Role of Ubiquitin and SUMO in Intracellular Trafficking

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Abstract

Precise location of proteins at a given time within a cell is essential to convey specific signals and result in a relevant functional outcome. Small ubiquitin-like modifications, such as ubiquitin and SUMO, represent a delicate and diverse way to transiently regulate intracellular trafficking events of existing proteins in cells. Trafficking of multiple proteins is controlled reversibly by ubiquitin and/or SUMO directly or indirectly via regulation of transport machinery components. Regulation is dynamic and multilayered, involving active crosstalk and interdependence between post-translational modifications. However, in most cases regulation appears very complex, and the mechanistic details regarding how ubiquitin and SUMO control protein location in cells are not yet fully understood. Moreover, most of the findings still lack in vivo evidence in multicellular organisms.

Posttranslational modifications in regulation of cellular processes

General principles of ubiquitination and SUMOylation

Posttranslational modification (PTM) of proteins is a powerful, fast and often transient way to control the fate of existing proteins and cell behaviour. In addition to e.g. chemical groups, such as phosphate groups, proteins can be modified by small polypeptides, such as ubiquitin and SUMO. Ubiquitin and SUMO share a similar three-dimensional structure and are principally covalently linked at lysine (K) residues of substrate proteins by a similar conjugation pathway (Hershko et al., 1998; Hay, 2013). Unlike ubiquitin, SUMO is preferentially attached at SUMO consensus sites \(\Psi Kx\epsilon\) in substrates \(\Psi =\) hydrophobic residue with high preference for I or V, \(x =\) any amino acid) under steady state conditions, but under stress conditions in particular more non-consensus sites are SUMOylated (Hendriks et al., 2016). Mammals express ubiquitously SUMO1, SUMO2 and SUMO3, whereas SUMO4 and SUMO5 are only expressed in some tissues and their functional role is unclear (Pichler et al., 2017). SUMO2 and SUMO3 are nearly identical and resemble approximately 50% to SUMO1 (Geiss-Friedlander et al., 2007).

Precursors of ubiquitin and SUMO are processed to mature forms prior to conjugation and activated by an E1 enzyme in a reaction depending on ATP. Subsequently ubiquitin and SUMO are transferred to an E2 enzyme, and E3 ligase alone or with E2 ligates them to substrates by an isopeptide bond (Hershko et al., 1998; Hay, 2013). Modification is reversible and specific proteases can cleave ubiquitin and SUMO from substrates (Williamson et al., 2013; Kunz et al.,...
Proteins can be tagged by a single ubiquitin or SUMO either at one or at multiple lysines. In addition, modifications can exist as chains. Ubiquitin contains seven internal lysine residues (K6, K11, K27, K29, K33, K48, K63) that are involved in the formation of ubiquitin-ubiquitin polymers, known as polyubiquitin chains (Pickart et al., 2004; Heride et al., 2014). In addition, the linkage between the amino-terminal amino group of methionine on a ubiquitin can be conjugated with a target protein and the carboxy-terminal carboxy group of the incoming ubiquitin for linear chains (Walczak et al., 2012). SUMOs can also form polymeric chains through internal lysine residues (Geiss-Friedlander et al., 2007). Monoubiquitination and different chain types determine the fate of the modified protein (Piper et al., 2014). For example, K48 ubiquitin chains are considered classical signals for proteasomal degradation and K63 ubiquitin chains are linked to trafficking and DNA damage response (Pickart et al., 2004). Recently basic principles of the field have been challenged by e.g. discoveries of mixed polyubiquitin chains and ubiquitination of non-lysine residues (Piper et al., 2014). Less is known regarding the consequences of attachment of either single or many SUMO moieties. SUMO chains are at least implicated in recruitment of SUMO-targeted ubiquitin ligases (Lallemand-Breitenbach et al., 2008).

Components, regulation and function of ubiquitin and SUMO machinery

Multiple enzymes involved in ubiquitin conjugation have been recognized. The human ubiquitin machinery comprises a network including two ubiquitin E1 enzymes, approximately 40 ubiquitin E2s, and more than 600 E3 ubiquitin ligases in the human genome (Heride et al., 2014). Three major types of E3 ligases are really interesting new gene (RING) type E3s comprising most of human E3s, homologous to E6-AP carboxyl terminus (HECT) and RING-between-RING (RBR) E3s ligases (Deshaies et al., 2009; Rotin et al., 2009; Wenzel et al., 2012). Ubiquitin is cleaved by approximately 100 deubiquitinating enzymes (DUBs) (Williamson et al., 2013; Heride et al., 2014). Intriguingly, only one heterodimeric E1 (Sae1/Aos1–Sae2/Uba2) and E2 (Ubc9) are known to be involved in SUMO conjugation (Hay, 2013). Three classes of SUMO E3s have been widely accepted and characterized including SP-RING Siz/PIAS ligases, RanBP2 and ZNF451 ligases (Geiss-Friedlander et al., 2007; Rytinki et al., 2009; Cappadocia et al., 2015). Both E2 and E3 can select substrates for SUMOylation, and spatial and temporal regulation of co-localization appears integral for substrate selection (Pichler et al., 2017). Cysteine proteases of the sentrin-specific protease (SENP) family members reverse SUMO conjugation in mammals (Kunz et al., 2018). Moreover, desumoylating isopeptides 1 and 2 and ubiquitin-specific protease-like 1 can deSUMOylate proteins (Shin et al., 2012; Schulz et al., 2012). Whereas ubiquitin machinery is widely expressed within a cell, components of SUMO conjugation pathway mainly localize at the nuclear pores and nucleus. Thus, most of SUMOylated substrates are nuclear proteins, although SUMO modified proteins outside of nucleus exist (Geiss-Friedlander et al., 2007).

Ubiquitination is regulated by extracellular stimuli including growth factors and cytokines, stress and cell cycle changes (Pickart et al., 2004; Heride et al., 2014). Different types of stress stimuli such as heat shock, hypoxia, reactive oxygen species, DNA damage and proteotoxic stress regulate the activity of SUMOylation machinery (Hieta-kangas et al., 2003; Shao et al., 2004; Bossis et al., 2006; Galanty et al., 2009; Morris et al., 2009; Seifert et al., 2015). Both ubiquitin and SUMO modifications can alter the function, activity, location and stability of their targets. Ubiquitin and SUMO are recognized by either ubiquitin-binding domains (UBD) or sumo-interacting domains (SIM), respectively, and serve as platforms for non-covalent protein–protein interactions (Seet et al., 2006). These domains have been identified in hundreds of proteins. PTMs are also involved in regulation of conjugation specificity and activation (Pichler et al., 2017). For example, SUMO-targeted ubiquitin ligase (STUbL) RNF4 contains multiple SIMs and a RING-domain to bind SUMOylated proteins and an E2 ubiquitin-conjugation enzyme (Sun et al., 2007; Tatham et al., 2008).
Ubiquitin and SUMO as signals regulating membrane trafficking and endocytosis

General principles of membrane protein trafficking and endocytosis in cells

Internalization and endocytosis of cell surface proteins including different receptors often occurs via the clathrin-dependent endocytic pathway. Cell surface receptors are clustered to pits coated with clathrin that pinch off of the membrane forming vesicles and early endosomes. From early endosomes cargo can be directed back to plasma membrane via recycling endosomes or destined to lysosomal degradation via late endosomes and multivesicular bodies (Mellman and Yarden, 2013). Also, other types of endocytic routes exist, including cholesterol-rich membrane structures, such as lipid rafts and caveolae (Barbieri et al., 2016). Several adaptor proteins and PTMs control these processes (Piper et al., 2014). Membrane protein trafficking and endocytosis system are tightly connected to protein homeostasis.

Ubiquitin and SUMO in receptor internalization at the cell surface and in endocytic compartments

Initial studies in yeast suggested that ubiquitin can function as a sorting signal regulating the internalization and endosomal targeting of cell surface receptors (Kölling et al., 1994; Hicke et al. 1996, Terrell et al., 1998). After that several studies in different systems and organisms have confirmed that ubiquitin is important regulator of endocytosis and its most critical functional role is likely at the sorting endosomes (Mellman and Yarden, 2013). Endocytosis of human receptor tyrosine kinases (RTKs) is suggested to be regulated by multimonoubiquitination and K63-polyubiquitination, and there is some controversy regarding the significance of specific ubiquitination type due to methodological challenges to address this complex regulatory system (Haglund et al., 2003; McCullough et al., 2004; Huang et al., 2006; Sundvall et al., 2008; Huang et al., 2013). During endocytosis the ubiquitin moieties of cargo are recognized by different endocytic adaptors and regulators via UBDs, such as Eps15, epsin and endosomal sortin complex required for transport (ESCRT) (Piper et al., 2014). Ubiquitin is also indirectly involved in control of endocytosis as components of endocytic machinery are actively regulated by ubiquitination (Piper et al., 2014).

SUMOylation has been implicated in the regulation of endocytic processes, although when compared to ubiquitin the evidence is less extensive, and more work is needed to make general conclusions. Nevertheless, endocytosis of kainate receptor GluR6 is regulated by SUMOylation and non-SUMOylated mutant of GluR6, GluR6-K886R, is endocytosis-impaired due to unknown mechanisms (Martin et al., 2007). SUMOylation of Smoothened (Smo) promotes its localization at the cell surface (Ma et al., 2016). Intriguingly, mechanistically SUMO interferes with efficient Smo ubiquitination by recruiting deubiquitinase UBPY/USP8 in a SIM-dependent manner (Ma et al., 2016). SUMOylation regulates also cell surface expression and activity of VEGFR2 receptor tyrosine kinase (Zhou et al., 2018). VEGFR2–SUMO1 fusion protein but not SUMOylation defective mutant VEGFR2 accumulated at the Golgi suggesting that mechanistically SUMO regulates exocytosis of VEGFR (Zhou et al., 2018). SUMOylation can also indirectly regulate endocytosis. Components of endocytic machinery are modified and regulated by SUMO, such as CIN85 (Tossidou et al., 2012) and arrestin (Wyatt et al., 2011). Interestingly, also dynamin interacts with several members of the SUMOylation machinery (Mishra et al., 2004). Moreover, SUMO can be important for membrane binding of proteins. SUMOylation of PTEN at lysine 266 within CBR3 loop fosters binding of PTEN to plasma membrane via electrostatic interactions (Huang et al., 2012).

Ubiquitin and SUMO as signals regulating nucleocytoplasmic shuttling and subnuclear targeting

General principles of nuclear import, subnuclear targeting and export

Passage of proteins in and out of the nucleus through nuclear pore complexes is tightly regulated.
In principle, many of the nuclear proteins contain nuclear localization signal (NLS) and nuclear export signal (NES) that facilitate trafficking via associations with karyopherins including importins and exportins (CRM1) together with Ran-GTP, respectively (Stewart M, 2007).

**Ubiquitin and SUMO in nuclear targeting and trapping**

Molecular mechanism by which ubiquitin machinery controls protein functions are very complex but, nevertheless, some evidence exists to suggest the role for ubiquitin as a signal controlling nucleocytoplasmic trafficking. Ubiquitination of p53 contributes to nuclear export, and although the regulation of p53 ubiquitination and trafficking has turned out to be very complicated and likely dependent of conditions, attachment of monoubiquitin in particular is suggested to play a role in trafficking (Lohrum et al., 2001; Li et al., 2003). Moreover, similar type of regulation has been suggested for another tumour suppressor protein, PTEN and NF-kB essential modulator NEMO (Huang et al., 2003; Trotman et al., 2007).

SUMO was initially discovered as a modifier of RanGap1 targeting it to nuclear pore complex (Matunis et al., 1996, Mahajan et al., 1997). Later SUMO has been implicated in both increasing and decreasing the nuclear accumulation of some proteins. Mechanistically, covalent linkage of SUMO may directly block protein interactions relevant for transport or generate SIM-mediated interaction platform facilitating or interfering with transport. SUMOylation is suggested to regulate nuclear import of full-length IGF receptor (Sehat et al., 2010). The levels of SUMOylation defective mutant IGFR are similar at the cell surface compared with wild type receptor, but the mutant receptor cannot translocate to nucleus unlike wild type (Sehat et al., 2010). Specifically IGFR is suggested to interact with RanBP2 at nuclear pores and that RanBP2 acts as an SUMO E3 ligase for IGFR (Pancham et al., 2015). SUMOylation may also increase the stability of IGFR (Pancham et al., 2015). Trafficking of SUMO machinery components is also under the control of SUMO. SUMOylation of Sae2 in the c-terminus within functional NLS efficiently increases nuclear accumulation (Truong et al., 2012).

SUMO has been suggested to regulate nuclear export of some proteins, including transcriptional repressor TEL, Kruppel-like transcription factor (KLF2), Serine hydroxymethyltransferase 1 (SHMT1), PTEN, p53, ErbB4 and Notch (Wood et al., 2003; Du et al., 2008, Anderson et al., 2009; Bassi et al., 2013, Santiago et al., 2013, Knittle et al., 2017; Antila et al., 2018). SUMOylation adjacent to NES of KLF5 interferes with its interaction with nuclear export receptor CRM1 resulting in the inhibition of efficient export and increased accumulation in the nucleus (Du et al., 2008). ErbB4 RTK undergoes regulated intramembrane proteolysis (RIP) releasing an intracellular domain (ICD) that can translocate to nucleus and regulate transcription. PIAS3 catalysed SUMOylation within NES of ErbB4 increases the nuclear accumulation of a tyrosine phosphorylated ICD by altering the interaction with CRM1 (Sundvall et al., 2012; Knittle et al., 2017). SUMOylation deficient mutant of ErbB4 accumulates less in nucleus and cannot convey efficiently nuclear signalling (Knittle et al., 2017). Another receptor undergoing RIP, Notch, is also SUMOylated in the nucleus and SUMOylation increases nuclear accumulation of the ICD, but mechanisms resulting in accumulation remain to be elucidated (Antila et al., 2018). SUMOylation of SHMT1 increases nuclear accumulation and mutation of the SUMO motif prevents translocation to the nucleus due to unknown mechanisms (Anderson DD et al., 2009). Interestingly, same sites are also ubiquitinated with K63 polyubiquitin chains increasing stability in the nucleus (Anderson et al., 2012). SUMOylation of PTEN (at K254) is suggested to regulate the efficient nuclear accumulation and subsequently DNA damage response. When compared to wild type, SUMOylation deficient mutant of PTEN localizes less into the nucleus and cannot efficiently regulate homologous recombination (Bassi et al., 2013). Nucleocytoplasmic distribution of SUMOylation-deficient transcriptional repressor TEL also changes compared to wild type (Wood et al., 2003). However, the direct mechanism how SUMO regulates subcellular localization of PTEN or TEL is not clear (Wood et al., 2003; Bassi et al., 2013). On the contrary, SUMOylation of p53 stimulates its nuclear export by increasing the
disassembly of p53 from the CRM1 in the cytosol (Santiago et al. 2013). Moreover, SUMOylation regulates nuclear export and intranuclear distribution of adenovirus E1B-55K protein (Kindsmüller et al., 2007). Altogether nuclear export and SUMOylation appear to be closely connected due to CRM1-mediated interaction of export complexes with SUMO E3 ligase RanBP2 (Ritterhoff et al., 2015) and SUMOylation also regulates nuclear transport via covalent modification of transport machinery (Rothenbusch et al., 2012).

Regulation of subnuclear targeting by ubiquitin and SUMO
Ubiquitin and SUMO system has been indicated in targeting proteins into certain subnuclear structures. Subnuclear targeting can direct proteins into locations essential for their functions, trap protein in locations where they are not available to regulate e.g. transcription or alter their susceptibility to regulators of stability. Both ubiquitin and SUMO are implicated in correct targeting of DNA repair factors to the sites of DNA damage (Ulrich, 2014).

For example, BRCA1 targeting to double-strand breaks is regulated by K63-linked ubiquitination and SUMO and the process involves RNF4 (Galanty et al., 2009; Morris et al., 2009; Guzzo et al., 2012). PML bodies are subnuclear structures involved in regulation of transcription and host a lot of transcription factors and their regulators (Zhong et al., 2000a). PML is strongly SUMOylated and SUMOylation regulates the integrity of PML bodies and stability of PML as SUMOylation deficient mutant PML does not form nuclear bodies when expressed in PML null cells (Ishov et al., 1999; Müller et al., 1998; Zhong et al., 2000a,b). Several nuclear proteins are hosted in PML bodies and SIM-mediated interactions are thought to be important for assembly (Shen et al., 2006). SUMO E3 ligases and deSUMOases have specific nuclear localizations and regulate substrate localization, but regulation is often not SUMO-dependent (Sachdev et al., 2001; Kotaja et al., 2002; Hietakangas et al., 2003). On the other hand, SUMO is suggested to be important in the regulation of subnuclear localization of Nuclear Factor of Activated T-cells (NFAT1) (Terui et al., 2004) or nucleolar localization of Proline-, glutamic acid- and leucine-rich protein 1 (PELP1) involved in ribosome biogenesis and regulation of transcription (Finkbeiner et al., 2011).

The genetic evidence in vivo and the significance of ubiquitin and SUMO mediated regulation of trafficking in human diseases
Genetic studies using targeted gene disruption in mice suggest that ubiquitin and SUMO pathways are essential, but a lot of redundancy is evident with many of the pathway components. For example, SUMO1 and SUMO3 knockout mice are viable, but SUMO2 knockout mice die during embryogenesis as well as Ubc9 knockout is lethal in mice (Nacerddine et al., 2005; Evdokimov et al., 2008; Zhang et al., 2008; Wang et al., 2014). Unfortunately, very little is known regarding genetic models of modification-deficient mutants and their phenotypes in vivo.

Deregulation of ubiquitin conjugation machinery and altered protein ubiquitination has been reported in diseases such as neurodegenerative diseases and cancer (Popovic et al., 2014). SUMOylation seems to be a general protective mechanism against the damage caused by stresses such as low oxygen and nutrient deprivation and may also protect neurons after stress and support the growth of cancer cells. Mutations targeting ubiquitin conjugation machinery, such as E3 ligases, and somatic mutations altering ubiquitin ligase binding and subsequent trafficking of targets, including deregulated endocytosis of RTKs, in cancer have been reported (Mellman and Yarden, 2013). SUMOylation seems to be up-regulated in cancer due to e.g. overexpression of the pathway components, such as Ubc9 and some E3 ligases (Seeler et al., 2017). Interestingly, it has been reported that the SUMOylation site is essential for leukemic transformation mediated by PML-RARalpha in acute promyelocytic leukemia (Zhu et al., 2005). Moreover, a germline variant of melanoma lineage-specific microphthalmia-associated transcription factor (MITF), MITF-E318K, increases predisposition to sporadic melanoma and renal cell carcinoma. This mutation disrupts SUMOylation site of MITF and results in increased transcriptional activity promoting tumourigenic properties in experimental models, but no changes
in trafficking were observed (Bertolotto et al., 2011; Yokoyama et al., 2011). However, despite extensive sequencing efforts, the evidence is so far lacking for ubiquitin or SUMO binding sites to be systematically targeted by somatic mutations in cancers. Development and research efforts using drugs that specifically inhibit the conjugation pathway activity and components will help to understand the role of SUMO and non-proteolytic functions of Ubiquitin in different contexts.

Conclusions

Emerging evidence strongly implicates both ubiquitination and SUMOylation in the regulation of intracellular trafficking of several proteins at multiple levels. However, these regulation systems are highly dynamic, complex and complicated with several overlapping compensatory mechanisms and the possibility of targeting same residues with different PTMs, making it challenging to design conclusive studies. In most cases the precise convincing mechanistic insights on how this regulation of trafficking happens remain to be elucidated. In addition, the field is still lacking evidence supporting many general findings in vivo. Nevertheless, conceptually ubiquitin and SUMO have already emerged as powerful and potent regulators of essential cellular processes, including protein trafficking to control temporal and special protein functions, and these PTMs are definitely an important focus for future research.

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References


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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.


