

**SE-DP-002**

**Cathepsin L processing of the Henipavirus F proteins**

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Hendra and Nipah are zoonotic viruses with high mortality rates in humans, and both have been placed on NIAID priority pathogens list. The fusion (F) proteins of Hendra and Nipah virus promote both virus-cell and cell-cell membrane fusion, thus mediating critical steps in viral infection. Recent work from our laboratory and others has shown that the cellular endosomal protease cathepsin L is critical for the proteolytic processing of both the Hendra and Nipah virus F proteins, delineating a novel mechanism for primary processing of viral glycoproteins. We have also demonstrated that cathepsin L-mediated proteolytic processing is necessary for promotion of cell-cell membrane fusion, making inhibition of cathepsin L a potentially viable strategy for antiviral therapy. Finally, endocytosis has been shown to play a role in the processing of both the Hendra F protein and the Nipah F protein, suggesting complex trafficking of the Henipavirus F proteins occurs prior to viral assembly. The reasons for this complex mechanism and its consequences for multiple facets of the viral life cycle are currently unknown, but the requirement for cathepsin L processing suggests that this host enzyme may be an effective target for antiviral therapy. *In this developmental grant, we are addressing the role of this unique activation mechanism in viral propagation and pathogenesis, and exploring the utilization of cathepsin inhibitors as antiviral therapeutics for the Henipaviruses.* In Aim 1, we are defining the impact of F protein trafficking and cathepsin cleavage on processes critical for viral propagation by examining whether viral particles containing uncleaved, non-fusogenic F protein can promote entry via endocytosis followed by cathepsin L cleavage, and assessing whether F protein trafficking and/or cleavage modulates subsequent critical F-attachment (G) protein associations or virus-like particle (VLP) formation. In Aim 2, we are examining the utilization of cathepsin inhibitors as Henipavirus antivirals by identification of new cathepsin inhibitors in a compound library screen. Our newly developed screen, which utilizes intracellular cleavage of a cathepsin substrate to generate a fluorescent product, will allow screening of inhibitors which efficiently block cathepsin activity in the context of cells which are permissive for Hendra and Nipah virus growth. We are also examining the effects of identified inhibitors on F protein function in cell-cell fusion assays, and have made the interesting discovery that inhibition of F protein cleavage occurs at a lower concentration than is needed for inhibition of all cellular cathepsin L activity. We are also determining the effect of these inhibitors in endothelial and neuronal cells, which play central roles in Henipavirus pathogenesis, but which have not been previously examined. Finally, we are examining the role of cathepsin L in bat cells, as bats serve as a reservoir species for the Hendra and Nipah viruses.