Chapter 18
Viral Interplay with the Host Sumoylation System

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Abstract Viruses have elaborate means to regulate cellular transcription in order to create a cellular environment that facilitates viral survival and reproduction. This includes enhancing viral macromolecular synthesis and preventing antiviral responses such as apoptosis and cell cycle arrest. There are numerous mechanisms by which viruses mediate their effects on the host cell, and this includes targeting various cellular post-translational modification systems, including sumoylation. The wide-ranging impact of sumoylation on cellular processes such as transcriptional regulation, apoptosis, and cell cycle control makes it an attractive target for viral dysregulation. To date, proteins from both RNA and DNA virus families have been shown to be modified by SUMO conjugation, and this modification appears critical for viral protein function. More interestingly, several members of the DNA virus families have been shown to modulate sumoylation, including papillomaviruses, adenoviruses, and herpesviruses. This chapter will focus on mechanisms by which these viruses disrupt sumoylation and the implications of this disruption for viral infection and disease.

Keywords SUMO · Herpesvirus · Cytomegalovirus · Epstein-Barr virus · Adenovirus · Papillomavirus

18.1 Modification of Viral Proteins by Sumoylation

Viral proteins were among the first defined substrates for sumoylation, beginning with the demonstration in 1999 that the human cytomegalovirus (HCMV) immediate-early 1 protein (IE1) was SUMO modified (Muller and Dejean, 1999). Since that time, 16 additional viral proteins (Table 18.1) have been shown to be

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Table 18.1 Sumoylated viral proteins

Virus	Protein	References
Cytomegalovirus (CMV)	IE1	(Muller and Dejean 1999)
Cytomegalovirus (CMV)	IE2	(Hofmann et al. 2000)
Papillomavirus	E1	(Rangasamy et al., 2000)
Epstein-Barr virus (EBV)	Zta (BZLF1)	(Adamson and Kenney, 2001)
Adenovirus (Ad)	E1B-55 K	(Endter et al., 2001)
Human herpesvirus type 6 (HHV6)	IE1B	(Gravel et al., 2002; Stanton et al., 2002)
Epstein-Barr virus (EBV)	Rta (BRLF1)	(Chang et al., 2004)
Epstein-Barr virus (EBV)	EBNA3C	(Rosendorff et al., 2004)
Adeno-associated virus (AAV)	Rep78	(Weger et al., 2004)
Human immunodeficiency virus (HIV)	p6	(Gurer et al., 2005)
Kaposi's sarcoma-associated herpesvirus (KSHV)	K-bZIP	(Izumiya et al., 2005)
Human T-cell leukemia virus (HTLV)	Tax	(Lamsoul et al., 2005)
Severe acute respiratory syndrome coronavirus (SARS-CoV)	N protein	(Li et al., 2005)
Vaccinia virus	A40R	(Palacios et al., 2005)
Moloney murine leukemia virus (MMLV)	CA	(Yueh et al., 2006)
Varicella-zoster virus	ORF29p	(Stallings and Silverstein, 2006)
Papillomavirus	E2	(Wu et al., 2008)

sumoylated. As expected for a predominantly nuclear modification process, the sumoylated viral proteins have been detected mostly for DNA viruses and retroviruses, as these all have an obligate nuclear phase for their life cycles. Among the DNA viruses, five major families (Parvoviridae, Papillomaviridae, Adenoviridae, Herpesviridae, and Poxviridae) have one or more sumoylated proteins, illustrating the widespread utilization of the sumoylation system by nuclear viruses. In particular, sumoylation is highly characteristic of herpesvirus family as HCMV (Muller and Dejean, 1999; Hofmann et al., 2000), Epstein-Barr virus (EBV) (Adamson and Kenney, 2001; Chang et al., 2004; Rosendorff et al., 2004), Human herpesvirus 6 (HHV6) (Gravel et al., 2002; Stanton et al., 2002), Kaposi's sarcoma-associated herpesvirus (KSHV) (Izumiya et al., 2005), and Varicella-zoster virus (VZV) (Stallings and Silverstein, 2006) all have 1 or more known sumoylated proteins. These five herpesviruses constitute five of the eight known human herpesviruses and include representatives of the alpha (VZV), beta (HCMV and HHV6), and gamma (EBV and KSHV) subgroups, further demonstrating that sumoylation of herpesvirus proteins is a frequent and common event. Based on these observations, it is likely that there will be sumoylated proteins identified for the other three human herpesviruses types, as well as for other DNA viruses.

Among the RNA viruses, sumoylation has mostly been observed to date for retroviruses, and the known targets include both virion antigens (Yueh et al., 2006; Gurer et al., 2005) and the Tax regulatory protein (Lamsoul et al., 2005). While the three known examples of sumoylated proteins in retroviruses are in human and murine

retroviruses, there is no reason to believe that other types of retroviruses would lack sumoylated proteins, and future studies will undoubtedly identify numerous SUMO targets among retroviruses of other species. Furthermore, the recent demonstration that the SARS nucleocapsid protein is sumoylated (Li et al., 2005) suggests that viral sumoylation is not necessarily confined to retroviruses and DNA viruses, but that RNA viruses in general may well have sumoylated proteins. Certainly for RNA viruses that have proteins with a nuclear phase, such as the nucleocapsid protein of coronaviruses, typical nuclear sumoylation is possible. Alternatively, cytoplasmic sumoylation has been demonstrated for several cellular proteins (Hilgarth et al., 2004), so even RNA virus proteins that never enter the nucleus may still be accessible for sumoylation. Much further study will be required to determine if sumoylation of RNA virus proteins is as common and widespread as it is for DNA viruses.

Functionally, the effect of sumoylation on viral proteins reflects the known effects on host proteins. Many host transcription factors are sumoylated, and this modification typically represses transactivation activity (Verger et al., 2003). Similarly, 10 of the 17 sumoylated viral proteins are involved in transcriptional regulation, though a direct effect on transcriptional activity by sumoylation has only been reported for six of these proteins: the HPV 16E2 protein (Wu et al., 2008), CMV IE1-p72 (Huh et al., 2008), CMV IE2-p86 (Hofmann et al., 2000), EBV RTA (Chang et al., 2004), EBV Z protein (Adamson, 2005), and KSHV K-bZIP (Izumiya et al., 2005). The HPV E2 protein has both transactivating and repressive activities, and sumoylation contributes positively to both activities in transient reporter assays. For both IE2-p86 and RTA, sumoylation enhances their activation activity; sumoylation of RTA is promoted by interaction of RTA with the host cell RanBPM protein which in turn recruits the SUMO conjugating enzyme, Ubc9 (Chang et al., 2008). In contrast, Z protein sumoylation decreases transactivation, and for K-bZIP sumoylation enhances its repressive activity. For CMV IE1-p72, sumoylation appears to have no direct effect on transcriptional activating activity of this protein (Nevels et al., 2004; Lee et al., 2004; Spengler et al., 2002), but more recently, sumoylation was shown to abolish IE1-p72's repressive activity on an interferon-responsive promoter, suggesting that sumoylation is antagonistic for the ability of CMV IE1 to down-regulate host innate immune responses (Huh et al., 2008). For the EBV EBNA3C protein (Rosendorff et al., 2004), its own sumoylation does not affect its ability to co-activate the LMP1 promoter with EBNA2, but the ability of EBNA3C to bind SUMO does contribute to transcriptional regulatory effects. Additionally, while the effect on transcriptional activity has not been tested, sumoylation enhances the intracellular stability of both the HHV6 IE1 protein (Stanton et al., 2002) and the AAV Rep78 protein (Weger et al., 2004), which could lead to a proportional increase in transcriptional activity for these proteins. Lastly, sumoylation affects the intracellular localization of both CMV IE1 (Sadanari et al., 2005) and human T-cell leukemia virus (HTLV) Tax protein (Lamsoul et al., 2005) and such sequestration away from promoters would be expected to reduce their transcriptional capacity.

Among the sumoylated viral proteins that are not transcription factors, the effects of sumoylation are diverse. There are three different viral structural proteins known to be sumoylated: the p6 protein of HIV (Gurer et al., 2005), the N protein of SARS

coronavirus (Li et al., 2005), and the Moloney murine leukemia virus (MMLV) capsid antigen, CA (Yueh et al., 2006). Sumoylation of p6 has a negative impact and correlates with reduced viral reproduction through an unknown mechanism. In contrast, sumoylation of CA or N protein has positive consequences for these two viruses. A CA mutant that cannot be sumoylated was unable to produce new virus and appeared blocked at an early step after reverse transcription, but before provirus formation. For N protein, sumoylation promotes homo-oligomerization, which is essential for new virion formation. Of the remaining known sumoylated viral proteins, three [the bovine papillomavirus E1 protein (Rangasamy et al., 2000), the adenovirus E1B-55 kDa protein (Endter et al., 2001), and the vaccinia virus A40R protein (Palacios et al., 2005)] require sumoylation for their proper intracellular localization and for two, the varicella-zoster virus ORF29 protein (Stallings and Silverstein, 2006) and the human papillomavirus E1 proteins (Rangasamy et al., 2000), the functional role of sumoylation has not been defined.

From the studies described above, it is clear that sumoylation of viral proteins is a fairly common event, and it is likely that many additional viral substrates for sumoylation will be detected in future studies. Furthermore, as for cellular substrates, sumoylation of viral proteins has myriad effects on protein function and overall viral replication. However, prediction of the specific effect that sumoylation will have on a particular viral protein has remained difficult and continues to require experimental verification. Importantly, since sumoylation is critical for normal function of many viral proteins, then modulation of the host sumoylation system may have some utility for antiviral therapeutics.

18.2 Viral Proteins that Affect Host Sumoylation

In the previous section, the sumoylation of viral proteins was examined, and the importance of this modification for viral protein function is now well established. Conversely, there is increasing evidence that many viruses can alter the sumoylation of host proteins, either for specific substrates or by globally affecting the sumoylation system. Modulation of host sumoylation presumably enhances the cellular environment for viral reproduction, either by facilitating sumoylation of the viral proteins or by affecting the activity of host proteins that are SUMO regulated. The following sections will examine individual viruses that can manipulate host sumoylation and will explore mechanisms to target specific host proteins or host sumoylation more generally.

18.3 Herpes Simplex Virus

The initial observations that viruses could impact host sumoylation were made for members of the herpesvirus family. The herpes simplex virus (HSV) immediate early gene product, ICP0 (also known as Vmw110), which is not itself sumoylated, causes loss of high molecular weight isoforms of PML (Everett et al., 1998). These

forms were eventually determined to be SUMO-modified versions of PML, a major component of nuclear ND10 bodies (Muller and Dejean, 1999). Similarly, ICP0 also decreases the amount of sumoylated Sp100, another major constituent of ND10s (Everett et al., 1998). ND10 disruption by HSV is necessary for effective lytic replication, and this disruption requires ICP0. Originally, this loss of sumoylated PML and Sp100 was believed to cause disruption of the ND10s, but subsequent studies showed that the SUMO protease, SENP1, could elicit similar loss of sumoylated PML and Sp100 without affecting ND10 structures (Bailey and O'Hare, 2002). Consequently, while loss of sumoylated ND10 components may be necessary for ND10 disruption, it is not sufficient, and other effects of ICP0 must also be required. Furthermore, a direct interaction between ICP0 and either unmodified or sumoylated PML does not occur, and ICP0 does not inhibit PML sumoylation or cause desumoylation of PML *in vitro* (Boutell et al., 2003), so its mechanism of action remains uncertain.

One possible mechanism for reduced sumoylation that has been explored is recruitment of a SUMO protease to the ND10s by ICP0. Bailey and O'Hare showed that ICP0 does recruit SENP1, at least under conditions of transient co-expression (Bailey and O'Hare, 2002). This recruitment of SENP to ND10s might explain why ICP0 expression leads to a generalized loss of nuclear sumoylated proteins (Bailey and O'Hare, 2002; Everett et al., 1998); if many of these unidentified proteins are transcription factors that traffic through the ND10 foci, they may be inadvertently desumoylated. An intriguing, though relatively unexplored, alternate model for ICP0 effects on sumoylated proteins relates to the known ubiquitin ligase activity of ICP0 (Boutell et al., 2002). A recent study in *S. pombe* identified mediator proteins that direct an ubiquitin ligase to sumoylated proteins and target these proteins for proteasomal degradation (Sun et al., 2007). The existence of comparable cellular mediators for ICP0 could lead to specific ubiquitinylation of sumoylated PML and Sp100 with their subsequent preferential degradation. A requirement for such mediators could also explain the observation that ICP0 increases ubiquitinylation of PML *in vivo*, but cannot function to modify PML or sumoylated PML in an *in vitro* system (Boutell et al., 2003). As for the SENP recruitment model, directed ubiquitinylation by ICP0 of sumoylated proteins that traffic to the ND10 sites could explain the generalized decrease in sumoylated nuclear proteins. Regardless of the mechanism, ICP0-mediated loss of sumoylated ND10 components appears to be at least part of the viral strategy for ND10 disruption. In addition, the collateral loss of other sumoylated proteins is likely to have significant effects on the host environment. Whether or not the loss of these other sumoylated proteins is essential or even somewhat beneficial to viral reproduction has not yet been investigated.

18.4 Cytomegalovirus

Like HSV, the human cytomegalovirus (CMV) also encodes an immediate early protein, IE1, which disrupts ND10s and reduces the level of sumoylated forms of PML (Muller and Dejean, 1999). However, there appear to be significant differences

between IE1 and ICP0 in properties and modes of action on PML. For example, ICP0-mediated reduction in sumoylated PML is abrogated by the proteasome inhibitor MG132, while MG132 is unable to protect sumoylated PML from IE1 (Lee et al., 2004). Furthermore, expression of ICP0, but not of IE1, also leads to loss of unmodified PML, An additional difference between IE1 and ICP0 is that ICP0 has ubiquitin ligase activity (Boutell et al., 2002) and does not bind PML (Boutell et al., 2003), whereas IE1 has no known ubiquitin ligase activity, but does interact with PML (Ahn et al., 1998). These major differences in properties between ICP0 and IE1 almost certainly indicate that their mechanisms for reducing sumoylated forms of PML will be distinct.

Mechanistically, there are several modes of possible IE1 action on sumoylated PML that are consistent with its known properties. First, direct binding of IE1 to PML could block access to the sumoylation enzymes, though no such effect is seen with an *in vitro* sumoylation assay (Kang et al., 2006). Alternatively, IE1 could have intrinsic SUMO protease activity, but again no such activity has been detectable *in vitro* (Kang et al., 2006). A variation of this second mechanism that has not been tested is that IE1 recruits a SUMO protease to the ND10s such as has been suggested for ICP0. A further variation is that IE1 might simply cause disaggregation and release of sumoylated PML from the ND10s, which might allow access to SENPs that were excluded from the ND10s. Another untested model is that IE1 recruits a cellular ubiquitin ligase with specificity for sumoylated proteins. This would target the sumoylated PML without affecting unmodified PML, consistent with lack of IE1 effect on the unsumoylated form of PML. Clearly, much additional work will be needed to clarify the processes by which both IE1 and ICP0 target sumoylated PML. Nonetheless, a thorough study of IE1 mutants by Lee et al. convincingly demonstrated that the ability of IE1 to cause loss of sumoylated PML forms is correlated with the transcriptional activating functions of IE1 and viral reproduction (Lee et al., 2004), so modulation of host sumoylation is an important function of this viral protein.

18.5 Human Herpesvirus 6

Like CMV, human herpesvirus 6 (HHV6) is a betaherpesviruses and expresses an IE1 protein, though the CMV IE1 and the HHV6 IE1 lack significant identity at the protein level (Gravel et al., 2002). Even without much relatedness to CMV IE1, the HHV6 IE1 still possesses a transcriptional activating function, is sumoylated, and localizes to ND10 bodies (Gravel et al., 2002; Stanton et al., 2002). Also like CMV IE1, sumoylation of HHV6 IE1 is not required for localization to ND10s (Gravel et al., 2004). Surprisingly, HHV6 IE1 does not cause disruption of ND10s when transiently expressed alone. Even during viral infection, ND10s did not disperse and instead condensed into a smaller number of larger bodies (Gravel et al., 2002). These results indicate that HHV6 does impact ND10s, but that other viral proteins must play a role instead of or in addition to IE1. Furthermore, it has not yet been reported

that HHV6 IE1 affects the sumoylation of PML or any other cellular proteins, so it remains to be determined if this virus in fact has any effect on host sumoylation.

18.6 Epstein-Barr Virus

The final member of the herpesvirus family with a reported effect on host sumoylation is the gamma herpesvirus, Epstein-Barr virus (EBV). During lytic infection, EBV causes disruption of ND10s just as do CMV and HSV (Bell et al., 2000). A subsequent study by Adamson and Kenney demonstrated that the EBV immediate early gene BZLF1 product, the Z protein, was sufficient to induce ND10 dispersion (Adamson and Kenney, 2001). As with its HSV and CMV counterparts, the Z protein is itself sumoylated and can reduce the amount of the sumoylated forms of PML. This reduction in sumoylated PML forms is not affected by proteasome inhibitors, which is similar to what has been observed for CMV IE1. Additionally, a mutant Z protein that cannot be sumoylated is still able to reduce the sumoylated forms of PML, but the loss of these modified forms can be overcome by over expression of SUMO1. Based on these observations, the author's proposed that the Z-mediated reduction of sumoylated PML is via a competition for limited quantities of SUMO1 (Adamson and Kenney, 2001). Somewhat paradoxically, the Z mutant that no longer affected the sumoylated forms of PML still caused ND10 disruption, so there was no direct correlation with loss of these forms and the status of the ND10s. Consequently, though Z does affect sumoylation of PML, the significance of this for viral reproduction is uncertain. Whether or not Z also affects the sumoylation of other proteins has not been examined, but would be predicted based on the proposed competition mechanism.

18.7 Adenovirus

The next viral family with members that affect sumoylation is the adenoviruses where two proteins, GAM1 and E1A, impact sumoylation of host proteins. GAM1 has the most dramatic inhibition of global sumoylation of all known viral proteins, and is also the most well-characterized mechanistically. Chicken Embryo Lethal Orphan (CELO) virus, also referred to as the avian adenovirus type 1 (Ad1), had its DNA completely sequenced in 1996 (Chiocca et al., 1996). GAM1, an early gene expressed by this virus, was first shown to have anti-apoptotic activity (Chiocca et al., 1997), but later this function was determined to be cell type specific (Wu et al., 2007). Interestingly, this 30 kDa viral protein has no homology to other known anti-apoptotic proteins such as E1B from the adenovirus type 5 or to the Bcl2 and Bax family of eukaryotic proteins. Moreover, a CELO Gam1 negative mutant is replication-defective, which clearly establishes GAM1 as an important protein for the viral life cycle with functions beyond anti-apoptotic activity.

A hallmark of viral infection is the increase of transcription to sustain viral replication. Cellular transcription is regulated by two mechanisms: (1) the level of transcription factors activity and (2) the acetylated state of the histone proteins. The

histones are the major chromatin components, and depending on their acetylation state they can regulate accessibility to transcription factors by locking promoters in a closed or open complex. Thus, transcription can be significantly up-regulated when transcription factor activity is increasing and when the histone deacetylase enzyme (HDAC) activity is decreasing. Interestingly, many transcription factors have been shown to be SUMO modified and their modifications repress their activities. Inversely, SUMO modification of HDAC enhances its activity (David et al., 2002). Thus, reduction of cellular sumoylation will have a positive effect on overall transcription. Since GAM1 is predominantly located in the nucleus, its role as a possible transcriptional regulator was investigated. Choccia and colleagues showed that GAM1 inhibits HDAC activity similar to treatment of cells with trichostatin A (TSA), a known inhibitor of HDAC (Chiocca et al., 2002). In an effort to determine the mechanism by which GAM1 inhibits HDAC activity, it was discovered that sumoylation was globally reduced when GAM1 was expressed in a dose dependent manner (Colombo et al., 2002; Boggio et al., 2004). In fact, GAM1 was also shown to redistribute SUMO from the nucleus to the cytoplasm, promoting transcription factor and HDAC desumoylation, thus positively influencing cellular transcription.

To address the mechanism of GAM1 action on sumoylation, Boggio et al. performed over expression experiments (Boggio et al., 2004). When the level of the SUMO activating enzyme, SAE1/SAE2, was increased in the presence of GAM1, the sumoylation levels of cellular proteins were restored. In contrast, over expression of SUMO or the conjugating enzyme, Ubc9, had no effect on the total sumoylated pool of cellular proteins. This clearly indicated a defect of the sumoylation system at an early step of the sumoylation cascade. The activating enzyme is a heterodimeric enzyme composed of two independent subunits, SAE1 and SAE2, and is essential for the initial step of the sumoylation process. After determining that GAM1 did not act as a protease by cleaving SUMO off of the activating enzyme, it was established that GAM1 binds to and inhibits the SAE1/SAE2 complex (Boggio et al., 2004). Moreover, the half-lives of both SAE1/SAE2 and Ubc9 were significantly reduced in the presence of GAM1. A subsequent study showed that this reduction in half-life of SAE1/SAE2 was due to proteasomal degradation resulting from increased ubiquitinylation of SAE1 mediated by GAM1 recruiting a cellular ubiquitin E3 ligase to the GAM1-SAE1/SAE2 complex (Boggio et al., 2007). The loss of SAE1 destabilizes both SAE2 and Ubc9, resulting in reduction in their levels as well. Together, the degradation of the activating and conjugating enzymes prevents any new sumoylation. Even though the desumoylation process is unaffected by GAM1, the inability to perform new SUMO modification greatly decreased the entire pool of sumoylated substrates. The net result of this GAM1 effect is an increase in overall cellular transcriptional activity which facilitates viral replication. While GAM1 may be an extreme example, it is likely that other viruses have evolved strategies to target the sumoylation enzymes in order to enhance the cellular transcriptional environment to favor their replication needs.

The other adenovirus proteins which influence host sumoylation are the E1A (Ledl et al., 2005) and E1B proteins (Muller and Dobner, 2008). E1A is an early protein that regulates viral genome transcription and contributes to cell transformation

(Berk, 2005). Unlike GAM1, E1A is no global modulator of sumoylation and instead affects only a single known substrate, pRB. pRB is a host regulatory factor that binds to E2F and masks the E2F transcriptional activation. In addition, pRB can recruit repressive chromatin remodeling factors to E2F bound promoters to further silence gene expression (Berk, 2005). Ledl et al. showed that SUMO is attached to pRB at a single residue, lysine 720, in the B-box motif which interacts with LxCxE-motif proteins such as E1A (Ledl et al., 2005). Mutation of lysine 720 results in a pRB protein with increased repressive activity on an E2F-responsive promoter, indicating that sumoylation negatively regulates pRB repressive activity. E1A is known to bind pRB leading to the release and activation of E2F. However, binding of E1A to pRB also prevents sumoylation of lysine 720, thereby providing another level of control of pRB by this viral protein (Ledl et al., 2005). Ultimately, E1A seeks to activate cellular S-phase and increase transcription of DNA replication related genes to promote viral genome reproduction, and influencing the sumoylation state of pRB may provide fine regulation of E2F activity. Furthermore, E1A binds to a variety of other cellular proteins (Berk, 2005), at least two of which are themselves sumoylated, CtBP (Lin et al., 2003) and p300 (Girdwood et al., 2003). This raises the possibility that E1A might also regulate the sumoylation state of binding partners other than pRB. While less global than the effects of GAM1, the reduction in sumoylation levels for specific substrates could still be an important mechanism by which E1A modulates the host cell environment. Similar substrate-specific reduction in sumoylation has also been observed for the papillomavirus E2 and E7 proteins (see below).

E1B-55 K is an important adenoviral regulatory protein that controls export of late viral RNAs, inhibition of cellular mRNA transport, and the proteasomal degradation of p53 [reviewed in (Flint and Gonzalez, 2003)]. E1B-55 K protein is sumoylated at a single lysine residue, amino acid 104, and this modification is required for transformation activity (Endter et al., 2001) and for proper intracellular localization through an effect on nuclear export (Kindsmuller et al., 2007). In addition to these effects of sumoylation on E1B-55 K itself, a recent report indicates that E1B-55 K stimulates sumoylation of p53 (Muller and Dobner, 2008). This effect requires direct interaction of p53 and E1B-55 K and also requires that E1B-55 K be sumoylated. While the effects of sumoylation on p53 function are complex and not clearly understood, it is likely that enhanced sumoylation of p53 by E1B-55 K represents another viral mechanism for modulating the activity of this critical host protein.

18.8 Human Papillomavirus

The human papillomaviruses (HPVs) are important pathogens that cause benign disease (warts) and promote certain epithelial tumors, particularly cervical cancer (Madkan et al., 2007). HPVs infecting the mucosa can be classified in two types based on their capacity to cause carcinogenesis: low risk and high risk. Interruption

of the normal virus life cycle and persistent expression of the two oncogene proteins, high risk HPV E6 and E7, underline the basis for cervical cancer progression. HPV E7 stimulates the cell cycle by promoting E2F release from pRb, while E6 promotes p53 degradation in an ubiquitin-dependent and independent manner (Doorbar, 2006). However, the molecular mechanism by which HPV hijacks the process of keratinocytes differentiation is much more complex and is defined by multiple virus-host interaction, some still undermined. There are three papillomavirus proteins that have been shown to affect host sumoylation: E2 (Y.-C. Wu and V. G. Wilson, unpublished results), E6 (A. Deyrieux and V. G. Wilson, unpublished results), and E7 (Ledl et al., 2005). All of these are early viral proteins with important roles in the viral life cycle, and their ability to affect host sumoylation likely contributes to their functional effects on the host cell.

The papillomavirus E2 protein is a multifunctional polypeptide with roles in viral DNA replication (Chiang et al., 1992), genome segregation (You et al., 2004), and transcription (Demeret et al., 1997). As a transcription factor, E2 is both an activator and repressor depending on the promoter context (Ledl et al., 2005). Similar to many transcription factors, E2 is itself sumoylated and this modification contributes to both transactivation and repressive ability (Wu et al., 2008). Furthermore, E2 can interact with a variety of other host cell transcription factors such as Sp1 (Steger et al., 2002), YY1 (Lee et al., 1998), and C/EBP (Hadaschik et al., 2003), all of which are themselves sumoylated (Spengler and Brattain, 2006; Deng et al., 2007; Kim et al., 2002). Recent preliminary results in our lab indicate that E2 reduces the sumoylation of C/EBP *in vivo*. Since sumoylation of C/EBP negatively regulates transcriptional synergy (Subramanian et al., 2003), E2-mediated reduction of C/EBP sumoylation may account for the observed enhancement of C/EBP activity by E2 protein (Hadaschik et al., 2003). Similar effects of E2 on its other sumoylated binding partners could be a general feature of E2 that contributes to its dysregulation of cellular transcription.

Like GAM1, the E6 protein was shown to interact with a component of the sumoylation system, but the target is different and the overall effect more subtle. In a study published in 2006, Dejean′s group showed that high risk HPV-E6, but not low risk E6, was capable of binding to and inhibiting PIASy activity (Bischof et al., 2006). Interestingly, PIASy is a SUMO ligase which enhances sumoylation of specific substrates such as p53 (Schmidt and Muller, 2003), and E6 binding blocks sumoylation of PIASy-specific substrates. Functionally, since PIASy acts as a promoter of cellular senescence (Bischof et al., 2006), this inhibition of PIASy by E6 may contribute to the ability of E6 to inhibit cellular senescence. Although E6 binds to PIASy and inhibits its ligase activity, it does not target this enzyme for degradation like GAM1 does for the SAE1/SAE2 enzyme. Mechanistically, E6 may be acting simply by sequestering or blocking PIASy in the complex. Whether or not E6 binds to other members of the PIAS family, or to other classes of SUMO ligases has not been reported. Recently, work in our lab has shown that E6 proteins can also bind to Ubc9, the SUMO conjugating enzyme and appear to promote its proteasomal degradation. While the consequences of this interaction for sumoylation function are under investigation, the ability of E6 to bind Ubc9 and reduce its levels

suggests that E6 may have multiple mechanisms for modulating host sumoylation activity.

The final HPV protein with known effects on sumoylation is the E7 protein. E7 is an early papillomavirus protein that both helps drive the host cell into a proliferative state and counteracts innate immunity (Hebner and Laimins, 2006). Like adenovirus E1A, the papillomavirus E7 protein is an LxCxE motif containing protein that binds pRB through this motif (Munger et al., 2001), and also like E1A, E7 binding inhibits sumoylation of pRB (Ledl et al., 2005). Additionally, E7 also binds many other cellular targets (Wilson and Rosas-Acosta, 2003) and may be able to modulate sumoylation of these proteins as well, though this has not yet been reported. Interestingly, many of the functions of E7 are inhibited by the cellular tumor suppressor, p14ARF (Pan et al., 2003), which is known to stimulate sumoylation of certain substrates (Woods et al., 2004; Rizos et al., 2005). This suggests that antagonism between the sumoylation inhibition by E7 and the enhancement of sumoylation by p14ARF may be an important determinant of the outcome of infections. Lastly, unpublished work in our lab has shown that like E6, E7 also binds to Ubc9. While no obvious effect by E7 on overall sumoylation *in vivo* has been observed, subtle effects mediated through Ubc9 are still possible. Much additional work will be needed to fully understand the interplay between sumoylation and E7 function.

18.9 Conclusion

The sumoylation system has been shown to be an important player in many biological processes, such as cellular differentiation, transcriptional regulation, and cell growth (Deyrieux et al., 2007; Gill, 2005; Ihara et al., 2007). Perturbing this biological system changes cellular response to diverse signaling pathways (Sharrocks, 2006). The large body of evidence presented here indicates that SUMO and its enzymatic pathway may also play an important role in viral-host interactions. Each of the viral proteins known to influence sumoylation causes either a global or substrate-specific reduction in sumoylation state. As sumoylation appears to be primarily a negative regulator of transcription, it is reasonable that many DNA viruses would seek to overcome this restraint to produce a more active cellular transcriptional environment that would favor viral gene expression. Additionally, sumoylation also contributes to senescence and apoptosis through at least the PIASy targets (Bischof et al., 2006), so viral inhibition of sumoylation should favor continued cell growth and survival. Thus, sumoylation has overall features that would contribute to an "anti-viral" state and which may need to be ameliorated in order for successful viral reproduction. Additionally, sumoylation modifies many non-transcription factors (Rosas-Acosta et al., 2005) which could be involved in more specific viral requirements, either positively or negatively. It is likely that many additional viral proteins that impact sumoylation wait to be discovered. Identifying and understanding these global and specific viral effects on sumoylation will provide new insight

into viral-host interactions and may highlight new targets for therapeutic treatment of viral infections.

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