Matching NLR Immune Receptors to Autoimmunity in camta3 Mutants Using Antimorphic NLR Alleles

To establish infection, pathogens deploy effectors to modify or remove host proteins. Plant immune receptors with nucleotide-binding, leucine-rich repeat domains (NLRs) detect these modifications and trigger immunity. Plant NLRs thus guard host "guardians." A corollary is that autoimmunity may result from inappropriate NLR activation because mutations in plant guardees could trigger corresponding NLR guards. To explore these hypotheses, we expressed 108 dominant-negative (DN) Arabidopsis NLRs in various lesion mimic mutants, including camta3, which exhibits autoimmunity. CAMTA3 was previously described as a negative regulator of immunity, and we find that autoimmunity in camta3 is fully suppressed by expressing DNs of two NLRs, DSC1 and DSC2. Additionally, expression of either NLR triggers cell death that can be suppressed by CAMTA3 expression. These findings support a model in which DSC1 and DSC2 guard CAMTA3, and they suggest that other negative regulators of immunity may similarly represent guardees.
CRISPR/Cas9 (clustered regularly interspaced palindromic repeats and the associated protein Cas9) techniques have made genome engineering and transcriptional reprogramming studies more advanced and cost-effective. For metabolic engineering purposes, the CRISPR-based tools have been applied to single and multiplex pathway modifications and transcriptional regulations. The effectiveness of these tools allows researchers to implement genome-wide perturbations, test model-guided genome editing strategies, and perform transcriptional reprogramming perturbations in a more advanced manner than previously possible. In this mini-review we highlight recent studies adopting CRISPR/Cas9 for systems-level perturbations and model-guided metabolic engineering.

General information
State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, Synthetic Biology Tools for Yeast
Authors: Jakociunas, T. (Intern), Jensen, M. K. (Intern), Keasling, J. (Intern)
Pages: 134-140
Publication date: 2017
Main Research Area: Technical/natural sciences

Publication information
Journal: Current Opinion in Biotechnology
Volume: 46
ISSN (Print): 0958-1669
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 8.55 SJR 3.331 SNIP 2.1
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 3.113 SNIP 2.143 CiteScore 7.99
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 3.271 SNIP 2.068 CiteScore 7.45
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 3.322 SNIP 2.198 CiteScore 7.93
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
Transcriptional reprogramming in yeast using dCas9 and combinatorial gRNA strategies

Transcriptional reprogramming is a fundamental process of living cells in order to adapt to environmental and endogenous cues. In order to allow flexible and timely control over gene expression without the interference of native gene expression machinery, a large number of studies have focused on developing synthetic biology tools for orthogonal control of transcription. Most recently, the nuclease-deficient Cas9 (dCas9) has emerged as a flexible tool for controlling activation and repression of target genes, by the simple RNA-guided positioning of dCas9 in the vicinity of the target gene transcription start site. In this study we compared two different systems of dCas9-mediated transcriptional reprogramming, and applied them to genes controlling two biosynthetic pathways for biobased production of isoprenoids and triacylglycerols (TAGs) in baker's yeast Saccharomyces cerevisiae. By testing 101 guide-RNA (gRNA) structures on a total of 14 different yeast promoters, we identified the best-performing combinations based on reporter assays. Though a larger number of gRNA-promoter combinations do not perturb gene expression, some gRNAs support expression perturbations up to ~threefold. The best-performing gRNAs were used for single and multiplex reprogramming strategies for redirecting flux related to isoprenoid production and optimization of TAG profiles. From these studies, we identified both constitutive and inducible multiplex reprogramming strategies enabling significant changes in isoprenoid production and increases in TAG. Taken together, we show similar performance for a constitutive and an inducible dCas9 approach, and identify multiplex gRNA designs that can significantly perturb isoprenoid production and TAG profiles in yeast without editing the genomic context of the target genes. We also identify a large number of gRNA positions in 14 native yeast target promoters that do not affect expression, suggesting the need for further optimization of gRNA design tools and dCas9 engineering.

General information
CRISPR/Cas9 advances engineering of microbial cell factories

One of the key drivers for successful metabolic engineering in microbes is the efficacy by which genomes can be edited. As such there are many methods to choose from when aiming to modify genomes, especially those of model organisms like yeast and bacteria. In recent years, clustered regularly interspaced palindromic repeats (CRISPR) and its associated proteins (Cas) have become the method of choice for precision genome engineering in many organisms due to their orthogonality, versatility and efficacy. Here we review the strategies adopted for implementation of RNA-guided CRISPR/Cas9 genome editing with special emphasis on their application for metabolic engineering of yeast and bacteria. Also, examples of how nuclease-deficient Cas9 has been applied for RNA-guided transcriptional regulation of target genes will be reviewed, as well as tools available for computer-aided design of guide-RNAs will be highlighted. Finally, this review will provide a perspective on the immediate challenges and opportunities foreseen by the use of CRISPR/Cas9 genome engineering and regulation in the context of metabolic engineering.
Saccharomyces cerevisiae is an established industrial host for production of recombinant proteins, fuels and chemicals. To enable stable integration of multiple marker-free overexpression cassettes in the genome of S. cerevisiae, we have developed a vector toolkit EasyClone-MarkerFree. The integration of linearized expression cassettes into defined genomic loci is facilitated by CRISPR/Cas9. Cas9 is recruited to the chromosomal location by specific guide RNAs (gRNAs) expressed from a set of gRNA helper vectors. Using our genome engineering vector suite, single and triple insertions are obtained with 90–100% and 60–70% targeting efficiency, respectively. We demonstrate application of the vector toolkit by constructing a haploid laboratory strain (CEN.PK113-7D) and a diploid industrial strain (Ethanol Red) for production of 3-hydroxypropionic acid, where we tested three different acetyl-CoA supply strategies, requiring overexpression of three to six genes each. Among the tested strategies was a bacterial cytosolic pyruvate dehydrogenase complex, which was integrated into the genome in a single transformation. The publicly available EasyClone-MarkerFree vector suite allows for facile and highly standardized genome engineering, and should be of particular interest to researchers working on yeast chassis with limited markers available.

**General information**

State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, Yeast Metabolic Engineering, Synthetic Biology Tools for Yeast, Research Groups, Applied Metabolic Engineering, Chalmers University of Technology
Number of pages: 11
Engineering an NADPH/NADP⁺ Redox Biosensor in Yeast

Genetically encoded biosensors have emerged as powerful tools for timely and precise in vivo evaluation of cellular metabolism. In particular, biosensors that can couple intercellular cues with downstream signaling responses are currently attracting major attention within health science and biotechnology. Still, there is a need for bioprospecting and engineering of more biosensors to enable real-time monitoring of specific cellular states and controlling downstream actuation. In this
study, we report the engineering and application of a transcription factor-based NADPH/NADP$^+$ redox biosensor in the budding yeast *Saccharomyces cerevisiae*. Using the biosensor, we are able to monitor the cause of oxidative stress by chemical induction, and changes in NADPH/NADP$^+$ ratios caused by genetic manipulations. Because of the regulatory potential of the biosensor, we also show that the biosensor can actuate upon NADPH deficiency by activation of NADPH regeneration. Finally, we couple the biosensor with an expression of dosage-sensitive genes (DSGs) and thereby create a novel tunable sensor-selector useful for synthetic selection of cells with higher NADPH/NADP$^+$ ratios from mixed cell populations. We show that the combination of exploitation and rational engineering of native signaling components is applicable for diagnosis, regulation, and selection of cellular redox states.

**General information**
State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, Synthetic Biology Tools for Yeast
Authors: Zhang, J. (Intern), Sonnenschein, N. (Intern), Pihl, T. P. B. (Intern), Pedersen, K. R. (Intern), Jensen, M. K. (Intern), Keasling, J. (Intern)
Number of pages: 11
Pages: 1546–1556
Publication date: 2016
Main Research Area: Technical/natural sciences

**Publication information**
Journal: A C S Synthetic Biology
Volume: 5
Issue number: 12
ISSN (Print): 2161-5063
Ratings:
Web of Science (2017): Indexed Yes
Scopus rating (2016): CiteScore 4.7 SJR 2.736 SNIP 1.024
Web of Science (2016): Indexed yes
Scopus rating (2015): SJR 2.269 SNIP 1.049 CiteScore 4.41
Web of Science (2015): Indexed yes
Scopus rating (2014): SJR 3.783 SNIP 1.219 CiteScore 3.84
Web of Science (2014): Indexed yes
Scopus rating (2013): SJR 1.796 SNIP 0.859 CiteScore 3.42
ISI indexed (2013): ISI indexed yes
ISI indexed (2012): ISI indexed no
Original language: English
Redox, Biosensor, Dosage-sensitive genes, Yeast
DOIs: 10.1021/acssynbio.6b00135
Source: FindIt
Source-ID: 2306572106
Publication: Research - peer-review › Journal article – Annual report year: 2016

**Engineering of synthetic, stress-responsive yeast promoters**
Advances in synthetic biology and our understanding of the rules of promoter architecture have led to the development of diverse synthetic constitutive and inducible promoters in eukaryotes and prokaryotes. However, the design of promoters inducible by specific endogenous or environmental conditions is still rarely undertaken. In this study, we engineered and characterized a set of strong, synthetic promoters for budding yeast *Saccharomyces cerevisiae* that are inducible under acidic conditions (pH≤ 3). Using available expression and transcription factor binding data, literature on transcriptional regulation, and known rules of promoter architecture we improved the low-pH performance of the YGP1 promoter by modifying transcription factor binding sites in its upstream activation sequence. The engineering strategy outlined for the YGP1 promoter was subsequently applied to create a response to low pH in the unrelated CCW14 promoter. We applied our best promoter variants to low-pH fermentations, enabling tenfold increased production of lactic acid compared to titres obtained with the commonly used, native TEF$^+$ promoter. Our findings outline and validate a general strategy to iteratively design and engineer synthetic yeast promoters inducible to environmental conditions or stresses of interest.

**General information**
State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, Synthetic Biology Tools for Yeast, iLoop, Yeast Cell Factories, Chalmers University of Technology
Authors: Rajkumar, A. S. (Intern), Liu, G. (Ekstern), Bergenholm, D. (Ekstern), Arsovska, D. (Intern), Kristensen, M. (Intern), Nielsen, J. (Intern), Jensen, M. K. (Intern), Keasling, J. (Intern)
Number of pages: 12
Engineering prokaryotic transcriptional activators as metabolite biosensors in yeast

Whole-cell biocatalysts have proven a tractable path toward sustainable production of bulk and fine chemicals. Yet the screening of libraries of cellular designs to identify best-performing biocatalysts is most often a low-throughput endeavor. For this reason, the development of biosensors enabling real-time monitoring of production has attracted attention. Here we applied systematic engineering of multiple parameters to search for a general biosensor design in the budding yeast Saccharomyces cerevisiae based on small-molecule binding transcriptional activators from the prokaryote superfamily of LysR-type transcriptional regulators (LTTRs). We identified a design supporting LTTR-dependent activation of reporter gene expression in the presence of cognate small-molecule inducers. As proof of principle, we applied the biosensors for in vivo screening of cells producing naringenin or cis,cis-muconic acid at different levels, and found that reporter gene output correlated with production. The transplantation of prokaryotic transcriptional activators into the eukaryotic chassis illustrates the potential of a hitherto untapped biosensor resource useful for biotechnological applications.

General information

State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, Synthetic Biology Tools for Yeast, Research Groups, Yeast Metabolic Engineering, iLoop, Bacterial Synthetic Biology, Technical University of Denmark, Evolva Biotech A/S, Evolva SA
Number of pages: 10
Pages: 951-958
Publication date: 2016
Main Research Area: Technical/natural sciences

Publication information

Journal: Nature Chemical Biology
Volume: 12
ISSN (Print): 1552-4450
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 2
Scopus rating (2016): SJR 8.778 SNIP 3.056 CiteScore 9.41
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 7.98 SNIP 2.821 CiteScore 9.11
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 7.136 SNIP 2.785 CiteScore 8.78
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 6.84 SNIP 2.894 CiteScore 8.55
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 2
CasEMBLR: Cas9-Facilitated Multiloci Genomic Integration of in Vivo Assembled DNA Parts in *Saccharomyces cerevisiae*

Homologous recombination (HR) in *Saccharomyces cerevisiae* has been harnessed for both plasmid construction and chromosomal integration of foreign DNA. Still, native HR machinery is not efficient enough for complex and marker-free genome engineering required for modern metabolic engineering. Here, we present a method for marker-free multiloci integration of *in vivo* assembled DNA parts. By the use of CRISPR/Cas9-mediated one-step double-strand breaks at single, double and triple integration sites we report the successful *in vivo* assembly and chromosomal integration of DNA parts. We call our method CasEMBLR and validate its applicability for genome engineering and cell factory development in two ways: (i) introduction of the carotenoid pathway from 15 DNA parts into three targeted loci, and (ii) creation of a tyrosine production strain using ten parts into two loci, simultaneously knocking out two genes. This method complements and improves the current set of tools available for genome engineering in *S. cerevisiae*.

**General information**

State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, Synthetic Biology Tools for Yeast, Yeast Metabolic Engineering, Yeast Cell Factories, Bacterial Cell Factories, Research Groups, Bacterial Cell Factory Optimization
Number of pages: 9
Pages: 1226-1234
Publication date: 2015
Main Research Area: Technical/natural sciences

**Publication Information**

Journal: A C S Synthetic Biology
Volume: 4
Issue number: 11
ISSN (Print): 2161-5063
Ratings:

Web of Science (2017): Indexed Yes
Scopus rating (2016): CiteScore 4.7 SJR 2.736 SNIP 1.024
Web of Science (2016): Indexed yes
Scopus rating (2015): SJR 2.269 SNIP 1.049 CiteScore 4.41
Web of Science (2015): Indexed yes
Scopus rating (2014): SJR 3.783 SNIP 1.219 CiteScore 3.84
Web of Science (2014): Indexed yes
CrEdit: CRISPR mediated multi-loci gene integration in Saccharomyces cerevisiae

Background: One of the bottlenecks in production of biochemicals and pharmaceuticals in Saccharomyces cerevisiae is stable and homogeneous expression of pathway genes. Integration of genes into the genome of the production organism is often a preferred option when compared to expression from episomal vectors. Existing approaches for achieving stable simultaneous genome integrations of multiple DNA fragments often result in relatively low integration efficiencies and furthermore rely on the use of selection markers. Results: Here, we have developed a novel method, CrEdit (CRISPR/Cas9 mediated genome Editing), which utilizes targeted double strand breaks caused by CRISPR/Cas9 to significantly increase the efficiency of homologous integration in order to edit and manipulate genomic DNA. Using CrEdit, the efficiency and locus specificity of targeted genome integrations reach close to 100% for single gene integration using short homology arms down to 60 base pairs both with and without selection. This enables direct and cost efficient inclusion of homology arms in PCR primers. As a proof of concept, a non-native beta-carotene pathway was reconstructed in S. cerevisiae by simultaneous integration of three pathway genes into individual intergenic genomic sites. Using longer homology arms, we demonstrate highly efficient and locus-specific genome integration even without selection with up to 84% correct clones for simultaneous integration of three gene expression cassettes. Conclusions: The CrEdit approach enables fast and cost effective genome integration for engineering of S. cerevisiae. Since the choice of the targeting sites is flexible, CrEdit is a powerful tool for diverse genome engineering applications.
Development of biosensors and their application in metabolic engineering

In a sustainable bioeconomy, many commodities and high value chemicals, including pharmaceuticals, will be manufactured using microbial cell factories from renewable feedstocks. These cell factories can be efficiently generated by constructing libraries of diversified genomes followed by screening for the desired phenotypes. However, methods available for microbial genome diversification far exceed our ability to screen and select for those variants with optimal performance. Genetically encoded biosensors have shown the potential to address this gap, given their ability to respond to small molecule binding and ease of implementation with high-throughput analysis. Here we describe recent progress in biosensor development and their applications in a metabolic engineering context. We also highlight examples of how biosensors can be integrated with synthetic circuits to exert feedback regulation on the metabolism for improved performance of cell factories.

General information
State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, Synthetic Biology Tools for Yeast
Authors: Zhang, J. (Intern), Jensen, M. K. (Intern), Keasling, J. (Intern)
Number of pages: 8
Publication date: 2015
Multiplex metabolic pathway engineering using CRISPR/Cas9 in *Saccharomyces cerevisiae*

CRISPR/Cas9 is a simple and efficient tool for targeted and marker-free genome engineering. Here, we report the development and successful application of a multiplex CRISPR/Cas9 system for genome engineering of up to 5 different genomic loci in one transformation step in baker's yeast *Saccharomyces cerevisiae*. To assess the specificity of the tool we employed genome re-sequencing to screen for off-target sites in all single knock-out strains targeted by different gRNAs. This extensive analysis identified no more genome variants in CRISPR/Cas9 engineered strains compared to wild-type reference strains. We applied our genome engineering tool for an exploratory analysis of all possible single, double, triple, quadruple and quintuple gene disruption combinations to search for strains with high mevalonate production, a key intermediate for the industrially important isoprenoid biosynthesis pathway. Even though we did not overexpress any genes in the mevalonate pathway, this analysis identified strains with mevalonate titers greater than 41-fold compared to the wild-type strain. Our findings illustrate the applicability of this highly specific and efficient multiplex genome engineering approach to accelerate functional genomics and metabolic engineering efforts. (C) 2015 International
Recent applications of synthetic biology tools for yeast metabolic engineering

The last 20 years of metabolic engineering has enabled bio-based production of fuels and chemicals from renewable carbon sources using cost-effective bioprocesses. Much of this work has been accomplished using engineered microorganisms that act as chemical factories. Although the time required to engineer microbial chemical factories has steadily decreased, improvement is still needed. Through the development of synthetic biology tools for key microbial hosts, it should be possible to further decrease the development times and improve the reliability of the resulting microorganism. Together with continuous decreases in price and improvements in DNA synthesis, assembly and sequencing, synthetic biology tools will rationalize time-consuming strain engineering, improve control of metabolic fluxes, and diversify screening assays for cellular metabolism. This review outlines some recently developed synthetic biology tools and their application to improve production of chemicals and fuels in yeast. Finally, we provide a perspective for the challenges that lie ahead.

General information
State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, Bacterial Cell Factories, Synthetic Biology Tools for Yeast
Authors: Jensen, M. K. (Intern), Keasling, J. (Intern)
Number of pages: 10
Publication date: 2015
Main Research Area: Technical/natural sciences

Publication information
Journal: FEMS Yeast Research
Volume: 15
Issue number: 1
ISSN (Print): 1567-1356
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.51 SJR 1.146 SNIP 0.847
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.193 SNIP 0.72 CiteScore 2.56
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.076 SNIP 0.838 CiteScore 2.37
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.225 SNIP 0.863 CiteScore 2.5
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.187 SNIP 0.844 CiteScore 2.56
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Transcriptome and genome size analysis of the venus flytrap

The insectivorous Venus flytrap (Dionaea muscipula) is renowned from Darwin's studies of plant carnivory and the origins of species. To provide tools to analyze the evolution and functional genomics of D. muscipula, we sequenced a normalized cDNA library synthesized from mRNA isolated from D. muscipula flowers and traps. Using the Oases transcriptome assembler 79,165,657 quality trimmed reads were assembled into 80,806 cDNA contigs, with an average length of 679 bp and an N50 length of 1,051 bp. A total of 17,047 unique proteins were identified, and assigned to Gene Ontology (GO) and classified into functional categories. A total of 15,547 full-length cDNA sequences were identified, from which open reading frames were detected in 10,941. Comparative GO analyses revealed that D. muscipula is highly represented in molecular functions related to catalytic, antioxidant, and electron carrier activities. Also, using a single copy sequence PCR-based method, we estimated that the genome size of D. muscipula is approx. 3 Gb. Our genome size estimate and transcriptome analyses will contribute to future research on this fascinating, monotypic species and its heterotrophic adaptations.

General information

State: Published
Organisations: Department of Systems Biology, Center for Biological Sequence Analysis, University of Copenhagen
Authors: Jensen, M. K. (Intern), Vogt, J. K. (Intern), Bressendorff, S. (Ekstern), Seguin-Orlando, A. (Ekstern), Petersen, M. (Ekstern), Sicheritz-Pontén, T. (Intern), Mundy, J. (Ekstern)
Number of pages: 13
Publication date: 2015
Main Research Area: Technical/natural sciences

Publication information

Journal: P L o S One
Volume: 10
Issue number: 4
Article number: e0123887
ISSN (Print): 1932-6203
Ratings:
BFI (2017): BFI-level 1
A DNA-binding-site landscape and regulatory network analysis for NAC transcription factors in Arabidopsis thaliana.

Target gene identification for transcription factors is a prerequisite for the systems wide understanding of organismal behaviour. NAM-ATAF1/2-CUC2 (NAC) transcription factors are amongst the largest transcription factor families in plants, yet limited data exist from unbiased approaches to resolve the DNA-binding preferences of individual members. Here, we present a TF-target gene identification workflow based on the integration of novel protein binding microarray data with gene expression and multi-species promoter sequence conservation to identify the DNA-binding specificities and the gene regulatory networks of 12 NAC transcription factors. Our data offer specific single-base resolution fingerprints for most TFs studied and indicate that NAC DNA-binding specificities might be predicted from their DNA-binding domain's sequence. The developed methodology, including the application of complementary functional genomics filters, makes it possible to translate, for each TF, protein binding microarray data into a set of high-quality target genes. With this approach, we confirm NAC target genes reported from independent in vivo analyses. We emphasize that candidate target gene sets together with the workflow associated with functional modules offer a strong resource to unravel the regulatory potential of NAC genes and that this workflow could be used to study other families of transcription factors.

General information
State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, Synthetic Biology Tools for Yeast, Department of Systems Biology, Center for Biological Sequence Analysis, Ghent University, University of Copenhagen
ATAF1 transcription factor directly regulates abscisic acid biosynthetic gene NCED3 in Arabidopsis thaliana.

ATAF1, an Arabidopsis thaliana NAC transcription factor, plays important roles in plant adaptation to environmental stress and development. To search for ATAF1 target genes, we used protein binding microarrays and chromatin-immunoprecipitation (ChIP). This identified T[A,C,G]CGT[A,G] and TT[A,C,G]CGT as ATAF1 consensus binding sequences. Co-expression analysis across publicly available microarray experiments identified 25 genes co-expressed with ATAF1. The promoter regions of ATAF1 co-expressors were significantly enriched for ATAF1 binding sites, and TTGCGTA was identified in the promoter of the key abscisic acid (ABA) phytohormone biosynthetic gene NCED3. ChIP-qPCR and expression analysis showed that ATAF1 binding to the NCED3 promoter correlated with increased NCED3 expression and ABA hormone levels. These results indicate that ATAF1 regulates ABA biosynthesis.

General information
State: Published
Organisations: Department of Systems Biology, Center for Biological Sequence Analysis, Max Planck Institute, University of Exeter, University of Copenhagen
Authors: Jensen, M. K. (Intern), Lindemose, S. (Forskerdatabase), De Masi, F. (Intern), Reimer, J. J. (Ekstern), Nielsen, M. (Ekstern), Perera, V. (Ekstern), Workman, C. (Intern), Turck, F. (Ekstern), Grant, M. R. (Ekstern), Mundy, J. (Forskerdatabase), Petersen, M. (Ekstern), Skriver, K. (Forskerdatabase)
Pages: 321-327
Publication date: 2013
Main Research Area: Technical/natural sciences
Structure, Function and Networks of Transcription Factors Involved in Abiotic Stress Responses.

Transcription factors (TFs) are master regulators of abiotic stress responses in plants. This review focuses on TFs from seven major TF families, known to play functional roles in response to abiotic stresses, including drought, high salinity, high osmolarity, temperature extremes and the phytohormone ABA. Although ectopic expression of several TFs has improved abiotic stress tolerance in plants, fine-tuning of TF expression and protein levels remains a challenge to avoid crop yield loss. To further our understanding of TFs in abiotic stress responses, emerging gene regulatory networks based on TFs and their direct targets genes are presented. These revealed components shared between ABA-dependent and independent signaling as well as abiotic and biotic stress signaling. Protein structure analysis suggested that TFs hubs of large interactomes with protein intrinsic disorder (ID), referring to their lack of fixed tertiary structures. ID is now an emerging topic in plant science. Furthermore, the importance of the ubiquitin-proteasome protein degradation systems and modification by sumoylation is also apparent from the interactomes. Therefore; TF interaction partners such as E3 ubiquitin ligases and TF regions with ID represent future targets for engineering improved abiotic stress tolerance in crops.

General information
State: Published
Organisations: University of Copenhagen
Authors: Lindemose, S. (Forskerdatabase), O'Shea, C. (Ekstern), Jensen, M. K. (Intern), Skriver, K. (Forskerdatabase)
Pages: 5842
Publication date: 2013
Main Research Area: Technical/natural sciences

Publication information
Journal: International Journal of Molecular Sciences (Online)
Volume: 14
Issue number: 3
ISSN (Print): 1661-6596
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 1.202 SNIP 1.147 CiteScore 3.73
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.156 SNIP 1.127 CiteScore 3.37
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.005 SNIP 1.157 CiteScore 3.06
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.762 SNIP 1.111 CiteScore 2.83
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.766 SNIP 1.197 CiteScore 2.86
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.781 SNIP 1.169 CiteScore 2.95
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.648 SNIP 0.915
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.357 SNIP 0.635
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 0.306 SNIP 0.4
Scopus rating (2007): SJR 0.26 SNIP 0.437
Scopus rating (2006): SJR 0.355 SNIP 0.565
Scopus rating (2005): SJR 0.666 SNIP 0.793
Ca\textsuperscript{2+} Induces Spontaneous Dephosphorylation of a Novel P5A-type ATPase

P5 ATPases constitute the least studied group of P-type ATPases, an essential family of ion pumps in all kingdoms of life. Although P5 ATPases are present in every eukaryotic genome analyzed so far, they have remained orphan pumps, and their biochemical function is obscure. We show that a P5A ATPase from barley, HvP5A1, locates to the endoplasmic reticulum and is able to rescue knock-out mutants of P5A genes in both Arabidopsis thaliana and Saccharomyces cerevisiae. HvP5A1 spontaneously forms a phosphorylated reaction cycle intermediate at the catalytic residue Asp-488, whereas, among all plant nutrients tested, only Ca\textsuperscript{2+} triggers dephosphorylation. Remarkably, Ca\textsuperscript{2+}-induced dephosphorylation occurs at high apparent [Ca\textsuperscript{2+}] (K\textsubscript{i} = 0.25 mM) and is independent of the phosphatase motif of the pump and the putative binding site for transported ligands located in M4. Taken together, our results rule out that Ca\textsuperscript{2+} is a transported substrate but indicate the presence of a cytosolic low affinity Ca\textsuperscript{2+}-binding site, which is conserved among P-type pumps and could be involved in pump regulation. Our work constitutes the first characterization of a P5 ATPase phosphoenzyme and points to Ca\textsuperscript{2+} as a modifier of its function.

General information
State: Published
Organisations: University of Copenhagen
Authors: Sorensen, D. M. (Ekstern), Moller, A. B. (Ekstern), Jakobsen, M. K. (Ekstern), Jensen, M. K. (Intern), Vangheluwe, P. (Ekstern), Buch-Pedersen, M. J. (Forskerdatabase), Palmgren, M. G. (Ekstern)
Pages: 28336-28348
Publication date: 2012
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Biological Chemistry
Volume: 287
Issue number: 34
ISSN (Print): 0021-9258

Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.17 SJR 2.755 SNIP 1.125
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 3.121 SNIP 1.184 CiteScore 4.4
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 3.254 SNIP 1.222 CiteScore 4.5
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 3.369 SNIP 1.231 CiteScore 4.87
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 3.361 SNIP 1.244 CiteScore 4.97
Order by disorder in plant signaling

Protein intrinsic disorder (ID), referring to the lack of a fixed tertiary structure, is an emerging topic in plant science. Proteins with ID challenge our perception of protein interactions because of their malleable behavior. They are abundant in highly regulated processes such as cellular signaling and transcription, where they exploit the flexibility of ID. In this opinion article we highlight trends in the field of protein ID and discuss its implications for interactions between plant transcription factors (TFs) and the cellular signaling hub protein RADICAL-INDUCED CELL DEATH 1 (RCD1). We envision RCD1–TF interactions as models for translating knowledge of ID-based interactions in vitro to the organismal level in vivo, and urge increased focus on ID in basic plant research and agricultural sciences.
Regulation of basal resistance by a powdery mildew‐induced cysteine‐rich receptor‐like protein kinase in barley

The receptor‐like protein kinases (RLKs) constitute a large and diverse group of proteins controlling numerous plant physiological processes, including development, hormone perception and stress responses. The cysteine‐rich RLKs (CRKs) represent a prominent subfamily of transmembrane‐anchored RLKs. We have identified a putative barley (Hordeum vulgare) CRK gene family member, designated HvCRK1. The mature putative protein comprises 645 amino acids, and includes a putative receptor domain containing two characteristic ‘domain 26 of unknown function’ (duf26) domains in the N-terminal region, followed by a rather short 17‐amino‐acid transmembrane domain, which includes an AAA motif, two features characteristic of endoplasmic reticulum (ER)‐targeted proteins and, finally, a characteristic putative protein kinase domain in the C‐terminus. The HvCRK1 transcript was isolated from leaves inoculated with the biotrophic fungal pathogen Blumeria graminis f.sp. hordei (Bgh). HvCRK1 transcripts were observed to accumulate transiently following Bgh inoculation of susceptible barley. Transient silencing of HvCRK1 expression in bombarded epidermal cells led to enhanced resistance to Bgh, but did not affect R‐gene‐mediated resistance. Silencing of HvCRK1 phenocopied the effective penetration resistance found in mlo‐resistant barley plants, and the possible link between HvCRK1 and MLO was substantiated by the fact that HvCRK1 induction on Bgh inoculation was dependent on Mlo. Finally, using both experimental and in silico approaches, we demonstrated that HvCRK1 localizes to the ER of barley cells. The negative effect on basal resistance against Bgh and the functional aspects of MLO‐ and ER‐localized HvCRK1 signalling on Bgh inoculation are discussed.

General information
State: Published
Organisations: Institute of Plant Genetics and Crop Plant Research, University of Copenhagen
Pages: 135-147
Publication date: 2012
Main Research Area: Technical/natural sciences

Publication information
Journal: Molecular Plant Pathology
Volume: 13
Issue number: 2
ISSN (Print): 1464-6722
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.45 SJR 1.826 SNIP 1.536
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 2.142 SNIP 1.652 CiteScore 4.68
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 2.08 SNIP 1.714 CiteScore 4.48
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 2.083 SNIP 1.641 CiteScore 4.7
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.773 SNIP 1.58 CiteScore 4.09
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.875 SNIP 1.839 CiteScore 4.2
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 2
Senescence-associated Barley NAC (NAM, ATAF1,2, CUC) Transcription Factor Interacts with Radical-induced Cell Death 1 through a Disordered Regulatory Domain

Senescence in plants involves massive nutrient relocation and age-related cell death. Characterization of the molecular components, such as transcription factors (TFs), involved in these processes is required to understand senescence. We found that HvNAC005 and HvNAC013 of the plant-specific NAC (NAM, ATAF1,2, CUC) TF family are up-regulated during senescence in barley (Hordeum vulgare). Both HvNAC005 and HvNAC013 bound the conserved NAC DNA target sequence. Computational and biophysical analyses showed that both proteins are intrinsically disordered in their large C-terminal domains, which are transcription regulatory domains (TRDs) in many NAC TFs. Using motif searches and interaction studies in yeast we identified an evolutionarily conserved sequence, the LP motif, in the TRD of HvNAC013. This motif was sufficient for transcriptional activity. In contrast, HvNAC005 did not function as a transcriptional activator suggesting that an involvement of HvNAC013 and HvNAC005 in senescence will be different. HvNAC013 interacted with barley radical-induced cell death 1 (RCD1) via the very C-terminal part of its TRD, outside of the region containing the LP motif. No significant secondary structure was induced in the HvNAC013 TRD upon interaction with RCD1. RCD1 also interacted with regions dominated by intrinsic disorder in TFs of the MYB and basic helix-loop-helix families. We propose that RCD1 is a regulatory protein capable of interacting with many different TFs by exploiting their intrinsic disorder. In addition, we present the first structural characterization of NAC C-terminal domains and relate intrinsic disorder and sequence motifs to activity and protein-protein interactions.

General information
State: Published
Organisations: Aarhus University, University of Copenhagen
Authors: Kjaersgaard, T. (Ekstern), Jensen, M. K. (Intern), Christiansen, M. (Forskerdatabase), Gregersen, P. L. (Forskerdatabase), Kragelund, B. B. (Forskerdatabase), Skriver, K. (Forskerdatabase)
Pages: 35418-35429
Publication date: 2011
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Biological Chemistry
Volume: 286
Issue number: 41
ISSN (Print): 0021-9258
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.17 SJR 2.755 SNIP 1.125
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 3.121 SNIP 1.184 CiteScore 4.4
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 3.254 SNIP 1.222 CiteScore 4.5
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 3.369 SNIP 1.231 CiteScore 4.87
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 3.361 SNIP 1.244 CiteScore 4.97
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 3.495 SNIP 1.26 CiteScore 4.97
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 3.923 SNIP 1.342
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 4.158 SNIP 1.344
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 4.289 SNIP 1.375
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 4.277 SNIP 1.373
Web of Science (2007): Indexed yes
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 4.08 SNIP 1.347
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 4.273 SNIP 1.426
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 4.445 SNIP 1.422
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 4.435 SNIP 1.426
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 4.87 SNIP 1.528
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 5.301 SNIP 1.572
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 5.91 SNIP 1.632

Original language: English

age-related cell death, protein-protein interaction, senescence, Monocotyledones Angiospermae Spermatophyta Plantae (Angiosperms, Monocots, Plants, Spermatophytes, Vascular Plants) - Gramineae [25305] Hordeum vulgare species barley common, Plantae (Fungi, Microorganisms, Nonvascular Plants, Plants) - Fungi [15000] yeast common, Hordeum vulgare HvNAC005 gene [Gramineae], Hordeum vulgare HvNAC013 gene [Gramineae], DNA, NAC, transcription factor, 03502, Genetics - General, 03504, Genetics - Plant, 10062, Biochemistry studies - Nucleic acids, purines and pyrimidines, 24500, Gerontology, Biochemistry and Molecular Biophysics, AK251058 GenBank, EMBL, DDJB nucleotide sequence, AK376297.1 GenBank, EMBL, DDJB nucleotide sequence, FR846236 GenBank, EMBL, DDJB nucleotide sequence, Aging, Molecular Genetics

DOIs:
Environmental stresses on both animals and plants impose massive transcriptional perturbations. Successful adaptations to such stresses are being orchestrated by both activating and repressing effects of transcription factors on specific target genes. We have recently published a systematic characterization of members of the large NAC gene transcription factor family in the model weed Arabidopsis thaliana. Our analysis revealed interesting sub-groupings of the Arabidopsis NAC genes, relating structure and function. Here we present a meta-analysis revealing distinct temporal expression profiles of NAC genes upon stimuli with seven phytohormones. Our analysis could be a first indication of NAC-centered transcriptional networks, which coordinate timely hormonal signaling in plants.

The arabidopsis thaliana NAC transcription factor family: Structure-function relationships and determinants of ANAC019 stress signalling

TFs (transcription factors) are modular proteins minimally containing a DBD (DNA-binding domain) and a TRD (transcription regulatory domain). NAC [for NAM (no apical meristem), ATAF, CUC (cup-shaped cotyledon)] proteins comprise one of the largest plant TF families. They are key regulators of stress perception and developmental programmes, and most share an N-terminal NAC domain. On the basis of analyses of gene expression data and the
phylogeny of Arabidopsis thaliana NAC TFs we systematically decipher structural and functional specificities of the conserved NAC domains and the divergent C-termini. Nine of the ten NAC domains analysed bind a previously identified conserved DNA target sequence with a CGT[GA] core, although with different affinities. Likewise, all but one of the NAC proteins analysed is dependent on the C-terminal region for transactivational activity. In silico analyses show that the NAC TRDs contain group-specific sequence motifs and are characterized by a high degree of intrinsic disorder. Furthermore, ANAC019 was identified as a new positive regulator of ABA (abscisic acid) signalling, conferring ABA hypersensitivity when ectopically expressed in plants. Interestingly, ectopic expression of the ANAC019 DBD or TRD alone also resulted in ABA hypersensitivity. Expression of stress-responsive marker genes [COR47 (cold-responsive 47), RD29b (responsive-to-desiccation 29b) and ERD11 (early-responsive-to-dehydration 11)] were also induced by full-length and truncated ANAC019. Domain-swapping experiments were used to analyse the specificity of this function. Chimaeric proteins, where the NAC domain of ANAC019 was replaced with the analogous regions from other NAC TFs, also have the ability to positively regulate ABA signalling. In contrast, replacing the ANAC019 TRD with other TRDs abolished ANAC019-mediated ABA hypersensitivity. In conclusion, our results demonstrate that the biochemical and functional specificity of NAC TFs is associated with both the DBDs and the TRDs. © The Authors Journal compilation © 2010 Biochemical Society.

General information
State: Published
Organisations: University of Copenhagen
Authors: Jensen, M. K. (Intern), Kjaersgaard, T. (Ekstern), Nielsen, M. M. (Ekstern), Galberg, P. (Forskerdatabase), Petersen, K. (Ekstern), O’Shea, C. (Ekstern), Skriver, K. (Forskerdatabase)
Pages: 183-196
Publication date: 2010
Main Research Area: Technical/natural sciences

Publication information
Journal: Biochemical Journal
Volume: 426
Issue number: 2
ISSN (Print): 0264-6021
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 2.341 SNIP 0.99 CiteScore 3.63
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 2.56 SNIP 1.123 CiteScore 3.85
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 2.865 SNIP 1.216 CiteScore 4.25
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 3.056 SNIP 1.343 CiteScore 4.99
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 3.091 SNIP 1.417 CiteScore 5.01
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 3.004 SNIP 1.264 CiteScore 4.66
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 3.248 SNIP 1.287
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 3.184 SNIP 1.226
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 2.838 SNIP 1.163
Scopus rating (2007): SJR 2.683 SNIP 1.101
Web of Science (2007): Indexed yes
Transcriptional regulation by an NAC (NAM-ATAF1,2-CUC2) transcription factor attenuates ABA signalling for efficient basal defence towards Blumeria graminis f. sp. hordei in Arabidopsis

ATAF1 is a member of a largely uncharacterized plant-specific gene family encoding NAC transcription factors, and is induced in response to various abiotic and biotic stimuli in Arabidopsis thaliana. Previously, we showed that a mutant allele of ATAF1 compromises penetration resistance in Arabidopsis with respect to the non-host biotrophic pathogen Blumeria graminis f. sp. hordei (Bgh). In this study, we have used genome-wide transcript profiling to characterize signalling perturbations in ataf1 plants following Bgh inoculation. Comparative transcriptomic analyses identified an over-representation of abscisic acid (ABA)-responsive genes, including the ABA biosynthesis gene AAO3, which is significantly induced in ataf1 plants compared to wild-type plants following inoculation with Bgh. Additionally, we show that Bgh inoculation results in decreased endogenous ABA levels in an ATAF1-dependent manner, and that the ABA biosynthetic mutant aao3 showed increased penetration resistance to Bgh compared to wild-type plants. Furthermore, we show that ataf1 plants show ABA-hyposensitive phenotypes during seedling development and germination. Our data support a negative correlation between ABA levels and penetration resistance, and identify ATAF1 as a new stimuli-dependent attenuator of ABA signalling for the mediation of efficient penetration resistance in Arabidopsis upon Bgh attack.

General information
State: Published
Organisations: Stochastic Systems and Signals Group, Theory Section, Department of Systems Biology, Ecosystems, Biosystems Division, Risø National Laboratory for Sustainable Energy, University of Exeter, University of Copenhagen
Authors: Jensen, M. K. (Intern), Hagedorn, P. (Intern), De Torres-Zabala, M. (Ekstern), Grant, M. R. (Ekstern), Rung, J. H. (Ekstern), Collinge, D. B. (Ekstern), Lyngkjær, M. F. (Intern)
Pages: 867-880
Publication date: 2008
Main Research Area: Technical/natural sciences

Publication information
Journal: Plant Journal
Volume: 56
Issue number: 6
ISSN (Print): 0960-7412
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 2
Scopus rating (2016): SJR 3.351 SNIP 1.508 CiteScore 5.93
BFI (2015): BFI-level 2
The Hv NAC6 transcription factor: a positive regulator of penetration resistance in barley and Arabidopsis

Pathogens induce the expression of many genes encoding plant transcription factors, though specific knowledge of the biological function of individual transcription factors remains scarce. NAC transcription factors are encoded in plants by a gene family with proposed functions in both abiotic and biotic stress adaptation, as well as in developmental processes. In this paper, we provide convincing evidence that a barley NAC transcription factor has a direct role in regulating basal defence. The gene transcript was isolated by differential display from barley leaves infected with the biotrophic powdery mildew fungus, Blumeria graminis f.sp. hordei (Bgh). The full-length cDNA clone was obtained using 5'-RACE and termed HvNAC6, due to its high similarity to the rice homologue, OsNAC6. Gene silencing of HvNAC6 during Bgh inoculation compromises penetration resistance in barley epidermal cells towards virulent Bgh. Complementing the effect of HvNAC6 gene silencing, transient overexpression of HvNAC6 increases the occurrence of penetration resistant cells towards Bgh attack. Quantitative RT-PCR shows the early and transient induction of HvNAC6 in barley epidermis upon Bgh infection. Additionally, our results show that the Arabidopsis HvNAC6 homologue ATAF1 is also induced by Bgh and the ataf1-1 mutant line shows decreased penetration resistance to this non-host pathogen. Collectively, these data suggest a conserved role of HvNAC6 and ATAF1 in the regulation of penetration resistance in monocots and dicots, respectively.
ATAF1, expression profiles, NAC transcription factor, penetration resistance, Blumeria graminis f.sp hordei (Bgh), HvNAC6
Interactions between plant RING-H2 and plant-specific NAC (NAM/ATAF1/2/CUC2) proteins: RING-H2 molecular specificity and cellular localization

Numerous, highly conserved RING-H2 domains are found in the model plant Arabidopsis thaliana (thale cress). To characterize potential RING-H2 protein interactions, the small RING-H2 protein RHA2a was used as bait in a yeast two-hybrid screen. RHA2a interacted with one of the plant-specific NAC [NAM (no apical meristem), ATAF1/2, CUC2 (cup-shaped cotyledons 2)] transcription factors, here named ANAC (abscisic acid-responsive NAC). The core RING-H2 domain was sufficient for the interaction. The ability of 11 structurally diverse RING-H2 domains to interact with ANAC was then examined. Robust interaction was detected for three of the domains, suggesting multi-specificity for the interaction. The domains that interacted with ANAC contain a glutamic acid residue in a position corresponding to a proline in many RING-H2 domains. Conversion of this glutamic acid residue into proline in RHA2a decreased its ability to interact with ANAC, most likely by changing the interaction surface. This suggested that a short, divergent region in RING-H2 domains modulate interaction specificity. ANAC contains a degenerate bipartite nuclear localization signal (NLS), while RHG1a, also identified as an ANAC interaction partner, contains a basic NLS. Both signals localized beta-glucuronidase reporter fusions to the nucleus. N-terminally truncated RHA2a also directed nuclear localization, apparently dependent on basic amino acids in the RING-H2 domain. Nuclear co-localization of the RING-H2 proteins and ANAC may enable their interaction in vivo to regulate the activity of the ANAC transcription factor.
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 3.184 SNIP 1.226
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 2.838 SNIP 1.163
Scopus rating (2007): SJR 2.683 SNIP 1.101
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 2.809 SNIP 1.138
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 2.552 SNIP 1.094
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 2.565 SNIP 1.126
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 2.711 SNIP 1.144
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 2.657 SNIP 1.157
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 2.538 SNIP 1.172
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 2.411 SNIP 1.224
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 2.563 SNIP 1.237
Original language: English
Abscisic acid-responsive nitrogen assimilation control protein (NAC), Cellular localization signal, Plant model, RING-H2 target protein, Ubiquitination pathway
Source: FindIt
Source-ID: 108529820
Publication: Research - peer-review › Journal article – Annual report year: 2003

Projects:

**Engineering of Polyketide Synthases for Production of Polyketides in Saccharomyces cerevisiae**

Technical University of Denmark
Period: 01/09/2017 → 31/08/2020
Number of participants: 4
Phd Student:
Romero Suarez, David (Intern)
Supervisor:
Keasling, Jay (Intern)
Weber, Tilmann (Intern)
Main Supervisor:
Jensen, Michael Krogh (Intern)

**Financing sources**
Source: Internal funding (public)
Name of research programme: Forskningsrådsstipendium
Project: PhD

**Biosensor development and next-generation sequencing approaches for studying molecular evolution in bacteria**

Technical University of Denmark
Period: 01/08/2017 → 31/07/2020
Number of participants: 3
Phd Student:
Capucci, Silvia (Intern)
Supervisor:
Jensen, Michael Krogh (Intern)
Main Supervisor:
Nørholm, Morten (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU)
Project: PhD

Directed evolution of small-molecule receptors and enzymes
Technical University of Denmark
Period: 15/02/2017 → 14/02/2020
Number of participants: 4
Phd Student:
D'ambrosio, Vasil (Intern)
Supervisor:
Keasling, Jay (Intern)
Lassen, Lærke Marie Münter (Intern)
Main Supervisor:
Jensen, Michael Krogh (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Marie Curie (EU-stipendium)
Project: PhD

Characterized Parts Libraries & Pathway Evolver
Technical University of Denmark
Period: 01/12/2016 → 30/11/2019
Number of participants: 4
Phd Student:
Petersen, Søren Dalsgård (Intern)
Supervisor:
Hillson, Nathan J. (Ekstern)
Keasling, Jay (Intern)
Main Supervisor:
Jensen, Michael Krogh (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU)
Project: PhD

Predictive and Accelerated Metabolic Engineering Network
PAcMEN is a European training network, which offers excellent training in biotech research and innovation for 16 talented young scientists. PhD students will carry out cutting-edge research in metabolic engineering, modeling, systems and synthetic biology. In collaboration with industrial partners, they will create novel solutions for sustainable production of fuels and chemicals. The graduates will be prepared through research, business, and entrepreneurship training to launch their careers in industry or academia.

Novo Nordisk Foundation Center for Biosustainability
Yeast Cell Factories
Research Groups
Yeast Metabolic Engineering
Synthetic Biology Tools for Yeast
Eukaryotic Molecular Cell Biology
Period: 01/10/2016 → 30/09/2020
Number of participants: 10
Biotechnology
Acronym: PAcMEN
Project participant:
Lohmann, Ricarda (Intern)
Phd Student:
Dahlin, Jonathan (Intern)
Petersen, Søren Dalsgård (Intern)
Olsson, Helén Emelie (Intern)
D'ambrosio, Vasil (Intern)
Marella, Eko Roy (Intern)
Supervisor:
Borodina, Irina (Intern)
Jensen, Michael Krogh (Intern)
Mortensen, Uffe Hasbro (Intern)
Project Coordinator:
Nielsen, Jens (Intern)

Financing sources
Source: EU research programme (public)
Name of research programme: MSCA-ITN - Marie Skłodowska-Curie actions – International Training Networks
Web address: http://www.pacmen-itn.eu

Systems-level evolutionary pathway engineering in yeast through growth-coupled selection
Technical University of Denmark
Period: 01/10/2016 → 30/09/2019
Number of participants: 4
Phd Student:
Hansen, Anne Sofie Lærke (Intern)
Supervisor:
Jensen, Michael Krogh (Intern)
Sonnenschein, Nikolaus (Intern)
Main Supervisor:
Herrgard, Markus (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Fonde
Project: PhD

Genome engineering, reprogramming and evolution of yeast-based cell factories
Technical University of Denmark
Period: 01/11/2015 → 31/10/2018
Number of participants: 4
Phd Student:
Damgaard Jensen, Emil (Intern)
Supervisor:
Andersen, Mikael Rørdam (Intern)
Keasling, Jay (Intern)
Main Supervisor:
Jensen, Michael Krogh (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Offentlig finansiering
Project: PhD
New Genetically-Encoded Biosensors for Yeast Cell Factory Optimization

Technical University of Denmark
Period: 01/11/2015 → 31/10/2018
Number of participants: 4
Phd Student:
Ambri, Francesca (Intern)
Supervisor:
Andersen, Mikael Rørdam (Intern)
Keasling, Jay (Intern)
Main Supervisor:
Jensen, Michael Krogh (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Offentlig finansiering
Project: PhD

Yeast Strain and Process Development

Technical University of Denmark
Period: 01/09/2015 → 31/08/2018
Number of participants: 4
Phd Student:
Lis, Alicia Viktoria (Intern)
Supervisor:
Keasling, Jay (Intern)
Schneider, Konstantin (Intern)
Main Supervisor:
Jensen, Michael Krogh (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Anden EU-finansiering
Project: PhD

Standardization of DNA Vector Design-Processes

Department of Systems Biology
Period: 01/03/2013 → 21/04/2016
Number of participants: 6
Phd Student:
Cavaleiro, Mafalda (Intern)
Supervisor:
Nielsen, Alex Toftgaard (Intern)
Main Supervisor:
Nørholm, Morten (Intern)
Examiner:
Jensen, Michael Krogh (Intern)
Hillson, Nathan J. (Ekstern)
Nour-Eldin, Hussam Hassan (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Marie Curie (EU-stipendium)
Project: PhD

Development of a Platform Yeast Cell Factory for Production of Aromatic Chemicals

Department of Systems Biology
Period: 01/02/2013 → 30/09/2016
Number of participants: 6
Phd Student:
Rodriguez Prado, Edith Angelica (Intern)
Supervisor:
Borodina, Irina (Intern)
Main Supervisor:
Nielsen, Jens (Intern)
Examiner:
Jensen, Michael Krogh (Intern)
Olesen, Kjeld (Ekstern)
Penttilä, Merja Elisa (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Eksternt finansieret virksomhed

Relations
Publications:
Development of a yeast cell factory for production of aromatic secondary metabolites
Project: PhD

Biosensors and genetic circuits for improved cell factory development
Department of Systems Biology
Period: 15/12/2012 → 30/06/2016
Number of participants: 6
Phd Student:
Rugbjerg, Peter (Intern)
Supervisor:
Förster, Jochen (Intern)
Main Supervisor:
Sommer, Morten Otto Alexander (Intern)
Examiner:
Jensen, Michael Krogh (Intern)
Jensen, Niels Bjerg (Intern)
Suess, Beatrix (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU)

Relations
Publications:
Cell Factory Stability and Genetic Circuits for Improved Strain Development
Project: PhD

Activities:

Standardized Yeast Cell Prototyping
Period: 18 May 2015
Michael Krogh Jensen (Speaker)
Novo Nordisk Foundation Center for Biosustainability
Synthetic Biology Tools for Yeast

Related event
Copenhagen Bioscience Conference 2015: Cell Factories & Biosustainability
17/05/2015 → 21/05/2015
Non-Native Small-Molecule Biosensors for Screening and Selection of Cell Factories
Period: 11 Jan 2015
Michael Krogh Jensen (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
Synthetic Biology Tools for Yeast

Related event
international conference on Biomolecular Engineering
11/01/2015 → 14/01/2015
Austin, United States
Activity: Talks and presentations › Conference presentations