Does the VSV M Protein Prevent NF-kB Activation by Blocking Protein Import?

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Vesicular stomatitis virus (VSV) is a member of the Rhabdovirus family that primarily infects cattle and other hoofed animals. We are investigating the mechanism used by VSV to regulate interferon (IFN) gene expression and NF-κB activation in mouse L929 cells. NF-κB is a transcription factor that is essential for IFN gene expression. Before activation, NF-κB is found in an inactive form in the cytoplasm, tightly bound to its inhibitory protein IκB-α. Upon activation, IkB-α is degraded leaving NF-κB free to localize to the nucleus. Once in the nucleus NF-κB binds to the promoter region of the IFN gene resulting in induction of the gene. Previous data generated in our lab indicates that wild type (Wt) VSV suppresses IFN production and prevents nuclear localization of NF-κB. Infection of L929 cells with an M-defective mutant strain of the virus (T1026R1) induces large amounts of IFN and leads to nuclear localization of NF-κB, indicating that the M protein is involved in these events. We isolate nuclei from infected cells and determine if NF-κB has been activation by quantifying NF-κB DNA binding. It is possible that the M protein is blocking an upstream step necessary for NF-κB activation, which then prevents nuclear localization of NF-κB. It is also possible that although NF-κB is activated in Wt-infected cells, the M protein blocks import of NF-κB through the nuclear pore. In support of the later hypothesis, recent reports show that the VSV M protein inhibits host transcription by blocking mRNA cytoplasmic import. To determine if the VSV M protein is blocking NF-κB nuclear import, we are investigating whether NF-κB is activated and therefore able to bind DNA in cytoplasmic extracts isolated from VSV-infected cells. We have compared NF-kB bindng in nuclear and cytoplasmic extracts using slightly different methods and conditions. Preliminary data suggests that the VSV M protein is blocking the nuclear pore cites inhibiting the activated NF-κB protein from entering the nucleus.