deduce the involvement of the members of the inﬂammosomal pathway (e.g. ASC/NALP3) as well as to exclude the involvement of other inﬂammatory pathways (Myd88) in the process of this unusual immunogenic response.

A2.20 Apoptotic human cells inhibit migration of granulocytes via release of lactoferrin

University of Edinburgh, MRC Centre for Inflammation Research, Edinburgh, UK

Apoptosis is a noninflammatory, programmed form of cell death. One mechanism underlying the non-phlogistic nature of the apoptosis program is the swift phagocytosis of dying cells. The objective of this study was to determine how apoptotic cells selectively attract mononuclear phagocytes and not granulocytes, the professional phagocytes that accumulate at sites of inﬂammation. In order to address this, Burkitt’s lymphoma (BL), a non-Hodgkin’s lymphoma, was employed as an in situ model of apoptosis. BL is characterised by a high rate of apoptosis and the selective inﬁltration of monocytes. However, no neutrophils are present in its stroma. In this study, we found that BL cells

Abstracts

as well as human cell lines of diverse lineages express lactoferrin, a 80 kDa pleiotropic glycoprotein, upon apoptosis induction. Lactoferrin was demonstrated to inhibit the chemotaxis of granulocytes but not mononuclear phagocytes, both in vitro and in vivo. This antiinflammatory activity of lactoferrin is independent of its iron-saturation status and does not alter intracellular calcium levels. Lactoferrin acts by preventing the acquisition of granulocyte activation status and morphology following agonist stimulation and affects granulocyte signaling pathways, such as phosphorylation of MAP kinases that regulate cell adhesion and motility. Together, our results identify lactoferrin as an antiinflammatory component of the apoptosis milieu and place it as one of the few till now identified molecules that act as negative regulators of granulocyte migration, eliciting in this way, tremendous therapeutic applications in the control of inflammatory conditions. J Clin Invest (2009)119(1):20–32.

A2.21 SKN-1 transcription factor required for pathogen resistance in Caenorhabditis elegans
D. Papp, P. Csermely and Csaba Soti
Department of Medical Chemistry, Semmelweis University, Budapest, Hungary

Oxidative stress is a major factor in aging, while antioxidant response is an important determinant of longevity. In recent years a relationship between oxidative stress and immunity has been revealed in humans and in various experimental models. Lately, oxidative stress during bacterial infection has been described in Caenorhabditis elegans. Reactive oxygen species are released both by invading bacterial pathogens and by NADPH oxidases from the C. elegans intestine. The mounting of oxidative stress response has been confirmed by the induction of antioxidant enzymes in worms during infection. Many of these enzymes are regulated by the stress inducible FOXO transcription factor, DAF-16. However, other antioxidant regulators such as the NRF2 ortholog SKN-1 transcription factor have not been investigated in C. elegans immunity. It is expressed both in ASI neurons and in the intestine of the nematode. Both our knock-out and RNAi knock-down experiments showed that in absence of all three isoforms nematodes displayed a highly elevated susceptibility to infection by Pseudomonas aeruginosa. To further investigate the role of SKN-1 in pathogen resistance, we employed oxidative stress (hydrogen peroxide pretreatment), which significantly enhanced the survival of worms against P. aeruginosa. The hormetic effect of oxidative stress was partially prevented in the absence of either SKN-1 or DAF-16. Moreover, the activation of SKN-1 during infection has been demonstrated by the induction of a SKN-1-dependent reporter. Thus, our data shows that SKN-1 is required for pathogen resistance and further strengthens the cross-talk between oxidative stress responses and immunity in C. elegans.

A2.22 Cytokine assessment in chronic kidney disease by xMAP technology
L. Albucescu1, E. Rusu1, C. Tanase1 and R. Albucescu1
1"Victor Babes" National Institute of Pathology, Biochemistry-Proteomics Laboratory, Bucharest, Romania, 2Fundeni Clinical Institute, Bucharest, Romania

Background: Endothelial dysfunction represents the initiating event in the atherosclerosis process, playing a crucial role in the development of cardiovascular and renal diseases, as a pathogenic link between vascular and renal involvement. Chronic kidney disease (CKD) can determine metabolic changes that may lead to increased oxidative stress or an enhanced inflammatory state, changes that can determine endothelial dysfunction.

Aim: To evaluate and validate new investigation methods for early stages of vascular dysfunction using new cellular and molecular biology techniques.

Methods: Multiplex analysis of cytokine levels using xMAP technology was performed on serum samples from 20 CKD patients and 20 controls; IL-6, IL-10 and TNFα were analyzed on Luminex® 200™ (Luminex Corp., USA) using Milliplex® MAP Human Cytokine/Chemokine Panel (Millipore, US). Multiplex data acquisition was performed using xSTARStation 2.3 (Applied Cytometry Systems, UK).

Results: IL-6 and TNFα serum levels were increased in CKD-patients (6.042 pg/ml ± 0.888 versus 3.163 pg/ml ± 0.473, p < 0.01, respectively 14.56 pg/ml ± 1.11 versus 7.463 pg/ml ± 0.883, p < 0.001). IL-10 was decreased in CKD samples (4.528 pg/ml ± 0.984 versus 12.11 pg/ml ± 4.964, p < 0.05). IL-6 and TNFα increase with stage, while for IL-10 no trend was visible.

Conclusions: The use of multiplex xMAP technology made possible the simultaneous quantitation of serum levels for 3 relevant molecules in CKD. IL-6 and TNFα showed a good potential of prediction with ROC areas of 0.76 with p = 0.02 for IL-6 and 0.865 with p = 0.001 for TNFα.

Acknowledgment: The present work was supported from Grant 4.2-171/2008.

A2.23 Correlation of the changes of blood plasma albumin and t-lymphocytes phenotype to tumor stage in gastrointestinal patients
I. Kalina1, E. Kirilova1, T. Zvagule2, G. Kirilov1 and N. Kurjane2
1Daugavpils University, Daugavpils, Latvia, 2Riga Stradinus University, Riga, Latvia

The original fluorescent probe ABM (an amino derivative of benzanilide) was used to characterize the membranes of lymphocytes and blood plasma albumin recovered from colorectal and gastric cancer patients with Stage II-IV. The fluorescence intensity of ABM in patients differ from the values seen from healthy control and reflected specific differences before and after medically indicated surgical treatment and corresponds to cancer stage. ABM fluorescence associated with select immunological parameters (CD4+/CD8+ ratios, lymphocyte counts etc.) in the cancer patients. Surgical treatment elevates immune state. With progress of cancer stage, CD4+, CD4+/CD8+ gradually decreased, while CD8+ gradually increased. The preoperative immune state of patients is negatively related to cancer stage. An aim of these studies was to elucidate criteria for clinical interpretation (i.e. of any alterations in albumin physicochemical parameters and/or lymphocytes functional activity) using ABM as a analytical agent. There was a seemingly excellent agreement between changes in ABM spectral parameters and both clinical and pathological estimate of the severity of disease in patients with solid tumors.

Acknowledgement: This work was supported by the European Structural Funds (Project Nr. 2009/0205/1DP/1.1.1.2.0/09/APIA/ V1AA/152)
A2.24 Glycoconjugates containing immunoactive LELTE peptide: Effect of glycosylation on cellular activation and natural killing

A. Kadk1, D. Adamek1, O. Renaudet2, K. Krenek3, I. Bossu2, P. Dumy2, O. Yanek1, D. Kavan1, R. Gazak1, V. Kren1 and K. Bezouska1
1Department of Biochemistry, Charles University, Prague, Czech Republic, 2Département de Chimie Moléculaire, Université Joseph Fourier, Grenoble, France, 3Institute of Microbiology, Academy of Sciences of Czech Republic, Prague, Czech Republic

CD69 is a widespread receptor of the immune system cells. Although a physiological ligand for this receptor is unknown, we have previously proved its affinity towards a range of ligands including calcium, carbohydrates, and charged compounds such as carboxylated calixarenes. Moreover, a pentapeptide sequence LELTE derived from a mycobacterial heat shock protein hsp65 has been recently identified as a ligand for CD69 representing a “danger” signal for the immune system. However, this peptide is not immunoactive per se, but only after its presentation within the multivalent environment of its parent protein, or after artificial dimerization using bifunctional reagents. Here we describe an entirely new way to present this peptide through attachment to a cyclopeptidic RAFT scaffold (K-K-P-G)2 through the ε-amino groups of lysine residues, alone or in combination with a carboxylated calixarene. The multivalent environment of such scaffolds to precipitate the CD69 receptor or to activate CD69-positive cells is enhanced in compounds, which possess both peptide and carbohydrate epitopes. These compounds efficiently activate natural killer lymphocytes, but are inactive from the point of view of activation-induced apoptosis of lymphocytes. These unique properties make the combined peptide / carbohydrate RAFTs highly suitable for evaluation in animal tumor therapies and predict them to be readily available and efficient immunoactivators. Supported by the Univ. Joseph Fourier, CNRS, Ministry of Education of Czech Republic (LC06010, MSM_0021620808, and 1M0505), Grant Agency of the Czech Academy of Science and Czech Grant Agency.

A2.25 Role of the mannose-binding lectin-2 X/Y (MBL-2 x/y) polymorphisms in patients with rheumatoid arthritis

H. Yaroglu Yildirim1, L. Ayaz1, A. Biçer2 and L. Tamer1
1Mersin University, Biochemistry, Mersin, Turkey, 2Mersin University, Physical Medicine, Mersin, Turkey

The mannose-binding lectin (MBL) pathway of innate immunity is part of the first line of defense against microorganisms. MBL recognizes and binds to carbohydrate patterns on the surface of microorganisms leading to complement activation by the MBL-associated serine protease2 (MASP-2). Rheumatoid arthritis (RA) is an immune disorder in which the immune system mistakes normal tissues for foreign ones and attempts to neutralize and rid the body of the perceived threat. Although some experts theorize that genetic and environmental factors play a role, the factors that lead to this self-attack and subsequent induction of the inflammatory process remain unknown. We aimed to investigate whether profile of MBL-2 X/Y genotyping may be associated with the risk of RA. The study population consisted of 59 patient with RA and 80 unrelated healthy individuals. Blood was collected in EDTA-containing tubes and DNA was extracted from leukocytes by High Pure PCR template preparation kit. Genotyping of MBL-2 polymorphisms were detected by using a LightCycler MBL-2 mutation detection kit in real-time PCR. No association was observed between the MBL-2 X/Y genotype and RA. The frequencies of XX, XY and YY genotypes were 50.8%, 44.1% and 5.1%; in cases and 56.3%, 38.8% and 5% in controls. Further studies on larger groups are needed to determine the prevalence of MBL-2 X/Y polymorphisms in patients with RA.

A2.26 Intravenous immunoglobulins as drug delivery system for target anticancer combined therapy

Z. Kejkl1, T. Briza1, V. Kraľ1, J. Králova1, P. Poučková4, A. Kraľ4 and P. Martasek4
1Institute of Chemical technology, Analytical Chemistry, Prague, Czech Republic, 2Zentiva R & D (sanofi-aventis group), Prague, Czech Republic, 3Academy of Sciences of the Czech Republic, Institute of Molecular Genetic, Prague, Czech Republic, 4Charles University, First Faculty of Medicine, Prague, Czech Republic

Immunotherapy can induce statistically significant inhibition of tumor growth, invasiveness, angiogenesis and prolongation of survival time. Its combination with anticancer destroying therapies (chemotherapy and photodynamic therapy) can be effective way for tumor destruction without next cancer recurrence. Therefore, we designed supramolecular multimodal system based on intravenous immunoglobulins for target transport of anticancer drugs and combined therapy. Their study in mice model showed its excellent anticancer activity.

Acknowledgment: This work was supported by grants from the Ministry of Education of the Czech Republic (Grants MSMT 1M 683705002, MSM600613707, MSM0021620857, AV0Z50520514; Projects LC 512, LC06077, and MSM 6036137307) and by the Grant Agency of the Czech Republic (Grant 203/09/1311) and, in part, by Project AV0Z50520514 and Grant KAN200200651 awarded by the Grant Agency of the Academy of Sciences of the Czech Republic.

A2.27 Adiponectin limits experimental autoimmune encephalomyelitis by suppressing the differentiation of CD4+ cells into Th17 cells

Y. Guo1, R. Zhang1, K. S. L. Lam2 and A. Xu3
1Department of Medicine, The University of Hong Kong, Hong Kong, Hong Kong, 2The University of Hong Kong, Department of Medicine; the Research Center of Heart, Brain, Hormone and Healthy Ageing (HBHA), Hong Kong, Hong Kong, 3The University of Hong Kong, Department of Pharmacology; Pharmacy, Hong Kong, Hong Kong

Experimental autoimmune encephalomyelitis (EAE) has been identified as an important and most commonly used animal model for investigating multiple sclerosis (MS), which is an inflammatory disease of the central nervous system (CNS). Previous studies showed that leptin can worsen the symptoms of EAE by increasing the production of pro-inflammatory cytokines. Since adiponectin is an anti-inflammatory adipokine which acts in an antagonistic manner to leptin, we hypothesize that adiponectin may reduce the symptoms of EAE and block the development of this disease. Our results showed that adiponectin knock-out mice are more susceptible to EAE development with higher clinical scores and disease incidence compared to wild type littermates. In addition, there are more inflammatory infiltrates into spinal cords in adiponectin knock-out mice. Multiple-cytokine
profiling of the splenic cells from both types of mice with EAE demonstrated that interleukin-17 (IL-17) plays an important role in this process. Accordingly, in the spinal cords of adiponectin knock-out mice, the gene expressions of IL-17 and its related cytokines were significantly elevated compared to the wild type littermates. Furthermore, our ex vivo study demonstrated that recombinant adiponectin can inhibit the differentiation of CD4^+ T cells into Th17 cells, resulting in reduced production of IL-17 and IL-6. In summary, these results demonstrate that adiponectin can limit EAE by suppressing the development of Th17 cells and the production of IL-17.

**A2.28**

**New hybridoma technology based on antigen-specific immunoglobulin receptors**

M. Tomita and K. Tsumoto

Mie University, Division of Chemistry for Materials, Graduate School of Engineering, Tsu, Japan

There are two types of immunological responses in vertebrates for defense against invading substances and pathogens. B lymphocytes, known to generate antibodies that can specifically bind to foreign antigens, are able to mature and express antigen-specific immunoglobulin receptors on their surfaces after repeated antigen stimuli. To generate hybridoma cells continuously secreting monoclonal antibodies, B lymphocytes must be somatically fused with cancerous myeloma cells. For this purpose, the expressed receptors play pivotal roles in selecting B lymphocytes generating specific antibodies to target antigens. We have established a new hybridoma technology to yield specific monoclonal antibodies against antigens of interest with high specificity and selectivity. This new technology consists of three critical steps. Antigen-sensitized B lymphocytes are pre-selected in advance for antigens based on immunoglobulin receptors on B lymphocytes. The antigen-selected B lymphocytes are then combined with myeloma cells by exploiting strong and specific interactions between biotin and avidin. Finally, B lymphocyte-myeloma cell complexes are selectively fused by electrical pulses. This entire pathway could be successfully confirmed on the basis of immunofluorescence analysis. The new technology confers at least a 5-40-fold increase in efficiency over that obtained with the poly(ethylene glycol)-mediated method. The advanced technology may also be applicable for large-scale generation of human antibodies for medical purposes using transgenic mice and for selective production of stereo-specific monoclonal antibodies against native structural antigens using antigen-expressing myeloma cells.

**A2.29**

**Dimeric thiourea linked GlcNAc are molecular switches that trigger the antitumor potential of natural killer cells due to a sequential cooperative engagement of activating receptor CD161 linking innate and adaptive immunity**

K. Bezouska^1, K. Karel^1, M. Hynek^1, K. Daniel^1,

K. Hofbauerova^2, N. Michal^2, R. Daniel^2, K. Marek^2, S. Jan^2,

F. Anna^2, K. Vladimir^2 and C. Michal^3

Charles University, Prague, Czech Republic, ^2 Institute of Microbiology, Praha, Czech Republic, ^3 Palacky University, Olomouc, Czech Republic

Activating lectin-type receptors on natural killer (NK) cells such as CD161 (NKR-P1) have been shown to react with N-acetyl-D-glucosamine (GlcNAc) conjugates resulting in partial protein against tumors in animal models. We describe here optimized compounds linking two GlcNAc residues to alkyl via thiourea bonds. GlcNAc dycl dimers can efficiently precipitate the A isoform of NKR-P1 in both rat and mouse NK cells, and activate NK cells at concentrations as low as 10–10 M. When administered into melanoma bearing mice, GlcNAc dimers can provide a permanent protection in 70% of animals. This is due to activation of NKT cells, and subsequent tumor infiltration by active CD8^+ T cell. The exceptional signaling efficiency of GlcNAc dimers is explained by sequential cooperative engagement of the target receptor leading to large signaling complexes of about 20 MDa containing G proteins, β-arrestin, phosphorylated dynamin, Src tyrosine kinases, Vav, Rac1, Grb2 and Ras. Supported by grants by Ministry of Education of Czech Republic (MSM_21620808 and I1M0505), by the Institutional Research Concept for the Institute of Microbiology (AVOZ50205051), by Czech Science Foundation (303/09/0477 and 305/09/1808), and by the European Commission (Project Spine 2 Complexes, contract LSHG-CT-2006-031220).

**A2.30**

**Conformation dependent continuous antigenic epitopes**

S. Tetin, Q. Ruan and S. Saldana

Abbott, Diagnostics Research, Abbott Park, IL, USA

Continuous, or linear, antigenic epitopes are common to proteins and peptides. The accessibility of continuous epitopes often depends on protein-peptide conformation and its proximity to disulfide bridges. Temperature dependence of the equilibrium binding constants and the kinetic rates were studied for mAb 106.3 and mAb3-631 by means of fluorescence spectroscopy. This antibody recognizes a relatively short amino acid sequence in the loop between cysteines 10 and 26 of human B-type natriuretic peptide (BNP) which is a cardiac hormone that regulates blood pressure and vascular water retention. Thermodynamic parameters including changes in the free energy, enthalpy and entropy measured at equilibrium are in a good agreement with the parameters calculated from kinetic data. The differences in thermodynamic parameters measured for the two antibodies under study support structural data obtained by NMR and X-ray crystallography.

**A2.31**

**Biotin-kodecytes – novel function-spacer-lipid (FSL) modified cells capable of being recovered from the circulation after 3 days**

C. Oliver^1, D. Blake^1, S. Ferguson^1, N. Bovin^2 and S. Henry^3

^1AUT University, Biotechnology Research Institute, Auckland, New Zealand, ^2Shemyakin Institute of Bioorganic Chemistry RAS, Moscow, Russian Federation

The ability to modify a population of blood cells with both an antigen of interest and a recovery label, infuse them into the circulation of an animal, and then visualize or recover a sample of the infused cells some days later for analysis, is now possible through the use of FSL (function-spacer-lipid) constructs. Murine kodecytes bearing both blood group A antigen (1) and biotin (A+biotin-kodecytes) were created by incubating murine red blood cells with a solution of FSL-biotin and FSL-A. These A+biotin-kodecytes were then infused into the circulation of laboratory mice. Blood was sampled (0.05 ml) at specific time points post transfusion and using the secondary reagent, avidinAlexFluor, the infused kodecytes could be identified in blood films for periods of up to 96 hours in naïve mice. When the same A+biotin-
kocytes were infused into laboratory animals with circulating anti-A (stimulated by immunization with A substance), the A+ biotin-kocytes were observed to have significantly reduced survival times. Control kocytes with either FSL-biotin, or FSL-biotin plus an innocuous FSL antigen (e.g. GB3), gave normal survival times in immunized and naïve animals. By using avidin coated microparticles, biotin-kocytes could be purified from whole blood samples and subjected to further in vitro analysis. The results of this work demonstrate a novel technique for both determining in vivo cell survival and for the recovery of cells that have been exposed to the circulation for several days.

Acknowledgement: Supported by KODE Biotech Ltd (kode-biotech.com)

Reference:

A2.32
Significant difference in antiviral unit of different interferon in stimulating expression of interferon stimulated gene 15
L. Yang1, S-M. Zeng2, B. Wang1, L-Y. Zhang1 and B. Liu1
1Department of Animal Science, Hebei University of Engineering, Handan, China, 2China Agricultural University, College of Animal Science and Technology, Beijing, China

Interferon stimulated gene 15 (ISG15), an ubiquitin cross-reactive protein, could conjugate to target proteins. Unlike ubiquitination, protein ISG15 modification did not target protein for degradation, but enhanced the cellular response to interferon, which played a key role in antiviral response. In this study, western blot and/or immunocytochemistry were performed to explore minimum antiviral units of interferon-α-β-τ in stimulating saturation expression of ISG15 by explants of bovine endometrium and mammary gland, as well as MadinDarby bovine kidney (MDBK), endometrial and mammary cells. Western blot indicated differential minimum antiviral units among recombinant human interferon-α (rhIFN-α, 100 IU/ml), rhIFN-β (1000 IU/ml) and recombinant bovine interferon-τ (rbIFN-τ, 10,000 IU/ml) in stimulating saturation expression of free and ISG15 conjugated proteins by MDBK cells, endometrial and mammary explants. The above results were further confirmed through immunocytochemical analysis by use of MDBK, endometrial and mammary cells. The expression patterns of ISG15 conjugated proteins by different explants were various at the same antiviral unit of the same interferon. In conclusion, there were 10 to 100 fold differences in minimum antiviral units of rhIFN-α, rhIFN-β, and rbIFN-τ in stimulating saturation expression of ISG15, and the different expression patterns of ISG15 conjugated proteins by different tissues might lead to different antiviral response on different tissues with the same interferon.

A2.33
The role of tyrosyl-tRNA synthetase in heart failure development
I. Kondratik1, V. Bobyk2, D. Ryabenko3, L. Sidork2 and O. Kornelyuk2
1Taras Shevchenko National University of Kyiv, Microbiology and General Immunology, Kyiv, Ukraine, 2Institute of Molecular Biology and Genetics, Kyiv, Ukraine, 3NSC ‘Institute of Cardiology named acad. N.D.Strazhesko’2, Kyiv, Ukraine

Background: Autoantibodies (aAbs) directed against the aminoacyl-tRNA synthetases are associated with myositis, arthritis, Raynaud’s phenomenon, fever and interstitial pneumonia, systemic lupus erythematosus and rheumatoid arthritis. N-terminal catalytic module of tyrosyl-tRNA synthetase (YRS) can function as a cytokine and act as a factor that stimulates angiogenesis. It is very promising in terms of new cardiotoxic drugs development, important for the treatment of common cardiovascular diseases such as myocardial infarction and heart failure.

Materials and methods: We developed of monospecific polyclonal anti-YRS antibodies directed against of full-length form of YRS using original immunization procedure. The level of specific anti-YRS autoantibodies were examined in sera of patients bearing of dilated cardiomyopathy (DCM) as chronic stage of heart failure progression in comparison with normal ones. The level of YRS expression in DCM-affected human hearts were identified by Western-blot analysis in comparison with normal samples. The time course changes of YRS expression were studied by immunoblotting in mouse hearts with experimental DCM-like autoimmune damage of myocardia.

Results: The increased level of YRS expression (both full-length enzyme and truncated N-terminal module) have been observed in cardiomyocytes from DCM-affected heart in comparison with normal ones. These changes accompanied by significant increase of anti-myosin autoantibodies level detected in DCM patients sera as well as in mouse model sera.

Conclusions: This results show a potential role of YRS in heart failure development and could be a real base for new diagnostic tools development.
Indirubin is an active compound of Polygonum tinctorum Lour. (P. tinctorum). Recently, we synthesized the novel indirubin derivative, 5'-nitro-indirubinoxime (5'-NIO), and demonstrated that it has more potent anti-tumor activity in vitro and in vivo than any other reported indirubin derivatives. Although indirubin is also known to have anti-inflammatory activity, its mechanism of action is poorly understood. In this study, we evaluated the effect of 5'-NIO on the TNF-α induced inflammatory conditions of human umbilical vein endothelial cells (HUVECs) to determine if it would be useful as an anti-atherosclerotic agent. We found that 5'-NIO significantly inhibited the TNF-α-induced expression of MCP-1 and IL-8 in HUVECs. Furthermore, 5'-NIO strongly inhibited the adhesion of U937 cells onto the endothelial cells by decreasing expression of the adhesion molecules, ICAM-1 and VCAM-1. We also demonstrated that 5'-NIO plays its anti-inflammatory effect through the inhibition of NF-κB nuclear translocation and JNK activation. In vivo study using Sprague-Dawley rats showed that 5'-NIO treatment markedly attenuates the LPS-induced inflammatory cell infiltration, confirming the anti-inflammatory effect of 5'-NIO in vivo. Taken together, these findings indicate that 5'-NIO is a novel candidate with the potential for the treatment of atherosclerosis.

A2.36
Development and preclinical test of novel attenuated recombinant bacillus Calmette-Guerin vaccine rBCG-ABM01 for the intravesical bladder carcinoma immunotherapy

C.-F. Lee1, Z. Ru-Wen2 and Y Dah-Shyong3
1National Defence Medical Center, Institute of Preventive Medicine, Taipei, Taiwan, 2Department of Health, Executive Yuan, Centers for Disease Control, Taipei, Taiwan, 3Department of Surgery, Tri-Service General Hospital, Taipei, Taiwan

Mycobacterium bovis bacillus Calmette Guerin (BCG)-immunotherapy has a well-documented and successful clinical history in the treatment of superficial bladder transitional-cell carcinoma (TCC). Nevertheless, regularly observed side effects, a certain degree of nonresponders and restriction to superficial cancers remain a major obstacle. Thus, alternative treatment strategies are intensively being explored. Here we reported the ability of multivalent vaccines of the recombinant BCG (rBCG-ABM01) in enhancing anti-cancer immunotherapy. Attenuated M. bovis BCG was engineered to secrete specific subunit antigens. The efficiency of protein was detected by flow cytometry and Western blot analysis. Antitumor effects were monitored by bioluminescence-imaging system (BLI), with measurement of cytokines and phenotyping of infiltrating lymphocytes in a murine orthotopic bladder-tumor model. Seven days after immunotherapy, mice treated with rBCG-ABM01 were found to have significantly higher Th1-polarized IFN-gamma concentrations than mice in the untreated controls. The frequencies of IL-2, IFN-gamma, and TNF-alpha producing cell were demonstrated higher than controls by cytomteric beads array and quantitative PCR, respectively. A highly immunopotent rBCG-ABM01 was developed and it elicited immune responses with a high serum IFN-gamma level, inhibited tumor growth and prolonged survival in tumor bearing mice. Conceivably, the studies should provide clues leading to elucidate the role of the potential usefulness of recombinant BCG vaccine in human bladder carcinoma.

Acknowledgement: This work was funded by the NSTP/ Biotechnology and Pharmaceuticals grants DOH99-TD-I-111-TM010.

A2.37
Interleukin-6, tumor necrosis factor-α polymorphisms and HBV infection status in two transplant patient groups
I. Alexiu1, G. Tarder1, C. Luca1, M. Chiva1, C. Sultana1, C. Gracea2, M. Stoian1 and C. Diaconu1
1Institute of Virology "Stefan S. Nicolau", Bucharest, Romania, 2Clinical Hospital for Infectious and Tropical Diseases "Dr. Victor Babes", Bucharest, Romania, 3Research base - Molecular Biology, University of Bucharest, Bucharest, Romania, 4Fundeni Clinical Institute for Digestive Disease and Liver Transplantation, Bucharest, Romania, 5Fundeni Clinical Institute for Urology and Kidney Transplantation, Bucharest, Romania

Introduction: Single nucleotide polymorphisms (SNPs) in regulatory regions of IL-6 (-174 G/C) and TNFα (-308 A/G) have been shown to affect their expression and, consequently, interfering infections in humans. The aim of this study was to investigate the frequency of genotypes associated with the mentioned SNPs of IL-6, TNF-α and to determine their relation with hepatitis B virus (HBV) infection in two transplant patient groups.

Materials and methods: Hundred twenty-two patients have been enrolled in the study, 50 liver transplant (LT) and 72 kidney transplant (KT) recipients. HBV infection status have been investigated by serology and quantitative PCR. Cytokines SNPs have been identified by sequencing. Results have been statistically reasoned with Pearson's chi-square test.

Results: Sequencing and viral results showed that GG genotype at position -174 in IL-6 gene is predominant in LT patients with HBV infection markers, and GC genotype is more frequent in LT patients HBV free. Statistics revealed a significant correlation between G allele at IL-6 SNP and the absence of HBV infection in LT recipients. For KT patients HBV+, GG genotype at IL-6 SNP is more frequent. In both patient groups GG genotype at position -308 in TNF-a gene was predominant. For KT patients results showed a TNF-a genotype distribution, considering HBV infection status, near statistical significance.

Conclusions: G allele at position -174 in IL-6 gene seems to correlate with the absence of HBV infection in liver transplant recipients and A allele at position -308 in TNF-a gene is possible to be more present in kidney transplant recipients HBV negative.

A2.38
Inhibitory effect of phenolic components from Sargassum thunbergii on allergic inflammatory response in human basophilic KU812F cells
J.-A. Kim1, V. T. Sang1, B. Ahn1, S.-S. Bak2, C.-S. Kong2 and S.-K. Kim1
1Department of Chemistry, Pukyong National University, Busan, Republic of Korea, 2Marine Bioprocess Research Center, Busan, Republic of Korea

In this study, we isolated three phenolic components, sargahydroquinic acid and sargachromanol from Sargassum thunbergii by diverse chromatographic methods and investigated their anti-inflammatory effects. The anti-allergic effect of three phenolic compounds was explored by measuring cytokine production and...
Results: Monocytic lineage markers CD14 and CD45 levels remained unchangeable for DD cells and control group. Promonocyte markers CD34, CD90 and CD115 in DD cells were significantly higher as compared to the controls. Considering mesenchymal stem cell markers CD29 and CD105, no significant difference between the two groups was observed. Dedifferentiated cells were successfully cultured and multiplied and could be stored under liquid nitrogen without losing their characteristics.

Conclusions: Leukapheresis filters-derived monocytes are capable of dedifferentiating into promonocytic cells committed in monocytic lineage, but they do not present mesenchymal stem cells markers. Given that the dedifferentiated cells can be further cultured and proliferated, these cells may represent an abundant human cell source that can be used for investigating dedifferentiation and transdifferentiation mechanisms.

A2.41 Calcium influx induced by the Adenylate Cyclase Toxin from Bordetella pertussis triggers endocytic trafficking of integrins
B. K. Uribe, C. Martin, G. Gomez-Bilbao and H. Ostolaza
Unidad de Biofisica (Centro Mixto CSIC-UPV/EHU) and Department of Biochemistry and Molecular Biology, University of Basque Country, Bilbao, Spain

The adenylate cyclase toxin (ACT) of Bordetella pertussis, is a 1706 residue-long polypeptide that contains a N-terminal adenylate cyclase activity bearing domain and a pore forming domain that harbours >40 calcium-binding nonapeptide repeats characteristic of the RTX (Repeats in Toxin) family. ACT targets primarily CD11b+ phagocytes and translocates across their cytoplasmic membrane the catalytic adenylate cyclase domain producing a rapid increase of intracellular cAMP that suppresses its bactericidal functions. Recently, we and others have reported that ACT induces intracellular calcium elevations that do not correlate with the toxin-induced cytotoxicity. The aim of the present work was to study the cellular consequences of the toxin-induced calcium influx. We show that the ACT-induced calcium rise is followed by CD11b/CD18 mobilization into raft-like microdomains. This movement is the result of the hydrolysis of a cytoskeletal protein, talin, likely through calpain activation. Migration of the β2 integrin seems to be consequence of calcium influx and not the result of a direct interaction with ACT, as the cation rise also induces the migration of β2 integrins, that are not specific receptors for the toxin. We also show that ACT and integrins, along with raft markers GM1 and flotillin-1, are rapidly internalised in a toxin-induced calcium rise-dependent process affecting an essential function of these immune cells i.e. adhesion. The removal from leukocyte plasma membrane of domains that contain key molecules such as integrins, may represent an advantageous strategy followed by pathogenic Bordetella to circumvent the host immune system.

A2.42 Structural studies of staphylococcal enterotoxin H in complex with T cell receptor and major histocompatibility complex class II
K. Rödström1, M. Saline2, G. Fischer3 and K. Lindkvist-Pettersson1
1University of Gothenburg, Cell and Molecular Biology, Gothenburg, Sweden, 2University of Gothenburg, Swedish NMR Centre, Gothenburg, Sweden, 3University of Gothenburg, Chemistry, Gothenburg, Sweden
Abstracts

Superantigens (SAgs) are bacterial toxins capable of cross-linking the immune receptors of the host, the T cell receptor (TCR) and major histocompatibility complex (MHC) class II, and thereby trigger a massive release of cytokines. This could lead to toxic shock syndrome, which can have a fatal outcome. Here, we present the crystal structure of the ternary complex between the superantigen, staphylococcal enterotoxin H (SEH), TCR and MHC, as well as the dimer complex, including only TCR and SEH. It is evident that SEH interacts with the variable α domain (Vα) of TCR, in sharp contrast to previously studied SAgs that interact with the Vβ domain. Due to the high structural conservation of amino acids in SEH that are crucial for the interaction, we propose that in addition to Vβ activation of T cells, there are SAgs, in addition to SEH, which are able to activate T cells through Vα as well. In addition to providing crucial information regarding the nature of TCR-mediated recognition of superantigens, the finding have central implications for future strategies aimed at preventing or modulating the often pathogenic response to superantigens.

A2.43

Abstract withdrawn

A2.44

Expression of rat NK cell receptor NKRPlB and its ligand Clrb in HEK293T cell line and their biophysical characterization

P. Čeládová1, J. Bláha1, P. Pompach1, D. Kavan2, K. Hofbauerova1, O. Vanek1 and K. Bezouska1

1Department of Biochemistry, Faculty of Science, Charles University, Prague, Czech Republic, 2Institute of Microbiology, AS CR, Prague, Czech Republic

Natural killer cells play a significant role in the immune response against tumor and infected cells. NK cells express a wide variety of surface receptors, including NKRPl, a C-type lectin-like family of both activating and inhibitory receptors. Recently, ligands have been found for some of these previously orphan molecules, some of them lying within the same family. This is also the case of rat Clrb as a cognitive ligand for rat NKRPlB. It has been shown that in rat, this inhibitory NKRPlB-Clrb mutual receptor system is subverted by rat cytomegalovirus protein RCTL, a viral version of Clrb, which serves as a decoy ligand for NK cells [1]. Here we present successful cloning and production of the above mentioned C-type lectin-like proteins based on transient transfection of HEK293T cell line in a suspension culture. This expression system allows us not only to obtain proteins of our interest with a satisfactory yield but also in their native conformation, removing the need for time consuming and often fruitless refolding procedures required in case of using the E. coli expression system. Moreover, the proteins are obtained in their glycosylated form. As glycosylation might participate on the lectin-lectin interaction this is considered an additional benefit of the system. Proteins were purified using IMAC followed by gel filtration, identified by mass spectrometry, characterized by analytical ultracentrifugation and Raman spectroscopy and crystallization trials were set up. This work is supported by the European Commission (Integrated project SPINE2-COMPLEXES, contract No. 031220).

Reference:

A2.45

Characterization of ZG16, a lectin-like protein found in the mucus layer of the intestine

J. H. Bergström1, M. E.V. Johansson1 and G. C. Hansson2

1Department of Medical Biochemistry, University of Gothenburg, Gothenburg, Sweden, 2Department of Medical Biochemistry, Institution of Biomedicine University of Gothenburg, Gothenburg, Sweden

The mammalian intestine harbors complex societies of beneficial bacteria coexisting with the host. The first line of defense preventing these microorganisms to invade the underlying epithelia is a mucus layer with the heavily O-glycosylated MUC2 mucin as the main structural component. In order to obtain a greater understanding of the regulation, function and structure of this barrier we analyzed the mucus using proteomics to find additional components. One of the proteins found and further studied were the lectin-like protein ZG16, a small protein with 167 amino acids containing a signal sequence and a jacalin-like lectin domain. To address the protein properties, ZG16 was cloned, expressed in mammalian cell system and an antisera was produced by the immunization of rabbits with the purified protein. Binding studies did not reveal any direct interaction with the MUC2 mucin or different glycoforms expressed on recombinant MUC1. Instead a strong binding to peptidoglycan, a bacterial cell wall element, was observed. As expected ZG16 also binds gram positive bacteria as they have peptidoglycan exposed to the surroundings. The viability of the bacteria after binding was unaffected in proliferation assays suggesting that ZG16 not have a direct bactericidal effect.

A2.46

Myelin basic protein peptide 46-62 ameliorates ongoing experimental allergic encephalomyelitis in DA rats

A. Belogurov, A. Stepanov, N. Ponomarenko and A. Gabibov

IBCh RAS, biocatalysis, Moscow, Russian Federation

The pathological role of antibodies raised against endogenous antigens in autoimmune diseases is widely accepted. Direct penetration of autoantibodies through the blood-brain barrier and their co-localization with neural tissue-specific autoantigens may explain their possible contribution in the neurodegeneration. Previously, we demonstrated that autoantibodies in multiple sclerosis (MS) reveal site-specific binding and cleavage activity toward myelin basic protein (MBP). Using recently created MBP-derived recombinant “epitope library” covering the entire protein sequence we determined epitopes of myelin basic protein specific for the autoantibodies isolated from MS patients. Further we administrated these peptides to DA rats with induced protracted relapsing experimental allergic encephalomyelitis (EAE) most closely related to MS. DA rats with EAE induced by syngenic spinal cord homogenate in complete Freund’s adjuvant were treated by human MBP fragments 46-62, 81–102, 124–139, and 147–170 in native and liposome form. MBP 124–139 and 147–170 displayed only mild therapeutic effect but MBP 46-62 significantly reduced EAE, reflected by lower clinical scores and shorter EAE duration compared to controls. Treatment of DA rats by respected peptides entrapped in liposomes increase their therapeutic efficiency. Thus, obtained data may be used for effective and directed treatment of Multiple Sclerosis opening new paths in struggle against neurodegenerative diseases.
A2.47 Neurotrophin-3 adaptation marker to chronic and acute brain hypoxia
M. Gheorghiu, D. Pasarica, B. Mahler, T. Trandafir and C. Bara
'Carol Davila' University of Medicine and Pharmacy, Pathophysiology and Immunology, Bucharest, Romania

Background: Chronic obstructive pulmonary disease (COPD) is a major cause of chronic hypoxia and cerebral ischemia involving a complex signaling cascade with an at least partial unremarked spatiotemporal pattern. In acute ischemia (ischemic stroke-IS), early excitotoxicity can lead to fast necrotic cell death, which produces the core of infarction. While brain cells are challenged by this deleterious mechanisms, they activate innate protective programs: synthesis of inflammatory cytokines and neuronal growth factors, members of neurotrophins family, suggesting that neuronal death is associated with an inflammatory reaction and a protective response. In chronic brain hypoxia, the inflammation intensity and the protective response efficiency are poorly characterized. The aim of this work was to begin the NT-3 comparative study between patients with COPD (chronic brain hypoxia) and patients with ischemic stroke (acute brain hypoxia), in order to establish a possible model of protective brain response to hypoxia.

Patients and methods: Forty-five patients were investigated after computed tomography-confirmed IS. We used ELISA to determine serum level NT-3 (as marker of the brain adaptation response). The results were compared with those obtained in 40 patients with confirmed COPD.

Results: Significant increase of NT-3 serum levels was obtained in COPD compared with normal and ischemic stroke lots.

Conclusion: Increased neurotrophin-3 serum level could represent an important marker of neuroprotection, this neurotrophic factor being a potential therapeutic agent in acute or chronic brain hypoxia.

A2.48 Interleukin 8 – leader of the inflammatory cytokines in chronic obstructive pulmonary disease
M. Gheorghiu, D. Pasarica, B. Mahler, T. Trandafir and C. Bara
'Carol Davila' University of Medicine and Pharmacy, Pathophysiology and Immunology, Bucharest, Romania

Background: Chronic obstructive pulmonary disease (COPD) is mainly characterized by inflammation throughout the central and peripheral airways, lung parenchyma, and pulmonary vasculature. Local defensive mechanisms are triggered by inflammatory stimuli, followed by signal transduction and activation of the transcription factor NF-κB, with synthesis of proinflammatory cytokines tumoral necrosis factor alpha (TNF-α), interleukin 1-beta (IL-1β), interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 10 (IL-10), etc. Inflammatory cytokines IL-1β, TNF-α, IL-6, IL-8 elicited defensive responses in lung parenchymal cells and bronchial cells, including activation of apoptosis. On the other hand, IL-10, the inhibitory cytokine, could also play an important antiapoptotic role, stimulating organ repair after injury. In addition, IL-8 promotes neutrophilia as well as neutrophils infiltration of bronchial tissue. We were interested in studying the cytokine signals which are involved in COPD development.

Patients and methods: Forty patients with confirmed COBP were investigated using blood samples. IL-1β, TNF-α, IL-6, IL-8 and IL-10 were measured using ELISA kits.

Results: Even all cytokines serum levels were increased comparing with normal, only IL-1β, TNF-α and IL-8 presented significant changes. The biggest concentration was obtained for IL-8.

Conclusion: Great serum level of IL-8 and its role of main chemoattractant factor for neutrophils strongly suggests that neutrophils activation and cytotoxicity are the main cause for the airways, lung parenchyma, and pulmonary vascular damage in COPD.

A2.49 Helicobacter pylori neutrophil-activating protein (HP-NAP) activates the MAPK and PI3K/Akt pathway in human mast cells
S.-Y. Du and H.-W. Fu
Department of Life Science, Institute of Molecular and Cellular Biology, National Tsing Hua University, Hsinchu, Taiwan

Helicobacter pylori (H. pylori), is a Gram-negative bacterium, is thought to infect over half of the human population. H. pylori infection induces acute and chronic inflammation and plays a key role in gastric mucosal diseases. The neutrophil-activating protein of Helicobacter pylori (HP-NAP), one of its virulence factors, has been identified to activate neutrophils, monocytes, and mast cells. However, the mechanism of HP-NAP activates mast cells is not fully understood. In this study, we show that HP-NAP induces histamine release and interleukin-6 (IL-6) production in human mast cells. We also found that HP-NAP induces extracellular regulated kinase (ERK), p38 mitogen-activated protein kinase (MAPK), and Akt activation in human mast cells. The kinase inhibitor of MAPK/ERK, p38 MAPK and phosphotydylinositol 3-kinase (PI3K) suppress HP-NAP-induced IL-6 production. These results indicate that HP-NAP activates human mast cells through MAPK and PI3K/Akt pathway.

A2.50 Human lubricin expresses the sialyl Lewis x determinant and has L-selectin ligand activity
C. Jin, A.-K. H. Ekwall, M. Bokarewa and N. G. Karlsson
1 Department of Medical Biochemistry, Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden, 2 Department of Rheumatology and Inflammation Research, Institute of Medicine, University of Gothenburg, Gothenburg, Sweden

Synovial fluid is responsible for lubricating the joint, which is the primary tissue for symptoms of osteoarthritis (OA) and rheumatoid arthritis (RA). Lubricin is an abundant mucin-like glycoprotein in synovial fluid. Except lubricating and protection of cartilage, lubricin also possesses other biological functions such as chondroprotection and control of synovial cell growth. In this study, lubricin’s O-linked oligosaccharides isolated from OA and RA synovial fluid were investigated by LC-MS. O-glycan on lubricin were found to be mainly sialylated core 1, but small amount of sialylated core 2 structures, containing both sulphate and fucose, was found. The presence of sulphated and fucosylated O-glycans on lubricin imply that its glycosylation may have specific function in addition to the lubricating property provided by simple core 1 structures. Further study showed L-selectin present on human lymphocytes was able to bind to lubricin. This indicates that lubricin may be involved in inflammatory cells recruitment influencing the L-selectin facilitated tethering.
A2.51 Promiscuity of binding/catalysis of antibodies – antidotes toward chemical weapons
I. Smirnov1, I. Kurkova1, E. Carletti2, P. Masson3, A. Belogurov1, N. Ponomarenko1, A. Tramontano4 and A. Gabibov1
1IBCh RAS, Moscow, Russian Federation, 2IBS CNRS, Grenoble, France, 3CRSSA, La Tronche, France, 4University of California, Davis, US

Immunoglobulins were shown to serve as an excellent template to generate de novo catalytic active centers. Both combinatorial and rational design approaches were used to improve their catalytic efficiency for numerous reactions. Organophosphorous poisons, (OP, nerve gases) may be regarded as one of the intriguing targets to develop antibody-based capturing machinery. Previously we obtained rationally designed antidiotypic antibodies 9A8 against acetylcholinesterase which was shown to have pronounced specificity of its active center toward OP analogs. Alternatively using combinatorial selection of human phage display library by non-toxic phosphorus compounds we selected A17 ScFv antibody able to capture OP analogs. This antibody was expressed as full length antibody and Fab-fragment of it was crystallized and two 3D structures of non-modified and modified by phosphonate residues were solved with resolution 1.5 and 1.36 Å respectively. The 3D structures of 9A8 and both A17 antibodies were compared and mechanism of OP capturing was described. The microcalorimetric studies allowed us to make quantitative conclusions concerning active sites of both antibodies. Rationally designed 9A8 has more dance active site, alternatively combinatorial selected A17 poses “primitive” rigid active center. 9A8 antibodies was interact with fluorescent analog of soman. The microcalorimetric studies allowed us to make quantitative conclusions concerning active sites of both antibodies. Rationally designed 9A8 has more dance active site, alternatively combinatorial selected A17 poses “primitive” rigid active center. 9A8 antibodies was interact with fluorescent analog of soman. 9A8 antibody hydrolyze paraoxon with low rate constant. These antibodies displayed certain specificity toward DNA. Rationally designed 9A8 was characterized as specific dsDNA binder and A17 was shown to catalyze dsDNA cleaving reaction. Thus both Abs has pronounced promiscuity of their catalytic centers.

A2.52 Molecular characterization of exhaled endogenous particles – a novel biological matrix non-invasively sampled from the respiratory tract
E. Milgrorodskaya1, A. Bredberg1, J. Gobom2, A.-C. Almstrand1, P. Larsson1 and A.-C. Olin1
1University of Gothenburg, Occupational & Environmental Medicine, Gothenburg, Sweden, 2University of Gothenburg, Institute of neuroscience and physiology, Gothenburg, Sweden

We have recently developed a novel non-invasive method to sample non-volatile material in exhaled air [1]. The method is based on sampling exhaled endogenous particles using a cascade impactor. At present, little is known about molecular composition of the exhaled particles (PEs). It is expected that PEs composition is similar to the one of the respiratory tract lining fluid (RTLF). Here we present initial results on molecular characterization of PEs. The major component of RTLF is respiratory surfactant, consisting of phospholipids and proteins. Therefore these classes of molecules were our primary investigation targets. PEs were collected on silica plates using in-house built sampling device [1]. For lipid analysis, individual samples (30 ± exhaled volume) were extracted and analyzed directly by MALDI TOF MS. For protein analysis, pooled samples from several individuals were collected (3000 l and 4400 l total exhaled volumes). Extracted proteins were separated by SDS-PAGE, subjected to in-gel trypsinolysis and analyzed by LC-MS for protein identification. Positive mode MALDI MS resulted in identification of several phosphatidylcholine (PC) species, with PC (16:0/16:0) being the most abundant as expected for pulmonary surfactant. Proteomic analysis of PEs resulted in identification of 235 proteins. A number of known pulmonary proteins, i.e. surfactant proteins, CC16, mucins were detected along with major plasma proteins. Identification of surfactant specific lipids and proteins in PEs confirms their origin to RTLF, making PEs a highly interesting biological matrix for monitoring biochemical changes in the distal airways.

Reference:

A2.53 Eukaryotic expression as an indispensable tool for preparation of native dimeric forms of NK cell C-type lectin-like receptors
O. Vanek1, P. Celadova1, J. Blaha1, D. Kavan2, P. Pompač2 and K. Bezouska1
1Department of Biochemistry, Faculty of Science, Charles University, Prague, Czech Republic, 2Department of Immunology and Genotobiology, Institute of Microbiology ASCR, Prague, Czech Republic

Natural killer cells are able to recognize and kill a variety of tumor and infected cells. The recognition is mediated by wide repertoire of cell surface receptors, both activation and inhibitory, belonging to two main structural families: immunoglobulin-like and C-type lectin-like. While ligands for the Ig-like receptors were shown to be MHC gp I proteins, ligands only for some of the lectin-like receptors are known up to date. Both families share relatively weak binding characteristics and in the case of lectin-like receptors, in vitro oligomerization clearly improves binding through increase in avidity. However, these lectin-like receptors were described mostly as dimeric in vivo and this minimal oligomer would be also the best for structural studies. Moreover, there is no crystal structure known of extracellular domain of any of these receptors in its full-length native dimeric form; probably because prokaryotic expression of native dimers is rather difficult. Here we present successful design, cloning and production of dimeric forms of some mouse, rat and human NK cell lectin-like receptors belonging to NKRPI and Clr families. Full-length extracellular receptor parts were expressed via transient transfection of HEK293 cell lines in suspension culture either alone or as a cleavable fusion with Fc fragment of human IgG1 to promote native dimerization. This fusion strategy resulted in cleaved pure covalent dimers of e.g. rat CIRb and purely dimeric Fc fusion of rat NKRPIB proteins and their structural characterization is currently under way. This work is supported by the European Commission (Integrated project SPINE2-COMPLEXES, contract No. 031220).

A2.54 New aspects of erlich ascites carcinoma development
H. Harutyunyan
H. Baniatyun Institute of Biochemistry of NAS RA, Yerevan, Armenia

Erlitch Ascites Carcinoma (EAC) cells growth kinetics in vitro and that correlation with infected mice surveillance are reported in present abstract. EAC cells were suspended in culture media
RPMI-1640, supplemented with 10% of bovine serum in penicillin vials. Incubation carried out in humidified chamber at 5% CO2 and 37°C. The growth kinetics described with classical phases: lag-phase 0–3 days; exponential growth phase 3–12 days; stationary phase 12–16 days; decline phase 16–24 days of cultivation. Cell concentration droppings had been detected three times during 24 days long cultivation: at 7th, 14th, and 21st days. The experiment had been repeated several times, every of which confirmed reliability of fined out result. Observed EAC cell dropping phenomenon had not been described earlier for in vitro conditions, but is in sound with the in vivo observation described by Zamai A.S. (2007). EAC cells infected mice surveillance was also examined. High level of mortality at 10–14 days was observed. Analysis of correlation between cells growth in vitro and mice surveillance in vivo revealed those considerable inverse value (Spearman r: -0.89, p value 0.0020**) at 7–15 days of the experiment. These results let us to assume that observed EAC cells growth kinetics reflect the EAC fate in mice organism. This may be a substantial contribution to the tumor diagnosis and specification of period of malignant process. The observed phenomenon of EAC cells concentration dropping at their cultivation is an object of our current researches. We hope it will contribute to the understanding of details of cancer development and suggest more purposeful treatment.

**A2.55**  
Effect of methylsulfonylmethane on LPS-IFN-gamma induced RAW 264.7 macrophage apoptosis  
A. Z. Karabay¹, T. Ozkan², A. Koc¹, A. Sunguroglu³ and Z. Bayukbingol⁴  
¹Department of Biochemistry, Ankara University Faculty of Pharmacy, Ankara, Turkey, ²Ankara University Institute of Biotechnology, Ankara, Turkey, ³Ankara University Faculty of Medicine, Medical Biology, Ankara, Turkey, ⁴Ankara University Faculty of Pharmacy, Ankara, Turkey

Natural organosulfur compound methylsulfonylmethane (MSM) is a metabolite of dimethyl sulfoxide which occurs at low levels in various foods. It has been extensively used as a dietary supplement for its potential to improve human health and to reduce arthritic pain. When activated with LPS-IFN-gamma, macrophages undergo apoptosis time and dose dependently. Abnormal release of NO and cytotoxicity mediated by excess NO may lead to inflammation and tissue injury. Therefore, besides reduction in NO generation, therapeutic strategies aimed at inhibiting NO-dependent cell apoptosis may contribute to improving the outcome of sepsis. In this study we aimed to show the effect of MSM on LPS-IFN-gamma stimulated RAW 264.7 macrophage apoptosis. Cells were seeded to 6-well plates and co-incubated with MSM for 1 hour. Following co-incubation, cells were incubated in the absence or presence of LPS-IFN-gamma for 24 hours. Proliferation of cells was estimated by MTT test. According to MTT results, treatment with LPS-IFN-gamma significantly decreased cell proliferation and MSM increased proliferation of activated macrophages. Cells were then stained with Annexin V-FITC and observed and counted under a fluorescence microscope to identify if this enhanced proliferative effect of MSM was a result of protection against apoptosis. Our results have shown that LPS-IFN-gamma significantly induced macrophages to apoptosis and MSM protected macrophages from LPS-IFN-gamma induced apoptosis (p < 0.05). This proliferative effect may elicit from inhibition of NO production by MSM. This study shows that MSM exerts permissive antiapoptotic activity and can be used in disorders related with NO dependent apoptosis.
A3.01
Abstract withdrawn

A3.02
Abstract withdrawn

A3.03
Abstract withdrawn

A3.04
Silybin improves liver injury in experimental non alcoholic fatty liver disease
G. Li Volpe1, I. Barbagallo1 and F. Salamone2
1Department of Biological Chemistry, University of Catania, Medical Chemistry and Molecular Biology, Catania, Italy,
2Department of Internal Medicine, University of Catania, Catania, Italy

Non Alcoholic Fatty Liver Disease (NAFLD) has a complex pathophysiology in which oxidative stress plays a pivotal role. Silybin, a flavonolignan extracted from milk thistle, exerts a marked liver protecting action in a variety of experimental liver injuries. We aimed to clarify if silybin treatment may favourably impact on liver injury progression in an animal model of NAFLD. We explored the effects of a 4-week daily (20 mg/kg i.p.) administration of silybin in 6-week-old db/db mice feeding a methionine-choline deficient diet. We evaluated liver histology by the NAFLD activity score (NAS); mitochondrial morphology by electron microscopy; liver 8-OHG, GSH and NFKB by ELISA; hepatic nitrite/nitrate by colorimetric assay; expression of iNOS and 3-nitrotyrosine by western blot and immunofluorescence; mitochondrial respiratory chain activity (MRC) by spectrophotometrical analysis. In db/db mice, silybin treatment markedly improved liver injury as measured by NAS (p < 0.01); significantly preserved cristae morphology and reduced hepatic triglycerides content (p < 0.001). Silybin administration strongly decreased liver TBARS, 8-OHG and increased GSH (p < 0.0001 for all). Strikingly, silybin completely restored hepatic MRC (p < 0.0001 for all the five complexes) while inhibited NFkB p65 and p50 subunits binding activity (p < 0.0001 for both); consistently, it abolished liver iNOS expression (p < 0.01) and reduced nitrite/nitrates (p < 0.05) and 3-nitrotyrosine levels. In conclusion, silybin exerts potent antioxidant and anti-inflammatory effects in murine NAFLD providing a strong rationale for the use of this compound in the management of patients with NAFLD.

A3.05
Abstract withdrawn

A3.06
Renal fibrosis and evidence for epithelial-mesenchymal transition in patients with nephrolithiasis
C. Boonla1, K. Kriegstein2, S. Bovornpadungkitti3, F. Strutz4, C. Predanon5 and P. Tosukhowong4
1Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, 2Department of Molecular Embryology, University of Freiburg, Institute for Anatomy and Cell Biology, Freiburg, Germany, 3Division of Urological Surgery, Khon Kaen Hospital, Khon Kaen, Thailand, 4Department of Nephrology and Rheumatology, Georg-August-University Medical Center, Göttingen, Germany

We quantified fibrotic lesion in renal tissues of nephrolithiasis patients and evaluated its association with renal function. Presence of epithelial-mesenchymal transition (EMT) in nephrolithiasic renal tissues was investigated. Patients diagnosed with large kidney stone formation (n = 50) and age and sex-matched healthy controls (n = 37) were recruited. Fifty-eight renal sections from 38 patients were stained and analyzed. Fibrosis was assessed by Masson’s trichrome staining. Co-expression of epithelial cytokeratins and mesenchymal markers (α-smooth muscle actin (αSMA) and vimentin) in renal tubular cells was detected by dual immunofluorescence staining. Expression of fibronectin, transforming growth factor-β1 (TGF-β1) and CD68 were investigated. Kidney function was significantly reduced in nephrolithiasis patients compared to healthy controls. Inflammation grading in nephrolithiasis renal tissues was correlated with percent fibrotic area. Renal fibrosis was inversely correlated with renal function. Cytokeratins co-expressed with αSMA and vimentin were observed in nephrolithicentric renal tubular cells, and these cells strongly expressed fibronectin and TGF-β1. Infiltration of CD68-positive cells was a common finding in inflamed renal sections. Conclusion, kidneys of nephrolithiasis patients displayed robust signs of inflammation and fibrosis. A close correlation of renal fibrosis with renal dysfunction was demonstrated. This is the first study to show evidence for renal tubular cells displaying signs of EMT in stone-containing kidneys. Plausibly, TGF-β1 triggers EMT which at least in part contributes to stone-induced renal fibrosis.

A3.07
Schedule dependency of insulin therapy, the four compartment model and mathematical modeling of blood glucose control
K. Pirkalani1 and Z. Talee Rad2
1Mehr Medical Group, Endocrinology/Internal Medicine/Molecular Biology, Tehran, Islamic Republic of Iran, 2Mehr Medical Group, Endocrinology/Gynecology, Tehran, Islamic Republic of Iran

Objective: To improve schedules for insulin (life’s central metabolic hormone) treatment with less needed dose and less hypoglycemic attacks, during instillation of a special preparation into the external auditory channel.

Research Design and Methods: BS was hourly measured in 20 and less frequently in an additional number of diabetics to plot...
an approximate curve of blood glucose level and the probable changes based on mathematical tools (interpolation, Autoregressive moving Average etc.). Data of twenty six patients were included in the design the mathematical model. Auditory instillation was done as reported before. Results: A series of unexpected hyper and hypoglycemia were encountered that confirmed release of glucose from a special reservoir (compartment III) rather than attributable to the counter regularity hormones. Conventional treatments act as contributory. The plotted curve is a sine curve with noise. Based on mathematical model intermittent treatment every 47 minutes where the second derivative is zero (wane of BS lowering mechanisms) is most effective with the lowest needed insulin dose and hence no hypoglycemic attacks. Patients showed excellent control with the intermittent schedule with doses to the range of 50–70% of their conventional dose. Conclusions: In diabetics (not normal persons) point of maximal effect and maximal need are not coincidental. Treatment based on the insulin need causes large fluctuations of BS levels and on the latter best BS control with less cumulative dose and less hypoglycemic attacks, respectively. This is the sign qua non that fixed intermittent treatment is more effective than event based schedule or continuous infusions.

A3.08
Continuous control of diabetes mellitus by infusion of Auditory Insulin (AI) preparation into the ear via a piezoelectric pump
K. Pirkalani and Z. Talaee Rad
Mehr Medical Group, Endocrinology/Internal Medicine/Molecular Biology, Tehran, Islamic Republic of Iran.

Objective: To find new routes of insulin treatment, we have used one of the most effective preclinical preparations with no accompanying toxic agent to instill into the external auditory channel.

Research design and methods: Two hundred and twelve patients with type I and II diabetes mellitus were enrolled. After discontinuation of all antidiabetic medications based on blood glucose levels 6–60 IU equivalent insulin was instilled into the ear of resting patients and blood glucose level were evaluated at 15, 30, 45, 60, 90, 120, 150 and 180 minutes and serum insulin at 120 minutes. Patients were monitored for local and systemic side effects. Ten patients received a 99mTc tagged insulin preparation and were scanned for site of absorption.

Results: BS levels decreased substantially in 185 patients (87%) and less pronounced in another 10 (4.7%) with an overall response rate of 91.7%. Type I patients showed a reduction of BS with relative higher insulin levels. No local or systemic side effects were encountered. Scintigraphy demonstrated sites of absorption.

Conclusions: We conclude that this treatment is feasible and extremely safe. Auditory cerumen (earwax) might contribute to absorption. In contrast to other routes external auditory channel has a residual bioavailability of 100% and an immediate bioavailability of 30–50% and can be regarded as the ideal route for both bolus and continuous treatments. It not only enables us immediate reactions to daily Physical activities, meals, stress and sex, many treatment schedules mimicking natural insulin oscillations become possible. Faster insulin preparations are underway.

A3.09
Treatment of 212 patients with Type I and II Diabetes Mellitus with a novel Insulin preparation which is avidly absorbed if instilled into the auditory channel: The Hamlet/Polonius effect
K. Pirkalani and Z. Talaee Rad
Mehr Medical Group, Endocrinology/Internal Medicine/Molecular Biology, Tehran, Islamic Republic of Iran.

Objective: To find new routes of insulin treatment, we have used one of the most effective preclinical preparations with no accompanying toxic agent to instill into the external auditory channel.

Research design and methods: Two hundred and twelve patients with type I and II diabetes mellitus were enrolled. After discontinuation of all antidiabetic medications based on blood glucose levels 6–60 IU equivalent insulin was instilled into the ear of resting patients and blood glucose level were evaluated at 15, 30, 45, 60, 90, 120, 150 and 180 minutes and serum insulin at 120 minutes. Patients were monitored for local and systemic side effects. Ten patients received a 99mTc tagged insulin preparation and were scanned for site of absorption.

Results: BS levels decreased substantially in 185 patients (87%) and less pronounced in another 10 (4.7%) with an overall response rate of 91.7%. Type I patients showed a reduction of BS with relative higher insulin levels. No local or systemic side effects were encountered. Scintigraphy demonstrated sites of absorption.

Conclusions: We conclude that this treatment is feasible and extremely safe. Auditory cerumen (earwax) might contribute to absorption. In contrast to other routes external auditory channel has a residual bioavailability of 100% and an immediate bioavailability of 30–50% and can be regarded as the ideal route for both bolus and continuous treatments. It not only enables us immediate reactions to daily Physical activities, meals, stress and sex, many treatment schedules mimicking natural insulin oscillations become possible. Faster insulin preparations are underway.

A3.10
Radix Paeoniae rubra is inhibiting PTP1B inhibitor through Pentagalloylglucose
Department of Pharmacognosy, University of Vienna, Vienna, Austria.

The protein tyrosine phosphatase 1B (PTP1B) is one of the main negative regulators of insulin signalling. PTP1B knockout mice not only show increased insulin sensitivity but also are resistant to weight gain when put on a high fat diet. Therefore, the inhibition of PTP1B is of substantial interest for the treatment of type 2 diabetes mellitus and the metabolic syndrome. We chose PTP1B as a target for bioassay-guided fractionation of root material of Paeonia rubra, a plant widely used in Traditional Chinese Medicine against the metabolic syndrome. After showing that the crude methanol extract of radix Paeoniae rubra displays PTP1B inhibitory activity in an in vitro enzyme assay we fractionated the methanolic extract with Sephadex® LH-20 column chromatography. Fractions were tested for their pharmacological activity in vitro and further separation on Sephadex® LH-20 and by preparative HPLC lead to the isolation of one active pure compound: Pentagalloylglucose (PGG),...
Abstracts

A3.11 Xanthinuria type I in two Czech families: Biochemical and molecular genetics analysis

B. Stiburkova1, J. Krijt1, J. Bartl1 and I. Sebesta2
1Institute of Inherited Metabolic Disorders, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic, 2Institute of Inherited Metabolic Disorders and Institute of Clinical Biochemistry and Laboratory Diagnostics, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic

Xanthinuria type I (OMIM 278300) is a rare disorder of purine metabolism caused by xanthine dehydrogenase deficiency. The affected individuals are characterised by strongly diminished production of serum uric acid (UA) and high urinary excretion of hypoxanthine and xanthine. The patients may develop calculi in the urinary tract, acute renal failure and myopathy due to deposits of xanthine. We report a two probands without clinical symptoms. Female (38 year.): the concentration of UA in serum was not detected and 0.4 pmol/hour/ml of plasma. The activity from heterozygous deletion was within the control ranges. Sequence analysis covered protein-coding regions of XDH. Both patients and his brother had normal biochemical findings. Second patient – male (25 year.): the concentration of UA in serum was not detected in two cases, in urine 0.03 mmol/l, excretion of xanthine 141 mmol/mol Cr (ref. < 25 mmol/mol Cr). Her two sons were investigated with normal biochemical findings. Second patient – male (25 year.): the concentration of UA in serum was not detected in urine 0.04 mmol/l, ref. 0.4-4.6) was detected, in five occasions UA was under the limit of detection. Excretion of xanthine in urine was 170 mmol/mol Cr (ref. < 25 mmol/mol Cr). Both parents and his brother had normal biochemical findings. Allopurinol loading test indicated xanthinuria type I, the xanthine oxidase activities in patients were 0 and 0.4 pmol/hour/ml of plasma. The activity from heterozygotes was within the control ranges. Sequence analysis covered promoter, 36 exons and intron-exon boundaries. We found three previously undescribed nonsense changes. Heterozygous deletion p.P214QfsX4 was found in female and her son, R825X in male and her mother. The haplotype and statistical analysis of consanguinity is in process. Supported by grant MSM0021620806 and MZO VN2005 Czech Republic.

A3.12 Abstract withdrawn

A3.13 Molecular mechanisms of plasma glucose-lowering effect of LVVYPW in streptozotocin-induced diabetic rats

F. Sarukhanyan1, J. Kellermann2 and N. Barkhudaryan1
1H. Bunianian Institute of Biochemistry NAS RA, Laboratory of Neuropeptides Biochemistry, Yerevan, Armenia, 2Max-Planck Institute of Biochemistry, Department of Protein Analysis, Martinsried, Germany

LVVYPW is a member of hemorphins family, an endogenous nonclassical opioid peptides derived from hemoglobin. Hemorphins were proposed to serve as homeostatic factors that switch on the compensatory systems in the organism during severe pathologies. Very recently we have revealed the plasma glucose-lowering effect of LVVYPW. Experiments were done on male Wistar rats aged 8–10 weeks (weight 200–220 g). Rats received single intraperitoneal (ip) injection of streptozotocin (STZ) at 60 mg/kg to induce diabetes. After ip injection of LVVYPW (1 mg/kg) into fasting STZ-induced diabetic rats, this peptide has been shown to decrease plasma glucose levels from 26.8 ± 0.61 mmol/l to 18.2 ± 1.88 mmol/l in 2 hour. Naloxone and naloxonazine, µ-opioid receptors (MORs) antagonists, abolished the plasma-lowering effect of LVVYPW. Thus, MORs are involved in anti-diabetic effect of LVVYPW. Earlier we have found out that Ca2+ /calmodulin(CaM)-dependent protein phosphatase calcineurin is involved in the molecular mechanisms of hemorphins action in physiological and pathophysiological conditions. LVVYPW recovered calcineurin activity, increased in STZ-induced diabetic rat plasma and brain. It is proposed that molecular mechanisms of hemorphins action in both physiology and pathology involve integration of Ca2+ /CaM/calcineurin pathway with MOR function.

A3.14 Aerobic metabolism in Rod outer segments: molecular basis for oxidative stress-related retinal pathologies

D. Calzia1, S. Barabino2, S. Ravera1, M. Rolando2, A. Morelli1, G. Calabria2 and I. Panfoli1
1University of Genova, Biology Department, Genova, Italy, 2Department of Neurosciences, University of Genova, Ophthalmology, and Genetics, Genova, Italy

Rods are associated with scotopic vision. Rod outer segment (OS), where phototransduction takes place [1], consists of a stack of flattened disks surrounded by the plasma membrane. Previous proteomic and biochemical analyses reported the functional expression of the respiratory chain complexes I-IV and F1Fo-ATP synthase in OS disks. Our present study confirmed the presence of an oxidative metabolism in rod OS, a more native sample than disks, isolated by a simpler procedure from bovine retinas [2]. Rod OS were characterized for purity. An oxygen consumption (stimulated by glucose and reverted by rotenone, antimycin A and KCN) and ATP synthesis (0.560 ± 0.084 µmol/min/mg), sensitive to the common ATP synthase inhibitors, was measured in rod OS. The presence of Cytochrome c oxidase (COX) and F1Fo-ATP synthase in the sample was verified by Semiquantitative Western-immunoblotting. Moreover COX was also catalytically active. Co-localization of COX and F1Fo-ATP synthase with Rodopsin in rod OS by immunohistochemical analysis on mouse ocular sections confirm results. Data indicate that an oxidative phosphorylation occurs in rod OS, which do not contain mitochondria, thank to the presence of ectopically located mitochondrial proteins. This study may shed light on the pathogenesis
of many retinal degenerative diseases that correlate with energy availability or oxidative stress in the photoreceptors and on the efficacy of empirical treatments, such as hyperbaric or vitamin therapies, in these pathologies.

References:

A3.15
Systemic redox modifications in acute diabetic foot ulcers
B. Virgolici1, M. Mohora1, I. Stoian1, L. Gaman1, D. Lixandru1, C. Muscurel1, A. Coman2, J. Vertommen3 and B. Manuel-Y-Keenoy3
1University of Medicine and Pharmacy Carol Davila, Bucharest, Romania, 2Institute of Diabetes Nutrition and Metabolic Diseases, Bucharest, Romania, 3University of Antwerp, Metabolic Research Unit, Antwerp, Belgium

Peripheral neuropathy and atherosclerotic peripheral arterial disease are implicated in diabetic foot ulcers pathogenesis. Oxidative stress is associated with diabetes mellitus. The aim of this study is to investigate oxidative stress, protein glycation and inflammation in diabetic foot patients considering the pathogenic mechanisms of acute diabetic foot ulcers. Thirty eight hospitalized type 2 diabetic foot patients were divided in a subgroup defined by the presence of both neuropathy and arteriopathy (n = 15) and another subgroup with neuropathy only (n = 23). Twenty healthy subjects were involved. Increased erythrocyte enzymes activities for glutathione S transferase (p < 0.05), catalase (p < 0.03), higher plasma levels for fasting glucose (p < 0.01), dicarbonyls (p < 0.01), C reactive protein (p < 0.04), malondialdehyde (p < 0.05) but lower superoxide dismutase activity (p < 0.02) were determined in the diabetic foot subgroup with both complications versus subgroup with neuropathy only. There weren’t any significant difference in the age, duration of diabetes, HbA1c and plasma lipids levels between subgroups. Studied parameters were modified in patients versus controls. In conclusion, the presence of atherosclerotic peripheral arterial disease in diabetic foot ulcers highlights important systemic redox modifications including: increased oxidative and carboxylic stress, increased levels of inflammatory markers and increased lipid peroxidation. Aggressive treatment focused on these pathogenic mechanisms should be instituted in order to prevent the development or evolution of coronary and renal arteries diseases in order to reduce cardiovascular mortality in diabetic foot patients.

A3.16
Evaluation of insulin-mimetic activity of new vanadyl-flavonoid complexes in alloxan-induced diabetes
D. Margina1, B. Veleescu, V. Uivarosi, V. Aldea, S. Negres and N. Mitrea
Faculty of Pharmacy, Bucharest, Romania

Aim: The aim of the study was the evaluation of the insulin-mimetic activity for new complexes of vanadyl with chrysin and querectin.

Materials and methods: Diabetes was induced to male adult Wistar strain albino rats with intraperitoneally administered alloxan. The animals were divided into five groups, each of six animals: one control group received water orally (1 ml/100 g body weight), two groups received complexes of vanadyl with chrysin and querectin (0.4 mmol/kg body weight) and two groups received the corresponding flavones (chrysin and querectin, 0.8 mmol/kg body weight). The treatment was continued for 5 days. Blood was drawn from the animals and serum was separated for the assay of glucose (with standard glucose-oxidase method) and insulin (ELISA method).

Results: The vanadyl-queretic complex determined a 42.89% decrease of the glucose level, even if the insulin level, compared to control, decreased with 7.01%. The vanadyl-chrysin complex leads to a 16.44% decrease of the glucose level, while the insulin level, compared to control, decreased with 5.26%. The insulin level increased with 56.14% under the effect of querectin (correlated with a 10.53% decrease of glucose level), while chrysin determined a 1.79% decrease of the insulin level (correlated with a 24.24% decrease of the glucose level).

Conclusions: The mechanism of action of the new complexes tested in this study was extra-pancreatic, based on the fact that complexes of vanadyl with querectin and chrysin determined the reduction of the glucose level which was not correlated with an increase of the insulin level.
A3.19
Alteration of the enzymatic activity of SDH upon glycation in diabetic conditions
A. Salari1, M. Habibi-Rezaei2, A. Bidmishkipoor3 and M. Amiriz
1Razi University, Tehran, Islamic Republic of Iran, 2University of Tehran, Tehran, Islamic Republic of Iran, 3Razi University, Kermanshah, Islamic Republic of Iran

Sorbitol dehydrogenase (SDH, EC 1.1.1.14) is a ubiquitous enzyme which catalyses the second step of polyol pathway. Here alteration of the enzymatic activity of SDH upon glycation in diabetic conditions has been investigated. After extraction and partial purification of SDH from sheep liver, the samples were incubated in defined concentrations of fructose and ribose and estimated for activity over time. The results indicate that the glycation can negatively affect the SDH functionality so that both the activity and the catalytic efficiency of this enzyme are reduced in comparison with the non-glycated control sample. These observations can explain the way of participation of the glycated SDH in the occurrence of diabetic complications.

A3.20
The impact of glycation on the kinetic parameters of sorbitol dehydrogenase
A. Salari1, M. Habibi-Rezaei2, A. Bidmishkipoor3 and M. Amiriz
1Razi University, Tehran, Islamic Republic of Iran, 2University of Tehran, Tehran, Islamic Republic of Iran, 3Razi University, Kermanshah, Islamic Republic of Iran

Sorbitol dehydrogenase (SDH) is one of the potential targets of nonenzymatic glycation in diabetic conditions. This protein catalyzes second step in polyol pathway and is known to be involved in cataract and some other diabetic complications. In this study, SDH was extracted and partially purified from sheep liver. The purified enzyme was incubated in defined concentrations of ribose and fructose, glycation processes were followed up and equilibrium constants (Keq) for SDH reactions were determined. The product of glycation shows declined equilibrium constant in comparison with the non-glycated SDH as control in enzymatic reaction. Parameters of sorbitol dehydrogenase in the occurrence of diabetic complications.

A3.21
Abstract withdrawn

A3.22
Screening of putative mutations in β-globin gene by DNA sequencing of β-thalassemia major patients from Izeh and Baghmalek cities of Iran
M. Oraki Kohshoor1, H. Gahedari2, M. Pedram3, K. Zandian3, B. Keikhaei1 and B. Salehi1
1Ahvaz Jundi Shapur University of Medical Sciences, Immunology, Ahvaz, Islamic Republic of Iran, 2Chamran University of Ahvaz, Genetics, Ahvaz, Islamic Republic of Iran, 3Ahvaz Research Center for Thalassemia and Hemoglobinopathies, Ahvaz, Islamic Republic of Iran

Beta thalassemia is a heterogeneous inherited disorder of β-globin synthesis with over 200 different β-globin reported mutations worldwide. The highest prevalence of the disease occur in the Mediterranean region, parts of the North and West Africa, the Middle East, the Indian subcontinent and the Southern Far East and Southeastern Asia, all together composing the so-called thalassemia belt. To establish the molecular spectrum of the beta thalassemia in the Izeh and the Baghmalek cities in the Iran, 110 unrelated beta thalassemia major patients with high Bakhtian ethnic background were studied. Genomic DNA from blood was extracted and after amplification of β-globin gene by specific primers, full length sequencing of β-globin gene was done by DNA sequencing. The remarkable results revealed the relative high frequency of beta thalassemia mild (β+) causing mutations within the subjects. These mutations were -88(C>A), -28(A>C), -101(C>T) and 5UTR + 20 locating in the promoter or untranslated region of the beta globin gene. The individuals were homozygous or compound heterozygous for the mentioned nucleotide changes. The Iran encompasses a multiethnic population with vast spectrum of beta thalassemia mutations. The result described here could greatly facilitate screening for β-thalassemia and prenatal diagnosis and also will be of valuable help in the development of successful prevention programs for the population, at least for the regions of Iran.

A3.23
Investigation on the anticancer properties and subacute toxicity of cultivated tiger milk mushroom (Lignosus rhinocerus)
M. L. Lee1, S. S. Lee1, N. H. Tan1, S. Y. Fung1, C. S. Tan2 and S. T. Ng2
1University of Malaya, CENAR and Department of Molecular Medicine, Faculty of Medicine, Kuala Lumpur, Malaysia, 2MARDI, Biotechnology Research Centre, Kuala Lumpur, Malaysia

The “Tiger-milk” mushroom (Lignosus rhinocerus) is an important medicinal mushroom used by the local tribal people in Malaysia. It is only found in a small geographic region, mainly Southeast Asia. This fungus was used by the locals to treat asthma, fever, cough, cancer and food poisoning. Recently, the mushroom has been successfully cultivated. We investigated the anti-cancer activities of the crude extracts of a cultivar of tiger milk mushroom (cultivar CH2) using lung (A549) and breast (MCF-7) carcinoma cell lines as well as on normal human bronchus (NL20) cell line by MTT method. The IC50 was determined to be 450 μg/ml and 3500 μg/ml respectively on A549, MCF-7 and NL20. DNA fragmentation assay showed a typical ladder pattern of DNA fragmentation, suggesting that the crude extract inhibits cancer cell growth via apoptosis. A 28-day sub-acute toxicity study using Sprague-Dawley rats was also carried out. The animals in control as well as in treated groups (50–1000 mg/kg) showed similar pattern of body weight gain, and the results of hematology and serum biochemistry also showed no statistically significant differences between the control and the treated groups.

A3.24
Grape-seed derived procyanidins inhibit dipeptidyl peptidase 4 (DPP4) activity
N. González-Albuin, N. Martínez-Micaelo, M. Blay, A. Ardèvol and M. Pinent
URV, Departament de Bioquímica i Bioteconomia, Tarragona, Spain

The plasma dipeptidyl peptidase 4 (DPP4) activity is increased in subjects with chronic hyperglycemia and type 2 diabetes, and
A3.25 The evaluation of LCAT concentration in patients with DM1

M. Wegner¹, A. Araszkiewicz², D. Zozulinska-Ziolkiewicz², B. Wierusz-Wysocka², A. Piorunska-Mikolajczak¹ and M. Piorunska-Stolzmann¹

¹Department of General Chemistry, University of Medical Sciences, Poznań, Poland, ²Department of Internal Medicine and Diabetology, University of Medical Sciences, Poznań, Poland

Introduction: Lecithin:cholesterol acyltransferase (LCAT) is one of the most important enzyme involved in HDL metabolism and promotes reverse cholesterol transport. Diabetes type 1 (DM1) is associated with lipoprotein abnormalities, therefore the aim of the study was to evaluate the LCAT concentration in DM1 patients.

Materials and methods: The studied group consisted of 81 DM1 (43.4 ± 6.3 years). It was divided into patients with ≤10 years (n = 54, subgroup1) and > 10 years (n = 27, subgroup 2) of DM1. 25 healthy people (31.2 ± 10.2 years) were a control group. Metabolic parameters were measured using standard laboratory procedures. IL-12 and LCAT concentrations were assayed by ELISA.

Results: DM1 patients had lower LCAT level than healthy group [5.5(4.1–6.3) versus 6.1(4.6–7.5) μg/ml]. In addition, the negative correlation between IL-12 and LCAT level has been shown in this group (r = −0.307, p < 0.05, n = 81). LCAT concentration was also decreased in patients of subgroup two in comparison to subgroup 1 [4.5(2.3–6.0) versus 5.6(4.5–6.4) μg/ml]. Moreover, higher ratio of LDL:HDL [1.7(0.7–3.7) versus 1.5(0.5–4.6)] and correlation between LCAT and IL-12 (r = −0.51, p < 0.05, n = 27) were found in subgroup 2.

Conclusion: Lower level of LCAT in DM1 patients could be one of the risks of atherosclerosis development. The LCAT concentration decreases upon DM1 duration, enhancing proatherosclerotic tendency in patients with long DM1 duration. The relation between the IL-12 and LCAT suggests, that low LCAT level inhibits the reverse cholesterol transport, what increases the chronic inflammatory process. The study was supported by PhD grant (No02476337).

A3.26 Glycation-induced changes in the molecule of glutathione S-transferase

I. Bousˇová, L. Trinková, L. Srbová, Z. Pruchová and J. Drišta

Charles University in Prague, Faculty of Pharmacy, Hradec Kralove, Czech Republic

Glycation is a non-enzymatic process resulting in the impairment of protein functions during diabetes mellitus and age-related diseases. The reactive alpha-dicarbonyl compounds (e.g. methylglyoxal) and advanced glycation end products (AGEs) are formed. Alpha-dicarbonyls cause protein cross-linking and formation of AGEs mainly on intracellular proteins, which usually lose their biological activity and may persist in tissues. Glutathione S-transferases (GST, EC 2.5.1.18), group of intracellular enzymes involved in detoxification of xenobiotics, belong to the most abundant cytosolic proteins. We suppose that GST may be modified by glycation in vivo, which would provide a rationale of its use as a model protein for studying glycation reactions. Glycation of GST by methylglyoxal, fructose or glucose was studied (37°C, for up to 28 days). The course of protein glycation was evaluated using following criteria: enzyme activity, formation of fluorescent AGEs, amino group’s content, protein conformation, cross linking and aggregation, carbonyl content, and changes in molecular charge of GST. The ongoing glycation by methylglyoxal 2 mM resulted in pronounced loss of GST enzymatic activity. It also led to the loss of 14 primary amino groups, which was accompanied by changes in protein mobility during native PAGE. Formation of cross-links with molecular weight of 65 and 135 kDa was observed. Obtained results can contribute to understanding of changes, which proceed in metabolism of xenobiotics during diabetes mellitus and ageing.

Acknowledgments: This study was realized with financial support from the Czech Science Foundation, Grant No. 524/09/P21.

A3.27 Adipocyte fatty acid binding protein (A-FABP) in Kupffer cell as a novel player in the pathogenesis of non-alcoholic fatty liver disease

Y. L. Wong¹, R. L. C. Hoo¹, K. S. L. Lam¹, P. M. Vanhoutte² and A. Xu¹

¹University of Hong Kong, Medicine, Hong Kong, ²University of Hong Kong, Pharmacology, Hong Kong

Obesity is a major risk factor for non-alcoholic fatty liver disease (NAFLD). Our recent study demonstrated that adipocyte fatty acid binding protein (A-FABP) is an early predictor for the development of obesity-related pathologies, including metabolic syndrome, type II diabetes and atherosclerosis. An increased level of A-FABP mRNA expression in patients with NAFLD has also been observed. This study aims to examine the role of A-FABP in the pathogenesis of NAFLD by using different mouse models. C57 male mice were intra-peritoneally injected with lipopolysaccharide (LPS) to induce acute liver injury or fed with high fat liquid diet to induce chronic liver injury. Depletion of Kupffer cell was performed by tail-vein injection of gadolinium chloride III to examine the hypothesis that AFABP is mainly expressed in hepatic macrophages. Hepatic A-FABP mRNA and protein levels were increased following acute injection of LPS or high-fat diet induction. It was accompanied by elevated levels of liver injury markers, alanine aminotransferase (ALT) and aspartate transaminase (AST) and increased production of pro-inflammatory cytokines. Depletion of
Kupffer cells reduced the LPS-induced over-expression of A-FABP in both mouse models, indicating Kupffer cells were the major cell population for A-FABP expression in liver. The pharmacological inhibitor of A-FABP blocked LPS-induced production of pro-inflammatory cytokines in macrophages. Both endothoxin and high-fat induced liver injury were associated with elevated A-FABP expression in Kupffer cells, indicating an etiological role of A-FABP in the pathogenesis of NAFLD.

A3.28 Calciphylaxis – A highly regulated process due to deregulation of mesenchymal cell differentiation
J. Beil1, B. Findeni1, C. Hauser-Kronberger1, M. Franzen2, S. Bastelberger1, L. Pradel1, E. Hinterseer1 and H. Salmhofer1,2
1General Hospital and Paracelsus Private Medical University Salzburg, Pathology, Salzburg, Austria, 2General Hospital and Paracelsus Private Medical University Salzburg, Internal Medicine I, Salzburg, Austria

Calciphylaxis is a rare and life-threatening disease and occurs in patients with renal failure and diabetes. It is characterized by vascular and soft-tissue calcifications which results from an active cell-mediated process of calcification regulating proteins. We investigate several calcification inhibitors (Fetuin-A, Matrix-GLA-Protein (MGP), bone morphogenic protein7 (BMP7), osteoprotegerin (OPG) and osteopontin (OPN)) which play a role in the differentiation of chondrocytes and osteoblasts by automated immunohistochemistry. Further regulating proteins like BMP2 and 4, Tenascin-C (TNC), SOX9 and Indian Hedgehog (Ihh) are investigated. Ihh is involved in the differentiation from proliferated chondrocytes to prehypertrophic chondrocytes. During the states of differentiation BMP7 is highly expressed. TNC is produced in association with the aggregating prechondrogenic mesenchymal cells in developing cartilages and it is involved in EMT. For this study formalin-fixed paraffin embedded (FFPE) subcutis tissue of 20 patient with calciphylaxis were compared with 22 normal controls. The vasculature, especially endothelial cells, intima, media and adventitia as well as the connective and fatty tissue were analysed. In calcified tissues BMP7 is over expressed in the vascularast as well as in the connective and fatty tissue. Furthermore Ihh and TNC was also higher expressed in calciphylaxis. MGP, OPG, OPN and BMP2 and 4 did not show any differences. Fetuin-A and Sox9 seems to be higher expressed. Calciphylaxis – A highly regulated process due to deregulation of mesenchymal cell differentiation

A3.29 The levels of IL-6, IL2R and IGFBP-3 in patient with atherosclerosis
H. Y. Yaroglu1, N. Muslu1, N. D. Unal2, L. Ayaz3, D. C. Yilmaz2, G. Polat1 and L. Tamer1
1Mersin University Medical Faculty, Medical Biochemistry, Mersin, Turkey, 2Mersin University Medical Faculty, Cardiology, Mersin, Turkey

Atherosogenesis is highly complex and is modulated by numerous genetic and environmental risk factors. A large body of basic scientific and clinical research supports the conclusion that inflammation plays a significant role in atherogenesis along the entire continuum of its progression. Inflammation adversely impacts intravascular lipid handling and metabolism, resulting in the development of macrophage foam cells, fatty streaks and atheromatous plaque formation. Interleukin-6 (IL-6) may play a direct role in endothelial activation, or an indirect role when fibrinogen synthesis is stimulated. Interleukin-2 receptors (IL-2R) are expressed on activated T cells, B cells, and monocytes. Insulin-like growth factor-binding protein-3 (IGFBP-3) inhibits cell growth, both directly by inhibition of DNA synthesis and indirectly by sequestration of IGFs, which stimulate cell growth. The aim of the present study was to examine the levels of IL-6, IL 2R and IGFBP-3 in atherosclerosis. Seventy patients with atherosclerosis (age 57.3 ± 13 years) and sixty control (age 60.9 ± 12 years) were included in our study. Blood samples were collected in the first 24 hours and at the end of the three month. Serum IGFBP-3, IL6 and IL2R levels were determined by electrochemiluminescence method. We found increased levels of IL-2R in the atherosclerosis group compared to control group (653.6 ± 322.4 U/ml versus 559.15 ± 297.3 U/ml, p = 0.04). There was no relationship between IGFBP-3 and IL-6 levels and atherosclerosis. Further investigations are required to disclose the mechanisms of elevation and the effects of increased serum levels of these factors in atherosclerosis.

A3.30 Effective delivery of encapsulated hydrophobic-type photosensitizers to the MCF-7 cancer cells
K. Zielinska1, J. Pietkiewicz2 and K. A. Wilk1
1Department of Chemistry, Wrocław University of Technology, Wrocław, Poland, 2Department of Medical Biochemistry, Medical University of Wrocław, Wrocław, Poland

The development of effective nanocarriers faces numerous challenges, reaching the target site preferably in a time controlled way, limiting the drug’s adverse effects and ensuring biocompatibility. Present work is focused on effective delivery of hydrophobic cyanines to the cancer cells by poly(n-butyl cyanoacrylate) (PBCA) nanocapsules. We report on the synthesis and imaging of PBCA nanocarriers, their in vitro biological evaluation and the cellular localization of cyanine IR-768 delivered in the nanocapsules to MCF-7 cancer cells. Oil-cored PBCA nanocapsules were prepared as in [1] by interfacial polymerization in o/w microemulsions of gemini sugar surfactants (N,N′-bis(C12LA)), N,N′-bis(3-aldonylamo)propyl]ethylenediamines (bisC12GA) or bisC12LA)), iso-butanol, iso-octane and water. Nanoparticles with diameters in the range of 200–600 nm were visualized by atomic force microscopy (AFM) and scanning electron microscopy (SEM). In vitro studies on the erythrocyte hemolysis and the cell viability of breast cancer MCF-7 cells showed that the PBCA nanocapsules are safe carriers of cyanines in the circulation, having a very low hemolytic potential and being non-toxic to the studied cells. Fluorescence microscopy visualized the cyanine intracellular distribution and demonstrated that PBCA-nanocarriers effectively delivered the cyanine molecules to the MCF-7 doxorubicin-sensitive and -resistant cell lines.

Reference:

A3.31 Acetic zinc influence on ethanol metabolism and basic membrane-bound enzymes activity in hepatic and brain cells under chronic alcoholic intoxication development
V. Chayka, O. Kharchenko, L. Bogun and O. Gadlia
Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

Deficiency of zinc in a number of organs is observed under conditions of a chronic alcoholic intoxication. For the purpose of
correction of the specified dissonance use zinc salts among which low toxicity characterizes acetic zinc. White laboratory rats-males (weight 180–200 g) kept to standard vivarium diet were used in experiments. Animals were parted on three groups: 1 group – control animals; 2 group – rats with a chronic alcoholic intoxication which caused behind standard procedure; 3 group – rats with a chronic alcoholic intoxication which follow-up administration of acetate in a dose of 0.2 g on 100 g of animal mass one times into day. Ethanol metabolism systems functioning (alcohol dehydrogenase, aldehyde dehydrogenase activity, cytochrome P450 content) and functional condition of plasmatic membranes of rats liver and brain cells were investigated for 4, 6, 11, 16 and 21 days after the experiment beginning. It was shown, that under the chronic alcoholic intoxication there was dysfunction of the basic systems of anol metabolism through the decrease of ADG/AIDG system activity, activation of microsomal oxidations (increase of the cytochrome P450 content) and increase of basic membrane-bound enzymes activity (Na⁺, K⁺-ATPase, Ca²⁺-Mg²⁺-ATPase and 5’-nucleotidase) of rats liver and brain cells. The acetic zinc treatment resulted in the reduction of ethanol influence on investigated metabolism indicators (normalization of cytochrome P450 content, decrease of membrane-bound enzymes activities, increase of ADG and AIDG activities) in rats liver and brain cells in comparison with animals with a chronic alcoholic intoxication.

**A3.32**

**Lebanese patients with Ornithine transcarbamoylase (OTC) deficiency:**

**Genotype–phenotype**

I. Saab, D. Daouk, H. Assi, H. Janom, K. Gharzeddine, K. Pascale and J. Usta

1 AUB, Medicine, Beirut, Lebanon, 2 AUB, Pediatrics and Adolescent Medicine, Beirut, Lebanon, 3 AUB, Biochemistry, Beirut, Lebanon

OTC deficiency is the most common enzyme deficiency among Urea Cycle Defects. It is an X-linked nuclear-encoded mitochondrial enzyme, with variable clinical presentation and age of onset. Homozygous males may present at any age while heterozygous females are asymptomatic in some cases. This study is the first report on the clinical presentation and genotype of Lebanese patients with OTC deficiency. Forty-nine subjects belonging to eight families were screened in our Metabolic Clinic. Patients presented at variable ages between 2 and 44 years. Clinical evaluation and metabolic work-up including ammonia level, plasma amino acids chromatography and urinary orotic acid quantification were done. Among late-onset cases, ten of the patients belonged to three consanguineous families from one village. Three of them passed away before diagnosis was made. The index patient was referred for incidental finding of moderate hyperammonemia (120 m/l) during a work-up for epigastric pain. All symptomatic patients had hyperammonemia, low ornithine and citrulline levels, and increased orotic aciduria. They are successfully controlled with protein restricted diet, arginine supplementation and sodium benzoate in acute decompensations. Genomic DNA from patients and their families was isolated. The 10 exons of the OTC gene were amplified by PCR, separated and sequenced for mutation analysis and SNPs. Dot blot analysis was also used to screen for carriers. Sequencing analysis revealed a point mutation Arg80His and a polymorphism AAG->AGA in Exon 2 in some of the patients. The remaining exons had normal sequence. Other genes will be screened in subjects with no identified mutations.

**A3.33**

Abstract withdrawn

**A3.34**

**Anti-inflammatory and liver protective action of bioactive complex rich in glycosaminoglycans obtained from small sea fish**

N. Rosoin, R. Nita, D. Ene and L. Olariu

1 «Ovidius» University, Constanta, Romania; Academy of Romanian Scientists, Biochemistry, Constanta, Romania, 2 S.C. BIOTEHNOS S.A, Research Department, Otopeni, Romania

By an original patented technology we have obtained a bioactive complex from small sea fish rich in sulfated glycosaminoglycans (44-60%) essential amino acids (2-6.5%), essential fatty acids (1-2% ω-6) and mineral salts (Ca, Na, K, Fe, Mg, Se, Ni, Cu, Si). This bioactive complex, besides anti-hyaluronidasic, decreasing of elastase and collagenase activity favor in vitro collagen fibrils formation. Also, GAG bioactive complex can interrupt collagenolysis activity of degeneration processes of cartilage, recovering extracellular matrix and manifests a strong antioxidant activity. In vivo on Wistar rats with carragenan-induced paw edema, and with CC14-induced liver toxicosis, we determinate an anti-inflammatory activity (81.4% in 24 hours) similar with diclofenac (83.7% in 24 hours) and liver protective effects by stimulation of hepatic catalase and inhibition of lipid peroxidation activity denoted by decreasing of MDA concentration. Taking into account the chemical composition of the GAG-bioactive complex obtained from small sea fish and the therapeutic effects highlighted by in vitro and in vivo experiments may be consider this a very good source for medicinal products with valuable therapeutic properties and minimal side effects.

**Acknowledgments:** This work was supported by CNMP PNCDI 2 research program 4, Project 6.1-043/2007–2010.

**A3.35**

Abstract withdrawn

**A3.36**

Abstract withdrawn

**A3.37**

**Three-dimensional quantitative structure-activity relationships (3D-QSAR) study of PPARγ partial agonists**

L. Gausch, E. Sala, G. Pujadas and S. Garcia-Vallvé

Universitat Rovira i Virgili, Biochemistry & Biotechnology, Tarragona, Spain

PPARγ is a member of the nuclear receptors superfamily that regulate the gene expression of proteins involved in glucose and lipid metabolism, regulation of adipocyte proliferation and differentiation and insulin sensitivity. Thiazolidinediones (TZDs) is one important class of synthetic agonist of PPARγ. TZDs are antidiabetic agents that target adipose tissue and improve insulin sensitivity, and are currently used in the treatment of type 2 diabetes. Despite the clinical benefit of these drugs, use of TZDs has been associated with adverse effects including weight gain, increased adipogenesis, renal fluid retention, and possible increased incidence of cardiovascular events. Therefore, new
Abstracts

A3.38  
**Antiplatelet activity of alpha-lipoic acid is mediated by PPARs**  
T.-C. Chou  
*Department of Physiology, National Defense Medical Center, Taipei, Taiwan*

Peroxisome proliferator-activated receptors (PPARs) including PPARα,β, and γ exist in human platelets and activation of PPARs inhibits platelet aggregation. Alpha-lipoic acid (ALA) occurring naturally in human diet exerts a protection against ath erosclerosis and thrombosis. However, little is known about the effect of ALA on platelet activation. Thus, the study was to investigate whether the antiplatelet activity of ALA is mediated by PPARs. ALA (200–800 μM) dose-dependently inhibited rabbit platelet aggregation induced by arachidonic acid (AA) or collagen. Addition of GW6471 (PPARα antagonist) or GW9662 (PPARγ antagonist), only the antiaggregative effect of ALA on AA-induced platelet aggregation was markedly reversed. However, GSK0660 (PPARγ antagonist) did not affect its antiplatelet activity. Similarly, the inhibitory effects of ALA on AA-induced thromboxane B2, O2− production, and Ca2+ mobilization were significantly reversed by simultaneous addition of GW6471 or GW6992. Moreover, addition of GW6471 or GW6992 markedly attenuated the increase of cyclic AMP formation by ALA. Interestingly, ALA also increased the interaction and immune-precipitation with PPARα,β,γ and protein kinase Ca (PKCa) accompanied by a decrease of PKCa activity both in resting or AA-stimulated platelets. Similarly, addition of PPARα,γ antagonists markedly reversed the inhibition of PKCa activity by ALA in resting or activated platelets. In conclusion, we first show a direct inhibitory effect of ALA on platelet aggregation, and provide a novel mechanism for its action through involvement of an association with PPARs and PKCa in platelets, by which ALA may subsequently produce antiplatelet activity.

A3.39  
**Fibrillation of human insulin is accelerated upon interaction with PVP-coated nanoparticles under in vitro conditions**  
A. A. Meratan1, H. Ostadi1, D. Morshedi2, A. Ghasemi2, S. Shariatizi1, A. Eshaghi1 and M. Nemat-Gorgani1  
1*Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Islamic Republic of Iran, 2Industrial and Environmental Biotechnology Department, National Institute of Genetic Engineering and Biotechnology, Tehran, Islamic Republic of Iran, 3Stanford Genome Technology Center, Stanford University, Palo Alto, US*

Proteins can interact with different biological and non-biological surfaces. However, the role of surface, as promoters of pathological aggregation of amyloidogenic proteins, is rarely studied. Nanoparticles are materials at sub-micrometer scales, usually 1–100 nm, and provide excellent systems for modeling protein surfaces. In particular, they can be readily fabricated with dimensions comparable to biological macromolecules. Moreover, the large surface area, small size of nanoparticles and the ability of nanoparticles to influence protein folding and aggregation make them as an interesting tool for understanding molecular events behind the processes leading from native to fibrillar states of proteins. Here the fibrillation process of human insulin was investigated in the absence and presence of Polyvinylpyrrolidone (PVP)-coated nanoparticles. Formation of amyloid fibrils and effect of PVP-coated nanoparticles were verified through some of the specific methods of amyloid detection such as Thioflavin-T (ThT) fluorescence, Congo red binding, circular dichroism and atomic force microscopy. The process was found to be affected by the presence of the nanoparticles, which accelerate human insulin fibrillation. Kinetic fibrillation data, as monitored by ThT fluorescence, suggested that nanoparticles caused shorter lag (nucleation) phase; which is the rate-determining step during amyloid fibrillation, possibly by increasing the local concentration of protein molecules. The present studies may open interesting directions for work on fibrillation-related diseases and for the discovery of agents (e.g. nanoparticles), which may interfere with such processes.

A3.40  
Abstract withdrawn

A3.41  
**Rage activation by advanced glycation end products (age) enhances collagen IV accumulation in human embryonic kidney (hek 293) cells**  
A. I. Serban1, M. C. Munteanu2, M. Costache2 and A. Dinischiotu2  
1*University of Agricultural Science and Veterinary Medicine, Biochemistry and Molecular Biology, Bucharest, Romania, 2University of Bucharest, Faculty of Biology, Biochemistry and Molecular Biology, Bucharest, Romania*

AGEs play major roles in the accumulation of extracellular matrix being involved in diabetic renal injuries. It seems that AGEs interaction with their receptor-RAGE leads to the activation of TGF-β and non-TGF-β pathways affecting the expression of several extracellular matrix proteins. Our aim was to evaluate the relationship between AGE, RAGE, TGF-β and collagen IV at the transcriptional and translational levels in HEK293 cells. The cells were incubated for 24 hours with AGE-BSA and BSA (control) at concentrations between 50–200 μg/ml in the presence or absence of 20 ng/ml anti-TGF-β or anti-RAGE antibodies. The mRNA expression of RAGE, TGF-β and procollagen γ1 was analyzed by quantitative real-time PCR. The protein levels of RAGE, type IV collagen and TGF-β were assessed by Western blot and ELISA. The concentration of 200 μg/ml AGE-BSA up-regulated the mRNA expression of RAGE, TGF-β and procollagen γ1 (IV), the relative expression ratio (R) being 1.83 ± 0.2, 3.4 ± 0.09 respectively 4 ± 0.12. At 100 μg/ml AGE-BSA, R decreased to 1.41 ± 0.12, 1.23 ± 0.05 and 2.68 ± 0.17 for RAGE, TGF-β, respectively procollagen γ1 (IV), whereas at 50 μg/ml AGE-BSA, R diminished more in all cases. The co-treatment with anti-TGF-β or anti-RAGE anti-
A3.42 Assessment of mutated apolipoprotein A5
J.-T. Kao, Y.-J. Huang, Y.-L. Lin and Y.-S. Wong
National Taiwan University, Taipei, Taiwan

Hypertriglyceridemia is an independent risk factor for coronary heart disease. According to the previous studies, triglyceride (TG) metabolic disorder was correlated with the apolipoprotein A5 (APOA5) gene polymorphisms. APOA5 can modulate the triglyceride hydrolyase activity of lipoprotein lipase (LPL) through direct activation or indirect effects to reduce the plasma TG. However, the mechanism of APOA5 polymorphisms is still unclear. We have identified a c.553G>T polymorphism, which was found in oriental populations only, causes a G185C substitution effect. Moreover, patients with c.553G>T polymorphism tend to suffer from hypertriglyceridemia. These phenomena inspired us that substitution of G185C might result in losing, or at least lowering the function of APOA5. The aim of this study is to assess the importance of glycine at 185th residue. Nineteen mutants of APOA5 protein were generated by site-directed mutagenesis. The mutant- and wild-type of APOA5 plasmid were transformed to E. coli BL21 and incubated at 37°C for 3 hour. Proteins produced were purified with nickel magnetic beads. A synthetic DMPC (1,2-Dimyristoyl-sn-Glycero-3-Phosphocholine), which simulated VLDL structure, was constructed. Different APOA5 protein was added to the DMPC vesicle preparation and incubated at 24°C for 3 hour. Then LPL activity was measured. The results showed that the activation ability of LPL by all mutant-type of APOA5 was lower than that of wild type of APOA5 (p = 0.0008). This might suggest that the importance of 185GG, any mutation will decrease its activation ability.

A3.43 Oxidative stress alterations in peritoneal macrophages and polymorphonuclear leukocytes for type I autoimmune diabetic mice
I. Adina Daniela1, S. Crina1, P. Ileana2, R. Beatrice Mihaela3, R. Dorel Lucian1 and R. Mihaela1
1‘Cantacuzino’ National Institute for Microbiology and Immunology, Laboratory of Cellular Immunity, Bucharest, Romania, 2Department of Health and Environmental Physics, ‘Horia Hulubei’ National Institute for Physics and Nuclear Engineering, Magurele, Romania, 3Department of Anatomy, University of Bucharest, Faculty of Biology, Animal Physiology and Biophysics, Bucharest, Romania

Diabetic hyperglycemia is associated with increased production of reactive oxygen species (ROS). The link between hyperglycemia and the complications of diabetes is unknown. Type I diabetic TCR-HA+/β-Ins-HA+/- mice are obtained by mating BALB/c mice-expressing transgenes encoding a TCR recognizing an immunodominant epitope of influenza PR8 virus hemagglutinin (HA110-120) in association with I-Ed (TCR-HA+/β-), and B10.D2 mice expressing the PR8 influenza virus HA transgene (Ins-HA+/-). Nitric oxide (NO) and O2- levels in peritoneal macrophages and polymorphonuclear leukocytes (PMN) are quantified. Respiratory burst of PMN was evaluated by flow cytometry. Three stimuli are used: N-formyl Met Leu Phe (fMLP), phorbol-12- myristate-13-acetate (PMA) and opsonised E. coli. Lymphocyte populations CD45R/B220+ , CD4+ and NK1.1 cells were investigated by flowcytometry. In plasma we have determined the carbonyl content in oxidative modified proteins and global antioxidant activity. In diabetic mice, O2- and NO production are higher compared to Balb/c mice. In peripheral blood and spleen of diabetic mice, a higher percentage of CD45R/B220+ and NK1.1 cells was detected by comparison with Balb/c mice. Plasma level of oxidized protein in diabetic mice was higher, while the antioxidant capacity was lower. In conclusion, all these characteristics indicate an altered oxidative stress in peritoneal macrophages and polymorphonuclear leukocytes associated with fulminant type I diabetes. This study was supported by PNII 41-07/2007.
Polymorphisms within prothrombotic genes in overweight patients with coronary artery disease

B. Sarcecka-Hajar1, I. Zak2 and J. Krauze3
1Department of Applied Pharmacy, Medical University of Silesia, Sosnowiec, Poland, 2Department of Biochemistry and Medical Genetics, Medical University of Silesia, Katowice, Poland, 3The First Department of Cardiology, Medical University of Silesia, Katowice, Poland

Background: Coronary artery disease (CAD) depends on a number of factors, genetic (including polymorphic variants encoding proteins involved in hemostatic mechanisms) and non-genetic (e.g. overweight or obesity). Methylene tetrahydrofolate reductase (MTHFR) catalyses remethylation of homocysteine (Hcy) to methionine. Increased levels of Hcy are related to decreased bioavailability of endothelial nitric oxide, well-known platelet aggregation inhibitor. Plasminogen activator inhibitor-I (PAI-I) is a fast-acting inhibitor of tissue and urokinase type (t-PA and u-PA) plasminogen activators. Its increased plasma levels are associated with different thrombotic disorders. The aim of the study was to establish associations between the MTHFR 677C>T and PAI-1 –675_–674insG polymorphisms and CAD in overweight patients.

Methods: We analysed 114 angiographically documented CAD patients with overweight and 118 blood donors with normal body mass index (BMI). Both polymorphisms were genotyped using PCR-RFLP method.

Results: The MTHFR T allele and T allele carriers (CT+TT) were slightly more frequent in patients than in controls. We observed higher prevalence of PAI-1 5G allele as well as 5G5G homozygotes in overweight cases compared to controls with normal BMI. The results were on bound of significance (p = 0.082, OR = 1.38 and p = 0.057, OR = 2.02 respectively). Contemporary carrier-state of both analyzed polymorphic variants did not differentiate analyzed groups.

Conclusions: There are possible interactions between PAI-1 –675_–674insG polymorphism and overweight in determining the risk of coronary artery disease.

Funding: NN-4-006/02, NN-1-060/03 and KNW-1-037/08 projects.

EPR evaluation of proteins engineered to carry Gd(III) contrast labels

J. Petrija1, J. Lagerstedt2, R. Sriam1, J. Desreux3, D. Thonon3, T. Jue1 and John Voss1
1University of California Davis, Biochemistry and Molecular Medicine, Davis, US, 2Department of Experimental Medical Science, Lund University, Lund, Sweden, 3University of Liege, Coordination and Radiochemistry, Liege, Belgium

Apo A-I is the major protein constituent of high-density lipoprotein (HDL) and plays a central role in phospholipid and cholesterol metabolism. We have engineered apoA-I protein (apoA-I) to contain a DOTA-chelated Gd(III) for the purpose of imaging reconstituted HDL (rHDL) biodistribution, metabolism, and regulation in vivo. Through the targeted substitution of cysteine in apo A-I, we have attached the thio-reactive MRI (magnetic resonance imaging) agent MTS-ADO3A to two distinct positions (55 and 76) in the protein. MRI imaging of infused mice shows apo A-I labeled with ADO3A migrates to both liver and kidney, the organs responsible for HDL catabolism, however the contrast properties of apo A-I particles when the ADO3A moiety is located at position 55, compared to the protein labeled at position 76. Interestingly, conventional X-band EPR spectroscopy can detect differences in the Gd(III) signal when comparing the labeled protein in lipid-free to the rHDL state. Furthermore, both the highest MR contrast and narrowest Gd(III) EPR spectrum are achieved with using rHDL-associated apoA-I containing the ADO3A probe attached to position 55. While room temperature EPR requires > 1 mM Gd(III)-labeled protein to resolve the spectrum, the volume requirement is low (~5 µl). Thus conventional X-band EPR spectroscopy provides a practical assessment for the suitability of imaging candidates containing the site-directed ADO3A contrast probe.

The placenta and the gut: Regulation of barrier functions and inflammation by the maternal gut flora

M. Al-Asmakh1, S. Pettersson1, M. Yip Shu Fen1, R. Fundele2 and I. Hedén1
1Karolinska Institutet, Microbiology, Tumor and Cell Biology, Stockholm, Sweden, 2Uppsala University, Physiology and Developmental Biology, Uppsala, Sweden, 3Qatar University, Pharmacy, Doha, Qatar

Pregnancy is characterized by an altered maternal metabolism, due to hormones produced by the placenta, and to the activation of the maternal immune system. The placenta plays a key role for maternal metabolism and immune responses, and regulates fetal nutrient supply, fetal growth and fetal programming. Impairment of placental functions, e.g. in gestational diabetes mellitus (GDM) and pre-eclampsia (PE), will affect not only the mother, but also the fetus with both short- and long-term consequences for perinatal morbidity and chronic diseases in adult hood (e.g. cardiovascular diseases, obesity, DM). The commensal gut flora regulates a number of activities related to protective-, structural- and metabolic functions within and outside of gut. The aim of the present project was to examine the role of the maternal microbiota on placental functions with implications for gestational diseases (e.g. GDM, PE).

Methods and results: Placentas were obtained from Germ-free (GF) and normal (SPF) mice at day 19 of pregnancy. Isolated mRNA were analysed with Illumina Sentrix® Beadchip Array MouseRef-8 V2 (23,600 probes) and demonstrated lower expression of components involved in tight junction signaling in GF compared to SPF mice. Transepithelial electrical resistance measurements showed that serum from conventionalized GF mice altered tight junctions in CaCo-2 cells. Furthermore, serum levels of IL-1β were elevated in pregnant GF mice compared to non-pregnant GF mice and to pregnant SPF mice.

Conclusions: The maternal gut flora affects properties of the placenta related to inflammatory responses and barrier functions with potential impact on feto-placental transport and metabolism.

Discovery and mechanistic characterization of neolignans as PPAR-gamma agonists

N. Fakhrudin1, A. Ladurner1, A. Atanasova1, E. Heiss1, L. Baumgartner2, P. Markt3, D. Schuster3, E. Ellmerer4, G. Wolber3, J. Rollinger2, H. Stuppner2 and V. Dirsch1
1Department of Pharmacognosy, University of Vienna, Vienna, Austria, 2University of Innsbruck, Institute of Pharmacy/Pharmacognosy and Center for Molecular Biosciences, Innsbruck, Austria, 3University of Innsbruck, Institute of Organic Chemistry, Innsbruck, Austria
A3 – Metabolic Diseases

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that play an important role in lipid and glucose metabolism. Among three subtypes of PPARs, PPAR-gamma is the best characterized and its agonists are useful for the treatment of insulin resistance and metabolic syndrome. Aim of our study was to identify new PPAR-gamma agonists by a pharmacophore-based virtual screening and characterize the activation profiles in the cellular level. This approach predicted several neolignans to bind to the PPAR-gamma ligand binding domain (LBD). To verify this prediction, the neolignans diedeugenol, tetrahydrodieugenol, and magnoisol were synthesized or isolated and PPAR-gamma binding studies were performed. Compared to the clinically used PPAR-gamma agonist pioglitazone, the neolignans exhibited a highly similar binding pattern and were binding to the PPAR-gamma LBD with EC50s in the nanomolar range. The activity of these compounds was confirmed in a cell-based luciferase reporter model, showing a binding pattern suggesting partial agonism. In a coactivator recruitment assay, diedeugenol, tetrahydrodieugenol, and magnoisol also behaved as partial agonists and showed different recruitment patterns compared to pioglitazone. These neolignans induced 3T3-L1 preadipocyte differentiation in PPAR-gamma-dependent manner confirming effectiveness in a cell model with endogenous PPAR-gamma expression. In summary, this study identified several neolignans as novel PPAR-gamma agonists. Their interesting activation profiles suggest them as potential pharmaceutical leads or dietary supplements for the treatment of type 2 diabetes and the metabolic syndrome.

A3.51 Detection of advanced glycation end products in the serum of diabetic patients with chronic renal failure

K. Madziaszka, W. A. Wyley, R. Danielewicz, A. Bronowicka-Szyldeko, Marian Klinger, A. Gamian

1Department of Nephrology and Transplantation Medicine, Wroclaw Medical University, Wroclaw, Poland, 2Department of Medical Biochemistry, Wroclaw Medical University, Wroclaw, Poland, 3Department of Medical Biochemistry, Wroclaw Medical University, Institute of Immunology and Experimental Therapy, Polish Academy of Science, Wroclaw, Poland

It is well known that advanced glycation end products (AGE) are correlated with an appearance of vascular complications in diabetic persons and have been identified as important modulators of the development and progression of diabetic nephropathy [1]. Most of the methods used for AGE determination in serum samples were able to identify a narrow spectrum of glycated molecules such as pentosidine, pyrraline, carboxymethyllysine-protein derivatives. The aim of the present study was using the new original antibodies which could identify AGE in serum of diabetics being in five stage (eGFR< 15 ml/min/1.73 m2) of chronic kidney disease (CKD). All patients were treated by insulin and had satisfactory glycemic control – HbAlc < 7.5%. Anti-AGE antibodies were obtained in our laboratory after rabbits immunization by selected fraction of glycated myoglobin [2]. The immunoblotting method allows to detect a wide array of AGE in 72% of serum samples, probably by recognizing the common epitopes presented in various glycating products. Control serum samples of healthy volunteers contain some physiological level of glycated albumin. It is intriguing that the diabetic patients with a similar clinical phenotype exhibited the significant differentiation of high molecular weight AGE products in serum. We concluded that the new anti-AGE antibodies may be useful in detection of wide spectrum of AGE in diabetic serum samples. This report was supported by Grant N 401 3564 33 from Polish Ministry of Science and Higher Education and Grant No 1601 from Wroclaw Medical University.

References:

A3.52 Multifunctional protein enolase as a model system to investigate the protein glycation

J. Pietkiewicz, A. Bronowicka-Szyldeko, R. Danielewicz, A. Gamian

1Department of Medical Biochemistry, Wroclaw Medical University, Wroclaw, Poland, 2Wroclaw Medical University, Institute of Immunology and Experimental Therapy, Polish Academy of Science, Department of Medical Biochemistry, Wroclaw, Poland

Hyperglycemia and oxidative stress generate the carbonyl shock in cells. Increase level of the reactive low molecular weight aldehydes, eg. methylglyoxal (MOG), 4-hydroxynonenal (4HNE), trans-2-nonenal (T2N) and acrolein (ACR) can modify protein during glycation. Enolase, such as one of most common intracellular proteins, is more susceptible for glycation than other glycosylation enzymes [1]. Probably, enolase protects others intracellular proteins against glycation by effective binding the reactive aldehydes [2]. The aim of this report is to study the ability of 4HNE, T2N, ACR and MOG to modify the human and mammalian enolase. The glycation was carried out at the conditions described previously for human muscle-specific enolase modified by MOG [3]. The incubation of human and pig muscle enolase with reactive aldehydes (1:300 molar ratio of enolase to glycation factor) caused the enzyme activity inhibition in the following order: 4HNE > MOG > ACR > T2N. The SDS/PAGE analysis showed that colostrinine (peptides mixture from sheep colostrum) as well as carnosine and pyridoxamine has ability to decrease level of the high molecular weight AGE. These factors are able to protect muscle enolase against the decreasing of catalytic function during irreversible glycation in hyperglycemia.

References:

A3.53 Studies of human enolase interaction with plasminogen


1Department of Medical Biochemistry, Wroclaw Medical University, Wroclaw, Poland, 2Department of Medical Biochemistry, Wroclaw Medical University, Institute of Immunology and Experimental Therapy, Polish Academy of Science, Wroclaw, Poland

Enolase, as a multifunctional protein plays a receptor role, e.g. for plasminogen, on the surface of many cells [1]. Previously we localized an enolase on the myosarcoma cells (MESSA-1) surface and in the isolated outer membrane of the human breast carcinoma cells (MCF-7) [2,3]. In this report we prove that the enolase catalytic activity on the surface of intact MCF-7 cells is at
about 25% higher level than in of MESSA-1 cells, which suggest more intensive expression of the enolase protein on the breast cancer cell surface. The enolase was localized immunocytochemically in the cytoplasm and on the MCF-7 cells surface. Moreover, we determined the MCF-7 cell ability to superficial binding of human plasminogen. The enolase-plasminogen complex in vitro was detected in non-denaturing PAGE and by immunoblotting with rabbit anti-human alpha-enolase specific antibodies. The dissociation constant value (36 nM) for enolase-plasminogen complex was determined by using the surface plasmon resonance (SPR) technique and indicates the high binding ligand-receptor affinity. Our studies suggest that enolase as a plasminogen receptor on the cancer cell surface can be an essential factor of the metastasis mechanisms. This report was supported by Grant No 1602 from Wroclaw Medical University.

Reference:

A3.54
The changes in serum adiponectin levels by oral administration of surfactants in mouse
K. Obara1, Y. Kiyotani1, R. Nagasaki2 and H. Ushio3
1Department of Food Science and Technology, Tokyo University of Marine Science and Technology, Tokyo, Japan; 2Department of Chemistry and Materials Science, Gunma National College of Technology, Gunma, Japan

Surfactants are amphiphilic and emulsify hydrophobic and hydrophilic compounds. They have been widely used for water-insoluble compounds in many food materials and pharmaceutical products. Some surfactants such as Cremophor EL have been considered to affect on biological and pharmacological activity. Bile acids, such as cholic acid, are biogenic amphiphilic molecules that play essential roles in the absorption of dietary lipids. Adiponectin, one of adipocytokines, is secreted from adipose tissue and is thought to play an important modulator of glucose and lipid metabolisms in insulin-sensitive tissues of both humans and animals. In this study, we investigated the influences of surfactants on mouse serum adiponectin levels. Mice are orally administered with ionic surfactants, such as cholic acid and 3-((3-Cholamidopropyl) dimetylammonio) propanesulfonate (CHAPS), and nonionic surfactants, such as Cremophor EL, Triton X-100, and Brij-35, separately. Serum adiponectin levels of mouse were measured by Western blotting with anti-adiponectin antibody. Cremophor EL, Triton X-100, and Brij-35 markedly increased adiponectin levels, while cholic acid reduced the level. Cremophor EL however failed to enhance adiponectin secretion from 3T3-L1 adipocytes. This paper will discuss the mechanisms of the function of surfactants.

A3.55
Changes in the energy status and glucose uptake rate of PC-12 cells induced by acute subcytotoxic or mildly cytotoxic hexavalent chromium insults
M. J. Goncalves, A. C. Calado Santos, P. Coelho, A. N. Costa, C. F. Dias Rodrigues, M. S. Santos, A. J. Guiomar, M. C. Alpoim and A. M. Urbano
Departamento de Ciências da Vida, Universidade de Coimbra, Coimbra, Portugal

In the 1920s, Otto Warburg reported that, contrary to most normal cells, cancer cells rely strongly on lactic fermentation for energy production, even in the presence of an adequate oxygen supply. Whether this metabolic reprogramming, known as the Warburg effect, is a causative effect in cancer or a mere epiphenomenon, has been a subject of intense debate, but, nowadays, it is increasingly viewed by cancer researchers as one of the fundamental alterations that occur during the process of malignant transformation. To investigate whether this metabolic reprogramming may underlie hexavalent chromium [Cr(VI)]-induced carcinogenesis, we have determined the effects of acute exposures to low levels of this well-established carcinogen on the rates of glucose uptake, lactate production and oxygen consumption in cultures of PC-12 cells. Moreover, we have also determined the effects of these exposures on the cells’ adenylate energy charge, an important indicator of the cellular energy status. Slight, but statistically significant, changes in the glucose uptake ratios and adenylate energy charge of the cells were observed, pointing to a potential role for the Warburg effect in Cr(VI)-induced carcinogenesis. This work was supported by Centro de Investigação em Meio Ambiente, Genética e Oncobiologia (Grant CIMAGO 26/07).

A3.56
The effect of extracts from purple sweet potato leaves in adipocyte differentiation
S.-L. Lee1, M.-S. Kuo2, M.-C. Kao1 and T.-Y. Chin2
1Department of Biological Science and Technology, China Medical University, Taichung, Taiwan; 2Department of Bioscience Technology, Chung Yung Christian University, Chung Li, Taiwan

Obesity is characterized by an increase of adipose tissue as a result of positive imbalance between food intake and energy expenditure. Previous research has suggested that decreased proliferation and adipogenesis are mechanisms to reduce obesity. Purple sweet potato leaves (PSPL) contain a high content of polyphenolics which have been shown to exhibit a number of biological activities. In this study, we examined the effect of PSPL extracts on differentiation of 3T3-L1 preadipocytes and investigated how they work. 3T3-L1 preadipocytes were differentiated from fibroblasts to mature adipocytes and served as experimental materials. The cell line was stimulated by a mixture of insulin, dexamethasone, and methylisobutyrate (IDM). The effect of PSPL extracts varied with dose administration and with time course. The results obtained from MTT assay and BrdU incorporation showed that PSPL extracts could inhibit 3T3-L1 cells proliferation. PSPL extracts altered mRNA expression of adipocyte factor-1 (PREF-1) and CCAAT/enhancer binding protein α (C/EBPα). Our data suggested that PSPL extracts may modulate proliferation and differentiation of 3T3-L1 cells.

A3.57
Oral administration of GABA and phytosteryl ferulates prevent decreasing stressed mouse plasma adiponectin levels
T. Kazama1, Y. Kiyotani2, K. Ohara1, R. Nagasaki2, H. Ushio3, H. Maehara4, S. Kanemoto5 and T. Satake6
1Department of Food Science and Technology, Tokyo University of Marine Science and Technology, Tokyo, Japan; 2Department of Chemistry and Materials Science, Gunma National College of Technology, Gunma, Japan; 5Satake Corporation, Hiroshima, Japan

Stress perturbs physiological functions of mammals and is believed to exaggerate metabolic syndrome such as type 2 diabetes mellitus. Adiponectin, one of adipocytokines, binds to receptors in skeletal muscle and liver, increasing metabolisms of fatty acid and glucose and consequently improving insulin resistance. We previously demonstrated that oral administration of gamma
aminobutyric acid (GABA, derived from rice germ) and phytosteryl ferulates (PSFs, derived from rice bran) effectively increased plasma adiponectin levels in normal mice. In the present study, we have examined the effects of immobilization stress on the plasma adiponectin levels and the ameliorating effects of GABA and PSFs on the stress-induced hypoadiponectinemia. Mouse adiponectin was estimated through IR-fluorescent-Western blotting using anti-adiponectin antibody. Immobilization stress markedly reduced mouse plasma adiponectin levels. Oral administration of GABA increased adiponectin levels into normal levels. PSFs also recovered the levels. Simultaneous administration of GABA and PSFs drastically augmented the adiponectin levels. Encapsulated GABA and PSFs preparation newly developed successfully prevented the stress-induced hypoadiponectinemia. These results suggest that simultaneous intake of GABA and PSFs rich in rice products would prevent us from stress-induced metabolic syndrome.

**A3.58**

**Robinia pseudo-acacia var. umbraclulifera and Amorphastilbol enhance the glucose and lipid metabolism in vivo mice models for obesity and diabetes**

W. Lee, Y.-S. Park, G. Yoon, J. Ham, H.-O Yang and S. N. Kim

**KIST Gangneung Institute, Gangneung, Republic of Korea**

**Introduction:** Obesity has been demonstrated to be associated with lipid metabolic disorders, which commonly increase the risk of insulin resistance. Type 2 diabetes is also characterized by insulin resistance and hyperlipidemia.

**Methods:** Hypolipidemic effects of *R. pseudo-acacia var. umbraclulifera* (RPA) and amorphastilbol (APH) were investigated in High-fat diet model (HFD). The anti-diabetic effects of RPA and APH were investigated in db/db mice model. After 8 weeks of administration, several biomarkers including body weight, food intake and blood glucose and mRNA levels for glucose and lipid metabolism were measured.

**Results:** Body, liver and adipose tissue weight were decreased in RPA and APH administered HFD mice than control. The mRNA expression level of UCP2 was increased but G6Pase, decreased in liver and adipose, adiponectin and UCP3 mRNA were increased in adipose tissue. Blood glucose, triglyceride and free fatty acid levels were decreased while HDL-cholesterol was increased in db/db mice model. It was also improved glucose tolerance without any body weight changes. The mRNA expression levels of UCP2 and G6Pase showed same pattern in HFD model, and PPARγ and UCP3 were increased in adipose tissue.

**Conclusions:** We suggested that RPA and APH have a potential for treatment of type 2 diabetes and obesity by enhancing the glucose and lipid metabolism.

**A3.59**

**Anti-inflammatory effects and mechanism of Hippocampus Kuda Bleeler derived compounds SE-1 and SE-2 on activated murine macrophages and microglial cells**

S.W.A. Himaya1, B. M. Ryu1, Z.-J. Qian2, Y. Li2 and S.-K. Kim1

1Department of Chemistry, Pukyong National University, Busan, Republic of Korea, 2Marine Bioprocess Research Center, Busan, Republic of Korea

**Introduction:** Inflammation has recently been implicated as a critical mechanism responsible for the neurodegenerative diseases. Macrophages and microglial cells are responsible for mediating inflammatory responses related to neurodegeneration. The two compounds isolated from sea horse Hippocampus Kuda Bleeler; 1-(5-bromo-2-hydroxy-4-methoxyphenyl) ethanone [SE-1] and 1-(2-hydroxy-4-methoxyphenyl) ethanone [SE-2] were studied as novel agents to suppress lipopolysaccharide (LPS) mediated inflammatory responses in cultured RAW264.7 and BV-2 cells. SE-1 and SE-2 significantly attenuated LPS induced release of inflammatory products such as nitric oxide, prostaglandin E2, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6). The compounds also down regulated the protein and gene levels of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), TNF-α, IL-1β and IL-6 in both cell lines. Molecular mechanism study shows that SE-1 and SE-2 inhibited the nuclear translocation of nuclear factor-κB (NF-κB) p65 and p50 subunits by attenuating the phosphorylation of IkBα. Also SE-1 and SE-2 suppressed the phosphorylation of MAPK molecules; JNK and p38. These results suggest the potential in vitro anti-inflammatory activity of SE-1 and SE-2, which inhibited the LPS stimulated inflammatory responses in RAW264.7 and BV-2 cells via suppressing MAPK and NF-κB signaling pathways.

**A3.60**

**Anti-obesity effect of carboxymethyl chitin via AMPK and aquaporin-7 signaling pathway in 3T3-L1 adipocytes**

C.-S. Kong1, J.-A. Kim2, B. Ahn2, M. Z. Karagozlu2,

H.-G. Byun1 and S.-K. Kim2

1Marine Bioprocess Research Center, Busan, Republic of Korea,

2Department of Chemistry, Pukyong National University, Busan, Republic of Korea

**Introduction:** Obesity is associated with metabolic syndrome. Type 2 diabetes is also characterized by insulin resistance and hyperlipidemia. The aim of this study was to investigate the anti-obesity effect of carboxymethyl-chitin (CM-chitin), a water-soluble derivative of chitin, by measuring lipid accumulation and adipogenesis related factors in 3T3-L1 adipocytes. CM-chitin was synthesized by means of carboxymethylation reaction. Its inhibitory effect on adipogenesis related transcriptional factors and adipocyte specific gene promoters. Moreover, the specific mechanism by CM-chitin was confirmed by transcriptional activations of the phosphorylated adenosine monophosphate-activated protein kinase (AMPK) and aquaporin-7. These results suggest that CM-chitin exerts anti-adipogenic effect on lipid accumulation through modulations of AMPK and aquaporin-7 signal pathways.

**A3.61**

**Glucosamine derivatives promote osteoblastic differentiation in osteoblastic MG-63 cells**

Y.-S. Cho, T.-K. Eom and S.-K. Kim

Department of Chemistry, Pukyong National University, Busan, Republic of Korea

In this study, the effects of glucosamin derivatives (glucosamine-6-phosphate: PGlc and glucosamine-6-sulfate: SGlc) on osteoblastic cell differentiation were evaluated in osteoblastic MG-63 cells. Our results provided evidences that glucosamin derivatives...
A3 – Metabolic Diseases

Abstracts

A3.62

Abstract withdrawn

A3.63

Structural properties of amyloidogenic apoA-I mutant L178H

A. Axelsson1, M. Cochran2, T. Duong3, M. Chokshi3, J. C. Voss4, L. M. Roberts4 and J. O. Lagerstedi1

1Lund University, Experimental Medical Science, Lund, Sweden, 2California State University, Sacramento, USA, 3University of California, Biochemistry and Molecular Medicine, Davis, USA

Apolipoprotein A-I (apoA-I) is the main protein constituent of high-density lipoprotein (HDL), commonly referred to as “the good cholesterol”. HDL and apoA-I have numerous atheroprotective functions including anti-oxidative and vasodilatory effects, and transport of cholesterol from peripheral tissues to the liver. For these processes to occur apoA-I must exhibit significant structural plasticity. However, mutant variants of apoA-I have been shown to be amyloidogenic. Here we focus on a natural apoA-I mutant that contains a leucine to histidine substitution at position 178 in the C-terminal region. It has previously been shown that this apoA-I mutant aggregates into amyloid fibrils together with the transport protein transthyretin and forms deposits in the osteoblastic differentiation and possibility for its application as a supplement in bone health.

A3.64

Abstract withdrawn

A3.65

Chronic high-fat-diet feeding effects on liver insulin resistance in male and female rats

A. Nadal-Casellas, A. M. Proenza, M. Gianotti and I. Lladó

Universitat de les Illes Balears, Metabolisme Energetic i Nutricio, Department Biologia Fonamental i Ciencies de la Salut and IUNICS, Ciberobn, ISCHL, Palma de Mallorca, Spain

Numerous studies point to oxidative stress as initial key event triggering obesity-induced insulin resistance in several tissues, but the etiology of insulin resistance in liver remains unclear. Taking into account the gender differences in the incidence of obesity-associated disorders, the aim of the present study was to investigate the effect of obesity on insulin signaling pathway in liver of male and female rats, as well as its relationship with gender differences in hepatic lipid accumulation. Ten week-old Wistar rats of both genders were fed a pelleted standard diet (3% w/w fat) or a palatable high-fat-diet (HFD) (24% w/w fat) for 26 weeks. Glucose, insulin and resistin levels were measured in serum. Lipid content as well as IRβ, IRS-2, AKT and JNK protein levels were measured in liver. Serum markers of insulin resistance were markedly lower in control female rats than in males and liver IRβ, IRS-2 and AKT protein levels showed the opposite profile. In response to the HFD feeding, female rats exhibited a greater increase of both body and fat depot weights than males; in contrast, hepatic lipid accumulation was higher in males. With the dietary treatment serum markers of insulin sensitivity were impaired in female rats although without reaching the levels of insulin resistance shown by males. Moreover, IRβ and AKT activation decreased markedly in female rats while IRS-2 inhibitory phosphorilation increased in this gender. In this context, the increased insulin resistance shown by HFD female rats, together with their greater adipose tissue expandability, could be adaptive mechanisms that prevent liver from lipotoxicity.

A3.66

HSD11B1 gene polymorphisms in Bosnian patients with type 2 diabetes and metabolic syndrome

T. Dujic1, T. Bego2, B. Mlinar2, S. Seniz2, M. Maleancia1, B. Prnjavorac3, J. Marc4 and A. Causevic1

1Department of Biochemistry and Clinical Analysis, Faculty of Pharmacy, University of Sarajevo, Sarajevo, Bosnia and Herzegovina, 2Faculty of Pharmacy, Department of Clinical Biochemistry, University of Ljubljana, Ljubljana, Slovenia, 3General Hospital of Tesanj, Tesanj, Bosnia and Herzegovina

Conversion of inactive cortisone to active cortisol by the enzyme 11beta-hydroxysteroid dehydrogenase type 1 (HSD11B1) enhances glucocorticoid receptor activation in target tissues. Increased expression of 11beta-HSD1 in adipose tissue is associated with obesity, glucose intolerance, type 2 diabetes and metabolic syndrome. In this study, we investigated association of two HSD11B1 gene polymorphisms with metabolic syndrome and type 2 diabetes. DNA samples from 80 diabetic patients with metabolic syndrome were genotyped for two polymorphisms in HSD11B1 gene: rs846910: G > A and rs45487298: insA by high resolution melting curve analysis. Influence of genotypes on clinical and metabolic parameters was assessed (fasting glucose, insulin, total cholesterol, LDL cholesterol, triglycerides, HbA1c, ALT, GGT, creatinine and urea levels). The mutated allele frequency was 0.06 and 0.24 for rs846910: G > A and rs45487298: insA by high resolution melting curve analysis. Influence of genotypes on clinical and metabolic parameters was assessed (fasting glucose, insulin, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, HbA1c, ALT, GGT, creatinine and urea levels). The mutated allele frequency was 0.06 and 0.24 for rs846910: G > A and rs45487298: insA by high resolution melting curve analysis. The polymorphism in the 3′UTR of HSD11B1 gene may be associated with better glycemic control in diabetic patients with metabolic syndrome.
**A3.67**

**Differential expression and localization of insulin receptor in renal tubule cells of diabetic rats**

R Gatica, J.C. Slebee, C. Caelles, and A. Yañez.

1 PhD program in Veterinary Medicine, Austral University of Chile, Cell Signalling Research Group, Institute for Research in Biomedicine Barcelona, Barcelona, Spain.

2 Enzimology laboratory, Institute of Biochemistry, Faculty of Science, Austral University of Chile, Valdivia, Chile.

3 Cell Signalling Research Group, Institute for Research in Biomedicine Barcelona, Barcelona, Spain.

Insulin is essential for energy management in the body; however, its role in kidney metabolism has not been extensively studied. Its binding to the insulin receptor leads to a cascade of intracellular signalling events, which lead to a regulation of multiple biological processes such as glucose metabolism. The insulin receptor (InsR) is a member of the receptor tyrosine kinase superfamily, and an integral glycoprotein of the plasma membrane in most mammalian cells. Here, we studied the expression, protein levels and localization of InsR in a long-term type 1 diabetes rat model induced by streptozotocin. Comparative qRT-PCR analysis demonstrated a significant increase in the expression of the InsR gene in renal tubule cells (RTC) of diabetic rats. In contrast, western blot analysis showed a significant reduction in the InsR subunit β level in these same cells. Furthermore, InsR immunoreactivity was localized in basolateral and apical plasma membrane of the RTC in renal cortex in control rats, while this pattern changed to a cytoplasmatic localization in the long term diabetic rats. This differential subcellular distribution and expression suggests the involvement of the InsR in the metabolic changes of the RTC of diabetic rats and its implication in the diabetic nephropathy (Foundation M. Botin; DID-UACh: 2006–19).

---

**A3.68**

**Hedera helix L. against prostate cancer metastasis**

H. Gumushan and S. Altun.

Department of Biology, Istanbul University, Istanbul, Turkey.

*Hedera helix* L., is a member of Araliaceae family, has cosmetic usage. The plant has such properties as antiproliferative, cytotoxic, antimicrobial, antifungal, antiprotozoal, and antiinflammatory effects. The aim of this study is to apply the extracts of leaf and unripe fruits of *H. helix* prepared with ethanol, on highly metastatic Mat-LyLu and weakly metastatic AT-2 cells, which are rat prostate cancer cells of the Dunning Model *in vitro* and to determine the effects of these extracts on the cell proliferation and migration of these cell lines of different metastatic abilities. Cell proliferation was detected spectrophotometrically by MTT (methyl thiazolyl diphenyl tetrazolium) assay. Cell migration, however, was investigated via wound healing assay. Our results showed that the ethanolic leaf extract of *H. helix* was inhibited both proliferation and migration depending on dosage and time in highly metastatic Mat-LyLu and weakly metastatic AT-2 cells. Ethanolic fruit extract of *H. helix*, however, suppressed the migration of highly metastatic Mat-LyLu cells where it didn’t produce a significant change on the proliferation of this cell line. Although AT-2 cells migration did not effected by the ethanolic fruit extract, their proliferation was inhibited. In conclusion, the ethanolic fruit extract of *H. helix* may suppress metastasis unaffected by proliferation. However effect of the ethanolic leaf extract of *H. helix* did not clear on metastatic ability of the cancer cells due to inhibition of cell proliferation.

---

**A3.69**

**Lipid trafficking alterations in acid ceramidase-deficient human fibroblasts**


1 Centre of Medical Genetics Dr. Jacinto Magalhães, National Health Institute Doutor Ricardo Jorge (INSA), Genetic Department, Oporto, Portugal.

2 Department of Chemistry and Biochemistry, Queens College of CUNY, Flushing, New York, USA.

3 Mayo Clinic and Foundation, Pulmonary and Critical Care Medicine, Rochester, Minnesota, USA.

4 Institut Fédératif de Biologie and INSERM U838, Laboratoire de Biochimie Métabolique, Toulouse, France.

5 University Fernando Pessoa, Health Sciences Faculty, Oporto, Portugal.

Farber disease (FD) is a fatal sphingolipidosis caused by the deficient activity of acid ceramidase (AC). This hydrolase is responsible for the degradation of ceramide (Cer) into sphingosine and free fatty acid in lysosomes. Although some advances have been made in the knowledge of the genetic and biochemical bases of AC-deficiency, little is known about the disease molecular pathogenesis. One pathogenetic common to several sphingolipidoses could reside in blocking of intracellular transport to or from lysosomes at several related cellular points with subsequent endosomal/lysosomal jam. The present study investigated whether intracellular trafficking of specific lipid molecules is blocked in FD. Increased filipin staining, indicative of high levels of intracellular unesterified cholesterol, was observed in AC-deficient cells and found to co-localize with a lysosomal marker. A similar observation was made for the ganglioside GM2. By fluorescence microscopy BODIPY-LacCer was found to be endocytosed from the plasma membrane to endosomes/lysosomes, in contrast to normal control cells where the lipid analog was transported to the Golgi complex. For BODIPY-C5-Cer, an increased level at the Golgi complex was seen in FD cells. Overexpression of AC corrected lipid traffic imbalances observed in AC-deficient fibroblasts. These data provide evidence for the importance of a steady-state level of lysosomal Cer for lipid homeostasis that, when disrupted, may lead to an unbalanced level of sphingolipids and cholesterol and subsequently, to alterations in cell signalling specific pathways with impact in intracellular lipid trafficking.

---

**A3.70**

**Abstract withdrawn**

---

**A3.71**

**No evidence for the involvement of carnitine palmitoyltransferase 1 transmembrane domains in the formation of an acylcarnitine pore**

S. Violante, L. IJlst, S. Denis, I. Tavares de Almeida, F. V. Ventura and R. J. Wanders.

1 Met&Gen Group, iMed.UL, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal.

2 Laboratory Genetic Metabolic Diseases, AMC, University of Amsterdam, Amsterdam, Netherlands.

Carnitine palmitoyltransferase 1 (CPT1) is recognized as the enzyme catalyzing the rate limiting reaction in the β-oxidation of long-chain fatty acids, converting long-chain acyl-CoA into the respective carnitine ester. It remains controversial how these acylcarnitine intermediates have access to the intermembrane space to be further transported by carnitine acylcarnitine translocase and redirected back to long-chain acyl-CoAs by carnitine palmitoyltransferase 2. It has been previously shown that rat
CPT1A has a hexamic quaternary structure arrangement, suggesting that this oligomeric complex could function as a pore channelling acylcarnitines through the outer mitochondrial membrane (OMM). We aimed to investigate if oligomerization of CPT1 to form such pore would indeed be the only pathway by which acylcarnitines enter into the intermembrane space. Oxidation of C16-carnitine was measured in control and CPT1 deficient intact human fibroblasts in order to disclose if this process is independent of CPT1 transferase activity but dependent on the hypothesized pore formation. We found that using C16-carnitine as substrate total β-oxidation in CPT1 deficient cell lines (carrying a nonsense mutation) is not affected. Thus, although both CPT1 function and structure are severely impaired in the deficient cell lines, C16-carnitine is still able to access the mitochondrial matrix to be further metabolized. Facing this, it seems that either the pore formed by oligomerization of CPT1 does not function as a channel for the produced acylcarnitines or this is not the exclusive route (and certainly not the major one) involved in acylcarnitines channelling through the OMM.

A3.72
Abstract withdrawn

A3.73
Molecular pathogenesis of a new glycogenesis caused by a mutation in glycogenin-1
J. Nilsson1, A.-R. Moslemi1, A. Halim2, A. Pedersen3 and A. Oldfors1
1Department of Pathology, University of Gothenburg, Institute of Biomedicine, Gothenburg, Sweden, 2Department of Clinical Chemistry, University of Gothenburg, Institute of Biomedicine, Gothenburg, Sweden, 3University of Gothenburg, Swedish NMR Centre, Gothenburg, Sweden
We recently described a new disease in a patient with cardiomyopathy and muscle weakness. Histochemical staining revealed a striking glycogen deficiency in skeletal muscle and an abnormal carbohydrate accumulation in the heart. Sequencing of the glycogenin-1 gene, GYG1, revealed a nonsense mutation in one allele and a missense mutation, Thr83Met, in the other. The allele with the missense mutation, the only allele expressed, resulted in inactivation of the autogcosylation of glycogenin-1. Glycogenin-1 is autogcosylated with approximately 10 glucose residues at tyrosine 195 and thereby initiates the glycogen-synthesis. To further analyze the pathogenesis of the Thr83Met mutation, human glycogenin-1 was expressed in CHO cells. Wild type glycogenin-1 was autogcosylated whereas the Thr83Met mutated form was not. Similar results were obtained when apo-glycogenin-1 was expressed in a cell-free system and autogcosylated in vitro. To test if threonine in position 83 is functionally important for autogcosylation we replaced it with other amino acids in the cell-free in vitro system. Serine in position 83 was functional whereas tyrosine and phenylalanine were not. The main difference between the functional and the non-functional amino acids, that we tested, is size suggesting that the amino acid in position 83 is of sterical importance. Two different catalytic activities are carried out by glycogenin-1: The linkage of glucose to tyrosine 195 and the elongation of α-1,4 linked glucose molecules. By mass spectrometry we demonstrated that Thr83Met mutated glycogenin-1 was unable to catalyze the tyrosine-O-glucose linkage explaining the inactivated autogcosylation.

A3.74
Lipin 1 gene polymorphisms in Bosnian population with type 2 diabetes and metabolic syndrome
T. Bego1, T. Dujic1, B. Milanar2, S. Semiz2, M. Malenica1, B. Prnjavorac1, J. Marc2 and A. Causevic1
1Department of Biochemistry and Clinical Analysis, Sarajevo, Faculty of Pharmacy, University of Sarajevo, Bosnia and Herzegovina, 2Department of Clinical Biochemistry, Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia, 3General Hospital of Tesanj, Tesanj, Bosnia and Herzegovina
Metabolic syndrome is a cluster of risk factors for atherosclerotic disease and type 2 diabetes mellitus comprising obesity, insulin resistance, hypertension, and dyslipidemia. The individual components of metabolic syndrome are strongly influenced by genetic factors. Lipin 1, a novel molecular protein expressed by adipocytes, has marked effects on adipose tissue mass, insulin sensitivity, and glucose homeostasis. Thus, we hypothesized that genetic variants within lipin 1 gene (LPIN1) are associated with traits of the metabolic syndrome. The study was done on 70 patients diagnosed with metabolic syndrome and type 2 diabetes. All subjects involved in the study were patients of General Hospital Tesanj (Bosnia and Herzegovina). DNA from those patients was isolated from the whole blood using the QIAGEN method. A total of two single nucleotide polymorphisms (SNPs) in LPIN1 gene, rs11693809 and rs2716610, were analyzed by real-time PCR with hydrolyzing probes. Correlations between biochemical parameters (glucose, HbA1c, insulin levels, HDL and LDL cholesterol, triglycerides, serum proteins, liver enzymes, etc) and rs11693809 and rs2716610 polymorphisms in LPIN1 gene were tested. Our analysis revealed correlation between AST (aspartate aminotransferase) activity and the rs11693809 polymorphism in diabetic patients with metabolic syndrome.

A3.75
Assessment of oxidative stress and cytokines in saliva and plasma of patients with oral aphthous ulcer lesions
E. Avci1, Z. Zafersoy Akarslan2, H. Erten3 and S. Coskun Cevher3
1Hitit University, Biology, Corum, Turkey, 2Gazi University, Dentistry, Ankara, Turkey, 3Gazi University, Biology, Ankara, Turkey
Recurrent aphthous ulcers (RAU) are common oral inflammatory lesions. Predisposing factors such as trauma, microbial factors, food, drug reactions, immune disorders, hormonal imbalance and smoking, all of which trigger free radical formation and upset the oxidant/antioxidant balance of an organism, as well as familial tendency, are believed to be involved in the etiology of RAU. With its contents of antioxidant molecules and enzymes, saliva and serum are the first step of the defensive mechanism against free radicals. The aim of the present study was to determine the salivary and serum malondialdehyde (MDA) level, glutathione (GSH) level, tumor necrosis factor-α (TNF-α) and interleukin (IL-2 and -10) levels in patients with RAU. Salivary and serum MDA and GSH levels were determined by using high performance liquid chromatography (HPLC) and also cytokines levels were evaluated by ELISA. Salivary and serum MDA and GSH levels were significantly elevated (p < 0.01) when compared to with control group. Although interleukin -2 and TNF-α levels were found high levels, IL-10 level was low with RAU in patients. The antioxidant system and levels of cytokines have been affected in the process of RAU.
A3.76 Effects of high-fat-diet feeding on skeletal muscle mitochondrial biogenesis in male and female rats
Y. Gomez-Perez, M. Gianotti, I. Llado and A. M. Proenza
Universitat de les Illes Balears, Biologia Fonamental i Ciències de la Salut, CIBERobn, Instituto de Salud Carlos III, Palma de Mallorca, Spain

High-fat-diet (HFD) feeding leads to overweight, insulin resistance and oxidative stress and has been associated to a decrease of mitochondrial mass and function. Since gender differences in mitochondrial function and oxidative stress have been reported, the aim of the present study was to investigate whether there is a gender dimorphism in the consequences of a HFD feeding on rat skeletal muscle mitochondrial biogenesis. Male and female Wistar rats of 2 months of age were fed a hypercaloric and hyperlipidic diet or a control pelleted diet for 26 weeks. Soleus and gastrocnemius muscle protein levels of PGC-1alpha, TFAM, COXII, UCP3, and antioxidant defences, as well as markers of oxidative damage were measured. In both genders, HFD feeding increased PGC-1alpha and TFAM levels in soleus and gastrocnemius muscles and COXII in the later. HFD fed male rats showed higher oxidative damage than their female counterparts in both muscles, and lower Mn-SOD and Cu-SOD levels in gastrocnemius. Soleus UCP3 levels were higher in control female rats than in males and increased with HFD feeding only in males, whereas HFD feeding increased UCP3 levels in gastrocnemius muscle of both genders. In conclusion, oxidative damage induced by HFD feeding is more marked in male rats, probably due to their lower antioxidant protection compared to female rats. In response to HFD feeding, male rats increased mitochondrial differentiation in a higher degree than females, suggesting gender differences in mitochondrial biogenesis in order to counteract the consequences of the increased availability of metabolic fuels.

A3.77 Determined to bile salts deconjugation ability of free and immobilized these strains and cholesterol removal by some Bifidobacterium sp
G. Alp1 and B. Aslan2
1Hitt University, Microbiology, Corum, Turkey, 2Gazi University, Biology, Ankara, Turkey

The aim of this study is primarily to evaluate the deconjugation of sodium taurocholate by four strains of Bifidobacterium sp. selected according to cholesterol removal capacity. Influence of different bile concentrations on cholesterol removal has been studied. Secondly, it has been targeted to determine the utility of immobilization effects on the deconjugation of sodium taurocholate of strains which have the highest deconjugation property. Bifidobacterium breve (A28, S4 and S9) and Bifidobacterium bifidum (A10) strains were used in this study. It has been confirmed that strains which have cholesterol removal capacity (A28, S4) were able to deconjugation of sodium taurocholate than those low cholesterol removal capacity (A10, S9) and the highest cholesterol removal (18%) was observed by strain A28 in 3 mg/ml bile concentration. Viability and deconjugation of sodium taurocholate of immobilized strains were determined. Deconjugation of sodium taurocholate and cholesterol removal by immobilized and free cells was studied and it has been determined that immobilized cells were more effective. According to these results, immobilized forms of B. breve A28 and S4 can be planned to be used in probiotic industry because of having higher cholesterol removing, viability and deconjugation ability.

A3.78 Gender differences in adipose tissue fatty acid composition and lipid management in response to changes in dietary fat
M. E. Estrany, A. M. Proenza, I. Llado and M. Gianotti
Universitat de les Illes Balears, Metabolisme Energetique i Nutricio, Departament Biologia Fonamental i Ciències de la Salut and IUNICS. Ciberobn, ISCHII, Palma de Mallorca, Spain

High-fat diet (HFD) feeding usually leads to hyperphagia and body weight gain, but macronutrient proportions in the diet can modulate energy intake and fat deposition. The mechanisms of fat accumulation and mobilization may differ significantly between depots, and gender can also influence these differences. The aim of the study was to determine gender differences in fatty acid (FA) composition of visceral and subcutaneous adipose tissues in response to changes in diet composition. We further analyzed the influence of both dietary FA and levels of proteins involved in tissue FA uptake and release on fat deposition. Wistar rats of both genders were fed a HFD (30% w/w fat) or a control diet (3% w/w fat) for 14 weeks. FA composition was analyzed by gas chromatography and levels of LPL, HSL, s2-AR, β3-AR and PKA were determined by Western blot. HFD feeding increased adiposity without changes in body weight and induced a fat accumulation in adipose tissues, which was gender- and depot-dependent. FA content of HFD was reflected in the tissue FA composition, with an increase in the proportion of MUFA s and a decrease in that of PUFA s in both fat depots. The sexual dimorphism in adiposity index could be associated to changes in the levels of proteins involved in tissue lipid management, and also related with a differential response to dietary FA content. Thus, the lower tissue fat accumulation of HFD female rats may be linked to lower LPL levels and higher levels of intermediates in the lipolytic pathway.

A3.79 Effect of ovariectomy and 17beta-estradiol on mitochondrial function and insulin response in rat skeletal muscle
G. Capilioni-Amor, M. Gianotti and F. J. García-Palmer
Universitat de les Illes Balears, Metabolisme Energetique i Nutricio, Departament Biologia Fonamental i Ciències de la Salut and IUNICS. Ciberobn, ISCHII, Palma de Mallorca, Spain

Sexual dimorphism in mitochondrial function has been evidenced in several tissues, including skeletal muscle. Sex hormones, especially estrogens, could play a key role in mitochondrial energy metabolism regulation, which is intimately linked to insulin response. In this context, estrogens seem to preserve mitochondrial structure and function by reducing oxidative stress and inhibiting apoptosis process. The aim of the present study was to evaluate the effects of ovariectomy and a subsequent estrogens supplementation on mitochondrial function, as well as its relationship with insulin sensitivity in skeletal muscle (soleus and gastrocnemius). 13-weeks old Wistar rats were used and divided into four experimental groups: controls both male and female, ovariectomized and ovariectomized supplemented with 17beta-estradiol. Soleus (prevalently oxidative) and gastrocnemius (prevalently glycolytic) muscles were homogenized and protein levels related with insulin response (GLUT-4, IRS, IRbeta) and mitochondrial function (ATPase, COXIV, TFAM) were measured by Western-blot. The results indicated that ovariectomy induced an insulin sensitivity decrease in soleus muscle, which was partially reversed by estradiol administration. On the other hand, the levels of marker proteins of mitochondrial function were diminished in gastrocnemius.
muscle of castrated animals, and estrogens replacement recovered the effect of ovariectomy. As a whole, results show an effect of hormonal environment on functionality of skeletal muscles, and they could also suggest a different response from muscle tissues to ovariectomy, depending on their prevalent metabolism.

A3.80 Synthesis of novel 1,3-Diarylpyrazol derivatives as potent inhibitors of COX-2 and thromboxane synthase

S. Baytas1, Y. Ozkan2, S. Unlu1 and M. F. Sahin1
1Department of Pharmaceutical Chemistry, Gazi University, Faculty of Pharmacy, Ankara, Turkey, 2Department of Biochemistry, Gazi University, Faculty of Pharmacy, Ankara, Turkey

It is well known that inhibition of the enzyme cyclooxygenase (COX) is the principal mechanism for the efficacy of NSAIDs. Among all the COX isomers selective inhibition of COX-2 which is being induced during inflammation is of interest since the resulting drugs lack the gastric side effects associated with classical NSAIDs. However, their prolonged use causes cardiovascular side effects which limit their clinical use. Researchers suggested that COX-2 inhibitors may increase the risk of thrombotic effects by reducing the production of prostacyclin (PGI2), while exerting a negligible effect on Thromboxane A2 (TXA2), level. PG12 formation is associated with vasodilatation and reduction in platelet aggregation. In contrast, TXA2 promotes vasoconstriction and platelet aggregation. While traditional NSAIDs were known to inhibit both PG12 and TXA2 formation, the more focused inhibition of COX-2 by selective inhibitors leaves TXA2 unaffected tipping the balance toward a prothrombotic effect. Based on these results, dual inhibition of COX-2 and thromboxane synthase emerged as a new strategy to provide effective and safer NSAIDs lacking the drug associated GI and cardiovascular side effects. The results obtained so far aid to understand the pathogenic mechanisms in AIP and will be presented at the conference.

A3.81 Characterization of the molecular basis of acute intermittent porphyria

H. Bastad Johannessen1, E. Romneseth2, K. Toska2, J. Underhaug2, A. Martinez1 and S. Sandberg2
1Department of Biomedicine, University of Bergen, Bergen, Norway, 2Haukeland University Hospital, NAPOS, Bergen, Norway

Acute intermittent porphyria (AIP) is an inherited disease caused by reduced activity in the third enzyme of the heme biosynthetic pathway, hydroxymethylbilane synthase (HMBS). The reasons for this reduction and the consequences of the mutations are not completely understood. However, valuable information about the phenotype-genotype correlations can be obtained by functional studies on the wild-type (wt) enzyme and certain disease causing mutations found in specific regions of HMBS. For these studies we have initially chosen R16W, K132N, R167W, R173W and V215E mutations, representing: i) mutations around the active site and/or interfering with proper binding of the co-factor dipyromethane, ii) mutations far from the active site and predicted to affect overall folding and flexibility, and iii) mutations affecting efficiency of transcription or translation. Presently, we have expressed recombinant human wt-HMBS and the 5 selected mutations in E. coli using pGEX expression vectors. The enzymes have been purified on Glutathione-Sepharose 4B and we have obtained high yields and purity for wt and most mutants. We are at present characterizing the steady-state kinetic parameters of the mutants comparing to the wt as well as their thermostability by differential scanning calorimetry, circular dichroism and fluorescence spectroscopy. Wt-HMBS shows a high thermostability (Tm = 79.5°C), and some of the mutants reveal a decreased thermostability, in addition to kinetic defects. Strategies aiming to stabilize the mutants are being investigated. The results obtained so far aid to understand the pathogenic mechanisms in AIP and will be presented at the conference.

A3.82 Research of antihypoxic drugs effect with myocardial disturbances

V. Moskalenko1, M. Zarytska1, A. Yagupova2, N. Gorchakova1 and I. Chekman3
1Bogomolets National Medical University, Sociale Medicine and Health Care, Kyiv, Ukraine, 2Bogomolets National Medical University, Bioorganic, Biological and Pharmaceutical Chemistry, Kyiv, Ukraine, 3Bogomolets National Medical University, Pharmacology, Kyiv, Ukraine

Cardiovascular diseases accompanied the phenomena a hypoxia and ischemia depending on the development of pathological state. In pathology of all these states matters, permeability of membranes, violation of energy and plastic metabolism, of vessels endothelia, intensification of lipid oxidation processes, increasing formation of superoxide anions and decline of activity of the free radical scavenging system. Therefore for prevention of harmful influence of the hypoxia states at ischemia, in case of occurring of extreme situations it is necessary to apply medications, which simultaneously influence on the indexes of energy metabolism, execute the function of free radicals scavengers, inhibit processes of lipids oxidation. In an experiment on rats, mise designed by hypoxia of various aethiology at previous introduction of acetylcystein, tau-rine, arginine, corargin, glutargin. Investigated influence of these preparations on the indexes of energy metabolism (content of adenylc nucleotides, creatine phosphate, activity of creatine phosphokinase), indexes of lipid oxidation, system of free radical scavenging (activity of catalase, super oxide dismutase) in tissues of myocardium, and also activity of indacible NOS (iNOS II) in the platelets of animals. Under hypoxia of various aethiology the proved preparations are different prevented the changes of energy metabolism indexes, lipid oxidation, system of free radical scavenging. As a result of high activity of iNOS the nitric oxide (NO), which executes a cytotoxic function under the pathology, is produced. For the correction of these changes applied donors of NO: arginine, corargin, glutargin.

A3.83 Abstract withdrawn

A3.84 Abstract withdrawn
A4 – Neurobiology

A4.01
Capsaicin is a key molecule in neuropathic pain induced by diabetes
B. M. Rada1, A. D. Iancu2, D. Rotaru3, D. L. Radu4, C. Stavaru5, C. Mustaciosu5 and M. Radu1
1Department of Animal Physiology and Biophysics, University of Bucharest, Faculty of Biology, Bucharest, Romania, 2/“Cantacuzeno” National Institute for Microbiology and Immunology, Laboratory of Cellular Immunity, Bucharest, Romania, 3/Department of Health and Environmental Physics, Romanian Authority, Food Quality, Standards and Marks, Bucharest, Romania, 4/National Institute for Microbiology and Immunology, “Cantacuzeno”, Laboratory of Cellular Immunity, Bucharest, Romania, 5/Department of Health and Environmental Physics, Horia Hulubei National Institute for Physics and Nuclear Engineering, Atominstitut, Magurele, Romania

Diabetic peripheral neuropathy is a common chronic complication of diabetes mellitus. Transient receptor potential (TRP) ion channels are involved in sensing physical and chemical stimuli. TRPVI is a Ca²⁺ permeant non-selective cation channel expressed predominantly by unmyelinated C-fibers and thinly myelinated A-δ fibers and plays a major role in inflammatory thermal sensation. Capsaicin is the active component of chili peppers (which are plants belonging to the genus Capsicum) and is the main compound responsible for TRPVI activation. In this study, we have used a transgene-mediated diabetes model to study the role of capsaicin in diabetic peripheral neuropathy. We have used sensory neurons from dorsal root ganglia prelevated from TCR-HA+/Ins-HA−/+ mice and Balb/c. Whole cell patch-clamp recording and immunofluorescence microscopy have been employed. The larger amplitude and reduced desensitization of TRPVI currents induced by capsaicin, obtained in TCR-HA+/Ins-HA−/+ mice, closely resemble changes identified in streptozotocin-induced diabetes in rats. Expression of TRPVI was significantly higher in the double-transgenic diabetic mice. Capsaicin treatment reduced the TRPVI expression. In diabetes, the link between abnormal pain sensitivity and hyperglycemia resulting from insulin deficiency is not clear, but our study proves that capsaicin plays a major role in regulating TRPVI function and expression in diabetes. This study was financed by the research grant PNCDI2 41-074/2007.

A4.02
The use of spect scintigraphy with I-123 ioflupane in differentiation of patients with parkinsonian syndromes and essential tremor
D. Fistouris1, N. Bousiou2, E. Karathanos2, D. Tsairidis3, A. Strataki3, V. Apostolidou3, A. Kyroudi3 and A. Zissimopoulos2
1Department of Biomedical Laboratory Science, Yonsei University, Wonju, Republic of Korea, 2Department of Biomedical Science, Hallym University, Chuncheon, Republic of Korea, 3Department of Biochemistry and Molecular Biology, University of Ulsan College of Medicine, Seoul, Republic of Korea

We have screened new drugs with a view to developing effective drugs against glutamate-induced excitotoxicity. In the present work, we show effects of a new drug, KHG25857 against glutamate-induced excitotoxicity in primary rat glial cultures. Pretreatment of glial cells with KHG25857 for 2 hour significantly protected glial cells against glutamate-induced excitotoxicity in a time- and dose-dependent manner with an optimum concentration of 100 μM. The drug significantly reduced production of proinflammatory cytokines, tumor necrosis factor-α, and interleukin-1β in glutamate-induced excitotoxicity. The drug also prevented glutamate-induced intracellular Ca²⁺ influx and reduced the subsequent overproduction of nitric oxide and reactive oxygen species. Furthermore, the drug preserved the mitochondrial potential and inhibited the overproduction of cytochrome c. In addition, the drug effectively attenuated the protein level changes of β-catenin and glycogen synthase kinase-3β. These results suggest that KHG25857 effectively protected primary cultures of rat glial cells against glutamate-induced excitotoxicity.

A4.03
Abstract withdrawn

A4.04
Protective effects of KHG25857 against glutamate-induced excitotoxicity in rat glial cultures
E.-A. Kim1, T. U. Kim1, S. Y. Choi3 and S.-W. Cho3
1Department of Biomedical Laboratory Science, Yonsei University, Wonju, Republic of Korea, 2Department of Biomedical Science, Hallym University, Chuncheon, Republic of Korea, 3Department of Biochemistry and Molecular Biology, University of Ulsan College of Medicine, Seoul, Republic of Korea

Patients & Method: Sixteen consecutive patients nine male and seven female (58 ± 9 years old) with movement disorders, were included in the study. The administered dose was 110MBq123I-Ioflupane. Lugol’s solution were given to each patient to minimize uptake of radioactive iodine by the thyroid gland. In each patient, SPECT scans were acquired on a gamma-camera at 3 hours post-injection. The acquired images were interpreted visually with consensus between two nuclear medicine physicians.

Results: Normal images are characterized by two symmetrical crescent-shaped areas of equal intensity. Abnormal images are either symmetric or asymmetric with unequal intensity and/or loss of crescent. Abnormal images with reduced DAT density in the striatum, were seen in nine patients and these patients had neurodegenerative Parkinsonism. The other seven patients showed homogeneous and symmetrical uptake in the striatum and in these patients Parkinson’s disease was ruled out. (images shown)

Conclusions: SPECT imaging with 123I-Ioflupane is a very useful tool to differentiate benign essential tremor from Parkinson’s disease.

A4.05
Antioxidant status in Schizophrenia
R. D. Rosolu
“Ovidius” University Constanța, Faculties of Medicine and Biology, Biochemistry, Constanța, Romania

Although the biochemical mechanisms involved in schizophrenia are not yet completely understood, there are mounting evidences...
that oxidative stress-mediated cell damage plays a role in the path physiology of schizophrenia. In the present research we have chosen to study the biochemical blood values of reduced GSH, GR, GPx, TAS, SOD, creatine and creatinine. For this reason we have selected one group of 21 male schizophrenic subjects with medication and a control group of 18 healthy subjects. For all the schizophrenic subjects, the creatininc index [IC = creatine/ (creatinine + creatine)] was considerably lower than the normal level of 0.5. It was also observed an important decrease below the normal range of the reduced GSH for all the patients in the schizophrenic patients group compared with the normal reduced GSH levels seen in the control group. For GR, TAS and GPx we have seen in the case of the schizophrenic patients lower decreases than in the case of GSH, but statistically significant when compared with the values seen for the control group. The SOD was found increased, possibly as an adaptive response to free radical overload. We may conclude that in schizophrenia there exists very probably some imbalances in the antioxidant defenses of the organism and some possible perturbations of the energy metabolism and of the processes of detoxification of ammonia that seems to be indicated by the imbalances of the creatinine/creatine ratio manifested in the reduction of the creatininc index.

A4.06
Minerval induces ER stress and apoptosis in astrocytoma 1321N1 cells but not in MRC-5 fibroblast cells
A. Marcilla-Etxenike, J. M. Barceló, X. Busquets and P. V. Escribá
University of the Balearic Islands, Biology, Palma de Mallorca, Spain
Minerval (2-hidroxi-9-cis octadecenoic acid) impairs lung cancer (A549) cell proliferation upon modulation of the plasma membrane lipid structure and subsequent regulation of protein kinase C localization and activity. However, this mechanism does not fully explain the regression of tumors induced by this drug in animal models of cancer. We have investigated the effect of palmitate and Minerval, on endoplasmic reticulum (ER) stress pathways and induction of apoptosis in 1321N1 astrocytoma cells and MRC-5 fetal fibroblast cells. For this purpose the cells were incubated in the presence or absence of Minerval and/or palmitate for 12, 24 and 48 hour under low-serum conditions. In this context, 1321N1 cells underwent apoptosis, as determined by caspase-8 activation and PARP degradation. Concomitantly, the levels of ER stress markers, phosphorylated eIF2, Irel-alfa, XBP-1, and CHOP, were increased compared with control (untreated) cells. In MRC-5 cells, Minerval did not induce ER stress whereas palmitate induced the expression of the above ER stress markers. In summary, Minerval induces ER stress and apoptosis in 1321N1 human astrocytoma cells, but not in MRC-5 human fibroblast cells. These data provide relevant data about the mechanism of action of Minerval and its high efficacy in the absence of toxic effects.

A4.07
Some biochemical changes in the brain of young rats subjected to Chlorpyrifos neurotoxicity
N. Talokha and Y. Salyha
Institute of Animal Biology, Laboratory of Metabolism, Lviv, Ukraine
Chlorpyrifos (CPF) is one of the most commonly used organophosphorus (OP) pesticides in the world. Like the other OP, CPF (O, O-diethyl-3, 5, 6-trichloro-2-pyridylyphosphorothioate) inhibits the enzyme acetylcholinesterase (AChE), which destroys acetylcholine, the neurotransmitter that activates cholinergic neurons. But number of scientist repeatedly demonstrated that CPF toxicity is not limited to cholinesterase inhibition alone but can act by other mechanisms. The aging brain shows selective neurochemical changes involving several neural cell populations. Increased brain metal levels have been associated with normal aging and a variety of neurodegenerative disorders. Copper is an important modulator of NMDA-receptor activity, zinc – of glutaminergic transmission. It is thus important to elucidate the mechanisms by which metal homeostasis of brain is maintained and how metals function in cellular processes, including neurotoxic damages. In present work we have studied levels of copper, zinc and iron in hippocampus, cerebellum and cerebral cortex of growing rats subjected to low doses of Chlorpyrifos. Simultaneously we have studied changes in activity of the antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase. Our data demonstrates age-dependent changes of investigated metal levels in different brain regions. We observed also some age-dependent and brain region-dependent changes in antioxidant enzymes activity. A whole series of biochemical analysis of different parts of rat brain and our previous experiments with Morris water maze confirm the CPF neurotoxicity and at the same time impel for investigations on cellular and molecular level.

A4.08
Energetic metabolism of myelinated axons: a new correlation among demyelination and axonal degeneration in Multiple sclerosis
S. Ravera¹, I. Panfoli¹, M. G. Aluigi¹, D. Calzia², E. Capello³, G. L. Mancardi¹ and A. Morelli¹
¹University of Genova, Biology Department, Genova, Italy,
²University of Genova, Neurosciences, Ophthalmology and Genetic Department, Genova, Italy
Multiple sclerosis (MS) is a demyelinating disease in which the myelin sheaths around the axons are damaged by immune system. During MS, the loss of myelin doesn’t simply cause a lowering of speed of conduction but also an axonal necrosis. Although, this is the major determinant of irreversible neurological disability, the mechanisms of axonal loss are poorly understood. Among the proposed hypotheses for degeneration of demyelinated axons, it was suggested that axonal degeneration depends on energy depletion [1]. Our recent study has demonstrated that the whole respiration chain is present in isolated myelin; moreover, these proteins are able to produce ATP, consuming oxygen [2]. This suggest that myelin sheath, not only surrounds the axon, but supplies it, through energy production. So we hypothesize that the demyeliniwheelization may imply a dramatic ATP depletion and consequent loss of functionality, axon survival and cytoarchitecture, suggesting a new neuro- trophic role for the sheath. By both biochemical and imaging techniques, we have observed that the presence and the functionality of the complete electron transport chain, normally present in myelin sheath, decrease drastically in the MS plaque. Moreover, this loss appears more serious in the active plaques respect the very early active plaques. These results could give a new input to the understanding of the correlation between the myelin loss and the axonal degeneration, clarifying the neuro-trophic role of myelin.

References:
A4.09
Involvement of proteases in oxidative stress-induced necrotic cell death
K. Glebova, S. Shram and N. Myasoedov
Institute of Molecular Genetics RAS, Moscow, Russian Federation

At present necrosis is considered as perfectly determinate process which can be delayed or switched to apoptosis at some defined stages. Formerly the involvement of proteolytic enzymes such as calpains and cathepsins in cells death processes was revealed in a number of investigations. The aim of our study is to elucidate the contribution of different protease types in oxidative stress (OS)-induced necrosis of cultured neuronal cells. An effect of three protease inhibitors – benzamidine (trisplin-like serine protease inhibitor), leupeptine (inhibitor of serine and cysteine proteases) and pepstatine A (inhibitor of aspartate proteases) on rat pheochromocytoma PC12 cells survival after OS was investigated. OS was performed by 30-min incubation of PC12 cells in medium with 1 mM H2O2. Necrotic cells were detected by staining with trypan blue dye 4 hour after OS induction. It was shown that benzamidine, leupeptine and pepstatine A suppress OS-induced necrosis of PC12 cells. At concentrations of 100 (benzamidine), 0.1 (leupeptine) and 50 mM (pestatine A) we observed the highest effect expressed in reducing the number of necrotic cells – 45, 25 and 43%, respectively. Using different schemes of inhibitors application it was established that benzamidine and pepstatine A were more effective when applied before and during H2O2-incubation. On the contrary cytoprotective effect of leupeptine was independent of application period. The data obtained indicate that benzamidine- and pepstatine A-sensitive proteases are involved in early stages of necrosis, whereas leupeptine-sensitive – in later stages. This study was supported by the Russian Foundation for Basic Research (Grant 08-04-01760).

A4.10
L-carnitine regulator – mildronate exerts acute anticonvulsant and antihypnotic effects
B. Svalbe1, R. Muceniece1, E. Liepinsh2, M. Dambrova2 and L. Zvejniece2
1University of Latvia, Riga, Latvia, 2Latvian Institute of Organic Synthesis, Riga, Latvia

A structural analogue of L-carnitine and an inhibitor of its bio-synthesis, mildronate is used in clinics as an anti-ischemic drug for neurological applications. The effects of mildronate on the nervous system have primarily been evaluated after long-term treatment, which significantly decreases the tissue concentrations of L-carnitine. However, the effects of acute administration of mildronate have not been systematically examined before. Mildronate (200 mg/kg, acute intraperitoneal administration) exerted dose-dependent anticonvulsant activity in a chemoconvulsant pentyleneetetrazole (PTZ)-induced clonic and tonic seizure test but did not change the effects of a convulsion-inducing dose of (+)-bicuculline, a γ-aminobutyric acid receptor antagonist. Mildronate also showed a dose-dependent antihypnotic effect on ethanol-induced loss of righting reflex, and it partially inhibited the reduction in cortical cerebral blood flow observed after ethanol administration. However, in a PTZ-induced seizure test, mildronate significantly stimulated the anticonvulsant activity of ethanol. The anticonvulsant activity of mildronate was completely blocked after pre-treatment with α2-adrenergic receptor antagonist yohimbine (2 mg/kg) and nitric oxide (NO) synthase inhibitor NG-nitro-L-arginine (10 mg/kg). These results demonstrate that acute administration of L-carnitine regulator mildronate exerts anticonvulsant and antihypnotic effects. The mildronate activity involves the α2-adrenergic receptor and NO-dependent mechanisms. These findings indicate that acute administration of mildronate could be beneficial for the treatment of seizures and alcohol intoxication.

A4.11
Dimensional alterations of planar Purkinje cell dendrites in reeler mice
J. K. Kim1, N. S. Kwon1, S. E. Chang1, K. T. Kim2, D. M. Lee1, S. Kim1, S. J. Yun1, D. Hwang1, J. W. Kim2, I. J. Ryhu2, Y. Hwu3, M. Giorgio4 and J. H. Je1
1POSTECH, Pohang, Republic of Korea, 2Korea University, Seoul, Republic of Korea, 3Academia Sinica, Taipei, Taiwan, 4Ecole Polytechnique Federale de Lausanne (EPFL), Lausanne, Switzerland

Reeler mice provide an animal model to understand mammalian brain development and brain pathologies. Particularly interesting are structural problems at the structural level: the atrophy of the cerebellum with disrupted cortical cytoarchitecture is a typical pathology in such a model, also observed in many neurological disorders. However, the important alteration patterns of Purkinje cell dendrites remain largely unknown. Here we provide direct evidence that the alignment of Purkinje cells in normal mice becomes disrupted in reeler mice. We observed this important alteration pattern by combining synchrotron X-ray microscopy with Golgi staining, visualizing the 3-dimensional (3-D) neuronal micro-architecture of ultrathick specimens (up to 1 mm). We specifically found dimensional alterations of planar Purkinje cell dendrites, i.e. abnormal 3-D arborization in reeler mice. The quantitative analysis of the branching patterns in a 3-D geometry revealed that the branching angle did not significantly change from 77 ± 8 (mean ± SEM) in spite of the dimensional alteration, whereas the length of branch segments strongly increased with a large variation with respect to normal specimens. The fractal dimension of the Purkinje cells, estimated in 3-D coordinates, decreases from 1.723 to 1.254, revealing a significant reduction of dendritic complexity. These dimensional alterations, in addition to abnormal Purkinje cell migration during cerebellar development, could be a factor in neurological disorders including motor malfunction in reeler mice.

A4.12
The N-terminus of amyloid-beta plays a crucial role in its aggregation and toxicity
B. Bolognesi1, T. R. Jahn1, A.-C. Borssson1, L. M. Luheshi1, J. J. Yerbury4, D. C. Crowther2 and C. M. Dobson1
1University of Cambridge, Chemistry, Cambridge, UK, 2University of Cambridge, Genetics, Cambridge, UK, 3Linköping University, Physics, Chemistry and Biology, Linköping, Sweden, 4University of Wollongong, School of Biological Sciences, Wollongong, Australia

The aggregation of Amyloid Beta (Aβ) peptide into insoluble amyloid fibrils that deposit in the brain is one of the primary pathogenic events in Alzheimer’s disease. We have previously shown, using a Drosophila model of Aβ toxicity, that the N-terminus of the Aβ peptide, despite being unstructured in the mature Aβ fibril, nonetheless affects Aβ induced neurodegeneration in vivo. In order to understand the contribution of the N-terminus of Aβ to its aggregation behaviour, we have investigated a number of rationally designed N-terminal mutants in vitro. We find that single amino acid mutations in this region affect significantly the kinetics of Aβ aggregation in vitro as measured by a range of spectroscopic techniques. Furthermore, we observe striking differences in the morphology of the aggregated species formed by these different Aβ mutants when imaged with TEM.
or AFM and also in the β-sheet content of their mature fibrils. Interestingly, mutants with an increased net charge or lower hydrophobicity tend to show slower aggregation kinetics, and to form more ordered aggregates whereas mutations that reduce net charge or increase hydrophobicity favour faster aggregation kinetics and poorly structured aggregates. In addition, the exposed hydrophobicity of aggregates formed in the early stages of aggregation is correlated to their toxicity. These findings demonstrate not only that the N-terminus of the Aβ peptide plays a crucial role in its aggregation and toxicity but also suggest that this region of Aβ may modulate in vivo toxicity by altering the conformations of aggregates that it forms.

A4.13
Selective PMCA silencing modulates calmodulin genes expression in cAMP-differentiated PC12 cells
T. Boczek, B. Ferenc, A. Kowalski and L. Zylinska
Department of Molecular Neurochemistry, Medical University of Lodz, Lodz, Poland
Transient Ca2+ waves or prolonged fluctuations in its concentration are well-established phenomenons observed during neuronal cell differentiation. Ca2+ action in the regulation of signaling pathways may be realized in both direct or indirect ways. However, to sustain proper signal transduction, Ca2+ concentration must be tightly controlled. Among others, the most sensitive mechanism is a plasma membrane Ca2+-ATPase (PMCA), existing in four functional isoforms. While PMCA1 and PMCA4 are widely distributed, PMCA2 and PMCA3 are unique for excitatory cells. The activity of PMCA is regulated by Ca2+/Calmodulin. Transient Ca2+ waves may be also mediated via CREB, a transcription factor that is able to activate numerous downstream genes. In our study, CREB phosphorylation correlated with activation of ERK and JNK, showing possible ways of CREB activation. These data indicate on the unique connection between PMCAs composition and expression profile of CaM genes during PC12 cell differentiation. Supported by: 502-16-809, 502-16-810 and 503-6086-2 from the Scientific and Technical Research Council of Turkey (TUBITAK), with a Project number of 107T763.

A4.14
Beta amyloid fibril destabilizing activities of some pyrazoline derivatives
A. Ercan1, S. Yabanoglu1, N. Gokhan-Kelekci2, B. Salih3 and G. Ucar1
1Department of Biochemistry, Hacettepe University Faculty of Pharmacy, Ankara, Turkey; 2Department of Pharmaceutical Chemistry, Hacettepe University Faculty of Pharmacy, Ankara, Turkey; 3Department of Chemistry, Hacettepe University Faculty of Pharmacy, Ankara, Turkey
Alzheimer’s Disease (AD) is a common progressive neurodegenerative disease characterized by intraneuronal neurofibrillary tangles and the extracellular deposition of the amyloid β-peptide. Aβ peptide is shown to be a proteolytic product of amyloid precursor protein (APP), and polymerization of Aβ (1–40) and Aβ (1–42) peptides lead to the formation of insoluble β-amyloid fibrils (fAβ) in AD brains. Destabilization of preformed fAβ is suggested to be an attractive therapeutic target for the treatment of AD. Increased brain and platelet monoamine oxidase-B (MAO-B) activities in AD patients suggested that oxidants formed by MAO catalysis may cause the neurodegeneration, and MAO inhibitors have some neuroprotective effects. Recent studies indicating that MAO-B inhibitors prevent the Aβ-fibril aggregation and senile plaque formation in AD are promising. On the basis of our preliminary studies showing that some N-substituted thio-carbamoyl derivatives had potent monoamine oxidase inhibitor activities, in the present study we synthesized 1-N-substituted thiocarbamyol-3-phenyl-5-(pyrrol-2-yl)-4,5-dihydro-(1H)-pyrazole derivatives as MAO inhibitors and evaluated their β-amyloid fibril destabilizing activities using spectroscopy, fluorescence spectroscopy, electron microscopy and MALDI Mass Spectroscopy. Our results showed that 1-N- substituted thiocarbamyol-3-phenyl-5-(pyrrol-2-yl)-4,5-dihydro-(1H)-pyrazole derivatives directly inhibited the deposition of fAβ in vitro. Although exact mechanism of the anti-amyloidogenic activity of these derivatives is unclear, pyrazolines appeared as the future promising molecules for development of new anti-Alzheimer drugs. This work was supported by the Scientific and Technical Research Council of Turkey (TUBITAK), with a Project number of 107T763.
**A4.16**

**Epigallocatechin-3-gallate (EGCG) binds to TTR and modulates its amyloidogenicity**


1IBMC - Instituto de Biologia Molecular e Celular, Grupo de Neurobiologia Molecular, Porto, Portugal, 2Departamento de Química, Universidade de Aveiro, Centro de Espectrometria de Massa, Aveiro, Portugal, 3Departamento de Química, Faculdade de Ciências, Universidade do Porto, Centro de Investigação em Química (UP) – CQUP (UP), Porto, Portugal

Transferrin (TTR) is an amyloidogenic protein being known more than one hundred TTR variants associated with hereditary amyloidosis. Although the details of the TTR amyloid fibril formation process are not completely known, the pathway involves TTR tetramer dissociation into monomers with altered conformation that will re-aggregate in an aggregated form, polymerize and form amyloid fibrils. Approaches for TTR amyloidosis that interfere with any step of this cascade of events have therapeutic potential. In this study we tested (-)-epigallocatechin-3-gallate (EGCG), the most abundant catechin of green tea, as inhibitor of TTR amyloid formation. We demonstrate, through biochemical and biophysical methods that EGCG binds strongly to isolated TTR, in vitro, and binds to TTR in human plasma, ex vivo. We also show, by stability assays through isoelectric focusing in partial denaturing conditions that EGCG acts as TTR stabilizer, either for wild type TTR or TTR V30M variant. In addition, we demonstrate by transmission electron microscopy (TEM) analysis that EGCG inhibits TTR aggregation in vitro and in a cell culture system. We also found that EGCG acts as amyloid fibril disruptor when tested in vitro for wild type TTR, TTR V78F and TTR L55P amyloid fibrils. The dual effect of EGCG as TTR stabilizer and fibril disrupter makes it a good candidate for therapy or as model for other molecules to be used as therapeutic agents in TTR amyloidosis. Based in these results, in vivo experiments in mice transgenic for human TTR V30M in a null background were performed and suggestive for a significant reduction of tissue TTR deposition.

---

**A4.17**

**The role of Phe329 in the inhibition of human butyrylcholinesterase by malachite green and methyl green**

K. Biberoglu, O. Tacal and H. Akbulut

1Department of Biochemistry, University of Hacettepe, School of Pharmacy, Ankara, Turkey, 2Department of Medical Oncology, University of Ankara, School of Medicine, Ankara, Turkey

Earlier kinetic studies in our laboratory have shown that cationic triarylmethane dyes are highly effective as inhibitors of cholinesterases with Ki in the micromolar range. In the present study, aiming to identify the binding sites of the dyes and their functional contacts with the active site, we have explored the role of Phe 329 in the inhibition of human butyrylcholinesterase (BChE) by malachite green (MG) and methyl green (MeG). The mutation Phe329Ala was introduced into human BChE by PCR-mediated site-directed mutagenesis with Pfu polymerase. Wild type and mutant BChE were stably expressed in human embryonic kidney cells and purified by anion exchange and procinamidine-Sepharose affinity chromatography. Studied at 25°C in 50 mM MOPS buffer (pH 8), with butyrylcholine as substrate, MG acted as single-occupancy, linear mixed type inhibitor of wild type BChE with Ki = 0.071 ± 0.004 μM. MeG caused competitive inhibition of wild type BChE with Ki = 0.17 ± 0.01 μM. Under the same experimental conditions, the two ligands caused complex, nonlinear inhibition of the Phe329Ala mutant and the kinetic pattern of inhibition indicated cooperative I binding at two sites. Intrinsieic K' values (= [I]2 extrapolated to [S] = 0) for MG and MeG were 2.0 ± 0.60 and 5.3 ± 1.1 μM, respectively. The differences in kinetic pattern and Ki values pointed to Phe 329 as a critical residue in determining enzyme conformation as well as ligand binding.

**Acknowledgement:** Supported by a grant (SBAG-3677) from Scientific and Technical Research Council of Turkey.

---

**A4.18**

**The dynamic of some plasma oxidative stress markers in stroke patients undergoing rehabilitation**

B. N. Manolescu, M. Berteau, E. Oprea, L. Dumitru, I. Farcașanu, A. Iliescu, R. Badea and Claudia Toma

1Department of Biochemistry, University of Medicine and Pharmacy “Carol Davila”, Bucharest, Romania, 2Department of Organic Chemistry, University of Bucharest, Bucharest, Romania, 3Department of Rehabilitation and Physical Medicine, University of Medicine and Pharmacy “Carol Davila”, Bucharest, Romania, 4Department of Rehabilitation and Physical Medicine, Elias Emergency Hospital, Bucharest, Romania

**Introduction:** Both in vitro and in vivo studies indicate oxidative stress is a key element in stroke etiology. Cerebral ischemic insult leads to overproduction of reactive oxygen and nitrogen species with deleterious consequences both for nervous tissue and other organs. Most of the studies investigated oxidative stress in acute stroke patients. Little attention was paid to post-acute stroke patients.

**Objective:** We investigated the dynamic of some oxidative and nitrosative plasma markers in stroke patients.

**Methods and patients:** The study group has 20 patients (8 males/12 females, 69.452.49 years old) who presented to our rehabilitation department from June 2009 till January 2010. Blood samples were taken at the beginning (moment 0) and at the end (moment 1) of the hospitalization time (131 days). We assessed the concentrations of total thiols, glutathione, advanced oxidation protein products (AOPP), protein carbonyls, and 3-nitro-tyrosine.

We also assessed the concentrations of total plasma proteins, albumin, and the activity of -glutamyl transpeptidase (GGT). A control group of 20 subjects (5 males/15 females, 62.783.15 years old) was created. Informed consent was obtained from all the participants in study or from their belongings.

**Results and discussions:** The concentration of all parameters decreased from moment 0 to moment 1 with the exception of total proteins and total thiols. We found statistical significant differences only for protein carbonyls and 3-nitro-tyrosine concentrations and GGT activity. This study will be completed with other oxidative and nitrosative stress markers. ±±

---

**A4.19**

**Plasma status of homocysteine and lipoprotein (a) during chronic phase of stroke**

B. N. Manolescu, M. Berteau, I. Farcașanu, L. Dumitru, E. Oprea, A. Iliescu, R. Badea and C. Toma

1Department of Biochemistry, University of Medicine and Pharmacy “Carol Davila”, Bucharest, Romania, 2Department of Rehabilitation and Physical Medicine, University of Medicine and Pharmacy “Carol Davila”, Bucharest, Romania, 3Department of Rehabilitation and Physical Medicine, Elias Emergency Hospital, Bucharest, Romania

**Introduction:** Homocysteine (Hcy) and lipoprotein (a) [Lp(a)] are two factors responsible for endothelium aggression. Both
A4.20 Manipulation of lipidic environment of prion protein-enriched domains isolated from rat cerebellar granule cells

D. Cunati, L. Botto, M. Masserini and P. Palestini
Department Experimental Medicine, Università degli Studi di Milano-Bicocca, Monza, Italy

The Prion protein is a GPI-anchored protein primarily concentrated in neuronal cells. Under certain conditions its cellular form, PrPC, can convert into the lethal isoform, PrPSc. This conversion occurs in particular regions of the membrane, called lipid rafts. These microdomains enriched with cholesterol and glycosphingolipids are thought to play a crucial role both in physiological functions and in alternative folding of the PrP. The aim of our project is to establish if the alteration of cell lipid composition can modify the membrane distribution of PrPC within raft or non-raft regions and to promote the activity of disintegrins (ADAM10/17) upon PrPC. Due to this reason, granule cells obtained from the cerebella of 8-days-old SD rats were incubated after 8 days in culture with 2 x 10^-6 M GM1 or GD1a at 4°C or 37°C for 4 hours. Detergent resistant fractions (DRM) preparation was accomplished according to Palestini et al., 2000 and proteins in all gradient fractions were separated and analysed by EF/WB. Lipids were separated by HPTLC and analysed by radiochromatoscaner. The treatment with GM1 at 4°C led to a remarkable enrichment of this ganglioside in DRM and slightly increased the content of PrPC and ADAM10/17 in this region. At 37°C GM1 was enriched in DRM and a moderate ganglioside incorporation was detectable in non-rafts membrane regions corresponding to gradient fractions from 8 to 12. In DRM PrPC and ADAM10/17 amounts decreased. Further experiments with GD1a are in progress; through the use of these ganglioside we mean to verify whether their major encumbrance can change into a bigger extent proteins distribution within raft and non-raft membrane regions.

A4.21 Evidence for a glial protective role in Mn2+-induced neurotoxicity

M. R. Sepulveda, D. Marcos, M. Berrocal and A. M. Mata
Universidad de Extremadura, Bioquímica y Biología Molecular y Genética, Facultad de Ciencias, Badajoz, Spain

Manganese is a vital element and cofactor of key enzymes in mammals. However, high levels of this ion in the body give rise to Mn2+ deposits in brain, inducing manganism, a Parkinson-like disease. Little is currently known about the mechanisms involved in this neurotoxicity, and potential treatments or transporters involved in Mn2+ detoxification. In this work we have used mouse primary cultures to explore Mn2+ effect on neural and glial cells. MTT assays showed that the presence of Mn2+ decreased the survival of both cell types, being glial cells more resistant to Mn2+ toxicity than neurons. Immunofluorescences showed that Mn2+ specifically affected cellular integrity and produced fragmentation of Golgi membranes, containing the secretory pathway Ca2+/Mn2+ -ATPase isoform 1 (SPCA1) as a functional candidate for Mn2+ detoxification. Besides, the cellular effects induced by Mn2+ were less pronounced in glia, which also prevented neurons against degeneration. On the other hand, the removal of Mn2+ from cultures or the addition of EDTA ceased the toxic effect of Mn2+ and allowed a partial reassembly of the Golgi complexes, mainly in glia. These findings lead to an understanding of the neurotoxicity linked to chronic-Mn2+ exposure in order to look for new therapeutic approaches. This work was supported by Grants from MICINN (BFU2008-00182) and Fundación Marcelino Botín, Spain.

A4.22 Abstract withdrawn

A4.23 Human cartilage glycoprotein gp-39 (YKL-40) is a putative oncogene

O. Balynska1, V. Baklaushev2 and V. Kavsan1
1Institute of Molecular Biology and Genetics, Biosynthesis of Nucleic acid, Kyiv, Ukraine, 2V. P. Serbsky National Research Centre for Social and Forensic Psychiatry, Laboratory of immunochemistry, Moscow, Russian Federation

Gliomas are the most frequent malignant primary brain tumors. They are highly aggressive, invasive, and neurologically destructive. Earlier it was found that HC gp-39 (human cartilage 39-KDa glycoprotein) gene was expressed at markedly higher level in glioblastomas compared to normal brain cells. Described results in accordance with previously published data indicate that YKL-40 may be used as a potential molecular marker for brain glial tumors. To determine whether YKL-40 possessed oncogenic properties we established human embryonic kidney HEK293 cell line that stably expressed YKL-40. Presence of YKL-40 in cells transfected by hybrid plasmid was tested by Western-blot and immunocytochemistry. The YKL-40-expressing clones grew as colonies in soft agar in contrast to the parental HEK293 cells or HEK293 cells transfected with the empty vector. To determine whether YKL-40 could influence tumor initiation and development in vivo, HEK293 cells that stably expressed YKL-40 and HEK293 cells transfected with the empty vector were used for stereotaxic intracerebral implantation to female wistar rats. On the 21th day after implantation tumor appeared in three rats with implanted HEK293 cells which stably expressed YKL-40, two rats had smaller tumor after 1 month. The volume of tumor was parameters are well known stroke risk factors. While the mechanisms of Hcy toxicity are somewhat understood, the exact mechanism relating Lp(a) with stroke is not well understood. Objective: We evaluated the level and dynamic of Hcy and Lp(a) concentration in ischemic stroke patients. Methods and patients: The study group has 10 patients (4 males/6 females, 73.33.6 years old) who presented to our rehabilitation department. Blood samples were taken at the hospitalization moment (moment 0) and at the discharge moment (moment 1). The hospitalization period of time was 131 days. We assessed plasma homocysteine and Lp(a) concentrations using commercially available kits. We also assessed routine biochemical analysis (blood glucose, triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol). The control group consists of 10 subjects (3 males/7 females, 62.83.7 years old). Informed consent was obtained from all the participants in our study or from their belongings. Results and discussion: We found that during the hospitalization period of time Hcy concentration increased, while Lp(a) concentration decreased. We found statistical significant difference only for Hcy. Also, we found a negative correlation between these two parameters. In order to obtain a better image about the dynamic of these parameters we need further investigation using larger study population and other parameters relevant for endothelium status after ischemic stroke.
A4.24 Identification of new interactions of tyrosine kinase Ack1 in mouse brain
J. M. Urena1, M. d. M. Masdeu2, A. La Torre3 and E. Soriano1
1Institut de Recerca Biomedica de Barcelona/ Unviersitat de Barcelona, Neurobiologia del Desarrollo y la Regeneración, Barcelona, Spain, 2Institut de Recerca Biomedica de Barcelona, Neurobiología del Desarrollo y la Regeneración, Barcelona, Spain

Ack is a tyrosine kinase having several protein-protein interaction domains: an SH3 domain, a region able to interact with Cdc42 (Cbd domains: Cdc42 binding domain) and several proline rich regions. In previous work we have shown the pattern of expression of Ack1 in adult and fetal tissues. Since this molecule has several protein-protein interaction domains, we have used two strategies in order to identify some of the putative molecules able to interact with Ack. In the first approach we have used immunoprecipitation followed by Mass-Spectrometry to identify the associated proteins; in the second strategy we have purified by affinity chromatography molecules interacting with GST-Ack1 proline-rich regions and we have identified them by Mass spectrometry. With these techniques we have described several new molecules, among others lymph bic associated protein, beta and gamma actin, keratin-6 alpha and several unnamed proteins. This data together with other molecules already described to interact with Ack can such as Grb2, Nck, beta-1 integrin and the nucleotide exchanger Db1 makes Ack1 to be a molecules with multiple possible partners. In addition we show how overexpression of Ack1 in cultured neurons from hippocampus or cerebellum increases the number of dendrites, and branching.

A4.25 Characterization of site specific O-glycan structures within the mucin-like domain of α-dystroglycan from human skeletal muscle
J. Nilsson1, J. Nilsson2, G. Larson2 and A. Grahn2
1Department of Pathology, Institute of Biomedicine, Göteborg, Sweden, 2Department of Clinical Chemistry and Transfusion Medicine, institute of Biomedicine, Göteborg, Sweden

The glycosylation of the extracellular protein α-dystroglycan is important for its ligand binding activity and altered or blocked glycosylation are associated with several forms of congenital muscular dystrophies. By immunoprecipitation and sialic acid capture-and-release enrichment strategies we isolated tryptic glycopeptides of α-dystroglycan from human skeletal muscle. Nano-LC/MS/MS was used to identify both glycopeptides and peptides corresponding to the mucin-like and C-terminal domain of α-dystroglycan. The O-glycans found had either Hex-O-Thr or HexNAc-O-Ser/Thr anchored structures, which were often elongated and frequently, but not always, terminated with sialic acid. The HexNAc-O-Ser/Thr but not Hex-O-Thr glycopeptides, displayed heterogeneity regarding glycans core structures and level of glycosylation site occupancy. We demonstrate for the first time glycans attachment sites of the NeuAcHexHexNAcHex-O structure, corresponding to the anticipated Neu5AcHex3Gal4GlcNAcβ2Man-O glycan (sLacNAc-Man), within the mucin-like domain of human α-dystroglycan from human skeletal muscle. In summary, 24 glycopeptides were characterized from human α-dystroglycan, which provide insight to the complex in vivo O-glycosylation of α-dystroglycan.

A4.26 Excess normal prion protein (PrPC) in rat central nervous system is responsible for the myelin damage associated with vitamin B12 deficiency
G. Scalabrin1, E. Mutti1, D. Veber1, A. Calligaro2 and G. Tredici2
1University of Milan, Milano, Italy, 2University of Pavia, Pavia, Italy, 3University of Milano-Bicocca, Monza, Italy

It has been widely accepted that the myelinolytic lesions associated with vitamin B12 (cobalamin, Cbl) deficiency in rats are caused by increased synthesis of tumour necrosis factor (TNF)-α in the central nervous system (CNS). However, TNF-α also regulates the PrPC synthesis in a cultured cell line and it has been suggested that the octarepeat region (OR) of PrPC has myelinolytic action. We therefore investigated whether the cascade of events responsible for the CNS myelinolytic lesions associated with Cbl deficiency may include abnormal PrPC levels in the CNS of totally gastrectomised (TGX) (i.e. Cbl-deficient) rats 2 months after total gastrectomy (TG) (when the myelin lesions are ultrastructurally most severe). We repeatedly injected anti-OR antibodies (Abs, bought from the University of Zürich), heat-inactivated anti-OR-Abs or anti-TNF-α Abs intracerebroventricularly (i.c.v.) for 2 months after TG, and injected recombinant PrPC i.c.v. into normal adult rats for 2 months. PrPC levels in spinal cord (SC) and cerebrospinal fluid (CSF) of TGX rats treated with saline alone were significantly increased in comparison with laparotomy controls. The SC myelin ultrastructure was normal in the anti-OR Ab-treated TGX rats, whereas SC myelinolytic lesions were still present in the TGX rats treated with heat-inactivated anti-OR Abs and in the PrPC-treated rats. The TGX rats treated with anti-TNF-α Abs showed a normal SC myelin ultrastructure and no increase in SC or CSF PrPC levels. Taken together, these data demonstrate that the SC myelinolytic lesions observed in Cbl-deficient rats are due to increased local PrPC levels caused by increased TNF-α synthesis.

A4.27 Imaged by LA-ICP-MS: metal accumulations after cerebral infants in course of time
D. Salter1, F. Uhlenbrock1, J. Manuelpillai1, B. Wu2, C. Palm3, A. Matusch1, K.-J. Langen1 and J. S. Becker2
1Forschungszentrum Jülich, Institute of Neurosciences and Medicine, Jülich, Germany, 2Forschungszentrum Jülich, Central Division of Analytical Chemistry, Jülich, Germany, 3University of Applied Sciences Regensburg, Faculty of Computer Science and Mathematics, Signal- and Image Processing in Medicine, Regensburg, Germany

In recent years accumulations of metals were increasingly described in context with brain diseases. Cerebral infants in particular cortical infants lead to secondary degeneration of neurons in thalamic areas corresponding to the damaged cortical part. In rats infants can be directed stereotactically to defined cortical sections by injection of the photo reactive substance rose bengal and adjustment of a light source to the skull. At different time points after infarct induction we prepared cryo sections of the rat brain. The slices were analyzed in respect of metal accumulation
Abstracts

A4.28
Abstract withdrawn

A4.29
Influence of Nanobodies on the aggregation properties of Alpha-synuclein
T. Guilliams¹, E. De Genst², E. Pardon², N. Cremañes¹, J. Steyaert¹, L. Wyns² and C. Dobson¹
¹University of Cambridge, Chemistry, Cambridge, UK, ²Vrije Universiteit Brussel, Natural Sciences, Brussels, Belgium

The fibrillation of α-synuclein (αS) and its deposition in Lewy Bodies is one of the key pathological hallmarks of Parkinson’s disease (PD). However, much remains to be understood about the mechanism of αS aggregation and its relationship to PD pathogenesis. The present work is focused on in vitro αS aggregation, using a number of novel conformation specific antibody fragments (Nanobodies) that have been determined by NMR to target different epitopes in monomeric αS. The binding of each Nanobody to αS has been characterised in atomic level detail and its effect on the kinetics of the αS aggregation pathway has been investigated closely. In the future, this approach will help to study the physical and structural properties of αS aggregates and understand how to prevent their formation.

A4.30
D-methionine in noise induced hearing loss
A. Wiktorow-Smugar, A. Rewerska, E. Rajkowska, P. Poltianski and M. Śliwińska-Kowalska
Nofer Institution of Occupational Medicine, Lodz, Poland

Oxidative stress in the cochlea is considered to play an important role in noise induced hearing loss. The purpose of this study is to understand the mechanism underlying the noise induced increase in reactive oxygen species (ROS) in the inner ear. The changes in superoxide dismutase (SOD), catalase, lipid peroxidation (LPO) and the auditory brainstem response (ABR) were measured in the cochlea 1, 7 and 14 after noise exposure (4 kHz octave band at the intensity of 118 dB SPL for 8 hours) in C57BL/6 mice. In addition we also studied the action of an antioxidant D-methionine (D-met) to investigate its role in preventing this noise induced oxidative stress and hearing loss. D-met was able to scavenge the free radicals resulting in a significant decrease in LPO levels from noise exposure controls. In summary, the findings of this study indicate that the time dependent alterations in scavenging enzymes facilitate the production of free radicals and D-met is effective in attenuating the noise-induced oxidative stress and associated functional loss in mice cochlea.

A4.31
Increased ERG amplitudes in a model of type 2 diabetes
M.-T. Perez, L. E. Johnson and M. Larsen
Department of Ophthalmology, Glostrup Hospital, Glostrup, Denmark

Purpose: To examine how retinal functional changes correlate with blood glucose levels.

Methods: Zucker diabetic fatty (ZDF) rats (8–22 weeks old) were compared with lean control rats using full field electroretinography (ERG). A group of ZDF rats received a long-acting insulin analog once daily from 17 weeks of age to reduce glucose levels to <10 mM. ERGs were performed in the three groups until 22 weeks. The expression of specific retinal cell markers was examined at 23 weeks of age.

Results: ZDF rats showed significantly higher scotopic a- and b-wave amplitudes (>10%) from 12 weeks of age compared to lean controls. A delay in the implicit times of all oscillatory potential (OP) wavelets of about 10% was also observed in ZDF rats at 14 weeks of age and onwards. Amplitudes were normalized within 3 hours after insulin administration, with no effect on OP latencies. Over time, insulin treatment produced a small shortening of OP latencies and a further decrease in a- and b-wave amplitudes, which became lower than observed in non-diabetic animals of the same age. Glial cell markers were up-regulated in diabetic rats, but not in insulin-treated animals.

Conclusions: Hyperglycemia led to higher ERG amplitudes, probably reflecting the ability of the outer retina to utilize the additional glucose as substrate. This is corroborated by the observation that insulin treatment reduced the retinal responses. The fact that insulin treatment produced sub-normal amplitudes may correlate with the initial worsening of retinopathy seen in some patients. The activation of glial cells noted in the untreated ZDF animals may, directly and/or indirectly, contribute to the effects observed.

A4.32
Abstract withdrawn

A4.33
Loss of megalin expression in the outer retina in models of photoreceptor degeneration
K. A Wunderlich¹, E. Zrenner² and M.-T. Perez¹
¹Department of Ophthalmology, Lund University, Lund, Sweden, ²University of Tuebingen, Center for Ophthalmology, Institute for Ophthalmic Research, Tuebingen, Germany

Purpose: Megalin, a member of the low-density lipoprotein receptor (LDLR) family, binds and takes up a range of ligands, including the neuroprotectants metallothionein I and II (MT-I + II). The purpose of the present study was to examine the distribution of megalin in rodent models of Retinitis Pigmentosa (RP) in relation to that of MT-I + II.

Methods: Megalin and MT-I + II expression were analyzed in normal mouse and rat retinas and in retinas of rd1 and rds mice and of RCS (Royal College of Surgeon) rats of various ages. The identity of labelled cells was verified by co-staining with various specific cell markers. Sites of possible interaction between megalin and MT-I + II were identified with an in situ proximity ligation assay (PLA).

Results: Strong up-regulation of MT-I + II was observed in Müller cells in degenerating retinas, which coincided with the onset
of glial fibrillary acidic protein expression (GFAP) in rd1 and rds mice. In normal animals, megalin was expressed in the nerve fiber layer, in the region of the inner and outer segments, and in Müller cell processes in older animals. In situ PLA revealed that interactions between megalin and MT-I+II might occur at the inner and outer margins in the normal retina. In degenerating retinas, however, megalin expression was lost in the outer retina early in the degenerative process in all three models.

**Conclusions:** Expression of MT-I+II appears to be controlled by different events in the three pathologies. A transfer of MT-I+II from e.g. Müller cells into photoreceptors appears less likely as megalin expression was progressively lost, suggesting that the potentially neuroprotective actions of metallothionein may be limited.

### A4.34

**Diosgenin-induced neuronal differentiation of rat neural stem cells requires ERK1/2**

T.-Y. Chin¹, C.-W. Cheng² and C.-Y. Wu³

¹Department of Bioscience Technology, Chung Yung Christian University, Chung-Li, Taiwan, ²Chung Shan Medical University, Institute of Biochemistry and Biotechnology, Taichung, Taiwan, ³Chung Yang Christian University, Institute of Biochemistry and Biotechnology, Taichung, Taiwan

Diosgenin, a steroidal sapogenin is extracted from the root of wild yam (Dioscorea villosa). It has been reported to have various biological activities, including antioxidant and anti-inflammatory effects. In this study, we examined the effect of diosgenin on neuronal differentiation of neural stem cells (NSCs). NSCs obtained from 14-day-old rat embryos were propagated as neuroepithelium and cultured under different conditions with or without diosgenin for 5 days. Diosgenin significantly increased the percentage of microtubulin associated protein 2 (MAP2) in positive neurons compared with the control cells. In addition, these results show that diosgenin promote the differentiation of NSCs into neuronal phenotype characterized by neurite outgrowth, the expression of neuronal marker protein TuJ1 and voltage-operated Ca2+ channel. Furthermore, diosgenin induced an increase in the phosphorylation of the extracellular signal regulated kinase 1/2 (ERK1/2). U0126, a selective ERK1/2 kinase inhibitor blocked both the phosphorylation of ERK1/2 and the ability of diosgenin to promote differentiation of NSCs into neurons. The structure of diosgenin is similar to that of estrogen. To investigate the possible role of the estrogen receptor (ER) in the course of a diosgenin-induced neurogenic effect, we used the highly specific ER antagonist ICI182,780 to blocked ER-related pathway. The addition of ICI182,780 did not alter diosgenin-induced neuronal differentiation. This data indicated that diosgenin does not require ER activation in neural differentiation. Taken together, our results suggest that diosgenin can induce NSCs differentiation through ERK1/2 activation in a ER-independent pathway.

### A4.35

**Abstract withdrawn**

### A4.36

**The chaperone-like protein HYPK acts together with NatA in cotranslational N-terminal acetylation and prevention of Huntingtin aggregation**

T. Arnesen¹, K. K. Starheim², P. Van Damme³, R. Evjenth⁴, H. Dihn⁵, M. Betts⁶, A. Rynningen⁷, J. Vandekerckhove⁸, K. Gevaert⁹ and D. Anderson⁵

¹Department of Molecular Biology, University of Bergen, Bergen, Norway, ²Department of Surgical Sciences, University of Bergen, Bergen, Norway, ³Department of Medical Protein Research, Department of Biochemistry, Ghent University, Ghent, Belgium, ⁴EMBL, Heidelberg, Germany, ⁵Department of Medicine, University of Bergen, Bergen, Norway, ⁶University of Oregon, Institute of Molecular Biology, Eugene, USA

The human NatA protein N-α-terminal-acetyltransferase complex is responsible for cotranslational N-terminal acetylation of proteins with Ser-, Ala-, Thr-, Gly-, and Val-N-termini. The NatA complex is composed of the catalytic subunit hNaa10p (hArd1) and the auxiliary subunit hNaa15p (hNat1/NATH). Using immunoprecipitation coupled with mass spectrometry we identified endogenous HYPK, a Huntingtin (Htt) interacting protein, as a novel stable interactor of NatA. HYPK has chaperone-like properties preventing Htt aggregation. HYPK, hNaa10p and hNaa15p were associated with polysome fractions indicating a function of HYPK when associated with the NatA complex during protein translation. Knockdown of both hNAA10 and hNAA15 decreased HYPK protein levels possibly indicating that NatA is required for the stability of HYPK. The biological importance of HYPK was evident from HYPK-knockdown HeLa cells displaying apoptosis and cell cycle arrest in the G0/G1 phase. Knockdown of HYPK or hNAA10 resulted in increased aggregation of an Htt-EGFP fusion with expanded polyglutamine stretches, suggesting that both HYPK and NatA prevent Htt aggregation. Furthermore, we demonstrated that HYPK is required for N-terminal acetylation of the known in vivo NatA substrate protein PCNP. Taken together, the physical interaction between HYPK and NatA seems to be of functional importance both for Htt aggregation and N-terminal acetylation.

### A4.37

**GABAB receptors are heteromultimers with a family of auxiliary subunits**

J. Schwenk¹, M. Metz², G. Zolles³, R. Turcek³, W. Bildl⁵, E. Tarusawa³, A. Kulik³, A. Unger³, M. Gassmann⁷, U. Schulte⁴, B. Fakler⁴ and B. Bettler²

¹University of Freiburg, Institute of Physiology II, Freiburg, Germany, ²Department of Biomedicine, University of Basel, Institute of Physiology, Basel, Switzerland, ³University of Freiburg, Institute of Anatomy and Cell Biology, Freiburg, Germany

Metabotropic GABAB receptors mediate the action of GABA (γ-aminobutyric acid) the main inhibitory neurotransmitter in the brain. They regulate synaptic transmission and signal propagation by controlling the activity of calcium and potassium channels. The GABAB receptor is a G-protein coupled receptor composed of two homologous proteins: GABAB1 and GABAB2. However, the heterologously expressed GABAB1(1,2) receptor heteromer failed to reproduce the functional diversity observed with native GABAB receptors. To test the hypothesis that functional diversity results from additional yet unknown partners we have used a proteomic analysis by combining affinity purifications of membrane protein complexes from rat brain with quantitative
Abstracts

A4.38
Retinoic acid effect on β-amyloid(25-35) toxicity in PC12 cells
A. Kowalski, L. Zylinska and E. Rebas
Department of Molecular Neurochemistry, Medical University of Lodz, Lodz, Poland

Beta-amyloid is a peptide known for constituting plaques in brains of Alzheimer disease patients. Formation of amyloid plaques results in overproduction of reactive oxygen species and is accompanied by a disturbed calcium homeostasis. There is an increasing amount of data concerning potential retinoids protection against Alzheimer disease. Retinoic acid attenuates accumulation of amyloid plaques and can support regeneration of cells by stimulation of neurodifferentiation. In this study we focused on the effect of one of the most toxic β-amyloid fragments (25-35) on cell viability. Moreover, we examined if retinoic acid can protect the cells against βA(25-35) cytotoxicity. Our models were pseudoneuronal PC12 control cells, and PC12 line with suppressed expression of PMCA3, a neuron-specific isoform of calcium pump. Viability of the cells was measured spectrophotometrically with WST-1 reagent. The results show that retinoic acid, in a concentration dependent manner, can protect control PC12 cells against toxic effect of βA(25-35). We did not observe this effect in PMCA3-reduced cells. It may suggest that the defensive effect of retinoic acid could depend on calcium homeostasis. This work was supported by The European Social Fund and the national budget within the framework of Measure 2.6 of the Integrated Regional Operational Programme (“Stypendia wspierajace innowacyjne badania naukowe doktorantow” Project) and from Medical University of Lodz grant 503-6086-2.

A4.40
Heat inactivation of tissue samples to stabilize proteins, peptides and their modifications
K. Alenäs1, M. Svensson1, M. Borén1, M. Fälth2, P. E. Andrén2, P. Svinningsson1 and K. Sköld3
1Denator AB, Biotech Center, Arvid Wallgrens Backe 20, Göteborg, Sweden, 2Biological and Medical Mass Spectrometry, Uppsala University, Uppsala, Sweden, 3Institute for Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

Removal of a sample from its natural surrounding leads to major disturbance of tissue homeostasis. The action of proteases and other enzymes can rapidly change the composition of the proteome and post-translational-modifications (PTMs). Subsequent analytical results reflect a mix of in vivo proteome and degradation products. Here, a novel stabilization system was used to treat fresh and frozen tissue samples to stop degradation and preserve the in vivo proteome. The system utilizes rapid heat inactivation to eliminate enzymatic activity in tissue and thereby enable detection of endogenous peptides and monitoring of important PTMs, such as phosphorylations. Stabilization was assessed by nano-LC-MS, MALDI, and western blotting on samples from brain, muscle, and liver. Inadequate sample handling cause an increase in degradation fragments which has been shown in these experiments. However, after immediate sample stabilization, no protein degradation fragments were detectable. The mass spectrometrically identified peptide peaks in stabilized samples consisted of several known neuropeptides, endogenous peptides, and novel, potentially biologically active, peptides. Most peaks detected in the untreated group originate from proteins such as hemoglobin, cyclophilin, NADH dehydrogenase, synuclein and other highly expressed proteins. Accordingly, the assayed functions of proteases, phosphatases and cytochrome C oxidase showed clear inactivation after stabilization. The levels of phosphorylated forms of CREB, GSK and MAPK remained unchanged after 2 hours in room temperature after stabilization as the levels of the same proteins in untreated samples decreased in only 10 minutes.

A4.39
Multiparametric fluorescence microscopy imaging of amyloid beta allows for direct observations of protein misfolding in vitro and in cells
E. K. Esbjörner1, C. Bertocini1, S. Schlachter2, D. Chris1, C. Kaminski3 and G. Kaminski Schierle2
1University of Cambridge, Chemistry, Cambridge, UK, 2University of Cambridge, Chemical Engineering and Biotechnology, Cambridge, UK

Misfolding and aggregation of amyloid beta peptides into oligomers and subsequently amyloid fibrils have detrimental effects on synapse function and cell viability, accounting in part for the memory loss and neuronal degeneration associated with Alzheimer’s disease. Whilst amyloid beta aggregation can be readily studied in vitro by established biophysical techniques, it has to date, been very difficult to directly monitor aggregation or identify the aggregation state of misfolded species in vivo with both spatial and temporal resolution. Multiparametric fluorescence microscopy allows for pixel-by-pixel acquisition of not only fluorescence intensities, but also of more responsive parameters such as fluorophore excited state lifetime and steady-state anisotropy and we have recently shown how these parameters sensitively report on the aggregation state of an engineered α-synuclein fusion protein with a C-terminal yellow fluorescent protein tag in vitro, in cells and in vivo. Here we have studied misfolding of amyloid beta (Ab40 and Ab42) in vitro and in cultures of neuronal cells. Cross-validation of multiparametric imaging data obtained in vitro with fluorescence spectroscopy, transmission electron microscopy and biochemical assays has allowed us to correlate certain fluorescence lifetime signatures and fluorescence anisotropy characteristics with the specific appearance of monomeric, oligomeric and fibrillar amyloid beta in our samples. Using this information we have then been able to identify the same species in a cellular context by examining the extracellular appearance, internalization and in-cell compartmentalization of Ab40 and Ab42.

mass spectrometry. The analysis identified GABA_B receptors as complexes of GABA_B1, GABA_B2 and a subset of the family of “potassium channel tetramerization domain-containing” (KCTD) proteins. Further biochemical, immunohistochemical and electrophysiological studies showed that KCTD isoforms 8, 12, 12b and 16 are tightly associated with the C-terminus of GABA_B2. They exhibit a distinct expression profile in the brain and localize to the plasma membrane of pre- and postsynaptic compartments in the hippocampal CA1 region. The receptor properties of the GABA_B1(2) heteromer were changed through coassembly with the KCTD proteins: the KCTD proteins increase agonist potency, alter the G-protein signaling of the receptors by inducing rapid onset and pronounced desensitization. Together, our results establish the KCTD proteins as auxiliary subunits of GABA_B receptors that determine the pharmacology and kinetics of the receptor response.
Poly-glutamine (poly-Q) diseases are neurodegenerative inherited disorders caused by the expansion over a pathological threshold of a poly-Q tract in the involved protein. In our work we have studied the poly-Q protein ataxin-3 (AT-3), the causative agent of spinocerebellar ataxia type 3. AT-3 consists of a globular N-term domain and a flexible tail containing the poly-Q tract. In particular, since the pathogenic mechanisms are still poorly understood, we investigated the relationship between AT-3 toxicity and protein aggregation in an intracellural environment. We used in our studies four human AT-3 variants expressed in E. coli to explore the correlation between proteins aggregation pathway and toxicity by biochemical and biophysical techniques. Growth curves analysis and structural investigations onto soluble and insoluble AT-3 protein fractions evidenced a multi-domain aggregation mechanism and a toxic effect dependent on soluble protein concentration. Considering that AT-3 deleterious effects on a bacterial host underlines a cytotoxic effect independent from organism complexity, we investigated general mechanisms by which toxicity can act. Even if no significant differences in ROS levels were observed, we have identified alterations in membrane integrity by SDS susceptibility test and propidium iodide staining. Furthermore, we employed our E. coli system as a model for evaluating the intracellular effects of redox status on aggregation and toxicity of AT-3, which contains six cysteins not involved in disulphide bridges. Our preliminary results demonstrated a possible role of disulphide bridges in aggregation process and oligomers generation.

Modified analogues of ACTH fragments as new candidates for effective drugs

The molecular mechanism underlying regulatory peptides biological action keeps unclear for the time being. In spite of the question, medical products based on peptides found wide application in clinics as effective drugs and prophylactics, for example neuropeptide Semax. As shown before Semax exposing to P2 membranes of different brain structure cells undergoes fast and aggressive protelysis by membrane enzymes. Some of peptide fragments appeared holds their own biological activity, individual specificity and directional influence. In this work we tried to clear two key processes underlying neuropeptide biological action. We determined the following: proteolysis and receptor specific binding on brain cells plasmatic membranes. Four original modified analogues of ACTH fragments containing in structure PGP C-terminal fragment been tested: ACTH (4-7)-Pro-Gly-Pro, ACTH (6-9)-Pro-Gly-Pro, ACTH (7-10)-Pro-Gly-Pro, ACTH (15-18)-Pro-Gly-Pro. All molecules shown different stability after exposition to plasmatic membranes of brain cells or nasal mucosa. It was shown that in time necessary for penetration sufficient for physiological action peptide quantities into the brain by intranasal way, the most part of molecules keeps stable. Exposed to P2 membranes of different brain structure cells, each of synthetic ACTH analogues undergoes individual proteolysis. The ACTH analogues influence on specific binding of well-known selective ligands (CP-55,940, WIN 55,212-2, Dihydrocapsaicin and other) was shown. This new artificial neuropeptides, we suppose, could give the best fit as a new drugs for clinical and prophylactic application.

Proteomic study of proteins tightly associated with tau polypeptide in neurofibrillary tangles in brains with Alzheimer’s disease

Alzheimer’s disease (AD) is the commonest cause of dementia in the elderly and is characterized by the presence of neurofibrillary tangles (NFTs) and neuritic plaques. Both structures represent dense accumulations of abnormal insoluble filaments. NFTs are formed by paired helical filaments (PHFs) composed by assembled tau polypeptides. The integral constituents of PHFs remain unknown mainly due to the high insolubility of NFTs. In this study we performed a proteomic analysis of NFTs to identify those potential polypeptides that could be closely bound to tau and that could have a possible role in the tau pathological assembly. Parafomaldehyde-fixed-brain tissue sections from three Mexican AD cases were immunolabeled with the conformational phosphor-dependent AT100 monoclonal antibody (Ser 199, Ser202, Thr205, Thr 212, Ser 214). We isolated 2300 NFTs from each case using a PALM system and pooled them for further studies. Proteins were extracted from pooled NFTs using several solubilization methods and were digested with trypsin. Generated peptides were separated by two-dimensional HPLC and analyzed by electro spray ionization and tandem mass spectrometry. Mass spectra were used to identify the polypeptides using different databases. We identified 57 polypeptides, which included some proteins related to metabolism, cytoskeleton, antioxidant proteins, protein kinases and ubiquitin. To verify the identification by proteomic analysis, we performed immunofluorescence and laser confocal microscopy for some polypeptides identified. This study demonstrates the advantages to combine techniques for high quality isolation of polypeptides and proteomics in fixed brain tissues.

Hyperphosphorylation of tau polypeptide and appearance of fibrillar amyloid-β deposits correlate with aging in a triple transgenic mouse model of Alzheimers disease

Hyperphosphorylated neurofibrillary tangles (NFTs) and fibrillar deposits of amyloid-β (Aβ) are the neuropathological hallmarks of Alzheimers disease (AD). Molecular mechanisms involved in
Abstracts

formation of such structures are not fully understood. However, the existence of animal models, such as the 3xTg-AD transgenic mouse, is very important to address this important issue. 3xTg-AD mouse carries gene mutations in the β-amyloid protein precursor (βAPPsw), presenilin-1 (PS1M146V) and tau P301L and its brain characteristically presents Aβ deposits and tau pathology, being cortex and hippocampus the most affected regions. Here we analyzed the presence of a variety of phosphor-dependent tau epitopes and tau C- and N-terminus markers at different ages of the 3xTg-AD mouse, and the temporal appearance of such epitopes and Aβ deposits using the 499, 46.1, Alz-50, 499, S199, S231, S396, S400, S404, and AD2 antibodies. Double immunolabeled tissues were counterstained with thiazin red and observed through a laser confocal microscope. Our results showed that appearance and distribution of hyperphosphorylated tau correlated with age. Tau aggregates were first observed at hippocampus in a 3 months old mouse. Interestingly, the appearance of tau aggregates in the cortex was observed at 7 months and they were very evident by the age of 18 months. By contrast, Aβ deposits were first observed at seven months and they were clearly identified at 18 months. This study revealed that the appearance of phosphor-tau epitopes preceded at least four months to Aβ deposits. This observation confirms the usefulness of the 3xTg-AD mouse in the study of AD neuropathology.

A4.46
Comparison of blood toxic and plasma essential elements of the autistic Turkish infants
C. Sezgin1, S. Kaya1 and S. Keskin2
1 Cerrahpasa Medical Faculty, Biochemistry, Istanbul, Turkey, 2 Cerrahpasa Medical Faculty, Pediatric Neurology, Istanbul, Turkey

The whole blood total mercury and lead levels plasma manganese, copper, zinc and selenium levels of autistic infants and their healthy siblings and casually selected healthy infants who are not autistic were measured and compared. Whole blood total mercury levels of autistic and control-1 are (11.97 ± 3.75 μg/l), (5.18 ± 2.24 μg/l), (p < 0.001), respectively, autistic and control-2 are (11.97 ± 3.75 μg/l), (6.06 ± 2.46 μg/l), (p < 0.001) respectively and significantly higher in autistics. Whole blood lead levels of autistic and control-1 are (16.14 ± 5.25 μg/l), (7.69 ± 2.17 μg/l), (p < 0.001) respectively, autistic and control-2 are (16.14 ± 5.25 μg/l), (9.62 ± 2.92 μg/l), (p < 0.001) respectively and significantly higher in autistics. Plasma manganese levels of autistic and control-1 are (6.41 ± 2.45 μg/l), (3.64 ± 0.92 μg/l), (p < 0.001) respectively, autistic and control-2 are (6.41 ± 2.45 μg/l), (4.11 ± 0.94 μg/l), (p < 0.001) respectively and significantly higher in autistics. Plasma copper levels of autistic and control-1 are (1557.48 ± 298.84 μg/l), (1274.09 ± 177.21 μg/l), (p = 0.004) respectively, autistic and control-2 are (1557.48 ± 298.84 μg/l), (1212.93 ± 271.41 μg/l), (p < 0.001) respectively and significantly higher in autistics. Plasma zinc levels of autistic and control-1 are (1469.80 ± 173.81 μg/l), (1611.01 ± 148.72 μg/l), (p = 0.048) respectively, autistic and control-2 are (1469.80 ± 173.81 μg/l), (1624.28 ± 201.76 μg/l), (p = 0.020) respectively and significantly lower in autistics. Plasma selenium levels of autistic and control-1 are (34.83 ± 7.14 μg/l), (34.83 ± 7.14 μg/l), (p < 0.001) respectively, autistic and control-2 are (34.83 ± 7.14 μg/l), (34.83 ± 7.14 μg/l), (p < 0.001) respectively and significantly lower in autistics. Whole blood total mercury and lead levels plasma manganese, copper, zinc and selenium levels of autistic infants and their healthy siblings and casually selected healthy infants who are not autistic were measured and compared. Whole blood total mercury levels of autistic and control-1 are (11.97 ± 3.75 μg/l), (5.18 ± 2.24 μg/l), (p < 0.001), respectively, autistic and control-2 are (11.97 ± 3.75 μg/l), (6.06 ± 2.46 μg/l), (p < 0.001) respectively and significantly higher in autistics. Whole blood lead levels of autistic and control-1 are (16.14 ± 5.25 μg/l), (7.69 ± 2.17 μg/l), (p < 0.001) respectively, autistic and control-2 are (16.14 ± 5.25 μg/l), (9.62 ± 2.92 μg/l), (p < 0.001) respectively and significantly higher in autistics. Plasma manganese levels of autistic and control-1 are (6.41 ± 2.45 μg/l), (3.64 ± 0.92 μg/l), (p < 0.001) respectively, autistic and control-2 are (6.41 ± 2.45 μg/l), (4.11 ± 0.94 μg/l), (p < 0.001) respectively and significantly higher in autistics. Plasma copper levels of autistic and control-1 are (1557.48 ± 298.84 μg/l), (1274.09 ± 177.21 μg/l), (p = 0.004) respectively, autistic and control-2 are (1557.48 ± 298.84 μg/l), (1212.93 ± 271.41 μg/l), (p < 0.001) respectively and significantly higher in autistics. Plasma zinc levels of autistic and control-1 are (1469.80 ± 173.81 μg/l), (1611.01 ± 148.72 μg/l), (p = 0.048) respectively, autistic and control-2 are (1469.80 ± 173.81 μg/l), (1624.28 ± 201.76 μg/l), (p = 0.020) respectively and significantly lower in autistics. Plasma selenium levels of autistic and control-1 are (34.83 ± 7.14 μg/l), (34.83 ± 7.14 μg/l), (p < 0.001) respectively, autistic and control-2 are (34.83 ± 7.14 μg/l), (34.83 ± 7.14 μg/l), (p < 0.001) respectively and significantly lower in autistics.

A4.45
Effect of Bacopa monniera extract on the expression of neuropeptide genes in SH-SY5Y neuroblastoma cells
S. Yothonsreuk and K. Petcharat
Naresuan University, Biochemistry, Phitsanulok, Thailand

The memory formation requires the action of several proteins and the alterations of protein expression have been attributed to memory functions. Neuropeptide Y (NPY) acts as a peptide neurotransmitter and neuroendocrine hormone in the nervous system. Several studies indicate that NPY and cAMP responsive element binding protein (CREB) played important roles in the formation of memory. Therefore, expression levels of both peptides also related with memory. Furthermore, it had been found that several herbs affect the memory; one is Bacopa monniera (brahmi). Thus far, there are little published scientific studies of the effects of brahmi on neuropeptide expression in neuronal cells. Therefore, the aim of this study was to examine the effect of brahmi on the expression of neuropeptide genes related to memory in SH-SY5Y neuroblastoma cells. SH-SY5Y neuroblastoma cells were cultured, then differentiated by 10 μM retinoic acid and treated with 100 μl/ml of brahmi extract for 2, 3 and 6 days to observe the duration of treatment that affects the mRNA expression. After mRNA extraction, cDNA was generated and the NPY and CREB genes were amplified by RT-PCR. Density of each band was evaluated by Scion software with paired samples t-Test statistics. Our data showed that the expression at gene level of NPY remained unchanged. On the contrary, our results showed that CREB mRNA level significantly increased (p < 0.05) by more than 2-folds after brahmi treatment for just 2 days. Alteration of CREB expression affected by brahmi could be one pathway in memory improvement, and this should be further analyzed.

A4.47
The blood-brain barrier permeability during convulsions in the rat pups born from folic acid supplemented mothers during their pregnancy
F. B. Seker1, H. Yorulmaz2, E. Kaptan3 and B. Oztas4
1 Department of Physiology, Istanbul University Faculty of Dentistry, Istanbul, Turkey, 2 T.C. Halic University, School of Nursing, Istanbul, Turkey, 3 Department of Biology, Istanbul University Faculty of Sciences, Istanbul, Turkey, 4 Istanbul Faculty of Medicine, Physiology Department, Istanbul, Turkey

Folic acid (FA) is used to prevent neural tube defects and takes part in important biochemical reactions in the body. In recent years it became a popular molecule to prevent cardiovascular and cerebrovascular diseases. In our study we investigated the effects of FA on the blood-brain barrier (BBB) permeability, brain water content (BWC), Zonula Occludens-1 (ZO-1) and Glial Fibrillary Acidic Protein (GFAP) immunoreactivity during convulsions in rat pups. We used 5 months old rat pups born from mothers supplemented 5 mg/kg/day FA intraperitoneally during their pregnancy. Evans blue method used for assessing BBB permeability. Penylenetetrazole (PTZ) induced convulsions caused a remarkable increase on the BBB permeability (p < 0.01). But in PTZ + FA group the BBB permeability significantly decreased when compared to PTZ group (p < 0.05).
BWC significantly increased in PTZ group when compared to controls (p < 0.05), it is not changed in PTZ + FA group. Although ZO-1 coloring power was found to be similar to control group, in PTZ + FA group ZO-1 immune reactivity was found to be increased. This result is original because it represents that FA might have an effect on the expression of ZO-1 protein during BBB development. GFAP immune reactivity increased in PTZ group when compared to control group. In PTZ + FA group GFAP immune reactivity was detected to be decreased when compared to PTZ group. The results of this study suggest that FA has protective effects on the BBB permeability decreasing paracellular passage of blood born molecules during convulsions in rat pups born from mothers supplemented FA during their pregnancy.

A4.48 Purification and mass spectrometric characterization of tau in human cerebrospinal fluid

J. Gobom1, E. Portelius1, S. F. Hansson1, H. Zetterberg1, P. Grognet2, E. Vanmechelen2, K. Höglund3, G. Brinkmalm1, A. Brinkmalm1 and K. Blienow1

1University of Gothenburg, Institute of Neuroscience and Physiology, Gothenburg, Sweden, 2Innogenetics, Ghent, Belgium

One of the typical brain lesions of Alzheimer’s disease (AD) is neurofibrillary tangles consisting of hyperphosphorylated forms of the microtubule-stabilizing protein tau. While significantly elevated concentrations, both of total tau and of phosphorylated tau, is found in the cerebrospinal fluid (CSF) of AD patients, it is still unknown which isoforms are present and which phosphorylation sites are utilized. Molecular characterization of tau in CSF presents an analytical challenge because of the high heterogeneity of the protein and its low concentration in CSF. We present a purification protocol based on immunoprecipitation that enabled, for the first time, mass spectrometric characterization of tau in human CSF. The tau purification method was optimized with regard to several experimental parameters including selection of antibody, antibody immobilization and CSF pre-treatment. ELISA was used to monitor tau recovery in each step. The IP samples were analyzed by Liquid chromatography (LC) coupled to either matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) or electrospray (ESI) hybrid linear ion trap Fourier transform ion cyclotron resonance mass spectrometry (QIT-FTICR MS). Nineteen tryptic fragments of tau were detected, identifying three tau isoforms. The ability to detect tau by MS in a 2-ml CSF sample paves the way for clinical CSF studies on the tauopathies. This study was supported by grants from the Swedish Research Council (projects 2006-6227 [HZ] and 2006-7240 [KB]), the Sahlgrenska University Hospital, the Göteborg Medical Society, Swedish Brain Power, Stiftelsen för Gamla Tjänarinnor, and the Alzheimer Foundation, Sweden.

A4.49 Excretion by urine of toxic and essential elements of the autistic Turkish infants

C. Sezgin, S. Kaya and S. Keskin

Cerrahpasca Medical Faculty, Biochemistry, Istanbul, Turkey

Urine total mercury, lead, manganese, copper, zinc and selenium levels of the autistic and their siblings were measured and compared. The binary comparison of age value results of autistic (n = 16), control-1 (n = 6) (their siblings) groups whose urine analyses were done are; autistic and control-1 are (5.93 ± 1.98), (8.37 ± 4.28), (p = 0.052) respectively and there is no significance. Urine total mercury levels of autistic and control-1 are (10.34 ± 5.81 μg/day), (18.41 ± 7.3 μg/day), (p = 0.019) respectively and significantly lower in autistics. Urine lead levels of autistic and control-1 are (10.34 ± 5.81 μg/day), (15.00 ± 8.11 μg/day), (p = 0.048) respectively and significantly lower in autistics. Urine manganese levels of autistic and control-1 are (2.34 ± 2.20 μg/day), (3.83 ± 2.96 μg/day), (p = 0.118) respectively and no significance. Urine copper levels autistic and control-1 are (18.90 ± 6.42 μg/day), (18.9 ± 7.67 μg/day), (p = 0.999) respectively and no significance. Urine zinc levels of autistic and control-1 are (1190.40 ± 613.10 μg/day), (1284.89 ± 501.19 μg/day), (p = 0.637) respectively and no significance. Urine selenium levels of autistic and control-1 are (47.22 ± 21.6 μg/day), (42.58 ± 25.9 μg/day), (p = 0.587) respectively and no significance. When the obtained results are evaluated, it gives rise to think that autistics are exposed to more neurotoxicity with toxic metals influence as especially mercury and lead and additionally copper and manganese levels are found to be higher in autistics however zinc and selenium levels were lower. Also, the lesser excretion of especially mercury and lead with urine when compared with healthy infants recurs to the mind that these metals accumulate in the body.

A4.50 TNF correlated with reelin in postmortem brain in depression patients

M. Lee and G. H. Han

Seoul National Hospital, Seoul, Republic of Korea

One of the hypothesis about the inflammatory factor involved in major depression converge to many proinflammatory substances such as TNF-α, IL-6. TNF-α which is balanced with other cytokines in brain takes part in synaptic plasticity, but it may be excitotoxic effect to brain when it excess. We search the correlation marker by using the Stanley Neuropathology Consortium Integrative Database (SNCID) which is a web based method to integrate the Stanley-Medical research Institute data sets. We found that TNF-α is significantly increased in major depression (n = 15) compared with normal controls (n = 15), not in schizophrenia, bipolar affective disorder in frontal lobe. Sperarman’s test showed that TNF-α level in frontal lobe is significantly correlated with dopamine D2 receptor mRNA in prefrontal lobe (p < 0.01), myelin oligodendrocyte glycoprotein mRNA in frontal lobe (ρ < 0.01), gial fibrillary acidic protein mRNA in frontal lob(ρ < 0.01). Also, IL-6 in frontal lobe (ρ < 0.01), reelin(ρ < 0.01) in frontal lobe is correlated with TNF-α. It is novel finding that TNF-α is correlated with reelin in human postmortem brain. Reelin helps migration of neuroblasts in developing brain as well as adult brain. It also participates in synaptic plasticity via modulate longterm potentiation(LTP). In summary, we suggest that mentioned correlated markers involve in synaptic plasticity, especially, correlation with reelin is novel finding.

A4.51 Hyperosmotic stress and UV radiation stimulate tau phosphorylation

M. Caballero Bermejo1, M. Olivera Santa-Catalina1, J. C. Alonso2, R. Argent1, S. dos Santos Pinheiro2, A. Cuenda2, M. J. Lorenzo1 and P. Centeno1

1Universidad de Extremadura, Cáceres, Spain, 2CNB, Madrid, Spain

Tau is a microtubule associated protein which has an important role in the development of Alzheimer’s Diseases (AD) and other...
diseases known as Tauopathies. When Tau is hyperphosphorylated, becomes in an insoluble form, the NFT. Although many protein kinases phosphorylate Tau in vitro, its phosphorylation in vivo is not well known yet. In this work we studied Tau phosphorylation by ERKs and SAPKs induced by osmotic stress (sorbitol) and Ultra-Violet (UV) exposure in HEK 293 cells transfected with human Tau (pCMV5-hTau40). The phosphorylation and activation of ERKs, p38s and JNKs by sorbitol and UV were analyzed by Western-blot using antibodies against the phosphorylated forms of these kinases and their substrates. Sorbitol and UV both induced the phosphorylation and activation of p38 and ERK 1/2 and both turned off in a time-dependent manner. JNKs showed a different behaviour remaining activated in the time scale used in this study, 2 hours. Sorbitol stress induced Tau phosphorylation at Thr-50 and Ser-235 in a time course very similar to the observed in ERKs and p38s activation. The use of inhibitors (PD98059 and PD184352) suggested that Tau phosphorylation at Thr-50 is ERKs dependent. UV stress induced Tau phosphorylation at Thr-50 and Ser-235 in a p38s and JNKs dependent manner, as revealed the use of SB203580 and SP600125.
Materials and Methods:

mitted diseases and simplifying the treatment of vaginitis. To combat the growing problem of sexually trans-

Endocrinology/Gynecology, Tehran, Islamic Republic of Iran

Ninety two patients responded completely after the first, after one week was performed.

Thorough pre-treatment and post treatment studies including cul-

tured and releases Vanadium with time. With strict eligibility crite-

ration, patients with refractory vaginal discharge (6 month duration,

 ped on V205 weight from which the material derived as it is pegylat-

pegylated Vanadium compound. The 3 concentrations were based

nal discharge of different etiologies with the most active of twelve

and toxicology studies; we treated patients with refractory vagi-

sion. Bacterial vaginitis responded best while fungal and Tricho-

and nobody showed major side effects. Symptoms due to PID

waned and cervical ulcers healed in almost all patients in ques-

tion. Biological vaginitis responded best while fungal and Tricho-

monas vaginitis responded later but completely as well.

Results:

No response to many local and systemic therapies) were treated.

Thorough pre-treatment and post treatment studies including cul-

tures were performed. Patients received a single session of local

against a series of bacteria, fungi and protozoan species: a sign of very

ancestral molecular target

K. Pirkanali1 and Z. Talaee Rad2

1 Mehr Medical Group, Endocrinology/Internal Medicine/Molecular Biology, Tehran, Islamic Republic of Iran, 2 Mehr Medical Group, Endocrinology/Gynecology, Tehran, Islamic Republic of Iran

Objectives: To combat the growing problem of sexually trans-

mitted diseases and simplifying the treatment of vaginitis.

Materials and Methods: In a phase I trial based on preclinical

and toxicology studies; we treated patients with refractory vagi-

nal discharge of different etiologies with the most active of twelve

pegylated Vanadium compound. The 3 concentrations were based

on V205 weight from which the material derived as it is pegylat-

ed and releases Vanadium with time. With strict eligibility crite-

ria, patients with refractory vaginal discharge (6 month duration,

no response to many local and systemic therapies) were treated.

Thorough pre-treatment and post treatment studies including cul-

tures were performed. Patients received a single session of local

vaginal washing with the solution. Re-examination and culture

after one week was performed.

Results: Ninety two patients responded completely after the first,

25 after the second and one patient after the third session with a

complete response rate of nearly 100%. Compliance was excellent

and nobody showed major side effects. Symptoms due to PID

waned and cervical ulcers healed in almost all patients in ques-

tion. Biological vaginitis responded best while fungal and Tricho-

monas vaginitis responded later but completely as well.

Conclusions: Concerning the current explosion of STD in recent

decades this single session treatment shows much promise and

study for viral and other germs inactivation is underway. Activity

against a series of bacteria, fungi, parasites (in vitro on intestinal

metazoans and worms) arises the question whether a very ancient

and ancestral molecule is targeted by this pegylated Vanadium

compound.

A5.04

Molecular methodology used in detection of influenza A H1N1 outbreak

S. Duvlis

Public Health Protection Institute, Virology and Molecular Diagnostics, Skopje, Macedonia, Pyramid

H1N1 subtype of influenza A type, as a cause of novel influenza A

outbreak is a challenge for using different molecular methodology. It

appears to be of swine origin and contains a unique combina-

tion of gene segments that has not been identified in the past. We

compared the different methodology used in typing and subtyping

of Influenza A H1N1 virus. Typing of influenza virus isolated

from nasopharyngeal swabs was performed using conventional

(qualitative) RT PCR and NASBA influenza A/B commercial kit. Real
time RT PCR methods recommended by CDC was used for

H1N1 subtyping using four sets of primers and probes for human

Influenza A-M (matrix gene), swine Influenza A, swine H1 and

human RNase P (as a internal control for RNA quality) gene seg-

ment. First 20 suspected patients for H1N1 were analysed using

the all three methodology. Twelve of them were confirmed as

Influenza A positive using Influenza conventional PCR reaction.

Using NASBA Nuclisens Easy Q influenza A/B method only two

were influenza A positive. CDC recommended real time RT PCR

protocol shows fourteen influenza A positives. Two of them were

seasonal Influenza A-H3N2 (the same one detected with NASBA)

and the rest were H1N1 subtypes. The rest of samples were tested

using only the CDC recommended methodology. Conventional

and NASBA methods failed to detect the novel H1N1 positive

samples giving negative results for influenza A type, maybe due to

a possible rearrangements on the novel virus and the less sensitiv-

ity of the methods. Recommended molecular method is the Real

time RT PCR able for simultaneously carry out the detection, typ-

ing, and subtyping of influenza viruses.

A5.05

Klebsiella pneumoniae with fucose synthesis

gene mutation displays reduced virulence in mice

B-J. Pan1 and J. H. Wu2

1 Chang Gang University, Institute of Biomedical Sciences, Kwei San, Tao Yuan 333, Taiwan, 2 Chang Gang University, Microbiology and Immunology, Kwei San, Tao Yuan 333, Taiwan

Human liver abscess caused by Klebsiella pneumoniae (KP) is a

recently emerging disease. One such strain from clinical isolate,

Kp5, induced high fatality in infected mice. A characteristic of

KP is its mucoid phenotype due to large capsule. The capsule of

Kp5 was found to possess high amount of fucose that was not

found in non-virulent strain causing urinary tract infection. The

Kp5 rarely interacted with mice peritoneal macrophages which

were induced by thioglycolate stimulation, indicating the fucose-

containing capsule could be used by bacteria to evade host

immune reaction. Mutant of Kp5 (Kp5m) was constructed by

inserting an apramycin resistant gene cassette to the gmd, one of

the fucose synthesis genes, and incorporated into Kp5 genome by

conjugation. The Kp5m displayed very low virulence to mice and

compared to the wild type Kp5, the Kp5m showed lower growth

rate, higher interaction with mouse peritoneal macrophages,

higher ability to form biofilm and lower toxicity to cultured

HepG2 cells. The bacterial morphology also showed some abnor-

mal filamentous phenotype which was not seen in Kp5. The

results suggested that the mutation reduced bacterial virulence in

mice, in addition also influenced bacterial growth and physiology.

A5.06

The implication of mtDNA mutations in HPV-

induced cervical carcinogenesis

C. D. Goia1, A. Botezatu1, D. Socolov2, I. V. Iancu1, I. Huica1, A. Pisca1 and G. Anton1

1 Stefan S. Nicolau Institute of Virology, Bucharest, Romania, 2 Grengre T. Popa University of Medicine and Pharmacy, Iasi, Romania

High risk human papillomaviruses (HPV) are known to be the eti-

ological agent of cervical cancer disease. On the other hand, other
cofactors are considered to be important in cervix carcinogenesis. Mutations in mitochondrial DNA (mtDNA) as well as alterations in mtDNA content have been reported in numerous cancers examined to date. The D-loop region has been shown to be a mutational hot spot in human cancer. In order to evaluate the role of mtDNA mutations in cervical lesions progression, cervical smears (collected in liquid cytology medium from 50 women, 29–55 years old) were investigated. Total genomic DNA (High Pure PCR Template, Roche Diagnostics) was isolated from scrape specimens from patients with different cytology (normal cervical epithelium, ASCUS, LSIL, HSIL and cervical cancer) and tested for HPV DNA presence (InnoLipa Genotyping kit- Innogenetics). To elucidate a causative role of mtDNA in cervical lesions, mtDNA mutations were investigated using Mutector mtDNA kit. In patients with normal cytology mtDNA mutations were detected in less than 15% of the cases, as compared with 50% of LSIL and HSIL cases. mtDNA mutations were detected in 60% cases of cervical cancer and only in 10% ASCUS. These studies provide strong evidence that instability in the D-loop region of mtDNA may be involved in cervical dysplasia. We suggested that mtDNA mutations may play a role in cervical precursor lesions and cancer but the mechanism of carcinogenesis remains to be solved.

A5.07 HIV V3 structures as novel HIV immunogens
O. Rosen, A. Moseri and J. Anglister
Weizmann Institute, Rehovot, Israel

A major objective in HIV-1 vaccine development is identifying immunogens that can induce antibody response against epitopes that are shared among all or many virus strains. An important HIV-gp120 epitope is the third variable (V3) region which binds to chemokine receptors CCR5 (“R5 viruses”) and CXCR4 (“X4 viruses”). Based on our previous NMR structure determination we discovered that V3 peptides from both X4 and R5 strains adopt a β-hairpin conformation. In the R5 conformation, two oppositely charged residues were juxtaposed, enabling a favorable electrostatic interaction that stabilized this conformation. In the X4 conformation, electrostatic repulsion between these residues forced a one register shift in the N-terminal strand of the V3. Despite this difference, both strains share a β-hairpin conformation. Peptide immunogens, mimicking these V3 structures were tested in mice. In patients with normal cytology mtDNA mutations were detected in less than 15% of the cases, as compared with 50% of LSIL and HSIL cases. mtDNA mutations were detected in 60% cases of cervical cancer and only in 10% ASCUS. These studies provide strong evidence that instability in the D-loop region of mtDNA may be involved in cervical dysplasia. We suggested that mtDNA mutations may play a role in cervical precursor lesions and cancer but the mechanism of carcinogenesis remains to be solved.

A5.08
Abstract withdrawn

A5.09 Redox and antioxidants status plant extract by oxidative stress related diseases
A. Sukiasyan, M. Yesayan, A. Dovlatyan and M. Aslanyan
Department of Chemical Technology and Environmental Engineering, State Engineering University of Armenia, Yerevan, Armenia

Natural antioxidants are extensively studied for their capacity to protect organisms and cell from damage induced by oxidative stress, the latter being considered a cause of infectious diseases. The major aim of the present study was to investigate the role of the plant extract (PE) as natural antioxidant in modeling oxidative stress (hemolysis of erythrocytes). We obtained changes by the measuring of redox potential and value of pH for extracts with different degree of ethanol. However calculation the value of pH pointed that all PE can be as restoring factor by free radical oxidation processes. It is spoken about fact, that our PE can be use as natural inhibitors in during oxidative damage in organism. Using electron spin resonance (ESR) technique we investigated in vitro free radical production by erythrocyte suspension with PE. We also studied the influence ethanol extracts on hemolysis kinetics induced by HCl solution in different situation. Antioxidant status of plant extracts was determination in erythrocyte suspensions by important products of lipid peroxidation. So, we try to develop novel approaches to correlate the level of lipid peroxidation and membrane damage for erythrocyte system. Our results shown that choose a method of extraction (time, temperature, using solution, etc.) can be determinate that pharmaceutical effect, which necessary for concrete case of organism damage. The method of peroxide hemolysis we suggest for using as specific test for detection of the presence different disease in an organism carried out by the activation of lipid peroxidation processes. This work was supported ANSEF Grant 07-NS-biochem-1440.

A5.10 Lipid chlorohydrins are bio logicaly active markers of inflammatory relevant oxidant – hypochlorite
A. Rohaszkiewicz, G. Bartosz and M. Soszynski
Department of Molecular Biophysics, University of Lodz, Lodz, Poland

Hypochlorite, a known pathologically relevant oxidant, gives the wide spectrum of his chlorination and oxidation products. Our previous experiments proved that chlorohydrins arise as major products upon the reaction of hypochlorite with unsaturated phosphatidylcholines. Having in mind their specificity, they are regarded to be a good marker of hypochlorite, but their possible biological role is still to be studied. Thus, we decided to check the effects of the presence of chlorohydrins in the cell membrane on the basic cell function. Fusion of liposomes labeled with NBD-PE, cell cycle, mitochondrial potential quantified with JC-1 and the level of caspase-3 stained stained with FITC-conjugated mAb was analysed with flow cytometry. Cell viability and proliferation ability was evaluated with MTT test and on the basis of the DNA content, respectively. Flow cytometry demonstrated fast fusion of chlorohydrin liposomes labeled with NBD-PE with cells in both cell suspension and monolayer. Incorporation of chlorohydrins into cells was followed by reduction of cell viability and proliferation ability depending on the order of chlorohydrin. Cell growth arrest was observed in G0/G1 phase with simultaneous reduction of cell population in S and G2/M phase. Contemporaneously, increase of the level of active form of caspase-3 and drop of mitochondrial potential was found in cells cultured in the presence of chlorohydrin liposomes. Phosphatidylcholine chlorohydrins, except having the quality to be a good hypochlorite marker, appear to be biologically active and the intensity of the effects were correlated with the number of HOCl-molecules substituted to the fatty acid chain.
A5.11
Sodium nitrite treatment reduce hepatic ischemic-perfusion injury
S. Yahaneghi1, A. Yalova2, G. Ucar1, P. Korkusuz2 and R. Gumusel3
1Department of Biochemistry, Hacettepe University, Faculty of Pharmacy, Ankara, Turkey, 2Department of Histology, Hacettepe University, Faculty of Medicine, Ankara, Turkey, 3Department of Pharmacology, Hacettepe University, Faculty of Pharmacy, Ankara, Turkey

Recent studies suggest that nitric oxide synthase (NOS)-independent NO sources mediate protecting effects towards ischemic injury on hypoxic or ischemic conditions. The purpose of this study is to investigate the cytoprotective effect of NO2-administration in various concentrations and different time periods, to hepatic ischemic-reperfusion injury. For the control group, hepatic I/R protocol (45 minutes I and 5 hour R) was performed on albino mice whereas for the study group, sodium nitrite (NaNO2, i.p.) was administrated in four different concentrations (1.65, 16.5, 165, 1650 µg/kg), 12 and 24 hours before the I/R protocol. Following reperfusion period, alanin aminotransferase (ALT) and aspartat aminotransferase (AST) levels in plasma and antioxidant enzyme levels; thus this can play a part in the protection towards I/R injury. Mophologic findings of hepatic protection by NO2- were evaluated histologi-}

A5.12
Abstract withdrawn

A5.13
Polyamine protective functions under antibiotic-mediated bacterial DNA damage
A. Akhova, M. Shumkov and A. Tkachenko
Institute of Ecology and Genetics of Microorganisms UB RAS, Perm, Russian Federation

Background: DNA is known to be the main target of reactive oxygen species that can be produced in response to a variety of antibiotics.

Aim: The investigation of polyamine defense functions being accomplished by their antioxidant activity and DNA protection in response to the impacts of two different antibiotic families.

Methods: Determination of hydroxy radicals with 3-(hydroxyphenyl)fluorescein cell staining followed by fluorescent detection on microplate rider. Registration of DNA damages by agarose gel electrophoresis of pDNA samples extracted out of E. coli cells exposed to antibiotics. Cell estimation by serial plating on LB media.

Results: (1) Fluorochinolone (levofloxacin) and aminoglycoside (amikacin) sublethal impacts on E. coli cells produced concentration effects on hydroxyl radical formation.

(2) Antibiotics investigated here resulted in cell DNA fragmentation.

(3) E. coli polyamines putrescine and spermidine were found to scavenge hydroxyl radicals produced by fluoroquinolone and aminoglycoside.

(4) Likewise, polyamines reduced the DNA fragmentation caused by fluoroquinolone and aminoglycoside.

(5) Furthermore, polyamines notably raised cell viability in response to all of investigated antibiotics.

Conclusions: Antibiotics referring to two investigated families, regardless of their specific intracellular targets produced cell hydroxyl radical formation and DNA damages. E. coli polyamines exerted their antioxidant and DNA-protective effects in response to fluoroquinolone and aminoglycoside. Polyamine positive effects on cell viability were exhibited in response to all of antibiotics investigated here.

A5.14
Prolyl oligopeptidase of Trypanosoma brucei hydrolyzes native collagen, peptide hormones and is active in the plasma of infected mice
I. Bastos1, F. Motta2, S. Charneau3, J. Santana4 and P. Grellier5
1University of Brasilia, Faculdade Ceilândia, Brasilia, Brazil, 2University of Brasilia, Faculdade de Medicina, Brasilia, Brazil, 3Museum National d'Histoire Naturelle, USM302, Paris, France

Proteases play important roles in many biological processes of parasites, including their host interactions. In sleeping sickness, Trypanosoma brucei proteases released into the host bloodstream could hydrolyze host factors, such as hormones, contributing to the development of the disease's symptoms. In this study, we present the identification of the T. brucei prolyl oligopeptidase gene (poph) and the characterization of its corresponding enzyme, POP Tb. Secondary structure predictions of POP Tb show a structural composition highly similar to other POPS. Recombinant POP Tb produced in E. coli was active and highly sensitive to inhibitors of T. cruzi POP Tc80. These inhibitors, which prevent T. cruzi entry into non-phagocytic cells, arrested growth of the T. brucei bloodstream form in a dose-dependent manner. POP Tb hydrolyzes hormone peptides containing Pro or Ala at the P1 position at a slightly alkaline pH, and also cleaves type I collagen in vitro and native collagen present in rat mesentery. Furthermore, POP Tb is released into the bloodstream of T. brucei infected mice where it remains active. These data suggest that POP Tb might contribute to the pathogenesis of sleeping sickness.

A5.15
Abstract withdrawn

A5.16
The HPLC (High Performance Liquid Chromatography) analysis of aflatoxins in maize from the main production areas in Iran
S. A. Ghiasian1, A. H. Maghsood1, G. S. Shephard2 and M. Bagher1
1Hamadan University of Medical Sciences, Hamadan, Islamic Republic of Iran, 2PROMEC Unit, Medical Research Council, Cape Town, South Africa

Fifty-one maize samples, intended for animal feed and human consumption, were collected from the four main maize production provinces in Iran and analyzed by high-performance liquid chromatography (HPLC) for contamination by four naturally-
occurring aflatoxin analogues (AFB1, AFB2, AFG1, and AFG2). Most of the maize samples obtained from Kermanshah and Mazandaran provinces were contaminated with aflatoxins, especially AFB1 and AFB2. The maximum AFB1 (276.3 μg/kg) and highest level of total aflatoxins (AFT) (316.9 μg/kg), were detected in a maize sample collected from Kermanshah province. The mean aflatoxin level from contaminated samples (52.60 μg/kg) from Kermanshah was significantly higher (p < 0.0001) than those in maize from the other three provinces, and exceeded all the maximum tolerated levels (MTLs) set for AFT in maize. The level of AFB1 in 15.8% of the total samples was above the MTL (5 μg/kg) for AFB1 in maize. The mean contamination level of AFT (23.86 μg/kg) was higher than MTL for maize in Iran (20 μg/kg). The levels of AFB1, AFB2, AFG1, and AFG2 ranged between not detected (<0.1 μg/kg) and 276.3, 30.4, 9.1, and 1.1 μg/kg in maize grains, respectively. The relatively high level of this carcinogenic mycotoxin indicated that the Iranain people are potentially endangered to hepatocarcinoma.

A5.17 Novel allosteric inhibitors of HIV-1 integrase on the base of multimodified oligonucleotides
T. Zatsepina, S. Korolev1, M. Smolov1, M. Gottikh2 and J. Agapkin1
1Moscow State University, Chemistry Department, Moscow, Russian Federation; 2Moscow State University, Belozersky Institute of Physical and Chemical Biology, Moscow, Russian Federation

The integration of HIV-1 DNA into the infected cell genome is one of the key steps in the viral infection cycle. The viral integrase is thus a key potential target for new antiviral drugs. Short single-stranded oligonucleotides conjugated with hydrophobic molecules inhibit integrase efficiently, inducing dissociation of the integrase–viral DNA complex. The dependence of the conjugate inhibitory activity on the oligonucleotide length and structure as well as on the structure of hydrophobic molecules has been studied. The nature of hydrophobic moiety plays the main role in the modulation of the conjugate inhibitory activity. They efficiently inhibit the catalytic activity of integrases with mutations in the catalytic core isolated from HIV-1 strains resistant to other—dimensional gel electrophoresis (2DE) were optimized. The average number of spots of these two saliva samples was around 130. Comparative analysis of the 2DE maps revealed very few divergences between the two species. The identification of the spots was made by peptide mass fingerprinting, peptide fragmentation and de novo sequencing using MALDI-TOF/TOF technology. The data revealed largely anti-haemostatic components, mainly lipocalins. Our results show that the R. brethesi and R. robustus saliva proteomes are rich in platelet aggregation inhibitors which promote the blood feeding of these insects. Support: PRONEX-FAPDF, CNPq, CAPES, FINATEC and FINEP.

A5.19 Bacterial proteases cleaves the MUC2 and may cause ulcerative colitis
D. B. Subramani, M. E. V. Johansson, G. C. F. and Hannson Svensson
Department of Medical Biochemistry, University of Gothenburg, Gothenburg, Sweden

The distal colon is covered with protective mucus layer, an outer loose and inner firm layer in mouse. The loose layer is heavily colonized by bacteria. Little is known about the role of bacterial proteases and break down of the mucus. MUC2 is major component of this mucus layer which is polymerized by dimerisation via C-termini and trimerization via N-termini producing a large network like structure. Our results shows that Bacteriodes fragilis and Porphyromonas gingivalis are found to cleave the N and C terminal of the MUC2 at specific cleavage sites without disrupting the intra cystine disulphide bond which stabilizes the mucus network. Other cleavages are predicted to dissolve the mucus gel. These findings suggest that the bacteria can degrade the mucus layer which causes the inflammation by contacting the epithelial cells directly may result in ulcerative colitis in human.

A5.20 Enzymes of the lysine biosynthetic pathway as targets for antifungals?
I. Gabrieli, P. Szweda, K. Kur, M. Prokop, M. Milewska and S. Milewski
1Department of Pharmaceutical Technology and Biochemistry, Gdansk University of Technology, Gdansk, Poland; 2Department of Organic Chemistry, Gdansk University of Technology, Gdansk, Poland

Systemic infections caused by human pathogenic fungi in immunocompromized patients continue to be one of the important clinical problems. Limited availability of safe and efficacious anti-fungal chemotherapeutics and emerging resistance to existing drugs stimulates search for novel molecular targets for antifungals. The L-lysine biosynthesis is unique in fungi and thus has been so far considered one of the potential targets for the rational design of antifungal drugs. In the present communication we report results of our studies on characterization of four enzymes catalyzing initial steps of AAP pathway. Their feeding success is greatly related to their salivary proteins that allow these insects to access their food by counteracting host haemostatic mechanisms, such as platelet aggregation, clotting and vasoconstriction, as well as host inflammatory reactions. Since these components are involved in the Trypanosoma cruzi transmission process, the salivas of epidemiologically significant Amazonian triatomin bugs, Rhodius brethesi and Rhodius robustus, were submitted to proteomic studies. Initially, the experimental conditions for wide-pH-ranged separation by two—dimensional gel electrophoresis (2DE) were optimized. The average number of spots of these two saliva samples was around 130. Comparative analysis of the 2DE maps revealed very few divergences between the two species. The identification of the spots was made by peptide mass fingerprinting, peptide fragmentation and de novo sequencing using MALDI-TOF/TOF technology. The data revealed largely anti-haemostatic components, mainly lipocalins. Our results show that the R. brethesi and R. robustus saliva proteomes are rich in platelet aggregation inhibitors which promote the blood feeding of these insects. Support: PRONEX-FAPDF, CNPq, CAPES, FINATEC and FINEP.
citrate synthase (HCS), homoaconitase (HA), homoisocitrate dehydrogenase (HICD) and α-aminoadipate aminotransferase (AadAT). The appropriate genes were cloned from the *C. albicans* genomic DNA, expressed in *E. coli*. their products were isolated as oligoHis-tagged proteins and characterized in terms of steady-state kinetic parameters, substrate specificity and cofactor requirements. Differences in the enzymatic properties of two isoforms of HCS encoded by the *LYS21* and *LYS22* genes were noted. Several potential inhibitors of the AAP enzymes were designed, synthesized and tested for their enzyme inhibitory potential. Finally, virulence of the constructed lys21A/lys22Δ mutant of *C. albicans* in the disseminated murine candidiasis model was compared to that of the wild-type strain. Conclusions questioning a possibility of exploitation of AAP enzymes as targets in chemotherapy of systemic fungal infections have been presented.

**A5.21**

**Isolation, cloning and sequence analysis of lactate dehydrogenase gene from *Theileria annulata***

A. Erdemir¹, M. Aktas², N. Dumanli² and D. Turgut-Balik³

¹Department of Bioengineering, Yıldız Technical University, Faculty of Chemical and Metallurgical Engineering, Istanbul, Turkey, ²Department of Parasitology, Firat University, Faculty of Veterinary Medicine, Elazığ, Turkey

Theileriosis is a serious animal disease that transmitted by ticks. It is caused by obligate intracellular parasitic protozoa of the genus *Theileria*. Infection by *Theileria* spp. parasites results in production losses and high mortality in susceptible animals. Currently available antiparasitic drugs are effective in animals but animals may remain carriers and treatment is most effective in the early stages of the disease. Isolation, cloning and analysis of lactate dehydrogenase from *T. annulata* which causes tropical theileriosis has been targeted in the present study with the ultimate aim of designing new antiparasitic drugs that will hopefully have wider mode of action in animals. The target sequence was isolated from the genomic DNA by PCR and cloned into the vector pGEM-T easy from a *T. annulata* strain isolated from the genomic DNA by PCR and cloned into the vector pGEM-T easy from a *Theileria* species for the first time, to our knowledge. Sequencing of the whole gene from both directions indicated that open reading frame was interrupted by two introns. There are also several single nucleotide exchanges in the sequence. Most remarkable is a five residue insertion in the active site loop region that might be an attractive target for inhibitors of the enzyme.

**A5.22**

**Association of first response antioxidant enzymes activities and course of blood infection***

L. Kocan¹, J. Vaškova ², J. Firment¹, L. Vaško² and J. Guzy³

¹1st Department of Anaesthesiology and Intensive Medicine, Louis Pasteur University Hospital, Košice, Slovakia, ²Department of Medical Chemistry, Biochemistry, Clinical Biochemistry, Pavol Jozef Šafárik University, Košice, Slovakia

Reacting free radicals act an essential part in pathogenesis of systemic inflammatory response syndrome, sepsis, severe sepsis and septic shock. Vitamins and trace elements as further enzyme cofactors redistribution is typical for sepsis which could be immediately concerned with relative deficit of circulating antioxidants. Deficiency of antioxidant enzyme glutathione peroxidase cofactor, selenium is commonly found in critically ill patients, particularly those suffering from severe infections mentioned and polytrauma. Glutathione peroxidase prevent organic substrate peroxidation by nonspecific reduction of peroxides following glutathione depletion. We attempt to improve survivance of critically ill patients by selenium supplementation. Oxidative stress become irreversible approximately in 6–24 hours from tissue hypoxia that’s way when shock arises, selenium intravenous injection supplementation has to be started as soon as possible. Optimal timing and dosage was 750 µg/day between 1 and 3 weeks depending on severity of the disease. Preliminary results showed increase in glutathione peroxidase activity in a group of selenium treated in comparison to placebo group treated by physiological solution from 0.19 µkat/l to 0.45 µkat/l. Inverse activity of glutathione reductase from 1.23 µkat/l to 0.65 µkat/l was estimated. According to improvement of patients clinical state evaluated by APACHE II score as well as 10% mortality decrease in treated group in comparison to placebo group we consider activation of another selenium containing antioxidant. Selenium supplementation could cause thioreredox reductase activation which uses the same reducing agent to reduce oxidized thiols.

**A5.23**

**Structural analysis of Toll-like receptor 2-activating lipoprotein from *Vibrio vulnificus***

S. Yu¹, N. Y. Lee², S-J. Park² and S. Rhee³

¹Seoul National University, Seoul, Korea, ²Yonsei University, Seoul, Korea

IlpA, a surface protein of the human pathogen *Vibrio vulnificus*, is the first lipoprotein to be characterized in *Vibrio* spp. as a major immunostimulant. Previously, it was characterized that IlpA was subject to lipidation at its N-terminal cysteine residue. The resulting IlpA then activates Toll-like receptor 2 in human cells, and induces overproduction of proinflammatory cytokines closely associated with septic shock in infected individuals. To identify structural features of IlpA, we determined the crystal structure of IlpA at 2.6 Å resolution. Specifically, IlpA consists of two homologous domains, each with αβ topology, similar to the structure of solute-binding protein which is a component of ATP-binding cassette transporter. In fact, binding of L-methionine was observed in the pocket between the two domains, suggesting that IlpA is an L-methionine-binding protein. The structural features of IlpA in this study, along with the immunological properties of IlpA identified previously and other solute-binding proteins, suggest that solute-binding lipoproteins of ATP-binding cassette transporter present at the bacterial cell surface could serve as pathogen-associated molecular patterns to Toll-like receptor 2, causing host immune responses against infection.

**A5.24**

**Comparative analysis of the Tn1546 element from newly isolated and identified vancomycin resistant *staphylococcus aureus* strain isolated from burn suffering human patients hospitalized at intensified care unit Sulaimani Central Hospital, Iraq***

D. A. Mohammed¹ and H. M. Said²

¹University of Sulaimani, College of Science, Biology, Sulaimani, Sulaimani-Kurdistan Region, Iraq, ²University of Würzburg, Medical Faculty, Radiation Oncology, Würzburg, Germany

Vancomycin-resistant Staphylococci was not analyzed extensively in Iraq yet. *Staphylococcus aureus*, a major cause of potentially...
life-threatening infections acquired in health care settings and has developed resistance to most classes of antimicrobial agents soon after their introduction into clinical use. In many health care institutions around the world, glycopeptidases such as vancomycin provide effective therapy against most strains of multi-drug-resistant S. aureus, including MRSA.

Materials and Methods: With PCR and DNA sequence analysis, the Tn1546 elements, a mobile genetic element that encodes vancomycin resistance proteins in enterococci and staphylococci was amplified and the sequences of which was compared to the prototype Tn1546 element from enterococci, and also to the previously sequenced Tn1546 of VRSA recorded in gene bank NCBI.

Results: In 2008, the first ten clinical isolates of vancomycin-resistant Staphylococcus aureus (VRSA) containing vanA gene with variable vancomycin MICs ranged from 32 to >128 g/ml were recovered in Sulaimani Emergency hospital for war victims/Sulaimani-Kurdistan region of Iraq. They possessed differences in its Tn1546 elements, which explain various expression level of VanA gene displayed by vancomycin MICs. The “Sulaimani-VRSA-Tn1546” element was different by distinct modifications in nucleotide sequences and showed partial homology with an enterococcal transposon Tn1546-like element.

Conclusions: S. aureus isolate has been shown to be vancomycin-resistant, due to a Tn1546 transposon analogue and proved to be effective as an alternative model approach for pathogenic bacterial characterization.

A5.26
Bacterial biofilms characterisation in human Iraqi patients suffering from urinary tracts infection
1University of Sulaimani, College of Science, Biology, Sulaimani, Sulaimani-Kurdistan Region, Iraq, 2Department of Medical Microbiology, College of Medicine - Al-Mustansyriah University, Baghdad, Iraq, 3Sulaimani University, College of Veterinary Medicine-, Sulaimani, Sulaimani-Kurdistan Region, Iraq, 4University of Würzburg, Medical Faculty, Gynaecology and Obstetrics, Würzburg, Germany, 5University of Würzburg, Medical Faculty, Radiation Oncology, Würzburg, Germany

Bacterial biofilm help clinicians explain some challenging areas in pathogenesis, diagnosis, and urinary tract infections (UTI) treatment. Biofilm formation needs two properties: cells adherence to surface & accumulation to form multilayered cell clusters Genetic and molecular biofilm formation basis in human pathogenic bacterial strains is multifaceted Microscopic observation confirmed that many types of UTIs (e.g. catheter-associated infections, struvite urolithiasis).

Methods: Causative agents isolation and identification of pres, per and post operative bacteriuria, biofilm formation on microtiter plates and urinary catheter, biofilm formation inhibition via chemical surfactants and biofilm antibiotic susceptibility to antimicrobial agents. Ionic surfactant formation analysis (SDS) and anionic surfactant (Tween 80) at 0.2% in TSB in addition to biofilm antibiotic susceptibility assay & bacterial isolates molecular analysis via Pulse Field Gel Electrophoresis (PFGE) & parallel biochemical & serologic samples analysis was done.

Results: SDS significantly inhibited biofilm formation on microtiter plate wells. Most isolated uropathogens were, E. coli, S. aureus and K. pneumoniae. Bacteriuria incidence in controls was 28%, E. coli were 10%, S. aureus 8%, P. aeruginosa 2% and 1% S. faecalis. Catheterization is most important risk factor for bacteriuria pre & post operatively. Antibiotics application did not eradicate bacterial biofilm on catheter surface.

Conclusions: The there was a domination of E. coli, S. aureus and K. pneumoniae isolated uropathogens. PFGE is suitable for pathogenic bacteria characterization analysis.

A5.25
Novel identification of different categories of regional species of diarrheagenic escherichia coli from human stool samples by using multiplex pcr technique
S. K. Arifi1, L. I. Faqi Salih1 and H. M. Said2
1University of Sulaimani, College of Science, Biology Department, Sulaimani, Sulaimani-Kurdistan Region, Iraq, 2University of Würzburg, Medical Faculty, Radiation Oncology, Würzburg, Germany

Enteropathogenic Escherichia coli (EPEC) are one of the oldest diarrheogenic E. coli and were recognized as an infantile diarrhea cause. EPEC strains are often the most frequently isolated bacteri- diarrheal pathogens. EPEC can be isolated from stools up to 2 weeks after cessation of symptoms. Presence of the eae gene responsible for A/E lesions and the bfpA gene that located on a plasmid, called the EPEC adherence factor (EAF), have been used to classify this group of bacteria into typical and atypical strains.

Material and Methods: DNA was extracted from Growing bacterial colonies. Amplification primers for (eaeE-p-f, eaeE-p-r, stEt-f, stEt-r, StrStEH-f, xEH-r, CVD-EA-f, MWG, Germany), were used to amplify E. Coli specific sequences that were separated on a 1X TBE Agarose gel. After electrophoresis, UV transilluminator was used to detect DNA binding ethidium bromide which appears as light fluorescent bands. Mann–Whitney U test for independent samples was used to analyze these data. p < 0.05 was considered as statistically significant. At least for three times experiments where repeated.

Results: PCR tested isolates from 111 EPEC patients only 45 (40.5%) were positive for the local eaeA gene and considered as true EPEC while, more categories of E. coli, 5/19 (26.3%) showed st and/or lt genes of ETEC, and 19 (21%) showed PCVD gene of EAEC.

Conclusions: E. coli characterization by Multiplex PCR is important because a considerable number of pathogenic E. coli remains non determinate by conventional diagnostic techniques during infection course.
A5.28 Detection of a new local endemic *V. cholerae* type as a result of a comparative molecular analysis screening approach of different natural infectious sources in Iraq


1University of Sulaimani, College of Science, Biology, Sulaimani, Sulaimani-Kurdistan Region, Iraq, 2Sulaimani University, College of Veterinary Medicine, Sulaimani, Sulaimani-Kurdistan Region, Iraq, 3Baghdad University, College of Medicine, Baghdad, Iraq, 4College of Medicine - Al-Mustanseryiah University, Medical Microbiology, Baghdad, Iraq, 5Department of Neurosurgery, University of Würzburg, Medical Faculty, Tumor Research Laboratory, Würzburg, Germany, 6University of Würzburg, Medical Faculty, Tumor Research Laboratory, Würzburg, Germany, 7University of Sulaimani, College of Science, Biology, Sulaimani, Sulaimani-Kurdistan Region, Iraq

In the time period between (2000 and 2009) serious cholera outbreaks occurred in different parts of Iraq. Here, pulse field gel electrophoresis (PFGE) was used as a DNA fingerprinting tool for first time in Iraq in order to detect possible infection sources and to compare between environmental & clinical isolates associated to cholera outbreaks.

**Methods:** Two sample groups, namely one of 100 acute diarrhea patients stool samples and another of 100 water samples from nine sites across Iraq were collected 2004–2006. Pathogen isolation & identification was according to standard methods. Gene Navigator System was used for PFGE, the running condition was: 100 mA;200 Volt / switching time 50 sec/20 hours using Not I restriction enzyme. Bacterial isolates molecular analysis was by Pulse Field Gel Electrophoresis (PFGE). Parallel biochemical and serological samples identification was done.

**Results:** *V. cholerae* isolation from stool of nine patients with profuse watery diarrhea, eight were O1 (six ogawa and two inaba, El Tor Biotype) & one isolate was NAG. Water samples were positive to Vibrio cholerae in Wast (four isolates) in Baghdad (one isolates). PFGE analysis showed two clinical isolates (Inaba) genetically identical therefore epidemiologically related while, NAG isolates one clinical & one environmental isolates were genetically identical therefore epidemiologically unrelated representing a novel local strain and indicated that water is the infection source.

**Conclusions:** Catheterization and surgery duration >45 minutes were found to be the most important risk factors for bacteriuria post operatively.

A5.29 Accounting for loop flexibility of SARS-CoV papain-like protease in computational drug discovery

R. Chaudhuri and M. Johnson

University of Illinois at Chicago, Center for Pharmaceutical Biotechnology, Chicago, USA

The papain-like cysteine protease (PLpro) encoded by the SARS coronavirus (SARS-CoV) replicase gene represents a potential target for antiviral drug development. The flexible loop around the PLpro active site shows substantial conformational adaptation between open and closed forms upon ligand binding, major challenge in computational drug discovery/design efforts. We used molecular dynamics to extensively sample the loop conformations and the phi-psi dihedral torsion change of Tyr269-Gln270 (loop residues) on inhibitor binding. Snapshots from molecular dynamics simulations of apo (open) and inhibitor bound (closed) forms (20ns each), of the loop were used to develop a two-pass pharmacophore model using computational solvent mapping, to eliminate false positives and account for protein flexibility in virtual screening. The dynamic model was trained on 101 PubChem compounds with 78% accuracy and tested on a test set of 59 in-house compounds with 78% accuracy. Hits retrieved from pharmacophore screening were docked into the two bound crystal structures of PLpro. We apply an adjusted scoring function (validated against the test set with an accuracy of 70%) to re-rank the docked compounds; top scoring compounds from both the docking runs were clustered based on 2D fingerprints and chosen for experimental testing. The results are quite promising, especially compared to traditional flexible docking approaches where a poor rank correlation of 16% was achieved between the experimental and predicted values. This model will be used for future database screening for discovery of novel leads against the SARS PLpro. Supported in part by NIH grant AI060915.

A5.30 Syphilis-kodecytes – novel function-spacer-lipid (FSL) modified red cells capable of sensitive and specific detection of syphilis antibodies

S. Komarraju1, S. Chesla2, N. Bovin3 and S. Henry4

1AUT University, Biotechnology Research Institute, Auckland, New Zealand, 2Immucor Inc., Atlanta, USA, 3Shemyakin Institute of Bioorganic Chemistry RAS, Moscow, Russian Federation

The ability to detect infectious agents is a prerequisite to the treatment and containment of transmittable diseases. Many diagnostics assays are based upon the detection of antibodies in serum against the infectious agent and often rely on ELISA or agglutination based assays to determine the infectious status. Using function-spacer-lipid (FSL) constructs bearing peptides (1) representative of an infectious agent attached to human red blood cells, offers a potential alternative diagnostic approach to the detection of antibody-defined infections. Using the syphilis spirochaete as a model, and a peptide selection algorithm, a series of FSL constructs were created and attached to human red cells creating syphilis-kodecytes. These koderces were then tested in routine blood typing platforms against serum from patients and blood donors of known syphilis status. This approach has
already been successfully used to detect ethnic specific blood group antibodies (1). It was found that syphilis-kodecytes used in blood grouping platforms are capable of the same sensitivity and specificity as ELISA and Particle Agglutination assays. The ability to engineer the FSL construct and replace the functional peptide for other infectious markers allows for a highly flexible and rapid-development technique for the detection of antibody-defined infectious agent markers, and may be an important low-technology tool for use in epidemics and pandemics, particularly in third world environments.

Acknowledgement: Supported by KODE Biotech Ltd (kode-biotech.com).

Reference:

A5.31
Elevated advanced oxidation protein products levels in chronic liver disease. Relation to severity of complication in liver cirrhosis
J. Zuwala-Jagiello1, M. Pazgan-Simon2, K. Simon2 and M. Warwas3

1Department of Pharmaceutical Biochemistry, Wroclaw Medical University, Wroclaw, Poland, 2Wroclaw Medical University, Clinic of Infectious Diseases, Liver Diseases and Acquired Immune Deficiency, Wroclaw, Poland

Background/Aims: Advanced oxidation protein products (AOPPs) and Nε-(carboxymethyl)lysine-modified advanced glycation end products (CML-modified AGEs) may contribute to progressive liver disease in the cirrhotic patients and influence the potency of oxidative stress. Little is known, however, regarding the relationship between the oxidative stress-related biomarkers and development of cirrhotic complications. The aim of this study was to evaluate the association between serum AOPPs and CML-modified AGEs levels and the severity of complications in liver cirrhosis.

Methods: Data of inpatients with cirrhotic complications were collected retrospectively. The serum AOPPs and CML-modified AGEs levels or levels as well as severity of complications of 45 inpatients was analyzed.

Results: The serum AOPPs level was strongly associated with the severity of liver function impairment as assessed by Child-Pugh and MELD scores (p < 0.001). Even a low (below median values) AOPPs or CML-modified AGEs levels (19.8 μmol/l and 1.4 μg/ml, respectively) indicated the existence of higher incidence of vascular disease (p < 0.001), and development of cirrhotic complications. The aim of this study was to evaluate the association between serum AOPPs and CML-modified AGEs levels and the severity of complications in liver cirrhosis.

Conclusions: High (above median values) AOPPs level may indicate the existence of severe complications associated with liver cirrhosis.

A5.32
Inhibition of dengue virus type-2 (DENV2) replication by flavanoid-derived compounds
M. Muhamad1, Y. K. Lee2, N. Abd. Rahman2 and R. Yusof3

1University of Technology MARA, Faculty of Medicine, Shah Alam, Malaysia, 2Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia, 3Department of Molecular Medicine, University of Malaya, Kuala Lumpur, Malaysia

Dengue viruses, mosquito-borne members of the Flaviviridae family, are the causative agents of dengue fever and its associated complications. More than 2.5 billion people in over 100 countries are at risk of infection. There is currently no treatment or vaccine available for dengue infection. This study employed whole-cell organism in vitro methods to study the inhibitory property of the flavanoid-derived compounds against DENV2 activity. Results showed that at concentration not exceeding the maximum non-toxic dose (MNTD), these compounds completely prevented DENV2 infection in HepG2 cells as indicated by the absence of cytopathic effects. The antiviral activity assessed by virus inhibition assay showed strong inhibition in a dose dependent manner. All five compounds exhibited inhibitory activity with a range of potency strengths of 72–100%. The plaque forming unit per ml (pfu/ml) was reduced prominently with a maximum reduction of 98%. Amplification of DENV2 protease (NS2B:NS3) RNA transcript harvested from in vitro inhibition assay showed decreased in the band intensity indicating reduced viral load. Quantitative RT-PCR results showed reduced copies of DENV2 protease/ng RNA relative to increased concentration of inhibitors. Thus, it was evident that synthesized compounds YK 51, YK 73 and YK 73x implicated their antiviral properties by inhibiting the DENV2 replication through reduction of the protease gene. The mode of actions is also strongly attributed to the functional groups of saturated piperidine ring and the carbamates or BOC. These highly potent inhibitors have great potential to become lead antiviral agent for dengue.

A5.33
Inflammatory and cytotoxic systemic effects induced by intratracheal instillation of pm10 and pm1
F. Farina, D. Cunati, G. Sancini and P. Palestini
Department of Experimental Medicine, University Milano-Bicocca, DIMS, Monza, Italy

The effects of particulate matter (PM) include asthma, cancer, cardiovascular and lung diseases. PM10, in the lungs, induces alveolar and systemic inflammation. Moreover, PM smaller than 100 nanometers can pass through cell membranes and migrate into other organs, could produce systemic inflammation and increase risk of thrombosis. Therefore, we evaluated the different inflammatory and cytotoxic potential of PM10 collected in an urban area in Milan in two seasons (summer’08 and winter’08) on the lungs, and the different systemic effects of PM1 collected in the same area and seasons. BALB/c mice were intratracheally aerosolized with suspensions of PM10 from different seasons (100 μg/100ml); after 3 hours, 24 hours, mice were sacrificed and BAL was collected, cytologically and biochemically characterized (total and differential cell counts, ALP, LDH, TNF-a, Hsp70, sPLA2), while lungs were excised for further analyses (HO-1, Casp8, NFKB). PM1 from different seasons (100 μg/100ml) was instead aerosolized once every 2 days for a total of three instillations; 24 hours after the last exposure, mice were sacrificed and BAL was collected, cytologically and biochemically characterized (total and differential cell counts, ALP, LDH, TNF-a, Hsp70, sPLA2). Lungs were excised for further analyses (HO-1, Casp8, NFKB, MDA), as brain (CD68, GFAP, TNF-a, MDA) and heart (HO-1, TNF-a, MPE, MDA). Blood samples were collected for coagulation markers analyses (Fibrinogen, sP-selectin, PAI-1). This strategy permits us to discriminate between stagionality pulmonary and systemic effects of PM10 and PM1, collected in the same Milan urban area. Supported by Fondazione Cariplo to P.Palestini.
A5.34 Interleukin 6 (IL-6) and Neurotrophin 3 (NT-3) dynamics in children with meningitis and encephalitis
D. Pasarica1, M. Gheorghiu1, T. Trandafir1, L. Ichim2, F. Toparceanu2 and C. Bleotu1
1University of Medicine and Pharmacy, Pathophysiology and Immunology, “Carol Davila” Bucharest, Romania, 2‘’Stefan S. Nicolau’’ Institute of Virology, Bucharest, Romania

The systemic inflammatory response induced by brain neuroinfections is an important target for the actual research. The activation of microglial cells represents the key element of the inflammatory response to viral aggression in acute viral meningitis (M_VIR) and encephalitis (E_VIR) by multiple etiologies. The inflammatory reactions and the efficacy of the protective mechanisms are two factors with control the course of neurological infections. The present work investigated the possible correlation of NT-3 with IL-6 from children with (M_VIR) and (E_VIR). NT-3 and IL-6 serum and CSF level were determined using ELISA kits. The patients were divided into two groups: -group I including children with M_VIR, HSV-, treated or not with dexamethasone (DEXA+ / DEXA-), n = 18 (20.3 ± 3.72 years) - group II including children with E_VIR, HSV+, DEXA+, n = 7 (20 ± 2.86 years). An inverse correlation was found between NT-3 and IL-6 serum levels in group I DEXA+ and in those of the group II DEXA- with a moderate course, while a direct correlation between the two serum parameters was determined in patients of group II DEXA- who died. In addition, an inverse correlation between the CSF NT-3 and IL-6 levels was found in survivor patients of the group I and II. The inverse correlation between NT-3 and IL-6 suggests that NT-3 may exert an immuno-modulatory action on IL-6 responses in survivor children after the viral neurological infections. The absence of this correlation corresponded to imminence of death. DEXA therapy did not influence the serum and CSF NT-3 dynamics.

A5.35 SNPs in MUC2 are expected to cause a nonfunctional protein and maybe an explanation for Ulcerative Colitis
P. Haridass1, M. E. V. Johansson and G. C. Hansson
Department of Medical Biochemistry, University of Gothenburg, Gothenburg, Sweden

Ulcerative colitis is an inflammatory bowel disease of unknown cause. MUC2 mucin is the major component of the intestinal mucus barrier. Nearly 180 SNPs were found in the MUC2 coding region of which 95 SNPs are non-synonymous. Five frame shift mutations in the N-terminal region of MUC2 caused by insertions or deletions disrupt the reading frame which leads to termination resulting in truncated protein. Mutations in the MUC2 gene are predicted to be linked to a dysfunctional MUC2 which could provide a genetic explanation for ulcerative colitis. Analysis of the SNPs which are evolutionarily stable makes them easier to use when the disease traits is followed in population studies. SNPs in MUC2 could probably be of help to determine the likelihood of an individual predisposed to ulcerative colitis disease.

A5.36 Transcriptional and protease activity analysis as tools for increasing rotavirus protein production in Saccharomyces cerevisiae
W. Rodriguez-Linas1, K. Tyo2, O. Ramirez1, L. Palomares1 and J. Nielsen1
1Instituto de Biotecnologia UNAM, Medicina Molecular y Bioprocesos, Cuernavaca, Mexico, 2Chalmers University of Technology, Chemical and Biological Engineering, Göteborg, Sweden

Rotavirus is the major etiological agent of diarrhea in children and young animals. Actual vaccines based on attenuated or inactivated virus could cause a disease transmission by reversing the attenuation or inefficient inactivation. Virus-like particles (VLP) have emerged as excellent vaccine candidates, as they are not infectious and their size and structure result in excellent immunostimulating properties. Rotavirus VLP assembly in the absence of other non-structural proteins have the same topological and functional characteristics as native viral particles. Therefore, the structural proteins possess the information required for particle assembly. A challenge that must be overcome in rotavirus VLP expression in yeast is increasing the heterologous protein production to promote self-assembly into VLP. The present work uses transcriptional and protease activity analysis for yeasts capable of expressing scaffold rotavirus protein for VLP formation. Various Saccharomyces cerevisiae strains variants expressing VPs rotavirus protein using constitutive promoters were used. Microarray analysis was used to access the overall state of the cell, linking phenotype to specific transcription factors, metabolites and overall biological shifts in the cell. In this regard, using DNA microarrays and protease activity tests, the yeast’s biological response to foreign protein production could be measured.

A5.37 Three-year prevalence survey of nosocomial infection in the same hospital
Z. Changran, L. Xia, T. Miaolian and L. Jiancong
Huang Pu Hospital of the First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China

Objective: To investigate the prevalence of nosocomial infection (NI) in the same hospital in the past 3 years.
Methods: The survey form of personal case was filled by adopting the method of combining clinical investigation and consulting inpatients medical records.
Results: The average rate of nosocomial infection (NI) was 4.8%; rate of NI was different in the different; there were not significant difference among age, hospitalization, invasive operation, infection sites, antibiotics using in the past three years, but, the rate of NI were changing in the different department in the 3 years.
Conclusions: The rate of NI were changing in the different hospitals and departments; The the survey and control of NI should be strengthened in the department and the season with high rate of nosocomial infection.

A5.38 A analysis on early diagnosis of nosocomial infection caused by pulmonary tuberculosis in patients with diabetes mellitus
Z. Changran, N. Yuangyuan, H. Ziming, X. Wenming and C. Weiling
Huang Pu Hospital of the First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China
Abstracts

Objective: To reduce the nosocomial infection caused by Mycobacterium Tuberculosis in patients with diabetes mellitus, some characteristics for early diagnosis of nosocomial infection was studied.

Methods: The clinical data of a total of 316 diabetes mellitus patients with pulmonary tuberculosis were made a retrospectively investigation from January 1998 to December 2007.

Results: (1) The age was 55.01 ± 12.77 and 61.92 ± 11.18 years old in the patients with active and inactive pulmonary tuberculosis respectively; (2) The pulmonary tuberculosis history was more frequent in the patients with inactive pulmonary tuberculosis than with active pulmonary tuberculosis; (3) The frequency of cough with profuse sputum and the sputum positive rate with Mycobacterium Tuberculosis were 48.4%, 80.0%, 25.2%, 10%; The GHBA1c, C peptide and the control of blood glucose level in patients with active and inactive pulmonary tuberculosis respectively, there were significant difference for these markers between the two groups (p < 0.05).

Conclusion: These clinical manifestations with frequent cough and profuse sputum, the sputum positive with Mycobacterium Tuberculosis and surveillance of blood glucose level are helpful to early diagnosis of pulmonary tuberculosis in the patients with diabetes mellitus. It is very important to screen the information for reducing the nosocomial infection of Mycobacterium Tuberculosis.

A5.39
A analysis on the clinical features of A/H1N1 influenza infection
Z. Changran, Z. Wu jun, L. Zhongjin, L. Jiancong and M. Li
Huang Pu Hospital of the First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China

Objective: To reduce stuffs and patients being infected in hospitals, the clinical features of A/H1N1 influenza infection were studied.

Methods: The clinical data of 17 patients with A/H1N1 influenza were made a retrospectively investigation in June 2009, the data included the change of body temperature, blood routine and chest X-rays check, 18 patients with ordinary influenza were studied as the control group.

Results: There were not significant difference for the change of body temperature, blood routine (p > 0.05); The chest X-rays check were normal in the two groups; All patients were confirmed positive for A/H1N1 RNA when they had fever, and were negative for A/H1N1 RNA when they had no fever in the A/H1N1 influenza group, those patients with ordinary influenza were all negative for A/H1N1 RNA when they had or not fever; These patients with A/H1N1 influenza or ordinary influenza had no complications, and all had good prognosis.

Conclusion: A/H1N1 influenza did not have any special clinical manifestations compared with ordinary influenza.

A5.40
A study on hospital infection in two parts of the same hospital
Z. Changran, L. Jiancong, L. Ming, Z. Hua and N. Yuanyuan
Huang Pu Hospital of the First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510700, China

Objective: To investigate the nosocomial infection characteristics in the two parts of the same hospital under the same administration mode.

Methods: The clinical data of patients with microbiologically documented nosocomial infection from January 2007 to December 2007 were retrospectively analyzed. Pathogens were identified by bacterial biochemical identification panel, antimicrobial susceptibility tests were done by disc agar diffusion test.

Results: The prevalence rate of nosocomial infection was 4.9% and 4.8% in the two parts of the same hospital. There were not significant difference in the distribution of infection site and bacilli, but there was significant difference in fungi among pathogens isolated from nosocomial infection cases(16.0% versus 8.0%), and the rate of nosocomial infection were changing in the different departments in the two parts of the same hospital.

Conclusion: There are different nosocomial infection characteristics even if in the same hospital. The nosocomial infection should be strengthened in the departments with more in-patients, according to the developing program of hospital.

A5.41
A clinical analysis of nosocomial fungal infection in same hospital for 4 years
Z. Changran, C. Weiling, X. Wenming, L. Jiancong and L. Ming
Huang Pu Hospital of the First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China

Objective: To investigate the nosocomial fungal infection in same a hospital. The pathogens, the infection routes, the high-risk factors, the drug-resistance of nosocomial fungal infection and its relationship with the use of antibiotics in same a hospital from January 2005 to December 2008.

Methods: The clinical data of patients with microbiologically documented nosocomial fungal infection were retrospectively analyzed. Pathogens were identified by fungal biochemical identification panel, antimicrobial susceptibility tests were done by disc agar diffusion test.

Results: The mean incidence rate of nosocomial fungal infections in a hospital from 2005 to 2008 was 8.04%. Most lower respiratory tract infection, accounting for 54.5%, followed by urinary tract and skin infection. Among high-risk factors, antibiotics use accounts for 42%, followed by ventilator use, accounting for 17.9%. Candida albicans is the major type of pathogens, accounting for 93.7%. 64 cases of drug sensitivity tests were carried out (30.9%), in which the strains were most sensitive to 5-FC, showing strongest resistance to Itraconazole.

Conclusions: Lower respiratory tract was the most frequent infection site; The main pathogens of nosocomial fungal infection were Candida albicans (93.7%); The management including rational use of antibiotics, microbiological culture and susceptibility test should be strengthened to reduce the nosocomial fungal infection.

A5.42
A analysis on the effect of the distribution of pathogens and their antimicrobial resistance by administrative intervention
Z. Changran, L. Xia, T. Miaolian, L. Xiaoyuan and L. Ming
Huang Pu Hospital of the First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China

Objective: To evaluate the effect of the distribution of pathogens and their antimicrobial resistance by controlling the use of the third generation cephalosporin.

Methods: Pathogens were identified by bacterial biochemical identification panel, antimicrobial susceptibility tests were done by disc agar diffusion test.
Hepatitis C virus infection induces pro- and antiapoptotic cell response: who is the winner?

M. Ripoll1, G. Quarta1, R. Scrima1, A. D’Aprile1, V. Pazienza2, D. Boffoli1, D. Moradpour3, N. Capitanio1 and C. Piccoli1

1Department of Biomedical Sciences, University of Foggia Medical School, Foggia, Italy, 2IRCCS Casa Sollievo della Sofferenza, Unit of Gastroenterology, San Giovanni Rotondo (FG), Italy, 3Centre Hospitalier Universitaire Vaudois, University of Lausanne, Unit of Gastroenterology and Hepatology, Lausanne, Switzerland

Abstract

Introduction: Apoptosis plays a central role in chronic hepatitis C virus (HCV) infection. Although the activation of cell death signals has been reported, HCV infection persists in most patients suggesting a pro-survival adaptation, eventually developing hepatocellular carcinoma. This study focused on the role of mitochondrial in the activation of pro- and antiapoptotic response in cells expressing HCV proteins.

Materials and Methods: Human Osteosarcoma U2-OS cells inducibly expressing the HCV polyprotein; huh7.5 hepatoma cells transfected with full length HCV genome.

Results: Long term induction of viral proteins in U2-OS cells induced a cyclosporine A-sensitive cytochrome c partial release from mitochondria, revealed by immunofluorescence, western blot and spectral analysis. In HCV-transfected Huh7.5 cells, release of the apoptosis inducing factor (AIF) with no apparent nuclear translocation was also observed. HCV positive cells displayed an HIF-dependent enhanced glycolysis, characterized by up-regulation of the mitochondria-bound HIF-1α (HII); preliminary data on signal transduction pathway revealed the i-perphosphorylation of Glycogen synthase kinase 3β (GSK-3β).

Conclusion: HCV causes a cell stress activating an early apoptotic response, the entity of which likely depends on the cell type. Nevertheless a wide series of cell survival mechanisms are also triggered resulting in a metabolic adaptation possibly favouring carcinogenesis. Based on our results, we propose a pro-survival mechanism linking HCV infection to inhibition of GSK-3β, stabilization of HIF-1α and up-regulation of HKII, the last events causing a glycolytic shift and protecting from apoptosis.
Diagnosis of trichomonas vaginalis infection: the sensitivities and specificities of PCR, microscopy and culture methods

A. H. Maghsoud1, M. Saidijam2, S. A. Ghiasian1 and N. Torkashvand1

1Department of Medical Parasitology & Mycology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Islamic Republic of Iran, 2Department of Molecular Medicine & Genetics, School of Medicine, Hamedan University of Medical Sciences, Hamedan, Islamic Republic of Iran

Trichomoniasis is a prevalent venereal protozoan infection which has been associated with human immunodeficiency virus acquisition and preterm birth. Currently, culture is considered the standard technique for the detection of Trichomonas vaginalis. The aim of this study was to compare wet-mount, culture and Polymerase Chain Reaction (PCR) methods to diagnose T. vaginalis in vaginal swab and urine specimens. For this, the vaginal and urine samples of 51 infected women were evaluated. The sensitivity and specificity of PCR using vaginal samples were 98 and 100%, respectively. Out of 51 infected women, 16 (31.37%) had vaginal and urine wet-preparation positive for T. vaginalis and 39 (76.47%) were positive by culture. The sensitivity and specificity of PCR using urine specimens were 58.82 and 100%, respectively. Our results showed that the use of urine-based detection of T. vaginalis is not suitable in women. But, the PCR-based detection of T. vaginalis using vaginal specimens would be an appropriate alternative to culture. The correct and precise diagnosis of trichomoniasis will cause to increase efficiency therapy and better control of this disease which reduce complications of infection.

Application of real-time TaqMan multiplex PCR in epidemiologic study of B. burgdorferi and A. phagocytophilum in tick samples

V. Captigina1, R. Ranka1, K. Brangulis1, A. Bormane2 and V. Baumanis1

1University of Latvia, Latvian Biomedical Research and Study Centre, Riga, Latvia, 2State Agency “Public Health Agency”, Riga, Latvia

For the detection of B. burgdorferi and A. phagocytophilum, etiologic agents of Lyme disease and human granulocytic ehrlichiosis respectively, large amount of methods has been described, which include antibody and antigen detection assays (immunofluorescence assay, enzyme-linked immunosorbent assay, Western blotting, culture isolation, and PCR-based (polymerase chain reaction) assays). The present study describes the validation of real-time TaqMan multiplex PCR assay for the detection of simultaneously infection of B. burgdorferi and A. phagocytophilum in field-collected tick samples from Latvia. DNA samples of 472 individuals field-collected ticks were examined by real-time TaqMan multiplex PCR assays, that amplify the 23S ribosomal RNA gene from B. burgdorferi and msp2 gene from A. phagocytophilum. Results of the study showed that the prevalence of B. burgdorferi in ticks was 44.5%, the prevalence of A. phagocytophilum – 1.0% and mixed infection was observed in 0.8% cases. In contrary to nested PCR, TaqMan PCR shows higher sensitivity and result in significant savings in time, reagents, thermal cycler usage and overall cost. The results confirm that TaqMan PCR assay is suitable for epidemiologic studies of B. burgdorferi and A. phagocytophilum in the tick samples. In this study the simultaneously infection of ticks with B. burgdorferi and A. phagocytophilum was observed. Further studies of other human tick-borne pathogens including TBE virus (tick-borne encephalitis) will be necessary in order to get the full picture of coinfection in regional ticks to assess the risk they pose to the human population.

Acknowledgement: This work has been supported by European Social Fund.

Mucoid phenotype switch in Burkholderia multivorans clinical isolates

I. Silva1, A. Ferreira1, J. Becker2 and L. Moreira1

1Instituto Superior Técnico, Institute for Biotechnology and Bioengineering, Centre for Biological and Chemical Engineering, Lisboa, Portugal, 2Instituto Gulbenkian de Ciência, Oeiras, Portugal

Burkholderia cepacia complex (Bcc) is a group of 17 closely related bacterial species from the Burkholderia genus that are important opportunistic pathogens in cystic fibrosis and chronic granulomatous disease patients. Bcc bacteria can lead to a rapid decline in lung function and septicaemia, causing the cepacia syndrome. Exopolysaccharides are one of the hypothesized virulence traits and are produced by strains from all Bcc species (1, 2). Phenotypic variation from mucoid to non-mucoid may occur during the course of infection and is hypothesized that the mucoid phenotype is correlated to persistence in the lung, whilst the nonmucoid phenotype is associated to increased disease severity (2). In order to understand which factors can trigger the mucoid phenotype switch, two isogenic mucoid and nonmucoid sequential clinical isolates from a cystic fibrosis patient's lung (Burkholderia multivorans D2095 and Burkholderia multivorans D2214) were grown under stress conditions: low iron availability, microaerophilia, oxidative stress and antibiotics. Switch from mucoid to nonmucoid was obtained under microaerophilic conditions. The strains were shown to be isogenic although their phenotypes differ in exopolysaccharide production, motility, antibiotic resistance and virulence in Galleria mellonella. A transcriptomic approach was performed in order to identify regulatory mechanisms underlying phenotype switch in Bcc bacteria. Gene expression profiling revealed altered expression of genes encoding cell envelope components, proteins involved in central metabolism, chemotaxis, motility and iron uptake. Further work to understand phenotype switch is being performed.

Vascular endothelial growth factor (VEGF) and lactate dehydrogenase (LDH) in the pleural effusions caused by different etiology

Z. Changran, X. Wenming, C. Weiling and W. Xiaoliang

Huang Pu Hospital of the First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China

Objective: To explore the clinical validity of vascular endothelial growth factor (VEGF) in the pleural effusions caused by different etiology.

Methods: VEGF in the pleural effusions caused by different etiology were measured by ELISA. LDH measurements were performed on a selective, discrete, multichannel analyzer using standard methodology.
RESULTS: (1) VEGF levels in the pleural effusions were 240.29 ± 11.52, 217.72 ± 49.51, 68.03 ± 50.70 pg/ml in the patients with parapneumonic effusions (PPE), tuberculosis effusions (TBE) and transudative pleural effusions respectively. There were significantly higher VEGF in the patients with PPE, TBE than those with transudative pleural effusion (p < 0.05). But, no significantly difference between the patients with PPE and TBE. (2) LDH levels in the sera were 156.75 ± 35.26, 142.94 ± 42.17, 128.57 ± 81.38 U/l with PPE, TBE and those with transudative pleural effusion respectively, there were not significantly difference among them(p > 0.05). (3) LDH levels in the pleural effusions were 1135.25 ± 747.85, 328.5 ± 178.89, 126.29 ± 60.16 U/l with PPE, TBE and those with transudative pleural effusion respectively, there were significantly difference between PPE, TBE and transudative pleural effusion (p < 0.05), and significantly difference between PPE and TBE (p < 0.05). The ratio of LDH in the pleural effusion and serum significantly difference between PPE and TBE (p < 0.05), but no difference in the transudative pleural effusion.

Conclusions: The detection of VEGF and LDH has diagnostic values in differentiating exudative and transudative pleural effusions, and PPE has more serious infection response than TBE.

A5.53
A analysis on the diagnosis value of thoracoscopy and closed pleural biopsy in the tuberculosis pleuritis
Z. Changran, L. Ming, Z. Hua and L. Jiancong
Huang Pu Hospital of the First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China

Objective: To evaluate the diagnosis value of thoracoscopy and percutaneous pleural needle biopsy in the pleural fluid patients with tuberculosis pleuritis.

Methods: One forty-nine patients with tuberculosis pleuritis and pleural fluid were analyzed from January 2003 to March 2007. They were allocated to thoracoscope group (n = 19), concluding operational biopsy (n = 2) and percutaneous pleural needle biopsy group (n = 130). Two examinations were evaluated and compared for the diagnosis of tuberculosis pleuritis.

Results: Among the 149 patients of tuberculosis pleuritis and pleural fluid, 16 patients were found granulation tissue, chronic inflammation and so on in their pleural tissue in the thoracoscope group (n = 19), which confirmed to the histological changes of tuberculosis pleuritis and the detection rate was 84.21%. There were 67 patients whose histological changes conformed to tuberculous pleuritis in the percutaneous pleural needle biopsy group and the detection rate was 51.54%. The difference of detection rate between the thoracoscope group and the percutaneous pleural needle biopsy group was significant (p < 0.05).

Conclusion: As examinations for tuberculosis pleuritis, the detection rate of thoracoscope and operational biopsy was larger than percutaneous pleural needle biopsy, which were related to field of vision, thus needs further investigation.

A5.54
Abstract withdrawn

A5.55
Contributions of nucleotide excision repair, DNA polymerase η and homologous recombination to replication of UV-irradiated herpes simplex virus type 1
I. Muylaert and P. Elias
University of Gothenburg, Medical Chemistry and Cell Biology, Gothenburg, Sweden

The effects of UV irradiation on herpes simplex virus type 1 (HSV-1) gene expression and DNA replication were examined in cell lines containing mutations inactivating the XPA gene product required for nucleotide excision repair, the DNA polymerase η responsible for translesion synthesis, or the Cockayne syndrome A and B (CSA and CSB) gene products required for transcription-coupled nucleotide excision repair. In the absence of XPA, CSA and CSB gene products, virus replication was reduced 10²-, 400-, and 100-fold, respectively. In DNA polymerase η mutant cells, HSV-1 plaque efficiency was reduced 10⁶-fold. Furthermore, DNA polymerase η was strictly required for virus replicaton at low multiplicities of infection but dispensable at high multiplicities of infection. Knock down of Rad51, Rad52, and Rad54 levels by RNA interference reduced replication of UV-irradiated HSV-1 150-, 100-, and 50-fold, respectively. We find that trans-cription-coupled repair efficiently supports expression of immediate early and early genes from UV-irradiated HSV-1 DNA. In contrast, the progression of the replication fork appears to be impaired, causing a severe reduction of late gene expression. Since the HSV-1 replisome does not make use of PCNA, we attribute the replication defect to an inability to perform PCNA-dependent translesion synthesis by polymerase switching at the fork. Instead, DNA polymerase η may act during postreplication gap-filling. Homologous recombination, finally, might restore the physical and genetic integrity of the virus chromosome.

A5.56
Impact of different parameters upon the expression of certain virulence factors in escherichia coli strains isolated from marine water
E. Panu1, C. Bleotu2, N. Rosoiu3, M. Popa4, L. Dascalu4, L.-M. Ditu4, V. Lazar3 and M.-C. Chifiriuc4
1I.P.H. (Institute of Public Health) Constanța, Constanța, Romania, 2Institute of Virology St. Nicolae, Bucharest, Bucharest, Romania, 3”Ovidius” University Constanța, Faculty of Medicine, Constanța, Romania, 4University of Bucharest, Faculty of Biology, Microbiology Immunology Department, Bucharest, Romania

Objective: To investigate the expression of 10 virulence factors (VF) in 100 E. coli strains isolated from Black Sea Coast, in different cultivation conditions simulating different environmental stress factors. Material and methods. Cell-associated (i.e. adherence to HeLa cells) and soluble (i.e. lecinthinase, lipase, gelatinase, caseinase, amylase, aesculin hydrolysis, DNase, sheep blood haemolysis, CAMP-like factor) VFs were investigated in variable incubation temperatures; NaCl and glucose concentration; pH; aerobic and anaerobic incubation. Results. The tested strains developed ability to grow at 22°C, 37°C, 44°C, irrespective to the salinity, pH and glucose concentration, in aerobic and anaerobic incubation conditions. The VF were better expressed at 37°C, followed by 22°C, especially adherence ability, siderophores, amylase and caseinase production. At 0% NaCl only amylase and siderophores production was expressed, at 5-6% NaCl sheep blood haemolysis and CAMP-like factors, while caseinase was expressed at 6% and 10% NaCl. The adherence to...
Abstracts

A5.57
Profiling of Mycobacterium tuberculosis gene expression during infection in genetically different mouse models
T. Skvortsov1, D. Ignatov1, A. Apt2 and T. Azhikina1
1Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russian Federation, 2Central Institute for Tuberculosis, Moscow, Russian Federation

Changes in gene expression in response to host defense mechanisms are necessary for the survival and functional activity of pathogenic bacteria. Analysis of these changes is crucial for a better understanding of the pathogenesis of infectious disease and development of effective treatment approaches. We performed the comparative investigation of transcriptomes of M. tuberculosis (H37Rv strain) in vivo. The transcriptomes were compared at different stages of infection in genetically susceptible (uneffective immune response) and genetically resistant (effective immune response) mice. Co-denaturation and co-renaturation of excess parallel sequencing of samples. Characterization of transcriptomes by high-throughput massive analysis allowed defining a number of genes, which expression do not depend on host resistance to tuberculosis. Among them there are 9 genes of lipid metabolism and 12 genes involved into cell wall processes. The corresponding proteins are the known or predicted virulence factors and could be further considered as potential therapeutic targets. This work was supported by the RFBR grant #08-04-01053 and the Russian Academy of Sciences grant (Physico-chemical biology. Structural, functional and evolutionary analysis of genomic cis-regulatory systems).

A5.58
Targeting the nucleotide metabolism of human pathogen T. brucei
M. Vodnala, A. Fijolek, K. Andersson Törnvist and A. Hofer
Umeå University, Medical Biochemistry and Biophysics, Umeå, Sweden

Trypanosoma brucei is a pathogen that infects humans (African sleeping sickness) and cattle (Nairobi). There are no vaccines available for this disease. The present drugs are toxic, expensive or not active against all variants of the parasite. When we have grown trypanosomes in the presence of 1 mM deoxyadenosine they died within a few hours. Nucleotide pools analyses showed intracellular accumulation of dATP and depletion of ATP pools in the trypanosomes. We have shown that T. brucei adenosine kinase is crucial for the accumulation of dATP and depletion of ATP1. However, when we used deoxyadenosine at lower concentrations than 1 mM, it was instead degraded by the trypanosomes. We found that T. brucei methylthioadenosine phosphorylase was responsible for this degradation and it was only when deoxyadenosine was used at a concentration high enough to saturate this enzyme it had a strong effect on parasite survival. These results suggest that deoxyadenosine analogs which are MTP-ase cleavage resistant can be used as antitrypanosomal drugs. Our preliminary experiments on trypanosomes infected mice with adenine arabinoside (Ara-A) showed that non cleavable deoxyadenosine analogs can be developed into new drugs against trypanosomes.

Reference:

A5.59
The levels of serum total antioxidant status (TAS) and cytokines in hepatitis B virus (HBV) infection
G. Akca1, E. Avel2, G. Alp3, S. Tuncbilek4 and S. Coskun Cevher5
1Gazi University, Microbiology, Ankara, Turkey, 2Hitit University, Biology, Corum, Turkey, 3Hitit University, Microbiology, Corum, Turkey, 4Ufuk University, Infection Diseases and Clinical Microbiology, Ankara, Turkey, 5Gazi University, Biology, Ankara, Turkey

Inflammatory-immune damage occurs in liver pertaining to Hepatitis B virus. Reactive oxygen species (ROS) are also considered to be the cause of this damage. However, antioxidant protection systems are formed against the deteriorating effects of ROS in organisms. It was aimed to detect and compare total antioxidant and cytokines(IL-2, TNF-α) levels of chronic and occasionally diagnosed inactive HBsAg carriers. In this study, 216 isolated anti-HBc total(+), 25HBsAg and anti-HBc total(+) patients’ sera, 20chronic HBV patients’ sera and 10 healthy people’s sera (control group) were used. The mean values of TAS were found 1.29 ± 0.20 mmol in chronic HBV patients, 1.24 ± 0.15 mmol in inactive HBsAg carriers, 1.16 ± 0.5 mmol in isolated anti-HBc (+) patients and 0.93 ± 0.10 mmol in control group as Trolox equivalent/l. The results revealed that there was no statistically significant difference among the patients’ groups as a statistically significant difference was found between the control and the patients’ groups (p < 0.05). Moreover, TAS values of isolated anti-HBc (+) and inactive HBsAg (+) carrier patients were found to be higher than control group as it was seen in HBV infected patients similarly. The result pointed out that the disease was progressing chronically. Besides, there was a significant increase in the levels of TNF-α and IL-2 in chronic HBV. It was concluded that the detection of serum TAS values and cytokines levels could be considered very important for the anticipation of the progression and ongoing to chronic hepatitis of HBV infected patients.

A5.60
The supramolecular organization of glycosphingolipid-receptors in lipid rafts affects the biological action of Shiga toxins
J. Betz, M. Bielaszewska, H. Karch, A. W. Friedrich and J. Müthing
University of Muenster, Institute for Hygiene, Muenster, Germany

Shiga toxin-producing Escherichia coli (STEC) are responsible for courses of disease from asymptomatic carriage to uncomplicated...
diarrhea, bloody diarrhea, and the life-threatening hemolytic-uremic syndrome (HUS). Shiga toxins (Stx) bind to the glycosphingolipid (GSL)-receptor globotriaosylceramide (Gb3Cer/CD77) and cause microvascular endothelial injury in specific target tissues. GSLs are located primarily in supramolecular cholesterol-enriched membrane microdomains, lipid rafts, being the prerequisite for efficient receptor-mediated endocytosis and retrograde transportation resulting in Stx-caused cellular injury. We aimed to characterize the microdomain-associated GSLs of endothelial cells from different vascular beds. In specific buoyant microdomain fractions of the plasma membrane, obtained by sucrose density gradient ultracentrifugation, Stx receptors were detected by thin-layer chromatography (TLC) overlay assays using specific anti-Gb3Cer antibodies or Stx in conjunction with anti-Stx antibodies. By means of immunofluorescence microscopy we could demonstrate that Stx receptor GSLs colocalize with different types of microdomains in micro- and macrovascular endothelial cells. To demonstrate the impact of the molecular assembly of microdomains on Stx receptor function, we disrupted the lipid rafts followed by cell viability assays. Importantly, the more sensitive microvascular endothelial cells showed a higher content of microdomains being resistant to cholesterol depletion, whereas the microdomains of less sensitive cells could be easily disrupted.

A5.61

Whole genome sequencing of a multiresistant bacterium isolated from a wastewater treatment plant receiving effluent from antibiotic drug manufacturing

A. Johnsson1, E. Kristiansson1, Y. S. Shouche2, C. Rutgersson1, E. Moore1 and D. G. J. Larsson1
1Department of Neuroscience and Physiology, University of Gothenburg, Gothenburg, Sweden, 2Pune University, National Centre for Cell Science, Ganeshkhind, Pune, India, 3Department of Clinical Bacteriology, University of Gothenburg, Gothenburg, Sweden

Antibiotics save millions of lives every year and are crucial for fighting disease worldwide. However, the heavy usage also leads to antibiotic resistance - one of the most important challenges for the health care sector. As bacteria can move between environments, and resistance genes often are transferred horizontally between species, it is important to protect also the environmental bacteria from excess antibiotic exposure. We have sequenced the genome of a multiresistant strain of Ochrobactrum intermedium, a common environmental bacterium and opportunistic pathogen. This particular strain was isolated inside a treatment plant in India receiving waste water from 90 bulk drug manufactures, and was found to be resistant to 36 of 39 tested antibiotics. Massively parallel pyrosequencing (454 sequencing) of its genome resulted in 500,000 reads compsing 250 million bases. Functional analysis revealed high levels of resistance genes providing resistance to different types of antibiotics, including sulfonamides, aminoglycosides and fluoroquinolones. Based on structural similarities, we also found several putative novel resistance genes previously not reported. The metagenomes also contained a high abundance of integrons and transposons facilitating the horizontal transfer of resistance genes. In addition, we found several high copy number resistance plasmids, two of which have previously not been described. We conclude that releases of high levels of antibiotics released into the environment strongly promote both resistance genes and elements supporting horizontal gene transfer. These results therefore stress the importance of a sustainable management of antibiotic waste world-wide.

A5.62

Antibiotic-contaminated effluent promotes mobile resistance in environmental bacteria

E. Kristiansson1, J. Fick2, A. Janzon1, R. Grabić3, C. Rutgersson1, B. Weisdärd1, S. Hannu1 and D. G. J. Larsson1
1Department of Neuroscience and Physiology, University of Gothenburg, Gothenburg, Sweden, 2Department of Chemistry, University of Umeå, Umeå, Sweden

The high use of antibiotics has caused an accelerating development of antibiotic resistance - one of the largest threats to human health. Bacteria defend themselves against antibiotics by acquiring resistance genes, i.e. mobile genetic elements that can be shared between species. The collection of resistance genes is particularly large in environmental bacteria and they may therefore constitute recruitment pools of resistance genes for pathogens. Recently, we have documented high environmental releases of antibiotics, but the effects on the bacterial communities and their resistance genes have not yet been studied. We sampled river sediments up and downstream from a treatment plant releasing high levels of fluoroquinolone antibiotics. The metagenomes were sequenced using massively parallel pyrosequencing generating 500,000 reads comprising 250 million bases. Functional analysis revealed high levels of resistance genes providing resistance to several classes of antibiotics, including sulfonamides, aminoglycosides and fluoroquinolones. Based on structural similarities, we also found several putative novel resistance genes previously not reported. The metagenomes also contained a high abundance of integrons and transposons facilitating the horizontal transfer of resistance genes. In addition, we found several high copy number resistance plasmids, two of which have previously not been described. We conclude that releases of high levels of antibiotics released into the environment strongly promote both resistance genes and elements supporting horizontal gene transfer. These results therefore stress the importance of a sustainable management of antibiotic waste world-wide.

A5.63

Glyco-bioinformatic and statistical analysis of inflammatory response in different tissue types

C. A. Hayes1, S. Nemes2 and N. G. Karlsson1
1Department of Medical Biochemistry, University of Gothenburg, Göteborg, Sweden, 2Department of Oncology, University of Gothenburg, Division of Clinical Cancer Epidemiology, Göteborg, Sweden

Glycosylation is one of the most important post-translational modifications of proteins, known to be involved in pathogen recognition, the innate immune response and protection of epithelial membranes. However, when compared to the tools available for the processing of high-throughput proteomic data, the glycomic domain is severely lacking. We are involved in development of glycomic solutions at many levels; the interpretation and assignment of oligosaccharide structure from LC-MS and MS/MS data; the development of methods for high-throughput comparison of samples; public-domain storage and presentation of LC/MS and MS/MS data for published oligosaccharide structures and the application of existing statistical tools to the analysis of oligosaccharide data-sets. Here we present our prototype database for public domain storage of published oligosaccharide structures. This is being developed to store our oligosaccharide LC-MS and MS/MS data. The peak lists are available for download and can be imported into a suitable program for visualisation. The database can be searched by parameters including
A5.64
ShotgunFunctionalizeR – an R-package for functional comparison of metagenomes
E. Kristiansson1, P. Hugenholtz2 and D. Dalevi3
1Department of Neuroscience and Physiology, University of Gothenburg, Gothenburg, Sweden, 2Joint Genome Institute, Microbial Ecology Program, Walnut Creek, US, 3Department of Computer Science And Engineering, Chalmers University of Technology, Gothenburg, Sweden

Microorganisms are ubiquitous in nature and constitute intrinsic parts of almost every ecosystem. A culture-independent and powerful way to study microbial communities is metagenomics. In such studies, functional analysis is performed on fragmented genetic material from multiple species in the community. The recent advances in high-throughput sequencing have greatly increased the amount of data in metagenomic projects. At present, there is an urgent need for efficient statistical tools to analyze these data. We have created ShotgunFunctionalizeR, an R-package for functional comparison of metagenomes. The package contains tools for importing, annotating and visualizing metagenomic data produced by shotgun high-throughput sequencing. ShotgunFunctionalizeR contains several statistical procedures for assessing functional differences between samples, both for individual genes and for entire pathways. In addition to standard and previously published methods, we have developed and implemented a novel approach based on a Poisson model. This procedure is highly flexible and thus applicable to a wide range of different experimental designs. We demonstrate the potential of ShotgunFunctionalizeR by performing a regression analysis on metagenomes sampled at multiple depths in the Pacific Ocean.

A5.65
Exploring the microbial resistome in river sediments exposed to extraordinary high levels of antibiotics
A. Janson1, C. Rutgersson1, E. Kristiansson1, E. B. Moore2 and D.G. J. Larsson1
1Department of Neuroscience and Physiology at the Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, 2Department of Biomedicine at the Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

The rapid development and propagation of antibiotic resistance in pathogenic and opportunistic bacteria is a major threat to public health worldwide. The phenomenon has been widely studied in the clinical setting, but comparatively little is known about the prevalence and diversity of antibiotic resistance in communities of environmental bacteria, often referred to as the environmental resistome. As the external environment may function as a reservoir of resistance genes to human pathogens, we are interested in how environmental bacteria are affected by antibiotic pollution. We have previously isolated microbial DNA from river sediments taken up- and downstream from a water treatment plant that processes waste water from several pharmaceutical plants producing antibiotics. In a previous study, we used deep sequencing to identify unprecedented frequencies of known resistance genes to several classes of antibiotics in these samples. In this study, we aim to functionally characterize the resistome in a more open and exploratory way by screening genomic DNA libraries transformed into sensitive hosts. To generate the libraries, several experimental strategies were explored, including mechanical shearing and enzymatic digestion of the isolated DNA followed by blunt- or sticky end cloning into different plasmids, subsequently transformed into sensitive E. coli. Pros and cons of the different strategies will be discussed along with preliminary results of the screening against selected antibiotics.

A5.66
Killing of Escherichia coli by Shigella flexneri
J.-H. Chen1, S.-C. Yang1, G.-H. Shen2 and S.-T. Hu3
1Institute of Molecular Biology College of Life Science, National Chung Hsing University, Taichung, Taiwan, 2Department of Internal Medicine, Division of Respiratory and Critical Care Medicine, Taichung Veterans General Hospital, Taichung, Taiwan, 3Institute of Microbiology & Immunology, National Yang-Ming University, Taipei, Taiwan

An outbreak of Shigella flexneri occurred in a Taiwan township in 1996 and extended to 2000. Of the 180 isolates recovered, 74 isolates (the epidemic strain isolates) had PFGE pattern N1X1, whereas the remaining 106 isolates (the non-epidemic strain isolates) had 51 PFGE patterns closely-related to N1X1. The non-epidemic strains likely resulted from deletions of the epidemic or other non-epidemic strains. From 1996 to 2000, 77%, 82%, 100%, 100%, and 100% of the epidemic strain isolates and 15%, 66.6%, 55%, 33%, and 0% of the non-epidemic strain isolates were found to be capable of killing E. coli strains recovered from rectal swabs of healthy persons. Thus, for the epidemic strain, isolates with the killing activity survived better yearly than those without the killing activity. But for the non-epidemic strains, deletions occurred continuously and isolates with killing activity disappeared yearly. A fosmid clone of an epidemic strain isolate (SH3160) was identified for the killing activity, and the colIb gene was further identified. The colIb gene located in a plasmid in SH3160 which could be transferred from S. flexneri to E. coli and vice versa by conjugation. Upon treatment of E. coli that carried plasmids containing colIb and the downstream immunity gene with mytomycin C, a 65-kd protein was induced. This protein could also be detected in cultured medium of E. coli that carried plasmids containing colIb under the control of lac promoter. A (His)6-tagged colicin Ib protein was purified and its killing activity was demonstrated in vitro.

A5.67
The Entamoeba histolytica EhTBP and EhTRF1 transcription factors are GAAC-box binding proteins in vitro
C. A. Castanon-Sanchez1, J. P. Luna-Arias2, M. E. Herrera-Aguirre3 and E. Orozco4
1Cinvestav-IPN, Programa Institucional de Biomedicina Molecular, Mexico, D.F., Mexico, 2Departamento de Biología Celular, Cinvestav-IPN, Mexico, D.F., Mexico, 3Universidad Autonoma de la Ciudad de Mexico, Posgrado en Ciencias Genomicas, Mexico, D.F., Mexico, 4Departamento de Infectomca y Patogonia Molecular, Cinvestav-IPN, Mexico, D.F., Mexico

Entamoeba histolytica is the protozoan parasite which causes human amoebiasis. In this organism, few transcription factors...
have been characterized. The Entamoeba histolytica TATA-box binding protein (EhTBP) is the first basal transcription factor that has been studied. Recently, we described the cloning and characterization of the Ehtrf1 encoding gene, another member of the TBP family. The E. histolytica core promoters have three conserved elements that control the transcription start site: the TATA-box, Inr and GAAC-box. It has been described that the GAAC element controls the transcription start site and the expression levels. It also directs a new transcription start site, which is independent of TATA or Inr elements. There has also been determined that the GAAC-box is recognized by an unknown nuclear protein named GAAC-binding protein (GBP).

In this work we describe the ability of EhTRF1 and EhTBP to bind to the GAAC element. Recombinant EhTRF1 and EhTBP polypeptides were expressed in bacteria as 26 kDa and 30 kDa, respectively, and were purified by IMAC using native conditions. To determine the GAAC-binding capacity of EhTRF1 and rEhTBP, we performed electrophoretic mobility shift assays that revealed the formation of EhTRF1- and rEhTBP-GAAC complexes, which were competed with 200-fold molar excess of unlabeled GAAC or TATTTAAA oligonucleotides, but not with an excess of mutated GAAC oligonucleotide or an unspecific competitor. We determined the dissociation constants of rEhTRF1 and rEhTBP for the GAAC-element. Our results indicated that EhTBP and EhTRF1 polypeptides are GAAC-binding proteins in vitro.

A5.68 Porphyromonas gingivalis HmuY protein as a potential marker of chronic periodontitis
H. Wojtowicz1, M. Radwan-Oczko2, M. Chomyszyn-Gajewska3 and T. Olczak4
1University of Wroclaw, Biotechnology, Wroclaw, Poland, 2Wroclaw Medical University, Periodontology, Wroclaw, Poland, 3Jagiellonian University, Medicine, Krakow, Poland

Porphyromonas gingivalis as a member of the red complex bacteria is considered one of the main etiological factors of chronic periodontitis. The bacterium has developed sophisticated mechanisms to communicate and evade host epithelial cells which are the first barrier preventing successful inflammation. The bacterium-host cell cross talk is a complex process which involves different types of interactions. Low iron accessibility is one of the first indicators that bacterium has reached host environment. As a response, several proteins are recruited to acquire iron or heme from host proteins. HmuY, an outer membrane lipoprotein, is able to sequester heme directly from hemoglobin and deliver it to a P. gingivalis outer-membrane heme receptor HmuR. High expression levels under iron-depleted conditions, a unique tertiary structure and low sequence homology to other bacterial proteins make HmuY protein a promising molecular marker of chronic periodontitis. Here we examined the presence of the hmuY gene and its expression levels in dental plaque samples of chronic periodontitis patients. Sequences of the hmuY gene found in different patient samples were compared. In order to determine the role of HmuY in the development of inflammation, we used an in vitro model consisting of epithelial cell line infected with P. gingivalis. We examined expression levels of the HmuY protein in bacterial cells interacting with epithelial cells and expression levels of several Toll-like receptor proteins in infected epithelial cells. We showed that HmuY protein is a specific marker of P. gingivalis that influence the bacterial-epithelial cell cross talk.

A5.69 The Entamoeba histolytica EhTBP and EhTRF1 transcription factors bind to different TATA variants in vitro
C. A. Castanon-Sanchez1, J. P. Luna-Arias2, M. E. Herrera-Aguirre1 and E. Orozco3
1Escuela Nacional de Medicina y Homeopatía-IPN, Programa Institucional de Bionciencia Molecular, Mexico, D.F., Mexico, 2Cinvestav-IPN, Departamento de Biologia Celular, Mexico, D.F., Mexico, 3Universidad Autonoma de la Ciudad de Mexico, Posgrado en Ciencias Genomicas, Mexico, D.F., Mexico

In eukaryotes, the TATA-box binding protein (TBP) forms the SL1, TFIIID and TFIIIB complexes, which are specific for the RNA polymerases (RNAP) I, II, and III, respectively. TBP in TFIIID is the first factor that binds to promoter to recruit TFH, TFIIA, RNAP II-TFIII, TFIIE and TFIIB transcription factors to assemble into the pre-initiation complex and initiate gene transcription. Mammalian TBPs can bind to a large number of diverse TATA elements. An exhaustive statistical genomic survey documented that the TATA-box is an A/T-rich eight bp segment, often flanked by G/C-rich sequences. In the case of Entamoeba histolytica, few basal transcription factors have been characterized. Previously, we described the in vitro EhTBP binding affinity for different TATA sequences found in E. histolytica gene promoters and others designed by us producing mutations in the TATA-box. Recently, we reported the Ehtrf1 gene cloning and demonstrated the ability of EhTRF1 to bind to TATTTAAA-box. In the present work, we describe the capacity of both EhTBP and EhTRF1 to bind to other TATA variants in vitro by electrophoretic mobility shift assays and determine their dissociation constants. Recombinant EhTRF1 and EhTBP proteins were expressed in bacteria as 28 kDa and 30 kDa 6His-tagged polypeptides, respectively, and purified by IMAC through Ni2+-NTA-agarose columns. Purified rEhTRF1 or rEhTBP polypeptides formed DNA-protein complexes with TATA-box variants containing C’s or C’s in the first, third, fifth and sixth positions, because these positions have been reported as important for DNA-binding activity for human and yeast TBPs.

A5.70 TT virus infection among blood donors in south of Iran
K. Solhjoo1, S. Azinfar2, A. Sotoodeh Jahromi3 and R. Yaqobi4
1Jahrom University of Medical Sciences, Microbiology, Jahrom, Islamic Republic of Iran, 2Jahrom Azad University, Microbiology, Jahrom, Islamic Republic of Iran, 3Transplantation Research Center, Virology, Shiraz, Islamic Republic of Iran

Introduction and Objective: Recently, a new infective agent of humans, TT virus (TTV), was isolated from the serum of a patient with posttransfusion hepatitis of unknown etiology, in Japan. The prevalence of TTV in different population groups, including apparently healthy individuals, is very high. The aim of this study was to determine the prevalence TTV in healthy blood donors.

Methods: Sera were collected from 499 healthy blood donors. None of them had clinical signs of acute or chronic hepatitis and serological markers for hepatitis A, hepatitis B, and hepatitis C viruses were negative. TTV DNA was amplified and detected using semi-nested PCR, followed by gel electrophoresis.

Results: Sixty-six of 499 (13.40%) samples had TTV DNA detected by PCR. From 66 TTV DNA positive, 63 were male
Serum alanine-aminotransferase and aspartate-aminotransferase levels in blood donors

K. Solbjo1, A. Sotoodeh Jahromi1, S. Azinfar2 and R. Yaqobi3
1Jahrom university of Medical sciences, Microbiology, Jahrom, Islamic Republic of Iran, 2Jahrom Azad university, Microbiology, Jahrom, Islamic Republic of Iran, 3Transplantion research center, virology, shiraz, Islamic Republic of Iran

Increased activity of serum aminotransferases such as alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) in blood donors may identify infection with non-A, non-B hepatitis, and is being used as a surrogate test for preventing post-transfusion viral hepatitis. We have studied ALT and AST levels in 499 consecutive blood donors to determine their association with gender, and hepatitis virus infection markers. Sera were collected from 499 healthy blood donors, tested for viral infection by ELISA and then ALT and AST levels were determined. Four hundred sixty-seven men and 32 women were studied; ALT activity was 26.80 ± 27.75 IU/l (mean ± SD) and ALT range was 10–142 IU/l. AST activity was 25.58 ± 24.12 IU/l (mean ± SD) and AST range was 10–124 IU/l. The upper normal value for men was 40 IU/l and 34 IU/l for women. From 499 cases, 71 people had an ALT level than normal value, and 65 (95.8%) of them were men and 3 (2.3%) of them were women (p = 0.417). From 499 cases, 67 people had an AST level than normal value, and 68 (95.8%) of them were men and 2 (3%) of them were women (p = 0.218). None of them had clinical signs of acute or chronic hepatitis and serological markers for hepatitis A, hepatitis B, and hepatitis C viruses were negative. It seems clear that different cutoff values should be considered for men and women, and ALT & AST elevation is influenced by many factors.

In-vitro inhibitory effect of BLIP derived peptides on TEM-1 beta-lactamase

N. Budeyri1, N. G. Avel1, E. Ozkirimli Olmez1 and B. Sariyar Akbulut1
1Marmara University, Bioengineering, Istanbul, Turkey, 2Bogazici University, Chemical Engineering, Istanbul, Turkey

Bacterial resistance against beta-lactam antibiotics has been a global health problem mainly caused by the presence of beta-lactamases. The combination of the beta-lactam with beta-lactamase inhibitors has proven to be a successful strategy to combat the escalating problem of beta-lactam resistance mediated by class A beta-lactamases. Most of the research on inhibitor binding to beta-lactamase has focused on TEM-1 class A beta-lactamase due to its prevalence. Beta-lactamase inhibitory protein (BLIP) has been shown to be a potent inhibitor of E. coli TEM-1 beta-lactamase. The interaction between beta-lactamase and BLIP leads to the design of candidate peptide based drugs for the inhibition of beta-lactamase. In this study, this approach has been used to investigate TEM-1 inhibition by the BLIP derived peptide (residues 45-53) and its variants. E. coli cells harboring the pUC18 plasmid that carries the bla gene of TEM-1 beta-lactamase, was used for periplasmic beta-lactamase synthesis. This enzyme was purified by osmotic shock method and followed by a combination of anion exchange (DEAE-cellulose) and gel filtration (Sephadex G-100) chromatographies. In vitro beta-lactamase inhibition by BLIP derived peptides was assayed on both pure TEM-1 and partially purified TEM-1 beta-lactamase using CENaTA as the substrate. This study has been supported by TUBITAK project 108M644.

Production of recombinant varicella zoster gE in CHO-K1 cells for improved serological diagnostics of VZV CNS infections

E. Thomsson1, E. Brunnhage2, M. Bäckström3, A. Persson2, M. Johansson2, F. Svensson3, M. Ekblad2, J.-Å. Liljeqvist2, G. C. Hansson1 and T. Bergrström3
1University of Gothenburg, Mammalian Protein Expression Core Facility, Gothenburg, Sweden, 2Department of Infectious Diseases, University of Gothenburg, Gothenburg, Sweden, 3Department of Medical Biochemistry, University of Gothenburg, Gothenburg, Sweden

Herpes simplex virus 1 and 2 (HSV-1, HSV-2) and varicella zoster virus (VZV) can all cause CNS disease. Discrimination between these infections is needed to optimize antiviral treatment. Initially, PCR detection of viral DNA in the cerebrospinal fluid (CSF) is the diagnostic method of choice. However, one week after onset the analysis usually has to rely on serological grounds. The present technique used – IgG analysis of paired CSF and serum samples by ELISA – is often based on crude preparations of viral antigens. It is characterised by low specificity and discrimination of the infections can not be accomplished due to serological cross reactivity. The VZV gE protein is an immunodominant and specific B-cell antigen for VZV and an ELISA candidate. Attempts using bacterial or baculovirus expressed VZV gE did not improve the diagnostic ability of the test. Instead, a plasmid carrying the His6-tagged extracellular part of VZVgE was transfected into mammalian CHO-K1 cells. Stable clones were generated and cultivated in a perfusion lab scale bioreactor (1.5 l) at pH 6.9, 37°C, and a dO2 of 40% under serum free conditions. Spent medium was concentrated and the recombinant protein was purified on a his-tag column. The product was used for serological diagnostics of IgG against VZV gE in ELISA. Preliminary results showed a high sensitivity and no cross reactivity between sera with high titres of IgG against only HSV-1 or only VZV. In paired serum-CSF samples the novel antigen could discriminate between VZV and HSV-1 encephalitis, which may solve a long-lasting diagnostic problem.

New details of inhibition of HCV helicase by lead compounds derived from benzotriazole

A. Polkowska-Nowakowska1, A. Godzick1, I. Zhukov2, A. Najda1, A. Boguszewska-Chachulska1, M. Bretnier2 and J. Poznanski3
1Polish Academy of Sciences, Institute of Biochemistry and Biophysics, Warsaw, Poland, 2National Institute of Chemistry, Lubljana, Slovenia, 3Institute of Pharmacy, Warsaw, Poland

Hepatitis C virus, an enveloped, highly mutable RNA virus is responsible for major cases of acute hepatitis and liver cancer. There is intense need for developing efficient treatments. Viral protein NS3 helicase/NTPase seems to be very attractive and still hardly examined molecular target, because blocking of its helicase activity prevents HCV replication in the hepatoma cell.
Nucleoside base - like compounds have been reported to act as HCV NS3 helicase inhibitors. 4,5,6,7-tetrahydroleurobenzotiazole (TBBT) and 5,6-dichloro-1-(B-D-ribofuranosyl)benzotrazole (DCBT) inhibit unwinding of DNA substrate with IC50 within μM range without any effect on the rate of ATP hydrolysis, catalysed by the enzyme. Several attempts were made to elucidate the mechanism of inhibition of HCV helicase activity by benzo-trazole derivatives. Recently we investigated binding of this two lead compounds to isolated domain 1 of HCV helicase using NMR spectroscopy. The results throw some light on the question of mode of action. From chemical shift mapping experiments we concluded, that DCBT binds to domain 1 in similar way as ATP. By contrary, TBBT binds in slightly different manner. Both compounds interacts with so called “spring helix”, which was recently shown to participate in coupling between ATP hydrolysis and DNA binding/unwinding. Basing on the rachet mechanism of DNA unwinding by NS3 helicase, the investigated benzotriazole derivatives can be expected to interfere with the transition states of the NS3 – DNA – ATP complex and freeze the helicase in a particular conformation.

A5.75

Fragmentation, ubiquitination and mosaicism of HIV/SIV genetic components in the context of thermo-stable adoviral (Ad) vectors as novel vaccine approaches against HIV-1/AIDS

T. Athanasopoulos1, T. Papagatsias2, A. Meiser2, J. Harris1, V. Buchy1, A. Benlahrech2, S. Herath3, S.-Y. Kwon4, K. Fischer5, L. Seymour6, R. Daniels7, N. Almond8, K. Logan2, L. Klavinskis2, S. Patterson2 and G. Dickson1


Broadening the immune response through development, optimisation and evaluation of HIV-1/SIV genetic components is a rational strategy towards HIV-1/SIV vaccination studies. We originally aimed to induce immunity to HIV-1 infection by developing a cohort of vectors in which HIV-1 genetic components have been engineered to stimulate broader CTL responses. Full-size gag (but also pol/env) genes including an across clade conserved/mosaic epitope were engineered, fused to mono- or tetra-ubiquitin (Ub) sequences and further tested on DC and non-DC cell lines by using plasmid, in vitro synthesised mRNA, or adeno-viral vectors. We subsequently constructed rAd vectors carrying genetically fragmented ubiquitinated fusions for either HIV (>10) or SIV (>7) gag genes in an attempt to reduce antigenic competition and alter epitopic dominance. To this context, sugar mixtures were identified which optimally preserved and thermostabilised adoviral-based vaccines infectivity while maintaining the strength and rigidity in a micro-needle array, aiming towards elimination of cold-chain distribution of a vaccine to Sub-Saharan African countries. Vectors retained substantial infectivity (i.e. >55% eGFP + cells) for up to 3 months trough a temperature range tested (20°C to +40°C). Transcutaneous injections of desiccated Ad OVA-GFP vectors (4E109 vg) in C57BL6 mice or footpad-mediated injection of Ad5-CMV-eGFP to lymph nodes demonstrated functionality of Ad-sugar formulations. We are currently extending our studies using either in vitro CTL epitope-mapping or in vivo plasmid prime/Ad boost strategies. SIV constructs will be forward tested in non-human primate models (cynomolgus macaques).

A5.76

Analysis of SPS-sensor dependent gene expression in Candida albicans

F. J. Alvarez, K. Ryman and P. O. Ljungdahl

Wenner-Gren Institute, Cell Biology, Stockholm, Sweden

Candida albicans is a commensal organism that lives as a benign member of the microflora of healthy individuals. Changes in the host environment can induce C. albicans to mount virulent infections. The majority of women experience at least one episode of acute vulvovaginal candidiasis (VVC), and 5% suffer recurrent chronic infections (>4 episodes annually). Importantly, chronic VVC affects nominally healthy women without measurable immunodeficiencies; the factors contributing to chronic infections remain elusive. C. albicans use the SPS-sensor to regulate the activity of two latent transcription factors Stp1 and Stp2. Activated Stp1 induces the expression of genes required for the catabolic utilization of host proteins, including the established virulence factor SAP2, a secreted aspartyl protease (SAP), and several oligopeptide transporters (OPTs). Activated Stp2 induces the expression of permeases that catalyze transport of amino acids into cells. Quantitative methods to monitor SPS-sensor dependent gene expression reveal that the induction of the SPS-sensor, or the introduction of a dominant activated STPI* allele greatly enhances the expression of SAP2 (>100 fold), OPT3 (12 fold), OPT4 (7-fold) and OPT5 (24 fold). Fluorescence reporter constructs that monitor Stpl (PSAP2-RFP) and Stp2 (PCAN1-GFP) regulated genes have been developed, enabling the integration of nutrient-based signals to be visualized in real-time in single cells. We are applying these tools to understand how fungal cells sense and respond to the vaginal environment, i.e. the nutrient content of cervical mucus, and the high content of amino acids and protein present in serum and semen.

A5.77

Abstract withdrawn

A5.78

Optimization of PCR protocol for the detection of genus Cronobacter

M. Blazkova, K. Mysakova, B. Javurkova, L. Kramonova, L. Fukal and P. Rauch

Institute of Chemical Technology Prague, Biochemistry and Microbiology, Praha, Czech Republic

A new bacterial genus Cronobacter of the family Enterobacteriaceae was established in 2008. Cronobacter spp. are Gram-negative, facultative-anaerobic, rod-shaped bacteria, which are recognised as causative agents of neonatal bacteremia, meningitis and necrotising enterocolitis. According to the Commission Regulation No 1444/2007, the food law of European Union insists on the absence of Cronobacter spp. (not detectable in 10 g of food product) in infant formulae. Although the gold standard for the detection of Cronobacter spp. in food is the conventional cultivation method, there is trend to develop rapid and user-friendly techniques. Here, we present data of the development of the PCR protocol with different sets of primers for the detection of Cronobacter spp. The reliability of protocols was evaluated with the range collection of 60 bacteria, including Cronobacter strains and other food relevant microorganisms, the detection limits were determined. The characteristics (specificity and sensitivity) of developed procedures are discussed.

Acknowledgement: This work was supported by the Grant of Ministry of Education MSM 6046137305, the Czech Grant Agency No. 525/09/1075 and the National Research Programme II 2B06048.
Adiponectin is an adipose tissue-derived mediator with significant antiatherogenic properties. A few studies were done in acute phase of myocardial infarction especially in non obese patients. We design a study to investigate the association between adiponectin concentration and acute phase of myocardial infarction in non obese patients. Adiponectin which is secreted by adipose tissues has been reported to have direct antiatherosclerotic effects also plasma adiponectin levels have been shown to be decreased in patients with cardiovascular diseases and hypertension. To determine the relationship between plasma adiponectin levels and serum triglyceride and cholesterol concentrations in patients with acute myocardial infarction and in healthy control group, this case-control study was carried out on 43 patients with acute myocardial infarction (AMI) and 43 age, sex, and other classic cardiovascular risk factors matched healthy persons with normal coronary artery angiography as control group. Blood samples were taken from the patients at first 24 hours of admission. Plasma adiponectin levels were measured by ELISA method in the patient and the control groups. Plasma adiponectin levels in the patients and the control group were 3.328 ±1.716 ig/ml and 5.014 ± 1.456 ig/ml, respectively (p < 0.001). Serum triglyceride concentrations in the patients and the control group were 146.05 ± 99.595 and 144.23 ± 99.824, respectively (p < 0.933). Serum cholesterol concentrations in the patients and the control group were 146.30 ± 45.209 and 171.84 ± 33.765 respectively (p < 0.046). There was a significant reverse relationship between plasma adiponectin levels and serum triglyceride (r = −0.356, p = 0.002) and cholesterol (r = −0.323, p = 0.035) concentrations in the patients, but this relationship was not found in the control group. The results suggest that low plasma adiponectin level may be an acute myocardial infarction risk factor. Further comprehensive studies are needed to explore effect of serum triglyceride and cholesterol concentrations on plasma adiponectin level.
cies and obese patients. We evaluated its level in patients with LUTS due to BPH.

**Methods:** Seventy five patients with BPH enrolled in this study and divided to three groups according to AUA symptom score. Serum adiponectin level checked and compared.

**Results:** In group I (Mild LUTS) 21 patients, Mean age was 66.8 years and mean adiponectin level 12.4 ug/ml, group II (Moderate LUTS) 30 patients, mean age 70.1 years, mean adiponectin 5.8 and group III (Severe LUTS) 24 patients, mean age 71.8 years and mean adiponectin 1.5, respectively.

**Conclusion:** Serum adiponectin level decline in patients with BPH, however it is a benign tumor and its level correlate with the symptom score (More severe LUTS has lower level).

**B1.05 Plasma homocysteine levels in woman with polycystic ovary syndrome**

A. Atanasova 1, G. Olive 2 and B. Seifdin 2

1. University Clinic for Gynecology and Obstetric, Skopje, Macedonia, FYROM, 2. University Clinic for Clinical Chemistry, Skopje, Macedonia, FYROM

Poly cystic ovary syndrome (PCOSy) is a condition characterized by oligomenorrhea and androgen excess, affects 6–10% of the women in reproductive period. Although the pathogenesis is still uncertain, many studies suggest that PCOSy may increase risk for insulin resistance, type 2 diabetes, dyslipidemia, cardiovascular risk. Woman with PCOSy would be expected to be at significantly increased risk for atherosclerotic disease. Evidence suggests that hyperhomocysteinemia may promote atherosclerosis by damaging the inner lining of arteries and promoting blood clots as a result of inflammation. Hyperhomocysteinemia has been shown as independent predictor of cardiovascular morbidity in patients with atherosclerosis. The aim of our study was to determinate levels of Hcy in woman with polycystic ovary syndrome. Thirty patients (age, 23, 5 ± 5.5) with PCOSy and twenty four (age, 25.5 ± 4.3) healthy, were involved in the study. Total Hcy was measured using fluorescent immunoassay. Statistically significant differences in serum concentration of Hcy were observed between groups. Mean Hcy level we found as (10.2 ± 2.9 versus 7.0 ± 1.5) in PCOSy and normal group respectively (p < 0.05). For Macedonian population we found statistically significant increased Hcy levels in woman with PCOS. Because an increased concentration of total Hcy has been shown as an independent risk factor for cardiovascular alterations, it is essential in this group of woman are taken measures for early prevention. Routine quantification of total Hcy in woman with proven PCOS would be simple and safe method for early prevention of cardiovascular diseases.

**B1.06 Cloning and characterization of a novel Nudix hydrolase encoded by nudC gene from plant pathogen Pseudomonas syringae pv. tomato str DC 3000**

M. Modzelan and E. Kraszewska

Institute of Biochemistry and Biophysics, Warsaw, Poland

Nudix hydrolases are widely distributed pyrophosphatases with a conserved amino acids motif GX5EX5UAIJRX2EEXGU (where U is a hydrophobic residue). They hydrolyze a variety of substrates, predominantly nucleoside diphosphate derivatives. The proposed role of Nudix enzymes is to maintain cellular homeostasis. However, it can not be excluded that some of these proteins from pathogenic organisms may play a role in pathogen invasion processes. The aim of this work was to characterize the first Nudix hydrolase from plant pathogenic bacteria Pseudomonas syringae pv. tomato str DC 3000. PCR reaction was used to amplify gene NP_792526 encoding the 278 aa NudC protein P.syringae genomic DNA. PCR product was cloned into expression vector. The recombinant NudC hydrolase was expressed in E. coli and purified by affinity chromatograhy. The enzymatic activity was tested with the purified recombinant NudC protein and a number of different substrates. At reaction conditions typical for Nudix hydrolases, at pH 8.0 and in the presence of Mg²⁺ ions, the preferred substrate of this enzyme was NADH. An attempt to elucidate the biological role of the NudC protein was undertaken by creating mutants with disruption of the gene encoding the NudC protein.
with 90% homology at the nucleotide level, coding for two poly-
peptides of 387 amino acids, respectively. One of them, diox1,
was located on a mega plasmid and the other, diox2, on the
chromosome. Both genes were subcloned in the pET29c vector
(Novagen), overexpressed in E. coli BL21(DE3) and purified to
electrophoretic homogeneity through Ni+-NTA chromatogra-
phy. Both purified protein fractions Diox1 and Diox2 exhibited
1-H2NA dioxygenase activity. The existence of two homologues
encoding for 1-H2NA with similar catalytic properties indicates a
gene duplication or transposition within the genome of Arthrob-
acter phenanthrenivorans Sphe3.

B1.09 Relationship of polyP metabolism and
bioinsecticide biosynthesis in Bacillus
thuringiensis
T. Doruk, U. Savaj, Y. Yalym and S. Tunca
Gebze Institute of Technology, Biology, Kocaeli, Turkey

Polyphosphate (polyP) is a linear polymer consisting of
tens to hundreds of phosphate molecules joined together by high-
energy anhydride bonds. PolyP is synthesized by polyP kinase
(PPK) using the terminal phosphate of ATP as the substrate. It
is degraded to phosphate by exopolyphosphatase (PPX) enzyme.
Although polyP polymer has very important functions (energy
storage, resistance to stress factors, regulation of gene expression
etc.) in the microorganisms studied, to our knowledge there is
only one study about the role of this polymer in Bacillus. In 2004
Shi et al., deleted the genes (ppk, ppx, pap) responsible for the
polyP metabolism and showed that polyP is necessary for the
motility, biofilm formation and sporulation in this microorgan-
ism. Bacillus thuringiensis, which produce δ-endotoxin, used for
biological control of insects. Some mineral elements especially
phosphate, which were added into the culture media, has been
shown to play very important stimulatory effect on toxin produc-
tion (Banerjee-Bhatnagar, 1999; Kurt et al., 2005). In this study,
it is aimed to obtain hypertoxic mutant strains by changing the
phosphate metabolism of B. thuringiensis israelensis. For this pur-
pose; ppk and ppx genes of B. thuringiensis were cloned separa-
ately and together and the effect of overexpression of these
genes on endotoxin production were determined. Optimum con-
ditions (pH, temperature, carbon source) for toxin production by
mutant strains and also resistance of these strains to different
stress factors (pH, temperature, oxidative stress, heavy metals)
were tested. This study will be the first example which relate
polyP metabolism with bioinsecticide biosynthesis.

B1.10 The effects of dexketoprofen on paraoxanase
and nitric oxide synthase activities in rat liver
 tissues
A. Cavunt Bayraktar1, M. Kavutcu1, H. Yagmurdur2 and
E. Ongen2
1Gazi University, Clinical Biochemistry, Ankara, Turkey, 2Ankara
Training and Research Hospital, Anesthesiology and Reanimation,
Ankara, Turkey

Dexketoprofen-trometamol is a water-soluble salt of the dextro-
rotatory enantiomer of nonsteroidal anti-inflammatory drug keto-
profen. In this study we investigate the relationship between
low-high dose of Dexketoprofen and enzyme activities such as
Paraoxanase (PON) and Nitric Oxide Synthase (NOS). Twenty-
six wistar albino rats in this study were randomly divided into 3
groups: Group-I = Control group (n = 10), Group-II = Low
dose group (treated i.p 10 mg/kg/day Dexketoprofen for 1 week;
n = 10), Group-III = High dose group (treated i.p 20 mg/kg/ day
Dexketoprofen for 1 week; n = 6). At the end of the study, rats
were sacrificed and their livers were removed. In the liver tis-
sues, NOS and PON activities were measured. Statistical analysis
were performed using Mann Whitney-U test. The PON activity
were significantly elevated in high dose group (6.78 ± 0.77 IU/
mg protein) compared to low dose (4.34 ± 0.64 IU/mg protein)
and control (3.80 ± 0.46 IU/mg protein) groups p < 0.05,
p < 0.025 respectively. There was no significant difference
between PON activities of the control and low dose groups.
NOS activity were significantly increased both high dose and low
dose groups (7.45 ± 0.84 and 5.61 ± 0.42 IU/mg protein) compared
to control group (2.70 ± 0.36 IU/mg protein), p < 0.025 and
p < 0.01 respectively. Also, NOS activity was found significantly
elevated in high dose group versus low dose group p < 0.05. In
conclusion, we suggested that high dose of dexketoprofen
induced the NO production by activating the NOS enzyme
expression. On the other hand, elevated PON activity, as an anti-
oxidant enzyme, could protect lipoproteins from oxidative stress.

B1.11 Characterisation of two new enzymes involved
in lysosomal phospholipid degradation
O. K. Greiner-Tollersrud1, G. Evjen2, T. Berg2 and
H. M. Stensland2
1University of Tromso, Institute of Medical Biology, Tromsø,
Norway, 2University of Tromso, Tromsø, Norway

The incomplete degradation of phospholipids plays an important
role in the aetiology of a variety of lysosomal storage disorder.
However, the array of lysosomal enzymes involved in this degra-
dation is incompletely known. Here we present the purification
and characterisation of two new enzymes involved in this process.
Lysosomal phospholipase A1 acting on phosphatidylcholine was
originally reported by Mellors and Tappel in 1967 and Fowler
deDuce in 1969; but never fully characterised biochemically or
genetically. The enzyme was purified to homogeneity from
bovine kidney as a 75 kDa glycoprotein composed of two
peptides of 25 and 50 kDa joined by noncovalent forces.
Remarkably this enzyme was absent from bovine brain suggest-
ing a tissue specific expression. In transfected COS-cells the
enzyme was transported to the lysosomes via the mannose-6-
phosphate receptor (MPR). Lysosomal lysosphingomyelinase is
an acid sphingomyelinase like protein that lacks the N-terminal
saposin-like domain, resulting in no intrinsic activity towards
sphingomyelin. The enzyme was purified from bovine kidney
and brain as a 55 kDa glycoprotein comprised of two
peptides of 387 amino acids 112
Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) are the principal enzymes responsible for ethanol metabolism in mammalian liver. To mimic ALDH2 deficiency which is found in 40–45% of East Asians, rat liver ALDH was inhibited by treatment with cyanamide. At doses of 0.2 mg/kg and 1.5 mg/kg cyanamide, rat liver ALDH activities were found to be inhibited by ~50% and ~75%, respectively. Steady-state metabolism of ethanol was determined in single-pass perfused rat liver inhibited by cyanamide. Concentrations of ethanol, acetaldehyde and acetate in the effluent perfusates were determined using GC or HPLC. The acetaldehyde concentrations were elevated from ~10 to ~70 and ~110 μM by 0.2 mg/kg and 1.5 mg/kg cyanamide, respectively, when perfused with 2 mM ethanol; and from ~20 to ~120 and ~200 μM when perfused with 10 mM ethanol. We compared the experimental data to those from computer simulations using the steady-state kinetic mechanism-based complete rate equations of rat ADH1.

The results indicate that the elimination rates of ethanol are decreased due to inhibition of ALDH activity and this can be quantitatively explained by build-up acetaldehyde with the computer simulations.

B1.13 The effect of polyphosphate kinase deficiency on antibiotic production in Streptomyces coelicolor
Y. Yalým, T. Doruk, Ü. Savaj and S. Tunca
Gebze Institute of Technology, Biology, Kocaeli, Turkey

Polyphosphate (polyP), which is found in every living cell, is a linear polymer consisting of tens to hundreds of phosphate molecules joined together by high-energy anhydride bonds. Polyphosphate, which is synthesized by the enzyme polyP kinase (PPK), is an important energy store and has been shown to have a crucial role in the regulation of adaptive responses of cells to physical and chemical stresses. Streptomyces are biotechnologically important bacteria and they are well known for their ability to produce a wide variety of bioactive compounds. Chouayekh and Virolle (2002) have reported that ppk gene of Streptomyces coelicolor has been disrupted and the effect of this study are discussed.

B1.14 Dequalinium inhibits autophagy in the human leukemic cell line NB4: apoptosis resistance mechanism or cell death inducer?
M. C. Estañ-Oñana, I. Gasañ-Gomez, K. A. Makowska, E. Moreno-Cubero, P. Sancho, V. Rubio, J. C. Diez and M. d. C. Boyano-Adanez
University of Alcalá, Biochemistry and Molecular Biology, Alcalá de Henares (Madrid), Spain

Autophagy is the process in which cellular compounds are degraded by the lysosome and then recycled. Although it can serve as a protective mechanism against apoptosis and starvation, excessive autophagy results in a non-apoptotic programmed cell death. However, there is evidence of a cross talk between autophagy and apoptosis. The tumoral suppressor p53 seems to inhibit autophagosome formation, preventing the mitochondrion recovery process and inducing apoptosis. Our group has demonstrated that the antitumoral agent dequalinium (DQA) induces apoptosis in the human acute promyelocytic leukemia cell line NB4. Previous results showed a partial inhibition of the DQA effects on apoptosis and on the intracellular levels of reactive oxygen species at a low dose of DQA in combination with the proteasome inhibitor MG-132, which induces apoptosis per se. Since MG-132 inhibits programmed protein degradation, it is possible that the proteins that accumulate in the cells then undergo a different type of degradation. Therefore, we studied apoptotic and autophagic events in NB4 cells treated with MG-132 and/or DQA and the possible implication of p53. Our results indicate that DQA induces apoptosis and inhibits autophagy, and that it triggers p53 nuclear translocation in NB4 cells. We suggest that MG-132 could promote autophagy, therefore resulting the combination with DQA in a competition with apoptosis. This might explain the reduction in apoptosis that occurs in co-treatment with DQA (10 μM, 48 hours). Moreover, we suggest that p53 could be, at least in part, responsible for the reversion of this effect at higher doses of DQA. This work was supported by UAH (GC2009-001). We thank JCCM & UAH for fellowships.

B1.15 Variations in the metabolic network of Saccharomyces cerevisiae during adaptive evolution on galactose
K.-K. Hong, G. Vemuri and J. Nielsen
Chalmers University of Technology, Chemical and Biological Engineering, Göteborg, Sweden

Exploring the changes in the metabolic network that improve fitness in a new niche is an essential step for evolutionary study. Galactose metabolism in S. cerevisiae is an interesting target for finding new metabolic network combinations because all GAL genes are well elucidated and their expression is strongly induced by galactose. We evolved wild-type S. cerevisiae using serial dilution on galactose minimal media. We subjected this yeast to adaptive evolution in three lineages, starting from a common ancestor, to increase the specific growth rate. After about 400 generations, the growth rate increased similarly in all the lineages, but the fermentative capacity of the evolved mutants from the lineages varied substantially. Glucose-1-phosphate consuming reactions were commonly induced in the mutants from the three lineages while the MAP kinase pathway was repressed, as revealed by transcriptome analysis. The intracellular concentration of glucose-1-phosphate significantly reduced while amount of glycogen and trehalose were considerably increased in all the mutants. We also sequenced the genomes of the three evolved mutants from each lineage. We will present an integrated analysis of the changes that are common to the three lineages and those that are specific to each lineage. The two most striking features of this study are that (i) the mutants evolved to possess very diverse phenotype, despite having the same growth rate and (ii) there were no transcriptional changes in the genes of the galactose pathway. The implications of the result on metabolic network analysis and impact of systems biology on evolutionary study are discussed.
B1.16
Effect of the antitumor agent dequalinium upon human prostate cancer cells
K. Makowska, I. Galan-Gomez, M. C. Estañ-Oñaña, E. Moreno-Cubero, M. d. C. Boyano-Adlanez and P. Sancho
University of Alcalá, Biochemistry and Molecular Biology, Alcalá de Henares, Madrid, Spain

Carcinomas are the most common cause of death due to cancer in humans, with prostate carcinoma as one of the most prevalent cancers. PC3 cell line established from human prostatic adenocarcinoma metastasis to bone, possesses characteristics of highly malignant neoplasm and therefore is an appropriate model to investigate carcinoma responses. Dequalinium (DQA), a member of delocalized lipophilic cations family, is accumulated and retained in mitochondria of carcinoma cells due to a higher negative mitochondrial transmembrane potential of these cells in comparison to normal cells. Mitochondrion, which makes an integral contribution on the regulation of main cellular events, is an adequate target for tumor cell eradication. Previous results have shown that DQA displays a different cytotoxic activity depending on the cell type. However, the exact mechanisms underlying DQA activity are not yet fully understood. Therefore, the aim of this study was to investigate the effect of this agent on the PC3 cell line in order to acquire a better knowledge about the DQA action mechanism in different cell lines. Preliminary results show a cytotoxic effect of DQA on PC3 cell line inducing the cell death by apoptosis or necrosis depending on the DQA concentration and the time of the treatment employed. Alterations of mitochondrial function have been evidenced by a loss of mitochondrial transmembrane potential, reactive oxygen species accumulation and other events. The current study improves the knowledge on DQA as a novel anticancer agent with a potential application in human prostate carcinoma therapy.

B1.17
Structural correlations in bacterial metabolic networks
P. Gerlee1, S. Bernhardsson2, L. Lizana1 and K. Sneppen1
1Center for Models of Life, Niels Bohr Institute, Copenhagen, Denmark, 2Institute of Theoretical Physics, Umeå University, Umeå, Sweden

Evolution of metabolism occurs through the acquisition and loss of genes whose products act as enzymes in metabolic reactions, and from a presumably simple primordial metabolism the organisms living today have evolved complex and highly variable metabolisms. We have studied this phenomenon by comparing the metabolic networks of 134 bacterial species with known phylogenetic relationships. We consider the “super-network” consisting of the union of all networks where each reaction-node is tagged with the number of organisms it belongs to (organism degree, OD). Network analysis shows that common reactions are much more likely to be connected to each other compared to a random null-model. This trend persists up to a distance of five reactions, which is similar to the average pathway length of 4.4 reactions, as defined in MetaCyc. Further, we propose a neutral model of metabolic evolution driven by horizontal gene transfer (HGT), which is only directed by biochemical constraints. The model is initialised with a single reaction-node, and by adding new nodes one at time metabolic networks are formed. Although these networks are unrelated and their only similarity stems from biochemical constraints, their “super-network” still exhibits the same structure as the bacterial counter-part. This suggests that bacterial metabolic network evolve through HGT and to a large extent are formed by biochemical constraints.

B1.18
Characterization of cellular composition of evolved strains of Lactococcus lactis
C. Price1, F. Santos2, D. Molenaar2, B. Teusink2, B. Poolman3 and O. Kuipers4
1Departments of Molecular Genetics and Biochemistry, University of Groningen, Haren, Netherlands, 2Vrije Universiteit Amsterdam, Systems Bioinformatics IBIVU, Amsterdam, Netherlands, 3Department of Biochemistry, University of Groningen, Groningen, Netherlands, 4Department of Molecular Genetics, University of Groningen, Haren, Netherlands

The lactic acid bacteria Lactococcus lactis has a well-characterized metabolism in which sugars are converted to mainly lactic acid. While L. lactis grows homofermentatively under anaerobic conditions with high concentrations of glucose, the bacteria switches to more efficient mixed fermentation in the presence of other sugars such as maltose and low concentrations of glucose. Inefficient and incomplete metabolism when a more efficient alternative appears available is a widespread phenomenon. In order to understand the evolutionary advantage of the inefficient use of substrates, L. lactis MG1363 has been evolved in four parallel chemostats in chemically defined medium, CDMPC, developed for long term cultivation. Since the evolutionary path is followed in four parallel experiments adaptive evolutionary paths can be compared to the final outcome. The evolved strains were extensively characterized with respect to gene reorganization, gene deletion/duplication as well as changes at the mRNA and protein level. Evolved strains were found to have an increased lag phase when revived in batch culture as well as having an increased stability during stationary phase. Also, strains have an increased secretion capacity when compared to the original strain. In addition to insights into overflow metabolism, constraints governing long-term mutation and adaptation in L. lactis can be analyzed and so contribute to our understanding of strain stability.

B1.19
Genome scale metabolic models of three bacteria in the human gut
Department of Chemical and Biological Engineering, Chalmers University of Technology, Göteborg, Sweden

Microbial intestinal metabolism is thought to affect many human disease states such as diabetes, obesity and inflammatory bowel diseases. Vast amounts of resources are spent on generating genotype data of the gut microbiota but the understanding of the link to phenotype is still limited. To fully understand the interplay between the microbes and the human host and to successfully hypothesize interventions, a more integrated approach is needed. Genome-scale metabolic models (GSMM) have successfully been used to link genotype and phenotype by predicting essential genes and describing the metabolism of several organisms. The formulation is mathematically tractable, scalable and requires a minimum of measured parameters. The construction of GSMM includes data base mining to annotate genes to reactions. Our automated GSMM framework will be used to infer reactions not yet annotated. We plan to construct GSMM for some of the most important species in the gut, namely B. thetaotaomicron, E. rectale and M. smithii. By flux balance analysis, it is possible to identify essential genes and interspecies metabolites. Integrated analysis of transcriptome data on GSMM highlights
otherwise unseen effects by identification of reporter metabolites. It will also be possible to simulate optimal conditions for butyrate production, important in inflammatory bowels diseases. Furthermore, the energy harvesting efficiency by the gut microbiome can be quantified with different microbiome compositions and substrates which are related to obesity development. Simulations can generate likely hypotheses for clinical interventions such as probiotics, prebiotics, therapeutics and altered diet.

B1.20
Aspects of sex-specific LRP-2 expression in kidney epithelial cells and the effect of modified LDL particles on receptor regulation
J. Plieschnig, E. T. Gensberger, W. Schneider and M. Hermann
Medical University of Vienna, Department of Medical Biochemistry, Vienna, Austria

Diseases including inflammation, diabetes, and obesity can lead to the generation of lipoproteins modified by oxidation, glycation, alkylation, and nitration. Uptake of modified LDL, without efficient degradation, leads to formation of foam cells, causing pathologies including atherosclerosis and glomerulosclerosis. Dyslipidemia accelerates renal damage. In our study we focus on the low-density lipoprotein receptor-related protein 2 (LRP2), which is highly expressed in the renal proximal tubule. We want to elucidate its function in the uptake of molecules involved in lipid metabolism in the kidney. Furthermore, we want to identify the underlying mechanisms of steroid hormone regulation of LRP-2 expression and test if estrogen has a protective effect. Two human kidney cell lines were used. Native HDL and LDL were isolated and modified LDL forms were generated. After incubation with lipoproteins, LRP2 expression was determined. Modified LDL particles caused an increase in LRP2 transcript levels. Furthermore, we were able to show that administration of estrogen enhances transcript expression. Now we address the question, if the incubation with estrogen alters the effect of lipoprotein particles. The inhibition of LRP2 by the receptor-associated protein (RAP), which prevents binding of other ligands, will elucidate receptor dependent regulations. The regulatory mechanisms of LRP2 and its roles in diseases leading to renal damage are not well established. Further studies of sex-related differences in kidney structure and functions will enable us to understand the development of various renal diseases, leading to a better knowledge of pathogenesis and prevention.

B1.21
Abstract withdrawn

B1.22
Reconstruction of a comprehensive Saccharomyces cerevisiae genome-scale metabolic and regulatory network
T. Österlund, I. Nookaew and J. Nielsen
Chalmers University of Technology, Chemical and Biological Engineering, Göteborg, Sweden

The yeast Saccharomyces cerevisiae is a well studied eukaryotic organism used in many model experiments. Several genome-scale models of the yeast metabolic network exist and these can be used for performing in silico simulations of the system and as a scaffold for omics data integration. All the previous reconstructions of the yeast metabolic network differ in scope and the way they were constructed and difference in metabolite nomenclature makes mapping between the different models difficult. Therefore, there is a need for a new, consensus model that describes as many cellular mechanisms as possible, and also suits good for simulations. We have constructed a new, more comprehensive, genome-scale metabolic model for yeast. The model was based on the consensus network, and a previous model, iN800 which includes useful information about lipid metabolism. Known transcription factors and gene regulations were also included in order to improve the prediction power of the model. In order to achieve an even more comprehensive description of the cellular mechanisms going on in yeast a stoichiometric model including the genes and reactions involved in the protein synthesis were constructed. This includes many processes, e.g. transcription, translation, splicing, mRNA degradation, post-translational modifications etc. The reconstruction of this network was made by database searches and literature mining. The model was validated by comparing simulations to experimental data from chemostat fermentations under different conditions, e.g. C-limited and N-limited growth. The result is a model that can simulate the system and also serve as a good platform for omics data integration.

B1.23
Energetic metabolism involved in the regulation of porcine spermatozoa motility
M. J. Bragado1, D. Martin-Hidalgo2, A. Hurtado de Llera2, M. C. Gil2 and L. J. García-Marín1
1Universidad de Extremadura, Bioquímica y Biología Molecular y Genética, Cáceres, Spain, 2Universidad de Extremadura, Medicina Animal, Cáceres, Spain, 3Universidad de Extremadura, SINTREP, Cáceres, Spain

There are two pathways for ATP production in mammalian spermatozoa, glycolysis and mitochondrial respiration. Their role in the control of one of the main spermatozoa functions, motility, remains under investigation. Our previous results showed that porcine spermatozoa motility is regulated by glycolgen synthase kinase-3γ (GSK3γ). Semen from 8 Duroc boars was used and spermatozoa motility was analyzed by computer assisted ISAS® program. GSK3β phosphorylation was evaluated by western blotting. Treatment with the glycolysis inhibitor, the glucose analogue 2-deoxy-D-glucose (DOG) led to a slight reduction in porcine spermatozoa motility (decreases in % motile and % rapid spermatozoa) together with a clearly more progressive movement (significant increases in LIN and STR coefficients and in the % of progressive spermatozoa). However, treatment with mitochondria activity inhibitors rotenone and cyanide caused almost a complete inhibition in porcine spermatozoa motility parameters. In parallel to a blockade in spermatozoa motility, mitochondria activity inhibitors led to a reduction in the phosphorylation levels of the GSK3γ isoform in these male germ cells with a non appreciable effect in GSK3β isoform. However, treatment of spermatozoa with DOG had no appreciable effect in GSK3γ/β phosphorylation. In summary, our results suggest that the porcine spermatozoa motility is clearly dependent on the mitochondrial activity. However, the glycolysis seems to be involved in the regulation of the porcine spermatozoa progressive movement. Supported: JUEX-PR107A100 (Spain).

B1.24
Proteome analysis of moderately halophilic bacteria response to oxygen limitation
S. Ceylan, B. S. Akbulut and D. Kazan
Marmara University, Bioengineering, Istanbul, Turkey

Halophilic microorganisms, which have ability to live in saline environments, provide a variety of applications in different fields
of biotechnology. The technical applications of compatible solutes, biopolymers and enzymes that are active and stable at high salt contents are important for industry. The biodegradation of some organic pollutants and the production of alternative energy are other fields of applications of these microorganisms. Moderately halophilic bacteria is interest because of its ability to grow in a wide range of salt concentration from 3% to 15%. Proteomics is the characterization of all proteins in a biological system. This study aims to investigate response mechanism of moderately halophilic bacteria, Halomonas sp. ADD12, to oxygen limitation using proteomic tools. Strain ADD12 was isolated from Camalti Saltern, Turkey, and has an ability to grow between 5% and 25% NaCl concentrations and it can degrade phenol. 16s rDNA analyses showed that isolate ADD12 was belong to Halomonas genus. To examine oxygen limitation response, Strain ADD12 was grown on aerobic and semi-aerobic conditions. Growth curves were determined at these conditions and microorganisms were harvested at the end of exponential phase. For both conditions two dimensional gel electrophoresis were conducted and proteome maps were visualized. It was seen from the maps, there was an inhibition of whole protein and enzyme production systems. The differently expressed proteins were analyzed by Proteome-Analyzer 4700. Proteins involved in oxygen limitation response were determined. This work was supported by Marmara University, Research Fund Projects FEN-C-DRP-181208-0286.

B1.25
Phytosteryl ferulates affect cross-talk between mTOR and NF-kappaB cascade
R. Nagasaka1, K. Ohara2 and H. Ushio3
1Gunma National College of Technology, Department of Chemistry and Materials Science, Gunma, Japan, 2Tokyo University of Marine Science & Technology, Tokyo, Japan
Hydroxycinnamic acid derivatives, observed ubiquitously in plants, have some physiological functions. We have recently reported that phytosterol ferulates inhibited DNA-binding of NF-xB. mTOR (mammalian target of rapamycin) was shown to be a key kinase acting downstream of the activation of the phosphatidylinositol 3 kinase (PI3K). Cumulative evidence supports the hypothesis that mTOR acts as a master switch of cellular catabolism and anabolism, signalling cells to expand, grow and proliferate. In this study, we investigated rapamycin effects upon mTOR and NF-kappaB cascade. The results will show population and resource utilization dynamics in different conditions and we will discuss implications of those results in the evolution of metabolic strategies.

B1.26
Metabolic trade-offs in competition for resources between Saccharomyces cerevisiae and Kluyveromyces lactis
S. Bratulic1 and G. Vemuri2
1Chalmers University of Technology, Gothenburg, Sweden, 2Chalmers University of Technology, Department of Chemical and Biochemical Engineering, Gothenburg, Sweden
One of the postulates of the theory of evolution is the competition for limited resources. Previous studies have shown that there is a trade-off between yield and rate in resource utilization and ATP production pathways, implying that different metabolic strategies may result in different competition outcomes. Two main metabolic strategies used by micro-organisms are respiration and fermentation. Respiration maximizes the efficiency of ATP production, resulting in increased yields at the expense of the rate. The whole population benefits from this cooperative use of resources, but individual fitness is reduced. In contrast, fermentation is an inefficient ATP production strategy, but it runs at a higher rate. This is a “cheat” strategy because the fitness of fermenting individuals is increased at the expense of the average population fitness. Our goal was to study competition between the two and find conditions, such as resource availability, population and spatial structure, that favor dominance of those strategies. We used Saccharomyces cerevisiae and Kluyveromyces lactis tagged with different fluorescent proteins as models for fermentation and respiration, respectively. We experimentally determined physiological parameters by individual cultivations and then used those parameters in dynamic spatial and non-spatial models to simulate competition experiments. To validate our models, we studied population dynamics in mixed batch, chemostat and spatially structured cultures. The results will show population and resource utilization dynamics in different conditions and we will discuss implications of those results in the evolution of metabolic strategies.

B1.27
Levosimendan protect against ischemia-reperfusion injury in the isolated perfused rat heart model
N. N. Turan1, G. Sahani1, T. Ozcelikay2, A. T. Ulas1 and M. Ark2
1Pharmacology Department, University of Gazi, Faculty of Pharmacy, Ankara, Turkey, 2Pharmacology Department, University of Ankara, Faculty of Pharmacy, Ankara, Turkey
Levosimendan, calcium-sensitizing agent, increases contractility by enhancing the sensitivity of myofilaments to calcium by binding to the C cardiac troponin in cardiac muscle in a calcium-dependent way. The aim of this study was to investigate whether levosimendan has protective effects on myocardial ischemia-reperfusion injury. After anesthesia heart were placed in cold Krebs-Heinseleit and mounted on Langendorff apparatus. Hearts were assigned into three groups: first, after 30 minute ischemia in order to induce ischemic postconditioning 3 times 10 second ischemia separated by 10 second reperfusion protocol was used; second, non-postconditioned group was used as control; and third, 10-8 mol levosimendan was added to the reperfusion fluid after 30 minute ischemia in order to induce chemical postconditioning. In all these groups, 30 minute global ischemia was followed by 120 minute reperfusion. cTnT and cTnI expression were made with Western Blot and apoptotic differences were matched with TUNEL method. 10-8 mol levo- simendan and ischemic post-conditioning significantly increased cTnT and cTnI expression compared with control hearts. Also, ischemic post-conditioning significantly reduced levosimendan and ischemic post-conditioning significantly increased cTnT expression compared with Levosimendan. Levosimendan significantly reduced apoptosis compared with control and ischemic post-conditioning groups. Levosimendan has a cardioprotective effect when administered after ischemia against ischemia-reperfusion injury. Protective effect is associated with decreased apoptosis.
B1.28 Genome sequencing, annotation and analysis of *Saccharomyces cerevisiae* from genotype to phenotype for industrial biotechnology applications


Department of Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden, Merck Research Labs, Bioprocess Research and Development, West Point, PA, USA

*Saccharomyces cerevisiae* is the most well characterized eukaryote and the preferred microbial cell factory for the industrial biotechnology applications. Genome sequencing of *S. cerevisiae* strain S288C, the first eukaryote genome sequence reported, provided a framework for gene annotation through functional genomics. Of particular relevance for metabolic engineering and industrial biotechnology, an annotated genome sequence was a prerequisite for genome-scale metabolic network reconstructions. Since the genome sequence of *S. cerevisiae* was released, the technologies have advanced and costs associated with whole genome sequencing have decreased substantially. Therefore this has opened an opportunity for the use of genome sequencing as a commonplace tool for advancing functional genomics and strain engineering. In this work we propose that high-throughput genome sequencing, annotation and analysis of *S. cerevisiae* strain CEN.PK 113-7D as commonly used in laboratory. We present the developed genome browser and single nucleotide polymorphism (SNP) database of *S. cerevisiae* strain CEN.PK 113-7D. To directly link genotype to phenotype information, then cellular behavior was characterized in well-controlled batch fermentations on glucose and galactose, complimented with transcriptome analysis. We demonstrate that S288C, the strain utilized for the publically available *S. cerevisiae* genome sequence, exhibits a typical *S. cerevisiae* behavior related to central carbon metabolism as compared to CEN.PK113-7D, a common laboratory strain for industrial biotechnology applications.

B1.29 Are Lewis rats less efficient than Sprague-Dawley rats to cope with heat stress?

V. Michel, J.-B. Drouet, A. Alonso, N. Fidier, R. Maury and F. Canini

IRIBA-La Tronche-CRSSA, La Tronche, France

Heat is an environmental factor threatening health and even life. The time during which an organism is able to cope efficiently with heat stress (heat tolerance) is highly variable among individuals. Glucocorticoid signalling is probably involved as hypothalamic-pituitary-adrenocortical (HPA) axis impairment is associated with decreased heat tolerance. Energy metabolism is one of the possible relay between HPA axis activation and heat tolerance. *Sprague-Dawley (SD)* and *Lewis (L)* rats, differing by their HPA axis regulation, were instrumented for body temperature (**T** body) recording, then exposed during 24 hours in warm (**Ta** = 33°C) or neutral (**Ta** = 22°C) environment. At the end, frontal cortex and peripheral metabolisms were analyzed. L rats had a lesser **T** body throughout heat exposure. Blood corticosterone was similar between strains in each condition. At neutrality, L rats had higher blood glycerol level than SD rats (**p < 0.05**). In the heat, L rats had lower glycemia (**p < 0.001**) and lactatemia (**p < 0.001**) levels but higher blood glycerol (**p < 0.10**) and free fatty acids (**p < 0.001**) levels than SD rats. At neutrality, no difference was observed between strains in brain content of 5-AMP-activated protein kinase (AMPKα2) and phosphorylated protein (p-AMPKα2), acetyl-CoA carboxylase protein (ACC) and phosphorylated protein (p-ACC). In the heat, L rats exhibited higher AMPKα2 (**p < 0.001**), lower p-AMPKα2/AMPKα2 level (**p < 0.10**), but similar ACC and p-ACC/ACC levels than SD rats. Altogether, it suggests that L rats favoured glycolytic pathway, whereas SD rats had shift to lipicid pathway, a more adapted metabolism. These metabolic changes are not associated with substantial difference in brain energy status.

B1.30 The effect of prenatal/postnatal nicotine administration on oxidant/antioxidant status in rats

S. Mjzrak, G. Erkan Alper, O. Caglayan and C. Yljma

Ege University School of Medicine, Medical Biochemistry, Izmir, Turkey, Kyrgyz Kale University School of Medicine, Medical Biochemistry, Kyrgyz Kale, Turkey, Ege University School of Medicine, Internal Medicine, Endocrinology, Izmir, Turkey

Cigarette smoking is common in societies worldwide and has been identified as injurious to human health. One difficulty lies in the fact that cigarette smoke is composed of lots of toxic chemicals including nicotine that causes addiction. Until now, rare studies have examined the effect of nicotine on oxidative stress in infants. However the results are sophisticated. For this reason, we aimed to determine the effect of long term nicotine exposure on oxidant and antioxidant system during prenatal and postnatal period. In our study, Swiss Albino rats were divided into three groups as control (**n = 10**), low dose nicotine (**n = 10** and high dose nicotine (**n = 9**). Nicotine hydrogen tartrate was given to rats in nicotine groups per oral in tap water freshly prepared everyday for 12 months in a dosage of 2.22 mg/kg body weight (BW) and 33.3 mg/kg BW, respectively. No significant difference in plasma level of total antioxidant status, 8-hydroxy-deoxyguanosine, total sulphhydril groups and erythrocyte lysate Malondialdehyde(MDA), Catalase and superoxide dismutase (SOD) levels in comparison of controls and nicotine groups. Only plasma cotinine, metabolite of nicotine levels (**p < 0.01**) and liver MDA levels higher (**p < 0.05**) and kidney SOD activity was lower (**p < 0.05**) in nicotine groups in correlation with nicotine dosage. Since, 12% of the pregnant population continue to smoke cigarettes, low dose nicotine replacement therapy during pregnancy may be better than smoking.

B1.31 Physiological characterization of *Aspergillus oryzae*

C. Knauf, W. Vongsangnak and J. Nielsen

Department of Chemical and Biological Engineering, Chalmers University of Technology, Göteborg, Sweden

The filamentous fungus *Aspergillus oryzae* is widely used as a microbial cell factory for large scale heterologous protein production. *A. oryzae* is also known as a natural organic acid producer. However, the metabolisms and regulations of organic acid production in *A. oryzae* are poorly characterized. Furthermore, the media composition has a major impact on the performance of this organism and the economic feasibility of an industrial fermentation process. We therefore evaluated the utilization of different nitrogen and carbon sources by *A. oryzae*. In this study, we aim for a deep investigation of the cellular mechanisms of the utilization of different nitrogen and carbon sources. Firstly, we performed batch cultivation on different carbon sources (e.g. glucose and glycerol) and nitrogen sources (e.g. peptone and di-ammonium phosphate). Further, genome-
wide expression analysis was performed to analyze the transcriptome under these different conditions and to identify key players in the metabolism and regulation. An updated genome-scale metabolic model of *A. oryzae* was used as a scaffold for simulations under different conditions. The embedding of the omics data into the *in silico* work was found to accelerate the optimization process of future industrial applications of this filamentous fungi. The derived results will be used for the construction of a strain that is performing as well on the cheaper nitrogen source.

**B1.32**

**Cloning and expression studies of putative Rhizopus oryzae hexokinase genes in Saccharomyces cerevisiae**

E. Alagöz, S. Acar, M. Yucel and H. Hamamec

1Middle East Technical University, Biotechnology, Ankara, Turkey, 2University Of California Davis, Molecular and Cellular Biology, California, USA, 3Middle East Technical University, Biological Sciences, Ankara, Turkey, 4Middle East Technical University, Food Engineering, Ankara, Turkey

*Rhizopus oryzae* is a filamentous fungus which can ferment sugar to ethanol, and lactic acid. Increasing demand of the world for use of renewable carbon sources has put this and similar organisms into a position where the biotechnology industries are more and more interested with these organisms. *R. oryzae* can grow on some renewable carbon sources which makes it a good candidate for production of ethanol as well as lactic acid. In the light of studies done for *Saccharomyces cerevisiae*, a well studied industrial and model organism, we are trying to investigate the regulation of glycolytic pathway of *R. oryzae*. In this study, we have used data from annotated genome database of *R. oryzae* to clone hexokinase genes of this organism. By doing blast search we obtained ten probable genes having two conserved domains “hexokinase 1” and “hexokinase 2”. These domains are present in all of the known hexokinase sequences cloned and characterized from other organisms including *S. cerevisiae*, *Aspergillus niger* and *Hansenula polymorpha*. We obtained PCR products of the five of the probable genes with the primers designed for them and these were also replicated by using *Taq* polymerase. At the end of the sequenc

**B1.33**

**Changes at transcriptome level during host-parasite interaction using Perkinsus olseni as a model**

R. B. Leite and M. L. Cancela

CCMAR, Centre of Marines Sciences, University of Algarve, Faro, Portugal

Bivalves represent one of the most important marine resources worldwide. Parasitic infections like the one caused by *Perkinsus sp.*, a member of the Alveolata group, which also comprises parasites highly relevant for medical and veterinary purposes like Plasmodium and Toxoplasma, significantly decrease shellfish production inducing severe economical/ecological damages. Here we report the transcriptomic response of *Perkinsus sp.* to its natural host the clam *Ruditapes decussatus*, revealing at the same time the complexity of the pathways present in this unicellular eukaryote. Illumina and 454 sequencing were used simultaneously to cover up an important part of Perkinsus transcriptomic. Two datasets, comprising *P. olseni* transcripts collected under normal culture conditions and when exposed to clam hemolymph, were sequenced using an illumina. Four hundred and fifty-four sequencing of a normalized cDNA library was used to fill up gaps between illumina contigs using hybrid sequencing assembling. This first attempt to characterize the mechanisms involved in host-parasite interaction using high-throughput sequencing unveiled the complexity behind these events, suggesting that this species might be less dependent on its host for its metabolism than its relatives and allowed the identification of some important chemotherapeutic target at metabolic level. The combination of this data, together with the annotated genome of *P. marinus*, will provide a set of genomic tools to study host-parasite interactions in this marine model system, for which there is a growing interest worldwide.

**Acknowledgments:** Project IsoPerk/PTDC/CVT/72083/2006 from FCT (Portuguese Science and Technology Foundation).

**B1.34**

**Combined effects of melatonin with epirubicin and doxorubicin in MCF-7 breast cancer cell lines**

B. Akpinar, Z. Altun, H. Ates and S. Kocturk

1Middle East Technical University, School of Medicine, Medical Biochemistry, Izmir, Turkey, 29 Eylul University, Institute of Oncology, Oncology, Izmir, Turkey, 3Eylul University, Hematology, Izmir, Turkey, 4Eylul University, Medical Biochemistry, Izmir, Turkey

Side effects of chemotherapeutic agents cause serious problems in cancer treatment. Thus new combined treatment strategies have been widely studied. Melatonin has been shown to inhibit cell proliferation in MCF-7 cell lines. The purpose of this study was to investigate antiproliferative effects of melatonin combined with epirubicin and doxorubicin in time and dose dependent manner on MCF-7. We have also aimed to determine the characteristics of combined effects of melatonin with epirubicin and doxorubicin. For these purposes MCF-7 cells were treated with different doses of melatonin (0.5–1500 nM), epirubicin (10–50 μM) and doxorubicin (10–50 μM) for 24–48–72 hours. MTT assays were carried out to investigate the effects of treatments on cell viability. MTT assays demonstrated that cell death was occurred in a dose and time dependent manner in epirubicin and doxorubicin treatments. Our results show that 30 μM concentrations of doxorubicin and epirubicin caused 41% and 50% inhibition of cell viability at 48 hours respectively. Combined effect of 1000 nM melatonin with 30 μM epirubicin or 30 μM doxorubicin enhanced cell death the ratio of 10–15% at 48 hours. Western blot data of caspase 3 also confirmed these results. Our data suggests that all treatments induce cell death in MCF-7 and combined effect of 1000 nM melatonin with 30 μM epirubicin or 30 μM doxorubicin enhance cell death. Although further studies needed we suggest that melatonin can be a candidate as an additive agent in chemotherapeutics and these results may be a basis for the researches to determine anticancer use of melatonin in breast cancer.
The antitumour derivative, C-1748, affects CYP3A4: crosstalk between drug metabolism, CYP3A4 expression and enzymatic activity

M. Niemira¹, A. Brillowska-Dabrowska¹, J. Konopa¹ and Z. Mazerska¹
¹Gdansk University of Technology, Department of Pharmaceutical Technology and Biochemistry, Gdansk, Poland, ²Gdansk University of Technology, Department of Microbiology, Gdansk, Poland

Individual variety in drug metabolism is one of the major causes of treatment failures during cancer therapy. Understanding of the relations between changes in the level of gene expression and catalytic activity of enzymes involved in metabolism of the drugs might lead to the development of novel strategies for regulation of these processes. Therefore, much attention has focused on the induction or inhibition of CYP3A4 cytochrome P450 isoenzyme, which is involved in the biotransformation of more than 50% of all drugs clinically used. It is known that expression of CYP3A4 is affected by different factors from genetic polymorphism to xenobiotic exposure. We have previously demonstrated that C-1748 was transformed in HepG2 cells and the number and concentration of metabolites were associated with CYP3A4 protein level. To confirm this findings, we determined the effect of C-1748 on the expression and enzymatic activity of CYP3A4 in HepG2 cells. Using a combination of reverse transcriptase RT-PCR and HPLC analysis, we found that the studied compound significantly decreased the expression of CYP3A4 mRNA and also enzymatic activity of this enzyme at EC50 concentration, after a short period of time. We demonstrated that this effect of C-1748 was reflected in the protein levels. However, cell incubation for a longer time with C-1748 resulted in an increase of CYP3A4 mRNA expression levels and its enzymatic activity relative to control. The presented studies showed for the first time that C-1748 affected the protein level, gene expression and enzymatic activity of CYP3A4 in HepG2 cell line in the different way that observed for other antitumour agents.
B2 – Signal Transduction

B2.01
Insulin and heparin bioactivation of 3D NWPF discs by water/O2 plasma for l929 fibroblast cell cultivation
H. Türköğlu, M. Manolache, M. Gümrükçerelolu
1Atılım Üniversitesi, Materials Engineering, Ankara, Turkey, 2Wisconsin/Madison University, Center for Plasma-Aided Manufacturing Unit (CPAM), Madison, USA, 3Hacettepe University, Chemical Engineering and Bioengineering Departments, Ankara, Turkey

The goal of this study was to obtain COOH functionalities on the surface of 3D, non woven polyester fabric (NWPF) discs by using low pressure water/O2 plasma assisted treatment. The plasma treatment was performed in a cylindrical, capacitively coupled RF-plasma-reactor in three steps: H2O/O2-plasma treatment; in situ (oxalyl chloride vapors) gas/solid reaction to convert –OH functionalities into –COCl groups; and hydrolysis for final-COOH functionalities. DeO (Design of Experiment) software program was used for the optimization of plasma modification. COOH and OH functionalities on modified surfaces were detected quantitatively by using fluorescent labeling technique and an UVX 300G sensor. Plasma treated and oxalyl chloride functionalized samples were biologically activated with insulin or heparin molecules by using spacer PEO (polyoxyethylene bis amine). Success of immobilization process was checked qualitatively with ESCA analysis. The average amount of immobilized insulin and heparin onto NWPF surfaces were determined as 146.09 and 4.81 nmol/cm², respectively. Our results showed that water/O2 plasma assisted treatment worked very well for functionalization (COOH) and bioactivation of 3D NWPF discs comparing with wet-chemistry methods. Cell culture experiments indicated that functionalization of NWPF discs and/or nanotopographies on the disc surfaces were effective on L929 cell adhesion and proliferation.

B2.02
Insulin like growth factor-1 stimulated erythropoietin production is mediated by ERK kinase pathway
P. Kohtary, H. Stier, T. Lees, B. Parker, P. Lee and M. Del Monte
University of Michigan, Ann Arbor, MI, USA

Purpose: Erythropoietin (Epo) is a known angiogenic factor. It is postulated that it may play a role in proliferative diabetic retinopathy (PDR). Since insulin like growth factor-1 (IGF-1) has also been implicated in the development of proliferative diabetic retinopathy, we have investigated the effect of IGF-1 on intracellular Epo production.

Methods: hRPE cell cultures were established from human eyes. Cell viability and proliferation were determined by the trypan blue exclusion method (T) and by thymidine incorporation (3H-thy). Localization of Epo in hRPE cells was examined by immunohistochemistry. Endogenous 14C-methionine-Epo (14C-Epo) production was quantitated by immunoprecipitation. hRPE cells were also treated with PD98059 (PD), a selective inhibitor of ERK kinase. Data were analyzed by Student t test.

Results: hRPE cell proliferation was stimulated by IGF-1 in a dose dependent manner as determined by 3H-thy and T. PD significantly inhibited IGF-1 stimulated 3H-thy incorporation in these cultures: (990.6 ± 187.46 versus 1296.4 ± 209.75, CPM ± SEM, p < 0.05, N = 4). IGF-1 also stimulated immunoprecipitated 14C-Epo in a dose dependant manner and this stimulation was also inhibited by PD (1481.93 ± 385.20 versus 5636.94 ± 2212.04, CPM ± SEM, p < 0.05, N = 5). Immunohistochemical studies demonstrated more densely stained Epo-positive cells in presence IGF-1 than in control and IGF-1 + PD.

Conclusions: These data suggest that IGF-1 is mitogenic in hRPE cells and the ERK kinase may be involved in mitogenic effect and in Epo synthesis. Development of an ERK Kinase inhibitor may be of therapeutic value in the treatment of proliferative ocular complications. Supported by the Skillman Foundation.

B2.03
Assigning the property of electricity conduction to the DNA molecule can explain many problems with signal transduction and transcription factors (TF)
K. Pirkalani, Z. Talea Rad, B. Khodabakhsh, R. Khodabakhsh, H. Haddadi, I. Khodadad, D. Safdarian and H. Bigdeli
1Mehr Medical Group, Endocrinology/Internal Medicine/Molecular Biology, Tehran, Islamic Republic of Iran, 2Mehr Medical Group, Endocrinology/Gynecology, Tehran, Islamic Republic of Iran, 3Mehr Medical Group, Internal Medicine/Mathematics/Statistics, Tehran, Islamic Republic of Iran, 4Mehr Medical Group, Mathematics, Tehran, Islamic Republic of Iran, 5Mehr Medical Group, Mathematical Biology, Tehran, Islamic Republic of Iran, 6Mehr Medical Group, Pharmacy, Tehran, Islamic Republic of Iran, 7Mehr Medical Group, Laboratory Medicine/Pathology, Tehran, Islamic Republic of Iran

Objective: To solve the problem of sequence recognition of TF by trial and error which is not economic we tried to find novel mechanical and electrical properties within the DNA molecules.

Materials and Methods: Based on data of Shirakawa et.al, we plotted new molecular conformations with different angles and axes rotations of DNA to find two molecular landmarks for electricity conduction namely alternating single and double bounds of the Sp2 hybridization nature and intermolecular channels that can harbor free electrons. We questioned whether tautomery of the bases can open the double stranded DNA long before topoisomerase enzymes come into effect. Besides, the distance between double and single bounds were compared between different electricity conducting polymers (polypyrole, polystyrene etc.) and DNA.

Results: DNA can cautiously be regarded an electricity conducting polymer which is doped with the histones. The wave of electricity causes a slower mechanical wave that melts (exposes) DNA at special sites to facilitates recognition by TFs and makes the histones. The time lag between electrical and mechanical waves is of utmost importance in this theory.

Conclusions: (1) DNA is probably an electricity conducting biopolymer which increases speed of response and facilitates recognition of targets by TFs.
(2) Entrance of specific TF through nuclear pores is the first signal for transcription in form of electrical excitation and gene exposure.
(3) This mechanism reduces the number of needed TF and makes general TFs more cell-specific; a few TFs can erase a series of gene inductions in different cells.
**B2.04 – Signal Transduction**

**B2.05**

The eIF2α kinase PKR modulates the hypoxic response by Stat3-dependent transcriptional suppression of HIF-1α expression

A. I. Papadakis1, E. Paraskeva2, P. Pois3, H. Muaddi1, L. Rapti3, K. Pantopoulos1, G. Simos2 and E. Koromilas A.6

1McGill University/Lady Davis Institute for Medical Research, 2Medicine: Division of Experimental Medicine, Montreal, Canada, 3University of Thessaly, Laboratory of Physiology, School of Medicine, Larissa, Greece, 4Lady Davis Institute for Medical Research, McGill University, Montreal, Canada, 5Queen’s University, Microbiology and Immunology and Pathology and Molecular Medicine, and Queen’s University Cancer Institute, Kingston, Canada, 6University of Thessaly, Laboratory of Biochemistry, School of Medicine, and Institute of Biomedical Research and Technology, Larissa, Greece, 7McGill University/Lady Davis Institute for Medical Research, Department of Oncology, Faculty of Medicine, McGill University, Montreal, Canada

Within the tumor microenvironment, hypoxic conditions trigger important cellular responses which promote angiogenesis, metabolic reprogramming and tumor progression. Hypoxia-inducible Factor-1α is a key transcription factor that is activated by hypoxic conditions to induce the expression of genes that contribute to tumor growth. In addition, cells respond to hypoxic stress not only by upregulating HIF-1α but also by inhibiting global protein synthesis through several mechanisms including the phosphorylation of the alpha subunit of eIF2α at serine (S) 51. In mammalian cells, phosphorylation of eIF2α is mediated by a family of kinases which respond to distinct forms of stress. We investigated whether these kinases are involved in the regulation of HIF-1α. Our results demonstrate for the first time that the double-stranded (ds) RNA-dependent protein kinase PKR plays a significant role in suppressing HIF-1α expression specifically at the transcriptional level. Transcriptional repression of the HIF1A gene by PKR is sufficient to impair the upregulation of HIF-1α and its downstream genes under hypoxic conditions. Inhibition of HIF1A gene transcription by PKR is independent of eIF2α phosphorylation but requires inhibition of the activity of the signal transducer and activator of transcription 3 (Stat3). We also provide evidence that transcriptional repression of HIF-1α requires the T cell protein tyrosine phosphatase (TC-PTP), which acts downstream of PKR to suppress Stat3. Our data reveal a novel tumor suppressor function of PKR through the inhibition of Stat3 activity and HIF-1α expression, which takes place independently of eIF2α phosphorylation.

**B2.06**

Screening a kinase inhibitor library for multiple target inhibitors of VEGFR2 and PDGFRβ

I. Kurko3, K. Penzes2, B. Szokol2, G. Nemeth2, C. Szantai-Kis2, L. Orfi2, T. Vantus2 and G. Keri2

1Pathobiotechnology Research Group, Hungarian Academy of Sciences, Semmelweis University, Budapest, Hungary, 2Vichem Chemie Research Ltd., Budapest, Hungary, 3Department of Pharmaceutical Chemistry, Semmelweis University, Budapest, Hungary

**Background:** VEGFR2 and PDGFRβ play an important role in tumor angiogenesis, in tumor growth and metastasis. Targeting these two signaling pathways may prevent the tumor progres-
B2.08 The antiproliferative effect of known and novel PLK1 inhibitors on different cancer cell lines

K. Penzes1, I. Kurko1, G. Nemeth2, B. Szokol2, C. Szantai-Kis2, A. Horvath1, T. Vantus3, G. Bokonyi1, L. Orfi4 and G. Keri5
1The Pathobiochemistry Research Group, Hungarian Academy of Sciences, Semmelweis University, Budapest, Hungary, 2Vichem Chemie Ltd., Budapest, Hungary

Polo like kinase 1 (PLK1) is essential in the regulation of mitotic entry, spindle formation, chromosome segregation and cytokinesis during mitosis. High expression and activity of this kinase occurs in many human tumor types. The inhibition of PLK1 activity results in their mitotic arrest and apoptosis thus the regression of tumors. We screened known and novel PLK1 inhibitors on 18 different cell lines to characterize their antitumor effect. Among these cancer cell lines were two (A431 and A549) with mutation in the PKL gene and one of them (Jurkat) showed high level of PLK1 protein. The viability of the cells was measured using a luciferase-coupled ATP quantification assay (CellTiter-Glo, Promega, Madison, WI). In this assay, luminescent signal is proportional to the amount of ATP and thus to the number of metabolically competent cells. The cells were treated with 10 μM of each of the PLK1 inhibitors, and the luminescent signal was recorded after 72 hour incubation. The compounds were further tested to define their EC50 values on all cell lines. These molecules have shown promising results in the inhibition of cell proliferation on each cell line with known genomic imprinting. There were no significant differences in the EC50 values in case of cells with or without PLK mutations. Our results indicate the effectiveness of the novel and known molecules and provide useful new data of their inhibition potential on 18 cancer cell lines.

B2.09 Biochemical characterization of novel PKD1 inhibitors using kinase assays and cellular studies

A. Varga1, B. Szokol2, G. Nemeth2, L. Orfi3, C. Szantai-Kis2, Z. Horvath2, Z. Varga2, G. Keri4 and T. Vantus5
1Pathobiochemistry Research Group, Hungarian Academy of Sciences, Semmelweis University, Budapest, Hungary, 2Vichem Chemie Ltd., Budapest, Hungary, 3Vichem Chemie Research Ltd., Department of Pharmaceutical Chemistry, Semmelweis University, Budapest, Hungary, 4Vichem Chemie Research Ltd., Pathobiochemistry Research Group, Hungarian Academy of Sciences, Semmelweis University, Budapest, Hungary

Background: Protein kinase D1 (PKD1) is the member of protein kinase D, a novel family of serine/threonine kinases targeted in development of novel therapeutics on PKD1. In this assay, luminescent signal is proportional to the amount of ATP and thus to the number of metabolically competent cells. The cells were treated with 10 μM of each of the PKD1 inhibitors, and the luminescent signal was recorded after 72 hour incubation. The compounds were further tested to define their EC50 values on all cell lines. These molecules have shown promising results in the inhibition of cell proliferation on each cell line with known genomic imprinting. There were no significant differences in the EC50 values in case of cells with or without PKD mutations. Our results indicate the effectiveness of the novel and known molecules and provide useful new data of their inhibition potential on 18 cancer cell lines.

B2.10 The antipsychotic drug haloperidol inhibits cholesterol biosynthesis, disrupts lipid rafts and impairs insulin signalling in SH-SY5Y cells

J. Sanchez-Wandelmen1, A. Canfran-Duque1, E. Casado M.2, G. de la Peña1, M. Lerma1, L. Huertas1, N. Quesada2, P. Mariscal1, A. Martin-Hidalgo3, O. Pastor3, M. A. Lasuncion4 and R. Busto5
1Servicio Bioquímica-Investigación, Hospital Ramón y Cajal, Madrid, Spain, 2Servicio Bioquímica-Investigación, Hospital Ramón y Cajal and CIBERONB, Madrid, Spain, 3Servicio Bioquímica Clínica, Hospital Ramón y Cajal, Madrid, Spain, 4Servicio Bioquímica-Investigación, Hospital Ramón y Cajal, CIBERONB and Universidad de Alcalá, Madrid, Spain

Conventional antipsychotics, such as haloperidol, are drugs used in schizophrenia, though they are not free of side effects. Cholesterol is concentrated in lipid rafts which are seen as major platforms for signal transduction events such as insulin receptor. Objective: To examine the effects of haloperidol on cholesterol synthesis, lipid raft composition and insulin signalling in a neuroblastoma cell line. Methods: Sterols were analyzed by reversed-phase HPLC. Lipid rafts were isolated by sucrose gradient fractionation and analyzed by western blot. The cells were incubated with Dil-LDL and immunodetected by fluorescence microscopy. Results: We found that haloperidol inhibited cholesterol synthesis affecting Δ7-reductase > Δ8, 7-isomerase > Δ14-reductase activities, in that order, which causes a change in the sterol composition of lipid raft and non-raft membrane domains. These effects were accompanied by profound alteration of lipid rafts functionality and impaired insulin signalling. Free cholesterol abrogated the effect of haloperidol on insulin signalling. In contrast, LDL was much less effective in preventing the effects of haloperidol, which is attributed to the drug's inhibition of intracellular vesicular trafficking. Conclusions: The disruption of lipid rafts and subsequent alteration of raft-dependent insulin signalling, as a consequence of both the inhibition of cholesterol synthesis and interference in intracellular cholesterol transport, should be considered as additional actions possibly involved in the undesirable effects of haloperidol. Acknowledgment: Supported by MICINN (SAF2008-01104) and CIBEROnb, ISCIII, Spain.

B2.11 Functional analysis of P2Y12 randomized libraries in yeast Saccharomyces cerevisiae

V. Ignatovica, K. Megnis and J. Klovins
Latvian Biomedical Research and Study centre, Riga, Latvia

G protein coupled receptors (GPCR) are targets of 40% of therapeutics currently used in clinical practice. Purinergic receptor 12 (P2Y12) belongs to GPCR and is a crucial factor for blood coagulation. Several antiplatelet agents: prasugrel, ticlopidine, clopidogrel that are widely used for preventing of stent thrombosis take their action via P2Y12. Functional characterization of P2Y12 regions that are involved in ligand binding is very important in development of novel therapeutics on P2Y12. In this study several amino acid positions of P2Y12 that are involved in ligand binding were characterised. First randomised libraries of P2Y12 were created in PCR using random primers. Obtained and determined their antiproliferative, apoptotic effects and ADME parameters.
randomised libraries of P2Y12 were cloned into yeast expression vector and transformed into corresponding Saccharomyces cerevisiae strain containing genetic modification of the mating-signal transduction pathway that enables agonist dependent growth on special selective medium. Variants of randomised P2Y12 were isolated, sequenced and tested for functional activity by natural agonist ADP. Analysis of randomised libraries showed that heterogeneity of obtained variants highly depends on primer heterogeneity that may affect occurrence of some P2Y12 variants more often than others. In summary some of analysed amino acids have important role in P2Y12 action and must be considered in design of new therapeutics on P2Y12.

B2.12
Abstract withdrawn

B2.13
The Drosophila EcR DNA-binding domain (EcRDBD) with its C-terminal extension possesses a hormone-dependent NLS activity

M. Orłowski1, B. Greb-Markiewicz1, T. Gwozdz1, J. Dutko-Gwozdz1, J. Dobrucki2 and A Ozyhar1
1Department of Biochemistry, Wrocław University of Technology, Faculty of Chemistry, Wrocław, Poland, 2Jagiellonian University, Division of Cell Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Krakow, Poland

The insect ecdysteroid receptor is a transcription factor complex comprised of two nuclear receptors, the 20-hydroxyecdysone receptor (EcR) and the Ultraspiracle protein (Usp). EcR and its ligands are attractive targets for development of gene switches in humans and in agricultural insect control. Our previous study suggested that N-terminal region of EcR has the significant impact on subcellular distribution of all EcR isoforms. In order to define nuclear localization signals (NLSs), series of EcR mutants tagged with yellow and cyan fluorescent proteins (YFP and CFP) were prepared and examined in different cell lines. Our data revealed the presence of the novel NLS proteins (YFP and CFP) were prepared and examined in different cell lines. Our data revealed the presence of the novel NLS sequence located close to the N-terminus. In THP-1 leukemic cells, dependent on the presence of nuclear localization signal sequence located close to the N-terminus, and its nuclear import is entirely dependent of caspase 3 cleavage. On the other hand, its effect on PARP cleavage was negligible suggesting that ANPs-induced PARP cleavage is not entirely dependent of caspase 3 and/or p38 kinase activation. Inhibition of ERK and JNK pathways did not prevent apoptosis induced by the ANPs indicating that they are not relevant for the apoptotic effects of these compounds. This work was supported by the Research project of the IOCB OZ40550506 and the Project #IM0508 by the Czech Ministry of Education, Youth and Sports of the Czech Republic.

B2.15
Calyculin-A induces phosphorylation and translocation of myosin phosphatase target subunit 1 in THP-1 leukemic cells

A. Kis, B. Loniat, P. Gergely and F. Erdődi
Department of Medical Chemistry, University of Debrecen, Debrecen, Hungary

Myosin phosphatase holoenzyme (MP) is composed of a PPI catalytic subunit (PP1c) and the myosin phosphatase target subunit (MYPT1). MYPT1 is generally distributed between the cytoplasm and the nucleus of cells, and its nuclear import is dependent on the presence of nuclear localization signal sequence located close to the N-terminus. In THP-1 leukemic cells MYPT1 is localized almost exclusively in the nucleus as revealed by confocal microscopy of the cells and by Western blots of subcellular fractions. The level of phosphorylation of MYPT1 at Thr695 and Thr850, which are implicated in the inhibition of MP activity, is low. Treatment of the cells with the phosphatase inhibitor calyculin-A (CL-A) induces translocation of MYPT1 from the nucleus to the cytoplasm followed by an increased phosphorylation level at the inhibitory sites and a decreased mobility of MYPT1. This supports the occurrence of additional phosphorylation events, since phosphorylation of MYPT1 at the two inhibitory sites only does not alter its mobility on SDS-PAGE. Inhibition of Rho-kinase did not influence the localization pattern, suggesting that Thr695 and Thr850 residues might not be involved in mediation nuclear transport. Contrarily, the distribution of PP1c isoforms between the subcellular fractions remains approximately the same upon CL-A treatment, suggesting that shuttling of MYPT1 between the nucleus and the cytoplasm requires its dissociation from PP1c. Further experiments are required to establish whether phosphorylation site(s) in MYPT1 close to the nuclear localization signal sequence might be involved in the regulation of the nuclear transport of MYPT1 and its association with PP1c.
B2.16
Diacylglycerol Kinase alpha mediates HGF-induced Rac activation and membrane ruffling by regulating atypical PKC and RhoGDI

F. Chianale1, E. Rainero2, C. Cianflone3, V. Bettiol4, A. Pighini1, P. Porporato5, N. Filigheddu1, A. Serini5, F. Sinigaglia3, G. Baldanzi3 and A. Graziani3
1University of Piemonte Orientale A. Avogadro, Clinical and Experimental Medicine Department, Novara, Italy, 2Department and Division of Molecular Angiogenesis, University of Torino and IRCC, Oncological Sciences Candiolò (TO), Italy

Diacylglycerol kinases (DGKs) convert diacylglycerol into phosphatidic acid (PA). We showed that DGKa is required for growth factor-induced cell migration and ruffling, by promoting Rac plasma membrane targeting and activation. Thus we investi-
gated the signalling pathway through which DGKa controls Rac activation at ruffling sites. The dynamics of Rac targeting to the plasma membrane are regulated by RhoGDI, which complexes with Rac and allows its localized activation. Moreover, atypical PKCz, which is directly regulated by PA, has been recently demon-
strated to regulate Rac activation through the release from RhoGDI. We show inhere that PKCz/α associates with RhoGDI and is required for both HGF- and constitutively-active myr-
DGKa-induced ruffle formation and Rac/RhoGDI recruitment to ruffling sites. In this context, we show that: (i) DGKa inhibi-
tion prevents HGF-induced PKCz/α recruitment to ruffling sites; (ii) myr-DGKa, as well as cell treatment with PA, is sufficient to induce PKCz/α activation and translocation to ruffling sites; (iii) DGKa is dispensable for ruffle formation and RhoGDI recruit-
ment to ruffling sites induced by constitutively-active myr-PKCz; (iv) DGKa is required for HGF-induced RhoGDI recruitment and for Rac/RhoGDI complex dissociation; (v) myr-DGKa is suf-
cient to induce Rac and RhoGDI recruitment at ruffling sites in a PKCz/α-dependent manner. Overall, our data demonstrate that DGKa, by producing PA, provides a key signal that, through atypical PKCz/α activation and plasma membrane recruitment, in complex with RhoGDI and Rac, allows Rac acti-
vation leading to formation of membrane ruffles, which constitute essential requirements for cell migration.

B2.17
Diacylglycerol kinase alpha regulates SDF1α-induced cell invasion by regulating atypical PKC and matrix metallo proteinases 9

C. Cianflone, E. Rainero, P. E. Porporato, F. Chianale, M. Gaggiani2, I. Locatelli, G. Ranaldo, G. Baldanzi and A. Graziani
Department of Clinical and Experimental Medicine, University of Piemonte Orientale Amedeo Avogadro, Novara, Italy

Diacylglycerol kinase enzymes convert diacylglycerol into phosphatidic acid. We demonstrated that that HGF and VEGF activate diacylglycerol kinase alpha (DGKα) in a Src-dependent manner and its activity is required for epithelial and endothelial cell migration, invasion, proliferation and angiogenesis. Recently we unveiled a new signalling pathway linking HGF receptor to Rac activation and formation of membrane protrusions, through DGKα-mediated regulation of atypical PKCs (αPKC) and Rho-
GDI. SDF1α, member of the chemokines CXC subfamily, and its receptor CXCR4, a G protein coupled receptor, mediate survival, proliferation, and invasion of breast carcinomas. Here we show that in MDA231, breast cancer cells, upon SDF1α stimulation, DGKα is activated and recruited to the plasma membrane in a pertussis toxin-sensitive manner. Both DGKα silencing and inhibi-
tion of its activity impair SDF1α-triggered invasion and chemotaxis. In particular, DGKα activity is required for SDF1α-induced matrix metallo proteinases 9 activation (MMP9), elongation of cell protrusions through extra cellular matrix, recruitment of aPKC at the plasma membrane and targeting of both β1 integrin and MMP9 at the tip of cell protrusions. Finally the expression of a constitutively membrane-bound active DGKα mutant is sufficient for formation of protrusions as well as targeting of both β1-inte-
grin and MMP9 at the protrusion tips and activation of MMP9 gelatinolytic activity in an aPKC-dependent manner. Altogether, these data indicate that DGKα activation stimulates SDF1α-
induced breast cancer cell invasion, by regulating aPKC-mediated protrusions in ECM and MMP9 targeting and activation.

B2.18
Generation and characterization of cell lines with stable suppression of key enzymes of poly(ADP-ribose) metabolism

K. Erdel1, P. Bai1, E. Szabo2 and L. Virag3
1Department of Medical Chemistry, University of Debrecen Medical and Health Science Center, Debrecen, Hungary, 2University of Debrecen Medical and Health Science Center, Department of Dermatology, Debrecen, Hungary

Poly(ADP-ribose)ylation, catalysed by poly(ADP-ribose) poly-

merases (PARP-s), is a reversible post-translational modification of proteins. The ADP-ribose polymer is formed by sequential attachment of ADP-ribose moieties from NAD+ to acceptor proteins. The major enzyme catalyzing poly(ADP-ribose) catab-
olysis is poly(ADP-ribose) glycohydrolase (PARG). Poly(ADP-ri-
bose)ylation is involved in the maintenance of genome integrity, DNA repair and regulation of gene expression. However, the exact mechanism of these regulations and the possible role of PARP in these processes is fairly unknown. Reports based on the use of PARP-1 knock out mice have demonstrated that inhibition or genetic ablation of PARP-1 provides remarkable pro-
tection from oxidative stress-related conditions. Little is known, however, about the role of poly(ADP-ribose) polymers and PARG protein in these cellular events. Here, we employed a len-
tiviral-mediated, gene-specific shRNA system to establish A549 cell lines with stable suppression of the PARG and PARP-1 genes. Our data demonstrate that shPARG and shPARP-1 cell lines display efficient reduction of targeted mRNAs and the corre-
spounding proteins. Oxidative stress induces transient nuclear synthesis of poly(ADP-ribose) polymers. In the shPARP-1 cell line, however, poly(ADP-ribose) synthesis was strongly sup-
pressed. Poly(ADP-ribose) synthesis was apparently unchanged in shPARG cells; however, degradation of the polymer was sig-
ificantly delayed. The efficient suppression of PARP-1 and PARP that has been achieved in A549 cells is a suitable tool for investigation of the role of poly(ADP-ribose) metabolism in oxii-
dative stress in special regard to cell death.

B2.19
Expression of TGF-beta/Smads signalling in human cervical cancers

I. V. Iancu1, D. Socolov2, A. Botezatu3, A. Plesa3, C. Ungureanu2, C. Goia3 and G. Anton3
1, Stefan S Nicolau-Institute of Virology, Viral Genetic Engineering, Bucharest, Romania, 2Gr. T Popa -University of Medicine, Iassy, Romania, 3Stefan S Nicolau-Institute of Virology, Bucharest, Romania

High risk human papillomaviruses (hrHPV) are considered etio-
logic agents of cervical cancer. TGF-b (transforming growth fac-

tor-beta) is well known for its anti-proliferative effects and the neoplastic cells often lose their sensitivity to TGF-b. A characteristic alteration associated with malignant progression is the loss of responsiveness to transforming growth factor-beta (TGF-b1)-induced cell growth inhibition. The aim of this investigation was to define the molecular basis of TGF-b1 function in cervical cancer. 30 cervix biopsies harvested from women (30–57 years old) who underwent surgical treatment for squamous cervical cancer (SSC), were included into the study. All samples were tested for hrHPV DNA presence (Linear Array, Roche). 5 biopsies from HPV DNA negative women (42–53 years old) who were subjected to hysterectomy for other causes were included as control group. In order, to explored TGF-b1 signaling pathway the expression of TGF-b1, TGF-b1 receptors and Smad2 were estimated in Real-Time RT-PCR (ABI) and in Western-blot investigations. The study revealed an abnormal overexpression of TGF-b1 in 36.66% (12/30) of SSC cases as compared with control group and an abnormal reduction of type I TGF-b1 receptor in 23.33% (7/30) of cervical cancer tissues. In addition, 16.66% (5/30) of the tumour samples presented a reduction in Smad2 expression (as confirmed by Real-Time and Western-blot investigations). Our results demonstrated that in human cervical cancer the disruption of TGF-b1/Smad signalling pathway that might contribute to the malignant progression of cervical dysplasia.

B2.20 Immunocytochemical analysis of subcellular localization and content of S6 kinase during cell cycle progression

V. Kukharchuk, A. Khoruzhenko, O. Cherednyk, I. Tykhonkova and V. Filonenko
Department of cell signalling, Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine

The ribosomal protein S6 kinases are important components of PI3K signal transduction pathway. S6Ks play a key role in the regulation of cell growth by stimulating protein synthesis in response to growth factors and nutrients. In the mammalian cells two forms of S6Ks are expressed, namely S6K1 and S6K2 that play both redundant and distinct functional role. It was shown, that selective inhibition of S6Ks led to proliferation delay. The goal of our work was to study the subcellular localization and content of S6Ks during cell cycle progression. Human breast cancer cells MCF-7 were cultured on the cover glass slides. Anti-S6K1 rabbit antibodies/anti-Ki-67 mouse antibodies and anti-S6K2 rabbit antibodies/anti-Ki-67 mouse antibodies mixtures were used to detect S6K1 and S6K2 content in proliferating cells. The patterns were examined using fluorescent microscopy. Our data shows, that S6K1 and S6K2 are localized in the cytoplasm during interphase, however weak positive reaction was revealed in cell nuclei as well. The population of studied cells demonstrated the same level of intracellular content of S6Ks. Immunocytochemical and immunofluorescent analysis revealed that the content of S6K1 and S6K2 increased during mitosis. In metaphase, anaphase and telophase the immunofluorescent reaction become much more bright and prominent. The statistical analysis confirmed the correlation between increased S6K1, S6K2 content and activation of proliferation process.

B2.21 Abstract withdrawn

B2.22 Glucocorticoid induced upregulation of apoptotic cell phagocytosis in human dendritic cells

Department of Biochemistry and Molecular Biology, University of Debrecen, Apoptosis and Genomics Research Group, Hungarian Academy of Sciences, Debrecen, Hungary

We have recently observed that transglutaminase 2 (TG2), a crosslinking enzyme, is involved in the regulation of the apoptotic phagocytic system in mouse macrophages; its deletion leads to impaired apoptotic cell phagocytosis and to the development of autoimmune disorders. Glucocorticoids represent powerful anti-inflammatory compounds due to their capacity to inhibit inflammatory cell recruitment and down-regulate production and responsiveness to pro-inflammatory cytokines. Long-term exposure of human monocytes to the synthetic glucocorticoid dexamethasone (Dex) reprograms their differentiation toward a pro-resolution phenotype, and this includes increased capacity for phagocytosis of apoptotic cells. In our study we have investigated the role of TG2 in phagocytosis of apoptotic cell by Dex treated immature dendritic cells (iDC). Dex increases the phagocytosis of apoptotic neutrophils by iDC. A Taqman Low Density Array with 95 apopto-phagocytic genes showed that Dex downregulated the expression of TG2 among other genes, but upregulates various phagocytosis receptors like MER/TK, CD14, cell surface molecules like adenosine A3 receptor, bridging molecules like C1QA and receptors that take part in signalling such as ALOX5 or effectors (e.g. DNASE2). Although TG2 is strongly downregulated by Dex, immature dendritic cells have increased phagocytic capacity, which is due to the use of alternative pathways upregulated by dexamethasone.

B2.23 TSC2/PP5 interaction: novel phosphatase for tumor suppressor?

O. Malanchuk 1, S. Palchevskyy 1, I. Gout 2 and V. Filonenko
1Department of Cell Signaling, Kyiv, Institute of Molecular Biology and Genetics of NASU, Ukraine, 2Department of Biochemistry and Molecular Biology, University College London, London, UK

Understanding the relationship between growth and proliferation in multicellular organisms requires identification of the key regulators of growth control and understanding of how they regulate growth and how growth is linked to cell proliferation. Recent progress in understanding the mechanisms of growth control indicates that the tumour-suppressor tuberous sclerosis complex TSC1/2 plays a major role in negative regulation of cell growth. TSC1/2 complex made of two proteins TSC1 and TSC2. Mutations in either TSC1 or TSC2 genes are genetically linked to TSC syndrome, connected with the development of hamartomas in numerous organ systems. Both protein products of TSC1 and TSC2 genes form an intracellular complex exerting GAP activity towards a small GTP binding protein Rheb. The activity of TSC1/2 complex is regulated by multiple phosphorylations of TSC2 mediated by several kinases, such as PKB, AMPK, etc. So far, very little is known about the molecular mechanisms of TSC2 dephosphorylation. In our previous research using the Y2H-screening we have identified a number of potential TSC2 binding partners including protein phosphatase 5 (PP5). The aim of this study was to characterize interactions between TSC2 and PP5. Data presented in this
work indicate that TSC2 interacts with PP5 in vivo and efficiency of such interaction depends on physiological status of cells. In addition, PP5 is capable of dephosphorylating TSC2 in vitro at AMPK potential sites. Our data suggest that the physiological relevance of TSC2/PP5 interaction should be in reversing the stimulatory signaling mediated by AMPK on TSC2 activity.

B2.24
Short and long-term regulation of the RhoA specific GEF isoforms Net1/Net1A during TGF-β mediated regulation of epithelial polarity and EMT
E. Papadimitriou,1 E. Vasilaki,2 A. Moustakas3, D. Kardassis2 and C. Stournaras1
1University of Crete, Medical School, Biochemistry, Heraklion, Greece, 2University of Crete, Medical School, Institute of Molecular Biology and Biotechnology, FORTH, Heraklion, Greece, 3Ludwig Institute for Cancer Research, Uppsala, Sweden

In the present study we analyzed the role of TGF-β in the regulation of the RhoA specific guanine exchange factor Net1. The two GEF isoforms Net1 and Net1A exist mainly in the nucleus and partly in the cytoplasm. Cytoplasmic Net1A localization is slightly higher than Net1 leading to an increased ability of Net1A to activate RhoA. We report here that TGF-β transcriptionally upregulated the Net1A but not the Net1 isoform in HaCaT keratinocytes. This was correlated with an upregulation of the cytoplasmic protein levels of Net1A that potently activated RhoA. Both Smad2 and Smad3 proteins are involved in the induction of Net1A by TGF-β. This was evident by overexpression experiments using adenoviral mediated gene expression of wtSmad2 and wtSmad3 as well as by silencing experiments using siRNAs targeting Smad2 or Smad3. The induction of Net1A by TGF-β was also completely abolished using the MEK1 inhibitor UO126, implying the involvement of the Erk pathway in this process. We also report that both isoforms were differentially downregulated upon long term TGF-β treatment. Indeed, Net1 was downregulated at the mRNA level while Net1A was targeted for degradation at the protein level, as indicated by using the MG132 proteasome inhibitor. Finally, siRNA mediated silencing of both Net1 and Net1A resulted in disruption of E-Cadherin mediated adherens junctions in HaCaT keratinocytes. Our results emphasize the role of Net1A in TGF-β mediated regulation of epithelial cell polarity and epithelial to mesenchymal transition.

B2.25
Identification of the cellular targets and mechanism of action of the glycerophosphoinositols
A. Varone1, S. Mariggio1, G. Nicolosi2, P. Pucci2 and D. Corda4
1Institute of Protein Biochemistry, CNR, Telethon Institute of Genetics and Medicine (TIGEM), Naples, Italy, 2Institute of Biomolecular Chemistry, CNR, Catania, Italy, 3CEINGE–Biotecnologie Avanzate, University of Naples Federico II, Department of Structural and Functional Biology, Napoli, Italy, 4Institute of Protein Biochemistry, CNR, Napoli, Italy

The glycerophosphoinositols (GPIs) are ubiquitous, bioactive metabolites produced by the phospholipase A2IVs activity on membrane phosphophosphoinositides. With their mechanism of action including modulation of adenylly cyclase and Rho-GTPase activities or increase of intracellular calcium levels, the glycerophosphoinositols have multiple effects: induction of cell proliferation in thyroid cells, stimulation of the chemotactic response in lymphocytes, modulation of actin cytoskeleton organisation in fibroblasts and reduction of the invasive potential of metastatic cells. In order to define the GPIs mechanism of action, we set out to identify their protein targets. Therefore, a proteomic approach based on high-throughput-differential-LC-MS/MS analysis was employed using modified GPIs, with a biotin moiety bound to their glycerol backbone (biotinylated-GPIs). The targets identified so far include proteins known to be involved in cell signalling, cytoskeleton organisation, protein folding and metabolic processes. We have focussed our attention on the Src-homology-phosphatase-1 (Shp-1), a well-known regulator of Src activation, since it could be related to the reported signalling pathway leading to the glycerophosphoinositol-mediated modulation of the actin cytoskeleton, that involves Src. We have first verified the binding between biotinylated-GPIs and recombinant Shp-1, in pull-down assays using streptavidin-coated beads and confirmed their direct interaction. Preliminary experiments using in-vitro phosphatase assays show that GPIs affect the Shp-1 enzymatic activity. With this approach we have defined the cascade involved in the GPIs-mediated control of the actin cytoskeleton.

B2.26
Decrease of thyrocyte functional activity in vitro is accompanied by subcellular redistribution of S6K1 and S6K2 in primary monolayer culture
O. Cherednyk, A. Khoruzhenko, L. Savinska and V. Filonenko
Institute of Molecular Biology and Genetics, Department of cell signalling, Kyiv, Ukraine

Thyroid function is closely related to its follicular structure. Loss of this specific organization in primary culture leads to the dedifferentiation of thyrocytes. TSH and insulin are the main regulators of thyroid function and realise their effect through PI3K signalling pathway. The ribosomal protein S6 kinase is an important member of this pathway. Depending on physiological status of tissue, S6K could be found in the cytoplasm or in the nucleus as well. The aim of this work was to determine subcellular localization of S6K1 and S6K2 forms of rpS6 kinase in the primary monolayer culture of thyrocytes obtained from undamaged follicles. Rat thyroid follicles were placed at low density into dishes coated with gelatine. At the 3rd, 6th and 10th day of cultivation the distribution of to S6K1 and S6K2 in cell compartments was subjected to immunocytochemical and Western blot analysis. Upon cultivation follicles completely lost their organization and differentiated into monolayer. This was accompanied by gradual decrease of intracellular thyroglobulin content. Follicles rearrangement was accompanied by the changes in cytoplasm/nucleus distribution of S6K. At the 3rd day of cultivation the S6K was found only in the cytoplasm, while after 6 and 10 days of cultivation, both S6K1 and S6K2 were detected in both cytoplasm and nuclei. Moreover, the cells with S6K1/2- positive nuclei appeared at the leading edge of outspreading follicle, whereas in the centre of sheet the nuclei were still non-stained. These results strongly suggest that the loss of thyrocyte follicular organization in monolayer culture is accompanied by subcellular redistribution of S6K1 and S6K2.
B2.27 Subcellular redistribution of rpS6 kinase is related to functional activity of rat thyrocytes in 2D and 3D culture
A. Khoruzhenko, O. Cheredynk and V. Filonenko
Department of cell signalling, Institute of Molecular Biology and Genetics, Kyiv, Ukraine

The kinase of ribosomal protein S6 is an important member of PI3K signal transduction pathway involved in control of protein synthesis and G1/S transition of the cell cycle. There are two forms of this kinase S6K1 and S6K2. Previously we have shown that in normal thyroid tissue they are detected predominantly in the cytoplasm of thyrocytes whereas in monolayer culture in course of follicle outspreading S6K1 and S6K2 appeared in nuclei as well. The goal of presented work was to detect which processes involved in follicle transformation from 3D structure to mono
culture colony migration (migration, proliferation, loss of follicle organization) is related to S6K subcellular redistribution. The cultivation of thyrocytes resulted in activation of cell proliferation. But there was not the correlation between S6K1/2 subcellular relocation and Ki-67 appearance in proliferating cells. To study possible effect of migration on subcellular localization of S6K1 and S6K2, cultured thyrocytes were stimulated to penetrate a porous membrane of Transwell. But immunocytochemical analysis revealed cytoplasmic localization S6K1/2. Detection of the content of these kinases in thyrocytes in 3 D culture with retention of follicle structure shown that in thyroglobulin-positive cells from follicle like structures S6K1/2 localized in cytoplasm, but in thyroglobulin-negative cells from solid areas of cultured aggregates of follicles S6K1/2 were observed in nuclei as well (like in monolayer cultures). Thus, the change of subcellular localization of S6K1/2 in cultured thyrocytes is directly related to change of level of functional activity, unlike the processes of proliferation and migration.

B2.28 Loss of RAF kinase inhibitor protein is a somatic event in the pathogenesis of therapy-related acute myeloid leukemias with C-RAF germline mutations
M. Haller1, A. Zebisch2, K. Hiden3, G. Hoefler4, H. Stil5 and J. Troppmair2
1Daniel-Swarovski Research Laboratory, Innsbruck Medical University, Innsbruck, Austria, 2Department of Oral Biology, Brain Korea 21 Project, Yonsei University College of Dentistry, Seoul, Korea

The kinase of C-RAF is an important member of PI3K signal transduction pathway involved in control of protein synthesis and G1/S transition of the cell cycle. There are two forms of this kinase S6K1 and S6K2. Previously we have shown that in normal thyroid tissue they are detected predominantly in the cytoplasm of thyrocytes whereas in monolayer culture in course of follicle outspreading S6K1 and S6K2 appeared in nuclei as well. The goal of presented work was to detect which processes involved in follicle transformation from 3D structure to mono
culture colony migration (migration, proliferation, loss of follicle organization) is related to S6K subcellular redistribution. The cultivation of thyrocytes resulted in activation of cell proliferation. But there was not the correlation between S6K1/2 subcellular relocation and Ki-67 appearance in proliferating cells. To study possible effect of migration on subcellular localization of S6K1 and S6K2, cultured thyrocytes were stimulated to penetrate a porous membrane of Transwell. But immunocytochemical analysis revealed cytoplasmic localization S6K1/2. Detection of the content of these kinases in thyrocytes in 3 D culture with retention of follicle structure shown that in thyroglobulin-positive cells from follicle like structures S6K1/2 localized in cytoplasm, but in thyroglobulin-negative cells from solid areas of cultured aggregates of follicles S6K1/2 were observed in nuclei as well (like in monolayer cultures). Thus, the change of subcellular localization of S6K1/2 in cultured thyrocytes is directly related to change of level of functional activity, unlike the processes of proliferation and migration.

B2.29 Life/death decisions in growth factor signaling: Role of mitochondrial events
K. Koziel, J. Smigelskaite and J. Troppmair
Daniel Swarovski Research Laboratory, Innsbruck Medical University, Innsbruck, Austria

Prolonged growth factor deprivation is a common physiological stimulus for the induction of apoptosis. Cell death under these conditions can be delayed through survival kinases or antiapop
totic members of Bcl-2 protein family. However, it is still largely unclear how the lack of a growth factor is translated to the death stimulus and how precisely survival proteins counteract these processes. We have been able to show that increased mitochondrial ROS production and mitochondrial Ca2+ overload following IL-3 withdrawal in 3D cells are essential intermediates in cell death induction, whose actions can be prevented by activated RAF and AKT or the overexpression of Bcl-2. To further define possible steps along the mitochondrial pathway to apoptotic cell death, which are susceptible to regulation by survival proteins, we analyzed changes in the mitochondrial membrane potential following growth factor removal. The drop in mitochondrial membrane potential (ΔΨ) after IL-3 removal was suppressed by activated RAF and overexpressed Bcl-2. Additionally, the treat
ment with the antioxidant N-acetyl-cysteine (NAC), which rescued cells from apoptosis, also prevented a drop in ΔΨ following IL-3 withdrawal in 3D cells. These findings further support the criti
cal role of mitochondrial events as targets for survival pathways.

B2.30 Optic Atrophy 1 is an A-kinase anchoring protein that mediates adrenergic control of lipolysis
G. Pidoux1, O. Witzcał2, E. Jarnaes3, L. Myrvold2, A.-J. Stokka2 and K. Tasken2
1Inserm U767, Paris, France, 2Biotechnology Centre of Oslo, Tasken group, Oslo, Norway

Adrenergic stimulation of adipocytes yields a cAMP signal that activates protein kinase A (PKA). PKA phosphorylates perilipin, a protein localised on the surface of lipid droplets that acts as a gatekeeper to regulate access of lipases converting stored tri
glycerides to free fatty acids and glycerol in a phosphorylation-depend
t manner. Here, we report a new function for Optic Atrophy 1 (OPA1), a protein known to regulate mitochondrial dynamics, as a dual-specificity A-kinase anchoring protein associ
ated with lipid droplets. By a variety of protein interaction assays, immunoprecipitation and immunolocalization experi
ments, we show that OPA1 organizes a supramolecular complex containing both PKA and perilipin. Furthermore, by a combina
tion of siRNA-mediated knockdown, reconstitution experiments using OPA1 with or without the ability to bind PKA and cellular delivery of PKA anchoring disruptor peptides we demonstrate that OPA1 targeting of PKA to lipid droplets is necessary for hormonal control of perilipin phosphorylation and lipolysis.

B2.31 Regulation of calcium signaling in non-excitable polarized exocrine cells from Homer2-Deficient mice
Y.-M. Yang, S.-I. Lee and D. M. Shin
Department of Oral Biology, Brain Korea 21 Project, Yonsei University College of Dentistry, Seoul, Korea

The Homers are scaffold proteins and consist of an N-terminal Ena/VASP homology 1 (EHV) protein-binding domain and
C-terminal leucin zipper/coiled-coil domain. The EVH domain recognizes the proline-rich motifs and binds many Ca\textsuperscript{2+} signaling proteins including G protein-coupled receptors, inositol 1,4,5-trisphosphate (IP\textsubscript{3}) receptors (IP\textsubscript{3}R)s, ryanodine receptors, and TRP channels. However, their role in Ca\textsuperscript{2+} signaling of non-excitable cells is not well known. In the present work, we investigated the role of Homer2 in Ca\textsuperscript{2+} signaling in parotid gland and pancreas acinar cells using Homer2\textsuperscript{-/-} mice. Homer2 showed polarized luminal localization in parotid acinar cells, but the deletion of Homer2 did not affect a localization of IP\textsubscript{3}R\textsubscript{s} or IP\textsubscript{R} channel activity. The protein expression level of plasma membrane Ca\textsuperscript{2+} ATPase (PMCA) was increased in parotid glands, whereas sarcoplasmic reticulum Ca\textsuperscript{2+} ATPase was increased in pancreas of Homer2\textsuperscript{-/-} mice. We found that deletion of Homer2 increased PMCA activity and Homer2 interacted with PMCAs in parotid cells. Moreover, the mRNA expression levels of TRPC3, Orai1 and STIM1 were increased and inhibitory effects of blockers on Ca\textsuperscript{2+} entry were diminished in pancreas cells of Homer2\textsuperscript{-/-} mice. These findings suggest that Homer2 may play an important role in regulation of Ca\textsuperscript{2+} signaling in non-excitable cells. This work was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Korea (A084007 and A090807).

B2.32
An integrated experimental workflow to increase throughput and data robustness for analysis of mammalian protein interaction networks
T. Glätzer\textsuperscript{1}, N. Lentze\textsuperscript{1}, T. Lampart\textsuperscript{1}, M. Gstaiger\textsuperscript{2}, R. Aebersold\textsuperscript{3} and D. Auerbach\textsuperscript{4}
1Dualsystems Biotech AG, Schlieren, Switzerland, 2Institute of Molecular Systems Biology, ETH, Zürich, Switzerland

To understand the organization of protein complexes and how biological information is propagated via protein-protein interaction is becoming increasingly important to get new functional insights on cellular processes. Affinity purification coupled to mass spectrometry (AP-MS) has been successfully used in the past to characterize protein complexes. However, mainly due to significant experimental limitations the success and power of the AP-MS strategy has been underrated to date. Here, we describe an integrated workflow for characterization of mammalian protein complexes. The experimental pipeline comprises rapid generation of isogenic mammalian cell lines, protein complex analysis using an isogenic mammalian cell line system in combination with high throughput nano-LC-Orbitrap-MS analysis. We have used the system to study interactions associated with the human protein phosphatase 2A and NF-kB systems. Analysis of these systems revealed new components of protein network structures that are linked to a number of important biological processes such as transcription, the cell proliferation, apoptosis and inflammation. The high performance of our strategy is well suited to identifying protein interaction partners that can subsequently be used as (i) entry points for focused research projects and (ii) to more globally map out whole signaling systems linked to important biological processes.

B2.33
Identification and functional study of CDK14/CCNY interacting proteins
J. Chen, S. Li and M. Jiang
Institute of Biochemistry and Cell Biology, SIBS, CAS, State Key Laboratory of Molecular Biology, Shanghai, China

The CDKs are a family of Ser/Thr protein kinases that share a highly conserved PSTAIRE motif which is involved in cyclin binding and has been used to classify CDK-related kinases. We have reported human PFTK1 (CDK14), which is highly expressed in brain, pancreas, kidney, heart, testis and ovary. To search its substrate and regulatory proteins, we screened a two-hybrid library and identified seven PFTK1 interacting proteins including four 14-3-3 isoforms, a septic family member KIAA0202 (SEPT8), PLZF protein and a novel cyclin, cyclin Y (CCNY). Identification of the CCNY interacting proteins by two-hybrid screening revealed PFTK1 (CDK14) and four 14-3-3 isoforms. The CCNY is a regulatory partner of PFTK1 (CDK14), which recruited PFTK1 (CDK14) to the plasma membrane and activated its associated kinase activity. Membrane-associated but not cytoplasm or nucleus localized CCNY is a substrate of CDK14-CCNY complex. 14-3-3s are a family of highly conserved acid proteins, which are expressed ubiquitously in all eukaryotic cells. The interaction between the 14-3-3 and CCNY or 14-3-3 and CDK14 was confirmed by co-immunoprecipitation and yeast two hybrid assay. The CCNY contains two putative 14-3-3 binding consensus motifs, RAS66T67IFLS and RKRS324AS326, the later is required for their interactions. The CDK14 contains a putative 14-3-3 binding consensus motif (RHS19IS120PSS), which overlapped with its second NLS. The Ser119 is crucial for the interaction between CDK14 and the 14-3-3 proteins. The effects of 14-3-3(CCNY, 14-3-3/CDK14 and CCNY/CDK14 interactions on their subcellular localization and phosphorylation were investigated.

B2.34
Roles of human Cyclin Y (CCNY) in cell cycle
S. Li, M. Jiang and J. Chen
Institute of Biochemistry and Cell Biology, SIBS, CAS, State Key Laboratory of Molecular Biology, Shanghai, China

Cyclins are regulatory subunits that associate with specific CDKs through the cyclin box to form functional protein kinase complexes. PFTK1 belongs to the subgroup of Cdc2-related kinase family and was characterized as a CDK that regulates cell cycle progression and cell proliferation. We identified a novel protein CCNY (Cyclin Y) as a PFTK1 (CDK14) interacting protein in a yeast two-hybrid screen. The cyclin box in CCNY and the PFTK1 interacting motif of PFTK1 (CDK14) are both required for the interaction which was confirmed by in vitro and in vivo assays. Two CCNY transcripts were detected in all tissues and cell lines. A 4.0 kb transcript is ubiquitously expressed at low level in human tissues, but 2.0 kb transcript is particularly abundant in pancreas, kidney, heart, testis and ovary. To confirm the interaction, we performed Co-IP assays. Two CCNY transcripts were detected in all tissues and cell lines. A 4.0 kb transcript is ubiquitously expressed at low level in human tissues, but 2.0 kb transcript is particularly abundant in testis, also expressed at high level in heart and skeleton muscle, and at low level in the other tissues. In cell lines, both the 4.0 kb and 2.0 kb transcripts are expressed at low level including 293T and NIH3T3, but higher level in HepG2. The CCNY was enriched at the plasma membrane due to an N-terminal myristoylation signal. The CCNY was also shown to affect the cell cycle progression. Ectopic expression of CCNY in 293T and HepG2 accumulated cells in S-phase and G2/M phase. Our data indicate that the CCNY is a regulatory partner of PFTK1 (CDK14), binding of CCNY to PFTK1 (CDK14) changes its intracellular location and regulates the PFTK1 (CDK14) activity.
B2.35
Vip induces NF-κB1 transactivation by different signaling pathways in human tumor and non-tumor prostate cells
A. B. Fernandez-Martinez1, E. Vacas1, A. M. Bajo2, J. C. Prieto2 and M. J. Carmona2
1Alcalá University, Physiology, Alcalá de Henares, Spain, 2Alcalá University, Biochemistry and Molecular Biology, Alcalá de Henares, Spain

Introduction: Vasoactive intestinal peptide (VIP) behaves as a pro-metastatic factor in human prostate cancer cells. The agent curcumin blocked VIP effects on the biomarkers related with the different steps of the metastatic cascade (Fernandez-Martinez et al., 2009). The main aim of this study was to test the signaling pathways involved in VIP-NF-κB1 (p50) transactivation in human prostate cells in order to characterize the VIP mechanism of action in the prostate gland.

Methods: We evaluated the effect of VIP on transactivation of NF-κB1 (p50) in three human prostate cell lines: RWPE1 (non-tumor cells) and LNCaP and PC3 (tumor cells). In our study, ELISA and confocal immunofluorescence microscopy were performed.

Results: VIP increases the binding of NF-κB1 to the promoter of its target genes in three human prostate cells and provokes faster responses according to the most aggressive status (RWPE1 < LNCaP < PC3 cells). We show, using specific kinase inhibitors and the EPAC agonist (O-CPT-2Met cAMP), that in control cells (RWPE1), the effect is mediated by activation of cAMP-dependent protein kinase (PKA) and implicates neither inhibitors and the EPAC agonist (O-CPT-2Met cAMP), that in (RWPE1 < LNCaP < PC3 cells). We show, using specific kinase inhibitors and the EPAC agonist (O-CPT-2Met cAMP), that in control cells (RWPE1), the effect is mediated by activation of cAMP-dependent protein kinase (PKA) and implicates neither control cells (RWPE1 < LNCaP < PC3 cells). We show, using specific kinase inhibitors and the EPAC agonist (O-CPT-2Met cAMP), that in (RWPE1 < LNCaP < PC3 cells). We show, using specific kinase inhibitors and the EPAC agonist (O-CPT-2Met cAMP), that in (RWPE1 < LNCaP < PC3 cells). We show, using specific kinase inhibitors and the EPAC agonist (O-CPT-2Met cAMP), that in (RWPE1 < LNCaP < PC3 cells). We show, using specific kinase inhibitors and the EPAC agonist (O-CPT-2Met cAMP), that in (RWPE1 < LNCaP < PC3 cells).

Conclusions: Our results indicate that VIP uses different pathways for NF-κB1 (p50) transactivation, transformed cells resort on pro-survival and pro-proliferative signaling pathways including ERK1/2, PI3-K and cAMP/EPAC.

B2.36
Thrombin induces inducible nitric oxide synthase expression via Ras, Raf-1, ERK, and NF-κB signaling pathways in NR8383 lung macrophages
B.-C. Chen1, C.-Y. Chi2 and C.-H. Lin2
1School of Respiratory Therapy, College of Medicine, Taipei Medical University, Taipei, Taiwan, 2Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, Taipei, Taiwan

Thrombin, a serine protease, is a well-known coagulation factor generated in vascular injury and which also plays an important role in lung inflammatory diseases. However, little is known about the signaling pathway in thrombin-induced iNOS expression in lung macrophages. In this study, we investigated the Ras/Raf-1/ERK and IKKα/β/NF-κB in thrombin-induced iNOS expression in NR8383 lung macrophages. Thrombin caused time- and dose-dependent increases in iNOS expression, which was attenuated by a Ras inhibitor (manumycin A), dominant negative mutant of Ras (RasN17), a Raf-1 inhibitor (GW 5074), and a mitogen-activated protein kinase kinase (MEK) inhibitor (PD 98059). Treatment of NR8383 macrophages with thrombin caused time-dependent activations of Ras, Raf-1, and extracellular signal-regulated kinase (ERK). The thrombin-induced increase in Ras activity was inhibited by manumycin A. Raf-1 phosphorylation at Ser338 by thrombin was inhibited by manumycin A and GW 5074. The thrombin-induced increase in ERK activity was inhibited by manumycin A, GW 5074, and PD 98059. Treatment of macrophages with a NF-κB inhibitor (PDTC), an IκBα phosphorylation inhibitor (Bay 117082), and IκB all inhibited thrombin-induced iNOS expression. Stimulation of cells with thrombin activated IKKα/β, IκBα phosphorylation, IκBα degradation, and κB-luciferase activity. The thrombin-mediated increase in the activities of IKKα/β and κB-luciferase were also inhibited by the manumycin A, GW 5074, and PD 98059. These results indicated the Ras/Raf-1/ERK pathway, which in turn initiates IKKα/β and NF-κB activation, and ultimately induces iNOS expression in NR8383 macrophages.

B2.37
Ganoderma tsugae extracts induce cell cycle arrest and apoptosis in lung cancer cells
Y.-C. Liu1, Y.-H. Yu1, C.-Y. Chen2, W.-H. Hsu2, H.-H. Hsieh2, J.-S. Chen3, K.-L. Ng1, Y.-C. Lin1 and M.-C. Kao3
1National Chung Hsing University, Taichung, Taiwan, 2China Medical University Hospital, Taichung, Taiwan, 3Asia University, Taichung, Taiwan, 4Chinese Culture University, Taipei, Taiwan, 5China Medical University, Taichung, Taiwan

Background: Lung cancer ranks the 1st cancer death in Taiwan. Improved therapeutic methods are thus especially needed. Bioactive botanical products are found to be useful in treatment of clinical diseases. Ganoderma, also known as Lingzhi, has long been used for human health in oriental countries. We have demonstrated that Tien-Shen-Lingzhi (TSLE) and Ganoderma tsugae (Gt) in Taiwan, is effective against colon and epidermoid carcinomas. Here, we focus on the growth inhibition effects of TSL extracts (TSLG) on lung cancer cells.

Objective: To evaluate the anticancer potential of TSLG on lung cancer cells in vitro and in vivo experiments.

Methods: The quality control (QC) of TSLG was monitored by chemical fingerprints (CF) using high performance liquid chromatography (HPLC) and electrospray ionization mass spectrometry (ESI-MS). A non-small cell lung cancer cell line, H23/0.3, was used for testing the anticancer effects of TSLG. In addition, MTT assay, flow cytometry, confocal microscopy, western blotting, and xenograft mouse model were also applied for this study.

Results: The CF profiles revealed identical patterns for TSLG from three individual batches of Gt. TSLG inhibited H23/0.3 cell proliferation with an IC50 of 0.37 mg/ml. TSLE-induced G1/S accumulation followed by cell apoptosis were observed. Western blotting results demonstrated decrease of cyclin A2 and increase of caspase-3 after TSLE treatment in a dose-dependent manner. Retardation of tumor growth was found in TSLE-treated mice.

Conclusion: TSLE demonstrated anticancer effects on lung cancer H23/0.3 cells. The active anticancer ingredient in TSLE remains to be investigated.

B2.38
A whole-genome microarray approach to signal pathway of growth inhibition effects of Ganoderma tsugae extracts on lung cancer cells
H.-H. Hsieh1, Y.-H. Yu2, C.-Y. Chen2, W.-H. Hsu2, J.-S. Chen3, K.-L. Ng1 and M.-C. Kao4
1Asia University, Taichung, Taiwan, 2China Medical University Hospital, Taichung, Taiwan, 4Department of Horticulture and Biotechnology, Chinese Culture University, Taichung, Taiwan, 5China Medical University, Taichung, Taiwan
Abstracts

Background: Lung cancers are among the neoplastic diseases with the worst prognosis. More treatment modalities are needed to improve clinical outcome. It's been a new trend to search for bioactive traditional Chinese medicines (TCM). Ganoderma, also known as Lingzhi, is a TCM and has been widely used for improving human health for centuries. We have addressed the antitumor effects of Ganoderma on various cancers. Here, a comprehensive genomic profiling was used to further explore the signal pathway for the antitumor effects of Ganoderma tsugae (Gt).

Objectives: To unravel the cellular effects of Tien-Shen-Lingzhi (TSL), an improved species of Gt in Taiwan, on non-small cell lung cancer (NSCLC) H23/0.3 cells.

Methods: A comparative genomics study was performed to search for TSL-mediated differential expressions on H23/0.3 cells. Bioinformatics tool, e.g. GeneGo MetaCore, was used to analyze the experimental results based on Affymetrix Human Genome U133 Plus 2.0 microarray.

Results: The whole-genome expression profiles of H23/0.3 with or without TSL treatment were resolved by multidimensional scaling (MDS) analysis. It indicated a TSL-mediated cell cycle perturbation. Bioinformatics analysis showed cell cycle was the most affected pathway. GeneGo MetaCore-derived cellular pathway map revealed dramatic hits (17/26) on the DNA replication. Some selected genes were confirmed by Q-PCR showing in concordance with the microarray results.

Conclusion: The genomic microarray data demonstrates the TSL-induced cell cycle deregulation in H23/0.3 cells. TSL-mediated cell cycle perturbation on H23/0.3 cells is consistent with the cellular pathway map resulting from Genego analysis.

B2.39 Molecular pathway of growth inhibition effects on breast cancer cells by Tien-Shen-Lingzhi

M.-C. Kao1, G.-R. You2, M.-S. Chang3, H.-P. Kuo1, J.-S. Chen4 and J.-Y. Liu5

1China Medical University, Department of Biological Science and Technology, Taichung, Taiwan, 2Chang Gung University, Graduate Institute of Medical Biotechnology, Taipie, Taiwan, 3National Defense Medical Center, Graduate Institute of Life Sciences, Taipie, Taiwan, 4National Defense Medical Center, Graduate Institute of Life Sciences, Taipie, Taiwan, 5National Defense Medical Center, Graduate Institute of Life Sciences, Taipie, Taiwan

Traditional Chinese Medicine (TCM) has been used to preserve the human vitality, to promote longevity, and for the prevention or treatment of a variety of diseases including cancer. Ganoderma, also known as Lingzhi, is a TCM and has been used for medicinal purposes for centuries. Among the eight common species of Ganoderma in Taiwan, Ganoderma tsugae (Gt) has been used in our study for fighting cancers. We have demonstrated that Tien-Shen-Lingzhi (TSL), an improved species of Gt in Taiwan, is effective against human breast cancer cell lines MCF7. The purpose of this study is to further unravel its molecular mechanism. Our results demonstrate that the ethanol extract of TSL (TSLEE) inhibits the proliferation of human breast cancer cells through the induction of cell cycle arrest. TSLEE-induced cell cycle arrest is associated with a dramatic decrease at the protein expression level of cyclin D1 and cyclin E in a dose-dependent manner, i.e. G1 arrest. Upon exposure of human breast cancer cells to TSLEE results in growth inhibition as evidenced by inhibiting PI3K/Akt pathway. Moreover, our data show that TSLEE can also reduce the chemoresistance of the anti-cancer drug Taxol, thereby increasing the clinical effectiveness of the treatment of breast cancer. In conclusion, our findings establish a mechanistic link between the PI3K/Akt pathway and TSLEE-induced cell cycle arrest. Our results also provide insightful understanding of using the TSL as a therapeutic TCM for the clinical treatment of human breast cancer.

B2.40 Polysulfate VEGF coreceptors: A novel approach for the development of anti-angiogenesis drugs

T. Bríza1, Z. Kejik2, M. Pavel2, J. Králova1, V. Kral4 and G. Petkova1

1Institute of Chemical Technology, Analytical Chemistry, Prague, Czech Republic, 2Charles University, 1st Medical Faculty, Prague, Czech Republic, 3Academy of Sciences of the Czech Republic, Institute of Molecular Genetics, Prague, Czech Republic, 4Zentiva R & D (sanofi-aventis group), Prague, Czech Republic

VEGF (Vascular endothelial growth factor) signaling pathway play one of the key roles in many physiological and pathological processes such as cancer. Important parts of this pathway are VEGF receptors and their polysulfate saccharide coreceptors. Their modulation and can be effective strategy of cancer treatment. Therefore we study recognition polysulfate saccharide motive by polysulfanthium salts. We observed strongly selectivity of polysulfanthium salts for this structure motive coupled with strongly spectral change. This work was funded by grant from the Grant Agency of the Czech Republic (Grant No. 203/09/131) and supported in part by project LC06077 awarded by the Ministry of Education of the Czech Republic, by project AV0Z50520514 awarded by the Academy of Sciences of the Czech Republic to J. Králová and by project MSM6046137307 to V. Kral.

B2.41 Sumoylation of ultraspiracle protein from Drosophila melanogaster

K. Bieška1, J. Séliga1, E. Wieczorek1, R. Niedenthal2 and A. Ozylar3

1Department of Biochemistry, Faculty of Chemistry, Wroclaw University of Technology, Wroclaw, Poland, 2Institute for Physiological Chemistry/Biochemistry, Medical School Hannover, Hannover, Germany

The ultraspiracle (USP) heterodimerizes with the ecdysone receptor (EcR) to form a functional complex EcR/Usp that mediates the effects of the steroid molting hormone ecdysone by activating and repressing expression of ecdysone response genes in insects. Complex EcR/Usp from Drosophila melanogaster provides an ideal model system for construct gene switches in humans. However, there is no information about posttranslational modification of Usp such as sumoylation. To investigate sumoylation of Usp we used Ub9 fusion-directed sumoylation (UFDS) system [Jakobs et al., 2007] which is based on fusion of conjugating enzyme Ub9 with substrate protein. We prepared constructs coding fusion proteins where Ub9 was placed to the N- or C-terminus of Usp (Ub9-Usp, Ub9-Ub9) expressed them in HEK293 cells and analyzed the protein extracts by western blotting with an Ub9 or an Usp antibody. The results revealed that Ub9-Usp is observed as two different migrating bands, of which the upper migrated slower when EGFP-SUMO1 was coexpressed, suggesting that the higher-molecular-weight protein consist of SUMO conjugated Ub9 fusion protein. Ub9-Ub9 is also sumoylated although less efficiently. To determine region of the modification, we prepared fragments of Usp including A/B region, DNA binding domain
B2 – Signal Transduction

(ABD) and ligand binding domain (LBD) fused to UbC9. For A/B region and LBD we showed that there are potential residues that can be sumoylated. We also used bio-informatics tools (SUMOplot, SUMOsp and PCI-SUMO) to predict and score potential sumoylation sites in Usp. Using this data, we prepared some point mutants. Identification of Usp sumoylation sites is currently underway.

B2.42 Effect of Ang II on the generation of superoxide anion via NAD(P)H oxidase enzyme complex in primary cultured vascular smooth muscle cells isolated from spontaneously hypertensive and normotensive rat aortas, and role of Src in this metabolic pathway

F. Uzuner, A. Tokay, A. Cetin and A. Yesilkaya
Akdeniz University Medical School, Department of Biochemistry, Antalya, Turkey

Ang II contributes to altered vascular tone, endothelial dysfunction, structural remodeling and vascular inflammation, characteristic features of vascular damage in hypertension. Ang II induces its effects via G-protein-coupled transmembrane receptor, Ang II receptor 1 (AT1R). The best characterized system in vascular cells is Ang II-stimulated NAD(P)H oxidase-mediated generation of superoxide anion, which appears to be upregulated in hypertension. Vascular smooth muscle cells (VSMC) were isolated from thoracic aortas of spontaneously hypertensive rats (SHR) and normotensive Wistar Kyoto rats (WKY). VSMC, to determine the AT1R-mediated Src phosphorylation and superoxide anion production via NADPH oxidase, were stimulated with Ang II with or without Losartan, diphenyl iodonium, DPI, and GF109203X. Src phosphorylation was determined by western blot method. Superoxide anion production was measured by cytochrome c reduction method. Src phosphorylation increased both SHR and WKY groups after Ang II stimulation compared with controls. On the other hand, Src phosphorylations were decreased both SHR and WKY groups after Ang II stimulation with inhibitors except DPI. Superoxide anion production was enhanced in both two groups in Ang II-stimulated cells. However superoxide anion production in SHR was significantly greater than WKY. All inhibitors reduced superoxide anion production both SHR and WKY groups in spite of Ang II stimulation. We found that Src and PKC phosphorylation may occur before the NAD(P)H activation. We showed that Ang II induced NADPH oxidase via Src.

B2.43 p38 MAPK phosphorylation by angiotensin II stimulation via NADPH oxidase in primary cultured vascular smooth muscle cells

S. Caglar, A. Cetin, A. Tokay and A. Yesilkaya
Department of Biochemistry, Akdeniz University Medical School, Antalya, Turkey

Angiotensin II (Ang II) has an important role in atherosclerosis, hypertension, pathogenesis of cardiovascular diseases and regulating blood pressure. It was shown that, in vascular smooth muscle cells (VSMC), Ang II activates various signal transduction pathways of p38 mitogen activated protein kinases (MAPKs). Ras can activate p38 MAPK through signal transduction pathway of NAD(P)H oxidase. VSMC cultured, used in the experiments, showed 99% positive immunostaining of smooth muscle α-actin antibody. This study was aimed to investigate whether or not Ras or NAD(P)H oxidase activation have a role in p38 MAPK phosphorylation after stimulation with Ang II in VSMC cultured. Phosphorylation was shown using western-blot techniques with specific phospho-antibodies against p38 MAPK proteins. In cultured rat vascular smooth muscle cells, angiotensin II (Ang II) induced a rapid increase in p38 mitogen activated protein kinase (MAPK) activity through the Ang II type 1 receptor. Ang II induced MAPK activity was abolished by the Ras inhibitor, FTS and NAD(P)H oxidase inhibitor DPI.

B2.44 Intracellular signaling cascades triggered by FPRL1 in human prostate cells

F. Cattaneo, S. Rea, A. Spina, L. M. Pavone and R. Ammendola
1Università degli Studi di Napoli Federico II, Dipartimento di Scienze Biomorfologiche e funzionali, Naples, Italy; 2Università degli Studi di Napoli Federico II, Dipartimento di Strutture e Funzioni, Naples, Italy; 3Università degli Studi di Napoli Federico II, Dipartimento di Biochimica e Biotecnologie Mediche, Naples, Italy

We investigated intracellular signaling pathways triggered by stimulation of formyl peptide receptor-like 1 (FPR1) with WKYMVm in human prostate cell line PNT1A. In growth-arrested cells, exposure to WKYMVm induced MEK-dependent ERKs activation as well as pertussis toxin-dependent (PTX) and ERK-dependent p47phox phosphorylation. We also examined the ability of the FPR1-induced signaling cascade to transactivate Met receptor, that is expressed in cancer cells and is implicated in cancer progression. We demonstrated that PNT1A cell line express a biologically functional FPR1 and that incubation of these cells with WKYMVm induce transactivation of Met receptor. Western blot experiments, performed with specific anti-pY antibody, showed that following FPR1 agonist stimulation Met receptor resulted phosphorylated on Y1313, Y1349 and Y1356 residues. These events are prevented by pretreatment with WRW4, an antagonist of FPR1, and by PTX, a selective inhibitor of Gi proteins. Moreover, the transphosphorylation of Met receptor on Y1313, Y1349 and Y1356 residues are also prevented by pretreatment with DPI, an inhibitor of NADPH oxidase, suggesting a key role of ROS in this process. We also demonstrated that stimulation with WKYMVm of PNT1A cells triggered the activation of further signaling pathways, involved in cell survivor, proliferation and neangiogenesis such as PI3K/Akt and STAT3 pathway. These intracellular signaling cascades are prevented by pretreatment with WRW4, PTX and SU11274, a selective inhibitor of TK activity of Met receptor. These studies suggest that stimulation of FPR1 with a specific agonist contribute to the progression of tumorigenesis in prostate.

B2.45 Acute modulation of the proteome by physiological hypoxia

1Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Instituto de Biomedicina de Sevilla-IBIS, Sevilla, Spain; 2Centro de Investigacion Cooperativa en Biocienicas, Unidad de Biologia Celular y Celasulas Madre, Derio, Spain

Suitable provision of the oxygen (O2) required for the aerobic synthesis of adenosine triphosphate (ATP) is a major physiological challenge since O2 deficit, even transient, can produce...
irreversible cellular damage. Hypoxia has a critical role in the pathogenesis of major causes of mortality, such as myocardial infarction, stroke, and chronic lung disease, as well as in reperfusion injury of transplanted organs and carcinogenesis. Acute hypoxia triggers respiratory and cardiovascular systemic reflexes to ensure fast O2 delivery to the brain or the heart, however upon exposure to protracted hypoxia cells generate homeostatic responses to minimize the deleterious effect of O2 deficiency. Cell adaptation to hypoxia depends on transcriptional and non-transcriptional mechanisms whose nature is as yet only partially known. In order to characterize the initial homeostatic responses elicited by the lack of O2 we have evaluated the proteomic changes associated with an acute and physiological drop in oxygen tension. Hypoxia leads to adaptive changes in the proteome that results in a tight control of the ATP expenditure. To evaluate the role of the well-know O2 sensors prolyl-4-hydroxylases (PHDs) in this fast regulation we have estimated the changes in the proteome produced by acute PHDs inhibition. A subset of the modifications observed under hypoxia was mimicked by PHDs activity inhibition. Here we present the results obtained with these two proteomics approaches and the initial characterization of the mechanisms underlying the acute responses to low O2 circumstances.

B2.46 Deriving Ca(2+) signals starting from puffs: The mechanism buffers genetic and environmental variability by functional robustness

K. Thurlay1, S. Tovey2, C. Taylor2 and M. Falcke1
1Max-Delbrueck-Center, Mathematical Cell Physiology, Berlin, Germany, 2University of Cambridge, Pharmacology, Cambridge, UK

Puffs are elemental events of Ca(2+) release from the ER through clusters of Ca(2+) channels. Much evidence now supports the idea that intracellular Ca(2+) dynamics are stochastic. However, stochastic mathematical models have so far been limited by the large number of states which emerge from the spatial heterogeneity of Ca(2+) channels. Furthermore, most existing models fail to match statistical properties of measured Ca(2+) signals. As a new concept, we formulate a theory in terms of interpuff interval and puff duration distributions, and verify it with a series of long-time measurements of single cell Ca(2+) spikes in HEK-293 cells. Our theory reproduces the typical spectrum of measured Ca(2+) signals like puffs, spiking and bursting, in analytically treatable test cases as well as in more realistic setups. We find criteria for spiking in terms of the strength of spatial coupling and the channel closing rate. As a main result, we formulate functional robustness in terms of the relation between average and standard deviation of interspike intervals. This relation describes the function of Ca(2+) signaling to spike faster and more regular upon stimulation. In the model, it does not depend on cluster properties and cluster arrangement, and is determined only by global characteristics of the Ca(2+) signaling pathway. This unifies robustness of function with sensitivity for feedback and control. Our findings were confirmed by \textit{in vivo} measurements of Ca(2+) spikes. In particular, we show that pharmacological reagents like PTH, U73122 and CPA fail to modulate spike statistics, as predicted by the model.

B2.47 Cancer cell-derived microvesicles constitute a novel mediator of hypoxia dependent induction of pro-angiogenic signaling

P. Kucharzewska, J. Welch, K. Svensson and M. Belting
Institution of Clinical Sciences, Oncology, Lund, Sweden

Hypoxia is a hallmark of solid tumors and a major driving force in tumor angiogenesis and metastasis. Although, the role of several hypoxia-induced growth factors, cytokines, and proteases has been clearly established, the exact mechanisms of how hypoxia promotes tumor development remain to be defined. Membrane-derived microvesicles (MVs) released from malignant cells may promote tumor progression by re-modelling the tumor microenvironment through effects on stromal cells. Here, we show that hypoxic alterations in the molecular composition of MVs derived from human glioblastoma cells (U87MG) are associated with a pro-angiogenic signalling response. Microarray analysis of mRNAs (approx. 27,000 sequences) in normoxic and hypoxic MVs demonstrated hypoxic regulation of a subset of transcripts, several of which have been implicated in angiogenesis. Moreover, comparative analysis of angiogenesis-related proteins showed up-regulation of specific pro-angiogenic proteins in hypoxic MVs in comparison to normoxic ones. MV stimulation of primary, human endothelial cells (HUVECs) resulted in the activation of key signalling pathways, most notably mTOR, p70 S6 Kinase and STATs. Interestingly, hypoxic alterations of the molecular composition of MVs correlated with significantly greater effects on proliferation and survival of HUVECs by hypoxic MVs as compared with normoxic MVs. These results provide important insight into how the oxygenation status of the tumor microenvironment may affect the composition of cancer cell-derived MVs and implicate MVs as an important communication pathway in the adaptive response of malignant cells to hypoxic stress.

B2.48 The PKCz-Plectin interaction controls the cytoskeleton assembly in PC12 cells differentiated with NGF and ATP

S. Corbalan-Garcia1, M. Guerrero-Valero2, C. Marin-Vicente2, J. C. Gomez-Fernandez2 and R. A. Zubarev2
1Universidad de Murcia, Biochemistry and Molecular Biology, Murcia, Spain, 2Karolinska Institute, Medical Biochemistry and Biophysics, Stockholm, Sweden

PKCz is a key mediator of neuronal differentiation mediated by NGF and ATP, but its downstream pathways largely remains to be elucidated. To identify signaling partners of PKCz, we analyzed proteins co-immunoprecipitated with this enzyme in PC12 cells differentiated with NGF and ATP and compared them with those obtained with NGF and growing media. Mass spectrometry analysis (LC-MS/MS) allowed the identification of several potential interacting proteins, being plectin and actin some of them. These putative PKCz-binding partners were validated by double-immunofluorescence and co-localization analysis of the endogenous proteins in the same cells. An increase in the co-localization of PKCz and plectin was detected when PC12 cells were differentiated with NGF and ATP. Furthermore, the intermediate filament organization of these cells was altered by small interfering RNA directed against PKCz, suggesting that this enzyme is participating in the intermediate filaments assembly during neuronal differentiation through plectin. In addition, cortical remodeling of actin associated with cell migration was reversed by downregulating PKCz synthesis, indicating the involvement of the enzyme in the dynamic reorganization of actin cytoskeleton during this process. Together, these data identify...
plectin and actin as new functional targets of the ATP-dependent PKCα signaling and suggest that this enzyme might control neuronal development at different levels by interacting with several cytoskeletal components. [This work was supported by grants from the Fundación Médica Mutua Madrileña, Fundación Séneca 08700/P1/08 and MICINN-Dirección General de Investigación (BFU/2008-01010)].

B2.49  
Study of proteasome implication in TGF-β1 and IGF-I effects on the production of IL-6, TIMP-1 and type-I collagen by nasal polyps fibroblasts  
S. D. Athanasiou¹, T. Stathas², S. Naxakis², E. Giannopoulou³ and A. J. Aletras¹ ¹Department of Chemistry, Laboratory of Biochemistry, University of Patras, Patras, Greece, ²Department of Otolaryngology, Medical School, University of Patras, Patras, Greece, ³Department of Pharmacology, Medical School, University of Patras, Patras, Greece

Nasal polyposis is a chronic inflammatory disease of the nasal mucosa, that characterized by inflammatory cells infiltration, ECM accumulation and oedema. High expression of TGF-β1 and IGF-I, factors which are implicated in fibrosis, in nasal polyps, has been reported. Proteasome is a large intracellular multi-subunit protease complex that selectively degrades intracellular proteins. The aim of this work was to study the effect of TGF-β1 and IGF-I on the production of factors, which may be implicated in nasal polyposis pathogenesis, such as IL-6, TIMP-1, and type-I collagen, as well as the contribution of proteasome in this effect. High expression of IL-6, TIMP-1 and type-I collagen in nasal polyps was ascertained by RT-PCR. Both TGF-β1 and IGF-I caused significant dose- and time-dependent stimulation of IL-6, TIMP-1 and type-I collagen expression in nasal polyps fibroblasts. When fibroblasts were cultured in the presence of proteasome inhibitors, a significant suppression of constitutive as well as of TGF-β1- and IGF-I-enhanced production of TIMP-1 and type-I collagen was observed, whereas the IL-6 expression was enhanced. Both TGF-β1 and IGF-I were able to enhance the proteasome activity and its subunits expression in a dose-dependent manner. When polyps explants were subjected to tissue culture in the presence of proteasome inhibitors, a significant suppression of IL-6, TIMP-1 and type-I collagen production was observed. In conclusion, it appears that TGF-β1 and IGF-I may be responsible for the high expression of IL-6, TIMP-1 and type-I collagen in nasal polyps, factors which are associated with ECM accumulation, through a process where the proteasome may be implicated.

B2.50  
The cAMP and TGF-β1 pathways, suppress the IL-1β and TNF-α-induced production of Matrix Metalloproteinase-1 from nasal polyps fibroblasts Acting on the NO and PKC  
I. Smirlik², S. D. Athanasiou¹, M. Giannakouli², E. Giannopoulou² and A. J. Aletras³ ¹Laboratory of Biochemistry, Department of Chemistry, University of Patras, Patras, Greece, ²Department of Pharmacology, Medical School, University of Patras, Patras, Greece

IL-1β and TNF-α, the main pro-inflammatory cytokines, stimulate the production of many MMPs by a variety of cells, whereas TGF-β1, an anti-inflammatory factor, induces the expression of TIMP-1 and in some cases it antagonizes the stimulatory effect of IL-1β on the expression of MMP-1 and MMP-3. The same effect has been also exerted by cAMP pathway. Nasal polyposis is a chronic inflammatory disease of the upper airways, in which the IL-1β, TNF-α, TGF-β1, MMPs and their inhibitors, are implicated. When polyps fibroblasts were cultured with IL-1β and TNF-α, a significant stimulation of MMP-1 expression was observed, which significantly inhibited by calphostin C, genistein, U0126, SP600125 and SB203580. The IL-1β- and TNF-α-induced production of MMP-1 was strongly suppressed in the presence of TGF-β1, forskolin and IBMX. The PKA inhibitor H-89 partially reversed the suppressive effect of forskolin but not that of TGF-β1. When fibroblasts were cultured for 2 or 48 hour with PMA, in the presence of TGF-β1 or forskolin, a significant suppression of MMP-1 expression was observed. The nitroprusside, a donor of NO, was also able to induce the MMP-1 expression in polyps fibroblasts, which was significantly inhibited by TGF-β1 or forskolin, while they had no effect on PMA-induced production of NO. In conclusion, TGF-β1 via of an unknown mechanism, and cAMP, possibly via activation of PKA, suppress the IL-1β- and TNF-α-induced production of MMP-1 by polyps fibroblasts. This effect may be mediated by influence on PKC and (or) on other factors which are involved in pathways activated by PKC and NO, but not directly on NO synthase.

B2.51  
The role of GCN2 in cell-cycle regulation in mammalian cells  
T. W. Abrahamsen¹, T. Tvegård¹, R. Syljusen², B. Grallert¹ and E. Boye¹ ¹Department Cell Biology, Oslo University Hospital, Radiumhospital, Oslo, Norway, ²Department Radiation Biology, Oslo University Hospital, Radiumhospital, Oslo, Norway

We have shown that fission yeast cells delay entry into S phase after ultraviolet (UVC) irradiation in G1 phase [1]. More recently, we found that the G1/S transition is totally dependent on the GCN2 kinase, which phosphorylates the translation initiation factor eIF2α and mediates a general depression of translation [2]. We are currently investigating how UVC irradiation affects the events of G1 phase in mammalian cells, and in particular the activation of GCN2 and its effects on cell cycle progression. It has previously been shown that also in mammalian cells translational repression in response to UV irradiation is dependent on GCN2 and phosphorylated eIF2α [3]. We have UVC-irradiated human U2OS and BJ cells, and confirmed previous findings of eIF2α phosphorylation after irradiation. In addition, we have begun investigating the role of GCN2 in the G1/S transition. GCN2 siRNA knockdown experiments indicate that eIF2α phosphorylation after UVC irradiation is indeed dependent on GCN2. These findings will be discussed and related to the possible checkpoint function of GCN2 in mammalian cells.

References:


B2.52
Characterization of insulin receptor substrate-4 expression in human colon tumors and tumoral colon lines
M. D. Fernández-Moreno1, I. D. Román1, E. Fernández-Contreras2, B. Hernández-Breijo3 and L. G. Guijarro1
1University of Alcalá, Department of Biochemistry and Molecular Biology, Alcalá de Henares, Spain, 2Hospital Universitario de La Princesa, Department of Gastroenterology and Hepatology, Madrid, Spain

Background and aims: Insulin receptor substrate proteins (IRSs), cytoplasmic adaptors that organize signalling complexes downstream of activated cell surface receptors, are implicated in mediating signals of tumour cells. Recently, it has been shown that IRS-1, IRS-2, and IRS-4 were each overexpressed in 80% of the HCC samples and we have demonstrated that IRS-4 is an essential protein for the proliferation/differentiation of HepG2 human hepatoblastome cells. In the other hand, colon-rectum cancers are one of the major ones in developed countries and their treatments remain poor and systemic therapies have not been efficient. Taking account the important role that IRS-4 shows in HCC and in other types of cancer, we decided to characterize for the first time IRS-4 expression in human colon tumours and tumour colon lines.

Methods: We have employed several tumour colon lines (HCT116, HT29, LOVO, MAWI, Colo205, RKO16) and samples of human colon tumours and adjacent tissue. IRS-4 was analysed by Western blotting using specific antibodies. Immunoprecipitation assays were carried out with anti-IRS-4, anti-pTyr and anti-phospho-IRS-4. Immunochemistry experiments were performed using anti-IRS-4.

Results and conclusions: Immunohistochemistry results show that in healthy adjacent tissue IRS-4 is located in colon crypts, mainly in crypt colonocytes nucleus. Interestingly, IRS-4 is expressed in every colon segments (ascending, transverse and descending colon) and dramatically expressed in tumour colon lines. Moreover, in human colon samples, IRS-4 is constitutively phosphorylated in tyrosine residues. Our results point to a potential role of IRS-4 in human colon tumours development.

B2.53
Comparative analysis of Sp1 gene regulation in an alternating oxygenating microenvironment of human brain tumors
H. M. Said1, B. Polat2, C. Hagemann3, A. Staab4, M. Flentje5 and D. Vordermark5
1Department of Radiation Oncology, University of Würzburg, Würzburg, Germany, 2University of Würzburg, Medical Faculty, Radiation Oncology, Würzburg, Germany, 3Department of Radiation Oncology, University of Würzburg, Medical Faculty, Tumor Research Laboratory, Würzburg, Germany, 4Paul-Scherrer-Institut, Proton Research, Villigen, Switzerland, 5Martin-Luther-University-Halle-Wittenberg, Radiation Oncology, Halle, Germany

Sp1 is a human transcription factor involved in gene expression in the early development of an organism. It belongs to the Sp/ KLF family of transcription factors. The protein is 785 amino acids long, with a molecular weight of 81 KDa. HIF-1α is the main the regulator most hypoxia induced genes. Studies related to the Sp1 regulation under hypoxic conditions where not conducted until now in human brain cancer.

Results and conclusions: Sp1 was over expressed via hypoxic oxygenation development in different glioblastoma cells micromilieu in vitro under extreme hypoxic conditions (0.1% oxygen) or reoxygenation after hypoxia, both on protein and mRNA level. An correlation was demonstrated between Sp1 expression and the hypoxia induced HIF-1α and the regulated genes in human glioblastoma tumor tissue specimens examined, in vitro.

Conclusions: Sp1 upregulation as an answer to hypocxotic development in glioblastoma micromilieu, both on protein and mRNA are considered as a coregulator of HIF-1α and as a consequence the genes that are regulated by the hypoxic HIF-1α pathway in cancer human cells.

B2.54
Luteolin induces apoptosis by ROS-mediated endoplasmic reticulum stress and mitochondrial dysfunction in mouse neuroblastoma cells
A. Y. Choi1, E.-J. Yeo2 and I. Kang3
1Kyung Hee University, Seoul, Korea, 2Gachon University, Seoul, Korea, 3Gachon University, Seoul, Korea

Neuroblastoma is the most common solid cancer that forms in nerve tissue and it is currently being treated by chemotherapy. In the present study, we investigated the anti-cancer effect of luteolin, a common flavonoid in Neuro2a mouse neuroblastoma cells. Luteolin induced activation of caspase-12, -9, and -3 and cleavage of poly (ADP-ribose) polymerase. Also, luteolin induced expression of ER stress-associated proteins, including glucose-regulated protein (GRP) 78 and 94, C/EBP homologous protein (CHOP/GADD153), the phosphorylation of eIF2α and the cleavage of poly (ADP-ribose) polymerase. Also, luteolin induced mitochondrial dysfunction in mouse neuroblastoma cells. Luteolin-induced ROS generation at early point and luteolin induced mitochondrial apoptotic pathway and induced mitochondrial dysfunction. In addition, antioxidants such as N-acetylcysteine and glutathione blocked luteolin-induced ROS generation, cell death, expression of CHOP and GRP78, and mitochondrial dysfunction. These results showed that luteolin-induced reactive oxygen species (ROS) accumulation might play an important role in ER stress-induced apoptosis and mitochondrial dysfunction in Neuro2a mouse neuroblastoma cells.

B2.55
JAK2/STAT pathway regulates HIF-1 activation and hypoxic gene expression via ROS and PI 3-kinase/Akt dependent pathways in hypoxia-stimulated microglial cells
K. Y. Hwang1, H. Yoon and I. Kang
1Kyung Hee University, Seoul, Korea

Hypoxia induces an inflammatory activation of microglia during cerebral ischemia. The transcription factor of hypoxia-inducible genes hypoxia-inducible factor-1 (HIF-1) is known to be involved in inflammation and immune response. JAK/STAT pathway is
shown to be activated in microglial cells from cytokines (IFN-γ) but its activation and effect on inflammatory responses are not understood in hypoxic microglia. Thus, we examined the roles of JAK2/STAT signaling in HIF-1 activation and inflammatory responses of hypoxia-stimulated microglial cells. Hypoxia increased phosphorylation of JAK2 and STAT pathways in microglia and treatment with a specific inhibitor of JAK2 (AG490) significantly inhibited hypoxia-induced expression of HIF-1α protein levels and transcription activity. Transfection with dominant-negative mutant of JAK2 (DN-JAK2) or STAT3 siRNA also blocked HIF-1 transactivation and expression of HIF-1 responsive genes such as iNOS, COX-2, and VEGF, suggesting that JAK2/STAT3 regulates hypoxia-induced HIF-1 activation. Treatment with antioxidant reduced hypoxia-induced JAK2/STAT3 phosphorylation. Moreover, we found that JAK2 pathway cross-talks with PI 3-kinase/Akt pathway in hypoxic BV2 microglia. Taken together, these results suggest that JAK2/STAT pathway is involved in hypoxia-induced HIF-1 activation and hypoxia gene expression and that ROS appears to act upstream of JAK2/STAT in hypoxic BV2 microglia.

B2.56
Abstract withdrawn

B2.57
Apicidin induces endoplasmic reticulum stress via reactive oxygen species generation in mouse Neuro 2A neuroblastoma cells

J. H. Choi and I. Kang
KyungHee University, Seoul, Korea

Neuroblastoma is the most common solid cancer of childhood that forms in nerve tissue. In the present study, we investigated the anti-cancer effect of apicidin, one of HDAC inhibitors, in Neuro 2A mouse neuroblastoma cells. Apicidin induced activation of caspase-12, a specific caspase in ER, as well as caspase-3. Apicidin induced unfolded protein responses (UPR) of ER, including expression of CHOP and splicing of XBP-1 and phosphorylation of EIF2α, JNK, p38MAPK, and ERK and the cleavage of ATF-6. Apicidin increased ROS accumulation and mitochondrial dysfunction in Neuro 2a cells. We found that treatment with salubrinal or CHOP siRNA blocked luteolin-induced cell death. We also found that an anti-oxidant N-acetyl cysteine (NAC) and a chelator of intracellular calcium ion BAPTA-AM inhibited apicidin-induced apoptosis and changes in UPR-associated proteins. Taken together, the results suggest that apicidin induces apoptotic cell death via ER stress through ROS accumulation and calcium ion.

B2.58
BDNF/TrkB axis regulates proliferation and invasion of cervical cancer cells

H. Yoon, K. Y. Hwang and I. Kang
KyungHee University, Seoul, Korea

Neurotrophin BDNF and Tropomyosin-related kinase B (TrkB) is a key signaling molecule in the development of the nervous system. BDNF and its receptor TrkB are often overexpressed in a variety of human cancers such as neuroblastoma and hepatocarcinoma, in which these are involved in tumor expansion and resistance to anti-tumor agents. In the present study, we examined the roles of BDNF/TrkB signaling pathways in proliferation and invasion of human cervical cancer cells. We found that BDNF and TrkB were overexpressed in human cervical cancer cell lines including HeLa, SiHa, Caski, and ME180 cells. Treatment with specific inhibitors of PKA (H89), TrkB (K252a), and NF-kappaB (SN50) significantly inhibited the expression of BDNF and TrkB, suggesting the involvement of these molecules in BDNF/TrkB axis in cervical carcinoma. We found that a CaMKII inhibitor KN93 increased BDNF secretion and increased the invasion of cervical cancer cells. We also found that inhibition of TrkB receptor by K252a inhibited the invasion of ME180 cervical cancer cells. Taken together, these results suggest that BDNF/TrkB axis may play roles in tumor growth and expansion in cervical cancer cells.

B2.59
Inhibiton of TRAIL-mediated apoptosis in non-adherently growing colon epithelial cells is connected with activation of pro-survival pathways

L. Koe, M. Hyzadalova, A. Vaculova, J. Hofmanova and A. Kozubik
Department of Cytokinetics, Institute of Biophysics ASCR, Brno, Czech Republic

In healthy colon, the colonic epithelial cells are shed into intestinal lumen at the top of the crypt and die by detachment-induced apoptosis (anoikis). Resistance of transformed epithelial cells to the anoikis promotes cancer cell invasion and metastasis. We studied the effects of TNF-related apoptosis inducing ligand (TRAIL) on cytokinetic parameters and adhesive properties of the cell lines derived from human foetal (FHC cells) and human adenocarcinoma (HT-29 cells) colon tissues in association with anoikis induction. We detected the significant decrease of TRAIL-induced apoptosis in the non-adherently growing HT-29 cells in comparison with the adherent cultivation. Based on this finding we focused our attention to detail mechanisms of the cell survival under TRAIL treatment. We confirmed our hypothesis of activation of pro-survival pathways, actually PI3K/Akt and MAPK/ERK, which are connected with focal adhesion kinase (FAK) phosphorylation. Increased phosphorylation of Akt and ERK kinases and also enhanced expression of FLIP and Mcl-1 proteins as downstream molecules of PI3K/Akt pathway were observed during non-adherent cultivation. Moreover, we detected significantly enhanced apoptotic parameters after treatment with TRAIL in combination with specific PI3K/Akt and MAPK/ERK inhibitors during non-adherent cultivation. Taken together, our data suggested that the decrease of the TRAIL-mediated apoptosis of colon epithelial cells induced by non-adherent type of cultivation is connected with the anchorage loss, FAK phosphorylation and with activation of pro-survival pathways. This work was supported by grants 305/09/1526 GACR, 303/09/H048 GACR, and 524/07/1178 GACR.

B2.60
Hypoxic induction of protease activated receptor-2 promotes angiogenic signaling in endothelial cells

K. Svensson1, S. Skölö2, T. Löfstedt1, R. Wolfram3 and M. Belting1
1Department of Clinical Sciences, Lund University, Division of Oncology, Lund, Sweden, 2Department of Oncology, Lund University, Lund University Hospital, Lund, Sweden, 3Department of Immunology and Microbial Science, The Scripps Research Institute, La Jolla, USA

Coagulation-dependent signaling through G-protein coupled protease activated receptors (PARs) has been implicated in tumor...
In vitro growth inhibitory effects of arsenic trioxide in non-small cell lung cancer with different k-ras mutations

F. He, Y. L. Kwong and C. M. Ho
The University of Hong Kong, Medicine, Hong Kong, Hong Kong

K-Ras mutation is frequently related to resistance of tyrosine kinase inhibitor (TKI) therapy for non-small cell lung cancer (NSCLC). Arsenic trioxide (ATO), as a novel anticancer drug, is primarily used to cure acute promyelocytic leukemia. However, for other types of cancers the effects of ATO are still unveiled. Here we test in vitro growth inhibitory effects of ATO in two NSCLC cell lines: H23 (K-Ras mutant) and HCC827 (K-Ras wild type) in hope of shedding light on overcoming the de novo resistance resulted by TKI treatments. ATO induces more cell death compared to Erlotinib (one small-molecule TKI in clinical use) as well as cell apoptosis. ATO leads to down-regulations of both phosphor-Akt and Akt while Erlotinib just diminishes phosphor-Akt. Of the Ras/Raf/Erk pathway, ATO enhances the phosphorylation of Erk1/2 significantly in H23 but not in HCC827. In order to prove that downstream signalings following activation of Erk1/2 are driven to apoptosis instead of cell proliferation in H23, we examined the change of Bax which is believed to play an important role in intrinsic apoptosis. Surprisingly, Bax is down-regulated in a dose- and time-dependent manner. Before the further explorations of this phenomenon, current acceptable explanation would be either that ATO is working through extrinsic apoptotic pathways or that ATO effects diversified signalings which combined together lead to final apoptosis at K-Ras mutant cell lines of NSCLC.

B2.62 Intracellular calcium content, phospholipase C and protein kinase C activity in rat colonicocytes under colitis-associated carcinogenesis

O. Kravchenko, O. Drobinska and L. Ostapchenko
Taras Shevchenko National University of Kyiv, Biology/ Biochemistry, Kyiv, Ukraine

The signaling pathway that increases cytoplasmic calcium concentration is the phospholipase C (PLC) pathway. PLC hydrolyses the membrane phospholipid PIP2 to form IP3 and diacylglycerol (DAG). DAG and Ca2+ activate the protein kinase C (PKC). A new study shows that calcium may lower the risk of colon cancer, but mechanisms of this effect remain vague. The aim of this study is to determine cytoplasmic Ca2+ concentration, PLC and PKC activity in colonicocytes during experimental colitis-associated carcinogenesis (CAC). CAC was modeled by water containing 1, 5% dextran sulfate sodium salt (DSS) and weekly injections of 1,2-dimethylhydrazine (DMG) during 10 weeks. The Ca2+ content was determined by Indo-1. The PKC and PLC activity was assessed by the standard methods. Changes of intracellular Ca2+ content were not determined on the 1-st day of DSS-colitis induction but PLC and PKC activities slightly increased. Maximal accumulation of Ca2+ was observed on the 3-rd and 7-th day of experiment, when the parameter was two times more than control value. PLC and PKC activities increased and were half as large (for PLC) and three times as much (for PKC) against the control on the 7-th day of DSS treatment. The DMG were accompanied by Ca2+ content gradually decreasing comparing with inflammation state but it remained significantly above the control. The PLC and PKC activity during DMG influence increased and reached maximum on the 4-th and 2-nd week respectively of carcinogenesis development. The changes in Ca2+ pathway may contribute to alterations in colonicocyte functions and cell cycle associated with colon inflammation-associated carcinogenesis.

B2.63 Nitric oxide synthase activity and expression its isoforms in gastric mucosa under stress-induced ulceration

I. Maksymovyych, O. Drobsinska and L. Ostapchenko
Taras Shevchenko University of Kyiv, Faculty of Biology/ Biochemistry, Kyiv, Ukraine

Nitric oxide (NO) as a secondary messenger has been shown to protect the gastrointestinal mucosa from a variety factors. However, production of excessive amount of NO has been implicated as a cytotoxic factor in a variety of pathophysiological processes. The exact role of NO in processes of gastric ulcer development induced by stress has not been established. The aim of the study was to determine the nitric oxide synthase (NOS) activity and expression its isoforms in gastric mucosa during development of ulcer induced by stress. Male rats weighing 250-270 g were starved for 24 hour prior to experiments, but were allowed free access to water. Gastric damages were induced by water immersion restraint stress. The animals were killed after application of 0.5; 1; 2; 3 hour WIR. NOS activity was measured in homogenate of gastric mucosa. Expression of NOS isoforms was analyzed by RT-PCR. The significant damages of gastric mucosa were not found after 0.5 hour of stress. However, the quantity and areas of ulcer increased on 1, 2, 3 hour of stress. The elevation of NOS activity in 3, 6, 7 times was observed during this experiment. Basal levels of endothelial and neuronal NOS mRNA expression were detected in normal tissue, but inducible mRNA expression wasn’t.
observed in this case. The levels of eNOS and nNOS mRNA expression hadn’t changed during ulcer development. Expression of iNOS was detected only after application 2 hours WIR. Further level of iNOS expression was increased.

B2.64
Functional analysis of Epithelial cell adhesion molecule (EpCAM) in breast cancer cell lines
A. Martowicz1, G. Untergasser2, M. Wurm1, G. Spizzo1 and G. Gastl
1Experimental Oncology Laboratory, Medical University Innsbruck, Hematology and Oncology, Innsbruck, Austria,
2Tumorbiology & Angiogenesis Laboratory, Medical University Innsbruck, Hematology and Oncology, Innsbruck, Austria

The Epithelial cell adhesion molecule (EpCAM) is a transmembrane molecule that inhibits cell-cell contact and adhesion by antagonizing E-cadherins. In primary and metastatic breast cancer EpCAM gene expression has been shown to be increased up to 1000 fold. In particular, overexpression in primary breast carcinomas correlates with poor overall survival in node-positive breast cancer patients. These observations suggest that EpCAM overexpression correlates with tumor metastasis and aggressive progression. In this study we investigated, whether EpCAM overexpression or downregulation by shRNA affects invasion, invasion and can promote tumor growth. Moreover, we screened for molecular targets that are modulated by overexpression or downregulation of EpCAM. Therefore, different breast cancer cell lines (n = 6) were analyzed for EpCAM, E-Cadherin and c-myc genes expression. Based on these data cell lines with low EpCAM expression were selected for overexpression by the use of a transposon based adeno viral system. On the other hand, cell lines with a strong EpCAM expression were transfected by a lentiviral system to generate a downregulation of this antigen. Subsequently, all generated cell lines will be used to evaluate differences in cell morphology, invasion and can promote tumor growth. Moreover, we screened for molecular targets that are modulated by overexpression or downregulation of EpCAM. Therefore, different breast cancer cell lines (n = 6) were analyzed for EpCAM, E-Cadherin and c-myc genes expression. Based on these data cell lines with low EpCAM expression were selected for overexpression by the use of a transposon based adeno viral system. On the other hand, cell lines with a strong EpCAM expression were transfected by a lentiviral system to generate a downregulation of this antigen. Subsequently, all generated cell lines will be used to evaluate differences in cell morphology, invasion and can promote tumor growth. Moreover, we screened for molecular targets that are modulated by overexpression or downregulation of EpCAM.

B2.65
Cytoplasm and organelle transfer between mesenchymal multipotent stromal cells and renal tubular cells in co-culture
T. Khryapenkova1, E. Plotaikov1, S. Galkina1, G. Sukikh2 and D. Zorov1
1A.N.Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russian Federation, 2Research Center of Obstetrics, Gynecology and Perinatology, Moscow, Russian Federation

One of the modern directions of nephroprotection is regenerative cell technology, i.e. transplantation of some stem and progenitor cells for improving kidney function after acute kidney injury. Co-culture of human mesenchymal multipotent stromal cells (MMSC) and rat renal tubular cells (RTC) were explored. We observed formation of different types of intercellular contacts between MMSC and RTC, including gap junction and so-called tunneling nanotubes. In a non-confluent monolayer when cells were at a distance one from another, TNT were formed very extensively, whereas after culture had nearly formed a monolayer, numerous short TNT between cells resembling threads connecting cloth patches were observed. We documented intercellular exchange with fluorescent probes specific to cytosol, plasmalemma and mitochondria. Initial transport of cellular components was revealed after 3 hour of co-culturing, and occurred in two directions – both direct and retrograde as referred to RTC. However, transport of probes toward MMSC was more efficient than toward RTC. Blocking of gap junction transport or nanotubes formation prevented transel of cytoplasm content. One significant result of coculture was appearance of renal-specific Tamm-Horsfall protein and cytokeratin in MMSC, indicating induction of their differentiation into kidney tubular cells. We conclude that transfer of cellular compartments between renal and stem cells could provide differentiation of MMSC when transplanted into kidney and result in therapeutic benefits in renal failure. Supported by Russian Foundation of Basic Research (# 08-04-01667 and 09-04-13663-ofi_c).

B2.66
Overexpressed Cyclophilin B protects cells from H2O2-mediated programed death
K. Kim and W. Choe
Department Biochemistry and Molecular Biology, Kyunghee University, Seoul, Korea

Cyclophilins (Cyps) were identified as cellular binding proteins for the immunosuppressive drug cyclosporine A (CsA) and are constitutively expressed in most tissues. Cyclophilins are multifunctional proteins that are involved in protein folding, mitochondrial functions, interaction with CD147, the immune system, and cancers. One of these family, Cyclophilin B is found mainly in the ER lumen and it has peptidyl-prolyl cis-trans isomerase (PPIase) activity that catalyzes protein folding reactions in cells. Reactive oxygen species (ROS) are generated as by-products of the mitochondrial respiratory process. They have emerged as an important signaling molecule based on their unique biochemical properties. It consists of various radicals, which might exert different effects on cellular signaling. Low levels of ROS regulate cellular signaling and play and important role in normal cell proliferation. In this study, we determined the potential role of overexpressed Cyclophilin B as a protector cancer cells from oxidative stress. With Huh7 (Human Hepatoma), we performed several experiments including Cell viability assay and western blot. According to our results, overexpressed CypB increased cell viability though activation of ERK pathways and PPIase activity was required for this function.

B2.67
5’-nitro-indirubinoxime induces G1 cell cycle arrest and apoptosis in salivary gland adenocarcinoma cells through the inhibition of Notch-1 signaling
1Chosan University College of Dentistry, Pathology, Gwangju, Korea, 2Dongguk University College of Oriental Medicine, Biochemistry, Gyeongju, Korea

5’-nitro-indirubinoxime (5’-NIO) is a novel indirubin derivative which exhibits anti-cancer activity in a variety of human cancer cells. Here, we report that 5’-NIO has a significant effect on growth inhibition and the induction of apoptotic processes in human salivary gland adenocarcinoma (SGT) cells by inhibiting the Notch-1 pathway. 5’-NIO significantly inhibited the mRNA levels of Notch-1 and Notch-3 and their ligands (Delta1, 2, 3 and Jagged-2) in SGT cells. Immunocytochemistry analysis showed that 5’-NIO specifically decreased the level of Notch-1 in the
Abstracts

B2.68
The interplay between benzo[α]pyrene and proinflammatory cytokine affects both metabolic activation of the procarcinogen and the inflammatory response in lung epithelial cells

I. Linaova1, M. Machala2, A. Kozubik1 and J. Vondracek1
1Institute of Biophysics ASCR, v.v.i., Brno, Czech Republic, 2Veterinary Research Institute, v.v.i., Brno, Czech Republic

Lung cancer is among the leading worldwide causes of the cancer-related death. Deregulated chronic lung inflammation plays an important role in cancer development and it can be induced both during pulmonary diseases and other external factors, such as tobacco smoking. Extensive production of proinflammatory cytokines, such as TNF, plays a key role in the pathophysiological events related to deregulate inflammatory reaction. In the present study, we analyzed mutual deregulation of enzymes involved both in xenobiotic metabolism and inflammatory responses, caused by environmental pollutant and important tobacco smoke constituent benzo[α]pyrene (BaP) and TNF in rat lung RLE-6TN alveolar type II cell line. Our results show that TNF may significantly modulate the expression of cytochrome P450 superfamily members, such as CYP1A1/1B1, and thereby affect xenobiotic clearance and toxicity. In similar manner, BaP modulates the expression/activity of enzymes induced by TNF that are involved in inflammatory responses such as prostaglandin-endoperoxide synthase 2 (COX-2) or inducible nitric oxide synthase (iNOS), leading to enhanced release of their products such as prostaglandin E2. Moreover, BaP increases expression of inflammatory cytokines induced by TNF in target cells. These results demonstrate that proinflammatory cytokines may significantly modulate metabolism of environmental pollutants, possibly contributing to tumor initiation. On the other hand, environmental pollutants affect signaling pathways related to inflammatory response and may thus contribute to deregulated inflammation in lung. [Supported by grant No. 524/09/1337 from the Czech Science Foundation.]

B2.69
The vitamin E analogue γ-Tocotrienol blocks osteoclastogenesis and promotes osteoblast maturation through the NF-KB and p38/MAPK pathway: evidence for combined antiosteolytic and bone reforming activity

A. Odysseos1, V. Liapis2, S. Hay3, Y. Christou1, E. Maltezou3, A. Keramidas1 and A. Evdokiou2
1EPOS-Iasis, R&D, Nicosia, Cyprus, 2University of Adelaide, Adelaide, Australia, 3University of Cyprus, Nicosia, Cyprus

We investigated the effects of the vitamin E analogue, γ-Tocotrienol (γ-T) on bone metastatic breast cancer cells survival and osteoclast differentiation and bone resorptive activity using three independent in vitro-model systems of osteoclastogenesis. When human peripheral blood mononuclear cells (PBMCs) and the RAW264.7 murine monocyctic cell line were cultured with the receptor activator of nuclear factor kappa B-ligand (RANKL), formation of tartrate-resistant acid phosphatase (TRAP) positive multinucleated cells and bone resorption were increased compared with untreated cells. γ-T dose-dependently inhibited RANKL-induced osteoclastogenic differentiation and bone resorption in both cell models. Similarly, it inhibited the bone resorptive activity of mature osteoclasts that were isolated from human Giant Cell Tumours of bone when cultured on dentine slices. RANKL activated osteoclastogenesis essential pathways NFkB, ERK1/2 and p38/MAPK, whereas γ-T profoundly inhibited RANKL-induced activation of NFkB and p38/MAPK. Effect of γ-T on osteoblast function was investigated in mineralized bone nodule-forming primary human osteoblast cultures. γ-T progressively increased matrix-containing mineralized nodules in osteoblast cultures with a concomitant increase in alkaline phosphatase activity. Comparable concentrations of γ-T induced profound apoptosis in highly metastatic breast cancer cell lines. Taken together these results demonstrate for the first time that γ-T has a combined inhibitory effect on osteoclast activity and on survival of bone metastatic cancer cells whereas it significantly promotes osteoblast activity, serving as promising treatment for osteolytic bone disease.

B2.70
Fluorescence polarization used in HTS in order to identify new antagonists of cAMP to inhibit PKA activity

A. David1, M. Andrej2, J. Klaveness3, K. Undheim4 and K. Tasken1
1The Biotechnology Centre of Oslo, Centre for Molecular Medicine Norway, Nordic EMBL Partnership, University of Oslo, Lauras AS, Oslo, Norway, 2Lauras AS, Oslo, Norway, 3University of Oslo, School of Pharmacy, Oslo, Norway, 4University of Oslo, Lauras AS, Department of Chemistry, Oslo, Norway

The second messenger cAMP (cyclic adenosine 3’,5’-monophosphate) is generated by ATP hydrolysis due to the G-protein-mediated activation of adenylate cyclase. An elevated level of cAMP leads to the inhibition of TCR signaling. The binding of cAMP to PKA regulatory subunits dissociates the holo-PKA, releasing active catalytic subunits. The active PKA C-subunits are then able to phosphorylate Csk, which phosphorylates Lck, leading to inhibition of T cell proliferation. Thus, antagonists able to keep the PKA under inactive form could play important role in treatment based on immunomodulation. We previously showed that the impaired proliferation of T cells from HIV infected patients can be improved up to 300% by the use of selective antagonist of PKA type I. To determine new antagonists of PKA type I, we optimized a Fluorescence Polarization High-Throughput Screen- ing (FP-HTS) assay (Saldanha et al. 2006) to test two types of small molecules: rationally designed analogues of cAMP and structural-based selected non-nucleotide compounds. Using this method, several hits were identified after the primary screen; cell based assays have been pursued to test the toxicity of the hits and their effect on the T cell proliferation.
B2.71 Design, synthesis and characterization of a highly effective Hog1 inhibitor: a powerful tool for analyzing MAP kinase signaling in yeast

P. Dineér1, J. Veide Vilg2, J. Kjellén2, M. Iwona1, T. Andersson1, S. Hohmann2, R. Wysocki3, M. J. Tamas2 and M. Grotti1

1 University of Gothenburg, Department of Chemistry, Medicinal Chemistry, Gothenburg, Sweden, 2 University of Gothenburg, Department of Cell and Molecular Biology, Gothenburg, Sweden, 3 University of Wrocław, Institute of Genetics and Microbiology, Wrocław, Poland

Cells utilize signal transduction pathways to sense and respond to internal and external cues. The Saccharomyces cerevisiae High-Osmolarity Glycerol (HOG) pathway is a conserved mitogen-activated protein kinase (MAPK) signal transduction system that is required for yeast to adapt to various stress conditions. HOG1, the MAPK of the HOG-pathway, is the yeast orthologue of mammalian p38, and it controls transcription, translation and cell cycle adaptations in response to environmental stress. Herein we describe design, synthesis, and biological application of a small molecule inhibitor that is cell-permeable, fast-acting, and highly selective against Hog1. We show that this molecule is a potent inhibitor of Hog1 kinase activity both in vitro and in vivo, and use it to demonstrate a crucial role of Hog1 in controlling the exit from stress-induced cell cycle arrest. Hence, this chemical genetic approach of signaling characterization provided novel insight into the role of Hog1.

B2.72 Chitinase 3-like 2 gene as a potential marker of human glial tumors

S. Avdieiev, P. Areshkov and V. Kavsan

Institute of molecular biology and genetics NAS of Ukraine, Kyiv, Ukraine

Identification and characterization of differentially expressed genes in astrocytic gliomas can be expected to provide important insights into the molecular determinants of carcinogenesis. Serial Analysis of Gene Expression found CHI3L1 (YKL-40) and CHI3L2 (YKL-39) genes among the most abundant transcripts in glioblastoma. Overexpression of CHI3L1 at the mRNA and protein levels in glioblastoma was shown in our previous study. Recklies et al. (2002) reported that CHI3L1 stimulated DNA synthesis and proliferation by activation of extracellular signal-regulated kinases (ERK1/ERK2) and protein kinase B (AKT)-mediated signaling cascades. Taking into account the lack of similar data for CHI3L2, closely related in amino acid and nucleotide sequences (52 and 56% homology) to CHI3L1, this study was initiated to analyze the expression and functional role of CHI3L2 in glial tumors. Northern hybridization and Western blot analysis showed the expression of CHI3L2 in the majority of glioblastomas. CHI3L2 activates ERK1/ERK2 in human embryonic kidney (HEK293) and human glioblastoma (U87 MG) cells. Unexpectedly, dose dependent decreases in total DNA content and [3H]thymidine incorporation were observed in HEK293 cells treated with CHI3L2. CHI3L2 induced sustained phosphorylation of ERK1/ERK2 in HEK293 cells in a very similar way to that was shown for PC12 cells treated with nerve growth factor (NGF). These data suggest that CHI3L2 may cause differentiation phenotype, but not proliferation. Thus, increased expression of CHI3L2 at the mRNA and protein levels in glioblastoma and its involvement in activation of MAPK signaling cascade may suggest its potential role in carcinogenesis.

B2.73 Abscisic acid released by quartz-stimulated macrophages plays a key role in the silica-induced inflammatory process

M. Magnone1, L. Sturla1, E. Iacchetti2, L. Guida1, S. Scarfi1, C. Usa2, A. De Flora1 and E. Zacchi1

1 Department of Experimental Medicine, University of Genova, Genova, Italy, 2 National Research Council, Institute of Biophysics, Genova, Italy

Exposure to airborne crystalline silica particles induces silicosis, an inflammatory disease of the lungs where alveolar macrophages release inflammatory mediators, like prostaglandin-E2 (PGE2) and tumor necrosis factor-α (TNF-α) eventually leading to tissue damage. The phytohormone Abscisic acid (ABA) has been recently discovered as a new human hormone involved in inflammation. Here we report the pivotal role of ABA in the silica-induced inflammatory process in murine RAW264.7 cells and in primary culture rat alveolar macrophages. Stimulation of RAW264.7 cells with quartz powder generates plasma membrane peroxidation responsible for ABA release. ABA then activates an autocrine loop sequentially involving binding of the hormone to its plasma membrane receptor LANCL2, activation of NADPH oxidase, production of reactive oxygen species, intracellular calcium rise, nuclear translocation of NF-kB and consequent increase of cyclooxygenase-2 expression, PGE2 production and TNF-α release. Silencing LANCL2 expression in RAW264.7 prior to stimulation with quartz results in an almost complete inhibition of PGE2 and TNF-α release, underlining a fundamental role of ABA in the early stage of quartz-induced inflammation. Studies on rat alveolar macrophages revealed early and late steps of ABA-induced responses strictly comparable to patterns of ABA signaling in murine RAW264.7 cells. These results identify ABA as a key mediator in quartz-induced pulmonary inflammation and provide a new possible target for anti-silicotic therapies.

B2.74 The Ser/Thr protein kinase activities in rat parietal cells under chronic atrophic gastritis development

M. Tymoshenko, L. Gayda, O. Drobsinska and L. Ostapchenko

Department of Biochemistry, Taras Shevchenko National University of Kyiv, Faculty of Biology, Kyiv, Ukraine

Chronic atrophic gastritis (CAG) has been associated with an increased risk of gastric cancer. Thus, investigation of mechanisms of gastric atrophic changes development remain problems of high importance. Disorders of apoptosis and cell proliferation in mucous are underlined in atrophy changes. Since the serine/threonine protein kinases play a commanding role in many signal pathways. The phosphorylation of cholinergic channel proteins by cAMP-dependent protein kinase A (PKA) results in increasing of its conductivity. Besides PKA Ca2+-dependent protein kinase C (PKC) phosphorylates cytoskeleton and membrane proteins thereby implicating in the processes for the regulation of parietal cell HC1 secretion. Rat model of CAG was performed by intragastric administration of 2% sodium salicylate, 20 mM/L deoxycholate sodium daily for 6 weeks. The parietal cells were isolated on 1st, 2nd, 3rd, 4th 5th and 6th weeks of model. Cell PKC and PKA activities have been evaluated by incorporation of 32P from labeled ATP to specific protein substrate. Histological findings showed a significant decrease of mucus gland thickness in CAG rats than that of control. The increasing of investigated PK activities was shown during initial four weeks of pathology development. These results suggested important role of Ser/Thr
phosphorylation in initiation of gastritis. Such disorders in intracellular signal transduction might result in functional changes, including HCl secretion and proliferation intensification. Besides, these effects served as additional pathogenesis factors of chronic atrophic gastritis development. Protein kinase A and C activities were decreased in a five week of pathology development.

**B2.75**

Sumoylation of the 20-hydroxyecdysone receptor from *Drosophila melanogaster*

J. Seliga1, K. Bielska1, E. Wieczorek1, R. Niedenthal2 and A. Ozjar1

1Department of Biochemistry, Wroclaw University of Technology, Wroclaw, Poland, 2Medical School Hannover, Institute for Physiological Chemistry/Biochemistry, Hannover, Germany

The 20-hydroxyecdysone receptor (EcR) is an insect transcription factor belonging to the nuclear receptor superfamily. EcR and its ligands, absent in vertebrates, are used in commercially available ecdysone-inducible expression systems and may have potential usage as gene switches with therapeutic application. It is little known and about EcR activity regulation especially by posttranslational modifications in mammalian cells and till now there has been no research about EcR sumoylation. In order to investigate SUMO attachment to EcR, we used the Ub9 fusion-directed SUMOylation (UFDS) system. Series of constructs coding all three isoforms of *Drosophila melanogaster* EcR (EcR-A, EcR-B1 and EcR-B2) fused with sumoylation conjugation enzyme (Ub9) on N- or C- termini were prepared, expressed in HEK293 cell line and examined by Western blotting. We observe electrophoretic mobility changes for all proteins suggesting SUMO conjugation, both with the endogenous SUMO and with the coexpressed EGFP-SUMO1. Dependent on the Ub9 fusion this sumoylation requires neither SUMO ligase nor any extracellular stimulation. We also observe diverse sumoylation pattern depending on location of Ub9 on N- or C- termini. Moreover we present here results of bioinformatic prediction of sumoylation sites of *Drosophila* EcR obtained with four predictors: PCI-SUMO, SUMOplotTM, SUMOpreg, SUMOsp2.0.

Acknowledgements: This work has been supported by the Polish Ministry of Science and Higher Education Grant N302 035 32/2827 and The Wroclaw University of Technology.

**B2.76**

Role of intracellular calcium in the control of boar sperm functions

D. Martin-Hidalgo, M. C. Gil, M. J. Bragado and L. J. Garcia-Marín

Universidad de Extremadura, SINTREP, Cáceres, Spain

To successfully achieve the fertilization of the oocyte, sperm cells undergo several processes that include activated motility, capacitation and the acrosome reaction. Our aim was to study the involvement of intracellular calcium in the regulation of these cellular processes in porcine spermatozoa. Semen from 8 Duroc boars was used and motility parameters were evaluated by computer assisted ISAS® program and the plasma membrane fluidity (MF), acrosome reaction (AR), cell viability and mitochondrial membrane potential (MMP) were analyzed by flow cytometry using as dyes Merocyanine-540/YO-PRO-1, FITC-PNA/PI, SYBR-14/PI and JC-1, respectively. Spermatozoa were incubated in an extracellular calcium (EGTA)-free medium (TBM) for different times at 39°C and 5% CO2 in presence/absence of the cell-permeable calcium chelator BAPTA-AM and cell permeable cAMP analogue 8Br-cAMP. BAPTA treatment led to a full inhibition of spermatozoa motility in TBM or stimulated by 8Br-cAMP. The reduction in spermatozoa viability and in the percentage of spermatozoa with high MMP observed in TBM or in presence of 8Br-cAMP is prevented by BAPTA incubation. Moreover, spermatozoa AR induced by 8Br-cAMP in TBM is inhibited by BAPTA treatment. However, intracellular calcium depletion did not modify the degree of MF in spermatozoa induced by 8Br-cAMP. In summary, our results suggest that intracellular calcium is necessary and sufficient to maintain the spermatozoa motility in a calcium free medium. Moreover, the intracellular calcium diminishes the spermatozoa viability and the MMP. However, the degree of MF in the porcine spermatozoa is independent of intracellular calcium. Supported by JUEX PR07A100.
sensation. Licking response of GSH was measured using the conditioned taste aversion mouse. The lingual epithelium of c57BL mouse was applied to in situ calcium imaging using Calcium Green 1. Fluorescein-5-maleimide-labelled GSH was alternatively loaded on the mouse lingual epithelium to monitor GSH distribution in taste buds. Ligand docking was simulated in a simulation software Sybyl 8.1 with a Surfex Dock program. GSH slightly elicited intracellular calcium responses in MSG responsive taste receptor cells. One type of the GSH responsive cells failed to respond MSG during GSH-inactivation state. Some taste bud cells were well stained with the fluorescein-labelled GSH. The conditioned taste aversion tests revealed that GSH synergistically enhanced mouse response to IMP. These results suggest that GSH would probably share the receptor on taste cell membrane with MSG.

B2.80
The growth and differentiation regulation of ornithine decarboxylase in Dictyostelium discoideum
S.-J. Park and S.-O. Kang
Laboratory of Biophysics, School of Biological Sciences, and Institute of Microbiology, Seoul National University, Seoul, Korea

The aliphatic polyamine such as putrescine, spermidine and spermine are normal cell constituents that play an important role in cell proliferation and differentiation. Although the precise action modes of polyamines are not understood until now, an observation has emerged which suggests the specific interaction of polyamine with nucleic acids to be very crucial in bringing about the actions in the cells at the molecular level. Ornithine decarboxylase (ODC, EC 4.1.1.17) catalyses the rate-limiting step of polyamine synthesis, in which L-ornithine is converted into putrescine. Dictyostelium discoideum offers an excellent model system to study the role of polyamines on the aspects of growth, differentiation and morphogenesis. This offered an interesting system for the present study roles of ODC in growth and differentiation and its relationship with methylglyoxal. To find out the role of ODC, odc was knockout in wide type cell by disruption. The odc knockout cells showed lower growth rate than the wild type. Moreover, when developed, differentiation of odc knockout cells changed. To understand role of methylglyoxal by polyamine, endogenous level of methylglyoxal was measured by HPLC in both odc knockout and wild type cells.

B2.81
Imatinib mesylate plus lithium chloride failed to treat glioblastoma in vitro: the effect and mechanism of midkine
M. Erguven1, N. Yazihan2, A. Sabanci3, E. Ermis4, M. Senyurek5 and A. Bilir5
1Yeni Yuzyil University Faculty of Medicine, Department of Biochemistry, Istanbul, Turkey, 2Ankara University Faculty of Medicine, Molecular Biology Research and Development Unit and Department of Pathophysiology, Ankara, Turkey, 3University, Istanbul Faculty of Medicine, Department of Neurosurgery, Istanbul, Turkey, 4Ankara University, Faculty of Medicine, Molecular Biology Research and Development Unit, Ankara, Turkey, 5Istanbul University Istanbul Faculty of Medicine, Department of Histology and Embryology, Istanbul, Turkey

Glioblastoma (GBM) is the most malignant primary brain tumor, characterized by its resistance to treatments and median survival times not much longer than one year. Failure of treatments relies on protective mechanisms against apoptosis. A heparin-binding growth factor Midkine (MK) has significant roles in anti-apoptotic processes and is highly expressed in GBM. In the present study, we aimed to investigate whether the combination of an effective and commonly used drugs in chemotherapy imatinib mesylate (IM) (10 μM) with an antipsychotic drug lithium chloride (LiCl) (100 μM) with new identified chemotherapeutic effects can be an effective treatment model for GBM by using monolayer cultures of human T98G human glioblastoma cell line and the role of MK levels in this treatment. The effects of drugs were investigated by evaluating cell proliferation index, MK levels by ELISA, and phospho glycogen synthase kinase 3-beta (p-GSK-3β), multi-dug resistance protein-1 (MRP-1) levels by western blotting at the 24th and 72nd hours. All drug treatments reduced cell numbers, and the levels of MK, MRP-1 and p-GSK-3β for 72 hours. LiCl was the most effective group to reduce cell numbers, MRP-1 and MK levels, and the combination group was the second one. The effective inhibition of p-GSK-3β levels were done by IM, the combination group and LiCl, respectively. Our results showed that IM plus LiCl failed to treat glioblastoma in vitro and IM decreased MK levels for the first time. Pleiotrophic effects of IM and LiCl on the crosstalk between MK, PI3K/AKT/GSK-3β-β-catenin signaling pathway and Wnt/Fz/GSK-3β-β-catenin pathway could lead to this failure.

B2.82
Crosstalk between the Ah receptor and cAMP signaling in an in vitro model of oval cells
J. Prochazkova1, L. Umannova1, A. Kozubik1, M. Machala2 and J. Vondracek1
1Institute of Biophysics AS CR, Department of Cytokinetics, Brno, Czech Republic, 2Veterinary Research Institute, Department of Toxicology, Pharmacology and Immunotherapy, Brno, Czech Republic

Aryl hydrocarbon receptor (AhR) has been originally described as a transcription factor mediating the toxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. Apart from its role in xenobiotic metabolism, AhR has been found to be important for liver development and it plays a crucial role in carcinogenic effects of AhR ligands. The AhR signaling can be triggered also by endogenous molecules like the second messenger cyclic adenosine monophosphate (cAMP). Using an in vitro model of liver progenitor cells, participating both in liver regeneration and in hepatocarcinogenesis, we tested the potency of cAMP and another protein kinase A activator, forskolin, to activate AhR signaling pathway, as analyzed at the level of: (i) AhR nuclear uptake; (ii) AhR protein degradation; and (iii) induction of model target gene Cyplal. As the expression of genes involved in intercellular communication can be deregulated by TCDD exposure, we also evaluated the potency of cAMP and forskolin, alone or in combination with TCDD, to modulate expression of Jup gene encoding adherens junctions/desmosomal protein plakoglobin. Our results indicate that cAMP and forskolin transiently activate AhR signaling in WB-F344 cells; however, no Cyplal induction has been detected and changes in Jup expression were contrary to those elicited by TCDD. Consequently, both compounds were able to modulate TCDD-induced changes in Cyplal and Jup expression. These results suggest a possible crosstalk between cAMP/forskolin-activated signaling and AhR in regulation of functions of liver progenitor cells. [This study is funded by the Czech Science Foundation, grant No. 524/09/1337].
Abstracts

B2.83  
Sphingosine kinase mediates resistance to the synthetic retinoid n-(4-hydroxyphenyl)retinamide in human ovarian cancer cells  
G. Iluzzi1, C. Bernacchioni1, M. Aureli2, S. Prioni2, G. Ferra1, V. Friso1, C. Donati2, P. Bruni2, S. Sonnino1 and A. Prinetti1  
1University Of Milan, Chemistry, Biochemistry And Biotechnology, Sezare (MI), Italy, 2University Of Florence, Biochemical Sciences, Florence, Italy

A2780/HPR is a HPR-resistant clonal cell line derived from A2780 human ovarian carcinoma cell line treated with the synthetic retinoid N-(4-hydroxyphenyl)retinamide (HPR). HPR induces in A2780 cells the production of (dihydro)ceramide with a concomitant reduction of cell proliferation and onset of apoptosis. Sphingosine kinase (SK) activity and SK-1 mRNA and protein levels resulted higher in A2780/HPR cells versus A2780. Beside this, the production of sphingosine-1-phosphate (SIP) was significantly higher in A2780/HPR versus A2780 cells, while the treatment with a potent and highly selective pharmacological sphingosine kinase inhibitor effectively reduced SIP production and resulted in a marked reduction of cell proliferation of A2780 and A2780/HPR cells. Moreover, the SK inhibitor sensitized A2780/HPR cells to the cytotoxic effect of HPR, due to the alteration of intracellular SIP and ceramide levels. On the other hand, the overexpression of SK-1 in A2780 cells was sufficient to induce HPR resistance in these cells. The role of SK in HPR resistance is not mediated by the SIP receptors, as suggested by the absence of effects after treatment of A2780 and A2780/HPR cells with agonists and antagonists of SIP receptors on the sensitivity to the drug. These data clearly demonstrate a role for SK in determining resistance to HPR in ovarian carcinoma cells, due to its effect in the regulation of intracellular ceramide/SIP ratio which is critical in the control of cell death and proliferation.

B2.84  
Quantitative analysis of the SNF1-pathway in Saccharomyces cerevisiae  
R. Garcia-Salcedo1, S. Frey2, G. Beltran1, D. Bosch1, S. Manchala1, K. Sott3, K. Elbing1, T. Ye1, M. Goksoyr3, O. Wolkenhauer1 and S. Hofmann1  
1Department of Cell and Molecular Biology, University of Gothenburg, Gothenburg, Sweden, 2Department of Computer Science, University of Rostock, Systems Biology and Bioinformatics Group, Rostock, Germany, 3Chalmers University of Technology, Department of Chemical and Biological Engineering, Molecular Biotechnology, Gothenburg, Sweden

In eukaryotic cells the SNF1/AMPK family of protein kinases plays an essential role in the control of the cellular response to changes in the energy balance. The activity of the baker’s yeast homologue Snf1 is dependent on the phosphorylation state of a single threonine (T210), which is regulated by a trio of activating kinases (Sak1, Tos3 and Elm1) and a single Type I phosphatase (Reg1-Gk7). In an effort to extend our knowledge of this process, we have integrated a large body of experimental data into a mathematical model. First, we have analysed the effect on Snf1 activation of SAK1 and REG1 over expression. We observed that over expression of Sak1 provokes a constitutive Snf1 phosphorylation and activation. However, in presence of glucose, the artificial Snf1 activation elicited by the increased SAK1 gene dosage does not have any effect on Mig1 phosphorylation and localization. Regulation of Snf1 phosphorylation was partially restored if REG1 was concomitantly over expressed. Second, we undertook time course studies on the activation/deactivation state of Snf1. Third, we quantified the main protein components of the pathway by means of immuno-qPCR revealing a 1:1:1 ratio amongst the SNF1 kinases components. Our mathematical model reliably simulated our observations and has been deployed to study the mechanisms that control Snf1 activation/deactivation.

B2.85  
Mapping cellular signal transduction  
C.-F. Tiger1, G. Cederlund2, R. Palmér2, S. Hofmann1, H. Kitano3 and M. Krantz1

1University Of Gothenburg, Cell and Molecular Biology, Gothenburg, Sweden, 2Linköping University, Department of Clinical and Experimental Medicine, Linköping, Sweden, 3The Systems Biology Institute, Tokyo, Japan

The survival, fitness and health of a living organism require the ability to perceive and adapt to changing external and internal conditions. On a cellular level, this ability is provided by the signal transduction network. The network encompasses all the interconnected signalling processes in the cell, and consists of a large number of proteins that convey information in the form of conformational changes propagating through this network. Due to the combinatorial nature of these state changes, the number of states that can potentially be assumed within the signal transduction network is staggering. This problem is referred to as the combinatorial explosion, and is perhaps the principal challenge in both visual representation and mathematical modelling of the signal transduction network. Here, we present a framework for comprehensive and unambiguous documentation of signal transduction systems, which is consistent with the format in which most biological data is generated. Furthermore, our framework can be used to derive an equation system for mathematical modelling of the network or selected parts. The framework breaks down the network into fundamental, binary reactions and states, and both known and unknown contingencies can be explicitly defined in a way that avoids any implicit assumptions. An important strength of our approach is that it allows for the automated conversion between different visualisation strategies. We illustrate the power of our framework by mapping the core characterised signalling system in the yeast Saccharomyces cerevisiae.

B2.86  
Genetic screening for suppressor mutations of the hyper- or non-filamentous growth of the yeast osmotic signalling mutants  
K. Furukawa and S. Hofmann  
University of Gothenburg, Cell and Molecular Biology/ Microbiology, Gothenburg, Sweden

In certain yeast Saccharomyces cerevisiae strains, diploid cells develop pseudohyphae under nitrogen starvation, while haploid cells produce invasive filaments that penetrate the agar in rich medium. We have recently reported that these morphological developments are strongly inhibited under hyper-osmotic conditions through the high-osmolarity glycerol (HOG) response MAPK pathway (Furukawa et al., 2009, Mol Microbiol). Deletion of the HOG1 MAPK gene enhances morphological developments, while expression of active Hog1 inhibits those even in the absence of hyper-osmotic stress. Moreover, it has been reported that HOG1 affects morphology and pathogenicity also in other fungal species. Candida albicans hog1 mutant cells form (pseudo)-hyphae and show related transcriptome changes even in the absence of morphogenetic signals. Cryptococcus neoformans hog1 mutant cells enhance production of capsule and melanin, which are crucial virulence factors. Hence, fungal Hog1 orthologues are...
thought to be central negative regulators of many aspects of morphological developments and virulence, and studies on the underlying mechanisms are relevant for the identification of novel targets for antifungal therapy. In order to more deeply understand the inhibitory role of Hog1 in morphological developments, the proteins or signalling pathways affected by Hog1 need to be identified. In this study, we attempted to screen suppressor mutations of the hyper- or non-filamentous growth which are caused by HOGL deletion or active Hog1, respectively. On the basis of the screening results, the mechanism by which Hog1 negatively regulates morphological developments will be discussed.

B2.87
Long-term inhibition of androgen receptor expression induces growth arrest in androgen sensitive and castration resistant prostate cancer cells through activating the intrinsic and extrinsic apoptotic pathways

M. Egger, H. Klocker and I. E. Eder
Medical University of Innsbruck, Experimental Urology, Innsbruck, Austria

The androgen receptor (AR) signaling pathway is one of the main mediators of prostate tumor growth and progression. Hence, androgen ablation is the standard treatment for patients in advanced stages, although most tumors eventually relapse by developing castration resistance. We have shown previously that blocking AR expression by small antisense molecules inhibits prostate tumor growth. The aim of this study was to elucidate cell death mechanisms after long-term inhibition of AR expression in androgen sensitive LNCaP and castration resistant LNCaPabl prostate cancer cells. AR expression was inhibited with a siRNA specifically targeting the AR (siAR). After short term treatment over 72 hours 10 nM of the siAR resulted in a significant knockdown of AR, associated with about 55% growth arrest in both cell lines and an increase in cleaved PARP and caspase 3 activity (2-fold induction). To investigate whether resistance mechanisms are induced upon long-term AR knockdown we treated the cells up to 7 weeks with very low siRNA doses of 0.5 nM. In both cell lines, growth inhibition of 44% was evident as from the second week of treatment and lasted over the whole treatment period reaching a 44% growth inhibition at 7 weeks. In vitro experiments have demonstrated that two members of the family, namely Spryl1 and Spryl2, regulate Ret activity in the developing kidneys and enteric nervous system, respectively. Ret functions as a receptor for the Glial-Derived Neurotrophic Factor (GDNF) family ligands (GFLs), which regulate several aspects of development of the autonomic nervous system and the urogenital system. Oncogenic mutations of Ret lead to development of medullary thyroid carcinoma (MTC), a malignancy of the calcitonin-producing cells of the thyroid (parafollicular cell or C-cells). In the present work we sought to elucidate whether Spryl restricts signaling by oncogenic Ret in MTC. Lentiviral-mediated expression of moderate amounts of Spryl1 in a MTC-derived human cell line (TT) reduced cell growth both in vitro and in subcutaneous xenografts in SCID mice. Expression of Spryl in TT cells resulted in up-regulation of p16 INK4a and down-regulation of several angiogenesis-related genes. Finally, genetic ablation of Spryl in mice led to development of C-cell hyperplasia, a precancerous lesion that precedes development of MTC. Taken together, these data suggest that Spryl might function as a tumor suppressor in MTC by acting as a cellular brake to oncogenic Ret signaling.

B2.89
C-cell hyperplasia in thyroid from Sprouty1 knockout mice

A. Macià1, P. Galle2, M. Santacana2, A. Basson M.3, X. Matias-Guiu2 and M. Encinas1
1Universitat de Lleida/IRB Lleida, Lleida, Spain; 2IRB Lleida, Lleida, Spain; 3King’s College, London, UK

Sprouty family members (Spryl1-4) are negative regulators of receptor tyrosine kinase signaling, especially of the ERK pathway. In vivo experiments have demonstrated that two members of the family, namely Spryl1 and Spryl2, regulate Ret activity in the developing kidneys and enteric nervous system, respectively. Ret functions as a receptor for the Glial-Derived Neurotrophic Factor (GDNF) family ligands (GFLs), which regulate several aspects of development of the autonomic nervous system and the urogenital system. Oncogenic mutations of Ret lead to development of medullary thyroid carcinoma (MTC), a malignancy of the calcitonin-producing cells of the thyroid (parafollicular cell or C-cells). In the present work we sought to elucidate whether Spryl restricts signaling by oncogenic Ret in MTC. Lentiviral-mediated expression of moderate amounts of Spryl1 in a MTC-derived human cell line (TT) reduced cell growth both in vitro and in subcutaneous xenografts in SCID mice. Expression of Spryl in TT cells resulted in up-regulation of p16 INK4a and down-regulation of several angiogenesis-related genes. Finally, genetic ablation of Spryl in mice led to development of C-cell hyperplasia, a precancerous lesion that precedes development of MTC. Taken together, these data suggest that Spryl might function as a tumor suppressor in MTC by acting as a cellular brake to oncogenic Ret signaling.

B2.88
Cyclin A/Cdk1 and CK2 cooperate to trigger degradation of the Stem-loop binding protein (SLBP) at the end of S phase inhibiting histone mRNA biosynthesis

M. M. Kosoglu1, L. M. Graves2 and W. F. Marzluff3
1Fatih University, Genetics and Bioengineering, Istanbul, Turkey; 2University of North Carolina at Chapel Hill, Department of Pharmacology, Chapel Hill, USA; 3University of North Carolina at Chapel Hill, Program in Molecular Biology and Biotechnology, Chapel Hill, USA

In metazoans, bulk of histone proteins is encoded by replication-dependent histone mRNAs. Expression of these histone mRNAs is cell cycle regulated and a major regulatory mechanism is S phase restriction of Stem-loop binding protein (SLBP) which is a necessary factor for histone pre-mRNA processing. SLBP is degraded at the end of S phase and this inhibits the histone mRNA biosynthesis until SLBP accumulates just before the beginning of the next S phase. SLBP degradation is signaled by phosphorylation of T60 and T61 in TTP (60-62) motif and a KRKL (95-98) sequence, which is a putative cyclin binding site. A fusion protein with 59-aa fragment of SLBP (51–108) fused to glutathione S-transferase, is sufficient to mimic SLBP degradation at the end of S phase. Using GST-SLBP fusion protein as substrate, we identified cyclina/Cdk1 as the major kinase for T61 in late S phase lysates. We also show that cyclina/Cdk1 but not cyclina/Cdk2 efficiently phosphorylates T61. Furthermore, knock down of Cdk1 stabilizes SLBP at the end of S phase. As a possible kinase for T60, CK2 inhibitors also prevent the degradation of SLBP at the end of S phase. In vitro phosphorylation of T60 by CK2 requires prior phosphorylation of T61 suggesting that T61 phosphorylation by cyclina/cdk1 primes the phosphorylation of T60 by CK2 and signals SLBP degradation. Based on these findings, we conclude that towards to end of S phase, increase in cyclina/Cdk1 activity in cooperation with CK2 triggers degradation of SLBP as a mechanism to shutdown histone mRNA biosynthesis.

B2.90
Abstract withdrawn
Abstracts

B2.91  
Network-based reconstruction of the glucose repression signaling pathway of yeast  
D. Hasdemir1, J. Nielsen2, Z. I. Ousa1 and B. Kirdar1  
1Bogaziçi University, Istanbul, Turkey, 2Chalmers University of Technology, Gothenburg, Sweden

Modeling of signaling pathways has proven to be a challenging task due to the large extent of the crosstalk between individual pathways. In most of the cases where the layout of the associated pathway is not believed to be explicitly shown, network-based approaches are excellent tools for the analysis of these signaling networks. A key element of the glucose repression signaling pathway which is subject to several ambiguities in its mechanism; Snf1 kinase is composed of a catalytic subunit Snf1p, a regulatory subunit Snf4p and a scaffolding β-subunit (Sip1p, Sip2p or GαB3p). In this study a network-based algorithm is developed for the integrated analysis of the genome wide expression data from SNF1 A, SNF4 A and SNF1 ASNF4 A mutants and the global PPI network of yeast. The linear paths between several key elements of the glucose repression signaling pathway were identified in the PPI network of yeast, following the elimination of the biases in the network. In the next step, the linear paths were scored based on the genome wide expression data. And, the significance of each binary interaction was calculated based on its occurrence frequency in the top scoring linear paths. The robustness of the algorithm was tested by using different PPI networks with varying coverage. On the other hand, the results were evaluated in a comparative manner through the identification of the functional modules in the network. As the final objective, the overview of the glucose repression mechanism in the absence of the Snf1 complex was achieved without being lost in the complexity of commonly used graph-theoretical approaches.

B2.92  
A correlation between TGF-β1-mediated JAM-A downregulation and cell invasiveness  
Y. Wang and W.-Y. Lui  
The University of Hong Kong, School of Biological Sciences, Pokfulam, Hong Kong

Transforming growth factor-beta (TGF-β) and junctional adhesion molecule-A (JAM-A) have been indicated as key regulators of cell migration, and an inverse relationship of JAM-A expression molecule-A (JAM-A) has been reported in breast cancer cells. Whether TGF-β1 signaling induces JAM-A downregulation leading to cancer cell invasion has not been investigated. In this study, we report that TGF-β1 significantly downregulates JAM-A expression at both mRNA and protein levels in MCF-7 cells that is slightly migratory and normally expresses high level of JAM-A. We showed that TGF-β1 downregulates JAM-A mRNA level via transcriptional regulation and the promoter region responsible to TGF-β1 stimulation is located between nt -367 and +1. In addition, TGF-β1 exerted its negative effect on JAM-A expression via post-translational control. There was a significant reduction (~60% reduction) in JAM-A protein levels in cyclohexamide-pre-treated TGF-β1-treated cells, indicating that TGF-β1 promotes JAM-A protein turnover. We showed that JNK inhibitor, but not Smad3 and Smad4 siRNA, could effectively abolish TGF-β1-mediated JAM-A protein degradation, although Smad activation alone could abolish JAM-A degradation in the absence of TGF-β1 stimulation. Furthermore, clathrin siRNA abolished TGF-β1-mediated JAM-A degradation, indicating that the degradation is mediated via clathrin-dependent endocytosis. Reduction of JAM-A in MCF-7 cells upon TGF-β1 treatment led to enhanced invasion in the invasion assays. Taken together, these results indicate a strong correlation between TGF-β1-mediated JAM-A downregulation and cell invasiveness. [Supported by Hong Kong Research Grants Council (HKU771507)]

B2.93  
Inhibition of TGF-β signaling by nuclear receptor TLX in neuroblastoma  
E. Johansson1, Z. Zeng2, T. Yoshida1 and K. Funa1  
1Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, 2School of Biological Science and Technology, Central South University, Molecular Biology Research Center, Changsha, China

Transforming growth factor beta (TGF-β) is a multifunctional cytokine that functions both as tumor suppressor and as inducer of tumor growth, depending on cancer type and stage of tumor development. TGF-β activates intracellular signaling through phosphorylation of Smad2 and Smad3 that form a complex with Smad4 and translocate to the nucleus, where they regulate transcription of various genes. TLX is an orphan nuclear receptor that is important for vertebrate brain functions. TLX has been shown to maintain adult neural stem cells in an undifferentiated and self-renewal state. We found that TLX also is highly expressed in several neuroblastoma cell lines. Although the role of TLX in adult neural stem cells is well established, its function in neuroblastoma is not well known. TGF-β does not have a significant effect on growth of wild-type IMR-32 neuroblastoma cells, but in IMR-32 cells where TLX expression was knocked down TGF-β treatment led to growth inhibition. Further, in TLX knockdown cells there is a significantly higher increase in mRNA levels of TGF-β target genes like Smad7 and p21 upon TGF-β treatment compared to control cells. TLX also binds several components of the TGF-β signal pathway, including Smad2, -3 and -4. We are now trying to unravel the mechanisms by which TLX regulates TGF-β signaling. Our results suggest that TGF-β and TLX have opposing effects on proliferation in neuroblastoma and insensitivity to TGF-β dependent growth inhibition due to high levels of TLX might be an important reason behind the high rate of proliferation of these cells.

B2.94  
Abstract withdrawn

B2.95  
Linearity range of the hyperosmotic stress response in Saccharomyces cerevisiae  
E. Petelcnz-Kardziel1, R. Babazadeh2, C. Beck3, M. Smeds2, E. Eriksson1, M. Goksa¨r2 and S. Hohmann1  
1University of Gothenburg, Cell and Molecular Biology, Gothenburg, Sweden, 2University of Gothenburg, Physics, Gothenburg, Sweden

The HOG (High Osmolarity Glycerol) pathway in Saccharomyces cerevisiae is a MAP kinase cascade activated upon hyperosmotic stress. The core element of this cascade is the Hog1 MAP kinase, which resides in the cytoplasm in the absence of hyperosmotic stress and is rapidly accumulated inside the nucleus, when the external osmolarity increases. Using fluorescent protein fusions we followed the sub-cellular localisation of Hog1 over time. We employed a single cell analysis platform, based on a fluorescence microscope coupled with micro-fluidics and optical tweezers, to acquire images of osmotically stressed cells. Using different treatments, which included a wide range of osmoticum concentrations
(100 mM to 1000 mM NaCl), applied to approximately 25 cells, we investigated threshold levels of the HOG response. We found that the widely claimed linear ratio between stress intensity and Hog1 nuclear enrichment is valid only up to a certain level of stress. Beyond this level the maximum in Hog1 nuclear retention is delayed. This delay is also reflected in cell population data (immuno-blotting) on Hog1 phosphorylation, as well as the direct transcriptional read-out (Gpd1 protein level). At present we can only speculate that this delay could be caused by the properties of the Hog1 translocation machinery.

**B2.96**

**Protein-protein interactions: identification, modulation and validation**

V. Hindjie, P. Tafelmeyer, J.-C. Rain, R. Lopez and E. Formstecher

Hybrigenics S.A Services, Paris, France

Yeast two-hybrid (Y2H) protein interaction screening is a key technology to identify new protein partners. To ensure reproducible and exhaustive Y2H results, our highly complex fragment libraries are screened to saturation using an optimized mating procedure that allows for testing on average 100 million interactions per screen, corresponding to a 10-fold coverage of the library. As a consequence, multiple, independent fragments are isolated for each interactant, enabling the immediate delineation of a minimal interacting domain and the computation of a confidence score. We have developed several methods to validate and further characterize interactions identified using Y2H screens or other protein interaction techniques. “One-by-One” assays allow for the pair-wise testing of the interaction between two selected proteins, either in vivo (in yeast) or in vitro using the powerful HTRF® (homogeneous time resolved fluorescence) technology of Cisbio International, which is based on time-resolved FRET. The smallest interacting domain on both the bait and the prey proteins, or individual amino acids critical for the interaction can be also rapidly delineated using yeast-based tools. This characterization is highly relevant for functional studies and drug discovery projects where a protein-protein interaction can be used as a target for the development of small therapeutic molecules. Here, we will present results that illustrate the strength of our Y2H screening and validation techniques.

**B2.98**

**Hydrogen peroxide generated during the RTKs signaling produces and acts locally confined**

P. Tyurin-Kuzmin1, N. Mishina2, V. Belousov2, C. Schultz1 and A. Vorotnikov2

1Moscow State University, Faculty of Basic Medicine, Moscow, Russian Federation, 2Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russian Federation, 3EMBL Heidelberg, Heidelberg, Germany

Cells produce hydrogen peroxide (H$_2$O$_2$) as a part of physiological response to many growth factors and cytokines. For instance, binding of epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) to their receptors, EGFR and PDGFR, respectively, activates superoxide anion radical and H$_2$O$_2$ production by NADPH oxidases. H$_2$O$_2$ acts as second messenger and inactivates tyrosine phosphatases. However, it remains unknown which cellular compartment is responsible for H$_2$O$_2$ production and what the diffusion properties of H$_2$O$_2$ within the cell contribute. In our work we aimed to study the intracellular localization of H$_2$O$_2$, which synthesized during the RTK stimulation of 3T3 fibroblast and HeLa cells. We produced the collection of HyPer biosensor variants with different intracellular localization. HyPer is genetically encoded ratiometric biosensor with a high H$_2$O$_2$ sensitivity and specificity. We found, that different cell types demonstrate different subcellular patterns of ROS production. In epithelial cells during the EGF stimulation H$_2$O$_2$ synthesise near endosomes with internalized EGFR. H$_2$O$_2$ level near the plasmic membrane is not changed. Fibroblasts after their PDGF stimulation generate H$_2$O$_2$ in the region of the plasmic membrane. Therefore cells generate H$_2$O$_2$ locally and it isn’t spread to other cell compartments. Both types of cells generate H$_2$O$_2$ also on endoplasmic reticulum membrane. Thus, our results show that during the stimulation of receptor tyrosine kinases H$_2$O$_2$ produces and acts locally in the particular cell compartments. H$_2$O$_2$ diffusion between compartments is limited. Our results provide the first visible evidence for a localized redox signaling model.

**B2.99**

**RGS2 and RGS4 proteins differentially modulate delta and kappa opioid receptor signaling**

M.-P. Papakonstantinou, L. J. Leontiadis, F. Nikolos, M. Sarris and Z. Georgoussi

N.C.S.R. Demokritos, Institute of Biology, Ag. Paraskevi, Athens, Greece

Regulators of G protein Signaling (RGS) comprise a large multifunctional protein family whose members possess intrinsic GAP activity that accelerates GTP hydrolysis from Gα subunits, thus modulating G protein coupled receptor (GPCR) signaling and also act as effector antagonists. We have previously shown that the RGS4 directly interacts with mu and delta opioid receptors to regulate their signaling (1-2). To deduce whether there is selectivity in coupling between members of RGS proteins and opioid receptors we tested the ability of other members of B/R4 RGS subfamily to interact with kappa (KOR) and delta (DOR) opioid receptors. Pull down experiments and co-immunoprecipitation studies indicated that RGS2 and RGS4 interact within C-terminus and third intracellular loop of both proteins.
B2.100
Analysis of carbonic anhydrase9 (CA9) expression in human brain cancer under different oxygenation conditions including hypoxia
H. M. Said1, C. Hagemann2, J. Stojic2, B. Schoemig2, B. Polat1, A. Staab3, G. Vince2, A. Katzer1, M. Flentje1 and D. Vordermark4
1University of Wuerzburg, Medical Faculty, Radiation Oncology, Wuerzburg, Germany, 2Department of Neurosurgery, University of Wuerzburg, Medical Faculty, Wuerzburg, Germany, 3Paul Schere Institute, Proton Research, Villingen, Switzerland, 4University of Halle – Wittenberg, Medical Faculty, Radiation Oncology, Wuerzburg, Germany

The hypoxia-inducible enzyme carbonic anhydrase 9 has recently been applied as a tumor hypoxia marker and an indicator of prognosis and a potential therapeutic target in malignant glioma. 

Material and methods: To characterize patterns of expression of CA9 in human malignant glioma cells, we studied CA9 protein, CA9 mRNA and hypoxia-inducible factor-1α (HIF-1α) protein levels in U87-MG, U251, U373 and GaMG cells exposed to in vitro hypoxia (1, 6 or 24 hour at 5%, 1% or 0.1% Oxygen). 

Results: Also CA9 mRNA and protein expression were examined in two groups of tumor patient specimens, namely low-grade astrocytoma (LGA; n = 15) and glioblastoma (GBM; n = 15) and compared to a negative control group of normal brain samples (n = 3). 

Conclusions: CA9 expression in GBM tumors occurs at a higher frequency, both on protein or mRNA levels, rendering CA9 as a suitable hypoxia-related diagnostic marker or target for therapeutic approaches.
Effects of pistacia atlantica extract on cell cycle regulatory proteins in breast cancer T47D cells

P. Fatih Rezaei1, S. Fouladzade2, Y. Y. Kamran2, T. Asgari1, G. Amin1, S. M. Ghaffari4 and E. Azizi2
1Department of Pharmacology and Toxicology and Department of Biochemistry, Faculty of Pharmacy and Institute of Biochemistry and Biophysics, Tehran, Islamic Republic of Iran, 2Department of Pharmacology and Toxicology, Faculty of Pharmacy, Tehran, Islamic Republic of Iran, 3Department of Pharmacognosy, Faculty of Pharmacy, Tehran, Islamic Republic of Iran, 4I.B.B., Department of Biochemistry, Tehran, Islamic Republic of Iran

Breast cancer is one of the most common cancers among Iranian women. Uncontrolled cell cycle plays a pivotal role in cancer incidence. Cell cycle is regulated by cyclins and cyclin-dependent kinases (cdks). Plants are important sources of active natural products which differ widely in terms of structure and biological properties. As a part of our studies on Iranian medicinal plants for new anticancer agents, in this project we evaluated the effects of Pistacia atlantica sub kurdica extract (with the local name of Baneh) on breast cancer T47D cells. The T47D cells were treated with 1 mg/ml of Baneh extract (IC50) and Doxorubicin 250 nM in the MTT cytotoxicity assay. Flow cytometry analysis of DAPI-stained cells showed significant arrest of cell cycle in G0/G1 phase of the cell cycle through down regulation of cyclin D1 and cdk4 in comparison to the control cells by treatment of Baneh extract and Dox for 12–72 hour. The G0/G1 phase in Baneh treated cells and G2/M accumulation of Dox treated cells was reduced in Baneh treated cells. The G0/G1 arrest of cell cycle was confirmed by the sharp down regulation of cyclin D1 and cdk4 in comparison to the control cells by increasing time. Moreover, protein level of cyclin E, cyclin A, cyclin B, cdk1 and cdk2 was reduced in Baneh treated cells. The effect of Dox on the level of these proteins was not as strong as the effect of Baneh. On the basis of these findings, Baneh extract showed antiproliferative activity led by arresting of the cells in G1 phase of the cell cycle through down regulation of the regulatory proteins of G1/S transition.

NF-kappaB activation and complex control of NOS2 gene promoter in K562 imatinib mesylate resistant CML cell line

T. Ozkan1, A. Karadag2, B. Altinok1, A. Z. Karabay3, A. Koc1, Z. Buyukbingol1 and A. Sunguroglu3
1Department of Biochemistry, Ankara University Faculty of Pharmacy, Ankara, Turkey, 2Ankara University Institute of Biotechnology, Ankara, Turkey, 3Department of Medical Biology, Ankara University Faculty of Medicine, Ankara, Turkey

Abnormal Wnt/β-catenin signaling pathway and increased expression of nitric oxide synthase2(NOS2) are both implicated in the development of various human cancers. Imatinib mesylate which is an Ahl kinase-selective inhibitor is a treatment option for most of the Chronic myeloid leukemia (CML) patients. Recently it has been identified that Bcr-Ab1-mediated Y phosphorylation of β-catenin is a cause of its increased protein stability and transcriptional signalling activity. In this study we aimed to analyse if NOS2 and cyclin D1 are target genes of β-catenin in K562 imatinib mesylate resistant CML cell line. For this purpose we first performed ChIP assay with anti β-catenin antibody and then PCR reactions were performed to examine β-catenin/Tcf-4 binding to NOS2 and cyclinD1 promoters. Using transfection assays we also investigated activation of NF-kappaB in K562 imatinib mesylate resistant CML cell line. Our results show that NOS2 and cyclinD1 are target genes of β-catenin. We also determined significantly increased NF-kB activation in K562 imatinib mesylate resistant CML cell line compared with non-resistant cells. In the present study, we have described the role of Wnt/β-catenin signaling in the regulation of NOS2 in K562 imatinib mesylate resistant CML cell line. And we have provided strong evidence that NOS2 is a novel downstream target of β-catenin suggesting the involvement of NO in Wnt/β-catenin signaling pathway that could contribute to imatinib resistant of CML. Increased NF-kB activation and binding of β-catenin to NOS2 gene indicates that human NOS2 promoter is under complex control and these observations present new targets to prevent imatinib resistance in CML.

Effect of wnt3a or lithium chloride preconditioning on nitric oxide production in RAW264.7 macrophages and K562 leukemic cell line

A. Z. Karabay1, T. Ozkan2, A. Koc1, A. Karadag3, B. Altinok2, Z. Buyukbingol1 and A. Sunguroglu3
1Department of Biochemistry, Ankara University Faculty of Pharmacy, Ankara, Turkey, 2Ankara University Institute of Biotechnology, Ankara, Turkey, 3Department of Medical Biology, Ankara University Faculty of Medicine, Ankara, Turkey

Wnt pathway regulates cell migration, proliferation and differentiation. In the absence of Wnt signal, β-catenin is degraded by a multiprotein complex containing GSK-3β. Binding of Wnt protein to its receptor leads to GSK-3β inhibition and nuclear accumulation of β-catenin, which binds to TCF/LEF to increase expression of Wnt target genes. LiCl is a mimicker of Wnt pathway via potent inhibition of GSK-3β. Wnt3a is also an activator of Wnt pathway. LPS/IFN-gamma synergically induce macrophages to produce various inflammatory mediators including nitric oxide. It was suggested that presence of LiCl potentiates nitrite production cell type selectively and LPS-dependent production of nitrite was modestly increased in RAW264.7 macrophages. In this study we aimed to analyse the effect of Wnt pathway activation on LPS/IFN-gamma induced or basal nitrite production in RAW 264.7 macrophages and K562 cell line. Our results show that LiCl induces nitrite production in both cytokine stimulated and unstimulated macrophages. 28 mM LiCl increased nitrite production by nearly two fold in both stimulated and unstimulated macrophages (p < 0.05). Wnt3A preconditioning did not significantly affect nitrite production when compared with LPS/IFN-gamma treated macrophages. K562 leukemic cells were also treated with LPS/LPS plus LiCl. Nitrite production was not significantly different between unstimulated, LPS and LPS plus LiCl stimulated cells which means that LiCl cell specifically effects nitrite production. It can be concluded that there is a strong link between Wnt β-catenin pathway and iNOS activation which must be kept in mind.
considering treatment options for diseases such as chronic inflammation and cancer.

B2.107
Hyperosmotic stress triggers the degradation of microtubule-associated protein Tau
M. Olivera Santa-Catalina, M. Caballero Bermejo, R. Argent, J. C. Alonso, S. dos Santos Pinheiro, A. Cuenda, F. Centeno and M. J. Lorenzo

1Universidad de Extremadura, Departamento de Bioquímica y Biología Molecular y Genética, Cáceres, Spain, 2Centro Nacional de Biotecnología (CNB), Consejo Superior de Investigaciones Científicas (CSIC), Departamento de Inmunología y Oncología, Madrid, Spain

Tau is a microtubule-associated protein which plays an important role in the structure and function of the neuronal cytoskeleton. Tau is also a major component of paired helical filaments (PHF), one of the pathological hallmarks of Alzheimer’s disease. Several alterations in the microtubule-associated protein Tau metabolism have been described in neurodegenerative disorders such as Alzheimer disease. Among these, hyperphosphorylation and cleavage are two of the most studied features. Although PHF formation mechanisms remain largely unknown it has been suggested that impaired proteolysis of Tau may be associated to its deposition. In the present work, we have studied the mechanisms underlying Tau degradation in response to hyperosmotic stress in the neuroblastoma cell line SH-SY5Y. Sorbitol treatment induces a time-dependent degradation of Tau and this effect is partially prevented by MG-132, suggesting a role for proteasome in this process. In addition, sorbitol also increases both calpain and caspase-3 activity and p38 MAPKs and ERK1/2 phosphorylation in a time dependent manner. Pre-treatment of cells with different concentrations of p38 MAPKs inhibitors (SB203580 and BIRB0796) does not prevent sorbitol-induced Tau degradation. However, this effect is partially prevented when cells are pre-treated with ERKs inhibitors (PD98059, PD184352 and U0126), which indicate the involvement of the ERK pathway in sorbitol-induced Tau degradation. Work supported by grants: PRI06A202, GRU09056 and BFU2007-67577-C02-02/ BMC. MCB and MOSC are recipients of Junta de Extremadura fellowships. S. S. P belongs to Erasmus Program.

B2.108
Estradiol as stimulator of syndecan-4 gene expression in human breast and colon cancer cells
N. Karamanos, C. Gialeli and A. Tsonis

Univ of Patras, Lab of Biochemistry/ Department of Chem, Patras, Greece

Syndecans are transmembrane cell surface heparan sulfate proteoglycans (HSPG) implicated in cancer development. Syndecan-4 is believed to have regulatory roles in cell adhesion and in the regulation of cell migration. Estrogens and particularly 17β-estradiol (E2) regulate several physiological processes and are implicated in the growth of solid tumors, such as breast and colon. E2 also influences estrogen receptor (ER) expression. ER is synthesized in many cell types as two protein forms, ERα and ERβ, which are products of separate genes. E2 signaling is supposed to be mediated via linear pathway involving insulin-like growth factor receptor (IGF-R) and epidermal growth factor receptor (EGFR). The aim of this study was therefore to examine whether estradiol affects the expression of ERs and proteoglycans (PGs) implicated in breast and colon cancers as well as whether this effect is associated with ER/IGF-R/EGFR pathways. In vitro studies were performed on a panel of breast and colon cell lines in the presence or absence of E2 and specific IGF-R, EGFR tyrosine kinase inhibitors. The results showed that E2 affected the gene expression of syndecan-4 via genomic and nongenomic pathway and is closely related with the expression balance between ERα/ERβ and the crosstalk between crucial ER/IGF-R/EGFR pathways.
B3 – Global Networks

B3.01
Androgen receptor signaling induced by supraphysiological doses of dihydrotestosterone in human peripheral blood lymphocytes
E. Imperlini1, A. Mancini1, S. Spaziani2, A. Alfieri2, P. Buono3 and S. Orru1
1Fondazione SDN-IRCCS, Naples, Italy, 2Facoltà di Scienze Motorie, Università di Napoli “Parthenope” & CEINGE Biotecnologie Avanzate srl, Naples, Italy, 3Fondazione SDN-IRCCS & Facoltà di Scienze Motorie, Università di Napoli “Parthenope”, Naples, Italy

Anabolic androgenic steroids (AASs), a class of steroid hormones related to testosterone, are natural ligands of androgen receptor (AR), a member of the nuclear receptor superfamily of ligand-activated transcription factors. AR binds specific DNA elements, known as androgen response elements (AREs). Testosterone, the main male sexual hormone, binds AR directly and indirectly, through conversion into dihydrotestosterone (DHT), its more active metabolite. AASs are frequently detected in the urine of doped athletes; their consumption is growing also among sport amateurs and adolescents. The effects of androgens can differ depending on the target cells and/or tissues. To gain insight into transcription activation mechanisms of AR, we investigated AR protein signaling in human peripheral blood lymphocytes treated with supraphysiological doses of DHT. We performed a comparative proteomic analysis and we identified about 30 differentially expressed proteins. At least five species contained a consensus ARE sequence in the promoter region of related coding genes. The analysis also revealed that high doses of DHT activate the drug detoxification process, stimulate cell motility and exert a prosurvival effect rather than an apoptotic one.

B3.02
Systems-level analysis of mitotic feedback controls
M. R. Domingo-Sananes and B. Novak
Department of Biochemistry, University of Oxford, Oxford Centre for Integrative Systems Biology, Oxford, UK

Entry into mitosis depends on the activation of the Mitosis Promoting Factor (MPF), a complex made up of cyclin-dependent kinase-I (CDK1) and cyclin-B. In many organisms, MPF activity is regulated by inhibitory phosphorylation of a CDK1-tyrosine residue by the Wee1 kinase. This phosphorylation is removed by the Cdc25 phosphatase once the cyclin component of MPF reaches a critical threshold. MPF in turn activates Cdc25 and inhibits Wee1, thus creating a positive and a double-negative feedback loop. These feedbacks are known to be important for proper mitotic control, since elimination of the Wee1 phosphorylation site on CDK1 can compromise the cell cycle control system. However, the individual contributions of the two feedback loops have not been separately assessed. We have constructed a mathematical model for the MPF tyrosine-phosphorylation switch that takes into account the multiple phosphorylations of Wee1 and Cdc25 by MPF and studied the effects of disrupting the feedback loops independently. We show that the Wee1-MPF double-negative feedback loop and the Cdc25-MPF positive feedback loop are not functionally equivalent. We also propose that MPF regulation of the Cdc25 phosphatase might have a more significant role than Wee1 regulation in setting the cyclin threshold for MPF activation and thus mitotic entry.

B3.03
Protein-protein interactions (PPIs) and disease
M. Duesbury
EBI, Proteomics Services, Cambridge, UK

While the complete sequence of the human genome is now available, the same state of completeness cannot be said to exist for the interactome or the proteome. Protein complexes are responsible for most processes within the cell, and modifications to their interactions perturbs the normal sequence of events, and contributes to many diseases. A more complete coverage of PPIs would help enable us to discover connections between disordered PPI networks and disease processes, identify new targets for drug therapy, and generate novel approaches to treatment or disease modification. IntAct is an freely available (open source) database for molecular interactions, containing over 100,000 protein-protein interactions (PPIs) from at least 63,000 proteins from many organisms. The captured data can displayed in a variety of formats and now includes drug-protein, and DNA-protein interactions. IntAct is a member of the IMEx consortium; a network of participating major public domain databases sharing information, and aiming to jointly capture all published molecular interaction data in a standardised format. IntAct source code and data are freely available at http://www.ebi.ac.uk/intact

B3.04
Comparison of three detection methods of Y chromosomal short tandem repeats (Y-STRs) in population genetics and forensic investigations
L. Pliss1, S. Rozanė2, A. Sabule2, V. Baumanis1 and A. Krumina3
1Latvian Biomedical Research and study centre, Riga, Latvia, 2Latvia State Centre for Forensic Medical Examination (VTMEC), Riga, Latvia, 3Riga Stradiņš University, Riga, Latvia

Introduction: One of the most frequently used approaches in forensic investigations and population genetics is detection of Y chromosome short tandem repeats (Y-STRs). A wide spectrum of analyzed polymorphic loci exhibits a high level of heterogeneity at the inter- and intra-population level. Molecular techniques for a length variation of Y-STRs are mostly based on multiplex PCR of 12 to 17 loci. The aim of the present study was to compare several multiplex Y-STRs detection methods.

Subjects and methods: Ninety unrelated males were analyzed using AmpFlSTR® Y-filer™ (Applied Biosystems) for forensic investigations as a routine procedure and 158 unrelated males for population genetics studies were typed using PowerPlex Y System (Promega). DYS392 was genotyping separately in several cases. Protein-protein interactions (PPIs) and disease processes, identify new targets for drug therapy, and generate novel approaches to treatment or disease modification. IntAct is an freely available (open source) database for molecular interactions, containing over 100,000 protein-protein interactions (PPIs) from at least 63,000 proteins from many organisms. The captured data can displayed in a variety of formats and now includes drug-protein, and DNA-protein interactions. IntAct is a member of the IMEx consortium; a network of participating major public domain databases sharing information, and aiming to jointly capture all published molecular interaction data in a standardised format. IntAct source code and data are freely available at http://www.ebi.ac.uk/intact
observed 0.9923 demonstrating the usefulness and informative power of this Y-STR set.

Conclusions: Based on series multiplex PCR, Y-STRs detection not always provide sufficient results for all loci. Additional molecular approaches in several cases should be applied, which include smaller number of analyzed loci.

Acknowledgment: Project has been granted from ESF program and LCS.

B3.05
A global protein Kinase and phosphatase interaction network in yeast

1Wellcome Trust Centre for Cell Biology, University of Edinburgh, School of Biological Sciences, Edinburgh, UK, 2Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada, 3University of Michigan, Michigan, USA, 4Department of Molecular Genetics, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada, 5Department of Pathology and Biostatistics, University of Michigan, Ann Arbor, Michigan, USA

Kinases and phosphatases regulate virtually all cellular processes through reversible phosphorylation of myriad substrates. The systematic identification of the proteins that associate with kinases is crucial for understanding the global regulatory network of the cell. However, due to the difficulty in detecting the often weak associations between kinases and their substrates or regulators, the kinase interactome (kinome) is only poorly charted. We systematically interrogated the complete set of kinases and most phosphatases in budding yeast using multiple epitope tags in small scale cultures. Protein complexes were captured on magnetic beads, followed by on-bead trypsin digestion and nano-scale LC-MS. We identified a kinase and phosphatase interaction (KPI) network of 1844 statistically significant interactions. The KPI network contained many dense local regions of interactions that suggested new functions. Notably, the cell cycle phosphatase Cdc14 associated with multiple kinases that revealed roles for Cdc14 in mitogen-activated protein kinase signaling, the DNA damage response and metabolism, while interactions of the target of rapamycin complex 1 (TORC1) uncovered new effector kinases in nitrogen and carbon metabolism. Our analysis reveals that kinase-kinase interactions were significantly enriched compared to all other kinase interaction partners and collectively formed a highly interconnected kinase network. This network was extremely robust and was far less modular than previous less complete interaction workgroup. This initiative is supported by an increasing number of major interaction databases and a number of practical applications are demonstrated.

B3.06
Do pneumatic tube systems affect hemolysis index?
B. Ongen1, 2, K. Seniha Mutlu1 and K. Ceyda2

1Department of Medical Biochemistry, Ege University School of Medicine, Izmir, Turkey, 2Department of Clinical Biochemistry, Ege University School of Medicine, Izmir, Turkey

Introduction: Pneumatic tube systems are key to our understanding of biological systems and there are many data resources, such as the IntAct molecular interaction database (www.ebi.ac.uk/intact) dedicated to capturing such experimental data. This increasing number of resources, however, presents the research worker interested in a specific biological domain with a significant data access challenge. To instantaneously access multiple interaction data resources, a common interface for computational access allowing software clients to interact with multiple sources using the same interface is required. This has lead to the development of PSICQUIC, the PSI Common Query InterfaCe, a community standard for computational access to molecular interaction resources. PSICQUIC has been jointly developed by major interaction data and tool providers in the context of the HUPO Proteomics Standards Initiative using the standard formats developed by the PSI Molecular interaction workgroup. This initiative is supported by an increasing number of major interaction databases and a number of practical applications are demonstrated.

B3.07
The PSICQUIC interface – a portal into the world of the interactome
S. Orchard, B. Aranda, S. Kerrien, R. Jimenez and H. Hermjakob
EMBL-EBI, Proteomics Services Team, Cambridge, UK

Molecular interactions are key to our understanding of biological systems and there are many data resources, such as the IntAct molecular interaction database (www.ebi.ac.uk/intact) dedicated to capturing such experimental data. This increasing number of resources, however, presents the research worker interested in a specific biological domain with a significant data access challenge. To instantaneously access multiple interaction data resources, a common interface for computational access allowing software clients to interact with multiple sources using the same interface is required. This has lead to the development of PSICQUIC, the PSI Common Query InterfaCe, a community standard for computational access to molecular interaction resources. PSICQUIC has been jointly developed by major interaction data and tool providers in the context of the HUPO Proteomics Standards Initiative using the standard formats developed by the PSI Molecular interaction workgroup. This initiative is supported by an increasing number of major interaction databases and a number of practical applications are demonstrated.

B3.08
Sulfate assimilation mediates tellurite accumulation and toxicity in yeast
L.-G. Ottosson1, K. Logg2, S. Isbister1, P. Sunnerhagen1, M. Käll1, A. Blomberg1 and J. Warringer1

1Department of Cell- and Molecular Biology, University of Gothenburg, Gothenburg, Sweden, 2Department of Applied Physics, Chalmers University of Technology, Gothenburg, Sweden

Despite a century of research and increasing environmental and human health concerns, the mechanistic basis for the toxicity and intracellular accumulation of derivates of the metalloid tellurium, in particular the oxygenion tellurite, remains an unsolved question in microbiology. We provide an unbiased view of the mechanisms of tellurium metabolism in the yeast Saccharomyces cerevisiae by
B3 – Global Networks

measuring deviations in tellurium tolerance and accumulation of a complete collection of gene knockouts. Reduction of tellurium and concomitant intracellular accumulation as metallic tellurium strongly correlated with loss of cellular fitness, demonstrating that tellurite reduction and toxicity are causally linked. The sulfate assimilation pathway upstream of Met17 and in particular the sulfite reductase and its cofactor siroheme were shown to be central to tellurite toxicity and its reduction to elemental tellurium. Gene knockouts with a deviation in tellurite tolerance also showed a similar deviation in the tolerance to both selenite and, interestingly, selenomethionine. This suggests that the bioassimilation of selenium and tellurium into seleno- and telluromethionine via the sulfate assimilation pathway, with potential further incorporation into proteins, is an important cause of tellurite and selenite toxicity in yeast. The here reported results represent a robust foundation to which to attack the mechanistic details of this molecular enigma.

B3.09
Comparison of histone H2A.X (Ser139) and ATM kinase (Ser1981) phosphorylation after one hour treatment of HL-60 cells with vanadocene dichloride and cisplatin; relation to the cell-cycle phases
R. Havelka1, J. Cmielova1, P. Siman1, J. Vinklarek2, J. Vavrova1 and M. Rezacova1
1Department of Medical Biochemistry, Charles University in Prague, Faculty of Medicine in Hradec Kralove, Hradec Kralove, Czech Republic, 2Department of General and Inorganic Chemistry, University of Pardubice, Faculty of Chemical Technology, Pardubice, Pardubice, Czech Republic

Aim: To assess difference in extent of DNA damage that involves formation of dsDNA breaks after 1 hour treatment of HL-60 cells with metalloocene agent vanadocene dichloride (VDC) and cisplatin (CP).

Methods: Phosphorylation of H2AX on Ser139 and ATM on Ser1981 in HL-60 cells were detected immunocytochemically. DNA was counterstained with propidium iodide; the cell immuno-fluorescence (IF) was measured by flow cytometry. The intensity of Ser1981P – labeled cells were evaluated by flow cytometry soft.

Results: Mean of gammaH2AX IF increase linearly with the dose of VDC in all cell-cycle phases. CP caused weak increase of gammaH2AX IF was less intense. Mean of gammaH2AX IF increase linearly with the dose of VDC in all cell-cycle phases. CP caused weak increase of gammaH2AX IF, in S phase was decrease observed. The cells treated with e.g. 1 and 0.5 mM/L VDC showed 99 resp. 96% gammaH2AX positivity. In case of same doses of cisplatin increase of gammaH2AX positivity was 98% negative. Slight increase of ATM-Ser1981P IF was observed after VDC treatment, whereas CP did not promote IF increase. Cells exposed to 1 and 0.5 mM/L VDC were 9 resp. 4% ATM-Ser1981P positive, control 2%.

Conclusions: The strong correlation between phosphorylation of ATM and H2AX after VDC treatment was observed. The data indicate that VDC cytotoxic effect can be particularly due to formation of dsDNA breaks in cells.

Acknowledgement: Supported by project MSM 0021620820 and Medica 84121.

B3.10
Mechanisms underlying anticancer effect of vanadocene complexes in comparison to cisplatin in p53 negative cells HL-60
P. Krejcirikova1, J. Cmielova1, R. Havelek1, P. Simon1, M. Rezacova1 and J. Vinklarek2
1Department of Medical Biochemistry, Faculty of Medicine in Hradec Kralove, Charles University in Prague, Hradec Kralove, Czech Republic, 2Department of General and Inorganic Chemistry, University of Pardubice, Pardubice, Czech Republic

Aim: The aim of the present study was to determine differences in molecular mechanism in action of known cytostatic drug cisplatin (CP) and a compound vanadocene dichloride (VDC) in HL-60 leukemia cell line.

Methods: We used WST test to evaluate toxicity of cytostatics (IC50 value) and trypan blue exclusion technique to determine cell viability. Changes in protein expression were analyzed by Western blotting. Caspase activity was determined by Caspase-Glo Assay set. Nuclei of infected cells were stained by DAPI.

Results: IC50 value was determined after 72 hours treatment as 3*10^-6 mol/l for CP and as 35*10^-6 mol/l for VD. Changes in protein expression of p21, Bid, Mcl-1 and gammaH2AX were evaluated. Expression of the protein p21 was detected mainly after VD treatment in the time interval 24, 48 and 72 hours. The induction of gammaH2AX was detected in samples influenced by CP after 48 and 72 hours of incubation, expression of Mcl-1 appeared after treatment by both drugs and the induction of the protein Bid was the most visible after influence by CP in the time interval 72 hours. The activity of caspases 3 and 9 in cells influenced by CP increased after 48 hours incubation, the activity of caspases 3, 8 and 9 stayed approximately the same in the same time interval after treatment by VD.

Conclusion: According to results of our study, there are differences in mechanism of action of both cytostatics.

Acknowledgement: This work was supported by The Ministry of Education of Czech Republic, project MSM 0021620820.

B3.11
The deletion of Raf Kinase-1 Inhibitor Protein (RKIP) produces an increase in the pancreas volume during development
F. Pardo1, J. Altimira2, A. Garcia2, A. Barbera3, C. Slebe J.1, A. Yañez1, A. Novials1 and R. Gomis3
1Universidad Austral de Chile, Instituto de Bioquimica, Valdivia, Chile, 2CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Barcelona, Spain, 3Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain

RKIP is a docking protein with the capacity to inhibit MAPK, GPCR and NFkB pathways, participating in cell proliferation and apoptosis. Previous studies, have demonstrated a downregulation of RKIP in a model of β cell regeneration. Also, we have seen the RKIP KO presents a bigger pancreas at 8-weeks old and a higher sensitivity to the glucose at 15 and 30 minutes in a glucose tolerance test. To understand the role of RKIP in the plasticity of the β cell, and a possible role in the inhibition in the replication, we have studied the pancreas in the whole KO mouse of RKIP during the development. The pancreas begins bigger in the KO mouse since the 28-days of development (1.11% ± 0.03 of BW in KO and 0.98% ± 0.05 in WT). Through morphometries studies we can observed that there are differences in the total beta cell mass (KO: 0.7 mg ± 0.01, WT: 0.41 mg ± 0.08) and in the distribution of different β cell areas. KO animals present a...
A comprehensive genetic-interaction map of a eukaryotic kinome

S. Sharifpoor1, D. van Dyk1, M. Costanzo1, H. Friesen1, A. Ralph1, J.-Y. Youn1, C. Myers2, B. Papp3, C. Boone1 and B. Andrews1

1University of Toronto, The Donnelly Centre, Toronto, Canada, 2University of Minnesota-Twin Cities, Computer Science & Engineering, Minneapolis, USA, 3Biological Research Center, Institute of Biochemistry, Szeged, Hungary

We have used functional genomic screens to interrogate the yeast kinome using combinatorial genetic perturbations. We systematically assessed the effects of either gene overexpression or gene deletion on the fitness of non-essential kinase deletion mutants to generate a meta-network of Synthetic Dosage Lethal (SDL) and Synthetic Lethal (SL) interactions of yeast kinases. The datasets are enriched for pathways known to be regulated by cognate kinases and analysis of the network schema reveals considerable new information about kinase function. The kinases that yielded informative SDL interactions were largely those with significant cell cycle or cell polarity roles and the SL hits were enriched for phosphoproteins, cell cycle regulated genes and inhibitory pathways regulated by the kinases. Condition-specific screens and analysis of kinase double mutants suggests that the apparent resistance of many kinases to genetic perturbation cannot be solely attributed to kinase redundancy but most likely reflects the requirement for many kinases in certain activating conditions. We also present the most complete list of literature-curated kinase targets to date. Using the curated kinase-substrate gold standard and the integrated SDL-SL meta-network we have identified triplet regulatory motifs, unraveling novel pathways regulated by kinases. Our functional map of the yeast kinome will provide a valuable resource that can be mined to identify roles of this important class of regulatory proteins.

B3.13
Systems biology approaches towards dynamic complexes of monogenic, aggregation-prone disorders: case study NCLs

S. Eno1, M.-L. Schmiedt², K. Uusi-Rauva2, A. Kyttälä2, M. Baumann1, A. Jalanko2 and M. Lalowski3

1University of Helsinki, Protein Chemistry/Proteomics/Peptide Synthesis and Array Unit; Biomedicum Helsinki, Helsinki, Finland, 2University of Helsinki, Public Health Genomics, National Institute for Health and Welfare; Biomedicum Helsinki, Helsinki, Finland

Protein-protein interaction networks are relevant to systems biology because they elucidate both spatial and temporal organization principles of the cellular networks. The Neuronal ceroid lipofuscinoses (NCLs) are the most common cause of inherited progressive encephalopathy of childhood, characterized by accrual of autofluorescent, ceroid-like lipopigment and degeneration of cortical neurons. NCLs are caused by mutations in 10 postulated genes (CLN1-10), eight of which have been cloned. Bioinformatic analyses and literature information demonstrates that NCL proteins are connected at the molecular level. Juvenile NCL (JNCL, Juvenile Batten disease) is caused by mutations in the CLN3 gene. To systematically characterize the NCL protein complexes we have created a “Gateway-compatible NCL entries library”, including all available disease genes and their direct interacting partners. At present our “NCL destination library” contains approximately 600 clones, with tags either at the N- or C-terminus of the human ORFs. As a proof of principle study, we chose to characterize CLN3 dynamic interactome by validating available binary yeast-two hybrid (Y2H) interaction data with affinity purifications combined with mass spectrometry. For validation of Y2H data we are utilizing various biochemical approaches, i.e. LUMIER-based coimmunoprecipitation assay (i). Human SH-SY5Y cells, stably expressing CLN3 protein with dual affinity tags (Protein G-Strep) have been generated as described (ii). The identity of isolated proteins will be determined by LC-MS/MS, and brought forward for comparative bioinformatic analyses and systematic RNAi studies to assess the dynamics of CLN3 complexes.
B4 – Gene Regulation

B4.01
Investigation of benzodiazepines and zolpidem effects on the expression of cytochromes P450 CYP1A1, CYP1A2 and CYP3A4 in primary cultures of human hepatocytes
R. Vrzal1, P. Pavék2 and Z. Dvorak1
1Palacky University Olomouc, Olomouc, Czech Republic, 2Charles University, Hradec Králové, Czech Republic

We have examined the potency of Alprazolam, Bromazepam, Clonazepoxide, Clonazepam, Diazepam, Lorazepam, Medazepam, Midazolam, Nitrazepam, Oxazepam, Triazolam and Zolpidem to induce CYP1A1, CYP1A2 and CYP3A4 in primary cultures of human hepatocytes. Compounds were tested in therapeutic concentrations and in concentrations corresponding to their plasma levels in intoxicated patients. We found weak but significant induction of CYP3A4 mRNA by Midazolam and Medazepam, while other drugs did not induce CYP3A4 expression. None of the tested compounds induced CYP1A1 and CYP1A2 mRNAs in three different human hepatocyte cultures. In addition, employing gene reporter assays with transiently transfected hepatocarcinoma cells, we found that tested compounds did not activate aryl hydrocarbon receptor (AhR), whereas Midazolam and Medazepam slightly activated pregnane X receptor (PXR). In conclusion, Medazepam and Midazolam are weak activators of pregnane X receptor and displayed weak potency to induce CYP3A4 mRNA in human hepatocytes. Our laboratory is supported by the grants from the Grant Agency of the Czech Republic GACR304/10/0149, GACR303/07/0128 and GACR305/08/P089.

B4.02
Valproic acid potentiates vitamin D receptor-mediated induction of CYP24 gene – A consequence for drug-induced osteomalacia
R. Vrač1, A. Dvoráková1, M. Bitmann2, P. Pavék2 and Z. Dvorak1
1Department of Cell Biology and Genetics, Palacky University Olomouc, Olomouc, Czech Republic, 2Department of Pharmacology and Toxicology, Charles University in Prague, Hradec Králové, Czech Republic

Valproic acid (VPA) found its use as a mood-stabilizing agent for treatment of epilepsy or bipolar disorders (brand names like Depakote, Depacon or Stavzor). On the molecular level, it was found to be an inhibitor of GABA transaminase or histone deacetylase 1 (HDAC1). Recently, our group described that VPA activated pregnane X receptor (PXR), which led to induction of cytochrome P450 3A4 (CYP3A4) in human hepatocytes. Since there are many drugs causing so-called drug-induced osteomalacia and these drugs act mainly via the PXR, we decided to investigate, whether the molecular mechanism might involve a vitamin D receptor (VDR)-mediated induction of CYP24, one of the major enzymes involved in the catabolism of active form of vitamin D (VD3). We found that VPA did not induce CYP24 mRNA in human hepatocytes next to the VD3, a typical inducer of CYP24. However, VPA potentiated CYP24 mRNA induction mediated by VD3. In human carcinoma cells, HepG2, it was able to transactivate a CYP24 promoter and this effect was independent on the presence of co-transfected expression vectors for VDR or PXR. The same potentiation was observed for endogenously present VDR in human embryonal kidney cells, HEK293.

B4.03
Evolutionary expansion of SPOP and associated TD/POZ gene family via chromosomal segmental duplication, retrotransposition and exonization of transposed elements
C.-J. Huang1, W.-Y. Lin2, C.-M. Chang1, Y.-H. Tsai2 and K.-B. Choo3
1Department of Animal Science, Chinese Culture University, Taipei, Taiwan, 2Department of Medical Research and Education, Taipei Veterans General Hospital, Taipei, Taiwan

Evolutionary expansion of a gene family may occur at both the DNA and RNA levels and may involve the highly abundant transposed elements (TEs). The rat testis-specific Rtdpoz-T2 and -T1 (rT2 and rT1) retrogenes are members of the TD/POZ gene family which also includes the well-characterized SPOP gene. We have previously shown frequent TE exonization in rT2/rT1 transcripts in developing embryos resulting in a complex set of transcripts as opposed to the relatively simple and TE-free transcripts in the tests. In this study, rT2/rT1 transcriptional activation in cancer cells is demonstrated; the cancer rT2/rT1 transcripts are structurally similar to the developmental transcripts in frequent TE exonization. An uncharacterized rT2/rT1-like SPOP paralog, designated as SPOP-like (SPOPL), and related retro-sequences have also been identified by sequence interrogation of the GenBank databases. Sequence alignment, synteny and phylogenetic analyses implicate that evolution of the TD/POZ family of genes and retrogenes from a common ancestor involves (i) at the DNA level: chromosomal segmental duplication and recruitment of TEs as tissue-specific promoters, and retrotransposition, and (ii) at the RNA level: TE exonization through alternative splicing to further expand the transcript population. TE involvement is particularly profound in embryonic and cancer cells and may have biological significance.

B4.04
Chromosomes open into two opposing semi-cones with projection of active translatable genes into the interchromatin domain: novel geometrical model explaining speed of recognition of binding sites by transcription factors TF
K. Pirkalani1, Z. Talae Rad2, H. Bigdel1, B. Khodabakhsh3 and B. Poulaki2
1Mehr Medical Group, Endocrinology/Internal Medicine/Molecular Biology, Tehran, Islamic Republic of Iran, 2Mehr Medical Group, Endocrinology/Gynecology, Tehran, Islamic Republic of Iran, 3Mehr Medical Group, Laboratory Medicine/Pathology, Tehran, Islamic Republic of Iran

We conclude that VPA significantly potentiates ligand-triggered induction of CYP24s and this may disturb the vitamin D homeostasis via increased catabolism. This work was supported by the grants from the Grant Agency of the Czech Republic GACR303/07/0128, GACR305/08/P089 and GACR304/10/0149.

Abstracts
Objective: Explaining the fast recognition of appropriate sequences by TFs Designing a 3d model highlighting microscopic organization of interphase chromosomes.

Materials and Methods: Incorporating nucleosome/beads on string model, we designed a topological model with six preconditions:
(1) Easy opening and condensation without risk of aberration.
(2) Retention of two longitudinal axes for long and short arms.
(3) Easy access of the TFs to the transcribed genes.
(4) Neighborhood of genes (chromosomes) that are translocated in cancer to each other.
(5) Availability of an interconnected channel system (interchromatin domain) in continuity with the nuclear membrane pores for TFs.
(6) Dynamic property of DNA molecule sensing the entrance of TFs and extending the transcribed genes to reduce the time of search.

Results: A topological model of two angled semi-cones (one for each chromosome arm) is the best fit. The potential space harbors genes for transcription. The central axes comprise of non transcribed genes seen as heterochromatin during interphase. The DNA molecule has alternating double and single bounds (nucleotide distance = 3.3 Angstrom) which is the major prerequisite for being electricity conductive. This property is used to extend specific genes outwards for easier recognition by transcription factors. Based on four color problem in topology, 63 frequent translocation seen in cancer were incorporated into a three dimensional neighborhood of chromosomes.

Conclusions: (1) Try and error of TFs is not an economic strategy.
(2) The interphase chromosome has a geometrical hierarchy.
(3) Two opposing angled semi-cone model best explains the potential space of transcribed genes.

B4.05
Overexpression of arabidopsis transcription factor AtICE2 enhance the expression of genes involved in the ABA-dependent cold response

M. Novokreshchenova1, A. Kurbidaeva1 and V. Tarasov2
1M.V.Lomonosov Moscow State University, Biotechnology, Moscow, Russian Federation, 2Vavilov Institute of General Genetics, Moscow, Russian Federation

An overexpression of AtICE2 (as we named the At1g12860 locus) in transgenic arabidopsis plants results not only in increased tolerance to deep freezing stress after cold acclimation (Fursova et al., 2009), but in development of stomatal cell lineages also (Novokreshchenova et al., 2009). In the current study, we characterized an expression pattern of Arabidopsis genes CBF1-3, RAB18, NCED3, RD29A, RD29B in arabidopsis plants constitutively over-expressed full-sized AtICE2 gene sequence (F-ICE2 line) and the deleted variant without 1st exon (D-ICE2). The expression level was examined by Real Time PCR analysis (F-ICE2 line) and the deleted variant without 1st exon (D-ICE2). The expression level was examined by Real Time PCR analysis (F-ICE2 line) and the deleted variant without 1st exon (D-ICE2). The expression level was examined by Real Time PCR analysis (F-ICE2 line) and the deleted variant without 1st exon (D-ICE2). The expression level was examined by Real Time PCR analysis (F-ICE2 line) and the deleted variant without 1st exon (D-ICE2). The expression level was examined by Real Time PCR analysis (F-ICE2 line) and the deleted variant without 1st exon (D-ICE2). The expression level was examined by Real Time PCR analysis (F-ICE2 line) and the deleted variant without 1st exon (D-ICE2). The expression level was examined by Real Time PCR analysis (F-ICE2 line) and the deleted variant without 1st exon (D-ICE2). The expression level was examined by Real Time PCR analysis (F-ICE2 line) and the deleted variant without 1st exon (D-ICE2). The expression level was examined by Real Time PCR analysis (F-ICE2 line) and the deleted variant without 1st exon (D-ICE2). The expression level was examined by Real Time PCR analysis (F-ICE2 line) and the deleted variant without 1st exon (D-ICE2).

B4.06
Micro-RNA regulation of SPOP in cancer cells

K.-B. Cho1, C.-J. Huang2, W.-Y. Lin3, H.-Y. Chen3 and S.-T. Yeh1
1Department of Medical Research and Education, Taipei Veterans General Hospital, Taipei, Taiwan; 2Department of Animal Science and Graduate Institute of Biotechnology, Chinese Culture University, Taipei, Taiwan; 3Department of Medical Research and Education, Taipei Veterans General Hospital and National Yang Ming University, Graduate Program, Department of Biotechnology and Laboratory Science in Medicine, Taipei, Taiwan

SPOP, speckle-type POZ protein, is a highly conserved protein found ubiquitously in human tissues, and has been shown to down-regulate the pancreatic transcription factor, PDX-1, in pancreas development. SPOP also participates in protein ubiquitination and degradation. This study aims to investigate regulation of SPOP expression. When real-time RT-PCR was performed to determine relative SPOP mRNA levels in a number of human cancer cell lines, a 14-fold difference was observed between different cell lines with the breast cancer MCF7 and cervical cancer SiHa cell lines expressing the highest and the lowest SPOP mRNA levels, respectively. Moreover, western blot analysis indicated relatively constant SPOP protein levels in all the cell lines analyzed except for the colon cancer cell line SK-CO1 which showed very low SPOP protein levels suggesting post-transcriptional regulation. microRNA database searches of the "1.5-kb 3’-UTR predict involvement of miRNAs 106 and 145 in SPOP regulation which was verified by luciferase reporter assays and direct miRNA knockdown and over-expression in selected cancer cell lines.

B4.07
Methylation status of miR-124a gene promoter and its tumor suppressor role in cervical oncogenesis

A. Botezatu1, I. V. Iancu1, C. D. Goia1, A. Plesa1, I. Huica1, D. Socolov2 and G. Anton1
1Institute of Virology “Stefan S Nicolau”, Bucharest, Romania, 2University of Medicine and Pharmacy “Gr.T.Popa”, Iasi, Romania

Identification of genes that undergo cancer-specific CpG island hypermethylation and correlation of these data with pre-neoplastic lesions, tumor stage, progression, and long-term prognosis are becoming increasingly common. Subsequent analysis proved that miR-124a is also frequently methylated in colon, breast and lung carcinoma cell lines. The hypermethylation of miR-124a promoter in cancer cells results in an increased expression of its target CDK6. This study was conducted to investigate the promoter methylation status of the miR-124a gene promoter in pre-neoplastic lesions and cervical cancer and his possible implication in regulation of p21 mRNA levels, and the CDK6 partner-cyclinD1. Promoter methylation was evaluated using a MS-PCR for bisulphite treated DNA (EpiTect Bisulfite Kit - Qiagen) samples. The DNA samples were isolated from 50 cervical swabs with abnormal cytology and 10 from normal patients. (High Pure PCR Template - Roche). RNA was isolated using Trizol and reverse-transcribed with Access Quick (Promega). The level of p21 and

References:
Human genome enhancers show tissue-specific and promoter-specific activity
A. Sorotokina and E. Prof. Sverdlov
Institute of Bioorganic Chemistry, Moscow, Russian Federation

The mammalian genome contains a great number of functional cis-regulatory non-transcribed elements, such as promoters, enhancers, silencers, and insulators which play a major part in regulation of genome activity. Enhancers are the DNA elements capable to increase the transcription initiated by the cognate promoter, and their activity is in most cases insignificantly depends on the promoter-enhancer distance and orientation. At the same time, it is believed that the activity of enhancer critically depends on tissue or cell type where this element is functioning. Earlier we identified and cloned 15 enhancers within one megabase region of human chromosome 19. Here we tested the activity of four of them in 5 cell lines of different origin and with respect to two different promoters (CMV and SV40). We demonstrated that the enhancers possess both pronounced tissue- and promoter-specificity. In addition, one of the enhancers was subjected to deletion analysis which allowed us to isolate its short core sequence responsible for the enhancer activity.

The role of nuclear receptors in statin-induced molecular mechanisms
M. Hafner1, T. Režen1, P. Juvan1, K. Monostory2, J.-M. Pascaux2 and D. Rozman1
1Faculty of Medicine, University of Ljubljana, Center for Functional genomics and Bio-chips, Ljubljana, Slovenia, 2Hungarian Academy of Science, Medical Research Center, Budapest, Hungary

Statins, cholesterol lowering drugs, act as competitive inhibitors of HMGCR which catalyses the rate limiting step of cholesterol synthesis. They activate different pathways not only via PXR but also via CAR-mediated mechanisms (rosuvastatin). The aim of this study is to get a better insight into the cross-talk between cholesterol homeostasis and metabolism of selected drugs in human primary hepatocytes and to define novel PXR and CAR-modulated genes. The effect of CAR on expression of cholesterol biosynthesis pathway will also be investigated. Human hepatocytes have been treated with rosuvastatin or atorvastatin for 12, 24 or 48 hour. Rifampicin has been applied as a well known PXR activator. Expression profiling was performed with Steroltalk v2 and Affymetrix HG-U133 Plus 2.0 arrays and data analysed with Orange and R Bioconductor software. To discriminate between targets of the two statins underlined genes common for the activity of both and may be activated by CAR. Overlap of the atorvastatin, rosuvastatin and rifampicin exposed genes involved in drug metabolism, cancer pathway and regulation of autophagy. Using expression profiling, bioinformatics and data mining we started to reveal novel pathways that are targeted by statins. More detailed information will be presented on the meeting.

B4.10 The role of orphan and ligand-dependent nuclear receptors in the regulation of the human apoM gene in the liver
I. Mosialou1 and D. Kardassis2
1Department of Biochemistry, University of Crete Medical School, Heraklion, Greece, 2Department of Biochemistry, and Institute of Molecular Biology and Biotechnology, University of Crete Medical School, FORTH-Hellas, Heraklion, Greece

Apolipoprotein M (apoM) plays an important role in High Density Lipoprotein (HDL) remodeling in plasma. Mice lacking HNF-1, the major regulator of apoM in the liver, are characterized by absence of apoM and preb HDL particles in plasma. We have previously reported that the proximal promoter of the human apoM gene is additionally regulated by orphan and ligand-dependent nuclear receptors such as HNF-4, RXR homodimers and RXR heterodimers with RAR, T3R, PPAR or LXR. In the present study, we further examined their binding characteristics to apoM promoter using site-directed mutagenesis, DNA affinity precipitation and chromatin immunoprecipitation assays. We show here that all receptors bind to a DR1 hormone response element (HRE) at position -33 to -21 albeit with different affinities while binding was abolished upon mutagenesis of this HRE. Interestingly, the same mutation did not affect the binding of RXR/T3R heterodimers or of LRH-1 shown previously by others to occupy the same region, suggesting the presence of two adjacent HREs in the proximal apoM promoter. In agreement with these, the HRE mutation abolished transactivation by all receptors except LRH-1. Adenosine-mediated overexpression of RXR or treatment of hepatic cells with retinoids, fibrates, oestrogens and T3 induced apoM gene expression. Retinoids, however, exhibited a biphasic mode of regulation with an early activation phase at 1–3 hour followed by a repression phase at 8–24 hour. In summary, our findings indicate that the apoM gene, similar to other genes of the HDL pathway, is a direct target of hormone nuclear receptors in hepatic cells.

B4.11 Macrophage specific regulation of apoCII gene expression via distal regulatory elements
I. C. Florea1, V. Trusca1, D. Kardassis2 and A. V. Gafencu1
1IC.B.P. "Nicolea Iorgulescu", Pharmacology and Molecular Biology, Bucharest, Romania, 2University of Crete, Medical School, Heraklion, Greece

Purpose: Apolipoprotein CII is involved in the conversion of VLDL into LDL by activating lipoprotein lipase, an enzyme found on the surface of endothelial cells. In atherosclerotic inflammation, monocyte derived macrophages become foam cells by ingesting ox-LDL and eventually die, further propagating the inflammatory process. This study aims to understand the mechanisms of apoCII gene regulation in macrophages, the data obtained may be useful for future therapeutic strategy.

Methods and Results: Transient transfection experiments on RAW 264.7 cells showed that the multienhancer 2 region (ME2) interacts with the proximal promoter of apoCII (+545/+18) and with its deletion mutant (~388/+18). Chromosome conformation capture experiments revealed that ME2 and apoCII promoter interact in antisense direction. Transient co-transfection experiments indicated that STAT1 transcription factor has a binding site in the region −354/−388. The interaction between ME2 and apoCII promoter allowed STAT1 to induce the transcription activation of the proximal promoter of apoCII. DNA precipitation experiments demonstrated the presence of a STAT1 binding site in the region 145-160 of ME2 that was also predicted by in silico analysis.

Conclusions: In macrophages, ApoCII gene expression is controlled by the cooperation between the promoter with ME2, which can bring different transcription factors in the proximity of apoCII promoter, but their effect depends on the interactions with factors acting on the proximal promoter.

Acknowledgment: This work was supported by the Romanian Ministry of Education and Research (CNCSIS PNII-Ideas grant no. 989/2008) and by the Romanian Academy.

B4.12 Inhibition of cornea angiogenesis by small interference RNA targeting vascular endothelial growth factor receptor2
M. Jafari Sani1, A. Zarei Mahmudabadi2, M. Bahabadi2, A. Mohseni2, and J. Alizadeh1
1Republic of Iran, 2Ahvaz Medical of Science University, Ahvaz, Iran

Introduction: Cornea neovascularization (NV) occurs in various ocular disorders including proliferate diabetic retinopathy, retinopathy of prematurity and secondary neurovascular glaucoma and chemical injuries which often result in blindness. Frequently vascular endothelial cell growth factors (VEGFs) are mainly responsible for the pathological neovascularization. Therefore, VEGF & VEGF Receptors (VEGFRs) could provide targets for therapeutic intervention on Cornea NV.

Material and method: In this study, we inspected the inhibitory effects of the specific small interfering RNAs (siRNA) targeting VEGFR (VEGF2) on the expression of VEGFR in human umbilical vein endothelial cells (HUVEC) in vitro. Three different siRNAs against the VEGFR2 were designed and chemically synthesized. Using GFP expression vector the specificity of siRNAs was investigated. Using GFP expression vector the specificity of siRNAs were transfected HUVEC cell using lipfecthaminth 2000. 24 hour after transfection the cells were harvested ant total RNA purified. RT-PCR and PCR Using VEGFR2 specific primers were done and PCR products were electrophoreses.

Results: The result has shown significantly decreased in VEGFR2 and mRNA. The clinical examination shown significantly reduces in corneal neovascularization.

Conclusion: These results indicate that VEGFR2-specific siRNAs can be use as a suitable therapy against corneal neovascularization in eye diseases.

B4.13 Characterization of a new signalling pathway controlling virulence in the bacterium Dickeya dadantii
S. Reverchon1, C. Dore1, F. Van Gijssegem2, G. Effantin1, J. Wawrzyniak1 and W. Nasser1
1MAP UMR 5240 CNRS-UCBL-INSa-BCS, INSa de Lyon, Villeurbanne, France, 2UMR217 INRA/INA-PG/UPMC, INAPG, Paris, France

Production of plant cell wall degrading enzymes (CWDE), the major virulence factors of Dickeya dadantii, is a highly regulated process involving multiple regulators that enable the information from different inputs to be assessed before virulence genes are expressed. We have characterized a 25-kb vfm cluster which is required for D. dadantii pathogenesis. The vfm cluster encodes a biosynthetic pathway for a metabolite composed of both amino acid and fatty acid moieties. Indeed, it encodes proteins displaying similarities with enzymes involved in amino acid activating process such as Ala-Ala ligases and amino acid decarboxylase (VfmO, VfmP, VfmK) and with enzymes involved in fatty acid biosynthesis such as acyl-CoA synthase, 3-oxoacyl-ACP synthases, acyl-carrier proteins, 4-phosphopantetheinyl transferase (VfmM, VfmA, VfmT, VfmW, VfmR, VfmB, VfmU, VfmN, VfmZ, VfmQ, VfmJ).

We suggested that the metabolite produced via the vfm cluster might act as a signal controlling virulence factor production in D. dadantii. This assertion is supported by the fact that, fatty acid derivatives serve as environmental cues to regulate virulence in several bacteria including Proteus mirabilis, Vibrio cholerae and Xanthomonas campestris. The vfm cluster contains two transcriptional regulators, VfmE of the AraC family and VfmH a two component response regulator which is associated to the Vfm sensor histidine kinase. Preliminary results suggested that VfmE controls signal production while VfmH-VfmJ may sense the signal and induce transcriptional activation of pathogenicity genes.

B4.14 Expression analysis of genes involved in epigenetic regulation and apoptosis in human myelodysplastic syndrome cell line treated by 5-azacitidine
K. Stankov1, G. Bogdanovic1, V. Kojic1, S. Stankov1 and K. Bajin-Katic1
1Department of Biochemistry, Medical faculty Novi Sad, University of Novi Sad, Novi Sad, Serbia; 2Oncology Institute of Vojvodina, Experimental Oncology, Srpska Kamenica, Serbia; 3Health Center Novi Sad, Novi Sad, Serbia

Expression analysis of genes involved in epigenetic regulation and apoptosis in human myelodysplastic syndrome cell line treated by 5-azacitidine

K. Stankov1, G. Bogdanovic1, V. Kojic1, S. Stankov1 and K. Bajin-Katic1
1Department of Biochemistry, Medical faculty Novi Sad, University of Novi Sad, Novi Sad, Serbia; 2Oncology Institute of Vojvodina, Experimental Oncology, Srpska Kamenica, Serbia; 3Health Center Novi Sad, Novi Sad, Serbia

PC-MDS is the first therapy-related myelodysplastic syndrome derived cell line, and based on its immunological, cytogenetic and molecular characterization, PC-MDS may be used as a new tool in evaluation of complex biology of MDS and a model for methylation studies. Therefore, our aim was to study the modulation of the expression status of 10 different genes involved in epigenetic regulation and apoptosis by the DNMT inhibitor 5-azacitidine (5-AZA), as markers of response to inhibition.

In our analysis we have used the SybrGreen technology and gene-specific primers for the qRT-PCR analysis of 10 genes, in cDNA of PC-MDS and K562 cells, treated by 1 micromole of 5-azacitidine, as markers of response to inhibition. The members of the bcl-2 fam-
ily of apoptosis-regulating genes bcl-2 and Bax, showed the statistically significant differences in expression, in comparison with non-treated PC-MDS cells. Our most interesting result was the significant re-expression of p15 in 5-AZA treated PC-MDS cells. p15, the inhibitor of cyclin-dependent kinases, is frequently hypermethylated in MDS. 5-AZA, reverts hypermethylation of this gene in vitro. Evaluation of our qRT-PCR results showed that the re-expression of p15 in PC-MDS cell line, implicate this novel cell line as a suitable model for the studies of pharmacologic demethylation as a possible mechanism resulting in hematologic response in MDS.

B4.15 Expression of the main virulence genes in Dickeya datanii is sensitive to the DNA supercoiling state

H. W. Nasser1, O. Zghidi-Abouzid1, G. Muskhelishvili2 and S. Reverchon1
1 CNRS-UCBL-INSa-BCS UMR 5240 Microbiologie, Adaptation, Pathogenie, INSa de Lyon, Villeurbanne, France, 2 Jacobs University, Bremen, Germany

Dickeya datanii (Erwinia chrysanthemi) is a bacterium that attacks a wide range of plant species. Soft rot, the visible symptom, is mainly due to the production of pectate lyases (Pels) that can destroy the cell walls. Transcription of the pel genes is modulated by various regulators in response to different stimuli such as KdgR (pectic compounds), Fis (growth phase), CRP (catabolic repression), H-NS (temperature and nutritional starvation). Most of these stimuli (growth phase, temperature, nutritional starvation...) also modulate the structural organisation of DNA and remarkably, two regulators of the pel genes, H-NS and Fis, are chromatin structuring proteins. We report that the expression of the pel genes is modulated in vivo by changes in the DNA topology. However the impact of the structural organization of DNA on the expression of these genes is less pronounced in the hns and fis mutants than in the parental strain. These findings therefore suggest that H-NS and Fis might adjust the global structural modifications of the chromatin at the pel gene promoters in order to optimize their expression. These data obtained in vitro were further confirmed in vivo by comparing the transcriptional capacity of DNA templates with various supercoiling states. Together, these data indicate that the transcription of the pel gene is regulated by a dynamic cooperation between the transcription machinery and the topological state of DNA.

B4.16 WT1 and its molecular partners modulate bag3 gene expression

G. Montano1, E. Cesaro1, R. Crescitelli1, L. Fattore1, A. Rosati2, P. Izzo1, C. Turco M.2 and P. Costanzo1
1 Università degli Studi di Napoli Federico II, Biochimica e Biotecnologie Mediche, Napoli, Italy, 2 Università degli Studi di Salerno, Scienze Farmaceutiche, Salerno, Italy

Wilms' tumour 1 protein, WT1, is a zinc finger transcriptional factor that controls the expression of a wide number of genes involved in tissue differentiation, in apoptosis, or in cell cycle control. Moreover, the transcriptional regulatory properties of WT1 are known to be influenced by interaction with an increasing number of molecular partners. Recently, we identified the Krüppel-like zinc finger protein, ZNF224, as a novel human WT1-interacting protein and demonstrated that ZNF224 may act as a transcriptional co-regulator of WT1 in order to modulate target gene activity. In the present study, we identify bag 3 as a novel target gene of WT1 protein, involved in apoptosis regulation. Bag3 gene expression is constitutive in some tumour types, including leukemias; in these cells, BAG3 protein has been demonstrated to sustain cell survival and down module cell apoptosis to drugs. We report that WT1 positively regulates bag3 gene expression through a transcriptional mechanism. WT1 knockdown affects bag3 expression and enhances apoptosis by 30% in the human erythroleukemia cell line K562. Instead, in HEK293 clones stably over-expressing ZNF224 we observe that WT1 is unable to activate bag3 gene expression, thus indicating that ZNF224 behaves as a transcriptional co-repressor of WT1 on bag 3 promoter. The study of the role of the proteins WT1 and ZNF224 in regulation of bag3 gene expression will provide novel insight into the understanding of apoptosis regulation.

B4.17 Gene expression profile in human white blood cells after ramp-type progressive exercise

D. Sakharov, E. Tonevitsky, E. Trushkin and A. Tonevitsky
Russian Research Institute of Physical Culture and Sport, Molecular Physiology, Moscow, Russian Federation

It is well known that high intensity (> 80% peak O2 uptake) endurance exercises alter gene expression in human white blood cells (WBCs). The purpose of present study was to investigate the effects of short ramp-type progressive exercise on immediate early gene (IEG) expression. Ten well trained skiers (21–24 year old) participated in ramp-type progressive exercise on treadmill until exhaustion. Average time in test and after anaerobic threshold was 14:30 and 5:15 minute respectively. Blood samples were obtained preexercise (T0) and immediately after exercise (T1). Exercise induced significant increase in extracellular heat-shock protein 70kDa (eHSP70), growth hormone (GH), cortisol concentration and creatine kinase (CK) activity in serum, so subjects endure high intensity physiological stress. Gene expression in WBCs was measured using GeneChip 1.0 ST Array (Affymetrix). With false discovery rate (FDR) <0.05 with 95% confidence, a total of 153 genes were differentially expressed (117 up regulated, 36 down regulated). Exercise influenced a variety of established gene pathways related to inflammation, stress response, signal transduction and apoptosis. But in present study we find a large group of differentially expressed ribosomal proteins (RP) and imprinted small nucleolar RNAs (snoRNA). In summary, even very short physiological stress (up to 7-15 min) lead to activation of stress response genes and previously unknown significant alteration of RP and snoRNA gene expression.

B4.18 E(y)2/Sus1 is required for PRE-blocking activity of the Wari insulator in Drosophila melanogaster

D. Chetverina, M. Erokhin, A. Parshikov and P. Georgiev
Department of the Control of Genetic Processes, Institute of Gene Biology RAS, Moscow, Russian Federation

Insulators are classically described by two experimentally defined properties, enhancer-blocking activity and barrier activity. Insulators operate in a position-dependent manner (enhancer-blocking activity), interfering with the activity of enhancers when inserted between these regulatory elements and a promoter but not when located upstream or downstream from them. The barrier activity of insulators blocks heterochromatinization and consequent silencing of a gene. Both activities of insulators are required for the anti-silencing function, i.e. for blocking the repressive effect
of a Polycomb response element (PRE). Recently we have found an insulator, named Wari, located between the white gene, which is responsible for eye pigmentation, and the CG32795 gene. The Wari insulator is also present downstream of the mini-white gene, which is widely used as a marker in transposons. Here, we show that the previously identified 368-bp core of this insulator is sufficient for blocking PRE-mediated silencing. Although Wari does not contain binding sites for known insulator proteins, the E(y)2 and CP190 proteins bind to Wari as well as to the Su(Hw)-containing insulators in vivo. It may well be that these proteins are recruited to the insulator by as yet unidentified DNA-binding protein. Partial inactivation of E(y)2 in a weak e(1)yg mutation impairs only the anti-silencing but not the enhancer-blocking activity of the Wari insulator. Thus, the E(y)2 protein in different Drosophila insulators serves to protect gene expression from silencing. This study is supported by the Russian Foundation for Basic Research (project no. 09-04-00903-a).

B4.19
Interaction of insulators with promoters results in gene looping in Drosophila melanogaster

M. Erokhin, A. Davydova, P. Georgiev and D. Chetverina
Department of the Control of Genetic Processes, Institute of Gene Biology RAS, Moscow, Russian Federation

Chromatin insulators are special regulatory elements involved in modulation of enhancer promoter interaction. Although much of the research was made to understand the main role of insulators in the regulation of gene expression in vivo, it’s still poorly known. Previously it was shown that Drosophila yellow and white genes contain insulators placed immediately downstream them, 1A2 and Wari, respectively. Here we report that both insulators are able to interact with their target promoters forming gene loops. Moreover, we show the interchangeability of interacting insulator-promoter pairs supporting that this could be a general property of insulators. We show that deletion of the Wari insulator downstream the white gene in transgenic constructs leads to significant decrease of the white gene expression what argues the important role of at least some insulators in gene transcription. Our results provide new insights in mechanism of insulator function and suggest their basic role in gene transcription. This study is supported by the Russian Foundation for Basic Research (project no. 09-04-00903-a).

B4.20
Genes expression in biofilm of Thermus spp

G.-H. Lin
Tzu Chi University, Microbiology, Hualien, Taiwan

At least 50% Thermophilic isolates from hot spring of Taiwan with biofilm forming ability. Biofilm is community of microorganism embedded in extracellular matrix which was secreted by the microorganism and it formed a physical and chemical barrier to keep clinical isolates from antibiotics and of macrophage attack. Following two-dimensional gel electrophoresis was applied to study the molecular mechanism of biofilm formation of Thermus spp. Results revealed that protein involved in general function prediction, transcription, and hypothetical proteins shared 37% of differential expressed proteins between the 8th hour planktonic and sessile cells. Meanwhile, hypothetical protein or protein with unknown function also played a role of 15% while comparing total protein from the 18th hour cells. We also found that 8% and 10% of protein involved in carbohydrate transport and metabolism were observed in the 8th and 18th hour samples, respectively. GalE protein is an UDP-galactose-4'-epimerase which involved in conversion UDP-galactose and UDP-glucose was found expressed differentially in biofilm cells. Chaperonin (GroESL) also expressed differentially between biofilm and planktonic cells. Overexpression of galE gene and groESL showed increasing biofilm formation ability indicating these genes were involved in biofilm forming. Meanwhile, we also found a two component regulator like protein expressed in sessile cells only while biofilm was formed. In order to study the complex regulatory networks of biofilm formation, we will clarify the function of those differentiate proteins and their contribution to biofilm formation.

B4.21
Rhythmic interaction between Period1 mRNA and HnRNP Q leads to circadian time-dependent translation

K.-H. Lee1, K.-C. Woo1, D.-Y. Kim1, S.-H. Kim2, H.-R. Lee2, T.-D. Kim3 and K.-T. Kim4
1Department of Life Science, POSTECH (Pohang University of Science and Technology), Division of Molecular and Life Science, Pohang, Korea, 2POSTECH (Pohang University of Science and Technology), School of Interdisciplinary Bioscience and Bioengineering, Pohang, Korea, 3Korea Research Institute of Bioscience and Biotechnology, Center for stem cell research, Daejeon, Korea, 4Department of Life Science, POSTECH (Pohang University of Science and Technology), Division of Molecular and Life Science / Division of Integrative Biosciences and Biotechnology, Pohang, Korea

The mouse PERIOD1 (mPER1) plays a crucial role in the circadian rhythm, and also provides an important link between the circadian and the cell cycle system. To date, the important issue of how mPER1 expression is rhythmic is explained by only limited information, transcriptional and posttranslational regulation. Here we show that the expression of mPER1 is regulated by rhythmic translation of mPER1 mRNA regardless of its mRNA profiles. This time-dependent translation is controlled by an internal ribosomal entry site (IRES) element in the 5'-untranslated region (5'UTR) of mPER1 mRNA along with the trans-acting factor, mouse heterogenous nuclear ribonucleoprotein Q (mHnRNP Q). Knockdown of mHnRNP Q caused a decrease and a delay in mPER1 expression. The rate of IRES-mediated translation exhibits phase-dependent characteristics through rhythmic interaction between mPER1 mRNA and mHnRNP Q. A mathematical model for describing the mPER1 expression also suggests that mHnRNP Q-mediated rhythmic translation is a key process in its circadian oscillation. Here, we demonstrate 5'UTR-mediated rhythmic mPER1 translation, and provide evidence for posttranscriptional regulation of the circadian rhythmicity of core clock genes.

B4.22
Nicotinamide N-Methyltransferase: a promising biomarker for early diagnosis and prognostic monitoring of oral squamous cell carcinoma

D. Sarini1, V. Pozzi1, A. Santarelli2, C. Rubini2, M. Tomasetti2, E. Renzi2, R. Rocchetti2, L. Lo Muzio3 and M. Emanueli1
1Università Politecnica delle Marche, Dipartimento di Biochimica, Biologia e Genetica, Ancona, Italy, 2Università Politecnica delle Marche, Dipartimento di Scienze Cliniche, Specialistiche e Odontostomatologiche, Ancona, Italy, 3Università Politecnica delle Marche, Dipartimento di Biochimica, Biologia e Genetica, Ancona, Italy
Oral squamous cell carcinoma (OSCC) is the most common malignancy of oral cavity. Up to now, reliable biomarkers for OSCC are still lacking. Therefore, it is necessary to identify target molecules for effective therapy and early diagnosis of OSCC. We analysed the expression of Nicotinamide N-Methyltransferase (NNMT), which catalyses the N-methylation of nicotinamide, in paired tumour and non-tumour tissues by RT-PCR, Real-Time PCR, and western blot. Compared with normal mucosa, OSCC exhibited significantly increased expression of NNMT in 50% of examined patients. NNMT was upregulated in most of the favourable OSCCs (N0). pT, pathological staging and lymph node metastasis showed an inverse correlation with NNMT mRNA levels. Immunohistochemical analyses seem to indicate a favourable prognosis in patients with tumours expressing high NNMT levels, whereas the absence of marked NNMT expression seems to constitute a hallmark of aggressive biological behaviour. We also evaluated the effect of shRNA-mediated inhibition of NNMT on the proliferation and apoptosis of oral cancer cell line PE/CA-PJ15. ShRNA vectors sufficiently suppressed NNMT expression. The shRNA-mediated gene silencing of NNMT resulted in a significant rise in apoptosis rate. Preliminary studies on salivary NNMT by western blot showed strong immunoreactive bands in samples of OSCC patients and negative or weakly positive bands in normal saliva samples. The present data support the hypothesis that NNMT plays a role in tumour expansion and represents a highly promising marker for early detection of oral cancer. Moreover, knowing the status of NNMT expression would be important in predicting the patient prognosis.

B4.23 Up-regulation of p21WAF1/CIP1 expression stimulates G1-phase arrest of the cell cycle in cladosporol A-treated HT-29 cells

D. Zurlo1, C. Leone1, G. Assante2, S. Salzano3, G. Renzone4, A. Scalonì1, C. Foresta1, V. Colantuoni1 and A. Lupo1
1Università del Sannio, Dipartimento di Scienze Biologiche ed Ambientali, Benevento, Italy, 2Università di Milano, Dipartimento di Patologia Vegetale, Milano, Italy, 3Istituto di Endocrinologia e Oncologia Sperimentale “Gaetano Salvatore” (IEOS)-CNR, Napoli, Italy, 4Laboratorio di Proteomica e Spettrometria di Oncologia Sperimentale “Gaetano Salvatore” (IEOS)-CNR, Napoli, Italy

Chemoprevention is a clinical practice that is gaining favour in the treatment of degenerative diseases including cancer. The list of compounds able to interfere with the various steps of the tumorigenic process is growing and includes new molecules isolated from plants, fungi and microorganisms. Cladosporols, a series of compounds purified as secondary metabolites from Cladosporium tenuissimum, display antifungal activity. In this study we sought to investigate the antiproliferative properties of cladosporol A, the major isoform of this family of compounds. By assessing cell viability we show that cladosporol A inhibits the growth of several human colorectal cancer-derived cell lines in a time- and dose-dependent manner. Administered to HT-29 cells, cladosporol A causes a G1-phase arrest of the cell cycle, accompanied by an early and robust induction of p21WAF1/CIP1. By RT-qPCR assays and transient transfections of a luciferase reporter gene, under the control of the p21WAF1/CIP1 promoter region, we demonstrate that the induction takes place at transcriptional level. Molecular dissection of this region, by deletion analysis, revealed the shortest fragment able to drive the full transcription. A series of Sp1 binding sites located at nucleotides were identified as crucial for the induction. Their site directed mutagenesis abolishes the induction. This is the first report of an antiproliferative effect induced by cladosporol A, mediated by transcription induction of p21WAF1/CIP1 in a p53-independent manner.

B4.24 Dynamics of histone modifications in actively transcribed locus

S. Varv, H. Sein, K. Kristjuhan and A. Kristjuhan
Department of Cell Biology, University of Tartu, Tartu, Estonia

Considering the importance of histone modifications in transcription control, the aim of our study is to determine the area and impact of transcription coupled histone modifications in the proximity of an actively transcribed locus. For that purpose we have constructed a yeast (S. cerevisiae) strain carrying a strong transcription termination sequence in the middle of an inducible model gene (VPS13) to detect different nucleosome modifications found on chromatin before and after RNA polymerase II transcription through the region. As shown previously, in actively transcribed loci, nucleosomes are constantly being exchanged giving rise to the question of the origin and maintenance of histone marks in that area. We show the spread of different acetylation and methylation marks on histones H3 and H4 on transcribed and untranscribed gene regions hence also indicating the origin of histone modifications and their relationship to RNA polymerase II dependent transcription. We also discuss the activity of histone deacetylase Rpd3 during transcription and its role in chromatin mark dynamics.

B4.25 Protein kinase Cα downregulates vaccinia related kinase 1 activity in DNA damage induced apoptosis

Pohang university of science and technology, Pohang, Korea

Vaccinia-related kinase 1 (VRK1) is a novel serine/threonine kinase, which plays an important role in cell proliferation. However, little is known about the upstream regulator of VRK1 activity. Here we provide evidence for a role of protein kinase C (PKC) in the regulation of murine VRK1. We show that PKC interacts with VRK1, phosphorylates its Ser355 residue in the putative regulatory region and negatively regulates its kinase activity in vitro. Intriguingly, PKCα-mediated induced cell death is facilitated by phosphorylation of VRK1 when cells were exposed to DNA damage agent. In addition, p53 plays a critical role in the regulation of DNA damage-induced cell death accompanied with PKCα-mediated modulation of VRK1. In p53-deficient cells, the effect of PKCα-mediated phosphorylation of VRK1 on cell viability did not appear. However, the cells overexpressing p53 exhibited significant reduction of cell viability when the cells were cotransfected with both VRK1 and PKCα. Taken together, these results indicate that PKC regulates phosphorylation and down-regulation of VRK1, thereby contributing to the cell cycle arrest and apoptotic cell death in p53-dependent manner.
B4.26
Decitabine in combination with SAHA causes apoptosis in cancer cells as well as in normal PBMC
B. Brodská, P. Otevrelová and A. Holoubek
Institute of Hematology and Blood Transfusion, Prague, Czech Republic

Decitabine (DAC) causing DNA hypomethylation and SAHA (vorinostat) inhibiting histone deacetylases represent epigenetic forces regulating gene and protein expression in cells, affecting cell cycle and cell death. Epigenetic agents are often used for developing novel methods of cancer therapy because of the reversibility and relatively slight mode of their action. Both DAC and SAHA has been approved by FDA for cancer therapy and combination of these two drugs has a great potential to make a considerable epigenetic changes. In our experiments, DAC alone causes substantial increase of p21 expression, higher levels of proteins p53 and Puma were also detected, but the viability of the cells, as measured by MTT test, decreased only minimally, and we did not detect any remarkable apoptotic effect. While in CML-T1 cells we observed slight decrease in McI-1 expression and PARP fragmentation, none of these effects have been found in lymphocytes. Addition of SAHA simultaneously with DAC suppressed DAC-induced p21 upregulation, p53 induction partially persisted only in lymphocytes. Substantial downregulation of anti-apoptotic McI-1 mRNA and protein expression and apoptosis-related features like mitochondrial membrane (MM) depolarization and PARP fragmentation were also detected. The extent of MM depolarization and viability decrease observed in CML-T1 cells after DAC+SAHA treatment implies that this combination has synergistic effect. Higher concentration of SAHA added with DAC was required for lymphocytes to lead them to apoptosis, designating this combination to be a promising therapeutic tool.

This work was supported by grant IGA NS 9637-4 from the MHCRA.

B4.27
Tumor hypoxia induced HIF-α regulated genes expression and its potential functionality as tumor marker genes in human brain cancer
H. M. Said1, C. Hagemann2, T. Supuran3, A. Scozzafava3, J. Anacker1, B. Polat1, A. Staab4, M. Flentje5 and D. Vordermark6
1University of Würzburg, Radiation Oncology, Würzburg, Germany, 2Department of Neurosurgery, University of Würzburg, 3Tumor Research Laboratory, Würzburg, Germany, 4University of Würzburg, Medical Faculty, Radiation Oncology, Würzburg, Germany, 5University of Würzburg, Medical Faculty, Tumor Research Laboratory, Department of Neurosurgery, Würzburg, Germany, 6Martin-Luther-University-Halle-Wittenberg, Radiation Oncology, Halle, Germany

CCAAT-enhancer-binding proteins (C/EBPs) are a family of transcription factors that promote the expression of certain genes through interaction with their promoter and comprise a family of basic region leucine zipper (bZIP) transcription factors that bind DNA through their basic regions, dimerize by their adjacent C-terminal leucine zippers to form homodimers or heterodimers with other C/EBP family members. Hypoxia induced activation of this regulatory protein in brain cancer had not been examined yet extensively.

Materials and Methods: C/EBP-α regulation level was examined in human glioblastoma cells lines like U373, U251, GaMG and U87-MG under extreme hypoxic oxygenation conditions (0.1% oxygen), reoxygenation after hypoxia for 24 and 48 hour and oxygenated conditions (21% oxygen and 5% carbon dioxide in vitro). Protein and mRNA level were detected via western blots and RT-PCR. Cells incubated for 24 hour with 100 μM DFO served as positive control for hypoxia and β-tubulin and β-actin served as loading control.

Results: C/EBP-α was up-regulated via hypoxic development in different glioblastoma cells (in vitro) under extreme hypoxic conditions (0.1% oxygen) or reoxygenation after hypoxia, both on protein and mRNA level. Further, there was an association between expression and the expression of hypoxia induced, HIF-1α regulated genes in human glioblastoma tumour specimens examined, in vitro.

Conclusions: C/EBP-α regulation as an answer to hypoxic development in glioblastoma, both on protein and mRNA level is a tumor cell specific hypoxic conditions influenced regulation in glioblastoma. HIF-1α is the main regulator under this conditions in human tumors.
Acute lymphoblastic Leukemia (ALL) is a severe blood disorder in which abnormal leukocytes are identified as immature forms of lymphocytes and is considered responsible for 25–30% of pediatric malignancies. Molecular approaches are used to detect various cancers types’ genomic alterations; one of these approaches is random amplified polymorphic DNA analysis (RAPD).

Methods: Whole blood genomic DNA extraction from ALL patients was performed with the later use of twenty three different arbitrary primers for RAPD-PCR analysis. Loss, gain, shift, and detectable band's intensity changes were scored and subjected to statistical analysis. Cluster analysis of RAPD-PCR methods were used for grouping bands into respective categories.

Results: 10 M 23 RAPD primers used in the study were able to amplify genomic sequences of ALL leukemia patients. The ten amplifying primers varied in their ability to detect genomic DNA instability of ALL patients. The obtained results revealed that one of amplifying primers showed novel DNA bands pattern in ALL patients, band number one (3162 bp) was absent in 78% of ALL patients, while this band was present in normal individuals. On the other hand, band number four (1 Kb) was absent in normal individuals but it was present in 55% of ALL patients. Moreover, dissimilarity matrix analysis of obtained RAPD-PCR bands of genomic DNA from whole blood of ALL patients provided essential information on genetic polymorphism among genomic DNA of ALL patients.

Conclusions: Genetic instabilities are frequent in ALL genomic DNA and could be detected quickly and accurately by using suitable RAPD primer.

B4.30
Potassium mediates Escherichia coli enzyme IIANtr/Vdependent regulation of sigma factor selectivity
K. Miri1, L. Chang-Ro1, C. Seung-Hyon1, K. Hyun-Jin1, A. Peterkofsky2 and S. Yeong-Jae1
1Seoul National University, Department of Biological Sciences and Institute of Microbiology, Seoul, Korea, 2National Institutes of Health, Laboratory of Cell Biology, National Heart, Lung, and Blood Institute, MD, USA

An Escherichia coli mutant devoid of enzyme IIANtr (EIAntr) of the nitrogen PTS is extremely sensitive to leucine-containing peptides due to decreased expression of acetylhydroxy acid synthase. This decreased expression is due to defective potassium homeostasis. We report here the mechanism for regulation of gene expression by the intracellular K+ level. The leucine hypersensitivity of a ptsN (encoding EIAntr) mutant was suppressed by deleting the rpoS gene, encoding the stationary phase σ factor. Despite intracellular levels of sigma factors comparable to the wild-type strain, most of the genes down-regulated in a ptsN mutant are controlled by σ70, while all the up-regulated genes are controlled by σS, implying that the balance of sigma activities is modified by ptsN deletion. This change of sigma factor activity was found to be due to increased levels of K+. In vitro transcription assays showed that a σ70 controlled gene and a σS controlled gene were differentially affected by potassium concentration. Biochemical studies revealed that K+ is responsible for sigma factor competition by differentially influencing the binding of σ70 and σS to core RNA polymerase. Taken together, the data indicate that EIAntr controls sigma factor selectivity by regulating the intracellular K+ level.
that P-TEFb may contribute to the stimulation of IL-8 gene expression by interacting with dengue viral core protein. The results of Immunostaining and co-immunoprecipitation assays demonstrated the association between P-TEFb and DENV core protein. The chromatin immunoprecipitation data provided evidence that the associated complex of P-TEFb and DENV core protein is recruited directly to the IL-8 gene promoter region, resulting in the activation of IL-8 gene expression. To our knowledge, these data provide the first evidence that P-TEFb and DENV core protein participate in the induction of IL-8 gene expression by DENV infection.

B4.33
Using siRNA and miRNA mimetics to obtain RNA-interference in cancer cells
C. Polonyi and S. K. C. Elmoth
Biochemistry and Structural Biology, Biochemistry, Lund, Sweden

It is a challenge to try to understand how gene expression is regulated in various eukaryotic organisms, including the human body. The cells in eukaryotic organisms contain small double-stranded hairpin-like RNA structures coded for by microRNA (miRNA) genes in the cell nucleus (Carthew and Sontheimer, 2009). These miRNA structures specifically base pair with coding mRNA after being processed, and hence hinder further expression of the targeted genes. This kind of gene silencing can also arise if a small double-stranded exogenous RNA, so called small interfering RNA (siRNA), is introduced into a eukaryotic cell. It would therefore be of interest to design and construct siRNA and microRNA mimetics and to specifically target their silencing effect on genes associated with the development of cancer. In the present project, we initially intend to explore the role of different microRNA candidates for the survival of various types of cancer cells. miR-146a is for example an interesting candidate for investigation, since this microRNA seems to influence the expression of the genes BRCa1 and BRCa2. These genes code for proteins that are associated with the development of not only female breast cancers, but for example also male breast cancers (Shen et al., 2008).

References:

B4.34
Role of cytokines in repair of DNA damages induced by alkylating agents
K. Kotsarenko, V. Lylo, L. Maciewicz and L. Lukash
Institute of Molecular Biology and Genetics of National Academy of Sciences of Ukraine, Kyiv, Ukraine

Repair of primary DNA damage induced by alkylating agents is realized by repair enzyme O6-alkylguanine-DNA alkyltransferase (or MGMT). MGMT repairs the most dangerous damages – formation of O6-alkylguanine in DNA. Mechanisms regulating expression of this enzyme are complicated and not studied enough nowadays. But the expression of the MGMT has been already known to be influenced by DNA hypermethylation and DNA breaks and also can be regulated through the mediated way, for example by protein p53, phosphorylation, ubiquitination. Moreover there are literature data that MGMT expression can be regulated by cytokines and growth factors IF-β, IL-3, GM-CSF. The purpose of our work was to research the possible influence of cytokines LIF, SCF and IL-3 on the MGMT expression in the human cell cultures. There were used the cells of different origin: original human cell lines BKF, 4BL2, standard tumor cell line Hep-2. Identification of MGMT protein in the cellular extract was carried out by the Western blot analysis. In our early works it has been shown that the MGMT protein may exist in cell cultures in two forms: the unmodified one with the molecular weight 22–24 kDa, and the modified of 48–50 kDa.

All the cytokines studied have been shown to inhibit MGMT expression in different way. The cytokin LIF almost did not affect on the amount of the modified protein, but caused the disappearance of the unmodified form. After the treatment of cells with IL-3 and SCF the protein in the unmodified form disappeared completely, and the amount of the modified protein considerably decreased.

B4.35
Next generation sequencing to reveal new sight into cancer research
X. Yang
BGI Shenzhen, Shenzhen, China

The human genome lies at the core of human disease research. Using next generation sequencing technology such as Illumina/ Solexa, we could get an all-round view of complex diseases including cancer at DNA, RNA and epigenetic level. With genome resequencing, somatic mutations from cancer genome can be obtained, including all point mutations, rearrangements and copy number changes. Exome capture sequencing is a relatively economical strategy. Cancer-specific genetic mutations will be identified by directly comparing exon sequences from a patient’s tumor to its non-disease exons. In addition, SNP genotypes will be calculated for each patient (both non disease and tumor tissue) as well as structural variations. Using transcriptome sequencing, digital gene expression profiling and small RNA sequencing technologies, cancer-specific alterations in expression levels, aberrant splicing and fusion transcripts could easily be defined. Whole-genome bisulfite sequencing, MeDIP-sequencing and ChIP-sequencing are used to discover aberrant DNA methylation and histone modifications. Epigenetic changes are now established in development and progression of cancer and other complex diseases. We are applying the strategies above to several solid tumors research. We are intended to identify the full range of somatically acquired genetic alteration, including point mutations on a genome-wide basis, insertions and deletions, copy number changes and genomic rearrangements, as well as characterizing the cancer cell transcriptome and epigenome. All these discoveries will promises a new era for cancer genomics.

B4.36
Peptidyl-prolyl isomerase cyclophilin B suppresses gastric carcinoma against hypoxia-induced apoptosis through attenuation of endoplasmic reticulum stress
W. Choo
Kyung Hee University, Department of Biochemistry and Molecular Biology, Seoul, Korea

Cyclophilins are protein chaperones that accelerate the rate of protein folding through their peptidyl-prolyl cis-trans isomerase (PPlase) activity. Cyclophilin B (CypB) is a 21-kDa protein with peptidyl-prolyl cis-trans isomerase activity. CypB is detected mainly in the ER lumen. Although there has been some speculation that CypB plays a role in protein folding in the endoplasmic
of murine melanoma B16F10 cells. Melanoma cells were stably analyzed the effect of T-cadherin overexpression on proliferation. cDNA exhibit decreased proliferative and invasive potential. We T-cadherin has a lower expression profile in many types of cancer.

B4.37 Overexpression of modified cyclophilin a regulated apoptosis through endoplasmic reticulum stress

W. Choe
Kyung Hee University, Department of Biochemistry and Molecular Biology, Seoul, Korea

The protein is guided to the ER by a signal sequence. signal sequence of preprolactin, after translocation into microsomes and cleavage by signal peptidase, is converted to an intermediate form. The KDEL sequence(lys-asp-glu-leu) is a signal for permanent retention of proteins in the endoplasmic reticulum(ER). Cytoplasmic A(CypA) is a relatively abundant small immunophilin exist in the cytoplasm of all mammalian cells. These immunophilins link to cytoplasmic dynine indirectly through the association of the immunophilin peptideprolyl isomerase (PPIase) domain with dynamitin, a component of the dynine-associated dynactin complex. Our study showed that the localization of cyclophilin A(CypA) is regulated by prolactin signal sequence and the KDEL sequence. The ER stress can be induced not only by alterations in physiological conditions such as proteins undergo post-translational modifications in the ER, which requires high levels of luminal Ca²⁺ and oxidative components. Exposure of cells to glucose starvation, inhibition of protein glycosylation, disturbance of Ca²⁺ homeostasis, or oxygen deprivation devastates ER environments and causes unfolded proteins to accumulate in the ER (ER stress). We identified ER existence of a modified cyclophilin A expressed in the Endoplasmic reticulum directed by the prolactin signal sequence and the KDEL sequence that resisted ER-stress-indexed cell death in charg cells.

B4.38 T-cadherin overexpression in culture of murine melanoma cells suppresses proliferation

E. Yurlova, K. Rubina, V. Sysoeva, E. Semina, Y. Parfyonova and V. Tkachuk
1 Department of Biological and Medical Chemistry, M.V. Lomonosov Moscow State University, Faculty of Basic Medicine, Moscow, Russian Federation, 2Cardiology Research Center of Russia, Institute of Experimental Cardiology, Laboratory of Angiogenesis, Moscow, Russian Federation

T-cadherin has a lower expression profile in many types of cancer and tumour cells. Tumour cells transfected with T-cadherin cDNA exhibit decreased proliferative and invasive potential. We analyzed the effect of T-cadherin overexpression on proliferation of murine melanoma B16F10 cells. Melanoma cells were stably transfected with the plasmid encoding human T-cadherin or control plasmid. For proliferation assay, cell numbers were determined daily for up to 6 days by counting in a cell counter. Distribution of melanoma cells at different stages of the cell cycle was determined with propidium iodide staining by flow cytometry. Expression of TGF-beta was measured by quantitative real-time RT-PCR. T-cadherin overexpression reduced proliferation rate of melanoma cells and decreased expression of TGF-beta compared to control. Flow cytometry confirmed the delay in the cell cycle progression in T-cadherin overexpressing melanoma cells since the percentage of those cells in G2/M phase was significantly lower than of control cells. In normal cells TGF-beta is known to inhibit proliferation, but in cancer various components of the TGF-beta signaling pathway could be mutated, making the cell resistant to the effects of TGF-beta. Although the mechanisms of T-cadherin-dependent suppression of melanoma cell growth have yet to be elucidated, our data indicate that there is a correlation between elevated T-cadherin and reduced TGF-beta expression. The downregulation of TGF-beta mRNA upon overexpression of T-cadherin suggests the possible implication of TGF-beta signaling in T-cadherin-mediated suppression of melanoma cell proliferation.

B4.39 Opposite effect of HIF-1 and HIF-2 on regulation of IL-8 expression in endothelial cells

U. Florezk1, A. Stachurska1, S. Czauderna1, K. Stalinska2, L. Poellinger3, A.-L. Levonen4, S. Yla-Herttuala1, A. Jozkowicz1, A. Loboda1 and J. Dulak1
1 Jagiellonian University, Faculty of Biochemistry, Biophysics and Biotechnology, Department of Medical Biotechnology, Krakow, Poland, 2Jagiellonian University, Faculty of Biochemistry, Biophysics and Biotechnology, Department of Cell Biochemistry, Krakow, Poland, 3Karolinska Institute, Department of Cell and Molecular Biology, Stockholm, Sweden, 4University of Kuopio, Department of Biotechnology and Molecular Medicine, A.I. Virtanen Institute, Kuopio, Finland

Hypoxic transcriptional response is primarily mediated by hypoxia-inducible factor (HIF)-1α and HIF-2, consisting of α and β subunits. The HIF-1α and HIF-2α are similar in their DNA binding and dimerization domains, but differ in transactivation domains, implying they may regulate distinct genes and require different transcriptional cofactors. It is well known that hypoxia is a potent inducer of angiogenesis by enhancing the expression of VEGF. Interestingly, recently we showed that interleukin-8 (IL-8), the other mediator of angiogenesis, tumor growth and metastasis, is down-regulated by HIF-1α. As HIFs may differ in their functions, we aimed to examine the effect of HIF-2α on IL-8 expression. Human microvascular endothelial cells (HMEC-1) were transduced with adenoviral vectors to express HIF-1α or HIF-2α. Our results indicate that in contrast to HIF-1α, overexpression of HIF-2α resulted in significantly increased expression of IL-8, as revealed by analysis of the IL-8 promoter activity as well as mRNA and protein level. Accordingly, HIF-2α was found to positively affect the activity of SP-1, transcription factor involved in IL-8 regulation, as shown by reporter gene analysis and EMSA assay. Moreover, exemplary gene, thymidine phosphorylase (TP), containing binding sites for SP-1, was shown to be upregulated by HIF-2α as well. Importantly, effect of HIF-2α on IL-8 as well as TP expression was reversed by SP-1 inhibitor, mithramycin A. Current study revealed for the first time opposite role of HIF-1α and HIF-2α in regulation of IL-8 expression in endothelial cells and the involvement of SP-1 in the positive regulation of IL-8 by HIF-2α.
B4.40
Heme oxygenase-1 overexpression leads to melanoma de-differentiation
H. Was1, A. Tejchman1, M. Dubiel1, W. Bobela1, P. Dominik1, J. Kotliński2, A. Zebzda2, J. Dulak2 and A. Jozkowicz2
1Department of Medical Biotechnology, Jagiellonian University, Faculty of Biochemistry, Biophysics and Biotechnology, Krakow, Poland, 2Jagiellonian University, Collegium Medicum, Transplantology Centre, Krakow, Poland

Heme oxygenase-1 (HO-1) is an inducible enzyme that degrades heme to biliverdin, carbon monoxide and iron. In our previous experiments we have demonstrated that HO-1 exerted pleiotropic pro-tumoral activities in B16(F10) melanoma cells both in vitro and in vivo. Therefore, the aim of the present study was to investigate mechanisms responsible for observed effects. Melanoma cell line B16(F10) were transduced with retroviral vectors encoding for luciferase, GFP and HO-1 [B16(F10)-luc-GFP-HO-1]. B16(F10)-luc-GFP cells were used as controls. B16(F10)-luc-GFP-HO-1 displayed a slight upregulation of antioxidative genes: biliverdin reductase, catalase and glutathione S-transferase. On the other hand, they expressed lower numbers of transcripts for p21 and Mdm2. Of importance, B16(F10)-luc-GFP-HO-1 cells exhibited less dendritic morphology and weaker pigmentation than their control counterparts. In parallel, they were characterized by significantly lower expression of melanoma markers: tyrosinase, TRP-1, TRP-2, gp100 and MART-1. Interestingly, de-differentiative activities of HO-1 were notably enhanced by hypoxic conditions. Finally, basal production of reactive oxygen species (ROS) was lower in B16(F10)-luc-GFP-HO-1 cells than in B16(F10)-luc-GFP cells and prolonged N-acetylcysteine (NAC) treatment reversed highly pigmented phenotype of B16(F10)-luc-GFP cells, suggesting role of HO-1 as a ROS scavenger in differentiation of melanoma cells. Altogether, these data suggest that HO-1 exerts strong de-differentiative activity probably through antioxidative mechanisms and in this way may induce more aggressive melanoma phenotype.

B4.41
The interaction of Snf1 with Tor1 in Saccharomyces cerevisiae
J. Zhang1, G. Vemuri1, J. Nielsen1, S. Vage2 and R. Aebbersold2
1Chalmers University of Technology, Gothenburg, Sweden, 2Institute of Molecular Systems Biology, ETU Zurich, Zurich, Switzerland

The yeast Saccharomyces cerevisiae attains energy homeostasis through complex regulatory events that are predominantly controlled by the Snf1 protein kinase. Snf1 senses the stress and energy starvation and activates the metabolic processes to produce ATP and inhibits anabolism, e.g., the biosynthesis of protein and lipid. Since its mammalian ortholog AMPK, a drug target for obesity and type II diabetes, also exerts analogous control of metabolism, there has been extensive interest to understand Snf1 activation and regulation in yeast to expedite human disease studies as well as fundamental understanding. Snf1 regulates the cellular growth and development in coordination with other signaling pathways such as the Tor1 pathway. When abundant and high quality nitrogen sources are present, Tor1 is active and initiates transcription of genes that are involved in ribosome biogenesis and protein translation. Although both Snf1 and Tor1 are key components in nutrition sensing and regulation of the cellular growth, little is known about how these two kinases coordinate in the regulation of cell growth. Here we apply a systems biology approach to investigate the interaction between Snf1 and Tor1. The yeast reference strain CEN.PK 113-7D and its derivative strains snf1Δ, tor1Δ and snf1Δtor1Δ were grown in chemostat in both carbon (glucose) and nitrogen (ammonia) -limited media. Samples were taken at the steady state for the analysis of transcriptome, phospho-proteome and metabolites such as free amino acids and lipids as well as glycogen/ trehalose. Integrative analysis was performed to combine the datasets at different levels and the preliminary results will be presented.

B4.42
MGST1 downregulation affects genes expression in PC12 cells
M. Sobczak1, T. Bozcek1, B. Ferenc1, J. Taha1, M. Wiktorska2, J. Niewiarowska2 and L. Zylinska2
1Department of Molecular Neurochemistry, Medical University, Lodz, Poland, 2Department of Molecular and Medical Biophysics, Medical University, Lodz, Poland

The microsomal glutathione transferase 1 (MGST1) belongs to the super family of Membrane Associated Proteins in Eicosanoid and Glutathione Metabolism (MAPEG). The enzyme is present in high amounts in the endoplasmic reticulum, mitochondrial and plasma membranes and possesses transferase and peroxidase activity. It may also play an important anti-apoptotic role. To test the MGST1 role in cell development we examined the effect of MGST1 suppression in PC12 cells. After transfection with eukaryotic vector containing specific antisense sequence, the expression of MGST1 mRNA decreased by 60% as was quantified by real-time PCR. The flow cytometry analyses revealed a higher percentage of necrotic cells. The total activity of glutathione transferases determined with non-specific substrate CDNB decreased by 15-20%, whereas in the presence of DCNB, a substrate more specific for cytosolic GSTs, the activity was similar to control cells. It may indicate that the decline in MGST1 cannot be compensated by a respective increase in cytosolic GSTs activity. Using microarray that profiles the expression of 263 genes we detected the altered expression of several groups of genes involved in the processes such as cell cycle and cell proliferation (S100A6, Rasgrf2, Cdk5), differentiation (Efnal1, Nocoaf, Pax2), cell adhesion (Dscam, L1cam, Ninj1, Ret, Sgg7) and apoptosis (Mch1, Ngrf, Rtn4, Ywhah). MGST1 appears to be essential for a proper PC12 cell development and its reduction enhanced necrosis; however, some adaptive processes caused by changes in genes expression could promote cell survival. Supported by the grants 502-16-809, 502-16-810 and 503-6086-2 from the Medical University of Lodz.

B4.43
Experimental rat model of heart failure induced by chronic hypoxia: gene expression profiles of HIF-1α and BNP-45
F. Ferdinal1, S. Hendrawan1 and M. Sadikin2
1Tarumanagara University School of Med, Biochemistry and Molecular Biology, Jakarta, Indonesia, 2University of Indonesia School of Med, Biochemistry and Molecular Biology, Jakarta, Indonesia

Despite significant therapeutic advances, incidence and mortality in heart failure (HF) remain unacceptably high. Therefore, novel insights into molecular mechanism of HF are required to develop novel therapeutic approaches. The progress made in our understanding of the pathophysiology of heart failure would not have been possible without a number of animal models, each one having unique advantages as well as disadvantages. The aim of study is to develop a rat model of HF induced by chronic hypoxia and...
explored the gene expression profiles of HIF-1α and BNP-45 as master regulatory gene of oxygen homeostasis and biomarker of HF, respectively. Rats, male, were randomized into seven groups (n = 4 per group), control normoxia exposed to room air, hypoxia group were housed in hypoxic chamber (028%) for 1, 3, 7, 14, 21 and 28 days respectively.

**Result:** Histopathologic examination shows: Massive hypertrophy of cardiomyocytes accompanied by alterations of intercalated disk, necrosis, fibrosis and apoptosis as a hallmark ventricular remodeling. A drastic increase in plasma BNP-45 levels at 21-day exposure. The BNP-45 mRNA and HIF-1α mRNA expression increased and the activities of HIF-1α were significantly increased compared with control group.

**Conclusions:** Chronic hypoxia causes; Ventricular hypertrophy accompanied by myocardial structural damage and then progress to heart failure. There were changes in the gene expression either in transcription and post-transcription level.

**B4.44**

**An early zygotic genome activator in tissue patterning formation, a multipotent Drosophila nuclear protein**

P. Giannios1, C. Delidakis1 and S. Tsitílo1

1University of Athens, Biochemistry and Molecular Biology, Athens, Greece, 2Foundation for Research and Technology, Institute of Molecular Biology and Biotechnology, Heraklion, Greece

The zinc finger protein Zelda, a key activator of the early zygotic genome in Drosophila, is shown to be necessary for patterning the wing structure during late developmental stages of the fly. This protein has been shown to play crucial roles during embryogenesis. Our data suggest that Zelda affects major components of signalling pathways such as Hedgehog and Notch. Spatiotemporal overexpression and knockout of zelda’s gene product in the larval stages of the fly, impair wing development resulting in abnormal adult tissue formation. In these experiments several changes in the production patterns of proteins such as Patched and Wingless are observed, indicating a direct involvement of Zelda in the signalling cascade involving these proteins. A prominent role of Zelda in cell cycle progression has been suggested by previous experiments. However, cell cycle promotion could be linked to or divorced from patterning decisions. Zelda is known to be involved in both processes and it remains to be elucidated the exact mechanism of this involvement.

**B4.45**

**Nuclear myc promoter-binding protein-1 (MBP-1) expression is a prognostic factor in invasive ductal breast carcinoma**

C. Mazzarella1, A. Ferro2, M. Lo Presti3, F. Contino2, S. Sbacchi2, E. Roz2, C. Lupo3, G. Perconti4, A. Giallongo4, A. Marrazzo2 and S. Feo3

1University of Palermo, Dipartimento di Oncologia Sperimentale e Applicazioni Cliniche, Palermo, Italy, 2University of Palermo, Department of Experimental Oncology and Clinical Applications, Palermo, Italy, 3La Maddalena Hospital, Anatomical Pathology Department, Palermo, Italy, 4CNR, Institute of Biomedicine and Molecular Immunology, Palermo, Italy

Myc Promoter-Binding Protein-1 (MBP-1) is a transcriptional repressor, generated by alternative translation of the α-enolase mRNA, that negatively regulates c-Myc gene expression (1) and plays a suppressive role on tumorogenesis (2, 3). We analyzed α-enolase and MBP-1 expression and localization in normal breast epithelium and primary infiltrating ductal breast carcinoma (IDC) from 177 patients by Western blotting and immunohistochemical analyses, using specific anti-α-enolase mAbs. A significantly increase in α-enolase expression was observed in 98% of the analysed tumors, compared to normal tissues. Nuclear MBP-1 has been found in almost all normal tissues while its expression is retained in only 35% of the matched tumours. Statistically significant inverse correlation was observed between expression and nuclear localization of MBP-1 and ErbB2 and Ki-67 expression, node positivity and tumor grade. Furthermore, nuclear MBP-1 is associated with good disease-free survival of patients with primary IDC. Transfection experiments in human breast SKBr3 cells (ErbB2+) demonstrated that MBP-1 ectopic expression results in down regulation of ErbB2 expression, and led us to identify the ErbB2 promoter region involved in the binding, indicating that, like c-Myc gene, ErbB2 is a direct target of MBP-1 transcriptional repression fuctions in breast cancer. Owing to the prognostic influence of nuclear-MBP-1 expression in a subgroup of small tumors and among patients with node-negative and ErbB2- cancers, where the need for prognostication is the greatest, MBP-1 nuclear expression may prove to be a clinically valuable prognostic variable for breast cancer patients.

**B4.46**

**GAPDH regulation in human tumor cells under hypoxic oxygenation conditions from different origin and their suitability as experimental housekeeping genes**

H. M. Said1, B. Polat1, C. Hagemann2, B. Schoenig2, A. Staab3, M. Flentje4 and D. Vordermark4

1University of Würzburg, Medical Faculty, Radiation Oncology, Würzburg, Germany, 2Department of Neurosurgery, University of Würzburg, Medical Faculty, Tumor Research Laboratory, Würzburg, Germany, 3Paul Scherer Institute, Proton Research, Villigen, Switzerland, 4Martin-Luther-University-Halle-Wittenberg, Radiation Oncology, Halle, Germany

Gene expression studies related to cancer diagnosis and treatment are important. In order to conduct such experiment accurately, absolutely reliable housekeeping genes are essential to normalize cancer related gene expression. However, no single gene of this group of genes manifests always stable expression levels under all experimental conditions. Incorrect choice of housekeeping genes leads to interpretation errors of experimental results including evaluation and quantification of pathological gene expression. Here, we examined (a) the degree of GAPDH expression regulation in Hep-1-6 mouse hepatoma and Hep-3-B and HepG2 human hepatocellular carcinoma cell lines as well as in human lung adenocarcinoma epithelial cell line (A-549) in addition to both HT-29, and HCT-116 colon cancer cell lines, and 4 glioblastoma cell lines under hypoxic conditions *in vitro* in comparison to other housekeeping genes like β-actin, serving as experimental loading controls.

**Results:** No hypoxia-induced regulatory effect on GAPDH expression was observed in cell lines studied *in vitro* that were; Hep-1-6 mouse hepatoma and Hep-3-B and HepG2 human hepatocellular carcinoma cell lines, Human lung adenocarcinoma epithelial cell line (A-549), both colon cancer cell lines HT-29, and HCT-116, glioblastoma U373, U31, U87-MG and GaMG. 

**Conclusions:** As it is the case for human hepatocellular carcinoma, mouse hepatoma, human colon cancer, and human lung adenoacarcinoma, brain cancer GAPDH represents an optimal choice of a housekeeping gene and (or) loading control to determine the expression of hypoxia induced genes in tumors of different origin.
Abstracts

B4.47
Glia/ischemia dual specific gene expression system for glioblastoma gene therapy
Hanyang University, Department of Bioengineering, Seoul, Korea

Glioblastoma multiforme (GBM) is the most aggressive malignant primary brain tumor in human. However, glioblastoma has a very poor prognosis despite advances of multimodality treatment such as surgical resection and radio-chemotherapy. In this study, we developed the glia and ischemia dual specific gene expression system for glioblastoma gene therapy. pN2-SV-Luc was constructed with the nestin intron 2 (N12)-SV40 promoter for glia cell specific gene expression. To evaluate the efficiency of the plasmid, pN2-SV-Luc was transfected into rat C6 and human U87 glioblastoma cells. Branched polyethyleneimine (PEI, 25KDa) was used as a gene carrier. Transfection assay showed that luciferase activity in the pN2-SV-Luc transfected cells was much higher than that in the pSV-Luc transfected cells. pEpo-N2-SV-Luc was constructed with an erythropoietin (Epo) enhancer for hypoxia specific gene expression. To confirm the glia and ischemia dual specific gene expression, pEpo-N12-SV-Luc was transfected into C6 cells and the cells were incubated under normoxia or hypoxia condition. The results showed that pEpo-N12-SV-Luc had higher gene expression than pN2-SV-Luc under hypoxia. Herpes simplex virus thymidine kinase/ganciclovir (HSV-TK/GCV) is the most common strategy for cancer suicide gene therapy. pEpo-N12-SV-Luc was constructed and transfected into C6 cells and the cells were maintained with GCV. As a result, the growth of the pEpo-N12-SV-TK transfected cells was significantly suppressed. Therefore, the glia and ischemia dual specific gene expression system with the N12-SV40 promoter and the Epo enhancer will be useful for glioblastoma specific gene therapy.

B4.48
Hypoxia-specific gene expression system using oxygen dependent degradation domain of activating transcription factor 4 for ischemia specific gene therapy
Hanyang University, Seoul, Korea

Angiogenic growth factor genes have been used for ischemic disease gene therapy. They are effective to protect the cells in ischemic tissues. However, they may induce tumor growth when they are expressed in normal tissues. Therefore, it is important to develop a gene regulatory system for hypoxia specific gene expression. In this study, the oxygen dependent degradation (ODD) domain from activating transcription factor 4 (ATF4) was evaluated for hypoxia specific gene expression. ATF4 is a member of the ATF/CREB family of the basic-leucine zipper transcription factors. ATF4 is induced post-translationally under hypoxia, which was mediated by the ODD domain. To evaluate the ATF4 ODD domain for gene therapy application, pSV-Luc-ATF4 ODD was constructed. For the construction, the luciferase cDNA without the stop codon was inserted into pSV vector. Then, the ODD domain was chemically synthesized and inserted at the downstream of the luciferase cDNA. In vitro transfection assay was performed and the cells were incubated under hypoxia or normoxia. The results showed that luciferase expression was induced under hypoxia in the pSV-Luc-ATF4 ODD transfected cells. The ATF4 ODD domain stabilized luciferase under hypoxia and promoted degradation of luciferase under normoxia. However, the pSV-Luc transfected cells did not show this effect.

B4.49
Gene regulation by histone-like modification of transcription factors
R. Gamsjaeger1, S. Webb2, J. Lamonica2, G. Blobel2 and J. Mackay3
1University of Sydney, School of Molecular Bioscience, Sydney, Australia, 2Children’s Hospital of Philadelphia, Division of Hematology, Philadelphia, USA

Gene regulation drives the development and homeostasis of every living organism; deregulation of this process has been implicated in the development of a range of severe diseases, including numerous cancers. It has become clear in recent years that an essential level of control of gene expression resides in reversible post-translational modifications of histone proteins that package DNA (“the histone code”). Recent data provide hints that analogous modifications in DNA-binding transcription factors exist and might provide a whole new level of complexity in gene regulation. However, our understanding of the molecular mechanisms through which these processes are regulated is far from complete. To address this problem, we focused on the essential transcription factor GATA-1 that regulates the expression of all erythroid and megakaryocyte-specific genes. It has recently been shown that GATA-1 is post-translationally acetylated at lysine residues located in the C-terminal portion of the protein and that mutations at these sites abrogate its biological activity. We have identified a bromodomain-containing protein that interacts with an acetylated GATA-1 peptide using two distinct acetylated lysines. We have characterized the binding mechanism structurally and biophysically using a combination of nuclear magnetic resonance spectroscopy (NMR) and surface plasmon resonance (SPR). Furthermore, we have used chromatin immunoprecipitation experiments (ChIP) to show that increases in GATA-1 occupancy are accompanied by increases in the bromodomain at important target sites confirming the biological significance of this interaction.

B4.50
Co-delivery of drug and gene using R3V6 and R3L6 peptides
Hanyang University, Department of Bioengineering, Seoul, Korea

R3V6 or R3L6 peptide, which is composed of 3 arginines and 6 valines or 6 leucines, formed self-assembled micelles in aqueous solution with a cationic arginine surface and a hydrophobic valine or leucine core. In this study, R3V6, R3A6, R3L6, and R3F6 peptides were synthesized and transfection efficiencies of the peptides or leucine core. In this study, R3V6, R3A6, R3L6, and R3F6 peptides were evaluated. In vitro transfection assays into 293 cells showed that R3V6 and R3L6 peptides had higher transfection efficiency than R3A6, R3F6 and poly-L-lysine (PLL). Since the peptide micelles had hydrophobic core, hydrophobic drug may be loaded into the core of the micelles. The incorporation of hydrophobic drug into the core of the peptide micelles may have two effects. First, the micelles will be more stable with tighter core in the presence of hydrophobic drug, which will increase the transfection efficiency. Second, the drug will be delivered into the cells more efficiently by endocytosis of the micelles rather than simple diffusion. In vitro transfection assay with the mixture of hydrophobic and promoted degradation of luciferase under normoxia. However, the pSV-Luc transfected cells did not show this effect.

drug, BCU, and the peptide micelles showed that the transfection efficiency of the micelles, suggesting that incorporation of hydrophobic drug increased the stability of the peptide micelles was improved, compared with the micelles without BCU. The weight ratio between BCU and R3V6 or R3L6 peptides for transfection was optimized. BCU and the peptide mixture had the highest transfection at a 0.8:1 weight ratio (BCU:R3V6) and a 1.2:1 weight ratio (BCU:R3L6). BCU is a strong anti-tumor drug. Therefore, the R3V6 and R3L6 peptides with BCU may be useful for anti-cancer drug and gene combinational therapy.

**B4.51**

**TISU, a common transcription and translation element specific to mRNAs with extremely short 5'UTR**

E. Roja, S. Hadar, H. Ora and D. Rivka
Weizmann Institute of Science, Rehovot, Israel

Regulation of gene expression at the transcriptional and translational levels occurs primarily at their initiation stages. Some proximal transcription regulatory elements are localized downstream to the transcription start site (TSS), and are also present in the mRNA where they could also influence translation. However there is little evidence for such composite transcription and translation elements. We have identified an element downstream to the TSS, which, remarkably, controls the initiation stages of both transcription and translation. This composite element is present in genes encoding for proteins involved in basic cellular functions such as respiration, protein metabolism and RNA synthesis. We showed this element to be essential for transcription and promoter strength. The core of the motif has an invariable ATG sequence, which serves also as the translation initiation codon. In these genes the initiating AUG is preceded by an unusually short 5'UTR. We demonstrated that translation in vitro and in vivo is initiated exclusively from the AUG of this motif, and that the AUG flanking sequences create a strong translation initiation context that is distinguished from the well-known Kozak in its unique ability to direct accurate translation initiation from mRNAs with a very short 5'UTR. We therefore named it TISU for Translation Initiator of Short 5'UTR. Our data suggest that TISU directs a new mode of translation initiation, which is cap-dependent but scanning independent. Moreover TISU is differentially regulated by translation initiation factor and under cellular stress. Thus coordinated transcription and translation can be mediated by a common regulatory element.

**B4.52**

**Regulation of expression of human intersectin 1 gene by alternative splicing and alternative transcription**

S. Kropyvko, O. Nikolaenko, L. Tsyba, D. Gerasymchuk and A. Rynditch
Institute of Molecular Biology and Genetics, NAS of Ukraine, Functional Genomics, Kyiv, Ukraine

Alternative splicing has recently become one of major mechanisms, increasing the diversity of transcriptome and has important application in physiology, development and genesis of diseases. Intersectin 1 (ITSN1) is an evolutionary conserved, multidomain protein involved in clathrin-associated endocytosis, signal transduction, and cytoskeleton rearrangement. ITSN1 mRNAs encode two major isoforms, a short and a long one. The short form is ubiquitously expressed and consists of two EH domains, a coiled-coil region, and five SH3(A-E) domains. The long form is predominantly expressed in neurons and includes three additional C-terminal domains, namely, DH, PH, and C2. Recently, it has been identified different variants of alternative splicing events affecting ITSN1 transcripts. Five of human transcripts have deletions in resulting protein and may affect binding properties and functions of ITSN1. Here we present results of cloning and characterization of fifteen full-length human ITSN1 transcripts comprising different combinations of alternatively spliced exons. Many eukaryotic genes contain multiple promoters. We have found alternative promoter in intron 5 of human ITSN1 gene that generates transcripts which encode proteins without first EH-domain. Their expression was found mostly in kidney, lung and liver tissues. We have cloned two full-length transcripts and found critical promoter region in LINE1-repeat using deletion mutants. Thus, tissue and development specific splicing may influence the interaction of ITSN1 with their partners and contribute to the regulation of ITSN1 protein function in endocytosis and signal transduction.

**B4.53**

**Elucidation of regulatory behaviour of Spo0A protein on bac operon**

O. Iriğul, T. E. Koroglu, H. Tayran and A. Karatas
Istanbul Technical University, Dr. Ocalgizar Molecular Biology-Biotechnology & Genetics Research Center (MOBGAM) and Molecular Biology and Genetics Department, Istanbul, Turkey

In *Bacillus subtilis*, a variety of responses are stimulated to allow the bacteria survive in the increasingly hostile environment. A very crucial response that makes *B. subtilis* form specialized cells, is sporulation. Sporulation initiation signals in *B. subtilis* result in the activation of the master regulatory protein Spo0A, by phosphorylation. Under such circumstances, phosphorylated and hence active Spo0A works directly or indirectly on the activation or inhibition of the transcription of many genes as sporulation accelerates. Besides sporulation, production of antibiotics is among the responses that lead bacteria to adapt the changing environment. In spo0A disrupted mutants of *B. subtilis*, the synthesis of antimicrobials and also resistance against other antibiotics were found to be deficient. These effects of spo0A mutations were associated with AbrB global regulator protein which is repressed by Spo0A. Bactilysin is a dipetide antibiotic and bac-ABCD*E* gene cluster of *B. subtilis* carries biosynthetic core functions on bactilysin production. In this study, spo0A gene, amplified from *B. subtilis*, was ligated to pQE60 expression vector and this recombinant plasmid was used to transform competent *E. coli* cells and Spo0A protein was isolated from these cells. Following purification, using electrophoretic mobility shift assay, direct or indirect regulatory behaviour of Spo0A protein was elucidated through the analysis of protein-DNA interaction between Spo0A protein and bac operon promoter region. This work is supported by The Scientific and Technological Research Council of Turkey (TUBITAK/TTBAG, 109T569).

**B4.54**

**HMG-box protein lxr1 is required for DNA-damage-induced expression of ribonucleotide reductase Rnr1**

O. Tsapolina and A. Chabes
Umea University, Department of Medical Biochemistry and Biophysics, Umea, Sweden

In *Saccharomyces cerevisiae*, Mec1 and Rad53 are essential and required, via the nonessential kinase Dun1, to activate ribonucleotide reductase (RNR), a key enzyme in the production of all four dNTPs. The activation of RNR by the Mec1-Rad53-Dun1
Abstracts

B4.55 Determination of regulatory mechanism of AbrB protein on bac operon
TE Koroglu, TAY, S. Mutlu and A. Karatas
Istanbul Technical University, MOBGAM Research Center and Molecular Biology and Genetics Department, Istanbul, Turkey

Bacilysin is a simple dipeptide antibiotic which is produced extracellularly by certain species of Bacillus subtilis that consists of L-alanine and L-anticapsin. Its synthesis seems to be under transcription regulation via the stringent response as well as under feedback regulation, and moreover it is a part of the global quorum-sensing control system. The loss of bacilysin production in spo0H and spo0A-blocked mutants as well as an increase in the production of bacilysin in abrB-disrupted mutants and the suppression of bacilysin-negative phenotype by an abrB mutation in spo0A-blocked mutants revealed that the transcription of some gene(s) involved in bacilysin formation is under the negative control of abrB gene product which is relieved by Spo0A protein.

Recently, it was found that ywfBCDEFG operon and a monocistronic gene ywfH are required for the biosynthesis of bacilysin and renamed as bacABCDGE operon. Under the light of these findings in this study, it was aimed to identify the binding properties of quorum sensing regulatory protein AbrB with the promoter region of bac operon whether it directly binds to the promoter region or not. For this purpose, abrB gene from B. subtilis genome was cloned into expression vector pQE60 and AbrB was expressed in E. coli. Obtained AbrB protein was used in Electrophoretic Mobility Shift Assay in order to identify the binding properties between AbrB and bac operon promoter. This work is supported by The Scientific and Technological Research Council of Turkey (TUBITAK/1BAG, 109TS69).

B4.56 Identification of SASP and spore coat protein profiles in bacA spores
ESaygili, E. Arslan, O. Irrigul and A. Karatas
Istanbul Technical University, MOBGAM Research Center and Molecular Biology and Genetics Department, Istanbul, Turkey

When exposed to nutrient deprivation, Bacillus subtilis cells cease growing exponentially and enter sporulation. During sporulation, cell division is asymmetric, generating two different cell types, forespore and mother cell. In subsequent stages, a variety of complex biosynthetic and morphogenic changes take place in the mother cell, in the forespore, and in the space between them. The proteinaceous coat which is produced by the mother cell, provides a thick, protective barrier that encases the mature spore. Bacterial spores are surrounded by a mechanically flexible protein coat that consists of many coat proteins, which protects the spore from environmental factors. Notwithstanding, forespore produces small-acid soluble proteins, SASPs. The chromosome of forespore is coated by some of these proteins that makes the spore gain resistance against UV. SASPs are also functional in dormancy and germination processes. In this study, a previously constructed B. subtilis strain (bacA::lacZ::erm), namely OGU1 containing transcriptional bacA-lacZ fusion at bacA locus was used for the ultimate purpose of the characterization of spore coat proteins and SASP profiles in O GU1 (bacA::lacZ::erm) and wild type PY79. DSM sporulation medium was used to drive cells into sporulation and then sporulated cells were used for SASP and coat protein isolations. In order to isolate SASP proteins, cells were ruptured in acidic environment and concentrated using low cut-off ultracentrifugation tubes and profiling of SASPs was done by using low pH polyacrylamide gel. Moreover, dialyzed and lyophilized spore coat proteins were also visualised by SDS-PAGE.

B4.57 SASP content and spore coat protein profile in yvfi spores
E. Arslan, E. Saygili, O. Irrigul and A. Karatas
Istanbul Technical University, MOBGAM Research Center and Molecular Biology and Genetics Department, Istanbul, Turkey

Under unfavorable nutritional conditions, Bacillus subtilis cells exhibit many differential behaviours, including sporulation. Sporulation in B. subtilis is intimately tied to the cell cycle. Sporulation process involves sequential and complex interactions between a large number of genes. Sporulation begins with an asymmetric cell division and then, the specialized spore septum is formed. Following that, the smaller prespore is then engulfed by mother cell. In forespore, small-acid-soluble proteins, known as SASPs, start to accumulate. These proteins, then act as amino acid storage proteins that to be used in case of germination for the resynthesis of biomolecules and they are stored to saturate spore DNA, therefore preventing DNA damage. Moreover, the proteinaceous spore coat begins to be deposited on the outside surface of the spore that consist of at least a dozen different proteins. Under the light of above-mentioned findings, the present project aims for the comparative clarification of the deposited SASP profiles and spore coat proteins between yvfi::Tn10::spc mutant and wild type PY79 strains. In this study, SASP proteins and spore coat proteins were isolated from the spores grown in DSM sporulation medium. Extracted spore coat proteins were concentrated using dialysis and visualized using SDS gel electrophoresis. Additionally, SASP proteins were extracted from cells through rupturing and proteins were ultrafiltrated using low cut-off ultrafiltration tubes in order to obtain a concentrated SASP protein mixture. Comparative analysis of SASP protein profiles were done after running those proteins on polyacrylamide gel electrophoresis at low pH.

B4.58 Regulatory profile of CodY protein on bacilysin biosynthetic operon
SMutlu, T. E. Koroglu, TAY and A. Karatas
Istanbul Technical University, MOBGAM Research Center and Molecular Biology and Genetics Department, Istanbul, Turkey

Regulatory profile of CodY protein on bacilysin biosynthetic operon
S. Mutlu, T. E. Koroglu, Tayyan and A. Karatas
Istanbul Technical University, MOBGAM Research Center and Molecular Biology and Genetics Department, Istanbul, Turkey

pathway leads to increased dNTP levels. Dun1 phosphorylates RNR inhibitors SmI1 and Dif1 promoting their degradation. In addition, Dun1 phosphorylates transcriptional repressor Cr1, leading to its dissociation from the promoters of RNR2, RNR3, and RNR4 genes. RNAI, which encodes the large subunit of yeast RNR, is also DNA-damage-inducible, but its promoter does not contain the Cr1 binding sites, and the mechanism of its activation by DNA damage has not been identified. We show that the induction of the Rnr1 levels by DNA damage requires transcription factor Ixr1. The Rnr1 levels increase in ixr1 mutants after DNA damage, instead of increasing as in the wild-type. In contrast, the DNA-damage-dependent elevation of the Rnr2, Rnr3 and Rnr4 levels is not affected by ixr1 mutation. In the absence of DNA damage, inactivation of IXR1 results in a moderate decrease of Rnr1 and dNTP levels, which is compensated by the Mecl-Rad53-Dun1-dependent upregulation of Rnr3 and Rnr4. The activation of RNR by Dun1 in ixr1 mutants is essential for cell viability. Deletion of SMLI or overexpression of RNRI or RNRII increases dNTP levels and rescues the ixr1 dun1 synthetic lethality. This work identifies Ixr1 as a novel player in regulation of DNA damage-inducible genes in yeast.

B4.57 SASP content and spore coat protein profile in yvfi spores
E. Arslan, E. Saygili, O. Irrigul and A. Karatas
Istanbul Technical University, MOBGAM Research Center and Molecular Biology and Genetics Department, Istanbul, Turkey

Under unfavorable nutritional conditions, Bacillus subtilis cells exhibit many differential behaviours, including sporulation. Sporulation in B. subtilis is intimately tied to the cell cycle. Sporulation process involves sequential and complex interactions between a large number of genes. Sporulation begins with an asymmetric cell division and then, the specialized spore septum is formed. Following that, the smaller prespore is then engulfed by mother cell. In forespore, small-acid-soluble proteins, known as SASPs, start to accumulate. These proteins, then act as amino acid storage proteins that to be used in case of germination for the resynthesis of biomolecules and they are stored to saturate spore DNA, therefore preventing DNA damage. Moreover, the proteinaceous spore coat begins to be deposited on the outside surface of the spore that consist of at least a dozen different proteins. Under the light of above-mentioned findings, the present project aims for the comparative clarification of the deposited SASP profiles and spore coat proteins between yvfi::Tn10::spc mutant and wild type PY79 strains. In this study, SASP proteins and spore coat proteins were isolated from the spores grown in DSM sporulation medium. Extracted spore coat proteins were concentrated using dialysis and visualized using SDS gel electrophoresis. Additionally, SASP proteins were extracted from cells through rupturing and proteins were ultrafiltrated using low cut-off ultrafiltration tubes in order to obtain a concentrated SASP protein mixture. Comparative analysis of SASP protein profiles were done after running those proteins on polyacrylamide gel electrophoresis at low pH.

B4.58 Regulatory profile of CodY protein on bacilysin biosynthetic operon
S. Mutlu, T. E. Koroglu, H. Tayyan and A. Karatas
Istanbul Technical University, MOBGAM Research Center and Molecular Biology and Genetics Department, Istanbul, Turkey

Regulatory profile of CodY protein on bacilysin biosynthetic operon
S. Mutlu, T. E. Koroglu, H. Tayyan and A. Karatas
Istanbul Technical University, MOBGAM Research Center and Molecular Biology and Genetics Department, Istanbul, Turkey

When exposed to nutrient deprivation, Bacillus subtilis cells cease growing exponentially and enter sporulation. During sporulation, cell division is asymmetric, generating two different cell types, forespore and mother cell. In subsequent stages, a variety of complex biosynthetic and morphogenic changes take place in the mother cell, in the forespore, and in the space between them. The proteinaceous coat which is produced by the mother cell, provides a thick, protective barrier that encases the mature spore. Bacterial spores are surrounded by a mechanically flexible protein coat that consists of many coat proteins, which protects the spore from environmental factors. Notwithstanding, forespore produces small-acid soluble proteins, SASPs. The chromosome of forespore is coated by some of these proteins that makes the spore gain resistance against UV. SASPs are also functional in dormancy and germination processes. In this study, a previously constructed B. subtilis strain (bacA::lacZ::erm), namely OGU1 containing transcriptional bacA-lacZ fusion at bacA locus was used for the ultimate purpose of the characterization of spore coat proteins and SASP profiles in OGU1 (bacA::lacZ::erm) and wild type PY79. DSM sporulation medium was used to drive cells into sporulation and then sporulated cells were used for SASP and coat protein isolations. In order to isolate SASP proteins, cells were ruptured in acidic environment and concentrated using low cut-off ultracentrifugation tubes and profiling of SASPs was done by using low pH polyacrylamide gel. Moreover, dialyzed and lyophilized spore coat proteins were also visualised by SDS-PAGE.
Bacilysin is one of the simplest peptide antibiotics which nonribosomally synthesized by certain strains of the *Bacillus subtilis*. Earlier studies revealed that quorum sensing mechanism and signal transduction phosphorelay control its biosynthesis. Bacilysin production is negatively regulated by GTP via the transcriptional regulator CodY which regulates stationary phase genes by detecting intracellular level of GTP results in an increase in their transcription. Moreover, it was demonstrated that bacilysin production in *rel*+ increased when the CodY protein was disrupted. Recently, *yvfBCDEF* operon and a monocistronic gene (*yvfH*) was shown to be essential biosynthetic genes taking role in the production of bacilysin. In this present study, the regulatory behaviour of CodY protein on bacilysin biosynthetic operon was identified by using Electrophoretic Mobility Shift Assay (EMSA) with the purpose of determination if CodY effects bacilysin biosynthesis through a direct or indirect interaction with the bac operon promoter. For this aim, *codY* gene was amplified from *B. subtilis* and cloned into pQE60 expression vector. CodY protein was expressed in *E. coli* cells and was purified. Interaction of this purified CodY protein with *hac* operon promoter was identified using EMSA, in order to reveal out regulatory pattern of CodY on this operon. This work is supported by The Scientific and Technological Research Council of Turkey (TUBITAK) (TBAG, 109T569).

**B4.59**

The effect of ComK transcriptional regulator on the expression of *yvfI* gene in *Bacillus subtilis*

T. Gun, O. Irigul and A. Karatás

Istanbul Technical University, MOBGAM Research Center and Molecular Biology and Genetics Department, Istanbul, Turkey

Quorum sensing mechanism is used by many bacterial species for establishing different bacterial behaviors. *Bacillus subtilis* is a good example in order to understand the signalling and regulation mechanism managed through a cell-density dependent manner. In *B. subtilis*, in the QS control of competence system, ComX binds to and activate a protein kinase, ComP. Then, ComP donates its phosphate group to ComA, ComA-P activates the production of ComS. ComS inhibits the proteolytic degradation of a transcriptional activator, ComK. After ComS transcription, ComK concentration increases quickly and competence genes become expressed which results in DNA-uptake into the cell. Recent studies showed that synthesis of bacilysin was controlled as a part of the global quorum-sensing control system. Recently, it has been shown that *yvfI* gene of *B. subtilis* was essential for the bacilysin biosynthesis. In this present study, we aimed to determine the effect of ComK, on the expression of *yvfI* gene. For this purpose, a *comK* inactivation vector was constructed through the ligation of *comK* gene into a spectinomycin cassette harbouring pGEMT vector. In order to analyze the effects of *comK* inactivation on *yvfI* expression, a *Bacillus* strain carrying lacZ fusion at *yvfI* locus, TEK7 (*yvfI::lacZ::erm*) was transformed by the constructed vector and this vector was driven into the chromosomal DNA of TEK7 by a single crossover event. In the following step, TEK7 and its *comK* inactivated derivative were grown in PA medium and *yvfI*-directed β-galactosidase activities were determined.

**B4.60**

The expressions of ADAMTS-1 and VEGF in DU-145, PC-3, MCF-7 and HT-29 cell lines

F. B. Sunay1, S. Aydogan Turkoglu2, F. Kockar3 and D. Okuyan1

1Department of Histology and Embryology, Balikesir University Faculty of Medicine, Balikesir, Turkey, 2Department of Molecular Biology, Balikesir University Faculty of Science and Art, Balikesir, Turkey, 3Department of Biology, Balikesir University Faculty of Science and Art, Turkey

The formation of new blood vessels from preexisting capillaries by the invasion and sprouting of the endothelial cells is called as angiogenesis. Angiogenesis is essential for tumor growth, progression and metastasis. Several studies proved that the microvascular intensity of the primer tumor is a very valuable prognostic determinant for breast, prostate and colorectal tumors. Angiogenesis is controlled by several endogenous stimulators and inhibitors of endothelial cell growth. Among these stimulators, the vascular endothelial growth factor (VEGF) owns a private value because of its importance and specificity in angiogenesis. As a result, suppression of the signal produced by VEGF inhibits angiogenesis and reduces tumor severity. The first described member of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin) family, ADAMTS-1, is an endogenous inhibitor of the angiogenesis. It is demonstrated that ADAMTS-1 binds and sequestrates VEGF and this results with the suppression of endothelial cell proliferation. There are several studies about the expression of ADAMTS-1 in different tissues and cell lines. The aim of this study was to determine the expression of ADAMTS-1 in breast, prostate and colorectal cancer cell lines namely MCF-7, DU-145, PC-3 and HT-29. The expression of the VEGF was also determined for the same cells. Total cellular mRNA were prepared from MCF-7, DU-145, PC-3 and HT-29 cells and subjected to RT-PCR using ADAMTS-1 and VEGF primers. GAPDH amplification was carried out as a positive control. VEGF expression was detected in all cells. ADAMTS-1 expression was also detected in PC-3 cell line.

**B4.61**

Abstract withdrawn

**B4.62**

Regulation of the genes for chlorate reductase (*Clr*) and chloride dismutase (*Cld*) in the chlorate-respiring bacterium *Ideonella dechlororatans*

N. Johansson, M. Hellberg, T. Nilsson and M. Rova

University of Karlstad, Chemistry and biomedical sciences, Karlstad, Sweden

Enzyme activities and mRNA levels of chlorate reductase and chloride dismutase was investigated in whole cell extracts of *Ideonella dechlororatans* grown under different growth conditions. This bacterium grows well both at aerobic and anaerobic conditions, using oxygen and chloride, respectively, as a terminal electron acceptor. It was found that preparations from cells grown in the absence of chlorate under aerobic conditions showed activity of both chlorate reductase, measured as chlorate dependent reduction of methyl viologen, and chloride dismutase, measured as chloride dependent oxygen production. At aerobic growth conditions, the addition of chlorate resulted in an increased activity of chlorate reductase. The highest activity of chlorate reductase was found in preparations from cells grown anaerobically in the pres-
ence of chlorate. No increase in enzyme activity could be detected for chlorite dismutase during anaerobic or aerobic growth in the presence of chlorate, compared to aerobic growth in the absence of chlorate. The mRNA levels for Clr and Cld, measured by real-time quantitative PCR using 16SrRNA as an intern standard, was found to be equal in preparations from cells grown anaerobically in the presence of chlorate compared to cells grown under aerobic conditions in the absence of chlorate. The results suggest that, in I. dechloratans, the activity of chlorate reductase is up-regulated by at least two factors, anaerobiosis and the presence of chlorate. Interestingly, the results also indicate that the studied regulation occurs at post-transcriptional level, while most examples of oxygen regulation in bacteria are reported to occur at transcriptional level.

**B4.63**

**Effect of Bacillus thuringiensis Cry3Aa intoxication on the expression of cysteine and serine peptidases in the midgut of the yellow mealworm, Tenebrio molitor**

E. Elpidina, A. Martynov and B. Oppert

1Moscow State University, Belozersky Institute of Physico-Chemical Biology, Moscow, Russian Federation, 2Moscow State University, Faculty of Bioengineering and Bioinformatics, Moscow, Russian Federation, 3USDA ARS Grain Marketing and Production Research Center, Manhattan, KS, USA

The yellow mealworm, *Tenebrio molitor*, is a stored-product pest found in stored grain products and is sensitive to the coleopteran-specific Cry3Aa toxin from *Bacillus thuringiensis* (Bt). Because insect digestive peptidases catalyze the processing of Cry protoxins to toxins, we evaluated the effect of Cry3Aa toxin on the expression of cysteine and serine mid gut peptidases genes in *T. molitor* larvae. High-throughput sequencing was used to obtain EST databases from the midguts of one month old *T. molitor* larvae fed for 24 hour with either a control diet or diet containing 0.1% Cry3Aa. Sequences encoding midgut cysteine peptidases from the C1 family were similar to four cathepsin B, four cathepsin L, and one cathepsin K. The expression of cysteine cathepsins was decreased overall in Bt-treated larvae, although one cathepsin L was found only in the treated animals and the expression of cathepsin K was 1.7-fold enhanced. Serine peptidases from the S1 family were represented by 18 predicted active peptidases and 19 serine peptidase homologs lacking functional amino acids. Three active peptidases were predicted as trypsins and five as chymotrypsins. Among the active serine peptidase sequences, five were found in both control and Bt-treated larvae, but eight were unique to control and five were unique to Bt-treated larvae. Serine peptidases common to both groups overall were expressed 1.5–10-fold lower in Bt-treated larvae. The data is the first application of high-throughput sequencing to the study of Bt intoxication and demonstrates that Cry3Aa intoxication in *T. molitor* induces widespread changes in peptidase gene expression. The work was supported by the RFBR.

**B4.64**

Abstract withdrawn

**B4.65**

**Tissue transglutaminase contributes to the all-trans retinoic acid induced differentiation syndrome phenotype in the NB4 model of acute promyelocytic leukemia**

K. Csomos, Z. Balajthy, L. Fesus and I. Nemet

University of Debrecen, Medical and Health Science Center, Department of Biochemistry and Molecular Biology, Debrecen, Hungary

Therapy of acute promyelocytic leukemia (APL) primarily consists of an all-trans-retinoic acid/ATRA-based treatment. Administration of ATRA leads to gene expression remodelling and results in the terminal differentiation of leukemic cells toward neutrophil granulocytes. One of the most induced genes by ATRA in APL NB4 cells is transglutaminase 2 (TG2). RNA interference-mediated stable silencing of TG2 in NB4 cells (TG2-KD NB4) coupled with whole genome microarray analysis revealed that TG2 contributes to the expression of large number of ATRA-regulated genes. In the TG2-KD NB4 cells several genes, related to neutrophil granulocyte function, stayed suppressed during ATRA-induced differentiation. Low induction of these genes led to reduced adhesive, migratory and phagocytic capacity of neutrophils and less superoxide production. ATRA-controlled down-regulation of those genes which are involved in cell cycle control and cell proliferation were held at higher expression level and found to be manifested in a higher proliferative rate of TG2-KD NB4 cells. Furthermore, in TG2-silenced NB4 cells we observed significantly lesser induction of CC-chemokines (CCL2, -3, -22, -24), which are responsible for the development of differentiation syndrome (DC) in ATRA treated APL patients. Based on our findings we suppose a complex regulatory effect of TG2 upon the ATRA-mediated differentiation of myeloid cells and propose that a reduced expression of TG2 in differentiating APL cells may suppress effector functions of neutrophil granulocytes and therefore moderate ATRA-induced hyper-inflammatory response in DS.
B5 – Regulation of Protein Function

B5.01
Abstract withdrawn

B5.02
β7-Strand of α-crystallin domain is important for monomer-monomer interaction in homo- and heterooligomers of human small heat shock protein
E. Mynrikov, O. Bukach, A. Seit-Nebi and N. Gusev
Moscow State University, Biochemistry, Moscow, Russian Federation

Members of the small heat shock proteins (sHsp) family play multiple roles in different cellular processes and αβ-crystallin, Hsp27, Hsp20, Hsp22 are expressed in practically all human tissues. All sHsp contain conservative α-crystallin domain and form small (dimers, tetramers) or large (16 or more subunits) oligomers. Heterogeneity and high mobility prevent successful crystallization of full-size human sHsp and hamper structural investigations. This makes desirable a search of new approaches for investigation of sHsp structure. Trying to solve this problem we used the methods of site-directed mutagenesis and replaced endogenous Cys residues of αβ-crystallin, Hsp20 and Hsp22 by Ser and simultaneously introduced a single Cys residue in position homologous to that of Cys137 of Hsp27. This residue is located in putative site involved in intersubunit interaction within sHsp oligomers. Point mutations do not affect the secondary, tertiary or quaternary structure and chaperone-like activity of investigated proteins and therefore mutated sHsp could be used for the study of monomer interaction. The wild type Hsp27 and the Cys-mutants of all other sHsp effectively form disulfide cross-linked homodimers after mild oxidation. αβ-crystallin, Hsp27, Hsp20 also effectively form disulfide crosslinked heterodimers, whereas formation of heterodimers containing Hsp22 and any other sHsp was markedly reduced. Thus, the β7-strand is essential for monomer interaction in homo- and heterooligomers of sHsp and Hsp22 is less effective than any other sHsp in formation of heterooligomeric complexes.

Acknowledgement: This investigation was supported by Russian Foundation for Basic Science.

B5.03
Alleles of APETALA1 gene with truncated C and K domains differently affect on the reproductive floral organ development in Arabidopsis thaliana abruptus mutant
E. Kupriyanova1 and U. Kawai-ooi2
1 Moscow State University, Faculty of Biology, Department of Genetics, Moscow, Russian Federation, 2Tyva State University, Kyzyl, Russian Federation

The AP1 gene is involved in determining floral organ development. It encodes a transcription factor with a MAD5, L, K and C domains and expresses in flower primordia. The functional role of the AP1 C-terminal domain is not well understood. The mutations which destroy different domains can help to elucidate their functions. The ap1-1 and ap1-3 mutations are the result of splice site acceptor changes at the third and fifth introns, respectively (Mandel et al., 1992); ap1-20 has deletion (21 bp) at the K domain region (Ondar et al., 2008). In the ap1-6 allele we have found the mutation in acceptor site in third intron (similar to ap1-1). PCR-amplified cDNA from ap1-6 mutant shows two splice variants: 671 bp without 4 exon; 626 bp without 4, 5 and GAG motif from 6th exon. Both cDNA variants contains 1-3 exons, which coding functional MAD5, L and a part of K domain. The lack of 4 exon leads to the frameshift and translation of incorrect amino acids of the C domain before a premature stop codon. A truncation of the C domain in ap1-1, ap1-6 and ap1-3 mutants correlates with the phenotype displayed by these mutants on the abruptus background (abr, allele of PID). The abr mutation leads to ectopic AP1 expression in the inner floral whorls. All double mutants lack floral organs in the 1 and 2d whorls and demonstrate strong reduction in stamens and carpel number. In contrast, double mutant ap1-20 abr does not demonstrate reproductive organ reduction. Since ap1-20 has functional C domain, it is suggested that this domain prevents the alteration of the process of determination of stamens and carpels when ectopically expressed in the inner whorls of abr mutant.

B5.04
Study of degradation processes of the AtNUDT7 Nudix regulatory protein from Arabidopsis thaliana
A. Lipko and E. Kraszewska
Institute of Biochemistry and Biophysics, Plant Biochemistry, Warsaw, Poland

Arabidopsis thaliana pyrophosphohydrolase AtNUDT7 belongs to a widely distributed Nudix family of proteins. It is a homodimeric protein that in vitro preferentially hydrolyzes ADP-ribose and NADH. It has recently been shown that AtNUDT7 is a very important factor modulating cellular response to biotic and abiotic stresses in Arabidopsis. It is well established that the cellular content of essential proteins is firmly regulated at different levels including controlled degradation of the proteins. Despite indications that some Nudix hydrolases may play compelling functions in the cell, no data were presented on mechanisms that regulate the cellular level of these proteins. During previous in-vitro studies we have established that the 26S proteasome non-ATPase regulatory subunit interacts with AtNUDT7 suggesting that the cellular level of this hydrolase may be regulated by proteasome. Therefore, the aim of this work was to show whether the AtNUDT7 protein is processed by this degrading complex. To address this question a cell-free degradation assay was used. We show here that in the presence of plant cell extract the AtNUDT7 protein diminish within two hours. The influence of ATP, protease inhibitors (PMSG, Complete, leupeptin) and specific proteasome inhibitor (MG115) on this process was studied. The obtained data led us to the conclusion that the AtNUDT7 Nudix hydrolase is degraded by the proteasomal complex.

B5.05
Hydrogen producing hydrogenases in Escherichia coli under glycerol fermentation at different pH
K. Trchounian1, T. Wood2 and A. Trchounian1
1 Yerevan State University, Biophysics, Yerevan, Armenia, 2Texas A&M University, Chemical Engineering, College Station, USA

Hydrogen is produced by E. coli Hyd-3 (hydC) and Hyd-4 (hydF) hydrogenases whereas Hyd-1 (hydA) and Hyd-2 (hydB) are uptake hydrogen-
Hyd-3 is responsible for H2 production and fhlA but strongly (~28-fold) in fold lower than in wild type. DCCD inhibited VH2 in all strains the above conditions.

Short collagen-related (SCR) peptides similar to

Introduction: Short collagen-related (SCR) peptides similar to collagen repeating sequences are known to have multiple biological effects including hypotensive, anticoagulant and antiathero-
genous. However molecular basis of such effects has not been elucidated so far. We supposed proteolytic enzymes to be potential targets for SCR peptides. Substrate-inhibitor specificity of human matrix metalloproteinase-2 (MMP-2), human thrombin and bovine angiotensin-converting enzyme (ACE) for thirteen human matrix metalloproteinase-2, human thrombin and bovine angiotensin-converting enzyme (ACE) for thirteen SCR peptides predominantly consisting of glycine and proline residues was investigated.

Methods: Protease activities were assayed using chromogenic and fluorogenic substrates. To determine protease hydrolysis products HPLC-analysis of dansylated SCR peptides and their derivates has been performed.

Results: As collagen breakdown can be a crucial event in SCR peptides formation we examined their inhibitory activity for MMP-2. All of SCR peptides (10 and 100 μM) tested appeared not to inhibit MMP-2. Meanwhile, several SCR peptides (10 and 1000 μM) reduced thrombin activity up to 80%. This effect occurred only in the presence of heparin, otherwise inhibition was decreased or lost. We have also shown that ACE breaks down peptides containing the C-terminal sequence Gly-Pro with release of H-Gly-Pro-OH as the major product.

Conclusions: SCR peptides are likely involved in regulating of release of H-Gly-Pro-OH as the major product.

Acknowledgements: This study was supported by the Russian Foundation for Basic Research (Grants No. 08-04-01760 and 09-04-013813).

In our presented work we investigated the influence of plant extract on free-radical oxidizing processes by changing of some enzymes activity. One of many aspects of oxidative damage to proteins includes mechanisms and consequences of oxidative stress. SOD is one of enzymes from antioxidant enzyme system for defense of cell from harmful. With this aim was investigated the value both the level of spontan chemiluminescence (ChL) and SOD activity by influence on biological target. Many attempts have been made to better characterize therapeutic properties of drug plant extracts for using in future by diseases. Herbs are harmless sources for obtaining natural antioxidants. Natural antioxidants are extensively studies for their capacity to protect organisms and cell from damage induced by oxidative stress. As experimental plant we were taken only drug store samples of Artemisia Absinthium L. Extraction of plant was carried out with using extractor of Soxlet in during long-term extraction. As experimental approaches had used a spontan and induced ChL analyze of lipid contained biological target Activity SOD was determinate by reaction of nitro blue tetrozolium reaction. We obtained that oxidation in plant extracts accompany by level of its ChL and SOD activity. Those results have high level of correlation. So, those investigations showed that these plant extracts were inhibitors of free radical oxidation with different power, and that must be provided by enzyme activity. These results were statistical processing with special computer program MatLab, and can be useful in clinical evaluation of actualities of drug plants by antioxidant therapy.
and CD44 exist as a tight biomolecular complex. Interestingly, this complex occurs in a RAP-independent manner. Moreover, we established by immunofluorescence experiments that LRP-1 and CD44 are colocalised at the plasma membrane and are partially located to cholesterol-rich membrane microdomains (caveolae). Inhibition of LRP-1 by RAP or by validated siRNA approach accelerates cell attachment. We demonstrated that the LRP-1-mediated control of cell adhesion occurs in a CD44 dependent manner. Finally, our results revealed for the first time that LRP-1 may act as an endocytic receptor for CD44 to support cell invasion.

B5.10

Polymorphisms of apolipoprotein(a) isoforms and lipoprotein(a) in a Macedonian population

K. Tosheska-Trajkovska¹, D. Labudovic¹, S. Alabakovska¹ and M. Spiroski²

¹Medical Faculty, Department of Medical and Experimental Biochemistry, Skopje, Macedonia, Fyrom; ²Medical Faculty, Institute of Immunobiology and Human Genetics, Skopje, Macedonia, Fyrom

High plasma levels of Lipoprotein(a) [Lp(a)] increase the risk of premature atherosclerosis. Lp(a) contains a unique protein, apolipoprotein(a) [apo(a)]. Apo(a) shows a high degree of genetic polymorphisms, resulting from variable number of tandem repeats of K-IV type 2 in the LPA gene. 180 healthy blood donors, aged between 18 and 60 years (94 males, 86 females), and 100 healthy children aged between 9 and 18 years (51 boys and 49 girls) were included in the study. Denaturing 3–15% gradient SDS-PAGE was used for the apo(a) isoforms separation. The results showed that the frequency distribution of apo(a) isoforms (B, S1, S3, S4, >S4) fit the expectation of the Hardy-Weinberg equilibrium. The frequencies of the six alleles were: B = 0.022, S1 = 0.028, S3 = 0.201, S4 = 0.397, >S4 = 0.110, and 0 = 0.242. The distribution of alleles was skewed towards alleles encoding large apo(a) isoforms associated with low Lp(a) levels. Large apo(a) isoforms (S4 and S3 from single banded and S4S3 from double-banded) were most prevalent in children and in adults. 164 of the adults (91.1%) and 98% of the children had a Lp(a) level beneath the 30 mg/dL, with 77% showing levels of 10 mg/dL or less. We found a significant inverse correlation (r = -0.3477, p < 0.001) between the Mr of apo(a) isoforms and plasma levels of Lp(a) in adults and in children (r = -0.4257, p < 0.001). Our results indicate that apo(a) isoforms are primarily genetically-determined, and are not affected by the plasma lipid and apolipoprotein concentration.

B5.11

Abstract withdrawn

B5.12

Distinct regulation of p53-mediated apoptosis by protein kinase C isoforms revealed by a yeast cell system

I. Coutinho¹, M. Leão¹, G. Pereira¹, J. Gonçalves², M. Córte-Real¹ and L. Saraiva¹

¹REQUIMTE/CEQU, Departamento de Ciências Biológicas, Laboratório de Microbiologia, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal; ²REQUIMTE/CEQU, Departamento de Ciências do Medicamento, Laboratório Farmacologia, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal; ³Centro de Biologia Molecular e Ambiental, Universidade do Minho, Campus Guimarães, Braga, Portugal

Apoptosis induced by p53 protein is firmly established as a central mechanism of tumour suppression. Therefore, the activation of p53 in tumour cells has been recognised as a promising strategy for cancer treatment. Several studies revealed that protein kinase C (PKC) isoforms are major regulators of p53. However, the complexity of the PKC family, namely the coexistence of several isoforms in a same mammalian cell, has hampered the elucidation of the role of individual PKC isoforms in the regulation of p53-mediated apoptosis. Hence, we used H2O2-treated yeast cells co-expressing the human wild-type p53 and a PKC isoform of the classical (PKCα), novel (PKCδ and -e) and atypical (PKCε) subfamilies. Effects of PKC isoforms on p53-mediated apoptosis were evaluated by analysis of cell viability, plasma membrane integrity, DNA fragmentation, metacaspase activation, reactive oxygen species production, mitochondrial transmembrane potential modification, mitochondrial network fragmentation, p53 phosphorylation and subcellular localization. A distinct regulation of p53-mediated apoptosis by PKC isoforms was detected. Whereas PKCα and -e had no effect, PKCδ and -ε stimulated a p53-mediated metacaspase and mitochondria-dependent apoptosis through p53 phosphorylation and partial mitochondrial p53 translocation. Together, this work reveals the conservation in yeast of a functional p53 transcription-independent mitochondria-mediated apoptosis. Also, it identifies PKCδ and -ε as activators of p53, and therefore as major targets in cancer therapy. We thank REQUI-MTE/CEQU, FCT (I&D/No 8/94) for financial support. I. Coutinho is recipient of a FCT PhD fellowship (SFRH/BD/36066/2007).

B5.13

Searching for small-molecule modulators of p53 activity and inhibitors of p53-MDM2 interaction, using yeast expression systems

M. Leão¹, I. Coutinho¹, M. Neves², H. Cidade², A. Paiva², E. Sousa², M. Pinto², J. Gonçalves³, C. Pereira¹ and L. Saraiva¹

¹REQUIMTE/CEQU, Departamento de Ciências Biológicas, Laboratório de Microbiologia, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal; ²CEQUIMED-UP, Departamento de Química, Laboratório Química Orgânica Farmacêutica da Faculdade Farmácia Universidade Porto, Porto, Portugal; ³REQUIMTE/CEQU, Departamento de Ciências do Medicamento, Laboratório de Farmacologia Faculdade de Farmácia Universidade do Porto, Porto, Portugal

The p53 tumour suppressor is a major regulator of cell proliferation and death. Activation of wild-type (wt) p53 and inhibition of p53 interaction to its endogenous negative regulator MDM2 represent attractive strategies in anticancer therapy [1]. Due to the high complexity of the mammalian p53 pathway, yeasts expressing the human wt p53 and co-expressing the human wt p53 and MDM2
proteins were used as simpler eukaryotic cell systems for a more efficient first-line screening of small-molecule modulators of p53 activity and inhibitors of p53-MDM2 interaction. We previously showed that expression of human wt p53 caused a marked growth inhibition [2]. Using this yeast p53 expression system, the effects of several flavonoids and xanthones on p53 activity were evaluated by growth assays and by analysis of several cell death markers. Some of the small-molecules tested behaved as potential p53 activators. Moreover, we detected that co-expression of human MDM2 with wt p53 significantly reduced the p53-induced growth arrest. The use of this yeast assay to search for small-molecule inhibitors of p53-MDM2 interaction was further validated by testing known inhibitors of p53-MDM2 interaction, RITA and Nutlin-3a [1]. Together, the developed yeast expression assays revealed to be promising drug screening approaches to identify modulators of p53 activity as well as inhibitors of p53-MDM2 interaction.

References:

We thank REQUIMTE/CEQUP I&D/No 8/94, CEQUIMED-UP (I&D/4040/2007), FEDER and POCTI for financial support. I.Coutinho (SRFH/BD/36066/2007) and M.Neves (SRFH/BD/21770/2005) are recipients of a FCT PhD fellowship.

B5.14 Sic1 plays a role in timing and oscillatory behaviour of B-type cyclins in yeast

M. Barbers1, C. Linke2, A. Adrover M.3, H. Lehrach2, F. Possas1, S. Krobitsch3 and E. Klipp1
1 Humboldt University Berlin, Institute for Biology, Theoretical Biophysics, Berlin, Germany
2 Max Planck Institute for Molecular Genetics, Berlin, Germany
3 Universitat Pompeu Fabra, Departament de CItàncies Experimentals i de la Salut, Barcelona, Spain

A number of dynamic models of cellular processes focus on cell cycle regulation. These models explain whether the dynamics of a network can be understood combining in vitro kinetics of its individual reactions. Moreover, biochemical feedback and feed-forward loops coordinate cell cycle oscillations occurring over a wide range of time scales.

The budding yeast cell cycle oscillates between states of low and high cyclin-dependent kinase activity, driven by the association of Cdk1 kinase with B-type (Clb) cyclins. Various Cdk1-Clb complexes are activated and inactivated in a fixed, temporally regulated sequence during cell cycle progression, inducing the characteristic behaviour known as “waves of cyclins”. The mechanism that regulates the appearance on schedule of these complexes is currently unknown. Here, we analyse the molecular basis of the oscillations of the Clbs, with major focus on the role of their inhibitor Sic1. We compare mathematical networks differing in the regulatory interactions that Sic1 may establish with Cdk1-Clb complexes. Our analysis suggests that the wave-like cyclins pattern derives from the binding of Sic1 to all three Clb pairs rather than from the degradation of Clbs. We show that a specific sequence of molecular interactions reproduces the oscillation-like behaviour of the Clb cyclins waves and verify experimentally the model predictions by protein-protein interaction techniques and time course experiments. Our results highlight a feed-forward regulation of Sic1 to synchronize the Cdk1-Clb complexes, acting as a timer in their appearance.

B5.15 Synthesis and biological activity of a new synthetic tea-derived compound that inhibits Dihydrofolate reductase in melanoma and breast cancer cells

University of Murcia, Department of Biochemistry and Molecular Biology (A.), Murcia, Spain

Tea catechins have emerged as promising chemopreventive agents because of their observed efficacy in various animal models. Tea polyphenols have shown antiproliferative and proapoptotic effects on human cancer cells. Recently, we have shown that the ester-bonded gallate catechins isolated from green tea, epigallocatechin-3-gallate (EGCG) and epicatechin-3-gallate (ECCG), are potent inhibitors of Dihydrofolate reductase (DHFR) activity. However, despite these excellent anticancer properties, tea catechins have at least one limitation: their low bioavailability, which is related to their low stability in neutral or slightly alkaline solutions and their inability to cross cellular membranes. In order to improve the stability and bioavailability of green tea polyphenols for cancer therapy, we successfully synthesized a new catechin-derived compound following three-step reaction sequence starting from the commercially available catechin. 3-O-(3,4,5-trimethoxybenzoyl)-(-)-catechin (TMCG) has shown significant antiproliferative activity against several cancer cell lines from breast, lung, colon and melanoma in a dose-dependent manner. As regards its action mechanism, we demonstrated that TMCG binds efficiently to DHFR and downregulates its expression in MDA-MB-231 breast cells and SK-MEL-28 melanoma cells. Disruption of the folate cycle by TMCG is a plausible explanation for its observed biological effects and suggests that, like other antifolate compounds, TMCG could be of clinical value in cancer therapy.

This study has been supported by Fundación Séneca, Región de Murcia, (08595.P1/08) and Ministerio de Ciencia e Innovación (SAF2009-12043-C02-01).

B5.16 Characterization of the phosphoinositide-binding site of C2 domains

J. Guillén-Casas, M. Guerrero-Valero, T. Coronado-Parrá, J. C. Gómez-Fernández and S. Corbalán-García
Universidad de Murcia, Departamento de Biología Móvil y Biología Molecular A, Murcia, Spain

C2 domains are considered peripheral proteins that are water-soluble and associate reversibly with lipid bilayers. Several evidences have demonstrated that some of these domains are able to interact with the inositol phospholipid PtdIns(4,5)P₂, which is able to directly participate in a myriad of functions, including cell signaling at the plasma membrane, regulation of membrane traffic and transport, cytoskeleton dynamics, and nuclear events. Recently, the crystal structure of PKCα-C2 domain in complex with Ca²⁺, PtdSer and PtdIns(4,5)P₂ has been described showing that the C2 domain of PKCα interacts specifically with PtdSer and PtdIns(4,5)P₂ through two independent motifs. Structure reveals that aromatic and cationic residues are able to interact directly with the PtdIns(4,5)P₂ moieties. Another important issue revealed in this work appeared when we analyzed the similarity of the C2 domains of classical PKCs with other C2 domains by structure-based sequence alignment enabling us to predict that there is a potential PtdIns(4,5)P₂ interacting-site. Moreover, we have characterized the binding of different C2
domains to PtdIns(4,5)P$_2$ and PtdSer using isothermal titration calorimetry and protein-to-membrane FRET, confirming the existence of a consensus sequence. This work was supported by grants from the Fundación Médica Mutua Madrilena and Fundación Séneca 08700/PI/08 (to S.C.-G.), and Ministerio de Ciencia e Innovacion BFU2008-01010 (to J.C.G.-F.). J.G.-C. belongs to the Programa Juan de la Cierva from the Ministerio de Ciencia e Innovacion.

B5.17

Cyt1Aa and Cyt2Ba toxins from *Bacillus thuringiensis* subsp. *israelensis*: activation by endogenous and exogenous proteases

M. Nisnevitch, 1 S. Sigawi, 1 S. Nikonov, 1 F. Nakonechny, 1 R. Cahani and 2 Y. Nitzan

1 Ariel University Center of Samaria, Department of Chemical Engineering, Biotechnology and Materials, Ariel, Israel, 2Bar-Ilan University, The Mina and Everard Goodman Faculty of Life Sciences, Ramat-Gan, Israel

The Gram-positive soil entomopathogenic bacterium *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) produces three parasporal Cyt 6-endotoxin species: Cyt1Aa, Cyt2Ba and Cyt1Ca in the form of protoxins. Cyt1Aa is the major and the most active component of the *Bti* parasporal crystal, comprising almost 50% of the toxin proteins. The other crystal component, Cyt2Ba, is highly homologous to Cyt1Aa. The Cyt1Aa proteotoxin exhibits some hemolytic and cytolytic activity, while the Cyt2Ba proteotoxin is almost completely inactive. Native activation of the protoxins occurs upon alkaline solubilization and further proteolysis from both the N- and the C-termini, which converts the protoxins into active toxins. We report on the activation of Cyt1Aa and Cyt2Ba by the endogenous zinc-containing metalloprotease camelysin, isolated from the *Bti*. Camelysin (23 kDa with a pI of 6.2) was extracted from intact bacterial cells by non-immunogenic agent. The isolated peptide can be applied as a natural cytotoxic test. When treated with camelysin, Cyt protoxins were truncated to a smaller form of protoxins. Cyt1Aa is the major and the most active component of the *Bti* parasporal crystal, comprising almost 50% of the toxin proteins. The other crystal component, Cyt2Ba, is highly homologous to Cyt1Aa. The Cyt1Aa proteotoxin exhibits some hemolytic and cytolytic activity, while the Cyt2Ba proteotoxin is almost completely inactive. Native activation of the protoxins occurs upon alkaline solubilization and further proteolysis from both the N- and the C-termini, which converts the protoxins into active toxins. We report on the activation of Cyt1Aa and Cyt2Ba by the endogenous zinc-containing metalloprotease camelysin, isolated from the *Bti*. Camelysin (23 kDa with a pI of 6.2) was extracted from intact bacterial cells by non-immunogenic agent. The isolated peptide can be applied as a natural cytotoxic test. When treated with camelysin, Cyt protoxins were truncated to a smaller form of protoxins. Cyt1Aa is the major and the most active component of the *Bti* parasporal crystal, comprising almost 50% of the toxin proteins. The other crystal component, Cyt2Ba, is highly homologous to Cyt1Aa. The Cyt1Aa proteotoxin exhibits some hemolytic and cytolytic activity, while the Cyt2Ba proteotoxin is almost completely inactive. Native activation of the protoxins occurs upon alkaline solubilization and further proteolysis from both the N- and the C-termini, which converts the protoxins into active toxins. We report on the activation of Cyt1Aa and Cyt2Ba by the endogenous zinc-containing metalloprotease camelysin, isolated from the *Bti*. Camelysin (23 kDa with a pI of 6.2) was extracted from intact bacterial cells by non-immunogenic agent. The isolated peptide can be applied as a natural cytotoxic test.

B5.18

A pH-dependent structural rearrangement of the human aromatase active site: implications in catalysis

G. Di Nardo, 1 M. Breitner, 1 J. Sadeghi S., 1 E. Nicolai, 2 G. Mesi, 2 A. Bandino, 3 D. Ghosh, 4 G. Panizza 4 and G. Gillardi 4

1 University of Torino, Department of Human and Animal Biology, Torino, Italy, 2 University of Rome Tor Vergata, Department of Experimental Medicine and Biochemical Sciences, Rome, Italy, 3 University of Torino, Department of Medicine and Experimental Oncology, Torino, Italy, 4 Hauptman-Woodward Medical Research Institute, Buffalo, USA

Aromatase is a cytochrome P450 catalyzing the conversion of androgens to estrogens. This enzyme, known to be an important target for cancer therapy, plays also a crucial role in the neuroendocrine brain system where is involved in the brain sexual differentiation, in the control of sexual behavior, neuroplasticity, neuroprotection, modulation of mood and affective status, aggressive behaviour, memory and cognitive function. The central role of aromatase in neuroprotection has been demonstrated in the cases of stroke, Alzheimer’s, Parkinson’s diseases and epilepsy. Like other neuroactive steroids, local estrogens levels may be controlled in the brain by a combination of both aromatase expression and activity. Nevertheless, the mechanisms responsible for aromatase activity modulation and therefore rapid changes in estrogens production are still unknown.

In this work, the effect of subtle changes in pH, known to be associated with neuronal activity, on human aromatase was investigated in vitro by using a recombinant form of human aromatase. A change of pH from 6.5 to 7.4 resulted in increase of the substrate dissociation constant and Michaelis-Menten constants, and conformational changes in the active site, as detected by circular dichroism and dynamic fluorescence spectroscopy. A pH dependence of substrate dissociation was observed as a high to low spin transition with an apparent pKa of 8.2 ± 0.3. The Asp309Asn mutant did not exhibit such pH dependence. These findings are consistent with protonation/deprotonation of Asp309 and structural rearrangements of the active site induced by subtle pH changes and altering substrate binding ability and catalysis of the enzyme.

B5.19

The protein N-terminal acetyltransferase NatA: A novel target for cancer chemotherapy?

T. Arnesen, 1 D. Gromyko, 1 J. E. Varhaug 2 and J. R. Lillevang 1

1 University of Bergen, Bergen, Norway, 2Haukeland University Hospital, Bergen, Norway

NAA15 (NATH) was originally identified by our group as upregulated in thyroid papillary carcinomas and has lately also been found to correlate with aggressiveness of neuroblastic tumours. hNaa15p protein interacts with the catalytic subunit hNaa10p (hARD1) forming the human NatA complex responsible for protein N-terminal acetylation in human cells. Using siRNA-knockdown of NatA in cancer cell lines combined with phenotype analysis and drug sensitization experiments we have characterized the biological impact of NatA. Knockdown of NatA in cancer cell lines induces apoptosis and G1-cell cycle arrest. We screened 11 different thyroid cell lines for hNAA10 RNAi phenotypes and observed mostly growth inhibition, which was independent of TP53 functional status and developed by several different mechanisms involving i) downregulation of cyclin D1, ii) increase in p27/Kip1 and iii) inactivation of Rb/E2F pathway. hNatA depletion in aggressive thyroid cancer cell lines (8305C, CAL-62, FTC-133) with mutated TP53 increased sensitivity to drug-induced cytotoxicity, but in a cell-type specific manner: 8305C and CAL-62 (Daunorubicin), and FTC-133 (Trotiglazone). Cells harbouring wild-type TP53 were also prone to apoptosis via the p53 pathway after hNatA downregulation. Importantly, in hNatA-depleted cells DNA-damage signalling was activated in the absence of exogenous DNA damage independent of TP53 status. Our findings indicate that several mechanisms of growth inhibition and apoptosis may be induced by hNatA knockdown and that hNatA knockdown could be exploited for use in combinatory cancer chemotherapy.
Abstracts

B5.20
The AtNUDT7 Nudix hydrolase from Arabidopsis thaliana interacts with two regulatory proteins RACK1A and 14.3.3
K. Olejnik1, M. Bucholc1, A. Anielska-Mazur1 and E. Kraszewska1
1Institute of Biochemistry and Biophysics, PAS, Plant Biochemistry, Warsaw, Poland, 2Institute of Biochemistry and Biophysics, PAS, Confocal and Fluorescence Microscopy Laboratory, Warsaw, Poland

The AtNUDT7 protein belongs to a widely distributed Nudix hydrolases that catalyze hydrolysis of a variety of nucleoside diphosphate derivatives. Previously, it has been shown that the protein exists as a dimer and that ADP-ribose and NADH are preferred substrates of this enzyme. Genetic analyses have indicated that the AtNUDT7 protein exerts negative control on the EDS1 complex that controls defense activation and programmed cell death. However, the molecular mechanism of the AtNUDT7 role in this process remains elusive. Finding proteins that interact with AtNUDT7 can help to understand this protein biological function. To address the question whether the AtNUDT7 protein interacts with other cellular polypeptides we have applied several in vitro and in vivo techniques. Using pull-down analysis we have identified two regulatory proteins, 14.3.3 and RACK1A, as the potential partners of AtNUDT7. We confirmed these interactions in yeast two-hybrid system. With a help of the bimolecular fluorescence complementation (BiFC) analysis, the interaction between AtNUDT7 and was also observed in Arabidopsis protoplasts. These data suggest that AtNUDT7 may function as part of a complex of regulatory proteins.

B5.21
Targeting the BAG-1/Hsp70 axis to inhibit tumor growth
M. Enthammer1, G. Wolber2, H. Stuppner3, M. Deutsch4 and J. Tropmpair4
1Daniel Swarowski Research Laboratory (DSL) and CAMD group, Department of Visceral, Transplant and Thoracic Surgery and Department of Pharmaceutical Chemistry, Innsbruck, Austria, 2Molecular Modeling, Institute of Pharmaceutical Chemistry, Berlin, Germany, 3Institute of Pharmacy, Department of Pharmacognosy, Innsbruck, Austria, 4Daniel Swarowski Research Laboratory (DSL), Department of Visceral, Transplant and Thoracic Surgery, Center of Operative Medicine, Innsbruck, Austria

BAG-1 is a multifunctional protein, which regulates cell growth, survival, intracellular signaling, and protein folding. Important for its function is the ability to associate with different proteins. These interactions are mainly mediated by the C-terminal BAG-domain comprising approx. 100 amino acids. Known interaction partners include the survival protein Bcl-2, heat shock protein 70 (Hsp70) and RAF kinases. BAG-1 expression is frequently altered in human cancer and depletion of BAG-1 negatively affected growth and survival of several cancer cell lines and prevented transformation by activated RAF in vivo and vitro. Hsp70 is present at constitutively elevated levels in many cancers and promotes tumor growth. Recently first evidence has been obtained that the disruption of BAG-1/Hsp70 interaction compromised tumor cell growth. Here we created a structure-based 3D-pharmacophore model combined with a GRID-based model using the crystal structure of BAG-1/Hsp70 as a basis to screen commercially available libraries of small molecular weight compounds. We were able to identify a putative ligand binding site in the interface of the BAG-1/Hsp70 complex and using several pharmacophore models with different degrees of restriction 40 virtual hits were selected following visual inspection and re-docking into the proposed binding site. These substances are currently screened in biochemical and cell-based assays for their ability to block BAG-1/Hsp70 interaction.

B5.22
Acetylcholinesterase expression in human colorectal adenocarcinoma cells (Caco-2)
A. Talavera, C. Luengos, F. J. Campoy, M. F. Montenegro, E. Muñoz-Delgado and C. J. Vidal
University of Murcia, Biochemistry and Molecular Biology, Murcia, Spain

Although the best known function of cholinesterases is the hydrolysis of the neurotransmitter acetylcholine, many studies indicate that acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) play non-catalytic actions, being implicated in morphogenesis, hematopoiesis, osteogenesis, apoptosis, and probably in tumorigenesis. We have previously reported that the expression of AChE and BuChE changes in brain tumours (meningiomas, gliomas, and neurinomas), as well as in those developed in breast, lymph node, gut, lung and kidney. These results prompted us to study AChE and BuChE expression on cell lines derived from human colon tumours and the effects that the under- and over-expression of AChE and BuChE genes have on cell proliferation, differentiation and apoptosis. For this purpose, cancerous intestinal cell lines (Caco-2 and HT-29) were transfected with AChE-siRNA and the effects of the silencing on the cell survival and apoptosis were studied. Our results show that Caco-2 cells contain little AChE activity and still less BuChE activity, whereas HT-29 cells have no detectable AChE or BuChE activity. Sedimentation analysis in sucrose gradients showed AChE activity of Caco-2 cells distributed between principal 4.6S forms and less 3.1S forms. According to our previous results, the above AChE forms were assigned to dimers and monomers. Caco-2 cells exhibited abundant AChE-H mRNA, four times lower levels of AChE-R and AChE-M mRNAs, and very few BuChE mRNA. Transfection with AChE-siRNA decreased AChE activity by about 90% and the content of AChE mRNAs by 70%. Current observations do not support severe effects of AChE silencing on Caco-2 survival.

B5.23
Phosducin family proteins in thyroid
U. Piotrowska and G. Adler
Medical Centre of Postgraduate Education, Department of Biochemistry and Molecular Biology, Warsaw, Poland

Phosducin-like orphan protein 1 (PhLOPI, 22,317 kDa), phosducin (Phd, 28,246 kDa) and phosducin-like protein 1 (PhlP1, 32,118 kDa) belong to phosducin-related regulatory phosphoprotein family. Their middle and C-terminal sequences are identical while differences occur in the length of their N termini. Despite of structure similarities, in the cell, each of the proteins plays different role. Phd binds and sequesters heterotrimeric G protein’s beta subunit, PhlP1 is involved in chaperoning of protein folding and PhLOPI, detected in nucleus, plays a role in transcription regulation. Although in various tissues the presence of the above phosducin family proteins was reported, their occurrence and function in the thyroid was never investigated. The aim of the present study was to determine the presence of phosducin-related proteins in postoperative thyroid and cultured thyroid cells. Two methods were used: the PCR which demonstr-
strated the presence of PhLP1 transcripts in thyroid, and Western blotting. Rabbit ap33 antibodies, against Phd and PhLP1 SQSLLEDFFEQATHTGPK sequence and ap34 as well as ap36 antibodies recognizing CIIKASNGAGDRFSIDVLPT and CMQDMHQFLSFGPRYGFPYE peptides respectively, present in Phd, PhLP1 and also PhLOP1, were obtained. In partially purified thyroid homogenate fractions Western blotting have shown the presence of 33 kDa immunoreactive band corresponding probably to PhLP1. Additionally a few other immunoreactive bands, stained also with anti 14-3-3 antibodies were found. They may reflect 14-3-3 - phosducin-like protein complexes present in thyroid in vivo or forms arisen during samples purification.

B5.24
Looking for a new anti-pertension therapy based on direct renin inhibition
D. Santucci1, S. Iannotti1, F. Sinibaldi2, C. Piro M 2, R. Santucci2 and N. Di Daniele1
1Università di Roma Tor Vergata, Dip.to di Medicina Interna, Rome, Italy, 2Università di Roma Tor Vergata, Dip.to Medicina Sperimentale e Scienze Biochimiche, Rome, Italy
Renin is a proteolytic enzyme of the hydrolases-class (aspartic proteases) appointed to the activation of the process in which ansiotensinogen is converted in angiotensin I and, successively, into the biologically active angiotensin II. Angiotensin II is the molecule stimulating the aldosterone secretion. Renin is a 37kD molecule constituted by 340 aminoacidic residues. Circulating are both pro-renin, which lacks enzymatic activity, and renin (Pro-Renin/Renin, 9:1 molar ratio). The renin-angiotensin system is often the target for clinical interventions relative to the hypertension treatment. Renin plays a primary role in the regulation of arterial pressure and it is involved in physiological processes inducing arterial hypertension. This explains the continuous research aimed to find new molecules to use as efficient renin inhibitors. Direct inhibition of renin represents a relatively new tool devoted to block the renin-angiotensin system and the body of cascade reactions leading to secretion of aldosterone. To this issue, we are undertaken a study on renin focused to achieve new renin inhibitors for therapeutic application in the hypertension field. At present, the structural and functional properties of the protein are under investigation under different experimental conditions; further, an expression system for renin inhibitors greatly differed in their ability to inhibit the apoptotic DNA fragmentation (2 to 10 μM Q-VD-OPh had larger effect than 50 μM zVADfmk while zDEVDfmk had no effect). We also analyzed the cellular adhesivity using a sensitive assay and found that only Q-VD-OPh (at least 10 μM) was able to prevent the apoptosis-related loss of the cell adhesiveness to fibronectin. This detailed analysis shows that “caspase” inhibitors have multiple targets in apoptotic cells and that all results obtained using these inhibitors at high concentrations have to be interpreted with caution. The work was supported by grant No 301,09/1026 from the Grant Agency of the Czech Republic.

B5.25
Abstract withdrawn

B5.26
Distinct effects of caspase inhibitors on caspase-3 activity, DNA fragmentation and cell adhesion to fibronectin in apoptotic cells
K. Kuželová, D. Grebečková and M. Pluskalová
Institute of Hematology and Blood Transfusion, Prague, Czech Republic
We studied in detail the effects of three caspase inhibitors (a relatively specific caspase-3 inhibitor zDEVDfmk, a general caspase inhibitor zVADfmk and a novel inhibitor Q-VD-OPh) on different apoptosis-related processes. The apoptosis was induced in the leukemic cell lines JURL-MK1 and HL60 using two unrelated drugs (SAHA and Glivec). In all these experimental systems, the inhibitor effects were markedly dose-dependent in the concentration range from 0.1 to 50 μM. All three inhibitors irreversibly bound to caspase-3 (as shown by anti-caspase-3 immunoblots) and prevented caspase-3, -9 and -2 activity in an in vitro assay at concentrations lower than 0.5 μM. On the other hand, the individual inhibitors greatly differed in their ability to inhibit the apoptotic DNA fragmentation (2 to 10 μM Q-VD-OPh had larger effect than 50 μM zVADfmk while zDEVDfmk had no effect). We also analyzed the cellular adhesivity using a sensitive assay and found that only Q-VD-OPh (at least 10 μM) was able to prevent the apoptosis-related loss of the cell adhesiveness to fibronectin. This detailed analysis shows that “caspase” inhibitors have multiple targets in apoptotic cells and that all results obtained using these inhibitors at high concentrations have to be interpreted with caution. The work was supported by grant No 301,09/1026 from the Grant Agency of the Czech Republic.

B5.27
The analog of diadenosine tetraphosphatse (JB 419) induces apoptosis in FHT-positive cells
R. Pecherzewska, A. Krakowiak, A. Tomaszewska, R. Kaczmarek, J. Baraniak and B. Nawrot
CMMS Polish Academy of Sciences, Bioorganic Chemistry, Lodz, Poland
The fragile histidine triad (FHT) protein is a diadenosine polyphosphate hydrolase belonging to the histidine triad family of nucleotide-binding proteins. FHT is a tumor suppressor protein which plays an important role in pro-apoptotic signaling, cell cycle control and sensitivity to DNA damaging agents. Loss of FHT is observed in a variety of tumors and premalignant states. Viral-mediated FHT gene therapy induces apoptosis and suppresses tumors in preclinical models. Some reports suggest that formation of a FHT-substrate complex, without subsequent hydrolysis of the substrate, induces cell death by apoptosis [1]. We have synthesized an analog of Ap4A called JB419 of the structure: ApsxpsA, where “ps” is a phosphorothioate linkage and “x” is the glycerol moiety. Also a fluorescent derivative of JB419 of the structure Fl-psxpsA (where Fl is a fluorescein residue) was prepared. ApsxpsA occurred to be an efficient inhibitor of FHT hydrolase activity [2]. We observed decrease of viability (MTT test) and induction of apoptosis (caspase-3 assay) after incubation of HEK293T cells with ApsxpsA. Fluorescence microscopy visualization showed that the fluorescent analog of JB419 accumulates in cytoplasmic compartments of the transfected cells. Moreover, co-localization studies indicated that Fl-psxpsA is partially present in mitochondrion. Our finding is relevant to recent findings that FHT may participate in mitochondrion-mediated apoptosis.
B5.28
Crystal structure of the N-terminal region of brain spectrin reveals a helical junction region, and a stable structural domain
S. Mebboob¹, Y. Song², M. Witek², F. Long², B. Santarsiero¹, M. Johnson¹ and L. Fung²

¹University of Illinois at Chicago, Center for Pharmaceutical Biotechnology, Chicago, USA, ²Department of Chemistry, University of Illinois at Chicago, USA

The crystal structure of a recombinant protein consisting of the first 147 residues of brain α-spectrin was solved to 2.3 Å. The N-terminal region consists of the partial domain (Helix C') and the anti-parallel, triple helical coiled-coil first structural domain (helices Aα, Bα, and Cα). The data revealed that each asymmetric unit contained two crystallographically independent structures (1 and 2). The crystal structure of the first structural domain resembled that of the first structural domain of erythroid α-spectrin, determined before by solution NMR studies, with some specific differences, especially at the N-terminal region, including Helix C' and the region connecting Helix C' with the first structural domain (the junction region). The first ten residues are in a disordered conformation, followed by Helix C' with an apparent, flexible bend. The junction region exhibits a helical conformation in contrast with an unstructured junction region in erythroid α-spectrin. A special feature that has not been reported in other spectrin domains is the long and flexible A1B1 loop of 13 residues. This loop is likely the recognition site for interaction with other proteins.

B5.29
Characterization of protein complexes subunits - using interaction and sequence identity
C.-H. Huang¹, H.-Y. Liao¹ and K.-L. Ng¹

¹Department of Computer Science and Information Engineering, National Formosa University, Yun-Lin, Taiwan, 2Department of Bioinformatics, Asia University, Taichung, Taiwan

Protein complexes play important roles in many cellular processes. There are several approaches have been developed for protein complexes prediction. Most of the approaches make use of the assumptions that (i) protein complexes have dense protein-protein interactions among their subunits, and (ii) high functional similarity for the subunits. In this paper, we suggest to investigate those assumptions by studying the subunits’ interaction topology and sequence similarity. Two topological parameters, density of protein-protein interactions (PPI) and connectedness, are defined to test whether protein complex are found in PPI dense region or not. The present data indicated that interaction dense regions represent protein complexes up to 20% of all the cases. A rather large proportion of protein complexes have a lower density of PPI, and connectedness. It is conjectured that prediction approaches based on the assumption that complexes are composed of highly PPI dense regions, connectedness can predict a rather limited numbers of the complexes. Computational approaches making use of the subunit sequence identity can identify complexes of a size less than 13 with reasonable evidence. In the future, we suggest computing variety aspects of protein complexes properties, i.e. static as well as the physiochemical properties, to describe protein complexes. These attributes include mRNA expression data, amino acid composition, length of protein subunits, half-life or instability index, and the dissociation constant, pKa, of a protein complex subunit. Such consideration can possibly provide better insights for our understanding of protein complexes architecture.

B5.30
Dephosphorylated NPr of the nitrogen-metabolic PTS regulates lipid A biosynthesis by direct interaction with LpxD
H.-j. Kim¹, M. G. Choi¹, C.-R. Lee¹, M. Kim¹, A. Peterkofsky², Y.-j. Soek¹ and Y.-j. Soek²

¹Department of Biological Sciences and Institute of Microbiology, Seoul National University, Seoul, Korea, ²National Institutes of Health, Laboratory of Cell Biology, National Heart, Lung, and Blood Institute, Bethesda, USA

Bacterial phosphoenolpyruvate-dependent phosphotransferase systems (PTS) play multiple roles in addition to sugar transport. Recent studies revealed that enzyme IIAntr of the nitrogen-metabolic PTS regulates the intracellular concentration of K⁺ by direct interaction with TrkA and KdpD. In this study, we show that NPr of the nitrogen-metabolic PTS directly interacts with and regulates Escherichia coli LpxD which catalyzes biosynthesis of lipid A of the lipopolysaccharide (LPS) layer and therefore is essential for growth. LpxD showed a preferential interaction with unphosphorylated NPr. Mutations in lipid A biosynthetic genes such as lpxD are known to confer hypersensitivity to hydrophobic antibiotics such as rifampin; in contrast, a ptsO (encoding NPr) deletion mutant showed increased resistance to rifampin and increased LPS biosynthesis. Taken together, our data show that unphosphorylated NPr decreases LPS biosynthesis by inhibiting LpxD activity.

B5.31
Biochemical and physiochemical characterization of thermostable direct hemolysin from Grimontia hollisae
Y.-K. Wang, S.-C. Huang, Y.-F. Wu and T.-K. Wu

National Chiao Tung University, Hsin-Chu, Taiwan

Thermostable direct hemolysin (TDH), a major virulence factor, is widely present in the clinical strain of Vibrio parahaemolyticus and a few other strains of Vibrio species. In this study, we describe biochemical and biophysical characterization of the recombinant TDH from Grimontia hollisae, and physiochemical determination of its cytotoxicity and virulence. The native polyacrylamide gel electrophoresis, gel filtration chromatography, analytical ultracentrifugation, and transmission electron microscopy (TEM) analysis revealed a possible tetrameric structure of Gh-rTDH in the absence of the host cell membrane environment, similar to that of the TDH purified from V. parahaemolyticus. The Gh-TDH exhibited the Arrhenius effect and changed its conformation into fibril form at around 57°C, as determined by the thermostability assay, circular dichroism, differential scanning calorimetry, and TEM. Further, apparent in vitro cytotoxicity and morphological changes including membrane bleeding, cell
B5.32 Effect of pseudophosphorylations on the structure and properties of 14-3-3:

N. Sluchanko, I. Chernik, A. Sei-Nebi and N. Gusev

Lomonosov Moscow State University, School of Biology, Moscow, Russian Federation

Adaptor proteins 14-3-3 are ubiquitously expressed in eukaryotes. The members of 14-3-3 family form dimers and predominately interact with phosphorylated proteins thus regulating multiple intracellular processes. 14-3-3 itself also undergoes phosphorylation catalyzed by a number of different protein kinases. For instance, 14-3-3ε is phosphorylated at Ser58, Ser184 and Thr232 both in vitro and in vivo. Phosphorylation affects interaction of 14-3-3 with certain client proteins, however effect of phosphorylation on physico-chemical properties of 14-3-3 remained uninvestigated. By replacing phosphorylated Ser/Thr residues by Glu we introduced mutations mimicking phosphorylation and analyzed effect of these mutations on the properties of purified recombinant 14-3-3. Mutation S58E increased intrinsic Trp fluorescence and binding of bis-ANS to 14-3-3. At low protein concentration mutation S58E induced dissociation of 14-3-3 dimer, increased its susceptibility to proteolysis and decreased its thermal stability, thus indicating potential role of Ser58 in regulation of 14-3-3 degradation in the cell. Mutation S184E slightly increased Stokes radius and thermal stability of 14-3-3. Mutation T232E induced only small changes in hydrodynamic properties probably reflecting the changes in the size or shape of 14-3-3 dimer. The properties of triple mutant (S58E/S184E/T232E) were intermediate between those of the wild type protein and its S58E mutant. Thus, pseudophosphorylation of 14-3-3ε at different sites has different effects on its structure and this might be important for regulation of interaction of 14-3-3 with different protein targets. This study was supported by RFBS grant.

B5.33 The modulation of the antioxidative system in MRC-5 cell line exposed to Fe3O4 nanoparticles

M. Radu1, M. C. Munteanu1, M. Costache1, A. I. Serban2, C. Sima3, A. Hermenean3, C. Grigoriu3 and A. Dinișchiută1

1University of Bucharest, Department of Biochemistry and Molecular Biology, Bucharest, Romania, 2University of Agricultural Sciences and Veterinary Medicine, Bucharest, Romania, 3National Institute of Laser, Plasma and Radiation Physics, Bucharest-Magurele, Romania, 4Vasile Goldis Western University of Arad, Arad, Romania

Particles generated from numerous anthropogenic and/or natural sources, such as crystalline alfa-Fe2O3 (hematite) nanoparticles, have the potential to damage the lung cells. In our study, the effects of these nanoparticles at a concentration of 12.5 μg/ml for 24, 48 and 72 hour on lipid peroxidation as well as, on the antioxidative system belonging to MRC-5 lung fibroblast cells were investigated. Exposure to alfa-Fe3O4 nanoparticles caused an increase in lipid peroxidation by 81%, 189% and 110% after 24, 48 respectively 72 hour of treatment. The reduced glutathione concentration decreased by 23.2% and 51.4% after 48 hour respectively 72 hour of exposure. An augmentation of superoxide dismutase, catalase, glutathione peroxidase, glutathione transferase and glutathione reductase within the interval between 48–72 hour was noticed. The proteic expression of Hsp 27 decreased by 50% and of Hsp 60 increased with 30% compared to control after 72 hour of treatment. Taking into account the increase of malondialdehyde level and the decrease of the reduced glutathione one and expression of the two Hsps, it can be concluded that the MRC-5 cells cannot efficiently counteract the Fe3O4 nanoparticles induced oxidative stress and pathological events could occur.

B5.34 Biochemical aspects of the cytotoxic effects of hematite nanoparticles in MRC-5 cells

M. C. Munteanu1, M. Radu1, A. I. Serban2, A. Hermenean3, C. Sima3, C. Grigoriu3, M. Costache2 and A. Dinișchiută1

1Department of Biochemistry and Molecular Biology, University of Bucharest, Bucharest, Romania, 2University of Agricultural Sciences and Veterinary Medicine, Bucharest, Romania, 3National Institute of Laser, Plasma and Radiation Physics, Bucharest, Romania, 4University of Bucharest, Bucharest, Romania

Ambient particulate matter (including nanoparticles) is an environmental factor that has been associated with respiratory morbidity and mortality. It is well known that the oxidative stress they generate induces pro-inflammatory effects in the pulmonary system. The aim of our study was to investigate the way of operation of the antioxidative system of MRC-5 lung fibroblast cell line after exposure to Fe3O4 nanoparticles for up to 72 hour. The MTT test revealed that a concentration of 12.5 micrograms of magnetite nanoparticles/ml has led to a decrease of MRC-5 cells viability of about 25% after 72 hours of exposure. The malondi-
extracellular matrix proteins. Immunoactive MMP-11 was detected in blood vessels and fibroblasts of the reactive tumor stroma. MMP-11 gene and protein expression was not upregulated after proangiogenic stimulation. Effects of MMP-11 overexpression on primary endothelial cells were studied by the use of recombinant adenoviruses. MMP-11 overexpression did neither affect in vitro proliferation of human endothelial cells nor angiogenic sprouting capacity in collagen. Although MMP-11 protein was clearly upregulated intracellularly, we did not observe an increase of the secreted active protease. To study the role of MMP-11 in tumor growth in vivo we generated MMP-11 overexpressing B16F10 melanoma cells by the sleeping beauty transposase system. B16F10 cells stably overexpressing MMP-11 secreted the active isoform of the protease into the supernatant. In comparison to mock-transfected cells, MMP-11 overexpressing B16F10 cells showed neither an increase in proliferation nor changes in expression levels of proangiogenic genes in vitro. Moreover, tumor cells were embedded in collagen and grafted into the chorioallantoic membrane of chicken embryos. In comparison to controls, MMP-11 overexpressing cells formed significantly larger tumors with strong invasion into host tissue as determined by IHC stainings for the melanoma marker S-100, alpha smooth muscle cell actin and desmin. Our data support the hypothesis, that MMP-11 secreted by stromal fibroblasts and endothelial cells supports invasion of tumor cells, tumor growth and metastasis in vivo.

B5.37
Neurotrophin 3 (NT-3) loses the immunomodulatory role in chronic diseases
D. Pasarica1, F. Toparcanu1, M. Gheorghiu1, B. Mahler1, M. Serban1, C. Berghia1, L. Iohnu2, C. Bleotu2 and C. Bara1
1IBMC, Porto, Portugal, 2Departamento de Quimica, Universidade de Coimbra, Coimbra, Portugal, 3Departamento de Química-Física, Faculdade de Farmácia, Porto, Portugal

Introduction: Chronic obstructive pulmonary disease (COPD) is characterized by the airflow limitation associated with increases of inflammatory proteins in systemic circulation. The inflammatory cells present in COPD have been shown to play important role in production of pro-inflammatory cytokines and neurotrophins who have been recognized as mediators of both inflammatory and/or airway remodeling processes. In the present work we investigated the possible correlation of NT-3 with pro-inflammatory cytokines IL-1β, TNF-α and IL-6 in serum of patients with COPD to find out if the expression of inflammatory cytokines, may be differently regulated by neurotrophins.

Patients and Methods: Twenty patients (four female and 16 male) with COPD (mean age 61.0 ± 14.19 years; range 17 to 77 years; mean smoking history, 28.4 ± 25.9 pack-years) were recruited to the study. Nine patients were classified as having stage II GOLD, seven patients as having stage III GOLD, and four patients as having stage IV GOLD. Serum samples were assessed by ELISA kits. The Pearson correlation coefficient (r) was calculated.

Results: NT-3 was positive correlated with IL-1β (r = 0.83 p = 0.05), TNF-α (r = 0.93 p = 0.05) and IL-6 (r = 0.94 p = 0.05) in patients with GOLD II. There are no correlation between these parameters in patients with GOLD III, IV.

Conclusions: Our results showed that NT-3 lost the immunomodulatory role on pro-inflammatory cytokines in patients with COPD. It suggests the potential pathophysiological role of NT-3 as mediators involved in airway inflammation and tissue remodeling in the pathogenesis COPD.

B5.38
Biological functions of Pir51
S.-C. Tsai, C.-E. Chen and P.-C. Tseng
China Medical University, Biological Science and Technology, Taichung, Taiwan

Pir51 (protein interacting with Rad51), is found highly expressed in aggressive mantle cell lymphoma (MCL) but not in indolent small lymphocytic lymphoma (SLL) patient samples. The expression level of Pir51 is regulated during the cell cycle in a pattern nearly identical to Rad51. RAD51 plays a major role in homologous recombination of DNA during double strand breaks and interacts with a lot of proteins. We find that pir51 interacts with RAD51. Therefore, we hypothesize that Pir51 exists in the protein complex. To explore the roles of the Pir51 protein complex, we used the BacterioMatch Two-Hybrid system in order to find the proteins that can interact with Pir51. After screening, we identified CDC20, one of the interesting clones. CDC20 is highly expressed in M phase of the cell cycle, and appears to act as a regulatory protein. To verify the interaction between Pir51 and CDC20, we performed IP-Western and immunostaining. Indeed, the data showed that CDC20 associates and colocalizes with Pir51. In addition, cdc20 functions as an activator of the anaphase promoting complex (APC). We are examining if Pir51 is ubiquitinated by APC and determining what roles of the ubiquitinated Pir51 protein are in the cells. In the long term goals, we will understand the biological functions of pir51, and may apply for the treatment of diseases.
Phosducin (Pd) is a regulator of G-protein-mediated signaling that is especially abundant in photoreceptors and pineal gland. In photoreceptors, Pd is phosphorylated in dark-adapted retina and undergoes dephosphorylation in response to light. Dephosphorylation of Pd allows for activation of the phototransduction cascade. Pd interacts with and regulates the activity of rhodopsin, a G protein-coupled receptor, and is involved in the response to light signals. The phosphorylation state of Pd depends on the light intensity, with dephosphorylation occurring in response to bright light. This regulatory mechanism contributes to the photoreceptor's ability to detect and respond to changes in light intensity.
Abstracts

B5.44

Human Naa50p (Nat5/San) displays both protein N- and N'-Acetyltransferase activity

R. Eivjenth1, K. Hole2, O. A. Karlsen1, M. Ziegler1, T. Arnesen2 and J. R. Lillehøj3
1Department of Molecular Biology, Bergen, Norway, 2Department of Molecular Biology, Department of Surgical Sciences and Department of Surgery, Bergen, Norway

Protein acetylation is a widespread covalent modification for eukaryotic proteins mediated by two distinct types of acetyltransferases. KATs (lysine N-acetyltransferases) modify the side chain of specific lysines on histones and other proteins, a central process in regulating gene expression, whereas NATs (N-terminal acetyltransferases) acetylate the amino group of nascent poly-peptides as they extrude from the ribosome. In yeast and humans, three different NAT complexes have been identified, NatA, NatB, and NatC. These complexes exhibit distinct substrate specificities. NatA is composed of the catalytic subunit Naa10p, and the auxiliary subunit Naa15p. In addition, Naa50p is physically associated with NatA, but its role in the complex is unknown. In fruitfly and humans Naa50p was shown to have acetyltransferase activity and play an important role in chromosome segregation. We have used recombinant purified hNaa50p and multiple oligopeptide substrates in order to identify the substrate specificity and characterize the acetyltransferase activity of hNaa50p. The results suggest that peptides with N-terminal Met-Leu-Xxx-Pro are acetylated by hNaa50p. In addition to this N-terminal acetyltransferase activity, we also found autoacetylation of hNaa50p in lysines 34, 37, and 140, which modulated protein acetyltransferase activity. Autoacetylation is consistent with a lysine N-acetyltransferase activity, and this was further supported by acetylation of histone H4 by hNaa50p in vitro. Our findings thus provide the first experimental evidence of an enzyme having both NAT and KAT activities.

B5.46

A novel cancer cell senescence-evasion mechanism involving GCIP inactivation by Rad


Sungkyunkwan University School of Medicine, Seoul, 135-230, Korea

Our work focuses on identifying factors critical to the carcinogenesis. Here, we investigated the possible role of Rad in this context. Rad is a Ras-related GTPase that promotes cell growth by accelerating cell cycle transitions. Rad knockdown induced cell cycle arrest and premature senescence without additional cellular stress in multiple cancer cells, indicating that Rad expression might be critical for the cell cycle. To investigate the precise function of Rad in this process, we used human Rad as bait in a yeast-two-hybrid screening system, and sought Rad-interacting proteins. We identified the Grap2 and Cyclin D Interacting Protein (GCIP)/DIP1/CCNDP1/HMM, a cell cycle inhibitory molecule, as a binding partner of Rad. Further analyses revealed that Rad binds directly to GCIP in vitro and coimmunoprecipitates with GCIP from cell lysates. Rad translocates GCIP from the nucleus to cytoplasm, thereby inhibiting the tumor suppressor activity of GCIP, which occurs in the nucleus. In the presence of Rad, GCIP loses its ability to reduce Rb phosphorylation and inhibit cyclin D1 activity. The function of Rad in transformation is also evidenced by increased telomerase activity and colony formation according to Rad expression level. In vivo tumorigenesis analyses revealed that tumors derived from Rad-knockdown cells were significantly smaller than those from control cells (p = 0.0131) and the pre-established tumors are reduced in size after the injection of siRad (p = 0.0064). Therefore we propose for the first time that Rad may promote carcinogenesis at least in part by inhibiting GCIP-mediated tumor suppression.
B5.48

Fish as a suitable model to unveil the function of the four and half LIM domains protein 2

S. M. Rafael1, V. Lai2, R. Schüle3 and M. L. Cancela4

1 CCMAR, University of Algarve, Faro, Portugal, 2Molecular Gynaecology Laboratory, Clinical Medical Centre, University of Freiburg, Freiburg, Germany

Four and a half LIM domains protein 2 (FHL2) is characterized by its structural LIM motifs, composed of two tandemly repeated zinc fingers, known to mediate protein-protein interactions. This particular structure is most probably responsible for FHL2 ability to function as a tissue-specific transcriptional co-regulator as well as a modulator of cytoskeleton architecture. Mammalian FHL2 gene expression is particularly abundant in the vascular system, namely in heart and blood vessels, but interestingly, knockout mice have been shown to develop an osteopenic (bone mass loss) phenotype. To better understand the physiologic relevance of FHL2, a vertebrate non-mammalian system was used. The gilthead seabream (Sparus aurata) is a marine teleost fish, economically relevant for aquaculture of Southern European countries, for which a large amount of molecular and cellular tools are available. Using a gain-of-function approach, by stably overexpressing FHL2 in sea bass VSA16 pre-osteoblast cells, we have observed an increase in matrix Gla protein (MGP) and Glarich protein (GRP) gene expression. Both genes are typically expressed in vascular smooth muscle cells while poorly expressed or absent, respectively, in wild type VSA16 cells. Overexpressing clones also exhibit a clear change of phenotype and a significant decrease in mineralization rate. Through in situ hybridization, we have identified in vivo expression of FHL2 in myoblasts, during early development as well as in adult muscle tissue. We hypothesized that FHL2 could act as a key determinant for cell fate by directing mesenchymal progenitor cells or transdifferentiating pre-osteoblast cells towards the myocyte lineage.

B5.49

Expression of chromosomal passenger protein in thyroid cancer

F. Shimamoto1, H. Tuncer2, G. Qi3, E. Aoki4, T. Takata3 and M. Tatsuka5

1Prefecture University of Hiroshima, Hiroshima, Japan, 2Department of Biophysics, Istanbul University Cerrahpasa Medical Faculty, Istanbul, Turkey, 3Department of Oral and Maxillofacial Pathology, Hiroshima University, Hiroshima, Japan, 4Department of Cellular and Molecular Biology, Hiroshima University, Hiroshima, Japan, 5Department of life science, Prefecture University of Hiroshima, Shobara, Kuwait

Survivin is a member of the inhibitor of apoptosis protein (IAP) family. Overexpression of survivin is commonly detected in various human cancers but not in normal adult tissue. The IAP function of survivin is believed to be critical for cancer progression. Survivin is also known to be a component of chromosome passenger protein (CPP) complex essential for chromosome segregation and cytokinesis as well as Aurora-B. Our previous study showed that the prognosis of colorectal cancer may depend on the localization of survivin. Here we show that by immunohistochemical analysis nuclear survivin and Aurora B overexpression in undifferentiated carcinoma of the thyroid were more frequently detected than those in papillary carcinoma and poorly differentiated carcinoma. Subcellular distribution of survivin in thyroid cancer was further demonstrated by Western blotting. Some of undifferentiated carcinomas with multiple metastasis expressed cytoplasmic and nuclear soluble survivin. These findings indicate that the expression of CPP may be deeply involved in the pathways of histogenesis of thyroid cancer, suggesting that cytoplasmic survivin may have a certain function to survive cancer cells.

B5.50

Altered ribonucleotide reductase (RNR) activity in yeast superoxide dismutase 1 (SOD1) deletion mutant

A. Keszthelyi and A. Chabes

Umea University, Department of Medical Biochemistry and Biophysics, Umea, Sweden

Deoxyribonucleoside triphosphates (dNTPs) as precursors for DNA synthesis play a fundamental role in DNA replication and repair. Their levels are precisely regulated during the cell cycle and after DNA damage, primarily by RNR, a key enzyme in dNTP biosynthesis. The transcription of the RNR subunits is highly upregulated after DNA damage and the activity of RNR is enhanced by degradation of its inhibitor, Sm1. The Sod1 protein is a cytosolic copper-zinc superoxide dismutase in yeast, which catalyzes the disproportionation of superoxide into oxygen and hydrogen peroxide and is important in antioxidative defence. Earlier, it has been demonstrated that loss of Sod1 leads to oxygen-dependent sensitivity to different DNA-damaging agents and that the induction of the RNR proteins after DNA damage was reduced in the sod1Δ mutant. We tested if lower dNTP levels in sod1Δ were responsible for the increased DNA damage sensitivity. Surprisingly, our preliminary data revealed that dNTP pools in the sod1Δ mutant were increased, even though the RNR protein levels were the same as in wild type. In this work we attempt to clarify what pathways are responsible for the elevated dNTP pools in sod1Δ and why the DNA damage checkpoint is not - or only partially - activated after damage.

B5.51

GABA-shunt enzymes mRNA expression in PMCA-depleted GH3 cells

A. Kowalski, L. Zylinska, B. Ferenc and E. Rebas

Medical University of Lodz, Lodz, Poland

Plasma membrane calcium ATPase (PMCA) is an enzyme maintaining low cytosolic calcium concentration. Our previous study on PC12 cells showed that reduction of neuron-specific isoforms - PMCA2 and PMCA3 resulted in the increase of intracellular calcium level and changed GABA metabolism under oxidative stress. In the present work we focused on rat anterior pituitary tumor GH3 cells – a widely used model in studies concerning calcium homeostasis. Using two stably transfected GH3 cell lines with suppressed expression of PMCA2 and PMCA3 we examined the intracellular calcium concentration and mRNA expression of all PMCA isoforms. Also, we determined the expression of mRNAs of GABA-shunt enzymes: glutamate decarboxylase, GABA-transaminase and succinic semialdehyde dehydrogenase. This pathway is considered to be one of protective mechanisms mobilized in response to stress conditions. Real-time PCR analysis revealed that expression of PMCA2 and PMCA3 mRNAs decreased up to 50% in the transfected GH3 cells. However, a significant increase of PMCA4 expression in PMCA3-suppressed cells indicated on some compensatory mechanism. The mRNA of GABA-shunt enzymes was altered only in PMCA3-suppressed cells: about 50% increase of GAD65 and about 80% decrease of SSADH. The reduction of PMCA isoforms caused increase of
Abstracts

B5.52
Study of the interaction between furosemide and human carbonic anhydrase II by spectroscopic methods
S. Ranjbar1, S. Ghobadi1 and R. Khodarahmi2
1Department of Biology, Razi University, Kermanshah, Islamic Republic of Iran, 2Department of Pharmacognosy and Biotechnology, and Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Islamic Republic of Iran

Human carbonic anhydrase (hCA, EC 4.2.1.1) is a Zn-dependent enzyme which catalyzes reversible interconversion of carbon dioxide to bicarbonate, with a proton release. In this study, the interaction of furosemide, an efficient diuretic and a sulfonamide drug, with hCA II was investigated by various spectroscopic methods such as intrinsic and extrinsic fluorescence, UV-Vis and circular dichroism (CD) spectroscopy, in 50 mM Tris buffer, pH 7.75. Fluorescence data indicated that furosemide acts as a potent quencher of hCA II emission. The Stern-Volmer analysis of quenching data at different temperatures elucidated that the quenching of intrinsic fluorescence of hCA II is occurred through a static quenching mechanism. Analysis of the thermodynamic parameters of binding indicated that hydrogen bonding and electrostatic forces play the major role in the interaction of furosemide with hCA II. Calculation of the protein surface hydrophobicity (PSH), using 1-anilinonaphthalene-8-sulfonic acid (ANS), indicated the decrement of PSH of hCA II in the presence of furosemide. The job’s plot confirmed that the binding of furosemide to the enzyme occurred via a 1:1 stoichiometry. The Far-UV CD results showed that furosemide caused slight increment in the α-helix content of hCA II whereas near-UV CD experiments in the presence of the drug indicated some decrement in the flexibility of the enzyme tertiary structure. Binding of furosemide to hCA II is accompanied with quenching the intrinsic fluorescence of the enzyme, decreasing the PSH of the complex, inducing some α-helical structure in the secondary structure of the enzyme and reducing its tertiary structure flexibility.

B5.53
Identification of a molecular switch regulated by phosphorylation in E2 ubiquitin-conjugating enzymes
E. Papaleo, P. Cocetti, V. Ranzani, F. Tripodi, I. Valimberti, C. Cirulli, P. Fantucci, L. Alberghina, M. Vanoni and L. De Gioia
University of Milano-Bicocca, Milano, Italy

Post-translational phosphorylation is an ubiquitous mechanism for cellular regulation and it is known it can modulate the proteins conformation by changing their energy landscape which is largely driven by the electrostatic perturbation induced by the phosphate group (1). Recently, it has been shown that several enzymes involved in the ubiquitination pathway can be affected by phosphorylations (2–4), as ubiquitin ligases and E2 ubiquitin conjugating enzymes. In the present contribution, we provide through molecular dynamics simulations, homology modelling, protein-protein docking, sequence comparisons and phylogenetic analysis of known E2 ubiquitin-conjugating enzymes, and biochemical assays, the first model of the structural effects and regulation mechanisms mediated by phosphorylation of the catalytic domain of E2 enzymes. Our investigation suggest that phosphorylations at catalytic domain of E2 ubiquitin conjugating enzymes can stabilize the whole protein structure and, in particular promote a conformation competent for ubiquitin binding of a loop in the surrounding of the catalytic cleft, which modulate the accessibility of the catalytic site.

References:

B5.54
Purification of the rat kidney glutathione reductase
B. Can1, G. Kalaksiz Erkmen1, O. Dalmizrak2, I. H. Ogus3 and N. Ozer2
1Department of Biochemistry, Faculty of Medicine, Hacettepe University, Ankara, 2Department of Basic Medical Sciences, Faculty of Medicine, Near East University, Mersin, 4Department of Biochemistry, Faculty of Medicine, Hacettepe University, Ankara, Turkey

Glutathione reductase (GR, E.C. 1.8.1.7, NADP oxidoreductase) is a member of flavoenzyme family. It catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) in the expense of nicotinamide adenine dinucleotide phosphate (NADPH). Pentose phosphate pathway is the main source of NADPH. GSH is the most abundant antioxidant in cells, its concentration is 100 times more than GSSG. GSH and GSSG forms thiol buffer and GSH fights against reactive oxygen species (ROS). Glutathione reductase (GR) is the main enzyme to maintain the thiol buffer system against oxidative stress. Thus, GR is one of the most important enzymes against oxidative damage. GR was purified and characterized from various sources but, according to our knowledge, there is no report on the purification of GR from rat kidney. In this study, rat kidney GR was purified 2356 fold with a yield of 16% by conventional chromatographic methods; Sephadex G-25 gel filtration, 2 dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE). The specific activity and the subunit molecular mass, Mr, of the purified GR were 250 units per milligram protein and 53 kDa, respectively.

B5.55
Comparative transcriptome and proteome analysis for the effect of berberine
C. Ozbalci1, K. Karaosmanoglu1, I. Aksan Kurnaz2, D. Kazan3 and B. Sirayir Akbulut1
1Marmara University, Bioengineering, Istanbul, Turkey, 2Yeditepe University, Bioengineering, Istanbul, Turkey, 3Marmara University, TUBITAK, Bioengineering, Genetic Engineering and Biotechnology Institute, Istanbul, Kocaeli, Turkey
The emergence of widespread drug resistance of pathogenic bacteria is a major threat to public health. In this respect, products derived from natural sources serve as invaluable sources for the development of novel anti-microbial agents. Berberine is an important plant derived alkaloid found in many medicinal plants including Hydrastis canadensis (goldenseal), Coptis chinensis, Berberis aquifolium, Berberis aristata, and Tinospora cordifolia, with medicinal uses focused on sub-acute and chronic inflammations including gastric disorders, respiratory affections and cancer during the 1800 second. In the late 1900 second, berberine was evaluated as a natural antibiotic to treat various bacteria-associated diarrheas. In the light of this information, the molecular response of Escherichia coli for the anti-microbial berberine has been investigated using comparative transcriptome and proteome analysis. Microarray analysis has been performed to study the transcriptional profiles of the cells exposed to berberine for identification of genes that were induced in response to the anti-microbial agent. 2-dimensional gel electrophoresis followed by mass spectrometric analysis has been performed for the characterization of differentially expressed proteins of the cells treated with berberine. Proteins and genes repressed or induced in the presence of berberine included those that are involved in transport and binding, membrane repair and maintenance, proteins synthesis, energy metabolism, regulation and replication. In addition, the comparative analysis of the transcriptome and proteome showed that there is significant correlation between them.

B5.56
Characterization of glutathione reductase purified from rat kidney
B. Can1, G. Kulaksiz Erkmen1, O. Dalmizrak2, I. H. Ogus1 and N. Ozer1
1Department of Biochemistry, Faculty of Medicine, Hacettepe University, Ankara, 2Department of Basic Medical Sciences, Faculty of Medicine, Near East University, Mersin, Turkey

Glutathione reductase (GR, E.C. 1.8.1.7) is a flavoenzyme which belongs to the pyridine nucleotide: disulfide oxidoreductase family. It reduces oxidized glutathione (GSSG), using nicotinamide adenine dinucleotide phosphate (NADPH), formed mainly by glutathione peroxidases. The final product, reduced glutathione (GSH) is the major component of thiol buffer ([GSH]/[GSSG] = 100/1) and it is used fighting against oxidative stress created by the reactive oxygen species (ROS). Glutathione reductase is responsible from the maintenance of thiol buffer in cells. Recently, GR was purified from rat kidney, using conventional chromatographic procedures; Such as gel filtration, affinity chromatography and ion exchange chromatography. In this study, it was aimed to characterize some features of rat kidney GR. The optimum pH and temperature were found to be 6.5 and 65°C, respectively. These data were in accordance with the previous studies characterizing GR from other sources. The activation energy, Ea, and temperature coefficient, Q10, for GR purified from rat kidney, were found to be 7.02 Kcal/mole and 1.42, respectively. The Michaelis-Menten constants were determined in 0.1 M phosphate buffer at pH 7.4 and 37°C. The Km(NADPH), at fixed [GSSG], 1 mM, and varied [NADPH], 20–1200 μM, and the Km(GSSG), at fixed [NADPH], 0.1 mM, and varied [GSSG], 10–200 μM, were found to be 15.3 ± 1.4 and 53.1 ± 3.4 μM, respectively.

B5.57
Defining the mitotic functions of San/Naa50 during Drosophila development
A. Pimenta-Marques1, P. Van Damme2, K. Gevaert3, T. Arnesen1 and R. Martinho4
1Gulbenkian Institute for Science, Rua da Quinta Grande, Oeiras, Portugal, 2VL1 Institute; Ghent University, Department of Medical Protein Research; Department of Biochemistry, Ghent, Belgium, 3University of Bergen, Department of Molecular Biology, Bergen, Norway, 4Gulbenkian Institute for Science, Early Fly Development, Oeiras, Portugal

N-terminal acetylation is a highly conserved and widespread protein modification. Increasing amount of data has linked this modification to cell cycle progression and cancer. Yet, little is known about the biological role of N-terminal acetylation during development. Others and we showed that san (separation anxiety), which encoded an N-terminal acetylated transferase (NAT), was essential for mitosis in Drosophila (in vivo and in vitro) and in HeLa cells (in vitro). San function was originally associated to centromeric sister chromatid cohesion. However our work suggested that San was also important for chromosome condensation/resolution, implying a more general mitotic function. Although the NAT activity of San is known to be required for its mitotic function, the relevant in vivo substrates are still unknown. We hypothesize that San regulates the activity (or stability) of unknown proteins whose function is crucial for mitosis. In order to identify these proteins we are currently using COF-RADIC proteomics technology. Preliminary results comparing the N-terminal acetylation profile of san mutant and wild type embryos identified eighteen in vivo substrates of San. Two of these proteins are required for mitosis and we are currently investigating if the San-dependent N-terminal acetylation is important for their mitotic function. Our long-term goal is to define the mitotic functions of San and other related NATs, and better understand the role of N-terminal acetylation during development.

B5.58
Biophysical studies on the structure and function of molecules from the vertebrate myelin sheath
C. Wang1, M. Myllykoski2 and P. Kursula2
1University of Oulu, Department of Biochemistry, Oulu, Finland, 2DESY, CSSB-HZI, Hamburg, Germany

Myelin is an insulating multilamellar membrane structure wrapped around selected axons in the vertebrate nervous system, allowing the rapid transmission of nerve impulses. Biochemically, myelin is a highly specialized plasma membrane produced by myelinating glial cells, harbouring several unique proteins and a specific lipid composition. De- or dysmyelination, caused e.g. by mutations in myelin-related genes or an autoimmune disease, can lead to severe neurological problems. Our goal is to use biophysical, biochemical, and structural biology methods to obtain information on the structure-function relationships in purified myelin proteins, and complexes thereof. Specifically, we mainly employ several synchrotron-based methods (X-ray crystallography, small-angle X-ray scattering, synchrotron radiation CD spectroscopy) together with complementary experiments, such as neutron scattering, fluorescence and oriented CD spectroscopy, chromatographic methods, calorimetry, surface plasmon resonance, enzyme kinetics, and light scattering to elucidate the details of molecular structure and interactions in myelin proteins. Eventually, we hope to better understand the details of the tightly packed myelin structure.
Abstracts

B5.59 Molecular properties of γ-glutamyl transpeptidases adapted to extreme environments
I. Castellano1, A. Di Salle1, A. Merlino2, M. Rossi1 and F. La Cara3
1Institute of Protein Biochemistry, C.N.R., Life Science, Naples, Italy, 2Department of Chemistry, University of Naples ‘Federico II’, Naples, Italy

γ-Glutamyl-transpeptidase (γ-GT) is an ubiquitous extracellular enzyme that plays a key role in glutathione metabolism. γ-GT catalyzes the hydrolysis of γ-glutamyl bonds in glutathione and glutamine and the transfer of the released γ-glutamyl group to amino acids and short peptides. γ-GT is synthesized as a precursor protein of 60 kDa, undergoing an intramolecular auto-cleavage to yield two subunits of 40-kDa and 20-kDa, respectively. However, the three extremophilic enzymes display γ-glutamyl hydrolase activity, but no transpeptidase activity. The comparison of their sequences and structural models with those of other bacterial and eukaryotic transpeptidase activity. The comparison of their sequences and eukaryal counterparts. Moreover a phylogenetic analysis suggests that several amino acids and short peptides could be used for molecular evolution for enhancing the stability of proteins.

B5.60 Phosphorylation is switch of L-PK cooperativity
I. Faustova1, A. Kuznetsov2, M. Loog3 and J. Jaak1
1University of Tartu, Institute of Chemistry, Tartu, Estonia, 2University of Tartu, Institute of Technology, Tartu, Estonia

Pyruvate kinase (ATP-pyruvate 2-O-phosphotransferase, EC 2.7.1.40) is the key enzyme in glycolysis and modification of its catalytic properties is critical for regulation of this metabolic pathway. There are four pyruvate kinase isoforms: M1, M2, R and L, whereas only M1 is considered as a non-allosteric enzyme. In our work we have shown that not only M1 is non-allosteric enzyme, but also non-phosphorylated L-type pyruvate kinase (L-PK) shows hyperbolic kinetics toward its substrate phosphoenolpyruvate (PEP) and can be characterized by hyperbolic Michaelis-Menten plot and KPEP = 0.11 mM. The L-PK is the only one isoenzyme which activity can be regulated by phosphorylation. It takes place at Ser(12)-Val(13). Phosphorylation of L-PK by cAMP-dependent protein kinase changes the catalytic properties of pyruvate kinase. We have discovered that the non-phosphorylated enzyme is not allosterically regulated and through phosphorylation the enzyme obtains cooperativity. This means that phosphorylation is a molecular switch between non-allosteric and allosteric forms of the enzyme. The phosphorylated allosteric enzyme was characterized by KADP = 0.1 mM, KPEP = 2.2 mM, and the Hill coefficient n = 2.5. Also it was observed that phosphorylation of the first subunit of the tetrameric enzyme switches on the allosteric mechanism, while further phosphorylation only modulates this effect. The discovery that phosphorylation is a switch between allosteric and non-allosteric states of L-PK seems to be important for understanding the interrelationship between allostery and the regulatory phosphorylation in general.

B5.61 C-terminal segment of yeast BMH proteins exhibits different structure compared to other 14-3-3 protein isoforms
D. Vlčová1, L. Rezáková2, M. Stepanek2, P. Herman3, J. Vexer1, T. Obsil1 and V. Obsilová1
1Institute of Physiology Academy of Sciences of the Czech Republic, Prague, Czech Republic, 2Faculty of Mathematics and Physics, Institute of Physics, Charles University, Prague, Czech Republic

14-3-3 proteins are abundant binding proteins involved in many biologically important processes. 14-3-3 proteins bind to other proteins in a phosphorylation-dependent manner and function as scaffold molecules modulating the activity of their binding partners. Yeast 14-3-3 protein isoforms BMH1 and BMH2 posses a distinctly variant C-terminal tail which differs them from the isoforms of higher eukaryotes. Their C-termini are longer and contains polyglutamine stretch of unknown function. It is now well established that the C-terminal segment of 14-3-3 proteins plays an important regulatory role by functioning as an autoinhibitor which can occupy the ligand binding groove and blocks the binding of inappropriate ligands. Whether the same holds true or not for the yeast isoforms is unclear. Therefore, we investigated the conformational behavior of the C-terminal segment of BMH proteins using various biochemical techniques. Dynamic light scattering, time-resolved fluorescence anisotropy decay and size exclusion chromatography measurements showed that the molecules of BMH proteins are significantly bigger compared to the human 14-3-3 zeta isoform. On the other hand, the sedimentation equilibrium analysis confirmed that BMH proteins form dimers. Time-resolved tryptophan fluorescence experiments revealed no dramatic structural changes of the C-terminal segment upon the ligand binding. Taken together, the C-terminal segment of BMH proteins adopts very open and extended conformation that increases their apparent molecular size. It seems, therefore, that the C-terminal segment of BMH proteins does not function as an autoinhibitor and does not block their ligand binding grooves.
N-α-terminal protein acetylation is one of the most common protein modifications in eukaryotic cells, occurring on approximately 80% of soluble human proteins. There are not many identified cases indicating the biological relevance of this protein modification but there is an increasing number of studies linking N-α-terminal acetylation to cell differentiation, cell cycle, cell survival, and cancer. The three major N-acetyltransferase complexes (NAT), NatA, NatB and NatC, are composed of a catalytic subunit and one or several accessory subunits, modifying a defined subset of cellular proteins in each one. NatB complex is characterized by acetylating proteins with Met-Asp, Met-Glu and Met-Gln termini. It consists of the catalytic subunit, Naa20p, and one accessory subunit, Naa25p, that are associated to the ribosome. It has been observed that this enzyme catalyzes N-terminal acetylation of actin and tropomyosin in yeast, being this modification necessary for a proper function of yeast actin cytoskeleton. It has also been demonstrated that mammalian actin and tropomyosin N-terminal acetylation is important for their proper biochemical activity. Therefore, we have analyzed hNatB downregulation function on mammalian actin cytoskeleton function. We have observed that reduction of any of hNatB subunits, hNaa20 and hNaa25, expression induces actin stress fibers disorganization, thus, affecting cell mechanics. This is accompanied by a diminution of cellular focal adhesions and a decrease in cellular motility.}

B5.64 dNTP pool imbalances: Mechanism of mutagenesis and effects on cell cycle

D. Kumar1, A. L. Abdulovic2, J. Viberg1, A. K. Nilsson1, T. A. Kunkel2 and A. Chabes1
1Department of Medical Biochemistry and Biophysics, Umeå University, Umeå, Sweden, 2National Institutes of Environmental Health Sciences, National Institutes of Health, Laboratory of Molecular Genetics and Laboratory of Structural Biology, DHHS, Research Triangle Park, NC, USA

Deoxyribonucleoside triphosphates–dNTPs (dATP, dTTP, dGTP, and dCTP)–are the building blocks of DNA and their balanced supply is essential for faithful genome duplication. Both the overall concentration and the balance among the individual dNTPs are tightly regulated, primarily by the enzyme ribonucleotide reductase (RNR). We introduced single amino acid substitutions in loop 2 of the allosteric specificity site of Saccharomyces cerevisiae RNR and obtained a collection of yeast strains with different but defined dNTP pool imbalances. We used these strains to study the mechanisms by which dNTP pool imbalances induce genome instability. Mutagenesis is enhanced even in the strains with mildly imbalanced dNTP pools, despite the availability of the two major replication error correction mechanisms: proof-reading and mismatch repair. However, the mutagenic potential of different dNTP pool imbalances does not directly correlate with their severity and the locations of the mutations in a strain with elevated dTTP and dCTP are completely different from those in a strain with elevated dATP and dGTP. We further investigated whether dNTP pool imbalances interfere with cell cycle progression and are detected by the S-phase checkpoint, a genome surveillance mechanism activated in response to DNA damage or replication blocks. The S-phase checkpoint is activated by the depletion of one or several dNTPs. In contrast, when none of the dNTPs is limiting for DNA replication, even extreme and mutagenic dNTP pool imbalances do not activate the S-phase checkpoint and do not interfere with the cell cycle progression.
Abstracts

B5.66 Determination of novel tumor markers in prostate carcinoma

M. Masařík1, J. Gumulec1, Š. Kuchtíková1, V. Kudláčková1, M. Jurád1, D. Pavlík2, A. Rovný3, R. Hrabec3, S. Kolčívá4 and R. Kízek4

1Department of Pathological Physiology, Masaryk University, Faculty of Medicine, Brno, Czech Republic, 2Department of Microelectronics, University of Technology, Brno, Czech Republic, 3Department of Urology, St. Anne’s University Hospital, Brno, Czech Republic, 4Department of Chemistry and Biochemistry, Mendel University, Brno, Czech Republic

Prostate cancer is second leading cause of death among men. In the present time, prostate specific antigen (PSA) is the only tumor marker used for carcinoma detection. This tumor marker has high level of sensitivity, but lower level of specificity, so it can be increased either in inflammation or in prostate hyperplasia. It is desirable to find a new markers with higher specificity. Metallothionein (MT) and alpha-methylacyl-CoA racemase could be such markers. Expression of both of these proteins is highly unregulated in prostate cancer. MT levels are elevated most likely due to disbalance of zinc metabolism in this disease, and reason of elevation of AMACR is still unknown. In our work we analyzed levels of MT, and AMACR in cell lines derived from prostate carcinoma – LNCaP, PC-3 and 22RVL, which were compared to prostate cell line PNT1A derived from normal prostate epithelium. For analysis we used immunoseparation techniques, western blotting and SDS-PAGE. In addition to this classical methods of molecular biology we used novel electrochemical detection and capillary electrophoresis on the chip. We found significant elevation of MT, AMACR and PSA in prostate tumor cell lines similarly as in real blood samples from patients with prostate carcinoma. This study provides important new information about expression of MT, PSA and AMACR in prostate tumor cell lines and describes application of electrochemical methods for their detection. Acknowledgement: Financial support from GACR 301/09/P436 and NS10200-3 is greatly acknowledged.

B5.67 Overexpressing ‘difficult proteins’ in escherichia coli

D. Vikström

CBR-Center for Biomembrane Research, Stockholm, Sweden

E. coli is the most widely used vehicle for the overexpression of proteins. Protein overexpression is usually driven by the T7 RNA polymerase. Finding the right strain and optimal conditions for the overexpression of a protein can be very time consuming and cumbersome. Therefore, we have developed a generic solution for E. coli based protein overexpression, the so-called Lemo21(DE3) strain. In this strain T7 RNA polymerase activity can be modulated precisely by its natural inhibitor T7 lysozyme, which is expressed from the titratable rhamnose promoter. The Lemo21(DE3) strain conveniently allows optimizing the overexpression of any given protein using only a single strain rather than a multitude of strains and is compatible with any pET vector. The Lemo21(DE3) strain is the platform of choice for the successful overexpression of membrane proteins and other ‘difficult-to-express’ proteins. In addition, we have developed a tag that forces the overexpression of any given protein in inclusion bodies. This tag greatly facilitates the expression and isolation of e.g., very toxic proteins and antigens.

B5.68 A subgroup of Ulcerative colitis patients with active disease has an altered MUC2 O-glycosylation pattern in sigmoid colon

J. Holmen Larson1, H. Karlsson1, J. Gräberger Crespo2, M. Johansson1, L. Eklund1, H. Sjövall2 and G. Hansson3

1University of Gothenburg, Medical Biochemistry and Cell biology, Gothenburg, Sweden, 2University of Gothenburg, Internal medicine, Gothenburg, Sweden

The mucus barrier plays a key role in colonic barrier function. Glycosylated mucins form a matrix that prevents entry of bacteria. One possible mechanism behind impaired barrier function in ulcerative colitis is a defective glycosylation pattern. Thus, novel glycoproteomic methods were used to study sigmoid colon biopsies obtained from ulcerative colitis patients (active and inactive disease), and control patients (n = 50). Mucins representing the inner attached mucus layer of colon were extracted from single biopsies and further separated by SDS-polyacrylamide/agarose composite gel electrophoresis. The three major mucin bands were tryptic digested and identified as oligomeric forms of MUC2 by proteomics. MUC2 is a highly glycosylated mucin and the major component of both mucus layers in the colon. The MUC2 O-glycans of the sigmoid colon were analyzed by nanoLC/mass spectrometry and more than 100 complex O-linked oligosaccharides were identified, mainly based on core 3 structures. A high degree of sialylation was observed and the substructure Galβ1-3/4-GlcNAcβ1-3 NeuAcα-6GalNAcβol was found in most glycans. The glycans present in more than 10 of the patients (26 glycans) were also semiquantified. In contrast to the O-glycans of other mucins, the sigmoid MUC2 O-glycan repertoire and relative amounts in normal individuals were relatively constant. However, a subpopulation of patients showed an accumulation of precursor glycans with a decrease of more complex glycans. This pattern was mainly found in active colitis but some patients in this group also had the normal pattern. The altered glycan pattern may contribute to deterioration of the barrier function in active colitis.

B5.69 Apoptosis-related cofilin phosphorylation at Ser3 is independent of ROCK activity

K. Kůželová, M. Pluskalová, D. Grebebová and Z. Hrkal

Institute of Hematology and Blood Transfusion, Prague, Czech Republic

The interactions of hematopoietic cells with the extracellular matrix (ECM) not only mechanically retain the immature cells in the bone marrow microenvironment but also regulate the activity of different intracellular signal cascades. We found that several anti-leukemic drugs (e.g. SAHA, Imatinib) modulated the cellular adhesiveness to fibronectin and we performed a proteomic analysis of JURL-MK1 cell line to uncover the proteins whose expression
level and/or posttranslational modifications are affected by these drugs. In this contribution, we focus mainly on collagen, the pivotal mediator of actin dynamics, whose activity is regulated by phosphorylation at Ser3. The phosphorylated (inactive) collagen was concentrated in small distinct spots distributed throughout the cytoplasm. The fraction of collagen phosphorylated at Ser3 was elevated by the cell treatment with adhesivity-stimulating drugs, in agreement with the need for preservation of strong actin structures. Surprisingly, collagen phosphorylation level also increased when apoptosis was triggered, in spite of a marked F-actin disassembly. We speculate that some prominent actin structures have to be protected from collagen-mediated destruction in order to assure the cell disintegration into apoptotic bodies. The apoptosis-related increase in collagen phosphorylation was at least partly inhibited by the caspase inhibitor Q-VD-OPh but not by Y-27632, an inhibitor of the protein ROCK which is known to play a major role in collagen regulation. This work was supported by the grant No 301/09/1026 from the Grant Agency of the Czech Republic and by the grant MZ0UHKT2005 from the Ministry of Health of the Czech Republic.

**B5.70** Nuclear localization of hepatic FBPase is impaired in the liver from fasted rats and mice


Universidad Austral de Chile, Biochemistry, Valdivia, Chile

Here, we find that the subcellular distribution of hepatic fructose 1,6-bisphosphatase (FBPase) is modulated by the nutritional state and diabetes, whereas the liver zonation was not affected. In healthy starved rats, FBPase was mainly localized in the cytoplasm of hepatocytes, adopting a uniformly pattern. However, in healthy re-fed rats, its subcellular localization dramatically changed and concentrated in the nucleus and the cell periphery. Interestingly, in hepatocytes of streptozotocin-induced diabetic rats FBPase was unable to translocate to the nucleus, indicating that insulin and not hyperglycemia plays a crucial role in the nuclear concentration of the enzyme. Moreover, hepatic FBPase was found phosphorylated only in the cytoplasm of both healthy and diabetic rats, suggesting that the phosphorylation state is involved in the nuclear translocation of FBPase. Despite the difference in primary structure between rat and mouse liver FBPase, the mouse enzyme showed a similar behaviour. Moreover, hepatic fructose 1,6 bisphosphate aldolase (aldolase B) presented virtually the same dynamic that FBPase in the liver of fasted and re-fed healthy rats and mice. The impaired nuclear translocation and the cytosolic FBPase concentration could account for the increased endogenous glucose production of liver of diabetic rats (Grants Funding: Fondecyt 1090740; 1090694; DID-UACh 2006-12; Fundacion Marcelino Botin).

**B5.71** Inadequate heat shock response of Mesothelial cells caused by Glucose degradation products contained in Peritoneal dialysis fluids

K. Kratochwill,1 H. Lederhuber1, R. Herzog2, A. Lichtenauer1, E. Salzer3 and C. Aufricht1

1Medical University of Vienna, Department of Pediatrics, Vienna, Austria; Zytosrotec GmbH, Vienna, Austria

Peritoneal dialysis (PD) is a convenient and cost-effective alternative to hemodialysis and the predominant treatment modality in pediatric patients. For decades glucose-based PD fluids (PDF) have been used in clinical practice. Because of their physicochemical properties these fluids harm the mesothelial cell (MC) layer. Glucose degradation products (GDP) are regarded as the major toxic compounds of conventional PDF, but their mode of cytopathic action is yet unclear. Heat shock proteins (HSP) have been shown to essentially contribute to mesothelial cell protection and repair following PDF exposure in *in-vitro* and *in-vivo* models. Using a proteomics and bioinformatics approach we could identify involved biological processes and molecular pathways leading to MC injury. In this study MC were exposed to PDF with or without GDP at normal culture conditions or elevated temperature (42°C) for varying exposure times. Cellular damage or viability was assessed using standard assays and HSP expression was measured using Western blot analysis. For deeper biological insight proteomic and transcriptomic analyses were performed. Our results showed increased cell damage of MC following exposure to PDF containing GDP, whereas HSP expression was not further elevated. Under heat stress conditions MC were only able to induce HSP when GDP were not present. Proteomic analysis confirmed a dampening effect of GDP on HSP expression over a range of normally stress-inducible chaperones. In follow-up experiments we could assign this effect to defined chemical compounds occurring in commercial PDF. This finding represents a novel pathomechanism, likely relevant in several glucose-associated diseases.

**B5.72** Serglycin interacts with type-1 collagen and affects the expression of matrix metalloproteinases from myeloma plasma cells

A. Skliris,1 V. Labropoulou2, M. Borset1 and A. Theocharis1

1University of Patras, Dep. of Chemistry, Patras, Greece; 2University of Patras, Dep. of Medicine, Patras, Greece; 3Institute of Cancer Research and Molecular Medicine, Trondheim, Norway

Serglycin is a small proteoglycan which can carry four to eight chains of chondroitin sulphate, which is the major serglycin form expressed by cells of hematopoietic origin. We have previously showed that serglycin is constitutively secreted but also present on the cell surface of myeloma plasma cells. Moreover, multiple myeloma, a B-cell malignancy, is characterized by clonal proliferation of plasma cells within the bone marrow, impaired hematopoiesis and osteolysis. The degradation of the extracellular matrix by proteolytic enzymes, such as matrix metalloproteinases, plays an important role in tumor progression, invasion, metastasis and bone remodeling. The aim of this study was to examine the role of serglycin, found on the membrane of multiple myeloma cells, as a surface receptor responsible for binding to type-I collagen and to analyze the expression of matrix metalloproteinases mediated through this interaction. Our results derived from solid phase binding assays showed that purified serglycin from multiple myeloma cell lines, has the ability to interact with type-I collagen. This interaction is mediated through the chondroitin sulphate chains of serglycin as after digestion with chondroitinase ABC the binding with collagen was decreased. In addition we have tested the ability of three different multiple myeloma cell lines to adhere to type-I collagen coated mictotiter plates in cell adhesion assays. Multiple myeloma cells can attach to type-I collagen suggesting a role for serglycin found on the cell surface of these cells. Finally we have showed that the expression of matrix metalloproteinases was affected by this interaction.

**B5.73** Abstract withdrawn
C1 – Molecular Machines

C1.01 Exploring the role of a conserved motif in the adenylation domain of a non-ribosomal peptide synthetase from Bacillus brevis
V. Bacevic-Popovic, M. Sprung, B. Soldo, S. Orhanovic and M. Pavela-Vrancic

Non-ribosomal peptide synthetases (NRPS) are molecular machines that synthesize peptide-based natural products, many of which are important pharmaceuticals with antibiotic, immunosuppressor and antitumor activities. A typical NRPS assembly line consists of a number of catalytic modules, each responsible for the incorporation of a single amino acid into the growing peptide product. Modules themselves comprise distinct catalytic domains, such as the adenylation domain (A) that, firstly, selects a specific amino acid monomer to be activated by ATP and, secondly, transfers it to the phosphopantetheinylation arm of the adjacent thiolation domain. Recent biochemical and structural evidence support the idea that the A domain uses a rotational movement of its two subdomains to adapt a single active site for the two partial reactions in the catalytic cycle. It has long been recognized that A domains share a set of 10 conserved motifs (A1-A10), most of which were assigned a particular role in subfunctional movement of the subdomains, the A9 motif drastically changes its position relative to the active site. The role of the conserved amino acid residues in this region and its importance for the two partial reactions of the catalytic cycle was examined by site-directed mutagenesis and biochemical characterization of mutant enzymes.

C1.02 Decavanadate effects in actin structure and function
S. Ramos1, J. J. G Moura2 and M. Aureliano1

Decameric vanadate (V10) is a vanadium (V) oligomer known to interact with many proteins and to affect their structure/function (Aureliano, 2009). Particularly with actin, this interaction prevents G-actin polymerization into F-actin filaments whereas decavanadate molecule is stabilized during the process, avoiding their decomposition into monomeric vanadate, V1 (Ramos et al, 2006). More recently, it was observed that upon incubation with decavanadate, protein cysteine oxidation occurred in G- and F-actin, whereas no effects were detected with V1 (Ramos et al, 2009). Moreover, only with V10 a concomitant vanadate reduction to vanadyl (vanadium (IV)) was observed by EPR (Ramos et al, 2009). These studies were further explored and it was observed that both cysteine oxidation and vanadate reduction are blocked by ATP. On contrary to V1, V10 up to 500 micraM (meaning 50 micraM decameric vanadate) induces an increase in G-actin intrinsic fluorescence, suggesting a change in protein conformation leading to tryptophan exposure to the solvent. An increase of actin hydrophobicity upon V10 incubation was confirmed by using the fluorescence probe ANSA. Upon incubation of F-actin with increasing V10 concentrations, a decrease of intrinsic fluorescence was detected, suggesting protein inactivation. Nucleotide exchange experiments point out that only decavanadate affects ATP interaction with actin. Putting it all together it is suggested that: (i) decavanadate interaction with actin induces cysteine oxidation and vanadate reduction being these effects prevented by ATP; (ii) decavanadate promotes actin conformational changes; iii) decavanadate have an effect on actin ATP binding site.

C1.03 Mycobacterial ubiquitin-like targeting – tagged for destruction
F. Striebel, M. Sutter, F. Inkamp, F. Damberger, E. Guth and E. Weber-Ban
ETH Zurich, Institute of Molecular Biology & Biophysics, Zurich, Switzerland

In eukaryotes, proteasomes represent the main degradation route for cellular proteins, however, in bacteria, the occurrence of the 20S proteasome is limited to the group of actinobacteria, amongst them the highly pathogenic Mycobacterium tuberculosis (Mtb). Actinobacteria use a tagging system functionally analogous to eukaryotic ubiquitylation, termed pupylation, to recruit substrates for proteasomal degradation. The pupylation-degradation pathway contributes to the virulence of Mtb supporting its persistence and proliferation inside the host macrophages. We have recently reconstituted and characterized the pupylation pathway that leads to conjugation of the prokaryotic ubiquitin-like protein (Pup) to proteasomal substrate proteins. We showed that Pup is rendered coupling-competent by deamidation of its C-terminal glutamine through the action of a previously uncharacterized protein we now call Dop (deamidase of Pup), then PafA catalyzes formation of an isopeptide bond between the glutamate and a substrate lysine. The latter step turns over one ATP per coupled Pup, suggesting that Pup is activated for conjugation via phosphorylation of its C-terminal glutamate. We have also investigated the events downstream of the pupylation pathway, which lead to degradation of pupylated substrate proteins by the mycobacterial proteasome complex. Mechanistic and structure/function principles of the complete pupylation-degradation pathway will be presented and in vivo relevance will be discussed.

C1.04 Abstract withdrawn

C1.05 Ceramide down-regulates the Na+/K+ ATPase in HepG2 cells
Z. Dakroub and S. Kreydiyyeh
American University of Beirut, Biology, Beirut, Lebanon

We showed previously that TNF-α down-regulates the Na+/K+ ATPase in HepG2 cells. TNF-α is known to cause the release of ceramide which enters the sphingomyelin pathway where it gets modified by several enzymes to other sphingolipids such as sphingosine, sphingosine 1-phosphate (S1P) and ceramide 1-phosphate (C1P) some of which have antagonistic effects to ceramide. The ratio of ceramide to the other sphingolipids determines the final outcome of the cell response. This work was
C1 – Molecular Machines

undertaken to study the role of ceramide and its metabolites on the Na+ / K+ ATPase in HepG2 cells. Cells were incubated for 15 minute with ceramide in presence and absence of CAY 10466 or SHK1, respective inhibitors of ceramidase and sphingosine kinase (SphK). The activity of the pump was assayed by measuring the amount of inorganic phosphate liberated, while changes in its expression were monitored by western blot analysis. Ceramidase reduced the activity and protein expression of the Na+ / K+ ATPase. This effect disappeared when ceramidase and sphingosine kinase were inhibited, suggesting that the effect of ceramide is mediated via sphingosine or sphingosine 1-phosphate. However, SphK1 added alone to cells, up-regulated the pump, revealing a stimulatory effect of the accumulated endogenous sphingosine. This hypothesis was confirmed by the observed up-regulation of the ATPase in HepG2 cells treated with sphingosine. The results suggest that S1P and ceramide (and/or ceramide -1P), exert a inhibitory effect on the pump while sphingosine has a stimulatory one.

C1.06 Nuclear import of RNA polymerase II requires the conserved GPN-loop GTpase XAB1/GPN1

D. Forget1, A.-A. Lacomba1, P. Cloutier1, R. Al-Khoury1, A. Bouchard1, M. Lavallée-Adam2, J. Archambault1, M. Blanchette2 and B. Coulombe1

Over the past three decades, many efforts have been made to identify and characterize the factors that regulate the activity of RNA polymerase II (RNAPII), the enzyme that synthesizes all the mRNA and many small nuclear RNA in eukaryotes. Quite surprisingly, very little is known about the cell machinery that regulates the fate of RNAPII before or after transcription. In this study, we report that XAB1/GPN1, a member of a unique class of GTpases containing a conserved GPN-loop, is involved in the biogenesis of RNAPII by regulating its nuclear import. XAB1/GPN1 is physically associated with RNAPII in the soluble cell fraction and is essential for cell growth. Accumulation of RNAPII in the cell’s cytoplasm was induced upon (i) depletion of XAB1/GPN1 by siRNA silencing, (ii) overexpression of a XAB1-GFP dominant negative fusion protein, (iii) mutation of the GTP-bind or GPN motifs of the XAB1/GPN1 homologue in yeast, NPA3, and (iv) treatment of cells with leptomycin B, an inhibitor of XPO1/CRM1-dependent nuclear export. Yeast strains with mutations in the NPA3 gene showed hypersensitivity to benomyl, a specific inhibitor of microtubule assembly, suggesting a role for microtubules in RNAPII biogenesis. Together, these results indicate that XAB1/GPN1 shuttles between the cytoplasm and the nucleus to mediate nuclear import of RNAPII.

C1.07 Nucleotide utilization requirements that render ClpB active as a chaperone

U. del Castillo1, I. Martín1, J. A. Fernandez-Higuero1, S. P. Acebron1, F. Moro1 and A. Muga1

1Universidad del País Vasco y Unidad de Biofísica (CSIC-UPV), 2Department of Bioquímica y Biología Molecular, Leioa, Spain, 3Universidad de Cantabria (UC) e Instituto de Biomedicina y Biotecnología de Cantabria, IBBTEC (CSIC-UC-IDICAN), Departamento de Biología Molecular, Cantabria, Spain

Conjugative systems contain an essential membrane protein that couples the relaxosome to the DNA transport apparatus called coupling protein (T4CP). TrwB is the T4CP of the conjugative plasmid R388. This protein, purified in the presence of detergents, binds preferentially purine over pyrimidine nucleotides, NTPs over NDPs, and ribo- over deoxyribonucleotides. In contrast, a soluble mutant, TrwBAN70, binds uniformly all tested nucleotides. Reconstitution of membrane proteins into liposomes is a widespread approach to analyze their biological function. Since, we are interested in the characterization of the activity of TrwB, we developed a reconstituted system that could be used to study the function of this protein. In this work, TrwB has been successfully reconstituted into liposomes being the protein preferentially oriented inside the membrane. The functional analysis of TrwB proteoliposomes demonstrate that when the protein is inserted into the lipid bilayer, TrwB is selective for ATP and the affinity is enhanced compared with the protein purified in detergent and with the soluble deletion mutant, TrwBAN70. From these results we conclude that the reconstitution of TrwB is the suitable way to study the biological activity of this coupling protein and that the transmembrane domain of TrwB could play a regulatory role in the biological activity of TrwB.

C1.08 Reconstitution into liposomes enhances nucleotide binding affinity of TrwB conjugative coupling protein

A. J. Vecino1, R. D. Segura1, B. Ugarte-Uribé1, S. Águila1, F. de la Cruz2, F. M. Goní1 and I. Alkorta1

1University of Pecs, Faculty of Medicine, Forensic Medicine, Pecs, Hungary, 2University of Pecs, Faculty of Medicine, Biophysics, Pecs, Hungary

Actin is involved in many cellular processes (force-generation, cell-division cycle etc.). The atomic structure of actin monomer (G-actin) consists of 4 subdomains. The interaction between the subdomains is dynamic; they might have different conformational and motional states depending on the bound cations and nucleo-

C1.09 Domain motions in skeletal G-actin

F. Kónczol1, J. Belagyi2 and D. Lőrinci2

1 University of Pecs, Faculty of Medicine, Forensic Medicine, Pecs, Hungary, 2University of Pecs, Faculty of Medicine, Biophysics, Pecs, Hungary

Actin is involved in many cellular processes (force-generation, cell-division cycle etc.). The atomic structure of actin monomer (G-actin) consists of 4 subdomains. The interaction between the subdomains is dynamic; they might have different conformational and motional states depending on the bound cations and nucleo-
C1.10 Comparative study of ZnO-MTCP and ZnO-CuMTCP nanoparticles in photodynamic therapy for breast cancer treatment in T47D cell line

S. S. Sadjadpour, R. Ghazaleh, S. I. Zargar and S. Safarian
Tehran University, Cellar & Molecular Biology, Tehran, Islamic Republic of Iran

Photodynamic therapy (PDT) is a method of clinical treatment whereby diseased cells and tissues are destroyed by a combination of light and special drugs called photosensitizers. Both the light and the photosensitizing agent are relatively harmless by themselves but combined, can result in selective tumor destruction. In this method for cancer treatment upon UV light exposed, light will emit from nanoparticles and this light can activate photosensitizers to generate singlet oxygen and caused itself but combined, can result in selective tumor destruction. In this method for cancer treatment upon UV light exposed, light will emit from nanoparticles and this light can activate photosensitizers to generate singlet oxygen and caused

C1.11 The oxygen reactive species implications in the efficiency of antitumoral treatment with complex combinations of ruthenium

M. I. Gruia\textsuperscript{1}, V. Uivaros\textsuperscript{2}, V. Negoita\textsuperscript{3}, M. Vastulescu\textsuperscript{1} and I. Gruia\textsuperscript{4}
\textsuperscript{1}Institute of Oncology, Biochemistry and Radiobiology, Bucharest, Romania, \textsuperscript{2}University of Medicine and Pharmacy, Chemistry, Bucharest, Romania, \textsuperscript{3}University of Bucharest, Physics, Bucharest, Romania

Introduction: The aim of our paper is to identify if the free radicals of oxygen plays an important role in the signaling pathway for the apoptotic transformations when we use as an antitumoral treatment new complex combinations of ruthenium.

Materials and methods: Our \textit{in vivo} studies were performed in Walker 256 carcinoma bearing Wistar rats treated with four new ruthenium complexes with fluorochinolones. The treatment started at 7 days from the tumor graft and we assayed in dynamics the apoptosis by flow-cytometry and the biochemical oxidative status parameters (such as lipid peroxides level, coo- per-oxidize activity, total thiol-groups level, ferric reducing ability of sample). The biological samples are represented by cells removed from liver, kidney, brain, pulmonary and tumor tissues, compared with the untreated control group. Results. The results show an increase of apoptosis from 14, 79\% to 59, 72\% in tumor cells treated with the most active combination of ruthenium with norflaxacin. Also we registered an increase of the oxidative status and the free radicals of oxygen production during the treatment.

Conclusions: Even the new compounds biological activities are at the DNA levels and they act as the oncosatic molecules with platinum (cisplatin, carboplatin, oxaliplatin, which can develop also a high cross-resistance) we found than our new synthesized complexes are less toxic and their activity is based also on the induction of oxidative stress.

C1.12 The C-terminal domain of TrwK, the VirB4 protein of the conjugative plasmid R388, displays a critical regulatory function

A. Pena, S. Zunzunegui, F. de la Cruz and I. Arechaga
IBBTEC, Departament of molecular biology, Santander, Spain

Type IV secretion systems (T4SS) translocate DNA and protein substrates across the cell envelope of bacteria to a widely distributed number of eukaryotic and prokaryotic target cells. Conjugative bacteria use T4SS to mediate the transfer of DNA and proteins to recipient cells. T4SS are macromolecular assemblies composed of 11 mating pair subunits (VirB1 to VirB11) and a coupling protein (VirD4) that span inner and outer bacterial membranes. Three of these subunits are ATPases that energize DNA and protein substrate transfer as well as pilus assembly. VirB4 proteins are the largest and most evolutionarily conserved proteins in T4SS and are essential for virulence and plasmid transfer.VirB4 proteins have been suggested to energize substrate translocation across the T4SS. Recently, we demonstrated that TrwK, the VirB4 homologue in the R388 conjugative system, is able to hydrolyze ATP in the absence of potential substrates. The atomic structure of VirB4 proteins is unknown. Based on computing predictions, a model of the C-terminal (residues 413 to 772) of Escherichia coli TrwK has been suggested using the atomic coordinates of the coupling protein TrwB of plasmid R388 as a template. Secondary structure predictions of TrwK
and TrwB revealed the presence of three $\alpha$-helices in the C-termi

nus that are conserved in all VirB4 proteins but are absent in TrwB. Therefore, we decided to generate truncated variants of TrwK where these $\alpha$-helical structures were sequentially removed and we have analyzed their in vitro and in vivo properties. The results suggest that the C-terminal end of VirB4 proteins could play a key functional regulatory role in the biological activity of T4SS.

C1.13

Abstract withdrawn

C1.14

Actinoporin structure at the lipid-water interface

K. Morante$^1$, E. A. Meschaly$^1$, A. Bellomio$^1$, D. Gli-Carton$^2$, M. Valle$^2$, J. M. González-Manás$^1$ and M. A. D. Guérin$^1$

$^1$University of the Basque Country, Biochemistry and Molecular Biology and University of Biofísica, Leioa, Spain, $^2$Cooperative Center for Research in Biosciences, Structural Biology Unit, Derio, Spain

Actinoporins are monomeric 20 kDa proteins produced by sea anemones that bind preferentially sphingomyelin-containing membranes and form oligomeric pores with a functional radius of ~10 Å. Pore formation leads to disruption of target cells and takes place in three steps: membrane binding, oligomerization and insertion of an amphipathic $\alpha$-helix located at the po-

rin N-terminus. Whereas monomer binding and insertion of the N-terminal helix have been extensively characterized, the struc-

ture of the oligomeric pore has remained elusive. The currently accepted model is the so-called toroidal pore in which the walls of tetrameric pores are lined by proteins and lipids. Fragacea-
toxin C is a novel actinoporin isolated from the sea anemone Actinia fragacea. Here we report on the crystallographic 1.8 Å resolution structure of oligomeric FraC in complex with deter-
gent. It consists of a crown-shaped oligomer with a diameter of about 110 Å and made up of 9 monomers. Cryo-electron microscopy studies of FraC pores in lipid bilayers show oligo-

ers composed of 8 subunits. Our results challenge the toroidal model since a cluster of 8-9 $\alpha$-helices can build the pore with-

out lids lining the walls. Actinoporins may thus be included in a new category of pore forming toxins: the $\alpha$-helical barrel toxins.

C1.15

Anti-cancerous effects of LaF$_3$: Tb$^{3+}$ nanoparticles on T47D cell line

G. Rafatian, S. S. Sadjadi-Pour, S. J. Zargar and S. Safarian

University of Tehran, Tehran, Islamic Republic of Iran

Cancer is a group of disease, in which cells become abnormal and divide without control. This accounts for 13% of human death. Recently nanotechnology is beginning to change the scien-
tific landscape in terms of disease diagnosis, treatment and pre-
vention. Nanoparticles are being used in nanomedicine as vehicles for drug delivery. One of the newly invented methods for cancer treatment is photodynamic therapy (PDT) which can be defined as the administration of a non-toxic drug known as a photosensitizer to a patient bearing a lesion, which is frequently, but not always cancer. After an incubation period this specific lesion is then usually illuminated with light which, in the presence of oxygen, leads to the generation of cytotoxic species and conse-

quently to cell death and tissue devastation. In our experiment we used LaF$_3$: Tb$^{3+}$ luminescent nanoparticles that get excited by UV lights so they would luminescence and activate a photo-
sensitizer drug called “MTCP” [meso- tetra (4-carboxyphenyl) porphine] which has been conjugated to the nanoparticles. When MTCP absorbs visible light it produces singlet oxygen which is toxic for cells. We have produced these nanoparticles and puri-

fied them by gel-exclusion chromatography. Then we examined their spectrum via spectrophluorimeter and spectrophotometer. Also we observed their uptake in T47D human breast cancer cell line via fluorescence microscope after incubation with 1 mM nanoparticles for 24 hours. UV light(256 nm, 100 µW/cm$^2$) was exposed to cells for 180 second and cell viability was measured using MTT test. MTT test shows 40% death resulting from sin-

glet oxygen produced by MTCP conjugated nanoparticles.

C1.16

Novel single stranded DNA binding proteins crucial for the DNA damage response

L. Cubedd$^1$, D. J. Richard$^2$, M. F. White$^1$ and K. K. Khanna$^2$

$^1$University of Sydney, School of Molecular Bioscience, Sydney, Australia, $^2$Queensland Institute of Medical Research, Signal Transduction Laboratory, Brisbane, Australia, $^3$University of St Andrews, Biomedical Sciences Research Complex, St Andrews, UK

Single stranded DNA binding (SSB) proteins are ubiquitous and essential for a variety of DNA metabolic processes including DNA replication, recombination, transcription and repair as well as for the recruitment of the repair machinery to sites of DNA damage. SSB proteins from the three domains of life are evolution-

tarily conserved and utilise oligonucleotide-binding (OB) domains for DNA binding. We describe two new human SSBs (hSSB1 and 2), with a domain organisation closer to the archaeal SSB than to the eukaryotic Replication Protein A (RPA). Building on our recent data (Richard et al. (2008) Nature 453(7195): 677–681), we show that hSSB1 is critical for the cellular response to double-stranded DNA breaks (DSBs) and directly interacts with the MRN (Mre11/Rad50/NBS1) complex to facilitate the repair of lethal DSBs. Preliminary data indicate that the second SSB, hSSB2 is involved in the cellular response to ultra violet radiation and we have embarked on the first biochemical charac-

terisation of this newly discovered protein. We propose that these novel hSSBs are functional homologues of the bacterial and ar-

cheal SSB family of proteins, placing them centrally in the DNA repair pathway. Like many early participants in the damage response pathway, hSSBs may therefore be involved in tumorigenesis and may significantly affect the response of patients to cancer therapies.

C1.17

A four-helix bundle model for E. coli PhoQ transmembrane domain

T. Lemmin and M. Dal Peraro

EPFL, IBI, Lausanne, Switzerland

In bacteria, two-component systems (TCS) detect the environ-
mental changes via a sensor kinase at the periplasmic level, which leads to a phosphorelay cascade mediating specific gene tran-
scription. PhoPQ is a TCS, which detects and responds to diva-

lent cations and antimicrobial peptides. It is composed of a che-

moperator (PhoQ) and a regulon (PhoP), which triggers gene regu-

lation. PhoQ assembly is characterized by a homodimer transmembrane protein. The X-ray crystal structure is largely unresolved, and consequently the mechanism that transmits the signal across the membrane remains unclear. Here, we use molec-
ular simulations to assemble an atomic model of PhoQ. First, the assembly of the transmembrane domain is investigated. We observed that few polar amino acids are crucial for the stability of this domain. Moreover, our results are consistent with new experimental cross-linking data, and support a four-helix bundle arrangement, which can be further exploited for the mechanistic understanding of PhoQ signaling response to external stimuli.

C1.18
Poly(A)-specific ribonuclease (PARN): Molecular mechanisms of mRNA cap and poly(A) tail recognition
N. Henriksson1, P. Nilsson1, W. Mousheng2, H. Song2 and A. Virtanen1
1Uppsala University, Department of Cell and Molecular Biology, Uppsala, Sweden, 2Institute of Molecular and Cell Biology, Singapore, Singapore

A critical step in eukaryotic mRNA degradation is removal of the poly(A) tail at the 3'-end of the mRNA. Poly(A)-specific ribonuclease (PARN) is an oligomeric, processive and cap-interacting 3'-5' exoribonuclease that efficiently degrades eukaryotic mRNA poly(A) tails. In addition to the exonuclease domain, PARN harbours two RNA-binding domains, an RNA recognition motif (RRM) and an R3H domain. We investigated the RNA binding properties of PARN and we could show that PARN binds poly(A) with high affinity and specificity. Furthermore, we showed that the RRM and R3H domains of PARN separately could bind to poly(A). To investigate specificity for and recognition of poly(A) in the active site of PARN, we performed a kinetic analysis on a repertoire of trinucleotide substrates in vitro. We showed that PARN harbors affinity for adenosines in the active site and that both the penultimate and the 3' end located nucleotide play an important role for providing adenosine-specificity in the active site of PARN. Moreover, we solved a crystal structure of PARN in complex with m'GppGpG cap analogue and showed that the cap binding and active sites overlap both structurally and functionally. By mutational analysis we identified residues in the active site that specifically recognize adenosines. Furthermore, biochemical data showed that the adenosine specificity in the active site is lost when Mn2+ is used instead of Mg2+ as divalent metal ion. Taken together, these results demonstrate that both RNA-binding properties of the RRM and R3H-domains in addition to base recognition in the active site contributes to PARN poly(A)-specificity.

C1.19
A model for the assembly and activation process of the pore-forming toxin Aerolysin
M. Degiacomi, I. Iacovache, G. van der Goot and M. Dal Peraro
EPFL, Lausanne, Switzerland

Pore-forming toxins are a complex virulence factor common to several bacterial families. These proteins are secreted as water-soluble but, upon binding to specific receptors on the target cell’s membrane, they transform into a transmembrane complex via oligomerisation and important conformational changes. This results in the creation of a pore, which induces a reaction cascade usually leading to membrane lysis. By means of all-atom molecular dynamics compared to in vitro experiments, we study the activation and assembly process of Aeromonas hydrophila aerolysin beta pore-forming toxin. We show here that the C-terminal peptide has a dual role. It is indeed both affecting the proper folding of the toxin and its activation. We ultimately identify specific point mutations affecting the correct functioning of this nanomachine.

C1.20
Transfection of cultured myoblast with mutant β-tropomyosin (TPM2EGFP)
S. Abdul-Hussein, A.-R. Moslemi and H. Tajsharghi
Institute of Biomedicine, Department of Pathology, Gothenburg, Sweden

Tropomyosins (TMs) belong to the family of actin-binding proteins. It provides stability and it is essential for myosin-actin interaction. The tropomyosin filament also plays an important role in maintaining the actin microfilament system. Mutations in the gene encoding β-tropomyosin (TPM2) result in a variety of inherited diseases, including nemaline myopathy, cap disease and distal arthrogryposis. We investigated the effects of E41K, K49del and G53ins mutations in TPM2 in cultured myoblasts. The E41K mutation is associated with a congenital myopathy characterized by either cap structures or accumulation of nemaline rods. The pathogenesis in patients with cap disease associated with K49del and G53ins mutations in TPM2 involves abnormal coarse-meshed intermyofibrillar network and the appearance of cap structures. A C-terminal tagged β-tropomyosin-enhanced green fluorescent protein (EGFP) fusion construct was generated. Human cultured myoblasts were transfected with wild-type and mutant β-tropomyosin-EGFP constructs (WT-TPM2EGFP, E41K-TPM2EGFP, K49del-TPM2EGFP and G53ins-TPM2EGFP) and stained with TRITC-phalloidin and DAPI. We also examined the ability of WT and mutant TMs to contribute to the sarcomeric thin filament by differentiation of myoblasts transfected with EGFP-tagged constructs. Confocal microscopy was performed using a Zeiss LSM 510 META. The transfection studies in cultured myoblasts and myotubes may provide a better insight into the pathogenetic mechanisms involved in microfilament system of the myoblasts and myotubes, which may be a part of the pathogenesis involved in the assembly of myofilaments and sarcomere structure in patients with mutations in TPM2.

C1.21
Interactions between TrwB protein and DNA substrates with secondary structures
M. F. Maria Inmaculada and E. Cabezón
IBBTEC, Molecular Biology, Santander, Spain

TrwB is a DNA-dependent ATPase involved in DNA transport during bacterial conjugation. The protein presents structural similarity to hexameric molecular motors such as F1-ATPase, FtsK or ring helicases, suggesting that TrwB also operates as a motor, using energy released from ATP hydrolysis to pump ssDNA through its central channel. During this year, we have carried out an extensive analysis with various DNA substrates to determine the preferred substrate for TrwB. Oligonucleotides with G-rich sequences forming G4 DNA structures were the optimal substrates for TrwB ATPase activity. The protein bound with 100-fold higher affinity to G4 DNA than to ssDNA of the same sequence. Moreover, TrwB formed oligomeric protein complexes only with oligonucleotides presenting such a G-quadruplex DNA structure, consistent with stoichiometry of 6 TrwB monomers to G4 DNA, as demonstrated by gel filtration chromatography and analytical ultracentrifugation experiments. G-quadruplex structures are widespread in the genomes and are thought to play a biological function in transcriptional regulation. They form very stable structures that can obstruct DNA replication, requiring the action of specific helicases to resolve them. These observations are discussed in terms of a possible role for TrwB in resolving G4 secondary structures that arise during conjugal DNA processing.
C1.22

The main pathways for obtaining abiotic stress-tolerant transgenic poplars

N. Kutsokon
Institute of Cell Biology and Genetic Engineering NAS of Ukraine, Kyiv, Ukraine

Populus is a very important genus in biology as well as in industry. Firstly, they are model woody plants with genome sequenced, secondly poplars are trees actively used for energy, paper and wood production because of their fast-growth and wide native area. Though many varieties were obtained through conventional breeding methods, genetic engineering has provided more effective tools for improving different traits. Creation of transgenic Populus lines is significant for fundamental physiological, biochemical and tree gene functions studies as well, as for applied fields, particularly for industry. One of the main fields for improving poplar phenotypes through gene transferring is tolerance to abiotic stresses – oxidative stress, drought, salinity, cold, environmental pollution etc. A number of processes are involved in the tolerance mechanism: osmolytes, polyamines, reactive oxygen species and antioxidant defense mechanisms (such as superoxide dismutase, catalase, peroxidase, ascorbate peroxidase, glutathione reductase), ion transport and compartmentalization of injurious ions. Thus, increasing the activity of antioxidant enzymes, levels of osmolites, metallothioneins at date are the main tasks for gene transformation of poplars to enhance stress-tolerance.

C1.23

Dissect and build: Reconstructing the cellulosome using X-ray crystallography and small angle X-ray scattering

M. Currie, J. Adams, S. Smith and Z. Jia
Queen’s University, Biochemistry, Kingston, Canada

With increasing energy consumption and the depletion of fossil fuels, development of alternative fuel sources is paramount. Plant cell wall (PCW) polysaccharides are the most abundant renewable carbon source. Therefore, conversion of plant biomass into biofuels is an attractive option to reduce our dependence on fossil fuels. However, the PCW is an insoluble and recalcitrant substrate, which makes the efficient breakdown of PCW polysaccharides into monosaccharides a major obstacle in bioethanol production. Several anaerobic microbes possess large cell surface bound multienzyme complexes, dubbed cellulosomes, which are capable of the synergistic degradation of PCW polysaccharides. Enhanced cellulolytic activity is conferred to hydrolytic enzymes that bind the core non-catalytic scaffoldin subunit of the cellulosome. However, understanding the structural elements responsible for the enzymatic synergy of the cellulosome is hindered by both the large size and inherent flexibility of these multi-protein complexes. Herein, we describe ongoing work to elucidate the three dimensional structure of the cellulosome using a dissect and build approach, which combines X-ray crystallography and small angle X-ray scattering (SAXS). Specifically, the crystal structure of a multimodular fragment of the cellulosome from Clostridium thermocellum containing three polypeptides will be presented along with SAXS structures of cellulosomal complexes that reveal the modular arrangement and flexibility of these complexes in solution. Our findings provide valuable insight into cellulosome structure and function that will aid in engineering designer cellulosomes for large scale bioethanol production.
C2.01
The urea cycle enzymes and several aminotransferases in fruit bodies maturation process of fungus *Pleurotus ostreatus*
A. Gasparyan¹, A. Nikoyan¹, S. Nanagulyan² and N. Eloyan¹
¹Yerevan State University, Biochemistry, Yerevan, Armenia,
²Yerevan State University, Botany, Yerevan, Armenia

In metabolism of some fungal species it was noted that ammonia (which is cellular poison) inhibits basidiospore formation. Ammonia removes from the organism by biosynthesis of urea in urea cycle. The urea cycle is integral part of ammonia-scavenging network. In higher fungi urea is source of reserve nitrogen but high levels of urea influence on taste and maturation process. The aspartate aminotransferase (AST) and alanin aminotransferase (ALT) play important role in nitrogen metabolism, particularly in nitrogen assimilation. In the present study activities of arginase, ornithine carbamoyltransferase (OCT), ALT, AST, content of urea and total protein in different stages of *Pleurotus ostreatus* fruit bodies have been investigated. The measurements were carried out every two days after primordia formation up to spore generation. We have noted significant changes of enzymes activity, content of urea and total protein during maturation. Arginase activity increased many times on the 2nd and 4th days of development. OCT and ALT activities increased up to basidiospore generation phase, after which it sharply dropped. In the last stages of development considerable rising of AST activity has been noted. Generally, the arginase and AST activities in the cap and gills exceeded stem 2–3 times. In contrast to arginase and AST, high levels of ALT and OCT activities were found in funnel stem. Highest amount of urea was pointed out in basidiospore generation phase. Total protein has increased throughout all stages of development.

C2.02
Prognostic significance of P53 abnormalities in Turkish colorectal cancer patients detected by PCR-SSCP
H. Tuncell¹, C. Ozkurt², S. Erdamar³, T. Kalkan⁴, M. A. Korpınar⁵, T. Hiyama⁶ and F. Shimamoto⁷
¹Istanbul University Cerrahpasa Medical Faculty, Biophysics, Istanbul, Turkey,
²Istanbul University Cerrahpasa Medical faculty, Pathology, Istanbul, Turkey,
³Hiroshima University Medical Faculty, Hiroshima, Japan,
⁴Prefectural University of Hiroshima, Hiroshima, Japan

Colorectal cancer is the second most frequent cause of cancer-related death. The p53 tumor suppressor gene, located on chromosome 17p13, is one of the most commonly mutated genes in all types of human cancer. The process of colorectal tumorigenesis is based on the concept of the ‘adenoma-carcinoma sequence’ and is believed to follow a multistep genetic model. Single-strand conformational polymorphism (SSCP) analysis can be used to detect abnormalities within the regions of p53. The study group comprised 40 patients admitted to Cerrahpasa Medical Faculty’s Hospital, Istanbul for curative surgical resection of colorectal cancer. Archival formalin-fixed, paraffin wax-embedded tissue was used. A single block representative of the tumor was selected in each case and the same block was used for PCR-SSCP. Ten-micrometer-thick tissue sections were placed on a glass slide and stained with HE. Then the tissue sections were dehydrated in graded ethanol solutions and dried without a cover glass. DNA was extracted from the tissues with 100 μl of extraction buffer at 55°C overnight. The tubes were boiled for 5 minutes to inactivate the proteinase K, and then 2 μl these extracts were used for polymerase chain reaction (PCR) amplification. All analyses were performed using the Statistical Package for the Social Sciences (SPSS). Non-parametric data were assessed using the Mann–Whitney U-test. Categorical variables were assessed by the χ² test. In conclusion, we observed a statistically significant relationship between the evaluation of p53 alterations by SSCP/sequencing and stage of tumors.
C3 – Extracellular Structures

C3.02
Alasan from Acinetobacter radioresistens S13 is an N-linked glycoprotein with a peculiar consensus sequence
P. Fattori, R. Mazzoli, M. Riva Violetta, C. Barello, M. G. Giuffrida, C. Giunta
1Department of Human and Animal Biology, University of Turin, Turin, Italy, 2CNR, ISPRA, Turin, Italy

Surfactants of biological origin are very requested in both the food industry and for cosmetic applications. In the present research the glycosylated nature of a surfactant protein, previously identified in the A. radioresistens S13 proteome, was demonstrated, and a multiplexed analysis was performed in order to establish the degree of homology with a known OmpA-like surfactant, produced by A. radioresistens KA53, named Alasan. The coverage study after N-terminal amino acid sequencing, peptide mass fingerprinting and MS-MS experiments revealed a 99.97% of identity, displaying only one residue change at the position 25 where a glycine of A. radioresistens KA53 alasan is replaced by a threonine in the protein isolated from A. radioresistens S13. Curiously, the mutation gives rise to a consensus N-glycosylation sequence absent in the previously characterized Alasan. Furthermore the residue 23 was detectable only after N-glycosidase treatment thus demonstrating that this position is consistent with the N-glycosylation site. The consensus sequence Asn-Asp-Thr proved to be peculiar since in general Asp, as well as Glu and Pro in the central position of the consensus site, gives rise to unstable glycosylations. All these results suggest that glycosylation processes in bacteria differ from those observed in eukaryotic cells and that the mechanisms and the significance of the glycosylation sites and that the mechanisms and the significance of the glycosylation site. The consensus sequence Asn-Asp-Thr, gives rise to a consensus N-glycosylation site. The consensus sequence Asn-Asp-Thr, gives rise to a consensus N-glycosylation site.

C3.03
NMR structural studies of the human interferon-α2 complex with human type I Interferon receptor
1Weizmann Institute of Science, Structural Biology, Rehovot, Israel, 2National University of Singapore, Department of Biological Sciences, Singapore, Singapore

Type I Interferons (IFNs) are a family of homologous helical cytokines initiating strong anti-viral and anti-proliferative activity. All type I IFNs bind to a common cell surface receptor consisting of two subunits, IFNAR1 and IFNAR2, associating upon binding of interferon. We studied inter-molecular interactions between IFNAR2-EC and IFNα2 using two novel approaches based on the use of deuterated and 2D homonuclear NOESY experiments for the detection of inter-molecular NOEs in large protein complexes which were developed and applied to the IFNAR2-EC/IFNα2 complex. Sequential and side-chain assignment of IFNAR2-EC and IFNα2 in their binary complex helped assign the inter-molecular NOEs to the corresponding protons. A docking model of the IFNα2-EC/IFNα2 complex was calculated based on the inter-molecular interactions found in the present study as well as four double mutant cycle constraints, previously observed NOEs between a single pair of residues and the NMR mapping of the binding sites on IFNAR2-EC and IFNα2. Our docking model doubles the buried surface area of the previous model and significantly increases the number of inter-molecular hydrogen bonds, salt bridges and Van der Waals interactions. Furthermore, the current model reveals participation of several new regions in the binding site such as the N-terminus and A-helix of IFNα2 and the C-domain of IFNAR2-EC. As a result of these additions, the orientation of IFNAR2-EC relative to IFNα2 has changed by 30° in comparison with the previous model. In addition, the new model strongly supports the recently proposed allosteric changes in IFNα2 upon IFNAR1-EC binding to the binary IFNα2/IFNAR2-EC complex.
C3.06 Involvement of hepatic stellate cell-derived myofibroblasts and portal myofibroblasts in liver fibrosis: A comparative study using proteomics and transcriptomics

N. Bosselut1, A. Cadoret2, P. Marcelo3, V. Barbu2, C. Rey2, J. Guéchot1, J. Vinh3, C. Housset2, M. Vaubourdolle1 and B Baudin1

1Hôpital Saint-Antoine, Biochimie A, Paris, France, 2Faculté de Médecine Saint-Antoine, INSERM UMR938, Paris, France, 3ESPCI, CNRS USR3149, Paris, France

In chronic liver diseases, the accumulation of extracellular matrix leading to fibrosis is caused by myofibroblasts, the origins of which are debatable. We compared rat hepatic stellate cell-derived myofibroblasts (MF-HSCs) and portal myofibroblasts (PMFs) using both proteomics (with two-dimensional electrophoresis and MALDI-TOF/TOF mass spectrometry) and transcriptomics (with DNA-microarrays). The aim of this study was to determine phenotypical and functional specificities of each kind of myofibroblasts and to define specific markers for each. Our results show that MF-HSCs would be involved in liver regeneration, angiogenesis, vascular tone, and express many proteins related to cellular response to stress, whereas MFPs showed proliferative and contractile features and could be involved in leucocytes chemoattraction. Using markers identified in this work, i.e. (i) cytoglobin and RGS4 for MF-HSCs; (ii) phosphocofilin-1, collagen-XV and calsequestrin-2 for PMFs; (iii) osteoprotegerin, which could be a serum marker of liver fibrosis. Score study also revealed that MF-HSCs expressed osteoprotegerin (OPG), which could be a serum marker of liver fibrosis. Score study also revealed that MF-HSCs expressed osteoprotegerin (OPG), which could be a serum marker of liver fibrosis. Score study also revealed that MF-HSCs expressed osteoprotegerin (OPG), which could be a serum marker of liver fibrosis. Score study also revealed that MF-HSCs expressed osteoprotegerin (OPG), which could be a serum marker of liver fibrosis.

C3.07 The content of glycosaminoglycans (GAG) as part of a diagnostic complex evaluation of treatment effect in ovarian cancer stages III-IV

V. Knazyeva Maryna1, V. Prokopyuk Alexandra1 and D. Pavlova Tamara1

1V. N. Karazin Kharkiv National University, biochemistry, Kharkov, Ukraine, 2Medical Radiology Institute, Oncogynecology, Kharkov, Ukraine, 3Public Organization “New thinking in medicine”, oncology, Kharkov, Ukraine

After the neoadjuvant polychemotherapy (NPChT) it becomes possible to make an operation to delete the original tumor in patients having ovarian cancer (OC) at the late (III-IV) stages. The complex of biochemical, clinical and ultrasound criteria for evaluating the effect of NPChT (1–6 courses) helps to find the optimal number of courses for each patient having OC stages III-IV and improve the efficiency of treatment. GAG fractions and total GAG in blood serum of 82 patients having OC at III-IV stages (serous adenocarcinoma) before treatment and after NPChT were determined by M. Stern et al. method. ELISA method was used to determine CA125 content. It was found that the decrease in level of total GAG in blood serum up to 75–70%, chondroitinsulphates up to 80–65%, II fraction GAG up to 75–65% of the original, I,III fractions, ratios of total GAGs and their fractions to normal, as well as the content of CA125 to 9.6–3.6% of its initial value suggests NPChT efficiency in patient having OC stages III-IV. These findings correlated with a reduction in size of a tumor conglomerate to 60–20%, of metastatic tumor in the recto-vaginal septum to 70–20%, the disappearance of ascites and pleural fluids (according to clinical and ultrasound data). Using the level GAGs and their fractions, their ratios,CA125 in diagnostic complex evaluation of the NPChT effect as well as clinical and ultrasound parameters improves the efficiency of this category patients treatment.

C3.08 Extracellutar synthesis of the polyol lipid - type bioemulsifiers by Rhodotorula glutinis

I. Maliszewksa, K. Zielinska and K. A. Wilk
Wroclaw University of Technology, Department of Chemistry, Wroclaw, Poland

Bioemulsifiers have attracted considerable interest in recent years, due to their unique properties e.g. mild production conditions and a variety of functions. Microbial emulsifiers have application in enhanced oil recovery operations (MEOR), in textiles, pharmaceuticals, cosmetics and the food industry. It was reported that production of surfactant can be stimulated in several microorganisms by depriving them essential nutrients different that carbon, such as phosphorus, iron, calcium and magnesium. This may lead to the synthesis and excretion of bioemulsifiers as a physiological response. Soil sample were collected at Polish sites historically contaminated by crude oil or its products. A strain Rhodotorula glutinis isolated in our laboratory was found to produce an extracellular emulsifier. The cultures grows rapidly after a lag time of about 12 hour but emulsifier production occurs during stationary phase. This observation suggest that compound production by isolated strain is a secondary microbial metabolic process. The highest bioemulsifier production was observed when C/N and C/Fe were combined. At high C/N of 30 and high C/Fe of 780, emulsification activity was 14U. High yeast extract concentration confirmed high Hyal-1 and -2 expression in cancerous samples. Hyaluronan-zymography revealed increased hyaluronidase activity mainly in cancerous samples. RT-PCR and Western Blotting confirmed high Hyal-1 and -2 expression in cancerous samples. PH-20 was found to be elevated mostly in normal samples. Hyal-1 and Hyal-2 seemed to be the major hyaluronidases expressed in cancerous samples, contributing to the formation of small angiogenic hyaluronan fragments. Hyal-3 was exclusively extracted in GdnHCl-Triton extracts of advanced cancer stage. These observations indicate that hyaluronidases are highly associated with tumor progression and may be used as targets in tumor therapy.

C3.09 The central cysteine-rich CysD domain of the MUC2 mucin mediates protein-protein interactions by dimer formation

Department of Medical Biochemistry, University of Gothenburg/ Institute of Biomedicine, Gothenburg, Sweden

After the neoadjuvant polychemotherapy (NPChT) it becomes possible to make an operation to delete the original tumor in patients having ovarian cancer (OC) at the late (III-IV) stages.
MUC2 is the major gel-forming mucin of the colon mucus in human. The MUC2 mucin gel is a branched net-like polymer assembled from monomers via covalently linked dimers in its C-termini and trimers in its N-termini. N- and C-terminal oligomerization is mediated via cysteine-rich parts that form covalent interchain disulfide bridges. The large central region of MUC2 contains two highly O-glycosylated mucin domains that are interrupted by another cysteine-rich region (CysD domain) of unknown function. We aimed at studying its function in a recombinant fusion protein that contained the CysD domain and a removable immunoglobulin G (IgG2a) Fc domain. When expressed in Chinese hamster ovary cells the fusion protein was secreted into the culture medium and subsequently purified by affinity chromatography. Analysis of the purified fusion protein by native gel electrophoresis and gel filtration chromatography showed that it formed tetrameric, octameric, dodecameric and 16-meric complexes. Individual analysis of the isolated CysD part and IgG2a part revealed that both parts formed dimers only. This finding was confirmed by analyzing another recombinant CysD fusion protein that encoded twice the general tandem repeat of the MUC2 mucin domain. In conclusion, oligomeric complexes were multiples of tetramers linked to each other via protein-protein interactions mediated by the CysD domain. These findings show that CysD domains of gel-forming mucins are inserting additional cross-links into the polymeric gel thereby determining its pore size. Due to the varying number of CysD domains in other gel-forming mucins distinct gel types are formed with different properties.

C3.10

Extracellular reactive oxygen species in aphids
A. Urbanska
University of Podlascie, Biochemistry and Molecular Biology, Siedlce, Poland

Examples of reactive oxygen species, ROS are superoxide anion, O$_2^\cdot$ and hydrogen peroxide, H$_2$O$_2$. ROS are intermediates in the reduction of O$_2$ into H$_2$O. In the red-ox homeostasis ROS are constantlyparallel released and removed. In general, the xenometabolism amplifies ROS production. ROS excess, inside/outside the cells is defined as oxidative stress. Although the dual nature of ROS is emphasized, in fact most research concern ROS toxicity. Recently, ROS appear as signaling molecules in biotic/abiotic stresses and plant-insect interactions. This paper presents extracellular ROS status in Rhopalosiphum padi and Sitobion avenae, in particular: (i) microscopic visualization of O$_2^\cdot$, and H$_2$O$_2$ in vitro and in vivo; (ii) H$_2$O$_2$ production consequently exogenous phenolics; (iii) enzymatic sources of O$_2^\cdot$ and H$_2$O$_2$ generation and decomposition. In vitro, H$_2$O$_2$ is produced in consequence of the salivary oxidative metabolism of dietary α-dihydroxyphenolics i.e. caffeic acid, catechin. In vivo, midgut appears major locus of O$_2^\cdot$ and H$_2$O$_2$ production. The phenolic xenobiotics show different prooxidant impact, α-dihydroxyphenolics and polyphenolics i.e. DOPA, catechin, caffeic, gallic and chlorogenic acid, in contrast with the monophenolics, enhance ROS production. Polyphenol oxidase (PPO) biocatalysis, located in midgut and saliva is recognized as fundamental oxidation of phenolic xenobiotics and enzymatic supply of O$_2^\cdot$, precursor of H$_2$O$_2$. Contrary to PPO, aphid peroxidase, POD eliminates H$_2$O$_2$. In addition, catalase, CAT decomposes H$_2$O$_2$ in midgut and impairs oxidative stress, in vivo. There is a direct link between ROS level/oxidative stress and PPO, POD and CAT of Aphididae.

C3.11

Isolation and characterization of α-Amylase obtained from thermophilic Anoxybacillus sp
S. Özdemir, S. Fincan and F. Matpan Bekler
1Department of Biology, Siirt University, Faculty of Science, Siirt, Turkey, 2Department of Biology, Dicle University, Faculty of Science, Diyarbakir, Turkey

α-Amylase (α1-4 D-glucanohydrolase EC 3.2.1.1) catalyzes endohydrolysis of α-1,4 glucosidic linkages in starch and any related oligosaccharides to make oligosaccharides and glucose. Amylases have a significant commercial importance and they occupied about 25-33% of the world enzyme. These enzymes are used in the textile and paper industries, detergents in starch liquefaction, as a food adhesive, and in sugar production. In this study, the morphological, biochemical, and 16 S rRNA analyses of the bacterium that was isolated from hot water springs of Ömerli, Afyonkarahisar in Turkey have been studied. The gram positive, spore-forming, motile, moderately thermophilic bacterium was found to be a strain of Anoxybacillus sp. analyzed by 16S rRNA comparison. The optimum growth time of Anoxybacillus sp. was determined to be the 20th hour, pH as 6.0 and temperature as 55°C. The extracellular amylase production of the bacterium was studied through SmF method. The highest α-amylase production was obtained in the 24th hour (1509.3 U/ml). The optimum enzyme activity was determined as 55 oC and pH as 7.0. The most stable α-amylase obtained from Anoxybacillus sp. can be used in industrial applications.

C3.12

Fluorophore-kodecytes – fluorescent function-space- lipid (FSL) modified cells for in vitro and in vivo analyses
D. Blake, A. Lan, D. Love, N. Bovin and S. Henry
1AUT University, Biotechnology Research Institute, Auckland, New Zealand, 2University of Auckland, Molecular, Cellular and Developmental Biology, School of Biological Sciences, Auckland, New Zealand, 3LabPLUS, Auckland City Hospital, Diagnostic Genetics, Auckland, New Zealand, 4Shemyakin Institute of Bioorganic Chemistry RAS, Moscow, Russian Federation

Most live cells are naturally poorly visible and so various secondary techniques, such as staining (including fluorescent tagging), are used to visualize them. However, a major limitation of most staining techniques is that they commonly compromise cell viability and/or functionality because the stain either covalently attaches to functional molecules or has toxic interactions. In contrast, FSL (function-spacer-lipid) constructs are designed to be dispersible in biological media, they can insert into cell membranes, and a range of synthetic molecules can be attached, such as fluorophores, without affecting the cells functionality or vitality (1–3). Two constructs for fluorescent tagging of live cells were investigated. In the first, FSL incorporated FITC as its functional moiety (FSL-FITC), and the second approach used biotin (FSL-biotin), which was then secondarily reacted with fluorophore labeled avidin. Both approaches were used to successfully label a variety of living cells including murine embryos, spermatozoa, epithelial and endothelial cells. FSL-FITC was additionally used to stain the digestive tract and circulation of zebrafish embryos. Acknowledgement: Supported by KODE Biotech Ltd (kodetech.com).

References:
C3.13 Proteome of chondrocyte-alginate constructs and monolayer cultures in relation to extracellular matrix modulation

S. Ab. Rahim¹, L. Selvarattan² and T. Kamatari²
¹University Technology MARA, Faculty of Medicine, Shah Alam, Malaysia, ²Monash University, Sunway Campus, Medicine and Health Sciences, Selangor, Malaysia, ³University of Malaya, Department of Orthopaedic Surgery, Kaula Lumpur, Malaysia

Chondrocyte-alginate constructs have been shown to produce differences in extracellular matrix organization and composition when compared to the monolayer culture system. The construct has been shown to change the collagen fibres orientation, increase glycosaminoglycan synthesis and retain chondrocytes isogenous ovoid morphology. Identification of proteins expressed by chondrocytes in these two culture systems is important to understand the modulation of the extracellular matrix and also regulation of its gene expression, cellular physiology and characteristics. Therefore, in this study, proteomic analysis was performed to compare protein profiles between chondrocyte-alginate construct and monolayer chondrocyte cultures, aimed at identification of key and novel proteins involved in the modulation of extracellular matrix synthesis. Using this investigative modality, we were able to establish a standard chondrocyte protein profile of chondrocyte monolayer cultures and chondrocyte-alginate constructs using 2-dimensional electrophoresis and mass spectrometry. Following the proteomic analysis, we have successfully determined expression changes on 8 proteins at lower molecular weight following the proteomic analysis, we have successfully determined using 2-dimensional electrophoresis and mass spectrometry. Following the proteomic analysis, we have successfully determined expression changes on 8 proteins at lower molecular weight region (CAPL, S100-A6, CHA, HPUBQ, CA1, CA2, CA3 and CA4). CAPL, S100-A6, CHA and HPUBQ proteins could be involved directly or indirectly in modulating chondrocyte extracellular matrix. Uncovering such specific proteins involved in the modulation of extracellular matrix formation will further lead to further identification of potential biomarkers for early detection of cartilage degeneration.

C3.14 Characterization of an extracellular β-galactosidase produced by thermophilic Bacillus licheniformis KG9 isolated from hot spring in Batman-Turkey

A. Kaplan¹, R. Gul Guven², F. Matpan Bekler³, O. Acer³ and K. Guven¹
¹Department of Biology, Dicle University, Faculty of Science, Diyarbakir, Turkey, ²Dicle University, Faculty of Education, Science Teaching Section, Diyarbakir, Turkey

In the present study, a thermophilic bacterial strain (KG9) was isolated from a hot spring in Batman (Turkey) and was found to be a member of Bacillus licheniformis following complete sequencing of 16s rRNA. This strain was found to produce high levels of extracellular β-galactosidase in NB at 50°C. The time course experiments of β-galactosidase synthesis in the cells show constitutive expression of enzyme, starting from 36 hour (with specific activity of 1.38 U/mg) up to 96 hour (2.45 U/mg) for lactose-free samples, whereas the enzyme production starts at 48 hour (1.39 U/mg) for lactose-containing samples, which increases sharply by increasing time up to 96 hour (3.25 U/mg) of cultivation at 50°C. The enzyme production levels were studied after addition of various carbon (galactose, glucose, lactose and soluble starch) and nitrogen sources (yeast extract glycine, ammonium sulphate, peptone, tripoline and beef extract) into liquid media. Galactose, glucose and soluble starch at 1% concentrations were found to inhibit the extracellular enzyme production to a great extent, whereas the nitrogen sources such as glycine and ammonium sulphate caused a slight increase in enzyme activity. Moreover, the extracellular β-galactosidase was partially purified and some properties of the enzyme and electrophoretic analysis were studied. The optimum temperature, pH and thermal stability for enzyme were found as 55°C, 8.0 and 90% retained activity at 60°C in 120 minute, respectively. This study is the first to report for the extracellular enzyme production in Bacillus licheniformis species, which may well be used in biotechnological processes.

C3.15 Modeling and molecular dynamics of α-dystroglycan’s C-terminus reveals the presence of an Ig-like domain encompassing its β-dystroglycan binding epitope

D. Piroli², F. Scandra², M. Bozzi², B. Giardina², A. Brancaccio² and M. C. De Rosa²
²Università Cattolica del Sacro Cuore, Istituto di Biochimica e Biochimica Clinica, Rome, Italy, ³Consiglio Nazionale delle Ricerche, Istituto di Chimica del Riconoscimento Molecolare, Rome, Italy

Dystroglycan (DG) is a cell surface receptor which is composed of two subunits that interact noncovalently, namely α- and β-DG. Given its sarcolemmal localization at the interface between the cytoskeleton and the basal lamina, DG is thought to be involved in the pathogenesis of several congenital muscular disorders [1]. To date only the 3D-structure of the N-terminal domain of α-DG has been solved by X-ray crystallography [2], and the structures of the other DG domains need still to be determined. We generated a first theoretical 3D-model on a portion of the α-DG’s C-terminal domain. An initial model was established using the I-TASSER algorithm which combines the methods of threading and ab initio modeling [3]. Subsequent refinement by molecular dynamics simulation led to an improved model structure which was analyzed by different protein analysis programs, including PROCHECK for the evaluation of Ramachandran plot quality, PROSA for testing interaction energies and VERIFY3D for identifying regions of improper folding. The resulting α-DG’s C-terminal domain (between residues 505–596) model is remarkably similar to the N-terminal domain (residues 60–158) solved at high resolution (PDB 1u2c), revealing the presence of another Ig-like domain within α-DG. Such domain model includes the β-DG binding epitope of α-DG and, confirming our previous experimental results, suggests that the epitope assumes a β-strand conformation [4].

References:

C3.16 High Tenascin-C expression marks active breast cancer tissue in neoadjuvantly treated patients

S. Bastelberger¹, E. Hinterseer¹, L. Pradel¹, J. Beil¹, G. Rendl², O. Dietze¹ and Hausser-C. Kronberger³
¹General Hospital and Paracelsus Private Medical University Salzburg, Pathology, Salzburg, Austria, ²General Hospital and Paracelsus Private Medical University Salzburg, Nuclear Medicine and Endocrinology, Salzburg, Austria

The extracellular matrix molecule Tenascin-C (TNC) in its many isoforms is highly expressed during tissue repair and pathological

conditions such as cancer. Utilising the selective pressure of neoadjuvant chemotherapy on carcinoma cells we screen for a subgroup of breast cancer patients with elevated TNC expression and a bad therapy response, evaluated by immunopathological markers, histopathological data (pTNM staging system), clinical data and F-18 FDG PET-CT. Sections of paraffin embedded samples from 68 preoperatively treated breast cancer patients are used for automated Immunohistochemical staining with appropriate antibodies targeting TNC, AE1/AE3, Ki67, CD3 and p53. In addition F-18 FDG PET-CT is performed to discriminate therapy response. We show that TNC expression in breast cancer is immensely reduced after neoadjuvant chemotherapy (43% of patients lost TNC expression) and that expression profiles after treatment correlate with histopathological data, especially the grade, indicating prognostic value. But we also show that TNC expression alone does not sufficiently correlate with therapy response. However, there is a strong correlation and colocalisation of the proliferation marker Ki67 in cancer cells and TNC in the surrounding tumour-stroma of patients having a less beneficial response to chemotherapy. The evaluation of expression levels revealed a direct linear correlation of TNC and Ki67. We expect TNC to be a dependable marker for active cancer tissue remaining after neoadjuvant treatment. These findings concur with the role TNC plays in tissue reorganisation, wound healing and its growth promoting properties in cancer.

C3.17 Glycoproteomic characterisation of O-glycosylated proteins from human cerebrospinal fluid
University of Gothenburg, Biomedicine, Gothenburg, Sweden

The glycosylation of proteins represents the most complex but also the most common post-translational modification of proteins. The glycans are either O-glycosidically linked to Ser or Thr or N-glycosidically linked to Asn. We have developed a capture-and-release method to enrich both tryptic N- and O-glycosylated peptides from proteomic samples, and then characterise them with tandem mass spectrometry (1). We mapped the glycan structures and identified 36 N-linked and 44 O-linked glycosylation sites on proteins from human cerebrospinal fluid (CSF). In order to more specifically study O-glycans we treated CSF samples with glycosidase PNGase F prior to the capture protocol, which removed most N-glycans. With this strategy 22 additional O-glycosylation sites on CSF glycoproteins were characterized. For instance, we identified two previously unknown minor glycosylation sites on ApoE, (Thr-26 and Thr-36). In addition, we found that the major glycosylation site in ApoE (Thr-212) showed microheterogeneity with respect to the glycan core structures. By using electron capture dissociation for the fragmentation of glycopeptides with intact O-glycans we pinpointed the second major glycosylation site of ApoE (1) to Ser-308 and not to Thr-307. ApoE interacts with amyloid beta peptides and the E4 variant of the ApoE gene is an established risk factor for developing Alzheimer’s disease. The biological implications of the presented ApoE glycosylations remain to be investigated.

Reference:
glycans were typically observed, in agreement with known biantennary complex-type and core 1 structures, respectively. Glycopeptide macro- and microheterogeneities were also revealed. This report demonstrates the applicability of hydrazide capture for comprehensive glycoproteomic characterization of urine samples.

Reference:

C3.20
Partial characterization of antimicrobial peptide produced by Bacillus cereus strain
E. Zboinska and K. Zielinska
Wroclaw University of Technology, Department of Chemistry, Wroclaw, Poland

Antimicrobial peptides (AMPs) are synthesized by most - if not all - living organisms. The AMPs consist of less than 100 amino acid residues, nearly all are amphiphilic and positively charged in neutral pH, that enables them to interact with target cell membrane. These interactions disturb membrane integrity, either by disruption or pore formation, resulting in cell death. Nowadays, when pathogens became resistant to known conventional antibiotics, antimicrobial peptides have attracted much attention as a promising class of therapeutics and biological control agents. While the AMPs from eukaryotic cells show broad inhibitory spectra against both gram-positive and gram-negative bacteria as well as fungi, protozoa, viruses, their procaryotic counterparts, and bacteria, with the highest activity against closely related strains. In this paper we describe bacteriocin-like substances produced by Bacillus cereus strain, belonging to food microflora, giving its preliminary characterization. The activity range of antimicrobial substance secreted by B. cereus is limited to gram-positive bacteria, with the highest activity against Bacillus spp. The inhibitory compound acts against microorganism phylogenetically related to the producer strain. B. cereus metabolite was classified as a bacteriocin-like inhibitory substance, without any lipid or carbohydrate moiety involved in the inhibitory activity. The activity was maintained after heating up to 90°C for 30 minute, but disappeared following treatment at 100°C. It was also stable in the pH range of 4.0–9.0 and relatively unaffected by organic chemicals.

C3.21
Optimization of cellulase production by newly isolated Bacillus sp
H. Mangotha, N. G. Avci, B. Sarıyar Akbulut and D. Kazan
Marmara University, Bioengineering, Istanbul, Turkey

Enzymes are widely used in various sectors as additives to improve the effectiveness of the processes they are involved in. These fields range from detergent- and textile-processing technologies to animal feed. Whereas treatment of fabrics with this enzyme helps to give a soft appearance, the use of this enzyme as an additive in animal feed improves the breakdown of raw materials for the animals to gain maximum benefit. The most significant bottleneck in the utilization of such enzymes is the dictated by the cost of their production. The aim of this work was to screen various newly isolated Bacillus strains for cellulose production and then improve the production of this enzyme by the selected microorganism. Among the isolates studied, Bacillus sp. 3 had the highest level of cellulase production with 1220 U/L at 45°C. Following the addition of different carbon and nitrogen sources to growth media for inducing enzyme production, cellulase production improved by about four fold to a value of 4380 U/L. When the time rate of enzyme production was monitored under the same conditions, it was found that there was no enzyme synthesis before 10 hours of growth and the maximum enzyme amount was detected after 15 hours of growth as cells entered stationary phase. Changing the growth temperature showed no significant improvement on total amount of enzyme produced. This work has been supported by Marmara University Research Fund Project FEN-C-DRP-171108-0267.

C3.22
A lectin histochemical study of the skin treated with Origanum hypericifolium essential oil in mice
P. III, N. Keskin, A. Celik and B. Sahin
Pamukkale University, Denizli, Turkey

Recently, herbal compounds such as phenolic acids, flavonoids, and high molecular weight polyphenols as botanical antioxidants have been extensively investigated in human health care. Origanum hypericifolium, the endemic species in the region of Denizli-Turkey, contains p-simene, γ-terpinene, cis- and trans-sabinene hydrate, borneole, terpinen-4-ol, α-terpinel, thymol and carvacrol. Monoterpenes like carvacrol have antioxidant and antitumor activities. Glycoconjugates (glycoproteins, glycolipids, proteoglycans) in cell, cell membrane, basement membrane and intracellular spaces, play an important role in many biological events. Lectins have been used for the detections of specific carbohydrates. In this study, the staining patterns of glycoconjugates in skin were investigated in mice topically treated with Origanum hypericifolium essential oil by means of lectin histochemistry. Two lectins were studied: Datura stramonium agglutinin (DSA) and Galanthus nivalis agglutinin (GNA). GNA specific for terminal mannose residues and DSA specific for Galβ1 → 4GlcNAc. In comparison with control, dense dermal connective tissue fibres were intensively stained using both lectins. On the other hand, epidermis was weakly stained. However, the sebaceous glands weakly stained by GNA. The results indicated that Origanum hypericifolium essential oil application can alter the carbohydrate compositions of skin.

C3.23
Expression levels of matrix metalloproteinase-7 and in situ localization of caseinolytic activity in colorectal cancer
D. Keles1, B. Arslan2, C. Terzi2, I. Tekmen3, E. Dursun3, O. Altungoz4 and G. Oktay5
1Dokuz Eylul University School of Medicine, Medical Biochemistry, Izmir, Turkey, 2Dokuz Eylul University School of Medicine, Surgery, Izmir, Turkey, 3Dokuz Eylul University School of Medicine, Histology and Embryology, Izmir, Turkey, 4Dokuz Eylul University School of Medicine, Medical Biology, Izmir, Turkey

Recent years it is believed that MMP-7 can contribute to tumor invasion and metastasis. The aim of our study is to investigate expression levels and latent-active forms of MMP-7, to determine local caseinolytic activity in colorectal cancer. 31 colorectal tumor and paired normal tissues were taken from patients with colorectal carcinoma. mRNA and protein expressions of MMP-7 were detected by Real Time PCR and ELISA, MMP-7 localization with immunohistochemistry. Casein Zymography and In Situ Zymography methods were used to detect proteolytic activity of MMP-7 and local caseinolytic activity, respectively. The quantitative results were compared with clinicopathological variables. MMP-7 mRNA expressions in tumor tissues were signifi-
cantly higher than normal tissues. Furthermore mRNA expression levels were positively correlated with Tumor and Pathological staging and negatively correlated with age. In MMP-7 protein expressions, there were no significant differences between tumor and normal tissues. We recorded a significant correlation between levels of MMP-7 protein expression and perineural invasion. In MMP-7 protein expressions, there were no significant differences between tumor and normal tissues.

In the results of immunohistochemical analysis, positive staining was observed in cytoplasm and cell membrane of tumor cells whereas there was no MMP-7 staining in normal cells. In addition, it was found that cascinolytic activity, localize epithelium region, was higher in tumor tissues than normal tissues. Our results indicated that MMP-7 influences tumor invasion of colorectal cancer by degrading extracellular matrix and promoting signal transduction pathways. Therefore MMP-7 may be selected as a target molecule in the use of new chemopreventive therapy in colorectal cancer.

C3.24
Comparison of MUC2 O-glycosylation from the colon of core 1- and core 3-knockout mice
K. Thomsson1, L. Xia2 and G. C. Hansson1
1University of Gothenburg, Medical Biochemistry, Gothenburg, Sweden, 2Oklahoma Medical Research Foundation, Cardiovascular Biology Research Program, Oklahoma City, USA

The major protein component of the mucosal layer in the colon is the multimerising MUC2 mucin, which has densely O-glycosylated domains, contributing to approximately 80% of its weight. The highly diverse O-glycans are linked via a GalNAc in a α1 linkage to serine or threonine residues, and mainly extended by core 1 or core 3 structures1. The biosynthesis of these core structures are regulated by specific glycosyltransferases. Mice lacking core-1 and core 3-derived glycans in colon by deletion of colonic epithelial glycosyltransferase activity, displayed increased susceptibility to colitis and colorectal tumours2. In this study we have analyzed the glycans present on colonic MUC2 from both the wild type and the knock-out mice. Mucins were purified from mucosal scrapings and the glycans released with reductive beta elimination. The reduced glycans were sequenced and relative amounts estimated using negative ion mode liquid chromatography-mass spectrometry (LC/MS) and MSn. Preliminary results confirmed the lack of the deleted core structures from the knock-out mice, however the mucins from the knockout-mice were still found to be densely glycosylated, and carrying oligosaccharides differing by both average size and terminal epitopes, compared to the wild type mouse.

References:

C3.25
Complete characterization of the MUC2 mucin C-terminal using a proteomics approach
S. van der Post, K. A. Thomson and G. C. Hansson
University of Gothenburg, Dept. of Medical Biochemistry, Gothenburg, Sweden

The highly glycosylated MUC2 mucin constitutes the major protein component of the mucus layer that covers and protects the epithelial surface of the intestinal tract. It was previously assumed that the mucus layers of the body served merely as a physical barrier, whereas today it is clear that the individual glycans and specific protein properties of the mucus layer are adapted to harbour the commensal bacterial flora, as well as suppressing and clearing pathogens. The aims of the current project are to characterize peptide modifications in the C-terminal part of MUC2, as well as obtaining complete sequence coverage in order to get a better insight in the protein function. Here we present a method to improve sequence coverage on a construct of the muc2 protein C-terminal (106 kDa). The approach is based on bottom-up proteomics using multiples enzymes for protein digestion prior to analysis by nanoLC-MS/MS on a hybrid linear ion trap/orbitrap mass spectrometer, using high resolution HCD fragmentation to obtain accurate peptide sequence information. Combined data from the differently digested samples resulted in a sequence coverage of approximately 60% as compared to a 30% coverage when using conventional trypsin digestion. The C-terminal is known to contain at least 9 potential N-glycosylation and multiple O-glycosylation sites. Using this approach 7 of these sites were characterized after manual interpretation of the fragmentation spectra. In addition, error tolerant protein database searches were used to gain further sequence coverage. By using the described method we managed to increase sequence coverage and characterized previously unknown protein modifications.
C4.01

The periplasmic protein YohN RcnB is involved in nickel and cobalt homeostasis in Escherichia coli

C. Bérriot, M.-A. Mandrand-Berthelot and A. Rodrigue
National Institute of Applied Sciences, Biosciences, Lyon, France

Metals are ubiquitous on Earth and become, with the evolution of Life, necessary for living organisms like bacteria. Otherwise, there is a subtle balance between the life-required amount and toxicity resulting from a too high quantity of metal ions. A strategy often used by cells is to regulate import and efflux of metal ions by the dynamic expression of specific proteins involved in this transport. In Escherichia coli, nickel and cobalt toxicity is counterbalanced by action of the RcnA efflux pump that permits transport. In this system, as shown by RT PCR experiments, this gene is a part of the operon renA-yohN and its expression is nickel and cobalt dependant. It encodes a small monomeric protein of 10 kDa localized in the periplasm. Databases do not contain any described homologs. By reporter gene studies, we show that, unlike renA, a renB mutant accumulates less nickel and cobalt ions suggesting a role in the transport or storage of these ions. However, RcnB seems not to function with the specific nickel transporter nik. Furthermore, several methods were employed to show that RcnB does not interact directly with nickel ions in vitro. Further investigations are required to precise its role but it could be the first member of a new family of accessorie proteins.

C4.02

Multidrug resistant P-glycoprotein positive cells are also cross-resistant to cisplatin

L. Gibalova
Institute of Molecular Physiology and Genetics, Laboratory of Biochemistry and Cytochemistry, Bratislava, Slovakia

P-glycoprotein (P-gp, a drug transporter in the plasma membrane) mediated multidrug resistance of neoplastic cells represents a real problem in the chemotherapeutic treatment. Our experimental model are mouse leukemia cells L1210/VCR in which the overexpression of P-gp was induced by vincristine (VCR). Cisplatin (cisPt) is a substance that is untransportable by P-gp. We found that L1210/VCR cells are also resistant to cisPt. However, resistance to this substance could not be reversed by addition of verapamil (P-gp inhibitor). CisPt induced more pronounced entry into apoptosis in P-gp negative sensitive L1210 cells than in L1210/VCR cells. While similar levels of Bax and Bcl-2 proteins were observed in resistant and sensitive cells, cisPt induced a more pronounced decrease of the Bcl-2 levels in L1210 cells than in L1210/VCR cells. Consistent with this observation, cisPt induced a larger decrease of the Bcl-2 content in the Bcl-2/Bax heterooligomer in L1210 cells than in L1210/VCR cells. Moreover, CisPt induced a similar apoptotic DNA fragmentation pattern in both resistant and sensitive cells. All observations indicated that multidrug resistant L1210/VCR cells are also resistant to cisPt (non-substrate of P-gp) and that this resistance is not connected with drug efflux activity of P-gp. This work was supported by: VVCE-0064-07, APVV-0084-07 a VEGA 2/7122, 2/0155/09.

C4.03

Overexpression of P-glycoprotein in L1210/VCR cells is associated with changes in several endoplasmic reticulum proteins

M. Seres
Institute of Molecular Physiology and Genetics, Laboratory of Biochemistry and Cytochemistry, Bratislava, Slovakia

Multidrug resistant cells L1210/VCR, which express membrane transporter, P-glycoprotein (P-gp, responsible to multidrug resistance), was found to be resistant to thapsigargin an inhibitor of SERCA, in contrast to parental sensitive cells L1210. L1210/VCR cells was obtained from P-gp negative cells L1210 by their gradual adaptation to vincristine (VCR). We have studied differences among L1210 and L1210/VCR cells in expression of endoplasmic reticulum proteins involved in the regulation of calcium homeostasis and calcium-dependent processes. Amounts of mRNA encoding RyR and IP3-receptor channels were found to be at similar levels in sensitive and resistant cells. However, mRNAs encoding IP3R1 or 2 were decreased in resistant cells cultivated in the presence of VCR, while mRNA encoding RyR remained unchanged. The amount of mRNA for SERCA2 was decreased in resistant cells when compared with sensitive cells. This decrease was more pronounced when resistant cells were cultivated in the presence of VCR. Calnexin was found to be associated with immature P-glycoprotein in resistant cells, but surprisingly, calnexin was found to be less expressed at the protein level in resistant as in sensitive cells. Thus, differences exist between sensitive and multidrug resistant cells in the expression of endoplasmic reticulum proteins involved in the control of intracellular calcium homeostasis or calcium-dependent processes. This work was supported by: VVCE-0064-07, APVV-0084-07 a VEGA 2/7122, 2/0155/09.

C4.04

Pyrenyl fluorescence to figure out bilayer polarity in diverse lipidic membranes and partitioning plus collisional quenching in fluid homogeneous bilayers

J. Martins, D. Arrais and M. Manuel
IBB-CBME, Universidade do Algarve, Faro, Portugal

As mixing the cholesterol into pure phospholipid bilayers, changes in the bilayer polarity occurs, depending on the chemical composition and on other thermodynamic parameters (e.g. T, P) [1]. Using the pyrene polarity scale, we characterized the polarity of pure POPC and egg-sphingomyelin, as well as of defined POPC/cholesterol and egg-sphingomyelin/cholesterol mixtures. When increasing cholesterol proportions, the polarity values for POPC decrease (as similarly for alcoholic solvents). For egg-sphingomyelin, an increased polarity occurs with augmenting cholesterol, and the results show no relation to the thermal behavior of homogeneous solvents (polar and apolar). We also quantified the partition of 1-pyrenesulfonate (PSA, anionic amphipile), into bilayers composed by POPC and zwitterionic/anionic POPC/POPS mixtures. Comparing with POPC at 25°C, we found increasing Kp values in parallel with relatively low molar.
proportions of anionic POPPS (until 10 mol %) in mixed fluid and flexible bilayers [2]. We studied then the quenching of PSA at low concentrations of a DOXYL quencher group, in fluid POPC. Steady-state fluorescence of PSA decreased with increasing proportions of quencher. However, the results are not well described by a collisional kinetic formalism for reactions occurring in two-dimensional (2D) media [3]. Furthermore, we used the pyrene excimer formation process to test the 2D formalism in analyzing steady-state fluorescence self-quenching in lipid bilayers.

References:

C4.05 The potassium uptake transporters of the yeast Hansenula polymorpha
M. C. Alvarez1, E. Cabrera2, Y. Martínez2, J. M. Siverio2 and J. Ramos3
1University of Cordoba, Microbiology, Cordoba, Spain, 2University of La Laguna, Biochemistry and Molecular Biology, Tenerife, Spain

Potassium is the most abundant intracellular cation in living cells, playing several important roles in vital physiological processes. Most of the information concerning K+ fluxes has been obtained in the usual model yeasts: the budding yeast Saccharomyces cerevisiae and the fission yeast Schizosaccharomyces pombe. In both of these, TRK1 and TRK2 genes are present and code for the main K+ uptake systems. However, further studies using the yeast Hansenula polymorpha, a member of the Saccharomyceseae, reveal the presence of two main K+ transporters belonging to the Hak and Trk families, respectively. Deletion mutants lacking HAK1 grow poorly at low K+, while trk1 shows Na+, Li+ and hygromycin sensitivity. Test growth of trk1hak1 at low K+ suggests at least a third K+ transporter. An in-depth kinetic analysis revealed the high affinity of HAK1 for K+. Furthermore, HAK1 expression is up-regulated under limited K+. Likewise, Hak1 levels are also posttranslationally regulated by K+.

C4.06 Hak1 and Trk1, two different systems mediating potassium uptake in Debaryomyces hansenii
J. L. Martínez and J. Ramos
University of Córdoba, Microbiology, Córdoba, Spain

Potassium is required for numerous cellular functions and cells have developed efficient systems to accumulate the cation against high concentration gradients. Two different systems for K+ uptake have been identified in most yeast. While Saccharomyces cerevisiae or Schizosaccharomyces pombe are endowed with two Trk type transporters (Trk1 and Trk2), other yeasts such as Hansenula polymorpha or the halotolerant Debaryomyces hansenii posses a Trk type plus a Hak (High Affinity K+) type transporter (1,2) which may be related to the limiting K+ amounts of the ecosystems where these yeasts can be found. We have studied the role of DhHAK1 and DhTrk1 in K+ transport. We have followed gene expression of DhTRK1 and DhHAK1 by qRT-PCR and, in addition, we have biochemically characterised K+ (Rb+) fluxes under conditions at which the genes coding for both transporters are differentially expressed. We conclude that whereas DhTRK1 expression is poorly regulated by external factors such as external potassium, sodium or pH, DhHAK1 expression is highly induced during potassium and sodium starvation. We propose that under potassium limiting conditions most, if not all, the potassium influx process observed in D. hansenii is mediated by DhHak1. Moreover, we found that the activity of the potassium transporters working in control cells (50 mM KCl grown cells) and in starved cells can be distinguished by the differential effect of potassium, sodium or pH on K+ (Rb+) transport.

C4.07 Lactococcus lactis, an alternative system for functional expression of peripheral and intrinsic plant membrane proteins
A. Barrand-Frelet1, S. Boutigny2, L. Moyet2, A. Deniaud3, D. Seigneurin-Berny2, D. Solvi4, F. Bernaudat4, E. Pebay-Peyroula1, J. Joyard4 and N. Rolland2
1Laboratoire de Physiologie Cellulaire Vegetale, CNRS, UJF, GRENOBLE, CEA Saclay/IBTeS/IBS/LSOD; 2Laboratoire de Physiologie Cellulaire Vegetale, CNRS, UJF, GRENOBLE, CEA, IBIS, Grenoble, France; 3Laboratoire d’Ingenierie des Macromolecules, CEA, IBIS, Grenoble, France

Despite their functional and biotechnological importance, the study of membrane proteins remains difficult due to their hydrophobicity and low natural abundance in cells. Moreover, into well-known heterologous systems, these proteins are often produced at very low levels, toxic and mis- or unfolded. Lactococcus lactis, a Gram-positive lactic bacterium, traditionally used in food fermentations, is now widely used in biotechnology for large-scale production of bacterial and eukaryotic proteins. We tested the expression, in L. lactis, of six Arabidopsis thaliana membrane proteins that could not be produced in sufficient amount using classical expression systems. In an effort to easily transfer genes from Gateway-based Arabidopsis cDNA libraries to the L. lactis expression vector pNZ8148, we first established a cloning strategy compatible with Gateway entry vectors. Interestingly, all the Arabidopsis proteins could be produced, in L. lactis, at levels compatible with further biochemical analyses. We then successfully developed solubilization and purification processes for three of them. Finally, we demonstrated that two proteins (one peripheral and one intrinsic protein) were active in this system. Altogether, these data suggest that L. lactis might be an attractive system for the efficient and functional production of difficult plant membrane proteins.

C4.08 The presence of P-glycoprotein in L1210 cells directly induces down-regulation of cell surface saccharide-targets of Concanavalin A
Z. Sulova1, P. Ditte2, T. Kurucova1, J. Sedlak3, J. Pastorek2 and A. Breier1

Overexpression of P-glycoprotein (P-gp), a plasma membrane drug transporter (an ABCB1 member of the ABC transporter family), is the most prevalent cause of multidrug resistance in cancer tissues. Lectin Concanavalin A (ConA) induces massive cell death of L1210 leukemia cells. Cell sublines of L1210 in which P-gp overexpression was induced by selection with vincristine (R) or by stable transfection with a plasmid encoding full-length human P-glycoprotein (T) were less sensitive to ConA.
Multidrug resistance (MDR) of neoplastic tissue represents a real obstacle to the effective chemotherapy of cancer. Several mechanisms of MDR were identified, from which the over-expression and efflux activity of P-glycoprotein (P-gp) – a plasma membrane ATPase (ABC1 member of ABC transporter family) – represents the most commonly observed reason for neoplastic disease chemotherapy malfunction. The process of P-gp-mediated MDR seems to be related to intracellular calcium homeostasis, at least indirectly, for the following reasons: (i) substances blocking calcium influx through L-type of calcium channels like verapamil were often found to antagonize P-gp-mediated MDR; (ii) calcium signal abnormalities were observed in cells over-expressing P-gp; (iii) cells with P-gp-mediated MDR were often resistant to thapsigargin; (iv) several differences in intracellular calcium localization were observed when P-gp-negative and P-gp-positive cells were compared; and (v) differences in the contents of several proteins of the endoplasmic reticulum involved in calcium homeostasis were observed to be associated with P-gp over-expression. This current study represents an attempt to summarize the knowledge about the possible relationship between P-gp-mediated MDR and intracellular calcium homeostasis.

C4.11 Interaction of lectins with specific binding affinity to surface of P-gp negative and P-gp positive cells L1210
T. Kurocova1, H. Kavcova1, K. Rogozanova1, D. Mislovicova2, A. Breier1 and Z. Sulova1
1Slovak Academy of Sciences, Institute of Molecular Physiology and Genetics, Bratislava, Slovakia, 2Slovak Academy of Sciences, Institute of Chemistry, Bratislava, Slovakia

Multidrug resistance (MDR) mouse leukemic cell line L1210 obtained by adaptation to vincristine is accompanied by expression of P-glycoprotein (P-gp). During adaptation give out to expression of P-gp and also to various kinds of metabolic changes. Our foregoing work shows decrease of UDP-saccharides in resistant cells, that are substrate of glycosylation reactions. We discovered changes in spectrum and levels of glycoproteins in resistant cells. Aim of this study was to observe changes in content of surface saccharides accompanied by expression of P-gp by lectins with affinity to different saccharides. Histochemical staining of negatively charged cell surface binding sites (mostly sialic acid) by ruthenium red (RR) revealed a compact layer of RR bound to the external coat of sensitive cells (S). In resistant cells cultivated in the absence (R) or presence of vincristine (V) the RR layer is either reduced or absent. Interaction of lectins to membrane proteins was observed by lectin blots. Application of some lectins caused changes in spectrum of marked proteins in membranes of sensitive and resistant cells. Lectins interact to sialic acid exerts almost non toxic effect to this cell lines. We can summarize that overexpression of P-gp caused also remodulation of another glycoproteins of membranes. This work was supported by: APVV-0808-07, VVCE-0064-07, VEGA-2/7122/27, VEGA-2/7028/27.
melanocytes. We show that the capacity to respond in different species correlates strongly with the degree of body transparency. Thus, the capacity to regulate internal chromatophores was larger in species where the effect would potentially be visible. Our study is the first to show evidence for a possible adaptive role of internal colour change, and one of few providing a function of melanin in internal tissues, and of non-cutaneous chromatophores in general.

C4.13
Beyond the membrane: Characterization of the oligopeptide binding protein from *Escherichia coli*

M. Klepsch¹, R. Berntsson², J.-W. de Gier¹ and D.-J. Slotboom²
¹Stockholm University, Stockholm, Sweden, ²University of Groningen, Groningen, Netherlands

*Escherichia coli* is often cultured on media that are based on rich peptone or tryptone mixtures supplying it with nutritious peptides. Specialized binding proteins in the periplasm facilitate the transfer of these substrates to permissive systems in the cytoplasmic membrane. One such binding protein is the oligopeptide binding protein OppA. Although OppA is one of the most abundant proteins in the periplasm of *E. coli*, its natural substrates have never been identified and our knowledge of its functioning is only limited. Therefore, we have initiated a comprehensive characterization of this peptide binding protein combining biochemical and biophysical approaches. So far, a crystal structure at 2.2 Å resolution of OppA was solved. In the binding cleft density fitting a tripeptide substrate with a Lysine at the second position was observed. Tripeptides appear to be the preferred substrates in vivo. To identify the natural substrates of OppA, we produced the protein in its native environment (the periplasm) and its natural substrates were analyzed using reverse-phase nano LC coupled to mass spectrometry. Finally, to understand the dynamics of the proposed “Venus-fly-trap” mechanism for ligand binding, we use multidimensional NMR spectroscopy complemented with CD studies. Eventually our studies will lead to insight in the structure of OppA, identification of its natural substrates and a detailed description of its modus operandi.

C4.14
Functional analysis of the *Ashbya gossypii* Fps1 homolog

C. Geiger, K. Lindkvist and S. Hohmann
CMB Microbiology, University of Gothenburg, Gothenburg, Sweden

Saccharomyces cerevisiae aquaglyceroporin Fps1 plays a central role in yeast osmoregulation, controlling the intracellular level of the compatible solute glycerol. When a cell encounter hypersmotic conditions, Fps1 closes rapidly to ensure retention and accumulation of glycerol. In adaptation to lower external osmolarity, Fps1 opens again to release glycerol and hence turgor pressure. Mutants lacking Fps1 cannot withstand a hypo-osmotic shock to the same extent as wild type cells. Fps1 has unusually long N- and C-terminal extensions, and amino acids that are crucial for the gating mechanism have been identified on both termini. Fps1 also facilitates passive uptake of other small molecules such as arsenite and acidic acid. The filamentous fungi *Ashbya gossypii* Fps1 homolog (AgFps1) has shorter termini than Fps1. The aim of this study is to determine the function of AgFps1 by heterologous expression in S. cerevisiae fps1 deletion mutants, and to study the physiological role of AgFps1 by deletion analysis in *Ashbya gossypii*. We can show that heterologous expression of AgFps1 in S. cerevisiae can substitute for Fps1 by releasing excessive glycerol upon a hypo-osmotic shock. AgFps1 expressed in S. cerevisiae appears to be hyperactive under hyperosmotic conditions, and exchanging the N- and C-terminal extensions for the corresponding termini of Fps1 is not sufficient to generate a regulated channel. Further, successful deletion of AgFps1 renders an *Ashbya gossypii* mutant more resistant to arsenite than wild type fungi, indicating that AgFps1 transports arsenite.

C4.15
Comparison of lipid and fatty acids composition of basolateral membrane from rat (*Rattus norvegicus*) and Atlantic cod (*Gadus morhua*) intestinal enterocytes: the question of homeoviscous adaptation

E. Knutsdottir, G. A. Gylfason and B. Asgeirsson
Science Institute, University of Iceland, Department of Biochemistry, Reykjavik, Iceland

The physical properties of biological membranes are sensitive to changes in temperature due to the phase behavior of the lipids. Therefore, it is important that membranes can adjust to different temperatures in order to fulfill their physiological role. Our aim was to compare the basolateral part from the intestinal endothelial cell membranes (BLMs) of a mesophilic species (rat, *Rattus norvegicus*) and a psychrophilic species (Atlantic cod, *Gadus morhua*) to see if it showed signs of homeoviscous adaptation in relation to cold-adaptation. Major lipid classes were analyzed by thin layer chromatography (TLC) and GC/MS spectrometry. TLC analyses showed similarity between the two species for neutral lipids. However for phospholipids, less phosphatidylethanolamine (PE) and phosphatidylserine was present in cod than in rat and higher amounts of phosphatidylcholine (PC) and sphingomyelin in the former. Results for fatty acids showed a lower amount of polyunsaturated fatty acids (PUFAs) in the cod BLM, but higher amount of mono-unsaturated fatty acids (MUFA). Furthermore, saturated fatty acids were fewer in the cod BLM fraction as expected, as such fatty acids decreases the viscosity of the membrane in the cold. Our findings show some characteristics expected in the maintenance of proper cell membrane fluidity at low temperatures. However, lower PE/PC ratio in cod and lower amount of PUFAs was unexpected, although it has been previously been observed in psychrophilic species. High amount of MUFA is instead likely a factor contributing to cold-adaptation by preventing perfect side-by-side packing of fatty acids in the BLM.

C4.16
Interaction of sanguinarine with the Na⁺/K⁺-ATPase

M. Janovska¹, M. Kubala¹, V. Simanek² and J. Ulrichova²
¹Department of Experimental Physics, Palacky University in Olomouc, Olomouc, Czech Republic, ²Department of Medical Chemistry and Biochemistry, Palacky University in Olomouc, Olomouc, Czech Republic

The balance between concentrations of Na⁺ and K⁺ ions across cell membranes is a fundamental property which is utilized in numerous physiological processes. The establishment of the concentration gradient is carried out by Na⁺/K⁺-ATPase. Sanguinarine (SG), the benzol[cd]phenanthridine alkaloid, exhibits a plethora of biological effects. SG is used in dental hygiene preparations, veterinary medicine and feed additives. On the other hand, SG is linked to adverse effects on humans such as the lu-
koplakia and the epidemic dropsy syndrome. In solution, at physiological pH, there is an acid-base equilibrium between the cation (SG+) and pseudobase (SGOH) forms of SG. In the mammalian gastrointestinal tract SG is converted to biologically inactive dihydrosanguinarine (DHSG). All three forms of SG exhibit fluorescence. Na+/K+-ATPase has been proposed as their possible cellular target. Our experiment, using a combination of steady-state and time-resolved techniques enabled separate the observation of individual SG forms present in the solution. It was shown that fluorescence spectra of SG or SGOH and to lesser extent also DHSG are sensitive to interactions that may be predicted from their binding to proteins. This study revealed that Na+/K+-ATPase was inhibited by SG+/SGOH. We did not find the evidence for DHSG/enzyme interaction. The fluorescence spectra analysis and fluorescence quenching experiments revealed that Na+/K+-ATPase binds SG+ and SGOH. For the former, the binding site on Na+/K+-ATPase was identified. <.

Acknowledgement: This work was supported by the grants of the Czech Science Foundation GACR 301/10/0883, and 522/08/H003.

C4.17 Interaction of fluorone dyes with Na+, K+-ATPase
K. Smidova and M. Kubala
Department of Experimental Physics, Palacky University in Olomouc, Olomouc, Czech Republic
Na+, K+-ATPase is a transmembrane protein which is found in each animal cell. It translocates sodium and potassium ions across the plasma membrane in 3Na+/2K+/in ratio, using the energy from ATP hydrolysis. It is one of the most important enzymes which is essential for establishing and maintaining high K+ and low Na+ in cytoplasm, required for various cellular activities such as a regulation of cell volume, maintenance of membrane potential and secondary nutrient transport. Malfunction of the Na+, K+-ATPase is connected with several diseases, like diabetes, hypertension, cataract, asthma and others. We investigated the interaction of Na+, K+-ATPase with fluorone dyes – eosin, erythrosine, bengal rose and fluorescein. These dyes are used in food industry or like a part of medicaments. The possible interaction between fluorone dyes and Na+, K+-ATPase could account some of adverse effects of drugs. Based on various steady-state fluorescence experiments we were able to observe binding of these fluorone dyes to Na+, K+-ATPase.

Acknowledgement: This work was supported by the grants of the Czech Science Foundation GACR 301/10/0883, and 522/08/H003.

C4.18 Abstract withdrawn

C4.19 Effects of dicyclohexylcarbodiimide (DCCD) treatment on H+ permeability of plasma membrane from marine microalga Dunaliella maritima
L. Popova, A. Genatulina, I. Andre'ev and Y. Balnokin
K.A. Timiryazev Plant Physiology Institute, Moscow, Russian Federation
DCCD is a carboxyl group modifier, an inhibitor of various membrane ion-translocating proteins. The effects of DCCD on electric potential difference (δΨ) across the vesicular plasma membranes isolated from marine microalga D. maritima were investigated. Transmembrane electric potential was created as Na+ or K+-diffusion potential. Na+-diffusion potential was imposed by a combination of Na+ and Na+-ionophore ETH 157. K+-diffusion potential was imposed by a combination of K+ and K+-ionophore valinomycin. Formation of δΨ across the vesicle membranes was monitored using optical δΨ-sensitive probe oxonol VI. The experiments revealed that DCCD induces proton permeability of the vesicle membrane in a Na+- and voltage-dependent manner. It was indicated by the following findings: In the vesicles pre-incubated with DCCD Na+-diffusion potential could be created upon Na+ addition which rapidly and spontaneously dissipated immediately after establishment. DCCD-induced dissipation of Na+-diffusion potential should be referred to proton leakage from the vesicle lumen since ΔΙNa across the vesicle membranes equals zero in the presence of ETH157. In the absence of Na+ the proton permeability of the membranes was not induced by DCCD. Thus, K+-diffusion potential sustained permanently in the vesicles treated with DCCD. The key mechanism for the DCCD-facilitated and Na+-dependent H+ efflux from the vesicle lumen is likely to be Na+/H+ antiporter which operates in D. maritima PM (Popova et al., 2005). Na+ protects the antiporter against interaction with DCCD since DCCD-induced dissipation of Na+-diffusion potential could be completely prevented by Na+ added before DCCD.

C4.20 Transport via cell membrane of the novel porphyrins enhanced by electroporation in vitro as anticancer therapy
J. Kulbacka1, J. Saezko1, N. Szkolacka1, M. Majkowski2, A. Choromanska1, A. Chwilkowska1, I. Kamińska1, M. Kotulska1 and B. Jachimska1
1Medical University, Department of Medical Biochemistry, Krakow, Poland, 2Wrocław University, Institute of Biochemistry and Molecular Biology, Laboratory of Cytobiology, Wrocław, Poland, 3Wrocław University of Technology, Institute of Biomedical Engineering and Instrumentation, Faculty of Fundamental Problems of Technology, Wrocław, Poland, 4Polish Academy of Sciences, Institute of Catalysis and Surface Chemistry, Krakow, Poland

Porphyrins are a large class of fluorescent crystalline pigments, with natural or synthetic origin, having in common a substituted aromatic macrocyclic ring consisting of four pyrrole-type residues, linked together by four methine bridging groups. Porphyrins are verified as an ideal photosensitizers because they are non-toxic, selectively retained in tumor tissue in high concentrations, water soluble to a certain level, cleared in a reasonable time from the body and rapidly from the skin which avoid photosensitive reaction. In current study we determined the cells viability (MTT assay) after standard photodynamic treatment (PDT) and after PDT combined with electroporation (EP). We applied two novel porphyrins CoTPPS and MnTPPS. We examined two cell lines of human colon adenocarcinoma (LoVo and LoVo/DX). The EP parameters were: 500 V/cm, 50 us, 5imp. The intracellular distribution of both porphyrins was also performed. With standard PDT cells were incubated 1 and 4 hours before irradiation, however the results were not efficient. The cells viability reached for both dyes about 60% for LoVo cells before irradiation, however the results were not efficient. The irradiation with 435 nm CoTPPS reached 33% of control cells and for LoVo cells viability reached for both dyes about 60% for LoVo cells and 25,9% for LoVo/DX. For irradiation with filter 530–550 nm for
LoVo/DX CottPPS reached 44.2% of cells viability. In case of the second porphyrin MnTPPS we obtained better results for 435 nm in both cell lines where cells viability was on the level of 50%.

C4.21

Abstract withdrawn

C4.22

Role of K+ efflux in phosphatidylserine exposure in platelets

D. Kerbiriou-Nabias1, A. Arachiche2 and J. Dachary-Prigent2
1INSERM U770, Hemostasis and Vascular Dynamics, Le Kremlin-Bicêtre, France, 2INSERM U688, Mitochondrial Physiopathology, Bordeaux, France

Phospholipid reorganization with translocation of phosphatidylserine (PS) to the external leaflet of cell membranes occurs in cell apoptosis and in platelets during coagulation. The aim of this work was to determine if Ca2+-stimulated PS exposure relies on cell shrinkage and K+ efflux leading to cell dehydratation and resulting in energetically favourable sites for phospholipid translocation. Immunoblotting studies demonstrated large- and small-intermediate-conductance Ca2+-activated K+ channels (Kca channels) in platelets. Flow cytometry analyses with Annexin V showed that in a high (120 mM) [K+] medium, PS exposure did not occur in platelets stimulated by thapsigargin, which releases Ca2+ from the endoplasmic reticulum and induces store-operated Ca2+ entry, or through receptors activation by a mixture of thrombin and convulxin. However, PS exposure induced by Ca2+-ionophores occurred in this medium, and also in a low (2.7 mM) K+ buffer in the presence of inhibitors of Kca channels (apamin, iberiotoxin, charybdotoxin and clotrimazole). Efflux of K+ occurred in low but not in high [K+] buffer in platelets stimulated by all agents, as measured with the fluorescence dye potassium-binding benzofuran isophthalate. Therefore, PS exposure is associated to K+ efflux in platelets stimulated by inducers of capacitative Ca2+ influx, whereas it occurs without Ca2+ movement with Ca2+-ionophores. Since PS exposure in EBV-immortalized B lymphocytes from Scott syndrome patients is defective under stimulation with Ca2+-ionophores despite normal Ca2+ influx, our results suggest that the founding element in this haemorrhagic pathology may not involve abnormal K+ movements.

C4.23

Cation exclusion in aquaporin water channels

B. Wu1, C. Steinbronn1, M. Alsterfjord2, T. Zeuthen2, A. Almasalmeh1 and E. Beitz2
1Christian-Albrechts-Universität zu Kiel, Pharmazeutisches Institut, Kiel, Germany, 2University of Copenhagen, Department of Cellular and Molecular Medicine, Copenhagen, Denmark

Aquaporin (AQP) water channels conduct water, glycerol and other small uncharged molecules across the plasma membrane along the respective osmotic/chemical gradient. Selective permeability for water and glycerol is achieved via the ar/R constriction and the NPA motif regions inside the monomer pores. These structural determinants render the pores highly effective in conducting water (water-specific aquaporins) or water plus solutes (aquaglyceroporins). Simultaneously, protons and cations are strictly excluded to maintain ionic transmembrane gradients. To experimentally address the mechanism of proton/cation exclusion in aquaporins, we conducted point mutagenesis within both constriction regions in AQPI and characterized their permeability for water and proton/cations in X. laevis oocytes and different yeast mutants. We showed that three point mutations turned the water-specific AQPI into a proton/alkali cation channel with reduced water permeability and the permeability sequence: H+ > K+ > Rb+ > Na+ > Cs+ > Li+. Contrary to theoretical models, our data indicated that electrostatic repulsion at the central NPA region does not suffice to exclude protons. Full proton exclusion was reached only in conjunction with the ar/R constriction at the pore mouth. In contrast, alkali cations were blocked by the NPA region but leaked through the ar/R constriction. Expression of alkali-leaking AQPs depolarized membrane potentials and compromised cell survival. Our results hint at the alkali-tight but solute-unselective NPA region as a feature of primary channels and the proton-tight and solute selective ar/R constriction variants as later adaptations within the AQP superfamily.

C4.24

A cellular fractionation approach to characterize the apical expression of SGLT1 in Caco2 and HT29 cells in response to glucose and insulin

C. Sa1, C. Lima2 and C. Pereira-Wilson1
1Department of Biology, CBMA – Centre of Molecular and Environmental Biology, University of Minho, Campus de Gualtar, Braga, Portugal, 2Department of Biology, CITAB – Centre for the Research and Technology of Agro-Environment and Biological Sciences, University of Minho, Braga, Portugal

Diabetes is a metabolic disorder caused by the lack of insulin production and/or a deficient response of target tissue cells to insulin (defined as insulin resistance). In addition to increased blood glucose levels, diabetes is also characterized by a raise in intestinal expression of the sodium-glucose cotransporter (SGLT1), a high affinity glucose/galactose transporter that requires the inward Na+ gradient to drive sugar transport across the brush border membrane (BBM) of the enterocytes. Caco2 and HT29 are cell lines often considered good in vitro models for the study of intestinal cellular adaptations. Here we report the effects of glucose and insulin on SGLT1 and GLUT2 protein expression in Caco2 and HT29 cells, which were submitted to a cellular fractionation procedure. Our results show that apical SGLT1 expression decreases with increasing media glucose, a response that is in contrast to the in vivo adaptations to luminal glucose. Studies are underway to evaluate the effect of apical versus basolateral exposure to glucose, insulin and short-chain fatty acids in these cell lines. CMS is supported by the Foundation for Science and Technology, Portugal, grant – SFRH/BD/42566/2007.

C4.25

Towards the structure-function-specificity relationship of glucose transporters

K. Elbing and K. Lindkvist-Peterson
University of Gothenburg, Cell and Molecular Biology, Gothenburg, Sweden

Glucose plays a central role in nutrient sensing and signalling and its uptake through facilitated diffusion is mediated by membrane associated glucose transporters, GLUT proteins in mammals and Hxt proteins in the yeast S. cerevisiae. These proteins belong to the Major Facilitator Superfamily (MFS), which is present in all studied organisms. They transport a wide range of solutes such as amino acids, sugars, nucleotides, drugs, peptides,
organic and inorganic anions, metabolites, neurotransmitters, polyols etc. All MFS proteins structurally investigated have been purified from their natural sources and successful heterologous production has not yet been reported. While there is a wealth of reports in the literature on mutagenesis studies, to analyze the structure-function relationship of glucose transporters, there are no three dimensional structures available. Our research goal is to achieve a more detailed understanding of the structure-function-specificity relationship of glucose transporters. To achieve this overall goal we have successfully expressed mammalian GLUTs and yeast Hxts in yeast. Initial solubilisation trials suggest that Brij35 solubilises GLUT4. Purification and crystallization analyses of the target proteins are ongoing.

C4.26 Morphological and biochemical changes in k562 lines at plasma membrane induced by TOM
M. D. Ene, L. Zglimbea, A. Netoiu, L. Olariu and E. Buse
SC Biotehnos SA, R&D, Otopeni - Ilfov, Romania

Determination of the relation between apoptosis and cellular process from the level of plasma membrane is important for in vitro evaluation of potentially chemotherapeutical agents. It represents an interesting trend for identification of events that can be used as indicators of tumor cell physiological disorders. The programmed cellular death is a fundamental physiological process observed during normal development but it is also involved in pathophysiological phenomena. Apoptosis is characterized by a variety of biochemical and morphological changes including chromatin condensation, DNA fragmentation and bleeding of the plasma membrane. Membrane potential is involved in the regulation of several immune functions developed in cells. The Na+/K+ gradient across the plasma membrane, mainly generated by the Na+/K+ pump plays a key role in the maintenance of membrane potential. This study is focused on the correlation between plasma membrane potential, apoptosis and membrane fluidity of a human chronic myelocytic leukemia cell line. After 24 hour of culture the K562 cells were exposed to different concentrations of TOM, a bio-compound isolated in Biotehnos laboratories. The cells are fluorescent marked in order to establish localization of the target proteins are ongoing.

C4.27 CFTR anion channel modulates expression of human transmembrane mucin MUC3 via the PDZ protein GOPC
T. Pelaseyed and G. C. Hansson
Biomedicine, University of Gothenburg, Medical Biochemistry, Gothenburg, Sweden

Mucins play a major role in maintaining epithelial protection in luminal organs. They act as lubricants against mechanical stress and they protect the underlying epithelial cells against pathogens. They have a rigid and extended structure due to their large mucin domains, rich in proline, threonine and serine residues that allow O-linkage of glycans. Three membrane-associated mucins, MUC3, MUC12 and MUC17, are unique in harboring Class I PDZ motifs, making them suitable ligands for PDZ-containing proteins. We performed a screening of 123 different human PDZ domains for binding to MUC3 and identified a strong binding between MUC3 and a PDZ protein GOPC. We demonstrated that this interaction was mediated by the C-terminal PDZ motif of MUC3, binding to a single PDZ domain on GOPC. GOPC acts as a binding partner of CFTR, down-regulating mature CFTR by degradation in post-Golgi compartments. Here, we show that GOPC down-regulated total levels of MUC3 and that this effect was reversed by introducing CFTR. Hence, these results demonstrate for the first time a regulative role of CFTR anion channel on expression of membrane-associated mucins.

C4.28 Lpe10p alters the activity of the Mrs2p-based yeast mitochondrial Mg2+ channel
G. Sponder1, S. Svɪdова1, R. Schindl2, R. J. Schweyen1, E. M. Froschauer1 and J. Weghuber2
1University of Vienna / Max F. Perutz Laboratories, Department for Microbiology, Immunobiology and Genetics, Vienna, Austria, 2University of Linz, Institute for Biophysics, Linz, Austria

The inner mitochondrial membrane forms a tight barrier for the passage of cations and protons. Mrs2p was found to be the first protein being involved in cation transport across the mitochondrial membrane. In previous studies we could show that it has the ability to form homo-oligomeric complexes in the inner mitochondrial membrane acting as a Mg2+ selective channel of high conductance. In this study we wanted to further characterize its homologue LPE10 also being a Mg2+ uptake system in bacteria. Deletion of either one or both genes results in a growth defect on non-fermentable carbon sources and the loss of rapid Mg2+ influx into mitochondria. Unexpectedly, only co-expression of both genes in a double deletion strain (mrs2Δ, lpe10Δ) leads to full complementation being the first indication ever that these two proteins cannot fully substitute for each other. In contrast to MRS2, deletion of LPE10 causes a considerable loss of the mitochondrial membrane potential (ΔΨ). Addition of the K+/H+ exchanger nigericin, which artificially increases the mitochondrial membrane potential, leads to restoration of Mg2+ influx into mitochondria from lpe10Δ, but not mrs2Δ, lpe10Δ cells. Thus, the loss of Mg2+ influx into lpe10Δ mitochondria is not due to the absence of a channel-forming protein, but rather because of the lack of a driving force essential for an active channel. Lpe10p therefore plays a dual role as constituent of a Msr2p-Lpe10p Mg2+ selective channel and as an essential regulator of the membrane potential in yeast mitochondria.

C4.29 Aquaglyceroporins selective for arsenite as alternative detoxification channels in some actinobacteria
J. Song, B. Wu and E. Beitz
Christian-Albrechts-Universität zu Kiel, Pharmazeutisches Institut, Kiel, Germany

Widespread inorganic arsenic compounds are potential toxins to almost all organisms. Pentavalent As (V), structurally mimicking phosphate ion, enters the cell via phosphate transporters and interferes with cellular metabolism involving phosphate; while As (III), with a pKa of 9.2, may pass through aquaglyceroporins in the form of neutral trihydroxide...
As(OH)₃ under normal pH. A major detoxification system common to most microbes is to rapidly reduce As (V) into As (III) by arsenite reductases ArsC or Acr2 and to excrete the reduced arsenite by exporters ArsB (or ArsBA complex) or Acr3 or even by aquaglyceroporins presumably. Although in recent years several aquaglyceroporins have been shown to conduct As (OH)₃ in heterologous expression systems, only one example is reported for physiological involvement of an aquaglyceroporin homolog in arsenic detoxification. Genome sequences of some actinobacteria reveal special aquaglyceroporin genes located in the ars operon replacing ArsB, suggesting a direct function of these isoforms in arsenic detoxification. We will present data regarding the physiological and biochemical functions and the selectivity features of these arsenite-selective aquaglyceroporin.

C4.30
Novel components of an active mitochondrial K⁺/H⁺ exchange
M. Aleschko¹, L. Zotova¹, G. Sponder¹, R. Baumgartner¹, S. Reipert², M. Prinz¹, R. J. Schweyen¹ and K. Nowikovsky¹
¹University of Vienna/Max F. Perutz Laboratories, Department of Microbiology, Immunobiology and Genetics, Vienna, Austria,
²University of Vienna/Max F. Perutz Laboratories, Department of Molecular Cell Biology, Vienna, Austria

Defects of the mitochondrial K⁺/H⁺ exchanger (KHE) result in increased matrix K⁺ content, swelling and autophagic decay of the organelle. We have identified the yeast Mdm38 and its human homologue LETM1, the candidate gene for seizures in Wolf-Hirschhorn syndrome. Two components of the KHE are essential for the cell’s survival. In a genome-wide screen for multi-copy suppressors of the petD phenotype we cloned the yeast Mdm38 and its human homologue LETM1 as moderate, and MRS7 and YDL183c as strong suppressors. Like Mdm38, MRS7 and YDL183c are mitochondrial inner-membrane proteins and constituents of ~500 KDa protein complexes. Triple mutant strain (mdm38Δ mrs7Δ ydl183cΔ) exhibits remarkably strong petD phenotype than mdm38Δ and a general growth reduction. They totally lack KHE activity, show a dramatic drop of mitochondrial membrane potential and heavy fragmentation of mitochondria and vacuoles. Nigericin, an ionophore with KHE activity, fully restores growth of the triple mutant, indicating that loss of KHE activity is the underlying cause of its phenotype. Mdm38 or overexpression of MRS7, YDL183c or LETM1 in the triple mutant rescues growth and KHE activity. A LETM1 human homologue, HCCR-1/LET-MD1, described as an oncogene, partially suppresses the yeast triple mutant phenotype. Based on these results, we propose that YDL183c and the Mdm38 homologues MRS7, LETM1 and HCCR-1 are involved in the formation of an active KHE system. Based on these results, we propose that MD1, described as an oncogene, partially suppresses the yeast triple mutant phenotype. Based on these results, we propose that MD1, described as an oncogene, partially suppresses the yeast triple mutant phenotype. Based on these results, we propose that MD1, described as an oncogene, partially suppresses the yeast triple mutant phenotype. Based on these results, we propose that MD1, described as an oncogene, partially suppresses the yeast triple mutant phenotype. Based on these results, we propose that MD1, described as an oncogene, partially suppresses the yeast triple mutant phenotype.

C4.31
Identification of the glycerol transporter-like protein in the halophilic black yeast Hortaea werneckii
T. Kogej, C. Gostincar and N. Gunde-Cimerman
Department of Biology, University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia

A halophilic black yeast Hortaea werneckii (Dothideomycetes, Ascomycota) inhabits hypersaline waters of marine salterns. H. werneckii grows at a wide range of salinities (0 – 5.2 M NaCl) and shows optimal growth at 0.85 – 1.7 M NaCl. Its ability to grow at such a wide range of salinities is rare amongst eukaryotic microorganisms and H. werneckii therefore represents a good model to study osmoadaptation. We have found previously that H. werneckii adapts to high NaCl concentration by accumulating glycerol and erythritol. In this study, we have identified a putative glycerol transporter protein of H. werneckii. A partial gene sequence was obtained from the expression library of H. werneckii and extended by genome walking. The gene was cloned and sequenced. It encodes a putative protein of 543 aa, which belongs to Major Facilitator Superfamily of transporters. BlastP showed its similarity to a number of putative sugar transporters and also to Stl1p from Saccharomyces cerevisiae. Stl1p is glycerol/H⁺ symporter of the plasma membrane with a role in osmo-adaptation. The putative protein of H. werneckii was named HwStl1 and it was characterized in silico. The studies of heterologous expression of HwStl1 in S. cerevisiae Astry1 mutant are currently under way. Additionally, we will evaluate the glycerol transporting ability of HwStl1 to conclude whether HwStl1 functions as a glycerol transporter in H. werneckii.

C4.32
The anterograde transport of the human Y2 receptor is regulated by the receptor C-terminus
C. Waither, K. Morl and A. G. Beck-Sickinger
Leipzig University, Faculty of Biosciences, Pharmacy and Psychology, Institute of Biochemistry, Leipzig, Germany

Neuropeptide Y receptors (YR) belong to the large superfamily of heptahelical G-protein coupled receptors. Four YR subtypes have been cloned from human tissue (Y1R, Y2R, Y4R and Y5R). These receptors are activated by the members of the NPY hormone family: neuropeptide Y (NPY), pancreatic polypeptide (PP) and peptide YY (PYY). Up to now the mechanism underlying intracellular trafficking processes, namely export, internalization, recycling and degradation, of the YRs is still unknown. In our work we investigated the cell surface transport of the Y2 receptor. We were able to identify a specific hydrophobic sequence motif in the proximal tail of the Y2 receptor C-terminus which is responsible for the export of the receptor from the endoplasmatic reticulum (ER) to the plasma membrane. Cell surface ELISA and fluorescence microscopy studies of alanine-substituted mutants revealed an accumulation and therefore retention of the receptor in the ER. Furthermore several C-terminal deletion mutants were generated by which we obtained a distinct accumulation of the receptor in the Golgi compartments. This implicates the presence of additional consensus sequences. These results demonstrate for the first time that a proximal C-terminal short sequence-motif is responsible for the export-regulation of the human Y2 receptor from intracellular compartments.

C4.33
Role of Rab GTPases in regulating neuronal membrane trafficking in C. elegans
N. Sasidharan¹, C. Oleniowicz¹, N. Wender¹, M. Sumakovic¹, S. Koenig¹, A. Audhya¹, A. Izen³, R. Goody¹, D. Fasshauer³, B. Grant¹ and S. Eimer¹
¹European Neuroscience Institute, Göttingen, Germany, ²University of Wisconsin, Madison, USA, ³Max Planck Institute for Molecular Physiology, Dortmund, Germany, ⁴Max Planck Institute for Biophysical Chemistry, Göttingen, Germany, ⁵Rutgers University, New Jersey, USA

The Rab family of small GTPases are molecular switches that regulate intracellular membrane trafficking in eukaryotes. As
opposed to the 61 Rab family members encoded in the mammalian genome, C. elegans has only 28 members facilitating a systematic analysis. To characterize the rabome in C. elegans, we determined the expression patterns of all C. elegans Rabs. To our surprise many Rab proteins were found to be highly cell-type specific. Rab GTPases seem to be particularly important for neuronal function, since they were found to be highly enriched in neurons. To analyze their roles in neurons, we determined their sub-cellular localizations. In addition, we systematically characterized all Rab mutants using a combination of tests ranging from a behavioral and biochemical analyses to high resolution imaging using High Pressure Freeze Electron Microscopy. From these studies, we have identified a novel Rab cascade specifically involved in the regulation of Dense Core Vesicle trafficking. Despite having identified specific functions for several of the Rabs, results have shown that there is significant functional redundancy between them. To address the redundancy among the Rab family members, we conducted large-scale synthetic RNAi screens. This study will provide for the first time a complete systematic analysis of Rab function within a multi-cellular organism leading to new insights on how intracellular membrane transport is dynamically regulated.

C4.34

Novel method for inhibitor screening of Plasmodium falciparum aquaporin

G. Fischer1, A. Moberg1, S. Tabaei2, M. Brändén2, F. Höök2 and R. Neutze1

1University of Gothenburg, Gothenburg, Sweden, 2Chalmers University of Technology, Gothenburg, Sweden

Malaria – causing millions of deaths every year worldwide – is caused by the protozoan parasite Plasmodium falciparium. As most forms of life, these contain proteins called aquaglyceroporins, which transport water and glycerol across the cell membrane. In particular, the transport of glycerol has been suggested to be essential to the parasite’s survival during its blood stage. We have developed a novel method to screen for potential inhibitors to this protein based on Surface Plasmon Resonance (Biacore®). Lipid vesicles containing the protein are immobilized on a gold surface and subjected to an osmotic gradient of sugar alcohols, which can be transported by the aquaglyceroporin. The transport kinetics of the solute are subsequently monitored via SPR and the differences in protein activity (NB: NOT binding) upon addition of a potential inhibitor are determined. The assay has been automated to be used in a 96-well format and capable to screen up to 50 compounds/day at very low protein consumption. First screening results yielded a compound which showed an effect on live Plasmodium falciparum cells in preliminary studies. To our knowledge, this is the first method, which is able to study the transport of uncharged solutes across membranes directly. The method also has the potential to easily be adapted to examine the function of other transporter proteins.
C5 – Energy Transduction

C5.01 On the rotary mechanism and ion binding specificity of F1F0-ATP synthases

D. Pogoryelov1, A. Krah2, J. Langer3, J. D. Faraldo-Gómez2 and T. Meier1
1Department of Structural Biology, Max Planck Institute of Biophysics, Frankfurt am Main, Germany, 2Max Planck Institute of Biophysics, Theoretical Molecular Biophysics Group, Frankfurt am Main, Germany, 3Department of Molecular Membrane Biology, Max Planck Institute of Biophysics, Frankfurt am Main, Germany

The mechanism of the F1F0 ATP synthase couples the downhill membrane translocation of H+ or Na+ to the rotation of an oligomeric ring of c-subunits (c-ring) in the F0 motor. The torque is transduced into the F1 motor, which causes sequential conformational changes in the catalytic centers, finally resulting in the generation of ATP. The design of the c-ring rotor provides the ion binding specificity and contributes to the translocation of the ions through the membrane during enzyme operation. The crystal structure of the c15 ring of the F1F0-ATP synthase from Spirulina platensis has been solved at 2.1 Å resolution (Pogoryelov et al., Nat. Struct. Mol. Biol., 2009). The way the proton is bound to this c-ring proposes that all ion binding sites of the c-ring remain in the proton-locked conformation while exposed to the membrane, whereas exposure to a more hydrophilic environment can unlock the ion binding site and promote ion release. This model is supported by combined structural, biochemical and in silico generated data of the proton binding site. A comparative study of the H+—binding c15 ring with the Na+-binding c11 ring from Ilyobacter tartaricus furthermore supports the notion that H+—selectivity is a property that is, in fact, shared by both F0 rotors but the structural arrangement of the c11 ring binding site is more prone to bind sodium ions instead of protons under physiological settings.

C5.02 Abstract withdrawn

C5.03 Antiporter activity of the complex I subunits NuoL, NuoM and NuoN from Escherichia coli analyzed in an in vivo model system

B. Kumar1, V. K. Moparthi1, C. Mathiesen2 and C. Hägerhäll2
1Lund University, Biochemistry and Structural Biology, Center for Molecular Protein Science, Lund, Sweden, 2The Norwegian University of Life Sciences, UMB, Aas, Norway

The complex I membrane spanning subunits NuoL, M and N are homologous to one particular class of Na+ or K+/H+ antiporters, encoded by the gene cluster denoted mrp/sha/pha/mnh in different bacteria. These subunits are prime candidates for harboring important parts of the proton pumping machinery of complex I. Deletion of mrpA or mrpD in Bacillus subtilis results in a Na+ and pH sensitive growth phenotype, making this a suitable model system to study antiporter function. Expression of MrpA in a B. subtilis ΔmrpD strain and vice versa did not result in any growth improvement under any condition tested. The expressed NuoL could rescue ΔmrpA to wild-type growth properties at pH 7.4, but enhanced the growth of ΔmrpD only to a lesser extent at this pH. The expressed NuoN could fully restore the wild type properties of ΔmrpD in the pH range from pH 6.5 to 7.5. In the ΔmrpA strain, expression of NuoN did not improve growth at pH 7.5 but resulted in some enhancement at pH 6.5. Cells expressing NuoM did not reach wild type growth levels in either deletion strain, but showed growth improvement under some of the conditions tested. At pH 6.5 no strain could be rescued by any complex I subunit. Taken together, this demonstrates that i.) the antiporter-like Nuo proteins can functionally replace real antiporters and ii.) each of the three complex I antiporter-like subunits have unique functional specializations and operate at different pH. The implications for the Complex I functional mechanism are discussed.

C5.04 Sodium interaction of the complex I antiporter-like subunits NuoL, M and N from Escherichia coli studied by 23Na NMR

K. Gorecki1, T. Drakenberg2, V. K. Moparthi1, E. Miklovyte1, M. Trané1 and C. Hägerhäll2
1Department of Biochemistry and Structural Biology, Lund University, Center for Molecular Protein Science, Lund, Sweden, 2Department of Biophysical Chemistry, Lund University, Center for Molecular Protein Science, Lund, Sweden

The complex I (NADH:quinone oxidoreductase) proteins NuoL, NuoM and NuoN are homologous to one type of Na+/H+ antiporters. These subunits are prime candidates for harbouring important parts of the proton pumping machinery. If they also retain antiporter function and/or the ability to translocate sodium is a matter of debate. In this work the sodium binding properties of the NuoL, NuoM and NuoN subunits from E. coli complex I was investigated and compared to those of the bona fide antiporters MrpA and MrpD from Bacillus subtilis using 23Na NMR spectroscopy. This technique is particularly suitable to monitor ion binding properties of macromolecules under conditions of very fast chemical exchange as expected from a transporter protein. High amounts of the individual proteins were obtained by expressing them as cytochrome c fusion proteins in E. coli. Purification was facilitated by a C-terminal histidine tag fused to the cytochrome c domain. The mobility of Na+ in the presence of the antiporter proteins was measured at gradually increasing concentrations. Cytochrome c alone was used as negative control protein. The specific binding constant for Na+ was estimated for each of the five proteins. The Na+ interaction was then assessed under different conditions and pH, in the presence of quinone and in the presence of the sodium-hydrogen exchange inhibitor 5-ethylisopropyl amiloride (EIPA). The sodium interaction was compared to the real antiporters MrpA and MrpD.

C5.05 Red complex I – using cytochrome c550 from Bacillus subtilis as a fusion domain to study NADH:quinone oxidoreductase

E. Miklovyte1, L. Moparthi1, T. Gustavsson2, M. Trané1 and C. Hägerhäll2
1Department of Biochemistry and Structural Biology, Lund University, Lund, Sweden, 2Novozymes A/S, Copenhagen, Denmark

Complex I (NADH:quinone oxidoreductase) contains four very large membrane spanning protein subunits that hitherto have...
been difficult to express individually in any appreciable amounts in *Escherichia coli*. The polypeptides contain no prosthetic groups or visual redox pigments and are poorly antigenic. In this work we have constructed fusion proteins where the C-terminal end of complex I protein subunits NuoH, NuoL, NuoM and NuoN from *E. coli* were genetically fused to the cytochrome c domain of Bacillus subtilis cytochrome c550. A naturally occurring transmembrane helix anchor was removed from the cytochrome c550 and was substituted by the membrane spanning polypeptide to be tagged. To facilitate purification of the proteins, a C-terminal his-tag was added to the cytochrome domain. The fusion proteins were expressed in *E. coli*, together with genes encoding cytochrome c maturation proteins, enabling holo-cytochrome c synthesis under aerobic conditions. The heme in cytochrome c is covalently bound to the polypeptide, renders the proteins visible by optical spectroscopy, and can be used to monitor and quantify the proteins, and to determine the orientation of the polypeptides when reconstituted in liposomes. Particularly the three large antiporter-like subunits NuoL, NuoM and NuoN, that previously had been particularly cumbersome to produce in *E. coli*, could be made in unprecedented amounts when expressed with a fused cytochrome c domain. Finally, a gene fragment encoding the NuoN-cytochrome c fusion protein was reintroduced into the nuo operon on the *E. coli* chromosome, allowing the production and characterization of cytochrome-tagged whole Complex I.

**C5.06**

**Low NAD(P)H fluorescence in rabbit skeletal myogenic stem cells increases upon differentiation**

V. Mildažiene, L. Degutytė-Fominis, O. Buzaite, R. Zukiene, Z. Naučiene and P. Džikas

Vytautas Magnus University, Centre of Environmental Research, Kaunas, Lithuania

Skeletal myoblasts have high potential for cell therapy based repair of the injured myocardium. Stem cell differentiation markers are important for searching appropriate cellular state optimal for engraftment. We followed differentiation induced changes in cellular distribution of mitochondria, cellular respiration, adenine and pyridine nucleotide content, NAD(P)H fluorescence, cellular size distribution in skeletal myogenic stem cell populations derived from rabbit skeletal muscle. Increase in fraction of larger size cells during differentiation was characteristic for all cell lines, although lines were heterogeneous by size and differentiation rate. Mitochondrial distribution changed from perinuclear in undifferentiated cells to even distribution in cytoplasm in differentiated cells. Cellular respiration rate decreased by 42–54% after 7 days differentiation compared to undifferentiated cells. The most remarkable feature of undifferentiated myoblasts was very low NAD(P)H fluorescence, that augmented after initiation of cell differentiation, due to metabolic and physiological changes but that did not correlate with increase in cellular size. Chromatographically determined concentration of the reduced and oxidized forms of pyridine dinucleotides and NAD(P)H fluorescence markedly increased on the 7th day of differentiation. Thus, an increase in NAD(P)H fluorescence may be useful for non-invasive detection of the onset of stem cell differentiation. The total amount of adenine dinucleotides (ATP, ADP, AMP) increased, but the ATP/ADP ratio decreased during differentiation. This work was supported by Lithuanian State Science and Studies Foundation, project No. B-26/2009.

**C5.07**

**Binding oxygen to heme d in cytochrome bd from Escherichia coli**

D. Bloch1, V. Borisov2 and M. Verkhovsky1

1University of Helsinki, Institute of Biotechnology, Helsinki, Finland, 2Lomonosov Moscow State University, Belyozersky Institute of Physical Chemical Biology, Moscow, Russian Federation

Cytochrome bd is expressed under low oxygen tension and has high affinity for O2. The enzyme as isolated is a mixture of two forms: (i) ferrous heme d bound to molecular oxygen ("state A"), and (ii) ferryl oxene heme d ("state F"), with the latter form contribution of 20–50%. Both forms can be reversibly destroyed either by oxidation (anaerobically or aerobically) or by depletion of oxygen at *Eh* where the enzyme remains in one-electron-reduced state. Binding of O2 to heme d was studied using the quasi-equilibrium OTTLE spectroelectrochemistry, where oxygen was allowed to equilibrate with the enzyme at a given *Eh*. Under the anaerobic conditions heme d has *E\textsubscript{m,app} ~ +260 mV* (vs. NHE, pH 7, 0.1% SML); at 1.2 mM O2, the *E\textsubscript{m,app} value becomes +495 mV. The *E\textsubscript{m,app} -dependence of *E\textsubscript{m,app} ~ +260 mV* (vs. NHE, pH 7, 0.1% SML); at 1.2 mM O2, the *E\textsubscript{m,app} value becomes +495 mV. The *E\textsubscript{m,app} -dependence of *E\textsubscript{m,app} ~ +260 mV* (vs. NHE, pH 7, 0.1% SML); at 1.2 mM O2, the *E\textsubscript{m,app} value becomes +495 mV. The *E\textsubscript{m,app} -dependence of the oxygen affinity is linear at the concentrations above 10 μM with the slope -60 mV/pO2 and the effective dissociation constant *Kd ~ 150 nM* O2. The latter value differs from the earlier data (*Kd ~ 280 nM* O2) where the oxygen affinity was directly measured in one-electron-reduced isolated enzyme. The difference is attributed to the fact that in the presence of O2, the enzyme catalyzes a steady-state flux of electrons supplied by the working electrode. Modeling proves that in the steady-state, the *K0* value of 280 nM is reached when the kinetics of the O → A and A → (F) → O transitions are the same and limited by the electron delivery from the working electrode, and the A → F transition is much faster. The O → A and A → F transitions show similar *E\textsubscript{m,app} values over the broad [O2] range. We propose that in the state F, heme d has an unusually low redox potential comparable to that of the state A-enzyme.
D1 – Secretory Pathways

D1.01
Functional analysis of the putative signal peptide of the PAR1 receptor
D. Zampatis1, N. Tsopanoglou2 and R. Schuelein1
1Leibniz institute for Molecular Biology, Berlin, Germany, 2Department of Pharmacology, University of Patras, Medical School, Patras, Greece

Protease-activated receptor-1 (PAR1) belongs to the family of G protein-coupled receptors which can be activated by proteolytic cleavage of their N-terminal extracellular domain. Cleavage at the R41/S42 bond of human PAR1 by thrombin unveils a tethered ligand with the recognition sequence SFLLRN. This sequence binds to conserved regions in the second extracellular loop of the receptor resulting in receptor activation and initiation of signal transduction. The N terminus of the PAR1 contains a hydrophobic domain (residues 1–23) which may represent a cleavable signal peptide according to bioinformatic analyses. Signal peptides have been shown to be necessary for the integration of G protein-coupled receptors into the ER membrane, the first step of their intracellular transport. Usually, these signal peptides are cleaved-off from the mature receptor following ER integration. However, this may not always be the case, as it has been shown recently in the case of the uncleaved pseudo signal peptide of the CRF2a (Rutz et al., 2006, J. Biol. Chem. 281, 24910-24921). In the present study we have investigated whether the hydrophobic sequence of PAR1 indeed represents a functional signal peptide. To address this question, we have constructed GFP marker proteins fusions with the putative signal peptide and have expressed these constructs in HEK 293 cells. Signal peptide cleavage was assessed by GFP secretion analyses using confocal microscopy and immunoprecipitation experiments. Our results indicate that the hydrophobic sequence 1-23 of PAR1 represents a functional signal peptide.

D1.02
Regulation of the COPI coat dependent trafficking by ubiquitination and phosphorylation in yeast S. cerevisiae
K. Jarmoszewicz1, K. Lukasiak1 and J. Kamińska1
1Institute of Biochemistry and Biophysics PAS, Department of Genetics, Warsaw, Poland, 2Institute of Biochemistry and Biophysics PAS, Warsaw, Poland

The trafficking of proteins between membrane limited organelles is mediated by vesicles which form from one membrane and fuse with the other. Formation of vesicles is mediated by coat proteins. One of the coat type is COPI that is involved in trafficking along the secretory and biosynthetic pathways. This coat consists of seven coatomer subunits: α, β1, β2, δ, ε, γ, ε-COP. It was shown that β′-COP is ubiquitylated and this ubiquitination has a regulatory role. Ligase responsible for β′-COP ubiquitination is unknown. Rsp5, an ubiquitin-protein ligase is involved in several intracellular trafficking steps. Because rps5 mutations interact genetically with genes encoding β-COP and ε-COP and Rsp5 cooperates with the COPI proteins in Golgi-ER trafficking we tested if Rsp5 can be responsible for β′-COP ubiquitination. We also found additional form of β′-COP subunit, probably phosphorylated one and focus our study on identification of kinases and phosphatases responsible for modification of β′-COP and on determination of the role of this modification on Golgi to ER trafficking. The NetPhos server predicted that β′-COP could be a substrate for protein kinase A, kinase C and casein kinase II, consequently strains with mutations affecting function of these kinases were tested for the presence of phosphorylated form of β′-COP. Additionally, strains lacking some phosphatases were examined for the accumulation of phosphorylated form of β′-COP protein.

D1.03
Biochemical and functional diversity of the yolk granule organelle present in the sea urchin egg and embryo
J. J. Robinson
Memorial University of Newfoundland, Biochemistry, St John’s, Canada

Using sucrose density gradient ultracentrifugation, we have identified two distinct populations of the yolk granule organelle resident in the sea urchin egg and embryo. Biochemical analyses of the higher and lower density granule populations revealed striking differences in their protein contents. Gelatin substrate gel analysis identified unique cleavage activities associated with each of the populations. Further characterization revealed that different forms of the Major Yolk Protein were housed in each of the granule populations. Structural differences between the Major Yolk Protein variants could be detected by their differing buoyant densities, susceptibilities to digestion with chymotrypsin and their interactions with the detergent, Triton X-100. Clearly, in the egg and developing embryo the yolk granule organelle exists in at least two biochemically distinct populations. The implications for the functional role(s) of the yolk granule during embryonic development will be discussed. Supported by a grant from the Natural Sciences and Engineering Research Council of Canada.

D1.04
The ultrastructure of the corpora allata of pimpla turionellae L. (Hymenoptera: Ichneumonidae)
A. Özli̇k1 and N. Gül2
1Hittit University Faculty of Science and Art, Biology, Corum, Turkey, 2Ankara University Faculty of Science, Biology, Ankara, Turkey

As a known endocrine gland, corpora allata (CA) of adult female Pimpla turionellae were examined ultrastructurally by Transmission Electron Microscopy (TEM). The gland was covered by thick fibrous connective tissue and it was dispersed in the gland as a stromal ramification. The ultrastructure of gland cells had the same characteristic ultrastructure of protein-secreting cells. These cells had a large and oval shape nucleus, numerous and little mixed together granules but occasionally had lysosome, mitochondria and vacuoles. There are electron-dense, electron-modarate, and electron-lucent type of these granules. These granules are actually membrane-limited secretory vesicles which are filled with the proteinaceous hormone produced by the corpora allata cells. Numerous secretory granules, microtubules and mitochondria were observed in the cytoplasm of axons of neurosecretory cells.
The paraldehyde fuchsin positive material in corpora cardiaca of pimpla turionellae L. (Hymenoptera: Ichneumonidae) during egg maturation

A. Özlik1 and N. Gül2

1Hıdı University Faculty of Science and Art, Biology, Corum, Turkey, 2Ankara University Faculty of Science, Biology, Ankara, Turkey

The purpose of this study is to examine the effects of the neurosecretory material in corpus cardiacum (CC) on reproduction of endoparasitic Pimpla turionellae L. For this purpose, the egg maturation and amount of the neurosecretory material in corpus cardiacum of the insects were examined by the serial cross sections. Egg maturation was determined by measuring the terminal oocyte length in the serial cross sections of the ovariole. The egg growth which has observed on the 3rd day, reached the maximum on 15th day and after that day the egg laying was observed. The sections of insect brains were stained by paraldehyde fuchsin. The amount of the paraldehyde fuchsin positive neurosecretory (PF+NS) material in CC was at a certain level in the first day of egg development. While terminal oocyte was reaching the maximum length, the amount of the PF+NS material in CC decreased the minimum level. During the egg laying phase, the amount of the PF+NS material in CC reached the maximum level. These observations give us the idea that the neurosecretory material in corpus cardiacum of this insect may be related to the egg development.

D1.06 Systems Biology of Protein Secretion in Saccharomyces cerevisiae: Mapping of Global Regulatory Structures

K. Tyo, Z. Liu, D. Petranovic and J. Nielsen

Chalmers University of Technology, Chemical and Biological Engineering, Göteborg, Sweden

The yeast Saccharomyces cerevisiae serves as an important eukaryotic model organism, both in the elucidation of molecular mechanisms underlying many human diseases and in the production of drugs, food products, industrial chemicals, and fuels. The secretory pathway, responsible for processing a third of all synthesized protein, has been studied extensively by reductionist approaches, but until recently, tools for systemic study of the secretory pathway have been inadequate. Here we undertook a global analysis of the protein secretion pathway in S. cerevisiae. Using network-based analysis of transcriptome data, key transcription factors and biological processes were identified related to disturbances in the secretory pathway. The disturbances, (i) recombination secretion of human insulin and α-amylase proteins, and (ii) deletion of the Unfolded Protein Response (UPR) transcription factor HAC1, caused significant physiological changes that could be mapped to (a) oxidative stress, (b) amino acid synthesis/salvage, and (c) overall transcriptional synthesis pathways. Through our analysis, we propose a holistic model for secretory pathway stress, linking endoplasmic reticulum folding to oxidative radical formation and NADPH consumption. This model should be useful as a scaffold for understanding protein secretion in higher eukaryotic cells and in the analysis of protein folding-related disease.

D1.07 Alpha amylase secretion from S. cerevisiae

Z. Liu, K. Tyo, D. Petranovic and J. Nielsen

Chalmers University of Technology, Gothenburg, Sweden

Being one of the best studied model organisms and armed with a vast array of molecular and biochemical technologies, S. cerevisiae has the potential to be an attractive cell factory for producing heterologous proteins. Here we evaluated α-amylase secretion using different expression strategies and aimed to gain a more detailed understanding of protein production mechanisms. The α-amylase from A. oryzae was directed by two kinds of leader sequence, the alpha factor leader and a synthetic leader and expressed separately in three different plasmids, POT plasmid with POT1 marker from S. pombe and TEF1 promoter for protein expression, CPOT plasmid with POT1 marker and TPII promoter, and p426GPD plasmid with URA3 marker and GAD-PH promoter. The synthetic leader in plasmid POT and p426GPD, compared to the alpha factor leader, produced more amylase, whereas the combination of the alpha factor leader and the CPOT plasmid showed the highest titer and specific production rate. This study sheds light on the effect of plasmid, promoter, and secretion leader sequence on protein secretion.

D1.08 The Pex11-protein family members control peroxisome proliferation

A. Huber, J. Koch, K. Pranjic, C. Brocard, F. Kragler and A. Hartig

University of Vienna, Max F. Perutz Laboratories, Center of Molecular Biology, Vienna, Austria

Peroxisomes are essential single membrane-bound organelles present in all eukaryotic cells. They enclose hydrogen peroxide-generating and degrading enzymes, their function is mainly associated with lipid metabolism and in yeasts, they are the exclusive site of fatty acid β-oxidation. These organelles quickly adapt their size, number and protein content to the cellular needs. Peroxisome biogenesis and proliferation is controlled by peroxins (PEX-proteins), more than 30 of these have been identified so far. Saccharomyces cerevisiae cells lacking PEX11 display fewer and larger peroxisomes and reduced utilization of oleic acid compared to wild type cells. Together with Pex25p and Pex27p, Pex11p represents the Pex11-protein family, orthologs of which have been identified in all eukaryotic organisms. Pex11-proteins participate in the proliferation of peroxisomes but their detailed molecular function is not known. Hence, we performed functional tests in S. cerevisiae wild type cells and deletion mutants lacking either one, two or all three PEX11-protein family members but expressing one of the yeast homologs from a plasmid. We recorded complementation of the deletion phenotypes, changes in size and number of peroxisomes and analysed mRNA levels using real-time PCR. Expression of homologs altered peroxisome number and/or re-established wild type levels of oleic acid consumption. Pex11p and Pex25p seem to play a crucial role regarding peroxisome abundance and function, while Pex27p might be a negative regulator. In summary our data suggest that these proteins influence each others expression thereby preparing the peroxisome for fission.
**D1.09 Understanding SNARE regulation via SM proteins: Insights from Sly1p/Sed5p interaction**

E. Demircioglu, G. Sicoli, M. Bennati and D. Fasshauer

Max Planck Institute for Biophysical Chemistry, Goettingen, Germany

Sec1/Munc18 (SM) proteins are important regulators of intracellular membrane fusion. They exert their function through interacting with the SNARE machinery, primarily with syntaxins. Despite the high structural homology among SM proteins, different modes are proposed for their association with syntaxins. Neuronal Munc18a binds to a “closed conformation” formed by Syntaxin1, whereas several SM proteins interact only with the N-peptide motifs of their cognate syntaxins. On the other hand, recent findings suggest that all SM proteins might exert a regulatory role, acting on both the N-peptide motifs and the closed conformation of syntaxins. So far, yeast Sly1p, the SM protein of ER-Golgi trafficking, is considered to interact solely with the N-peptide portion of its cognate syntaxin Sed5p. We investigated the Sly1p/Sed5p association in light of the proposed common binding model through biochemical and biophysical methods. The current analysis shows that N-peptide binding is indeed the major contributor to the high affinity, yet the remainder of Sed5p contributes as well. Interestingly, individual Sed5p seems to exist in a stable closed conformation, which slows the formation of a SNARE complex. Therefore, we are interested to know how Sed5p switches to a SNARE-accessible conformation and whether Sly1p has a role on this.

**D1.10 Membrane-localized chaperone interactions in the folding of polytopic membrane proteins**

N. Hörnge, P. O. Ljungdahl and C. Andréasson

Department of Cell Biology, Stockholm University, Wenner-Gren Institute, Stockholm, Sweden

In eukaryotic cells polytopic membrane proteins fold to attain their native three-dimensional conformations within the lipid bilayer of the endoplasmic reticulum (ER). Folding requires specific interactions between the transmembrane segments. In analogy with the folding process of soluble proteins, chaperones might transiently associate with the transmembrane segments during synthesis and thereby facilitate folding by preventing non-productive interactions. Shr3 is an integral membrane protein of the ER of Saccharomyces cerevisiae that potentially functions as a specialized membrane-localized chaperone for members of the amino acid permease family. In shr3A cells the 12 membrane-spanning segments of amino acid permeases insert into the membrane resulting in the correct topology but the permeases do not exit from the ER and instead accumulate in large detergent-resistant aggregates. Currently, we are investigating the structural and temporal requirements for the interaction between Shr3 and the amino acid permeases. Specifically, we are implementing an in vitro cross-linking approach using unnatural photo-reactive amino acids incorporated into the transmembrane segments of Shr3. Moreover, we have set up conditional expression of Shr3 via fusions to regulated degrons that target the protein for proteasomal degradation. By employing these two methodologies we hope to define how and when Shr3 interacts with its substrates.
ated by the VGFAEAA peptide was not. Given the outcomes of this study we propose a model for the trafficking of cardosin A to the vacuole depending on each of the described domains. The validation of cardosin A vacuolar sorting determinants and of its trafficking pathways can provide helpful tools for the study of vacuolar transport of other proteins.
D2 – Mitochondria

D2.01 Human mitochondrial transcription and replication proteins are regulated via differential pathways
F. Brunì1, P. Loguercio Polosa, M. N. Gadaleta, P. Cantatore and M. Roberti
Department of Biochemistry and Molecular Biology, University of Bari, Bari, Italy

The expression of many nuclear genes encoding mitochondrial proteins is regulated by several nuclear transcription factors. NRF-2 is a transcription factor involved in the control of cell cycle progression, protein synthesis and mitochondrial biogenesis. Concerning NRF-2 control on mtDNA transcription and replication proteins, the few available data indicate that it positively controls the transcription initiation factors TFAM and TFB2M. In the current study, we investigated the dependence on NRF-2 of the genes for other proteins of the minimal mitochondrial transcription and replication machineries: the transcription termination factor mTERF, the RNA polymerase (POLRMT), the transcription repressor MTERF3, the helicase TWINKLE, the single-stranded binding protein (mtSSB) and the catalytic (POL-yA) and accessory (POL-yB) subunits of DNA polymerase γ. By EMSA and ChIP assays, we demonstrated the functionality of in silico predicted binding sites for NRF-2 in the promoters of mTERF, POLRMT, TWINKLE, mtSSB and POL-yB genes. A putative site predicted in POL-yA promoter was unable to interact with NRF-2. No binding was predicted and experimentally observed for MTERF3 gene promoter. Moreover, knock-down and overexpression experiments definitively ruled out the NRF-2 control on MTERF3 and POL-yA genes, and confirmed the positive regulation on the expression of other proteins. In conclusion, the current study extends the collection of mitochondrial genes controlled by NRF-2. Interestingly, NRF-2 does not regulate the genes for POL-yA and for MTERF3; this evidence arouses interest in the study of differential pathways responsible for the regulation of these two proteins.

D2.02 Is mitochondrial genome chicken or egg of carcinogenesis?
A. Czarniecka1, A. Klemba1, P. Katarzyna2, K. Tomasz2, J. Lubinski3, M. Zdrozny2, B. Kofler6, M. Mossakowska7, E. Bartnik2 and J. Petros8
1Department of Oncology, Military Institute of the Health Services, Laboratory of Molecular Oncology, Warsaw, Poland, 2University of Warsaw, Institute of Genetics and Biotechnology, Warsaw, Poland, 3Institute of Genetics, Polish Poland, 4Institute of Medical University, International Hereditary Cancer Center, Clinical Pathology, Lodz, Poland, 5Department of Oncology and Breast Diseases, Institute of Polish Mothers Health Center, Szczecin, Poland, 6Department of Oncology and Breast Diseases, Institute of Polish Mothers Health Center, Lodz, Poland, 7Department of Pediatrics, Paracelsus Medical University, Salzburg, Austria, 8International Institute of Molecular and Cell Biology, Warsaw, Poland, 9Department of Urology, Emory University School of Medicine, Atlanta, US

Objectives: Investigation of the role of mtDNA mutations and inherited polymorphisms in breast cancer development.
Methods: mtDNA NADH-dehydrogenase genes, D-loop sequence and haplogroup distribution analysis was performed in breast cancer patients, centenarians and women with cancer free history cohorts. Subsequent comparison with general Polish population was performed.

Results: The mitochondrial haplogroup distribution in patients with breast cancer differs from a group of cancer-free controls and general population as haplogroup I is overrepresented. Breast cancer patients in comparison to cancer free control cohort abundantly carry 10398G (p < 0.001). This polymorphism is cancer development factor with OR = 9.510 and RR = 7.576. A10398G RFLP test has high sensitivity = 0.769 and specificity = 0.740. Moreover in breast cancer population A1811G and A4529T are overrepresented (p = 0.001) if compared with centenarians. Patients with biopsy-proven breast cancer carry specific sets of mtDNA polymorphisms including G3915A, A4727G, A4769G, T3082C, A8860G, T10034C, T10238C, A10398G, A10859G, T11233C, A11467G, G11718A and G12372A. Some of the polymorphisms are very rare (i.e. T11233C) or not reported before (i.e. T5082C) in general population and may be classified as breast cancer bio-markers.

Conclusions: mtDNA polymorphisms establish a specific genetic background for cancer development and may enable selection of populations at high cancer risk and support the process of prevention and early diagnosis.

D2.03 Consequences of the activation of mitochondrial ATP-sensitive potassium channel on the physico-chemical properties of mitochondria from rat myomertum
O. Vadyuk and S. Kosterin
Department of Muscle Biochemistry, Palladin Institute of Biochemistry, Kiev, Ukraine

Mitochondrial ATP-sensitive potassium channel is a main factor of regulation of K+ exchange in mitochondria. It is assumed, that activation of this structure leads to improvement of mitochondrial physiology. MitoKATP channel from cardiac muscle is relatively well described, but functioning of this channel in other tissue, in particular in smooth muscles, is not fully understood yet. Aim of this work was to determine the consequences of activation of mitoKATP on the functioning of mitochondria from the rat myomertum. Using photon correlation spectroscopy we measured an average diameter of mitochondrial particles. It was found that average size of mitochondria in KCl-containing medium was 470.6 ± 23 nm, while ATP decreased their size to 416.4 ± 21.7 nm. Addition of channel activator diazoxide restored mitochondrial size to 459 ± 32.3 nm, while blocking of the channel with selective blocker glibenclamide decreased an average mitochondrial size to 430 ± 28 nm. And none of the above mentioned effects were showed in medium which did not contain KCl. Thereby, using selective activator and blocker of mitochondrial ATP-sensitive potassium channel we showed that activation of this structure in mitochondria from myomertum increased their average diameter and leaded to partial depolarization of the inner membrane.
Diabetes mellitus may lead to a number of complications, which determine the clinical progression of the disease and affect various tissues. Streptozotocin-induced diabetes mellitus (STZ) is widely used to induce experimental diabetes due to its ability to selectively target and destroy insulin-producing pancreatic beta-cells. Several groups have investigated mitochondrial function in type 1 diabetes, reporting mitochondrial oxidative stress and impairment of mitochondrial respiration and OXPHOS complex activities. Inhere, protein expression profiles of rat brain mitochondria were compared between animal models of streptozotocin-induced diabetes mellitus (STZ) and age matched controls (AMC) at 3 weeks after induction of hyperglycaemia. Protein samples from four STZ and four AMC rat brain mitochondria were analyzed with a quantitative 2D-gel electrophoresis, to assess statistically significant expression changes. First tests were conducted on a wide range of pH 3-10, and later ones using zoomed pH range, as 6-11 and 4-7, and only in the latter range proteins show a statistically significant variation. A total of four spots were differentially expressed between the two experimental conditions, and all were decreased in the diabetic condition. These spots were cut from the gel, digested with trypsin, and subsequently analyzed by HPLC-ESI-MS/MS. Among these identified proteins a decrease of more than a half of NDUFS3 expression is of particular interest, as this is a core subunit of complex I, fundamental for both respiration and OXPHOS complex activities. Inhere, protein expression profiles of rat brain mitochondria were compared between animal models of streptozotocin-induced diabetes mellitus (STZ) and age matched controls (AMC) at 3 weeks after induction of hyperglycaemia. Protein samples from four STZ and four AMC rat brain mitochondria were analyzed with a quantitative 2D-gel electrophoresis, to assess statistically significant expression changes. First tests were conducted on a wide range of pH 3-10, and later ones using zoomed pH range, as 6-11 and 4-7, and only in the latter range proteins show a statistically significant variation. A total of four spots were differentially expressed between the two experimental conditions, and all were decreased in the diabetic condition. These spots were cut from the gel, digested with trypsin, and subsequently analyzed by HPLC-ESI-MS/MS. Among these identified proteins a decrease of more than a half of NDUFS3 expression is of particular interest, as this is a core subunit of complex I, fundamental for both its assembly and function. These results underline the involvement of complex I in type 1 diabetes. We are carrying out further studies in order to confirm the other identified proteins.
events in the cytoplasm, mitochondria and nucleus involved in the apoptosis resistance to cisplatin (10 μM and 50 μM) in MCF7 cells WT and MCF7 that overexpress bcl-2. The compartmentation of GSH, mitochondrial distribution, apoptosis detection and [Ca^{2+}] were studied by confocal microscopy. The cell cycle, ROS level and the mitochondrial transmembrane potential (mmp) was studied by flow cytometry and the cellular level of reduced glutathione (GSH) by spectrophotometry. Mitochondrial perinuclear rearrangement was an early event, followed by the decrease in mmp. The doses dependent increase in [Ca^{2+}] was followed by its perinuclear distribution, and release to the cytoplasm. The GSH level and its nuclear compartmentation were increased in the cells that resisted apoptosis. All these events were delayed or less significant in MCF7 bcl-2 cells. Bcl-2 overexpression conferred increased apoptosis resistance and was demonstrated to increase nuclear glutathione concentration, thus providing protection at nuclear level, apart from its well documented effect on mitochondria. The mitochondrial morphology and distribution could represent a key factor in the nucleus-mitochondrional cross talk.

D2.08 cAMP dependent protein kinase regulates post-translational processing and expression of complex I subunits in mammalian cells

S. Papa1, S. Sacco1, D. De Rasmo1, A. Signorile1, F. Papa1, D. Panelli1, A. Nicastro1, R. Scaringi1, A. Santeramo1, E. Roca1, R. Trentadue1 and M. Larizza1

1University of Bari “A. Moro”, Department of Medical Biochemistry, Biology and Physics, Bari, Italy; 2Department of Odontostomatological and Surgery, University of Bari “A. Moro”, Bari, Italy, 3Italian Research Council, Institute of BiOMEMBRANES and Bioenergetics, Bari, Italy

Work is presented on the role of cAMP-dependent protein phosphorylation in post-translational processing and biosynthesis of complex I subunits in mammalian cell cultures [1,2]. PKA-mediated phosphorylation of the NDUFS4 subunit of complex I promotes in cell cultures in vivo import/maturation in mitochondria of the precursor of this protein [3]. The import promotion appears to be associated with the observed cAMP-dependent stimulation of the catalytic activity of complex I. These effects of PKA are counteracted by activation of protein phosphatase(s). PKA and the transription factor CREB play a critical role in the biosynthesis of complex I subunits. CREB phosphorylation by PKA and/or cAMKs activates, at nuclear and mitochondrial level a transcriptional regulatory cascade which promotes the concerted expression of nuclear and mitochondrial encoded subunits of complex I and other respiratory chain proteins [4].

Acknowledgement: National Project on “Italian Human Proteome Net” RBRN07BMCT_014 (MIUR).

References:
branes. Respiratory Control, ΔΨ generation). Then, the amounts of flavins in aliquots of neutralized perchloric extracts of both spheroplasts and mitochondria were measured by HPLC. SCM capability to metabolise externally added and endogenous FAD and FMN was investigated both spectroscopically and via HPLC. FAD deamination and FMN dephosphorylation were studied with respect to following features: dependence on substrate concentration, pH profile and inhibitor sensitivity. The existence of two novel mitochondrial FAD pyrophosphatase (diphosphatase) (EC 3.6.1.18) and FMN phosphohydrolase (EC 3.1.3.2) activities, which catalyse FAD + H₂O → FMN + AMP and FMN + H₂O → riboflavin + Pi conversion, respectively, is here shown. In the light of cytosolic riboflavin, FMN and FAD concentrations, probably mitochondria play a major role in regulating the flavin pool in yeast cell under glucose limitation and in relation to flavoprotein deflavination and reconstitution.

D2.12 The functions and mitochondrial targeting of NADH dehydrogenase (ubiquinone) flavoprotein 2 (NDUFV2) subunit in human mitochondrial complex I
H.-Y. Liu and M.-C. Kao
National Tsing Hua University, Institute of Molecular Medicine, Hsinchu, Taiwan

NADH dehydrogenase (ubiquinone) flavoprotein 2 (NDUFV2), containing one iron sulfur cluster ([2Fe-2S] binuclear cluster N1a), is one of the core nucleus-encoded subunits existing in human mitochondrial complex I. Defects in this subunit have been associated with Parkinson’s disease, Alzheimer’s disease, Bipolar disorder and Schizophrenia. In this study, we applied the RNA interference (RNAi) technology in human T-REx 293 cells to investigate the function of NDUFV2 subunit. The results showed that suppression of NDUFV2 expression in the cells would slow down the growth rate of cell in galactose medium, decrease the oxygen consumption rate, reduce mitochondrial membrane potential and increase reactive oxygen species (ROS) generation, but did not affect complex I assembly. These observations provided the evidences that NDUFV2 plays an essential role for energy transduction in cells. In addition, we also generated various truncated constructs and point-mutated mutants to investigate the mitochondrial targeting mechanism of NDUFV2. It was found that the cleavage site of NDUFV2 was located around amino acid residue 32 and the first 22 residues of NDUFV2 were enough to function as a mitochondrial targeting sequence (MTS) to carry the passenger protein into mitochondria. In a recent study, the patients with hypertrophic cardiomyopathy and encephalomyopathy were found to contain a 4 bp-deletion in the second intron of NDUFV2 gene (IVS2 + 5_+ 8delGTAA) thus cause the exon 2 losing. To dissect the pathogenic mechanism of this mutation, we established the human disease model and found that lost of this exon 2 causes NDUFV2 to lose its mitochondrial targeting ability.

D2.13 Mitochondria are a biochemical target of the perfume chemical components: Lilial, Lyral and Hedione
J. Usta1, H. Yassmine2, Y. Bou-Moughlabay1, O. Al-Rifi1, P. Barnabe1, H. Chmaisse-Nacchache2, R. Fakhury4 and D. Griffiths4
1AUB, Biochemistry, Beirut, Lebanon, 2Beirut Arab University, Environmental Biology, Beirut, Lebanon, 3Beirut Arab University, Pharmacy, Beirut, Lebanon, 4Beirut Arab University, Faculty of Science, Beirut, Lebanon, 5Warwick University, Physiological Chemistry, Coventry, UK

Fragrance chemicals are among the basic components of commercial perfumes formulation and are extensively used in cosmetics and food products. The majority of fragrance chemicals are lipophilic molecules that cross the plasma membrane and interact with intracellular or intra-organelle sites initiating thus, many events that may lead to cell death. In this study the biological effect of lilial, lyral and hedione, widely used chemicals in perfume industry, was investigated on several cell lines in culture including: MCF7, Caco2, Hek293, NIH3T3, HepG2 and HaCat. Cells were treated for 24 hours with each chemical at concentration varying between 10–100 μM. Viability was evaluated using MTT assay. No significant effect was found with MCF7, Caco2, Hek293, NIH3T3, HepG2 or HaCat. A decrease of 87%, 76% and 89%, in the viability of HaCat (primary keratinocytes) cell was obtained with 50μM of each lilial, lyral and hedione and an estimated EC50 of 59 nM, 100 nM and 5 μM respectively. Further investigations have shown a significant decrease in ATP level of: 40%, 69%, and 50% and a significant increase in reactive oxygen species level of: 50%, 50%, 53% following HaCat treatment with lilial, lyral and hedione at their EC50 value respectively. The effect of these chemicals on the activity of NADH dehydrogenase and Succinate dehydrogenase (complexes I & II) and complex V (ATPase) of mitochondria isolated from HaCat cells is currently being investigated. Our preliminary data suggest mitochondria as a target of these chemicals.

D2.14 Effect of minocycline and ATP on structure and function of non-native conformations of cytochrome c
A. Patriarca1, M. C. Piro1, M. Barl1, F. Politeccll2 and L. Fiorucci1
1Department of Experimental Medicine and Biochemical Sciences, University of Roma Tor Vergata, Rome, Italy, 2University of “Roma Tre”, Biology, Rome, Italy

Cytochrome c (cyt c) is a peripheral membrane protein that is associated with the inner mitochondrial membrane via the interaction with the phospholipid cardiolipin (CL). Beyond the role as electron carrier in the respiratory chain, cyt c is involved in the triggering of apoptosis. Chronologically, the latest function attributed to cyt c has been associated to its ability to be activated and fulfill the task of a peroxidase. It’s been demonstrated that post-translational modifications, such as oxidative or nitrosative chemical modifications of the protein or its interaction with detergents, denaturant agents and lipids, can trigger peroxidase activation. The disruption of the M80-Fe ligation increases the peroxidase activity of cyt c by increasing the access of H₂O₂. We studied the structural features of a) cyt c bound to cardiolipin (CL)-containing liposomes, b) Y67H and H26Y cyt c mutants. In particular we have used the weak absorption band at 695 nm to assess the conformational transitions in cyt c associated with the disruption of Fe-S(M80) bond, that brings about the acquirement.
D2.15
Proanthocyanidins in acute dose affect mitochondrial respiration in muscle of Wistar rats
Universitat Rovira i Virgili, Biochemistry and Biotechnology, Tarragona, Spain

Proanthocyanidins are some of the main polyphenols found in plant derived food and beverages. They have a protective effect against metabolic and cardiovascular diseases. Mitochondrion is the principal energy source of cell that converts nutrients into energy through cellular respiration. Compromised mitochondrial function has been linked to numerous diseases, including those of the metabolic and cardiovascular systems. To evaluate if the proanthocyanidins affect mitochondrial activity, we measured the levels of mitochondrial respiration ex-vivo from skeletal muscle of Wistar rats treated with an acute dose of 250 mg of GSPE (Grape Seed Proanthocyanidins extract) Kg. For this, male Wistar rats were fasted prior to oral gavage of GSPE using lard oil as a vehicle and after 5 hours rats were sacrificed and the gastrocnemius muscle was extracted for isolation of mitochondria. We used these mitochondria to measure their respiration using pyruvate as substrate in an oxygen-2k (OROBOROS). Mitochondrial states were achieved adding pyruvate to get the state 2, state 3 was obtained with the addition of adenosine 5'-diphosphate (ADP), the state 4o was achieved by adding oligomycin and, finally, uncoupling state (Unc.) was evaluated with the addition of carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP). The results obtained indicate that the proanthocyanidins may affect mitochondrial respiration in gastrocnemius muscle, causing an increase of oxygen consumption statistically significant in all mitochondrial states. We may conclude that an acute dose of GSPE improve muscle mitochondrial functions. This work was supported by Grant AGL2008-00387 from the Spanish government.

D2.16
The ischemic tolerance signaling may be involved in nephroprotective effects of mitochondria-targeted antioxidant SkQR1
I. Pevzner, E. Plotnikov, S. Jankauskas, V. Skulachev and D. Zorov
A. N. Belozersky Institute of Physico-Chemical Biology and Institute of Mitoengineering, Moscow State University, Moscow, Russian Federation

Acute kidney injury (AKI) is a pathology, which apparently impairs the life of patients after surgery or kidney transplantation. The main cause of AKI is accepted to be an oxidative stress in kidney tissue, which can be observed in ischemia/reperfusion (IR) model. Mitochondria play important role in renal cell damage as they produce large amount of reactive oxygen species (ROS). We explored the mechanism implied in the nephroprotective action of mitochondria-targeted antioxidant SkQR1 (10-(6'-plastoquinonyl)decyloRhodamin) after rat kidney IR. Single injection of SkQR1 before ischemia and four times in post-ischemic period resulted in significant restoration of renal function. Survival rate after IR rose up from 15% to 80%. SkQR1 also decreased ROS production (revealed by confocal microscopy of vital kidney slices) and the level of lipid peroxidation products in kidney tissue exposed to IR. We observed increased level of erythropoietin (EPO) and phosphorylated GSK-3b in kidney 3 hour and 24 hour after SkQR1 injection. The amount of EPO and p-GSK-3b were also higher in rats obtaining SkQR1 after IR. EPO and p-GSK3b are known to be crucial elements in ischemic tolerance mechanisms resulting in better cell survival. We observed that ischemic and hypoxic preconditioning of kidney also provided elevation of p-GSK3b and EPO and decreased oxidative damage of kidney. Thus we demonstrated the efficiency of mitochondria-targeted antioxidant SkQR1 as a new nephroprotector in ischemic AKI. The mechanism of SkQR1 action apparently includes induction of preconditioning signaling and abolishing of oxidative burst in mitochondria. Supported by RFBR 08-04-01667 and Rostok Group (Mr. A.V. Chikunov).

D2.18
mtDNA and electron transport chain integrity in lifespan extention mediated by calorie restriction in Saccharomyces cerevisiae
E. B. Tahara1, K. Cezário2, N. C. Souza-Pinto1, M. H. Barros2 and A. J. Kowaltowski1
1Institute of Chemistry, University of São Paulo, Biochemistry, São Paulo, Brazil, 2Institute of Biomedical Sciences, University of São Paulo, Microbiology, São Paulo, Brazil

Aging involves a decline in metabolic function and efficiency of biological systems over time. Our group elected S. cerevisiae as a model organism and identified that calorie restriction (CR) extends lifespan, improves mitochondrial DNA (mtDNA) integrity, and decreases mtDNA synthesis. In order to analyze if mtDNA is a key factor in CR-mediated aging, we generated mtDNA-deletion (ΔmtDNA) and mtDNA-replication-deficient (Δ) Saccharomyces cerevisiae strains. Analysis of mtDNA integrity showed a significant decrease in the mutant strains, indicating that mtDNA is a key factor in CR-mediated aging. Further, we observed that CR significantly reduces oxidative stress and mitochondrial dysfunction in ΔmtDNA and Δ strains. This suggests that mtDNA integrity is crucial in CR-mediated aging, providing a potential target for the development of interventions to prevent age-related diseases.
model organism and calorie restriction (CR) as an energetic intervention to uncover relevant aging hallmarks in eukaryotic cells. Since oxidative metabolism plays a role in *S. cerevisiae* viability during stationary growth phase, we investigated the influence of the mitochondrial genome – mtDNA is responsible for the synthesis of a number of electron transport chain constituent proteins – and respiratory pathways on this phenotype. We determined lifespans and the respiratory growth capacity in *ahb2D* (mutants harboring marked mitochondrial genome instability) and of rho–*0* (mutants in which mtDNA is partially or totally absent) and observed that neither of them responded to CR by extending lifespan, nor exhibited respiratory growth. CR was also unable to increase lifespan in cyt1D (mutants in which cytochrome c1 is absent and are, therefore, unable to grow in respiratory medium). Interestingly, the lpd1D mutant (which does not display dihydrodiolipoyl dehydrogenase activity, an important enzyme involved in oxidative metabolism and nucleoid assembly) showed not only a residual respiratory growth capacity, but also a response to CR. Moreover, we also found mtDNA is less stable in the absence of dihydrodiolipoyl dehydrogenase when in CR and aconitate – also a nucleoid protein – under standard conditions. Altogether, our data present evidence that mitochondrial electron transport chain functional integrity is required for lifespan extension mediated by CR and that glucose governs mtDNA-protein interactions in *S. cerevisiae*. Supported by FA-PESP/CNPq.

D2.19
Malignant transformation of human bronchial epithelial cells (BEAS-2B) by Cr(VI) is associated with altered mitochondria and bioenergetic phenotype
A. Sampaio1, L. Mendes1, A. C. Gonçalves2, M. Abrantes2, A. B. Sarmento2, F. Botelho2, P. Oliveira3, R. Carvalho1, A. Urbano1 and more malignant phenotypes correlate with increased mitochondrial membrane potential and the mitochondria content in a normal bronchial epithelial cell. The transformation of bronchial epithelial cells was paralleled by increased glycolysis. Aiming to establish whether the malignant transformation of bronchial epithelial cells (BEAS-2B) by Cr(VI) is associated with altered mitochondria and bioenergetic phenotype. Our results revealed that the number, and 1H and 13C NMR spectroscopy was used to assess the mitochondria’s bioenergetic phenotype. The mitochondrial electron transport chain functional integrity is required for lifespan extension mediated by CR and that glucose governs mtDNA-protein interactions in *S. cerevisiae*. Supported by FA-PESP/CNPq.

D2.20
The mitochondrial response to photodynamic reaction mediated by Photofrin in A549 cells
A. Chwilkowska1, J. Saczko1, J. Kulbacka1, A. Choromanska1, N. Skolucka1 and M. Majkowski1
1Department of Medical Biochemistry, Medical University, Wrocław, Poland, 2Wrocław University, Institute of Biochemistry and Molecular Biology, Laboratory of Cytobiology, Wrocław, Poland
Photodynamic therapy (PDT) is an effective cancer treatment that induces cytotoxicity through intercellular generation of reactive oxygen species (ROS). Photofrin (Ph) mediated photodynamic reaction was applied to the human lung carcinoma cell line A549. There were studied colocalization of photosensitizer with Mito-Tracker Green. The mitochondrial cells’ response to the PDT was examined by changes in mitochondrial superoxide dismutase (SOD2) activity and measurement of mitochondrial oxidoreductive potential. The colocalization studies have shown that Ph was mainly localized in mitochondrial membranes and also in the intracellular compartments. Photodynamic reaction induced dynamic changes in SOD2 activity. Its level raised immediately after PDT reaction. Work of mitochondria was evaluated via measuring mitochondrial dehydrogenases in MTT assay. The A549 cells showed significant disorder in the mitochondria function immediately after PDT and it decreased with increasing time post treatment. These results demonstrated that A549 cells respond to PDT -Ph by involving the mitochondria.

D2.21
Effect of oxidized lipid containing hydroperoxide or carboxylic acid group on interaction of cytochrome c to model mitochondrial membrane
C. Kawai1, I. L. Nantes2 and M. D. S. Baptista1
1Universidade de São Paulo, Instituto de Química, São Paulo, Brazil, 2Universidade Federal do ABC, Centro de Ciências Naturais e Humana, São Paulo, Brazil
In the inner mitochondrial membrane (IMM) lipid oxidation can affect the binding and activity of cytochrome c (cyc) with repercussions for the cell respiration and apoptosis. The binding of cyc to IMM mimetic liposomes containing the oxidized phosphatidylcholine was investigated at pH 6.2 and 7.4, which are expected to be present in the intermembrane space of respiring and uncoupled mitochondria. The binding was assessed by fluorescence quenching promoted by resonance energy transfer from the PPDC pyrene from membrane (donor) to heme group of cyc (acceptor). The binding isotherms were treated using a model based in Hill plots presented two binding sites *Kd*1 and *Kd*2, representing two distinct states in the membrane, and a cooperativity coefficient n. The presence of PazPC (1-O-Hexadecyl-2-Azelaoyl-sn-Glycerol-3-Phosphocholine) decreased *Kd*1 value one thousand fold at pH 6.2 and promoted a decrease in *Kd*2 at pH 7.4. We characterized a strong interaction of PazPC with cyc, which may explain the decrease in the *Kd*2 values. The presence of POPCox (POPC containing hydroperoxide) also diminished the *Kd*1 value but caused a significant increased *Kd*2 values in both pHs. The expression in *Kd*1 in the presence of POPCox can be related to detachment process of cyc from IMM and consequently to trigger the apoptotic process. We have also
observed that POPCox reacts with heme iron of cyt c bound to membrane, generating hydroxyl radical, which may further damage the IMM facilitating the release of cyt c. Supported by FAPESP and CNPq.

D2.22
Self-loading of the mitochondrial replicative helicase TWINKLE onto circular ssDNA
E. Jent1, M. Falkenberg1 and G. Farge2
1 Sahlgrenska Academy, Gothenburg University, Gothenburg, Sweden, 2Vrije Universiteit, Department of Physics and Astronomy and Laser Centre, Amsterdam, Netherlands

Helicases are motor proteins that catalyze the separation of nucleic acid duplexes using energy released by the hydrolysis of nucleoside triphosphate (NTP). This unwinding activity is crucial for many nucleic acid metabolic processes, especially for DNA replication. Replicative helicases assemble into ring-shaped hexamers that typically have a ring structure where the central channel accommodates one DNA strand while the second strand is excluded. One consequence of the ssDNA binding in the central channel is that replicative helicases have to be loaded onto the ssDNA during initiation of replication. Many helicases need accessory proteins, helicase loaders, to assemble the monomers into hexamers around ssDNA or to break up the hexameric ring structure. There are also examples of helicases that can load onto DNA without assistance of a loading factor. One of these helicases is the bacteriophage helicase T7 gene 4 protein (gp4). TWINKLE is the only known replicative helicase involved in replication of human mitochondrial DNA (mtDNA) and displays high sequence similarity to T7gp4. TWINKLE can unwind short stretches of dsDNA in a 5’ to 3’ direction. Together with other mitochondrial replicative helicases TWINKLE form a processive replisome in vitro that can synthesize ssDNA molecules of 16 kb. Moreover, TWINKLE is a very stable hexameric helicase in solution which raises the question of how TWINKLE is loaded on ssDNA. We show that TWINKLE is able to load onto circular ssDNA in vitro without help from a loading factor and support DNA replication on a circular dsDNA substrate.

D2.23
Propolis reduces mitochondrial oxidative stress but not cell death induced by doxorubicin in melanoma cells
L. Benguedouar1, P. Bernard2, M. Lahouel3 and F. Antonicelli4
1 Laboratoire de Dermatologie, UMR CNRS-6237, UFR Médecine, URCA, Attached to Laboratoire de Toxicologie Moléculaire, Université de Jijel Algérie, Reims, France, 2Laboratoire de Dermatologie, UMR CNRS-6237, UFR Médecine, URCA, Reims, France, 3Laboratoire de Toxicologie Moléculaire, Faculté des Sciences, Université de Jijel Algérie, Jijel, Algeria, 4F. Antonicellia A-Laboratoire de Dermatologie, UMR CNRS-6237, UFR Médecine, URCA, Reims, France

Propolis is a natural honeybee product resinous and dark coloured, gathered by bees from leaf buds of various tree species. We recently demonstrated that propolis extract protected heart and liver tissues from oxidative stress by protecting the mitochondria. Besides, it has also been shown that propolis induced human melanoma cell death and that this effect is comparable to doxorubicin effect, a clinical anticancer drug. Then, we wondered whether propolis extract would modulate doxorubicin-induced toxicity by reducing oxidative stress in melanoma cells. In this study, we first confirmed that propolis extract inhibited significantly superoxide anion O₂⁻ production from isolated mitochondria. We then demonstrated that propolis extract had a strong capability to scavenge O₂⁻ production using the WST-1 test on human and murine cultured melanoma cells. Additionally, propolis extract at 10 µg/ml exhibited a cytotoxic effect on melanoma cells comparable to doxorubicin used at 3 µM. However, although the combined effects of propolis extract and doxorubicin reduced mitochondria O₂⁻ production, co-treatment of melanoma cells with propolis extract did not protect from doxorubicin-induced cell death. These results suggest that mechanisms not dependent on the production of ROS are involved in doxorubicin induced apoptosis in melanoma cells.

D2.24
Mitochondrial RNA polymerase is needed for activation of the origin of light-strand DNA replication
J. Miralles Fuste1, S. Wanrooij2, E. Jent3, C. E. Grancyme1, T. Claett1, Y. Shi2, N. Atanassova1, I. J. Holt1, C. M. Gustafsson2 and M. Falkenberg2
1 Karolinska Institutet, Division of Metabolic Diseases, Stockholm, Sweden, 2Göteborg Universitet, Medical biochemistry and Cell Biology, Göteborg, Sweden, 3MRC, Mitochondrial Biology Unit, Cambridge, UK

Mitochondrial DNA is replicated by a unique enzymatic machinery, which is distinct from the replication apparatus used for copying the nuclear genome. We have examined the mechanisms of origin-specific initiation of lagging-strand DNA synthesis in human mitochondria. We demonstrate that the mitochondrial RNA polymerase (POLRMT) is the primase required for initiation of DNA synthesis from the light-strand origin of DNA replication (OriL). Using only purified POLRMT and DNA replication factors, we can faithfully reconstitute OriL dependent initiation in vitro. Leading strand DNA synthesis is initiated from the heavy-strand origin of DNA replication and passes OriL. The single stranded OriL is exposed and adopts a stem-loop structure. At this stage, POLRMT initiates primer synthesis from a poly-dT stretch in the single-stranded loop region. After about 25 nt, POLRMT is replaced by DNA polymerase γ and DNA synthesis commences. Our findings demonstrate that POLRMT can function as an origin specific primase in mammalian mitochondria.
D3.01 Functional Caveolin-1 is required for Hepatitis B virus infection
A. Macovei, C. Radulescu, C. Lazar and N. Nichita
Institute of Biochemistry, Viral Glycoproteins, Bucharest, Romania

Investigation of the entry pathways of hepatitis B virus (HBV) has been hampered by the lack of versatile in vitro infectivity models. Most concepts on HBV infection come from the more robust duck HBV system; however, whether the two viruses use the same mechanisms to invade target cells is still a matter of controversy. In this study, we investigate the role of an important plasma membrane component, caveolin-1 (Cav-1), in HBV infection. Caveolins are the main structural components of caveolae, planar membranous invaginations. These lipid rafts may be involved in the endocytosis of numerous ligands and complex signaling pathways within the cell. We used the HepaRG cell line permissive for HBV infection to stably express dominant-negative Cav-1 and dynamin-2, a GTPase involved in vesicle formation at the plasma membrane and other organelles. The endocytic properties of the newly established cell lines, HepaRG(Cav-1), HepaRG(Cav-1Δ81), HepaRG(Dyn-2), and HepaRG(Dyn-2K44A), were validated using specific markers for different entry routes. The cells maintained their properties during cell culture, supported differentiation, and were permissive for HBV infection. The levels of both HBV transcripts and antigens were significantly decreased in cells expressing the mutant proteins, while viral replication was not directly affected. Chemical inhibitors that specifically inhibit clathrin-mediated endocytosis had no effect on HBV infection. We therefore concluded that functional Cav-1 is essential for HBV to initiate productive infection in HepaRG cells which strongly suggest that the virus is using Cav-1-mediated endocytosis to enter the host cells.

D3.02 Evaluation of hyperbranched polyester as a drug carrier system for intestinal cells
S. Cohen1, Z. Sideratou2, C. M. Paleos3 and R. Korenstein4
1Tel Aviv University, Pharmacology and Physiology, Tel Aviv, Israel, 2NCSR "Demokritos", Institute of Physical Chemistry, Attiki, Greece, 3NCSR, Institute of Physical Chemistry, Attiki, Greece, 4Tel Aviv University, Physiology and Pharmacology, Tel Aviv, Israel

Boltorn® H40 (BH-40) is a biodegradable, non-toxic polymer with 64 terminal hydroxy groups (7,300 MW) that has a highly branched polyester structure. When this dendritic polymer functionalized with polyethylene (PEG) it becomes a water-soluble unimolecular micelle. This in vitro enriched to: (i) Evaluate sphingolipids, which are involved in the endocytosis of numerous ligands and complex signaling pathways within the cell. We used the HepaRG cell line permissive for HBV infection to stably express dominant-negative Cav-1 and dynamin-2, a GTPase involved in vesicle formation at the plasma membrane and other organelles. The endocytic properties of the newly established cell lines, HepaRG(Cav-1), HepaRG(Cav-1Δ81), HepaRG(Dyn-2), and HepaRG(Dyn-2K44A), were validated using specific markers for different entry routes. The cells maintained their properties during cell culture, supported differentiation, and were permissive for HBV infection. The levels of both HBV transcripts and antigens were significantly decreased in cells expressing the mutant proteins, while viral replication was not directly affected. Chemical inhibitors that specifically inhibit clathrin-mediated endocytosis had no effect on HBV infection. We therefore concluded that functional Cav-1 is essential for HBV to initiate productive infection in HepaRG cells which strongly suggest that the virus is using Cav-1-mediated endocytosis to enter the host cells.

D3.03 Characterization of murine macrophages eliminating different types of dying neutrophil granulocytes
Z. Doroš, G. Buchan, A. Mägi and F. László
Department of Biochemistry and Molecular Biology, University of Debrecen, Debrecen, Hungary

Macrophages can efficiently eliminate dying neutrophil granulocytes preventing development of inflammation. It is well known how macrophages can engulf apoptotic and necrotic cells separately; however, it is still unclear how they behave when different types of dead cells are present in their environment simultaneously. We have investigated both bone-marrow derived and peritoneal murine macrophages and have initiated different types of cell death forms: 1. Spontaneous apoptosis, 2. Spontaneous necrosis induced by serum deprivation; 2. Heat-induced necrosis; 3. Necroptosis induced by a pan-caspase inhibitor, the ZVAD.fmk. We have characterized the death processes of neutrophils, and performed phagocytosis assays. It has been demonstrated that cells undergoing spontaneous necrosis are engulfed by macrophages most efficiently compared to other death types. We have found that different forms of dead cells have different influences on each other’s uptake by macrophages when they were applied simultaneously in phagocytosis assays. Our results suggest that the engulfment of several phagocytosis substrates present together is a more complex molecular process than just a simple competition between the substrates and uptake could be inhibited by recombinant Annexin V. We have also investigated how production of pro-inflammatory cytokines (IL-6 and IL-1β) is changed during the phagocytosis processes in co-incubation assays. Our results may contribute to our better understanding on the development of some inflammatory and autoimmune diseases.

D3.04 Structural studies of the human tetraspan vesicle membrane proteins
C. Loew and P. Nordlund
Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

Integral membrane proteins of the tetraspan vesicle protein (TVP) family are highly abundant components of different vesicle types. They are believed to be involved in various aspects of the synaptic vesicle cycle, including vesicle biogenesis, exocytosis and endocytotic recycling. It is suggested that TVPs provide a core set of components required for a minimal vesicle that could serve as a matrix for assembly of specialized vesicular carriers in different cells. This class of proteins is characterized by four trans-
membrane segments with an M-shaped topology. They are grouped into three distinct families: (i) gysins, (ii) physisins and (iii) secretory carrier-associated membrane proteins (SCAMPs). Multiple interactions of TVPs with lipids (mainly cholesterol) and components of the recycling machinery like the SNAREs, dynamin, adaptor proteins and eps15 homology (EH)-domain proteins have been described. To succeed in structure determination of membrane proteins, a robust expression and purification protocol for TVPs was established to guarantee homogeneous high quality material for characterization and crystallization. New methods like automated analytical gelfiltration and static light scattering were implemented for that purpose, which allow the expression and purification of mg quantities of members of the gynin and SCAMP family up to now. TVPs lack the presence of larger folded soluble domains, which otherwise might be favourable in crystallization. To increase the chance for crystal formation, antibody fragments were selected and complexed with TVPs for crystallization. Known interactions partners were cloned and used together with TVPs to improve their crystallizability.

D3.05

Abstract withdrawn

D3.06

ITSN2 adaptor protein: functional comparison and impact on early development
O. Novokhatska1, L. Tsyba1, I. Skrypkina1, O. Nikolaenko1, O. Dergai1, Jacques Moreau2 and A. Rynditch1
1Institute of Molecular Biology and Genetics NASU, Kyiv, Ukraine, 2Jacques Monod Institute, Paris, France

Adaptors of intersectin (ITSN) family serve as scaffolds for multiprotein complex assembly during endocytosis. Whereas most analyses dealt with ITSN1, participating in cytoskeleton rearrangements, cell signalling and survival, little is known about ITSN2. Our research is focused on ITSN2. We demonstrated significant colocalization of ITSN2 and ITSN1, and showed that endogenous proteins coimmunoprecipitated but did not interact. Study of ITSN2 interactions revealed that most protein partners of ITSN1 are common – dynamin 1, SOS1, Synj1, N-WASP, c-Src. Our research is focused on ITSN2. We demonstrated significant colocalization of ITSN2 and ITSN1, and showed that endogenous proteins coimmunoprecipitated but did not interact. Study of ITSN2 interactions revealed that most protein partners of ITSN1 are common – dynamin 1, SOS1, Synj1, N-WASP, c-Src. Our research is focused in brain whereas ITSN1-22a could be regulator of ITSN1-22a. Thus, neuron-specific isoform modifies binding of intramolecular binding controls interactions of the SH3A domain with specific SH3 domains of ITSN1 and Src towards dynamin 1. Inclusions encoded by the microexons affect distribution of charged amino acids in the interface of interaction of the SH3 domain. Also the novel isoform ITSN1-22a with alternative C-terminus was characterized. Expression analysis of ITSN1-22a revealed its presence in all tissues tested, but in comparison to ITSN1-s levels of expression were significantly lower. The appearance of the ITSN1-22a was presumably due to mobile elements transpositions during early evolution of mammals. We showed that ITSN1-22a forms complex in vivo and codistributes with ITSN1-s isoform and is also engaged in clathrin-mediated endocytosis but possesses distinctive features. Alternative C-terminus of ITSN1-22a is engaged in isoform homodimerization via disulphide bonds. Moreover, it provides specific interactions with amphiphysin 1 and by intramolecular binding controls interactions of the SH3A domain of ITSN1-22a. Thus, neuron-specific isoform modifies binding of ITSN1 in brain whereas ITSN1-22a could be regulator of ITSN1-s function in mammals.

D3.07

Functional characterization of adaptor protein ITSN1 isoforms
M. Dergai1, O. Dergai1, I. Skrypkina1, L. Tsyba1, I. Zlatkiy2 and A. Rynditch1
1Institute of Molecular Biology and Genetics, Kyiv, Ukraine, 2A. V. Damansky Institute of Colloid and Water Chemistry, Kyiv, Ukraine

Our research is focused on investigation of adaptor protein intersectin 1 (ITSN1) and plethora of its isoforms in cells of vertebrae. ITSN1 is engaged in clathrin-mediated endocytosis, mitogenic signaling, axonal growth, spine development and actin cytoskeleton rearrangements. We investigated neuron-specific isoform of ITSN1. It is expressed since the beginning of nervous system development and is conserved from fish to human. Neuron-specific inclusion of microexon changes binding specificity of the SH3A domain of ITSN1. We studied how neuron-specific alternative splicing controls specificity of the SH3 domains of ITSN1 and Src towards dynamin 1. Inclusions encoded by the microexons affect distribution of charged amino acids in the interface of interaction of the SH3 domain. Also the novel isoform ITSN1-22a with alternative C-terminus was characterized. Expression analysis of ITSN1-22a revealed its presence in all tissues tested, but in comparison to ITSN1-s levels of expression were significantly lower. The appearance of the ITSN1-22a was presumably due to mobile elements transpositions during early evolution of mammals. We showed that ITSN1-22a forms complex in vivo and codistributes with ITSN1-s isoform and is also engaged in clathrin-mediated endocytosis but possesses distinctive features. Alternative C-terminus of ITSN1-22a is engaged in isoform homodimerization via disulphide bonds. Moreover, it provides specific interactions with amphiphysin 1 and by intramolecular binding controls interactions of the SH3A domain of ITSN1-22a. Thus, neuron-specific isoform modifies binding of ITSN1 in brain whereas ITSN1-22a could be regulator of ITSN1-s function in mammals.
ferrin was also cholesterol-dependent as resulted after methyl-
beta-cyclodextrin treatment. Bafilomycin A1 treatment demon-
strated that the internalization of lactoferrin might use of acidic
endosome-like organelles. Following lactoferrin internalization,
we observed partial co-localization of lactoferrin and early endo-
some antigen in vesicular/endosomal – like structures. Our results
suggest that lactoferrin is internalized primarily via clathrin-
dependent pathways in THP-1 cells, but need further studies to
address this issue.

D3.09
Molecular studies and 3D electron microscopy
to reveal components of phagosome
maturation
M. Osinska, R. Bartosiewicz and E. Wyroba
Nencki Institute of Experimental Biology, Warsaw, Poland

Our extensive bioinformatic analysis of evolution and phylogeny
of Rab7 proteins indicate that they evolved before radiation of
main eukaryotic supergroups and numerous Rab7 copies were
identified in some unicellular eukaryotes suggesting both the
functional specialization of these proteins and complexity of
membrane trafficking in these species (Mackiewicz and Wyroba
2009). A model to study this problem is Paramecium in which
paralogous genes encode 98% identical Rab7 copies. Mass spec-
troscopy, 2D gels, immunoprecipitation with specific antibodies
(Abs) and 3D localization were used to study Rab7 role in
phagolysosomal system. MS confirmed that each Ab precisely
recognized only one of the two Rab7 proteins and their localiza-
tion was distinct. RNAi of rab7a gene inhibited phagocytosis and
impaired acidification due to the lack of proton pomp. Silencing
of rab7b gene had no this effect and its protein product is modi-
fied postranslationally and seems to acquire a new function.
Rab7a was detected in the phagosomal compartment where it co-
localized with its putative effector RILP1-like protein in the pri-
mary and secondary lysosomes. The nature of these Rab7a
positive vesicles was also confirmed by the presence of the lyso-
somal markers with Abs against LAMP2 and cathepsin L in dou-
ble immunogold labeling that was proved by tomographic
analysis in JEM1400 (Olympus iTEM software). Taken together
these results show that tripartite machinery: Rab7, RILP and
LAMP2 required for phagosome maturation in mammalian cells,
as proposed by Huynh and coworkers (2007), seems to appear
early in eukaryote evolution. Supported by grant N N301 256836
of the Ministry of Science and Higher Education.
D4 – Nucleus

D4.01
Abstract withdrawn

D4.02
RNA polymerase I directed synthesis and expression of polycistronic viral RNA

A. Makarov¹, T. Komarova¹, A. Svarts² and Y. Dorohov¹
¹A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russian Federation, ²N.I. Vavilov Institute of General Genetics, Russian Academy of Science, Moscow, Russian Federation

During last years we gathered enough evidence that RNA synthesized under control of different promoter types recognized by polymerase (Pol) types I, II and III in plants share different synthetic and export pathways. Contrary to this RNA synthesized by same Pol type tend to compete for processing and export, and this is particularly true with Pol II-directed RNAs (mRNA, miRNA). The pathways of RNA processing are closely linked to those of pre-mRNA. Here we put forward the question whether Pol I could provide synthesis of mRNA (mRNAPol I), would it be then properly processed, exported to cytoplasm, and translated. To study this we designed Pol I promoter-based transient system for Tobacco mosaic virus RNA – a GFP encoding TMV-based viral vector that is delivered to a host plant by Agrobacterium tumefaciens. Our studies demonstrated that Pol I can provide synthesis of significant amount of 6 kb mRNAPol I, and that it can be successfully transported to the cytoplasm, since GFP synthesis can be observed. TMV genomic mRNAPol I neither shares mRNAPol II processing pathway nor undergoes splicing and capping which is likely to explain low level of GFP accumulation. Yet joint expression of TMV genomic mRNAPol II with Pol II-directed TMV 126k capping protein and silencing suppressor TBSV p19 stimulated virus-directed GFP production. TMV replication and GFP production.

D4.03
Vinerolbine can affect the chromatin proteins of alveolar macrophages

A. Chadeegani Rabban, M. Babaei and N. Shahmir
I.B.B, Tehran, Islamic Republic of Iran

Vinerolbine is a semi synthetic vinea alkaloid antitumor drug, which is used in the treatment of some cancers such as advanced nonsmall cell lung cancer, breast cancer and ovarian epithelial cancers. Vinerolbine suppresses depolymerization of mitotic microtubules and destroys mitotic spindle so it can block division of cancer cells in mitosis. In the present study the effect of vinerolbine on chromatin components of alveolar macrophages was investigated using SDS and agarose gel electrophoresis and western blot techniques. Alveolar macrophage were obtained by lavage, and after precubation, the cells were treated with various concentrations of vinerolbine for 6 hours. Histones and high mobility group non histone proteins (HMG) were then extracted and analyzed. The results show that increasing drug concentration reduced viability of the cells thus at 80 mic g/ml nearly 68% viability is obtained. SDS-PAGE demonstrates aggregation of histone proteins by drug action, when vinerolbine concentration exceeded higher than 20 mic g/ml. Western blot analysis against histones and HMG proteins antisera confirmed the result. DNA extracted from the drug treated and the controls also exhibited compaction of DNA in a dose dependent manner. From the results it is concluded that the effect of vinerolbine on chromatin proteins of alveolar macrophage is dose dependent and binding of drug to chromatin induces chromatin compaction thus the proteins are not extractable with usual procedures.

D4.04
Comparision of the effect of heavy metals lead, nickel and cobalt on chromatin yproteins of hepatocyte nuclei

A. Rabbani Chadeegani, N. Shahmir and M. Babaei
I.B.B, Tehran, Islamic Republic of Iran

Heavy metals are environmental pollutants and their application in developing technology and citizens have increased. In the present study we have compared the effects of lead, nickel and cobalt on chromatin proteins in hepatocytes nuclei employing SDS, agarose gel electrophoresis, and western blotting techniques. The results showed that upon addition of metals to hepatocytes nuclei turbidity is occurred which could be monitored at 400 nm. Turbidity is increased as metal concentrations is raised and lead and nickel were more potent compared to cobalt. Analysis of the histone proteins extracted from the metal treated samples and the control revealed that the content of core histones, H1 and H4 were decreased upon addition of lead and nickel whereas in the case of cobalt the histones pattern remained unchanged. Western blots were also confirmed the results. The metals also bind to high mobility group proteins, HMG B, but not to HMG N. In the presence of metals the DNA pattern of nuclei digested with MNase was decreased as metals concentration, were increased. From the results it is concluded that lead and nickel bind to chromatin proteins and reduced their extractability by common procedures, suggesting that their binding to chromatin may proceed the chromatin into condensation which inhibits DNA and RNA synthesis in the cell.

D4.05
Abstract withdrawn

D4.06
Induction of the cold shock protein YB-1 as a result of the hypoxic development in human brain cancer

H. M. Said¹, C. Hagemann², B. Polar³, A. Staab⁴, A. Katzer⁵, J. Anacker⁶, D. Vordermark⁶ and M. Flentje¹
¹University of Würzburg, Medical Faculty, Radiation Oncology, Würzburg, Germany, ²University of Würzburg, Medical Faculty, Tumor Research Laboratory, Department of Neurosurgery, Würzburg, Germany, ³Medical Faculty - University of Würzburg, Department of Radiation Oncology, Würzburg, Germany, ⁴Paul Scherer Institute, Proton Research, Villigen, Switzerland, ⁵University of Würzburg, Medical Faculty, Gynaecology and Obstetrics, Würzburg, Germany, ⁶Martin-Luther-University-Halle-Wittenberg., Radiation Oncology, Halle, Germany

Y-box proteins belong to a family of DNA- and RNA-binding factors, also called cold shock proteins that are highly conserved

D4 – Nucleus Abstracts

FEBs Journal 277 (Suppl. 1) 37–271 (2010) © 2010 The Authors Journal compilation © 2010 Federation of European Biochemical Societies 229
dental evolution and have been shown to function as regulators of gene transcription and translation. A wide range of nucleic acid structures are reported to be specifically bound by Y-box proteins, most of which harbor an inverted CCAAT-box (ATT-GG) as the core binding site. HIF-1α is the main the regulator of several hypoxia induced genes.

**Materials and methods:** YB-1 regulation level was examined in human glioblastoma cells lines like U373, U251, GaMG and U87-MG under extreme hypoxic oxygenation conditions (0.1% oxygen), reoxygenation after hypoxia for 24 and 48 hour and oxygenated conditions (21% oxygen and 5% carbon dioxide) in vitro. Protein and mRNA level were detected via western blots and RT-PCR. Cells incubated for 24 hour with 100 µM DFO served positive control for hypoxia and β-actin served as loading control, respectively.

**Results:** YB-1 was over expressed via hypoxic oxygenation development in different glioblastoma cells micromilieu in vitro under extreme hypoxic conditions (0.1% oxygen or reoxygenation after hypoxia, both on protein and mRNA level. Further, there was an association between YB-1 expression and the hypoxia induced HIF-1α and the regulated genes in human glioblastoma tumor specimens examined, in vitro.

**Conclusions:** YB-1 is upregulated as an answer to hypoxic oxygenation conditions development in glioblastoma micromilieu, both on protein and mRNA level and can be considered as a coregulator of HIF-1α and as a consequence the genes that are regulated by the hypoxic HIF-1α pathway in cancer human cells.

**D4.07**

**THAP11 and HP1BP3 proteins are component of the HB-2.8 enhancer-blocker located in the human c-Myc chromatin domain**

F. Continò, L. Carramusa, C. Mazzarella, G. Di Cara and S. Feo

Department of Experimental Oncology and Clinical Applications, University of Palermo, Palermo, Italy

Insulators are DNA elements that block the extension of a condensed chromatin domain into a transcriptionally active region or prevent the interaction of a distal enhancer with a promoter when placed between the two. Human c-Myc and Ptt-I genes map close each other and are separated by an intergenic region rich of DNAseI hypersensitivity sites. Starting from the observation that PVT1 expression is restricted to a low number of normal tissues compared to the wide distribution of c-Myc mRNA we focus our studies on the function and structure of the regions surrounding the DHS present between the two genes. Stable and transient transfection, indicate that one of the regions (HB-2.8) has enhancer blocking activity. Further evidences indicate that HB-2.8 blocks the activity of c-Myc distal enhancer (PA-A) on the Ptt-1 promoter, suggesting that c-Myc and Ptt-1 genes are insulated in two distinct chromatin. A Matrix-attachment region (MAR) has been mapped in close proximity of the HB-2.8 region and we found it is associated to the core element responsible for the HB-2.8 enhancer-blocking activity. By means of DNA affinity chromatography and proteomic analysis we characterized two proteins interacting with HB-2.8, from nuclear extract of Hela cells: THAP11 and HP1BP3. Overexpression of THAP11 inhibits growth of different cells types and its function is mediated by its ability to repress transcription of c-Myc. HP1BP3 is a component of heterochromatin and may be involved in chromatin structure. Functional characterization of these proteins may contribute to define MYC chromatin domain.

**D4.08**

**The nuclear compartmentation of glutathione: effect on cell cycle progression and chromatin condensation**

J. Markovic1, N. J. Mora2, A. M. Broseta2, A. Gimeno3, N. de la Concepcion2 and F. V. Pallardo4

1University of Valencia, Core Research Facility, Valencia, Spain
2University of Valencia, Valencia, Spain
3The Biomedical Network Research Centre for Rare Diseases, Valencia, Spain
4University of Valencia, Physiology, Valencia, Spain

One of many functions of GSH is control of cell proliferation, possibly by modulation of redox sensitive cell cycle regulatory proteins and telomerase activity. We have studied the importance of nuclear compartmentation of glutathione (GSH) in the proliferation of 3T3 fibroblasts for a period of 5 days. Cells were treated with diethyl maleate (DEM) that depletes GSH in the whole cell and buthionine sulfoximine (BSO) which preserves the nuclear pool of GSH. The cell cycle progression was delayed in the cells treated with DEM, when the nuclear GSH was depleted. We found lower level of proliferating cells, Id2 protein expression, and glutathionylated nuclear proteins and increase in level of oxidized nuclear proteins comparing to control and BSO treated cells. The cells with high nuclear GSH, in G1/S phase, showed sparse chromatin pattern, while the depletion of nuclear GSH caused chromatin condensation, which was reverted by GSH ester. Our results demonstrate for the first time that nuclear GSH levels plays a decisive role in cellular proliferation, possibly by favouring the chromatin decompaction during G1/S transition. The pattern and level of glutathiolation of nuclear proteins change both along the cell cycle and with the depletion of nuclear GSH, which points to the possible implication of this modification in the control of chromatin structure dynamics. Our report underscores the important role of nuclear glutathione in cell physiology and suggests that manipulation on nuclear GSH levels could be of paramount importance during development and cancer.
D5 – Chloroplasts

D5.01 Chloroplast thylakoid transporters
L. Yin1, P. Karlsson1, C. Spetza1, E.-M. Ar3 and B. Schoefs1
1Linköping University, Linköping, Sweden, 2University of Turku, Turku, Finland, 3Université de Bourgogne, Dijon, France

The aim of this study is to identify and functionally characterize solute transporters from the chloroplast thylakoid membrane of Arabidopsis thaliana. As compared to chloroplast envelope transporters, much less information is available for transport processes across the thylakoid membrane, which is mostly studied as the site of light-driven photosynthetic reactions coupled to ATP synthesis. Although there are many reported examples of transport activities, only a few thylakoid transporters have been identified at the gene level. Using bioinformatics analyses, we have predicted the existence of approx. fifteen thylakoid transporters. For experimental validation, we have carried out immuno-localization studies used peptide-specific antibodies, functional analyses in heterologous system and validation using knockout mutants. We have recently identified one ATP/ADP carrier (Thuswaldner et al. JBC 2007) and one Na(+)-dependent phosphate transporter (Ruiz-Pavon et al. JBC 2008). They are proposed to participate in the nucleotide metabolism in the thylakoid lumen (Spetza et al. PNAS 20004) as well as to balance the trans-thylakoid proton electrochemical gradient storage. Based on phenotypic analyses of knockout mutants, we will present novel data about the key physiological role of the two transporters during environmental stress. For experimental validation, we have carried out immuno-localization studies used peptide-specific antibodies, functional analyses in heterologous system and validation using knockout mutants. We have recently identified one ATP/ADP carrier (Thuswaldner et al. JBC 2007) and one Na(+)-dependent phosphate transporter (Ruiz-Pavon et al. JBC 2008). They are proposed to participate in the nucleotide metabolism in the thylakoid lumen (Spetza et al. PNAS 20004) as well as to balance the trans-thylakoid proton electrochemical gradient storage. Based on phenotypic analyses of knockout mutants, we will present novel data about the key physiological role of the two transporters during high-light-induced repair of photosystem II complex in the thylakoid membrane. Subsequently, we will make a survey on the outlook of thylakoid activities awaiting identification of responsible proteins. Such knowledge is necessary to understand the thylakoid network of transporters, and its role in photosynthesis and adaptation to environmental stress.

D5.02 Chloroplast protease AtDeg5 is involved in wounding-related degradation of PsbF apoprotein
R. Lucinski1, L. Misztal2 and G. Jackowski1
1Department of Plant Physiology, Adam Mickiewicz University, Poznań, Poland, 2Department of Biotechnology, Adam Mickiewicz University, Poznań, Poland

Deg5 is a serine type chloroplast protease found to be peripheraly attached to the lumen side of thylakoid membrane. Knowledge of the enzyme’s function is limited to its possible involvement in degradation of photodamaged PsbA in Arabidopsis thaliana. Given the lack of knowledge concerning potential AtDeg5 involvement in disposal of chloroplast proteins damaged during plant exposition to stresses other than elevated irradiance we have sought to determine the function of the enzyme in degradation of PsbA-F apoproteins mediated by brief (3 hour) treatment with high salt, wounding, desiccation, cold and heat. In order to reach this goal insertion line devoid of AtDeg5 was prepared. Immunotitration of PsbA-F in wild type plants showed that all the apoproteins except for PsbF underwent remarkable decrease in abundance in response to prevailing majority of stresses. When thylakoids isolated from leaves of wild type plants which had previously faced brief treatment with the five stresses were incubated in darkness for 6 hour further disappearance of PsbA and C-F took place. It was demonstrated that PsbA decline in thylakoids isolated from high salt, desiccation and wounding – treated leaves as well as PsbF decline in thylakoids isolated from wound-exposed leaves was remarkably reduced in the presence of aprotinin, a typical inhibitor of serine-type proteases, including AtDeg5. In contrast to a pronounced decline in PsbF which took place in vivo in leaves of wide type plants exposed to wounding, the apoprotein resisted disappearance in deg5 mutant. Clearly, therefore, AtDeg5 is specifically involved in wounding-related hydrolysis of PsbF.

D5.03 Modeling and Mutational analysis of Anion transporter 1 protein of Arabidopsis thaliana
L. Ruiz-Pavon1, P. Karlsson1, J. Carlsson1, D. Samyn1, B. Persson2, B. L. Persson1 and C. Spetza2
1Linnaeus University, School of Natural Sciences, Kalmar, Sweden, 2Division of Molecular Genetics, Chemistry and Biology, Linköping, Sweden, 3Division of Bioinformatics, Department of Physics, Chemistry and Biology, Linköping University, Linköping, Sweden

The thylakoid anion transporter 1 (ANTR1) from Arabidopsis thaliana, has been characterized as a Na-dependent Pi transporter when expressed in E. coli (1), but no data is yet available for the protein structure and amino acids involved in transport of Pi. In this study a three-dimensional structural model of ANTR1 was constructed in silico using the crystal structure of glycerol-3-phosphate/phosphate antipporter from E. coli as a template. Based on Multiple Sequence Alignments (MSAs) with other plant ANT-Rs and mammalian SLC17 homologues, five highly conserved amino acids involved in Pi transport have been identified, namely Arg-120, Ser-124 and Arg-201 inside the putative translocation pathway, Arg-228 and Asp-382 exposed at the cytoplasmic surface of the protein. The activity of the protein as a Na-dependent Pi transporter in the wild type and mutants was analyzed by heterologous expression and uptake of radioactive Pi into E. coli cells. Substitution of the three Arg (120, 201 and 228) for Glu residues and of Asp-382 for an Asn residue resulted in an inactive ANTR1 transporter. All other mutants had sufficient activity to allow measurement of kinetic parameters, attesting that the mutated proteins were functional. Based on our results, we propose that Arg-201 is a critical residue for substrate binding and translocation, whereas Ser-124 may function as periplasmic gateway for Na+ ions. Residue Arg-120 plays an important role in Pi binding and associated conformational changes, and finally that Arg-228 and Asp-382 only weakly participate in interactions allowing conformational changes to occur at the cytoplasmic surface of the transporter.

D5.04 The chloroplast protein CPSAR1, dually localized in the stroma and the inner envelope membrane, is involved in thylakoid biogenesis
C. García1, N. Zaman Khan1, U. Nannmark2 and H. Aronsson1
1Department of Plant and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden, 2University of Gothenburg, Institute for Biomedicine, Sahlgrenska Academy, Gothenburg, Sweden

Thylakoid biogenesis is a crucial step for plant development involving the combined action of many cellular actors. CPSAR1 is shown here to be required for the normal organization of mature thylakoid stacks and ultimately for embryo development. CPSAR1 is a chloroplast protein that has a dual localization in...
the stroma and the inner envelope membrane according to microscopy studies and subfractionation analysis. CPSAR1 is close to the Obg nucleotide binding protein subfamily and displays GTPase activity as demonstrated by in vitro assays. Disruption of CPSAR1 gene via T-DNA insertion results in an arrest of embryo development. In addition, transmission electron microscopy analysis indicates that mutant embryos are unable to develop thylakoid membranes and remain white. Unstacked membrane structures resembling single lamellae accumulate in the stroma and do not assemble into mature thylakoid stacks. CPSAR1 RNA interference induces partially developed thylakoids leading to pale green embryos. Altogether, the presented data demonstrate that CPSAR1 is a protein essential for the formation of normal thylakoid membranes and suggest a possible involvement in the initiation of vesicles from the inner envelope membrane for the transfer of lipids to the thylakoids.

**D5.05**

Characterization of CPARF1 and its potential role in vesicular membrane transport in chloroplasts

M. Alezzawi, Christel Garcia and H. Aronsson
Department of Plant and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden

The aim of the project is to better understand how the thylakoid membrane in initially formed and maintained. More specifically, we aim to further scrutinize the chloroplast localized vesicular transport system, similar to that responsible for membrane transport between the ER and the Golgi. The thylakoid membrane of higher plant chloroplast is made of membrane lipids synthesized in the chloroplast envelope. As the inner envelope membrane and the thylakoid are separated by the aqueous stroma, a system for transporting newly synthesized membrane material from the inner envelope membrane to the thylakoid is required. A study using the Arabidopsis genome and web-based subcellular localization prediction tools, reported several putative chloroplast-localized proteins with a high sequence similarity to components of cytosolic vesicular trafficking. After having established the predicted chloroplast-localization of the putative chloroplast vesicular trafficking components CPARF1, the major goal is to identify the molecular events that drive chloroplast localized vesicular transport. Both in vivo and in vitro experiments are to be used.

**D5.06**

Function and regulation of the protein import translocon of the inner envelope membrane of chloroplasts: translocation channel(s)??

E. Kovács-Bogdán, P. Benz, M. Balsera, J. Soll and B. Bölter
Ludwig-Maximilians-Universität München, Department Biologie I, Plant Biochemistry, Planegg-Martinsried, Germany

Most chloroplast proteins are encoded for on the nucleus and have to be transported into the organelle after translation in the cytoplasm. The Toc and Tic machineries (Translocon at the outer/inner envelope membrane of chloroplasts) mediate the import of these proteins across the chloroplast membranes. Tic110 is the central component of the Tic translocon. We were able to demonstrate that it constitutes a cation-selective channel that is sensitive to the presence of chloroplastic transit peptides. With the help of computational studies, limited proteolysis experiments coupled with mass spectrometry and cysteine modification analysis, we were furthermore able to establish a topological model for Tic110. Finally, we could identify Tic110 as a Trx target, as it was shown to possess a redox active disulfide bridge, which can be reduced by stromal thiorodoxins in vitro. Another Tic component, Tic20 has also been proposed to form a channel in the inner envelope membrane of chloroplasts. Both Tic110 and Tic20 are clearly important for plant viability and preprotein translocation, but there are neither electrophysiologocal nor biochemical data supporting that Tic20 can form a channel. Here we demonstrate that we successfully established systems to express and purify full-length Tic20 and were able to determine its secondary structure and topology. Further experiments were designed to verify the notion that Tic110 and Tic20 function together in the import process.

**D5.07**

An interplay between chloroplastic N-terminal methionine excision and FtsH protease is essential for normal chloroplast development and function

Z. Adam1, F. Frottin2, C. Espagne2, T. Meinnel2 and C. Giglione1
1The Hebrew University of Jerusalem, Institute of Plant Sciences, Rehovot, Israel. 2Centre National de la Recherche Scientifique, ISV, UPR2355, Gif-sur-Yvette Cedex, France

N terminal Met excision (NME) is the earliest proteolytic event affecting proteins whilst they are still bound to the ribosome. About two thirds of all proteins in any organism are subjected to this type of processing, and all cell compartments where protein synthesis occurs contain dedicated NME machinery. Recent studies have shown that developmental defects, induced in the model plant Arabidopsis thaliana by inhibition of cytoplasmic or chloroplastic NME, are accompanied by an increase in cellular proteolytic activity. Whereas there is increasing evidence to support a tight connection between NME and protein degradation, the identity of the proteases involved has been unknown. Here we report that chloroplastic NME acts upstream of the nuclear-encoded chloroplastic FtsH metalloprotease. The chloroplastic developmental defects and their higher sensitivity to photoinhibition, observed in plants lacking the FtsH2 protease, are abolished when chloroplastic NME is inhibited, either genetically or chemically. Moreover, the characterization of accumulation of D1 and D2 proteins of PSII reaction center in different genetic backgrounds reveals that their level is strongly dependent on the previous action of NME enzymes. Under standard light conditions, correctly NME-processed D1 and D2 are turned-over primarily by FtsH2 protease. Absence of FtsH2 induces accumulation of the correctly NME-processed D1 and D2. Inhibition of chloroplastic NME compromises the recognition of D1 and D2 by FtsH2 and the unprocessed forms seems to be mainly recognized by other chloroplast proteases. These results highlight the tight functional interplay between NME enzymes and the FtsH protease.
E1 – Synthetic Biology

E1.01
Abstract withdrawn

E1.02
Lactic acid bacteria with surface-displayed binding proteins can bind antibodies and tumor necrosis factor α

A. Berleč1, M. Ravnikar2, N. Obermajer1, M. Lunder2 and B. Strukelj3

1Jožef Stefan Institute, Ljubljana, Slovenia, 2Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia

Lactic acid bacteria (LAB) are widely used in the food industry for the fermentation of vegetable, dairy and meat products. LAB also have beneficial health effects, which mainly include alleviation of intestinal diseases and are widely used as probiotics. Their health effects can be strengthened by the introduction of new properties via genetic modification. We have designed LAB which can be directed against various targets in gastrointestinal tract. We have used B domain of staphylococcal protein A, which is capable of binding Fc region of antibodies, as a model protein. We have prepared and compared several gene constructs for displaying the B domain on the surface of the model lactic acid bacterium Lactococcus lactis. Its surface display and functionality were confirmed by flow cytometry and fluorescence microscopy of recombinant lactococcal cells stained with specific or nonspecific antibody. The maximum capacity of the recombinant lactococci for antibody binding was estimated at 0.146 μg of antibody per 109 cells. The stability of the displayed B domain was tested against simulated gastric juice and a maximum 14% decrease in binding of antibody was observed after treatment. A TNFα-binding affibody was also displayed, in the same manner. Its ability to bind fluorescently labelled TNFα was confirmed by flow cytometry and fluorescence microscopy. L. lactis with TNFα binding capability could be used in amelioration of chronic intestinal inflammations such as ulcerative colitis or Crohn’s disease, which have a high long-term risk of colon cancer development.

E1.03
Genotoxicity and cytotoxicity effect of cadmium chloride on male mice kidney

M. Jafari Sani1, A. Zarei Mahmodabadi2, M. Adelipur1, J. Alizadeh3, B. Rezavand1 and A. Mohseni3

1Baqiyatallah Medical of Science University, Tehran, Islamic Republic of Iran, 2Tehran Medical of Science University, Tehran, Islamic Republic of Iran, 3Laboratory Technologist, Tehran, Islamic Republic of Iran

Introduction: Cadmium is one of the most toxic heavy metals in our environments having a very strong ability to accumulate in body organs especially in kidney. Our aim of present study was to determine the Genotoxicity and cytotoxicity in mice kidneys exposed to cadmium.

Material and method: We sacrificed 30 male mice in this study that randomly divided into three different groups (1control & 2treatment). Every five mice in one cage and remain in standard conditions. After 1 week, we applied peritoneal injection to exposed mice with Cd (300 μg/Kg.b.wt) on 0, 6, 12, 24, 48 hours. Twenty hour after latest injection the mice were killed and their kidney obtained. Then Oxidative stress markers (Malondialdehyde (MDA), Glutathione (GSH) and Superoxide Dismutase (SOD)) were assayed on homogenized kidney for studying cytotoxicity effect, and for Genotoxicity DNA damage studies, we used Comet assay on separated kidney cells. Also for cytotoxicity effect we employed histological tests. Finally, to analyze statistical data we used SPSS15 software.

Results: Present results shown that MDA and GSH concentration and SOD activity in treated group have a significantly increase in compare with control group (p < 0.01). The comet assay results obviously showed DNA breakage in treated group that was stimulated by Cd where this is not seen in the control group. Even in histological experiments, we were able to see cellular damage obviously.

E1.04
Oxidative stress and DNA damage in mice brain exposure to Cadmium Chloride

M. Jafari Sani1, A. Zarei Mahmodabadi2, M. Adelipur1, N. Shariaty Far3, J. Alizadeh3, B. Rezavand1 and A. Mohseni3

1Baqiyatallah Medical of Science University, Tehran, Islamic Republic of Iran, 2Tehran Medical of Science University, Tehran, Islamic Republic of Iran, 3Ahvaz Medical of Science University, Ahvaz, Islamic Republic of Iran

Introduction: Cadmium is one of the most toxic heavy metals in our environments having a very strong ability to accumulate in body organs especially in kidney. Our aim of present study was to determine the Genotoxicity and cytotoxicity in mice kidneys exposed to cadmium.

Material and method: We sacrificed 30 male mice in this study that randomly divided into three different groups (1control & 2treatment). Every five mice in one cage and remain in standard conditions. After 1 week, we applied peritoneal injection to exposed mice with Cd (300 μg/Kg.b.wt) on 0, 6, 12, 24, 48 hours. Twenty-four hour after latest injection the mice were killed and their brain removed. Then Oxidative stress markers (Malondialdehyde (MDA), Glutathione (GSH) and Superoxide Dismutase (SOD)) were assayed on homogenized kidney for studying cytotoxicity effect, and for Genotoxicity DNA damage studies, we used Comet assay on separated kidney cells. Also for cytotoxicity effect we employed histological tests. Finally, to analyze statistical data we used SPSS15 software.

Results: Present results shown that MDA and GSH concentration and SOD activity in treated group have a significantly increase in compare with control group (p < 0.01). The comet assay results obviously showed DNA breakage in treated group that was stimulated by Cd where this is not seen in the control group. Even in histological experiments, we were able to see cellular damage obviously.

E1.05
Endo-α-1,4-polygalactosaminidase structure and evolution

D. Naumoff1 and O. Stepuschenko2

1Institute for Genetics and Selection of Industrial Microorganisms, Laboratoy of Bioinformatics, Moscow, Russian Federation, 2Department of Genetics, Kazan State University, Kazan, Russian Federation

Family GH114 of glycoside hydrolases (or COG3868) includes endo-α-1,4-polygalactosaminidase [EC 3.2.1.109] from Pseu-
Metabolic engineering of *Saccharomyces cerevisiae* for overproduction of plant isoprenoids

Y. Chen\(^1\), V. Siewers\(^2\), M. Schalk\(^2\), L. Daviet\(^2\) and J. Nielsen\(^1\)

\(^1\)Chalmers University of Technology, SE, Göteborg, Sweden, \(^2\)Firmenich SA, Corporate R&D Division, CH Geneva, Switzerland

Isoprenoids are becoming industrially important compounds because of their potential applications as anticancer, antitumor, antiviral, antibiotic, nutraceuticals, feed supplements as well as their characteristic flavors and aromas. Because of limitation in synthetic means or low amounts and complex mixtures of natural sources, heterologous microbial production of isoprenoids is therefore considered as a suitable alternative to extraction from plants or chemical synthesis. Here we engineered the yeast *Saccharomyces cerevisiae* as a microbial host for heterologous biosynthesis of two different plant isoprenoids, namely santalene and patchoulol. By (i) introducing genetic modifications of the mevalonate pathway that enable the yeast to accumulate high levels of the key intermediate farnesyl diphosphate, (ii) enhancing the sesquiterpene synthase expression level by using codon optimized gene and changing promoter, and (iii) optimized fed-batch fermentation, titer in the g/L range were achieved. The results revealed the potential of yeast as a cell factory for production of plant isoprenoids.

E1.06

Yeast perfume factory: metabolic engineering of *Saccharomyces cerevisiae* for plant isoprenoid biosynthesis

S. Gionata\(^1\), C. Knuf\(^1\), P. Siavash\(^1\), Y. Chen\(^1\), J. Maury\(^2\), M. Schalk\(^1\), L. Daviet\(^1\), J. Nielsen\(^1\) and V. Siewers\(^1\)

\(^1\)Chalmers University of Technology, Chemical and Biological Engineering, Gothenburg, Sweden, \(^2\)Firmenich SA, Geneva, Switzerland

The production of plant isoprenoids in microbial systems is an environmentally friendly and attractive alternative to the commonly used chemical synthesis and plant extraction. This class of compounds is mainly used by the chemical industry for production of fragrances and aroma chemicals. Isoprenoids represent a diverse class of secondary metabolites, natively present in the metabolic network of *Saccharomyces cerevisiae* and used to satisfy macromolecule precursor demand for biomass production. Isoprenoids are naturally produced in *S. cerevisiae* through the mevalonate pathway (MVA). Aim of this project is the de-regulation of the MVA pathway in *S. cerevisiae* to increase the precursor pool for isoprenoid synthesis resulting in an efficient *S. cerevisiae* cell factory for biosynthesis of target compounds. Yeast is metabolically engineered for isoprenoids accumulation by introducing genetic modifications that enable to channel the flux through the isoprenoid pathway thus increasing the levels of the pathway products. Throughout the project different approaches for optimal isoprenoid production modulating the expression of key metabolic steps are investigated and the production capacity of the engineered strains is evaluated through a fermentation process coupling biochemical production of these compounds to biomass formation.
With the ultimate goal of constructing an artificial recombination enzyme we are interested in the mechanism of DNA strand exchange. This process, in vivo performed by proteins in the RecA family, is despite extensive studies not understood in full detail. We therefore want to study the molecular parameters involved in the exchange using an in vitro platform. Positively charged liposomes have been widely used as non-viral gene-carriers, where the positive charges attract the negatively charged DNA. We here show that DNA strand exchange is significantly enhanced, both in rate and yield, on the surface of cationic liposomes. The fastest exchange is seen for liposomes containing 35% cationic lipid and when the positive charges on the outer leaflet of the liposomes equal the amount of negative charges on the added DNA. Both increasing and decreasing the amount of DNA slows down the exchange rate, either due to competition for the binding sites or due to that the single-strands on average are further away from destabilized duplexes. By studying the exchange of mismatched DNA we conclude that the DNA opens in a “zipper-like” manner on the liposome surface, since a mismatched base-pair in the end of the sequence affects the exchange rate much more than a mismatched base-pair in the middle of the sequence. The liposome surface can be easily functionalized with lipids bearing specific functionalities and we intend to modify the surface to study the effects of hydrogen bonding and/or hydrophobic interactions.

References:

E1.10 Engineering of the fungal phosphoketolase pathway in the yeast Saccharomyces cerevisiae, effects on glycerol overproduction and flux distributions

M. Papini, I. Nisokaw, V. Siewers and J. Nielsen
Chalmers University of Technology, Systems Biology, Gothenburg, Sweden

As the science of metabolic engineering becomes more and more a reliable tool for reprogramming microbial cellular machinery, we attempted to force the boundaries of a novel pathway to increase intracellular levels of cytosolic acetate by expressing the fungal genes from the phosphoketolase pathway (phk). This pathway is used by the species Aspergillus to deviate the PP fluxes towards the production of the intermediates AcetylCoA or Acetate. Through an elegant approach, whose efficacy was partially evaluated with simulation based on a genome scale metabolic model, we expressed the genes from the phosphoketolase pathway (xpk and ack), together with other enzymatic activities, in the yeast S. cerevisiae and physiologically characterized the recombinant strain. To our knowledge, this is the first time that this pathway is expressed in yeast. The effect of this pathway on the yeast’s metabolism was studied both in a wild type S. cerevisiae CEN.PK 113-5D strain and in a strain deleted in the glycerol-phosphomutase gene (gpm1). As previously shown, such a deletion mutant, is not capable of utilizing glucose as carbon source. In the work we evaluated whereas the expression of the phk genes could restore this growth defect. This has the potential to lead to the development of a first generation strain able to produce glycerol with a theoretical maximum yield from glucose higher as those reported before.

E1.11 A simple, robust and highly efficient transient expression system for production of secreted proteins

T. Viik, M. Oudshoorn, M. Roza, E. van Voskuilen, J. Gerritsen, P. van Berkel and P. Parren
Genmab, Utrecht, Netherlands

We have developed an optimized mammalian transient expression system, capable of producing up to 700 mg/l of native secreted (glyco)proteins in less than a week. The system is composed of commercially available components and is based on transient expression in the fast growing suspension cell line, HEK-293F. The method depends on an optimal combination of a gene transfer method, an efficient expression vector and cotransfection with expression enhancing plasmids. Optimization of all components of the expression system by Experimental Design Techniques, yielded expression levels of up to 400 mg/l for antibodies (including IgG, IgA, IgM and Fab fragments of various species) and of up to 700 mg/l for over 100 other (glyco)proteins. Expression volumes were scalable from 0.1 ml up to 1.2 l in a simple shaker flask system in animal component free, low protein, medium enabling straightforward purification of relatively large amounts of native protein in a short, 4–5 days, production time. Details of the production method and extensive protein analysis in comparison to material produced from stably transfected CHO cell lines will be presented.

E1.12 Function-Spacer-Lipid (FSL) constructs enable inkjet printing of blood group antigens

K. Barr, O. Diegel, S. Parker, N. Bovin and S. Henry
1 AUT University, Biotechnology Research Institute, Auckland, New Zealand, 2 AUT University, Creative Industries Research Institute, Auckland, New Zealand, 3 Shemyakin Institute of Bioorganic Chemistry RAS, Moscow, Russian Federation

A limitation of inkjet printing antigens to solid surfaces is that the molecule to be printed must be dispersible in solution, retain antigenicity, and remain on the printed surface when exposed to biological solutions. FSL (function-spacer-lipid) constructs were designed to be dispersible in water and to attach a range of synthetic molecules to cell membranes. FSL constructs currently include carbohydrates, peptides, fluorophores, ligands and biotin and are being used in the manufacture of modified cells called “kodecytes” for use in blood group typing laboratories and for research purposes (1–2). FSL constructs have now been used to create prototype diagnostic assay kits, using standard desktop inkjet printers, by simply replacing the ink in a spent cartridge with a solution of FSL construct. The FSL constructs can then be printed onto a substrate in much the same way one might print a letter or drawing on a sheet of paper. Blood group FSLs have been printed onto silica and nitrocellulose surfaces, and used to identify blood group antibodies in diagnostic and research samples. Combining FSL technology with existing inkjet printing technology provides a cost effective alternative to existing methods of manufacturing diagnostic test kits, including microarrays which, for the most part, use robotics. In addition to cost savings the versatility of this manufacturing method provides additional benefits for research laboratories and micro-analyses.

Acknowledgement: Supported by KODE Biotech Ltd (kodebiotech.com)

References:
E1.13 Antiradical activity in hydroxylated betalains

Department of Biochemistry and Molecular Biology A, University of Murcia, Murcia, Spain

Betalains are natural water soluble pigments present in most plant families belonging to the order Caryophyllales. They can be classified as either betacyanins or betaxanthins. Betaxanthins contain different amino acid or amine side chains, and exhibit yellow-orange coloration. Selected betaxanthins were designed to explore the effect of the presence of phenolic hydroxyl groups in the free radical scavenging activity of betalains under the ABTS+ radical assay. Activity shown by betaxanthins was compared with that of Trolox. Effect of pH on the free radical scavenging activity of betalains was studied. The antioxidant activity of betaxanthins was characterized under the FRAP assay. Betaxanthins present high antioxidant and free radical scavenging activities not linked to the presence of phenolic hydroxyls. This may be general to all betalains, which contain a similar electronic resonance system. In addition to their intrinsic activity, a clear enhancing effect on the scavenging of the free radical ABTS+ and in the antioxidant power of betaxanthins has been demonstrated to be linked to the presence of one or two phenolic hydroxyl groups in their structure. This work was supported by the Project AGL2007-65907, and by Programa de ayudas a Grupos de Excelencia de la Región de Murcia, Murcia, Spain, de la Fundación Seneca, Agencia de Ciencia y Tecnología de la Región de Murcia (Plan Regional de Ciencia y Tecnología 2007/2010) (GERM 2007-2010). F. Gandía-Herrero holds a contract with the "Programa Ramón y Cajal" (MICINN, FEDER, Spain).

E1.14 Stability analysis of the main pigment in cactus pears

Department of Biochemistry and Molecular Biology A, University of Murcia, Murcia, Spain

Betalains are nitrogenous plant pigments which are present in plants belonging to the order Caryophyllales. Betalains replace anthocyanins and exhibit yellow (betaxanthins) or violet (betacyanins) coloration. Interest in these molecules(6,7),(992,994)

E1.15 Stability and antioxidant activity of a maltodextrin encapsulated betalain

Department of Biochemistry and Molecular Biology A, University of Murcia, Murcia, Spain

Betalains are water-soluble nitrogen-containing secondary metabolites which are present in plants belonging to the order Caryophyllales. They are pigments widely used as additives in the food industry and are classified in betacyanins (violet) or betaxanthins (yellow). In cactus pears, the characteristic betalain is indicaxanthin, a proline derived betaxanthin first isolated from Opuntia ficus indica fruits. In the present work, indicaxanthin has been obtained pure and its stability has been highly promoted by its encapsulation into a maltodextrin matrix. A suitable spray-drying procedure for encapsulation has been described, obtaining a bright yellow powder. Stability of the encapsulated pigment has been analysed under different conditions. In the absence of light, pure encapsulated pigment can be stored at 20°C for months without appreciable loss of the bioactive substance and colour variation. Free radical scavenging and antioxidant properties of the encapsulated pigment have been studied under the ABTS+ radical and FRAP assays. These results demonstrate the high degree of stability of betalains under encapsulation and the maintenance of the antioxidant properties. This work was supported by MEC (Spain) and FEDER (Project AGL2007-65907), and by Programa de Ayudas a Grupos de Excelencia de la Región de Murcia, de la Fundación Seneca, Agencia de Ciencia y Tecnología de la Región de Murcia (Plan Regional de Ciencia y Tecnología 2007/2010). F. Gandía-Herrero holds a contract with the “Programa Ramón y Cajal” (MICINN, FEDER, Spain).

E1.16 Synthesis and physicochemical characterisation of hemoglobin anionic polyelectrolyte conjugates

B. Mansuroğlu, S. Derman, K. Kızilbey and Z. Mustafaeva Akdeste
Yıldız Technical University, Bioengineering, Istanbul, Turkey

Blood is an important biologic fluid with many diverse properties and functions. One important and unique function of blood is to transport oxygen to tissues [1]. Hemoglobin (Hb), a tetrameric protein consisting of ab dimers, is responsible for the transport of O2 and has been evaluated as an ideal blood substitute. However, stroma-free Hb solutions suffer from disadvantages of high O2 affinity, tetrameric dissociation, short intravascular retention time and high colloid oncotic pressure (COP) [2–3]. To circumvent these disadvantages, recent attentions were focused on increasing the molecular dimension of Hb through chemical modification [3–4]. Polymer conjugated Hb is, therefore, one strategy to enlarge Hb molecules [4]. The aim of synthesis of Hemoglobin-Polymer conjugates is to produce oxygen carriers with a high molecular weight and low oxygen affinity. In this study, we conjugated Hemoglobin (Hg) to different anionic polymers. Polymer-Hemoglobin conjugate reaction was prepared with the water-soluble 1-ethyl-3-(3-dimethylaminopropyl) carbodimide hydrochloride (EDC). We investigated the mechanism for the formation of Hemoglobin-Polymer conjugates different ratio of components by physicochemical analyses methods (High Performance Chromatography, Furier Transfor Infrared Spectroscopy, Ultra-violet Visible Spectroscopy, Refractive Index, Light Scattering, Viscosity Quadruple Detector System ‘VISCOTEX’).
E1.17 Plasma membrane vesicles as a cell-like model system for studying cell-penetrating peptide – membrane interactions
H. Amand1, C. Bostrom1, Per Lincoln1, B. Norden1 and E. Esbjorner-Winters2
1 Division of Physical Chemistry, Department of Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden, 2 Department of Chemistry, University of Cambridge, Cambridge, UK

Efficient delivery of gene-targeted drugs faces difficult problems due to the hydrophobic and impermeable nature of the cell’s plasma membrane. This creates the need for delivery vectors that can promote uptake at the cell surface. Cell-penetrating peptides (CPPs) have emerged as promising vector candidates, due to their ability to deliver macromolecular cargo into cells in a non-toxic manner. The uptake mechanisms of these peptides are despite extensive studies not well-described, but today endocytosis is considered a major pathway. However, it remains to be established which interactions on the cell surface that are responsible for triggering endocytosis. In this study, we have investigated the membrane interactions of the basic and amphipathic CPP penetratin, using plasma membrane vesicles (PMVs) derived directly from live mammalian cells. This is a new model system in CPP research and is attractive because of its cell-representative membrane composition, including cell surface-attached proteoglycans, which are believed to be crucial for CPP uptake. Specifically this study addresses the influence of basic residues by comparing penetratin to its arginine- and lysine-enriched analogues PenArg and PenLys. We show, using confocal microscopy imaging and a quantitative assay to determine binding affinity, that these peptides bind to PMVs in an arginine-dependent manner that correlates directly with uptake levels in live cells. Such correlation has to date only been obtained in simpler model systems such as lipid vesicles and recycling the used TTaspAT, optimizing conditions for the amino-transferring reaction in producing HPA, precipitating the product and recycling the used TTaspAT. Kinetic analyses showed that the Km and kcat of TTaspAT against HPP was 13.8 mM and 10.9 s-1, respectively. TTaspAT is of advantage in high thermostability and better activity against HPP. TTaspAT may be another good biocatalyst suitable for the production of unnatural amino acids in biopharmaceuticals.

E1.18 Thermal Biosynthesis of Homophenylalanine by Thermus thermophilus aspartate aminotransferase
S.-P. Wu1, H.-R. Luo2 and T.-S. Hwang2
1 Department of Biotechnology, Yuanpei University, Hsinchu City, Taiwan, 2 Chinese Culture University, Graduate Institute of Biotechnology, Taipei, Taiwan

Aspartate aminotransferases (AspAT) can catalyze the reversible transamination reaction from aspartate to a-ketoglutarate to form glutamate and oxaloacetate. AspAT plays an important role in the physiological metabolism of amino acids. It presents a substrates of amino acids with carbonyl group and is also found to possess significant activity against aromatic amino acids. Therefore, AspAT can be used to produce L-aspartate for synthesizing sweeter aspartame, and also to produce L-aromatic amino acids for nutrition addition in foods or medicinal purpose. Thermus thermophilus is a well-known thermophilic bacterium from hot spring. T. thermophilus AspAT (TTaspAT) was cloned and expressed in E. coli. We found that TTaspAT possesses high activity (1.64 U/mg at 37°C and 7.66 U/mg at 70°C) against homophenylpyruvate (HPP) to form homophenylalanine (HPA), an important compound in synthesizing some sweetener aspartame, and also to produce L-aromatic amino acids in biopharmaceuticals.

E1.19 Cell-free protein synthesis for NMR spectroscopy applications
A. Pedersen, J. Enberg, K. Hellberg and G. Karlsson
University of Gothenburg, Swedish NMR Centre, Göteborg, Sweden

Cell-free protein synthesis is a convenient method for producing preparative amounts of protein for structural biology. Highly specific isotope incorporation for NMR spectroscopy is especially efficient as the absence of an active metabolism decreases the amount of isotopic scrambling. For certain amino acids, however, scrambling as well as unproductive consumption in the cell-free reaction is significant. Only about 15% of the supplied amino acids are currently incorporated into the target protein even in the best reported systems, suggesting a significant potential for improvement by optimization of the existing system as well as by introducing new additives. This is important as the amino acids account for more than half of the costs associated with producing a 15N,13C-labelled sample for NMR. Deleting genes responsible for these processes enhances the labelling specificity. Here, we show that by adding exogenous inhibitors it is possible to increase expression yield as well as promote selective labelling of amino acids.

E1.20 Lysozyme microbubbles with new properties for smart applications
S. Melino1, M. Zhou2, M. Tortora1, M. Paci1, M. Ashokumar2 and F. Cavaliere1
1 Department of Sciences and Chemical Technologies, University of Rome Tor Vergata, Rome, Italy, 2 University of Melbourne, School of Chemistry, Melbourne, Australia

Microbubbles (MB) are small gas-filled colloidal particles that have specific acoustic properties that make them useful as a contrast agent in ultrasound imaging. The use of the MBs in clinical practice led to the development of more sensitive imaging techniques both in cardiology and radiology, including subharmonic and multipulse imaging, pulse inversion and harmonic power Doppler. Protein-based microbubbles are typically obtained by dispersing of gas phase in the protein solution. The protein deposited/cross-linked on the gas/liquid interface stabilizes the gas core. Innovative applications of protein-MBs prompt the investigation on the properties of MBs obtained using different proteins that are able to confer them specific properties and functionality. Recently, growth factor-releasing scaffolds have been also fabricated by incorporating growth factor-releasing BSA-MBs in the scaffold manufacturing processes. BSA-MBs have been used as a new porogen to produce BSA-coated cell-friendly surfaces in preserving the bioactivity of loaded growth factor. In a previous work, we have synthesized stable lysozyme microbubbles (Lys-MB) using high intensity ultrasound-induced emulsification of a partly reduced lysozyme in aqueous solutions. Both the
Abstracts

E1.21
Environmental factors enhancing in vitro catalysis of DNA strand exchange
B. Feng, K. Frykholm, B. Norden and F. Westerlund
Department of Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden

Strand exchange involving single and double stranded DNA is a well-known biological phenomenon and the basis for DNA repair and maintenance of genetic diversity as performed by recombination proteins like RecA and Rad51 in vivo. Artificial systems, including lipid vesicles and polypeptides, have also been reported to catalyze strand exchange. However, the basic mechanisms behind strand exchange biocatalysis are not understood despite intense structural and functional studies of both recombination proteins and simple model systems. In order to get a better understanding of the role of environment and water activity at active catalytic sites, we here study the strand exchange kinetics of matched and mismatched fluorescently labeled DNA oligonucleotides, using FRET and CD spectroscopy. Taking advantage of critical effects in crowded media, we have systematically investigated the significance of various factors for catalysis, including balancing of water activity, volume of exclusion, ionic strength and temperature. We report the discovery of specific conditions at which the DNA strand exchange rate is increased by several orders of magnitude, while maintaining B-DNA conformation.

Our results are discussed in terms of fundamental principles governing strand exchange, and relationship between stability and activity of the DNA double helix. They may also find practical applications in DNA nanotechnology and gene therapy.

E1.22
Abstract withdrawn

E1.23
Effects of PEGylation and acetylation of PAMAM dendrimers on DNA binding, cytotoxicity and in vitro transfection efficiency
K. Fant, E. K. Esbjörner, A. Jenkins, M. Grossel, P. Lincoln and B. Nordén
Chalmers University of Technology, Chemical and Biological Engineering/Physical Chemistry, Gothenburg, Sweden
Southampton University, Chemistry, Southampton, UK

Poly(amideamine) (PAMAM) dendrimers are promising multipotent gene delivery vectors, providing favourable DNA condensation properties and also the possibility of conjugation of different targeting ligands to their surface. They have been used for transfection both in vitro and in vivo, but their application is limited due to cytotoxicity. In this work we investigate two kinds of surface modifications, acetylation and PEGylation, and how they affect the DNA binding characteristics, the cytotoxicity and the in vitro transfection efficiency of generation 4 and 5 PAMAM dendrimers. Further, we evaluate how the morphology of DNA-dendrimer complexes changes upon dilution in cell growth medium, which occurs before the complexes reach the cell surface. We find that the surface-modified dendrimers have excellent biocompatibility profiles. However, their transfection efficiency is very low, which is explained by our observation that DNA is not sufficiently condensed by these dendrimers. Although small particles are obtained, the availability for ethidium intercalation and nuclease degradation is significantly higher in the modified dendrimer-DNA complexes than in unmodified ones. Dilution in cell growth medium has a dramatic effect on these electrostatically assembled complexes. Therefore we want to emphasize the importance of performing biophysical characterization under conditions as close to the transfection situation as possible, to enable conclusions regarding structure-activity relations of gene delivery vectors.

E1.24
Uptake mechanisms of cell penetrating peptides: the role of tryptophans and arginines in cell internalization efficiency
H. Rydberg, P. Thorén, E. K. Esbjörner and B. Nordén
Division of Physical Chemistry, Department of Chemical and Biological Engineering, Chalmers University of Technology, Göteborg, Sweden

Cell-penetrating Peptides (CPPs) are naturally derived peptides that have been revealed as promising candidates for intracellular delivery of proteins and other macromolecules such as DNA and RNA. Endocytosis is recognized as a major entry pathway for many CPPs, but the cell surface interactions leading to efficient internalization via this route have still not been fully characterized. Our goal is to deepen the understanding of how CPPs enter cells. Many studies suggest that arginines and tryptophans are important for CPP function. We are studying the influence these amino acids have on cell internalization, by comparing a series of new peptides, consisting of arginines and tryptophans in different combinations, with the well studied CPP penetratin in CHO-K1 cells by use of confocal microscopy as well as by cytotoxicity measurements. Our results propose that tryptophan content has influence on internalization and cytotoxicity, but also that the patterning of the tryptophans and arginines is of great importance.

E1.25
CIChE: Exploiting stabilized tandem repeats for heterologous expression
K. Kocharin, K. E.J. Tyo and J. Nielsen
Chalmers University of Technology, Chemical and Biological Engineering, Göteborg, Sweden

Episomal plasmids have been used to acquire high copy expression of many valuable products and have been widely used in biotechnology industries. Plasmid expression systems require a selective pressure such as antibiotics or auxotrophic markers in order to maintain a plasmid in the cell for consistent production of target products. Here, we present progress in developing a novel, stable, high copy expression system for heterologous genes in Saccharomyces cerevisiae. Chemically Induced Chromosomal Evolution (CIChE) achieves high copy number through tandem gene duplication in the genome. By virtue of being on the genome, no selective pressure is required and genetic stability is significantly improved. Homologous recombination facilitates both integration of the CIChE construct into the genome and evolu-
A. Nicolae

Faculty of Pharmacy, Biochemistry, Bucharest, Romania

Ru-nf and logarithmic for Ru-oflo.

of fluorescence intensity for Ru-cpx, exponential variations for double stranded calf thymus DNA, expressed as linear variations that there are interactions between the studied complexes and the fluorescence emission spectrophotometry. Our results showed ligands studied with double stranded calf thymus DNA through cin. In this regard we investigated DNA-binding properties of some ruthenium (III) complexes with also its important toxicity, drove to new transitional metal damage in tumor cells, blocking their division and resulting in action between small molecules and DNA, which cause DNA

E1.26

In vitro studies regarding the interactions of some ruthenium (III) fluoroquinolones complexes with double stranded calf thymus DNA

A. L. Arsene1, V. Uivarosti2, N. Mitrea1, C. Dragoi1 and A. Nicolae1

1Faculty of Pharmacy, Biochemistry, Bucharest, Romania,
2Faculty of Pharmacy, Inorganic Chemistry, Bucharest, Romania

Cancer is the second major cause of death after cardiovascular diseases. Therefore developing new antitumor therapies represents a target for the pharmaceutical research worldwide. Numerous biological experiments have demonstrated that DNA is the primary intracellular target of anticancer drugs, due to the interaction between small molecules and DNA, which cause DNA damage in tumor cells, blocking their division and resulting in neoplastic cell death. Cisplatin success in cancer treatment, but also its important toxicity, drove to new transitional metal research, like ruthenium (III). The present paper presents the DNA-binding properties of some ruthenium (III) complexes with fluoroquinolones, namely ciprofloxacin, ofloxacin and norfloxacin. In this regard we investigated in vitro the interactions of the ligands studied with double stranded calf thymus DNA through fluorescence emission spectrophotometry. Our results showed that there are interactions between the studied complexes and the double stranded calf thymus DNA, expressed as linear variations of fluorescence intensity for Ru-cpx, exponential variations for Ru-nf and logarithmic for Ru-oflo.

E1.27

Abstract withdrawn

E1.28

Cell-free production of integral membrane proteins for incorporation into nanodiscs and micelles

J. Enberg, I. Isaksson, A. Pedersen and G. Karlsson

Göteborgs Universitet, Svenskt NMR Centrum, Göteborg, Sweden

One bottleneck in obtaining structures by NMR or X-ray crystallography is that the protein needs to be purified in high concentration. This problem often occurs when the protein is expressed in low concentration in the natural source or causing toxicological problems when overexpressed. Cell-free protein synthesis is not subject to cell toxicity and offers the possibility of manipulating the reaction conditions to stabilize the produced protein. We have produced and purified wide range of integral membrane proteins, including also θ-helical and β-barrel integral membrane proteins. By supplying detergents or lipids directly to the reaction mixture the environment can be fine tuned allowing the protein to be synthesised in a solubilized and functionally folded state. Hence, avoiding the need for refolding or extraction from membranes. To find the best condition for a protein one needs to evaluate different lipids and detergents to see which maximizes expression levels of solubilized. An attractive alternative to lipids and detergents are nanodiscs. Nanodiscs allow for easy purification and allows access to both sides of the membrane. We have found that nanodiscs can be added to the cell-free reaction allowing proteins to be incorporated as they are synthesized without inhibiting the protein synthesis. Cell-free protein synthesis also provides the possibility of selective incorporation of isotope labelled amino acids. This makes it possible to only label one or a few types of amino acid, greatly reducing the amounts of peaks in a NMR spectra, thus minimizing the amount of overlapping signals and simplifying sequential assignment.

E1.29

[FeFe]-hydrogenases for hydrogen gas production: Evolving natural bio-catalysts for artificial devices

F. Valetti, S. Morra and G. Gilardi

Department of Human and Animal Biology, University of Torino, Torino, Italy

A recombinant expression system for [FeFe]-hydrogenase was used to produce and characterize Chlamydomonas reinhardtii hydrogenase (CrHydA1) and Clostridium acetobutylicum hydrogenase (CaHydA). The suitability of these enzymes as bio-catalysts in artificial devices was tested. In such devices, electrons and protons are supplied to the hydrogenase through nanostuctured electrodes. Stability and optimized interaction/electron transfer with electrodes are the key elements to exploit the highly evolved ability of [FeFe]-hydrogenases to catalyze hydrogen production without the need for expensive noble metals. CrHydA1 and CaHydA were expressed in the active form in E. coli and affinity purified with a yield of milligrams of pure protein per litre of culture. The whole process was performed under strict anaerobic conditions to avoid inactivation of the enzymes. Functional characterization confirmed that recombinant CrHydA1 and CaHydA are highly thermo stable: activity is conserved up to 60–70°C with a half-life of several hours at high temperatures. These features will be particularly desirable in artificial devices and other future applications. Preliminary trials of immobilization on different types of electrodes are under study to identify the best targets for the construction of the cathodic hydrogen evolving electrode. Directed evolution techniques are being applied to engineer new variants of the recombinant [FeFe]-hydrogenases with improved catalytic properties and enhanced stability to oxygen.
E2 – Molecular Recognition

E2.01
Canonical serine protease inhibition: last stand of the lock-and-key hypothesis?
Z. Gaspari1, V. Peter2, S. Balazs1 and P. András1
1Eotvos Lorand University, Institute of Chemistry, Budapest, Hungary, 2Department of Chemistry and Biochemistry, University of Sussex, Brighton, UK

Canonical serine protease inhibitors interact with their target enzyme in a substrate-like manner. Their efficiency is conventionally attributed to the rigidity of their protease binding loop with no conformational change upon enzyme binding, yielding an example of the rigid lock-and-key model for biomolecular interactions. However, solution-state structural studies reveal that the protease binding loop displays considerable flexibility in the free state and structural differences between the free and protease-bound states are observable. We resolve this apparent contradiction by showing that the ps-ns timescale dynamics of two related small, 35-residue inhibitors map a relatively large conformational space that includes conformers close to the enzyme-bound ones [1]. This type of fast time-scale dynamics enables the association rate to be solely diffusion-controlled just like in the rigid-body model. Eliminating the necessity for an induced fit means that enzyme binding requires no energetic costs related to conformational rearrangements of the inhibitor backbone. Our results suggest that conformer selection is a general phenomenon that might occur on multiple time scales depending on the specific dynamical properties of the partners. This reinforces the importance of internal dynamics as a key factor in fine-tuned biomolecular interactions, which must be considered in inhibitor design.

Reference:

E2.02
Human galectin-3 interacts with two anticancer drugs
V. Bogoeva1, A. Variale2, J. Constance1 and S. D’Auria2
1Institute of Molecular Biology, Molecular Biology of Cell Cycle, Sofia, Bulgaria, 2Institute of Protein Biochemistry, Lab for Molecular Sensing, Naples, Italy, 3MandalMed, Inc, California, San Francisco, US

Human galectin-3 (hGal-3) is a mammalian lectin involved in regulation of RNA splicing, apoptosis, cell differentiation, and proliferation. Multimerized extracellular hGal-3 is thought to crosslink cells by binding to glycoproteins and glycosylated cancer antigens on the cell surface or extracellular matrix. Fluorescence spectroscopy and circular dichroism (CD) were used to study the interaction of hGal-3 with two anticancer agents: bohemine and Zn porphyrin (ZnTPPS4). The dissociation constant for binding of bohemine with hGal-3 was $K_D = 0.23 \pm 0.05 \mu M$. The hyperbolic titration curve indicated the presence of a single bohemine binding site. The binding of ZnTPPS4 to hGal-3 (with and without lactose) is of high affinity, having $K_D = 0.18-0.20 \mu M$ and not inhibited by lactose, indicating that the ZnTPPS4 binding site and the carbohydrate site are different. CD spectra of the complexes with hGal-3 suggested that the binding of the hydrophobic compounds changed the hGal-3 secondary structure. In summary, we show that two compounds with anticancer activity, bohemine and ZnTPPS4, have high affinity for hGal-3 at a site that is distinct from its carbohydrate site. Since hGal-3 binds to several carbohydrate cancer antigens, the results suggest that it may have utility in the targeted delivery of drugs for cancer.

E2.03
Crystal structure of the C-terminal carbohydrate recognition domain of human galectin-9
H. Yoshida1, M. Teraoka1, N. Nishi1, T. Nakamura2, M. Hirashima3 and S. Kamitori1
1Kagawa University, Life Science Research Center and Faculty of Medicine, Kagawa, Japan, 2Faculty of Medicine, Department of Endocrinology, Kagawa University, Kagawa, Japan, 3Faculty of Medicine, Departments of Immunology and Immunopathology, Kagawa University, Kagawa, Japan

Galectin-9 (Gal-9) is a member of the family of β-galactoside-binding lectins and belongs to the tandem-repeat-type galectins having two carbohydrate recognition domains (CRDs) joined by amino acid linker peptide. Gal-9 is associated with various biological functions and known to be an eosinophil chemotaxant, T-cell immunoglobulin domain and mucin domain3 ligand, and inducer of T helper 1 cell apoptosis. The sequence identity between N- and C-terminal CRD (N-CRD, 1-148; C-CRD, 210-355) is 39%. The C-CRD has higher similarity with CRD of chimera-type Galactin-3 with 42%. To elucidate the relationship between the structure and biological activity of Gal-9, each structure of N-CRD and C-CRD is important. So far, the crystal structure of N-CRD in complexes with various ligands have been reported, however, the structure of C-CRD was not available. Here we report the crystal structure of C-CRD of Gal-9. The structure of C-CRD was solved by a molecular replacement using the structure of Gal-3 (PDB ID, 1KJL) and refined at 1.7 Å resolution. A comparison of monomeric structure of C-CRD with that of N-CRD does not show a major difference but reveals a significant difference in ligand binding sites which would be responsible for a difference of carbohydrate binding activity. Unexpectedly, N-terminal region (210-219) of C-CRD was invisible due to not only poor electron density but also insufficient space, since the three monomeric structure forms a trimmer. Though it is not clear whether C-CRD forms timer only in crystal structure or also in vivo, it might be related in any biological activity of Gal-9.

E2.04
The HIV-1 gp120 interaction with Nt-CCR5 – New insights from NMR
E. Schur1, T. Scherl4, F. Naider2 and J. Anglister1
1Weizmann Institute of Science, Structural Biology, Rehovot, Israel, 2College of Staten Island of the City University of New York, Chemistry and Macromolecular Assembly Institute, New York, US

Human immunodeficiency virus type 1 (HIV-1) is the retrovirus that causes the acquired immunodeficiency syndrome (AIDS). HIV infection in humans is a pandemic affecting millions of people around the world. HIV-1 entry into target cells is mediated by the successive interaction of the viral envelope glycoprotein gp120 with the cellular receptor CD4 and with a G protein-cou-
E2 – Molecular Recognition

pled chemokine co-receptor, mainly CCR5 or CXCR4. Interaction of CCR5 with the HIV-1 gp120-CD4 complex involves the amino-terminal domain of the CCR5 receptor (NT-CCR5) and requires posttranslational sulfation of its tyrosine residues. We studied a 27-residue peptide corresponding in sequence to NT-CCR5 (residues 1-27) and containing two sulfated tyrosine residues at positions Y10, Y14, in complex with a truncated, homogeneously glycosilated, gp120 JR-FL (residues 88–492, ΔY1, ΔY2) and a CD4-mimic miniprotein. 1H,1H-filtered NOE experiments revealed evidence for a helical conformation in the center of the peptide, induced upon gp120 binding. Saturation Transfer Difference (STD) experiments allowed identification of the NT-CCR5 residues that participate in gp120 binding and highlight the importance of the sulfated tyrosine residues.

E2.05
Abstract withdrawn

E2.06
cAbVIM4, a nanobody inhibiting the metallo-β-lactamase VIM-4
J.-S. Sohier1, E. Pardon2, C. Laurent1, P. Lassaux1, N. Buys2, K. Willibal2, L. Wynn2, J. Steyaert2 and M. Galleni1
1University of Liege, Biological Macromolecules, Center for Protein Engineering, Liege, Belgium, 2Department of Molecular and Cellular Interactions (VIB), Free University of Brussels, Structural Biology Brussels lab, Brussels, Belgium

The introduction of β-lactam antibiotics like penicillins in medical practice is one of the biggest clinical successes. Unfortunately, bacteria evolved rapidly to develop resistances. The most common resistance mechanism is the bacterial production of β-lactamase enzymes which cleave the β-lactam ring. Besides serine-β-lactamases of classes A, C and D, metallo-β-lactamases (MBL) of class B are emerging as a problematic group of enzymes challenging the efficiency of modern antibacterial therapy. Notably, MBLs of the VIM family hydrolyse a broad range of β-lactam substrates including the last generation carbapenems. These enzymes can also be observed during nosocomial infections caused by multiresistant pathogens. A successful strategy to counter β-lactamases mediated resistance is the use of β-lactamase inhibitors which restore bacterial susceptibility to the antibiotic. To date, there is no clinically useful inhibitor of class B MBL. In the field of drug discovery, camel heavy chain antibodies are of particular interest. These antibodies are devoid of light chains so that their heavy chain’s variable domains (VHH or nanobody) are actually the smallest intact antigen-binding fragments derived from functional immunoglobulin. Here we present cAbVIM4, a dromedary VHH inhibiting the MBL VIM-4 which was isolated by phage display from an immune library. Its μM level inhibitory ability spans substrates of different types. In an effort to identify binding “hot spots” of this VHH, alanine scanning mutagenesis has been realised. Measurements of the binding potency and the inhibition percentage of those mutants reveal insights into the interaction and the inhibition mechanisms.

E2.07
Abstract withdrawn

E2.08
Construction of a tethered lipid bilayer for membrane protein research
F. Inci1 and F. N. Kok2
1 Istanbul Technical University, Molecular Biology-Genetics and Biotechnology Program, Istanbul, Turkey, 2Department of Molecular Biology and Genetics, Istanbul, Istanbul Technical University, Turkey

Membrane proteins have a wide range of important functions in cell and deficiencies related to their function cause a number of diseases. They could not be studied in aqueous solutions due to their high hydrophobic content; hence it is necessary to develop cell membrane mimicking systems (artificial lipid membranes) to study them. In our work, we developed an artificial lipid membrane for the integration of a multi-drug resistance protein called P-glycoprotein (P-gp). To form the first layer, different concentrations of 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino polyethylene glycol]-2000 (DSPE-PEG) were incubated with 3,3-dithiodipropionic acid di(N-hydroxy succinimide ester) (DTSP) self-assembled gold slides. Liposomes were then spread to the surface for the formation of the second layer. For the formation of P-gp incorporated membranes, liposomes with different P-gp content (Tris-EGTA, pH 7.0) were used. According to surface plasmon resonance and quartz crystal microbalance analysis, liposome spreading was significantly affected by DSPE-PEG amount on the surface and 0.03 and 0.04 mg/ml of DSPE-PEG concentrations were observed to be optimum for bilayer construction. Preliminary studies showed that 1–10 μl P-gp addition gave better results when compared to higher concentrations.

Acknowledgement: The grants from TUBITAK TBAG-108T933 and TUBITAK 2211 - Ph.D. Fellowship are highly acknowledged.

E2.09
The epitope on monomeric beta-actin recognized by anti – phosducin antibodies
U. Piotrowska and G. Adler
Medical Centre of Postgraduate Education, Warsaw, Poland

The peptide SQSLEEDFEGQATHTGPK (p33) originates from protein phosducin-like protein 1 (PhLP1, MW 32 kDa) taking part in protein chaperoning. This peptide linked to Keyhole limpet hemocyanin was used for rabbits immunization. It was found that ap33 antibodies obtained recognize apart from Phd and PhLP1 also 43 kDa protein. Mass spectroscopy analysis of the 43 kDa protein, purified from human thyroid tissue homogenate, identified it as cytoplasmic beta-actin, the cell cytoskeleton element different in respect of sequence from the phosducin family proteins. The aim of the present study was to characterize epitope common for phosducin and beta-actin. Analysis of beta-actin sequence performed by conformational epitope prediction servers let to choose amino acids L D F E Q A T K as those which build the epitope. Most of them belong to the 221–241 peptide (p37) located on the actin – actin interface of the fibrillar form of actin. The p37 peptide was synthesized and used in reactions with ap33. Good affinity of the soluble p37 to ap33 confirmed participation of this region of actin molecule in formation of immunological complex with ap33. In polymeric actin the p37 localization site is not accessible for antibodies, so ap33 recognizes only monomeric form of this molecule. This observation was confirmed by immuno-nostaining of thyroid cells by ap33 and anti actin antibodies. The existence of the epitope common for actin and phosducin may reflect a topological similarity of small region of their surfaces.
Abstracts

E2.10
Interaction of α-crystallin with argininosuccinate lyase
Y.-H. Chen, Y.-W. Cheng and H.-J. Lee
National Defense Medical Centre, Biochemistry, Taipei, Taiwan
α-Crystallin is the member of the small heat shock protein family and present ubiquitously in all vertebrate to maintain the transparency of lens. Argininosuccinate lyase (ASL) is a urea cycle enzyme that catalyzes the reversible conversion of argininosuccinate into arginine and fumarate. In the present study, the protein-protein interaction behaviors of α-crystallin and ASL were investigated. Incubation with different weight ratio of α-crystallin led to ~30% increase in the catalytic efficiency of ASL. α-Crystallin protected the ASL from low and high temperature induced inactivation. An apparent conformational change and extensive exposure of hydrophobic patches was observed when ASL was incubated with α-crystallin at room temperature. Real-time measurement of protein-protein interaction by using surface plasmon resonance showed that the association of α-crystallin with ASL at different weight ratio did not perform a simple interaction mechanism.

E2.11
The lactococcin G immunity protein recognizes specific regions in both peptides constituting the two-peptide bacteriocin lactococcin G
Department of Molecular Biosciences, University of Oslo, Oslo, Norway
Ribosomally synthesized antimicrobial peptides produced by lactic acid bacteria are often termed bacteriocins. Bacteriocins are secreted by the producer cell and kill target cells by permeabilizing the membrane. To avoid being killed by its own bacteriocin the producer strain also produces an immunity protein. Lactococcin G and enterocin 1071 are two so-called two-peptide bacteriocins, i.e. they both consist of two different peptides and both peptides need to be present to obtain optimal antimicrobial activity. The two lactococcin G peptides (LcnG-α and LcnG-β) are thought to form a transmembrane helix-helix structure in target membranes, thereby causing membrane leakage. Lactococcin G and enterocin 1071 show about 55% amino acid sequence identity, whereas their immunity proteins show 38% amino acid sequence identity. Despite their sequence similarity, lactococcin G and enterocin 1071 recognize and kill different target bacteria. Hybrid peptides consisting of the N-terminal part of lactococcin G and the C-terminal part of enterocin 1071 were constructed and combinations of these hybrid peptides and wild type lactococcin G and enterocin 1071 peptides were assayed against two strains expressing the lactococcin G immunity protein. The results indicate that the N-terminal end of LcnG-α (residues 1–13) and the mid region of LcnG-β (residues 14–24) are important for the recognition between lactococcin G and its immunity protein. Both regions are positioned adjacent to each other in the proposed transmembrane helix-helix structure. Moreover, the results indicate that the recognition between the immunity protein and the cognate bacteriocin depends on a cellular component.

E2.12
The cytochrome c-cardiolipin interaction: looking for a model of protein binding to the mitochondrial membrane
F. Sinibaldi1, B. D. Howes2, M. C. Piro1, T. Ferri1, G. Smulevich2 and R. Santucci1
1Department Experimental Medicine and Biochemical Sciences, Rome, University of Rome Tor Vergata, Italy, 2Department of Chemistry, University of Florence, Sexto Fiorentino, FI, Italy
Several models have been proposed to explain the interaction of cytochrome c (cyt c) with cardiolipin (CL), a component of the mitochondrial membrane. One hypothesis proposes that an acyl chain of the phospholipid accommodates into a hydrophobic channel of the protein located close the N52 residue. An alternative model considers the insertion of the acyl chain in the region of the M80-containing loop, in which the K72 and K73 residues seem to play a primary role. Recent studies indicated that two distinct transitions take place in the cyt c-CL binding reaction and data obtained on the N52I variant of the protein provided support to a model in which two (instead of one) close acyl chains of the CL liposome are inserted into the same cyt c molecule, one at each of the hydrophobic sites of the protein, to form the cyt c-CL complex. To clarify better this still controversial point, we have undertaken a study on the K72N, K73N and the K7273N double mutant of cyt c to establish the role played by lysines K72 and K73 in the cyt c-CL binding reaction. Preliminary results show that these variants bind to CL by a two-state transition and that M80, i.e. the axial ligand of the heme-iron in the native protein, is replaced by an intrinsic residue, likely a histidine (lysine) residue upon complex formation for the K7273N (K73N) mutant. Conversely, binding of CL to K72N has relatively little effect on the heme pocket structure, suggesting that the lysine residue replacing M80 upon complex formation is primarily K72.

Acknowledgements: Research funded in part by grants from MIUR (PRIN 2007KAWXCL to R.S.).

E2.13
Nanofluidics for single DNA molecule manipulation and analysis
F. Persson, C. Freitag, E. Sandlund, J. Fritzsche, F. Westerlund and J. Tegenfeldt
Department of Physics, University of Gothenburg, Gothenburg, Sweden
During the last decades the advances in nanotechnology has enabled us to fabricate artificial structures and fluidic channels that are on the order of the natural size scales for DNA (~1 nm – 100 nm). When placing a DNA molecule into a nanofluidic channel with a diameter smaller than the natural coiled up state of the molecule it stretches out along the channel. Positions along one of these linearized DNA molecules directly correspond to positions along the sequence of the DNA. This has enabled investigations and visualizations of DNA-protein interactions as well as genomic information within single DNA molecules. In addition, it has revealed many new and important insights into the conformational changes of DNA molecules in crowded and confined environments. The advantages of using nanofluidics over other means of stretching DNA, for example molecular combing, shear flow or optical/magnetic tweezers, is that it supports massive parallelization, is compatible with Lab-On-a-Chip technology and that the DNA is in equilibrium during measurements, ensuring reproducibility. We will present an overview of new techniques and appli-
E2.14 Detection of the ligand binding/release state of membrane protein bacteriorhodopsin by the change of solubility in Dodecyl-β-D-maltoside
T. Sasai¹, M. Demura² and Y. Mukai²
¹Meiji University, Kanawaga, Japan, ²Hokkaido University, Hokkaido, Japan

A seven transmembrane protein bacteriorhodopsin (bR) exists on the biological membrane of Halobacterium salinarum, and forms two-dimensional lattice structure called purple membrane (PM) by specific interaction between bR molecules or bR and lipids. bR that functions as light-driven proton pump shows a large shift of λmax and a subtle tertiary structural change accompanied by the binding/release of a ligand, retinal. In this study, we investigated the correlation between the ligand binding/release and the solubility in a non-ionic detergent, Dodecyl-β-D-maltoside (DM) on bR. When PM was treated by 20 mM-DM at 25°C for 24 hour, only about 20% of bR was solubilized. Contrary to this, all protein molecules were solubilized from the completely photo-bleached, that is, retinal-released membrane by hydroxylamine. In addition, the plots of solubilization ratio of the protein by DM versus bleaching ratio of PM were well fitted by a simple exponential curve, indicating that the ratio of apo-protein in PM can be calculated from the solubilization ratio. Interestingly, the solubility of the bleached membrane in DDM was decreased by re-addition of the retinal to the with that of intact PM. These results suggest that the change of solubility of bR in DDM is attributed to the tertiary or quaternary structural changes accompanied by the ligand binding/release.

E2.15 Evolutionarily conserved furofuran region of aflatoxin B₁ and related micotoxins is electrostatically preorganized to bind DNA
U. Bren
National Institute of Chemistry, Laboratory for Molecular Modeling, Ljubljana, Slovenia

Aflatoxin B₁ and related furofuran micotoxins produced by the common molds of Aspergillus are of great interest due to their role in the etiology of human liver cancer. After intake they get epoxidized by cytochrome P450 to corresponding ultimate carcinogens which alkylate DNA, evidently after intercalation. Aflatoxin B₁, exo-8,9-epoxide intercalation free energy of -4.1 and -4.9 kcal/mol was calculated using Molecular Dynamics (MD) simulations in conjunction with Linear Interaction Energy (LIE) and Linear Response Approximation (LRA) methods, respectively, and compares favorably with the range of -3.9 to -4.9 kcal/mol obtained experimentally using various techniques and nucleobase sequences. Moreover, to provide an insight into interactions responsible for intercalation, the electrostatic, dispersion, preorganization as well as various group contributions to the calculated free energy were explicitly determined. The furofuran region is electrostatically preorganized to bind DNA, which has the potential to explain, why this structural element was evolutionarily conserved in a variety of micotoxins. On the other hand, the large planar body enhances intercalation solely through dispersion interaction, which again explains, why this region of related micotoxins could have been evolutionarily optimized in a different way. Finally, comparing generation of Near Attack Configurations (NAC) during the course of MD simulations involving charged and uncharged aflatoxin B₁, exo-8,9-epoxide, respectively, revealed that preorganized electrostatics plays a vital role also in the subsequent chemical step and speeds up the corresponding alkylation by a factor of 4.5.

E2.16 New designs of protein binding aptamers with LNA and UNA
B. Vester
Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark

Synthetic nucleic acid aptamers can recognize a wide variety of targets, from simple ions to whole cells. Even so, the "classical" aptamer targets are proteins. Aptamers typically bind with a dissociation constant in the low nanomolar range and with high specificity comparable to those from antibodies. New nucleic acid aptamers are often created by selection from a large random sequence pool. The final aptamer can be synthesized in a reproducible manner in short time and modified to improve thermodynamic stability, decrease nuclease sensitivity or contain reporter groups. We are investigating advantages of incorporation of LNA (locked nucleic acid) and UNA (unlocked nucleic acid) in aptamers. LNA is an RNA-like structure with a bridge in the ribose ring providing a stiffness that significantly increases melting temperatures of short duplexes and confers nuclease resistance. UNA has an opened ribose ring and therefore work the opposite way of LNA thus providing flexibility and decreasing melting temperatures of duplexes. Some of our studies benefit from already known aptamers that we redesign and modify to investigate changes in various features and we study in the molecules modifications are allowed and how advantageous they are. Furthermore, we have developed template-directed enzymatic methods to incorporate LNA in nucleic acid strands using LNA triphosphates with DNA polymerases. In combination with other methodologies this allow us to make in vitro selection of protein binding aptamers from LNA- and UNA modified nucleic acid libraries.

In vitro selection of LNA-containing aptamers
H. Doessing and B. Vester
University of Southern Denmark, Biochemistry and Molecular Biology, Odense, Denmark

Aptamers are structured RNA or DNA molecules with high affinity for various molecular targets, and they can be obtained through a process known as in vitro selection. In many cases aptamer stability and chemistry needs further refinement to achieve optimal properties, but ad hoc modifications frequently interfere with the desired aptamer structure. Inclusion of modified nucleotides in the selection scheme yields aptamers that are tailored for the desired nucleotide derivatives. The use of modified nucleotides is limited, however, by the specificity of the enzymes used in the in vitro selection process and occasionally also by specific properties of the nucleotide derivative in question. Locked nucleic acid (LNA) is a nucleoside analogue with a 2'-4' linker that locks the ribose in the 3'-endo form. LNA is known to confer nuclease resistance as well as excellent thermal stability to secondary structures. LNA is therefore well suited for application in the aptamer field. We present an outline of in vitro selection of DNA aptamers in the presence of LNA triphosphates and how we have managed to overcome the challenges associated with each step in the selection scheme.
E2.18

Abstract withdrawn

E2.19

Molecular recognition mechanism of p63 by Itch-e3 ligase: advances and effects of a p63 mutation related to ectodermal dysplasias

A. Bellomaria1, G. Barbato1, G. Melino2, M. Paci1 and S. Melino1

1Department of Chemical Sciences and Technologies, University of Rome “Tor Vergata”, Rome, Italy, 2Department of biochemistry and experimental medicine, University of Rome, Rome, Italy

Recently, it has been shown that Itch mediates the degradation of TAp63 and ΔNp63 proteins1. Itch-e3-ligase contains four WW domains important in the recognized process. Several signaling complexes, that these domains mediate, have been implicated in human diseases (Muscular Dystrophy, Alzheimer’s Disease, Huntington Disease etc.). WW domains are highly compact protein-protein binding modules that interact with short proline-rich sequences. Based on their ligand-binding specificity they have been categorized into four groups. WW domains fold into stable three-stranded antiparallel β-sheet structures, and their primary sequence share two conserved tryptophan residues spaced 20–22 amino acids apart. The four WW domains of Itch are considered belonging to the Group I, which binds polypeptides with a PY motif characterized by a PPXY consensus sequence, where X can belong to the Group I, which binds polypeptides with a PY motif characterized by a PPXY consensus sequence, where X can be any residue. It is likely that the Itch-p63 interaction results from a direct interaction of Itch-WW2 domain with the PY motif of p63. Here, we present a structural characterization of the interaction by fluorescence, CD and NMR spectroscopy of the Itch-WW2 domain. Interaction studies in vitro between Itch-WW2 domain and pep63, which correspond to the fragment of the p63 protein including the PY motif, were performed. Moreover, the effects of a site specific mutation of p63, that has been reported in both Hay-Wells syndrome and Rapp–Hodgkin syndrome, was also evaluated both on the conformation of pep63 and on the WW-pep63 interaction.

Reference:

E2.20

Trapping conformational states along ligand binding dynamics: The impact of induced-fit on enzyme catalysis

T. Meinnel1, S. Fieuxlaine1, A. Boularot1, I. Artaud2, M. Desmadris1, F. Dardele and C. Giglione1

1CNRS, ISV, UPR2355, Gif-sur-Yvette Cedex, France, 2Université Paris V, CNRS, UMR8601, Paris, France

It is now accepted that the functions of proteins are governed by their dynamic nature, especially in relation to catalysis or their interactions with other proteins. Protein-ligand induced-fit is one example of such a dynamic mechanism that mediates molecular recognition. However, a detailed molecular description and demonstration of this biochemical phenomenon has remained largely obscure, primarily due to the difficulty of capturing the steps of this transient process. To identify the structural and dynamic bases governing this conformational change, we took advantage of the “slow tight-binding mechanism” induced by the potent antibiotic actinomycin, which is a pseudopeptide inhibitor, on its specific target enzyme peptide deformylase. We solved more than six distinct crystal structures showing the kinetics of the conformational change from the initial open state, including the encounter complex, to the final closed state of the enzyme. These structural determinations included intermediates that were trapped during the process, allowing us to monitor the action of an enzyme in real time at atomic resolution. Our data along with further biochemical and biophysical analyses will enable a coherent causal reconstruction of the sequence of events leading to inhibition of enzyme activity. Moreover, we provide new insight on how enzymes achieve a catalytically competent conformation in which the reactive groups are brought into close proximity, resulting in catalysis.

E2.21

Separation of monomer, multimer and complex forms of IGFBP-1 from human serum using lectin-affinity chromatography

D. Lagundzin, D. Robajac, G. Miljus, R. Masnikosa and O. Nedie

Institute for the application of nuclear energy-INEP, University of Belgrade, Belgrade, Serbia

Insulin-like growth factor–binding protein 1 (IGFBP-1) is a phosphoprotein which regulates the action of the insulin-like growth factors and, thus, the metabolism. The majority of IGFBP-1 in the circulation is bound to a glycoprotein α2-macroglobulin (α2M) which protects IGFBP-1 from proteolysis, and a minor portion is found in a monomer or multimer form. In order to separate these molecular species, a panel of eleven agarose-immobilised lectins was used. Electrophoresis and immunoblotting were employed for identification of the IGFBP-1 forms. IGFBP-1/α2M complexes bound selectively to agarose immobilised Con A, SNA, RCA I, PHA-E, WGA and LCA lectin, whereas IGFBP-1 multimers (having similar electrophoretic mobility as IGFBP-1 multimers) remained unbound. Monomer IGFBP-1 was not expected to interact with lectins, however it bound to Con A and was eluted only with the pH 3.0 buffer. It is known that phosphoproteins interact with metal ions and the presence of Ca (II), Mn (II) and Mg (II) is needed for the Con A activation. In this study, it was shown that the presence of all three ions was necessary to obtain such a strong bond between IGFBP-1 and Con A. The absence of Mg (II) reduced the binding to some extent, whereas the absence of either Ca (II) or Mn (II) abolished the interaction. Therefore, chromatography with the immobilised SNA, RCA I, PHA-E, WGA or LCA (or a mixture of these lectins) in the first step may be used to isolate IGFBP-1/α2M complexes from the serum, chromatography with Con A in the second step may be used to isolate IGFBP-1 monomers, whereas IGFBP-1 multimers would remain among the unbound proteins.

E2.22

New avenues to malaria diagnosis – nucleic acid aptamers against P. falciparum histidine rich protein 2 and lactate dehydrogenase

Y. W. Cheung and J. A. Tanner

Department of Biochemistry, The University of Hong Kong, Hong Kong, Hong Kong

Malaria diagnosis is an ongoing challenge, particularly in many malaria-endemic regions that have inadequate access to traditional microscopic diagnostic approaches. Self-diagnosis and mis-diagnosis are causing overuse of antimalarial drugs leading to increased drug resistance and other problems. Non-microscopic
tests have been developed including rapid dipstick immunoassays and polymerase chain reaction, but immunoassays have issues with antibody stability and PCR requires equipment, electricity and technical expertise. We are developing nucleic acid aptamers against plasmidium proteins histidine rich protein 2 (HRP2) and lactate dehydrogenase (LDH) with a view to developing such aptamers for incorporation into a more robust rapid diagnostic test. We present the cloning and purification of these targets, and demonstrate specific selection of nucleic acid aptamers against these targets. This work lays a foundation for further development of applications of nucleic acid aptamers for malaria diagnosis.

E2.23
Do intramolecular CH...O hydrogen bonds really contribute to the DNA shaping?
Quantum chemical and AIM analysis
Y. Yurenko¹, R. Zhurakivsky², S. Samijlenko¹ and D. Hovorun¹
¹Institute of Molecular Biology and Genetics, Molecular Biophysics, Kiev, Ukraine, ²Institute of Molecular Biology and Genetics, Kiev, Ukraine

The aim of this work is to cast some light on the H-bonds in double stranded DNA in A1 and B1 forms. For this purpose, there were performed the MP2 and DFT quantum chemical calculations of the canonical nucleoside conformers, relative to the A1 and B1 DNA forms, and their Watson-Crick pairs, which were regarded as the simplest models of DNA. Based on the AIM analysis, five types of the CH...O hydrogen bonds, involving bases and sugar, were detected numerically from 1 to 3 per a conformer: C2'H...O5', C1'H...O2, C6'H...O5', C8'H...O5', and C6'H...O4'. Strengths of H-bonds occupy the range of 2.3 – 5.6 kcal/mol, i.e. surely exceeding the kT value (0.62 kcal/mol) at room temperature 298.15 K. The nucleoside CH...O bonds appeared to “survive” changes in mutual turns of bases and sugar, determined by angle chi, in rather large ranges, admissible for a given conformation. That points out to the source of the DNA liability, lowering it to adapt necessary conformatations in processes of its functioning. The calculation of the interaction in the dA T nucleoside pair gives evidence that between the bases adenine and thymine, additionally to the N6H...O4 and N1...N3H canonical H-bonds, the C2'H...O2 third one is formed, which, being rather weak (about 1 kcal/mol), satisfies the AIM criteria of H-bonding and should be classified as a true H-bond. Interesting, total energy of all the CH...O nontraditional intramolecular H-bonds in nucleoside pairs proved to be commensurable (from 0.5 up to 0.8) with the energy of H-bonds between the bases in Watson-Crick pairs, which implies their significant role in the DNA shaping and in processes of protein-nucleic acid recognition.

E2.24
Relationship between apoptosis and total sialic acid level in regressing and remodeling organs during amphibian metamorphosis
E. Kaptan, S. Sancar Bas and M. Sengezer Inceli
Faculty of Science, Department of Biology, Istanbul University, Istanbul, Turkey

Amphibian metamorphosis contains several cellular processes such as proliferation and cell death. Thus, it is accepted as a significant model organism for study of vertebrate development and different cellular events. In this study, we aim to investigate changes of total sialic acid level and apoptosis during metamorphosis of Rana ridibunda larvae. For this purpose, it was composed of four groups namely premetamorphosis, pro-metamorphosis, metamorphic climax and postmetamorphosis in terms of larval developmental stages of the frog. In these groups, it was spectrophotometrically estimated sialic acid level in regressing (tail) and remodeling (intestine/liver) organs. Also, apoptotic cells were stained by TUNEL technique and the apoptotic index was determined in metamorphosing organs. Our findings showed that apoptotic cell death increased during metamorphic climax and decreased after metamorphosis in the larval organs. Sialic acid content of intestine and liver did not show any changes during developmental stages but increased after metamorphosis completed. However, there was a decline in sialic acid content of the tail in climax. Frog tail contains extensively cell death process and undergoes complete degeneration. In spite of increased apoptotic index, decreased sialic acid level in tail at this stage can be an indication of enhanced cell death. At the same time, this situation may be related to elimination of sialic acid's antigenic blockage during apoptosis. Intestine and liver contain both cell death and proliferative process. They remodel into their adult forms. Consequently, their sialic acid profiles during metamorphosis were different from the tail’s profile.

E2.25
Computational studies on the interaction of ABO-active saccharides with the norovirus VA387 capsid protein can explain experimental binding data
A. K. K. Chaitanya¹, N. Waqas², S. Francesco², E. R. Gustaf³, L. Göran and N. Per-Georg
¹Department of Computer Science and Engineering, Chalmers University of Technology, Gothenburg, Sweden, ²University of Gothenburg, Institute of Biomedicine, Göteborg, Sweden, ³Department of Clinical Chemistry and Transfusion Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden, ⁴University of Gothenburg, Institute of Biomedicine, Gothenburg, Sweden

Norovirus strains are known to cause recurring epidemics of winter vomiting disease. The crystal structure of the capsid protein of VA387, a representative of the clinically important GII.4 genocluster, was recently solved in complex with histo-blood group A- and B-trisaccharides (2OBS and 2OBT). However, the VA387 strain is known to bind also to other natural carbohydrate ligands for which detailed structural information of the complexes is not available. In this study, we have computationally explored the fit of the VA387 with a set of naturally occurring carbohydrate ligands containing a terminal α1,2-linked fucose. Molecular dynamics (MD) simulations show that type 1 and 3 extensions of the ABO-active saccharides with the norovirus VA387 capsid protein can explain experimental binding data.
Abstracts

E2.26
Lectin staining patterns in metamorphosing organs of the frog (Rana ridibunda)
E. Kapitan, M. Sengoz Inceli and S. Sancar Bas
Faculty of Science, Department of Biology, Istanbul University, Istanbul, Turkey

Carbohydrate chains conjugated with proteins or lipids on cell surface and/or cytoplasm have an important role in various cellular functions such as cell interaction, signal transduction, cell adhesion and proliferation. In this present study, localizations and developmental variations of specific sugar moieties in the terminal carbohydrate chains of glycoconjugates were investigated during metamorphosis of Rana ridibunda larvae. For this purpose, it was composed of four groups so called premetamorphosis, prometamorphosis, metamorphic climax and postmetamorphosis in terms of larval developmental stages of the frog. In order to determine the composition of terminal sugar residues such as L-fucose, N-acetyl-β-D-glucosamine, β-galactose, α-N-acetyl-D-galactosamine and α-D-mannose in metamorphosing organs, five horseradish peroxidase conjugated lectins were employed. It was found that Con A and PNA lectins gave no significant reaction in larval tissues, WGA, DBA and UEA-I lectins exhibited strong affinity to white blood cells in the liver. There were also an affinity in intestinal mucosal cells and tail epidermal cells. The binding activity of lectins showed some changes during distinct metamorphic developmental stages. Especially, it was decreased in climax stage and postmetamorphic period. These results suggest that the changes of lectin binding in metamorphosis can be indicate the presence of some cellular events in larval metamorphic differentiation such as cell differentiation and damages of cell adhesion between death and differentiating cell. Also they can be useful model for detection of white blood cell in amphibian hematopoietic argans and metamorphic changes.

E2.27
cpSRP43 is a LHCP specific chaperone and targeting factor
S. Falk and I. Sinning
Universität Heidelberg, Biochemiezentrum (BZH), Heidelberg, Germany

The chloroplast signal recognition particle (cpSRP) differs significantly from the canonical SRPs. It can act both co- and posttranslationally in the insertion of membrane proteins into the thylakoid membrane. The cpSRP consists of cpSRP44 homolog of cytosolic SRP54, however instead of the SRP RNA a new protein component, cpSRP43, is present. cpSRP43 is involved in the posttranslational targeting of light harvesting chlorophyll a/b binding proteins (LHCs) to the thylakoid membrane. LHCs serve as antenna systems in photosynthesis and contain three transmembrane helices (TM1 to 3). After synthesis in the cytosol LHCs are imported into the stroma where they form a soluble complex with cpSRP. LHCP recognition involves a highly conserved internal signal sequence consisting of the so-called L18 region and TM3. Subsequently the transit complex is targeted to the thylakoid membrane where the LHCs are inserted in an Alb3 dependent process. However, it is not clear yet how the transit complex recognizes Alb3 and how the substrate transfer to Alb3 is regulated. We show that cpSRP43 is as a highly specific chaperone for LHCPs and cpSRP43 alone is sufficient to form a soluble complex with its membrane protein cargo. Furthermore, Alb3 recruits cpSRP43 to the thylakoid membrane using its intrinsically disordered stromal C-terminus (A3CT) in a coupled binding and folding mechanism. We identify two positively charged motifs in A3CT that mediate the interaction with cpSRP43. These motifs are absent in the C-terminal domain of the paralog Alb4, which therefore does not interact with cpSRP43.

E2.28
Abstract withdrawn

E2.29
Structural characterization of the SRP system
P. Grudnik, G. Bange, G. Stjepanovic, A. Hendricks and I. Sinning
Heidelberg University, Biochemistry Center (BZH), Heidelberg, Germany

Cotranslational targeting of proteins destined for membrane integration or secretion is mediated by the universally conserved signal recognition particle (SRP) and its receptor (SR). SRP is a ribonucleoprotein complex and binds to hydrophobic signal sequences of nascent polypeptide chains as they emerge from ribosomal exit tunnel. The SRP/RNC (ribosome nascent chain) complex interacts with the membrane associated SR. The delivery of the RNC to the translocation channel in the membrane leads to dissociation of the SRP/SR complex. In order to coordinate the presence of a signal sequence at the ribosome with the availability of a vacant translocation channel, two GTPases present in SRP and SR form a unique complex in which GTP hydrolysis is activated in a composite active site. We present recent progress in the structure and function analysis of the SRP systems from the crenarchaeon Sulfolobus solfataricus and the gram-negative bacterium Escherichia coli.

E2.30
Aza-β3-amino acid containing peptidomimetics as cAMP-dependent protein kinase substrates
K. Kisseljova1, A. Kuznetsov1, M. Baudy-Floch2 and J. Jarv1
1University of Tartu, Tartu, Estonia, 2University de Rennes 1, Rennes, France

Peptidomimetic analogs of RRASVA, known as the “minimal substrate” for the catalytic subunit of cAMP dependent protein kinase (PKA), were synthesized by consecutive replacement of natural amino acids by their azaβ3-analogs. These peptidomimetics were tested as PKA substrates and kinetic parameters of phosphorylation reaction were determined. It was found that binding of these peptidomimetics with the enzyme active center was sensitive to location of the backbone modification, while the maximal rate of the reaction was practically not affected by the position of azaβ3-analog residue in pseudopeptide. The kinetic parameters of phosphorylation reaction were in correlation with calculated data of structure modeling and computational studies of peptide docking in enzyme active center. It was concluded that certain structural fragments, modification of which influences binding kinetics can be distinguished for consensus sequence azaβ3-analogs. Defining these structural features might open new perspectives for pharmacophore design of peptide and peptide-like PKA ligands.
Puchellin, a RIP class lectin: elucidation of isoforms toxicity using biomembrane models
T. M. Nobre, A. P. U. Araújo and L. M. Beltrami
Universidade de São Paulo, Instituto de Física de São Carlos, São Carlos, Brazil

Puchellin, a heterodimeric lectin of Ribosome Inactivating Protein class, formed by two chain, toxic A-chain and a D-galactose-binding B-chain. Recently, our group described four puchellin isoforms (PI, PII, PIII, PIV) that presented different toxicity degree. PI and PII are more toxic than PIII and PIV, spite high identity between them. This difference in toxicity was investigate using biomembrane models systems content different glycolipids in their compositions. Langmuir Monolayers results indicated that PII and PIV bind to DPPC monolayers in the same extent, but, with addition of 5% of GalCer, this interaction was very different. PII remains strongly interacting with the mixed monolayer, but PIV seems to be inhibited by the presence of the glycolipids. To better evaluate this possible competition between specific interaction to the sugar and hydrophobic interactions we study the proteins using fluorescence spectroscopy with the ANS dye in presence of different sugars. Interestingly, PII was very dependent on the sugar type, since a blue shift was observed in the following sequence: lactose (4 nm), D-Gal (10 nm) and NAc-Gal (25 nm). In other hand, PIV present a high exposure of hydrophobic regions, which was not dependent of the sugar, since for both carbohydrates, the blue shift was equal to 55 nm. In conclusion we attribute the differences in puchellin isoforms toxicity to possibility of PII isoform to strongly interact to the membrane, by sugar and hydrophobic interaction, differently of PIV, which seems to be more selective to one of the possible interaction with the membrane. Supported by Brazilian Agencies: FAPESP, CNPq.

E2.32
Abstract withdrawn

E2.33
Abstract withdrawn
Biosensors are a type of sensors in which the recognition system utilizes a biochemical mechanism. The biological recognition system translates information from the biochemical domain, usually an analyte concentration, into a chemical or physical output signal with a defined sensitivity. A biosensor consists of two components: a bioreceptor and a transducer. The bioreceptor is a biomolecule that recognizes the target analyte whereas the transducer converts the recognition event into a measurable signal. The main areas of application of biosensors are health care, food industry and environment. In our laboratory, we are developing amperometric sensors which have immobilized oxidoreductase enzymes on thin-layer gold planar electrodes. During amperometric transduction, a constant potential was maintained with respect to a reference electrode. The current generated by the oxidation or reduction of molecules at the surfaces of the working electrode was measured. Amperometric signals were linearly dependent on analyte concentration. Present biosensors serve for an analysis of wine. We were able to set the concentration of lactic acid, malic acid, glucose + fructose, alcohol and some other components in wine samples. Our goal is selling our devices to wine-makers. To be commercially successful, biosensors have to meet specific requirements. Our biosensors showed suitable high sensitivity and resolution, specificity, dynamic range and rapid response. This work was supported by Slovak research and development agency APVV-VMSP-P-0073-09.

E3.02 Dissociation and re-complementation of Alpha Keratin and Melanin from hair to build a novel biopolymer that can be used as different biocompatible prostheses at wide polymerization ratios with a spectrum of tensile and impact strength

K. Pirkalani1 and Z. Talea Rad2
1Mehr Medical Group, Endocrinology/Internal Medicine/Molecular Biology, Tehran, Islamic Republic of Iran, 2Mehr Medical Group, Endocrinology/Gynecology, Tehran, Islamic Republic of Iran

Objectives: Alpha-Keratin is a ubiquitous polymer of low tensile and impact strength found in many tissues with different functions. Melanin also is a ubiquitous macromolecule whose function in organs like brain and adrenals is not well understood but it is the most effective UB absorbing agents in nature. During treatment of patients with alopecia universalis, we encountered patients who regained only white hair upon treatment and never relapsed. Besides all patients show regrowth of white hair at beginning and the disease never attacks white hair. We postulated that interaction of Melanin and Keratin is not only a physical co-impaction as microscopic studies of the hair show but a chemical co-polymerization that gives rise to new antigenic determinants.

Materials and Methods: In two different chemical environments, detergent treated black hair without fat was treated in a way that once Keratin and once Melanin could be extracted. After drying, the two were let to interact in a very delicate basic microenvironment that enabled growth of the crystals. Different non stochiometric proportions were used to evaluate chemical and physical properties.

Results: Co-polymerization of the two compounds is a real event with birth of compounds distinct from the parents. The largest product is at present 30 mm × 20 mm × 7 mm. Some of them are very resistant to tension and/or impact.

Conclusion: As the condition of co-polymerization is not optimum shape and physical properties cannot be controlled yet but as they are biocompatible they open a new horizon for prostheses in plastic surgery.

E3.03 In-silico evidence of a pAO1 encoded tagatose-derivate catabolic pathway in Arthrobacter nicotinovorans

M. Mihasan
Alexandru Ioan Cuza University, Faculty of Biology, Molecular and Experimental Biology, Iasi, Romania

Based on similarity searches, two putative pathways were previously described as being encoded by the pAO1 megaplasmid of Arthrobacter nicotinovorans: an almost fully established nicotine-degrading pathway and a yet unknown putative sugar-catabolic pathway. The general organization of the latter indicated three possible targets for docking experiments: a periplasmatic binding protein part of an ABC-type sugar transport system, a transcriptional control factor and an putative sugar dehydrogenase. In an effort to investigate a possible substrate of sugar-catabolic pathway, the tridimensional structures of the three target proteins were built by homology modeling using 3D-JIGSAW. In silico blind docking experiments using a database of 90 sugar as flexible ligands and the three structures as receptors were performed using Chimera/Dock6 suite. The results were ranked according to their potential binding place and grid score. Surprisingly 1,6-di-O-phosphono-D-tagatose proved to be the preferred ligand for all three proteins, indicating that the second pathway coded by the pAO1 megaplasmid is a tagatose-derivate catabolic pathway.

E3.04 Characterization of water-soluble conjugates of enzymes with dextran

M. Alitikoglu, Y. Basaran and H. Kuzu
Yildiz Technical University, Istanbul, Turkey

Introduction: Protein–polysaccharide complexes are also important in industrial applications such as micro- and nano-encapsulation processes, the design of multi-layers structures, the formation and stabilization of food emulsions, the formation of new food gels and the recovery of proteins from industrial by-products. In biotechnology, stability of the enzymes in vitro are still being one of the most important issues. Storage and operational stabilities are both important for the usage of enzymes. The modification of the enzyme surface with a hydrophilic polymer could be a good strategy to form a shell around the enzyme molecule. In general, these modifications increase the stability of
the enzymes in high temperature, different pH and organic media.

Methods: The covalently-formed conjugates of HRP (Horseradish Peroxidase) and GOD (Glucose oxidase) with dextran have been proposed as an alternative modified enzyme towards industrial application and storage stability. The contribution of conjugate formations to the thermal stability of enzymes were evaluated with the activities determined. Interactions of industrial enzymes (HRP and GOD) with dextran in different molar ratios of components were systematically investigated by Fourier Transform Infrared (FT-IR) spectrophotometer, fluorescence spectrometer, gel-permeation chromatography (GPC), and Atomic force microscope (AFM) methods.

Results and Conclusions: Multipoint covalent bonding of dextran to horseradish peroxidase caused the formation of a conjugate whose thermal stability was increased and long storage lifetimes for these conjugates were obtained.

E3.06
Polysaccharides and glycolipids from probiotic bacteria
G. Novik1, Y. Knirel2 and E. Szewczak Dez1
1Institute of Microbiology BNAS, Belarus Collection of Microorganisms, Minsk, Belarus; 2N. D. Zelinsky Institute of Organic Chemistry RAS, Moscow, Russian Federation, 3Lund University, Pure and Applied Biochemistry, Lund, Sweden

Extensive investigations of biological active compounds from probiotic bacteria are of the interest for the preparation of therapeutic products. Among these substances the bacterial polysaccharides and polar lipids. Therefore, it is important to identify and characterise the biologically active compounds. A branched glucogalactan with a heptasaccharide repeating unit was found to be specific for the strain B. bifidum BIM B-465. Polar lipids were effectively extracted by supercritical carbon dioxide. One of the major components of interest was glycolipids. The highest glycolipids yield was obtained from dry bacterial biomass at process parameters 250 bar, 40°C, CO2 flow rate 10 g/min and hydromethanolic co-solvent 9.3%v/v (methanol-water 9:1 v/v). The thin layer chromatography revealed presence of two major glycolipid fractions. The fractions were identical to those obtained with classical organic solvents extraction. Fatty acids identification of glycolipids subjected to acid methanolysis and analyzed by gas liquid chromatography showed presence of palmitic acid, myristic acid and erucic acid. The sugar analysis after hydrolysis of carbohydrate moiety and acetylation was determined by gas liquid chromatography. 249

E3.07
A bioinformatics study of the human phosphofructokinase-1
A. Ivorsan1, V. Ostafe1 and A. Ciorsac2
1West University of Timisoara, Chemistry, Timisoara, Romania, 2Politehnica University Timisoara, Physical Education and Sport, Timisoara, Romania

Phosphofructokinase-1 (PFK-1) is a kinase enzyme involved in glycolysis, a process that requires 12 enzymatic reactions for the breakdown of glycogen to lactic acid. The glycolytic system works predominantly within skeletal muscle tissue. The inhibition of PFK-1 by ascorbate in resting muscle of mouse was already shown. When muscle is in activity, this enzyme combines with muscle proteins being protected by inhibition and glycolysis is possible. The Protein Data Bank contains only a few structures for PFK-1 for invertebrate’s organisms. The aim of this work is to perform a bioinformatics study to reveal if there are sequential and/or structural similarities between vertebrates and invertebrates PFK-1 in order to determine the active site for ascorbate binding and to perform computational drug design for the PFK-1 inhibition.

E3.08
The thermodynamic investigation of T5 bacteriophage ejected DNA
T. Partskhaladze, T. Mdzinarashvili and N. Shengelia
Tbilisi State University, Biophysics, Tbilisi, Georgia

The calorimetric measurements were used to investigate the thermal stability of T5 phage DNA under the physiological environmental condition and to check the existence of DNA-associated protein, which is thought to play protective role of virus genome against bacterial intracellular barrier (restriction enzymes). From the experimental data it have been observed that calorimetric curves of T5 phage ejected DNA contains two peaks, with melting temperature Tm ~85°C and Tm ~97.5°C. Judging by the shape, the first one is typical for ds-DNA denaturation. As for the second thermal melting peak, supposedly it should belong to a protein. In order to check the existence of protein in T5 phage DNA solution, it was previous treated with trypsin at 37°C, after which it has been shown that only one heat absorption peak (Tm~85°C) was remained on the thermal denaturation curve. It has revealed that second melting peak corresponds to the protein. It was interested to investigate if the thermal denaturation of phage DNA is reversible, in case of associated or without protein, i.e. to check the ability of heat-induced melted phage DNA to restore its double structure (at least partially) and the possible role of DNA-associated proteins in this process. As it was seen from experimental data thermal denaturation of T5 phage DNA is partially reversible (~90%), when it is associated with protein and irreversible - without it.

E3.09
Monomerization of the viral entry inhibitor griffithsin yields insights into the relationship between multivalent binding to high mannose oligosaccharides and antiviral activity
National Cancer Institute, Frederick, USA

Mutations were introduced to the domain-swapped homodimer of the antiviral lectin griffithsin (mGRFT) with the aim of preventing dimerization and thus making the molecule smaller and better suited to potential applications as an anti-HIV agent. Whereas several single and double mutants remained dimeric, insertion of either two or four amino acids at the dimerization interface resulted in a monomeric form of the protein (mGRFT). Several additional mutated forms of mGRFT were designed, expressed, and purified, with the goal of improving purification and eliminating monomer-monomer interactions. The monomeric character of the modified proteins was confirmed by sedimentation equilibrium ultracentrifugation and by their high resolution X-ray crystal structures, whereas their binding to carbohydrates
was assessed by isothermal titration calorimetry. Cell-based antiviral activity assays utilizing different variants of mGRFT indicated that the monomeric form of the lectin is essentially inactive against HIV-1, suggesting that the antiviral activity of GRFT stems from crosslinking and aggregation of viral particles via multivalent interactions between GRFT and oligosaccharides present on HIV envelope glycoproteins. Atomic resolution crystal structure of a complex between mGRFT and nonanamannoside revealed that a single mGRFT molecule binds to two different nonanamannoside molecules through all three carbohydrate-binding sites present on the monomer. Fusion proteins containing mGRFT and other antiviral lectins were also prepared.

E3.10 Camel beta-casein, a novel nano emulsifier for dissolving curcumin in pharmaceutical and nutritional industries

M. Esmaeili1, S.M. Ghaffari1, M. S. Atri1, Z. Moosavi-Movahedi2, A. Sharifizadeh1, M. Farhadi1, R. Yousefi4 and A. A. Moosavi-Movahedi1
1Institute of Biochemistry and Biophysics, Tehran, Islamic Republic of Iran, 2Department of Chemistry, Faculty of Science, Tarbiat Modares University, Tehran, Islamic Republic of Iran, 3ENT-HNS Research Center of Iran University of Medical Science, Tehran, Islamic Republic of Iran, 4Department of Biology, Faculty of Science, Shiraz University, Shiraz, Islamic Republic of Iran

Beta casein is an amphiphilic protein with self-assembling behavior that can form micellar nanostructures and could be exploited as a safe vehicle for hydrophobic therapeutic agent such as curcumin. Curcumin is a potent antioxidant and antibiotic natural polyphenol, which is almost insoluble at water. Critical association concentration of camel beta casein was determined at 25, 30 and 37°C using pyrene fluorescence and the solubility of curcumin was evaluated according to the solvent-evaporation technique. Beta-casein can improve the solubility of curcumin at least 2570- and 37 times. Analysis of fluorescence emmission of curcumin reveal that hydrophobic interactions are mainly contributed. We claim that these observations provide promise in the design of the suitable GRAS carrier for curcumin in nutritional industries. Curcumin encapsulated in camel beta-casein micelles can be a novel vehicle for hydrophobic drugs.

E3.11 CoNSEnsX: an ensemble view of protein and peptide structures and NMR-derived experimental data

A. F. Angyan1, B. Seppanen2, A. Perczel3 and Z. Gaspari1
1Eotvos Lorand University, Institute of Chemistry, Budapest, Hungary, 2Biological Research Center, Institute of Biochemistry, Széged, Hungary, 3Eotvos Lorand University, Institute of Chemistry, ELTE-HAS Protein Modelling group, Budapest, Hungary

Representation of the dynamical features of proteins becomes more and more important with the recognition of the role of internal motions in biological function. One of the most common way for such representation is the use of structural ensembles instead of individual conformers. However, the evaluating the relevance of such ensembles is far from being standardized. We developed a tool named CoNSEnsX (Consistency of NMR-derived Structural Ensembles with eXperimental data) that allows fast, simple and convenient assessment of the accuracy of protein structural ensembles. By analyzing the correspondence of the ensemble to chemical shift, NOE, $^{1}H$ order parameter, RDC and scalar coupling data together, the ability of the conformer set to represent the structure and dynamics of the protein can easily be judged. We provide thorough evaluation of various ensembles of human ubiquitin available publicly or generated in this study and show that most of them are in good agreement with the majority of the NMR data used, but there are parameters not matched by either of the conformer sets. This reveals that no current method is able to reflect all available NMR parameters.

E3.12 Genetic engineering of allosteric β-lactamases

B. Baudoux and P. Soumillion
Life Science Institute, Université Catholique de Louvain, Biochemistry, Louvain-La-Neuve, Belgium

In nature, enzymes activities can be regulated by many ways, one of them being the allostery. This phenomenon received attention from protein engineering scientists since many years. The design of an allosteric site de novo within the scaffold of enzymes may be of different interests. From a fundamental point of view, this kind of engineering allows to better understand the molecular basis of signal transmission through the protein as well as the evolution mechanisms underlying the apparition and adaptation of allosteric regulation systems. In the applied field, the design of proteins regulated by molecules of interest could lead to the development of biosensors based on the measurement of activity modulation. In the lab, we chose the TEM1 β-lactamase as a scaffold protein to create a binding site by random peptides insertion within three loops. Those three loops are in close contact and not far from the active site. Taken together they might mimic the binding site found within the camel monochain antibodies. Using phage display, this strategy has already leaded to the selection of enzymes regulated by various ligands. Nevertheless, the strategy suffered from different disadvantages and a new collection of mutated β-lactamases was rebuilt. By modifying the construction strategy and by stabilizing the β-lactamase, we obtained a library of better quality and diversity. The library is of 6.10$^6$ clones, with at least 30% presenting a β-lactamase activity. The next step will be the selection by phage display of enzymes that bind to various targets (proteins, small molecules).

E3.13 Delta-sleep inducing peptide entrapped in polymer matrices for biomedical applications

T. Sukhanova1, M. Semenikhina1, A. Art’yukhov1, I. Prudchenko1, M. Shitlman2 and E. Markvicheva2
1Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry, Laboratory Polymers for Biology, Moscow, Russian Federation, 2D. Mendeleiev University of Chemical Technology of Russia, Research and Teaching Center “Biomaterials”, Moscow, Russian Federation

Polymer scaffolds providing enhanced cell attachment and proliferation can be used for tissue engineering. Bioactive molecules entrapped in the scaffolds could stimulate cell growth and secreting biomolecules for tissue repair. The aim of the study was to develop peptide entrapment in various matrices and to estimate its in vitro release kinetics. Delta-sleep inducing peptide (DSIP, WAGG-DASGE, pI 4.7) which possesses neuroprotective properties was used as a model peptide. A series of polymer matrices differed by their composition and charge were proposed, namely positively charged poly(dimethylylaminoethyl methacrylate) (PDMAEMA),
negatively charged polyacrylic acid (PAA), noncharged poly(vinyl alcohol) (PVA) and poly(vinyl alcohol-co-glycidyl methacrylate) (PVA-GMA). Two techniques were developed: 1) peptide adsorption onto the preprepared macroporous matrix (PDMAEMA, PAA, PVA-GMA) from the aqueous solution and 2) adding DSIP solution to prepared matrix (PVA) or monomer solution before polymerization. The amount of DSIP released from the matrices was estimated by reverse phase HPLC. DSIP entrapment was 100% in case of PDMAEMA, PVA, PVA-GMA matrices while the peptide amount in PAA matrix didn’t exceed 6% of initial quantity. Peptide release in MillQ water and aqueous solutions (PBS, pH 7.4, 0.9% NaCl) from PDMAEMA matrix increased with increasing solution ionic strength and pH from acidic to neutral values. DSIP release kinetics from PVA matrix didn’t depend on ionic strength while from PVA-GMA matrix no release was observed. Thus, DSIP release depended upon composition, structure and charge of the matrix as well as on the solution used for release study.

E3.14
The effect of deleting a putative salt bridge on the properties of the thermostable subtilisin-like proteinase, aqualysin I
J. Arnaðsdóttir1, M. Magnúsdóttir1, Ó. Fridjónsson2 and M. Kristjánsson1
1Department of Biochemistry, Science Institute, University of Iceland, Reykjavik, Iceland, 2Matís-Prokaria, Reykjavik, Iceland
Aqualysin I is a subtilisin-like serine proteinase, from the thermophilic bacterium Thermus aquaticus. The enzyme is predicted to contain a salt bridge between Asp17 and Arg259, connecting the N- and C-terminal regions of the enzyme. We have previously reported on the stabilizing effect of incorporating a putative salt bridge at a corresponding site into the structure of VPR, a molecular analog. It is known that the specific protein binding sites usually are placed in the cavities on protein surfaces and to carry out the analysis of distribution hydrophobic and hydrophilic areas on protein surfaces. At the first step we imported the 3D structure of Apo-E with PDB ID 1GS9 from Protein Data Bank (PDB; http://www.rcsb.org/pdb/). Then using our program complex we built Apo-E surface and defined two cavities with rank one which are adjacent and form highly charged regions. One of the cavities is formed by residues Arg150, Asp151, Asp153 and Asp154; another cavity is formed by residues Gln156, Lys157 and Arg158. Both defined cavities are placed on a critical for Apo-E binding region (region 140–160, [Wilson C. et.al. 1991. Science. Vol. 252. P. 1817–1822]). We think that the defined cavities form receptor-binding site of Apo-E. Now we are synthesizing their peptide’s analogues and investigating their ability to LDLR binding.

E3.15
Detection of the binding site of apolipoprotein E
I. Shutova and V. Golubovich
Institute of Bioorganic Chemistry of the National Academy of Sciences of Belarus, Minsk, Belarus
Apolipoprotein E is low-density lipoprotein and together with its receptor LDLR plays important role in the development of various cardiovascular diseases. The aim of our study was to detect binding site of Apo-E and to give a forecast of its peptide’s low-molecular analogs. It is known that the specific protein binding sites usually are placed in the cavities on protein surfaces and form hydrophobic and hydrophilic clusters. Therefore assigned task we solved with the help of designed by us program complex which allows: to build the molecular protein surfaces, to detect cavities on the protein surfaces and to carry out the analysis of distribution hydrophobic and hydrophilic areas on protein surfaces. At the first step we imported the 3D structure of Apo-E with PDB ID 1GS9 from Protein Data Bank (PDB; http://www.rcsb.org/pdb/). Then using our program complex we built Apo-E surface and defined two cavities with rank one which are adjacent and form highly charged regions. One of the cavities is formed by residues Arg150, Asp151, Asp153 and Asp154; another cavity is formed by residues Gln156, Lys157 and Arg158. Both defined cavities are placed on a critical for Apo-E binding region (region 140–160, [Wilson C. et.al. 1991. Science. Vol. 252. P. 1817–1822]). We think that the defined cavities form receptor-binding site of Apo-E. Now we are synthesizing their peptide’s analogues and investigating their ability to LDLR binding.

E3.16
Chimeric allene oxide synthase-lipoxygenase fusion proteins
H. Lõhelaid, R. Järving, I. Järving and N. Samel
Tallinn University of Technology, Tallinn, Estonia
In plants, the conversion of fatty acids into allene oxides is catalyzed by two distinct enzymes: a lipoxygenase (LOX) and a cytochrome P450 type allene oxide synthase (AOS). In corals, the formation of hydroperoxide (HpETE) and its further conversion into the allene oxide is catalyzed by a unique fusion protein where 8R-LOX is fused to a catalase-related AOS. While crystal structure of AOS-LOX of the Caribbean coral Plexaura homomalla is available, it is not certain how two domains of the fusion protein work together. Another naturally occurring AOS/8R-LOX fusion protein has been cloned from the arctic coral Gersenia fruticosa. The other LOX present is arachidonate 11R-lipoxygenase (11R-LOX). By using domain swapping between two coral enzymes we created several chimeras and characterized their catalytic properties. Chimeric constructs of sequences encoding for P. homomalla AOS plus G. fruticosa 8R- or 11R-LOX were prepared by PCR cloning, sequenced and expressed in E. coli. The incubation products of arachidonic acid with recombinant fusion proteins were identified by RP-HPLC and mass-spectrometry. Our results show that the fusion of P. homomalla AOS with G. fruticosa 8R- or 11R-LOX does not alter the expression level of the co-expressed enzymes. The LOX domains of the chimeras convert arachidonic acid into 8R-HpETE and 11R-HpETE, respectively. In addition to its natural substrate 8R-HpETE, the AOS domain of the fusion protein converts 11R-HpETE into the corresponding allene oxide. In conclusion, results obtained indicate that at least in vitro conditions AOS and LOX domains operate independently and AOS tolerates different LOX partners.

E3.17
Influence of Cd707 Ile → Leu substitution in N-terminal deletional variant of Taq DNA polymerase on stability and activity of the enzyme
K. Davalieva and G. Efremov
Research Centre for Genetic Engineering and Biotechnology, Macedonian Academy of Sciences and Arts, Skopje, Macedonia, FYROM
The success, yield and specificity of PCR are affected by the DNA polymerase activity at room temperature. One way of pre-
Venting this activity is by genetic modifications of the DNA polymerase. For Taq DNA polymerase, mutations in the gene (Glu626Lys, Trp706Arg, Ile707Leu and Glu708Asp), were found to contribute to the significant decrease of the enzyme activity at room temperature. The aim of this study was to evaluate the usefulness of Ile707Leu cold-sensitive mutation in the N-terminal deletional variant of Taq DNA polymerase (Klentaq278) in PCR reaction and the influence of the mutation on overall stability of the enzyme. The Ile(707) to Leu substitution was introduced to Klentaq278 by site-directed mutagenesis. Normal and mutant DNA polymerases were expressed under tac promoter and purified to homogeneity. The mutant polymerase showed reduced polymerase activity at room temperature for 12 times and approximately 88% of Klentaq278 thermostability. This substitution in the enzyme reduced twice the maximum tolerance of K and Mg ions, ten times the tolerance of Na ions and ten times the enzyme tolerance of blood and phenol as PCR inhibitors. The major effect of the amino acid substitution was the reduction of the amplification capacity of the polymerase. Mutant polymerase is suitable for amplification of single or multiple fragments without hot start but only up to 1 kb length. The mutation Ile707Leu in Klentaq278 DNA polymerase reduces the overall processivity and stability of the enzyme and limits the application of this DNA polymerase in PCR only to small fragments.

**E3.18**

When is an amino acid residue buried? Why should we care?

B. A. van der Molen and P. J. Winn

*University of Birmingham, CSB, School of Biosciences, Birmingham, UK*

Determining “when an amino acid is buried” is important to our understanding of protein function, protein-protein interactions and protein evolution. This may appear a trivial question, as solvent accessibility has been discussed in literature for almost 40 years. However there is no universally accepted definition or value of solvent accessibility that is used to decide if a residue is buried in the molecular structure of a protein, or exposed to solvent. To quantify this a better statistical and physical understanding of this property is needed. Here we developed a method to analyse the amino acid distribution in individual proteins and their preferred solvent accessibilities. This is in contrast to the more common method of performing a statistical analysis of the solvent accessibilities of all amino acids in an entire data-set of proteins. We show that each amino acid residue has a statistical preference for a range of solvent-accessibilities in a sample of approximately 25,000 structures from the Protein Data Bank. Our data gives information about how exposed different residue types should ordinarily be, which is important for a statistical analysis of the role amino acids play in molecular recognition and in the design of optimal protein structures.

**E3.19**

Abstract withdrawn
Nonlinear analysis of glycolytic enzymes sequences

A. Isvoran¹, A. Ciorsac², D. Craciun³ and V. Ostafe¹
¹West University of Timisoara, Chemistry, Timisoara, Romania,
²Politehnica University Timisoara, Physical Education and Sport, Timisoara, Romania,
³Teacher Training Department, West University of Timisoara, Timisoara, Romania

Glycolysis is an ancient, universal pathway of obtaining metabolic energy. During glycolysis glucose is broken down in ten steps to two molecules of pyruvate, which then enters the mitochondria where it is oxidised through the tricarboxylic acid cycle to carbon dioxide and water. The different enzymes involved in glycolysis act as kinases, mutases, and dehydrogenases, cleaving enzymes, isomerases or enolases. Within this study we apply the linear and non-linear analysis methods, such as spectral analysis (SA), detrended fluctuations analysis (DFA) and Hurst exponent calculation to reveal if there are non-linear aspects concerning the sequences of enzymes involved in glycolysis process. Our study shows that even if the enzymes do not share sequence or structural homology with one another, and are therefore found in different protein families, they reveal long-range correlation concerning the physicochemical properties of the amino acids within their sequences. This result sustains other published data revealing non-randomness of protein sequences with direct consequences on protein folding.
stand the biology of wt and mutant (G2019S) LRRK2 and to search for selective small-molecule inhibitors, human wt and mutant LRRK2 were individually expressed in the yeast Saccharomyces cerevisiae. High copy number expression of wt or mutant LRRK2 in yeast caused significant growth inhibition, which was more pronounced for the PD-causing mutant G2019S. This work represents the first attempt to express wt and mutant LRRK2 in yeast. This system may help uncovering basic aspects of both normal and pathogenic LRRK2 biology and may be further used as a first-line drug screening approach for selective pharmacological inhibitors with promising therapeutic applications in neurodegenerative disorders, such as PD. We thank REQUIMTE/CEUP, FCT (IF&D/No8/94) and U.Porto/Santander Totta for financial support. C. Pereira is recipient of a FCT fellowship (SFRH/BPD/44209/2008).

E4.05 Role of aggregation conditions in structure, and toxicity of Yeast Hexokinase aggregates

H. Ramshini1, C. Parrini2, A. Relini2, M. Zampagni3, B. Mannini3, A. A. Saboury4, M. Nemat-Gorgani1 and F. Chiti3
1Payam noor University-branch of Sabzevar, Biology, Sabzevar, Islamic Republic of Iran, 2Dipartimento di Fisica, Università di Genova, Via Dodecaneso, Genova, Italy, 3Dipartimento di Scienze Biochimiche, Università di Firenze, Viale Morgagni, Florence, Italy, 4Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Islamic Republic of Iran

Formation of well-ordered fibrillar protein deposits is common to a large group of amyloid-associated disorders, including Alzheimer’s, Parkinson’s, type II diabetes and prion diseases. Disease-unrelated proteins are also able to form amyloid assemblies in vitro displaying comparable structures and cytotoxicity. The nature of the pathogenic species and the mechanism by which the aggregation process results in cell damage are, however, the subject of intense debate. In the present study, we have investigated the propensity of hexokinase type B from Saccharomyces cerevisiae (YHKB) to form amyloid-like and amorphous aggregates under various conditions and determined their cytotoxicity. Amyloid-like aggregation was induced under conditions known to be most effective for globular proteins (i.e. low pH in the presence of salts or TFE). Under both conditions; the resulted products were either globular or amorphous, while in the presence of TFE, they were either globular or amorphous, but in the presence of TFE, an irregular morphology was observed. Thermal aggregation of the enzyme was also investigated in 50 mM phosphate buffer, pH 7 at 60°C. The aggregates were not found toxic to human neuroblastoma cells, as indicated by the MTT reduction assay while those formed at acidic pH and in the presence of Congo red. Atomic force microscopy indicated morphologies distinct from typical amyloid. At low pH and in the presence of salts or TFE, they were either globular or amorphous, while in the presence of TFE, they were either globular or amorphous, but in the presence of TFE, an irregular morphology was observed. Thermal aggregation of the enzyme was also investigated in 50 mM phosphate buffer, pH 7 at 60°C. The aggregates were not found toxic to human neuroblastoma cells, as indicated by the MTT reduction assay while those formed at acidic pH and in the presence of TFE indicated cytotoxicity. The differences in cytotoxicity appear to arise from variations in the nature and morphology of the aggregates in the conditions tested.

E4.06 Importance of leucine 134 to the co-chaperone activity of Bacillus licheniformis nucleotide exchange factor GrpE

L.-L. Lin1, M.-G. Lin2, B.-E. Chen1, W.-C. Liang1 and M.-C. Chi1
1Department of Applied Chemistry, National Chiayi University, Chiayi, Taiwan, 2Department of Biochemical Science and Technology, National Chiayi University, Chiayi, Taiwan

GrpE proteins function as a nucleotide exchange factor of the eubacterium DnaK molecular chaperone system. The co-chaperone GrpE accelerates ADP dissociation from DnaK and cooperatively facilitates the DnaK chaperone cycle with another co-chaperone, DnaJ. To elucidate the role of leucine 134 of Bacillus licheniformis nucleotide exchange factor (BGrpE), site-saturation mutagenesis was employed to generate all possible replacements for this residue. Wild-type and mutant proteins were purified by nickel-chelated chromatography and had a molecular mass of approximately 30 kDa. BGrpE and 9 mutant proteins synergistically stimulated the ATPase activity of B. licheniformis DnaK (BIdnaK), whereas L134H, L134K, L134D, L134E, L134E, L134N, L134Q, L134S, and L134P had no co-chaperone activity. In vitro binding assay revealed that the functional BGrpE variants mainly interacted with the monomer of BIdnaK, but non-specific interaction was observed for the remaining mutant proteins. Comparative analyses of the far-UV CD spectra revealed that the α-helical content of the inactive mutant BGrpEs was reduced significantly with respect to wild-type protein. Moreover, the inactive mutant proteins also exhibited a more sensitivity towards the temperature-induced denaturation. These results suggest that Leu134 plays an important role in the structural integrity of BGrpE.

E4.07 Production and refolding of soluble forms of mouse NK cell receptors NKR-P1F and Clrg

K. Kotynkova1, P. Ham1, Ondrej Vaneck1, M. Holubova2, P. Celadova2 and K. Bezouska1
1Academy of Sciences of the Czech Republic, Institute of Microbiology, Prague, Czech Republic, 2Department of Biochemistry, Faculty of Science, Charles University Prague, Prague, Czech Republic

Mouse NKR-P1F: Clrg receptor:ligand pair is important component of the receptor “zipper” that occurs at the contact between natural killer cell and its target cell, and represent a recently discovered example of lectin-lectin interactions important for recognition of immune cell subsets. Previously, immunologists have brought evidence for the involvement of the NKR-P1F:Clrg pair in cellular activation such as T cell proliferation. In future we want to study structure of these proteins and interactions between them, but the first task was expression and refolding. We have amplified the individual cDNA clones for the receptors by RT-PCR from spleens of C57BL/6 mice, and transferred DNA fragments coding for the extracellular ligand binding domains of the two receptors into pET-30 bacterial expression vector. After induction of protein production with IPTG, the protein precipitated into inclusion bodies, from which they could be refolded in vitro. NKR-P1F construct produced a small amount of soluble protein using the standard protocol, but we have experienced difficulties with the reproducibility of the refolding protocol. In the case of Clrg the standard protocols for protein refolding were not sufficient. In order for the Clrg to fold properly, the fourth cysteine which does not fit into the pattern usual for this family of receptors was substituted for serine and resulting C64S construct was shown to be more useful. Supported by grants from Ministry of Education of Czech Republic (MSM 21620808 and 1M0505), and from The Grant Agency of Czech Rep. (GACR 305/09/H008 and 305/09/0477).
E4.08 Interaction of iron ion with camel alpha-lactalbumin
M. S. Atri and A. A. Saboury
Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Islamic Republic of Iran

Enrichment of foods with iron is a common vehicle for delivering iron to the consumer. However, when food products are fortified with minerals some reactions of minerals with food components may influence their structure. In the present study, the interaction between iron ion and alpha-lactalbumin (α-La), which is one of the major whey proteins in milk, has been studied by spectroscopic techniques. By the analysis of fluorescence spectrum, it was observed that the iron ion has an ability to quench the intrinsic fluorescence of α-La. The association equilibrium constant of iron with camel holot (α-La) is about 104 M−1 based on the fluorescence quenching results. Experimental results showed that the binding of iron to α-La induced conformational changes in α-La. Circular dichroism spectra showed that the alpha-helix of α-La decreased about 8% when the molar ration of iron ion to α-La was ten. Hence, the secondary structure of the protein is altered by iron ion. The conformational change of α-La in the presence of iron ion was also confirmed by UV absorption spectra.

E4.09 Abstract withdrawn

E4.10 Amyloid-like aggregation of α-chymotrypsin in different aqueous-organic solvents
M. Kotormán, I. Kun and L. M. Simon
University of Szeged, Biochemistry and Molecular Biology, Szeged, Hungary

The amyloidoses are a group of protein misfolding disorders characterized by the accumulation of insoluble fibrillar protein. The mechanism by which amyloidogenic proteins undergo conversion from a soluble globular form to the cross-β conformation has not yet been elucidated. Several observations suggest that amyloid represents a generic form of polypeptide conformation, and most proteins have the potential to form amyloid-like structures. It has recently been demonstrated that α-chymotrypsin is driven toward amyloid aggregation by the addition of 2,2,2-trifluoroethanol and at high temperature. The aim of this work was to study the aggregation of α-chymotrypsin in ethanol, methanol, tert-butanol and dimethylformamide (DMF). The formation of aggregates was followed via the binding of thioflavin T (ThT) and 1-anilino-8-naphthalenesulfonic acid (ANS), with intrinsic fluorescence and turbidity measurements. The role of the surface charges of the enzyme in aggregation was studied through the use of chemically modified enzyme forms. α-Chymotrypsin underwent amyloid-like fibril formation in all of the studied organic solvents. The ThT binding and turbidity measurements revealed that the aggregation was maximal after incubation in 55% ethanol for 5 hour at 24°C. The highest fibrillation after incubation for 24 hour at 24°C occurred in methanol and in DMF at 60–70%, and in tert-butanol at 60–80%. The ThT and ANS binding and intrinsic fluorescence results showed that the hydrophobic residues are more solvent-exposed in the aggregated form of α-chymotrypsin. The chemical modifications indicated that the surface charges of the enzyme play an important role in the aggregation process.

E4.11 The effect of calcium ions and phosphorylation on the Starmaker conformation – FRET studies
M. Wojtas1, A. Ozyhar2, M. Orłowski1, J. Mazurek2 and P. Dobrzyński1
1Department of Biochemistry, Wroclaw University of Technology, Wroclaw, Poland, 2Department of Animal Physiology, University of Wroclaw, Wroclaw, Poland

Biomineralization is the process by which living organisms produce biominerals such as shells, teeth and otoliths. Starmaker (Stm) is involved in biomineralization of otoliths in Danio rerio. Stm belongs to a class of intrinsically disordered proteins (IDPs). Stm demonstrates a low content of ordered secondary structure and no tightly packed hydrophobic core. Typical IDPs are very flexible, but may adopt rigid conformations in the presence of ligands or other proteins. Stm has a significantly extended rod-shaped conformation, however calcium ions, which are putative ligands of Stm, induce compaction of the extended conformation of Stm. We have applied Förster resonance energy transfer (FRET) to examine the flexibility of Stm. FRET D-A pairs spanning the protein molecule were created by inserting tryptophan (donor) and cysteine residues (labeled with IAE-DANS probe as an acceptor) by site-directed mutagenesis. The Stm conformational changes detected by the distances measurements between chosen D-A pairs were monitored in the presence of calcium ions. Phosphorylation of Stm by PKA and GSK-3 kinases has been shown. We have examined the effect of phosphate groups on the Stm conformation.

E4.12 A native-like fold of region 4 of initially unfolded α70 subunit of E. coli RNA polymerase induced by TFE
P. Kaczka1, A. Polkowska-Nowakowska1, K. Bolewski1, I. Zhukov2, K. L. Wierzchowski1 and J. Poznański1
1Institute of Biochemistry and Biophysics PAS, Warszawa, Poland, 2Slovenian NMR Centre, National Institute of Chemistry, Ljubljana, Slovenia

Recombinant protein rECr70 comprises domain 4 of E. coli RNA polymerase α70 subunit, which is responsible for recognition of DNA promoter -35 region. It was found sufficiently soluble only unfolded in low-pH solution. Its folding, accompanying addition of 2,2,2-trifluoroethanol (TFE), was monitored by TFE-induced migration of signals in a series of 15N HSQC spectra. A common trend of uniform upfield shift of resonances breaks down for some residues at 10–15% TFE, evidencing the buildup of non-helical regions separating initially induced helices. The cooperative reorganization of non-helical regions suggests that rECr70 somehow folds into a structure compatible with canonical DNA-recognizing HLHTH motif existing in homologous domains of thermophilic bacteria. The sequential distribution of TFE-induced secondary chemical shifts (CSI) clearly indicates that TFE acts not only as a canonical helix inducer, but also tends to stabilize a spatial arrangement of the initially induced secondary structure elements. Analysis of 15N relaxation data at 10 and 30% of TFE content demonstrated that the whole HLHTH motif displays increased values of the order parameter, indicating reduced backbone flexibility in the nanosecond time scale and some contributions from slow motional process on the micro- to millisecond time scale. For protein in 30% TFE solution, 23 structural cross-peaks unequivocally assigned in 15N- and 13C-edited NOESY spectra were used to build low-resolution model of protein structure.
**E4.13**
Crystal structure of an intramolecular chaperone mediating triple-β-helix folding
E. C. Schalz1, A. Dickmanns1, H. Urlaub2, A. Schmitt1, M. Mühlenhoff2, K. Stummeyer2, D. Schwarzer3, R. Gerardy-Schahn1 and R. Ficner1
1University of Goettingen, Molecular Structural Biology, Goettingen, Germany, 2Max Planck Institute for Biophysical Chemistry, Bioanalytical Mass Spectrometry, Goettingen, Germany, 3Hannover Medical School, Cellular Chemistry, Hannover, Germany

Protein folding is often mediated by molecular chaperones. Recently, a novel class of intramolecular chaperones has been identified in tailspike proteins of evolutionarily distant viruses, which require a C-terminal chaperone for correct folding. The highly homologous chaperone domains are interchangeable between pre-proteins and release themselves after protein folding. We solved the crystal structures of two intramolecular chaperone domains in either the released or the pre-cleaved form, revealing the role of the chaperone domain in the formation of a triple-β-helix fold. Tentacle-like protrusions enclose the polypeptide chains of the pre-protein during the folding process. After the assembly, a sensory mechanism for correctly folded triple β-helices triggers a serine-lysine catalytic dyad to autoproteolytically release the mature protein. Sequence analysis shows a conservation of the intramolecular chaperones in functionally unrelated proteins presumably sharing triple β-helices as a common structural motif.

**E4.14**
Production of single chain antibodies against prion protein in *Escherichia coli* and *Bacillus subtilis*
N. Cavallarin, M. Vicario and A. Negro
University of Padova, Experimental Veterinary Science CRIBI, Padova, Italy

Prion diseases or transmissible spongiform encephalopathies are fatal neurodegenerative diseases that affect humans but also several mammalian species. Monoclonal antibodies with high affinity are used as immunodiagnostic and therapeutic reagents against prion diseases. Antibodies directed against PrPC, when added to scrapie-infected cell culture or delivered *in vivo*, can result in elimination of PrPSc or prevent its replication. Here we reported the expression and purification from *E. coli* and from *Bacillus subtilis* of a single chain antibody derived from 8H4 monoclonal antibody. The two systems were evaluated in order to obtain corrected and folded antibodies since 8H4 antibodies contain three disulphide bridge necessary for its functional activity. The expression of the only single chain 8H4 protein was very low and insoluble in *E. coli* and therefore we have produced it as fusion protein to thioredoxin or chaperone Dnak. Thioredoxin fusion protein when expressed in Origami *E. coli* strain has shown the formation of correct disulphide bonds in cytoplasm and enhances the solubility of fusion protein. The fusion protein is able to recognize prion protein in vitro in western blot and also it is internalized by the cells via PrPC in bovine prion protein CHO expressing cells. The chaperone fusion Dnak although gives large amount of fusion protein it is largely insoluble and refolding system will be reported. *B. subtilis* is a gram positive bacteria most popular in the field of biotechnology for its high capacity of production and secretion of recombinant protein at low cost. Here we reported the expression of secreted protein via shuttle *E. coli/B. subtilis* expression plasmid pHTr43.

**E4.15**
Assembly capacity of major capsid protein VP1 of hamster polyomavirus are influenced by size and position of truncations at its carboxy-terminal region
A. Gedvilaite and A. Aleksaite
Institute of Biotechnology, Vilnius, Lithuania

The VP1 protein of polyomaviruses is composed of a central globular tightly packed domain (about 250 amino acids in length) and of more loosely packed domains at both ends. The carboxy-terminal region of VP1 is responsible for the interaction of pentameric subunits in the capsids of polyomaviruses. Therefore, it was considered that the carboxy-terminal region cannot be deleted or substituted by foreign sequences without disrupting the assembly process. The objective of this study was to investigate the potential influence of the carboxy-terminal region of hamster polyomavirus (HaPyV) VP1 protein on the assembly of virus-like particles (VLPs) using carboxy-terminally truncated or modified VP1 proteins expressed in yeast. The HaPyV-VP1 was modified at its carboxy-terminal region by consecutive truncations and single amino acid exchanges. The ability of yeast-expressed VP1 variants to form VLPs strongly depended on size and position of truncations. VP1 variants lacking 21, 69 and 79 amino acid (aa) residues at its carboxy-terminal region efficiently formed VLPs similar to those formed by the unmodified VP1 (diameter 40–45 nm). On the contrast, VP1 derivatives with carboxy-terminal truncations of 35 to 56 aa residues failed to form VLPs. VP1 mutants with a single A339G aa exchange or internal deletions of aa 335 to aa 346 and aa 335 to aa 363 resulted in the formation of VLPs of a smaller size (diameter 20 nm). These data indicate that certain parts of the carboxy-terminal region of VP1 are not essential for pentamer-pentamer interactions in the capsid, at least in the yeast expression system used.

**E4.16**
Zn(II) ions inhibit fibrillization of monomeric insulin
N. Anda, J. Gavrillova, J. Smirnova, V. Tougu and P. Palumaa
Department of Gene Technology, Tallinn University of Technology, Tallinn, Estonia

Insulin, a 51-residue peptide hormone is an intrinsically amyloidogenic peptide, forming amyloid fibrils in vitro. In the secretory granules insulin is densely packed into Zn2Insulin6 crystals that assures osmotic stability of vesicles and prevents the peptide fibrillization. However, after release from the pancreatic β-cells insulin dissociates into active monomers, which tend to fibrillize not only at acidic but also at physiological pH values. We studied the effect of Zn(II) that are co-secreted with the peptide on the fibrillization of insulin by monitoring the increase in the fluorescence of the fibril reactive dye Thioflavin T. Zn(II) inhibited the fibrillization of monomeric insulin at physiological pH values. At pH 7.3 the inhibitory effect of Zn(II) was very strong (IC50 = 3.5 μM), whereas Zn(II) ions decreased the fibrillization rate constant and increased the lag phase of the process. At pH 5.5 the inhibitory effect of Zn(II) was much weaker since 20 μM Zn(II) caused only a slight increase in the lag period. The pH dependence points towards participation of His residue(s) in the Zn(II)-induced inhibition of insulin fibrillization. It is known that His residue at position B10 participates in binding of Zn(II) ions. The results indicate that Zn(II) may be physiologically relevant suppressor of the fibrillization of insulin at its release sites and in circulation. It is hypothesized that misfolded oligomeric intermediates occurring in insulin fibrillization pathway, especially under
the conditions of zinc deficit, may induce autoantibodies against insulin that may lead to β-cell damage and autoimmune type I diabetes.

E4.17 Interrelationships between coiled-coils, intrinsically disordered sequences and charged single α-helices

B. Szappanos1, D. Suveges2, L. Nyitray2, A. Perczel1, G. Toth1 and Z. Gaspari1

1Eotvos Lorand University, Institute of Chemistry, Budapest, Hungary, 2Department of Biochemistry, Eotvos Lorand University, Budapest, Hungary, 3Agricultural Biotechnology Center, Godollo, Hungary

Both intrinsically disordered proteins and coiled-coil sequences are predicted to constitute a significant fraction of both prokaryotic and eukaryotic proteomes. Using a number of prediction algorithms, we present a detailed analysis of cross-predictions of such sequences. Our results show that coiled-coils are usually predicted to be unstructured, which is in agreement with the fact that they form dimers or multimers and are not folded as a monomer. Reverse cross-predictions are less frequent presumably due to the (usually) heptad repeat signature characteristic of coiled-coils. To achieve acceptable prediction accuracy while minimizing cross-predictions, we suggest the use of the programs Coils and IUPred. In addition, we have developed a web server for the prediction of charged single α-helices based on the consensus of two methods. We suggest that in some cases, cross-predictions might mark evolutionary transitions along the common α-helix evolution. Furthermore, a simple but powerful rule-based approach allows a general and accurate analytical solution that can be applied to almost all cases of interest. Furthermore, a simple but detailed experimental protocol is laid down and validated to guide experiment design, so to obtain the best possible estimates of the parameters. The fitting algorithm is implemented on a publicly-accessible web server for use by experimentalists worldwide. Numerical and experimental validation of the model is presented.

E4.18 The tube model in Gromacs: a platform for the study of protein folding and aggregation

M. Sandal and M. Vendruscolo

Department of Chemistry, University of Cambridge, Cambridge, UK

Protein aggregation occurs on timescales that are extremely difficult to explore by using full atomistic simulations. In the last 20 years, however, it has been realized that many essential features of protein behaviour, including for example the importance of the initial hydrophobic collapse in protein folding and aggregation, emerge in a very simple and elegant fashion by using simplified models. Here we take advantage of this approach by adopting the so-called “tube model”, which includes excluded volume and self-attractive interactions that capture very effectively hydrophobic effects and hydrogen bonding. The tube model has been already used to model successfully the essential features of folding landscapes and of the process of amyloid formation. In this study we present an implementation of the tube model in the Gromacs simulation package, which is one of the most used molecular dynamics software suites. Our specific objectives are: (i) To create a tool based on a commonly used software platform to facilitate the use of the tube model; (ii) To combine the computational advantages of the tube model with the integration of experimental data (including e.g. NMR chemical shifts) to obtain structural and dynamical information on systems with large time and size scales, such as those involved in protein aggregation; (iii) To augment the tube model with residue-specific interactions, including electrostatic and van der Waals interactions. We finally present an initial application of the current implementation to the modeling of formation of oligomeric assemblies.
Abstracts

E4.21 Oligomerization and conformation of amyloidogenic protein human stefin B. Insight from ESI MS
T. Kumn1, A. Taler-Vereie2, K. Dkerget2, M. Friedemann1, E. Jerovnik2 and P. Palumaa1
1Department of Gene Technology, Tallinn University of Technology, Tallinn, Estonia, 2Department of Biochemistry, Jofep Stefan Institute, Molecular and Structural Biology, Ljubljana, Slovenia

Human stefin B, an 11 kDa intracellular protein, is a member of the cystatin family of cysteine protease inhibitors. Cystatins have a tendency to form domain-swapped dimers, higher oligomers and amyloid fibrils. Misfolding, aggregation and fibril formation of peptides and proteins is a common feature of amyloidogenic diseases including several major neurodegenerative diseases. The aggregation pathway of amyloidogenic proteins into the fibrils is thought to pass through transitional oligomeric forms, which are considered to be the toxic entities and their structure is in focus of intensive research. In the current study we demonstrate that electrospray ionisation mass spectrometry (ESI MS) with preliminary separation by size exclusion chromatography (SEC) is well applicable to study oligomerization and conformational states of human stefin B. We demonstrate that interpretation of ESI MS spectra allows clear determination of distinct stefin B oligomeric forms, starting from dimers up to dodecamers. Application of charge-state distribution analysis of ESI MS spectra indicated opening of compact conformational state of stefin B monomer in TFE solutions. We think that conformational changes of monomeric stefin B, which was shown to form molten globular intermediate before, might be crucial for oligomerization and fibrillation of this protein. We also show the applicability of ESI MS for determination a complex between amyloid β (Aβ) peptide implied in Alzheimer’s disease and the dimers of stefin B. We propose that the ESI MS approach can be used for the study of aggregation of certain type of amyloidogenic proteins, especially those with rather stable oligomers.

E4.22 Characterization of the aggregation competent state of the acylphosphatase from Sulfolobus solfataricus
F. Bemporad1, A. De Simone1, F. Chitì2 and C. M. Dobson1
1Department of Chemistry, University of Cambridge, Cambridge, UK, 2Dipartimento di Scienze Biochimiche, Universita’ di Firenze, Firenze, Italy

Aggregation of proteins into amyloid-like aggregates is linked to the onset of several human diseases, ranging from neurodegenerative diseases to systemic amyloidoses. The first step of this process is the conversion of proteins from their functional state into an aggregation competent conformation. Understanding the properties of this conformation is fundamental in order to develop therapies aimed to combat these tremendous diseases. The acylphosphatase from Sulfolobus solfataricus (Sso AcP) is a globular protein, commonly used as a model for aggregation studies, characterized by the presence of an 11 residue long unfolded N-terminal segment. The protein aggregates starting from an ensemble of native-like conformations formed with no need of unfolding events across the major folding energy barrier. The crucial event in the process is the establishment of an interaction between the N-terminal segment and the globular unit of two different Sso AcP molecules. When separated, neither the N-terminal segment nor the globular unit of the molecule are able to aggregate. These features make this system particularly suitable to study amyloidogenic properties of proteins. Experiments monitored through NMR we are characterizing the aggregation competent conformational ensemble populated by the globular unit of the Sso AcP molecule. This approach will allow the determination of the regions that become more flexible under aggregation promoting conditions. The comparison between data measured in the presence/absence of peptides corresponding to the N-terminal segment will lead us to the determination of the regions implied in the establishment of the amyloidogenic interaction.

E4.23 An orientational preference of a transmembrane helix in a multistranded membrane protein can influence the membrane insertion efficiency of a marginally hydrophobic neighbour helix
K. Öjemalm, K. K. Hälling, I. Nilsson and G. von Heijne
Center for Biomembrane Research, Stockholm University, Stockholm, Sweden

The efficiency with which a segment of a nascent polypeptide chain is incorporated into the ER membrane via the Sec61 translocon is to a great extent dependent on its overall hydrophobicity. Nevertheless, previous studies of natural and engineered membrane proteins have clearly shown that also features extrinsic to a specific polypeptide segment may influence its membrane insertion efficiency. In the current study, we analyze the effect of one such feature. In a transmembrane polypeptide, segments rich in positively charged amino acid residues show a strong tendency to be located on the cytosolic side of the membrane. We investigate how a lysine-induced orientational preference of a transmembrane helix influences the membrane insertion efficiency of a preceding marginally hydrophobic segment. For this purpose, we analyze the topology of leader peptidase variants synthesized in E. coli. The translocon experiments in the presence of rough microsomal membrane. We found that the level of hydrophobicity required for efficient membrane incorporation of a polypeptide segment was altered by lysines inserted into the C-terminal flank of the subsequent transmembrane helix. The hydrophobicity threshold decreased when the N-terminal end of the segment oriented towards the cytosolic side of the membrane and increased when the N-terminal end oriented towards the luminal side. In summary, this study constitutes a systematic characterization of how a transmembrane helix may influence the insertion efficiency of a neighbouring one. Additionally, it exemplifies a long-range effect of positively charged amino acids on the topology of a membrane-spanning protein.
E4.24 Structure studies of human frataxin and its higher order structures: implication in Friedreich's ataxia

E.-C. Ahlgren and S. Al-Karadaghi
Department of Biochemistry and Structural Biology, Lund University, Lund, Sweden

Iron is an essential part of prosthetic groups such as heme and iron sulphur clusters, which are involved in various redox reactions of central importance for life. Iron is not easily available to organisms, mainly due to the low solubility of its most common form Fe$^{3+}$. The Fe$^{2+}$ variant has higher solubility however it is easily oxidized by atmospheric oxygen to Fe$^{3+}$ as well as being toxic to organisms, since it promotes the generation of highly reactive oxygen species (ROS), capable of causing severe damage to proteins and nucleic acids. Therefore, cells require mechanisms in iron metabolism and one of them is the neurodegenerative disease Friedreich's ataxia. Sufferers of this disease have extensive frataxin which disrupts and reduces the production of frataxin. Frataxin is involved in iron detoxification, storage and delivery in the mitochondrial matrix and when being recombinantly expressed monomers as well as oligomers are produced. Only the 3D structure of the monomer has been resolved, this by X-ray crystallography to a resolution of 1.8 Å. The oligomers are well suited for studies with Transmission Electron Microscopy (TEM) and in combination with negative staining; single particle reconstruction can be performed. We have been working on optimising conditions and stains needed in order to perform TEM and do single particle reconstruction of frataxin, which presently is being carried out.

E4.25 Integration of chemical information with protein sequences and 3D structures from PDB

G. Kleywegt and A. Golovin
EMBL-EBI, Cambridge, UK

PDBeMotif is an extremely fast and powerful search tool that facilitates exploration of the Protein Data Bank (PDB) by combining protein sequence, chemical structure and 3D data in a single search. Currently it is the only tool that offers this kind of integration at this speed. PDBeMotif can be used to examine the characteristics of the binding sites of single proteins or classes of proteins such as Kinases and the conserved structural features of their immediate environments either within the same specie or across different species. For example, it can highlight a conserved activation loop common to protein kinases, which is important in regulating activity and is marked by conserved DFG and APE motifs at the start and end of the loop, respectively. The predictability of the effect of modifications to small molecules that bind to the active and/or regulatory sites of proteins on their efficacy can be based on the outcome of analytic work done using PDBeMotif. It can be ported to all major operating system platforms such as MS Windows, LINUX, Apple Mac and Solaris as it is written in Java and uses Oracle and the free source PostGreSQL database server. PDBeMotif can be used online or downloaded and installed locally where public and private PDB files (including libraries of theoretically derived 3D structures) can be loaded and analyzed. There is also the capability to load protein site annotations, families and domains from Distributed Annotation System (DAS) servers.

E4.26 Reactive Oxygen Species stimulate phosphorylation of eIF2α independent of protein folding in ER-stressed cells

N. S. Wong and D. Lam
The University of Hong Kong, Biochemistry, Hong Kong, Hong Kong

The eukaryotic initiation factor-2 (eIF2) is an oligomeric protein that recruits the methionyl initiator transfer RNA (met-tRNA) to the 40S small ribosomal subunit during translational initiation. The α-subunit of eIF2 (eIF2α) can be phosphorylated by several eIF2α kinases during cellular stress. eIF2α kinases such as PKR and GCN2 could be activated by oxidative stress, suggesting that reactive-oxygen-species (ROS) produced within cells may lead to phosphorylation of eIF2α. However, it is currently uncertain if protein folding had any role to play in ROS-induced phosphorylation of eIF2α. We had addressed this issue by studying the effect of ROS on eIF2α phosphorylation in HeLa cells treated with tunicamycin (TUN) that interrupts protein folding in the endoplasmic reticulum. Incubation of HeLa cells with TUN resulted in a significant increase in the intracellular level of ROS, phosphorylation of eIF2α, activation of PERK, and splicing of XBP-1 mRNA. In the presence of cycloheximide (CHX), the intracellular level of ROS was still elevated and the phosphorylation of eIF2α was still observed in response to TUN. However, there was no activation of PERK or the splicing of XBP-1, suggesting that protein misfolding is not the only cause for TUN-induced production of ROS and phosphorylation of eIF2α. The addition of dithiothreitol (DTT) to cells treated with both CHX and TUN resulted in a marked reduction of eIF2α phosphorylation. Our data suggested that protein folding does not have to be involved in ROS-mediated phosphorylation of eIF2α.

E4.27 A potassium switch of ATP-induced GroEL conformational changes

J. Chen, M. Makabe and K. Kuwajima
National Institute of Natural Sciences, Okazaki Institute for Integrative Bioscience, Okazaki, Japan

Bacterial chaperonin GroEL utilizes the co-chaperone GroES to assist folding of cellular proteins in an ATP-dependent manner. An ATP-induced allosteric communication through GroEL double rings is prerequisite for the overall chaperonin cycle. It has been shown by Horovitz et al. (1995) that ATP-induced allosteric transitions of GroEL consist of two phases with one at relatively low ATP concentrations (≦ 100 μM) and the second at higher concentrations of ATP with a midpoint in the range of 16 to 160 μM. Here we studied the effect of essential metal cofactor, potassium ion, on the ATP-driven allosteric transitions of GroEL by using a tryptophan mutated GroEL. Within the test range of potassium concentration, two distinct patterns of ATP-induced allosteric transitions were observed. We showed that at 50 mM potassium concentration, a remarkable increasing second-phase of ATP-induced allosteric transition was observed which is contrary to the decreasing phase at 10 mM potassium concentration. References:

E5 – Catalytic Mechanisms

E5.01
Abstract withdrawn

E5.02
Quantification of the primary antioxidant capacity of different molecules

J. L. Munoz-Munoz1, F. Garcia-Molina1, R. Varon2, J. Tudela1, F. Garcia-Canovas1 and J. N. Rodriguez-Lopez1
1GENZ: Grupo de Investigacion Enzimologia, Murcia, Spain, 2Departamento de Quimica-Fisica, Universidad de Castilla la Mancha, Albacete, Spain

We have developed a kinetic method for determining the antioxidant capacity of a variety of molecules. In this method, denominated the enzymatic kinetic method, the free radical of ABTS is generated continuously in the reaction medium by the peroxidase/ABTS/H2O2 system. The presence of an antioxidant in the solution provokes a lag period in the accumulation of the free radical in the medium, and by studying this lag period it is possible to calculate the antioxidant capacity of the molecule in question. This antioxidant capacity will be quantified by n, the number of electrons donated per molecule of antioxidant in the primary antioxidant capacity, efficient concentration, EC50, ratio of the antioxidant concentration necessary to decrease the initial concentration of ABTS radical to 50% [EC50 = 1/(2n)] and primary antioxidant or antiradical power, (ARP), (ARP = 1/EC50 = 2n). This work has been partially supported by grants from several Spanish organizations. Ministerio de Educacion y Ciencia (Madrid, Spain) Project BIO2009-12956, from the Fundacion Seneca (CARM, Murcia, Spain) Projects 08856/P1/08 and 08595/P1/08, from the Consejeria de Educacion y Bienestar Social de la Junta de Comunidades de Castilla La Mancha, Project FISCAM PI-2007/53.

E5.03
Alteration of catalytic mechanism of tomato allene oxide synthase LeAOS3 and alfalfa hydroperoxide lyase MtHPL by site-directed mutagenesis

Y. Toporkova1, L. Muchtarova1, Y. Gogolev2 and A. Grechkin2
1Kazan Institute of Biochemistry and Biophysics of KSC of RAS, Laboratory of Oxylipins, Kazan, Russian Federation, 2Kazan Institute of Biochemistry and Biophysics of KSC of RAS, Group of Molecular Biology, Kazan, Russian Federation

Crucial enzymes of lipoxigenase pathway of fatty acids metabolism are cytochromes P450 of the CYP74 family comprised of allene oxide synthases (AOS), hydroperoxide lyases (HPL) and divinyl ether synthases (DES). For substitution with site-directed mutagenesis we have chosen amino acids forming conservative substitutions for all cytochromes P450 oxygen-binding domain and ERR-triad. Substitutions K302S,F295I,S297A and T366Y in LeAOS3 sequence led to changes in catalysis. Along with product characteristic for LeAOS3 reaction – α-ketol – there are product of HPL reaction, particularly 9-oxononanoic acid. Substitutions of K302S,S297A and T366Y possess both activities of AOS and HPL. The α-ketol and 9-oxononanoic acid are detected in different ratios. In the case of F295I substitution activity of AOS is absent. Along with 12-oxododecenonic acid – product characteristic for reaction of wild type enzyme – MtHPL mutant forms F284I,G288I,N285A,N285T and F287Y catalyze formation of novel products 13-oxotridecadienoic and 11-oxoundecenoic acids due to catalysis disruption. Results of site-directed mutagenesis prove the CYP74 family origin from one ancestor with HPL activity. Amino acid substitution in any significant for catalysis site of LeAOS3 led to conversion it into HPL, but not DES as one could expect in some cases. Substitution in the same sites of MtHPL led to appearance of novel products due to catalysis disruption, but not to exchange of catalysis type.

E5.04
Quantification of the secondary antioxidant capacity of different molecules and their kinetic antioxidant efficiency

1GENZ: Grupo de Investigacion Enzimologia, Murcia, Spain, 2Universidad de Castilla la Mancha, Albacete, Spain

When we study the antioxidant capacity of a compound and any deviations appears in the kinetic, it will reflect chemical reactions of the reaction products, in which more free radical molecules are consumed, reflecting a secondary antioxidant capacity. To compare the secondary antioxidant capacity of different antioxidants, we propose a non-enzymatic kinetic test consisting of the kinetic analysis of free radical consumption at fixed initial antioxidant concentration and excess free radical concentration. This excess of free radical would be the result of the initial concentration minus the concentration consumed in its reaction with the antioxidant. The secondary antioxidant capacity is characterized by n, the number of electrons donated per molecule of antioxidant in the chemical reactions of the reaction products, efficient concentration, EC50, ratio of the antioxidant concentration necessary to decrease the initial concentration of ABTS radical to 50% in the secondary antioxidant capacity [EC50 = 1/(2n)] and secondary antioxidant or antiradical power (ARP), (ARP = 1/EC50 = 2n). This work has been partially supported by grants from several Spanish organizations. Ministerio de Educacion y Ciencia (Madrid, Spain) Project BIO2009-12956, from the Fundacion Seneca (CARM, Murcia, Spain) Projects 08856/P1/08 and 08595/P1/08, from the Consejeria de Educacion y Bienestar Social de la Junta de Comunidades de Castilla La Mancha, Project FISCAM PI-2007/53.

E5.05
New links between protein biosynthesis and nonribosomal peptide synthesis

M. Mocibob1, N. Ivicec1, S. Bilokapic1, T. Maier2, M. Luic1, N. Ban1 and I. Weygand-Durasevic1
1Faculty of Science, Department of Chemistry, Zagreb, University of Zagreb, Croatia, 2Rudjer Boskovic Institute, Division of Physical Chemistry, Zagreb, Croatia, 3ETH Zurich, Institute of Molecular Biology and Biophysics, Zurich, Switzerland

Aminoacyl-tRNA synthetases (aaRS) catalyze ATP-dependent attachment of amino acids to tRNA in protein synthesis. Similar role play adenyltransfer domains in template independent nonribosomal peptide synthesis (NRPS), by attachment of activated pre-
cursors to dedicated carrier proteins. Although they catalyze analogous reactions in mechanistically similar manner, using aminoacyl-adenylates as intermediates, adenylation domains and aaRS are structurally and evolutionarily unrelated. The genomes of many bacteria are sprinkled with putative ORFs encoding the proteins homologous to class II aaRS catalytic core, but lacking tRNA binding domain. We have cloned and characterized three such aaRS truncated homologs. Sequence analysis shows clear homology, while crystal structure determination confirmed remarkable structural similarity to seryl-tRNA synthetases (SerRS) from methanogenic archaea. In spite of close resemblance to SerRS, these enzymes have different amino acid specificity. Even more surprising, the SerRS homologs do not aminoacylate tRNA, but instead they transfer amino acids to thiol group of phosphopantetheinyl prosthetic arm of carrier proteins found in their genomic vicinity. Thus, the reaction they catalyze is essentially the same as the reaction catalyzed by adenylation domains in NRPS. It has been long speculated about possible evolutionary relations between programmed ribosomal protein synthesis and template independent NRPS, based on weak thiol acylation activity of contemporary aaRS. Our findings of SerRS homologs engaged exclusively in carrier protein acylation and thioester bond formation provide direct experimental evidence that further supports this hypothesis.

E5.06
A QM/MM study of mechanism of antidote action in reactivation of tabun inhibited AChE
G. Šinčo\textsuperscript{1} and I. Vinković Vreček\textsuperscript{2}
\textsuperscript{1}Institute for Medical Research and Occupational Health, Biochemistry and Organic Analytical Chemistry Unit, Zagreb, Croatia, \textsuperscript{2}Institute for Medical Research and Occupational Health, Zagreb, Croatia

The world food production is closely related with growing use of pesticides in agriculture. Pesticides based on neurotoxic organophosphorous compounds (OPC) and related chemical warfare agents have long attracted attention of the scientists to the development of efficient antidotes for the poisoning with OPC. OPC inhibit irreversibly acetylcholinesterase (AChE), a key enzyme in cholinergic neurotransmission, by phosphorylation. Oximes are in use for reactivation of AChE and regeneration of its catalytic activity as antidotes. To design new reactivators a better understanding of the reactivation process is needed. Our aim was to study the reaction mechanism of reactivation using a QM/MM methodology. We selected the AChE-tabun conjugate since tabun is one of the most potent nerve agent, and very resistant to reactivation. We selected oxime reactivator K027 (4-carbamoyl-1-(3-[4-[(E)-(hydroxyimino)methyl]pyridin-1-yl]propyl)pyridinium bromide) due to its effectiveness in reactivation of tabun or pesticide inhibited AChE. QM/MM approach allows computation of the reaction energy profile for the active site atoms trapped inside the native protein environment. First we modelled reactivation reaction on a truncated system using a quantum mechanical (QM) calculation at the BP86/6-311+G(d,p) level of theory. For the molecular mechanical (MM) part, the CHARMM force field was used. We showed that during reactivation oxime needs to change its position inside active site of AChE to form transition state with AChE-tabun conjugate. Analysis of AChE-tabun-K027 transition state structure identified the critical residues not only for binding of oximes but also for reactivation of enzyme.

E5.07
Investigation of inhibition effect of human placental fructose 1,6 bisphosphate aldolase with bivalent ion
N. H. Aksoy\textsuperscript{1} and P. Dogan\textsuperscript{2}
\textsuperscript{1}Aksaray University, Aksaray, Turkey, \textsuperscript{2}Hacettepe University, Ankara, Turkey

Fructose-1,6-bisphosphate aldolase (FBPA) is a major glycolytic enzyme found in most cells. FBPA, a homotetramer with a molecular mass of 160 kDa, Fructose-1,6-bisphosphate aldolase reversibly catalyses the cleavage of fructose 1,6 bisphosphate into the triose phosphates: glyceralddehyde phosphate and dihydroxyacetone phosphate. In this study, we carried out inhibition effects of bivalent ion on Fructose-1,6-bisphosphate aldolase. We have investigated the interaction of human placental aldolase with bivalent Mg\textsuperscript{2+} metal. It was defined that, Mg\textsuperscript{2+} is competitive inhibitor of healthy human placental FBPA. Ki values of healthy human placental aldolase for Mg\textsuperscript{2+} ion was confirmed 14.678 ± 2.702 mM

E5.08
Oxidative protein folding catalyzed by a selenocysteine variant of DsbA
A. Davis\textsuperscript{1}, M. Hansson-Edalat\textsuperscript{2}, G. Malojcic\textsuperscript{1}, R. Glockshuber\textsuperscript{1} and D. Hilvert\textsuperscript{1}
\textsuperscript{1}ETH Zurich, Zurich, Switzerland, \textsuperscript{2}Stockholm University, Stockholm, Sweden

The Escherichia coli dithiol oxidase DsbA, one of the most oxidizing thioredoxin-like disulfide oxidoreductases characterized to date (E\textsuperscript{m} = -122 mV), catalyzes formation of structural disulfide bonds in the bacterial periplasm. Previous studies with the redox enzymes E. coli thioredoxin (E\textsuperscript{m} = -270 mV) and glutaredoxin 3 (E\textsuperscript{m*} = -198 mV) in which the catalytic cysteine was replaced with selenocysteine showed a marked decrease in redox potential and, in the case of glutaredoxin 3, an increase in the rate of reoxidation by an oxidized substrate. To extend the natural properties of DsbA, we replaced the nucleophilic Cys30 from the active site with selenocysteine using cotranslational selenocysteine insertion via a designed SECIS element in the dsbA gene and coexpression of the selA, selB, and selC genes. The resulting enzyme (C30U DsbA*) shows remarkably little change in structure, redox potential, or kinetics of in vitro substrate oxidation and reoxidation by DsbB compared to the control variant harboring Cys30 (DsbA*) and wild type DsbA. Nevertheless, C30U DsbA* only partially complements DsbA in a dsbA deletion strain, most likely due to its low expression level. This new selenoenzyme provides a framework for investigating the differing reactivities of selenol and thiol groups in a protein context, and for evolving tailored enzymes for studying oxidative protein folding in vivo.

E5.09
Kinetic and dynamic properties of interface mutants of alkaline phosphatase from E. coli
M. Sprung, S. Orhanovic, V. Bucevic-Popovic, B. Soldo and M. Pavela-Vrancic
Faculty of Science, Croatia

Although alkaline phosphatase (APase) from E. coli crystallizes as a symmetric dimer, it displays deviations from Michaelis-Menten kinetics, supported by a model describing a dimeric enzyme with unequal subunits. The proposed model, describing the mechanism of substrate hydrolysis, encompasses a conformational change mediated by subunit interactions. Role and mechanism of
possible communication between subunits of this dimeric enzyme are not resolved. Rigid segments in the polypeptide structure supposedly accomplish transfer of conformational information, between the active sites, across the subunit interface. The importance of the subunit interface and the β-sheeted sheet, stretching from underneath an active site to the subunit surface, in the catalytic mechanism has been probed by site-directed mutagenesis. The mutation replacing Thr-81 with alanine and Gln-83 with leucine were introduced into the APase gene. Amino acid residues Thr-81 and Gln-83 are located within the β-sheeted sheet at the contact surface between the subunits, and form hydrogen bonds with analogous amino acids from the adjacent subunit. Two kinds of mutant proteins were prepared, a single T81A and double T81A/Q83L mutant. Dynamic properties and rigidity of Trp-109 environment of WT APase and mutant enzymes were assessed by acrylamide fluorescence quenching and by measuring phosphorescence lifetime of Trp-109 located in the vicinity of the mutation site and β sheeted sheet. Influence of mutations on the kinetic properties determined in 1 M Tris/HCl, pH 8, and in 0.35 M 2A2M1P, pH 10.5 was compared with the effects that mutations had on rigidity of a protein structure.

E5.10 Nano-sized horseradish peroxidase cross-linked aggregates

L. Hadidi, M. Habibi Rezaei and A. Badiei
University of Tehran, Tehran, Islamic Republic of Iran

Horseradish peroxidase has been considered as a useful enzyme towards remediation purposes such as removal of oil contamination. However various criteria limit soluble enzyme applications which can be relieved in immobilized preparations. In this study carrier free immobilization of horseradish peroxidase (HRP EC 1.11.1.7) is used and cross-linked enzyme aggregates (CLEAs) were prepared. Nano-CLEAs products were characterized for size and shape using dynamic light scattering (DLS) and scanning electron microscopy, respectively. Optimization of nano-scaled CLEAs preparation was carried out upon stoichiometric considerations on safe enzyme cross-linking using glutaraldehyde as a bifunctional cross-linking agent. The products of immobilization have resulted with improved kinetic features and stability. For example catalytic performance of horseradish peroxidase improved and thermal stability of nano-sized CLEAs was increased. However, the pH profile of cross-linked enzyme aggregates did not show observable difference in comparison with the free one.

E5.11 Immobilization of horseradish peroxidase aggregates into porous silica

L. Hadidi, M. Habibi Rezaei and A. Badiei
University of Tehran, Tehran, Islamic Republic of Iran

Enzyme stabilization is one of the major challenges in the industrial biocatalytic processes. In this investigation cross linked horseradish peroxidase aggregates (EC 1.11.1.7) were immobilized into porous silica with wide range pore size diameters. To reach this goal, cross-linked enzyme aggregates were prepared and then effective parameters on particle size including enzyme concentration, time of aggregation and pH were investigated. The particle size of CLEAs became bigger with increasing the concentration of enzyme. Hence, the loaded amount of CLEAs into porous silica decreased. CLEAs formed at different pH also immobilized into porous silica. The immobilized amount was much larger in acidic environment due to the fact that particle size of CLEAs was smaller in acidic condition. Time of aggregation that is the interval between adding the precipitant and the bifunctional cross linker had an direct influence on particle size, increasing this time led to formation of big particles and consequently low small loading into porous silica. The size of CLEAs was determined by dynamic light scattering.

E5.12 Abstract withdrawn

E5.13 Engineering of Bacillus sp. strain TS-23 α-amylase for improving its thermal and oxidative stabilities

M.-C. Chi, Y.-H. Chen and L.-L. Lin
National Chiayi University, Chiayi, Taiwan

BACGNC/EGRS is a thermostable variant derived from the truncated α-amylase (BACGNC) of alkalophilic Bacillus sp. strain TS-23. With the aim of enhancing its resistance towards chemical oxidation, Met231 of BACGNC/EGRS was replaced by leucine to create BACGNC/EGRS/M231L. The functional significance of the 31 C-terminal residues of BACGNC/EGRS/M231L was also explored by site-directed mutagenesis of the 483rd codon in the gene to stop codon (TAA), thereon the engineered enzyme was named BACGNC/EGRS/M231L/EGC31. BACGNC/EGRS/M231L and BACGNC/EGRS/M231L/EGC31 were very similar to BACGNC in terms of specific activity, kinetic parameters, pH V activity profile, and the hydrolysis of raw starch; however, the engineered enzymes showed an increased half-life at 70°C. The intrinsic fluorescence and circular dichroism spectra were nearly identical for wild-type and engineered enzymes, but they exhibited a different sensitivity towards GdnHCl-induced denaturation. Performance of the engineered enzymes was evaluated in the presence of commonly used detergent compounds and some detergents from the local markets. A high compatibility and performance of both BACGNC/EGRS/M231L and BACGNC/EGRS/M231L/EGC31 may be desirable for their practical uses in the detergent industry.

E5.14 Catalysis of immobilised human flavin-containing monooxygenase

S. Castrignano, S. Sadeghi and G. Gilardi
Department of Animal and Human Biology, University of Turin, Turin, Italy

Human flavin-containing monooxygenase isofrom 3 (hFMO3) is a hepatic enzyme that catalyses the oxygenation of a large number of structurally diverse drugs and xenobiotics transforming them into benign and readily excretable products. Since the design and development of new therapeutic drugs may take advantage of these detoxifying properties, much interest has been focused on the development of novel techniques suitable for pharmacological research applied to hFMO3 catalytic properties. With this aim, an hFMO3 electrochemical sensor, developed by modifying a glassy carbon electrode with an entrapping gel obtained by glutaraldehyde co-crosslinking of hFMO3 with bovine serum albumin, is reported in this work. Redox properties of hFMO3 sensor have been compared with those of FAD entrapped gel electrode by cyclic voltammetry, revealing for the protein bound FAD, a significant shift of redox peaks towards negative potentials and a total reversibility of the redox reaction. The redox potential measured for the entrapped protein was −411 ± 10 mV.
E5 – Catalytic Mechanisms

The responsiveness of the sensor was investigated with four different substrates, trimethylamine, ammonia, triethylamine and benzylamine (nonsteroidal anti-inflammatory drug) by calculating kinetic parameters including the apparent Michaelis-Menten constant \( k_{\text{M}} = 82.3 \pm 4.3 \), \( 94.1 \pm 6.4 \); \( 120.7 \pm 11.2 \) and \( 115.9 \pm 6.8 \mu M \), respectively, sensitivity \( 39–45 \text{ mAM-lcm}^{-2} \) and response linearity from 2–80 \( \mu M \). The data obtained confirm that the hFMO3 sensor has good characteristics in terms of substrate detection, reproducibility and stability, therefore can be employed for catalytic activity measurements of new chemical entities turned over by hFMO3.

E5.15

Least-motion mechanism in enzyme catalysis

P.-O. Syren and K. Hult
Department of Biochemistry, Royal Institute of Technology (KTH), AlbaNova University Centre, Stockholm, Sweden

Enzymes are dynamic catalysts that lower the activation barrier compared to the corresponding reaction in solution by utilizing a set of catalytic strategies. These include proximity effects, electrostatic stabilization of the transition state (TS), symmetrical hydrogen bonds and tunneling. An additional possibility is that enzymes have evolved to use an economy in atomic motion upon the enzyme-substrate complex (ES) to TS transition, as has been shown for example for benzoyl formate decarboxylase (EC 4.1.1.7) as well as for serine proteases. Here we present data consistent with a least-motion mechanism for lipase catalysis based on experimentally determined \( k_{\text{cat}} \), molecular dynamics simulations and ab initio B3LYP calculations. Lipases are of particular industrial interest due to their high stability in organic media, substrate tolerance and stereoselectivity. It could be beneficial to take the least-motion mechanism into consideration in protein engineering and rational design projects since certain transition state conformations violate the least-motion mechanism and can thus be disregarded as unproductive.

E5.16

The effects of selected flavonoids on the adenosine deaminase activity

M. Kavutcu, S. Sengoren and O. Canbolat
Gazi University Medical Faculty, Medical Biochemistry, Ankara, Turkey

Adenosine Deaminase (ADA, EC 3.5.4.4) was purchased from Sigma-Aldrich (A-1155, 1.000 Units) and its kinetic properties were appraised in this study, ADA catalyses the irreversible deamination of adenosine and 2'-deoxyadenosine to inosine and 2'-deoxyinosine, respectively. Quercetin, apigenin, kaempferol, naringenin, rutin, luteolin, kaffeic acid as selected flavonoids, and chloroform, carrot extract were evaluated whether they have inhibitory potencies on the kinetic properties of ADA or not. Km and Vm values of the enzyme against adenosine substrate was found to be 105\( \mu M \) and 89.4\( \mu M/l \) respectively by using Lineweaver-Burk graphics. The effects of above-mentioned flavonoids, chloroform, carrot extract and some factors like temperature and \( pH \) on the enzyme kinetics were observed. Quercetin, caffeic acid and naringenin were exhibited most potent inhibitor for ADA, and IC50 values were found 6.1, 6.06 and 6.15 \( \mu M \) respectively. Kinetic analysis showed a mixed type inhibition for quercetin, caffeic acid and naringenin. Ki values were found 314.2, 429.6 and 435.7 \( \mu M \) respectively. The other flavonoids and chloroform had no important inhibitory potencies on the ADA activity. Interestingly, carrot extract were exhibited strong inhibitory effect on the ADA activity, and IC50 values was found 105\( \mu M \). The ranges of optimal \( pH \) and temperature were found 6.5–8.0 and 25–50\(^\circ\)C respectively. As a result, inhibitory potencies of flavonoids seem to be structurally related (hydroxyl-ation pattern), and this effect may be due to structural/pharmacological properties.

E5.17

Further evolution of \( \beta \)-lactamase activity from glyoxalase II to different quasi-species

W. Zhang\(^1\), S. Sun\(^2\), D. Andersson\(^3\) and B. Mannervik\(^4\)
\(^1\) Department of Biochemistry and Organic Chemistry, Uppsala University, Uppsala, Sweden; \(^2\) Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden

The global use of antibiotics is dominated by \( \beta \)-lactam derivatives, like penicillins and cephalosporins. However, widespread use of \( \beta \)-lactam antibiotics leads to the explosion of antibiotic resistance. Moreover, resistance is emerging rapidly after the introduction of new antibiotics into clinical practice. A major mechanism of \( \beta \)-lactam resistance involves the rapid evolution of \( \beta \)-lactamases, which hydrolyse the \( \beta \)-lactam ring, thus sabotaging antibacterial properties. In a previous study, human glyoxalase II (GlyH) was converted into a metallo-\( \beta \)-lactamase (MBL) by a combined approach involving rational design and directed evolution. The engineered enzyme (evMBL8) exhibited enhanced resistance with cefotaxime (a 3rd-generation cephalosporin antibiotic). However, catalytic efficiency with cefotaxime compared to IMP-1, the natural counterpart of evMBL8, was low and evMBL8 did not show any observable activity with other \( \beta \)-(lactam antibiotics. Based on sequence homology analysis and homology modeling, a triple mutant of evMBL8 (evMBL9) was constructed. A preliminary screening in Salmonella typhimurium indicated that evMBL9 conferred resistance to different \( \beta \)-lactam scaffolds, including penicillins, cephalosporins and carbapenems. A combined approach to multiple site-directed mutagenesis and random mutagenesis by error-prone PCR was performed to further evolve evMBL9 to different quasi-species. And a systematic study of enzyme activity, enzyme stability and expression is being conducted.
Glutathione reductase (GR) from baker’s yeast (EC 1.6.4.2) catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) by NADPH. We covalently bound GR to chitin particles (CP) Tyser 35 prepared from crab carapace. The aim of this work is to compare the properties of free and immobilized GR and to check whether there is any evidence of altered catalytic mechanism after immobilization. Furthermore, this is the first step of a new method for specific quantification of glutathione. The immobilization method includes chitin activation with hexamethylenediamine (HEMDA) and glutaraldehyde. CP (1 g) were activated with 12.8 ml 2% (v/v) HEMDA at 40°C for 2 hour. The excess of HEMDA was removed, and then 3 ml of 3% (v/v) glutaraldehyde were added. After 30 minutes at 30°C, active CP were washed five times with 10 ml of absolute ethanol. Subsequently, the material was washed three times with 10 ml of 100 mM phosphate buffer pH 7.0. For immobilization, GR (0.22 U) was added to 250 mg of active CP and incubated at 4°C, overnight. We show that GR was successfully immobilized on chitin with retention of its catalytic activity and could be reused. Its optimal pH and temperature were 6.5 and 35°C respectively. For the unbound GR, optimal pH and temperature were 7.0 and 25°C respectively. One factor that contributes to the shift in pH optimum value is the modification imposed on the ionizing groups of the enzyme caused by the immobilization process. If the reaction involves amino groups near the catalytic center, this may change the kinetic behavior of the enzyme, in particular in relation to pH.

The luciferase from Renilla catalyzes an oxidative decarboxylation of coelenterazine with the generation of the coelenteramide and CO2 releasing light on calcium binding. Apo-obelin is one of the widely used bioluminescent reporters for in vivo imaging. Bioluminescence of marine coelenterates is initiated by increase in intracellular Ca2+. In sea pansy Renilla, Ca2+-induced bioluminescence involves two distinct proteins, Ca2+-triggered coelenterazine-binding protein (CBP) and luciferase. CBP contains one molecule of a tightly bound coelenterazine which is released for reaction with luciferase and O2 only subsequent to Ca2+ binding. The stabilization of coelenterazine and its analogs within CBP against autooxidation and Ca2+-triggered substrate release has a remarkably simple explanation from the spatial structures: CBP with bound coelenterazine and Ca2+-loaded apo-CBP. We also demonstrate that CBP with bound coelenterazine and its analogs reveals greater thermostability and at least two times higher light output in reaction with both wild type luciferase and its mutants than that with free coelenterazine. Thus, in prospect CBP might be used as a carrier and protector of coelenterazine (or its analogs) in imaging experiments in vivo. Supported by RFBR grant 09-04-12202 and Lavrentiev grant of SB RAS.
codon. These constructs were used to test peptide bond formation by tRNA\textit{Ser}(CUA)-2'dA as compared to tRNA\textit{Ser}(CUA)-A in a coupled \textit{in vitro} transcription/translation system. The results indicate that 2'-OH of both, peptide-tRNA and aminoacyl tRNA can be omitted in the peptide bond formation reaction. The former observation that tRNA\textit{Ser}(CUA)-2'dA is inactive in elongation is probably related to applied experimental system and low processivity during translation initiation, before the nascent peptide chain is being localized in the ribosomal exit channel.

E5.24  
**Kinetic characterization of the** \textit{Drosophila} \textit{lebanonensis} \textit{ADH} T114V mutant  
Y. Wuxiuer1, N. Cols2, R. González-Duarte2, R. Ladenstein3, I. Sylte1 and J.-O. Winberg1  
1Faculty of Health Sciences, Department of Medical Biology, University of Tromsø, Tromsø, Norway, 2Department of Genetics, Barcelona, University of Barcelona, Spain, 3Karolinska Institutet NOVUM, Center for Structural Biochemistry, Huddinge, Sweden  

\textit{Drosophila lebanonensis} alcohol dehydrogenase (D\textit{ADH}) belongs to the short-chain dehydrogenase/reductase (SDR) superfamily. D\textit{ADH} contains three conserved amino acid residues Ser138, Tyr151 and Lys155 in the active site and an eight-membered water chain that connects the buried active site with the bulk solvent. The OH-group in the side-chain of Thr114 is a part of the water chain. It has been proposed that this water chain functions as a proton relay system during catalysis. We have therefore produced a T114V mutant in D\textit{ADH} and investigated the kinetic properties of the mutant. The results were compared with the results of the wild type D\textit{ADH}. Kinetic studies revealed that the reaction mechanism for the mutant enzyme like the wild type enzyme was consistent with a compulsory ordered mechanism where the coenzyme binds to the free enzyme. As for the wild type D\textit{ADH}, prop-2-ol is a better substrate than ethanol. For the mutant enzyme, the reaction mechanism for the mutant compared to the wild type enzyme. Furthermore, the pKa value for the binding of NAD+ was reduced for the mutant compared to the wild type enzyme. These results will be discussed in view of the water chain as a proton relay during catalysis.

E5.25  
**Novel Inhibitors of Matrix Metalloprotease-9 and -14**  
S. Sjøli1, O. Adekoya2, Y. Jiang3, Ø. Akselsen3, V. T. Hansen1, C. Sivertsen1, J.-O. Winberg1  
1Faculty of Health Sciences, Department of Medical Biology, University of Tromsø, Tromsø, Norway, 2Faculty of Health Sciences, Department of Pharmacy, University of Tromsø, Tromsø, Norway, 3Department of Pharmaceutical Chemistry, University of Oslo, School of Pharmacy, Oslo, Norway  

Matrix metalloproteinases (MMPs) have the capability to cleave any component of the extracellular matrix (ECM) and process a large number of "non-ECM" proteins. MMPs have complicated biological functions playing a role in several normal and pathological conditions. Cleavage of a substrate by one MMP may promote disease while cleavage of another substrate by the same or another MMP may prevent disease. As sites outside the catalytic region are involved in cleavage of macromolecular substrates, it is pertinent to develop compounds that bind either to these sites or to sites in the catalytic domain. In the present work, various compounds have been tested for their capacity to inhibit MMP-9 and MMP-14 activity. The compounds varied in size, structure and putative zinc chelating properties. Enzyme kinetic studies were performed with a fluorogenic substrate to determine both IC50 values and the type of inhibition caused by the compounds. Molecular modeling was done by docking the compounds into the X-ray crystal structures of MMP-9 and MMP-14 using the Glide docking program. The results revealed that the strongest inhibitors showed IC50 values in the lower \(\mu\text{M}\) region, while those with the weakest binding had IC50 values > 1000 \(\mu\text{M}\). There were no striking differences between the two enzymes with respect to obtained IC50 values. Surprisingly, some of the compounds gave a mixed type of inhibition while others gave a competitive inhibition pattern against the fluorogenic substrate. When the compounds were docked into the free enzyme, they interacted with the catalytic zinc ion of both enzymes.

E5.26  
**Enzymatic modification of manno-glycans**  
A. Rosengren1, O. Hekmat1, P. Pavon Orozco2, L. Lo Leggio3 and H. Stålbrand1  
1Department of Biochemistry and Structural biology, Lund University, Lund, Sweden, 2Department of Chemistry, Copenhagen University, Copenhagen, Denmark  

The interest of using hemicellulose as a renewable resource for novel oligomeric and polymeric products is currently increasing. With this in mind we combine molecular biology, protein crystallography, enzyme kinetics and mass spectrometry in our studies of enzymes active against the major softwood hemicellulose: O-acetyl galactoglucomanannan (GGM). We have recently developed an enzyme triggered drug delivery gel on the basis of GGM. The hydrolytic properties of hemicellulose degrading enzymes from Aspergilli are being studied in detail. \(\alpha\)-Galactosidases from family 27 and family 36 are compared in terms of substrate specificity and efficiency in removal of galactose side groups. \(\beta\)-Mannanases are interesting not only because of backbone cleavage of GGM. As retaining glycoside hydrolases they are theoretically able to catalyze transglycosylation reactions, useful for the biosynthesis of new hemicellulose-based glyco conjugates. Results on the enzyme substrate interactions important for transglycosylation capacity, based on comparison of kinetic properties, hydrolysis pattern and 3D structures of wild-type and mutated active site variants of a family 26 \(\beta\)-mannanase (CiMan26A) will be discussed. Several family 5 \(\beta\)-mannanases have strong aglycone (acceptor) binding and we have generated a range of mutants in this region to study differences in tolerance of various acceptors, ranging from sugar- to non-sugar acceptors such as alcohols. Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry and high performance anion exchange chromatography (HPAEC) is used to analyze the transfer capacity of the enzymes being studied.

E5.27  
**Application of reverse-phase HPLC to quantify oligopeptide acetylation eliminates interference from unspecific acetyl CoA hydrolysis**  
R. Evjenth, K. Hole, M. Ziegler and J. R. Lillehaug  
Department of Molecular Biology, University of Bergen, Bergen, Norway  

Protein acetylation is among the most common covalent modification in eukaryotic cells and is performed by different acety-
E5.28
Mutagenetic and crystallographic characterization of rHint1, the phosphoramidates hydrolase with the P-S cleavage activity
M. Ozga, R. Dolot, A. Krakowiak, R. Kaczmarek and W. J. Stec
CBMM PAS, Bioorganic Chemistry, Lodz, Poland
The histidine triad nucleotide binding protein1 (Hint 1) is an enzyme of the first branch of the HIT superfamily that catalyzes the hydrolysis of P-N bond in AMP-N-e-lysine, AMP-N-alanine, AMP-N-morpholidate substrates with the rate of ca. 1 mmol/min/µg and adenosine-5'-monophosphoramidate (AMP-NH2) with the rate of 1.71 mmol/min/µg [1]. Recently we reported that in the cleavage reaction of the P-diastereoisomers of 5'-O-[N-(tryptophanamide)phosphoramidothioate catalyzed by the wt rHint1, the hydrolysis of P-N bond was accompanied by a parallel loss of the sulfur from the resulting AMPS [2]. In an independent experiment we have demonstrated that the wt Hint1 hydrolyzes the AMPS with the rate of ca. 0.2 nmol/min/µg resulting in formation of AMP and H2S as final products. We have also noticed that other ribonucleotide phosphorothioates and corresponding deoxyribonucleotides are also substrates for Hint1 hydrolase. In our extended studies on desulfuration process and P-S cleavage ability of Hint1 enzyme, we have used mutagenetic approach to generate Hint1 cystein mutants. Since one of the product of desulfuration reaction is H2S, it was of great interest to measure the activity of three Hint1 mutants: C38A, C84A, C38/84A towards AMPS and AMP-Lys substrates in order to exclude that H2S (formed in the reaction with AMPS) is the product from cystein residues present in polypeptide chain of Hint1. We have also crystallized the cystein mutants and compared their structure with native Hint1 protein. The trials to co-crystallize the Hint1 enzyme with AMPS are in progress.

E5.29
Substrate binding mechanism of type I extradiol dioxygenase from Rhodococcus sp. strain DK17
H. J. Cho1, K. Kim2, E. Kim2 and B. S. Kang1
1Kyungpook National University, Daegu, Republic of Korea, 2Yonsei University, Seoul, Republic of Korea
Rhodococcus sp. strain DK17 is known to employ a single extradiol dioxygenase in alkylbenzene metabolism. The crystal structures of AkbC and the enzyme-substrate (3-methylcatechol) complex were determined. AkbC was found to be composed of eight identical subunits, each containing one ferrous ion. A monomeric AkbC molecule has two structural elements, N-domain (residues M1 – G138) and C-domain (K139 – G300) including the C-terminal tail (H285 – G300), which covers its active site. In each monomer, the ferrous iron is coordinated by the typical 2-His-1-carboxylate facial triad motif plus an additional amino acid residue Y253 in the C-domain. In the substrate bound AkbC structures, 3-MC interacts the iron with single hydroxyl group representing an intermediate state in the substrate binding process. Structure-based mutagenesis revealed that the C-terminal tail and the β-hairpin structure ([β11-β12] form parts of the substrate-binding pocket that is responsible for correct positioning of the substrate at the active site for subsequent cleavage and also for its substrate specificity. AkbC's longer C-terminal region compared to dihydroxybenzophenones restricts the substrate range. Widening the opening of the active site results in an increase of the enzyme activity against dihydroxyphenyl. We propose a substrate binding mechanism which includes a sequential interaction of two hydroxyl groups of 3-methylcatechol for bidentate binding to the iron and a rearrangement of the substrate bound position in the octagonal iron coordination to be deprotonated and open the site for an oxygen.

E5.30
Mechanism of photoprotein bioluminescence
E. Vysotski1, J. Lee2 and E. Vysotski2
1Institute of Biophysics Russian Academy of Science, Siberian Branch, Photobiology laboratory, Krasnoyarsk, Russian Federation, 2Department of Biochemistry and Molecular Biology, University of Georgia, Athens, US
Ca2+-regulated photoproteins are responsible for the light emission of a variety of marine coelenterates. Direct information concerning the bioluminescence mechanism derives from X-ray structures of ligand-dependent conformational states of photoproteins. The bioluminescence results from the Ca2+-triggered decomposition of a high energy intermediate, the 2-peroxycoelenteramide observed to reside in the binding cavity and stabilized in place by a H-bond network. We have proposed that movement of residues occurring as a result of accommodating the Ca2+ in the nearby Ca2+-binding loops, would perturb these H-bonds, destabilizing this intermediate and allowing the decarboxylation reaction that generates the product coelenteramide in its excited state. In the cavity structure of the Ca2+-discharged photoprotein, a water molecule nearby the coelenteramide amide is so positioned that, if it were in this same place in the intermediate conformation, it could donate a proton to the first formed dioxytane anion, prior to its decomposition to the excited state product. Thus the primary product would be the neutral excited coelenteramide (λmax = 390–405 nm). It is proposed that the lower energy excited state giving rise to the blue bioluminescence (λmax = 460–495 nm), results from excited state proton transfer from the OH bound to the 6-phenyl substituent, to a nearby His residue as a proton acceptor. Supported by: ONR, NIH, Georgia
Nitrones have the general chemical formula X-CH=NO-Y. They were first used to trap free radicals in chemical systems and then subsequently in biochemical systems. The nitron-based free radical traps have significant potential in the treatment of many harmful diseases such as neurodegenerative complaint, cancer, ischaemia/reperfusion injury, endotoxaemia. It has been focused on free radical scavenging activity of new synthetic molecules named as Nitron 5.1.1 and 5.1.2. For this aim, N-Methyl Nitrones free radical trapping activities tested with based on the reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH). Due to the presence of an odd electron DPPH gives a strong absorption at 517 nm. As this electron becomes paired off in the presence of a hydrogen donor, i.e. a free radical scavenging antioxidant, the absorption strength is decreased, and the resulting decolorization is stoichiometric with respect to the number of electrons captured. Result showed that nitrons 5.1.1 and 5.1.2 have more powerful free radical trapping activity compare with electrons captured. Result showed that nitrons 5.1.1 and 5.1.2 have more powerful free radical trapping activity compare with electrons captured. This is also confirmed by the results of the study. In two of the most active experimented nitrones, the radical is delocalized on the carbon bound to methyl groups and this leads to using up of a second DPPH.

**E5.31**

Antioxidant properties of new novel synthetic molecules; N-Methyl Nitrones

O. Canbolat1, S. Yavuz2, S. Senogoren1, Y. Yildirir2 and M. Kavutcu1

1Gazi University Medical Faculty, Medical Biochemistry, Ankara, Turkey, 2Gazi University Science and Art Faculty, Chemistry Department, Ankara, Turkey

Nitrones have the general chemical formula X-CH = NO-Y. They were first used to trap free radicals in chemical systems and then subsequently in biochemical systems. The nitron-based free radical traps have significant potential in the treatment of many harmful diseases such as neurodegenerative diseases, cancer, ischaemia/reperfusion injury, endotoxaemia. It has been focused on free radical scavenging activity of new synthetic molecules named as Nitron 5.1.1 and 5.1.2. For this aim, N-Methyl Nitrones free radical trapping activities tested with based on the reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH). Due to the presence of an odd electron DPPH gives a strong absorption maximum at 517 nm. As this electron becomes paired off in the presence of a hydrogen donor, i.e., a free radical scavenging antioxidant, the absorption strength is decreased, and the resulting decolorization is stoichiometric with respect to the number of electrons captured. Result showed that nitrons 5.1.1 and 5.1.2 have more powerful free radical trapping activity compared with electrons captured. Result showed that nitrons 5.1.1 and 5.1.2 have more powerful free radical trapping activity compared with electrons captured. This is also confirmed by the results of the study. In two of the most active experimented nitrones, the radical is delocalized on the carbon bound to methyl groups and this leads to using up of a second DPPH.

**E5.32**

Catalasal and peroxidatic mechanisms of *Mycobacterium tuberculosis* catalase-peroxidase (KatG)

P. Nicholls, J. Worrall and D. Svistunenko

University of Essex, Biological Sciences, Colchester, UK

Catalase-peroxidases (KatG haem enzymes) share the catalytic characteristics of classical catalases and peroxidases, but function by different mechanisms. M. tuberculosis KatG, in its catalatic reaction with peroxide, undergoes the reaction cycle of Eqs. (1) & (2): (1) KatGFe(II) + HOOH -> I + HOH -> IS -> KatGFe(II) + HO2. The overall catalatic reaction involves at least three intermediate species, a transient form “I*”, a persistent form “I”, and the rate-limiting “Michaelis” complex “IS”. Complex IS is populated only at high peroxide levels and decays as peroxide concentration falls to zero, but the compound I-like species “I*” persists, as both formation and removal require reaction with peroxide. It decays slowly by secondary reactions with endogenous or added hydrogen donors. The three species have been partially characterised using stopped flow spectrophotometry and rapid free electron paramagnetic resonance (EPR). Their UV-visible spectra are unique, and their EPR signals indicate involvement of at least two distinct protein radicals. None of these intermediates seems to be chemically closely analogous to the corresponding compounds involved in catalysis by classical catalases and peroxidases. Reaction of KatG with peracetic acid produces a somewhat different population of compound I-like species. Both peracetae-induced and peroxide-induced forms can also react peroxidatically with hydrogen donors such as guaiacol. The three catalatic intermediates however show different reactivities towards such donors. A model mechanism will be proposed for the roles of haem iron compounds and protein radicals in both types of catalysis.

**E5.33**

Abstract withdrawn

**E5.34**

Enzymatic mechanism of bacterial NAD kinases

L. Assairi1, G. Poncet-Montange2, S. Pochet3 and G. Labesse2

1Institut Curie, INSERM U759, 91405 Orsay Cedex, France, 2CNRS UMR5048, INSERM U554, Université Montpellier, Montpellier, France, 3Institut Pasteur, CNRS URA 2128, Unité de Chimie et Biocatalyse, Paris, France

We determined the crystal structures of NADK1 from Listeria monocytogenes (Lm) in free state and bound to NAD and NADP. The conserved GGDGT motif of NADKs has been suggested to be catalytically important and to be involved in nucleotide binding or in phosphate transfer. Our analysis revealed that the Aspartate in the conserved GGDGT motif has a different role in Phosphofructokinase (PFK) and NADKs. In PFKs this conserved residue is involved in ATP activation, whereas in NADK it is involved in ADP activation. In the LmNADK1-NAD the side chain of Asp-45 becomes hydrogen-bonded to 2'-hydroxyl group of NAD. To investigate the role of Asp-45, we determined the crystal structure of LmNADK1-D45N mutant. The active site of this mutant was similar of the wild-type enzyme with the hydrophobic present in the same conformation. The Aspartate adopted the same buried conformation as Aspartate but did not form any hydrogen bond with NAD. The mutation D45N resulted in a 10-fold decrease in activity. Thus Asp-45 appears as a key residue for catalytic activity of LmNADK1.

**E5.35**

Effects of various inhibitors on α-Amylase in *Anoxybacillus sp.* AH1 isolated from hotspring

O. Acet1, F. Matpan Bekler1, R. Gul Guven2, H. Princciochlu3, A. Kaplan1 and K. Guven1

1Department of Biology, Dicle University, Faculty of Science, Diyarbakir, Turkey, 2Dicle University, Faculty of Education, Science Teaching Section, Diyarbakir, Turkey

α-Amylase in *Anoxybacillus sp.* AH1 was partially purified and some properties such as optimum temperature, pH and thermal stability were studied. The optimum temperature and pH for this enzyme were found at 7.0 and 60°C, respectively, and found to be sensitive to temperature. The thermal stability were enhanced by 30% glycerol. Km and Vmax values were calculated from Lineweaver-Burk plot as 0.102 and 0.929, respectively. The enzyme is sensitive to temperature. The thermal stability were enhanced by 30% glycerol. Km and Vmax values were calculated from Lineweaver-Burk plot as 0.102 and 0.929, respectively. The enzyme is sensitive to temperature.

The optimum temperature and pH for this enzyme were found at 7.0 and 60°C, respectively, and found to be sensitive to temperature. The thermal stability were enhanced by 30% glycerol. Km and Vmax values were calculated from Lineweaver-Burk plot as 0.102 and 0.929, respectively. The enzyme is sensitive to temperature. The thermal stability were enhanced by 30% glycerol. Km and Vmax values were calculated from Lineweaver-Burk plot as 0.102 and 0.929, respectively. The enzyme is sensitive to temperature.
eating that this enzyme is a metalloenzyme. 2-Mercaptoethanol (64% at 10 mM) and DTT (more than 100% at 10 mM) were found to enhance α-amylase activity to a great extent, while p-chloromercuribenzoic acid (PCMB) inhibited the enzymatic activity (52% at 4 mM), indicating the presence of at least one essential Cys residue modified by the reagents in the active site of the enzyme. Iodoacetamide and N-ethylmaleimide had a little effect on the enzyme. Phenylmethylsulfonyl fluoride (PMSF) inhibited the enzyme strongly (60% at 4 mM), also showing the participation of serine at the enzyme activity. This study is one of the first studies to report for the characterisation of an α amylase in A. oxyxynyx species.

E5.36 Structural analysis of active site of cytokinin oxidase/dehydrogenase
D. Kopcenc1, H. Popelkova2, P. Briozzo3, C. Madzak4, I. Frechot1, A. Majira1, M. Laloue5, N. Houba-Herin1 and M. Sebela1
1 Department of Biochemistry, Palacky University, Olomouc, Czech Republic, 2 Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, US, 3 INRA-AgroParisTech, UMR206 Laboratoire de Chimie Biologique, Grignon, France, 4 INRA-CNRS-INAPG, Laboratoire de Microbiologie et Génétique Moléculaire, Grignon, France, 5 INRA, Laboratoire de Biologie Cellulaire, Versailles, France

The flavoenzyme cytokinin oxidase/dehydrogenase (CKO) irreversibly deactivates plant hormones cytokinins and thus participates in controlling their metabolic level. CKOs catalyse the oxidative breakdown of isoprenoid cytokinins to adenine/adenosine and the corresponding unsaturated aldehydes. Oxygen as well as 2,6-dichlorophenolindophenol (DCIP) or para-quinone derivatives function as electron acceptors. An extracellular CKO from Zea mays (ZmCKO1) was expressed in the yeast Yarrowia lipolytica. Subsequently, several active-site mutants were constructed and their kinetic properties were analyzed to clarify the importance for catalysis of amino acid residues like H105, D169, E288, E381, P427, V378, L492, W397 and others located at the importance for catalysis of amino acid residues like H105, D169, E288, E381, P427, V378, L492, W397 and others located at the import of Education, Youth and Sports of the Czech Republic.

E5.37 Structural analysis of plant aminoaidehyde dehydrogenases
M. Tylichova1, D. Kopcenc1, P. Briozzo2, S. Moréra2, J. Snegovaf2 and M. Sebela1
1 Department of Biochemistry, Palacky University, Olomouc, Czech Republic, 2 INRA-AgroParisTech, UMR206 Laboratoire de Chimie Biologique, Grignon, France, 3 CNRS, Laboratoire d’Enzymologie et de Biochimie Structurales, Gif-sur-Yvette, France

Aminoaidehyde dehydrogenases (AMADH, EC 1.2.1.19) catalyze the terminal step in polyamine catabolism. They oxidize various α-aminoaidehydes like 4-aminobutyraldehyde (ABAL) and 3-amino-propanaldehyde (APAL) to the corresponding α-amino acids that individually participate in processes of defense against drought, salinity and other stress events and biosynthesis of car-nitine and secondary metabolites (fragrance compounds, alka-loids). Based on their amino acid sequence, the enzymes belong to the same group as stress-related betaine aldehyde dehydrogenases (BADHs, EC 1.2.1.8). There are commonly two AMADH isoenzymes present in plants. To understand their function, we expressed two AMADHs from pea (Pisum sativum) and performed an X-ray crystallographic study together with kinetic analysis using a large set of natural and synthetic aminoaidehydes. The crystal structures of pea AMADHs in complex with NAD+ coenzyme were refined at 2.4 and 2.15 Å resolution, respectively. They revealed a dimeric shape of the enzymes and provided a detailed description of the coenzyme and substrate binding site. Likewise, we analyzed kinetic properties of two AMADHs from tomato (Lycopersicon esculentum) and two isoenzymes from maize (Zea mays). Our results show that all six studied enzymes preferred APAL and ABAL to betaine aldehyde as substrates. Although substrate preference can vary among species, in terms of wider substrate specificity the above-mentioned enzymes should be considered as AMADHs and not BADHs. This work was supported by grant 522/08/0555 from the Czech Science Foundation and grant MSM 6198959215 from the Ministry of Education, Youth and Sports of the Czech Republic.

E5.38 Assay of insect polyphenol oxidase-educational aspect
A. Urbanska
University of Podlasie. Biochemistry and Molecular Biology, Siedlce, Poland

Polyphenol oxidase, PPO catalyses the hydroxylation of monophenols and the oxidation of o-diphenols to o-quinones. PPO is named phenolase, catecholase, tyrosinase still, although the EC assigns two names, monophenol monoxygenase and o-diphenol oxidase. Principally, PPO is involved in: synthesis of melamins, biogenic amines, betalains; detoxification and toxification of xenobiotics; protein-phenol binding; carcinogenesis, ROS production; plant food processing. PPO analysis is applicable to biochemistry; clinical diagnostics; chemical resistance of plants; food biochemistry/technology and other fields. PPO assay appears significant practice for science and medicine students. Usually, students study potato PPO with pyrogallol/quaiacol. I propound staining procedure for aphid PPO (EC 1.10.3.1), with L- and D-DOPA, catechol, catechin, quercetin, caffeic, chlorogenic, gallic, protocathecyclic acids. For detection of salivary PPO aphids (other Homoptera or Heteroptera) puncture agarose (1.25%)-sucrose (30%) gels incorporating 0.1% phenolics. Colouring of the saliva inside gels demonstrates PPO reaction. For PPO of salivary glands, digest system, cuticle and hemolymph (1–10µl) these are freshly dissected/collected and incubated with 0.1% L-DOPA, in 0.1 M Na-phosphate buffer, pH 7.4 for 1hour at 30°C. Darkening is visible at the light microscope. Controls incorporate 0.005 M phenylthiourea. In my opinion this practice is appropriate to explain/discuss theoretical and analytical subjects on insect PPO i.e. exogenous/endogenous, extracellular/ intracellular occurrence; lack of specificity; melanin synthesis pathway; microscopic visualization; analysis in vitro, in vivo and in situ.
E5.39 Structural parameters of Zoanthus yellow fluorescent protein chromophore from molecular dynamics in gas phase and condensed phase

E. Walczak and T. Andruniow
Wroclaw University of Technology, Institute of Physical and Theoretical Chemistry, Wroclaw, Poland

Fluorescent proteins are very useful for tracking the localization and dynamics of proteins in living cells and as reporters for gene expression studies. They are commonly used in resonance energy-transfer applications and in multicolour labelling. For this reason, it is important to understand how the protein functions and its chromophore on the atomic scale, explaining the role of particular atoms, residues and hydrogen bonds. Green fluorescent protein GFP (PDB entry code 1EMB), purified from the jellyfish Aequorea victoria, is the best known and most well described fluorescent protein. The properties of the protein, e.g. a structure, chromophore geometry, absorption and emission spectra have been widely investigated for years. In recent years, there has been an increase in published results from quantum mechanical (ab initio and DFT) analyses of the GFP chromophore in vacuo and condensed phase. Another GFP like protein is Zoanthus yellow fluorescent protein (zFP538). zFP538 (PDB entry code 2GOR), purified from coral, is less known and examined. It is believed, however, that the zFP538 chromophore comprises an additional six membered ring, causing a shift in the absorption and emission maxima to longer wave lengths compared to GFP chromophore. The zFP538 chromophore was investigated by molecular mechanics (MM), using the CHARMM27 force field. MM energy minimisations and molecular dynamics (MD) simulations were applied. Analyses were carried out in the gas phase, water and a protein environment. The calculated structural parameters were compared to ab initio and DFT data available from the literature.

E5.40 Towards the structural understanding of the catalytic activity acquisition Application to autoimmune diseases

D. Le Minoux, S. Padoleau-Lefevre, B. Bihan-Avalle, D. Thomas and A. Friboulet
Université de Technologie de Compiègne, Compiègne, France

Since the catalytic antibody discovery, they are often found in an autoimmune disease background. Today, the catalytic antibody production and their role are still unclear. This work has two goals: To define the amino acids which confer a catalytic function to an antibody and to understand more precisely the relation catalytic antibody-autoimmune disease. For this purpose, a phage display system is used as a selection tool of single chain fragment variable (scFv) expressed in fusion with gH1p. Four phage banks have been synthesized from spleen-cell of four various background mice (Healthy and nonimmunized; autoimmune and immunized). First of all, scFv banks derived from spleen-cell repertoires of mice were constructed. The heavy chain variable regions (VH) and light chain variable regions (VL) of antibodies were amplified by specific primer mix. The VH and VL sequences have been randomly associated by a sequence homology. The hypothetical catalytic scFvs will be selected against a specific compound and tested to characterize a catalytic activity. The comparison of catalytic scFv sequences between themselves will provide insights to define the amino acids which confer a catalytic function. The comparison of catalytic scFv repertoires from nonimmunized mice would give us information about the presence of catalytic sequence in the antibody germ line and the comparison between nonimmunized and immunized mice would bring insights about the role of somatic mutations for the acquisition of catalytic function by antibody.

E5.41 Substrate specificity and catalytic mechanism of the Escherichia coli FrlB amadoriase

A. Atanasova1, S. Mittelmaier2, Y. Handzhiyski1, A. Sredovska1, I. Ivanov1 and R. Ivanov1
1Institute of Molecular Biology “Roumen Tsanev”, Bulgarian Academy of Sciences, Gene Regulations, Sofia, Bulgaria, 2Department of Chemistry and Pharmacy, University of Erlangen-Nuremberg, Food Chemistry, Erlangen, Germany

Amadoriases are a class of enzymes catalyzing the decomposition of Amadori products. The latter are formed in a spontaneous chemical reaction known as non-enzymatic glycosylation (glycation) of the Maillard reaction. Recently, an amadoriase enzyme, the FrlB amadoriase, has been identified in Escherichia coli (Wiane et al. JBC 2002; 277 42523-42529). This enzyme has been shown to catalyze the hydrolysis of fructoselysine 6-phosphate to lysine and glucose 6-phosphate. In the reverse reaction the enzyme has failed to catalyze the attachment of six other amino acids (glycine, valine, glutamine, methionine, arginine and isoleucine) to glucose 6-phosphate. Accordingly, the enzyme has been considered to contribute to bacterial utilization of fructoselysine in the intestine. In the present study we provide evidence for broader substrate specificity of the enzyme. In both the forward and the reverse reaction the enzyme was found to use as substrates predominantly charged and polar amino acids. Importantly, high molecular mass polypeptides such as Poly-L-lysine also served as substrates for the FrlB amadoriase. Thus the FrlB amadoriase appears the first enzyme capable of deglycating polypeptides by acting directly on the bond between the sugar moiety and the amino group. In addition, we obtained data arguing for an oxidative rather than hydrolytic mode of action of the enzyme. Based on these result we suggest a different physiological role for the E. coli FrlB amadoriase.

E5.42 Analysis of self-catalytic maturation capability of galactose oxidase by site-directed mutagenesis

B. Kocuklu1, Z. B. Ogel2 and M. J. McPherson3
1Middle East Technical University, Biotechnology, Ankara, Turkey, 2Middle East Technical University, Food Engineering, Ankara, Turkey, 3University of Leeds, Structural Molecular Biology, Leeds, UK

In this study, self-processing mechanism of galactose oxidase was investigated in E. coli by altering some amino acids which were supposed to play a crucial role in pro-peptide removal. Galactose oxidase from F. graminearum (NRRL 2903) is a monomeric, copper- containing 68 kDa enzyme with an unusual thioether bond. It has a 41 aa leader peptide, including a signal peptide, an 8 aa long putative pre-peptide, and a 17 aa N-terminal pro-peptide which is autocatalytically cleaved by the aerobic addition of Cu2+ to yield a 639 amino acid mature protein. Pro-peptide cleavage is followed by the formation of a thioether

bond at the Cu²⁺-containing active center. Following the expression of mutant GOase constructs, mutated at the junction of the 25 aa long pre-pro peptide region and the mature protein region, protein samples were purified and analyzed. Results have shown that all mutant proteins were slightly above 70 kDa, which is above the size of the mature enzyme. N-terminal sequencing has shown that all constructs retained the 25 aa long pre-pro-peptide region, suggesting absence of cleavage. However, these mutant pre-pro-GOases were all active. Accord- ingly, it was shown for the first time that the cleavage of the pro-peptide is not essential for the formation of the thioether bond, hence, activity of the enzyme. Comparison of the activi- ties of the pre-pro and mature forms of GOase showed that there is not any significant difference between them. It is sug-gested that the 8 aa long putative pre-peptide is responsible from the lack of cleavage, thus if deleted, autocatalytic cleavage is expected to take place. This hypothesis is now under investigation.

E5.43
Multiscale QM/MM modelling and enzyme catalysis
P. Fernandes
REQUIMTE, Chemistry, University of Porto, Porto, Portugal

Enzymes are catalysts with a very peculiar structure. They are macromolecules with usually tens of thousands of atoms, which have a small active site that participates directly in the reaction and a large scaffold that, among other functions, may create an electrostatic field that promotes catalysis. The way computational chemists deal with these systems is to build up a hybrid QM/MM model [1]. However, beyond the different size scales of the two regions, the enzyme structure changes within large time scales, which can not be efficiently explored with a QM/MM model that used a high accuracy hamiltonean in the quantum region. Here we review recent work in enzymatic catalysis, using coupled and decoupled methodologies, emphasizing the strategies that decouple the time-scales and size-scales of the system [2, 3]. The decoupling approximation may be very rewarding as it allows for the efficient exploration of all aspects of catalysis. It consists in the use of pure classical mechanics, pure quantum mechanics and hybrid methods, each one in a different model of the same enzyme, to study the same catalytic mechanism, and make use of the information of a given size scale to model/calibrate the adjacent size scale.

References:

E5.44
Cloning, expression and purification of two yeast genes (ADH, FDH) in E. coli and determination of their catalytic properties
M. Madyagol, P. Utekal, Z. Levarski, L. Panciova, S Stuchlik and J. Turna
Department of Molecular Biology, Comenius University in Bratislava, Bratislava, Slovakia

Natural flavoring additives of plant origin such as six carbon aldehydes and alcohols are currently used as additives in foods, beverages and cosmetics. In recent decades, enzymes are used for their processing, especially oxidoreductases. The most preferred enzymes of the group oxidoreductases is alcohol dehydrogenase (ADH), which in the presence of cofactors NADH/NAD⁺ catalyzes the interconversion of C6-aldehydes and C6-alcohols. In vi- tro conditions were successfully used to reduce the trans-2- hexenal to trans-2-hexenol ADH from Saccharomyces cerevisiae ADH, as most of the enzymes of the group oxidoreductases is dependent on nicotinamide cofactor β1,4-nicotinamide adenine dinucleotide (NADH) or β1,4-nicotinamide adenine dinucleotide phosphate (NADPH). Unlike enzymes, cofactors are consumed in stoichiometric amounts together with the substrates. Because of the high price of these compounds there is considerable inter- est in developing effective regeneration methods. Presently in enzyme bioreactors for the in vitro regeneration of NADH from NAD⁺ intensively used enzyme is NAD⁺ dependent format dehydrogenase (FDH) from methylotroph bacterial species Candida boidinii is used. In this work we have cloned the two above mentioned yeast enzymes in various expression vectors. After the optimization of the expression in E. coli cells, initial fermentation runs were carried out, followed by purification in preparative amounts using different affinity tags. The determination of enzyme activity and stability was carried out and the possibility of their subsequent application in bioconversion of C-6-aldehyde in the form of free or immobilized enzymes was verified.

E5.45
Conserved regions among azathioprine-active glutathione transferase chimeras show high sensitivity to mutations and give a 10-fold activation when inserted into low-active wild type enzymes
O. Modin, W. Zhang and B. Mannervik
University of Uppsala, Biochemistry and Organic chemistry, Uppsala, Sweden

Upon conjugation of azathioprine with glutathione, 6-mercaptopurine is released, which induces DNA damage. GSTs with improved azathioprine activity have potential uses as selectable markers in gene therapy and in antibody-directed enzyme pro-drug therapy. Chimeric glutathione transferases (GSTs) were previously generated by DNA shuffling and two recombin- ed segments were identified as primary determinants of azathi- oprine activity. These segments correspond to part of the sub- strate-binding H-site and they were examined with respect to sufficiency for azathioprine activity and tolerance to point mutations. Insertion of these two small regions from the active human GST A2-2 into the low-active human GST A3-3 and rat GST A3-3 generated stable chimeras with 10-fold enhanced azathioprine activity. Two potential active site residues in these regions were mutated to produce a library with all possible resi- due combinations. 1920 colonies were screened and 20 purified mutants were characterized and all of them showed decreased azathioprine activity, further emphasizing the importance of the region and its susceptibility to mutations with respect to azathi- oprine activity. Homologous recombination keeps conserved regions intact which the results show is not only beneficial for the stability of the chimeras but also for maintaining the regions with low tolerance to mutations, regarding the targeted property, and to acquire more variation in other parts of the sequence into the pool of active enzymes. Further modifications in directed evolution should thus be focused to regions with more variation present among the most active mutants. (Sup- ported by the Swedish Cancer Society).
E5.46
Perspectives of application of Armenian highland plants in cancer treatment
S. Sharoyan, A. Antonyan, S. Mardanyan and H. Harutyunyan
H. Buniatyan Institute of Biochemistry, Yerevan, Armenia

At cancer, the purine metabolism is accelerated, and the level of key enzyme of purine nucleotide pathway, adenosine deaminase (ADA), is enhanced in serum and tissues. The regulation of ADA activity seems prospective for cancer treatment. For this purpose the specific plant extracts might be a promising approach in the quest of remedy against cancer. We studied the influence on ADA activity of aqueous extracts of 25 plants, traditionally used in folk medicine and/or as food. The numbers of total and alive cells of mice Erlich Ascites Carcinoma (EAC), cultivated in the presence and absence of plant extracts significantly inhibiting ADA, were daily monitored. The kinetics of cells growth evidenced the decreasing of total and alive cells number in the presence of plant extracts down to 40–60% of control. To understand if the cells growth inhibition is consequence of enzyme inhibition by the extracts, the experiments in the presence of known ADA inhibitor, Erythro-9-(2-hydroxy-3-nonyl) adenine, EHNA, were performed. The influence of EHNA on the cell samples had been amplified by addition of cytotoxic substrate of ADA, 2’-deoxyadenosine (dAdo). The observed analogous kinetics of inhibition of EAC cells growth by EHNA+dAdo and by oregano (Origanum vulgare)+dAdo allow us suggesting that ADA inhibiting ability of oregano is a cause of inhibition of EAC cells growth. Hence, the plant extracts inhibiting ADA activity might be recommended for clinical testing as new, mild, and non-expensive remedy against cancer.

E5.47
Characterization of cytochrome c-554 from Methylosinus trichosporium OB3b, a heme protein exhibiting HALS EPR signals
E. Harbitz and K. K. Andersson
Department of molecular biosciences, University of Oslo, Oslo, Norway

A novel low-spin cytochrome c-554 has been purified from the methane oxidizing bacteria Methylosinus trichosporium OB3b. This cytochrome exhibits a ferric HALS (highly axial low-spin) low-temperature EPR signal, with a typical HALS lineshape and a g-max value of 3.41. The EPR spectrum changes slightly with pH. MALDI-TOF-MS has shown that this cytochrome has a molecular weight of 12 230 Da, and that it only contains one heme group. The CD spectrum of the oxidized form of this cytochrome shows a peak at around 730 nm. Together with light absorption experiments this indicates a His/Met coordination of the heme iron. The sequence of the first 39 N-terminal amino acids and 27 C-terminal amino acids has been determined and sequence alignments performed. The most similar hits represent c-type cytochromes from bacteria belonging to the phylum Bradyrhizobiaceae, in which Methylosinus trichosporium OB3b is included. C-type cytochromes have similar His/Met coordination of the heme iron they can exhibit different EPR signals. The correlation between different EPR signals and structure is not known for His/Met coordinated hemes. Characterization of this protein aims to provide a broader basis for understanding this correlation. As EPR signals reflect the immediate surroundings of the unpaired electron, the HALS signal could indicate a difference in the electron transport pathway to and from the heme group.