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**Title:** Endocytosis of Foot-and-mouth Disease Virus  
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### **Project Abstract**

Infection by foot-and-mouth disease virus (FMDV) is “acid-activated” and relies upon the low pH within endosomes. Field strains of the virus use integrin molecules as receptors for their delivery to acidic, early-endosomes via clathrin-mediated endocytosis. Conversely, cell-culture adapted FMDV can initiate infection using heparan sulphate proteoglycans (HSPG) as alternative receptors to the integrin. The ability of the virus to switch between integrin and HSPG receptors has been linked to long-term, virus persistence in the animal host. HSPG are not internalised by the clathrin pathway; instead they are taken up by a number of lipid-raft mediated processes, such as the caveolae or flotillin pathways. These pathways normally deliver ‘cargo’ to pH neutral endosomes where FMDV uncoating and infection would not be expected to result. However, HSPG-mediated virus uptake clearly results in infection. It has been recognised recently that “acid-activated” viruses can be delivered to acidic compartments via alternative ‘non-clathrin’ routes. This is achieved by a process known as endocytic-pathway merging. These observations suggest that HS-binding FMDV could use a novel, non-clathrin uptake-mechanism that results in virus delivery to acidic endosomes. Recently, we have discovered an FMDV that lacks a functional integrin binding site and can infect HS-negative cells. The entry mechanism used by this virus is also unknown. This project will investigate the cell-entry mechanism used for infection by HS-binding, and by the novel FMDV described above. Specifically, we will identify the key regulatory molecules and vesicles that are required for virus uptake to acidic endosomes. This work will provide new insight into FMDV cell-entry and possibly important information of the mechanism on virus persistence and/or cellular endocytosis.

### **Full description of project**

Research on foot-and-mouth disease (FMD) has a long history as it was the first animal disease for which a viral aetiology was demonstrated more than 100 years ago. Nevertheless, the threats posed by the disease have not been removed due to the difficulties in controlling the virus (FMDV) as it exists as multiple non-cross protective serotypes, is highly contagious and has the ability to infect a wide range of domesticated and wild species of ruminants which can remain persistently infected for long periods. The disease has been largely eradicated in developed countries but remains endemic in many regions of the world where it results in significant loss of trade, and is a major barrier to economic and social prosperity.

FMDV is a non-enveloped virus consisting of a protein capsid, surrounding a genome of one linear, positive-strand of RNA (vRNA). The outer capsid surface is formed by 3 structural proteins, VP1-3. A fourth structural protein, VP4 is located inside the capsid where it likely interacts with the vRNA. FMDV relies upon acid-mediated uncoating, to release of the vRNA which is then transferred across the endosomal membrane to the cell cytosol, where virus replication occurs.

FMDV field strains use cell-surface molecules (known as integrins) for cell attachment. Attachment is mediated by an arginine-glycine-aspartic acid (RGD) motif, which is located at the apex of a conformationally flexible loop of VP1 (the VP1 GH-loop). Several integrins are known to bind the RGD motif and we have identified three (avb1, avb6 and avb8) as receptor for FMDV (1). A fourth RGD-binding integrin, avb3 has also been reported by others as an FMDV receptor. Using avb6 as our model, we have shown that the virus is taken up by clathrin-mediated endocytosis (CME) (2) and delivered to acidic, early-endosomes. Further, we have recently identified early endosomes as the site of infection (i.e. virus uncoating and vRNA membrane penetration) using expression of dominant-negative (DN) versions of rab proteins, which block specific steps of intracellular vesicle trafficking through the clathrin pathway (3).

Several other clathrin-independent endocytosis pathways operate within mammalian cells which deliver cargos to distinct intracellular locations. One such pathway is the caveolae-mediated uptake which is dependent on caveolin, lipid-rafts and cholesterol. Cargos that are internalised by caveolae are delivered to neutral pH endosomes called caveosomes. A number of viruses use this pathway for entry and infection and much of our knowledge of caveolae comes from studies with simian virus-40. A number of non-caveolae uptake pathways also originate from lipid-rafts, and novel clathrin- and raft-independent processes have also been recently identified. These pathways have different requirements for cellular proteins such as dynamin, flotillin, small GTPases, and for cholesterol and other specific lipids, and can also be exploited by viruses for infection.

Most acid-activated viruses (e.g. FMDV field strains) are taken up by 'classical' CME and delivered to acidic early- or late-endosomes where membrane penetration is triggered. However, it has recently been recognised that "acid-activated" viruses can also be delivered to acidic compartments using alternative 'non-clathrin' routes. This is achieved by a process known as endocytic pathway merging. Adaption of FMDV to cell culture is accompanied by an acquired ability to bind heparan sulphate (HS) and use HS-proteoglycans to mediated entry (4). This ability enables the virus to infect a wide range of cell types without mediation by integrins. The ability of the virus to switch between integrin and HSPG receptors has been linked to long-term, virus persistence in the animal host. Recently we have discovered a novel FMDV that lacks a functional integrin binding site and is infectious for HS negative cells suggesting that the virus can use a third entry mechanism which may be important for infection in the animal host.

This project will investigate the cell entry mechanisms used by HS-binding, and the novel FMDV described above, to infect cells. Viruses that have only integrin or HS binding will be generated by site-directed mutagenesis using our established reverse genetics system. We also have a range of cell lines which express defined RGD-binding integrins and others that specifically lack HS expression. You will investigate entry using a range of pharmacological and DN inhibitors of specific endocytosis pathways, in combination with state-of-the-art Bio-imaging (including immunofluorescence confocal, live-cell and electron microscopy). Assays for the effects of several of the DN proteins on specific endocytosis pathways are already established whereas others will need to be developed. This work will provide new insight into

FMDV cell-entry and could provide novel information of the mechanism of virus persistence and/or cellular endocytosis.

### **Research activity of the Group**

The main focus of our research is the mechanisms of virus endocytosis. We have identified the cellular receptors used by FMDV to initiate infection and identified the key viral determinants of receptor specificity. We have identified the endocytic pathway used by field-strains of FMDV and identified early-endosomes as the key site of infection. Recently, we have identified the cell-entry route used by Bluetongue virus for infection. We also undertake research to understand how FMDV uses host-cell membranes for viral genome replication.

### **References to project for further reading**

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