Karolinska Institutet

**Department of Biosciences and Nutrition**

# Activation of retrovirus spikes for membrane fusion

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# ABSTRACT

Retroviruses are enveloped viruses that use reverse transcriptase to convert their RNA genome into DNA which is integrated into the host DNA. They enter into the cell by a membrane fusion process. In this the viral membrane fuses with that of the cell so that the internal capsid of the virus with the genome can be released into the cell cytoplasm. The fusion is mediated by the spike protein in the viral membrane. In my thesis work I have studied how the spike proteins of two retroviruses, the human immunodeficiency virus typ 1 (HIV-1) and a murine leukemia virus (MLV), are able to catalyze the fusion reaction. I found that defined changes in the spike structure mediated different steps in the fusion process. As the spikes constitute the major target for neutralizing antibodies against the virus the structures of the spikes and its natural intermediate forms should facilitate development of retroviral vaccines and drugs, which are so heavily needed in the case of HIV-1.

The spike is made as a trimeric transmembrane protein in the infected cell and incorporated into virus by budding at the cell surface. The spike then guides the virus to an uninfected cell for virus entry by membrane fusion. These tasks put several demands on the spike function. A key event is its binding to a receptor molecule of the target cell. This triggers, or activates the spike for membrane fusion. However, the activation should not occur in the cell where the virus is produced. Therefore, premature activation is prevented by synthesis of the spike in a precursor form, unable to fuse. Proteolytic cleavage of the spike, before receptor binding, then creates a mature form of the spike, which can be activated by the receptor. I have studied the maturation process of the Moloney (Mo)-MLV spike.

The Mo-MLV spike precursor matures by two proteolytic cleavages. The first one is by the furin enzyme of the producer cell and this forms the receptor binding peripheral subunit (SU) and the fusion active transmembrane subunit (TM) of the spike. The second cleavage is made by the viral protease and occurs in newly released virus. It separates a short peptide, called the R-peptide, from the membrane internal cytoplasmic tail (CT) of TM. I have studied the R-peptide cleavage using biochemical techniques and found that the cleavages in the trimeric spike occur by positive cooperativity, i.e. the cleavage of one subunit facilitates the cleavages of the two other TM subunits in the spike. This helps the spike to reach full fusion activity soon after virus budding. The activation of the spike by the receptor involves dissociation of SU from TM so that the latter can fuse the viral and the cell membranes. I also found that the R-peptide interfered with this dissociation step by inhibiting isomerization of the intersubunit disulfide in the Mo-MLV spike, which normally occurs soon after receptor binding.

Using HIV-1 I studied how the primary receptor CD4 activated the spike for binding to the coreceptor, a chemokinreceptor. In this case I used cryo-electron microscopy and image processing to determine the 3D structures of the native unliganded and the CD4 bound spike. The spikes were released from virus, with or without bound CD4, by solubilization with TX-100 and isolated for EM analysis by density gradient centrifugation. I found that the native spike had an open cage-like structure, where the protomeric unit formed a common roof and a lobe, and a leg on the side. This structure was verified by the unique fitting of the earlier determined atomic structure of the core portion of the peripheral subunit. CD4 binding caused the roof part to lift up probably to enable coreceptor binding and to open the roof for the fusion activated TM.



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