ROLE OF THE VSV M PROTEIN ON NF-κB REGULATION IN L929 CELLS

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We previously reported that NF-κB activation does not occur until later times postinfection in mouse L929 cells infected with interferon (IFN)-inducing wild-type (wt) vesicular stomatitis virus (VSV). In contrast, NF-κB is activated in cells infected with the interferon-inducing R1 strain that encodes a defective matrix (M) protein. An M-defective recombinant virus also activated NF-κB, although the timing varied slightly from activation by R1. Real-Time PCR analysis of RNA isolated from whole cells showed that very little IFN mRNA is produced in wt infected cells, while IFN mRNA was produced in cells infected with R1 and M-defective recombinant virus. The M protein has been shown to inhibit host transcription by blocking cytoplasmic export of mRNA, however IFN mRNA trapped in the nucleus should have been amplified from our whole cell RNA extracts. These findings suggest that the M protein blocks induction of the IFN gene by preventing NF-κB activation, or by blocking nuclear import of activated NF-κB protein. To determine if the M protein prevents NF-κB from entering the nucleus, we measured NF-κB activation in cytoplasmic extracts from wt infected cells using the TransAM assay (Active Motif). No significant NF-κB DNA binding was detected in these cytoplasmic extracts, suggesting that the M protein is not preventing nuclear import of activated NF-κB. We next measured NF-κB activation in cells coinfected with R1 and r1026M. Preliminary data indicates that there is not a component in r1026M that is able to restore R1's ability to suppress NF-κB, suggesting that the M protein alone regulates NF-κB activity. To determine if the M protein alone, in the absence of other viral proteins, is responsible for NF-κB regulation, we examined the ability of transfected Wt or R1 M protein to suppress NF-κB activation. We found that the Wt M protein alone was not able to suppress TNF-α mediated-NF-κB activation, however this protein did slightly limit R1-mediated NF-κB activation. These studies will help define the role of the M protein in regulation of NF-κB in VSV-infected cells.