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.
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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Foot-and-mouth disease virus capsid assembly

Foot-and-mouth disease (FMD) is caused by a highly contagious virus (FMDV) that infects cloven-hoofed animals, including both domesticated and wildlife. FMD is one of the most important threats to the agricultural industry. Europe is usually free of FMD, however sometimes outbreaks occur and can result in large productivity losses and severe trade restrictions. One example of such an outbreak of FMD occurred in the United Kingdom (UK) in 2001, where the total costs were estimated to around 8 billion pounds. An outbreak of FMD has not occurred in Denmark since 1983. However, introduction of the disease back into Denmark would have huge consequences for the Danish industrialized pig production, and remains a continuing threat.

FMDV belongs to the picornavirus family. The virus particles consist of a capsid surrounding a single-stranded, positive sense genomic RNA of approximately 8,400 nucleotides. The capsids consist of 60 copies of four different structural proteins, termed VP1, VP2, VP3 and VP4. These capsid proteins are generated by cleavage of the capsid precursor P1-2A into VP0, VP3, VP1 and a short 2A peptide by the viral encoded 3C protease (3Cpro). The 2A peptide is not included in the virus particle. During assembly of the capsid proteins, VP0 is cleaved to generate VP4 and VP2 by a process that is currently unknown. The processing of the capsid precursor into the capsid proteins is necessary for virus capsid assembly, and thus essential in the virus life cycle. Blocking the processing of the junctions between the capsid proteins, might be fatal for the virus, and thus might be a possible antiviral target.

The focus of this Ph.D. thesis has been to determine the importance of the amino acid sequences flanking the junctions within the FMDV P1-2A precursor in relation to correct processing by the 3Cpro. Furthermore, studies have also been performed on analyzing a specific motif within VP1, which is important for correct capsid precursor processing.

Manuscript 1: In an earlier study it was found that the VP1 K210E (the P2 position in the VP1/2A junction) in FMDV O1 Manisa, blocked cleavage of this junction. The mutant virus acquired a second site mutation, VP1 E83K. Introduction of this VP1 E83K alone caused the virus to generate a new second-site mutation near the VP1/2A junction, at the 2A L2P (the P2´ position). Interestingly, this substitution also severely inhibited cleavage of this junction. In this manuscript virus adaptations within these two mutant viruses (e.g. FMDV VP1 K210E and FMDV VP1 E83K) were analyzed by next generation sequencing. This identified variants within the FMDV VP1 E83K, where the VP1 K210N was observed. To identify which amino acids could be tolerated at this site for processing of the VP1/2A junction, various alternative amino acids (aa) were introduced at VP1 K210 in the FMDV A22 Iraq. Interestingly, only the presence of a positively charged aa (e.g. lysine or arginine) at this position permitted efficient cleavage of the VP1/2A junction by the 3Cpro, whereas the presence of either an aa with neutral or negative charge severely blocked cleavage of this junction.

Manuscript 2: In this manuscript, the impact of different substitutions near the junctions separating the capsid proteins within the capsid precursor, was investigated. The VP0/VP3- and the VP3/VP1 junctions were modified with different aa substitutions within a construct encoding the P1-2A area. These constructs were expressed in a transient expression assay in either the presence or absence of 3Cpro. The VP2 K217R and the VP3 I2P (2 aa upstream and downstream the VP0/VP3 junction respectively), strongly reduced cleavage of this junction. The VP2 K217E substitution completely prevented cleavage of this junction by the 3Cpro. Both the VP3 Q221P and the VP1 T1P (1 aa upstream and downstream the junction) each severely inhibited cleavage of the VP3/VP1 junction. Interestingly, blocking either of these junctions, did
not prevent processing of any other junction within the capsid precursor. Thus, it seems that there is no strict order of 3Cpro processing of the capsid precursor. The modifications were also introduced into full-length FMDV RNA. However, only the wt and the mutant having the VP2 K217R substitution could be rescued as infectious virus. Higher levels of the VP0-VP3 intermediate were observed within cells infected with the mutant virus. The VP0-VP3 intermediate was not incorporated in either empty capsids or virus particles. Thus, processing of this junction seems necessary for capsid assembly.

Manuscript 3: This manuscript identified a short motif required for correct FMDV capsid precursor processing. Various modifications were introduced into the VP1-coding region of FMDV P1-2A cDNA. Truncations removing the 2A peptide and 27 aa of the C-terminus of VP1, resulted in severe blocking of the processing of both the VP0/VP3- and VP3/VP1 junctions within the capsid precursor. Introducing small deletions identified the region VP1 185-190, as being necessary for correct processing by the 3Cpro. Five of these aa (YCPRP) are highly conserved among all seven serotypes of FMDV. Furthermore, these residues are also conserved in many other picornaviruses, including poliovirus. Single aa substitutions in the motif revealed that VP1 Y185A and VP1 R188A individually impeded cleavage of both the VP0/VP3- and the VP3/VP1 junctions, which are more than 400 aa and around 200 aa away from the site of modification. Interestingly, this conserved motif can also explain earlier observations on the poliovirus capsid precursor, which is highly resistant to cleavage following truncation (which removed this motif). The results suggest that the identified motif, is important for correct folding of picornavirus capsid precursors and their subsequent processing.
Space-Time mapping of terahertz-induced electron field emission

We present simulations and experiments showing how THz induced electron field emission from gold antennas can be mapped indirectly in space and time. Our simulations predict the spatiotemporal electron distribution after single-cycle THz-induced field emission. Two different experiments validate this through a time-accumulated, spatial electron mapping due to both short- and long-time irradiation with THz transients.