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Deletion of a conserved LLRKxGxKG motif in Murine Hepatitis virus non-structural protein 1 attenuates the virus in mice

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Coronaviruses are enveloped, positive-stranded RNA viruses of the order *Nidovirales*, family *Coronaviridae*, subfamily *Coronavirinae* . Coronaviruses infect many species of animal including humans, and they cause severe disease in livestock animals leading to high economic losses. In 2002-2003, the appearance of severe acute respiratory syndrome (SARS), caused by a formerly unknown coronavirus, *Severe acute respiratory syndrome-related coronavirus* (SARSr-CoV), renewed interest in this group of viruses.

Coronaviruses are classified into four genera, with alpha- and beta-coronaviruses infecting mammals, for example *Human coronavirus* strain 229E (HCoV-229E), SARSr-CoV and *Murine coronavirus* (MHV) virus, and gamma- and deltacoronaviruses infecting avian species.

Coronavirus genomes are extraordinarily large with sizes ranging from 27 to 32 kilobases (kb). One-third of the genome consists of open reading frames (ORF) encodong the structural proteins spike (S), envelope (E), membrane (M), and nucleocapsid (N), as well as group-specific accessory proteins, and approximately two-thirds of the genome encodes the nonstructural replicase (nsp) proteins that are involved in viral RNA synthesis. Many of the coronavirus nsp proteins have been shown, or are predicted, to have enzymatic functions, including papain-like cysteine proteinases (nsp3), 3C-like cysteine proteinase (nsp5), RNA-primase (nsp8), RNA-dependent RNA polymerase (nsp12), 5'-3' helicase (nsp13), S-adenosyl-L-methionine-dependent (guanine-N7)-methyltransferase (N7-MTase, nsp14), 3'-5' exonuclease (nsp14), endoribonuclease (nsp15) and S-adenosyl-L-methionine-dependent (nucleoside-2'*O*)-methyltransferase (2'*O*-MTase, nsp16).

Nsp1 is the N-terminal cleavage product of the replicase polyprotein and is the first mature viral protein expressed in the host cell cytoplasm. Deletion of the nsp1-coding region in infectious clones of MHV yielded viruses that were unable to productively infect cultured cells. Furthermore, exogenous expression of MHV nsp1 in mammalian cells arrested the cell cycle in the G0/G1 phase and inhibited cell proliferation. SARS-CoV Nsp1 protein induces host mRNA degradation and translational suppression both in nsp1-expressing cells and in SARSr-CoV-infected cells. *In vivo* studies suggest that SARSr-CoV nsp1 may counteract the host innate immune responses[18], which may provide a survival advantage for the virus. Overall, these observations indicate that nsp1 might participate in multiple stages of the coronavirus life cycle, and they implicate this protein as a potentially important virulence factor.

Nsp1 sequences are relatively divergent among the different coronaviruses. However, sequence alignment revealed that there is a region (LLRKxGxKG:aa191-199), which is conserved in the nsp1 proteins of different coronaviruses such as MHV, SARSr-CoV and Human coronavirus strain OC43[15,16]. In order to investigate the function of this conserved domain, we expressed, *in vitro, a* version of the MHV nsp1 protein where the LLRKxGxKG region has been deleted, and using a reverse genetics approach, we rescued a recombinant mutant virus in which the same region had beed deleted. We then studied the biological properties of wildtype and mutant nsp1 proteins *in vitro* and the virulence of wildtype and mutant viruses *in vivo*. Our results show that the MHV nsp1 LLRKxGxKG region has a modulatiry role in the biological function of the protein and a attenuating effect on the pathogenesis of virus infection in mice.