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## Studies on the Role of Mss1 During HSV-1 Infection

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The 26S proteasome is a cellular machine that degrades ubiquitinated proteins. The proteasome-associated proteins in the 19S cap capture and unfold these proteins. The 19S cap proteins (specifically the ATPases) have been shown to possess supporting functions in DNA repair, transcription, and translation when uncoupled from the core proteasome. We have previously shown that Herpes Simplex Virus type-1 (HSV-1) infection alters the localization of the proteasome subunit Mss1—an ATPase protein of the 19S cap. We will study the importance this cellular protein during infection by artificially reducing levels of Mss1. Analysis of Mss1 levels of after cyclohexamine treatment indicates that this protein is long-lived ( $t_{1/2} = 20$  hours). In order to target Mss1 uniquely for degradation, we have constructed a lentiviral delivery system for Mss1-specific shRNA molecules. Titers of the lentiviral stocks were determined by using quantitative real-time PCR and the efficiency of Mss1 knockdown was determined by Western Blot analysis. Western Blot analysis results suggest that efficient and sustained knockdown was only achieved at an MOI of 5 and required re-infection 12 hours later. Twelve hours later, these cells were infected with HSV-1 at an MOI of 3. Similar levels of virus were produced from cells with reduced levels of Mss1 as compared to the controls. At this MOI, it is possible that we may overlook subtle kinetic differences in virus replication. We are currently addressing these possibilities. These studies will help us better understand the auxiliary functions of Mss1 during infection.