Viral Replication

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Activation of influenza viruses by type II transmembrane serine proteases from non-human primates

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Annually recurring influenza epidemics and occasional pandemics are responsible for substantial global morbidity and mortality. Antiviral therapy available at present is plagued by frequent resistance development and novel therapeutic approaches are urgently needed. Cleavage of the influenza virus hemagglutinin (HA) by host cell proteases is essential for viral infectivity and the responsible enzymes constitute potential targets for antiviral intervention. The type II transmembrane serine proteases TMPRSS2, TMPRSS4 and HAT activate influenza viruses in cell culture and expression of TMPRSS2 has recently been shown to be essential for the spread and pathogenesis of H1N1 viruses in mice. However, the role of TMPRSS2 and other TTSPs in influenza virus infection of primates is unknown. We investigated whether TTSP orthologous of non-human primates can activate HA and support influenza virus spread in respiratory epithelium. We cloned the sequences of TMPRSS2, TMPRSS4 and HAT of rhesus (*Macaca mulatta)* and cynomolgus (*Macaca fascicularis*) macaques as well as TMPRSS2 from marmosets (*Callithrix jacchus*) and showed that these proteins activate HA in transfected cells. In addition, by employing precision cut lung slices (PCLS), we show that influenza viruses efficiently replicate in macaque and marmoset respiratory epithelium and that spread is suppressed by the serine protease inhibitor camostat mesylate, which is known to block activity of TMPRSS2 and other TTSPs. We are currently investigating whether influenza viruses indeed target TMPRSS2 positive cells in macaque and marmoset PCLS and the results of these studies will be presented. In sum, our findings indicate that macaques and marmosets are adequate models to study proteolytic activation of influenza viruses by type II transmembrane serine proteases (TTSPs) and its inhibition.

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Two alternative ways of start site selection in human norovirus reinitiation of translation

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Translation started by reinitiation after termination is one mechanism that allows expression of bicistronic RNAs. In eukaryotes cases of reinitiation after termination of translation of long ORFs have so far only been found in viral RNAs. The prototype of this mechanism has been described for caliciviruses.

The calicivirus minor capsid protein VP2 is expressed via a termination/reinitiation process depending on an upstream sequence element denoted "termination upstream ribosomal binding site" (TURBS). We have shown for feline calicivirus (FCV) and rabbit hemorrhagic disease virus (RHDV) that the TURBS contains three short sequence motifs essential for reinitiation. Motif1 is conserved among all caliciviruses and is complementary to a sequence in the 18S rRNA leading to the model that hybridization between motif1 and 18S rRNA tethers the post-termination ribosome to the mRNA. Motif2 and motif2* are proposed to establish a secondary structure positioning the ribosome relative to the start site of the 3´-terminal ORF.

Here we analyzed human norovirus (huNV) sequences for the presence of these motifs and their importance for reinitiation. The 3 motifs were identified by sequence analyses in the region upstream of the VP2 start site and we showed that these motifs are essential for reinitiation of huNV VP2 translation. More detailed analyses showed that the site of reinitiation is not obviously fixed to a single codon and does not need to be an AUG, even though this codon is clearly preferred. Interestingly, we were able to show that reinitiation can occur at AUG codons downstream of the canonical start/stop site in huNV and FCV, but not in RHDV. While reinitiation at the original start site is independent of the Kozak context, downstream initiation exhibits the requirements for linear scanning. These analyses on start codon recognition give a more detailed insight into this fascinating mechanism of gene expression.