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Interfering with the Sumoylation of PML by Herpesviruses

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The promyelocytic leukemia protein (PML) is present in both the nucleoplasm and discrete subnuclear multiprotein structures known as PML oncogenic domains (PODs), nuclear domain 10 (ND10), or PML nuclear bodies. These structures are believed to be important in the intrinsic cellular response to viral infection, because (i) they bind foreign DNA that enters the nucleus and appear to prevent its transcription, (ii) interferon stimulates synthesis of proteins that comprise PODs, (iii) $PML^{-/-}$ mice and cells are resistant to apoptosis induced by different stimuli including interferon, and (iv) overproduction of PML inhibits the replication of some viruses. Two major components of PODs, PML and Sp100, are post-translationally modified by covalent conjugation (sumoylation) with the small ubiquitin-like modifier proteins (SUMO). Sumoylation occurs through a pathway distinct from but analogous to the ubiquitin (Ub) conjugation system. The sumoylation of PML is essential for formation of the mature PODs where other cellular proteins such as Sp100, Daxx and CBP are recruited.

The integrity of PODs is affected by infection of most herpesviruses, including herpes simplex virus type-1 (HSV-1), human cytomegalovirus (HCMV), human herpesvirus-6 (HHV6) and Epstein-Barr virus (EBV), leading to disruption of PODs at very early times after lytic infection. The viral regulatory proteins responsible for POD disruption are ICP0 in HSV-1, IE1 in HCMV, IE1B in HHV-6 and ZTA(BZLF1) in EBV. In HSV-1 infected cells, ICP0, which itself encodes an intrinsic Ub E3 ligase, causes the rapid proteasome-dependent degradation of both sumoylated and unmodified forms of PML. The exact role of POD disruption by the regulatory proteins of herpesvirus is not clear. However, they appear to do so, in part as a measure to override global repression of POD-bound input viral genome. The POD disruption may facilitate viral DNA replication because initiation of formation of viral DNA replication compartments (or center) also occurs at the periphery of PODs.

In HCMV infection, IE1 transiently targets to the PODs within the first two hours after infection, and subsequently PML, other POD components and IE1 are all displaced from the PODs into the nucleoplasm within three to four hours. Recent studies have suggested that IE1 both binds to PML and may disrupt PODs by causing the loss of sumoylated forms of PML. In the present study we set out to characterize the ability of HCMV IE1 to desumoylate PML in comparison with this property of HSV-1 ICP0. We found that, in contrast to ICP0, the desumoylation of PML by cotransfected IE1 was resistant to the proteasome-inhibitor MG132, suggesting that IE1 may use a different mechanism to desumoylate PML than does the ICP0 protein. Reduced sumoylation of PML was also observed in permissive U373 cells after infection with wild-type HCMV and proved to require IE1

protein. Mutational analysis revealed that the central hydrophobic domain of IE1 is required for both PML binding and for desumoylation of PML, and confirmed that all IE1 mutants tested that were deficient in these functions also failed to both target to PODs and to disrupt PODs. These same mutants were also inactive in several reporter gene transactivation assays and in inhibition of PML-mediated transcriptional repression. To study whether the abilities of IE1 to interfere with sumoylation of PML and to regulate transcription in target reporter gene assays may also be required for efficient viral growth, we generated mutant HCMV-BAC clones that encode mutant forms of IE1 that are deficient in PML-associated activities. Importantly, a viral DNA genome containing a deleted IE1(Δ290-320) gene that was defective in these activities was not infectious when transfected into permissive human fibroblast (HF) cells, but the mutant IE1(K450R), which is defective in IE1 sumoylation, remained infectious. These results suggest that the correlated deficiencies in IE1(Δ290-320) activity in desumoylating PML and in regulating transcription may be required functions for efficient initiation of viral growth in cultured HF cells. Taken together, our mutational analysis strengthens the idea that interference by HCMV IE1 with both the sumoylation of PML and its repressor activity requires a physical interaction with PML that also leads to disruption of PODs. These activities of IE1 also correlate with several transactivation functions of IE1 and may be requirements for efficient initiation of the lytic cycle in vivo.