Modification of FMDV anti-host defense mechanism

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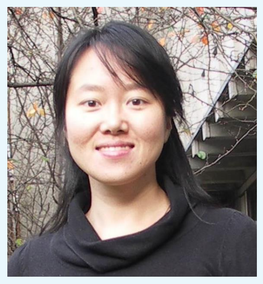

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INTRODUCTION

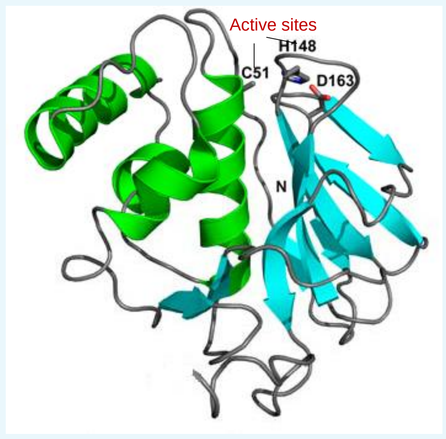
Foot-and-mouth disease virus (FMDV) is the etiologic agent of FMD, an infectious and sometimes fatal viral disease that affects cloven-hoofed animals. The FMDV genome encodes a large polyprotein, the first component of which is the Leader protein.

Unusually, within the picornavirus family, the FMDV Leader protein (L^{pro}) 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. L^{pro} 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.

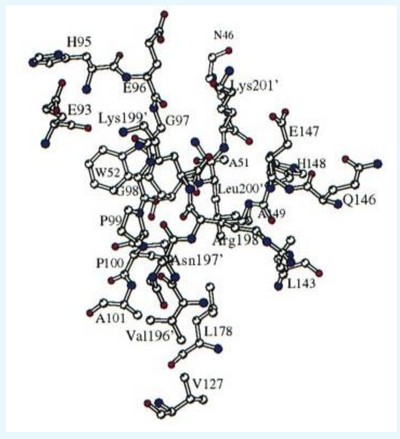
METHODS

1. Substitute the charged and conserved amino acids as well as those at substrates binding sites

For now, around 80 mutants have been made using alanine scanning mutagenesis.

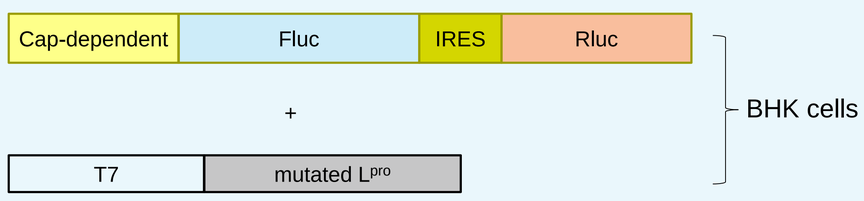


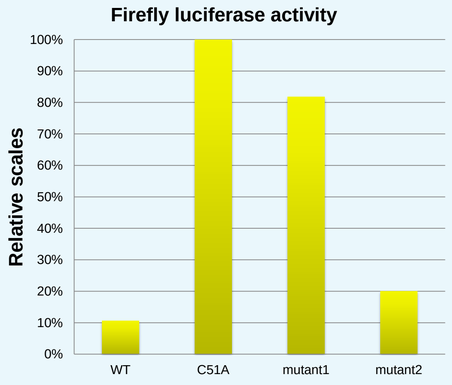
Lb^{pro} 3D structure



Residues at substrates binding sites

2. Test the impact of L^{pro} mutants on cap-dependent translation



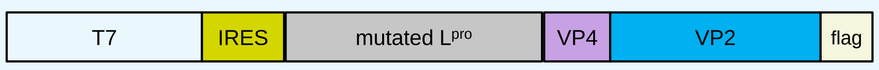


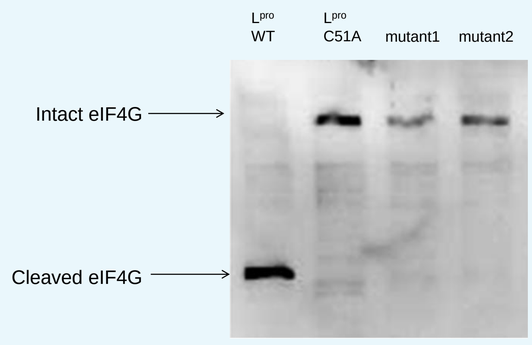
The firefly and renilla luciferase (Fluc and Rluc) activities are measured in the transient expression system within BHK cells. The firefly luciferase activity is normalized to the renilla luciferase to compensate for differences in transfection efficiency.

Wild type L^{pro} ⟶ cleaved eIF4G ⟶ low Fluc expression

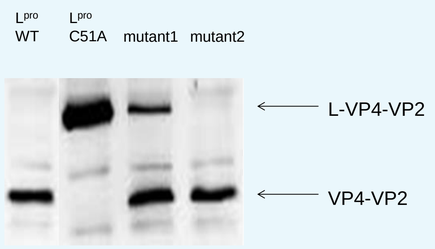
Inactive L^{pro} ⟶ Intact eIF4G ⟶ normal Fluc expression (eg: L^{pro} C51A)

3. Confirm the cleavage activities of L^{pro} mutants in BHK cells



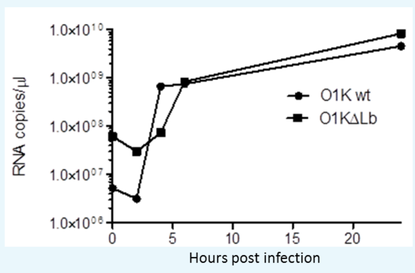


Anti-eIF4G

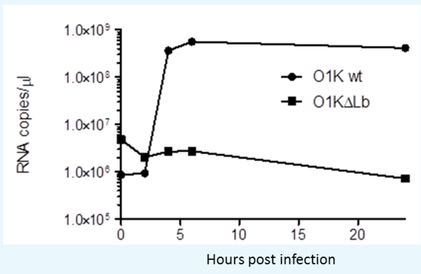


Anti-flag

4. Determine the effect of mutated L^{pro} of FMDV on viral replication



Baby Hamster Kidney cells



Primary bovine thyroid cells

The mutations in L^{pro} that destroy the eIF4G cleavage activity but not self-processing will be introduced into the full length FMDV cDNA and viruses rescued in order to understand the impact of these mutations on viral replication in different cell cultures.

PROSPECTIVES

1. Dissect the two main cleavage activities of L^{pro}
2. Better understand the interactions between L^{pro} and its substrates
3. Understand the host immune responses of the mutant virus

Reference

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