Conserved elements within the genome of foot-and mouth disease virus; their influence on virus replication

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Title: **Conserved elements within the genome of foot-and mouth disease virus; their influence on virus replication**

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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.