CLONING AND SEQUENCING OF VIRAL PROTEINS RESPONSIBLE FOR INHIBITING HOST TRANSCRIPTION IN VESICULAR STOMATITUS VIRUS MUTANTS 22-20 AND 22-25. ***J. Filby,*** *L. Golebiewski, M.C. Ferran*, Department of Biological Sciences, jaf0798@rit.edu, lxg4044@rit.edu, mcfsbi@rit.edu*

Type I interferons, IFN α and β, are cytokines, which constitute the primary antiviral response of the mammalian immune system. Once the host cell detects that a viral infection has occurred, a complicated signal transduction cascade is activated, which results in transcription of the IFN gene. IFN is then secreted and binds to adjacent cells' chemical receptors, activating a cascade of antiviral genes. To counteract this host antiviral response, viruses have evolved ways to prevent IFN expression, including blocking IFN receptors and inhibiting host transcription. Wild Type VSV successfully blocks IFN expression in host cells, whereas VSV mutant R1 is a good inducer of IFN. Data collected in our laboratory demonstrates that Wt VSV might block IFN gene expression by preventing activation of NF-κB, a host transcription factor that is essential for induction of the IFN gene. Several virus strains (R1 for example) that contain a mutation in the Matrix protein induce IFN, and we have shown that these viruses also activate NF-κB. These results suggest that the M protein is solely responsible for regulation of IFN production. Two other mutants, 22-20 and 22-25, which were isolated from the same plaque, vary in their ability to regulate IFN production. 22-20 is a good inducer of IFN, whereas 22-25 suppresses IFN production. It has been reported that the sequence of the Matrix protein in 22-20 and 22-25 are identical, indicating that another viral protein is involved in regulation of IFN production in VSV-infected cells. Interestingly, sequencing results generated in our laboratory demonstrated that the M protein of the IFN-inducing 22-20 strain does contain a mutation. The goal of the project was to clone genes from VSV mutants 22-20 and 22-25 and sequence them in order to identify if a second protein is also be responsible for inhibiting IFN production. Each of the viral proteins were cloned into eukaryotic expression vectors and transformed into bacteria. The plasmids were extracted and purified, then digested with restriction enzymes for verification. Sequencing to fill gaps in coverage of 22-20 and 22-25 genomes is currently being done so they can be properly aligned and compared. These expression constructs will be transfected into mammalian cells and the ability of the transfected viral protein to regulate NF-κB IFN gene activation will be monitored.