



Viruses, SUMO, and immunity: the interplay between viruses and the host SUMOylation system

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Abstract

The conjugation of small ubiquitin-like modifier (SUMO) proteins to substrates is a well-described post-translational modification that regulates protein activity, subcellular localization, and protein–protein interactions for a variety of downstream cellular activities. Several studies describe SUMOylation as an essential post-translational modification for successful viral infection across a broad range of viruses, including RNA and DNA viruses, both enveloped and un-enveloped. These viruses include but are not limited to herpes viruses, human immunodeficiency virus-1, and coronaviruses. In addition to the SUMOylation of viral proteins during infection, evidence shows that viruses manipulate the SUMO pathway for host protein SUMOylation. SUMOylation of host and viral proteins greatly impacts host innate immunity through viral manipulation of the host SUMOylation machinery to promote viral replication and pathogenesis. Other post-translational modifications like phosphorylation can also modulate SUMO function. For example, phosphorylation of COUP-TF interacting protein 2 (CTIP2) leads to its SUMOylation and subsequent proteasomal degradation. The SUMOylation of CTIP2 and subsequent degradation prevents CTIP2-mediated recruitment of a multi-enzymatic complex to the HIV-1 promoter that usually prevents the transcription of integrated viral DNA. Thus, the “SUMO switch” could have implications for CTIP2-mediated transcriptional repression of HIV-1 in latency and viral persistence. In this review, we describe the consequences of SUMO in innate immunity and then focus on the various ways that viral pathogens have evolved to hijack the conserved SUMO machinery. Increased understanding of the many roles of SUMOylation in viral infections can lead to novel insight into the regulation of viral pathogenesis with the potential to uncover new targets for antiviral therapies.

Keywords SUMOylation · Virus · Post-translational modifications · Infection

Introduction

The conjugation of the small ubiquitin-like modifier (SUMO) to proteins increases the diversity and complexity of the proteome in eukaryotic cells. The *Saccharomyces cerevisiae* SUMO homolog Smt3 is the earliest identification of SUMO and was characterized as a suppressor of the centromeric protein Mif2. Still, the exact mechanism of suppression remained unclear even after its discovery (Meluh and Koshland 1995). The field grew in 1996 when several laboratories independently identified SUMO in mammals. One group identified SUMO as an interacting partner of DNA repair proteins RAD51/RAD52 in the nucleus (Shen et al. 1996), providing

more evidence regarding the potential role of SUMO in cellular functions. In addition, another group identified SUMO as a novel binding partner of the pro-apoptotic protein, Fas, during their studies of extrinsic apoptotic pathways (Okura et al. 1996). SUMO was also identified as the promyelocytic leukemia protein (PML) associated tumor-suppressor protein, regulating its localization and function (Boddy et al. 1996; Zhong et al. 2000). Together, these studies provided early evidence of SUMO as a binding partner for nuclear proteins. However, the functional consequences of protein SUMOylation remained unclear until two groups independently described that covalent attachment of SUMO to a substrate functioned as a post-translational modification (PTM) (Mahajan et al. 1997; Matunis et al. 1996). During investigations of the nuclear pore complex (NPC), both groups discovered that unmodified Ran-GTPase-activating protein (RanGAP1) was cytosolic and diffuse, but that SUMO-modified RanGAP1 was bound to the NPC through its interaction with a nucleoporin

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protein, RanBP2 (Mahajan et al. 1997). This interaction was also found to protect ran-GAP1 from SUMO-specific protease (SENPs)-mediated de-SUMOylation (Zhu et al. 2009). These findings were significant for at least two reasons: (1) this was the first evidence of SUMO as a covalent, reversible protein modifier and (2) SUMO conjugation (SUMOylation) resulted in a change in protein function (i.e., subcellular localization).

Our current knowledge of SUMOylation as a covalent PTM stems from these early studies on nuclear transport. The human genome encodes four SUMO proteins, SUMOs 1–4. SUMO2 and SUMO3 share significantly more sequence similarity to one another than to SUMO1 or SUMO4, and this is reflected in the specificity of SUMOylated targets. For example, SUMO1 and SUMO2/3 appear to have distinct targets (Vertegaal et al. 2006). On the other hand, SUMO4 is predicted to have 87% amino acid identity with SUMO2 and is expressed in lymph nodes and spleen, although some reports suggest that SUMO4 is a pseudogene (Guo et al. 2004; Hammoudi et al. 2016). The functional capabilities, specificity, and expression profiles of SUMO4 remain unclear. SUMO1 is the most divergent, sharing only 50% sequence identity with SUMO2/3 (Gareau and Lima 2010). Additional studies on cell type, subcellular localization, protein substrates, and disease versus normal state are required to understand how different SUMOs function.

SUMO proteins are expressed in a precursor form that requires C-terminal proteolytic processing by SUMO-specific proteases (SENPs) to expose a diglycine motif essential for conjugation to the target protein (Hickey et al. 2012). There are six mammalian SENP family members (SENPs 1–3 and SENPs 5–7), each with distinct substrate specificity and tissue distribution (Kolli et al. 2010). The conjugation of mature SUMO1 and SUMO2/3 to protein substrates is mediated by a pathway similar to ubiquitin and consists of E1, E2, and E3 enzymes (Fig. 1). Mature SUMO is activated by the E1-activating enzyme, a heterodimer composed of SUMO-activating enzymes (SAE)1 and SAE2 (Desterro et al. 1999; Okuma et al. 1999). SUMO is then transferred from the E1 heterodimer-activating enzyme to the E2-conjugating enzyme, Ubc9 (Lee et al. 1998; Okuma et al. 1999). Finally, it is transferred to substrates with or without catalytic assistance of SUMO E3 ligases (Reverter and Lima 2005). Extensive analysis of several SUMO substrates confirmed that most SUMO-accepting lysine residues (K) lie within a consensus sequence, Ψ KXE, with Ψ representing an aliphatic amino acid, preferably leucine, isoleucine, or valine (Johnson 2004). Other studies reported that this SUMOylation consensus sequence is sufficient to target some substrates, such as RanGAP, through direct interaction with the SUMO-conjugating enzyme Ubc9 (Sampson et al. 2001). However, since only one E2-conjugating enzyme exists in the SUMO pathway, SUMO E3 ligases are required to modify other

substrates, including SUMOylated at non-consensus sites. On the other hand, the ability of SUMO4 to conjugate to substrates in vivo is still questioned, as SENPs are unable to process the SUMO4 precursor protein (Guo et al. 2004; Owerbach et al. 2005).

SUMO can be attached to substrates as a single SUMO moiety (mono-SUMOylation), at multiple lysine residues (multi-SUMOylation), or consist of a chain of SUMOs attached to one lysine residue (poly-SUMOylation); SUMO2/3 forms polymeric chains, in which SUMO-SUMO linkages occur at Ω KXE sequences in their N-terminal extensions (Fig. 1) (Johnson 2004; Tatham et al. 2001). SUMOylation may affect protein localization, stability, and/or activity by altering the dynamics of protein–protein interactions (Johnson 2004). These interactions are primarily mediated by the recruitment of binding partners that harbor SUMO-interacting motifs (SIMs) and are characterized by a stretch of acidic and/or serine residues and a hydrophobic core (Minty et al. 2000; Song et al. 2004). SUMOylation is a highly dynamic and reversible process, and the SENP family of proteins mediates the removal of SUMO from conjugated proteins, the same proteins that are required for the production of mature SUMO (described above). As a result, SENP enzymes maintain the balance between SUMO processing and conjugation/deconjugation. SUMOylation plays significant roles in development, signal transduction, epigenetic processes, and DNA repair (Qu et al. 2014; Sarangi and Zhao 2015; Smith et al. 2011).

Dysregulated SUMOylation has been implicated in several disease states, including cancer, neurodegeneration, and viral infection. In prostate cancer, overexpression of SUMO isopeptidases is accompanied by cellular stress (Bawa-Khalife et al. 2010; Han et al. 2010; Kaikkonen et al. 2009). In neurodegenerative diseases, including Huntington's, Parkinson's, and Alzheimer's diseases, SUMOylation increases plaque formation and aggregation of key proteins like APP and Tau, contributing to neuronal cytotoxicity (Princz and Tavernarakis 2020; Sarge and Park-Sarge 2009). Several studies report SUMOylation as an essential post-translational mechanism for successful viral infection (Gurer et al. 2005; Lamsoul et al. 2005; Yueh et al. 2006). In addition to viral proteins being SUMO substrates, there is evidence that viruses can manipulate the SUMO pathway to modulate host antiviral responses and promote viral replication and pathogenesis (Lowrey et al. 2017). For example, viruses including Epstein-Barr virus (EBV), hepatitis virus, human immunodeficiency virus (HIV), and herpesviruses (HSV) are among the numerous known viral infections that take advantage of host SUMOylation machinery to promote infection and replication (Brown et al. 2016; Conn et al. 2016; Li et al. 2012; Schaller et al. 2011; Sengupta et al. 2017). Given the complexity of the SUMO pathway, the diverse processes that SUMOylation can affect, and its importance in health and

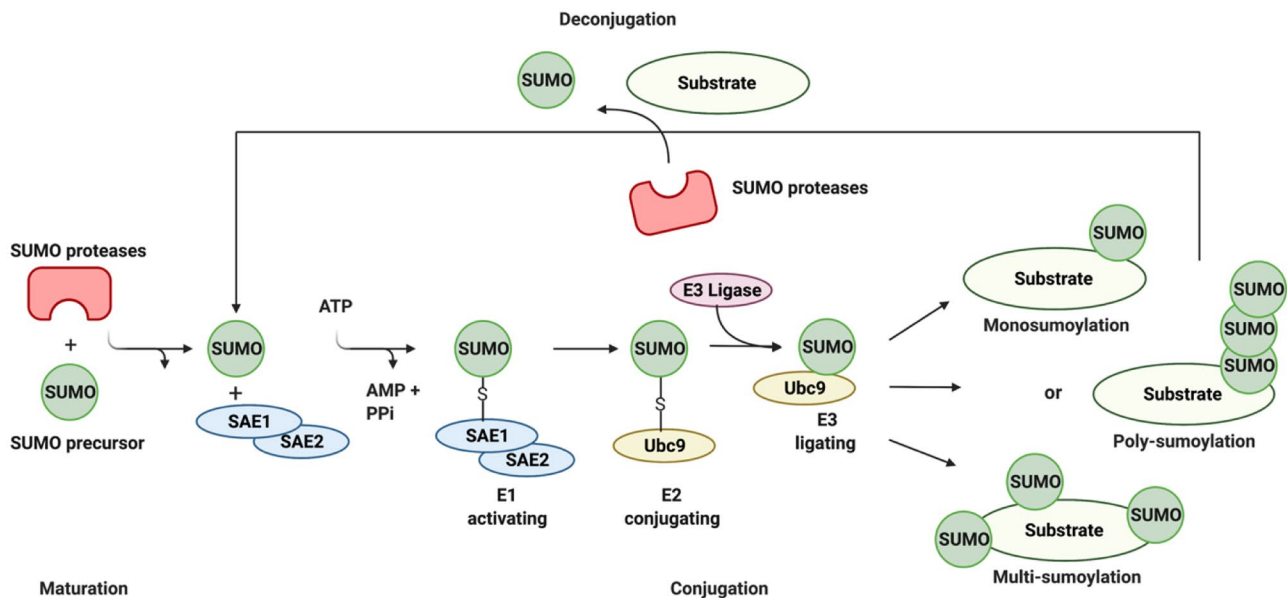


Fig. 1 SUMOylation: the Small Ubiquitin-related Modifier (SUMO) conjugation-deconjugation pathway. SUMO proteins are processed by a SUMO-specific protease to expose a diglycine (-GG) motif, resulting in a mature SUMO peptide (~12 kDa). Mature SUMO is activated in an ATP-dependent reaction, creating an intermediate thioester bond between the active site of SUMO and the heterodimeric E1-activating enzyme (Aos1/Uba2). Following activation, SUMO

is transferred from the E1 enzyme to Ubc9, the central E2 enzyme. Ubc9 may then directly facilitate the binding of SUMO to the target substrate (not shown), or may recruit an E3 enzyme, which functions in substrate recognition and specificity. SUMO is removed from target proteins (deconjugation) by SUMO proteases, making SUMOylation a reversible process. AMP adenosine monophosphate, PPI pyrophosphate, SAE1/SAE2 SUMO-activating enzyme

disease, increased understanding of the potential impacts of substrate/SUMO status on host response to challenge is critical for developing interventions. Thus, in this review, we describe the implications of SUMO in innate immunity and then focus on the various ways that viral pathogens have evolved to hijack the conserved SUMO machinery. This review concentrates on some of the mechanisms by which viruses manipulate the enzymatic components (E1, E2, E3) of the host SUMOylation machinery to promote viral replication and pathogenesis.

SUMOylation and innate immunity

The innate immune system is the first line of defense against viral infection. It plays a vital role in early detection, initiating interferon (IFN) synthesis, and promoting an antiviral environment. IFN is primarily secreted by activated T-cells and binds to interferon receptors on infected cells, activating the Jak/STAT pathway and the subsequent expression and activity of interferon-stimulated genes (ISGs) (Robertsen 2018; Tau and Rothman 1999). SUMOylation is suggested to play a role in regulating innate immunity by altering the production of type I interferons (Kim and Ahn 2015; Liu, et al. 2013a, b). Protein inhibitor of activated STAT-1 (PIAS1), a negative regulator of NF- κ B signaling, was also identified as a putative

SUMO E3 ligase, further implicating SUMOylation as a modulator of innate immunity (Liu et al. 2005). Additional analyses revealed that SUMO-conjugating enzyme Ubc9 conjugated SUMO1 to I κ B α (Desterro et al. 1998). Remarkably, SUMOylation of I κ B α occurs at lysine 21 residue, which is also required for its ubiquitination. Thus, SUMO1-modified I κ B α is resistant to ubiquitin-mediated proteasomal degradation (Desterro et al. 1998), providing early evidence for viral manipulation of host SUMO machinery. This antagonistic effect of SUMOylation results in the inhibition of NF- κ B transcription. Conversely, SUMO2/3 conjugation to NF- κ B essential modifier (NEMO/IKK γ) prevents binding of the deubiquitinase CYLD and potentiates IKK activation, leading to constitutive activation of the NF- κ B pathway (Liu et al. 2013a, b). This modification is reversed explicitly by the de-SUMOylating enzyme SENP6, indicating that the SUMOylation pathway can have both synergistic and antagonistic effects on the ubiquitination of a given substrate. Loss of SENP6 significantly potentiated TLR-mediated NF- κ B activation and inflammation (Liu et al. 2013a, b).

Given the dynamic role of protein SUMOylation on innate immunity, it is not surprising that pathogens have evolved to exploit the host SUMOylation machinery. During infection and replication, viruses can use the SUMOylation process to ensure viral persistence and replication. Some viruses mimic SUMO-targeted ubiquitin ligases (STUBLs),

a group of ubiquitin ligases that contain SUMO-interacting motifs (SIMs), targeting the ligases to SUMOylated proteins (Perry et al. 2008). For example, the HSV early viral protein ICP0 (infected cell polypeptide 0) is responsible for initiating lytic infection. HSV has STUbL properties and targets SUMOylated PML and Sp100 proteins that are important for tumor suppression (Boutell et al. 2003). ICP0 expression in a stable cell line resulted in a decrease in global SUMO conjugates, including SUMOylated forms of PML (Boutell et al. 2011). ICP0 was also shown to target PML more efficiently than SUMO in general (Boutell et al. 2011). These findings suggest that ICP0 counteracts the intrinsic anti-HSV defense by specifically targeting SUMO substrates and inducing proteasomal degradation.

A growing number of viruses have also been shown to target the SUMOylation enzymes to modulate global SUMOylation levels. Some viruses benefit from increased protein SUMOylation, while others benefit from inhibition of SUMOylation processes. For example, the EBV protein kinase BGLF4 suppresses global cellular SUMOylation to enhance extracellular virus production during lytic viral replication (Li et al. 2012). In contrast, influenza virus type A (IAV) infection significantly increases the SUMOylation of cellular substrates by both SUMO1 and SUMO2/3 (Domingues et al. 2015). These studies illustrate the importance of viral modulation of global SUMOylation processes during infections. While these studies provide a greater understanding of global changes in SUMOylation during viral infections, it is also essential to understand the specific cellular targets of SUMOylation processes that viruses use to promote proliferation and persistence. Understanding viral manipulation of specific components of the SUMOylation machinery may lead to new therapeutic targets that restore normal SUMOylation levels, thus inhibiting viral replication and pathogenesis.

Viral targeting of SUMOylation enzymes

Viruses and the E1 SUMO-activating enzyme

The adenovirus protein Gam1 is the only example of a viral protein that targets the SUMO-activating SAE1/2 heterodimer (Boggio et al. 2004). Gam1 is critical for viral replication and decreases the histone deacetylase, HDAC1 (Colombo et al. 2002). A later study revealed that Gam1 expression binds to SAE1/2 and inhibits the formation of the E1-SUMO intermediate *in vitro* (Boggio et al. 2007). Gam1 expression also significantly reduced the intracellular levels of SAE1/2-activating enzymes and the SUMO-conjugating enzyme Ubc9, although the mechanism is unknown (Boggio et al. 2007). Gam1 induces the reduction in SAE1/2 by recruiting two cellular ubiquitin ligases

to the SAE1/2 complex (Boggio et al. 2007). The ubiquitin ligase ubiquitinates SAE1, resulting in its degradation by the proteasome (Boggio et al. 2007). It was further revealed that the degradation of SAE2 was not directly related to Gam1 but is instead a consequence of SAE1 degradation. The stability of the SUMO E1 heterodimer is closely associated with the presence of both subunits, and the loss of the SAE1 subunit results in proteasome-mediated degradation of SAE2 (Boggio et al. 2007). Consequently, loss of the SUMO E1 heterodimer leads to the accumulation of unmodified SUMO substrates and accumulation of SUMO1 in the cytoplasm, creating a favorable cellular environment for the virus (Colombo et al. 2002). Although the avian adenovirus is currently the only virus reported to target the SUMO-activating enzyme, the capacity to modulate SUMOylation processes by interfering with the E1 heterodimer is likely for multiple viruses.

Viruses and Ubc9

Given the importance of the only reported E2 SUMO-conjugating enzyme in the SUMOylation pathway, Ubc9 is an ideal target for viruses (Fig. 2). Early reports identified the Adenoviral protein E1A as a binding partner of mouse Ubc9, which potentiates the effects of E1A on PML-NB size and quantity (Hateboer et al. 1996). This finding was significant because it was later discovered that differences in PML-NB size and number correlate with infection states of α -herpesviruses (Boutell and Everett 2013). Similarly, interactions with Ubc9 and the HIV-1 envelope protein gp120 stabilizes gp120, resulting in increased viral infectivity of HIV-1 (Jaber et al. 2009). In addition, during latent viral infections of EBV, latent membrane protein 1 (LMP1) interacts with Ubc9 resulting in increased SUMOylation of cellular proteins and subsequent modulation of innate immune responses (Bentz et al. 2011, 2012).

Many viral proteins are SUMOylated and target Ubc9 to facilitate their modification and subsequent localization and/or activity. For example, during human cytomegalovirus (HCMV) infection, the DNA polymerase subunit UL44 binds Ubc9 and is significantly modified by both SUMO1 and SUMO2/3, resulting in decreased UL44 expression and increased viral replication (Sinigalia et al. 2012). The nucleocapsid protein (NP) of Hantavirus mediates viral assembly of structural proteins, interacts with Ubc9, and is SUMOylated (Alfadhli et al. 2001). Similar to UL44, NP SUMOylation alters its subcellular localization and influences Hantavirus replication (Alfadhli et al. 2001; Maeda et al. 2003). In addition, the human papillomavirus (HPV) protein E2, which supports viral replication, also interacts with Ubc9 (Wu et al. 2008). The E2/ubc9 interaction results in the SUMOylation and increased transcriptional activity of HPV E2 (Wu et al. 2008). On the other hand,

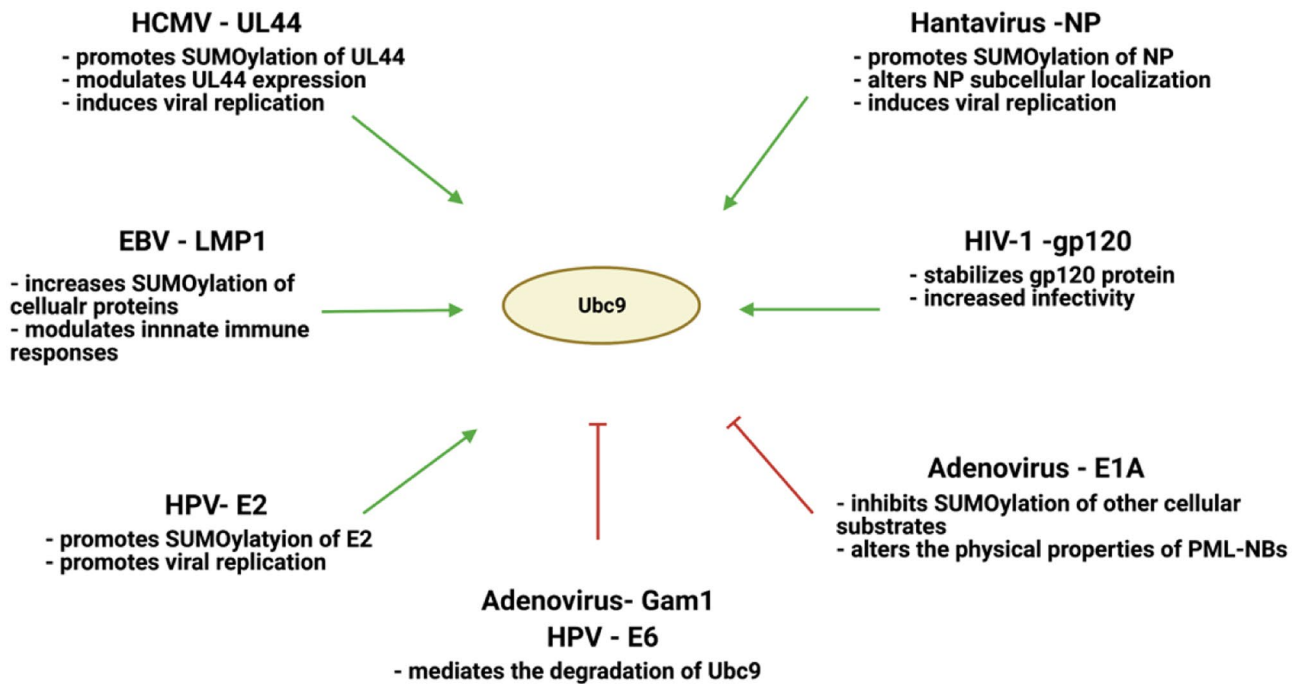


Fig. 2 Viral targeting of Ubc9. ADV Gam1 and HPV E6 induce the degradation of Ubc9, promoting viral replication. ADV E1A interaction with Ubc9 blocks SUMOylation of other cellular substrates and restricts innate immune responses. Conversely, EBV LMP1, HCMV UL44, Hantavirus NP, HPV E2, and HIV gp120 increase

the SUMOylation of cellular substrates, modulating viral replication. ADV adenovirus, Gam1; HPV human papilloma virus; EBV Epstein-Barr virus; LMP1 latent membrane protein 1; HCMV human cytomegalovirus; NP nucleocapsid protein

some viruses target Ubc9 to induce the degradation of this crucial SUMO-conjugating enzyme. As previously mentioned, the adenovirus protein Gam 1 expression also greatly reduced Ubc9 stability, promoting transcriptional activation and a favorable cellular environment for viral replication.

In addition, human papillomavirus (HPV) E6 protein targets Ubc9, resulting in proteasome-mediated degradation of Ubc9 (Heaton et al. 2011).

The nucleocapsid (N) protein of severe acute respiratory syndrome coronavirus (SARS-CoV) was also identified as an interacting partner of Ubc9 (Fan et al. 2006; Li et al. 2005). The coronavirus N protein is a multifunctional protein that plays an essential role in replicating genomic RNA and nucleocapsid assembly (Chang and Brian 1996). Biochemical analysis revealed that SUMO1 modified the N protein at the lysine 62 residue, resulting in the homo-oligomerization of the N protein (Li et al. 2005). Given that homo-oligomerization is essential for the proper activity of the N protein, SUMOylation of this protein could play a functional role in SARS-CoV replication (Fan et al. 2006). It is not known whether the N protein of SARS-CoV-2, which causes COVID-19, is also SUMOylated. However, given the significant similarity of the N protein sequence among coronaviruses, it is likely that the SARS-CoV-2 N

protein is modified by SUMO (Marra et al. 2003; Zhou et al. 2020). Notably, the N protein can stimulate strong immune responses, indicating the N protein as a potential target for vaccine development.

There is still much to be understood as to how different viral proteins influence Ubc9. Although several viral proteins target Ubc9 and are themselves substrates for SUMOylation, how these interactions affect the signaling pathways of the viral protein and Ubc9 requires further investigation. It is possible that in addition to direct interactions with Ubc9, viral proteins can target regulators of Ubc9. Deciphering the many ways viral proteins alter the expression and function of Ubc9 may identify potential targets for the treatment of viral-induced pathologies.

Viruses and SUMO E3 ligases

Substrate specificity is determined by SUMO E3 ligases and is essential in mediating conjugation to non-consensus lysine residues. However, SUMO E3 ligases are not always required for SUMOylation processes. Still, several viruses can manipulate SUMO E3 ligases to regulate viral replication (Fig. 3). The protein inhibitor of activated STATs (PIAS) family and RANB2 are among the most studied viral targeted SUMO E3 ligases. PIAS family members

E3 ligases

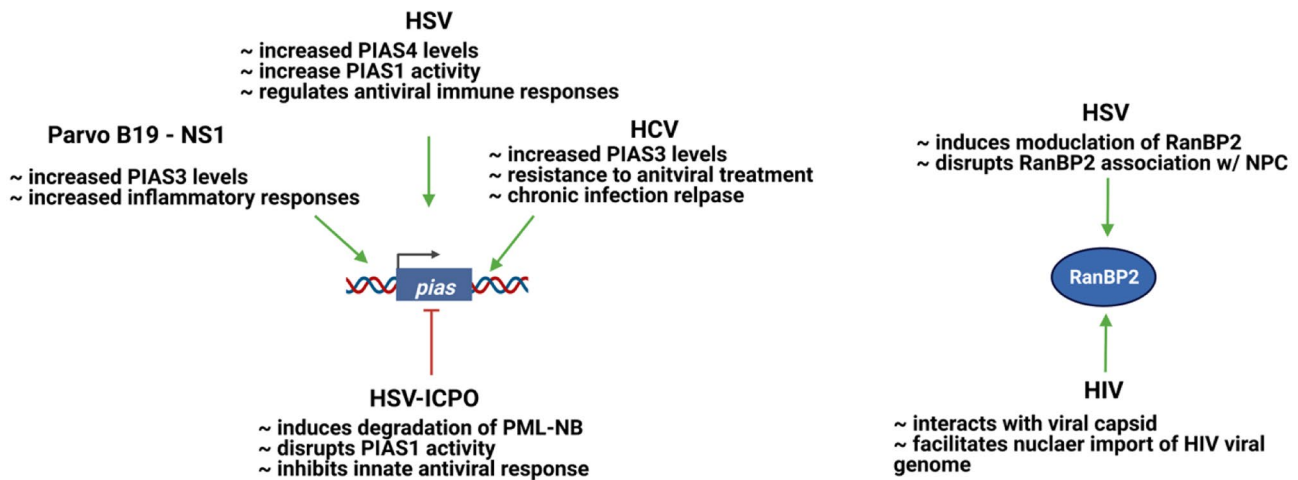


Fig. 3 Viral targeting of SUMO E3 ligases. The HSV ICP0 protein disrupts the activity of PIAS family members and counteracts innate immune responses. Parvovirus B19, HSV, and HCV induce the expression of PIAS1, 3, and/or 4 resulting in regulation of host immune responses and inflammation. The HIV capsid protein interacts with RanBP2 to facilitate nuclear import of HIV DNA. HSV

interacts with RanBP2 and disrupts its association with other NPC members, but the overall functional consequence has not been described. HSV herpes simplex virus, ICP0 infected cell polypeptide 0, PIAS protein inhibitor of activated STAT, HCV hepatitis C virus, NS1 nonstructural protein

participate in several cellular processes, including immune regulation, DNA repair, and cell proliferation (Shuai and Liu 2005). During HSV-1 infection, SUMO modifications play a role in PML-NB-mediated intrinsic antiviral immunity. PIAS4 is recruited to nuclear foci containing the viral genome to positively regulate antiviral immune responses to HSV-1 (Conn et al. 2016). Similarly, PIAS1 is recruited to sites adjacent to HSV/PML-NB associated protein colocalization (Brown et al. 2016). This recruitment of constituent antiviral PML-NB factors correlates well with a cooperative restriction of viral gene expression (Everett et al. 2008; Glass and Everett 2013). The innate antiviral response to HSV-1 infection is guided by SUMO-dependent recruitment of PIAS4 and PIAS1. HSV-1 counteracts this component of host immunity via the expression of ICP0, the viral E3 ubiquitin ligase with SUMO-targeted ubiquitin ligase (STUbL)-like properties (Boutell et al. 2011). ICP0 induces proteasomal-mediated degradation of PML-NB-associated factors, inhibiting the cellular restriction of viral gene expression (Boutell and Everett 2013).

Conversely, contrasting roles of PIAS as positive regulators of the innate antiviral immune response are reported, as well. Overexpression of duck PIAS2 (duPIAS2) promotes viral replication of the highly pathogenic avian influenza virus (HPAIV) H₅N₁ (Zu et al. 2020). PIAS2 influences papillomavirus replication in mammalian cells by interacting with the viral helicase E1 protein, stimulating its SUMOylation (Rosas-Acosta et al. 2005). The parvovirus

B19 viral protein NS1 transactivates several cellular promoters, including *pias3*, and elevated PIAS3 levels are implicated in exacerbated inflammatory responses and endothelial cell dysfunction during infection (Duechting et al. 2008). Increased PIAS3 levels are also associated with relapse of chronic hepatitis C viral (HCV) infection and resistance to antiviral treatment (Heim et al. 1999). Taken together, these studies revealed a dynamic role of PIAS proteins as intrinsic factors that can restrict or promote viral infection.

While fewer studies address the viral manipulation of the nuclear pore protein RanBP2 compared to PIAS family members, the viral-induced modulation of RanBP2 is thought to regulate the shuttling of proteins and viral genomes into and out of the nucleus. HSV infection induces the modulation of RanBP2 by disrupting its association with other members of the nuclear pore complex (Hofemeister and O'Hare 2008). RanBP2 is also essential for the nuclear import of the HIV-1 viral genome via interactions with the viral capsid (Bichel et al. 2013; Zhang et al. 2010). Consistent with this finding, specific RanBP2 mutations bear strong signatures of virus-driven positive selection during primate evolution (Meyerson et al. 2014). Mutations of a positively selected residue in RanBP2 were shown to affect its interactions with the HIV capsid. However, the mechanistic link between capsid-RanBP2 binding and the nuclear import of the viral genome is unknown (Schaller et al. 2011).

Viruses and SENPs

SENPs are the central element of the SUMOylation machinery as they are responsible for regulating the deconjugation of SUMO from its substrates (de-SUMOylation) and overall levels of free SUMO. However, limited data exist on the effects of viral manipulation of SENPs (Fig. 4). In hepatitis B virus (HBV)-infected cells, the HBV X protein (HBx) alters crucial cellular pathways and promotes de-SUMOylation of the host transcription factor Sp110 (Sengupta et al. 2017). Mechanistically, HBx promotes the formation of an Sp110-SEN1-HBx complex, thereby translocating the Sp110 transcription factor to reprogram host gene expression and promote viral proliferation (Sengupta et al. 2017). Kaposi's sarcoma-associated herpesvirus (KHSV) uses a more intricate mechanism that exploits both SUMOylation and de-SUMOylation machinery to promote its replication. During the latent phase, the latent-associated nuclear antigen (LANA) interacts with SUMO2/3 modified proteins to silence viral gene expression (Cai et al. 2013). Interestingly, LANA itself is SUMOylated, and its expression levels are explicitly regulated by SENP6 (Lin et al. 2017). Chromatin immunoprecipitation coupled with sequencing (ChIP-seq) experiments revealed that LANA binds the SENP6 promoter, repressing SENP6 expression (Lin et al. 2017). SENP6 overexpression impeded the establishment of latency as a result of the decrease in the abundance of LANA. These data suggest that LANA inhibits SENP6 from regulating its own SUMOylation and expression levels as a means of maintaining KHSV latency (Lin et al. 2017).

The previously discussed EBV oncoprotein LMP1 also inhibits SUMO-protease activity, resulting in the accumulation of SUMOylated proteins (Selby et al. 2019). Specifically,

LMP1 induces the SUMOylation of the SUMO-protease SENP2 at lysine residues K48 and K447, resulting in reduced SENP2 function, trafficking, and stability (Selby et al. 2019). Interestingly, while mutations of SENP2 K447 reversed LMP1-mediated inhibition of SENP2 activity, mutation of SENP2 K48 failed to abrogate LMP1-mediated inhibition. However, SENP2 localization and stability were not altered when SENP2 K48 and/or K447 were mutated, demonstrating that targeting these residues by LMP1 is not required for SENP2 turnover or distribution (Selby et al. 2019). However, these effects appeared to depend on a functional C-terminal activating region (CTAR) domain of LMP1. Researchers examined if SENP2 was ubiquitinated during EBV latency to better understand this variance, given that SUMO can, directly and indirectly, compete with ubiquitin (Kerscher et al. 2006; Kroetz 2005). Lower levels of ubiquitinated SENP2 were detected in LMP1-expressing lysates as compared to control-expressing lysates. Inhibition of ubiquitination processes also exhibited the observed changes in SENP2 biology as LMP1-mediated SUMOylation (Selby et al. 2019). These results demonstrate that LMP1 modulates the activity, stability, and trafficking of SENP2 by manipulating both SUMOylation and ubiquitin machinery during latent EBV infections.

Much like LANA's role in KHSV latency, these findings highlight the importance of post-translational modifications in latent viral infections. However, the functional role of PTMs like SUMOylation in viral latency remains largely unexplored. For example, the co-repressor COUP-TF interacting protein 2 (CTIP2) recruits a multi-enzymatic complex to the HIV-1 promoter, preventing the transcription of integrated viral DNA (Marban et al.

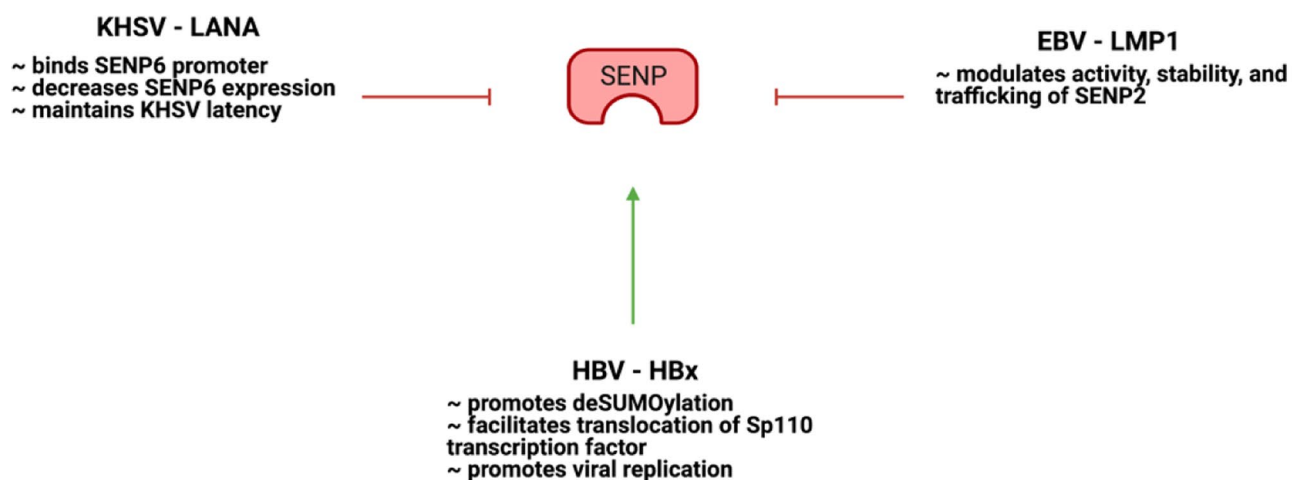


Fig. 4 Viral targeting of SUMO-specific proteases (SENPs). KHSV LANA and EBV LMP1 block cellular SENP by inhibiting its transcription and facilitating its degradation, respectively. The HBV HBx protein induces SENP activity and facilitates de-SUMOylation of

cellular substrates. KHSV Kaposi's sarcoma-associated herpesvirus, LANA latency-associated nuclear antigen, EBV Epstein-Barr virus, LMP1 latent membrane protein 1, SENP SUMO-specific protease, HBV hepatitis B virus, HBx hepatitis B virus protein X

2007). However, the mechanisms associated with establishing and maintaining this complex at the HIV-1 promoter remain unclear. Studies have shown that this major regulator protein, CTIP2, is SUMOylated by SUMO1 and that PTMs, including phosphorylation and SUMOylation, mediate its interactions with other proteins and complexes (Dubuissez et al. 2016; Tirard et al. 2012). Specifically, CTIP2 contains a motif, also known as a phosphorylation-dependent SUMOylation motif (PDSM), that, when phosphorylated, leads to enhanced SUMOylation of CTIP2 (Hietakangas et al. 2006). The SUMOylation of CTIP2 ultimately results in its proteasomal degradation (Zhang et al. 2012). This “SUMO switch” for CTIP2-mediated transcriptional repression of HIV-1 in microglia or other target cells is of great interest.

Maintaining viral latency in cells with incorporated viral DNA is essential to prevent reactivation. In the case of people with HIV (PWH), lifelong antiretroviral therapy (ART) is required, as latently infected cells of the CNS are from time to time reactivated and can produce virus or viral proteins (Wallet et al. 2019). Previous studies investigating HIV-1 latency in microglia found that the tripartite motif-containing 28 (TRIM28) protein, a known SUMO E3 ligase, associates with CTIP2, a known SUMO substrate, and contributes to the establishment and persistence of HIV-1 latency (Ait-Ammar et al. 2021; Ivanov et al. 2007). Mechanistically, CTIP2 directs Tat relocation from the Tat/TAR complex to the viral latency complex, while TRIM28 interacts and colocalizes with Tat to promote its degradation via the proteasome pathway (Ait-Ammar et al. 2021). Interestingly, a recent study has shown that HIV-1 Vpr mediates the depletion of CTIP2 to counteract viral gene silencing (Forouzanfar et al. 2019). Whether Vpr hijacks the SUMOylation machinery to promote the degradation of CTIP2 is unknown. The recognition of TRIM28 as a SUMO E3 ligase and CTIP2 as a SUMO substrate may have profound therapeutic implications.

Summary

Since its discovery, SUMO modification has been established as an important regulator of several biological pathways. Here, we have outlined the implications of SUMOylation in innate immunity and the interplay between viruses and the host SUMOylation machinery. Given that SUMOylation helps regulate antiviral immune responses, pathogens have evolved various means of subverting this innate host defense. Viruses have adapted several mechanisms to manipulate different steps of the SUMOylation machinery for their benefit. A growing number of viral proteins have been identified that can control SUMOylation, both on global and substrate-specific levels. Numerous studies have addressed

the manipulation of these targets as a regulatory adaptation for viral replication and pathogenesis. However, it is clear that viruses target the SUMOylation machinery to modulate both lytic and lysogenic stages of their life cycle.

In most cases, the overall effect of viral manipulation of host SUMOylation pathways is unclear. Many unidentified viral proteins are likely capable of impacting host protein SUMOylation pathways. In fact, the role of PTMs, like SUMOylation, during latent infections has also been largely unexplored. Identifying these players will provide novel insight into the regulation of viral pathogenesis with the potential to uncover new targets for antiviral therapies.

Abbreviations ADV: Adenovirus; ART: Antiretroviral therapy; CTAR: C-terminal activating region; CTIP2: COUP TF-interacting protein 2; CYLD: CYLD lysine 63 deubiquitinase; gp120: Envelope glycoprotein 120; EBV: Epstein-Barr virus; HBV: Hepatitis B virus; HBx: Hepatitis B virus X protein; HCV: Hepatitis C virus; HSV: Herpes Simplex virus; HPAIV: Highly pathogenic avian influenza virus; HCMV: Human cytomegalovirus; HIV: Human immunodeficiency virus; HPV: Human papillomavirus; ICP0: Infected cell polypeptide 0; IKK: IκB kinases; KHSV: Kaposi's sarcoma-associated herpesvirus; LMP1: Latent membrane protein 1; LANA: Latent-associated nuclear antigen; NEMO: NF-κB essential modulator; NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; IκBa: Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; NPC: Nuclear pore complex; NP: Nucleocapsid protein; PDSM: Phosphorylation-dependent SUMOylation motif; PTM: Post-translational modification; PML-NB: Promyelocytic leukemia protein nuclear bodies; PIAS: Protein inhibitor of activated STATS; RanBP2: RAN binding protein 2; RanGAP1: Ran-GTPase activating protein; RING: Really Interesting New Gene; SARS-CoV: Severe acute respiratory syndrome coronavirus; SUMO: Small ubiquitin-related modifier; Sp100: Speckled 110 kDa; SAE1/2: SUMO-activating enzyme 1 and 2; SIM: SUMO-interacting motifs; SENP: SUMO-specific protease; TLR: Toll-like receptor; Ubc9: Ubiquitin-conjugating 9

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Declarations

Conflicts of interest The authors declare no competing interests.

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