

Regulation of the nucleocytoplasmic trafficking of viral and cellular proteins by ubiquitin and small ubiquitin-related modifiers

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Nucleocytoplasmic trafficking of many cellular proteins is regulated by nuclear import/export signals as well as post-translational modifications such as covalent conjugation of ubiquitin and small ubiquitin-related modifiers (SUMOs). Ubiquitination and SUMOylation are rapid and reversible ways to modulate the intracellular localisation and function of substrate proteins. These pathways have been co-opted by some viruses, which depend on the host cell machinery to transport their proteins in and out of the nucleus. In this review, we will summarise our current knowledge on the ubiquitin/SUMO-regulated nuclear/subnuclear trafficking of cellular proteins and describe examples of viral exploitation of these pathways.

Introduction

The replication of many viruses (particularly DNA viruses and retroviruses) involves nuclear steps (Griffin et al., 2007). Even for viruses that replicate in the cytoplasm (*e.g.* many negative-stranded RNA viruses), it is not uncommon for specific viral proteins to enter the nucleus during certain stages of infection to achieve specific functions such as antagonising the host's immune activation (Katze

et al., 2002; Ramachandran and Horvath, 2009; Goodbourn and Randall, 2009) or global inhibition of host gene expression (Petersen et al., 2000; von Kobbe et al., 2000). The nuclear import and export of viral proteins depend on the host cell machinery and are subject to regulation by pathways that normally modulate the trafficking of cellular proteins, including post-translational modifications. Conjugation of modifying groups such as phosphate, acetyl group, ubiquitin (Ub), and Ub-like molecules is a rapid and efficient way of modulating the properties of proteins after their synthesis (Ulrich, 2005). Particularly, Ub and small ubiquitin-related modifiers (SUMOs) have drawn much attention in recent years due to their roles in diverse biological functions (Denuc and Marfany, 2010; Liu and Walters, 2010). Although the regulatory functions of Ub and SUMO in the nucleocytoplasmic trafficking of cellular proteins have been extensively studied (Wilson and Rangasamy, 2001; Pichler and Melchior, 2002; Shcherbik and Haines, 2004), their involvement in viral protein trafficking has been only recently appreciated. In this review, we will summarise our current knowledge on Ub- and SUMO-regulated nucleocytoplasmic trafficking of cellular proteins and give a few examples of how these pathways are exploited by viruses.

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Key words: Post-translational modification, Proteasomes/ubiquitin, Protein sorting/trafficking/targeting, Viruses.

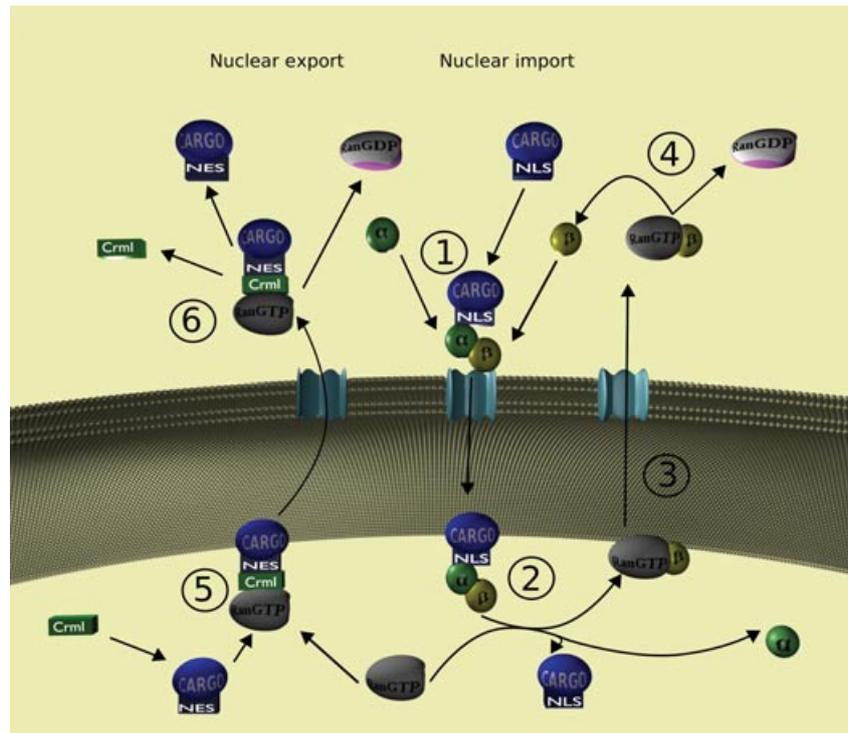
Abbreviations used: Ad5, adenovirus type 5; BPV, bovine papillomavirus; CCT-a, CTP-phosphocholine cytidyltransferase; CREB, cAMP response element-binding protein; CRM-1, chromosome region maintenance 1; CtBP, C-terminal binding protein of adenovirus E1A; EBNA, Epstein-Barr virus nuclear antigen; EBV, Epstein-Barr virus; ESCRT, endosomal sorting complex required for transport; HCMV, human cytomegalovirus; HHV-6, human herpesvirus 6; HIF- α , hypoxia inducible factor alpha; HPV, human papillomavirus; HTLV-1, human T-cell leukaemia virus type 1; IGF-1R, insulin-like growth factor 1 receptor; IKK, I κ B kinase; I κ B α , inhibitor of NF- κ B alpha; KLF5, Krüppel-like factor 5; KSHV, Kaposi's sarcoma-associated herpesvirus; LMB, leptomycin B; NEMO, NF- κ B essential modulator; NES, nuclear export signal; NIV, Nipah virus; NLS, nuclear localisation signal; PIAS, protein inhibitor of activated STAT; PML, promyelocytic leukaemia protein; PTEN, phosphatase and tensin homolog on chromosome 10; RanGAP1, Ran GTPase-activating protein 1; RARA, retinoic acid receptor alpha; Smad3, Smad and MAD-related protein 3; STUbL, SUMO-targeted ubiquitin ligase; SUMO, small ubiquitin-related modifier; TSS, Tax speckled structure; Ub, ubiquitin; VHL, von Hippel-Lindau protein.

Figure 1 | Simplified illustration of Ran-dependent nuclear import and export pathways

Steps (1) to (4) illustrate importin α/β -dependent nuclear import; steps (5) and (6) illustrate CRM-1-dependent nuclear export.

(1) Importin α binds to the NLS of the cargo protein and forms a complex with importin β . The complex is transported into the nucleus through the nuclear pore complex. (2) In the nucleus, binding of RanGTP to importin β results in its dissociation from importin α and the release of the cargo. (3) RanGTP-bound importin β is exported to the cytoplasm. (4) Importin β is released upon hydrolysis of RanGTP to RanGDP, allowing for a new cycle of nuclear import.

(5) CRM-1 binds to the NES of the cargo protein and forms a complex with RanGTP. (6) The complex is transported into the cytoplasm, where hydrolysis of RanGTP results in the release of the cargo.

**Nuclear import and export machinery**

For proteins larger than 30 kDa, efficient trafficking across the nuclear membrane requires transporters and depends on the presence of specific nuclear import/export signals (Güttler and Görlich, 2011; Jamali et al., 2011). In most cases, these signals are recognised by import or export receptors of the karyopherin- β family either directly or via an adapter (Sorokin et al., 2007). The best-characterised nuclear import pathway involves importin α/β , which mediates the nuclear translocation of nuclear localisation signal (NLS)-containing cargoes in a Ran-dependent manner, as illustrated in Figure 1. Similarly, the export transporter chromosome region maintenance 1 (CRM-1; or exportin 1) mediates the nuclear export of a large number of cargoes containing nuclear export signals (NESs) [steps (5) and (6) in Figure 1]. For

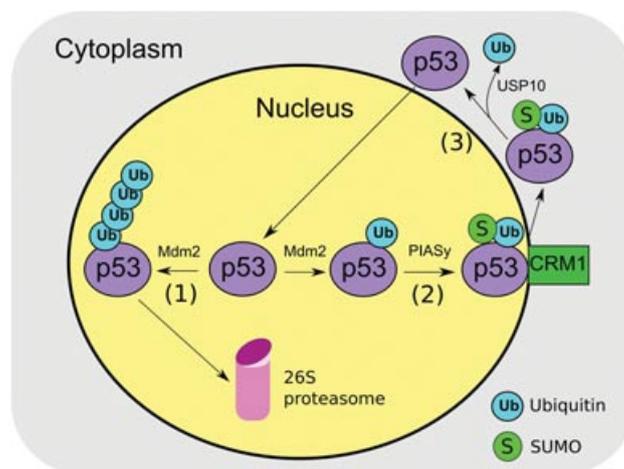
a comprehensive review of the nuclear import/export machinery, readers are referred to Moroianu (1999), Sorokin et al. (2007) and Jamali et al. (2011). In addition, the nucleocytoplasmic trafficking of many proteins is also subject to regulation by post-translational modifications such as phosphorylation, ubiquitination and SUMOylation (Jans and Hübner, 1996; Wilson and Rangasamy, 2001; Shcherbik and Haines, 2004).

Ub and SUMO pathways

Ubiquitin is a small 76-amino-acid protein modifier that is covalently attached to the substrate protein usually via an acceptor lysine residue. Ub conjugation occurs by the sequential action of three enzymes: (i) a Ub-activating enzyme (E1), (ii) a

Figure 2 Schematic illustration of p53 nucleocytoplasmic trafficking regulated by Ub and SUMO

(1) p53 polyubiquitination by Mdm2 leads to protein degradation (Li et al., 2003). (2) p53 monoubiquitination by Mdm2 and SUMOylation by PIASy cooperatively lead to nuclear export (Li et al., 2003, Carter et al., 2007). (3) Demodified p53 translocates back to the nucleus (Yuan et al., 2010) (see text for details).



Ub-conjugating enzyme (E2) and (iii) a Ub ligase (E3), which determines substrate specificity and the type of conjugation (*e.g.* monoUb versus polyUb, K48- versus K63-linked Ub). The ubiquitination machinery has been reviewed in Kerscher et al. (2006). Though initially described as a regulator of protein degradation (Wilkinson et al., 1980), Ub is now known to play important roles in a broad spectrum of cellular activities such as intracellular protein trafficking, transcriptional regulation, DNA repair, cell-cycle progression and apoptosis (Shcherbik and Haines, 2004; Liu and Walters, 2010).

SUMO's involvement in nuclear trafficking was initially suggested by its first known substrate, Ran GTPase-activating protein 1 (RanGAP1), which is a part of the nuclear import/export machinery (Cole and Hammell, 1998; Fried and Kutay, 2003). Unmodified RanGAP1 is diffused in the cytoplasm, whereas SUMO conjugation targets it to the nuclear pore complex, where it regulates protein/RNA trafficking across the nuclear membrane (Matunis et al., 1996; Mahajan et al., 1997; Matunis et al., 1998). Since then SUMO has been associated with the nuclear/subnuclear targeting of various cellular proteins (discussed below). The vertebrate genome encodes at least four SUMO isoforms, namely SUMO1-4. Similar to ubiquitination, SUMOylation is a

three-step process involving an E1-activating enzyme heterodimer Aos/Uba2, an E2-conjugating enzyme Ubc9 and substrate-specific E3 ligases (reviewed in Gareau and Lima, 2010).

Given that proteins can be modified by both Ub and SUMO, sometimes on the same lysine residue, it is not surprising that these two modification systems communicate. Indeed, the interaction between Ub and SUMO pathways is complex and could be either competitive, cooperative or differential (Ulrich, 2005; Denuc and Marfany, 2010). In the following section, we will briefly summarise examples of cellular proteins whose nucleocytoplasmic trafficking is regulated by Ub and/or SUMO and discuss the crosstalk between the two pathways so as to provide a better context when we later review viral exploitation of these pathways.

Ub/SUMO-regulated nucleocytoplasmic trafficking of cellular proteins

Ub and nuclear import/export

Ubiquitination is usually associated with cytoplasmic localisation via promoting either nuclear export or cytoplasmic retention. The best-characterised example is the tumour suppressor gene p53 (reviewed in Shcherbik and Haines, 2004; Stehmeier and Muller, 2009; Lee and Gu, 2010). P53 is

ubiquitinated on its six C-terminal lysine residues by the E3 Ub ligase Mdm2 (Honda et al., 1997; Rodriguez et al., 2000; Lohrum et al., 2001; Nakamura et al., 2002). Although polyubiquitination results in proteasomal degradation, monoubiquitination by the same E3 ligase leads to nuclear export (Li et al., 2003) [pathways (1) and (2) in Figure 2]. It has been postulated that Ub modification induces a conformational change, leading to unmasking of the NES which resides in the proximity of the Ub sites (Shcherbik and Haines, 2004). Once in the cytoplasm, p53 can be deubiquitinated by the deubiquitinase USP10, resulting in its translocation back to the nucleus (Yuan et al., 2010) [pathway (3) in Figure 2]. Polyubiquitination of transcription factors Smad and MAD-related protein 3 (Smad3) and hypoxia inducible factor alpha (HIF- α) has also been linked to their export from the nucleus and termination of transcriptional activation (Fukuchi et al., 2001; Groulx and Lee, 2002). Alternatively, ubiquitination can favour cytoplasmic localisation by inhibiting nuclear import, which is exemplified in the case of CTP-phosphocholine cytidyltransferase (CCT- α). The regulatory enzyme CCT- α can switch between an inactive form in the cytoplasm and an active form in the nucleus. It possesses an NLS located in close proximity of the putative ubiquitination site. By using CCT- α -Ub hybrid constructs that vary in the intermolecular distance between Ub and the NLS, the authors showed that monoubiquitination masks the NLS, resulting in the disruption of importin- α binding and consequently a blockage in the nuclear import (Chen and Mallampalli, 2009).

There are currently few examples of Ub playing a direct role in the nuclear import of target proteins. A good example is the tumour suppressor phosphatase and tensin homolog on chromosome 10 (PTEN) (Trotman et al., 2007). Though no conventional NLSs have been identified in PTEN, the tumour suppressor is able to translocate into the nucleus. Ubiquitination on lysine residue K289 likely plays a role in the nuclear import of PTEN because the K289E mutant, associated with Cowden syndrome, is defective in nuclear import, monoubiquitination, as well as its tumour suppression ability. The authors further identified another monoubiquitination site K13, which is also found to be mutated in spontaneous cancer, as being important for nuclear import.

A list of cellular proteins whose nuclear import/export is regulated by ubiquitination can be found in Table 1.

SUMO and nuclear import/export

In contrast to Ub, SUMO is usually associated with nuclear/subnuclear targeting. The first identified SUMO substrate RanGAP1 is targeted to the nuclear pore complex and binds to the nucleoporin RanBP2/Nup358 upon SUMOylation (Matunis et al., 1996; Mahajan et al., 1997; Matunis et al., 1998). Because RanBP2 is part of the nucleocytoplasmic transport machinery and itself has SUMO E3 ligase activity (Mattaj and Englmeier, 1998; Görlich and Kutay, 1999; Pichler et al., 2002), it has been postulated that SUMOylation might be coupled to translocation through the nuclear pore (Pichler and Melchior, 2002).

Examples of proteins whose intracellular distribution is affected by SUMOylation are listed in Table 1. In the case of Krüppel-like factor 5 (KLF5), SUMOylation on a site close to the endogenous NES inhibits the NES function and leads to nuclear retention (Du et al., 2008). SUMO also enhances the nuclear accumulation of insulin-like growth factor 1 receptor (IGF-1R) (Sehat et al., 2010), cAMP-response-element-binding protein (CREB) (Comerford et al., 2003) and C-terminal binding protein of adenovirus E1A (CtBP) (Lin et al., 2003b). SUMO site mutation favours cytoplasmic localisation in all three cases; however, whether this is mediated through promoting nuclear import or inhibiting nuclear export is not clear. On the other hand, nuclear targeting of promyelocytic leukaemia protein (PML) *per se* does not seem to require SUMOylation, as mutating the SUMO site does not prevent its nuclear accumulation. However, SUMOylation of PML is important for its localisation in nuclear bodies (Zhong et al., 2000; Lallemand-Breitenbach et al., 2001).

The involvement of SUMO in regulating nuclear export has been suggested by studies of the retinoic acid receptor alpha (RARA) (Zhu et al., 2009) and the E-twenty-six family transcriptional repressor TEL (Wood et al., 2003). In both cases, the putative SUMO site mutants are restricted to the nucleus. However, one caveat is that ubiquitination was not examined in these studies and the observed effect, therefore, could potentially be due to the lack of ubiquitination.

Table 1 | Nucleocytoplasmic trafficking of cellular proteins regulated by Ub and/or SUMO

Protein	Major functions	Ub regulation	SUMO regulation	References
CCT α	Enzyme involved in the synthesis of phosphatidylcholine	Ub site is in close proximity of the NLS. Ub results in masking of the NLS and disruption of importin- α binding, leading to an inhibition of nuclear import.	Not examined	Chen and Mallampalli, 2009
CREB	Transcriptional regulation	Phosphorylation-dependent ubiquitination results in proteasomal degradation in hypoxia	SUMO conjugation on a lysine residue within the NLS is important for nuclear import	Taylor et al., 2000; Comerford et al., 2003
CtBP	Transcription repressor	Not examined	Mutation of the SUMOylation site shifts CtBP from the nucleus to the cytoplasm and abolishes its transcriptional repression activity	Lin et al., 2003b
DdMEK1	Kinase involved in the chemotaxis of dictyostelium	Ub contributes to nuclear targeting	Chemoattractant stimulation induces SUMOylation of MEK1, which is required for its translocation from the nucleus to the cytosol and cortex	Sobko et al., 2002
DEDD	Apoptosis regulator I	Ub induces cytoplasmic localisation and higher proapoptotic capacity	Not examined	Lee et al., 2005
HIF- α	Transcription factor	Under hypoxic conditions, HIF- α disassociates from the E3 ligase complex and accumulates in the nucleus. Upon return to normoxia, it binds to the ligase complex and polyUb leads to nuclear export and degradation.	SUMOylation increases HIF- α 's stability and its transcriptional activity	Groulx and Lee, 2002; Bae et al., 2004
HIPK2	Transcriptional regulation	PolyUb leads to protein degradation	SUMOylation is important for HIPK2 localisation to nuclear speckles	Kim et al., 1999; Choi et al., 2008
IGF-1R	Receptor tyrosine kinase	Phosphorylation-dependent ubiquitination is important for downstream signalling	Ligand-dependent SUMOylation is required for nuclear translocation	Sehat et al., 2007; Sehat et al., 2010
I κ B α	Inhibitor of NF- κ B	Ub results in proteasomal degradation, leading to NF- κ B activation	SUMOylation of I κ B α results in its resistance to Ub-mediated proteasomal degradation, resulting in the retention of NF- κ B in the cytoplasm	Desterro et al., 1998
KLF5	Transcription factor	Ub leads to rapid proteasomal degradation	SUMO site is close to the NES. SUMOylation enhances nuclear localisation by inhibiting the NES function.	Chen et al., 2005; Du et al., 2008
Lef1	Transcription factor	Not examined	SUMOylation by E3 ligase PIASy promotes Lef1 localisation to PML bodies and represses its function	Sachdev et al., 2001
NEMO	Regulatory subunit of the IKK complex	Upon genotoxic stress, sequential modification of NEMO by SUMO and Ub cooperatively leads to NF- κ B activation		Huang et al., 2003

(Continued)

Table 1 | Continued

Protein	Major functions	Ub regulation	SUMO regulation	References
p53	Tumor suppressor	MonoUb on C-terminal lysine residues by Mdm2 promotes nuclear export, whereas polyUb leads to proteasomal degradation.	SUMOylation by PIASy E3 ligase promotes its dissociation from Mdm2 and nuclear export	Honda et al., 1997; Rodriguez et al., 2000; Lohrum et al., 2001; Nakamura et al., 2002; Li et al., 2003; Carter et al., 2007
PML	Major component of the PML nuclear bodies	SUMO-dependent E3 Ub ligase RNF4 ubiquitinates PML and causes its proteasomal degradation	SUMO modification is important for nuclear body localisation	Müller et al., 1998; Duprez et al., 1999; Tatham et al., 2008
PTEN	Tumor suppressor	MonoUb by Nedd4 on residues K13 and K289 is required for nuclear import. Nuclear localisation is also controlled by the deubiquitinating enzyme HAUSP.	Not examined	Trotman et al., 2007; Song et al., 2008
RanGAP1	Nucleocytoplasmic transport	Not examined	SUMO modification is required for its interaction with RanBP2 and targeting to the nuclear pore complex	Matunis et al., 1996; Mahajan et al., 1997; Matunis et al., 1998
RARA	Transcriptional regulation	Not examined	SUMO2 modification is involved in the nucleocytoplasmic shuttling of RARA	Zhu et al., 2009
Smad3	Transcription factor	PolyUb by ROC1-SCF complex leads to nuclear export and degradation, resulting in the termination of Smad3-induced transcriptional activation	PIASy-mediated SUMOylation stimulates nuclear export	Fukuchi et al., 2001; Imoto et al., 2008
Smad4	Transcriptional regulation	MonoUb of Smad4 enhances its transcriptional activity, whereas polyUb leads to proteasomal degradation	SUMOylation is mediated by PIAS family E3 ligases and occurs in the nucleus. SUMO enhances nuclear retention of Smad4 and increases its stability.	Lin et al., 2003a; Lee et al., 2003; Morén et al., 2003
Sp3	Transcription factor	Not examined	SUMO modification by PIAS1 is required for inactivation of Sp3 and its translocation to nuclear periphery and nuclear dots	Ross et al., 2002; Sapetschnig et al., 2002
TEL	Transcriptional repressor	Not examined	SUMOylation is important for nuclear speckle targeting. SUMO might also be important for nuclear export.	Chakrabarti et al., 2000; Wood et al., 2003
VHL	Tumor suppressor	Ub contributes to nuclear export	SUMOylation by PIASy E3 ligase promotes nuclear localisation	Cai and Robertson, 2010

Crosstalk between Ub and SUMO systems

Because proteins can be modified by both Ub and SUMO, the interaction between the two systems determines the eventual outcome of the modification.

When the same lysine residue serves as both Ub and SUMO sites, as seen in the examples of the inhibitor of NF- κ B alpha (*I κ B α*) (Desterro et al., 1998) and

von Hippel–Lindau protein (VHL) (Cai and Robertson, 2010), modifications by Ub and SUMO usually have opposing effects on the localisation and functions of the target protein, although there may be exceptions (discussed below). For example, although ubiquitination of *I κ B α* leads to its degradation and consequently the nuclear translocation of NF- κ B subunits, SUMOylation leads to its resistance to

Ub-mediated proteasomal degradation, resulting in retention of NF- κ B in the cytoplasm (Desterro et al., 1998). VHL is SUMOylated by the PIASy E3 ligase, which promotes its nuclear localisation, whereas ubiquitination on the same lysine residue contributes to nuclear export (Cai and Robertson, 2010).

However, ubiquitination and SUMOylation occurring on the same lysine residue do not necessarily lead to antagonistic effects on the function of the target protein, as exemplified by the sequential modifications of the NF- κ B essential modulator (NEMO) during genotoxic stress (Huang et al., 2003). After treatment with DNA-damaging reagents, I κ B kinase (IKK) complex activation requires the passage of the regulatory subunit NEMO through the nucleus. Although SUMOylation seems to target NEMO to the nucleus, ubiquitination, which occurs at a later time point, results in the shuttling of the protein back to the cytoplasm. In this case, the sequential modifications by SUMO and Ub cooperatively lead to the activation of IKK.

Ubiquitin and SUMO also appear to cooperate in regulating the nuclear export of p53. As discussed in the section 'Ub and nuclear import/export,' p53 nuclear export depends on monoubiquitination on C-terminal lysine residues (Lohrum et al., 2001; Li et al., 2003). Carter et al. (2007) have shown that SUMOylation also plays a role in this process. According to the model proposed by these authors, the attachment of a single Ub serves as an initiation event, allowing for access to the NES and recognition of p53 by SUMO E3 ligases such as PIASy, which leads to further modification of p53 to release Mdm2 and allow nuclear export [pathway (2) in Figure 2].

Recent identification and characterisation of SUMO-targeted ubiquitin ligases (STUbLs) in yeast (reviewed in Geoffroy and Hay, 2009), fly (Barry et al., 2011; Abed et al., 2011a) and mammalian cells (Lallemand-Breitenbach et al., 2008; Tatham et al., 2008; Mukhopadhyay et al., 2010) have added yet another layer of complexity to the Ub/SUMO network. STUbLs selectively ubiquitinate SUMOylated proteins and proteins that contain SUMO-like domains, resulting in their deSUMOylation and/or degradation (Prudden et al., 2007). The presence of STUbLs helps to maintain the delicate balance between SUMOylation and ubiquitination (Abed et al., 2011b).

Viral exploitation of the Ub/SUMO pathways

Viruses are obligate parasites. Because of the extremely limited protein encoding ability of the viral genome, viruses have evolved ways to utilise the host cell machinery, including the Ub/SUMO pathways. One example of viruses exploiting the Ub pathway is the involvement of cellular endosomal sorting complex required for transport (ESCRT) in the budding of viruses from the multivesicular bodies, which was initially shown for retroviruses (Martin-Serrano et al., 2001; Garrus et al., 2001) and then extended to other viral families (Schmitt et al., 2002; Yasuda et al., 2003; Wirblich et al., 2008). The interaction between viral proteins and the ESCRT complexes has been extensively studied, and there are a few excellent reviews on this subject (Bieniasz, 2006; Martin-Serrano, 2007; Chen and Lamb, 2008; McDonald and Martin-Serrano, 2009; Gustin et al., 2011). Here, we will focus on a much lesser known aspect of viral interaction with the Ub/SUMO system: the regulation of nucleocytoplasmic trafficking of viral proteins by Ub and SUMO.

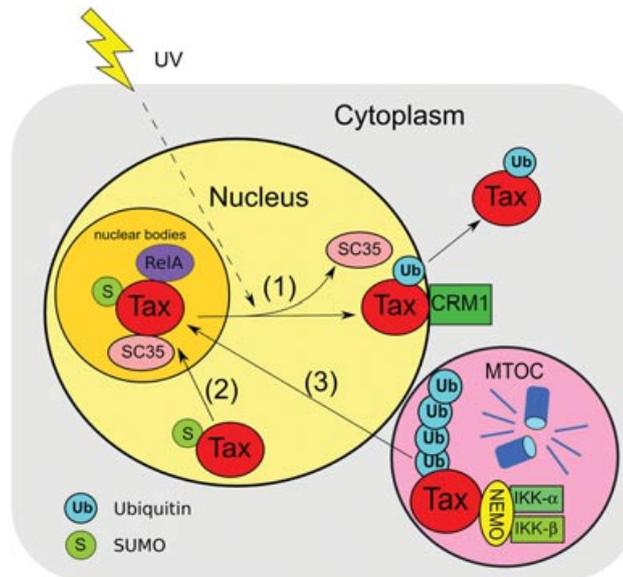
Retrovirus

To date, the best-characterised example of a viral protein whose nucleocytoplasmic trafficking is regulated by the Ub/SUMO pathways is the Tax protein of the human T-cell leukaemia virus type 1 (HTLV-1). HTLV-1 is the etiological agent of adult T-cell leukaemia/lymphoma, and tropical spastic paraparesis/HTLV-associated myelopathy (Jeang, 2010; Shembade and Harhaj, 2010). Tax, a 40 kDa phosphoprotein encoded by the pX region of the virus, is a multifunctional protein that plays key roles in viral replication as well as T-cell transformation (Matsuoka and Jeang, 2011). It promotes cell survival by constitutively activating the NF- κ B family transcription factors via interactions with the components of the pathway at both cytoplasmic and nuclear steps (Nasr et al., 2006).

Tax shuttles between the nuclear and cytoplasmic compartments (Burton et al., 2000; Kfoury et al., 2011). In the nucleus, Tax is predominantly localised to heterogeneous nuclear foci known as Tax speckled structures (TSS), which contain a variety of cellular proteins including transcription factors, splicing cofactors, and DNA damage recognition and cell cycle regulatory factors (Semmes et al.,

Figure 3 | Schematic illustration of HTLV-1 Tax nucleocytoplasmic trafficking regulated by Ub and SUMO

(1) UV induces monoubiquitination of Tax, its dissociation from nuclear body proteins and nuclear export (Gatza et al., 2007). (2) SUMOylation targets Tax to the nuclear bodies (Lamsoul et al., 2005). (3) K63-linked polyubiquitination targets Tax to microtubule organising center as well as nuclear bodies (Kfoury et al., 2011). Polyubiquitination is also important for targeting the IKK signalosome to the centrosome and nuclear translocation of RelA (Kfoury et al., 2008, Nasr et al., 2006) (see text for details).



1996; Bex et al., 1997; Haoudi et al., 2003). In the cytoplasm, a major fraction of Tax localises to perinuclear structures colocalising with the centrosome or microtubule-organising center in close association with the *cis*-Golgi compartment (Nejmeddine et al., 2005; Kfoury et al., 2008; Kfoury et al., 2011). Using a photoconvertible fluorophore (Dendra-2) coupled with live cell confocal microscopy, Kfoury et al. (2011) recently showed that the same Tax molecule can shuttle amongst Tax nuclear bodies as well as between Tax nuclear bodies and the centrosome. This dynamic shuttling is important for its function in interaction with NEMO and activation of NF- κ B (Kfoury et al., 2011). Cellular stress such as UV irradiation induces the translocation of Tax from TSS to the cytoplasm (Gatza and Marriott, 2006; Gatza et al., 2007). The nucleocytoplasmic shuttling of Tax is mediated by a non-canonical NLS at the N-terminal (Smith and Greene, 1990; Smith and Greene, 1992; Meertens et al., 2004) and a leucine-rich NES (Burton et al., 2000; Alefantis et al., 2003). However, Tax also undergoes different forms of post-translational modification, includ-

ing phosphorylation (Bex et al., 1999), acetylation (Lodewick et al., 2009), ubiquitination (Nasr et al., 2006; Kfoury et al., 2008) and SUMOylation (Nasr et al., 2006; Kfoury et al., 2011), all of which are implicated in its functions. Particularly, ubiquitination and SUMOylation have been shown to be involved in its nucleocytoplasmic trafficking (Figure 3).

Tax is modified by monoUb as well as K48- and K63-linked Ub chains (Chiari et al., 2004; Peloponese et al., 2004; Kfoury et al., 2008). Although K48 Ub chains lead to Tax degradation, K63 Ub chains mediate binding to NEMO, recruitment of the IKK signalosome to the centrosome and activation of NF- κ B (Peloponese et al., 2004; Kfoury et al., 2008). The ubiquitination sites have been mapped to the C-terminal lysine residues (K263, K280 and K284). Mutating these three K residue results in a dramatic reduction of polyUb and monoUb of Tax, whereas the three N-terminal K residues have minimal effect on Tax Ub (Chiari et al., 2004). Several groups have shown that ubiquitination of Tax is associated with its cytoplasmic localisation, although whether this is effectuated through promoting nuclear export or

cytoplasmic retention is still controversial (discussed below).

Gatza et al. (2007) showed that monoUb promotes the nuclear export of Tax in response to DNA damage [pathway (1) in Figure 3]. Following UV irradiation, a rapid translocation of Tax from the nucleus to the cytoplasm was observed. The UV-induced translocation correlates with an increase in monoUb of Tax at K280 and K284 residues. The K280/284R double mutant is not ubiquitinated and does not translocate to the cytoplasm after UV treatment. Moreover, fusing a copy of Ub to either the N-terminal or C-terminal of Tax to mimic monoubiquitination induces nuclear export. Interestingly, the nuclear export of the Tax–Ub fusion protein is blocked by leptomycin B (LMB), an inhibitor of CRM-1-dependent pathway, indicating that the Ub-induced nuclear export of Tax is still dependent on a functional NES. The authors proposed that monoUb facilitates the dissociation of Tax from TSS in the nucleus, promoting its nuclear export (Gatza et al., 2007). Lamsoul et al. (2005) also reported that Ub favours cytoplasmic localisation of Tax. Overexpression of Ub results in increased cytoplasmic localisation of Tax, which is phenocopied by fusing one copy of Ub to the C-terminal of the protein. Because co-localisation between Tax and exogenously expressed Ub is observed only in the cytoplasm, the authors concluded that ubiquitination is involved in the cytoplasmic retention of Tax. However, the absence of detectable co-localisation between Tax and Ub in the nucleus does not preclude the possibility that the ubiquitinated species are rapidly exported out of the nucleus.

Tax is also SUMOylated on lysine residues that overlap the Ub conjugation sites (Lamsoul et al., 2005; Nasr et al., 2006). Ub and SUMO seem to have opposing effects on Tax subcellular distribution. Although Ub favours cytoplasmic localisation, as discussed above, SUMO overexpression leads to predominantly nuclear body localisation. SUMOylation seems to be required for targeting Tax to nuclear bodies [pathway (2) in Figure 3] because Ub/SUMO site mutants fail to localise to nuclear speckles, and nuclear body formation can be restored by C-terminal fusion of SUMO but not Ub (Lamsoul et al., 2005). Kfoury et al. (2011) showed later that Ub fusion to the same K mutants can restore nuclear body localisation as well, suggesting that Ub can also target Tax to nuclear bodies [pathway (3) in Figure

3]. The discrepancy between the two studies possibly stems from the different cell types used (293T cells in the Lamsoul paper versus HeLa cells in the Kfoury paper) because the localisation of Tax is known to be cell type dependent (Alefantis et al., 2003). The nuclear retention induced by SUMOylation is possibly via NES masking, in agreement with an earlier report suggesting that the Tax NES likely exists as a conditionally masked signal (Alefantis et al., 2003).

Despite their opposing effect on Tax localisation, Ub and SUMO are both required for NF- κ B activation. They control critical cytoplasmic and nuclear steps of NF- κ B activation, respectively. Ubiquitinated Tax binds to the IKK complex and induces its activation, leading to the degradation of I κ B and nuclear translocation of Rel A, whereas SUMOylated Tax recruits Rel A to Tax nuclear bodies, promoting its complete transcriptional activation (Nasr et al., 2006).

Besides HTLV-1 Tax, the nucleocytoplasmic trafficking of a few other viral proteins has also been linked to Ub and/or SUMO. Examples are listed in Table 2 and will be briefly discussed below.

DNA viruses

Adenovirus type 5 E1B-55K protein

The 55 kDa phosphoprotein encoded in early region 1B (E1B-55K) from adenovirus type 5 (Ad5) is required for efficient viral DNA replication, selective viral late mRNA nuclear export and inactivation of the tumour suppressor protein p53 (Berk, 2005). E1B-55K shuttles between the nuclear and cytoplasmic compartments. It possesses a C-terminal NLS and an N-terminal leucine-rich NES (Krätzer et al., 2000). In addition, nucleocytoplasmic trafficking may also involve covalent conjugation of SUMO1, which appears to facilitate efficient nuclear import and/or subnuclear targeting. Overexpression of SUMO1 in transformed rat cells expressing E1B-55K causes the accumulation of E1B-55K at defined subnuclear structures, whereas arginine substitution of the major SUMOylation site, K104, results in a defect in nuclear import and accumulation in cytoplasmic inclusions (Endter et al., 2001).

SUMOylation also seems to be involved in regulating the nuclear export of E1B-55K. The nuclear export of E1B-55K is mediated by CRM-1-dependent and -independent pathways. Inactivation

Table 2 | Nucleocytoplasmic trafficking of viral proteins regulated by Ub and/or SUMO

Protein	Major functions	Ub regulation	SUMO regulation	References
Ad5 E1B-55K	Viral replication, viral mRNA export, inactivation of p53	Not examined	SUMO is involved in targeting E1B-55K to subnuclear structures. SUMOylation also negatively regulates nuclear export by promoting nuclear retention.	Endter et al., 2001; Kindsmüller et al., 2007
BPV E1	Viral replication	Free E1 is readily degraded via Ub-mediated proteasomal degradation. It is stabilised when bound to cyclin E/Cdk2.	SUMOylation is potentially involved in nuclear import/export	Rangasamy et al., 2000; Malcles et al., 2002; Rosas-Acosta and Wilson, 2008
EBV EBNA3C	Viral latent antigen	Not examined	SUMO-3 modification might be involved in localisation to nuclear dots	Rosendorff et al., 2004
HTLV-1 Tax	Viral replication, T-cell transformation	In response to DNA damage, Tax is monoubiquitinated on K280 and K284, inducing nuclear export. Ub may also favour cytoplasmic localisation by promoting cytoplasmic retention.	SUMO is important for targeting Tax to nuclear bodies, and it promotes its nuclear retention	Lamsoul et al., 2005; Gatza et al., 2007; Kfoury et al., 2011
NiV Matrix	Viral assembly and budding, possible nuclear functions	Ubiquitination on a lysine residue within the NLS is important for nuclear export. Ub might also be involved in membrane targeting and budding of NiV matrix.	Not examined	Wang et al., 2010

of CRM-1-dependent pathway by mutating the NES or LMB treatment causes redistribution of the viral protein from the cytoplasm to the nucleus and its accumulation at the viral replication centers, suggesting that CRM-1 is a major export receptor. However, this nuclear restriction is relieved by concurrent mutation of the SUMOylation site, suggesting that deconjugation of SUMO1 allows nuclear export via CRM-1-independent pathways (Kindsmüller et al., 2007). The authors proposed that SUMO facilitates nuclear retention of E1B-55K, thus negatively regulating nuclear export.

Bovine papillomavirus E1 protein

The E1 protein of papillomaviruses (PV) is the major initiator protein for viral DNA replication and plays important roles in the establishment of stable episomal viral genomes in the host cell nucleus (Wilson et al., 2002). Similar to E1B-55K, the E1 protein of bovine papillomavirus (BPV) has been shown to be SUMOylated by host cell machinery, although the role of SUMOylation in the intracellular distribution of BPV E1 is controversial (see below). SUMOyla-

tion of BPV E1 is possibly mediated through the host SUMO conjugating enzyme Ubc9 and PIAS family E3 SUMO ligases (Rangasamy and Wilson, 2000; Rosas-Acosta et al., 2005). SUMOylation on lysine residue 514 seems to be important for nuclear targeting. In transfected COS-1 cells, although wild-type E1 accumulates in intranuclear foci in addition to diffused nucleoplasmic distribution with little or no cytoplasmic staining, the SUMO site mutant K514R as well as the Ubc9-binding defective mutants are unable to localise to the nucleus (Rangasamy and Wilson, 2000; Rangasamy et al., 2000). However, Fradet-Turcotte et al. (2009) found that in C33A cells, the nuclear localisation of the E1 proteins of human papillomavirus (HPV) 11, HPV16 and BPV is independent of SUMOylation. These authors showed that HPV11 E1 binds to Ubc9 similar to BPV E1, as reported previously, but the nuclear accumulation of HPV11 E1 is not affected by mutations that weaken Ubc9 binding and by inhibition of the SUMO pathway using a SUMO-activating enzyme inhibitor Gam1, a dominant negative version of Ubc9 or shRNA depletion of Ubc9 (Fradet-Turcotte et al.,

2009). Quite surprisingly, the authors also found that contrary to the previous report by Rangasamy et al. (2000), the SUMOylation site mutants of BPV E1 are not defective in the nuclear localisation in C33A cells (Fradet-Turcotte et al., 2009). Interestingly, another report by Rosas-Acosta et al. (2005) suggested that SUMOylation might mediate nuclear export of BPV E1 by enhancing its interaction with CRM-1 (Rosas-Acosta and Wilson, 2008). According to this report, the non-SUMOylatable mutant is almost exclusively nuclear and is more enriched in close proximity to the nuclear envelope as compared with the wild-type form, supporting a role for SUMO in the intracellular distribution of BPV E1 albeit different from the one previously suggested (Rangasamy et al., 2000).

Epstein–Barr virus proteins

Epstein–Barr virus (EBV) is a human gammaherpesvirus that has been associated with a variety of cancers (de Oliveira et al., 2010). Multiple proteins encoded by EBV have been shown to be modified by Ub or SUMO, including Rta, Zta and Epstein–Barr virus nuclear antigen (EBNA) 3C, but a role for Ub/SUMO in regulating the cellular localisation of these proteins has not been established in most cases (see below).

Rta and Zta are two immediate early proteins that mediate the switch between the latent and lytic forms of EBV infection (Speck et al., 1997). SUMO-1 modification of Rta by the E3 ligases PIAS1 and RanBPM enhances its transactivation activity, although the effect of SUMO-1 on the subcellular localisation of Rta was not examined in these studies (Chang et al., 2004, 2008). Rta can also be modified by SUMO2/3 in the presence of viral protein LF2. LF2 binding induces the translocation of Rta from the nucleus to the cytoplasm and represses its activity, but this redistribution of Rta seems to be independent of its SUMOylation (Calderwood et al., 2008; Heilmann et al., 2010). Similarly, Zta can be modified by SUMO-1, 2 and 3, but in contrast to Rta, SUMO-1 modification of Zta seems to repress its transactivation activity (Adamson and Kenney, 2001; Adamson, 2005; Hagemeyer et al., 2010). SUMOylation does not seem to play a role in the subcellular localisation of Zta because its localisation pattern remains unaltered when SUMO-1 is overexpressed or when its SUMO-1 conjugation site is mutated (Adamson, 2005; Hagemeyer et al., 2010).

EBNA3C is a viral latent antigen involved in primary B cell transformation by EBV (Saha et al., 2009). Rosendorff et al. (2004) found that EBNA3C is modified by SUMO2/3. SUMO-3 modification might be involved in the subnuclear localisation of EBNA3C because the wild-type protein localises to nuclear dots, whereas truncation mutants defective in SUMO-3 conjugation exhibit diffused localisation patterns in the nucleus. However, the SUMOylation site in EBNA3C has not been pinpointed and a definitive role for SUMO-3 in the subnuclear targeting of ENBA3C is yet to be confirmed.

LMP2A, a transmembrane protein expressed during EBV latency, was found to be ubiquitinated by Nedd4 family ubiquitin E3 ligases on amino terminal non-lysine residues (Ikeda et al., 2002). This modification affects protein stability (Ikeda et al., 2001), but its functional relevance is not clear.

Immediate early proteins of other herpesviruses

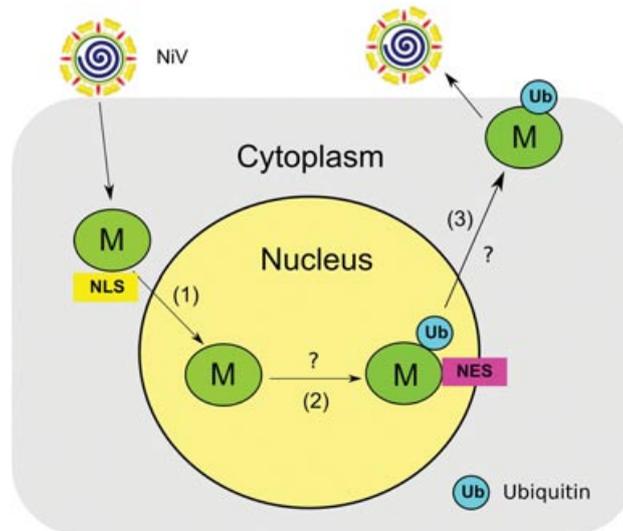
Besides EBV, the immediate early proteins of a few other human herpesviruses (HHVs) have been shown to be modified by SUMO, including the K-bZIP protein of Kaposi's sarcoma-associated herpesvirus (KSHV) (Izumiya et al., 2005), the IE1 and IE2 proteins of human cytomegalovirus (HCMV) (Hofmann et al., 2000; Ahn et al., 2001; Spengler et al., 2002; Nevels et al., 2004) and the IE1 protein of human herpesvirus 6 (HHV-6) (Gravel et al., 2004). SUMOylation modulates the substrate protein's transcriptional activation or repression function in some cases (*e.g.* KSHV K-bZIP and HCMV IE2); however, in all cases examined, SUMO does not seem to affect the subcellular localisation of the substrate protein (Hofmann et al., 2000; Ahn et al., 2001; Spengler et al., 2002; Nevels et al., 2004; Gravel et al., 2004; Izumiya et al., 2005).

Negative-stranded RNA virus

Most negative-stranded RNA viruses replicate in the cytoplasm, and there is no nuclear stage required to complete the viral life cycle (Griffin et al., 2007). However, both non-structural and structural proteins of these cytoplasmic viruses have been shown to translocate to the nuclear compartment. Examples include the matrix protein of the vesicular stomatitis virus (Petersen et al., 2000), the W proteins of

Figure 4 | Proposed model for NiV matrix protein (NiV M) nucleocytoplasmic trafficking

(1) NiV M translocates to the nucleus in an NLS-dependent manner. (2) NiV M is ubiquitinated via an unknown mechanism. (3) Ubiquitination of M promotes its nuclear export via an NES-dependent mechanism. Ubiquitination of M may also be important for its membrane targeting and budding, although the mechanism is currently unclear (Wang et al., 2010).



paramyxoviruses (Shaw et al., 2004) and the matrix proteins of several paramyxoviruses (Yoshida et al., 1976; Peeples et al., 1992; Ghildyal et al., 2003). The nuclear translocation is usually associated with host antagonistic activities of these viral proteins to facilitate viral replication (Petersen et al., 2000; Ghildyal et al., 2003; Shaw et al., 2005).

We recently demonstrated that the matrix protein of Nipah virus (NiV), a lethal member of the family *Paramyxoviridae*, shuttles between the nuclear and cytoplasmic compartments (Wang et al., 2010). During live NiV infection, the matrix protein is first found in the nucleus of infected cells before it localises to cytoplasmic and plasma membrane locations at later time points. The nucleocytoplasmic trafficking of NiV matrix is regulated by ubiquitination in addition to its bi-partite NLS and leucine-rich NES (Figure 4). Ub depletion by proteasome inhibitors including MG132 and bortezomib restricts matrix to the nucleus, which is phenocopied by mutating the putative ubiquitination site (K258) from lysine to arginine. Moreover, fusing a copy of Ub to mimic monoubiquitination restores nuclear export to the K258R mutant, supporting the hypothesis that monoubiquitination of matrix promotes its nuclear export. However, our current data are also compati-

ble with an alternative model in which ubiquitination inhibits nuclear import of the protein, favouring cytoplasmic retention. Further investigation is needed to distinguish between these two possibilities. We also showed that nuclear translocation of NiV matrix is functionally correlated with matrix-mediated viral budding. The ubiquitination site mutants are defective in plasma membrane targeting and viral budding, implying a potential role of ubiquitination in these processes.

Very interestingly, sequence alignment of matrix proteins from viruses across *Paramyxoviridae* reveals that the putative Ub site in NiV (K258) is absolutely conserved in all viruses examined (Table 3). Previous studies as well as our own research have shown that NiV matrix is by no means the only paramyxoviral matrix protein to translocate to the nucleus. The matrix proteins of Sendai virus (Yoshida et al., 1976), Newcastle disease virus (Peeples, 1988; Peeples et al., 1992), human respiratory syncytial virus (Ghildyal et al., 2003), mumps virus, Hendra virus and tupaia paramyxovirus (last three are our unpublished data) all exhibit nuclear localisation under viral infection or cell transfection conditions. It would be interesting to test whether the nucleocytoplasmic trafficking of other paramyxoviral matrix proteins might

Table 3 | Sequence alignment of matrix proteins of different paramyxoviruses

Genus	Species	Matrix protein
Henipavirus	Nipah virus	GNFVR--RAGKYYSVDYCRRKIDRMK
	Hendra virus	GNFVR--RAGKYYSVEYCKRKIDRMK
Unassigned	Tupaia paramyxovirus	GNFVR--KGGDVYSNSYCKKIDRMD
Morbillivirus	Measles virus	GNFRR--KKSEVYSADYCKMKIEKMG
	Canine distemper virus	GNFCR--KKNQAYSADYCKLIEKMG
Respirovirus	HPIV-1	GIIRR--KVGKIYSVEYCKNIEKMK
	Sendai virus	GLIRR--KVGKIYSVEYCKSIEKMR
	HPIV-3	GLIKR--KVGGRMYSVEYCKQIEKMR
Rubulavirus	Mumps virus	CNLCCKGRNKLRSDENYFASKCRKMN
	Tioman virus	CNLYRGNKPFKAYDDTYFSQKCRAMQ
Avulavirus	Simian virus 41	CNILKNKKIKQRGVDSYFSSKAISMQ
	Newcastle Disease virus	GLMTTVDRKGGKVTDFDKLEKIRSLD

The matrix protein sequences of 12 viruses from different genera within the family *Paramyxoviridae* were aligned using CLUSTAL W (version 1.83). Positively charged amino acid residues that conform to the consensus for bipartite NLSs are coloured green. The red arrow points to the lysine residue conserved amongst all 12 viruses (Wang et al., 2010).

also be regulated by ubiquitination similar to NiV matrix.

Conclusions and perspectives

The nucleocytoplasmic trafficking of many cellular proteins is regulated by post-translational modifications including ubiquitination and SUMOylation in addition to their endogenous NLSs and NESs, as discussed above. However, although the role of Ub/SUMO in regulating the intracellular localisation and functions of cellular proteins has been well established, to our knowledge, their involvement in viral protein trafficking remains largely unknown, with the exception of only a few examples (*e.g.* HTLV-1 Tax, Ad5 E1B-55K, BPV E1 and NiV matrix). Nevertheless, these past few years have witnessed a fast-growing list of viruses that utilise the host's Ub/SUMO system to facilitate various steps of their life cycle [for a complete review of viral takeover of the Ub/SUMO system, readers are referred to references Boggio and Chiocca (2006); Gustin et al. (2011)]. Therefore, targeting the Ub/SUMO system (via either global inhibition of the pathways or modulating the functions of individual components such as Ub/SUMO ligases and deubiquitinating/deSUMOylating enzymes) can potentially be a way to develop broad-spectrum antivirals. Indeed, the US Food and Drug Administration-approved protea-

some inhibitor, bortezomib, has been shown to inhibit different families of viruses including Hepatitis B virus (*Hepadnaviridae*) (Bandi et al., 2010), HIV (*Retroviridae*) (Yu et al., 2009), vesicular stomatitis virus (*Rhabdoviridae*) (Neznanov et al., 2008; Dudek et al., 2010), influenza A virus (*Orthomyxoviridae*) (Dudek et al., 2010), NiV (*Paramyxoviridae*) (Wang et al., 2010), coronaviruses (*Coronaviridae*) (Raaben et al., 2010; Ma et al., 2010) and orthopoxviruses (*Poxviridae*) (Teale et al., 2009). However, most of these studies have been done in *in vitro* models, and it is important to evaluate the *in vivo* efficacy of bortezomib and its oral equivalents such as MLN9708 and PR-047 (Zhou et al., 2009; Kupperman et al., 2010; Dick and Fleming, 2010) as potential broad-spectrum antivirals.

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Conflict of interest statement

The authors have declared no conflict of interest.

References

- Abed, M., Barry, K.C., Kenyagin, D., Koltun, B., Phippen, T.M., Delrow, J.J., Parkhurst, S.M. and Orian, A. (2011a) Degringolade, a SUMO-targeted ubiquitin ligase, inhibits hairy/groucho-mediated repression. *EMBO J.* **30**, 1289–1301
- Abed, M., Bitman-Lotan, E. and Orian, A. (2011b) A fly view of a SUMO-targeted ubiquitin ligase. *Fly (Austin)* **5**, 340–344
- Adamson, A.L. (2005) Effects of SUMO-1 upon Epstein–Barr virus BZLF1 function and BMRF1 expression. *Biochem. Biophys. Res. Commun.* **336**, 22–28
- Adamson, A.L. and Kenney, S. (2001) Epstein–Barr virus immediate-early protein BZLF1 is SUMO-1 modified and disrupts promyelocytic leukemia bodies. *J. Virol.* **75**, 2388–2399
- Ahn, J.H., Xu, Y., Jang, W.J., Matunis, M.J. and Hayward, G.S. (2001) Evaluation of interactions of human cytomegalovirus immediate-early IE2 regulatory protein with small ubiquitin-like modifiers and their conjugation enzyme Ubc9. *J. Virol.* **75**, 3859–3872
- Alefantis, T., Barmak, K., Harhaj, E.W., Grant, C. and Wigdahl, B. (2003) Characterization of a nuclear export signal within the human T cell leukemia virus type I transactivator protein Tax. *J. Biol. Chem.* **278**, 21814–21822
- Bae, S., Jeong, J., Park, J.A., Kim, S., Bae, M., Choi, S. and Kim, K. (2004) SUMOylation increases HIF-1 α stability and its transcriptional activity. *Biochem. Biophys. Res. Commun.* **324**, 394–400
- Bandi, P., Garcia, M.L., Booth, C.J., Chisari, F.V. and Robek, M.D. (2010) Bortezomib inhibits hepatitis B virus replication in transgenic mice. *Antimicrob. Agents Chemother.* **54**, 749–756
- Berk, A.J. (2005) Recent lessons in gene expression, cell cycle control, and cell biology from adenovirus. *Oncogene* **24**, 7673–7685
- Barry, K.C., Abed, M., Kenyagin, D., Werwie, T.R., Boico, O., Orian, A. and Parkhurst, S.M. (2011) The drosophila STU β L protein degradingolade limits HES functions during embryogenesis. *Development* **138**, 1759–1769
- Bex, F., McDowall, A., Burny, A. and Gaynor, R. (1997) The human T-cell leukemia virus type 1 transactivator protein Tax colocalizes in unique nuclear structures with NF- κ B proteins. *J. Virol.* **71**, 3484–3497
- Bex, F., Murphy, K., Wattiez, R., Burny, A. and Gaynor, R.B. (1999) Phosphorylation of the human T-cell leukemia virus type 1 transactivator Tax on adjacent serine residues is critical for Tax activation. *J. Virol.* **73**, 738–745
- Bieniasz, P.D. (2006) Late budding domains and host proteins in enveloped virus release. *Virology* **344**, 55–63
- Boggio, R. and Chiocca, S. (2006) Viruses and SUMOylation: Recent highlights. *Curr. Opin. Microbiol.* **9**, 430–436
- Burton, M., Upadhyaya, C.D., Maier, B., Hope, T.J. and Semmes, O.J. (2000) Human T-cell leukemia virus type 1 Tax shuttles between functionally discrete subcellular targets. *J. Virol.* **74**, 2351–2364
- Cai, Q. and Robertson, E.S. (2010) Ubiquitin/SUMO modification regulates VHL protein stability and nucleocytoplasmic localization. *PLoS ONE* **5**, e12636.
- Calderwood, M.A., Holthaus, A.M. and Johannsen, E. (2008) The Epstein–Barr virus LF2 protein inhibits viral replication. *J. Virol.* **82**, 8509–8519
- Carter, S., Bischof, O., Dejean, A. and Vousden, K.H. (2007) C-terminal modifications regulate MDM2 dissociation and nuclear export of p53. *Nat. Cell Biol.* **9**, 428–435
- Chakrabarti, S.R., Sood, R., Nandi, S. and Nucifora, G. (2000) Posttranslational modification of TEL and TEL/AML1 by SUMO-1 and cell-cycle-dependent assembly into nuclear bodies. *Proc. Natl. Acad. Sci. U. S. A.* **97**, 13281–13285
- Chang, L., Lee, Y., Cheng, T., Hong, Y., Lu, P., Wang, J.J., Wang, W., Kuo, C., Li, S.S. and Liu, S. (2004) Post-translational modification of Rta of Epstein–Barr virus by SUMO-1. *J. Biol. Chem.* **279**, 38803–38812
- Chang, L., Liu, S., Kuo, C., Wang, W., Chuang, J., Bianchi, E. and Hong, Y. (2008) Enhancement of transactivation activity of Rta of Epstein–Barr virus by RanBPM. *J. Mol. Biol.* **379**, 231–242
- Chen, B.B. and Mallampalli, R.K. (2009) Masking of a nuclear signal motif by monoubiquitination leads to mislocalization and degradation of the regulatory enzyme cytidylyltransferase. *Mol. Cell Biol.* **29**, 3062–3075
- Chen, B.J. and Lamb, R.A. (2008) Mechanisms for enveloped virus budding: Can some viruses do without an ESCRT? *Virology* **372**, 221–232
- Chen, C., Sun, X., Ran, Q., Wilkinson, K.D., Murphy, T.J., Simons, J.W. and Dong, J. (2005) Ubiquitin-proteasome degradation of KLF5 transcription factor in cancer and untransformed epithelial cells. *Oncogene* **24**, 3319–3327
- Chiari, E., Lamsoul, I., Lodewick, J., Chopin, C., Bex, F. and Pique, C. (2004) Stable ubiquitination of human T-cell leukemia virus type 1 Tax is required for proteasome binding. *J. Virol.* **78**, 11823–11832
- Choi, D.W., Seo, Y., Kim, E., Sung, K.S., Ahn, J.W., Park, S., Lee, S. and Choi, C.Y. (2008) Ubiquitination and degradation of homeodomain-interacting protein kinase 2 by WD40 repeat/socs box protein WSB-1. *J. Biol. Chem.* **283**, 4682–4689
- Cole, C.N. and Hammell, C.M. (1998) Nucleocytoplasmic transport: Driving and directing transport. *Curr. Biol.* **8**, R368–R372
- Comerford, K.M., Leonard, M.O., Karhausen, J., Carey, R., Colgan, S.P. and Taylor, C.T. (2003) Small ubiquitin-related modifier-1 modification mediates resolution of CREB-dependent responses to hypoxia. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 986–991
- de Oliveira, D.E., Ballon, G. and Cesarman, E. (2010) NF- κ B signaling modulation by EBV and KSHV. *Trends Microbiol.* **18**, 248–257
- Denuc, A. and Marfany, G. (2010) SUMO and ubiquitin paths converge. *Biochem. Soc. Trans.* **38**, 34–39
- Desterro, J.M., Rodriguez, M.S. and Hay, R.T. (1998) SUMO-1 modification of I κ B α inhibits NF- κ B activation. *Mol. Cell* **2**, 233–239
- Dick, L.R. and Fleming, P.E. (2010) Building on bortezomib: Second-generation proteasome inhibitors as anti-cancer therapy. *Drug Discov. Today* **15**, 243–249
- Du, J.X., Bialkowska, A.B., McConnell, B.B. and Yang, V.W. (2008) SUMOylation regulates nuclear localization of Krüppel-like factor 5. *J. Biol. Chem.* **283**, 31991–32002
- Dudek, S.E., Luig, C., Pauli, E., Schubert, U. and Ludwig, S. (2010) The clinically approved proteasome inhibitor PS-341 efficiently blocks influenza A virus and vesicular stomatitis virus propagation by establishing an antiviral state. *J. Virol.* **84**, 9439–9451
- Duprez, E., Saurin, A.J., Desterro, J.M., Lallemand-Breitenbach, V., Howe, K., Boddy, M.N., Solomon, E., de Thé, H., Hay, R.T. and Freemont, P.S. (1999) SUMO-1 modification of the acute promyelocytic leukaemia protein pml: Implications for nuclear localisation. *J. Cell Sci.* **112** (Pt 3), 381–393
- Endter, C., Kzhyshkowska, J., Stauber, R. and Dobner, T. (2001) SUMO-1 modification required for transformation by adenovirus type 5 early region 1B 55-kDa oncoprotein. *Proc. Natl. Acad. Sci. U. S. A.* **98**, 11312–11317
- Fradet-Turcotte, A., Brault, K., Titolo, S., Howley, P.M. and Archambault, J. (2009) Characterization of papillomavirus e1 helicase mutants defective for interaction with the SUMO-conjugating enzyme Ubc9. *Virology* **395**, 190–201
- Fried, H. and Kutay, U. (2003) Nucleocytoplasmic transport: Taking an inventory. *Cell Mol. Life Sci.* **60**, 1659–1688
- Fukuchi, M., Imamura, T., Chiba, T., Ebisawa, T., Kawabata, M., Tanaka, K. and Miyazono, K. (2001) Ligand-dependent degradation of Smad3 by a ubiquitin ligase complex of ROC1 and associated proteins. *Mol. Biol. Cell* **12**, 1431–1443

- Gareau, J.R. and Lima, C.D. (2010) The SUMO pathway: Emerging mechanisms that shape specificity, conjugation and recognition. *Nat. Rev. Mol. Cell Biol.* **11**, 861–871
- Garrus, J.E., von Schwedler, U.K., Pornillos, O.W., Morham, S.G., Zavitz, K.H., Wang, H.E., Wettstein, D.A., Stray, K.M., Côté, M., Rich, R.L., Myszkowski, D.G. and Sundquist, W.I. (2001) Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. *Cell* **107**, 55–65
- Gatza, M.L., Dayaram, T. and Marriott, S.J. (2007) Ubiquitination of HTLV-1 Tax in response to DNA damage regulates nuclear complex formation and nuclear export. *Retrovirology* **4**, 95
- Gatza, M.L. and Marriott, S.J. (2006) Genotoxic stress and cellular stress alter the subcellular distribution of human T-cell leukemia virus type 1 Tax through a CRM1-dependent mechanism. *J. Virol.* **80**, 6657–6668
- Geoffroy, M. and Hay, R.T. (2009) An additional role for SUMO in ubiquitin-mediated proteolysis. *Nat. Rev. Mol. Cell Biol.* **10**, 564–568
- Ghildyal, R., Baulch-Brown, C., Mills, J. and Meanger, J. (2003) The matrix protein of human respiratory syncytial virus localises to the nucleus of infected cells and inhibits transcription. *Arch. Virol.* **148**, 1419–1429
- Goodbourn, S. and Randall, R.E. (2009) The regulation of type I interferon production by paramyxoviruses. *J. Interferon Cytokine Res.* **29**, 539–547
- Görlich, D. and Kutay, U. (1999) Transport between the cell nucleus and the cytoplasm. *Annu. Rev. Cell Dev. Biol.* **15**, 607–660
- Gravel, A., Dion, V., Cloutier, N., Gosselin, J. and Flamand, L. (2004) Characterization of human herpesvirus 6 variant b immediate-early 1 protein modifications by small ubiquitin-related modifiers. *J. Gen. Virol.* **85**, 1319–1328
- Griffin, D.E., Lamb, M.A., Martin, M.A., Roizman, B. and Straus, S.E. (2007) Fields virology. In *Virus Replication Strategies*. Knipe D.M. and Howley P.M. (eds.). Wolters Kluwer, Lippincott, Williams & Wilkins, Philadelphia, PA, 119–140
- Groulx, I. and Lee, S. (2002) Oxygen-dependent ubiquitination and degradation of hypoxia-inducible factor requires nuclear-cytoplasmic trafficking of the von Hippel–Lindau tumor suppressor protein. *Mol. Cell Biol.* **22**, 5319–5336
- Gustin, J.K., Moses, A.V., Früh, K. and Douglas, J.L. (2011) Viral takeover of the host ubiquitin system. *Front. Microbiol.* **2**, 161
- Güttler, T. and Görlich, D. (2011) Ran-dependent nuclear export mediators: A structural perspective. *EMBO J.* **30**, 3457–3474
- Hagemeyer, S.R., Dickerson, S.J., Meng, Q., Yu, X., Mertz, J.E. and Kenney, S.C. (2010) SUMOylation of the Epstein–Barr virus BZLF1 protein inhibits its transcriptional activity and is regulated by the virus-encoded protein kinase. *J. Virol.* **84**, 4383–4394
- Haoudi, A., Daniels, R.C., Wong, E., Kupfer, G. and Semmes, O.J. (2003) Human T-cell leukemia virus-I Tax oncoprotein functionally targets a subnuclear complex involved in cellular DNA damage-response. *J. Biol. Chem.* **278**, 37736–37744
- Heilmann, A.M.F., Calderwood, M.A. and Johannsen, E. (2010) Epstein–Barr virus LF2 protein regulates viral replication by altering rta subcellular localization. *J. Virol.* **84**, 9920–9931
- Hofmann, H., Flöss, S. and Stamminger, T. (2000) Covalent modification of the transactivator protein IE2-p86 of human cytomegalovirus by conjugation to the ubiquitin-homologous proteins SUMO-1 and hSMT3b. *J. Virol.* **74**, 2510–2524
- Honda, R., Tanaka, H. and Yasuda, H. (1997) Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor p53. *FEBS Lett.* **420**, 25–27
- Huang, T.T., Wuerzberger-Davis, S.M., Wu, Z. and Miyamoto, S. (2003) Sequential modification of NEMO/IKK γ by SUMO-1 and ubiquitin mediates NF- κ B activation by genotoxic stress. *Cell* **115**, 565–576
- Ikeda, M., Ikeda, A. and Longnecker, R. (2001) PY motifs of Epstein–Barr virus LMP2A regulate protein stability and phosphorylation of LMP2A-associated proteins. *J. Virol.* **75**, 5711–5718
- Ikeda, M., Ikeda, A. and Longnecker, R. (2002) Lysine-independent ubiquitination of Epstein–Barr virus LMP2A. *Virology* **300**, 153–159
- Imoto, S., Ohbayashi, N., Ikeda, O., Kamitani, S., Muromoto, R., Sekine, Y. and Matsuda, T. (2008) SUMOylation of Smad3 stimulates its nuclear export during PIASy-mediated suppression of TGF- β signaling. *Biochem. Biophys. Res. Commun.* **370**, 359–365
- Izumiya, Y., Ellison, T.J., Yeh, E.T.H., Jung, J.U., Luciw, P.A. and Kung, H. (2005) Kaposi's sarcoma-associated herpesvirus K-bZIP represses gene transcription via SUMO modification. *J. Virol.* **79**, 9912–9925
- Jamali, T., Jamali, Y., Mehrbod, M. and Mofrad, M.R.K. (2011) Nuclear pore complex: Biochemistry and biophysics of nucleocytoplasmic transport in health and disease. *Int. Rev. Cell Mol. Biol.* **287**, 233–286
- Jans, D.A. and Hübner, S. (1996) Regulation of protein transport to the nucleus: Central role of phosphorylation. *Physiol. Rev.* **76**, 651–685
- Jeang, K. (2010) HTLV-1 and adult T-cell leukemia: Insights into viral transformation of cells 30 years after virus discovery. *J. Formos. Med. Assoc.* **109**, 688–693
- Katze, M.G., He, Y. and Gale, M.J. (2002) Viruses and interferon: A fight for supremacy. *Nat. Rev. Immunol.* **2**, 675–687
- Kerscher, O., Felberbaum, R. and Hochstrasser, M. (2006) Modification of proteins by ubiquitin and ubiquitin-like proteins. *Annu. Rev. Cell Dev. Biol.* **22**, 159–180
- Kfoury, Y., Nasr, R., Favre-Bonvin, A., El-Sabban, M., Renault, N., Giron, M., Setterblad, N., Hajj, H.E., Chiari, E., Mikati, A.G., Hermine, O., Saib, A., de Thé, H., Pique, C. and Bazarbachi, A. (2008) Ubiquitylated Tax targets and binds the IKK signalosome at the centrosome. *Oncogene* **27**, 1665–1676
- Kfoury, Y., Setterblad, N., El-Sabban, M., Zamborlini, A., Dassouki, Z., El Hajj, H., Hermine, O., Pique, C., de Thé, H., Saïb, A. and Bazarbachi, A. (2011) Tax ubiquitylation and SUMOylation control the dynamic shuttling of Tax and NEMO between Ubc9 nuclear bodies and the centrosome. *Blood* **117**, 190–199
- Kim, Y.H., Choi, C.Y. and Kim, Y. (1999) Nuclear modification of the homeodomain-interacting protein kinase 2 (HIPK2) by the ubiquitin-like protein SUMO-1. *Proc. Natl. Acad. Sci. U. S. A.* **96**, 12350–12355
- Kinds Müller, K., Groitl, P., Härtl, B., Blanchette, P., Hauber, J. and Dobner, T. (2007) Intranuclear targeting and nuclear export of the adenovirus E1B-55K protein are regulated by SUMO1 conjugation. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 6684–6689
- Krätzer, F., Rosorius, O., Heger, P., Hirschmann, N., Dobner, T., Hauber, J. and Stauber, R.H. (2000) The adenovirus type 5 E1B-55K oncoprotein is a highly active shuttle protein and shuttling is independent of E4orf6, p53 and Mdm2. *Oncogene* **19**, 850–857
- Kupperman, E., Lee, E.C., Cao, Y., Bannerman, B., Fitzgerald, M., Berger, A., Yu, J., Yang, Y., Hales, P., Bruzzese, F., Liu, J., Blank, J., Garcia, K., Tsu, C., Dick, L., Fleming, P., Yu, L., Manfredi, M., Rolfe, M. and Bolen, J. (2010) Evaluation of the proteasome inhibitor MLN9708 in preclinical models of human cancer. *Cancer Res.* **70**, 1970–1980
- Lallemand-Breitenbach, V., Jeanne, M., Benhenda, S., Nasr, R., Lei, M., Peres, L., Zhou, J., Zhu, J., Raught, B. and de Thé, H. (2008) Arsenic degrades PML or PML-RAR α through a SUMO-triggered RNF4/ubiquitin-mediated pathway. *Nat. Cell Biol.* **10**, 547–555
- Lallemand-Breitenbach, V., Zhu, J., Puvion, F., Koken, M., Honoré, N., Doubeikovskiy, A., Duprez, E., Pandolfi, P.P., Puvion, E.,

- Freemont, P. and de Thé, H. (2001) Role of promyelocytic leukemia (Pml) SUMOylation in nuclear body formation, 11s proteasome recruitment, and as2o3-induced Pml or Pml/retinoic acid receptor alpha degradation. *J. Exp. Med.* **193**, 1361–1371
- Lamsoul, I., Lodewick, J., Lebrun, S., Brasseur, R., Burny, A., Gaynor, R.B. and Bex, F. (2005) Exclusive ubiquitination and SUMOylation on overlapping lysine residues mediate NF-kappaB activation by the human T-cell leukemia virus Tax oncoprotein. *Mol. Cell Biol.* **25**, 10391–10406
- Lee, J.C., Wang, G.X., Schickling, O. and Peter, M.E. (2005) Fusing DEDD with ubiquitin changes its intracellular localization and apoptotic potential. *Apoptosis* **10**, 1483–1495
- Lee, J.T. and Gu, W. (2010) The multiple levels of regulation by p53 ubiquitination. *Cell Death Differ.* **17**, 86–92
- Lee, P.S.W., Chang, C., Liu, D. and Derynck, R. (2003) SUMOylation of Smad4, the common Smad mediator of transforming growth factor-beta family signaling. *J. Biol. Chem.* **278**, 27853–27863
- Li, M., Brooks, C.L., Wu-Baer, F., Chen, D., Baer, R. and Gu, W. (2003) Mono- versus polyubiquitination: Differential control of p53 fate by Mdm2. *Science* **302**, 1972–1975
- Lin, X., Liang, M., Liang, Y., Brunicardi, F.C. and Feng, X. (2003a) SUMO-1/Ubc9 promotes nuclear accumulation and metabolic stability of tumor suppressor Smad4. *J. Biol. Chem.* **278**, 31043–31048
- Lin, X., Sun, B., Liang, M., Liang, Y., Gast, A., Hildebrand, J., Brunicardi, F.C., Melchior, F. and Feng, X. (2003b) Opposed regulation of corepressor CtBP by SUMOylation and pdz binding. *Mol. Cell* **11**, 1389–1396
- Liu, F. and Walters, K.J. (2010) Multitasking with ubiquitin through multivalent interactions. *Trends Biochem. Sci.* **35**, 352–360
- Lodewick, J., Lamsoul, I., Polania, A., Lebrun, S., Burny, A., Ratner, L. and Bex, F. (2009) Acetylation of the human T-cell leukemia virus type 1 Tax oncoprotein by p300 promotes activation of the NF-kappaB pathway. *Virology* **386**, 68–78
- Lohrum, M.A., Woods, D.B., Ludwig, R.L., Bálint, E. and Vousden, K.H. (2001) C-terminal ubiquitination of p53 contributes to nuclear export. *Mol. Cell Biol.* **21**, 8521–8532
- Ma, X., Bartczak, A., Zhang, J., Khattar, R., Chen, L., Liu, M.F., Edwards, A., Levy, G. and McGillivray, I.D. (2010) Proteasome inhibition *in vivo* promotes survival in a lethal murine model of severe acute respiratory syndrome. *J. Virol.* **84**, 12419–12428
- Mahajan, R., Delphin, C., Guan, T., Gerace, L. and Melchior, F. (1997) A small ubiquitin-related polypeptide involved in targeting rangap1 to nuclear pore complex protein ranbp2. *Cell* **88**, 97–107
- Malces, M., Cueille, N., Mechali, F., Coux, O. and Bonne-Andrea, C. (2002) Regulation of bovine papillomavirus replicative helicase e1 by the ubiquitin-proteasome pathway. *J. Virol.* **76**, 11350–11358
- Martin-Serrano, J. (2007) The role of ubiquitin in retroviral egress. *Traffic* **8**, 1297–1303
- Martin-Serrano, J., Zang, T. and Bieniasz, P.D. (2001) HIV-1 and ebola virus encode small peptide motifs that recruit tsg101 to sites of particle assembly to facilitate egress. *Nat. Med.* **7**, 1313–1319
- Matsuoka, M. and Jeang, K. (2011) Human T-cell leukemia virus type 1 (HTLV-1) and leukemic transformation: Viral infectivity, Tax, hbz and therapy. *Oncogene* **30**, 1379–1389
- Mattaj, I.W. and Englmeier, L. (1998) Nucleocytoplasmic transport: The soluble phase. *Annu. Rev. Biochem.* **67**, 265–306
- Matunis, M.J., Coutavas, E. and Blobel, G. (1996) A novel ubiquitin-like modification modulates the partitioning of the ran-GTPase-activating protein rangap1 between the cytosol and the nuclear pore complex. *J. Cell Biol.* **135**, 1457–1470
- Matunis, M.J., Wu, J. and Blobel, G. (1998) SUMO-1 modification and its role in targeting the ran GTPase-activating protein, rangap1, to the nuclear pore complex. *J. Cell Biol.* **140**, 499–509
- McDonald, B. and Martin-Serrano, J. (2009) No strings attached: The ESCRT machinery in viral budding and cytokinesis. *J. Cell Sci.* **122**, 2167–2177
- Meertens, L., Chevalier, S., Weil, R., Gessain, A. and Mahieux, R. (2004) A 10-amino acid domain within human T-cell leukemia virus type 1 and type 2 Tax protein sequences is responsible for their divergent subcellular distribution. *J. Biol. Chem.* **279**, 43307–43320
- Morén, A., Hellman, U., Inada, Y., Imamura, T., Heldin, C. and Moustakas, A. (2003) Differential ubiquitination defines the functional status of the tumor suppressor Smad4. *J. Biol. Chem.* **278**, 33571–33582
- Moroianu, J. (1999) Nuclear import and export pathways. *J. Cell Biochem. Suppl* **32–33**, 76–83
- Mukhopadhyay, D., Arnaoutov, A. and Dasso, M. (2010) The SUMO protease senp6 is essential for inner kinetochore assembly. *J. Cell Biol.* **188**, 681–692
- Müller, S., Matunis, M.J. and Dejean, A. (1998) Conjugation with the ubiquitin-related modifier SUMO-1 regulates the partitioning of Pml within the nucleus. *EMBO J.* **17**, 61–70
- Nakamura, S., Roth, J.A. and Mukhopadhyay, T. (2002) Multiple lysine mutations in the c-terminus of p53 make it resistant to degradation mediated by Mdm2 but not by human papillomavirus e6 and induce growth inhibition in Mdm2-overexpressing cells. *Oncogene* **21**, 2605–2610
- Nasr, R., Chiari, E., El-Sabban, M., Mahieux, R., Kfoury, Y., Abdulhay, M., Yazbeck, V., Hermine, O., de Thé, H., Pique, C. and Bazarbachi, A. (2006) Tax ubiquitylation and SUMOylation control critical cytoplasmic and nuclear steps of NF-kappaB activation. *Blood* **107**, 4021–4029
- Nejmeddine, M., Barnard, A.L., Tanaka, Y., Taylor, G.P. and Bangham, C.R.M. (2005) Human t-lymphotropic virus, type 1, Tax protein triggers microtubule reorientation in the virological synapse. *J. Biol. Chem.* **280**, 29653–29660
- Nevels, M., Brune, W. and Shenk, T. (2004) SUMOylation of the human cytomegalovirus 72-kilodalton IE1 protein facilitates expression of the 86-kilodalton IE2 protein and promotes viral replication. *J. Virol.* **78**, 7803–7812
- Neznanov, N., Dragunsky, E.M., Chumakov, K.M., Neznanova, L., Wek, R.C., Gudkov, A.V. and Banerjee, A.K. (2008) Different effect of proteasome inhibition on vesicular stomatitis virus and poliovirus replication. *PLoS ONE* **3**, e1887
- Peebles, M.E. (1988) Differential detergent treatment allows immunofluorescent localization of the Newcastle disease virus matrix protein within the nucleus of infected cells. *Virology* **162**, 255–259
- Peebles, M.E., Wang, C., Gupta, K.C. and Coleman, N. (1992) Nuclear entry and nucleolar localization of the Newcastle disease virus (NDV) matrix protein occur early in infection and do not require other NDV proteins. *J. Virol.* **66**, 3263–3269
- Peloponese, J.J., Iha, H., Yedavalli, V.R.K., Miyazato, A., Li, Y., Haller, K., Benkirane, M. and Jeang, K. (2004) Ubiquitination of human T-cell leukemia virus type 1 Tax modulates its activity. *J. Virol.* **78**, 11686–11695
- Petersen, J.M., Her, L.S., Varvel, V., Lund, E. and Dahlberg, J.E. (2000) The matrix protein of vesicular stomatitis virus inhibits nucleocytoplasmic transport when it is in the nucleus and associated with nuclear pore complexes. *Mol. Cell Biol.* **20**, 8590–8601
- Pichler, A., Gast, A., Seeler, J.S., Dejean, A. and Melchior, F. (2002) The nucleoporin ranbp2 has SUMO1 e3 ligase activity. *Cell* **108**, 109–120
- Pichler, A. and Melchior, F. (2002) Ubiquitin-related modifier SUMO1 and nucleocytoplasmic transport. *Traffic* **3**, 381–387
- Prudden, J., Pebernard, S., Raffa, G., Slavina, D.A., Perry, J.J.P., Tainer, J.A., McGowan, C.H. and Boddy, M.N. (2007)

- SUMO-targeted ubiquitin ligases in genome stability. *EMBO J.* **26**, 4089–4101
- Raaben, M., Posthuma, C.C., Verheije, M.H., te Lintelo, E.G., Kikkert, M., Drijfhout, J.W., Snijder, E.J., Rottier, P.J.M. and de Haan, C.A.M. (2010) The ubiquitin-proteasome system plays an important role during various stages of the coronavirus infection cycle. *J. Virol.* **84**, 7869–7879
- Ramachandran, A. and Horvath, C.M. (2009) Paramyxovirus disruption of interferon signal transduction: Status report. *J. Interferon Cytokine Res.* **29**, 531–537
- Rangasamy, D. and Wilson, V.G. (2000) Bovine papillomavirus e1 protein is SUMOylated by the host cell Ubc9 protein. *J. Biol. Chem.* **275**, 30487–30495
- Rangasamy, D., Woytek, K., Khan, S.A. and Wilson, V.G. (2000) SUMO-1 modification of bovine papillomavirus e1 protein is required for intranuclear accumulation. *J. Biol. Chem.* **275**, 37999–38004
- Rodriguez, M.S., Desterro, J.M., Lain, S., Lane, D.P. and Hay, R.T. (2000) Multiple C-terminal lysine residues target p53 for ubiquitin-proteasome-mediated degradation. *Mol. Cell Biol.* **20**, 8458–8467
- Rosas-Acosta, G., Langereis, M.A., Deyrieux, A. and Wilson, V.G. (2005) Proteins of the pias family enhance the SUMOylation of the papillomavirus e1 protein. *Virology* **331**, 190–203
- Rosas-Acosta, G. and Wilson, V.G. (2008) Identification of a nuclear export signal sequence for bovine papillomavirus e1 protein. *Virology* **373**, 149–162
- Rosendorff, A., Illanes, D., David, G., Lin, J., Kieff, E. and Johannsen, E. (2004) EBNA3c coactivation with EBNA2 requires a SUMO homology domain. *J. Virol.* **78**, 367–377
- Ross, S., Best, J.L., Zon, L.I. and Gill, G. (2002) SUMO-1 modification represses sp3 transcriptional activation and modulates its subnuclear localization. *Mol. Cell* **10**, 831–842
- Sachdev, S., Bruhn, L., Sieber, H., Pichler, A., Melchior, F. and Grosschedl, R. (2001) Piasy, a nuclear matrix-associated SUMO e3 ligase, represses lef1 activity by sequestration into nuclear bodies. *Genes Dev.* **15**, 3088–3103
- Saha, A., Murakami, M., Kumar, P., Bajaj, B., Sims, K. and Robertson, E.S. (2009) Epstein-Barr virus nuclear antigen 3c augments Mdm2-mediated p53 ubiquitination and degradation by deubiquitinating mdm2. *J. Virol.* **83**, 4652–4669
- Sapetschnig, A., Rischitor, G., Braun, H., Doll, A., Schergaut, M., Melchior, F. and Suske, G. (2002) Transcription factor sp3 is silenced through SUMO modification by pias1. *EMBO J.* **21**, 5206–5215
- Schmitt, A.P., Leser, G.P., Waning, D.L. and Lamb, R.A. (2002) Requirements for budding of paramyxovirus simian virus 5 virus-like particles. *J. Virol.* **76**, 3952–3964
- Sehat, B., Andersson, S., Vasilcanu, R., Girnita, L. and Larsson, O. (2007) Role of ubiquitination in IGF-1 receptor signaling and degradation. *PLoS ONE* **2**, e340
- Sehat, B., Tofigh, A., Lin, Y., Trocmé, E., Liljedahl, U., Lagergren, J. and Larsson, O. (2010) SUMOylation mediates the nuclear translocation and signaling of the IGF-1 receptor. *Sci. Signal.* **3**, ra10
- Semmes, O.J., Majone, F., Cantemir, C., Turchetto, L., Hjelle, B. and Jeang, K.T. (1996) HTLV-I and HTLV-II Tax: Differences in induction of micronuclei in cells and transcriptional activation of viral LTRs. *Virology* **217**, 373–379
- Shaw, M.L., Cardenas, W.B., Zamarin, D., Palese, P. and Basler, C.F. (2005) Nuclear localization of the Nipah virus w protein allows for inhibition of both virus- and toll-like receptor 3-triggered signaling pathways. *J. Virol.* **79**, 6078–6088
- Shaw, M.L., García-Sastre, A., Palese, P. and Basler, C.F. (2004) Nipah virus v and w proteins have a common stat1-binding domain yet inhibit stat1 activation from the cytoplasmic and nuclear compartments, respectively. *J. Virol.* **78**, 5633–5641
- Shcherbik, N. and Haines, D.S. (2004) Ub on the move. *J. Cell Biochem.* **93**, 11–19
- Shembade, N. and Harhaj, E.W. (2010) Role of post-translational modifications of HTLV-1 Tax in NF- κ B activation. *World J. Biol. Chem.* **1**, 13–20
- Smith, M.R. and Greene, W.C. (1990) Identification of HTLV-I Tax trans-activator mutants exhibiting novel transcriptional phenotypes. *Genes Dev.* **4**, 1875–1885
- Smith, M.R. and Greene, W.C. (1992) Characterization of a novel nuclear localization signal in the HTLV-I Tax transactivator protein. *Virology* **187**, 316–320
- Sobko, A., Ma, H. and Firtel, R.A. (2002) Regulated SUMOylation and ubiquitination of ddmek1 is required for proper chemotaxis. *Dev. Cell* **2**, 745–756
- Song, M.S., Salmena, L., Carracedo, A., Egia, A., Lo-Coco, F., Teruya-Feldstein, J. and Pandolfi, P.P. (2008) The deubiquitylation and localization of pten are regulated by a HAUSP-Pml network. *Nature* **455**, 813–817
- Sorokin, A.V., Kim, E.R. and Ovchinnikov, L.P. (2007) Nucleocytoplasmic transport of proteins. *Biochemistry (Mosc.)* **72**, 1439–1457
- Speck, S.H., Chatila, T. and Flemington, E. (1997) Reactivation of Epstein-Barr virus: Regulation and function of the BZLF1 gene. *Trends Microbiol.* **5**, 399–405
- Spengler, M.L., Kurapatwinski, K., Black, A.R. and Azizkhan-Clifford, J. (2002) SUMO-1 modification of human cytomegalovirus IE1/IE72. *J. Virol.* **76**, 2990–2996
- Stehmeier, P. and Muller, S. (2009) Regulation of p53 family members by the ubiquitin-like SUMO system. *DNA Repair (Amst.)* **8**, 491–498
- Tatham, M.H., Geoffroy, M., Shen, L., Plechanovova, A., Hattersley, N., Jaffray, E.G., Palvimo, J.J. and Hay, R.T. (2008) Rnf4 is a poly-SUMO-specific e3 ubiquitin ligase required for arsenic-induced pml degradation. *Nat. Cell Biol.* **10**, 538–546
- Taylor, C.T., Furuta, G.T., Synnestvedt, K. and Colgan, S.P. (2000) Phosphorylation-dependent targeting of camp response element binding protein to the ubiquitin/proteasome pathway in hypoxia. *Proc. Natl. Acad. Sci. U. S. A.* **97**, 12091–12096
- Teale, A., Campbell, S., Van Buuren, N., Magee, W.C., Watmough, K., Couturier, B., Shipclark, R. and Barry, M. (2009) Orthopoxviruses require a functional ubiquitin-proteasome system for productive replication. *J. Virol.* **83**, 2099–2108
- Trotman, L.C., Wang, X., Alimonti, A., Chen, Z., Teruya-Feldstein, J., Yang, H., Pavletich, N.P., Carver, B.S., Cordon-Cardo, C., Erdjument-Bromage, H., Tempst, P., Chi, S., Kim, H., Misteli, T., Jiang, X. and Pandolfi, P.P. (2007) Ubiquitination regulates pten nuclear import and tumor suppression. *Cell* **128**, 141–156
- Ulrich, H.D. (2005) Mutual interactions between the SUMO and ubiquitin systems: A plea of no contest. *Trends Cell Biol.* **15**, 525–532
- von Kobbe C, van Deursen JM, Rodrigues, J.P., Sitterlin, D., Bachi, A., Wu, X., Wilm, M., Carmo-Fonseca, M. and Izaurralde, E. (2000) Vesicular stomatitis virus matrix protein inhibits host cell gene expression by targeting the nucleoporin nup98. *Mol. Cell* **6**, 1243–1252
- Wang, Y.E., Park, A., Lake, M., Pentecost, M., Torres, B., Yun, T.E., Wolf, M.C., Holbrook, M.R., Freiberg, A.N. and Lee, B. (2010) Ubiquitin-regulated nuclear-cytoplasmic trafficking of the Nipah virus matrix protein is important for viral budding. *PLoS Pathog.* **6**, e1001186
- Wilkinson, K.D., Urban, M.K. and Haas, A.L. (1980) Ubiquitin is the atp-dependent proteolysis factor i of rabbit reticulocytes. *J. Biol. Chem.* **255**, 7529–7532
- Wilson, V.G. and Rangasamy, D. (2001) Intracellular targeting of proteins by SUMOylation. *Exp. Cell Res.* **271**, 57–65

- Wilson, V.G., West, M., Woytek, K. and Rangasamy, D. (2002) Papillomavirus e1 proteins: Form, function, and features. *Virus Genes* **24**, 275–290
- Wirblich, C., Tan, G.S., Papaneri, A., Godlewski, P.J., Orenstein, J.M., Harty, R.N. and Schnell, M.J. (2008) Ppey motif within the rabies virus (rv) matrix protein is essential for efficient virion release and rv pathogenicity. *J. Virol.* **82**, 9730–9738
- Wood, L.D., Irvin, B.J., Nucifora, G., Luce, K.S. and Hiebert, S.W. (2003) Small ubiquitin-like modifier conjugation regulates nuclear export of tel, a putative tumor suppressor. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 3257–3262
- Yasuda, J., Nakao, M., Kawaoka, Y. and Shida, H. (2003) NEDD4 regulates egress of ebola virus-like particles from host cells. *J. Virol.* **77**, 9987–9992
- Yoshida, T., Nagai Y^Yoshii, S., Maeno, K. and Matsumoto, T. (1976) Membrane (m) protein of hvj (sendai virus): Its role in virus assembly. *Virology* **71**, 143–161
- Yu, L., Mohanram, V., Simonson, O.E., Smith, C.I.E., Spetz, A. and Mohamed, A.J. (2009) Proteasome inhibitors block HIV-1 replication by affecting both cellular and viral targets. *Biochem. Biophys. Res. Commun.* **385**, 100–105
- Yuan, J., Luo, K., Zhang, L., Cheville, J.C. and Lou, Z. (2010) Usp10 regulates p53 localization and stability by deubiquitinating p53. *Cell* **140**, 384–396
- Zhong, S., Müller, S., Ronchetti, S., Freemont, P.S., Dejean, A. and Pandolfi, P.P. (2000) Role of SUMO-1-modified Pml in nuclear body formation. *Blood* **95**, 2748–2752
- Zhou, H., Aujay, M.A., Bennett, M.K., Dajee, M., Demo, S.D., Fang, Y., Ho, M.N., Jiang, J., Kirk, C.J., Laidig, G.J., Lewis, E.R., Lu, Y., Muchamuel, T., Parlati, F., Ring, E., Shenk, K.D., Shields, J., Shwonek, P.J., Stanton, T., Sun, C.M., Sylvain, C., Woo, T.M. and Yang, J. (2009) Design and synthesis of an orally bioavailable and selective peptide epoxyketone proteasome inhibitor (pr-047). *J. Med. Chem.* **52**, 3028–3038
- Zhu, L., Santos, N.C. and Kim, K.H. (2009) Small ubiquitin-like modifier-2 modification of retinoic acid receptor-alpha regulates its subcellular localization and transcriptional activity. *Endocrinology* **150**, 5586–5595

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