Identification of a short, highly conserved, motif required for picornavirus capsid precursor processing at distal sites

Many picornaviruses cause important diseases in humans and other animals including poliovirus, rhinoviruses (causing the common cold) and foot-and-mouth disease virus (FMDV). These small, non-enveloped viruses comprise a positive-stranded RNA genome (ca. 7-9 kb) enclosed within a protein shell composed of 60 copies of three or four different capsid proteins. For the aphthoviruses (e.g. FMDV) and cardioviruses, the capsid precursor, P1-2A, is cleaved by the 3C protease (3Cpro) to generate VP0, VP3 and VP1 plus 2A. For enteroviruses, e.g. poliovirus, the capsid precursor is P1 alone, which is cleaved by the 3C protease to generate just VP0, VP3 and VP1. The sequences required for correct processing of the FMDV capsid protein precursor in mammalian cells were analyzed. Truncation of the P1-2A precursor from its C-terminus showed that loss of the 2A peptide (18 residues long) and 27 residues from the C-terminus of VP1 (211 residues long) resulted in a precursor that cannot be processed by 3Cpro although it still contained two unmodified internal cleavage sites (VP0/VP3 and VP3/VP1 junctions). Furthermore, introduction of small deletions within P1-2A identified residues 185-190 within VP1 as being required for 3Cpro-mediated processing and for optimal accumulation of the precursor. Within this C-terminal region of VP1, five of these residues (YCPRP), are very highly conserved in all FMDVs and are also conserved amongst other picornaviruses. Mutant FMDV P1-2A precursors with single amino acid substitutions within this motif were highly resistant to cleavage at internal junctions. Such substitutions also abrogated virus infectivity. These results can explain earlier observations that loss of the C-terminus (including the conserved motif) from the poliovirus capsid precursor conferred resistance to processing. Thus, this motif seems essential for maintaining the correct structure of picornavirus capsid precursors prior to processing and subsequent capsid assembly; it may represent a site that interacts with cellular chaperones.
A reply to "A comment on "Inter-laboratory study to characterize the detection of serum antibodies against porcine epidemic diarrhoea virus"
I have read with very much interest the paper of Strandbygaard et al. recently published in Veterinary Microbiology (2016, 197, 151–160). As a veterinary diagnostic laboratory Biovet recognizes the interest of such kind of study which evaluates the performances of diagnostic assays in various laboratories.
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Cleavages at the three junctions within the foot-and-mouth disease virus capsid precursor (P1–2A) by the 3C protease are mutually independent

The foot-and-mouth disease virus capsid precursor, P1–2A, is cleaved by the 3C protease (3Cpro) to VP0, VP3, VP1 and 2A. The P1–2A precursor (wt or mutant) was expressed alone or with 3Cpro and processing of P1–2A was determined. The VP2 K217R and VP3 I2P substitutions (near the VP0/VP3 junction) strongly reduced the processing at this junction by 3Cpro while the substitution VP2 K217E blocked cleavage. At the VP3/VP1 junction, the substitutions VP3 Q2221P and VP1 T1P each severely inhibited processing at this site. Blocking cleavage at either junction did not prevent processing elsewhere in P1–2A. These modifications were also introduced into full-length FMDV RNA; only wt and the VP2 K217R mutant were viable. uncleaved VP0-VP3 and the processed products were observed within cells infected with the mutant virus. The VP0-VP3 was not incorporated into empty capsids or virus particles. The three junctions within P1–2A are processed by 3Cpro independently.

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Complete genome sequence of an African swine fever virus (ASFV POL/2015/Podlaskie) determined directly from pig erythrocyte-associated nucleic acid

African swine fever (ASF) is an important disease of domestic pigs and wild boar. The disease is caused by African swine fever virus (ASFV). In 2014, ASFV was introduced into Eastern Europe, and it has since then continued to spread within various Eastern European countries. Investigating differences in sequences between ASFV isolates may be a valuable tool to understand differences in virulence among them, however currently, no complete genome sequences of the viruses responsible for the Eastern European outbreaks have been reported. In this study, the complete genome sequence of a highly virulent ASFV was determined directly from erythrocyte-associated nucleic acids obtained from a pig experimentally infected with an isolate from Poland (ASFV POL/2015/Podlaskie). The sequence (ca. 189 kb) of this recent European ASFV showed 95 nt differences (99.95 % identity) from the ASFV Georgia 2007/1 genome. The complete sequence of ASFV/Pol/2015/Podlaskie should assist further studies on the genetic diversity and evolution of the European ASFVs.

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Detection and Characterization of Distinct Alphacoronaviruses in Five Different Bat Species in Denmark

Bat populations harbour a multitude of viruses; some of these are pathogenic or potentially pathogenic in other animals or humans. Therefore, it is important to monitor the populations and characterize these viruses. In this study, the presence of coronaviruses (CoVs) in different species of Danish bats was investigated using active surveillance at different geographical locations in Denmark. Faecal samples were screened for the presence of CoVs using pan-CoV real-time RT-PCR assays. The amplicons, obtained from five different species of bats, were sequenced. Phylogenetic analysis revealed a species-specific clustering with the samples from Myotis daubentonii, showing a close resemblance to coronavirus sequences obtained from the same species of bat in Germany and the United Kingdom. Our results show, for the first time, that multiple, distinct alphacoronaviruses are present in the Danish bat populations.

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Porcine epidemic diarrhoea virus, strain CV777, was initially characterized in 1978 as the causative agent of a disease first identified in the UK in 1971. This coronavirus has been widely distributed among laboratories and has been passaged both within pigs and in cell culture. To determine the variability between different stocks of the PEDV strain CV777, sequencing of the full-length genome (ca. 28kb) has been performed in 6 different laboratories, using different protocols. Not surprisingly, each of the different full genome sequences were distinct from each other and from the reference sequence (Accession number AF353511) but they are >99% identical. Unique and shared differences between sequences were identified. The coding region for the surface-exposed spike protein showed the highest proportion of variability including both point mutations and small deletions. The predicted expression of the ORF3 gene product was more dramatically affected in three different variants of this virus through either loss of the initiation codon or gain of a premature termination codon. The genome of one isolate had a substantially rearranged 5’-terminal sequence. This rearrangement was validated through the analysis of sub-genomic mRNAs from infected cells. It is clearly important to know the features of the specific sample of CV777 being used for experimental studies.
Infection of pigs with African swine fever virus via ingestion of stable flies (Stomoxys calcitrans)

Within Eastern Europe, African swine fever virus (ASFV) has unexpectedly spread to farms with high biosecurity. In an attempt to explain this process, pigs were allowed to ingest flies that had fed on ASFV-spiked blood, which had a realistic titre for an infected pig. Some of the pigs became infected with the virus. Thus, ingestion of blood-sucking flies, having fed on ASFV-infected wild boar before entering stables, represents a potential route for disease transmission.
Modifications to the foot-and-mouth disease virus 2A peptide; influence on polyprotein processing and virus replication

Foot-and-mouth disease virus (FMDV) has a positive-sense ssRNA genome that includes a single, large, open reading frame encoding a polyprotein. The co-translational "cleavage" of this polyprotein at the 2A/2B junction is mediated by the 2A peptide (18 residues in length) using a non-proteolytic mechanism termed "ribosome skipping" or "StopGo". Multiple variants of the 2A polypeptide with this property among the picornaviruses share a conserved C-terminal motif (D(V/I)E(S/T)NPG↓P). The impact of 2A modifications within this motif on FMDV protein synthesis, polyprotein processing and virus viability were investigated.

Amino acid substitutions are tolerated at residues E₁₄, S₁₅ and N₁₆ within the 2A sequence of infectious FMDVs despite their reported "cleavage" efficiencies at the 2A/2B junction of only ca. 30-50% compared to wt. In contrast, no viruses were rescued containing substitutions at residues P₁₇, G₁₈ or P₁₉ that displayed little or no "cleavage" activity in vitro, but wt revertants were obtained. The 2A substitutions impaired the replication of a FMDV replicon. Using transient expression assays, it was shown that certain amino acid substitutions at residues E₁₄, S₁₅ and N₁₆ resulted in partial "cleavage" of a protease-free polyprotein indicating that these specific residues are not essential for co-translational "cleavage". Immunofluorescence studies, using full-length FMDV RNA transcripts encoding mutant 2A peptides, indicated that the 2A peptide remained attached to adjacent proteins, presumably 2B. These results show that efficient "cleavage" at the 2A/2B junction is required for optimal virus replication. However, maximal StopGo activity does not appear to be essential for the viability of FMDV. Importance

Foot-and-mouth disease virus (FMDV) causes one of the most economically important diseases of farm animals. Co-translational "cleavage" of the FMDV polyprotein...
precursor at the 2A/2B junction, termed StopGo, is mediated by the short 2A peptide through a non-proteolytic mechanism which leads to release of the nascent protein and continued translation of the downstream sequence. Improved understanding of this process will not only give a better insight into how this peptide influences the FMDV replication cycle but may also assist the application of this sequence in biotechnology for the production of multiple proteins from a single mRNA. Our data show that single amino acid substitutions in the 2A peptide can have a major influence on viral protein synthesis, virus viability and polyprotein processing. It also indicates that efficient "cleavage" at the 2A/2B junction is required for optimal virus replication. However, maximal StopGo activity is not essential for the viability of FMDV.

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Foot-and-mouth disease virus (FMDV) is responsible for one of the most economically important infectious diseases of livestock. The virus spreads very easily and continues to affect many countries (mainly in Africa and Asia). The risks associated with the introduction of FMDV result in major barriers to trade in animals and their products. Seven antigenically distinct forms of the virus are known, called serotypes, but serotype C has not been detected anywhere for many years and may now be extinct. The serotypes have been further divided into topotypes (except for serotype Asia-1 viruses, which comprise a single topotype), genotypes, lineages and sub-lineages, which are usually restricted to specific geographical regions. However, sometimes, trans-regional spread of some strains occurs. Due to the error-prone replication of the RNA genome, the virus continuously evolves and new strains frequently arise (e.g. with modified antigenicity). Using nucleotide sequencing technologies, this rapid evolution of the viral genome can be followed. This allows the tracing of virus transmission pathways within an outbreak of disease if (near) full-length genome sequences can be generated. Furthermore, the movement of distinct virus lineages, from one country to another can be analyzed. Some important examples of the spread of new strains of FMD virus are described.
Selection of functional 2A sequences within foot-and-mouth disease virus; requirements for the NPGP motif with a distinct codon bias

Foot-and-mouth disease virus (FMDV) has a positive-sense ssRNA genome including a single, large, open reading frame. Splitting of the encoded polyprotein at the 2A/2B junction is mediated by the 2A peptide (18 residues long) which induces a non-proteolytic, co-translational, "cleavage" at its own C-terminus. A conserved feature among variants of 2A is the C-terminal motif N16P17G18/P19 where P19 is the first residue of 2B. It has been shown previously that certain amino acid substitutions can be tolerated at residues E14, S15 and N16 within the 2A sequence of infectious FMDVs but no variants at residues P17, G18 or P19 have been identified. In this study, using highly degenerate primers, we analysed if any other residues can be present at each position of the NPG/P motif within infectious FMDV. No alternative forms of this motif were found to be encoded by rescued FMDVs after 2, 3 or 4 passages. However, surprisingly, a clear codon preference for the wt nucleotide sequence encoding the NPGP motif within these viruses was observed. Indeed, the codons selected to code for P17 and P19 within this motif were distinct; thus the synonymous codons are not equivalent.
Short time window for transmissibility of African swine fever virus from a contaminated environment

Since the introduction of African swine fever virus (ASFV) into the Baltic states and Poland in 2014, the disease has continued to spread within these regions. In 2017, the virus spread further west and the first cases of disease were reported in the Czech Republic and Romania, in wild boar and domestic pigs, respectively. To control further spread, knowledge of different modes of transmission, including indirect transmission via a contaminated environment, is crucial. Up until now, such an indirect mode of transmission has not been demonstrated. In this study, transmission via an environment contaminated with excretions from ASFV-infected pigs was investigated. Following euthanasia of pigs that were infected with an isolate of ASFV from Poland (POL/2015/Podlaskie/Lindholm), healthy pigs were introduced into the pens, in which the ASFV-infected pigs had been housed. Introduction was performed at 1, 3, 5 or 7 days, following euthanasia of the infected pig groups. Pigs, that were introduced into the contaminated environment after 1 day, developed clinical disease within 1 week, and both ASFV DNA and infectious virus were isolated from their blood. However, pigs introduced into the contaminated pens after 3, 5 or 7 days did not develop any signs of ASFV infection and no viral DNA was detected in blood samples obtained from these pigs within the following 3 weeks. Thus, it was shown that exposure of pigs to an environment contaminated with ASFV can result in infection. However, the time window for transmissibility of ASFV seems very limited, and, within our experimental system, there appears to be a rapid decrease in the infectivity of ASFV in the environment.

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Strategy for efficient generation of numerous full-length cDNA clones of classical swine fever virus for haplotypeing

Background: Direct molecular cloning of full-length cDNAs derived from viral RNA is an approach to identify the individual viral genomes within a virus population. This enables characterization of distinct viral haplotypes present during infection.

Results: In this study, we recover individual genomes of classical swine fever virus (CSFV), present in a pig infected with vKos that was rescued from a cDNA clone corresponding to the highly virulent CSFV Koslov strain. Full-length cDNA amplicons (ca. 12.3 kb) were made by long RT-PCR, using RNA extracted from serum, and inserted directly into a cloning vector prior to detailed characterization of the individual viral genome sequences. The amplicons used for cloning were deep sequenced, which revealed low level sequence variation (<5%) scattered across the genome consistent with the clone-derived origin of vKos. Numerous full-length cDNA clones were generated using these amplicons and full-genome sequencing of individual cDNA clones revealed insights into the virus diversity and the haplotypes present during infection. Most cDNA clones were unique, containing several single-nucleotide polymorphisms, and phylogenetic reconstruction revealed a low degree of order. Conclusions: This optimized methodology enables highly efficient construction of full-length cDNA clones corresponding to individual viral genomes present within RNA virus populations.

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Survival and localization of African swine fever virus in stable flies (Stomoxys calcitrans) after feeding on viremic blood using a membrane feeder

Since 2014, African swine fever virus (ASFV) has been spreading within Eastern Europe. Within affected regions, the virus has infected some farms with high biosecurity and a marked seasonality of outbreaks in domestic pigs has been observed. ASFV transmission from stable flies, Stomoxys calcitrans, has previously been shown both mechanically and via ingestion of whole flies. Hence, blood-feeding flies may offer one explanation for the introductions into high biosecurity farms and for the observed seasonality. The aim of this study was to further elucidate the potential role of stable flies in ASFV transmission. Different parts of flies were analyzed for the presence of viral DNA and infectious virus at different time points following in vitro feeding of the flies on blood from an ASFV-infected pig. Using qPCR, ASFV DNA was detectable in mouth parts of flies for at least 12 h and remained in head and body samples from the flies for up to three days following feeding. Infectious virus was detected in fly body samples prepared at 3 h and 12 h after feeding. The presence of infectious ASFV in stable flies following feeding on viremic blood means that such flies are capable of transporting infectious virus. The detection of ASFV DNA in the flies for up to three days following feeding suggests that qPCR analysis of blood-feeding flies during ASFV outbreaks could be a useful method to elucidate the role of these flies in ASFV transmission under field conditions.

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Transmission of foot-and-mouth disease from persistently infected carrier cattle to naïve cattle via transfer of oropharyngeal fluid.

Control and eradication of foot-and-mouth disease (FMD) is impeded by the existence of a persistent, subclinical, phase of infection in ruminants; animals with this status are referred to as carriers. However, the epidemiological significance of these FMD virus (FMDV) carriers is uncertain. In the current investigation, the contagion associated with FMDV carrier cattle was investigated by exposure of susceptible cattle and pigs to oropharyngeal fluid (OPF) or tissues harvested from persistently infected cattle. Naïve cattle were inoculated through intranasopharyngeal deposition of unprocessed OPF that had been collected from FMDV carriers at 30 days post infection. These inoculated cattle developed clinical FMD of similar severity as animals that had been infected with a high-titer inoculum. In contrast, pigs exposed via intraoropharyngeal inoculation of the same OPF, or by ingestion of nasopharyngeal tissues harvested from the same cohort of persistently infected cattle, did not develop FMD. These findings indicate that there is demonstrable contagion associated with FMDV carrier cattle despite the lack of evidence for transmission by direct contact. The findings presented herein provide novel information that should be considered for FMD risk mitigation strategies.
Kit-of-parts for use in a prime-boost vaccination strategy to protect cloven-footed animals against foot-and-mouth disease virus infection

The present invention relates to a kit-of-parts for use in immunizing an animal against foot-and-mouth disease virus (FMDV) infection. In particular, the present invention relates to a kit-of-parts containing a priming composition and a boosting composition for use in a prime-boost FMDV-vaccination strategy.

Determinants of the VP1/2A junction cleavage by the 3C protease in foot-and-mouth disease virus infected cells

The foot-and-mouth disease virus (FMDV) capsid precursor, P1-2A, is cleaved by FMDV 3C protease to yield VP0, VP3, VP1 and 2A. Cleavage of the VP1/2A junction is the slowest. Serotype O FMDVs with uncleaved VP1-2A (having a K210E substitution in VP1; at position P2 in cleavage site) have been described previously and acquired a second site substitution (VP1 E83K) during virus rescue. Furthermore, introduction of the VP1 E83K substitution alone generated a second site change at the VP1/2A junction (2A L2P, position P2’ in cleavage site). These virus adaptations have now been analysed using Next Generation Sequencing to determine sub-consensus level changes in the virus; this revealed other variants within the E83K mutant virus population that changed residue VP1 K210. The construction of serotype A viruses with a blocked VP1/2A cleavage site (containing K210E) has now been achieved. A collection of alternative amino acid substitutions were made at this site and the properties of the mutant viruses determined. Only the presence of a positively charged residue at position P2 in the cleavage site permitted efficient cleavage of the VP1/2A junction, consistent with analyses of diverse FMDV genome sequences. Interestingly, in contrast to the serotype O virus results, no second site mutations occurred within the VP1 coding region of serotype A viruses with the blocked VP1/2A cleavage site. However, some of these viruses acquired changes in the 2C protein that is involved in enterovirus morphogenesis. These results have implications for the testing of potential antiviral agents targeting the FMDV 3C protease.
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Distinct roles for the IIId2 sub-domain in pestivirus and picornavirus internal ribosome entry sites

Viral internal ribosomes entry site (IRES) elements coordinate the recruitment of the host translation machinery to direct the initiation of viral protein synthesis. Within hepatitis C virus (HCV)-like IRES elements, the sub-domain IIId(1) is crucial for recruiting the 40S ribosomal subunit. However, some HCV-like IRES elements possess an additional sub-domain, termed IIId2, whose function remains unclear. Herein, we show that IIId2 sub-domains from divergent viruses have different functions. The IIId2 sub-domain present in Seneca valley virus (SVV), a picornavirus, is dispensable for IRES activity, while the IIId2 sub-domains of two pestiviruses, classical swine fever virus (CSFV) and border disease virus (BDV), are required for 80S ribosomes assembly and IRES activity. Unlike in SVV, the deletion of IIId2 from the CSFV and BDV IRES elements impairs initiation of translation by inhibiting the assembly of 80S ribosomes. Consequently, this negatively affects the replication of CSFV and BDV. Finally, we show that the SVV IIId2 sub-domain is required for efficient viral RNA synthesis and growth of SVV, but not for IRES function. This study sheds light on the molecular evolution of viruses by clearly demonstrating that conserved RNA structures, within distantly related RNA viruses, have acquired different roles in the virus life cycles.
Experimental Infection of Young Pigs with an Early European Strain of Porcine Epidemic Diarrhoea Virus and a Recent US Strain

Outbreaks of porcine epidemic diarrhoea (PED) were reported across Europe during the 1980s and 1990s, but only sporadic outbreaks occurred in recent years. PED virus (PEDV) spread for the first time into the USA in 2013 and has caused severe economic losses. Retrospectively, it was found that two different strains of PEDV have been introduced into the United States, both are closely related to strains circulating in China where a new wave of the disease occurred from 2010 onwards. Since autumn 2014, new outbreaks of PED have occurred in Europe. In this study, weaned piglets were inoculated with an early European isolate (Br1/87) or faecal/intestinal suspensions derived from pigs infected with a recent European strain of PEDV (from Germany) or a US strain of PEDV. No evidence for infection resulted from inoculation of pigs with the German sample that contained high levels of PEDV RNA; there were no clinical signs, excretion of viral RNA or anti-PEDV antibody production. In contrast, all the pigs in the other two groups showed evidence of infection. Mild clinical signs of disease, mainly diarrhoea, occurred in piglets inoculated with the Br1/87 and US PEDV strains. PEDV RNA was detected throughout the intestine in euthanized animals at 4 days post-inoculation. In addition, within these animals, low levels of viral RNA were detected in lungs and livers with higher levels in spleens. Serocconversion against PEDV occurred in all surviving infected animals within 10 days. PEDV RNA excretion occurred for at least 2 weeks. The US PEDV RNA was detected at low levels in serum samples on multiple days. It is apparent that current diagnostic systems can detect infection by the different virus strains.

General information
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Scopus rating (2014): CiteScore 2.23 SJR 1.048 SNIP 1.207
Web of Science (2014): Impact factor 2.944
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BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 2.05 SJR 0.939 SNIP 1.124
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BFI (2010): BFI-level 1
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BFI (2008): BFI-level 1
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Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 0.456 SNIP 0.777
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Genome organization, translation and replication of foot-and-mouth disease virus RNA

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Overview of Foot-and-Mouth Disease and Its Impact as a Re-emergent Viral Infection

General information
Separation of foot-and-mouth disease virus leader protein activities; identification of mutants that retain efficient self-processing activity but poorly induce eIF4G cleavage

Foot-and-mouth disease virus (FMDV) is a picornavirus and its RNA genome encodes a large polyprotein. The N-terminal part of this polyprotein is the Leader protein, a cysteine protease, termed Lpro. The virus causes the rapid inhibition of host cell cap-dependent protein synthesis within infected cells. This results from the Lpro dependent cleavage of the cellular translation initiation factor eIF4G. Lpro also releases itself from the virus capsid precursor by cleaving the L/P1 junction. Using site-directed mutagenesis of the Lpro coding sequence, we have investigated the role of 51 separate amino acid residues in the functions of this protein. These selected residues are either highly conserved or are charged and exposed on the protein surface. Using transient expression assays within BHK cells, it was found that residues around the active site (W52, L53 and A149) of Lpro and others located elsewhere (K38, K39, R44, H138 and W159) are involved in the induction of eIF4G cleavage but not in the processing of the L/P1 junction. Modified viruses, encoding such amino acid substitutions within Lpro can replicate in BHK cells but did not grow well in primary bovine thyroid cells. This study characterizes mutant viruses that are deficient in blocking host cell responses to infection (e.g. interferon induction) and can assist in the rational design of antiviral agents targeting this process and in the production of attenuated viruses.
Transmission of African swine fever virus from infected pigs by direct contact and aerosol routes

In 2014, African swine fever virus (ASFV) was introduced into the Baltic states and Poland. Since then, the disease has continued to spread within these regions, and recently, cases were reported in the Czech Republic and Romania.
Currently, there is an increasing risk of ASFV introduction into Western Europe. Hence, there is an urgent need to assess current contingency plans. For this purpose, knowledge of modes-of-transmission and clinical outcome in pigs infected with new European ASFV strains is needed. In the present study, two experiments were conducted in pigs using an isolate of ASFV from Poland (designated here POL/2015/Podlaskie/Lindholm). In both studies, pigs were inoculated intranasally with the virus and contact pigs were exposed to the experimentally infected pigs, either directly (contact within and between pens) or by air. Pigs exposed to the virus by intranasal inoculation, by direct contact to infected animals and by aerosol developed acute disease characterized by viremia, fever and depression. Infectious virus was first detected in blood obtained from the inoculated pigs and then sequentially among the within-pen, between-pen and air-contact pigs. ASFV DNA and occasionally infectious virus was found in nasal-, oral-, and rectal swabs obtained from the pigs, and ASFV DNA was detected in air samples. No anti-ASFV antibodies were detected in sera.

In conclusion, the study shows that the currently circulating strain of ASFV can be efficiently transmitted via direct contact and by aerosols. Also, the results provide quantitative transmission parameters and knowledge of infection stages in pigs infected with this ASFV.

**General information**

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Scopus rating (2015): CiteScore 2.56 SJR 1.413 SNIP 1.21  
Web of Science (2015): Impact factor 2.564  
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BFI (2014): BFI-level 2  
Scopus rating (2014): CiteScore 2.54 SJR 1.291 SNIP 1.256  
Web of Science (2014): Impact factor 2.511  
Web of Science (2014): Indexed yes  
BFI (2013): BFI-level 2  
Scopus rating (2013): CiteScore 3 SJR 1.459 SNIP 1.471  
Web of Science (2013): Impact factor 2.726  
ISI indexed (2013): ISI indexed yes  
Web of Science (2013): Indexed yes  
BFI (2012): BFI-level 2  
Scopus rating (2012): CiteScore 3.18 SJR 1.441 SNIP 1.569  
Web of Science (2012): Impact factor 3.127  
ISI indexed (2012): ISI indexed yes  
Web of Science (2012): Indexed yes  
BFI (2011): BFI-level 2
Foot-and-mouth disease (FMD) remains one of the most economically important infectious diseases of production animals globally. Vaccination can successfully control this disease, however, current vaccines are imperfect. They are made using chemically inactivated FMD virus (FMDV) that is produced in large-scale mammalian cell culture under high containment conditions. Here, we have expressed the FMDV capsid protein precursor (P1-2A) of strain O1 Manisa alone or with the FMDV 3C protease (3Cpro) using a "single cycle" packaged alphavirus self-replicating RNA based on Semliki Forest virus (SFV). When the FMDV P1-2A was expressed with 3Cpro then processing of the FMDV capsid precursor protein is observed within cells and the proteins assemble into empty capsid particles. The products interact with anti-FMDV antibodies in an ELISA and bind to the integrin αvβ6 (a cellular receptor for FMDV). In cattle vaccinated with these rSFV-FMDV vectors alone, anti-FMDV antibodies were elicited but the immune response was insufficient to give protection against FMDV challenge. However, the prior vaccination with these vectors resulted in a much stronger immune response against FMDV post-challenge and the viremia observed was decreased in level and duration. In subsequent experiments, cattle were sequentially vaccinated with a rSFV-FMDV followed by recombinant FMDV empty capsid particles, or vice versa, prior to challenge. Animals given a primary vaccination with the rSFV-FMDV vector and then boosted with FMDV empty capsids showed a strong anti-FMDV antibody response prior to challenge, they were protected against disease and no FMDV RNA was detected in their sera post-challenge. Initial inoculation with empty capsids followed by the rSFV-FMDV was much less effective at combating the FMDV challenge and a large post-challenge boost to the level of anti-FMDV antibodies was observed. This prime-boost system, using reagents that can be generated outside of high-
containment facilities, offers significant advantages to achieve control of FMD by vaccination.

**General information**

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Contributors: Gullberg, M., Lohse, L., Bøtner, A., McInerney, G. M., Burman, A., Jackson, T., Polacek, C., Belsham, G.
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- Scopus rating (2017): CiteScore 3.01 SJR 1.164 SNIP 1.111
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- Scopus rating (2014): CiteScore 3.54 SJR 1.559 SNIP 1.148
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- Scopus rating (2013): CiteScore 3.94 SJR 1.772 SNIP 1.153
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- Web of Science (2013): Indexed yes
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- Scopus rating (2012): CiteScore 4.15 SJR 1.982 SNIP 1.156
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- Scopus rating (2011): CiteScore 4.58 SJR 2.425 SNIP 1.233
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- Web of Science (2010): Indexed yes
- BFI (2009): BFI-level 1
- Scopus rating (2009): SJR 2.614 SNIP 1.046
- Web of Science (2009): Indexed yes
- BFI (2008): BFI-level 1
- Scopus rating (2008): SJR 2.506 SNIP 1.006
Assessing the potential spread and maintenance of foot-and-mouth disease virus infection in wild ungulates: general principles and application to a specific scenario in Thrace

Foot-and-mouth disease (FMD), due to infection with serotype O virus, occurred in wild boar and within eleven outbreaks in domestic livestock in the south-east of Bulgaria, Thrace region, in 2011. Hence, the issue of the potential for the spread and maintenance of FMD virus (FMDV) infection in a population of wild ungulates became important. This assessment focused on the spread and maintenance of FMDV infection within a hypothetical wild boar and deer population in an environment, which is characterized by a climate transitional between Mediterranean and continental and variable wildlife population densities. The assessment was based on three aspects: (i) a systematic review of the literature focusing on experimental infection studies to identify the parameters describing the duration of FMDV infection in deer and wild boar, as well as observational studies assessing the occurrence of FMDV infection in wild deer and wild boar populations, (ii) prevalence survey data of wild boar and deer in Bulgaria and Turkey and (iii) an epidemiological model, simulating the host-to-host spread of FMDV infections. It is concluded, based on all three aspects, that the wildlife population in Thrace, and so wildlife populations in similar ecological settings, are probably not able to maintain FMD in the long term in the absence of FMDV infection in the domestic host population. However, limited spread of FMDV infection in time and space in the wildlife populations can occur. If there is a continued crossover of FMDV between domestic and wildlife populations or a higher population density, virus circulation may be prolonged.

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Web of Science (2017): Impact factor 3.504
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Scopus rating (2016): CiteScore 2.16 SJR 1.046 SNIP 0.998
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During a severe outbreak of diarrhoea and vomiting in a pig herd in Central Eastern Europe, faecal samples were tested positive for porcine epidemic diarrhoea virus (PEDV) and negative for transmissible gastroenteritis virus (TGEV) using a commercial RT-qPCR assay that can detect both of these coronaviruses. However, further analyses, using other TGEV- and PEDV-specific RT-qPCR assays, provided results inconsistent with infection by either of these viruses. Sequencing of an amplicon (ca. 1.6 kb), generated by an RT-PCR specific for the PEDV S-gene, indicated a very close similarity (ca. 99% identity) to recently described chimeric viruses termed swine enteric coronaviruses (SeCoVs). These viruses (with an RNA genome of ca. 28 kb) were first identified in Italy in samples from 2009 but have not been detected there since 2012.
A closely related virus was detected in archived samples in Germany from 2012, but has not been detected subsequently. Building on the initial sequence data, further amplicons were generated and over 9 kb of sequence corresponding to the 3′-terminus of the new SeCoV genome was determined. Sequence comparisons showed that the three known SeCoVs are ≥98% identical across this region and contain the S-gene and 3a sequences from PEDV within a backbone of TGEV, but the viruses are clearly distinct from each other. It is demonstrated, for the first time, that pigs from within the SeCoV-infected herd seroconverted against PEDV but tested negative in a TGEV-specific ELISA that detects antibodies against the S protein. These results indicate that SeCoV is continuing to circulate in Europe and suggest it can cause a disease that is very similar to PED. Specific detection of the chimeric SeCoVs either requires development of a new diagnostic RT-qPCR assay or the combined use of assays targeting the PEDV S-gene and another part of the TGEV genome.
Conserved elements within the genome of foot-and-mouth disease virus; their influence on virus replication

Objectives:
Several conserved elements within the genome of foot-and-mouth disease virus (FMDV) have been identified, e.g. the IRES. Such elements can be crucial for the efficient replication of the genomic RNA. Previously, SHAPE analysis of the entire FMDV genome (Poulsen et al., 2016 submitted) has identified a conserved RNA structure within the 3Dpol coding region (the RNA-dependent RNA polymerase) which might have an important role in virus replication. The FMDV 2A peptide, another conserved element, is responsible for the primary “cleavage” at its own C-terminus (2A/2B junction). It is believed that this “cleavage” is achieved by ribosomal skipping, in which the 2A peptide prevents the ribosome from linking the next amino acid (aa) to the growing polypeptide. The nature of this “cleavage” has so far not been investigated in the context of the full-length FMDV RNA within cells. Through reverse genetics, this study aims to identify how these distinct conserved elements influence the replication of FMDV RNA.

Methods:
Changes were made within the predicted 3Dpol RNA structure and the 2A peptide coding sequence which were expected to be detrimental for their function. These were:
1) Silent mutations, to disrupt the 3Dpol RNA secondary structure, were generated in a FMDV replicon containing Gaussia luciferase.
2) Sequence changes encoding selected modifications of the 2A peptide (as described by Donnelly et al., 2001) were introduced into a full-length FMDV cDNA and in a FMDV replicon cDNA containing Gaussia luciferase. RNA transcripts were generated in vitro from the plasmids, and introduced into BHK cells by electroporation. The replication efficiency was assessed by measurement of luciferase activity or by rescue of mutant viruses. The rescued viruses derived from the 2A mutant cDNAs were passaged 3 times and the rescued RNAs were sequenced.

Results:
Initial results indicate that 3 different replicon mutants, with the disrupted 3Dpol RNA structure, had very similar RNA replication efficiencies as the wt FMDV replicon. Furthermore, the replicon system showed that the 2A mutants were also able to undergo replication, although at a lower rate than for the wt FMDV replicon. One mutant which previously (Donnelly et al., 2001) was found not to undergo “cleavage” was still replication competent. Analysis of rescued viruses by sequencing of the third passage revealed that the 2A mutants with the lowest “cleavage” activity had reverted to the wt but some mutants with defective “cleavage” activity were viable.

Conclusions:
Initial results confirm that efficient “cleavage” at the 2A/2B junction is required for optimal replication. Rescue of viable mutant viruses with mutants previously characterized as “non-cleaving” indicates a discrepancy between in vitro and cell-based experiments.
Detrimental changes to the 3Dpol RNA structure did not change the replication efficiency in a replicon system. However, these results do not eliminate a possible effect of this structure on virus replication; such analyses are in progress. Further study of these two conserved elements will provide more valuable insights into mechanisms underlying FMDV virus replication.

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**Development and evaluation of tailored specific real-time RT-PCR assays for detection of foot-and-mouth disease virus serotypes circulating in East Africa**

Rapid, reliable and accurate diagnostic methods provide essential support to programmes that monitor and control foot-and-mouth disease (FMD). While pan-specific molecular tests for FMD virus (FMDV) detection are well established and widely used in endemic and FMD-free countries, current serotyping methods mainly rely either on antigen detection ELISAs or nucleotide sequencing approaches. This report describes the development of a panel of serotype-specific real-time RT-PCR assays (rtRT-PCR) tailored to detect FMDV lineages currently circulating in East Africa. These assays target sequences within the VP1-coding region that share high intra-lineage identity, but do not cross-react with FMD viruses from other serotypes that circulate in the region. These serotype-specific assays operate with the same thermal profile as the pan-diagnostic tests making it possible to run them in parallel to produce CT values comparable to the pan-diagnostic test detecting the 3D-coding region. These assays were evaluated alongside the established pan-specific molecular test using field samples and virus isolates collected from Tanzania, Kenya and Ethiopia that had been previously characterised by nucleotide sequencing. Samples (n = 71) representing serotype A (topotype AFRICA, lineage G-I), serotype O (topotypes EA-2 and EA-4), serotype SAT 1 (topotype I (NWZ)) and serotype SAT2 (topotype IV) were correctly identified using these rtRT-PCR assays. Furthermore, FMDV RNA from samples that did not contain infectious virus could still be serotyped using these assays. These serotype-specific real-time RT-PCR assays can detect and characterise FMDVs currently circulating in East Africa and hence improve disease control in this region.

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Experimental infection of piglets with an early European strain of PED virus and a recent US PEDV strain

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Contributors: Lohse, L., Krog, J. S., Strandbygaard, B., Rasmussen, T. B., Kjær, J., Belsham, G., Bøtner, A.
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Foot-and-mouth disease virus capsid proteins; analysis of protein processing, assembly and utility as vaccines

Foot-and-mouth disease (FMD) remains one of the most economically important infectious diseases of production animals globally. The infection is caused by foot-and-mouth disease virus (FMDV), a member of the picornavirus family. The positive sense RNA genome of the virus includes a single, large, open reading frame that encodes a polyprotein. The intact polyprotein is never observed as it is processed, both during and after translation, to 15 different mature proteins plus a variety of precursors. The FMDV capsid protein precursor, P1-2A, is cleaved by the virus encoded 3C protease (3Cpro) to generate VP0, VP3, VP1 and the peptide 2A. Sixty copies of each of the capsid proteins “self-assemble” into empty capsid particles or with the RNA genome into infectious viruses. These particles normally lack 2A but it is possible to construct and isolate mutant FMDVs in which the cleavage of the VP1/2A junction is greatly inhibited, leading to the production of “self-tagged” virus particles that retain the 2A peptide. Interestingly, such mutant viruses acquire “second site” changes elsewhere within VP1.

Recent studies have shown that reducing the expression level of the 3Cpro relative to the P1-2A capsid precursor enhances the yield of processed capsid proteins and their assembly into empty capsid particles within mammalian cells. Such particles can potentially form the basis of a vaccine but they may only have the same properties as the current inactivated vaccines. We have expressed the FMDV P1-2A alone or with FMDV 3Cpro using a “single cycle” alphavirus vector based on Semliki Forest virus (SFV). Cattle vaccinated with these rSFV-FMDV vectors alone, produced anti-FMDV antibodies but the immune response was insufficient to give protection against FMDV challenge. However, vaccination with these vectors primed a much stronger immune response against FMDV post-challenge. In subsequent experiments, cattle were sequentially vaccinated with a rSFV-FMDV followed by recombinant FMDV empty capsid particles, or vice versa, prior to challenge. Animals given a primary vaccination with the rSFV-FMDV vector and then boosted with FMDV empty capsids showed a strong anti-FMDV antibody response prior to challenge; they were protected against disease and no FMDV RNA was detected in their sera post-challenge. Initial inoculation with empty capsids followed by the rSFV-FMDV was much less effective at combating the FMDV challenge. This prime-boost system, using reagents that can be generated outside of high-containment facilities, offers significant advantages to achieve control of FMD by vaccination.

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High diversity of picornaviruses in rats from different continents revealed by deep sequencing

Outbreaks of zoonotic diseases in humans and livestock are not uncommon, and an important component in containment of such emerging viral diseases is rapid and reliable diagnostics. Such methods are often PCR-based and hence require the availability of sequence data from the pathogen. Rattus norvegicus (R. norvegicus) is a known reservoir for important zoonotic pathogens. Transmission may be direct via contact with the animal, for example, through exposure to its faecal matter, or indirectly mediated by arthropod vectors. Here we investigated the viral content in rat faecal matter (n=29) collected from two continents by analyzing 2.2 billion next-generation sequencing reads derived from both DNA and RNA.
Among other virus families, we found sequences from members of the Picornaviridae to be abundant in the microbiome of all the samples. Here we describe the diversity of the picornavirus-like contigs including near-full-length genomes closely related to the Boone cardiovirus and Theiler's encephalomyelitis virus. From this study, we conclude that picornaviruses within R. norvegicus are more diverse than previously recognized. The virome of R. norvegicus should be investigated further to assess the full potential for zoonotic virus transmission.
Occurrence of Swine Enteric Coronavirus (SeCoV) infection during 2016 within Central Eastern Europe

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Prevention of foot-and-mouth disease in cattle using a prime-boot-vaccination strategy
Foot-and-mouth disease (FMD) is one of the most economically important infectious diseases of production animals globally. Vaccination can help to control this disease, however, current vaccines are imperfect. They are made using chemically inactivated FMD virus (FMDV) that is produced in mammalian cell culture under high containment. Here, we have expressed the FMDV capsid protein precursor (P1-2A) of strain O1 Manisa alone or with the FMDV 3C protease (3Cpro) using a "single cycle" packaged alphavirus self-replicating RNA based on Semliki Forest virus (SFV). When the FMDV P1-2A was expressed with 3Cpro then processing of the FMDV capsid precursor protein is observed within cells and the proteins assemble into empty capsid particles. In cattle vaccinated once with these rSFV-FMDV vectors alone, anti-FMDV antibodies were elicited but the immune response was insufficient to give protection against FMDV challenge. However, the prior vaccination with these vectors resulted in a much stronger immune response against FMDV post-challenge and the viremia observed was decreased in level and duration. In subsequent experiments, cattle were sequentially vaccinated with a rSFV-FMDV followed by recombinant FMDV empty capsid particles, or vice versa, prior to challenge. Animals given a primary vaccination with the rSFV-FMDV vector and then boosted with FMDV empty capsids showed a strong anti-FMDV antibody response prior to challenge. Following challenge with FMDV, the cattle were protected against disease and no FMDV RNA was detected in their sera. Initial inoculation with empty capsids followed by the rSFV-FMDV was much less effective at combating the FMDV challenge and a large post-challenge boost to the level
of anti-FMDV antibodies was observed and clinical disease occurred. This prime-boost system, using reagents that can be generated outside of high-containment facilities, offers significant advantages to achieve control of FMD by vaccination.

QA prime-boost vaccination strategy in prevent serotype O FMDV infection using a "single-cycle" alphavirus vector and empty capsid particles

Introduction
Foot-and-mouth disease (FMD) remains one of the most economically important infectious diseases of production animals globally. Vaccination can help to control this disease, however, current vaccines based on chemically inactivated FMDV, are imperfect and there is a need for new, safe and effective vaccines to control FMD. There is no cross protection between the 7 serotypes but serotype O is the most abundant globally.

Material and methods
The FMDV capsid protein precursor (P1-2A) of strain O1 Manisa has been expressed with the FMDV 3C protease (3Cpro) using a "single cycle" packaged alphavirus self-replicating RNA based on Semliki Forest virus (SFV). Purified O1 Manisa empty capsid particles (ECs) have been prepared using a recombinant vaccinia virus expression system. Cattle have been vaccinated with the SFV-FMDV vectors and boosted subsequently with the ECs and then challenged with serotype O FMDV. The immune response against FMDV achieved by vaccination and infection status following challenge has been determined.

Results
In cattle vaccinated once with these rSFV-FMDV vectors alone, anti-FMDV antibodies were elicited but the immune response was insufficient to give protection against FMDV challenge. However, the vaccination with these vectors resulted in a much stronger immune response against FMDV post-challenge than in naïve animals. In subsequent experiments, cattle were sequentially vaccinated with the rSFV-FMDV vector and then boosted with FMDV empty capsids prior to challenge. Animals given a primary vaccination with the rSFV-FMDV vector and then boosted with FMDV empty capsids showed a strong anti-FMDV antibody response prior to challenge. Following challenge with serotype O FMDV, the cattle were protected against disease and no FMDV RNA was detected in their sera.

Discussion
This prime-boost system, using reagents that can be generated outside of high-containment facilities, offers significant advantages to achieve control of FMD by vaccination.

Sequence adaptations during growth of rescued classical swine fever viruses in cell culture and within infected pigs
Classical swine fever virus (CSFV) causes an economically important disease of swine. Four different viruses were rescued from full-length cloned cDNAs derived from the Paderborn strain of CSFV. Three of these viruses had been modified by mutagenesis (with 7 or 8 nt changes) within stem 2 of the subdomain IIIf of the internal ribosome entry site (IRES) that directs the initiation of protein synthesis. Rescued viruses were inoculated into pigs. The rescued vPader10 virus, without modifications in the IRES, induced clinical disease in pigs that was very similar to that observed previously with the parental field strain and transmission to in-contact pigs occurred. Two sequence reversions, in the NS2 and NSSB coding regions, became dominant within the virus populations in these infected pigs. Rescued viruses, with mutant IRES elements, did not induce disease and only very limited circulation of viral RNA could be detected. However, the animals
inoculated with these mutant viruses seroconverted against CSFV. Thus, these mutant viruses were highly attenuated in vivo. All 4 rescued viruses were also passaged up to 20 times in cell culture. Using full genome sequencing, the same two adaptations within each of four independent virus populations were observed that restored the coding sequence to that of the parental field strain. These adaptations occurred with different kinetics. The combination of reverse genetics and in depth, full genome sequencing provides a powerful approach to analyse virus adaptation and to identify key determinants of viral replication efficiency in cells and within host animals.
Unrecognized circulation of SAT 1 foot-and-mouth disease virus in cattle herds around Queen Elizabeth National Park in Uganda

Foot-and-mouth disease (FMD) is endemic in Uganda in spite of the control measures used. Various aspects of the maintenance and circulation of FMD viruses (FMDV) in Uganda are not well understood; these include the role of the African buffalo (Syncerus caffer) as a reservoir for FMDV. To better understand the epidemiology of FMD at the livestock-wildlife-interface, samples were collected from young, unvaccinated cattle from 24 pastoral herds that closely interact with wildlife around Queen Elizabeth National Park in Uganda, and analysed for evidence of FMDV infection. In total, 37 (15 %) of 247 serum samples had detectable antibodies against FMDV non-structural proteins (NSPs) using a pan-serotypic assay. Within these 37 sera, antibody titres ≥ 80 against the structural proteins of serotypes O, SAT 1, SAT 2 and SAT 3 were detected by ELISA in 5, 7, 4 and 3 samples, respectively, while neutralizing antibodies were only detected against serotype O in 3 samples. Two FMDV isolates, with identical VP1 coding sequences, were obtained from probang samples from clinically healthy calves from the same herd and are serotype SAT 1 (topotype IV (EA-I)). Based on the VP1 coding sequences, these viruses are distinct from previous cattle and buffalo SAT 1 FMDV isolates obtained from the same area (19-30 % nucleotide difference) and from the vaccine strain (TAN155/71) used within Uganda (26 % nucleotide difference). Eight herds had only one or a few animals with antibodies against FMDV NSPs while six herds had more substantial evidence of prior infection with FMDV. There was no evidence for exposure to FMDV in the other ten herds. The two identical SAT 1 FMDV VP1 sequences are distinct from former buffalo and cattle isolates from the same area, thus, transmission between buffalo and cattle was not demonstrated. These new SAT 1 FMDV isolates differed significantly from the vaccine strain used to control Ugandan FMD outbreaks, indicating a need for vaccine matching studies. Only six herds had clear serological evidence for exposure to O and SAT 1 FMDV. Scattered presence of antibodies against FMDV in other herds may be due to the occasional introduction of animals to the area or maternal antibodies from past infection and/or vaccination. The evidence for asymptomatic FMDV infection has implications for disease control strategies in the area since this obstructs early disease detection that is based on clinical signs in FMDV infected animals.
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Scopus rating (2010): SJR 1.076 SNIP 1.396
Web of Science (2010): Impact factor 2.371
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Scopus rating (2009): SJR 0.969 SNIP 0.985
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 0.529 SNIP 0.811
Virulence determinants within the E2 glycoprotein of Classical Swine Fever Virus

Classical Swine Fever is a highly contagious disease of pigs caused by Classical Swine Fever Virus (CSFV), a member of the pestivirus genus within the family Flaviviridae. The E2 glycoprotein of CSFV has been shown to be an important factor for the virulence of the virus. In a recent study, we have identified a specific motif within the E2 glycoprotein that contributes to the virulence of the highly virulent CSFV strain Koslov (Fahnøe et al. 2014). This motif comprises residues S74 and L75 in the N-terminal domain of E2 (S763 and L764 in the polyprotein). Evidence points towards involvement of this motif in virulence. CSFV strains encoding L763 and P764 represent the predominant alleles across all published full-length CSFV genomes, whereas the S763/L764 combination is only seen in highly virulent strains. In this study, mutations were introduced into the consensus cDNA clone of the highly virulent CSFV strain Koslov to evaluate the virulence of a set of E2 mutants with modifications in the encoded residues 763 and 764; these mutants are termed; vKos_SP, vKos_LP and vKos_LL, respectively. Animal infection experiments were performed to compare virulence of these E2 mutants in comparison to vKos (with the SL motif). The results indicate that the E2 residues 763-64 play an important role in CSFV virulence.

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Virulence determinants within the E2 glycoprotein of Classical Swine Fever Virus

Classical Swine Fever is a highly contagious disease of pigs caused by Classical Swine Fever Virus (CSFV), a member of the pestivirus genus within the family Flaviviridae. The E2 glycoprotein of CSFV has been shown to be an important factor for the virulence of the virus. In a recent study, we have identified a specific motif within the E2 glycoprotein that contributes to the virulence of the highly virulent CSFV strain Koslov (Fahnøe et al. 2014). This motif comprises residues S74 and L75 in the N-terminal domain of E2 (S763 and L764 in the polyprotein). Evidence points towards involvement of this motif in virulence. CSFV strains encoding L763 and P764 represent the predominant alleles across all published full-length CSFV genomes, whereas the S763/L764 combination is only seen in highly virulent strains. In this study, mutations were introduced into the consensus cDNA clone of the highly virulent CSFV strain Koslov to evaluate the virulence of a set of E2 mutants with modifications in the encoded residues 763 and 764; these mutants are termed; vKos_SP, vKos_LP and vKos_LL, respectively. Animal infection experiments were performed to compare virulence of these E2 mutants in comparison to vKos (with the SL motif). The results indicate that the E2 residues 763-64 play an important role in CSFV virulence.

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Analysis of Recent Serotype O Foot-and-Mouth Disease Viruses from Livestock in Kenya: Evidence of Four Independently Evolving Lineages

Foot-and-mouth disease (FMD) is endemic in Kenya where four serotypes (O, A, SAT 1 and SAT 2) of the virus are currently in circulation. Within 2010 and 2011, the National Laboratory recorded an increase in the number of FMD outbreaks caused by serotype O virus. The characteristics of these viruses were determined to ascertain whether these were independent outbreaks or one single strain spreading throughout the country. The sequences of the complete VP1-coding region were analysed from viruses sampled within different areas of Kenya during 2010 and 2011. The results indicated that the 2010 to 2011 outbreaks in Kenya were caused by four independent strains. By comparison with earlier type O isolates from Eastern Africa, it was apparent that the outbreaks were caused by viruses from three different lineages of topotype EA-2 and a fourth virus strain belonging to topotype EA-4. The topotypes EA-1 and EA-3 were not detected from these outbreaks. Implications of these results for FMD control in Eastern Africa are discussed.

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Bat Coronaviruses circulating in Danish bats

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Bat Coronaviruses circulating in Danish bats

General information
Challenges for Serology-Based Characterization of Foot-and-Mouth Disease Outbreaks in Endemic Areas; Identification of Two Separate Lineages of Serotype O FMDV in Uganda in 2011

Control of foot-and-mouth disease (FMD) in Uganda by ring vaccination largely depends on costly trivalent vaccines, and use of monovalent vaccines could improve the cost effectiveness. This, however, requires application of highly specific diagnostic tests. This study investigated outbreaks of FMD in seven Ugandan districts, during 2011, using the PrioCHECK(R) FMDV NS ELISA, solid-phase blocking ELISAs (SPBEs) and virus neutralization tests (VNTs), together with virological analyses for characterization of the responsible viruses. Two hundred and eighteen (218) cattle and 23 goat sera as well as 82 oropharyngeal fluid/epithelial tissue samples were collected. Some 50% of the cattle and 17% of the goat sera were positive by the PrioCHECK(R) FMDV NS ELISA, while SPBEs identified titres 80 for antibodies against serotype O FMD virus (FMDV) in 51% of the anti-NSP positive cattle sera. However, 35% of the anti-NSP positive cattle sera had SPBE titres 80 against multiple serotypes, primarily against serotypes O, SAT 1 and SAT 3. Comparison of SPBEs and VNTs for the detection of antibodies against serotypes O, SAT 1 and SAT 3 in 72 NSP positive cattle sera showed comparable results against serotype O (P=0.181), while VNTs detected significantly fewer samples positive for antibodies against SAT 1 and SAT 3 than the SPBEs (P
Characterisation of recent foot-and-mouth disease viruses from African buffalo (Syncerus caffer) and cattle in Kenya is consistent with independent virus populations

Background
Understanding the epidemiology of foot-and-mouth disease (FMD), including roles played by different hosts, is essential for improving disease control. The African buffalo (Syncerus caffer) is a reservoir for the SAT serotypes of FMD virus (FMDV). Large buffalo populations commonly intermingle with livestock in Kenya, yet earlier studies have focused on FMD in the domestic livestock, hence the contribution of buffalo to disease in livestock is largely unknown. This study analysed 47 epithelia collected from FMD outbreaks in Kenyan cattle between 2008 and 2012, and 102 probang and serum samples collected from buffalo in three different Kenyan ecosystems; Maasai-Mara (MME) (n=40), Tsavo (TSE) (n=33), and Meru (ME) (n=29).

Results
Antibodies against FMDV non-structural proteins were found in 65 of 102 (64%) sera from buffalo with 44/102 and 53/102 also having neutralising antibodies directed against FMDV SAT 1 and SAT 2, respectively. FMDV RNA was detected in 42% of the buffalo probang samples by RT-qPCR (Cycle Threshold (Ct) ≤32). Two buffalo probang samples were positive
by VI and were identified as FMDV SAT 1 and SAT 2 by Ag-ELISA, while the latter assay detected serotypes O (1), A (20), SAT 1 (7) and SAT 2 (19) in the 47 cattle epithelia. VP1 coding sequences were generated for two buffalo and 21 cattle samples. Phylogenetic analyses revealed SAT 1 and SAT 2 virus lineages within buffalo that were distinct from those detected in cattle.

Conclusions
We found that FMDV serotypes O, A, SAT 1 and SAT 2 were circulating among cattle in Kenya and cause disease, but only SAT 1 and SAT 2 viruses were successfully isolated from clinically normal buffalo. The buffalo isolates were genetically distinct from isolates obtained from cattle. Control efforts should focus primarily on reducing FMDV circulation among livestock and limiting interaction with buffalo. Comprehensive studies incorporating additional buffalo viruses are recommended.

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Scopus rating (2015): CiteScore 1.86 SJR 0.981 SNIP 1.009
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Web of Science (2012): Impact factor 1.861
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Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1

To investigate the foot-and-mouth disease virus (FMDV) serotypes circulating in Uganda’s cattle population, both serological and virological analyses of samples from outbreaks that occurred during 2012-2013 were performed. Altogether, 79 sera and 60 oropharyngeal fluid (OP)/tissue/oral swab samples were collected from herds with reported FMD outbreaks in seven different Ugandan districts. Overall, 61/79 (77%) of the cattle sera were positive for antibodies against FMDV by PrioCHECK® FMDV NS ELISA and solid phase blocking ELISA detected titres ≥ 80 for serotypes O, SAT 1, SAT 2 and SAT 3 in 41, 45, 30 and 45 of these 61 seropositive samples, respectively. Virus neutralisation tests detected the highest levels of neutralising antibodies (titres ≥ 45) against serotype O in the herds from Kween and Rakai districts, against SAT 1 in the herd from Nwoya district and against SAT 2 in the herds from Kiruhura, Isingiro and Ntungamo districts. Consistent with the detection of high levels of neutralising antibodies against SAT 2, was the isolation of a SAT 2 FMDV from Isingiro; sequencing (for the VP1 coding region) indicated that this virus belonged to lineage I within this serotype, like the currently used vaccine strain. From the Wakiso district 11 tissue/swab samples were collected; serotype A FMDV, genotype Africa (G-I), was isolated from the epithelial samples. This study shows that within a period of less than one year, FMD outbreaks in Uganda were caused by four different serotypes namely O, A, SAT 1 and SAT 2. Therefore, to enhance the control of FMD in Uganda, there is need for efficient and timely determination of outbreak virus strains/serotypes and vaccine matching. The value of incorporating serotype A antigen into the imported vaccines along with the current serotype O, SAT 1 and SAT 2 strains should be considered.

General information
State: Published
Organisations: National Veterinary Institute, Section for Virology, Ministry of Agriculture, Animal Industry and Fisheries, Makerere University, University of Copenhagen
Number of pages: 17
Publication date: 2015
Peer-reviewed: Yes
Development and Characterization of Probe-Based Real Time Quantitative RT-PCR Assays for Detection and Serotyping of Foot-And-Mouth Disease Viruses Circulating in West Eurasia.

Rapid and accurate diagnosis of foot-and-mouth disease (FMD) and virus serotyping are of paramount importance for control of this disease in endemic areas where vaccination is practiced. Ideally this virus characterization should be achieved without the need for virus amplification in cell culture. Due to the heterogeneity of FMD viruses (FMDVs) in...
different parts of the world, region specific diagnostic tests are required. In this study, hydrolysable probe-based real time reverse transcription quantitative polymerase chain reaction (RTqPCR) assays were developed for specific detection and serotyping of the FMDVs currently circulating in West Eurasia. These assays were evaluated, in parallel with pan-FMDV diagnostic assays and earlier serotype-specific assays, using field samples originating from Pakistan and Afghanistan containing FMD viruses belonging to different sublineages of OPanAsia, A-Iran05 and Asia-1 (Group-II and Group-VII (Sindh-08)). In addition, field samples from Iran and Bulgaria, containing FMDVs belonging to the O-PanAsia ANT-10 subline-agewere also tested. Each of the three primer/probe sets was designed to be specific for just one of the serotypes O, A and Asia-1 of FMDV and detected the RNA from the target viruses with cycle threshold (C<sub>T</sub>) values comparable with those obtained with the serotype independent pan-FMDV diagnostic assays. No cross-reactivity was observed in the assays between the heterotypic viruses circulating in the region. The assays reported here have higher diagnostic sensitivity (100% each for serotypes O and Asia-1, and 92% [95% CI = 81.4–100%] for serotype A positive samples) and specificity (100% each for serotypes O, A and Asia-1 positive samples) for the viruses currently circulating in West Eurasia compared to the serotyping assays reported earlier. Comparisons of the sequences of the primers and probes used in these assays and the corresponding regions of the circulating viruses provided explanations for the poor recognition of some of the viruses by the earlier assays. These new assays should help in the early detection and typing of serotype O, A and Asia-1 FMDVs circulating in West Eurasia to enable improved disease control.

**General Information**

**State:** Published

**Organisations:** National Veterinary Institute, Section for Virology, University of Malakand

**Contributors:** Jamal, S. M., Belsham, G.

**Publication date:** 2015

**Peer-reviewed:** Yes

**Publication Information**

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- Web of Science (2019): Indexed yes

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- BFI (2017): BFI-level 1

- Scopus rating (2017): CiteScore 3.01 SJR 1.164 SNIP 1.111
- Web of Science (2017): Indexed yes

- BFI (2016): BFI-level 1
- Scopus rating (2016): CiteScore 3.11 SJR 1.236 SNIP 1.101
- Web of Science (2016): Indexed yes

- BFI (2015): BFI-level 1
- Scopus rating (2015): CiteScore 3.32 SJR 1.427 SNIP 1.136
- Web of Science (2015): Indexed yes

- BFI (2014): BFI-level 1
- Scopus rating (2014): CiteScore 3.54 SJR 1.559 SNIP 1.148
- Web of Science (2014): Indexed yes

- BFI (2013): BFI-level 1
- Scopus rating (2013): CiteScore 3.94 SJR 1.772 SNIP 1.153
- ISI indexed (2013): ISI indexed yes

- Web of Science (2013): Indexed yes

- BFI (2012): BFI-level 1
- Scopus rating (2012): CiteScore 4.15 SJR 1.982 SNIP 1.156
- Web of Science (2012): Impact factor 3.73

- ISI indexed (2012): ISI indexed yes

- Web of Science (2012): Indexed yes

- BFI (2011): BFI-level 1
- Scopus rating (2011): CiteScore 4.58 SJR 2.425 SNIP 1.233
- Web of Science (2011): Impact factor 4.092
Foot-and-Mouth Disease

Foot-and-mouth disease (FMD) is an economically important, highly contagious disease of cloven-hoofed animals characterised by the appearance of vesicles (blisters) on the feet and in, and around, the mouth. The causative agent, foot-and-mouth disease virus (FMDV), was the first mammalian virus to be discovered. It has a ribonucleic acid (RNA) genome enclosed within a protein coat (capsid). The virus replicates very rapidly within the cytoplasm of cells. The RNA genome has to function both as a messenger RNA (mRNA) and as a template for RNA replication. The RNA encodes a single large polyprotein that is processed, by virus-encoded proteases, to about 12 mature products (plus functionally important precursors) that are required for virus replication and assembly. Some of these viral proteins modify host cell activities to block antivirus defence systems. Thus, this small virus displays a remarkably complex array of biological activities.

General information
State: Published
Organisations: National Veterinary Institute, Section for Virology, The Pirbright Institute
Contributors: Belsham, G., Charleston, B., Jackson, T., Paton, D. J.
Number of pages: 9
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Publisher: Wiley
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DOIs: 10.1002/9780470015902.a0001024.pub
Source: PublicationPreSubmission
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Research output: Research - peer-review > Encyclopedia chapter – Annual report year: 2015

Foot-and-Mouth Disease Virus Serotype SAT 3 in Long-Horned Ankole Calf, Uganda

After a 16-year interval, foot-and-mouth disease virus serotype SAT 3 was isolated in 2013 from an apparently healthy long-horned Ankole calf that grazed close to buffalo in Uganda. The emergent virus strain is ≈20% different in nucleotide sequence (encoding VP1 [viral protein 1]) from its closest relatives isolated previously from buffalo in Uganda.

General information
Hvordan ser afrikansk svinepest ud i danske grise II?: Rapport over smitteforsøg i drægtige søer 2014


General information
State: Published
Organisations: National Veterinary Institute, Section for Virology
Contributors: Lohse, L., Strandbygaard, B., Nielsen, J., Uttenthal, Å., Rasmussen, T. B., Belsham, G., Bøtner, A.
Pages: 21-23
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Peer-reviewed: Unknown

Publication information
Journal: Dansk Veterinaertidsskrift
Volume: 9
ISSN (Print): 0106-6854
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
BFI (2017): BFI-level 1
Web of Science (2017): Indexed yes
We identified novel viruses in feces from cattle with diarrhea collected in 2009 in Hokkaido Prefecture, Japan, by using a metagenomics approach and determined the (near) complete sequences of the virus. Sequence analyses revealed that they had a standard picornavirus genome organization, i.e. 5' untranslated region (UTR) - L - P1 (VP4- VP3- VP2- VP1) - P2 (2A- 2B- 2C) - P3 (3A- 3B- 3C- 3D) - 3'UTR- poly(A). They are closely related to other unclassified Chinese picornaviruses; bat picornaviruses group 1-3, feline picornavirus, and canine picornavirus, sharing 45.4-51.4% (P1), 38.0-44.9% (P2), and 49.6-53.3% (P3) amino acid identities, respectively. The phylogenetic analyses and detailed genome characterization showed that they, together with the unclassified Chinese picornaviruses, grouped as a cluster for the P1, 2C, 3CD and VP1 coding regions. These viruses had conserved features (e.g. predicted protein cleavage sites, presence of a leader protein, 2A, 2C, 3C, and 3D functional domains), suggesting they have a common ancestor. Reverse-transcription-PCR assays, using specific primers designed from the 5'UTR sequence of these viruses, showed that 23.0% (20/87) of fecal samples from cattle with diarrhea were positive, indicating the prevalence of these picornavirus in the Japanese cattle population in Hokkaido Prefecture. However, further studies are needed to investigate the pathogenic potential and etiological role of these viruses in cattle.
Long Range RNA-RNA interactions with the genome of classical swine fever virus

General information
State: Published
Organisations: National Veterinary Institute, Section for Virology
Contributors: Hadsbjerg, J., Rasmussen, T. B., Belsham, G.
Long Range RNA-RNA interactions with the genome of classical swine fever virus

General information
State: Published
Organisations: National Veterinary Institute, Section for Virology
Contributors: Hadsbjerg, J., Rasmussen, T. B., Belsham, G.
Number of pages: 1
Publication date: 2015
Peer-reviewed: Yes
Event: Poster session presented at 10th International Congress for Veterinary Virology, Montpellier, France.
Electronic versions:
PosterEpiESVV_final300dpi.pdf

Use of recombinant capsid proteins in the development of a vaccine against foot-and-mouth disease virus (FMDV).

Foot-and-mouth disease remains one of the world’s most economically important diseases of livestock. It is caused by foot-and-mouth disease virus, a member of the picornavirus family. The virus replicates very rapidly and can be efficiently transmitted between hosts by a variety of routes. The disease has been effectively controlled in some parts of the world but remains endemic in many others, thus there is a constant risk of introduction of the disease into areas that are normally free of foot-and-mouth disease with potentially huge economic consequences. To reduce the need for large-scale culling of infected, and potentially infected, animals there has been significant effort to develop new vaccines against this disease which avoid some, or all, of the deficiencies of current vaccines. A major focus has been on the use of systems that express the structural proteins of the virus that self-assemble to generate “empty capsid” particles which share many features with the intact virus but lack the ribonucleic acid genome and are therefore non-infectious. Such particles can be “designed” to improve their stability or modify their antigenicity and can be produced without “high containment” facilities. The development and use of such improved vaccines should assist in the global efforts to control this important disease
Characteristics of a foot-and-mouth disease virus with a partial VP1 G-H loop deletion in experimentally infected cattle

Previous work in cattle illustrated the protective efficacy and negative marker potential of a A serotype foot-and-mouth disease virus (FMDV) vaccine prepared from a virus lacking a significant portion of the VP1 G-H loop (termed A(−)). Since this deletion also includes the arginine-glycine-aspartate (RGD) motif required for virus attachment to the host cell in vivo, it was hypothesised that this virus would be attenuated in naturally susceptible animals. The A(−) virus was passaged three times in cattle via needle inoculation of virus suspension delivered into the intradermal space of the tongue (intradermolingual: IDL). Included in the study were three direct contact cattle, two of which were used for the third cattle passage (by inoculation) after direct contact exposure for three days. Cattle were monitored for clinical signs and samples were collected for sequencing as well as antibody and viral genome detection by ELISA and qRT-PCR. Following needle inoculation with the A(−) virus, naïve cattle developed typical clinical signs of FMDV infection, diagnostic assays also provided positive serological and virological results. However, the contact cattle did not develop clinical signs or generate serological or virological markers indicative of FMDV infection even when the cattle were subsequently needle inoculated with 10^5 TCID50 A(−) FMDV delivered IDL following three days of direct contact exposure. The results suggest that the A(−) virus is not attenuated in cattle when inoculated IDL. This virus could be useful as a tool to understand further the natural pathogenesis, receptor usage and internalisation pathways of FMDV.
**Coupled adaptations affecting cleavage of the VP1/2A junction by 3C protease in foot-and-mouth disease virus infected cells**

The foot-and-mouth disease virus (FMDV) capsid protein precursor P1-2A is cleaved by the 3C protease to produce VP0, VP3, VP1 and 2A. It was shown previously that modification of a single amino acid residue (K210) within the VP1 protein, close to the VP1/2A cleavage site, inhibited cleavage of this junction and resulted in the production of "self-tagged" virus...
particles containing the 2A peptide. A second site substitution (E83K) within VP1 was also observed within the rescued virus (Gullberg et al., 2013). It is now shown that introduction of this E83K change alone into a serotype O virus resulted in the rapid accumulation of a second site substitution within the 2A sequence (L2P) that also blocked VP1/2A cleavage suggesting a linkage between the E83K change in VP1 and cleavage of the VP1/2A junction. In a serotype A background, the K210E substitution in VP1 rapidly reverted to wild type. However, introduction of the 2A L2P substitution alone, or with the VP1 K210E change, into this virus resulted in the production of viable viruses. Cells infected with viruses containing the VP1 K210E and/or the 2A L2P substitutions contained the uncleaved VP1-2A protein; the 2A L2P substitution rendered the VP1/2A junction totally resistant to cleavage by 3C protease. The basis for the linkage between amino acid substitutions that are well separated on the surface of the virus particle will be discussed.

Detection of European bat lyssavirus type 2 in Danish Daubenton’s bats
European bat lyssavirus (EBLV) is considered to be endemic in the Danish bat populations, but limited information exists about the types of EBLV strains currently in circulation. EBLV type 1 (EBLV-1) is seen as the predominant type in the Serotine bats (Eptesicus serotinus) with the latest case identified in 2009.

Development of tailored real-time RT-PCR assays for the detection and differentiation of serotype O, A and Asia-1 foot-and-mouth disease virus lineages circulating in the Middle East
Rapid and accurate diagnosis is essential for effective control of foot-and-mouth disease (FMD). In countries where FMD is endemic, identification of the serotypes of the causative virus strains is important for vaccine selection and tracing the source of outbreaks. In this study, real-time reverse transcription polymerase chain reaction (rRT-PCR) assays using primer/probe sets designed from the VP1 coding region of the virus genomes were developed for the specific detection of serotype O, A and Asia-1 FMD viruses (FMDVs) circulating in the Middle East. These assays were evaluated using representative field samples of serotype O strains belonging exclusively to the PanAsia-2 lineage, serotype A strains of the Iran-05 lineage and serotype Asia-1 viruses from three relevant sub-groups. When RNA extracted from archival and contemporary field strains was tested using one- or two-step rRT-PCR assays, all three primer/probe sets detected the RNA from homotypic viruses and no cross-reactivity was observed with heterotypic viruses. Similar results were obtained using both single- and multiplex assay formats. Using plasmid standards, the minimum detection level of these tests was found to be lower than two copies. The results illustrate the potential of tailored rRT-PCR tools for the detection and categorization of viruses circulating in the Middle East belonging to distinct subgroups of serotypes O, A and Asia-1. These assays can also overcome the problem of serotyping samples which are found positive by the generic rRT-PCR diagnostic assays but negative by virus isolation and antigen-detection ELISA which would otherwise have to be serotyped by nucleotide sequencing. A similar approach could be used to develop serotyping assays for FMDV strains circulating in other regions of the world.
Experimental infection of pregnant sows with African swine fever (ASFV Georgia 2007): Clinical outcome, pathogenesis and vertical transmission

African swine fever virus (ASFV) causes a severe hemorrhagic fever in domestic pigs. The disease was introduced from the African continent to Georgia in 2007 and has since spread throughout the Caucasus and the Russian Federation. ASF is now established in Eastern Europe and outbreaks have occurred in domestic pigs and wild boar in Poland and the Baltic countries in 2014. Therefore, there is an increased risk of further transmission across Europe. The present study investigates the properties and the effect of the circulating ASF virus strain in Danish pregnant sows.

General information

State: Published
Organisations: National Veterinary Institute, Section for Virology
Contributors: Lohse, L., Strandbygaard, B., Nielsen, J., Uttenthal, Å., Rasmussen, T. B., Belsham, G. J., Bøtner, A.
Number of pages: 1
Publication date: 2014
Peer-reviewed: No
Event: Poster session presented at 8th Annual Meeting of Epizone, Copenhagen, Denmark.
Electronic versions:

FMDV-induced stress granules are disrupted by the viral L-protease

Eukaryotic cells respond to environmental stress by entering a state of reduced protein synthesis, redirecting resources to damage control and defense. This reduced translation is closely linked to the formation of cytoplasmic stress granules (SGs). SGs are multicomponent foci, which contain stalled translation preinitiation complexes, including polyadenylated mRNAs, and several aggregation-prone RNA binding factors, such as the Ras-GAP SH3 domain-binding protein (G3BP) that enable their formation. Once the stress is lifted, the stalled complexes from the SGs are believed to re-engage in translation, facilitating cellular recovery.

A growing body of evidence shows that various viruses can trigger SG formation. However, the presence of SGs may not be beneficial to the virus and many viruses have found ways to circumvent, disrupt or even utilize these granules, suggesting a role for SGs as a general cellular defense mechanism. For picornaviruses, poliovirus have been shown to disrupt SGs by the 3C-protease dependent cleavage of G3BP (3) and for cardioviruses (Theiler’s murine encephomyelitis virus and mengovirus), SG formation is inhibited by the presence of the viral L-protein (1, 2).
We have found that foot-and-mouth disease virus (FMDV) triggers SG formation early during infection in IBRS-2 cells. These SGs contain G3BP and TIA-1, but not dsRNA. However, the presence of the FMDV-induced SGs is transient due to the cleavage of G3BP by the viral L-protease (Lpro), which results in subsequent SG dispersal. Cells infected with an Lpro-deficient mutant FMDV are not subjected to G3BP cleavage and the SGs formed upon infection with this mutant maintain throughout the infection. In vitro studies using different variants of the Lpro show different G3BP cleavage efficiencies, suggesting a superior function of the full length Lpro for this substrate. Furthermore, the Lpro-directed G3BP cleavage is not dependent on virus replication, as investigated by transfecting FMDV RNAs lacking a functional 3D-polypolymerase. Finally, FMDV RNAs that contain Lpro, but lack the FMDV 3C-protease, also induce cleavage of G3BP, showing that both FMDV and poliovirus target the same SG component but with different proteases.

**General information**
State: Published
Organisations: National Veterinary Institute, Section for Virology, Karolinska Institutet
Contributors: Polacek, C., Belsham, G., McInerney, G.
Number of pages: 1
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Electronic versions:
prod11396000402702.Polacek_Abstract EUROPIC2014final.pdf

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Oral presentation.
Source: dtu
Source-ID: u::10942
Research output: Research - peer-review › Conference abstract in proceedings – Annual report year: 2014

**Full-length genomic analysis of korean porcine sapelovirus strains.**
Porcine sapelovirus (PSV), a species of the genus Sapelovirus within the family Picornaviridae, is associated with diarrhea, pneumonia, severe neurological disorders, and reproductive failure in pigs. However, the structural features of the complete PSV genome remain largely unknown. To analyze the structural features of PSV genomes, the full-length nucleotide sequences of three Korean PSV strains were determined and analyzed using bioinformatic techniques in comparison with other known PSV strains. The Korean PSV genomes ranged from 7,542 to 7,566 nucleotides excluding the 3' poly(A) tail, and showed the typical picornavirus genome organization; 5'untranslated region (UTR)-L-VP4-VP2-VP3-VP1-2A-2B-2C-3A-3B-3C-3D-3'UTR. Three distinct cis-active RNA elements, the internal ribosome entry site (IRES) in the 5'UTR, a cis-replication element (CRE) in the 2C coding region and 3'UTR were identified and their structures were predicted. Interestingly, the structural features of the CRE and 3'UTR were different between PSV strains. The availability of these first complete genome sequences for PSV strains will facilitate future investigations of the molecular pathogenesis and evolutionary characteristics of PSV.

**General information**
State: Published
Organisations: National Veterinary Institute, Section for Virology, Chonnam National University, Korea Basic Science Institute
Contributors: Son, K., Kim, D., Kwon, J., Choi, J., Kang, M., Belsham, G., Cho, K.
Publication date: 2014
Peer-reviewed: Yes

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Volume: 9
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Article number: e107860
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BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Genetic diversity of serotype A foot-and-mouth disease viruses in Kenya from 1964 to 2013; implications for control strategies in eastern Africa

Serotype A is the most genetically and antigenically diverse of the foot-and-mouth disease virus (FMDV) serotypes. Records of its occurrence in Kenya date back to 1952 and the antigenic diversity of the outbreak viruses in this region is reflected by the current use of two different vaccine strains (K5/1980 and K35/1980) and previous use of two other strains (K18/66 and K179/71). This study aimed at enhancing the understanding of the patterns of genetic variation of serotype A FMDV in Kenya. The complete VP1 coding region sequences of 38 field isolates, identified as serotype A FMDV, were determined. Coalescent-based methods were used to infer times of divergence of the virus strains and the evolutionary rates alongside 27 other serotype A FMDV sequences from Genbank and the World Reference Laboratory (WRL). This study represents the first comprehensive genetic analysis of serotype A FMDVs from Kenya. The study detected four previously defined genotypes/clusters (termed G-I, G-III, G-VII and G-VIII), within the
Africa topotype, together with a fifth lineage that has apparently emerged from within G-I; these different lineages have each had a countrywide distribution. Genotypes G-III and G-VIII that were first isolated in 1964 are now apparently extinct; G-VII was last recorded in 2005, while G-I (including the new lineage) is currently in widespread circulation. High genetic diversity, widespread distribution and transboundary spread of serotype A FMDVs across the region of eastern Africa was apparent. Continuous surveillance for the virus, coupled to genetic and antigenic characterization is recommended for improved regional control strategies.

**General information**

State: Published
Organisations: National Veterinary Institute, Section for Virology, Foot-and-Mouth Disease Laboratory, Makerere University, Instituto Gulbenkian de Ciência, University of Copenhagen
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**Publication information**

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Volume: 21
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BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.67 SJR 1.278 SNIP 1.031
Web of Science (2017): Impact factor 2.545
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.77 SJR 1.334 SNIP 1.033
Web of Science (2016): Impact factor 2.885
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 2.85 SJR 1.441 SNIP 1.079
Web of Science (2015): Impact factor 2.591
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 2.99 SJR 1.391 SNIP 1.125
Web of Science (2014): Impact factor 3.015
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 3.26 SJR 1.563 SNIP 1.178
Web of Science (2013): Impact factor 3.264
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 2.87 SJR 1.22 SNIP 0.989
Web of Science (2012): Impact factor 2.768
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 3.11 SJR 1.189 SNIP 1.185
Web of Science (2011): Impact factor 3.128
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.33 SNIP 1.048
Web of Science (2010): Impact factor 3.086
Web of Science (2010): Indexed yes
Modification of FMDV anti-host defense mechanism
Foot-and-mouth disease virus (FMDV) is the etiologic agent of FMD, an infectious and sometimes fatal viral disease that affects cloven-hoofed animals. The FMDV genome encodes a large polyprotein, the first component of which is the Leader protein. Unusually, within the picornavirus family, the FMDV Leader protein (Lpro) is a protease. This protease induces a very rapid inhibition of host cell cap-dependent protein synthesis within infected cells. This results from cleavage of the cellular translation initiation factor eIF4G. Translation of the viral RNA is unaffected since it is dependent on an internal ribosome entry site (IRES) that directs cap-independent translation initiation. Lpro also releases itself from the virus capsid precursor (at the L/P1 junction). The aim of this project is to identify amino acids that are essential for eIF4G cleavage but not for the self-processing. This study may allow design of mutant viruses that are deficient in blocking host cell responses to infection (e.g. interferon induction) and assist in the rational design of antiviral agents targeting this process.

Rapid Spread of Schmallenberg Virus-infected Biting Midges (Culicoides spp.) across Denmark in 2012
Detection of Schmallenberg virus RNA, using real-time RT-PCR, in biting midges (Culicoides spp.) caught at 48 locations in 2011 and four well-separated farms during 2012 in Denmark, revealed a remarkably rapid spread of virus-infected midges across the country. During 2012, some 213 pools of obsoletus group midges (10 specimens per pool) were examined, and of these, 35 of the 174 parous pools were Schmallenberg virus RNA positive and 11 of them were positive in the heads. Culicoides species-specific PCRs identified both C. obsoletus and C. dewulfi as vectors of Schmallenberg virus.
Rescue of the CSFV Koslov strain from a cloned cDNA

General information
State: Published
Organisations: National Veterinary Institute, Section for Virology, Friedrich-Loeffler-Institute
Contributors: Fahnøe, U., Belsham, G., Höper, D., Beer, M., Rasmussen, T. B.
Publication date: 2014
Peer-reviewed: Yes
Event: Abstract from Workshop on Laboratory Diagnosis of African and Classical Swine Fever (ASF and CSF), Madrid, Spain.

Rescue of the highly virulent classical swine fever virus strain "Koslov" from cloned cDNA and first insights into genome variations relevant for virulence

Classical swine fever virus (CSFV) strain "Koslov" is highly virulent with a mortality rate of up to 100% in pigs. In this study, we modified non-functional cDNAs generated from the blood of Koslov virus infected pigs by site-directed mutagenesis, removing non-synonymous mutations step-by-step, thereby producing genomes encoding the consensus amino acid sequence. Viruses rescued from the construct corresponding to the inferred parental form were highly virulent, when tested in pigs, with infected animals displaying pronounced clinical symptoms leading to high mortality. The reconstruction therefore gave rise to a functional cDNA corresponding to the highly virulent Koslov strain of CSFV. It could be demonstrated that two single amino acid changes (S763L and P968H) in the surface structural protein E2 resulted in attenuation in the porcine infection system while another single amino acid change within the nonstructural protein NS3 (D2183G) reduced virus growth within cells in vitro.

General information
State: Published
Organisations: Molecular Evolution, National Veterinary Institute, Section for Virology, Systems Biotechnology, Department of Systems Biology, Center for Biological Sequence Analysis, Technical University of Denmark, Friedrich-Loeffler-Institute
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Peer-reviewed: Yes

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Journal: Virology
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Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
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Scopus rating (2017): CiteScore 3.14 SJR 1.728 SNIP 0.93
Web of Science (2017): Impact factor 3.374
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Sequence Adaptation during Growth of Modified Classical Swine Fever Viruses in Cell Culture

General information
Sequence adaptations affecting cleavage of the VP1/2A junction by the 3C protease in foot-and-mouth disease virus-infected cells.

The foot-and-mouth disease virus (FMDV) capsid protein precursor P1-2A is cleaved by the virus-encoded 3C protease to VP0, VP3, VP1, and 2A. It was shown previously that modification of a single amino acid residue (K210E) within the VP1 protein and close to the VP1/2A cleavage site, inhibited cleavage of this junction and produced 'self-tagged' virus particles. A second site substitution (E83K) within VP1 was also observed within the rescued virus [Gullberg et al. (2013). J Virol 87, 11591-11603]. It was shown here that introduction of this E83K change alone into a serotype O virus resulted in the rapid accumulation of a second site substitution within the 2A sequence (L2P), which also blocked VP1/2A cleavage. This suggests a linkage between the E83K change in VP1 and cleavage of the VP1/2A junction. Cells infected with viruses containing the VP1 K210E or the 2A L2P substitutions contained the uncleaved VP1-2A protein. The 2A L2P substitution resulted in the VP1/2A junction being highly resistant to cleavage by the 3C protease, hence it may be a preferred route for 'tagging' virus particles.
Foot-and-mouth disease virus (FMDV) is responsible for one of the most economically important diseases of farm animals (estimated annual costs are about US$10 billion globally). The virus is the prototypic Aphthovirus within the family Picornaviridae and has a positive sense RNA genome (ca. 8.3kb) encoding a single large polyprotein that is processed to generate about 15 mature proteins plus precursors. The virus particle comprises 60 copies of 4 separate capsid proteins (VP1-VP4) plus a single copy of the genome. By modifying full length cDNAs, producing RNA transcripts in vitro, and...
introducing these into susceptible cells it is possible to rescue specifically altered FMDVs. We have used this approach to generate modified viruses that have particular properties; these studies can assist in the development of improved and safer vaccines to protect against FMDV. For example, we have made changes to the leader (L) protein coding sequence. The L protein is the first component of the viral polyprotein and is produced in two forms, termed Lab and Lb as the result of use of alternative initiation codons, 84 nt apart. Both forms have protease activity (which separates the L protein from the capsid precursor) and induce the shut-off of host cell protein synthesis. When the shorter form, Lb, is precisely deleted then FMD viruses that grow well in cell culture are produced (Belsham, 2013). However such viruses are attenuated within cattle. In contrast, when the entire Lab coding sequence is deleted then no viable viruses are generated. In an alternative approach, we have modified a processing site within the viral polyprotein so that incomplete processing occurs. It has been shown that a single amino acid substitution that blocks cleavage of the VP1/2A junction within the capsid precursor results in the production of modified “self-tagged” virus particles that contain the VP1-2A precursor (Gullberg et al., 2013). This approach works for two of the most common FMDV serotypes (O and A) and offers the possibility of a single approach to purifying virus particles from different serotypes using reagents targeted to the conserved 2A peptide.

General information
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Contributors: Gullberg, M., Polacek, C., Bøtner, A., Belsham, G.
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Electronic versions:
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The diagnostic utility of stabilized blood for detection of foot-and-mouth disease virus RNA by RT-qPCR
In Europe, clinical signs indicative of foot-and-mouth disease (FMD), would immediately lead to collection of blood and relevant organ material for further laboratory examination for this vesicular disease virus. Today, the first line system for detection of virus in the sample material is real time RT-PCR (RT-qPCR). The aim of this study was to investigate the diagnostic utility of stabilized blood for detection of FMDV RNA in this system.

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Contributors: S. Fontél, K., Bøtner, A., Belsham, G., Lohse, L.
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Analysis of classical swine fever virus RNA replication determinants using replicons
Self-replicating RNAs (replicons), with or without reporter gene sequences, derived from the genome of the Paderborn strain of classical swine fever virus (CSFV) have been produced. The full-length viral cDNA, propagated within a bacterial artificial chromosome (BAC), was modified by targeted recombination within E. coli. RNA transcripts were produced in vitro and introduced into cells by electroporation. The translation and replication of the replicon RNAs could be followed by the accumulation of luciferase (from Renilla reniformis or Gaussia princeps) protein expression (where appropriate), as well as by detection of the CSFV NS3 protein production within the cells. Inclusion of the viral E2 coding region within the replicon was advantageous for the replication efficiency. Production of chimeric RNAs, substituting the NS2 and NS3 coding regions (as a unit) from the Paderborn strain with the equivalent sequences from the highly virulent Koslov strain or the vaccine strain Riems, blocked replication. However, replacing the Paderborn NS5B coding sequence with the RNA polymerase coding sequence from the Koslov strain greatly enhanced expression of the reporter protein from the replicon. In contrast, replacement with the Riems NS5B sequence significantly impaired replication efficiency. Thus these replicons provide a system for determining specific regions of the CSFV genome required for genome replication without the constraints of maintaining infectivity.

General information
State: Published
Assembly and characterization of foot-and-mouth disease virus empty capsid particles expressed within mammalian cells

The foot-and-mouth disease virus (FMDV) structural protein precursor, P1-2A, is cleaved by the virus-encoded 3C protease (3Cpro) into the capsid proteins VP0, VP1 and VP3 (and 2A). In some systems, it is difficult to produce large amounts of these processed capsid proteins since 3Cpro can be toxic for cells. The expression level of 3Cpro activity has now been reduced relative to the P1-2A, and the effect on the yield of processed capsid proteins and their assembly into empty capsid particles within mammalian cells has been determined. Using a vaccinia-virus-based transient expression system, P1-2A (from serotypes O and A) and 3Cpro were expressed from monocistronic cDNA cassettes as P1-2A-3C, or from dicistronic cassettes with the 3Cpro expression dependent on a mutant FMDV internal ribosome entry site (IRES) (designated P1-2A-mIRES-3C). The effects of using a mutant 3Cpro with reduced catalytic activity or using two different mutant IRES elements (the wt GNRA tetraloop sequence GCGA converted, in the cDNA, to GAGA or GTTA) were analysed. For both serotypes, the P1-2A-mIRES-3C construct containing the inefficient GTTA mutant IRES produced the highest amount of processed capsid proteins. These products self-assembled to form FMDV empty capsid particles, which have a related, but distinct, morphology (as determined by electron microscopy and reconstruction) from that determined previously by X-ray crystallography. The assembled empty capsids bind, in a divalent cation-dependent manner, to the RGD-dependent integrin αvβ6, a cellular receptor for FMDV, and are recognized appropriately in serotype-specific antigen ELISAs.

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Contributors: Gullberg, M., Muszynski, B., Organtini, L. J., Ashley, R. E., Hafenstein, S. L., Belsham, G. J., Polacek, C.
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Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.93 SJR 1.544 SNIP 0.891
Web of Science (2016): Impact factor 2.838
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.26 SJR 1.738 SNIP 0.998
Web of Science (2015): Impact factor 3.192
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.25 SJR 1.69 SNIP 1.057
Web of Science (2014): Impact factor 3.183
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
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ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 3.28 SJR 1.525 SNIP 1.034
Web of Science (2012): Impact factor 3.127
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 3.6 SJR 1.684 SNIP 1.145
Web of Science (2011): Impact factor 3.363
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.678 SNIP 1.053
Web of Science (2010): Impact factor 3.568
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.662 SNIP 1.127
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.648 SNIP 1.068
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 1.593 SNIP 1.131
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.709 SNIP 1.128
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 1.654 SNIP 1.137
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 1.55 SNIP 1.215
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 1.58 SNIP 1.145
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 1.369 SNIP 1.083
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 1.488 SNIP 1.109
Efficient generation of recombinant RNA viruses using targeted recombination-mediated mutagenesis of bacterial artificial chromosomes containing full-length cDNA

Background
Infectious cDNA clones are a prerequisite for directed genetic manipulation of RNA viruses. Here, a strategy to facilitate manipulation and rescue of classical swine fever viruses (CSFVs) from full-length cDNAs present within bacterial artificial chromosomes (BACs) is described. This strategy allows manipulation of viral cDNA by targeted recombination-mediated mutagenesis within bacteria.

Results
A new CSFV-BAC (pBeloR26) derived from the Riems vaccine strain has been constructed and subsequently modified in the E2 coding sequence, using the targeted recombination strategy to enable rescue of chimeric pestiviruses (vR26_E2gif and vR26_TAV) with potential as new marker vaccine candidates. Sequencing of the BACs revealed a high genetic stability during passages within bacteria. The complete genome sequences of rescued viruses, after extensive passages in mammalian cells showed that modifications in the E2 protein coding sequence were stably maintained. A single amino acid substitution (D3431G) in the RNA dependent RNA polymerase was observed in the rescued viruses vR26_E2gif and vR26, which was reversion to the parental Riems sequence.

Conclusions
These results show that targeted recombination-mediated mutagenesis provides a powerful tool for expediting the construction of novel RNA genomes and should be applicable to the manipulation of other RNA viruses.
Efficient production of foot-and-mouth disease virus empty capsids in insect cells following down regulation of 3C protease activity

Foot-and-mouth disease virus (FMDV) is a significant economically and distributed globally pathogen of Artiodactyla. Current vaccines are chemically inactivated whole virus particles that require large-scale virus growth in strict biocontainment with the associated risks of accidental release or incomplete inactivation. Non-infectious empty capsids are structural mimics of authentic particles with no associated risk and constitute an alternate vaccine candidate. Capsids self-assemble from the processed virus structural proteins, VP0, VP3 and VP1, which are released from the structural protein precursor P1-2A by the action of the virus-encoded 3C protease. To date recombinant empty capsid assembly has been limited by poor expression levels, restricting the development of empty capsids as a viable vaccine. Here expression of the FMDV structural protein precursor P1-2A in insect cells is shown to be efficient but linkage of the cognate 3C protease to the C-terminus reduces expression significantly. Inactivation of the 3C enzyme in a P1-2A-3C cassette allows expression and intermediate levels of 3C activity resulted in efficient processing of the P1-2A precursor into the structural proteins which assembled into empty capsids. Expression was independent of the insect host cell background and leads to capsids that are recognised as authentic by a range of anti-FMDV bovine sera suggesting their feasibility as an alternate vaccine.

General information

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BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 1.82 SJR 0.858 SNIP 0.817
Web of Science (2017): Impact factor 1.756
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 1.78 SJR 0.873 SNIP 0.729
Web of Science (2016): Impact factor 1.693
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 1.68 SJR 0.87 SNIP 0.802
Web of Science (2015): Impact factor 1.508
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 1.87 SJR 0.898 SNIP 0.933
Web of Science (2014): Impact factor 1.781
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 1.99 SJR 0.866 SNIP 0.9
Web of Science (2013): Impact factor 1.883
ISI indexed (2013): ISI indexed yes
Foot-and-mouth disease: past, present and future

ABSTRACT: Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals including cattle, pigs, sheep and many wildlife species. It can cause enormous economic losses when incursions occur into countries which are normally disease free. In addition, it has long-term effects within countries where the disease is endemic due to reduced animal productivity and the restrictions on international trade in animal products. The disease is caused by infection with foot-and-mouth disease virus (FMDV), a picornavirus. Seven different serotypes (and numerous variants) of FMDV have been identified. Some serotypes have a restricted geographical distribution, e.g. Asia-1, whereas others, notably serotype O, occur in many different regions. There is no cross-protection between serotypes and sometimes protection conferred by vaccines even of the same serotype can be limited. Thus it is important to characterize the viruses that are circulating if vaccination is being used for disease control. This review describes current methods for the detection and characterization of FMDVs. Sequence information is increasingly being used for identifying the source of outbreaks. In addition such information can be used to understand antigenic change within virus strains. The challenges and
opportunities for improving the control of the disease within endemic settings, with a focus on Eurasia, are discussed, including the role of the FAO/EuFMD/OIE Progressive Control Pathway. Better control of the disease in endemic areas reduces the risk of incursions into disease-free regions.

**General information**

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**Contributors:** Jamal, S. M., Belsham, G.
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- BFI (2018): BFI-level 2
- Web of Science (2018): Indexed yes
- BFI (2017): BFI-level 2
- Scopus rating (2017): SJR 1.266 SNIP 1.139
- Web of Science (2017): Impact factor 2.903
- Web of Science (2017): Indexed yes
- BFI (2016): BFI-level 2
- Scopus rating (2016): SJR 1.44 SNIP 1.303
- Web of Science (2016): Impact factor 2.798
- Web of Science (2016): Indexed yes
- BFI (2015): BFI-level 2
- Scopus rating (2015): CiteScore 2.66 SJR 1.537 SNIP 1.153
- Web of Science (2015): Impact factor 2.928
- Web of Science (2015): Indexed yes
- BFI (2014): BFI-level 2
- Scopus rating (2014): CiteScore 2.46 SJR 1.453 SNIP 1.423
- Web of Science (2014): Impact factor 2.815
- Web of Science (2014): Indexed yes
- BFI (2013): BFI-level 2
- Scopus rating (2013): CiteScore 3.13 SJR 1.681 SNIP 1.701
- Web of Science (2013): Impact factor 3.383
- ISI indexed (2013): ISI indexed yes
- Web of Science (2013): Indexed yes
- BFI (2012): BFI-level 2
- Scopus rating (2012): CiteScore 2.97 SJR 1.461 SNIP 1.45
- Web of Science (2012): Impact factor 3.426
- ISI indexed (2012): ISI indexed yes
- Web of Science (2012): Indexed yes
- BFI (2011): BFI-level 2
- Scopus rating (2011): CiteScore 3.85 SJR 1.712 SNIP 1.655
- Web of Science (2011): Impact factor 4.06
- ISI indexed (2011): ISI indexed yes
- Web of Science (2011): Indexed yes
- BFI (2010): BFI-level 2
- Scopus rating (2010): SJR 1.531 SNIP 1.606
- BFI (2009): BFI-level 2
Foot-and-Mouth Disease Virus Serotype O Phylodynamics: Genetic Variability Associated with Epidemiological Factors in Pakistan

One of the most challenging aspects of foot-and-mouth disease (FMD) control is the high genetic variability of the FMD virus (FMDV). In endemic settings such as the Indian subcontinent, this variability has resulted in the emergence of pandemic strains that have spread widely and caused devastating outbreaks in disease-free areas. In countries trying to control and eradicate FMD using vaccination strategies, the constantly evolving and wide diversity of field FMDV strains is an obstacle for identifying vaccine strains that are successful in conferring protection against infection with field viruses. Consequently, quantitative knowledge on the factors that are associated with variability of the FMDV is prerequisite for preventing and controlling FMD in the Indian subcontinent. A hierarchical linear model was used to assess the association between time, space, host species and the genetic variability of serotype O FMDV using viruses collected in Pakistan from 2005 to 2011. Significant (P < 0.05) amino acid and nucleotide variations were associated with spatial distance, but not with differences in host species, which is consistent with the frequent multi-species infection of this serotype O FMDV. Results from this study will contribute to the understanding of FMDV variability and to the design of FMD control strategies in Pakistan. Viruses sequenced here also provide the earliest reported isolate from the Pan Asia IANT-10 sublineage, which has caused several outbreaks in the Middle East and spread into Europe (Bulgaria) and Africa (Libya).

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Web of Science (2018): Indexed yes
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Scopus rating (2017): CiteScore 2.87 SJR 1.147 SNIP 1.488
Web of Science (2017): Impact factor 3.504
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.16 SJR 1.046 SNIP 0.998
Web of Science (2016): Impact factor 3.585
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 2.29 SJR 1.305 SNIP 1.249
Web of Science (2015): Impact factor 2.714
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 2.23 SJR 1.048 SNIP 1.207
Web of Science (2014): Impact factor 2.944
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 2.33 SJR 0.975 SNIP 1.123
Web of Science (2013): Impact factor 3.116
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 2.04 SJR 0.847 SNIP 1.178
Web of Science (2012): Impact factor 2.096
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 2.05 SJR 0.939 SNIP 1.124
Web of Science (2011): Impact factor 1.809
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.761 SNIP 0.983
Web of Science (2010): Impact factor 2.448
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.601 SNIP 0.907
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 0.363 SNIP 0.707
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 0.456 SNIP 0.777
Scopus rating (2006): SJR 0.425 SNIP 0.756
Scopus rating (2005): SJR 0.394 SNIP 0.852
Scopus rating (2004): SJR 0.296 SNIP 0.571
Scopus rating (2003): SJR 0.298 SNIP 0.68
Scopus rating (2002): SJR 0.268 SNIP 0.635
Scopus rating (2001): SJR 0.33 SNIP 0.658
Identification of the determinants of efficient Pestivirus replication

The key for the survival of a virus is to copy its own genome into progeny genomes that allows continued reproduction. The mechanism behind this "copy function" or "replication" is a well-organized process that involves the formation of a replication complex in the cell and interactions between the viral proteins. The replication process in single-stranded RNA viruses of positive polarity requires a particular enzyme, an RNA dependent RNA polymerase, that has no direct counterpart elsewhere in nature. The variable nature of rapidly evolving viral genomes pose a constant challenge to the host, and in depth knowledge of the traits that determine the fitness of the virus in this regard are highly valuable. Recent advances in the field of molecular virology with methods to manipulate viral genomes have significantly helped to uncover these core mechanisms responsible for exploitation of the host. This includes aspects of the infection, evasion from host antiviral defense, genome replication and viral assembly. With special reference to a particular RNA virus, Classical swine fever virus (CSFV), this thesis deals with the elucidation of traits involved in replication of the viral genome. This is accomplished via the application of precisely bioengineered viral constructs and through the use of state-of-the-art virological methods. The presence of full-length cDNA sequences of RNA viruses within stable vectors has been the "holy grail" for the reverse genetics approaches, and for the rescue of bioengineered mutants. The availability, in our lab, of bacterial artificial chromosomes (BACs) containing full-length cDNA sequences which can be used to rescue three different CSFV strains with a spectrum of virulence, have been a central resource for this work. The thesis is composed of four parts: Part 1, gives a general introduction to RNA viruses, with the focus on viruses classified within the Flaviviridae. Next, pestiviruses are described with special attention to classical swine fever virus and the disease it is responsible for. A brief history of types of viral vaccines is provided, finishing with a description of the molecular methods used for viral cDNA manipulation, bio-engineering approaches, description of viral reporters and so forth. Part 2, "Pestiviruses: Infection and requirements for viral RNA replication" is meant as a walk through the literature describing Pestivirus/Classical swine fever virus replication determinants, including a thorough presentation of the viral proteins, and the involvement of these in the infection progress. Part 3, "The manuscripts", includes the papers published and submitted on this work. These describe the outcome of experiments performed during the three years. Manuscript I is a coauthored paper that describes a summary of the work I have been doing in my thesis dealing with the application of the Red/ET mediated homologous recombination method to modify viral cDNA. For proof of this method, CSFV/BDV chimeric clones were produced and characterized (Submitted paper, BMC genomics). Manuscript II describes the generation of replicons that express two different types of luciferases (Rluc and Gluc), and their application as a tool for easy monitoring of replication competence (published paper, Journal of General Virology (94), 1739-1748). Manuscript III describes the properties of chimeric replicons and infectious clones that include a RNA dependent RNA polymerase (NS5B) from one of three different CSFV strains with distinct virulence properties. The entire NS5B proved to influence replication competence and key residues for replication competence was identified as judged by reporter protein expression kinetics and from using infectious clones. Furthermore, evidence is provided that these specific single amino acid substitutions in the NS5B fingertip region, can influence the rate of viral RNA replication and virus spread. Part 4, is a summary and discussion of the general and overall conclusions and a walk through the milestones that have been achieved. Future perspectives and work that should be carried out are addressed as well.
Influence of the Leader protein coding region of foot-and-mouth disease virus on virus replication

The foot-and-mouth disease virus (FMDV) Leader (L) protein is produced in two forms, Lab and Lb, differing only at their amino-termini, due to the use of separate initiation codons, usually 84 nt apart. It has been shown previously, and confirmed here, that precise deletion of the Lab coding sequence is lethal for the virus, whereas loss of the Lb coding sequence results in a virus that is viable in BHK cells. In addition, it is now shown that deletion of the ‘spacer’ region between these two initiation codons can be tolerated. Growth of the virus precisely lacking just the Lb coding sequence resulted in a previously undetected accumulation of frameshift mutations within the ‘spacer’ region. These mutations block the inappropriate fusion of amino acid sequences to the amino-terminus of the capsid protein precursor. Modification, by site-directed mutagenesis, of the Lab initiation codon, in the context of the virus lacking the Lb coding region, was also tolerated by the virus within BHK cells. However, precise loss of the Lb coding sequence alone blocked FMDV replication in primary bovine thyroid cells. Thus, the requirement for the Leader protein coding sequences is highly dependent on the nature and extent of the residual Leader protein sequences and on the host cell system used. FMDVs precisely lacking Lb and with the Lab initiation codon modified may represent safer seed viruses for vaccine production.

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BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.68 SJR 1.325 SNIP 0.877
Web of Science (2017): Impact factor 2.514
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.93 SJR 1.544 SNIP 0.891
Web of Science (2016): Impact factor 2.838
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.26 SJR 1.738 SNIP 0.998
Web of Science (2015): Impact factor 3.192
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.25 SJR 1.69 SNIP 1.057
Web of Science (2014): Impact factor 3.183
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 3.64 SJR 1.764 SNIP 1.154
Web of Science (2013): Impact factor 3.529
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 3.28 SJR 1.525 SNIP 1.034
Web of Science (2012): Impact factor 3.127
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 3.6 SJR 1.684 SNIP 1.145
Low levels of foot-and-mouth disease virus 3C protease expression are required to achieve optimal capsid protein expression and processing in mammalian cells

The foot-and-mouth disease virus (FMDV) capsid protein precursor (P1-2A) is processed by the virus-encoded 3C protease (3Cpro) to produce VP0, VP3, VP1 and 2A. Within the virus-encoded polyprotein, the P1-2A and 3Cpro can be expected to be produced at equivalent concentrations. However, using transient-expression assays, within mammalian cells, it is possible to modify the relative amounts of the substrate and protease. It has now been shown that optimal production of the processed capsid proteins from P1-2A is achieved with reduced levels of 3Cpro expression, relative to the P1-2A, compared with that achieved with a single P1-2A-3C polyprotein. Expression of the FMDV 3Cpro is poorly tolerated by mammalian cells and higher levels of the 3Cpro greatly inhibit protein expression. In addition, it is demonstrated that both the intact P1-2A precursor and the processed capsid proteins can be efficiently detected by FMDV antigen detection assays. Furthermore, the P1-2A and the processed forms each bind to the integrin αvβ6, the major FMDV receptor. These results contribute to the development of systems which efficiently express the components of empty capsid particles and may represent the basis for safer production of diagnostic reagents and improved vaccines against foot-and-mouth disease.

General information
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Organisations: National Veterinary Institute, Section for Virology, Lanzhou Veterinary Research Institute
Contributors: Polacek, C., Gullberg, M., Li, J., Belsham, G.
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Occurrence of Schmallenberg virus in Danish biting midges (Culicoides spp.)

General information
State: Published
Organisations: National Veterinary Institute, Section for Virology, Section for Epidemiology
Contributors: Rasmussen, L. D., Kirkeby, C., Kristensen, B., Rasmussen, T. B., Belsham, G., Bødker, R., Bøtner, A.
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Electronic versions:
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Research output: Research - peer-review : Poster – Annual report year: 2013

Processing of the VP1/2A Junction Is Not Necessary for Production of Foot-and-Mouth Disease Virus Empty Capsids and Infectious Viruses: Characterization of "Self-Tagged" Particles

The foot-and-mouth disease virus (FMDV) capsid protein precursor, P1-2A, is cleaved by 3Cpro to generate VP0, VP3, VP1, and the peptide 2A. The capsid proteins self-assemble into empty capsid particles or viruses which do not contain 2A. In a cell culture-adapted strain of FMDV (O1 Manisa [Lindholm]), three different amino acid substitutions (E83K, S134C, and K210E) were identified within the VP1 region of the P1-2A precursor compared to the field strain (wild type [wt]). Expression of the O1 Manisa P1-2A (wt or with the S134C substitution in VP1) plus 3Cpro, using a transient expression system, resulted in efficient capsid protein production and self-assembly of empty capsid particles. Removal of the 2A peptide from the capsid protein precursor had no effect on capsid protein processing or particle assembly. However, modification of E83K alone abrogated particle assembly with no apparent effect on protein processing. Interestingly, the K210E substitution, close to the VP1/2A junction, completely blocked processing by 3Cpro at this cleavage site, but efficient assembly of "self-tagged" empty capsid particles, containing the uncleaved VP1-2A, was observed. These self-tagged particles behaved like the unmodified empty capsids in antigen enzyme-linked immunosorbent assays and integrin receptor binding assays. Furthermore, mutant viruses with uncleaved VP1-2A could be rescued in cells from full-length FMDV RNA transcripts encoding the K210E substitution in VP1. Thus, cleavage of the VP1/2A junction is not essential for virus viability. The production of such engineered self-tagged empty capsid particles may facilitate their purification for use as diagnostic reagents and vaccines.

General information
State: Published
Organisations: National Veterinary Institute, Section for Virology
Contributors: Gullberg, M., Polacek, C., Bøtner, A., Belsham, G.
Pages: 11591-11603
The comparative utility of oral swabs and probang samples for detection of foot-and-mouth disease virus infection in cattle and pigs

Foot-and-mouth disease virus (FMDV) RNA was measured using quantitative reverse transcription-PCR (qRT-PCR) assays in oral swabs and probang samples collected from cattle and pigs during experimental infections with serotype O FMDV. During acute infection, FMDV RNA was measurable in oral swabs as well as in probang samples from both species. FMDV RNA could be detected in oral swabs and probang samples from a time point corresponding to the onset of viremia in directly inoculated animals, whereas animals which were infected through contact exposure had low levels of FMDV RNA in oral swabs before viral RNA could be measured in serum. Analysis of samples collected from cattle persistently infected with FMDV showed that it was not possible to detect FMDV RNA in oral swabs harvested beyond 10 days post infection (dpi), despite the presence of FMDV RNA in probang samples that had been collected as late as 35 dpi. An interesting feature of the persistent infection in the cattle was the apparent decline in the level of FMDV RNA in probang samples after the acute phase of infection, which was followed by a marked rise again (in all the carrier animals) by 28 dpi.
Results from this study indicate that qRT-PCR analysis of oralswabs is a useful approach in order to achieve a time efficient and reliable initial diagnosis of acute FMD in cattle and pigs, whereas probang sampling is essential for the detection of cattle that are persistently infected "carriers" of FMDV.
Transplacental transmission of field and rescued strains of BTV-2 and BTV-8 in experimentally infected sheep

Transplacental transmission of bluetongue virus has been shown previously for the North European strain of serotype 8 (BTV-8) and for tissue culture or chicken egg-adapted vaccine strains but not for field strains of other serotypes. In this study, pregnant ewes (6 per group) were inoculated with either field or rescued strains of BTV-2 and BTV-8 in order to determine the ability of these viruses to cross the placental barrier. The field BTV-2 and BTV-8 strains was passaged once in Culicoides KC cells and once in mammalian cells. All virus inoculated sheep became infected and seroconverted against the different BTV strains used in this study. BTV RNA was detectable in the blood of all but two ewes for over 28 days but infectious virus could only be detected in the blood for a much shorter period. Interestingly, transplacental transmission of BTV-2 (both field and rescued strains) was demonstrated at high efficiency (6 out of 13 lambs born to BTV-2 infected ewes) while only 1 lamb of 12 born to BTV-8 infected ewes showed evidence of in utero infection. In addition, evidence for horizontal transmission of BTV-2 between ewes was observed. As expected, the parental BTV-2 and BTV-8 viruses and the viruses rescued by reverse genetics showed very similar properties to each other. This study showed, for the first time, that transplacental transmission of BTV-2, which had been minimally passaged in cell culture, can occur; hence such transmission might be more frequent than previously thought.
Analysis of viremia and transplacental transmission of field and rescued strains of BTV-2 and BTV-8 following inoculation of pregnant sheep

Objectives
Live bluetongue virus (BTV) vaccine-strains and also, surprisingly, the European strain of BTV-8 can cross the placental-barrier and thus pass from one generation of animals to another without involvement of the insect vector. A better understanding of the genetic basis for the transmission characteristics of the virus would help to identify the risks posed by further BTV incursions and facilitate the design of better control strategies. The development of reverse genetics for BTV enables investigation of the genetic traits conferred by individual genome segments within rescued viruses by making defined reassortants. To date, only a few experiments have investigated whether field and rescued virus strains behave similarly in vivo.

Methods
Twenty-four sheep (in 4 groups of 6) were inoculated (s.c.) with 4 strains of BTV in late pregnancy (approx. 1 month before lambing). The viruses used were: BTV-2 wt (Italian field strain), BTV-2 (rescued), BTV-8 wt (field strain from the Netherlands) and BTV-8 (rescued). Four sheep were non-inoculated controls. Blood samples from the sheep were tested frequently for viremia and anti-BTV antibodies (by ELISA) in the period until lambing. Pre-colostral blood samples were collected from all newborn lambs, except for one born dead, to determine if transplacental transmission had occurred. Milk from ewes was collected daily for 7 days after lambing and blood samples from the lambs were collected on days 0, 3 and 7 after birth. All samples have being tested for the presence of anti-BTV antibodies and for virus (RT-qPCR).

Results
All inoculated animals developed viremia. The viremia was significantly higher at all sampling points following inoculation (p<0.01 or p< 0.05, Mann-Whitney's U Test) in animals inoculated with BTV-2 wt compared to animals inoculated with BTV-2 rescued, whereas no significant difference was detected between BTV-8 wt and BTV-8 rescued. Wild type virus infected animals had a longer lag phase before antibodies were detected but the response increased at a faster rate. Some of the animals displayed clinical signs of infection, e.g. fever and panting. All the ewes delivered one lamb each, a few lambs born early did not thrive and were euthanized but most appeared healthy. Seven of the 28 lambs had been infected transplacentally; 2 from ewes inoculated with BTV-2 wt, 3 from ewes inoculated with BTV-2 rescued and 1 from a ewe inoculated with BTV-8 wt. The last infected lamb was from a non-inoculated control sheep, in the same stable but physically separated from, the BTV-2 wt inoculated ewes and became viremic with BTV-2 10 days after the others were inoculated.

Conclusion
Both wild-type and rescued BTVs induced viremia. Surprisingly, transplacental transmission occurred more frequently in ewes inoculated with BTV-2, both wt and rescued, than in ewes inoculated with BTV-8. The BTV-2 wt was passaged once in Kc and once in CPT-Tert cells. These very few passages may be enough to introduce changes enabling the virus to cross the placental barrier. This experiment indicates it will be difficult to identify a single BTV segment responsible for transplacental transmission in sheep using rescued BTV-2 and BTV-8 strains.
Capsid coding sequences of foot-and-mouth disease viruses are determinants of pathogenicity in pigs

The surface exposed capsid proteins, VP1, VP2 and VP3, of foot-and-mouth disease virus (FMDV) determine its antigenicity and the ability of the virus to interact with host-cell receptors. Hence, modification of these structural proteins may alter the properties of the virus. In the present study we compared the pathogenicity of different FMDVs in young pigs. In total 32 pigs, 7-weeks-old, were exposed to virus, either by direct inoculation or through contact with inoculated pigs, using cell culture adapted (O1K B64), chimeric (O1K/A-TUR and O1K/O-UKG) or field strain (O-UKG/34/2001) viruses. The O1K B64 virus and the two chimeric viruses are identical to each other except for the capsid coding region. Animals exposed to O1K B64 did not exhibit signs of disease, while pigs exposed to each of the other viruses showed typical clinical signs of foot-and-mouth disease (FMD). All pigs infected with the O1K/O-UKG chimera or the field strain (O-UKG/34/2001) developed fulminant disease. Furthermore, 3 of 4 in-contact pigs exposed to the O1K/O-UKG virus died in the acute phase of infection, likely from myocardial infection. However, in the group exposed to the O1K/A-TUR chimeric virus, only 1 pig showed symptoms of disease within the time frame of the experiment (10 days). All pigs that developed clinical disease showed a high level of viral RNA in serum and infected pigs that survived the acute phase of infection developed a serotype specific antibody response. It is concluded that the capsid coding sequences are determinants of FMDV pathogenicity in pigs.
Comparison of the pathogenicity of two serotype O foot-and-mouth disease viruses (chimeric and field strain viruses) in pigs

General information
State: Published
Organisations: National Veterinary Institute, Section for Virology, Pirbright Institute
Contributors: Lohse, L., Jackson, T., Bøtner, A., Belsham, G.
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Event: Poster session presented at EuFMD meeting 2012, Jerez de la Frontera, Spain.
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Culicoids as Vectors of Schmallenberg Virus

General information
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Organisations: National Veterinary Institute, Division of Virology, Sektion for Eksotiske Virussygdomme, Division of Veterinary Diagnostics and Research, Section for Veterinary Epidemiology and public sector consultancy
Contributors: Rasmussen, L. D., Kristensen, B., Kirkeby, C., Rasmussen, T. B., Belsham, G., Bødker, R., Bøtner, A.
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Scopus rating (2017): CiteScore 4.78 SJR 3.278 SNIP 1.916
Web of Science (2017): Impact factor 7.422
Web of Science (2017): Indexed yes
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Scopus rating (2016): CiteScore 4.92 SJR 3.428 SNIP 2.198
Web of Science (2016): Impact factor 8.222
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 4.23 SJR 3.101 SNIP 2.012
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 4.59 SJR 3.509 SNIP 2.406
Web of Science (2014): Impact factor 6.751
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 4.68 SJR 3.254 SNIP 2.266
Web of Science (2013): Impact factor 7.327
Detection and genetic characterization of foot-and-mouth disease viruses in samples from clinically healthy animals in endemic settings

A total of 1501 oral swab samples from Pakistan, Afghanistan and Tajikistan were collected from clinically healthy animals between July 2008 and August 2009 and assayed for the presence of foot-and-mouth disease virus (FMDV) RNA. The oral swab samples from two (of four) live animal markets in Pakistan (n = 245), one (of three) live animal market in Afghanistan (n = 61) and both the live animal markets in Tajikistan (n = 120) all tested negative. However, 2 of 129 (~2%) samples from Gondal and 11 of 123 (9%) from Chichawatni markets in Pakistan were positive for FMDV RNA. Similarly, 12 of 81 (15%) samples from Kabul and 10 of 20 (50%) from Badakhshan in Afghanistan were found to be positive. Serotypes A and O of FMDV were identified within these samples. Oral swab samples were also collected from dairy colonies in Harbanspura, Lahore (n = 232) and Nagori, Karachi (n = 136), but all tested negative for FMDV. In the Landhi dairy colony, Pakistan, a cohort of 179 apparently healthy animals was studied. On their arrival within the colony, thirty-nine (22%) of these animals were found positive for FMDV RNA (serotype A was identified), while 130 (72.6%) had antibodies to FMDV non-structural proteins. Thus, newly introduced animals may be a significant source of the disease in
the colony. Only two animals from the cohort were detected as becoming positive for FMDV RNA during a follow-up period of 4 months; however, only 10 animals remained negative for anti-NSP antibodies during this period.

**General information**

State: Published

Organisations: Division of Virology, Sektion for Eksotiske Virussygdomme, National Veterinary Institute, Food and Agriculture Organization of the United Nations, FAO Tajikistan, National Veterinary Laboratory, Quaid-I-Azam University


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Scopus rating (2017): CiteScore 2.87 SJR 1.147 SNIP 1.488

Web of Science (2017): Impact factor 3.504

Web of Science (2017): Indexed yes

BFI (2016): BFI-level 1

Scopus rating (2016): CiteScore 2.16 SJR 1.046 SNIP 0.998

Web of Science (2016): Impact factor 3.585

Web of Science (2016): Indexed yes

BFI (2015): BFI-level 1

Scopus rating (2015): CiteScore 2.29 SJR 1.305 SNIP 1.249

Web of Science (2015): Impact factor 2.714

Web of Science (2015): Indexed yes

BFI (2014): BFI-level 1

Scopus rating (2014): CiteScore 2.23 SJR 1.048 SNIP 1.207

Web of Science (2014): Impact factor 2.944

Web of Science (2014): Indexed yes

BFI (2013): BFI-level 1

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BFI (2012): BFI-level 1

Scopus rating (2012): CiteScore 2.04 SJR 0.847 SNIP 1.178

Web of Science (2012): Impact factor 2.096

ISI indexed (2012): ISI indexed yes

Web of Science (2012): Indexed yes

BFI (2011): BFI-level 1

Scopus rating (2011): CiteScore 2.05 SJR 0.939 SNIP 1.124

Web of Science (2011): Impact factor 1.809

ISI indexed (2011): ISI indexed yes

BFI (2010): BFI-level 1

Scopus rating (2010): SJR 0.761 SNIP 0.983

Web of Science (2010): Impact factor 2.448

Web of Science (2010): Indexed yes
Detection of foot-and-mouth disease virus RNA in pharyngeal epithelium biopsy samples obtained from infected cattle: Investigation of possible sites of virus replication and persistence

Foot-and-mouth disease (FMD) is a highly contagious viral infection of significant financial importance to the export and trade of agricultural products. The occurrence of persistently infected "carriers" of FMD-virus (FMDV) in ruminant species adds further complications to disease control. There have been significant discrepancies in reports regarding the pathogenesis of FMDV infection in cattle with specific emphasis on the anatomical sites involved in early and persistent virus replication. In this study, collection of small biopsy samples from the dorsal soft palate (DSP) of live animals was used to investigate the level of FMDV RNA present at this site at sequential time points during the infection. Results were compared to measurements of virus excretion in samples of oropharyngeal fluid collected at corresponding time points. Possible sites of virus persistence were investigated through measurements of the levels of FMDV RNA in the DSP as well as mandibular and retropharyngeal lymph nodes beyond 28 days after infection. Results indicated only low levels of FMDV RNA present in samples of pharyngeal epithelia during both early and persistent phases of infection with significantly higher levels of virus detected in pharyngeal excretions. It is concluded that the targeted area for sampling within the DSP does not harbour significant levels of virus replication during acute or persistent FMDV infection in cattle. Furthermore, the DSP and the mandibular and retropharyngeal lymph nodes cannot be concluded to be principal sites for persistence of FMDV.
Differences in detection of foot-and-mouth disease virus RNA in oral swabs and probang samples during experimental infection of cattle and pigs

General information
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Organisations: Division of Virology, Sektion for Eksotiske Virussygdomme, National Veterinary Institute, Section for Virology
Contributors: Stenfeldt, A. C., Lohse, L., Belsham, G.
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Research output: Research › Poster – Annual report year: 2012

EFSA Panel on Animal Health and Welfare (AHAW); Scientific Opinion on foot-and-mouth disease in Thrace: EFSA-Q-2010-01238

Following a request from the Commission, the Panel on Animal Health and Welfare was asked to deliver a Scientific Opinion on: 1) the expected prevalence (design prevalence) under different circumstances, and, 2) an updated scientific assessment of the size of the relevant geographical area for the purpose of monitoring and surveillance programmes for bluetongue. A systematic literature review and a review of monitoring and surveillance data from European Union Member States was performed in order to estimate the prevalences observed in the Member States. The prevalences observed in areas that have been infected for several years were slightly lower than the design prevalence of 2 % currently used for monthly testing of sentinel animals, but much lower than the design prevalences of 20 % and 10 % for annual surveys in populations of unvaccinated and vaccinated ruminants, respectively. Currently there is no scientific evidence that suggests an optimal size of the relevant geographic unit for BTV monitoring and surveillance, since it depends on many factors, including the goal of the surveillance programmes. Early warning based on passive surveillance will take place irrespective of the size of the geographical unit but, when based on active surveillance, it is best targeted at regions considered at risk for introduction, using small geographical units, a high sampling frequency and sample size. For estimating the impact of interventions on the prevalence of infected animals, smaller areas result in more precise estimates of the prevalence and also take better account of local differences. For establishing freedom from infection, smaller areas result in lower design prevalence for a region as a whole and take better account of local differences in infection dynamics.

General information
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Organisations: National Veterinary Institute, Section for Virology, European Food Safety Authority
Contributors: Bøtner, A., Thulke, H., Salman, M., Belsham, G., Khomenko, S., Depner, K., Alexandrov, T., Ozyoruk, F., Chondrokouki, H.
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Evidence for Culicoides obsoletus group as vector for Schmallenberg virus in Denmark

Schmallenberg virus (SBV) was first identified in Germany in late 2011 by the Friedrich Loeffler Institute and has now been found in several European countries including Holland, France, Belgium, U.K. and Spain. The disease, which affects sheep, cattle and goats, was first recognized due to transient clinical symptoms including fever, diarrhea and loss of milk production. However, a more significant consequence of infection in pregnant animals is the production of severe congenital malformations in newborn animals, especially lambs. The virus is a member of the Orthobunyavirus genus within the Bunyaviridae family and is closely related to Shamonda and Akabane viruses. These viruses are transmitted by insect vectors (including biting midges (Culicoides sp.) and mosquitoes). To determine whether these insects may act as vectors for SBV, biting midges (Culicoides spp.) caught in October 2011, in the south-west of Denmark (close to the German border), were sorted into pools and tested for the presence of Schmallenberg virus RNA by RT-qPCR. From 18 pools of 5 midges from the C. obsoletus group, 2 pools were both found positive in two separate assays, targeting the L- and S- segments of the SBV RNA. However, 4 pools of C. punctatus s.str were negative. The sequence of 80bp (excluding the primer sequences) from the amplicons (ca. 145bp) was identical to that published for the expected region of the SBV L-segment. The levels of SBV RNA detected in the biting midges were much higher than could be accounted for due to the residue of a blood meal and no ruminant actin mRNA could be detected either. These results strongly suggest that SBV has replicated within specimens of the C. obsoletus group and indicates that these biting midges can act as vectors for this virus. To date (end of March), no cases of disease due to SBV have been detected in sheep, cattle or goats in Denmark.

General information
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Organisations: National Veterinary Institute, Division of Virology, Sektion for Eksotiske Virussygdomme, Division of Veterinary Diagnostics and Research, Section for Veterinary Epidemiology and public sector consultancy
Contributors: Rasmussen, L. D., Kristensen, B., Kirkeby, C., Rasmussen, T. B., Belsham, G., Bødker, R., Bøtner, A.
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Modulation of Cytokine mRNA Expression in Pharyngeal Epithelial Samples obtained from Cattle Infected with Foot-and-Mouth Disease Virus

A novel technique of endoscopical collection of small tissue samples was used to obtain sequential tissue samples from the dorsal soft palate (DSP) of individual cattle infected with foot-and-mouth disease virus (FMDV) at different phases of the infection. Levels of mRNA encoding interferon (IFN)-a and IFN-b as well as tumour necrosis factor (TNF)-a were measured in these samples by quantitative reverse transcriptase polymerase chain reaction. Expression of IFN-b mRNA was significantly down-regulated in the biopsy samples harvested during the acute phase of infection, while there was no statistically significant effect on the expression of IFN-a mRNA compared with baseline levels. In contrast, the mRNA encoding TNF-a was significantly up-regulated in samples collected during both acute and late (>28 days post infection) phases of infection. There were also significantly higher levels of TNF-a mRNA expressed in samples derived from animals that were identified subsequently as persistently infected FMDV-carriers. It was concluded that there was a significant difference in the host-response in the DSP of calves that were identified as persistently infected, subclinical carriers of FMDV.

General information
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Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Innate Immunology, Division of Veterinary Diagnostics and Research, DTU Data Analysis
Contributors: Stenfeldt, A. C., Heegaard, P. M. H., Stockmarr, A., Belsham, G.
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Publication information
Modulation of Translation Initiation Efficiency in Classical Swine Fever Virus

Modulation of translation initiation efficiency on classical swine fever virus (CSFV) RNA can be achieved by targeted mutations within the internal ribosome entry site (IRES). In this study, cDNAs corresponding to the wild type (wt) or mutant forms of the IRES of CSFV strain Paderborn were amplified and inserted into dicistronic reporter plasmids encoding Fluc and Rluc under the control of a T7 promoter. The mutations were within domains II, IIIId1 and IIIf of the IRES. The plasmids were transfected into BHK cells infected with the recombinant vaccinia virus, vTF7-3, which expresses the T7 RNA polymerase. IRES mutants with different levels of IRES activity were identified and then introduced by homologous recombination into bacterial artificial chromosomes (BACs), containing CSFV Paderborn cDNA downstream of a T7 promoter. From the wt and mutant BACs, full-length CSFV RNA transcripts were produced in vitro and electroporated into porcine PK15 cells. Rescued mutant viruses were obtained from RNAs that contained mutations within domain IIIf which retained more than 75% of wt translation efficiency. Sequencing of cDNA generated from these rescued viruses verified the maintenance of the introduced changes within the IRES. The growth characteristics of each rescued mutant virus were compared to that of the wt virus. It was shown that viable mutant viruses with reduced translation initiation efficiency can be designed and generated and that viruses containing mutations within domain IIIf of the IRES have reduced growth in cell culture compared to the wt virus.

General information
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Organisations: National Veterinary Institute, Division of Virology, Sektion for Eksotiske Virussygdomme
Contributors: Friis, M. B., Rasmussen, T. B., Belsham, G.
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Web of Science (2018): Indexed yes
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Web of Science (2017): Impact factor 4.368
Web of Science (2017): Indexed yes
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Scopus rating (2016): CiteScore 4.42 SJR 3.114 SNIP 1.124
Web of Science (2016): Impact factor 4.663
Web of Science (2016): Indexed yes
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Scopus rating (2015): CiteScore 4.42 SJR 3.282 SNIP 1.132
Web of Science (2015): Impact factor 4.606
Web of Science (2015): Indexed yes
Monitoring the determinants of efficient viral replication using Classical Swine Fever Virus-reporter replicons

Classical swine fever virus (CSFV) is the etiological agent of the severe porcine disease, classical swine fever. Unraveling the molecular determinants of efficient replication is crucial for gaining improved knowledge of the pathogenic features of this virus. Monitoring the replication competence of the CSFV genome within cells can be achieved using autonomously
replicating constructs (replicons) containing a reporter gene that expresses a readily quantifiable enzyme. Here, a newly implemented cloning technique was applied to genome modification of the fulllength CSFV cDNA previously inserted into a single-copy bacterial artificial chromosome (BAC). This technique, the Red/ET counter-selection method, is based upon homologous recombination, thus obviating the need for internal restriction sites or complex cloning strategies. Several CSFV replicons with deletions in regions encoding virus structural proteins considered non-essential for RNA replication were constructed and these deletions were replaced with an in-frame insertion of the Renilla luciferase (Rluc) sequence. RNA transcripts from these replicons should be translated as a single functional open reading frame. Full-genome cDNAs (~10-12,3 kb) were amplified from the BACs using a stable long-PCR method and in vitro transcripts were assayed in permissive cells. The CSFV-Rluc replicons were evaluated for their ability to replicate using immunofluorescence staining (-NS3 and -E2), and the Renilla luciferase assay. We conclude that Rluc expression is an efficient way of monitoring replication of these constructs.
Picornaviruses
Diseases of Swine, Tenth Edition is a fully revised and updated version of this indispensable reference for detailed and comprehensive information on diseases in the pig. Now published in association with the American Association of Swine Veterinarians, this new edition adds new knowledge throughout in a more consistent, reorganized format for more intuitive access to information, with new chapters on the cardiovascular system, food safety and zoonotic diseases, and performing clinical trials. Diseases of Swine remains an essential resource on swine production, health, and management for swine practitioners at all levels, including students, swine veterinarians, and researchers.

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Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
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Reconstruction of the Transmission History of RNA Virus Outbreaks Using Full Genome Sequences: Foot-and-Mouth Disease Virus in Bulgaria in 2011
Improvements to sequencing protocols and the development of computational phylogenetics have opened up opportunities to study the rapid evolution of RNA viruses in real time. In practical terms, these results can be combined with field data in order to reconstruct spatiotemporal scenarios that describe the origin and transmission pathways of viruses during an epidemic. In the case of notifiable diseases, such as foot-and-mouth disease (FMD), these analyses provide important insights into the epidemiology of field outbreaks that can support disease control programmes. This study reconstructs the origin and transmission history of the FMD outbreaks which occurred during 2011 in Burgas Province, Bulgaria, a country that had been previously FMD-free-without-vaccination since 1996. Nineteen full genome sequences (FGS) of FMD virus (FMDV) were generated and analysed, including eight representative viruses from all of the virus-positive outbreaks of the disease in the country and 11 closely-related contemporary viruses from countries in the region where FMD is endemic (Turkey and Israel). All Bulgarian sequences shared a single putative common ancestor which was closely related to the index case identified in wild boar. The closest relative from outside of Bulgaria was a FMDV collected during 2010 in Bursa (Anatolia, Turkey). Within Bulgaria, two discrete genetic clusters were detected that corresponded to two episodes of outbreaks that occurred during January and March-April 2011. The number of nucleotide substitutions that were present between, and within, these separate clusters provided evidence that undetected FMDV infection had occurred. These conclusions are supported by laboratory data that subsequently identified three additional FMDV-infected livestock premises by serosurveillance, as well as a number of antibody positive wild boar on both sides of the border with Turkish Thrace. This study highlights how FGS analysis can be used as an effective on-the-spot tool to support and help direct epidemiological investigations of field outbreaks.

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Serotype identification and VP1 coding sequence analysis of foot-and-mouth disease virus from outbreaks in Eastern and Northern Uganda in 2008/9

In April 2008, foot-and-mouth disease (FMD) outbreaks were reported in Kamuli district of the eastern region of Uganda. Soon after lifting the quarantines in this area, further FMD outbreaks were reported in northern Uganda, which spread to more than 10 districts. The aim of this study was to identify the serotype and compare the variable protein (VP)1 coding sequences of the viruses responsible for FMD outbreaks during 2008 and 2009, to trace the transmission pathways of the disease in Uganda. Probang and epithelial swab samples were collected from cattle with clinical signs of FMD. From the total of 27 positive samples, the VP1 coding region was amplified and sequenced. Each of these sequences showed >99% identity to each other, and just five distinct sequences were identified. BLAST searches and phylogenetic analysis of the complete variable protein (VP)1 coding sequences revealed that they belonged to serotype O, topotype EA-2. The close similarity between the virus sequences suggested introduction from a single source. We therefore conclude that FMD in the northern region of Uganda was most likely introduced from the outbreak in the eastern region across Lake Kyoga through movement of live animals. This has significant implications for the effectiveness of the current...
FMD control measures.

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Organisations: Sektion for Eksotiske Virusssygdomme, Division of Virology, National Veterinary Institute, Makerere University, Ministry of Agriculture, Animal Industry and Fisheries, University of Copenhagen
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Scopus rating (2010): SJR 0.761 SNIP 0.983
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Virulence in pigs of vPader10 rescued from an infectious cDNA clone of the CSFV strain Paderborn

The BAC clone, pBeloPader10, contains a complete cDNA of the CSFV strain Paderborn. Virus, named vPader10, was rescued from this construct by electroproporation of RNA transcripts into porcine PK15 cells. To further study the characteristics of vPader10, we evaluated the virulence of this virus in vivo in pigs. An animal experiment was performed where three pigs were inoculated with vPader10 and housed in-contact with two non-inoculated pigs for 5 weeks. Following inoculation with vPader10, two out of three pigs displayed severe clinical signs of CSF from PID 14 that progressed until death of the pigs at PID 21 and PID 22, respectively. High fever (>41°C) was observed in these pigs from PID 14 and remained at a high level until day of death. One of two contact pigs developed similar clinical disease that initiated at PID 21 and progressed until it was euthanised at PID 32 due to severe clinical signs. One inoculated and one in-contact pig showed little or no clinical symptoms. Virus was detected in blood by RT-qPCR from PID 3-4 in all inoculated pigs and from PID 14 in both contact pigs. In the severely diseased pigs the viral loads reached high levels (Ct ≈ 20) whereas the two pigs without clinical symptoms displayed transient viral loads that peaked at Ct ≈ 30. The results from this experiment demonstrate that vPader10 rescued from pBeloPader10 is virulent and transmissible in pigs.

Virus survival in slurry: Analysis of the stability of foot-and-mouth disease, classical swine fever, bovine viral diarrhoea and swine influenza viruses

Farm slurry can be highly contaminated with viral pathogens. The survival of these pathogens within slurry is important since this material is often distributed onto farm land either directly or after heat treatment. There is clearly some risk of spreading pathogens in the early stages of an outbreak of disease before it has been recognized. The survival of foot-and-mouth disease virus, classical swine fever virus, bovine viral diarrhoea virus and swine influenza virus, which belong to three different RNA virus families plus porcine parvovirus (a DNA virus) was examined under controlled conditions. For each RNA virus, the virus survival in farm slurry under anaerobic conditions was short (generally ≤1h) when heated (to 55°C) but each of these viruses could retain infectivity at cool temperatures (5°C) for many weeks. The porcine parvovirus survived considerably longer than each of the RNA viruses under all conditions tested. The implications for disease spread are discussed.

General information
Host-response to foot-and-mouth disease in cattle; possible implications for the development of persistently infected “carriers”

General purpose and objectives Foot-and-mouth disease (FMD) is a viral infection of implicit financial importance for countries, such as Denmark, which rely on a significant trade in agricultural products. The disease is highly contagious with rapid spread amongst susceptible animals, causing substantial economical implications for farmers and live-stock industries of affected countries. The occurrence of persistently infected, so called “carriers” of FMD-virus (FMDV) which may shed infectious virus for prolonged periods of time following exposure to the virus, causes significant complications for effective disease control. The main purpose of this PhD-project has been to investigate the host response to FMD infection in cattle, with further objectives of elucidating any detectable differences in the measured immune response between animals that developed into FMDV carriers and those that did not. Experimental studies The thesis is based on results obtained from seven separate animal experiments with FMDV serotype O, which have been performed at DTU-Vet, Lindholm. In five out of the six experiments that were performed in cattle, animals were infected with FMDV O UKG 34/2001, representing the virus isolate responsible for the FMD outbreak in the UK and northern Europe in 2001. One cattle experiment was performed with an FMDV serotype O isolated from samples collected from a cattle farm in Uganda during an outbreak in 2006, whilst one additional experiment was designed to investigate the clinical course of infection with FMDV O UKG 34/2001 in sheep. An experimental study design involving endoscopical collection of small biopsies of pharyngeal mucosa from live cattle was developed. This technique enables collection of sequential tissue samples from infected animals, allowing investigation of the local tissue response to infection within this specific anatomical region of individual animals, at different time points following infection. This sampling system was used to investigate the pathogenesis of FMD infection in cattle through quantification of the levels of FMDV RNA present within the pharyngeal epithelia during early infection. Similar analyses were performed on samples of pharyngeal epithelia and associated lymph nodes collected during post mortem examinations performed at around 32-35 days post infection in order to investigate possible sites of virus persistence. The early host response to FMDV O in cattle was investigated through measurements of systemic parameters consisting of the acute phase proteins, serum amyloid A (SAA) and haptoglobin (HP), as well as type 1 interferon (IFN). The local tissue response within the pharyngeal epithelia was investigated through measurements of mRNA levels of inflammatory cytokines in sequential biopsy samples. Structure of Thesis The first chapter contains general background information on the host response to virus infections, as well as characteristics of FMDV and the pathogenesis of the infection. Detailed aims and objectives of the project are stated at the end of chapter 1. Chapter 2 contains overall descriptions of the animal experiments included in the project. The general concepts of the experimental procedures are described, as well as the clinical characteristics of infection caused by the two different FMDV O isolates in cattle. The clinical description of the experiment performed with FMDV O UKG 34/2001 in sheep includes results of measurements of viremia and the development of specific anti-FMDV O antibodies, as these results are not presented in the included manuscripts. The third chapter of the thesis contains three manuscripts of research articles for publication in peer-reviewed scientific journals. The first manuscript is based on serological measurements of the acute phase proteins SAA and HP, together with the bioactivity of type 1 IFN, in three out of the performed cattle experiments. Measurements of
the systemic response to early infection with FMDV is related to the observed development of clinical signs of infection as well as the occurrence of viremia and development of anti-FMDV antibodies. Observed variations in the acute phase response of HP between carriers and non-carriers are discussed. The second manuscript contains results from measurements of mRNA levels of inflammatory cytokines IFNα- and –β as well as tumor necrosis factor –α (TNF-α), in collected biopsy samples. The type 1 interferon response in the analyzed tissue samples is discussed in relation to the previously reported systemic interferon response. The measured cytokine responses, as well as an observed variation in the TNF-α response between carriers and non-carriers, are discussed in relation to previous publications within the subject area. The third manuscript deals with investigations of possible sites of virus replication during early and persistent phases of infection. Levels of FMDV RNA was quantified in sequential biopsy samples of pharyngeal mucosa harvested during early infection, as well as in corresponding tissue samples collected post mortem. The final chapter of the thesis contains a general discussion of the obtained results, together with overall conclusions and future perspectives for continued research within the specific area.

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Contributors: Stenfeldt, A. C., Belsham, G.
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Analysis of the acute phase responses of Serum Amyloid A, Haptoglobin and Type 1 Interferon in cattle experimentally infected with foot-and-mouth disease virus serotype O
A series of challenge experiments were performed in order to investigate the acute phase responses to foot-and-mouth disease virus (FMDV) infection in cattle and possible implications for the development of persistently infected "carriers". The host response to infection was investigated through measurements of the concentrations of the acute phase proteins (APPs) serum amyloid A (SAA) and haptoglobin (HP), as well as the bioactivity of type 1 interferon (IFN) in serum of infected animals. Results were based on measurements from a total of 36 infected animals of which 24 were kept for observational periods exceeding 28 days in order to determine the carrier-status of individual animals. The systemic host response to FMDV in infected animals was evaluated in comparison to similar measurements in sera from 6 mock-inoculated control animals. There was a significant increase in serum concentrations of both APPs and type 1 IFN in infected animals coinciding with the onset of viremia and clinical disease. The measured parameters declined to baseline levels within 21 days after inoculation, indicating that there was no systemically measurable inflammatory reaction related to the carrier state of FMD. There was a statistically significant difference in the HP response between carriers and non-carriers with a lower response in the animals that subsequently developed into FMDV carriers. It was concluded that the induction of SAA, HP and type 1 IFN in serum can be used as markers of acute infection by FMDV in cattle.

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Analysis of the acute phase responses of Serum Amyloid A, Haptoglobin and Type 1 Interferon in cattle experimentally infected with foot-and-mouth disease virus serotype O
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Analysis of the acute phase responses of Serum Amyloid A, Haptoglobin and Type 1 Interferon in cattle experimentally infected with foot-and-mouth disease virus serotype O
A series of challenge experiments were performed in order to investigate the acute phase responses to foot-and-mouth disease virus (FMDV) infection in cattle and possible implications for the development of persistently infected "carriers". The host response to infection was investigated through measurements of the concentrations of the acute phase proteins (APPs) serum amyloid A (SAA) and haptoglobin (HP), as well as the bioactivity of type 1 interferon (IFN) in serum of infected animals. Results were based on measurements from a total of 36 infected animals of which 24 were kept for observational periods exceeding 28 days in order to determine the carrier-status of individual animals. The systemic host response to FMDV in infected animals was evaluated in comparison to similar measurements in sera from 6 mock-inoculated control animals. There was a significant increase in serum concentrations of both APPs and type 1 IFN in infected animals coinciding with the onset of viremia and clinical disease. The measured parameters declined to baseline levels within 21 days after inoculation, indicating that there was no systemically measurable inflammatory reaction related to the carrier state of FMD. There was a statistically significant difference in the HP response between carriers and non-carriers with a lower response in the animals that subsequently developed into FMDV carriers. It was concluded that the induction of SAA, HP and type 1 IFN in serum can be used as markers of acute infection by FMDV in cattle.

General information
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Capsid proteins from field strains of foot-and-mouth disease virus confer a pathogenic phenotype in cattle on an attenuated, cell-culture-adapted virus

Chimeric foot-and-mouth disease viruses (FMDVs) have been generated from plasmids containing full-length FMDV cDNAs and characterized. The parental virus cDNA was derived from the cell-culture-adapted O1Kaufbeuren B64 (O1K B64) strain. Chimeric viruses, containing capsid coding sequences derived from the O/UKG/34/2001 or A/Turkey 2/2006 field viruses, were constructed using the backbone from the O1K B64 cDNA, and viable viruses (O1K/O-UKG and O1K/A-Tur, respectively) were successfully rescued in each case. These viruses grew well in primary bovine thyroid cells but grew less efficiently in BHK cells than the rescued parental O1K B64 virus. The two chimeric viruses displayed the expected antigenicity in serotype-specific antigen ELISAs. Following inoculation of each virus into cattle, the rescued O1K B64 strain proved to be attenuated whereas, with each chimeric virus, typical clinical signs of foot-and-mouth disease were observed, which then spread to in-contact animals. Thus, the surface-exposed capsid proteins of the O1K B64 strain are responsible for its attenuation in cattle. Consequently, there is no evidence for any adaptation, acquired during cell culture, outside the capsid coding region within the O1K B64 strain that inhibits replication in cattle. These chimeric infectious cDNA plasmids provide a basis for the analysis of FMDV pathogenicity and characterization of receptor utilization in vivo.

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Classical Swine Fever Virus-Rluc Replicons: A Tool for Monitoring the Determinants of Efficient Viral Replication

Classical swine fever virus (CSFV) is the etiologic agent of the severe porcine disease, classical swine fever. Unraveling the molecular determinants of efficient replication is crucial for gaining proper knowledge of the pathogenic traits of this virus. Monitoring the replication competence within cells can be achieved using autonomously replicating genome constructs (replicons) containing a reporter gene that expresses a readily quantifiable enzyme. Here, a newly implemented cloning technique was applied to genome modification of the full-length CSFV cDNA previously inserted into a single-copy bacterial artificial chromosome (BAC). This technique, the Red/ET counter-selection method, is based upon homologous recombination, thus obviating the need for internal restriction sites. Several CSFV replicons with deletions in regions encoding structural viral proteins considered non-essential for RNA replication were constructed and these deletions were swapped with an in-frame insertion of the Renilla luciferase (Rluc) sequence. RNA transcripts from these replicons should be translated as a single functional open reading frame. Full-genome cDNA's (~10-12.3 kb) were amplified from the BACs using a stable long-PCR method and in vitro transcripts were assayed in permissive cells. The CSFV-Rluc replicons were evaluated for their replication competence using antibody staining (against NS3), qRT-PCR and the Renilla luciferase assay. A CSFV-Rluc replicon with similar replication kinetics compared to the wild type CSFV-Paderborn strain, as judged by qRT-PCR, was picked as the candidate and could potentially be useful as a tool for further downstream applications including investigation of CSFV non-structural proteins involvement in viral replication.

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Contributors: Risager, P. C., Belsham, G. J., Rasmussen, T. B.
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Foot-and-mouth disease (FMD) is endemic in Pakistan and Afghanistan. Three different serotypes of the virus, namely O, A and Asia-1, are responsible for the outbreaks of this disease in these countries. In the present study, the nucleotide-coding sequences for the VP1 capsid protein (69 samples) or for all four capsid proteins (P1, seven representative samples) of the serotype A FMD viruses circulating in Pakistan and Afghanistan were determined. Phylogenetic analysis of the foot-and-mouth disease virus (FMDV) VP1-coding sequences from these countries collected between 2002 and 2009 revealed the presence of at least four lineages within two distinct genotypes, all belonging to the Asia topotype, within serotype A. The predominant lineage observed was A-Iran05 but three other lineages (a new one is named here A-Pak09) were also identified. The A-Iran05 lineage is still evolving as revealed by the presence of seven distinct variants, the dominant being the A-Iran05AFG-07 and A-Iran05BAR-08 sublineages. The rate of evolution of the A-Iran05 lineage was found to be about 1.2x10–2 substitutions per nucleotide per year. This high rate of change is consistent with the rapid appearance of new variants of FMDV serotype A in the region. The A22/Iraq FMDV vaccine is antigenically distinct from the A-Iran05BAR-08 viruses. Mapping of the amino acid changes between the capsid proteins of the A22/Iraq vaccine strain and the A-Iran05BAR-08 viruses onto the A22/Iraq capsid structure identified candidate amino acid substitutions, exposed on the virus surface, which may explain this antigenic difference.

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Contributors: Jamal, S. M., Ferrari, G., Ahmed, S., Normann, P., Curry, S., Belsham, G.
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Scopus rating (2004): SJR 1.55 SNIP 1.215
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 1.58 SNIP 1.145
Web of Science (2003): Indexed yes
Foot-and-mouth disease (FMD) is endemic in Pakistan and Afghanistan; serotypes O, A and Asia-1 of the virus are responsible for the outbreaks in these countries with FMDV type O usually being the most common. In the present study, the nucleotide sequences encoding the FMDV capsid protein VP1 from virus samples were determined. Phylogenetic analysis of the serotype O FMD viruses circulating in Pakistan and Afghanistan between 1997 and 2009 revealed the presence of at least three different lineages within the ME-SA (Middle East South Asia) topotype. The three lineages detected in this study are Pak98, Iran2001 and PanAsia. The PanAsia lineage is currently dominant in the area and is evolving with time as revealed by the appearance of distinct variants e.g. PanAsia-II and a new variant designated here as PanAsia-III. The rates of evolution of the O-PanAsia-II and III sublineages prevalent in the region were found to be $6.65 \times 10^{-3}$ (95% CI=5.49–7.80×10$^{-3}$) and 7.80×10$^{-3}$ (95% CI=6.72–8.89×10$^{-3}$) substitutions per nucleotide per year, respectively. The present study reveals the presence of multiple (sub-)lineages of FMDV serotype O co-circulating in the region and that significant new variants are frequently emerging.
Low diversity of foot-and-mouth disease serotype C virus in Kenya: evidence for probable vaccine strain re-introductions in the field

Most viruses are maintained by complex processes of evolution that enable them to survive but also complicate efforts to achieve their control. In this paper, we study patterns of evolution in foot-and-mouth disease (FMD) serotype C virus isolates from Kenya, one of the few places in the world where serotype C has been endemic and is suspected to remain. The nucleotide sequences encoding the capsid protein VP1 from eight isolates collected between 1967 and 2004 were analysed for patterns of sequence divergence and evolution. Very low nucleotide diversity ($\pi=0.0025$) and remarkably little change (only five segregating sites and three amino-acid changes) were observed in these isolates collected over a period of almost 40 years. We interpret these results as being suggestive of re-introductions of the vaccine strain into the field. The implications of these results for the maintenance of serotype C FMD virus and the use of vaccination as a control measure in Kenya are discussed.

General information
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Organisations: Division of Virology, National Veterinary Institute, Makerere University, University of Copenhagen
Contributors: Sangula, A., Siegismund, H., Belsham, G., Balinda, S., Masembe, C., Muwanika, V.
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Modulation of Translation Initiation Efficiency in Classical Swine Fever Virus

Modulation of translation initiation efficiency on classical swine fever virus (CSFV) RNA can be achieved by targeted mutations within the internal ribosome entry site (IRES). In this study, the nucleotides 47 to 427, including the IRES region of the wt CSFV strain Paderborn, were amplified and inserted, under T7 promoter control, into mono- and dicistronic plasmids containing the reporter genes rLuc and fLuc. Mutant fragments of the IRES sequence were generated by overlap PCR and inserted into the reporter plasmids. To evaluate IRES functionality, translation of the rLuc was placed under the control of the wt or mutant CSFV IRES and transfected into BHK cells infected with vTF7-3 which expresses the T7 RNA polymerase. rLuc activity was measured in cell lysates. A series of IRES mutants representing different levels of IRES activity (20% - 100%) were selected and inserted by homologous recombination into Bacterial Artificial Chromosomes (BAC) clones, containing the full-length Paderborn sequence under the transcriptional control of a T7 promoter and a selection marker in place of the IRES. RNA transcripts were produced in vitro and electroporated into porcine PK15 cells. Rescued mutant viruses were obtained after one cell culture passage from constructs with more than 75 % translation efficiency compared to the wildtype IRES. cDNA was generated from these clones and sequenced to verify the maintenance of the changes in the IRES. These results show that full-length viable mutant viruses of the CSFV strain Paderborn with modulated translation initiation efficiency can be designed and generated.

General information
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Contributors: Friis, M. B., Rasmussen, T. B., Belsham, G. J.
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Molecular characterization of serotype Asia-1 foot-and-mouth disease viruses in Pakistan and Afghanistan; emergence of a new genetic Group and evidence for a novel recombinant virus
Foot-and-mouth disease (FMD) is endemic in Pakistan and Afghanistan. The FMD virus serotypes O, A and Asia-1 are responsible for the outbreaks in these countries. Diverse strains of FMDV, even within the same serotype, co-circulate. Characterization of the viruses in circulation can facilitate appropriate vaccine selection and tracing of outbreaks. The present study characterized foot-and-mouth disease serotype Asia-1 viruses circulating in Pakistan and Afghanistan during the period 1998–2009. Phylogenetic analysis of FMDV type Asia-1 revealed that three different genetic Groups of serotype Asia-1 have circulated in Pakistan during this time. These are Group-II, -VI and, recently, a novel Group
(designated here as Group-VII). This new Group has not been detected in neighbouring Afghanistan during the study period but viruses from Groups I and II are in circulation there. Using near complete genome sequences, from FMD viruses of serotypes Asia-1 and A that are currently circulating in Pakistan, we have identified an interserotypic recombinant virus, which has the VP2-VP3-VP1-2A coding sequences derived from a Group-VII Asia-1 virus and the remainder of the genome from a serotype A virus of the A-Iran05AFG-07 sub-lineage. The Asia-1 FMDVs currently circulating in Pakistan and Afghanistan are not efficiently neutralized by antisera raised against the Asia-1/Shamir vaccine strain. Thus, new Asia-1 vaccine strains may be required to block the spread of the current Asia-1 viruses.

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- Web of Science (2015): Impact factor 2.591
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- Scopus rating (2014): CiteScore 2.99 SJR 1.391 SNIP 1.125
- Web of Science (2014): Impact factor 3.015
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- Scopus rating (2012): CiteScore 2.87 SJR 1.22 SNIP 0.989
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Rescue of foot-and-mouth disease viruses that are pathogenic for cattle from preserved viral RNA samples

Background: Foot and mouth disease is an economically important disease of cloven-hoofed animals including cattle, sheep and pigs. It is caused by a picornavirus, foot-and-mouth disease virus (FMDV), which has a positive sense RNA genome which, when introduced into cells, can initiate virus replication. Principal Findings: A system has been developed to rescue infectious FMDV from RNA preparations generated from clinical samples obtained under experimental conditions and then applied to samples collected in the "field". Clinical samples from suspect cases of foot-and-mouth disease (FMD) were obtained from within Pakistan and Afghanistan. The samples were treated to preserve the RNA and then transported to National Veterinary Institute, Lindholm, Denmark. Following RNA extraction, FMDV RNA was quantified by real-time RT-PCR and samples containing significant levels of FMDV RNA were introduced into susceptible cells using electroporation. Progeny viruses were amplified in primary bovine thyroid cells and characterized using antigen ELISA and also by RT-PCR plus sequencing. FMD viruses of three different serotypes and multiple lineages have been successfully rescued from the RNA samples. Two of the rescued viruses (of serotype O and Asia 1) were inoculated into bull calves under high containment conditions. Acute clinical disease was observed in each case which spread rapidly from the inoculated calves to in-contact animals. Thus the rescued viruses were highly pathogenic. The availability of the rescued viruses enabled serotyping by antigen ELISA and facilitated genome sequencing. Conclusions: The procedure described here should improve the characterization of FMDVs circulating in countries where the disease is endemic and thus enhance disease control globally.
Structural Features of the Seneca Valley Virus Internal Ribosome Entry Site (IRES) Element: a Picornavirus with a Pestivirus-Like IRES

The RNA genome of Seneca Valley virus (SVV), a recently identified picornavirus, contains an internal ribosome entry site (IRES) element which has structural and functional similarity to that from classical swine fever virus (CSFV) and hepatitis C virus, members of the FLAVIVIRIDAE: The SVV IRES has an absolute requirement for the presence of a short region of virus-coding sequence to allow it to function either in cells or in rabbit reticulocyte lysate. The IRES activity does not require the translation initiation factor elf4A or intact elf4G. The predicted secondary structure indicates that the SVV IRES is more closely related to the CSFV IRES, including the presence of a bipartite IIId domain. Mutagenesis of the SVV IRES, coupled to functional assays, support the core elements of the IRES structure model, but surprisingly, deletion of the conserved IIId2 domain had no effect on IRES activity, including 40S and elf3 binding. This is the first example of a picornavirus IRES that is most closely related to the CSFV IRES and suggests the possibility of multiple, independent recombination events between the genomes of the Picornaviridae and Flaviviridae to give rise to similar IRES elements.
Bluetongue in Denmark during 2008

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Co-circulation of two extremely divergent serotype SAT 2 lineages in Kenya highlights challenges to foot-and-mouth disease control.
Amongst the SAT serotypes of foot-and-mouth disease virus (FMDV), the SAT 2 serotype is the most widely distributed throughout sub-Saharan Africa. Kenyan serotype SAT 2 viruses have been reported to display the highest genetic diversity for the serotype globally. This complicates diagnosis and control, and it is essential that patterns of virus circulation are known in order to overcome these difficulties. This study was undertaken to establish patterns of evolution of FMDV serotype SAT 2 in Kenya using complete VP1 coding sequences in a dataset of 65 sequences from Africa, collected over a period of 50 years. Two highly divergent lineages were observed to co-circulate, and occasional trans-boundary spread was inferred, emphasizing the value of constant monitoring and characterization of field strains for improved diagnosis and appropriate vaccine application as well as the need for regional approaches to control.

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Scopus rating (2011): CiteScore 2.17 SJR 0.902 SNIP 1.044
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Detection of myxoma viruses encoding a defective M135R gene from clinical cases of myxomatosis; possible implications for the role of the M135R protein as a virulence factor

Background: Myxoma virus is a member of the Poxviridae and causes disease in European rabbits. Laboratory confirmation of the clinical disease, which occurs in the autumn of most years in Denmark, has been achieved previously using antigen ELISA and electron microscopy. Results: An unusually large number of clinically suspected cases of myxomatosis were observed in Denmark during 2007. Myxoma virus DNA was detected, using a new real time PCR assay which targets the M029L gene, in over 70% of the clinical samples submitted for laboratory confirmation. Unexpectedly, further analysis revealed that a high proportion of these viral DNA preparations contained a frame-shift mutation within the M135R gene that has previously been identified as a virulence factor. This frame-shift mutation results in expression of a greatly truncated product. The same frame-shift mutation has also been found recently within an avirulent strain of myxoma virus (6918). However, three other frame-shift mutations found in this strain (in the genes M009L, M036L and M148R) were not shared with the Danish viruses but a single nucleotide deletion in the M138R/M139R intergenic region was a common feature. Conclusions: It appears that expression of the full-length myxoma virus M135R protein is not required for virulence in rabbits. Hence, the frame-shift mutation in the M135R gene in the nonpathogenic 6918 virus strain is not sufficient to explain the attenuation of this myxoma virus but one/some of the other frame-shift mutations alone or in conjunction with one/some of the thirty two amino acid substitutions must also contribute. The real time PCR assay for myxoma virus is a useful diagnostic tool for laboratory confirmation of suspected cases of myxomatosis.
Diversity and transboundary mobility of serotype O foot-and-mouth disease virus in East Africa: Implications for vaccination policies.

Foot-and-mouth disease (FMD) virus serotype O has been responsible for most reported outbreaks of the disease in East Africa. A sustained campaign for the past 40 years to control FMD mainly by vaccination, combined with quarantine and zoosanitary measures has been undertaken with limited success. We investigated the genetic relationships among
serotype O strains in eastern Africa using complete VP1 coding region sequences obtained from 46 FMD virus isolates collected in Kenya in the years 1964–2008 and 8 Ugandan isolates collected between 1999 and 2006. In addition, 21 selected FMDV sequences from Genbank representing reference strains from eastern Africa and elsewhere were included in the Bayesian inference analyses and the detection of selection forces. The results confirmed previous observations that eastern Africa harbours four distinct topotypes (clades with >15% sequence divergence). All but one strain isolated post-2000 belonged to topotypes EA-2, EA-3 and EA-4, while all three vaccines have been based on strains in the EA-1 topotype. The estimated dN/dS ratios across the individual codons of the entire VP1 coding region revealed that purifying (negative) selection constituted the dominant evolutionary force. Cross-border disease transmission within the region has been suggested with probable incursions of topotypes EA-3 and EA-4 into Kenya and Uganda from neighboring Ethiopia and Sudan. We conclude that the vaccines have probably been effective in controlling EA-1, but less so for the other topotypes and propose a more comprehensive representation of topotypes in the development of new vaccines in recognition of the considerable diversity and transboundary nature of serotype O.

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Evolutionary analysis of foot-and-mouth disease virus serotype SAT 1 isolates from east africa suggests two independent introductions from southern africa

Background: In East Africa, foot-and-mouth disease virus serotype SAT 1 is responsible for occasional severe outbreaks in livestock and is known to be maintained within the buffalo populations. Little is known about the evolutionary forces underlying its epidemiology in the region. To enhance our appreciation of the epidemiological status of serotype SAT 1 virus in the region, we inferred its evolutionary and phylogeographic history by means of genealogy-based coalescent methods using 53 VP1 coding sequences covering a sampling period from 1948-2007. Results: The VP1 coding sequence of 11 serotype SAT 1 FMD viruses from East Africa has been determined and compared with known sequences derived from other SAT 1 viruses from sub-Saharan Africa. Purifying (negative) selection and low substitution rates characterized the SAT 1 virus isolates in East Africa. Two virus groups with probable independent introductions from southern Africa were identified from a maximum clade credibility tree. One group was exclusive to Uganda while the other was present within Kenya and Tanzania. Conclusions: Our results provide a baseline characterization of the inter-regional spread of SAT 1 in sub-Saharan Africa and highlight the importance of a regional approach to trans-boundary animal disease control in order to monitor circulating strains and apply appropriate vaccines.
Foot-and-Mouth Disease Virus 2C Is a Hexameric AAA+ Protein with a Coordinated ATP Hydrolysis Mechanism.

Foot-and-mouth disease virus (FMDV), a positive sense, single-stranded RNA virus, causes a highly contagious disease in cloven-hoofed livestock. Like other picomaviruses, FMDV has a conserved 2C protein assigned to the superfamily 3 helicases a group of AAA+ ATPases that has a predicted N-terminal membrane-binding amphipathic helix attached to the...
main ATPase domain. In infected cells, 2C is involved in the formation of membrane vesicles, where it co-localizes with viral RNA replication complexes, but its precise role in virus replication has not been elucidated. We show here that deletion of the predicted N-terminal amphipathic helix enables overexpression in Escherichia coli of a highly soluble truncated protein, 2C(34–318), that has ATPase and RNA binding activity. ATPase activity was abrogated by point mutations in the Walker A (K116A) and B (D160A) motifs and Motif C (N207A) in the active site. Unliganded 2C(34–318) exhibits concentration-dependent self-association to yield oligomeric forms, the largest of which is tetrameric. Strikingly, in the presence of ATP and RNA, FMDV 2C(34–318) containing the N207A mutation, which binds but does not hydrolyze ATP, was found to oligomerize specifically into hexamers. Visualization of FMDV 2C-ATP-RNA complexes by negative stain electron microscopy revealed hexameric ring structures with 6-fold symmetry that are characteristic of AAA+ ATPases. ATPase assays performed by mixing purified active and inactive 2C(34–318) subunits revealed a coordinated mechanism of ATP hydrolysis. Our results provide new insights into the structure and mechanism of picornavirus 2C proteins that will facilitate new investigations of their roles in infection.
Host response to Foot- and Mouth Disease infection in cattle; possible implications for the development of "carriers".

FMD is a viral disease with severe implications for agricultural trade in affected countries. Any cloven hoofed animal species may become infected, and ruminants, especially cattle and buffalo, may develop into "carriers" persistently shedding low amounts of virus for several years after exposure to the disease. The FMDV infection is defined as persistent when live virus can be detected for more than 28 days post infection. FMD infection in ruminants involves initial viral replication in pharyngeal epithelia, from where the virus spreads systemically. Characteristic vesicular lesions develop in the cornified stratified squamous epithelia of the coronary bands and oral cavity within a few days of infection. Viremia occurs within 2-3 days of infection, but is rapidly cleared through the effect of circulating antibodies generated by the adaptive immune response. The host response involves initial activation of the innate immune response, with activation and recruitment of effector-cells, and subsequent activation of T- and B-cells, leading to the production of circulating antibodies, as well as activation of cytotoxic T-cells. Previous experiments have indicated that the site of persistent replication of FMDV is located in pharyngeal lymphoid tissue, as well as the basal epithelia of the dorsal soft palate. A series of animal experiments, with the aim of investigating the host immune response, and sites of viral replication at different time points during both acute and persistent phases of FMDV infection in cattle has been performed. During these experiments, bull calves of 4-5 months of age were infected with FMDV O UKG 34/2001, and disease development was monitored for 32 days. Disease progression was monitored through observation of clinical signs, and analysis of serum for the presence of viral genomes as well as FMDV-specific antibodies. Viral shedding was measured through qPCR of mouth swabs and oropharyngeal fluid (probang samples). Tissue samples derived from endoscopical collection
of biopsies of the dorsal soft palate from live animals at different times post infection, as well as samples of lymphoid tissue derived from staged post mortems were analysed for the presence of viral proteins through indirect immunoflorescence. These samples have also been analysed for the presence of specific populations of immune cells such as CD8+ T-cells and Dendritic cells. Biopsy samples are collected at different time points during acute and persistent infection in order to monitor the progress of viral replication, as well as the local cellular immune response, at specific sites over time. In order to measure the systemic response to infection, serum concentrations of acute phase proteins Serum Amyloid A (SAA) and Haptoglobin, as well as biologically active type 1 interferon (IFN 1) are being quantified. These markers of host immune response are also being used in order to detect any possible differences in host response throughout the infection in animals that become persistently infected compared to those that clear the infection effectively.

General information
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Contributors: Stenfeldt, C., Heegaard, P. M. H., Tjørnehøj, K., Belsham, G.
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Insights into Cleavage Specificity from the Crystal Structure of Foot-and-Mouth Disease Virus 3C Protease Complexed with a Peptide Substrate

Foot-and-mouth disease (FMD) is a serious, widespread viral disease of cloven-hoofed animals, including important agricultural species such as cattle, sheep, pigs and goats (19, 45). The virus spreads rapidly and, although endemic and epidemic situations can be controlled using vaccines that are based on inactivated virus particles, political and technical difficulties with the maintenance and use of vaccine stocks has stimulated the search for alternative means of tackling the disease, such as anti-viral drugs (16). The development of such treatments will demand a detailed knowledge of the molecular basis of viral replication. In this paper we focus on the structural basis of the cleavage activity of FMDV 3Cpro; as a highly conserved viral enzyme (11), FMDV 3Cpro is a potential drug target.

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Scopus rating (2015): CiteScore 3.97 SJR 3.005 SNIP 1.099
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Molecular characterization of SAT 2 foot-and-mouth disease virus from post-outbreak slaughtered animals: implications for disease control in Uganda.

In Uganda, limiting the extent of foot-and-mouth disease (FMD) spread during outbreaks involves short term measures such as ring vaccination and restrictions to the movement of livestock and their products to and from the affected areas. In
this study, the presence of FMD virus RNA was investigated in cattle samples, three months after FMD quarantine measures had been lifted in the area in 2004 following an outbreak. Oropharyngeal tissue samples were obtained from 12 cattle slaughtered in a small town abattoir of Kiboga. FMD virus RNA was detected by diagnostic RT-PCR in 9 of the 12 tissue samples. Part of the coding region for the capsid protein VP1 was amplified and sequenced. All samples were identified as belonging to the SAT 2 serotype. The implications for FMD control of both virus introductions into Uganda and the presence of carrier animals following outbreaks are discussed.

**General information**

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BFI (2010): BFI-level 1
Phylogenetic analyses of the polyprotein coding sequences of serotype O foot-and-mouth disease viruses in East Africa: evidence for interserotypic recombination

Background Foot-and-mouth disease (FMD) is endemic in East Africa with the majority of the reported outbreaks attributed to serotype O virus. In this study, phylogenetic analyses of the polyprotein coding region of serotype O FMD viruses from Kenya and Uganda has been undertaken to infer evolutionary relationships and processes responsible for the generation and maintenance of diversity within this serotype. FMD virus RNA was obtained from six samples following virus isolation in cell culture and in one case by direct extraction from an oropharyngeal sample. Following RT-PCR, the single long open reading frame, encoding the polyprotein, was sequenced. Results Phylogenetic comparisons of the VP1 coding region showed that the recent East African viruses belong to one lineage within the EA-2 topotype while an older Kenyan strain, K/52/1992 is a representative of the topotype EA-1. Evolutionary relationships between the coding regions for the leader protease (L), the capsid region and almost the entire coding region are monophyletic except for the K/52/1992 which is distinct. Furthermore, phylogenetic relationships for the P2 and P3 regions suggest that the K/52/1992 is a probable recombinant between serotypes A and O. A bootscan analysis of K/52/1992 with East African FMD serotype A viruses (A21/KEN/1964 and A23/KEN/1965) and serotype O viral isolate (K/117/1999) revealed that the P2 region is probably derived from a serotype A strain while the P3 region appears to be a mosaic derived from both serotypes A and O. Conclusions Sequences of the VP1 coding region from recent serotype O FMDVs from Kenya and Uganda are all representatives of a specific East African lineage (topotype EA-2), a probable indication that hardly any FMD introductions of this serotype have occurred from outside the region in the recent past. Furthermore, evidence for interserotypic recombination, within the non-structural protein coding regions, between FMDVs of serotypes A and O has been obtained. In addition to characterization using the VP1 coding region, analyses involving the non-structural protein coding regions should be performed in order to identify evolutionary processes shaping FMD viral populations.

General information
State: Published
Organisations: Division of Virology, National Veterinary Institute, Makerere University, Ministry of Agriculture, Animal Industry and Fisheries, University of Copenhagen
The role of African buffalos (Syncerus caffer) in the maintenance of foot-and-mouth disease in Uganda

Background To study the role of African buffalos (Syncerus caffer) in the maintenance of foot-and-mouth disease in Uganda, serum samples were collected from 207 African buffalos, 21 impalas (Aepyceros melampus), 1 giraffe (Giraffa camelopardalis), 1 common eland (Taurotragus oryx), 7 hartebeests (Alcelaphus buselaphus) and 5 waterbucks (Kobus ellipsiprymnus) from four major National Parks in Uganda between 2005 and 2008. Serum samples were screened to detect antibodies against foot-and-mouth disease virus (FMDV) non-structural proteins (NSP) using the Ceditest FMDV NS ELISA. Solid Phase Blocking ELISAs (SPBE) were used to determine the serotype-specificity of antibodies against the seven serotypes of FMDV among the positive samples. Virus isolation and sequencing were undertaken to identify circulating viruses and determine relatedness between them. Results Among the buffalo samples tested, 85% (95% CI = 80-90%) were positive for antibodies against FMDV non-structural proteins while one hartebeest sample out of seven (14.3%; 95% CI = -11.6-40.2%) was the only positive from 35 other wildlife samples from a variety of different species. In the buffalo, high serotype-specific antibody titres (equal to or greater than 80) were found against serotypes O (7/27 samples), SAT 1 (23/29 samples), SAT 2 (18/32 samples) and SAT 3 (16/30 samples). Among the samples titrated for antibodies against the four serotypes O, SAT 1, SAT 2 and SAT 3, 17/22 (77%; CI = 59.4-94.6%) had high titres against at least two serotypes. FMDV isolates of serotypes SAT 1 (1 sample) and SAT 2 (2 samples) were obtained from buffalo probang samples collected in Queen Elizabeth National Park (QENP) in 2007. Sequence analysis and comparison of VP1 coding sequences showed that the SAT 1 isolate belonged to topotype IV while the SAT 2 isolates belonged to different lineages within the East African topotype X. Conclusions Consistent detection of high antibody titres in buffalos supports the view that African buffalos play an important role in the maintenance of FMDV infection within National Parks in Uganda. Both SAT 1 and SAT 2 viruses were isolated, and serological data indicate that it is also likely that FMDV serotypes O and SAT 3 may be present in the buffalo population. Detailed studies should be undertaken to define further the role of wildlife in the epidemiology of FMDV in East Africa.

General information
State: E-pub ahead of print
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Ministry of Agriculture, Animal Industry and Fisheries, Makerere University, Ministry of Agriculture, Water and Forestry, University of Copenhagen
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BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
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Scopus rating (2017): CiteScore 2.16 SJR 0.934 SNIP 1.108
Web of Science (2017): Impact factor 1.958
Web of Science (2017): Indexed yes
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Scopus rating (2016): CiteScore 1.83 SJR 0.87 SNIP 1.011
Web of Science (2016): Impact factor 1.75
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 1.86 SJR 0.981 SNIP 1.009
Web of Science (2015): Impact factor 1.643
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 1.81 SJR 0.943 SNIP 1.018
Web of Science (2014): Impact factor 1.777
Web of Science (2014): Indexed yes
A dominant negative mutant of rab5 inhibits infection of cells by foot-and-mouth disease virus; implications for virus entry.

Foot-and-mouth disease virus (FMDV) can use a number of different integrins (alphavβ1, alphavβ3, alphavβ6, and alphavβ8) as receptors to initiate infection. Infection mediated by alphavβ6 is known to occur by clathrin-mediated endocytosis and is dependent on the acidic pH within endosomes. On internalization, virus is detected rapidly in early endosomes (EE) and subsequently in perinuclear recycling endosomes (PNRE), but not in late endosomal compartments. Due to the extreme sensitivity of FMDV to acidic pH, it is thought that EE can provide a pH low enough for infection to occur; however, definitive proof that infection takes place from within these compartments is still lacking. Here we have investigated the intracellular transport steps required for FMDV infection of IBRS-2 cells, which express vβ8 as their FMDV receptor. These experiments confirmed that FMDV infection mediated by alphavβ8 is also dependent on clathrin-mediated endocytosis and an acidic pH within endosomes. Also, the effect on FMDV infection of dominant-negative (DN) mutants of cellular rab proteins that regulate endosomal traffic was examined. Expression of DN rab5 reduced the number of FMDV-infected cells by 80%, while expression of DN rab4 or DN rab7 had virtually no effect on infection. Expression of DN rab11 inhibited infection by FMDV, albeit to a small extent (35%). These results demonstrate that FMDV infection takes place predominantly from within EE and does not require virus trafficking to the late endosomal compartments. However, our results suggest that infection may not be exclusive to EE and that a small amount of infection could occur from within PNRE.

General information

State: Published
Organizations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, The Pirbright Institute
Contributors: Johns, H., Berryman, S., Monaghan, P., Belsham, G., Jackson, T.
Pages: 6247-6256
Publication date: 2009
Bluetongue in Denmark 2008

General information
State: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Rasmussen, L. D., Rasmussen, T. B., Belsham, G., Strandbygaard, B., Bøtner, A.
Publication date: 2009
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Event: Poster session presented at 3rd Annual Meeting of EPIZONE, Antalya, Turkey.
Electronic versions:
Bluetongue in Denmark 2008 (EPIZONE poster).pdf
Source: orbit
Source-ID: 236907
Research output: Research - peer-review › Poster – Annual report year: 2009

Divergent picornavirus IRES elements
Internal ribosome entry site (IRES) elements were first identified about 20 years ago within the 5’ untranslated region of picornavirus RNAs. They direct a cap-independent mechanism of translation initiation on the viral RNA. Within the picornavirus family it is now known that there are four classes of IRES element which vary in size (450-270nt), they also have different, complex, secondary structures and distinct requirements for cellular proteins to allow them to function. This review describes the features of each class of picornavirus IRES element but focuses on the characteristics of the most recently described group, initially identified within the porcine teschovirus-1 RNA, which has strong similarities to the IRES elements from within the genomes of hepatitis C virus and the pestiviruses which are members of the flavivirus family. The selection of the initiation codon by these distinct IRES elements is also discussed.

General information
State: Published
Organisations: Division of Virology, National Veterinary Institute
Contributors: Belsham, G.
Pages: 183-192
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Publication information
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Foot-and-mouth disease

Foot-and-mouth disease is an economically important, highly contagious, disease of cloven-hoofed animals characterized by the appearance of vesicles (blisters) on the feet and in and around the mouth. The causative agent, foot-and-mouth disease virus, was the first mammalian virus to be discovered. It has a ribonucleic acid (RNA) genome enclosed within a protein coat. The virus replicates very rapidly within the cytoplasm of cells. The RNA genome has to function both as a messenger RNA and as a template for RNA replication. The RNA encodes a single polyprotein which is processed, by virus-encoded proteases, to about 12 mature products which are required for virus replication and assembly. Some of these viral proteins modify host cell activities to block anti-virus defence systems. Thus, this small virus displays a remarkably complex array of biological activities.

General information
State: E-pub ahead of print
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute, Pirbright Institute
Contributors: Belsham, G., Charleston, B., Jackson, T., Paton, D. J.
Publication date: 2009
Peer-reviewed: Yes

Persistence of foot-and-mouth disease virus in ruminants.

During the spring of 2008, a new clinical project, with the aim of investigating mechanisms involved in development of FMD carrier animals, has been launched in the new FMD facilities of the Danish Veterinary Institute located at Lindholm Island. The project is based on a series of animal experiments, investigating the host response to FMD infection in sheep and cattle. FMD infection in ruminants involves initial viral replication in pharyngeal epithelia, from where the virus spreads systemically via the lymphatic system. Characteristic vesicular lesions develop in the cornified stratified squamous epithelia of the coronary bands and oral cavity within a few days of infection. Viremia occurs within 2-3 days of infection, but is rapidly cleared through the effect of circulating antibodies of the adaptive immune response. The host response involves initial activation of the innate immune response, with activation and recruitment of effector-cells, and subsequent activation of T- and B-cells, leading to the production of circulating antibodies, as well as activation of cytotoxic T-cells. In ruminants, approximately 50% of animals infected with FMDV develop into persistently infected carrier animals, with intermittent excretion of live virus, whilst remaining animals clear the infection effectively. Previous experiments have indicated that the site of persistent viral replication is located in pharyngeal lymphoid tissue, as well as the basal epithelia of the dorsal soft palate. In these locations, FMDV is capable of persistent replication, without being detected by the host cellular immune response, which would normally be expected to clear virus infected cells. In an ongoing series of experiments, animals of 4-5 moths of age are infected with FMD O UKG 34/2001, either through subepidermo-lingual injection or direct contact with inoculated animals. Animals are kept for approximately 2 to 4 months, and the progression of infection is monitored through samples of oropharyngeal fluid (probang samples) and serum, which are analysed for presence of live virus and development of antibodies. During different fixed time points of the infection, biopsy samples of epithelial and lymphoid tissues from the pharynx and dorsal soft palate are collected with the use of an endoscope equipped with biopsy forceps. Biopsy samples are used to investigate the host's cellular immune response at different time points during the infection, as well as the presence of FMDV antigen using immunohistochemistry. Samples will also be used to investigate expression of genes related to the innate and adaptive immune responses through qPCR at the mRNA level.

General information
State: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Stenfeldt, C., Belsham, G., Tjørmehej, K., Alexandersen, S.
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Translation initiation on picornavirus RNA

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Organisations: Unknown
Contributors: Belsham, G., Jackson, R. J.
Pages: 869-900
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Volume: Vol. 39
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Publisher: Cold Spring Harbor Laboratory Press
Editors: Sonenberg, N., Hershey, J. W. B., Mathews, M. B.
Source-ID: 316417
Research output: Research - peer-review : Book chapter – Annual report year: 2000

Dynamics of picornavirus RNA replication within infected cells.
Replication of many picornaviruses is inhibited by low concentrations of guanidine. Guanidine-resistant mutants are readily isolated and the mutations map to the coding region for the 2C protein. Using in vitro replication assays it has been determined previously that guanidine blocks the initiation of negative-strand synthesis. We have now examined the dynamics of RNA replication, measured by quantitative RT-PCR, within cells infected with either swine vesicular disease virus (an enterovirus) or foot-and-mouth disease virus as regulated by the presence or absence of guanidine. Following the removal of guanidine from the infected cells, RNA replication occurs after a significant lag phase. This restoration of RNA synthesis requires de novo protein synthesis. Viral RNA can be maintained for at least 72 h within cells in the absence of apparent replication but guanidine-resistant virus can become predominant. Amino acid substitutions within the 2C protein that confer guanidine resistance to swine vesicular disease virus and foot-and-mouth disease virus have been identified. Even when RNA synthesis is well established, the addition of guanidine has a major impact on the level of RNA replication. Thus, the guanidine-sensitive step in RNA synthesis is important throughout the virus life cycle in cells.

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Organisations: Division of Virology, National Veterinary Institute
Contributors: Belsham, G., Normann, P.
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Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.68 SJR 1.325 SNIP 0.877
Web of Science (2017): Impact factor 2.514
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.93 SJR 1.544 SNIP 0.891
Web of Science (2016): Impact factor 2.838
BFI (2015): BFI-level 1
Foot-and-mouth disease virus, but not bovine enterovirus, targets the host cell cytoskeleton, via the non-structural protein 3Cpro.

Foot-and-mouth disease virus (FMDV), a member of the Picornaviridae, is a pathogen of cloven-hoofed animals and causes a disease of major economic importance. Picornavirus-infected cells show changes in cell morphology and rearrangement of cytoplasmic membranes, which are a consequence of virus replication. We show here, by confocal immunofluorescence and electron microscopy, that the changes in morphology of FMDV-infected cells involve changes in the distribution of microtubule and intermediate filament components during infection. Despite the continued presence of centrosomes in infected cells, there is a loss of tethering of microtubules to the microtubule organizing center (MTOC) region. Loss of labeling for -tubulin, but not pericentrin, from the MTOC suggests a targeting of -tubulin (or associated proteins) rather than a total breakdown in MTOC structure. The identity of the FMDV protein(s) responsible was determined by the expression of individual viral nonstructural proteins and their precursors in uninfected cells. We report that the only viral nonstructural protein able to reproduce the loss of -tubulin from the MTOC and the loss of integrity of the microtubule system is FMDV 3Cpro. In contrast, infection of cells with another picornavirus, bovine enterovirus, did not affect -tubulin distribution, and the microtubule network remained relatively unaffected.

General information
State: Published
Organisations: Division of Virology, National Veterinary Institute, The Pirbright Institute, Queen's University Belfast, University of St Andrews
Contributors: Armer, H., Moffat, K., Wileman, T., Belsham, G., Jackson, T., Duprex, P., Ryan, M., Monaghan, P.
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Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 4.24 SJR 2.853 SNIP 1.096
Web of Science (2017): Impact factor 4.368
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.42 SJR 3.114 SNIP 1.124
Web of Science (2016): Impact factor 4.663
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 4.42 SJR 3.282 SNIP 1.132
Web of Science (2015): Impact factor 4.606
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 4.4 SJR 3.187 SNIP 1.208
Web of Science (2014): Impact factor 4.439
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 4.92 SJR 3.496 SNIP 1.251
Web of Science (2013): Impact factor 4.648
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): CiteScore 5.2 SJR 3.19 SNIP 1.222
Monocistronic mRNAs containing defective hepatitis C virus-like picornavirus internal ribosome entry site elements in their 5' untranslated regions are efficiently translated in cells by a cap-dependent mechanism

The initiation of protein synthesis on mRNAs within eukaryotic cells is achieved either by a 5’ cap-dependent mechanism or through internal initiation directed by an internal ribosome entry site (IRES). Picornavirus IRES elements, located in the 59 untranslated region (5'UTR), contain extensive secondary structure and multiple upstream AUG codons. These features can be expected to inhibit cap-dependent initiation of translation. However, we have now shown that certain mutant hepatitis C virus-like picornavirus IRES elements (from porcine teschovirus-1 and avian encephalomyelitis virus), which are unable to direct internal initiation, are not significant barriers to efficient translation of capped monocistronic mRNAs that contain these defective elements within their 5'UTRs. Moreover, the translation of these mRNAs is highly sensitive to the expression of an enterovirus 2A protease (which induces cleavage of eIF4G) and is also inhibited by hippuristanol, a specific inhibitor of eIF4A function, in contrast to their parental wild-type IRES elements. These results provide a possible basis for the evolution of viral IRES elements within the context of functional mRNAs that are translated by a cap-dependent mechanism.

General information
The picornavirus avian encephalomyelitis virus possesses a hepatitis C virus-like internal ribosome entry site element

Avian encephalomyelitis virus (AEV) is a picornavirus that causes disease in poultry worldwide, and flocks must be vaccinated for protection. AEV is currently classified within the hepatovirus genus, since its proteins are most closely related to those of hepatitis A virus (HAV). We now provide evidence that the 494-nucleotide-long 5' untranslated region of the AEV genome contains an internal ribosome entry site (IRES) element that functions efficiently in vitro and in mammalian cells. Unlike the HAV IRES, the AEV IRES is relatively short and functions in the presence of cleaved eIF4G and it is also resistant to an inhibitor of eIF4A. These properties are reminiscent of the recently discovered class of IRES elements within certain other picornaviruses, such as porcine teschovirus 1 (PTV-1). Like the PTV-1 IRES, the AEV IRES
shows significant similarity to the hepatitis C virus (HCV) IRES in sequence, function, and predicted secondary structure. Furthermore, mutational analysis of the predicted pseudoknot structure at the 3’ end of the AEV IRES lends support to the secondary structure we present. AEV is therefore another example of a picornavirus harboring an HCV-like IRES element within its genome, and thus, its classification within the hepatovirus genus may need to be reassessed in light of these findings.

General information
State: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Bakhshesh, M., Groppelli, E., Willcocks, M., Royall, E., Belsham, G., Roberts, L.
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Peer-reviewed: Yes

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Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 4.24 SJR 2.853 SNIP 1.096
Web of Science (2017): Impact factor 4.368
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.42 SJR 3.114 SNIP 1.124
Web of Science (2016): Impact factor 4.663
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 4.42 SJR 3.282 SNIP 1.132
Web of Science (2015): Impact factor 4.606
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 4.4 SJR 3.187 SNIP 1.208
Web of Science (2014): Impact factor 4.439
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 4.92 SJR 3.496 SNIP 1.251
Web of Science (2013): Impact factor 4.648
ISI indexed (2013): ISI indexed yes
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BFI (2012): BFI-level 2
Scopus rating (2012): CiteScore 5.2 SJR 3.19 SNIP 1.222
Web of Science (2012): Impact factor 5.076
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): CiteScore 5.37 SJR 3.429 SNIP 1.282
Web of Science (2011): Impact factor 5.402
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 3.596 SNIP 1.277
Development of a novel recombinant encapsidated RNA particle: evaluation as an internal control for diagnostic RT-PCR

This report describes the generation of novel encapsidated RNA particles and their evaluation as in-tube internal controls in diagnostic real-time reverse-transcription PCR (rRT-PCR) assays for the detection of RNA viruses. A cassette containing sequences of 2 diagnostic primer sets for foot-and-mouth disease virus (FMDV) and a set for swine vesicular disease virus (SVDV) was engineered into a full-length cDNA clone containing the RNA-2 segment of Cowpea Mosaic Virus (CPMV). After co-inoculation with a plasmid that expressed CPMV RNA-1, recombinant virus particles were rescued from cowpea plants (Vigna unguiculata). RNA contained in these particles was amplified in diagnostic rRT-PCR assays used for detection of FMDV and SVDV. Amplification of these internal controls was used to confirm that rRT-PCR inhibitors were absent from clinical samples, thereby verifying negative assay results. The recombinant CPMVs did not reduce the analytical sensitivity of the rRT-PCRs when amplification of the insert was performed in the same tube as the diagnostic target. This system provides an attractive solution to the production of internal controls for rRT-PCR assays since CPMV grows to high yields in plants, the particles are thermostable, RNase resistant and simple purification of RNA-2 containing capsids yields a preparation which is non-infectious.

General information
State: Published
Organisations: Section of Vesicular virus diseases, Division of Virology, National Veterinary Institute, The Pirbright Institute, John Innes Centre
Pages: 218-225
Publication date: 2007
Peer-reviewed: Yes
Identification of minimal sequences of the Rhopalosiphum padi virus 5' untranslated region required for internal initiation of protein synthesis in mammalian, plant and insect translation systems

Rhopalosiphum padi virus (RhPV) is a member of the family Dicistroviridae. The genomes of viruses in this family contain two open reading frames, each preceded by distinct internal ribosome entry site (IRES) elements. The RhPV 5' IRES is functional in mammalian, insect and plant translation systems and can form 48S initiation complexes in vitro with just the mammalian initiation factors eIF2, eIF3 and eIF1. Large regions of the 5' untranslated region (UTR) can be deleted without affecting initiation-complex formation. The minimal sequences required for directing internal initiation in mammalian (rabbit reticulocyte lysate), plant (wheatgerm extract) and insect (Sf21 cells) translation systems have now been defined. A fragment (nt 426–579) from the 3' portion of the 5' UTR can direct translation in each of these translation systems. In addition, a distinct region (nt 300–429) is also active. Thus, unstructured regions within the 5' UTR seem to be critical for IRES function.
Inhibition of the Secretory pathway by Foot-and-Mouth disease virus 2BC protein is reproduced by co-expression of 2B with 2C, and the site of inhibition is determined by the subcellular location of 2C

Infection of cells with picornaviruses can lead to a block in protein secretion. For poliovirus this is achieved by the 3A protein, and the consequent reduction in secretion of proinflammatory cytokines and surface expression of major histocompatibility complex class I proteins may inhibit host immune responses in vivo. Foot-and-mouth disease virus (FMDV), another picornavirus, can cause persistent infection of ruminants, suggesting it too may inhibit immune responses. Endoplasmic reticulum (ER)-to-Golgi apparatus transport of proteins is blocked by the FMDV 2BC protein. The observation that 2BC is processed to 2B and 2C during infection and that individual 2B and 2C proteins are unable to block secretion stimulated us to study the effects of 2BC processing on the secretory pathway. Even though 2BC was processed rapidly to 2B and 2C, protein transport to the plasma membrane was still blocked in FMDV-infected cells. The block could be reconstituted by coexpression of 2B and 2C, showing that processing of 2BC did not compromise the ability of FMDV to slow secretion. Under these conditions, 2C was located to the Golgi apparatus, and the block in transport also occurred in the Golgi apparatus. Interestingly, the block in transport could be redirected to the ER when 2B was coexpressed with a 2C protein fused to an ER retention element. Thus, for FMDV a block in secretion is dependent on both 2B and 2C, with the latter determining the site of the block.

General information
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Organisations: Section of Vesicular virus diseases, Division of Virology, National Veterinary Institute, The Pirbright Institute , University of St Andrews
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BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
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Scopus rating (2017): CiteScore 4.24 SJR 2.853 SNIP 1.096
Web of Science (2017): Impact factor 4.368
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.42 SJR 3.114 SNIP 1.124
Web of Science (2016): Impact factor 4.663
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 4.42 SJR 3.282 SNIP 1.132
Web of Science (2015): Impact factor 4.606
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 4.4 SJR 3.187 SNIP 1.208
Web of Science (2014): Impact factor 4.439
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 4.92 SJR 3.496 SNIP 1.251
Significance of Arginine 20 in the 2A protease for swine vesicular disease virus pathogenicity

Pathogenic and attenuated strains of swine vesicular disease virus (SVDV), an enterovirus, have been characterized previously and, by using chimeric infectious cDNA clones, the key determinants of pathogenicity in pigs have been mapped to the coding region for 1D–2A. Within this region, residue 20 of the 2A protease is particularly significant. Inoculation of pigs with mutant viruses containing single amino acid substitutions at this residue leads to the appearance of revertants, often containing an arginine at this position encoded by an AGA codon, one of six codons for this residue. The properties in pigs of two chimeric viruses, each with an arginine residue at this position but encoded by different
codons, have been investigated in parallel with the parental pathogenic and attenuated strains. Presence of the arginine residue, but not of the AGA codon, is essential for induction of high viraemia and appearance of significant disease.

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Organisations: Section of Vesicular virus diseases, Division of Virology, National Veterinary Institute, National Institute of Animal Health, The Pirbright Institute
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Peer-reviewed: Yes
Caliciviruses differ in their functional requirements for eIF4F components

Two classes of viruses, namely members of the Potyviridae and Caliciviridae, use a novel mechanism for the initiation of protein synthesis that involves the interaction of translation initiation factors with a viral protein covalently linked to the viral RNA, known as VPg. The calicivirus VPg proteins can interact directly with the initiation factors eIF4E and eIF3. Translation initiation on feline calicivirus (FCV) RNA requires eIF4E because it is inhibited by recombinant 4E-BP1. However, to date, there have been no functional studies carried out with respect to norovirus translation initiation, because of a lack of a suitable source of VPg-linked viral RNA. We have now used the recently identified murine norovirus (MNV) as a model system for norovirus translation and have extended our previous studies with FCV RNA to examine the role of the other eIF4F components in translation initiation. We now demonstrate that, as with FCV, MNV VPg interacts directly with eIF4E, although, unlike FCV RNA, translation of MNV RNA is not sensitive to 4E-BP1, eIF4E depletion, or foot-and-mouth disease virus Lb protease-mediated cleavage of eIF4G. We also demonstrate that both FCV and MNV RNA translation require the RNA helicase component of the eIF4F complex, namely eIF4A, because translation was sensitive (albeit to different degrees) to a dominant negative form and to a small molecule inhibitor of eIF4A (hippuristanol). These results suggest that calicivirus RNAs differ with respect to their requirements for the components of the eIF4F translation initiation complex.
Functional analyses of RNA structures shared between the internal ribosome entry sites of hepatitis C virus and the picornavirus porcine teschovirus 1 Taifan

The internal ribosome entry site (IRES) of porcine teschovirus 1 (PTV-1), a member of the Picornaviridae family, is quite distinct from other well-characterized picornavirus IRES elements, but it displays functional similarities to the IRES from hepatitis C virus (HCV), a member of the Flaviviridae family. In particular, a dominant negative mutant form of eIF4A does not inhibit the activity of the PTV-1 IRES. Furthermore, there is a high level (ca. 50%) of identity between the PTV-1 and HCV IRES sequences. A secondary-structure model of the whole PTV-1 IRES has been derived which includes a pseudoknot. Validation of specific features within the model has been achieved by mutagenesis and functional assays. The differences and similarities between the PTV-1 and HCV IRES elements should assist in defining the critical features of this type of IRES.

General information
State: Published
Organisations: The Pirbright Institute, National Institute of Animal Health
Contributors: Chard, L. S., Kaku, Y., Jones, B., Nayak, A., Belsham, G.
Pages: 1271-1279
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Peer-reviewed: Yes

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Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 4.24 SJR 2.853 SNIP 1.096
Web of Science (2017): Impact factor 4.368
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.42 SJR 3.114 SNIP 1.124
Web of Science (2016): Impact factor 4.663
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 4.42 SJR 3.282 SNIP 1.132
Web of Science (2015): Impact factor 4.606
Original language: English

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Source: orbit
Source-ID: 315851

Research output: Research - peer-review › Journal article – Annual report year: 2006
Functional Characterization of IRESes by a Inhibitor of the RNA helicase eIF4A

RNA helicases are molecular motors that are involved in virtually all aspects of RNA metabolism. Eukaryotic initiation factor (eIF) 4A is the prototypical member of the DEAD-box family of RNA helicases. It is thought to use energy from ATP hydrolysis to unwind mRNA structure and, in conjunction with other translation factors, it prepares mRNA templates for ribosome recruitment during translation initiation. In screening marine extracts for new eukaryotic translation initiation inhibitors, we identified the natural product hippuristanol. We show here that this compound is a selective and potent inhibitor of eIF4A RNA-binding activity that can be used to distinguish between eIF4A-dependent and -independent modes of translation initiation in vitro and in vivo. We also show that poliovirus replication is delayed when infected cells are exposed to hippuristanol. Our study demonstrates the feasibility of selectively targeting members of the DEAD-box helicase family with small-molecule inhibitors.

General information
State: Published
Organisations: McGill University, University of the Ryukyus, Harvard Medical School, Pirbright Institute
Pages: 213-220
Publication date: 2006
Peer-reviewed: Yes

Publication Information
Journal: Nature Chemical Biology
Volume: 2
Issue number: 4
ISSN (Print): 1552-4450
Ratings:
BFI (2019): BFI-level 2
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 10.12 SJR 8.604 SNIP 2.973
Web of Science (2017): Impact factor 13.843
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 9.41 SJR 8.946 SNIP 3.061
Web of Science (2016): Impact factor 15.066
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 9.11 SJR 7.984 SNIP 2.804
Web of Science (2015): Impact factor 12.709
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 8.78 SJR 7.184 SNIP 2.78
Web of Science (2014): Impact factor 12.996
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 8.55 SJR 6.939 SNIP 2.886
Web of Science (2013): Impact factor 13.217
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): CiteScore 9.14 SJR 7.595 SNIP 3.424
Web of Science (2012): Impact factor 12.948
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): CiteScore 8.61 SJR 7.486 SNIP 3.569
Hepatitis C virus-related internal ribosome entry sites are found in multiple genera of the Picornaviridae

The internal ribosome entry site (IRES) elements from porcine enterovirus 8 and simian virus 2, two members of a proposed new genus within the family Picornaviridae, were characterized. These IRES elements, in common with the porcine teschovirus 1 IRES, were found to be related functionally and structurally to the IRES element from Hepatitis C virus, a member of the family Flaviviridae. Partial secondary structure predictions were derived and functional assays demonstrated that these IRES elements continued to be active when eIF4G was cleaved and when the activity of eIF4A was blocked.

General information
State: Published
Organisations: The Pirbright Institute, McIntyre Medical Sciences Building, University of the Ryukyus
Contributors: Chard, L. S., Bordeleau, M., Pelletier, J., Tanaka, J., Belsham, G.
Pages: 927-936
Publication date: 2006
Peer-reviewed: Yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.25 SJR 1.69 SNIP 1.057
Web of Science (2014): Impact factor 3.183
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 3.64 SJR 1.764 SNIP 1.154
Web of Science (2013): Impact factor 3.529
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 3.28 SJR 1.525 SNIP 1.034
Web of Science (2012): Impact factor 3.127
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 3.6 SJR 1.684 SNIP 1.145
Web of Science (2011): Impact factor 3.363
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.678 SNIP 1.053
Web of Science (2010): Impact factor 3.568
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.662 SNIP 1.127
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.648 SNIP 1.068
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 1.593 SNIP 1.131
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.709 SNIP 1.128
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 1.654 SNIP 1.137
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 1.55 SNIP 1.215
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 1.58 SNIP 1.145
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 1.369 SNIP 1.083
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 1.488 SNIP 1.109
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 1.416 SNIP 1.065
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 1.423 SNIP 1.074

Original language: English
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Source: orbit
Source-ID: 315849
Research output: Research - peer-review > Journal article – Annual report year: 2006
Role of RNA structure and RNA binding activity of foot-and-mouth disease virus 3C protein in VPg uridylylation and virus replication

The uridylylation of the VPg peptide primer is the first stage in the replication of picornavirus RNA. This process can be achieved in vitro using purified components, including 3B (VPg) with the RNA dependent RNA polymerase (3D(pol)), the precursor 3CD, and an RNA template containing the cre/bus. We show that certain RNA sequences within the foot-and-mouth disease virus (FMDV) 5' untranslated region but outside of the cre/bus can enhance VPg uridylylation activity. Furthermore, we have shown that the FMDV X protein alone can substitute for 3CD, albeit less efficiently. In addition, the VPg precursors, 3B(3)C and 3B(123)C, can function as substrates for uridylylation in the absence of added 3C or 3CD. Residues within the FMDV 3C protein involved in interaction with the cre/bus RNA have been identified and are located on the face of the protein opposite from the catalytic site. These residues within 3C are also essential for VPg uridylylation activity and efficient virus replication.

General information
State: Published
Organisations: Sektion for Eksotiske Virussygdomme, Division of Virology, National Veterinary Institute
Contributors: Nayak, A., Goodfellow, I. G., Woolaway, K. E., Birtley, J., Curry, S., Belsham, G.
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Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 4.24 SJR 2.853 SNIP 1.096
Web of Science (2017): Impact factor 4.368
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.42 SJR 3.114 SNIP 1.124
Web of Science (2016): Impact factor 4.663
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 4.42 SJR 3.282 SNIP 1.132
Web of Science (2015): Impact factor 4.606
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 4.4 SJR 3.187 SNIP 1.208
Web of Science (2014): Impact factor 4.439
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 4.92 SJR 3.496 SNIP 1.251
Web of Science (2013): Impact factor 4.648
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): CiteScore 5.2 SJR 3.19 SNIP 1.222
Web of Science (2012): Impact factor 5.076
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): CiteScore 5.37 SJR 3.429 SNIP 1.282
Stabilized baculovirus vector expressing a heterologous gene and GP64 from a single bicistronic transcript

The efficient scale-up of recombinant protein production in insect-cell bioreactors using baculovirus expression vectors is hampered by reductions in yield with increasing viral passage, the so-called passage effect. This phenomenon is characterized by the generation and subsequent accumulation of defective interfering baculoviruses (DIs), which interfere with the replication of genomically intact virus. A novel baculovirus expression vector is presented equipped with a bicistronic expression cassette that allows the simultaneous expression of the recombinant gene (GFP, first cistron) and an essential baculovirus gene (GP64, second cistron) from a single messenger RNA (mRNA). The translation of GP64 is mediated by an internal ribosome entry site (IRES) element from Rhopalosiphum padi virus (RhPV) while the native GP64 gene is deleted. In this way, a dominant selection pressure is placed on the entire bicistronic mRNA and hence on the maintenance of the foreign gene. The bicistronic expression vector was superior to the control baculovirus vector in that GFP expression remained at much higher levels upon continued virus passage. The versatility of this stabilized vector was demonstrated by its ability to propagate in a number of cell lines including Sf21, Sf9 and High Five cells. This novel baculovirus vector is especially valuable for large-scale recombinant protein production in insect-cell bioreactors where the number of viral passages is high. (c) 2005 Elsevier B.V. All rights reserved.

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Organizations: Wageningen IMARES, University of Surrey, Pirbright Institute
Pages: 13-21
Publication date: 2006
Publication information
Journal: Journal of Biotechnology
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Ratings:
  BFI (2019): BFI-level 1
  Web of Science (2019): Indexed yes
  BFI (2018): BFI-level 1
  Web of Science (2018): Indexed yes
  BFI (2017): BFI-level 1
  Scopus rating (2017): CiteScore 2.64 SJR 0.929 SNIP 0.86
  Web of Science (2017): Impact factor 2.533
  Web of Science (2017): Indexed yes
  BFI (2016): BFI-level 1
  Scopus rating (2016): CiteScore 2.88 SJR 1.004 SNIP 0.929
  Web of Science (2016): Impact factor 2.599
  Web of Science (2016): Indexed yes
  BFI (2015): BFI-level 1
  Scopus rating (2015): CiteScore 2.87 SJR 1.068 SNIP 0.988
  Web of Science (2015): Impact factor 2.667
  Web of Science (2015): Indexed yes
  BFI (2014): BFI-level 1
  Scopus rating (2014): CiteScore 2.95 SJR 1.116 SNIP 1.13
  Web of Science (2014): Impact factor 2.871
  Web of Science (2014): Indexed yes
  BFI (2013): BFI-level 1
  Scopus rating (2013): CiteScore 3.22 SJR 1.183 SNIP 1.175
  Web of Science (2013): Impact factor 2.884
  ISI indexed (2013): ISI indexed yes
  Web of Science (2013): Indexed yes
  BFI (2012): BFI-level 1
  Scopus rating (2012): CiteScore 3.4 SJR 1.238 SNIP 1.312
  Web of Science (2012): Impact factor 3.183
  ISI indexed (2012): ISI indexed yes
  Web of Science (2012): Indexed yes
  BFI (2011): BFI-level 1
  Scopus rating (2011): CiteScore 2.87 SJR 1.165 SNIP 1.043
  Web of Science (2011): Impact factor 3.045
  ISI indexed (2011): ISI indexed yes
  Web of Science (2011): Indexed yes
  BFI (2010): BFI-level 1
  Scopus rating (2010): SJR 1.135 SNIP 1.175
  Web of Science (2010): Impact factor 2.97
  Web of Science (2010): Indexed yes
  BFI (2009): BFI-level 1
  Scopus rating (2009): SJR 1.224 SNIP 1.231
  Web of Science (2009): Indexed yes
  BFI (2008): BFI-level 1
  Scopus rating (2008): SJR 1.147 SNIP 1.265
  Web of Science (2008): Indexed yes
  Scopus rating (2007): SJR 1.133 SNIP 1.27
  Web of Science (2007): Indexed yes
A cross-kingdom internal ribosome entry site reveals a simplified mode of internal ribosome entry

Rhopalosiphum padi virus (RhPV) is an insect virus of the Dicistroviridae family. Recently, the 579-nucleotide-long 5’ untranslated region (UTR) of RhPV has been shown to contain an internal ribosome entry site (IRES) that functions efficiently in mammalian, plant, and insect in vitro translation systems. Here, the mechanism of action of the RhPV IRES has been characterized by reconstitution of mammalian 48S initiation complexes on the IRES from purified components combined with the toeprint assay. There is an absolute requirement for the initiation factors eIF2 and eIF3 and the scanning factor eIF1 to form 48S complexes on the IRES. In addition, eIF1A, eIF4F (or the C-terminal fragment of eIF4G), and eIF4A strongly stimulated the assembly of this complex, whereas eIF4B had no effect. Although the eIF4-dependent pathway is dominant in the RhPV IRES-directed cell-free translation, omission of either eIF4G or eIF4A or both still allowed the assembly of 48S complexes from purified components with similar to 23% of maximum efficiency. Deletions of up to 100 nucleotides throughout the 5’-UTR sequence produced at most a marginal effect on the IRES activity, suggesting the absence of specific binding sites for initiation factors. Only deletion of the U-rich unstructured 380-nucleotide region proximal to the initiation codon resulted in a complete loss of the IRES activity. We suggest that the single-stranded nature of the RhPV IRES accounts for its strong but less selective potential to bind key mRNA recruiting components of the translation initiation apparatus from diverse origins.

General information
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Organisations: Lomonosov Moscow State University, University of Surrey, Pirbright Institute
Pages: 7879-7888
Publication date: 2005
Peer-reviewed: Yes
Factors required for the uridylylation of the foot-and-mouth disease virus 3B1, 3B2 and 3B3 peptides by the RNA dependent RNA polymerase (3D(pol)) in vitro

The 5′ terminus of picornavirus genomic RNA is covalently linked to the virus-encoded peptide 3B (VPg). Foot-and-mouth disease virus (FMDV) is unique in encoding and using 3 distinct forms of this peptide. These peptides each act as primers for RNA synthesis by the virus-encoded RNA polymerase 3Dpol. To act as the primer for positive-strand RNA synthesis, the 3B peptides have to be uridylylated to form VPgpU(pU). For certain picornaviruses, it has been shown that this reaction is achieved by the 3Dpol in the presence of the 3CD precursor plus an internal RNA sequence termed a cis-acting replication element (cre). The FMDV cre has been identified previously to be within the 5′ untranslated region, whereas all other picornavirus cre structures are within the viral coding region. The requirements for the in vitro
uridylylation of each of the FMDV 3B peptides has now been determined, and the role of the FMDV cre (also known as the 3B-uridylylation site, or bus) in this reaction has been analyzed. The poly(A) tail does not act as a significant template for FMDV 3B uridylylation.

General information
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Organisations: The Pirbright Institute, University of Reading
Contributors: Nayak, A., Goodfellow, I. G., Belsham, G.
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BFI (2019): BFI-level 2
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Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 4.24 SJR 2.853 SNIP 1.096
Web of Science (2017): Impact factor 4.368
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.42 SJR 3.114 SNIP 1.124
Web of Science (2016): Impact factor 4.663
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 4.42 SJR 3.282 SNIP 1.132
Web of Science (2015): Impact factor 4.606
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 4.4 SJR 3.187 SNIP 1.208
Web of Science (2014): Impact factor 4.439
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 4.92 SJR 3.496 SNIP 1.251
Web of Science (2013): Impact factor 4.648
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): CiteScore 5.2 SJR 3.19 SNIP 1.222
Web of Science (2012): Impact factor 5.076
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): CiteScore 5.37 SJR 3.429 SNIP 1.282
Web of Science (2011): Impact factor 5.402
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 3.596 SNIP 1.277
Web of Science (2010): Impact factor 5.189
Web of Science (2010): Indexed yes
Importance of arginine 20 of the swine vesicular disease virus 2A protease for activity and virulence

A major virulence determinant of swine vesicular disease virus (SVDV), an Enterovirus that causes an acute vesicular disease, has been mapped to residue 20 of the 2A protease. The SVDV 2A protease cleaves the ID-2A junction in the viral polyprotein, induces cleavage of translation initiation factor eIF4GI, and stimulates the activity of enterovirus internal ribosome entry sites (IRESs). The 2A protease from an attenuated strain of SVDV (Ile at residue 20) is significantly defective at inducing cleavage of eIF4GI and the activation of IRES-dependent translation compared to the 2A protease from a pathogenic strain (J1/73, Arg at residue 20), but the two proteases have similar ID-2A cleavage activities (Y. Sakoda, N. Ross-Smith, T. Inoue, and G. J. Belsham, J. Virol. 75:10643-10650, 2001). Residue 20 has now been modified to every possible amino acid, and the activities of each mutant 2A protease has been analyzed. Selected mutants were reconstructed into full-length SVDV cDNA, and viruses were rescued. The rate of virus growth in cultured swine kidney cells reflected the efficiency of 2A protease activity. In experimentally infected pigs, all four of the mutant viruses tested displayed much-reduced virulence compared to the J1/73 virus but a significant, albeit reduced, level of viral replication and excretion was detected. Direct sequencing of cDNA derived from samples taken early and late in infection indicated that a gradual selection-reversion to a more efficient protease occurred. The data indicated that extensive sequence change and selection may introduce a severe bottleneck in virus replication, leading to a decreased viral load and reduced or no clinical disease.

General information
State: Published
Organisations: National Institute of Animal Health, Pirbright Institute
Contributors: Inoue, T., Alexandersen, S., Clark, A. T., Murphy, C., Quan, M., Reid, S. M., Sakoda, Y., Johns, H. L., Belsham, G.
Pages: 428-440
Publication date: 2005
Peer-reviewed: Yes

Publication information
Journal: Journal of Virology
Volume: 79
Issue number: 1
Translation and replication of FMDV RNA

The seven chapters in this volume provide an account of the present knowledge and understanding of FMD pathogenesis and global epidemiology, the detailed structure of the virus itself and the properties of its RNA genome, the immune response of the host and the state of the art in vaccine production, and the nature of FMD virus evolution. It is clear that in all these areas there is still much more to learn about this fascinating virus. Because of its highly contagious nature research work on FMD is restricted to a small number of laboratories worldwide that have adequate containment facilities. Despite this restriction, the recent progress in research on FMD which is described in this volume has provided a remarkable level of understanding of this unique virus.

General information
State: Published
Organisations: Pirbright Institute
Contributors: Belsham, G.
Number of pages: 178
Pages: 43-70
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Title of host publication: Foot and mouth disease virus : Current Topics in Microbiology and Immunology
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Place of publication: New York
Publisher: Springer Berlin Heidelberg
Editor: Mahy, B. W. J.
ISBN (Print): 3-540-22419-x
Source: orbit
Source-ID: 315857
Research output: Research - peer-review › Book chapter – Annual report year: 2005

Cleavage of eukaryotic translation initiation factor 4GII within foot-and-mouth disease virus-infected cells: Identification of the L-protease cleavage site in vitro

Foot-and-mouth disease virus (FMDV) induces a very rapid inhibition of host cell protein synthesis within infected cells. This is accompanied by the cleavage of the eukaryotic translation initiation factor 4G (elF4G). The cleavage of the related protein elF4GII has now been analyzed. Within FMDV-infected cells, cleavage of elF4G and elF4GII occurs with similar kinetics. Cleavage of elF4GII is induced in cells and in cell extracts by the FMDV leader protease (L-pro) alone, generating cleavage products similar to those induced by enterovirus and rhinovirus 2A protease (2A(pro)). By the use of a fusion protein containing residues 445 to 744 of human elF4GII, it was demonstrated that the FMDV L pro specifically cleaves this protein between residues 6700 and 5701, immediately adjacent to the site (V699/G700) cleaved by rhinovirus 2A(pro) in vitro. By the use of a fusion protein containing residues 445 to 744 of human elF4GII, it was demonstrated that the FMDV L pro specifically cleaves this protein between residues 6700 and 5701, immediately adjacent to the site (V699/G700) cleaved by rhinovirus 2A(pro) in vitro. The G700/S701 cleavage site does not correspond, by amino acid sequence alignment, to that cleaved in elF4G by the FMDV L-pro in vitro. Knowledge of the cleavage sites and the three-dimensional structures of the FMDV L-pro and rhinovirus 2A(pro) enabled mutant forms of the elF4GII sequence to be generated that are differentially resistant to either one of these proteases. These results confirmed the specificity of each protease and showed that the mutant forms of the fusion protein substrate retained their correct sensitivity to other proteases.

General information
Functional and structural similarities between the internal ribosome entry sites of hepatitis C virus and porcine teschovirus, a picornavirus

Initiation of protein synthesis on picornavirus RNA requires an internal ribosome entry site (IRES). Typically, picornavirus IRES elements contain about 450 nucleotides (nt) and use most of the cellular translation initiation factors. However, it is now shown that just 280 nt of the porcine teschovirus type 1 Talfan (PTV-1) 5′ untranslated region direct the efficient internal initiation of translation in vitro and within cells. In toeprinting assays, assembly of 48S preinitiation complexes from purified components on the PTV-1 IRES was achieved with just 40S ribosomal subunits plus eIF2 and Met-tRNAiMet. Indeed, a binary complex between 40S subunits and the PTV-1 IRES is formed. Thus, the PTV-1 IRES has properties that are entirely different from other picornavirus IRES elements but highly reminiscent of the hepatitis C virus (HCV) IRES. Comparison between the PTV-1 IRES and HCV IRES elements revealed islands of high sequence identity that occur in regions critical for the interactions of the HCV IRES with the 40S ribosomal subunit and eIF3. Thus, there is significant functional and structural similarity between the IRES elements from the picornavirus PTV-1 and HCV, a flavivirus.
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Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.42 SJR 3.114 SNIP 1.124
Web of Science (2016): Impact factor 4.663
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 4.42 SJR 3.282 SNIP 1.132
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Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 4.4 SJR 3.187 SNIP 1.208
Web of Science (2014): Impact factor 4.439
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 4.92 SJR 3.496 SNIP 1.251
Web of Science (2013): Impact factor 4.648
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): CiteScore 5.2 SJR 3.19 SNIP 1.222
Web of Science (2012): Impact factor 5.076
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): CiteScore 5.37 SJR 3.429 SNIP 1.282
Web of Science (2011): Impact factor 5.402
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 3.596 SNIP 1.277
Web of Science (2010): Impact factor 5.189
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 3.631 SNIP 1.306
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 3.846 SNIP 1.26
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 3.598 SNIP 1.307
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 3.809 SNIP 1.252
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 3.443 SNIP 1.244
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 3.452 SNIP 1.33
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 3.464 SNIP 1.328
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 3.219 SNIP 1.253
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 3.569 SNIP 1.354
Web of Science (2001): Indexed yes
Genome organisation, translation and replication of foot-and-mouth disease virus RNA

General information
State: Published
Organisations: Pirbright Institute
Contributors: Belsham, G., Martinez-Salas, E.
Number of pages: 600
Pages: 19-52
Publication date: 2004

Host publication information
Title of host publication: Foot-and-Mouth Disease: Current Perspectives
Volume: Chapter 2
Publisher: Horizon Press
Editors: Domingo, E., Sobrino, F.
ISBN (Print): 0849329515
Source: orbit
Source-ID: 315859
Research output: Research - peer-review › Book chapter – Annual report year: 2004

Sequential modification of translation initiation factor eIF4GI by two different foot-and-mouth disease virus proteases within infected baby hamster kidney cells: identification of the 3C(pro) cleavage site

Infection of cells by foot-and-mouth disease virus (FMDV) causes the rapid inhibition of cellular cap-dependent protein synthesis that results from cleavage of the translation initiation factor eIF4G, a component of the cap-binding complex eIF4F. Two FMDV proteases, the leader (L) and 3C proteases, have been shown individually to induce cleavage of eIF4GI at distinct sites within baby hamster kidney (BHK) cells. Here, sequential cleavage of eIF4GI by the L and 3C proteases was demonstrated in FMDV-infected BHK cells. The FMDV 3C cleavage site within hamster eIF4GI was localized to a small region (about 40 aa) of the protein, between the sites cleaved by the poliovirus 2A protease and the human immunodeficiency virus type 2 protease. Human eIF4GI was found to be resistant to the action of the FMDV 3C protease. On the basis of amino acid sequence alignments, it was predicted and then verified that substitution of a single amino acid residue within this region of human eIF4GI conferred sensitivity to cleavage by the FMDV 3C protease within cells. Full-length eIF4GI and both forms of the C-terminal cleavage product must be capable of supporting the activity of the FMDV internal ribosome entry site in directing translation initiation.

General information
State: Published
Organisations: Pirbright Institute
Contributors: Strong, R., Belsham, G.
Pages: 2953-2962
Publication date: 2004
Peer-reviewed: Yes

Publication information
Journal: Journal of General Virology
Volume: 85
Issue number: Part 10
ISSN (Print): 0022-1317
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
The Rhopalosiphum padi virus 5' internal ribosome entry site is functional in Spodoptera frugiperda 21 cells and in their cell-free lysates: implications for the baculovirus expression system

Cap-independent internal initiation of translation occurs on a number of viral and cellular mRNAs and is directed by internal ribosome entry site (IRES) elements. Rhopalosiphum padi virus (RhPV) is a member of the Dicistroviridae. These viruses have single-stranded, positive-sense RNA genomes that contain two open reading frames, both preceded by IRES elements. Previously, the activity of the RhPV 5' UTR IRES has been demonstrated in mammalian, Drosophila and wheat germ in vitro translation systems. It is now shown that this IRES also functions within Spodoptera frugiperda (Sf21) cells which are widely used in the baculovirus expression system, and in a novel Sf21 cell-based lysate system. Inclusion of the RhPV IRES in a dicistronic reporter mRNA transcript increased translation of the second cistron 23-fold within Sf21 cells. In contrast, the encephalomyocarditis virus IRES was inactive in both systems. The RhPV IRES therefore has the potential to be utilized in insect cell expression systems.

General information
State: Published
Organisations: University of Surrey, RiNA GmbH, Pirbright Institute
Contributors: Royall, E., Woolaway, K. E., Schacherl, J., Kubick, S., Belsham, G., Roberts, L. O.
Pages: 1565-1569
Publication date: 2004
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Publication information
Journal: Journal of General Virology
Volume: 85
Issue number: Part 6
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Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.68 SJR 1.325 SNIP 0.877
Web of Science (2017): Impact factor 2.514
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.93 SJR 1.544 SNIP 0.891
Web of Science (2016): Impact factor 2.838
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.26 SJR 1.738 SNIP 0.998
Web of Science (2015): Impact factor 3.192
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.25 SJR 1.69 SNIP 1.057
Web of Science (2014): Impact factor 3.183
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 3.64 SJR 1.764 SNIP 1.154
Web of Science (2013): Impact factor 3.529
Conserved nucleotides within the J-domain of the encephalomyocarditis virus internal ribosome entry site are required for activity and for interaction with eIF4G

The internal ribosome entry site (IRES) elements of cardioviruses (e.g., encephalomyocarditis virus [EMCV] and foot-and-mouth disease virus) are predicted to have very similar secondary structures. Among these complex RNA structures there is only rather limited complete sequence conservation. Within the J domain of the EMCV IRES there are four highly conserved nucleotides (A704, C705, G723, and A724), which are predicted to be unpaired and have been targeted for mutagenesis. Using an IRES-dependent cell selection system, we have isolated functional IRES elements from a pool of up to 256 mutants. All changes to these conserved nucleotides resulted in IRES elements that were less efficient at directing internal initiation of translation than the wild-type element, and even some of the single point mutants were highly defective. Each of the mutations adversely affected the ability of the RNAs to interact with the translation initiation factor eIF4G.
Rinderpest virus lineage differentiation using RT-PCR and SNAP-ELISA

An RT-PCR/ELISA system has been developed that detects and differentiates Rinderpest virus (RPV) from the other closely related morbillivirus of ruminants, Peste des petits Ruminants virus (PPRV). In addition, using lineage specific probes, it is possible to determine whether the virus sample is wild-type or vaccine, and the likely origin of the outbreak if it is wild-type. It involves carrying out a RT-PCR with one digoxygenin (Dig)-labelled primer followed by a hybridisation step with a virus-specific, biotin-labelled, probe. The hybridisation step is carried out in an ELISA format on a streptavidin-coated plate. The DIG-labelled products are detected using a specific anti-DIG monoclonal antibody and an anti-mouse horseradish peroxidase conjugate. The hybridisation step replaces nucleotide sequencing or nested PCR for confirmation of the identity of DNA product. The assay is fast and easy to carry out and can give semi-quantitative estimates of the virus content of samples. (C) 2002 Published by Elsevier Science B.V.

General information
State: Published
Organisations: Pirbright Institute
Contributors: Forsyth, M. A., Parida, S., Alexandersen, S., Belsham, G., Barret, T.
Pages: 29-36
Publication date: 2003
Peer-reviewed: Yes

Publication information
Journal: Journal of Virological Methods
Volume: 107
Issue number: 1
ISSN (Print): 0166-0934
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 1.82 SJR 0.858 SNIP 0.817
The foot-and-mouth disease virus cis-acting replication element (cre) can be complemented in trans within infected cells. A temperature-sensitive (ts) mutation was identified within the 5′-untranslated region of foot-and-mouth disease virus (FMDV) RNA. The mutation destabilizes a stem-loop structure recently identified as a cis-acting replication element (cre). Genetic analyses indicated that the ts defect in virus replication could be complemented. Thus, the FMDV cre can function in trans. It is suggested that the cre be renamed a 3B-uridylylation site (bus).

General information
State: Published
Organisations: Pirbright Institute
Contributors: Tiley, L., King, A. M. Q., Belsham, G.
Pages: 2243-2246
Publication date: 2003
Peer-reviewed: Yes

Publication information
Journal: Journal of Virology
Volume: 77
Issue number: 3
ISSN (Print): 0022-538X
Ratings:
BFI (2019): BFI-level 2
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 4.24 SJR 2.853 SNIP 1.096
Web of Science (2017): Impact factor 4.368
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.42 SJR 3.114 SNIP 1.124
Web of Science (2016): Impact factor 4.663
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 4.42 SJR 3.282 SNIP 1.132
Web of Science (2015): Impact factor 4.606
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 4.4 SJR 3.187 SNIP 1.208
Web of Science (2014): Impact factor 4.439
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 4.92 SJR 3.496 SNIP 1.251
Web of Science (2013): Impact factor 4.648
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): CiteScore 5.2 SJR 3.19 SNIP 1.222
Web of Science (2012): Impact factor 5.076
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
Conservation of L and 3C proteinase activities across distantly related aphthoviruses

The foot-and-mouth disease virus (FMDV) leader (L) proteinase is an important virulence determinant in FMDV infections. It possesses two distinct catalytic activities: (i) C-terminal processing at the L/VP4 junction; and (ii) induction of the cleavage of translation initiation factor eIF4G, an event that inhibits cap-dependent translation in infected cells. The only other member of the Aphthovirus genus, equine rhinitis A virus (ERAV), also encodes an L protein, but this shares only 32% amino acid identity with its FMDV counterpart. Another more distantly related picornavirus, equine rhinitis B virus (ERBV), which is not classified as an aphthovirus, also encodes an L protein. Using in vitro transcription and translation analysis, we have shown that both ERAV and ERBV L proteins have C-terminal processing activity. Furthermore, expression of ERAV L, but not ERBV L, in BHK-21 cells resulted in the efficient inhibition of cap-dependent translation in these cells. We have shown that the ERAV. and FMDV L proteinases induce cleavage of eIF4GI at very similar or identical positions. Interestingly, ERAV X also induces eIF4GI cleavage and again produces distinct products that co-migrate with those induced by FMDV 3C. The ERBV L proteinase does not induce eIF4GI cleavage, consistent with its inability to shut down cap-dependent translation. We have also shown that another unique feature of FMDV L, the stimulation of enterovirus internal ribosome entry site (IRES) activity, is also shared by the ERAV L proteinase but not by ERBV L. The functional conservation of the divergent ERAV and FMDV proteinases indicates the likelihood of a similar and important role for these enzymes in the pathogenesis of infections caused by these distantly related aphthoviruses.
Detection of all seven serotypes of foot-and-mouth disease virus by real-time, fluorogenic reverse transcription polymerase chain reaction assay

A fluorogenic RT-PCR (5'-nuclease probe-based) assay using a primer/probe set designed from the internal ribosomal entry site region of the virus genome was developed for the specific detection of all seven serotypes of foot-and-mouth disease (FMD) virus in epithelial suspensions and cell culture virus preparations. The reverse transcription polymerase chain reaction (RT-PCR) specifically detected FMD virus in sample submissions from the UK 2001 FMD outbreak with greater sensitivity than our conventional RT-PCR procedure and our routine diagnostic procedures of ELISA and virus isolation in cell culture. The fluorogenic RT-PCR provides relatively fast results, enables a quantitative assessment to be made of virus amounts and can handle more samples and/or replicates of samples in a single assay than the conventional RT-PCR procedure. Therefore it is seen as a valuable tool to complement the routine diagnostic procedures for FMD virus diagnosis. (C) 2002 Published by Elsevier Science B.V.

General information
State: Published
Organisations: Pirbright Institute
Contributors: Reid, S. M., Ferris, N. P., Hutchings, G. H., Zhang, Z., Belsham, G., Alexandersen, S.
Pages: 67-80
Publication date: 2002
Peer-reviewed: Yes

Publication information
Journal: Journal of Virological Methods
Volume: 105
Issue number: 1
ISSN (Print): 0166-0934
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 1.82 SJR 0.858 SNIP 0.817
Web of Science (2017): Impact factor 1.756
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 1.78 SJR 0.873 SNIP 0.729
Web of Science (2016): Impact factor 1.693
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 1.68 SJR 0.87 SNIP 0.802
Web of Science (2015): Impact factor 1.508
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 1.87 SJR 0.898 SNIP 0.933
Web of Science (2014): Impact factor 1.781
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 1.99 SJR 0.866 SNIP 0.9
Web of Science (2013): Impact factor 1.883
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 2.08 SJR 0.873 SNIP 0.929
Web of Science (2012): Impact factor 1.9
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 2.23 SJR 0.908 SNIP 0.987
Web of Science (2011): Impact factor 2.011
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.91 SNIP 1.001
Web of Science (2010): Impact factor 2.139
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.973 SNIP 1.059
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 0.926 SNIP 1.072
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 0.963 SNIP 1.025
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 0.886 SNIP 1.073
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 0.873 SNIP 1.021
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 0.723 SNIP 1.079
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 0.759 SNIP 1.012
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 0.714 SNIP 1.058
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 0.755 SNIP 0.948
Scopus rating (2000): SJR 0.628 SNIP 0.899
Scopus rating (1999): SJR 0.635 SNIP 0.836
Original language: English
DOIs:
10.1016/S0166-0934(02)00081-2
Unique characteristics of a picornavirus internal ribosome entry site from the Porcine Teschovirus-1 Talfan

The teschoviruses constitute a recently defined picornavirus genus. Most of the genome sequence of the porcine teschovirus-1 (PTV) Talfan and several other strains is known. We now demonstrate that initiation of protein synthesis occurs at nucleotide (nt) 412 on the PTV Talfan RNA and that nt 1 to 405 contains an internal ribosome entry site (IRES) that functions efficiently in vitro and within mammalian cells. In comparison with other picornavirus IRES elements, the PTV IRES is relatively short and lacks a significant polypyrimidine tract near the 3' end. Expression of an enterovirus 2A protease, which induces cleavage of eIF4G within the translation initiation complex eIF4F, has little effect on the PTV IRES activity within BHK cells. The PTV IRES has a unique set of properties and represents a new class of picornavirus IRES element.

General information
State: Published
Organisations: National Institute of Animal Health, Pirbright Institute
Contributors: Kaku, Y., Chard, L. S., Inoue, T., Belsham, G.
Pages: 11721-11728
Publication date: 2002
Peer-reviewed: Yes

Publication information
Journal: Journal of Virology
Volume: 76
Issue number: 22
ISSN (Print): 0022-538X
Ratings:
BFI (2019): BFI-level 2
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 4.24 SJR 2.853 SNIP 1.096
Web of Science (2017): Impact factor 4.368
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.42 SJR 3.114 SNIP 1.124
Web of Science (2016): Impact factor 4.663
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 4.42 SJR 3.282 SNIP 1.132
Web of Science (2015): Impact factor 4.606
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 4.4 SJR 3.187 SNIP 1.208
Web of Science (2014): Impact factor 4.439
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 4.92 SJR 3.496 SNIP 1.251
Web of Science (2013): Impact factor 4.648
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): CiteScore 5.2 SJR 3.19 SNIP 1.222
Web of Science (2012): Impact factor 5.076
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
An attenuating mutation in the 2A protease of swine vesicular disease virus, a picornavirus, regulates cap- and internal ribosome entry site-dependent protein synthesis

Virulent and avirulent strains of swine vesicular disease virus (SVDV), a picornavirus, have been characterized previously. The major determinants for attenuation have been mapped to specific residues in the 1D-2A-coding region. The properties of the 2A proteases from the virulent and avirulent strains of SVDV have now been examined. Both proteases efficiently cleaved the 1D/2A junction in vitro and in vivo. However, the 2A protease of the avirulent strain of SVDV was much less effective than the virulent-virus 2A protease at inducing cleavage of translation initiation factor eIF4G1 within transfected cells. Hence the virulent-virus 2A protease is much more effective at inhibiting cap-dependent protein synthesis. Furthermore, the virulent-virus 2A protease strongly stimulated the internal ribosome entry sites (IRESs) from coxsackievirus B4 and from SVDV, while the avirulent-virus 2A protease was significantly less active in these assays. Thus, the different properties of the 2A proteases from the virulent and avirulent strains of SVDV in regulating protein synthesis initiation reflect the distinct pathogenic properties of the viruses from which they are derived. A single amino acid substitution, adjacent to His21 of the catalytic triad, is sufficient to confer the characteristics of the virulent-strain 2A protease on the avirulent-strain protease. It is concluded that the efficiency of picornavirus protein synthesis, controlled directly by the IRES or indirectly by the 2A protease, can determine virus virulence.
A novel protein-RNA binding assay: functional interactions of the foot-and-mouth disease virus internal ribosome entry site with cellular proteins

Translation initiation on foot-and-mouth disease virus (FMDV) RNA occurs by a cap-independent mechanism directed by a highly structured element (similar to 435 nt) termed an internal ribosome entry site (IRES). A functional assay to identify proteins that bind to the FMDV IRES and are necessary for FMDV IRES-mediated translation initiation has been developed. In vitro-transcribed polyadenylated RNAs corresponding to the whole or part of the FMDV IRES were immobilized on oligo-dT Dynabeads and used to deplete rabbit reticulocyte lysate (RRL) of IRES-binding proteins. Translation initiation factors eIF4G, eIF4A, and eIF4B bound to the 3' domain of the FMDV IRES. Depletion of eIF4G from RRL by this region of the FMDV IRES correlated with the loss of translational capacity of the RRL for capped, uncapped, and FMDV IRES-dependent mRNAs. However, this depleted RRL still supported hepatitis C virus IRES-directed translation. Poly (rC) binding protein-2 bound to the central domain of the FMDV IRES, but depletion of RRL with this IRES domain had no effect on FMDV IRES-directed translation initiation.

General information
State: Published
Organisations: Pirbright Institute
Contributors: Stassinopoulos, I. A., Belsham, G.
Pages: 114-122
Publication date: 2001
Peer-reviewed: Yes

Publication information
Journal: R N A
Volume: 7
Issue number: 1
ISSN (Print): 1355-8382
Ratings:
BFI (2019): BFI-level 2
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 3.66 SJR 3.219 SNIP 0.94
Cleavage of translation initiation factor 4AI (eIF4AI) but not eIF4AII by foot-and-mouth disease virus 3C protease: identification of the eIF4AI cleavage site

The translation initiation factor eIF4A is cleaved within mammalian cells infected by foot-and-mouth disease virus (FMDV). The FMDV 3C protease cleaves eIF4AI (between residues E143 and V144), but not the closely related eIF4AII.
Modification of eIF4AI, to produce a sequence identical to eIF4AII around the cleavage site, blocked proteolysis. Alignment of mammalian eIF4AI onto the three-dimensional structure of yeast eIF4A located the scissile bond within an exposed, flexible portion of the molecule. The N- and C-terminal cleavage products of eIF4AI generated by FMDV 3C dissociate. Cleavage of eIF4AI by FMDV 3C is thus expected to inactivate it. (C) 2001 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.
Diagnosis of foot-and-mouth disease by real time, fluorogenic polymerase chain reaction assay

General information
State: Published
Organisations: Pirbright Institute
Contributors: Reid, S. M., Ferris, N. P., Hutchings, G. H., Zhang, Z., Belsham, G., Alexandersen, S.
Pages: 621-623
Publication date: 2001
Peer-reviewed: Yes

Publication information
Journal: Veterinary Record
Volume: 149
ISSN (Print): 0042-4900
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 0.39 SJR 0.464 SNIP 0.858
Web of Science (2017): Impact factor 2.05
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 0.3 SJR 0.482 SNIP 0.745
Web of Science (2016): Impact factor 1.737
Eukaryotic initiation factors 4A (eIF4A) and 4G (eIF4G) mutually interact in a 1 : 1 ratio in vivo

mRNA translation in eukaryotic cells involves a set of proteins termed translation initiation factors (eIFs), several of which are involved in the binding of ribosomes to mRNA. These include eIF4G, a modular scaffolding protein, and eIF4A, an RNA helicase, of which two closely related forms are known in mammals, eIF4A(I) and eIF4A(II). In mammals, eIF4G possesses two independent sites for binding eIF4A, whereas in other eukaryotes (e.g. yeast) only one site appears to be present, thus raising the issue of the stoichiometry of eIF4G-eIF4A complexes in different eukaryotes. We show that in human embryonic kidney cells eIF4G is associated with eIF4A(I) or eIF4A(II) but not with both simultaneously, suggesting a stoichiometry of 1:1 rather than 1:2. To confirm this, eIF4A(I) or eIF4A(II) was expressed in a tagged form in these cells, and complexes with eIF4G were again isolated. Complexes containing tagged eIF4A(I) or eIF4A(II) contained no endogenous eIF4A, supporting the notion that eIF4G binds only one molecule of eIF4A. Each binding site in eIF4G can bind either eIF4A(I) or eIF4A(II). The data imply that the second binding site in mammalian eIF4A does not bind an additional eIF4A molecule and that initiation factor complexes in different eukaryotes contain one eIF4A per eIF4G.
Foot-and-mouth disease

General information
State: Published
Organisations: Pirbright Institute
Contributors: King, A. M. Q., Belsham, G., Donaldson, A. I.
Publication date: 2001

Host publication information
Title of host publication: Encyclopaedia of the Life Sciences
Publisher: Macmillan Press Ltd.
Source: orbit
Source-ID: 316525
Research output: Research - peer-review › Book chapter – Annual report year: 2001
Induction of a protective response in swine vaccinated with DNA encoding foot-and-mouth disease virus empty capsid proteins and the 3D RNA polymerase

This work focuses on the development of a potential recombinant DNA vaccine against foot-and-mouth disease virus (FMDV). Such a vaccine would have significant advantages over the conventional inactivated virus vaccine, in particular having none of the risks associated with the high security requirements for working with live virus. The principal aim of this strategy was to stimulate an antibody response to native, neutralizing epitopes of empty FMDV capsids generated in vivo. Thus, a plasmid (pCDNA3.1/P1-2A3C3D) was constructed containing FMDV cDNA sequences encoding the viral structural protein precursor P1-2A and the non-structural proteins 3C and 3D. The 3C protein was included to ensure cleavage of the P1-2A precursor to VPO, VP1 and VP3, the components of self-assembling empty capsids. The non-structural protein 3D was also included in the construct in order to provide additional stimulation of CD4(+) T cells. When swine were immunized with this plasmid, antibodies to FMDV and the 3D polymerase were synthesized. Furthermore, neutralizing antibodies were detected and, after three sequential vaccinations with DNA, some of the animals were protected against challenge with live virus. Additional experiments suggested that the antibody response to FMDV proteins was improved by the co-administration of a plasmid encoding porcine granulocyte-macrophage colony-stimulating factor. Although still not as effective as the conventional virus vaccine, the results encourage further work towards the development of a DNA vaccine against FMDV.

General information
State: Published
Organisations: INRA Institut National de La Recherche Agronomique, Pirbright Institute
Contributors: Cedillo-Barron, L., Foster-Cuevas, M., Belsham, G., Lefevre, F., Parkhouse, R. M. E.
Pages: 1713-1724
Publication date: 2001
Peer-reviewed: Yes

Publication information
Journal: Journal of General Virology
Volume: 82
Issue number: Part 7
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BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.68 SJR 1.325 SNIP 0.877
Web of Science (2017): Impact factor 2.514
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.93 SJR 1.544 SNIP 0.891
Web of Science (2016): Impact factor 2.838
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.26 SJR 1.738 SNIP 0.998
Web of Science (2015): Impact factor 3.192
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.25 SJR 1.69 SNIP 1.057
Web of Science (2014): Impact factor 3.183
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 3.64 SJR 1.764 SNIP 1.154
Web of Science (2013): Impact factor 3.529
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 3.28 SJR 1.525 SNIP 1.034
Web of Science (2012): Impact factor 3.127
ISI indexed (2012): ISI indexed yes
The 5′ untranslated region of Rhopalosiphum padi virus contains an internal ribosome entry site which functions efficiently in mammalian, plant, and insect translation systems

Rhopalosiphum padi virus (RhPV) is one of several picorna-like viruses that infect insects; sequence analysis has revealed distinct differences between these agents and mammalian picornaviruses. RhPV has a single-stranded positive-sense RNA genome of about 10 kb; unlike the genomes of Picornaviridae, however, this genome contains two long open reading frames (ORFs). ORF1 encodes the virus nonstructural proteins, while the downstream ORF, ORF2, specifies the structural proteins. Both ORFs are preceded by long untranslated regions (UTRs). The intergenic UTR is known to contain an internal ribosome entry site (IRES) which directs non-AUG-initiated translation of ORF2. We have examined the 5′ UTR of RhPV for IRES activity by translating synthetic dicistronic mRNAs containing this sequence in a variety of systems. We now report that the 5′ UTR contains an element which directs internal initiation of protein synthesis from an AUG codon in mammalian, plant, and Drosophila in vitro translation systems. In contrast, the encephalomyocarditis virus IRES functions only in the mammalian system. The RhPV 5′ IRES element has features in common with picornavirus IRES elements, in that no coding sequence is required for IRES function, but also with cellular IRES elements, as deletion analysis indicates that this IRES element does not have sharply defined boundaries.

General information
State: Published
Organisations: University of Surrey, The Pirbright Institute
Contributors: Woolaway, K. E., Lazaridis, K., Belsham, G., Carter, M. J., Roberts, L. O.
The requirement for eukaryotic initiation factor 4A (eIF4A) in translation is in direct proportion to the degree of mRNA 5' secondary structure

Eukaryotic initiation factor (eIF) 4A functions as a subunit of the initiation factor complex eIF4F, which mediates the binding of mRNA to the ribosome, eIF4A possesses ATPase and RNA helicase activities and is the prototype for a large family of putative RNA helicases (the DEAD box family). It is thought that the function of eIF4A during translation initiation is to unwind the mRNA secondary structure in the 5' UTR to facilitate ribosome binding. However, the evidence to support this hypothesis is rather indirect, and it was reported that eIF4A is also required for the translation of mRNAs possessing minimal 5' UTR secondary structure. Were this hypothesis correct, the requirement for eIF4A should correlate with the degree of mRNA secondary structure. To test this hypothesis, the effect of a dominant-negative mutant of mammalian eIF4A on translation of mRNAs with various degrees of secondary structure was studied in vitro. Here, we show that mRNAs containing stable secondary structure in the 5' untranslated region are more susceptible to inhibition by the eIF4A mutant. The mutant protein also strongly inhibits translation from several picornavirus internal ribosome entry sites (IRES), although to different extents. UV crosslinking of eIF4F subunits and eIF4B to the mRNA cap structure is dramatically reduced by the eIF4A mutant and RNA secondary structure. Finally, the eIF4A mutant forms a more stable complex with eIF4G, as compared to the wild-type eIF4A, thus explaining the mechanism by which substoichiometric amounts of mutant eIF4A inhibit translation.
ABC50 interacts with eukaryotic initiation factor 2 and associates with the ribosome in an ATP-dependent manner

Eukaryotic initiation factor 2 (eIF2) plays a key role in the process of translation initiation and in its control. Here we demonstrate that highly purified mammalian eIF2 contains an additional polypeptide of apparent molecular mass of 110
kDa. This polypeptide co-purified with eIF2 through five different chromatography procedures. A cDNA clone encoding the polypeptide was isolated, and its sequence closely matched that of a protein previously termed ABC50, a member of the ATP-binding cassette (ABC) family of proteins. Antibodies to ABC50 co-immunoprecipitated eIF2 and vice versa, indicating that the two proteins interact. The presence of ABC50 had no effect upon the ability of eIF2 to bind GDP but markedly enhanced the association of methionyl-tRNA with the factor. Unlike the majority of ABC proteins, which are membrane-associated transporters, ABC50 associates with the ribosome and co-sediments in sucrose gradients with the 40 and 60 S ribosomal subunits. The association of ABC50 with ribosomal subunits was increased by ATP and decreased by ADP. ABC50 is related to GCN20 and eEF3, two yeast ABC proteins that are not membrane-associated transporters and are instead implicated in mRNA translation and/or its control. Thus, these data identify ABC50 as a third ABC protein with a likely function in mRNA translation, which associates with eIF2 and with ribosomes.
Analysis of the c-myc IRES; a potential role for cell-type specific trans-acting factors and the nuclear compartment

The 5' UTR of c-myc mRNA contains an internal ribosome entry segment (IRES) and consequently, c-myc mRNAs can be translated by the alternative mechanism of internal ribosome entry. However, there is also some evidence suggesting that c-myc mRNA translation can occur via the conventional cap-dependent scanning mechanism. Using both bicistronic and monocistronic mRNAs containing the c-myc 5' UTR, we demonstrate that both mechanisms can contribute to c-myc protein synthesis. A wide range of cell types are capable of initiating translation of c-myc by internal ribosome entry, albeit with different efficiencies. Moreover, our data suggest that the spectrum of efficiencies observed in these cell types is likely to be due to variation in the cellular concentration of non-canonical translation factors. Interestingly, the c-myc IRES is 7-fold more active than the human rhinovirus 2 (HRV2) IRES and 5-fold more active than the encephalomyocarditis virus (EMCV) IRES. However, the protein requirements for the c-myc IRES must differ significantly from these viral IRESs, since an unidentified nuclear event appears to be a pre-requisite for efficient c-myc IRES-driven initiation.

General information
State: Published
Organisations: University of Leicester
Contributors: Stoneley, M., Soubkhankulova, T., Le Quesne, J. P. C., Coldwell, M., Jopling, C. L., Belsham, G., Willis, A. E.
Pages: 687-694
Publication date: 2000
Peer-reviewed: Yes

Publication information
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<td>SJR 5.092</td>
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<td>2005</td>
<td>BFI-level 2</td>
<td>SJR 5.092</td>
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Caspases are not involved in the cleavage of translation initiation factor eIF4GI during picornavirus infection

Infection of cells by many picornaviruses results in the rapid inhibition of cellular protein synthesis due to cleavage of the translation initiation factor eIF4G. The poliovirus (PV) 2A and foot-and-mouth disease virus (FMDV) L proteases are each sufficient to mediate this cleavage, but the cleavage mechanism may be indirect, involving an unidentified cellular protease(s). eIF4G is also targeted for cleavage by caspase-3 during apoptosis. Here, it is shown that caspase inhibitors do not inhibit the cleavage of eIF4GI during PV or FMDV infection. Similarly, in transient-expression studies, the cleavage of eIF4GI induced by PV 2A or FMDV L was unaffected by these inhibitors. Furthermore, the cleavage of eIF4GI was observed in PV-infected MCF-7 cells lacking caspase-3. These data, and the fact that induction of apoptosis yields different eIF4GI cleavage fragments, indicate that caspases do not have a major role in the cleavage of eIF4GI during PV or FMDV infection.

General information
State: Published
Organisations: University of Surrey, Pirbright Institute
Pages: 1703-1707
Publication date: 2000
Peer-reviewed: Yes

Publication information
Journal: Journal of General Virology
Volume: 81
Issue number: Part 7
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Ratings:
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Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.68 SJR 1.325 SNIP 0.877
Web of Science (2017): Impact factor 2.514
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.93 SJR 1.544 SNIP 0.891
Web of Science (2016): Impact factor 2.838
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.26 SJR 1.738 SNIP 0.998
Web of Science (2015): Impact factor 3.192
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Development of reverse transcription-PCR (oligonucleotide probing) enzyme-linked immunosorbent assays for diagnosis and preliminary typing of foot-and-mouth disease: A new system using simple and aqueous-phase hybridization

A reverse transcription-PCR (RT-PCR)-enzyme-linked immunosorbent assay system that detects a relatively conserved region within the RNA genome of all seven serotypes of foot-and-mouth disease virus (FMDV) has been developed. The high specificity of the assay is achieved by including a rapid hybridization step with a biotin-labeled internal
oligonucleotide. The assay is highly sensitive, fast, and easy to perform. A similar assay, based on a highly variable region of the FMDV genome and employing a single asymmetry: RT-PCR and multiple hybridization oligonucleotides, was developed to demonstrate the method's ability to type FMDV. Based on our theoretical and practical knowledge of the methodology, we predict that similar assays are applicable to diagnosis and strain differentiation in any system amenable to PCR amplification.

**General information**

State: Published  
Organisations: Pirbright Institute  
Contributors: Alexandersen, S., Forsyth, M. A., Reid, S. M., Belsham, G.  
Pages: 4604-4613  
Publication date: 2000  
Peer-reviewed: Yes

**Publication information**

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Volume: 38  
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Ratings:  
BFI (2019): BFI-level 1  
Web of Science (2019): Indexed yes  
BFI (2018): BFI-level 1  
Web of Science (2018): Indexed yes  
BFI (2017): BFI-level 1  
Scopus rating (2017): CiteScore 3.55 SJR 2.256 SNIP 1.443  
Web of Science (2017): Impact factor 4.054  
Web of Science (2017): Indexed yes  
BFI (2016): BFI-level 1  
Scopus rating (2016): CiteScore 3.57 SJR 2.196 SNIP 1.4  
Web of Science (2016): Impact factor 3.712  
Web of Science (2016): Indexed yes  
BFI (2015): BFI-level 1  
Scopus rating (2015): CiteScore 3.56 SJR 2.206 SNIP 1.431  
Web of Science (2015): Indexed yes  
BFI (2014): BFI-level 1  
Scopus rating (2014): CiteScore 3.84 SJR 2.231 SNIP 1.528  
Web of Science (2014): Impact factor 3.993  
Web of Science (2014): Indexed yes  
BFI (2013): BFI-level 1  
Scopus rating (2013): CiteScore 4.18 SJR 2.438 SNIP 1.63  
Web of Science (2013): Impact factor 4.232  
ISI indexed (2013): ISI indexed yes  
Web of Science (2013): Indexed yes  
BFI (2012): BFI-level 1  
Scopus rating (2012): CiteScore 4.11 SJR 2.148 SNIP 1.626  
Web of Science (2012): Impact factor 4.068  
ISI indexed (2012): ISI indexed yes  
Web of Science (2012): Indexed yes  
BFI (2011): BFI-level 1  
Scopus rating (2011): CiteScore 4.27 SJR 2.346 SNIP 1.699  
Web of Science (2011): Impact factor 4.153  
ISI indexed (2011): ISI indexed yes  
Web of Science (2011): Indexed yes  
BFI (2010): BFI-level 1  
Scopus rating (2010): SJR 2.343 SNIP 1.731
Foot-and-mouth disease virus 3C protease induces cleavage of translation initiation factors eIF4A and eIF4G within infected cells

Infection of cells by foot-and-mouth disease virus (FMDV) results in the rapid inhibition of host cell protein synthesis. This process is accompanied by the early cleavage of the translation initiation factor eIF4G, a component of the cap-binding complex eIF4F. This cleavage is mediated by the leader (L) protease. Subsequently, as the virus proteins accumulate, secondary cleavages of eIF4G occur. Furthermore, eIF4A (46 kDa), a second component of eIF4F, is also cleaved in these later stages of the infection cycle. The 33-kDa cleavage product of eIF4A has lost a fragment from its N terminus. Transient-expression assays demonstrated that eIF4A was not cleaved in the presence of FMDV L or with the poliovirus 2A protease (which also mediates eIF4G cleavage) but was cleaved when the FMDV 3C protease was expressed. The FMDV 3C protease was also shown in such assays to induce cleavage of eIF4G, resulting in the production of cleavage products different from those generated by the L protease. Consistent with these results, within cells infected with a mutant: FMDV lacking the L protease or within cells containing an FMDV replicon lacking L-P1 coding sequences it was again shown that eIF4A and eIF4G were cleaved.
IRES element with activity in plants, insects and mammalian expression systems

General information
State: Published
Organisations: Unknown
Contributors: Roberts, L. O., Belsham, G.
Publication date: 2000

Picornavirus RNA translation: roles for cellular proteins
Picornavirus RNA is translated within cells even when cellular cap-dependent protein synthesis is blocked. The efficiency of recognition of the viral RNA by the translational apparatus can determine viral tropism. The roles of cellular translation-initiation factors and other RNA-binding proteins in viral RNA-mediated protein synthesis are discussed.

General information
State: Published
Organisations: McGill University, Pirbright Institute
Contributors: Belsham, G., Sonenberg, N.
Pages: 330-335
Publication date: 2000
Peer-reviewed: Yes
Replication-competent foot-and-mouth disease virus RNAs lacking capsid coding sequences

RNA transcripts were prepared from plasmids encoding an infectious cDNA of foot-and-mouth disease virus (FMDV) or derivatives in which the leader (Lab and Lb) and capsid protein coding sequences were deleted or replaced by sequences encoding chloramphenicol acetyltransferase (CAT). The transcripts were electroporated into BHK cells and the expression of CAT and the FMDV 3C protease was monitored. Detection of CAT and 3C was dependent on the ability of the transcript to replicate. All of the Lb coding sequence and 94% of P1 (the capsid protein precursor) coding sequence could be deleted without any apparent effect on the ability of the RNA to replicate. Thus, no cis-acting replication element is present within this region of the FMDV genome. Trans-encapsidation of these FMDV replicons was very inefficient, which may
explain the lack of production of defective-interfering particles in FMDV-infected cells.
A selection system for functional internal ribosome entry site (IRES) elements: Analysis of the requirement for a conserved GNRA tetraloop in the encephalomyocarditis virus IRES

Picornavirus internal ribosome entry site (IRES) elements direct cap-independent internal initiation of protein synthesis within mammalian cells. These RNA elements (about 450 nt) contain extensive secondary structure including a hairpin loop with a conserved GNRA motif. Such loops are important in RNA-RNA and RNA-protein interactions. Plasmids that express dicistronic mRNAs of the structure GUS/IRES/HOOK have been constructed. The HOOK sequence encodes a cell-surface-targeted protein (sFv); the translation of this open reading frame within mammalian cells from these dicistronic mRNAs requires a functional IRES element. Cells that express the sFv can be selected from nonexpressing cells. A pool of up to 256 mutant encephalomyocarditis virus IRES elements was generated by converting the wild-type hairpin loop sequence (GCGA) to NNNN. Following transfection of this pool of mutants into COS-7 cells, plasmids were recovered from selected sFv-expressing cells. These DNAs were amplified in Escherichia coli and transfected again into COS-7 cells for further cycles to enrich for plasmids encoding functional IRES elements. The sequence of individual selected IRES elements was determined. All functional IRES elements had a tetraloop with a 3' terminal A residue. Optimal IRES activity, assayed in vitro and within cells, was obtained from plasmids encoding an IRES with the hairpin loop sequence fitting a RNRA consensus. In contrast, IRES elements containing YCYA tetraloops were severely defective.
Localization of Foot-and-Mouth Disease Virus RNA by in situ hybridization within bovine tissues

Foot-and-mouth disease is a highly contagious disease of cloven hooved animals. In cattle, both acute and long-term persistent infections occur. Foot-and-mouth disease virus (FMDV), a picornavirus, has been shown, using virus isolation procedures, to replicate in the pharynx and soft palate of cattle. In this study, in situ hybridization has been used to detect FMDV RNA within the cells of tissues removed from infected bovines. A digoxigenin-labelled anti-sense RNA probe was prepared corresponding to a region of the FMDV genome encoding part of the RNA-dependent RNA polymerase (3D). The efficacy and specificity of this probe for in situ hybridisation was determined using virus-infected cells in tissue culture. Strong cytoplasmic staining was only detected in FMDV-infected cells. Various tissue samples were collected from FMDV-infected cattle between 5 and 17 days post-infection. Viral RNA was detected by in situ hybridisation within cells of the soft palate, tonsil and pharynx up to 17 days post-infection. This technique is useful for the study of FMDV localization in cattle both during and after the acute clinical phase of disease and may assist in identifying specific sites of virus persistence.

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General information
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Organisations: Pirbright Institute
Contributors: Murphy, M. L. P., Forsyth, M. A., Belsham, G., Salt, J. S.
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Publication date: 1999
Peer-reviewed: Yes

Publication information
Journal: Virus Research
Volume: 62
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BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.58 SJR 1.147 SNIP 0.901
Web of Science (2017): Impact factor 2.484
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.55 SJR 1.208 SNIP 0.917
Web of Science (2016): Impact factor 2.628
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 2.56 SJR 1.253 SNIP 0.9
Web of Science (2015): Impact factor 2.526
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 2.63 SJR 1.213 SNIP 0.926
Web of Science (2014): Impact factor 2.324
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 2.94 SJR 1.3 SNIP 1.105
Web of Science (2013): Impact factor 2.827
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 2.9 SJR 1.21 SNIP 1.05
Web of Science (2012): Impact factor 2.745
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 3.04 SJR 1.271 SNIP 1.216
Web of Science (2011): Impact factor 2.941
Differentiating infection from vaccination in foot-and-mouth disease using a panel of recombinant, non-structural proteins in ELISA

A profiling ELISA was developed to detect antibody to the non-structural (NS) proteins Lb, 2C, 3A, 3D, and the polyprotein 3ABC, of foot-and-mouth disease virus (FMDV). The assay was used to examine panels of serum from naive cattle, and from experimentally infected or vaccinated animals. All sera from cattle experimentally infected with any of the seven serotypes of FMDV were positive for antibody to 2C, 3A, 3D and 3ABC, and the majority were positive for Lb. The three categories of sera could be differentiated on the basis of the presence or absence of antibody, to the structural and/or NS proteins of FMDV. The assay is simple, rapid and reproducible and can be used to identify prevaccination infection in animals which are seropositive for antibody to the structural proteins of the virus. Validating the assay with field sera demonstrated that antibody to 3ABC, and usually one or more of the other non-structural proteins, was detected only in animals reported to have shown clinical signs of FMD. Vaccinated cattle which had received less than five vaccinations, were frequently positive for antibody to 30 but were negative for antibody to 3ABC. Occasional animals which had received more than ten vaccinations had NS protein antibody profiles which were similar to those seen following infection. (C) 1998 Elsevier Science Ltd. All rights reserved.
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 3.19 SJR 1.863 SNIP 1.124
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.33 SJR 1.985 SNIP 1.142
Web of Science (2016): Impact factor 3.235
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.45 SJR 2.073 SNIP 1.248
Web of Science (2015): Impact factor 3.413
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.57 SJR 2.105 SNIP 1.218
Web of Science (2014): Impact factor 3.624
Web of Science (2014): Indexed yes
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Scopus rating (2013): CiteScore 3.43 SJR 1.752 SNIP 1.115
Web of Science (2013): Impact factor 3.485
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 3.56 SJR 1.656 SNIP 1.154
Web of Science (2012): Impact factor 3.492
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 3.86 SJR 1.744 SNIP 1.269
Web of Science (2011): Impact factor 3.766
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.663 SNIP 1.21
Web of Science (2010): Impact factor 3.572
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.453 SNIP 1.21
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.355 SNIP 1.027
Scopus rating (2007): SJR 1.299 SNIP 1.114
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.328 SNIP 1.167
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 1.219 SNIP 1.068
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 1.17 SNIP 1.172
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 1.153 SNIP 1.125
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 1.277 SNIP 0.997
Recognition of picornavirus internal ribosome entry sites within cells; influence of cellular and viral proteins

The ability of different picornavirus internal ribosome entry site (IRES) elements to direct initiation of protein synthesis has been assayed in different cell lines in the presence and absence of viral proteases that inhibit cap-dependent protein synthesis. Reporter plasmids that express dicistronic mRNAs, containing different IRES elements, with the general structure CAT/IRES/LUC, have been assayed. In each plasmid, the CAT sequence encodes chloramphenicol acetyl transferase and the LUC sequence encodes luciferase. The poliovirus (PV) 2A protease and the foot-and-mouth disease virus (FMDV) Lb protease induce the cleavage of the translation initiation factor elF4G and hence inhibit the activity of the cap-binding complex, elF4F. In human osteosarcoma (HTK-143) cells, each of the various IRES elements functioned efficiently. In these cells, the co-expression of the viral proteases severely inhibited the expression of CAT, but the proteases had little effect on the activities of the various IRES elements. In contrast, in baby hamster kidney (BHK) cells, the efficiencies of the different IRES elements varied significantly, whereas, in normal rat kidney (NRK) cells, each of the IRES elements was relatively inefficient. In both BHK and NRK cells, the activities of those IRES elements that functioned inefficiently were strongly stimulated by the co-expression of the PV 2A or FMDV Lb proteases. This stimulation was independent of the loss of cap-dependent protein synthesis and was not achieved by the co-expression of the C-terminal fragment of elF4G. The results suggest that the PV 2A and FMDV Lb proteases induce the cleavage of another cellular protein, in addition to elF4G, which influences IRES function.

General information
State: Published
Organisations: Pirbright Institute
Contributors: Roberts, L. O., Seamons, R. A., Belsham, G.
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Publication date: 1998
Peer-reviewed: Yes

Publication information
Journal: R N A
Volume: 4
Issue number: 5
ISSN (Print): 1355-8382
Ratings:
BFI (2019): BFI-level 2
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 3.66 SJR 3.219 SNIP 0.94
Web of Science (2017): Impact factor 4.49
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 3.69 SJR 3.658 SNIP 1.032
Web of Science (2016): Impact factor 4.605
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 4.49 SJR 4.489 SNIP 1.053
Web of Science (2015): Impact factor 4.344
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 4.68 SJR 4.428 SNIP 1.106
Web of Science (2014): Impact factor 4.936
Vaccinia virus protein synthesis has a low requirement for the intact translation initiation factor eIF4F, the cap-binding complex within infected cells

The role of the cap-binding complex, eIF4F, in the translation of vaccinia virus mRNAs has been analyzed within infected cells. Plasmid DNAs, which express dicistronic mRNAs containing: a picornavirus internal ribosome entry site, produced within vaccinia virus-infected cells both beta-glucuronidase and a cell surface-targeted single-chain antibody (sFv), Cells expressing sFv were selected from nonexpressing cells, enabling analysis of protein synthesis specifically within the transfected cells. Coexpression of poliovirus 2A or foot-and-mouth disease virus Lb proteases, which cleaved translation initiation factor eIF4G, greatly inhibited cap-dependent protein (beta-glucuronidase) synthesis. Under these conditions, internal ribosome entry site-directed expression of sFv continued and cell selection was maintained. Furthermore, vaccinia virus protein synthesis persisted in the selected cells containing cleaved eIF4G. Thus, late vaccinia virus protein synthesis has a low requirement for the intact cap-binding complex eIF4F. This may be attributed to the short unstructured 5' noncoding regions of the vaccinia virus mRNAs, possibly aided by the presence of poly(A) at both 5' and 3' termini.

General information
State: Published
Organisations: Pirbright Institute
Contributors: Mulder, J., Robertson, M. E. M., Seamons, R. A., Belsham, G.
Analysis of picornavirus internal ribosome entry site function in vivo

Complementation of defective picornavirus internal ribosome entry site (IRES) elements by the coexpression of fragments of the IRES

Mutant forms of the encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES) have been produced and shown to be severely defective in directing internal initiation of protein synthesis within cells using the vaccinia/T7 RNA polymerase system. Mutants in different regions of the IRES were complemented in trans by coexpression of the intact EMCV IRES but not by coexpression of the related IRES elements from Theiler's murine encephalomyelitis virus (another cardiovirus) or from foot-and-mouth disease virus. Distinct, truncated regions of the EMCV IRES, insufficient to direct internal initiation, were also shown to complement defective EMCV IRES elements. It was necessary for the complementing molecule, whether truncated or full length, to be expressed in the positive sense orientation. RT-PCR analysis provided no support for the idea that any recombination event was responsible for the complementation. The data suggest that multiple activities are performed by distinct functional entities within the IRES in the process of internal initiation of protein synthesis. At least some of these different functions may be achieved by different molecules acting in trans. (C) 1997 Academic Press
The La autoantigen contains a dimerization domain that is essential for enhancing translation

The La autoantigen is an RNA-binding protein that is involved in initiation and termination of RNA polymerase III transcription. It also binds several viral RNAs, including those of poliovirus and human immunodeficiency virus (HIV). Binding of the La protein to these RNAs enhances their translation in vitro (K. Meerovitch, Y., V., Svitkin, H., S., Lee, F., Lejbkowicz, D., J., Kenan, E., K. L., Chan, V., I., Agol, J. D., Keene, and N. Sonenberg, J. Virol., 67:3798-3807, 1993, and Y. V. Svitkin, A. Pause, and N. Sonenberg, J. Virol. 68:7001-7007, 1994). Here, a functional domain in the carboxy-terminal half of the La protein that is distinct from the RNA-binding domain is described. Deletion of this domain abrogated the ability of La protein to enhance translation of poliovirus RNA and a hybrid HN trans-activation-response element-chloramphenicol acetyltransferase mRNA. Far-Western assays indicated that the La protein homodimerized in vitro, and the C-terminal deletions that caused a loss of activity in translation also abrogated the dimerization signal. Gel filtration chromatography of recombinant La protein confirmed that La protein exists as a dimer under native conditions. Addition of the purified dimerization domain resulted in a loss of translation stimulatory activity of La protein in cell-free-translation reactions.
Activation of the translational suppressor 4E-BP1 following infection with encephalomyocarditis virus and poliovirus

Infection of cells with picornaviruses, such as poliovirus and encephalomyocarditis virus (EMCV), causes a shutoff of host protein synthesis. The molecular mechanism of the shutoff has been partly elucidated for poliovirus but not for EMCV. Translation initiation in eukaryotes is facilitated by the mRNA 5' cap structure to which the multisubunit translation initiation factor eIF4F binds to promote ribosome binding. Picornaviruses use a mechanism for the translation of their RNA that is independent of the cap structure. Poliovirus infection engenders the cleavage of the eIF4G (formerly p220) component of eIF4F and renders this complex inactive for cap-dependent translation. In contrast, EMCV infection does not result in eIF4G cleavage. Here, we report that both EMCV and poliovirus activate a translational repressor, 4E-BP1, that inhibits cap-dependent translation by binding to the cap-binding subunit eIF4E. Binding of eIF4E occurs only to the underphosphorylated form of 4E-BP1, and this interaction is highly regulated in cells. We show that 4E-BP1 becomes dephosphorylated upon infection with both EMCV and poliovirus. Dephosphorylation of 4E-BP1 temporally coincides with the shutoff of protein synthesis by EMCV but lags behind the shutoff and eIF4G cleavage in poliovirus-infected cells. Dephosphorylation of 4E-BP1 by specifically inhibiting cap-dependent translation may be the major cause of the shutoff phenomenon in EMCV-infected cells.
RNA-protein interactions in regulation of picornavirus RNA translation
The translation of picornavirus RNA occurs by a cap-independent mechanism directed by a region of about 450 nucleotides from the 5' untranslated region, termed an internal ribosome entry site (IRES). Internal initiation of protein synthesis occurs without any requirement for viral proteins. Furthermore, it is maintained when host cell protein synthesis is almost abolished. By using in vitro translation systems two distinct families of IRES elements which have very different predicted RNA secondary structures have been defined. The cardiovirus and aphthovirus elements function very efficiently in rabbit reticulocyte lysate, whereas the enterovirus and rhinovirus elements function poorly in this system. However, supplementation of this translation system with additional cellular proteins can stimulate translation directed by the enterovirus and rhinovirus RNAs and reduce production of aberrant initiation products. The characterization of cellular proteins interacting with the picornavirus IRES is a major focus of research. Many different protein species can be observed to interact with regions of the IRES by in vitro analyses, e.g., UV cross-linking. However, the function and significance of many of these interactions are not always known. For two proteins, La and the polypyrimidine tract-binding protein, evidence has been obtained for a functional role of their interaction with IRES elements.

A hybrid baculovirus-bacteriophage T7 transient expression system
A hybrid recombinant baculovirus-bacteriophage T7 expression system was developed for transient expression in insect cells of plasmids with foreign genes provided with a T7 promoter. The coding sequence for T7 RNA polymerase, with or without a nuclear localization signal, was inserted into the genome of Autographa californica nuclear polyhedrosis virus. Recombinant viruses stably expressed T7 RNA polymerase in insect cells. Upon transfection of infected insect cells with plasmids containing the genes for chloramphenicol acetyltransferase (CAT), the hepatitis B virus precore-, core- or e-antigens under control of the T7 promoter, transient expression of these genes was detected by ELISA. The results obtained indicate that this baculovirus/T7 system provides a simple and widely applicable tool for transient gene expression studies.
Assembly of foot-and-mouth disease virus empty capsids synthesized by a vaccinia virus expression system
cDNA cassettes encoding the foot-and-mouth disease virus (FMDV) structural protein precursor (P1-2A) together with the
3C protease, which cleaves this molecule to 1AB, 1C and 1D, were constructed. These cassettes were introduced into
vaccinia virus (VV) transfer vectors. Attempts to isolate recombinant VVs constitutively expressing these cassettes were
unsuccessful. However, when the P1-2A-3C cassette was placed under the control of the bacteriophage T7 promoter,
stable VV/FMDV recombinants were isolated. Co-infection with recombinant VV vTF7-3 (which expresses T7 RNA
polymerase) led to the production of correctly processed FMDV capsid proteins. Analysis by sucrose gradient
centrifugation showed that material which co-sedimented with natural empty capsid particles (70S) was formed. Electron
microscopy revealed empty capsid-like particles with diameters of about 30 nm. Studies using monoclonal antibodies
specific for conformational epitopes indicated that the antigenicity of the synthetic particles was similar to whole virions
and natural empty capsid particles. Surprisingly, merely the modification of a single amino acid residue within the
myristoylation consensus sequence at the N terminus of P1-2A allowed the isolation of a recombinant VV which
constitutively expressed the correctly processed proteins. However, the capsid proteins expressed from this mutant
cassette failed to assemble into 70S empty particles.

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Contributors: Abrams, C. C., King, A. M. Q., Belsham, G.
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Scopus rating (2016): CiteScore 2.93 SJR 1.544 SNIP 0.891
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BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.26 SJR 1.738 SNIP 0.998
Web of Science (2015): Impact factor 3.192
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BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.25 SJR 1.69 SNIP 1.057
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Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 3.64 SJR 1.764 SNIP 1.154
Web of Science (2013): Impact factor 3.529
ISI indexed (2013): ISI indexed yes
Defective point mutants of the encephalomyocarditis virus internal ribosome entry site can be complemented in trans

Point mutations were introduced at random into cDNA corresponding to nucleotides 260-833 of the encephalomyocarditis virus (EMCV) 5' noncoding region. This region contains the internal ribosome entry site (IRES). The mutations were identified by sequence analysis and the effect on the activity of the IRES was determined using in vitro translation reactions in rabbit reticulocyte lysate. Significantly defective mutants each contained multiple point mutations. These mutants were constructed into a dicistronic mRNA expression plasmid and the activities of the mutant IRES elements were determined using the vaccinia virus/T7 RNA polymerase transient expression system in vivo. The most severely defective of these mutants displayed about 5% of wild-type activity. The activities, relative to wild type, of these mutant IRES elements determined using in vitro and in vivo assays were similar. Two deletion mutants, lacking sequences from the 5' terminus to nt 411 and 484, were also constructed. Each of these deletions inactivated the IRES in vivo (to less than 1% of wild-type activity). Coexpression within cells of the wild-type EMCV IRES, either alone or linked to another coding sequence, enhanced the activity of each of the defective IRES elements except that deleted to nt 484. The results are
consistent with a model in which different regions of the IRES participate in a discontinuous transfer of an initiation complex to the 3' end of the IRES element for initiation of protein synthesis to occur. (C) 1995 academic Press, Inc.
Identification of critical amino acids within the foot-and-mouth disease virus Leader protein, a cysteine protease
The Leader protein of foot-and-mouth disease virus (FM DV) is the first component of the virus polyprotein. It is synthesized in two forms, Lab and Lb, both of which display the ability to cleave the L/P1 junction in trans and to induce the cleavage of the cap-binding complex component eIF-4G (p220). The L protease has weak homology to the family of cysteine proteases, which have a catalytic dyad composed of a cysteine and a histidine. Mutations have been introduced into FMDV cDNA to modify each of the four cysteine residues and the three conserved histidine residues within the Lb species. The activities of the mutant L proteins have been determined. Modification of a single cysteine residue (residue 51) or of a single histidine residue (residue 148) abolished the abilities of L to cleave the L/P1 junction and to inhibit cap-dependent protein synthesis. In contrast, modification of each of the other cysteine residues and other conserved histidine residues had no apparent effect on these activities. (C) 1995 Academic Press, Inc.
Role of La in Internal Initiation

General information
State: Published
Organisations: McGill University
Contributors: Belsham, G., Sonenberg, N., Svitkin, Y.
Pages: 85-98
Strong buffering capacity of insect cells. Implications for the baculovirus expression system

Insect cells are widely used for expression of a variety of different proteins by using the baculovirus expression system. The applicability of this system depends on production of proteins which have biological properties similar to their native counterparts. One application has been the expression of viral capsid proteins and their assembly into empty capsid structures to provide new viral immunogens which retain complex antigenic sites. An important parameter for efficient folding and assembly of proteins into viral procapsids may be the intracellular pH, particularly for acid-labile particles such as foot-and-mouth disease virus (FMDV). Benzoic acid was used as an effective indicator of intracellular pH in insect cells and 3-O-methyl glucose to measure cell volumes. We have determined the intracellular volume of the Spodoptera frugiperda IPLB-Sf21 insect cells 0.50 +/- 0.08 pL per cell. Using the distribution of [C-14]-benzoic acid, we show that the intracellular pH remains constant at pH 7.0 when the cells are grown in media with pH values ranging from 6.2 to 6.8 and, moreover, is not affected by baculovirus infection. These results suggest that insect cells are suitable to express and produce acid-labile structures via the baculovirus expression system and that assembly of proteins and viral procapsids could occur.
Viral RNA modulates the acid sensitivity of foot-and-mouth disease virus capsids

Foot-and-mouth disease virus (FMDV) manifests an extreme sensitivity to acid, which is thought to be important for entry of the RNA genome into the cell. We have compared the low-pH-induced disassembly in vitro of virions and natural empty capsids of three subtypes of serotype A FMDV by enzyme-linked immunosorbent assay and sucrose gradient sedimentation analysis. For all three subtypes (A22 Iraq 24/64, A10(61), and A24 Cruzeiro), the empty capsid was more stable by 0.5 pH unit on average than the corresponding virion. Unexpectedly, in the natural empty capsids used in this study, the precursor capsid protein VP0 was found largely to be cleaved into VP2 and VP4. For picornaviruses the processing of VP0 is closely associated with encapsidation of viral RNA, which is considered likely to play a catalytic role in the cleavage. Investigation of the cleavage of WO in natural empty capsids failed to implicate the viral RNA. However, it remains possible that these particles arise from abortive attempts to encapsidate RNA. Empty capsids expressed from a vaccinia virus recombinant showed essentially the same acid lability as natural empty capsids, despite differing considerably in the extent of VP0 processing, with the synthetic particles containing almost exclusively uncleaved VP0. These results indicate that it is the viral RNA that modulates acid lability in FMDV. In all cases the capsids dissociate at low pH directly into pentameric subunits. Comparison of the three viruses indicates that FMDV A22 Iraq is about 0.5 pH unit more sensitive to low pH than types A10(61) and A24 Cruzeiro. Sequence analysis of the three subtypes identified several differences at the interface between pentamers and highlighted a His-cu-helix dipole interaction which spans the pentamer interface and appears likely to influence the acid lability of the virus.
Insulin dependent stimulation of protein synthesis by phosphorylation of a regulator of 5’-cap function

The cloning is described of two related human complementary DNAs encoding polypeptides that interact specifically with the translation initiation factor elf-4E, which binds to the messenger RNA 5’-cap structure. Interaction of these proteins with elf-4E inhibits translation but treatment of cells with insulin causes one of them to become hyperphosphorylated and dissociate from elf-4E, thereby relieving the translational inhibition. The action of this new regulator of protein synthesis is therefore modulated by insulin, which acts to stimulate the overall rate of translation and promote cell growth.

General information
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Organisations: McGill University, Washington University St. Louis, The Pirbright Institute
Contributors: Pause, A., Belsham, G., Gingras, A., Donze, O., Lin, T., Lawrence, J. J. C., Sonenberg, N.
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Scopus rating (2016): CiteScore 13.33
Web of Science (2016): Impact factor 19.304
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 14.38
Web of Science (2015): Impact factor 17.184
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
PHAS-1 as a link between mitogen-activated protein kinase and translation initiation

PHAS-1 is a heat-stable protein (relative molecular mass approximate to 12,400) found in many tissues. It is rapidly phosphorylated in rat adipocytes incubated with insulin or growth factors. Nonphosphorylated PHAS-1 bound to initiation factor 4E (eIF-4E) and inhibited protein synthesis. Serine-64 in PHAS-1 was rapidly phosphorylated by mitogen-activated (MAP) kinase, the major insulin-stimulated PHAS-1 kinase in adipocyte extracts. Results obtained with antibodies, immobilized PHAS-1, and a messenger RNA cap affinity resin indicated that PHAS-1 did not bind eIF-4E when serine-64 was phosphorylated. Thus, PHAS-1 may be a key mediator of the stimulation of protein synthesis by the diverse group of agents and stimuli that activate MAP kinase.
TARGET-SPECIFIC ARREST OF MESSANGER-RNA TRANSLATION BY ANTISENSE 2'-O-ALKYLOLIGORIBONUCLEOTIDES

We describe a novel experimental approach to investigate mRNA translation. Antisense 2'-O-allyl oligoribonucleotides (oligos) efficiently arrest translation of targeted mRNAs in rabbit reticulocyte lysate and wheat germ extract while displaying minimal non-specific effects on translation. Oligo/mRNA-hybrids positioned anywhere within the 5' UTR or the first similar to 20 nucleotides of the open reading frame block cap-dependent translation initiation with high specificity. The thermodynamic stability of hybrids between 2'-O-alkyl oligos and RNA permits translational inhibition with oligos as short as 10 nucleotides. This inhibition is independent of RNase H cleavage or modifications which render the mRNA untranslatable. We show that 2'-O-alkyl oligos can also be employed to interfere with cap-independent internal initiation of translation and to arrest translation elongation. The latter is accomplished by UV-crosslinking of psoralen-tagged 2'-O-methyloligoribonucleotides to the mRNA within the open reading frame. The utility of 2'-O-alkyloligoribonucleotides to arrest translation from defined positions within an mRNA provides new approaches to investigate mRNA translation.

General information
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Organisations: The Pirbright Institute
Contributors: Johansson, H. E., Belsham, G., Sproat, B. S., Hentze, M. W.
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BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 10.84 SJR 9.025 SNIP 3.028
Web of Science (2017): Impact factor 11.561
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 9.28 SJR 7.883 SNIP 2.744
Web of Science (2016): Impact factor 10.162
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 9.48 SJR 7.358 SNIP 2.631
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 8.74 SJR 6.64 SNIP 2.552
Web of Science (2014): Impact factor 9.112
Web of Science (2014): Indexed yes
TRANS COMPLEMENTATION BY RNA OF DEFECTIVE FOOT-AND-MOUTH-DISEASE VIRUS INTERNAL RIBOSOME ENTRY SITE ELEMENTS

A region of about 435 bases from the 5' noncoding region of foot-and-mouth disease virus RNA directs internal initiation of protein synthesis. This region, termed the internal ribosome entry site (IRES), is predicted to contain extensive secondary structure. Precise deletion of five predicted secondary structure features has been performed. The mutant IRES elements have been constructed into vectors which express bicistronic mRNAs and assayed within cells. Each of the modified IRES elements was defective in directing internal initiation when assayed alone. However, coexpression of an intact foot-and-mouth disease virus IRES complemented four of these defective elements to an efficiency of up to 80% of wild-type activity. No complementation was observed with the structurally analogous element from encephalomyocarditis virus. The role of RNA-RNA interactions in the function of the picornavirus IRES is discussed.
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Contributors: Drew, J., Belsham, G.
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BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 4.24 SJR 2.853 SNIP 1.096
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Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.42 SJR 3.114 SNIP 1.124
Web of Science (2016): Impact factor 4.663
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Scopus rating (2015): CiteScore 4.42 SJR 3.282 SNIP 1.132
Web of Science (2015): Impact factor 4.606
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 4.4 SJR 3.187 SNIP 1.208
Web of Science (2014): Impact factor 4.439
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 4.92 SJR 3.496 SNIP 1.251
Web of Science (2013): Impact factor 4.648
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): CiteScore 5.2 SJR 3.19 SNIP 1.222
Web of Science (2012): Impact factor 5.076
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): CiteScore 5.37 SJR 3.429 SNIP 1.282
Web of Science (2011): Impact factor 5.402
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 3.596 SNIP 1.277
Web of Science (2010): Impact factor 5.189
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 3.631 SNIP 1.306
Web of Science (2009): Indexed yes
Translation of encephalomyocarditis virus RNA: parameters influencing the selection of the internal initiation site
The initiation of encephalomyocarditis virus translation is by internal ribosome entry exclusively at the 11th AUG codon from the 5'-end, which is the central of the three AUG codons in the sequence...ACGAUGAUAAUAUGGCCACAACCAUG... and is located some 25 nt downstream from an oligopyrimidine tract conserved amongst related viruses. As the sequences between the oligopyrimidine tract and AUG-10/11 are poorly conserved and thus possibly serve only as a spacer, the influence of this spacer length on initiation frequency at the three AUG codons was examined in vitro and in vivo. Deletion of 11 residues resulted in initiation almost exclusively at AUG-12 but at significantly reduced overall efficiency. Insertion of eight residues caused a 15-fold increase in initiation frequency at AUG-10 and a decrease at AUG-11. Longer insertions reduced overall efficiency without changing the initiation site preferences. With the wild-type spacing, complete substitution of the oligopyrimidine tract by purines caused a 30-35% decrease in initiation efficiency, and partial substitution only a 10-15% decrease. Thus the internal initiation mechanism selects the initiation site partly on the basis of its distance from upstream elements, of which the oligopyrimidine tract is not the most critical, but for reasons not yet understood a preference for AUG-11 is superimposed on this selection.

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Contributors: Kaminski, A., Belsham, G., Jackson, R. J.
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BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 7.53 SJR 7.058 SNIP 1.622
Unprocessed foot-and-mouth disease virus capsid precursor displays discontinuous epitopes involved in viral neutralization
A foot-and-mouth disease virus (FMDV) cDNA cassette containing sequences encoding the capsid precursor P1, peptide 2A and a truncated 2B (abbreviated P1-2A) of type C FMDV, has been modified to generate the authentic amino terminus and the myristoylation signal. This construct has been used to produce a recombinant baculovirus (AcMM53) which, upon infection of Spodoptera frugiperda insect cells, expressed a recombinant P1-2A precursor with a high yield. This polyprotein reacted with neutralizing monoclonal antibodies (MAbs) that bind to continuous epitopes of the major antigenic site A (also termed site 1) of capsid protein VP1. Unexpectedly, it also reacted with neutralizing MAbs which define complex, discontinuous epitopes previously identified on FMDV particles. The reactivity of MAbs with P1-2A was quantitatively similar to their reactivity with intact virus and, in both cases, the reactivity with MAbs that recognized discontinuous epitopes was lost upon heat denaturation of the antigen. The finding that a capsid precursor may fold in such a way as to maintain discontinuous epitopes involved in virus neutralization present on the virion surface opens the possibility of using unprocessed capsid precursors as novel antiviral immunogens.

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Scopus rating (2017): CiteScore 4.24 SJR 2.853 SNIP 1.096
Web of Science (2017): Impact factor 4.368
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.42 SJR 3.114 SNIP 1.124
Web of Science (2016): Impact factor 4.663
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 4.42 SJR 3.282 SNIP 1.132
Web of Science (2015): Impact factor 4.606
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 4.4 SJR 3.187 SNIP 1.208
Web of Science (2014): Impact factor 4.439
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 4.92 SJR 3.496 SNIP 1.251
Web of Science (2013): Impact factor 4.648
ISI indexed (2013): ISI indexed yes
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BFI (2012): BFI-level 2
Scopus rating (2012): CiteScore 5.2 SJR 3.19 SNIP 1.222
Web of Science (2012): Impact factor 5.076
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): CiteScore 5.37 SJR 3.429 SNIP 1.282
A single nucleotide substitution in the internal ribosome entry site of foot-and-mouth-disease virus leads to enhanced cap-independent translation in vivo

Mutants of foot-and-mouth disease virus (FMDV) with altered biological properties can be selected during the course of persistent infection of BHK-21 cells with FMDV C-S8c1 (J. C. de la Torre, E. Martinez-Salas, J. Diez, A. Villaverde, F. Gebauer, E. Rocha, M. Davila, and E. Domingo, J. Virol. 62:2050-2058, 1988). Two nucleotide substitutions, U to C at position -376 and A to G at position -15, (counting as +1 the A of the first functional AUG), were fixed within the internal ribosome entry site (IRES) of R100, the virus rescued after 100 passages of the carrier BHK-21 cells. IRES-directed cap-independent protein synthesis was quantitated by using bicistronic constructs of the form chloramphenicol acetyltransferase gene-IRES-luciferase gene. The IRES from R100 was 1.5- to 5-fold more active than that of C-S8c1 in directing cap-independent luciferase synthesis. This enhanced translational activity was observed when the RNAs were transcribed either in the nucleus or in the cytoplasm by a weak or a strong promoter, respectively. C-S8c1 and R100 IRES elements were functional in both FMDV-sensitive and FMDV-resistant cells (including persistently infected R cells), indicating that factors mediating cap-independent protein synthesis are not limited in any of the analyzed cell lines. Constructs in which each of the two mutations in the R100 IRES were analyzed separately indicate that the transition at position -376 is responsible for the enhanced activity of the R100 IRES. By estimating the effect that an increase in the initial translation efficiency may have on subsequent RNA replication steps, we suggest that the modifications in the IRES elements can account for the previously described hypervirulence of FMDV R100 for BHK-21 cells. The results show that a single point mutation in an IRES element of a picornavirus can cause an increase in translation efficiency.

General information
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Organisations: Universidad Autónoma de Madrid, The Pirbright Institute
Contributors: Martinez-Salas, E., Saiz, J., Davila, M., Belsham, G., Domingo, E.
Distinctive features of foot-and-mouth disease virus, a member of the picornavirus family; aspects of virus protein synthesis, protein processing and structure

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Contributors: Belsham, G.
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Scopus rating (2017): CiteScore 3.23 SJR 1.446 SNIP 1.195
Web of Science (2017): Impact factor 3.427
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.7 SJR 1.341 SNIP 0.877
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BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 2.49 SJR 1.095 SNIP 0.93
Web of Science (2015): Impact factor 2.581
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The 2 species of the foot-and-mouth-disease virus leader protein, expressed individually, exhibit the same activities

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Publication information
Journal: Virology
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BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
TRANSCOMPLEMENTATION OF CAP-INDEPENDENT TRANSLATION DIRECTED BY POLIOVIRUS 5' NONCODING REGION DELETION MUTANTS - EVIDENCE FOR RNA-RNA INTERACTIONS

Poliovirus (PV) RNA is translated by a cap-independent mechanism involving the internal entry of ribosomes onto the 5' noncoding region (NCR). Using the vaccinia virus-T7 RNA polymerase transient expression system, we showed previously that deletion of certain individual predicted secondary structures within the PV 5' NCR rendered the element defective in directing internal initiation when assayed alone. However, these defective 5' NCRs were functional when coexpressed within cells with full-length PV cDNA (N. Percy, G. J. Belsham, J. K. Brangwyn, M. Sullivan, D. M. Stone, and J. W. Almond, J. Virol. 66:1695-1701, 1992). We have extended the study to demonstrate that when these predicted secondary structures are deleted in combination, the enhanced activity in the presence of the full-length PV cDNA is still observed. Indeed, a poliovirus 5' NCR devoid of all predicted secondary structures is capable of initiating protein synthesis under these conditions. Surprisingly, we also found that this enhancement of activity requires neither any PV protein nor the inhibition of cap-dependent translation. The results indicate that the defective PV 5' NCR elements can be complemented in trans by functional 5' NCRs in a highly sequence specific manner.

General information
State: Published
Organisations: University of Reading, Pirbright Institute
Contributors: Stone, D. M., Almond, J. W., Brangwyn, J. K., Belsham, G.
Pages: 6215-6223
Publication date: 1993
Peer-reviewed: Yes

Publication information
Journal: Journal of Virology
Volume: 67
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Ratings:
BFI (2019): BFI-level 2
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 4.24 SJR 2.853 SNIP 1.096
Web of Science (2017): Impact factor 4.368
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.42 SJR 3.114 SNIP 1.124
Web of Science (2016): Impact factor 4.663
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 4.42 SJR 3.282 SNIP 1.132
Web of Science (2015): Impact factor 4.606
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 4.4 SJR 3.187 SNIP 1.208
Web of Science (2014): Impact factor 4.439
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 4.92 SJR 3.496 SNIP 1.251
Web of Science (2013): Impact factor 4.648
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): CiteScore 5.2 SJR 3.19 SNIP 1.222
Dual initiation sites of protein-synthesis on foot-and-mouth-disease virus-RNA are selected following internal entry and scanning of ribosomes invivo

The initiation of protein synthesis on foot-and-mouth disease virus RNA occurs at two sites separated by 84 nucleotides. Immediately upstream from the first of these sites is the internal ribosome entry site (IRES), which directs the translation of this RNA to be cap-independent. The utilization of these two initiation sites has been examined using artificial fusion genes in vivo under a variety of conditions. Additional in-frame AUG codons have been introduced between these two authentic start sites to determine the mechanism by which ribosomes recognize the second start site. The results indicate that following internal entry of ribosomes on the 5' side of the first initiation codon, many fail to initiate protein synthesis at this position and scan along the RNA to the second initiation site. In the presence or absence of the IRES both initiation sites are efficiently used but the utilization of the two sites is slightly biased towards the second initiation site by the IRES. Furthermore, in the presence of the IRES, protein synthesis initiates at both sites independently of the activity of the cap-binding complex.

General information
State: Published
Organisations: Pirbright Institute
Contributors: Belsham, G.
Intracellular modifications induced by poliovirus reduce the requirement for structural motifs in the 5' noncoding region of the genome involved in internal initiation of protein-synthesis

A series of genetic deletions based partly on two RNA secondary structure models (M. A. Skinner, V. R. Racaniello, G. Dunn, J. Cooper, P. D. Minor, and J. W. Almond, J. Mol. Biol. 207:379-392, 1989; E. V. Pilipenko, V. M. Blinov, L. I. Romanova, A. N. Sinjakov, S. V. Maslova, and V. I. Agol, Virology 168:201-209, 1989) was made in the cDNA encoding the 5' noncoding region (5' NCR) of the poliovirus genome in order to study the sequences that direct the internal entry of ribosomes. The modified cDNAs were placed between two open reading frames in a single transcriptional unit and used to transfect cells in culture. Internal entry of ribosomes was detected by measuring translation from the second open reading frame in the bicistronic mRNA. When assayed alone, a large proportion of the poliovirus 5' NCR superstructure including several well-defined stem-loops was required for ribosome entry and efficient translation. However, in cells cotransfected with a complete infectious poliovirus cDNA, the requirement for the stem-loops in this large superstructure was reduced. The results suggest that virus infection modifies the cellular translational machinery, so that shortened forms of the 5' NCR are sufficient for cap-independent translation, and that the internal entry of ribosomes occurs by two distinct modes during the virus replication cycle.

General information
State: Published
Organisations: University of Reading, Pirbright Institute
Pages: 1695-1701
Publication date: 1992
Peer-reviewed: Yes

Publication information
Journal: Journal of Virology
Volume: 66
Issue number: 3
ISSN (Print): 0022-538X
Ratings:
BFI (2019): BFI-level 2
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 4.24 SJR 2.853 SNIP 1.096
Web of Science (2017): Impact factor 4.368
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.42 SJR 3.114 SNIP 1.124
Web of Science (2016): Impact factor 4.663
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 4.42 SJR 3.282 SNIP 1.132
Web of Science (2015): Impact factor 4.606
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Sequence of genome segment-9 of bluetongue virus (serotype-1, South-Africa) and expression analysis demonstrating that different forms of VP6 are derived from initiation of protein-synthesis at 2 distinct sites

Bluetongue virus (BTV) VP6 is often resolved into two closely migrating bands by SDS-PAGE (VP6 and VP6a). RNA segment 9 of BTV-serotype 1 South Africa (encoding VP6) has been cloned as cDNA, and the complete sequence has been determined. Expression of this clone both in vitro and in tissue culture produced the same polypeptide doublet as
seen previously in extracts from BTV-infected cells. Modification of the cDNA, including the removal of the first initiation
codon, demonstrated that the two forms of VP6 are derived from initiation of protein synthesis at two distinct sites and not
by post-translational modification.

General information
State: Published
Organisations: The Pirbright Institute
Contributors: Wade-Evans, A. M., Mertens, P. P. C., Belsham, G.
Pages: 3023-3026
Publication date: 1992
Peer-reviewed: Yes

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BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.68 SJR 1.325 SNIP 0.877
Web of Science (2017): Impact factor 2.514
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.93 SJR 1.544 SNIP 0.891
Web of Science (2016): Impact factor 2.838
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.26 SJR 1.738 SNIP 0.998
Web of Science (2015): Impact factor 3.192
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.25 SJR 1.69 SNIP 1.057
Web of Science (2014): Impact factor 3.183
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 3.64 SJR 1.764 SNIP 1.154
Web of Science (2013): Impact factor 3.529
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 3.28 SJR 1.525 SNIP 1.034
Web of Science (2012): Impact factor 3.127
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 3.6 SJR 1.684 SNIP 1.145
Web of Science (2011): Impact factor 3.363
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.678 SNIP 1.053
Web of Science (2010): Impact factor 3.568
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Myristoylation of foot-and-mouth-disease virus capsid protein precursors is independent of other viral-proteins and occurs in both mammalian and insect cells

The myristoylation of the foot-and-mouth disease virus (FMDV) capsid precursor P1-2A and its amino-terminal cleavage product 1AB, expressed from subgenomic cDNA, has been analysed. The modification reaction is independent of other FMDV proteins and occurs in both mammalian and insect cells. Blocking of the myristoylation site does not prevent efficient processing of the FMDV capsid precursor. A cDNA cassette in which the leader protease sequence is substituted by an ATG codon produces myristoylated 1AB, indicating correct removal of the novel N-terminal methionine residue.

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State: Published
Organisations: Pirbright Institute, Agricultural University
Pages: 747-751
Publication date: 1991
Peer-reviewed: Yes

Publication information
Journal: Journal of General Virology
Volume: 72
Issue number: Part 3
ISSN (Print): 0022-1317
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.68 SJR 1.325 SNIP 0.877
Web of Science (2017): Impact factor 2.514
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The mechanism of translation of cowpea mosaic-virus middle component RNA - no evidence for internal initiation from experiments in an animal-cell transient expression system

The possibility that internal initiation of translation is responsible for the synthesis of the middle component (M) RNA-encoded 95K protein of cowpea mosaic virus (CPMV) has been investigated by constructing plasmids in which the entire sequence of CPMV M RNA was cloned downstream of a chloramphenicol acetyltransferase gene. Expression of these plasmids in an animal cell expression system revealed that no synthesis of the proteins encoded by the downstream CPMV open reading frame takes place from RNA derived from these constructs under conditions where the internal ribosome entry site of foot-and-mouth disease virus is functional. The results indicate that internal initiation is not responsible for the synthesis of the 95K protein in this system.
The molecular biology of the morbilliviruses

General information
State: Published
Organisations: Pirbright Institute
Contributors: Barrett, T., Subbarao, S. M., Belsham, G., Mahy, B. W. J.
Number of pages: 618
Pages: 83-102
Publication date: 1991

Host publication information
Title of host publication: The paramyxoviruses
Place of publication: New York
Publisher: Plenum Publishing Corporation
Editor: Kingsbury, D. W.
A region of the 5' noncoding region of foot-and-mouth-disease virus-RNA directs efficient internal initiation of protein-synthesis within cells - the role of L-protease in translational control

Plasmids encoding bicistronic mRNAs have been constructed and used to identify a region from the 5' noncoding region of foot-and-mouth disease virus (FMDV) which directs efficient internal initiation of protein synthesis within cells. The loss of about 30 nucleotides (nt) from the 5' terminus or about 50 nt from the 3' terminus of the 435-nt region completely abolished the activity of this region. The expression of the FMDV L protease severely inhibited the expression of other genes unless they were preceded by this element. The regulation of protein synthesis mediated by FMDV is discussed.

General information
State: Published
Organisations: The Pirbright Institute
Contributors: Belsham, G., Brangwyn, J. K.
Pages: 5389-5395
Publication date: 1990
Peer-reviewed: Yes

Publication information
Journal: Journal of Virology
Volume: 64
Issue number: 11
ISSN (Print): 0022-538X
Ratings:
BFI (2019): BFI-level 2
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 4.24 SJR 2.853 SNIP 1.096
Web of Science (2017): Impact factor 4.368
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.42 SJR 3.114 SNIP 1.124
Web of Science (2016): Impact factor 4.663
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 4.42 SJR 3.282 SNIP 1.132
Web of Science (2015): Impact factor 4.606
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 4.4 SJR 3.187 SNIP 1.208
Web of Science (2014): Impact factor 4.439
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 4.92 SJR 3.496 SNIP 1.251
Web of Science (2013): Impact factor 4.648
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): CiteScore 5.2 SJR 3.19 SNIP 1.222
Web of Science (2012): Impact factor 5.076
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
EXPRESSION OF CAULIFLOWER MOSAIC-VIRUS GENE-1 USING A BACULOVIRUS VECTOR BASED UPON THE P10 GENE AND A NOVEL SELECTION METHOD

A new baculovirus expression vector based upon the p10 gene of Autographa californica nuclear polyhedrosis virus (AcNPV) and a novel system for the screening of p10 recombinants have been developed. The insertion of a cassette containing the lacZ gene under the control of a heat-shock promoter of Drosophila melanogaster downstream from the cloning site in p10 transfer vectors allows the convenient identification of putative recombinants by virtue of their expression of β-galactosidase. Using this p10 transfer vector an AcNPV recombinant was engineered with a cDNA copy of gene I of cauliflower mosaic virus (CaMV) in place of the p10 coding sequence. This pi 0 recombinant expressed CaMV gene I protein at levels equivalent to those of p10 and polyhedrin, and was shown to be as effective in producing this protein as recombinants exploiting the polyhedrin promoter. CaMV gene I protein formed large numbers of hollow fiber-like structures in the cytoplasm of infected cells. Because the polyhedrin gene remains intact, these p10 expression vectors may be exploited for the expression of heterologous proteins in insects infected per os and for the enhancement of baculovirus pathogenicity for insect control.

General information
State: Published
Organisations: Wageningen University & Research, Agricultural and Food Research Council, Pirbright Institute
Pages: 312-320
Publication date: 1990
Peer-reviewed: Yes
Expression of cauliflower mosaic-virus gene-I in insect cells using a novel polyhedrin-based baculovirus expression vector

An improved polyhedrin-based baculovirus expression vector was constructed to expedite distinguishing infections by putative baculovirus recombinants from infections by wild-type (wt) baculovirus. The vector utilizes the Escherichia coli β-galactosidase gene (lacZ) as a genetic marker for positive recombination between wt Autographa californica nuclear polyhedrosis virus and the baculovirus transfer vector. The marker gene/expression cassette was constructed so that lacZ and the deleted polyhedrin gene were transcribed in opposite orientations, both terminating in a simian virus 40 DNA fragment which acts as a bidirectional terminator. In the constructed vector, lacZ is transcribed from the Drosophila melanogaster heat-shock promoter (hsp70), which is constitutively expressed in baculovirus-infected Spodoptera frugiperda (Sf) cells, thereby making the site of the deleted polyhedrin gene available for the insertion and expression of foreign genes under the control of the polyhedrin promoter. Recombinant baculoviruses are readily selected in plaque assays by the development of a blue colour upon the addition of X-Gal. The colour selection renders the retrieval of recombinants less dependent on a high frequency of recombination between the transfer vector and wt baculovirus DNA. The usefulness of this new vector was illustrated by expressing gene I of cauliflower mosaic virus, which encodes a protein of Mr 46000. Expression of gene I was at the same level as in cells infected with a conventional polyhedrin-based expression vector. Gene I protein formed large hollow fibre-like structures in the cytoplasm of infected Sf cells. This is the first plant virus protein to be expressed in insect cells by a recombinant baculovirus.
Intracellular expression and processing of foot-and-mouth-disease virus capsid precursors using vaccinia virus vectors - Influence of the L protease
cDNA cassettes of FMDV have been constructed which encode the capsid precursor (P1-2A) alone or with the proteases L and 3C which are required for processing of this precursor to the products 1 AB, 1 C, and 1 D. These cassettes have been analyzed using in vitro transcription and translation reactions and within cells using recombinant vaccinia viruses. Processing of the precursors occurred more efficiently in cells than in cell-free systems but similar properties were observed. It was not possible to isolate recombinant vaccinia viruses containing FMDV cassettes which included the intact coding sequence for the L protein. Deletion of part of the L sequence, which abolished its proteolytic activity, also abolished this incompatibility with vaccinia virus. The vaccinia recombinant, vTF7-3, which expresses the bacteriophage T7 RNA polymerase was used in transient expression studies using plasmids containing a T7 promoter upstream of the FMDV cassettes. Under these conditions it was possible to coexpress L, P1-2A, and 3C in the vaccinia-infected cells; each of the proteolytic activities was observed and correctly processed 1AB, 1C, and 1D were produced.
Sequence analysis of monoclonal antibody resistant mutants of type O foot-and-mouth-disease virus - evidence for the involvement of the 3 surface exposed capsid proteins in 4 antigenic sites

Sequence analysis of monoclonal antibody resistant mutants of type O foot and mouth disease virus has been performed. Distinct clusters of amino acid substitutions conferring resistance to neutralization at each of the four previously defined antigenic sites (McCahon et al., 1989, J. Gen. Virol. 70, 639–645) have been identified. One site corresponds to the well-known 140–160 region of VP1, a second site is also on VP1, one site is on VP2, and the fourth site is on VP3. All of the amino acid substitutions identified are located on the surface of the virus. Despite the differences in three-dimensional structure between FMDV and other picornaviruses the neutralizing antigenic sites occur in analogous positions on the capsid surface.

General information
State: Published
Organisations: The Pirbright Institute
Contributors: Kitson, J. D. A., McCahon, D., Belsham, G.
Pages: 26-34
Publication date: 1990
Peer-reviewed: Yes

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Journal: Virology
Volume: 179
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ISSN (Print): 0042-6822
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BFI (2019): BFI-level 1
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BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 3.14 SJR 1.728 SNIP 0.93
Web of Science (2017): Impact factor 3.374
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.47 SJR 1.937 SNIP 0.955
Web of Science (2016): Impact factor 3.353
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.2 SJR 1.796 SNIP 0.899
Web of Science (2015): Impact factor 3.2
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.14 SJR 1.705 SNIP 0.915
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 3.37 SJR 1.78 SNIP 0.948
Web of Science (2013): Impact factor 3.278
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 3.57 SJR 1.768 SNIP 0.976
Web of Science (2012): Impact factor 3.367
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 3.32 SJR 1.686 SNIP 0.929
Web of Science (2011): Impact factor 3.351
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.73 SNIP 0.856
Web of Science (2010): Impact factor 3.305
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.772 SNIP 0.907
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 2 SNIP 0.964
Scopus rating (2007): SJR 1.893 SNIP 0.987
Scopus rating (2006): SJR 1.827 SNIP 0.889
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 1.684 SNIP 0.903
Scopus rating (2004): SJR 1.613 SNIP 0.967
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 1.706 SNIP 0.97
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 1.543 SNIP 0.891
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 1.751 SNIP 0.902
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 2.103 SNIP 0.986
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 1.957 SNIP 0.99
Original language: English
DOIs:
10.1016/0042-6822(90)90269-W
Source: orbit
Source-ID: 317076
Research output: Research - peer-review › Journal article – Annual report year: 1990
Synthesis of foot-and-mouth-disease virus capsid proteins in insect cells using baculovirus expression vectors

Foot-and-mouth disease virus (FMDV) cDNA cassettes containing sequences encoding the capsid precursor P1-2A with and without those encoding the proteases L and 3C were introduced into Autographa californica nuclear polyhedrosis virus (AcMNPV) expression vectors. Procapsid proteins 1AB, 1C and 1D were produced in cells infected with recombinant baculoviruses, when L and 3C were present in the constructs, indicating that these FMDV proteases were active in insect cells. Unlike P1 processing in poliovirus, which has been shown to be catalysed mainly by the 3CD gene product, the 3C protease of FMDV was able to process P1 independently of 3D. Cytotoxicity of the L protease for insect cells prevented the use of the optimized transfer vector, pAcRP23, for inserting L-containing cassettes into AcMNPV. By contrast, viable AcMNPV-FMDV recombinants could be made without restriction on choice of the transfer vector when the L gene was either not expressed or inactivated by an inframe deletion. In the latter case, normal cleavage at the L-P1 junction no longer occurred in cis, and a new processing event, probably catalysed by 3C, was observed within the C-terminal region of the residual L protein. Analysis of baculovirus-expressed products in sucrose gradients showed that a fraction of the capsid proteins is present in an aggregated form, migrating at 70S and possibly resembling FMDV empty capsid particles.

General information
State: Published
Organisations: Wageningen IMARES, The Pirbright Institute
Contributors: Roosien, J., Belsham, G., Ryan, M., King, A. M. Q., Vlak, J.
Pages: 1703-1711
Publication date: 1990
Peer-reviewed: Yes

Publication information
Journal: Journal of General Virology
Volume: 71
Issue number: Part 8
ISSN (Print): 0022-1317
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.68 SJR 1.325 SNIP 0.877
Web of Science (2017): Impact factor 2.514
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.93 SJR 1.544 SNIP 0.891
Web of Science (2016): Impact factor 2.838
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.26 SJR 1.738 SNIP 0.998
Web of Science (2015): Impact factor 3.192
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.25 SJR 1.69 SNIP 1.057
Web of Science (2014): Impact factor 3.183
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 3.64 SJR 1.764 SNIP 1.154
Web of Science (2013): Impact factor 3.529
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 3.28 SJR 1.525 SNIP 1.034
Web of Science (2012): Impact factor 3.127
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 3.6 SJR 1.684 SNIP 1.145
The role of the 5′-nontranslated region of the fusion protein messenger-RNAs of canine-distemper virus and rinderpest virus

The mRNAs which code for the fusion proteins of the morbilliviruses (measles virus, canine distemper virus, and rinderpest virus) have unusually long 5′ untranslated regions (UTRs) which are GC-rich and are capable of folding into extensive secondary structures. In measles virus the first AUG codons in the fusion (F) protein mRNA are in close proximity at nucleotide positions 574 and 583 and protein translation is initiated at the second position. In the canine distemper virus (CDV) and rinderpest virus (RPV) F gene transcripts the analogous initiation codons are preceded by several other AUG codons many nucleotides upstream either in the same reading frame or at the beginning of other short open reading frames. We have studied the effect of deleting these upstream regions on the production of the fusion proteins of both CDV and RPV from cDNA constructs. Within the cells the presence of these regions enhances the production of the F protein while, in contrast, the production of the authentic F protein from in vitro translations using RNA transcripts is inhibited by these sequences.

General information
State: Published
Organisations: The Pirbright Institute
Contributors: Evans, S. A., Belsham, G., Barrett, T.
Pages: 317-323
Publication date: 1990
Peer-reviewed: Yes
Evidence for at least 4 antigenic sites on type-O foot-and-mouth-disease virus involved in virus neutralization - identification by single and multiple site monoclonal antibody-resistant mutants

Neutralizing monoclonal antibodies raised against type O foot-and-mouth disease virus have been characterized on the basis of their reactivity with a panel of single site monoclonal antibody-resistant mutants which had defined three antigenic sites. Five antibodies neutralized all these mutants, but by selecting further single site mutants with one of these antibodies it was possible to define a fourth site involved in virus neutralization. Two monoclonal antibodies still neutralized these mutants and all multiple site resistant mutants. One multiple site resistant mutant was resistant to neutralization at each of four antigenic sites but was still efficiently neutralized by type O convalescent cattle sera. The relationship between sites recognized by different monoclonal antibodies generated in different laboratories is discussed.

General information
State: Published
Organisations: The Pirbright Institute, Laboratoire Roger Bellon, State Veterinary Institute for Virus Research, Central Veterinary Institute, United States Department of Agriculture, Instituto Zooprofilattico Sperimentale delle Venezie
Pages: 639-645
Publication date: 1989
Peer-reviewed: Yes

Publication information
Journal: Journal of General Virology
Volume: 70
Issue number: Part 3
ISSN (Print): 0022-1317
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.68 SJR 1.325 SNIP 0.877
Web of Science (2017): Impact factor 2.514
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.93 SJR 1.544 SNIP 0.891
Web of Science (2016): Impact factor 2.838
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.26 SJR 1.738 SNIP 0.998
Web of Science (2015): Impact factor 3.192
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.25 SJR 1.69 SNIP 1.057
Web of Science (2014): Impact factor 3.183
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 3.64 SJR 1.764 SNIP 1.154
Immune-response and protection of cattle and pigs generated by a vaccinia virus recombinant expressing the F-protein of rinderpest virus

General information
State: Published
Organisations: The Pirbright Institute
Pages: 655-658
Immunization with a vaccinia recombinant expressing the F-protein protects rabbits from challenge with a lethal dose of rinderpest virus

A cDNA clone containing the complete coding sequence of the rinderpest fusion protein (F) gene was inserted into the thymidine kinase gene of vaccinia virus (WR strain) under the control of the 7.5K early/late vaccinia virus promoter. All forms of the F protein, i.e., the glycosylated Fo precursor, the unglycosylated F1 protein, and the glycosylated F2 protein, were detected in cells infected with the recombinant virus. Vaccination of rabbits with the recombinant virus induced antibodies which reacted in an ELISA system specific for rinderpest. The rabbit sera contained neutralizing antibodies against rinderpest virus and precipitated the F protein from lysates of rinderpest infected cells. Rabbits vaccinated with the recombinant rinderpest F gene vaccinia virus were protected from a lethal challenge with the lapinized Nakamura 3 strain of rinderpest virus. Variations in the severity of clinical symptoms correlated with the level of anti-F protein antibodies produced.

General information
State: Published
Organisations: The Pirbright Institute
Contributors: Barret, T., Belsham, G., Subbarao, S. M., Evans, S. A.
Pages: 11-18
Publication date: 1989
Peer-reviewed: Yes

Publication information
Journal: Virology
Volume: 170
Issue number: 1
ISSN (Print): 0042-6822
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 3.14 SJR 1.728 SNIP 0.93
Web of Science (2017): Impact factor 3.374
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.47 SJR 1.937 SNIP 0.955
Web of Science (2016): Impact factor 3.353
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Specificity of enzyme substrate interactions in foot-and-mouth-disease virus polyprotein processing

A series of transcripts derived from FMDV cDNA plasmids containing defined regions of the genome were translated in a rabbit reticulocyte lysate system. The products were analysed directly or following incubation with an FMDV-infected cell processing extract. Processing by the L proteinase at the U1 A cleavage site occurred when most of the P1–2A protein was absent. Substitution of sequences upstream of the 2C/3A cleavage site showed that the 3C proteinase was also able to cleave at an entirely novel cleavage site, apparently at K-I amino acid pairs. Cleavage at the 2A/2B site was not only independent of L and 3C proteinases, but was shown to occur when 2A and as few as four 2B N-terminal amino acids were present. Thus, the disparate proteolytic activities responsible for all three primary processing events that give rise to the products L, P1–2A, 2BC, and P3 were highly resistant either to major deletion or substitution of protein sequences.
adjacent to, or at, the site of cleavage. By contrast, secondary processing in trans was sensitive to changes at remote sites. For example, removal of the C-terminal regions of P1–2A and 2BC precursors impaired their ability to act as substrates for 3C proteinase activity. Processing of P1–2A, particularly of the 1 D/2A cleavage site, was enhanced by inclusion of sequences from the 3D region of the genome.

General information
State: Published
Organisations: The Pirbright Institute
Contributors: Ryan, M., Belsham, G., King, A. M. Q.
Pages: 35-45
Publication date: 1989
Peer-reviewed: Yes

Publication information
Journal: Virology
Volume: 173
Issue number: 1
ISSN (Print): 0042-6822
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 3.14 SJR 1.728 SNIP 0.93
Web of Science (2017): Impact factor 3.374
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.47 SJR 1.937 SNIP 0.955
Web of Science (2016): Impact factor 3.353
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.2 SJR 1.796 SNIP 0.899
Web of Science (2015): Impact factor 3.2
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.14 SJR 1.705 SNIP 0.915
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 3.37 SJR 1.78 SNIP 0.948
Web of Science (2013): Impact factor 3.278
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 3.57 SJR 1.768 SNIP 0.976
Web of Science (2012): Impact factor 3.367
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 3.32 SJR 1.686 SNIP 0.929
Web of Science (2011): Impact factor 3.351
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.73 SNIP 0.856
Web of Science (2010): Impact factor 3.305
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.772 SNIP 0.907
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 2 SNIP 0.964
Vaccines '89 Modern Approaches to New Vaccines including Prevention of AIDS

General information
State: Published
Organisations: Unknown
Contributors: Belsham, G., Ryan, M., Brangwyn, J. K.
Number of pages: 556
Pages: 445-448
Publication date: 1989

Host publication information
Title of host publication: Vaccines '89 Modern Approaches to New Vaccines including Prevention of AIDS
Publisher: Cold Spring Harbor Laboratory Press
Editors: Lerner, R. A., Ginsberg, H., Chanock, R. M., Brown, F.
ISBN (Print): 08-79-69323-1
Source: orbit
Source-ID: 317085
Research output: Research - peer-review > Book chapter – Annual report year: 1989

Studies on the infectivity of foot-and-mouth-disease virus-RNA using microinjection

General information
State: Published
Organisations: The Pirbright Institute
Contributors: Belsham, G., Bostock, C. J.
Pages: 265-274
Publication date: 1988
Peer-reviewed: Yes

Publication information
Journal: Journal of General Virology
Volume: 69
Issue number: Part 2
ISSN (Print): 0022-1317
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.68 SJR 1.325 SNIP 0.877
Web of Science (2017): Impact factor 2.514
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.93 SJR 1.544 SNIP 0.891
Web of Science (2016): Impact factor 2.838
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.26 SJR 1.738 SNIP 0.998
Web of Science (2015): Impact factor 3.192
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.25 SJR 1.69 SNIP 1.057
Web of Science (2014): Impact factor 3.183
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 3.64 SJR 1.764 SNIP 1.154
Web of Science (2013): Impact factor 3.529
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 3.28 SJR 1.525 SNIP 1.034
Web of Science (2012): Impact factor 3.127
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 3.6 SJR 1.684 SNIP 1.145
Web of Science (2011): Impact factor 3.363
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.678 SNIP 1.053
Web of Science (2010): Impact factor 3.568
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.662 SNIP 1.127
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.648 SNIP 1.068
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 1.593 SNIP 1.131
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.709 SNIP 1.128
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 1.654 SNIP 1.137
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 1.55 SNIP 1.215
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 1.58 SNIP 1.145
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 1.369 SNIP 1.083
Web of Science (2002): Indexed yes
Neutralization of foot-and-mouth-disease virus can be mediated through any of at least 3 separate antigenic sites
Expression of polyoma virus middle-T-antigen in Saccharomyces-cerevisiae

The polyoma middle-T gene, lacking its intron, was inserted into a yeast expression plasmid containing the phosphoglycerate kinase promoter. Such plasmids transformed yeast at low frequency and these transformants expressed middle-T antigen at a level of approximately 0.1% cell protein. Furthermore, expression of this protein was frequently lost during growth in liquid culture and this loss of middle-T was accompanied by a twofold increase in the rate of growth. The spontaneous production of a truncated middle-T antigen, lacking the C terminus, was also observed; the expression of this protein did not inhibit the growth rate of the cells. Recovery and analysis of the expression plasmids encoding the truncated molecule showed that a single C · G base pair had been deleted from a run of nine consecutive C · G base pairs (Pyr nucleotide 1239–1247) within the middle-T coding region. This frame-shift mutation results in premature termination of the protein and loss of the strongly hydrophobic region of the molecule believed to be responsible for the membrane association of middle-T antigen.
The expression and properties of polyoma-virus middle-T antigen in simian cells

SV40 late replacement vectors containing the polyoma middle-T coding sequences have been constructed. Mixed hybrid virus stocks have been obtained through complementation with a defective SV40 helper genome (dl 1055) following DNA transfection into CV-1 cells. Middle-T antigen is expressed in the infected simian cells at about 5–10 fold higher levels than in polyoma virus-infected mouse cells and has the pp60c-sec-associated tyrosine-specific protein kinase activity in vitro. However, the 'specific activity' of the kinase in extracts of the infected CV-1 cells is lower than that observed in polyoma infected 3T6 cell extracts. The half-life of middle-T antigen in the CV-1 cells is about 4 h but the in vitro kinase activity associated with middle-T has a half-life of at least 8 h and hence appears to be stabilized. The in vivo phosphorylated species of middle-T has been shown by sucrose gradient analyses to be largely distinct from the middle-T with associated protein kinase activity in vitro.
An antibody to a synthetic peptide recognizes polyomavirus middle-T antigen and reveals multiple in vitro tyrosine phosphorylation sites

Antibodies were raised against three synthetic peptides corresponding to sequences surrounding tyrosine 315, a putative in vitro phosphorylation site in polyomavirus middle-T antigen. Only one of the peptides (called C and corresponding to residues 311 to 330) elicited antibodies that recognized middle-T efficiently. Middle-T present in immunoprecipitates formed with purified anti-C serum still accepted phosphate on tyrosine in an in vitro kinase reaction. This implies that tyrosines other than 315 and 322 that lie within the antibody binding region are phosphorylated under these conditions. This conclusion was supported by the altered partial V8 proteolysis fingerprint of the labeled middle-T. Two-dimensional tryptic fingerprint analysis of 32P-labeled middle-T showed that several tryptic peptides identified as including tyrosine 315 and 322 were missing from middle-T labeled in anti-C immunoprecipitates compared with middle-T labeled in immunoprecipitates made by using anti-tumor cell serum. However, one major labeled peptide remained. This peptide was also present in fingerprints of 32P-labeled middle-T coded by M45, dl23, pAS131, and dl1013, but a peptide with altered mobility was present in dl8 middle-T. This identified the peptide as including tyrosine 250. We deduce from these data that (i) the presence of the antibody against peptide C inhibits phosphorylation of tyrosines 315 and 322; (ii) middle-T labeled in the kinase reaction after immunoprecipitation with anti-C serum is phosphorylated on tyrosine 250; and (iii) when anti-tumor cell serum is used in the in vitro kinase reaction, middle-T is phosphorylated at multiple sites, including residues 250, 315, and 322.
Protein-kinases and insulin action in fat-cells

General information
State: Published
Organisations: University of Bristol
Contributors: Denton, R. M., Brownsey, R. W., Hopkirk, T. J., Belsham, G.
Pages: 768-771
Publication date: 1984
Peer-reviewed: Yes

Publication information
Journal: Biochemical Society. Transactions
Volume: 12
Issue number: 5
ISSN (Print): 0300-5127
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
Biochemical properties of the 145,000-dalton Super-T antigen from Simian-Virus 40-transformed BALB/c 3T3 Clone 20 cells

General information
Reversibility of the insulin-stimulated phosphorylation of ATP citrate lyase and a cytoplasmic protein of subunit Mr 22 000 in adipose-tissue

General information
State: Published
Organisations: University of Bristol
Contributors: Belsham, G., Brownsey, R. W., Denton, R. M.
Pages: 345-352
Publication date: 1982
Peer-reviewed: Yes

Publication information
Journal: Biochemical Journal
Volume: 204
Issue number: 1
ISSN (Print): 0264-6021
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 3.62 SJR 2.224 SNIP 0.953
Web of Science (2017): Impact factor 3.857
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.63 SJR 2.402 SNIP 0.981
Web of Science (2016): Impact factor 3.797
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.85 SJR 2.572 SNIP 1.12
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
A partial view of the mechanism of insulin action

General information
State: Published
Organisations: University of Bristol
Contributors: Denton, R. M., Brownsey, R. W., Belshaw, G.
Pages: 347-362
Publication date: 1981
Peer-reviewed: Yes

Publication information
Journal: Diabetologia
Volume: 21
Issue number: 4
ISSN (Print): 0012-186X
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 5.09 SJR 3.228 SNIP 1.619
Web of Science (2017): Impact factor 6.023
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 5.23 SJR 3.25 SNIP 1.721
Web of Science (2016): Impact factor 6.08
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 5.57 SJR 3.61 SNIP 1.933
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 5.57 SJR 3.243 SNIP 1.964
Web of Science (2014): Impact factor 6.671
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 6 SJR 3.259 SNIP 2.035
Web of Science (2013): Impact factor 6.88
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 5.76 SJR 3.235 SNIP 1.914
Web of Science (2012): Impact factor 6.487
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): CiteScore 5.47 SJR 3.177 SNIP 1.857
Web of Science (2011): Impact factor 6.814
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 3.345 SNIP 1.847
Web of Science (2010): Impact factor 6.973
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 2.985 SNIP 1.644
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 3.268 SNIP 1.845
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 2.8 SNIP 1.609
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 2.677 SNIP 1.459
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 2.332 SNIP 1.58
Scopus rating (2004): SJR 2.492 SNIP 1.883
Scopus rating (2003): SJR 1.977 SNIP 1.814
Scopus rating (2002): SJR 1.948 SNIP 1.76
Evidence for phosphorylation and activation of acetyl CoA carboxylase by a membrane-associated cyclic AMP-independent protein kinase – relationship to the activation of acetyl CoA carboxylase by insulin

General information
State: Published
Organisations: University of Bristol
Contributors: Brownsey, R. W., Belsham, G., Denton, R. M.
Pages: 145-150
Publication date: 1981
Peer-reviewed: Yes

Publication information
Journal: F E B S Letters
Volume: 124
Issue number: 2
ISSN (Print): 0014-5793
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 3.43 SJR 1.991 SNIP 0.916
Web of Science (2017): Impact factor 2.999
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.48 SJR 1.967 SNIP 0.89
Web of Science (2016): Impact factor 3.623
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.49 SJR 2.022 SNIP 0.923
Web of Science (2015): Impact factor 3.519
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.19 SJR 1.859 SNIP 0.87
Web of Science (2014): Impact factor 3.169
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 3.71 SJR 2.356 SNIP 0.982
Web of Science (2013): Impact factor 3.341
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 3.67 SJR 2.291 SNIP 0.913
Web of Science (2012): Impact factor 3.582
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
The role of fat cell plasma membrane proteins in the mechanism of insulin action

General information
State: Published
Organisations: Unknown
Contributors: Belsham, G.
Publication date: 1981

Publication information
Original language: English
Source: orbit
Source-ID: 317099

Anti-insulin receptor antibodies mimic the effects of insulin on the activities of pyruvate-dehydrogenase and acetylCoA carboxylase and on specific protein-phosphorylation in rat epididymal fat-cells
Autoantibodies against insulin [I] receptors found in certain patients with severe I resistance stimulate glucose transport and metabolism in fat cell and muscle preparations. Preincubation of rat epididymal adipose tissue with 1:1000 dilution of one such serum apparently results in a 2-fold increase in the initial activities of pyruvate dehydrogenase and acetylCoA carboxylase. These increases are similar to the maximum effects of I. Incubation of isolated fat cells with the serum at the same concentration also resulted in the increased phosphorylation of 3 intracellular proteins with subunit MW of 130,000,
35,000 and 22,000 to the same extent as observed with I. The short term effects of I apparently do not involve the entry of the I molecule (or part thereof) into cells of target tissues.

General information
State: Published
Organisations: University of Bristol
Contributors: Belsham, G., Brownsey, R. W., Hughes, W. A., Denton, R. M.
Pages: 307-312
Publication date: 1980
Peer-reviewed: Yes

Publication information
Journal: Diabetologia
Volume: 18
Issue number: 4
ISSN (Print): 0012-186X
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 5.09 SJR 3.228 SNIP 1.619
Web of Science (2017): Impact factor 6.023
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 5.23 SJR 3.25 SNIP 1.721
Web of Science (2016): Impact factor 6.08
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 5.57 SJR 3.61 SNIP 1.933
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 5.57 SJR 3.243 SNIP 1.964
Web of Science (2014): Impact factor 6.671
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 6 SJR 3.259 SNIP 2.035
Web of Science (2013): Impact factor 6.88
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 5.76 SJR 3.235 SNIP 1.914
Web of Science (2012): Impact factor 6.487
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): CiteScore 5.47 SJR 3.177 SNIP 1.857
Web of Science (2011): Impact factor 6.814
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 3.345 SNIP 1.847
Web of Science (2010): Impact factor 6.973
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 2.985 SNIP 1.644
BFI (2008): BFI-level 2
The effects of insulin and adrenaline on the phosphorylation of a 22 000 Mr protein within isolated fat cells; possible identification as the 'inhibitor-1 of the general phosphatase'

General information
State: Published
Organisations: Unknown
Contributors: Belsham, G., Denton, R. M.
Pages: 382-383
Publication date: 1980
Peer-reviewed: Yes

Publication information
Journal: Biochemical Society. Transactions
Volume: 8
ISSN (Print): 0300-5127
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 3.23 SJR 1.776 SNIP 0.696
Web of Science (2017): Impact factor 3.394
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.99 SJR 1.865 SNIP 0.629
Web of Science (2016): Impact factor 2.765
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.04 SJR 1.976 SNIP 0.713
Web of Science (2015): Impact factor 2.679
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.23 SJR 2.09 SNIP 0.779
Web of Science (2014): Impact factor 3.194
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 3.23 SJR 2.022 SNIP 0.763
Use of a novel rapid preparation of fat-cell plasma-membranes employing Percoll to investigate the effects of insulin and adrenaline on membrane-protein phosphorylation within intact fat-cells

General information
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Projects:

Porcine coronavirus - pathogenesis and control
Lazov, C. M., PhD Student, National Veterinary Institute
Bøtner, A., Main Supervisor, National Veterinary Institute
Belsham, G., Supervisor, National Veterinary Institute
Rasmussen, T. B., Supervisor, National Veterinary Institute
Institut stipendie (DTU)
01/09/2016 → 13/06/2020
Award relations: Porcine coronavirus - pathogenesis and control
Project: PhD

FMDV capsid assembly
Kristensen, T., PhD Student, National Veterinary Institute
Belsham, G., Main Supervisor, National Veterinary Institute
Rasmussen, T. B., Supervisor, National Veterinary Institute
Jungersen, G., Examiner, National Veterinary Institute
Scheel, T., Examiner
Stonehouse, N. J., Examiner
Institut stipendie (DTU)
01/10/2015 → 30/09/2018
Award relations: FMDV capsid assembly
Project: PhD

Host range selection, virulence determinants and pathogenesis of influenza A viruses: Towards the identification of new antiviral drugs and vaccines
Andersen, M. R., PhD Student, National Veterinary Institute
Larsen, L. E., Main Supervisor, National Veterinary Institute
Kvisgaard, L. K., Supervisor, National Veterinary Institute
Belsham, G., Examiner, National Veterinary Institute
Pedersen, F. S., Examiner
Zohari, S., Examiner
Institut stipendie (DTU)
01/08/2015 → 31/12/2018
Award relations: Host range selection, virulence determinants and pathogenesis of influenza A viruses: Towards the identification of new antiviral drugs and vaccines
Project: PhD

Investigation og the genetic basis for virus tropism and virulence of classical swine fever virus
Johnston, C. M., PhD Student, National Veterinary Institute
Rasmussen, T. B., Main Supervisor, National Veterinary Institute
Belsham, G., Supervisor, National Veterinary Institute
Pedersen, A. G., Supervisor, Department of Health Technology
Heegaard, P. M. H., Examiner, National Veterinary Institute
Bukh, J., Examiner
Hulst, M. M., Examiner
Institut stipendie (DTU)
15/08/2015 → 14/08/2018
Award relations: Investigation og the genetic basis for virus tropism and virulence of classical swine fever virus
Project: PhD

Molecular biology of foot-and-mouth disease virus
Kjær, J., PhD Student, National Veterinary Institute
Belsham, G., Main Supervisor, National Veterinary Institute
Rasmussen, T. B., Supervisor, National Veterinary Institute
Larsen, L. E., Examiner, National Veterinary Institute
Bukh, J., Examiner
Ryan, M. D., Examiner
Offentlig finansiering
15/12/2014 → 20/06/2018
Award relations: Molecular biology of foot-and-mouth disease virus
Project: PhD

Animale influenza virus
Fobian, K., PhD Student, National Veterinary Institute
Larsen, L. E., Main Supervisor, National Veterinary Institute
Breum, S. Ø., Supervisor, National Veterinary Institute
Belsham, G., Examiner, National Veterinary Institute
Bragstad, K., Examiner
Harder, T. C., Examiner
Institut stipendie (DTU) Samf.
01/06/2010 → 05/11/2014
Award relations: Animale influenza virus
Project: PhD

Identification of the determinants of efficient pestivirus replication
Risager, P. C., PhD Student, National Veterinary Institute
Belsham, G., Main Supervisor, National Veterinary Institute
Rasmussen, T. B., Supervisor, National Veterinary Institute
Larsen, L. E., Examiner, National Veterinary Institute
Becher, P., Examiner
Lindberg, M., Examiner
Institut, samfinansiering
01/01/2010 → 18/12/2013
Award relations: Identification of the determinants of efficient pestivirus replication
Project: PhD

Animal Virology, studies on the development of Carrier animals or airborne spread
Stenfeldt, A. C., PhD Student, National Veterinary Institute
Belsham, G., Main Supervisor, National Veterinary Institute
Jungersen, G., Examiner, National Veterinary Institute
De Clercq, K., Examiner
Valarcher, J. P., Examiner
Institut, samfinansiering
01/02/2008 → 21/09/2011
Award relations: Animal Virology, studies on the development of Carrier animals or airborne spread
Project: PhD

Functional analysis of replication determinants in classical swine fever virus
Hadsbjerg, J., PhD Student, National Veterinary Institute
Belsham, G., Main Supervisor, National Veterinary Institute
Rasmussen, T. B., Supervisor, National Veterinary Institute
Larsen, L. E., Examiner, National Veterinary Institute
Becher, P., Examiner
Knudsen, C. R., Examiner
Institut stipendie (DTU)
01/09/2013 → 05/04/2017
Award relations: Functional analysis of replication determinants in classical swine fever virus
Project: PhD

Activities:

Journal of General Virology (Journal)
Graham Belsham (Editor)
National Veterinary Institute
Section for Virology
Description
Guest Editor