Identification of nuclear antiviral factors counteracted by ICP0 of herpes simplex virus

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Viruses hijack ubiquitin machinery to modify the cellular proteome and subvert host intrinsic defenses. The HSV-1 protein ICP0 is a viral E3 ubiquitin ligase required for efficient infection. Although ICP0 is known to ubiquitinate some cellular antiviral factors and induce their proteasomal degradation, the extent to which HSV-1 utilizes ubiquitination is not well understood. We employed multiple complementary proteomic approaches to identify substrates for ICP0 ubiquitin ligase activity. We compared infections for wild-type HSV-1 and an inactivate ICP0 mutant using quantitative proteomics to identify cellular substrates by (i) defining the ubiquitinome through antibody enrichment of modified peptides, (ii) determining changes in protein abundance by whole-cell proteomics, and (iii) identifying proteins associated with replicating viral genomes. Integrating these datasets allowed us to identify potential ICP0 substrates and predict effects of ubiquitination on protein abundance. Our approach revealed 45 ubiquitinated and degraded proteins (including known substrates PML, ATRX, DNA-PK, and USP7), as well as proteins that are ubiquitinated but not decreased in abundance during infection. We identified SLFN5 as a novel degraded substrate of ICP0 and characterized its effect on HSV-1 infection. We observed rapid loss of SLFN5 during HSV-1 infection and confirmed ICP0 is necessary and sufficient to promote proteasome-mediated degradation. Depletion of SLFN5 enhanced transcription of viral genes and RNA polymerase II loading on viral promoters. SLFN5 associated with viral genomes in the absence of ICP0, and ectopic expression repressed HSV-1 replication. These data suggest SLFN5 is a restriction factor of HSV-1 that inhibits viral gene transcription. Degradation of cellular proteins removes intrinsic defenses, while ubiquitination without degradation may promote virus infection by altering cellular processes. Proteomic approaches with wild-type and ICP0 mutant HSV-1 provide a global view of viral and host proteomes during infection and help to explain functions of ICP0 that promote viral takeover of cellular processes.