SUMOYLATION – AN EMERGING PLAYER IN PROTEIN MODIFICATION AND CELL FUNCTION

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Introduction

With the advent of refined mass spectrometry techniques, there is an unprecedented opportunity to identify post-translational modifications at a proteome-wide level. Various post-translational modifications including phosphorylation, methylation, acetylation, sumoylation and ubiquitination are known and some of them have been attributed to regulate cellular function. While unravelling the precise role of post-translational modifications is non-trivial, several studies highlight the interdependency of the modifications in both their occurrence and regulatory function. The scope of this review is limited in describing the role and regulation of sumoylation in process of cell differentiation and cell cycle.

The Small Ubiquitin like Modifier (SUMO)

In 1996 Guenter Bolbel's lab reported for the first time that the nuclear translocation of RanGAP1 (Ran-GTPase-activating protein) is modulated by a novel ubiquitin-like modification (Matunis et al., 1996). Following this, another observation was made by Frauke Melchior's group which revealed that SUMO modification targets RanGAP1 to the nuclear pore complex (NPC) where it interacts with Ran binding protein RanBP2, to play a role in nuclear import through GTPase activity of the Ran protein (Mahajan et al., 1997). Since then hundreds of protein till date have been shown to be regulated by sumoylation. The list is ever increasing, indicating the fundamental and widespread role of sumoylation in regulating cellular events.

SUMOs and the sumoylation pathway

Four different kinds of SUMO, SUMO-1, 2, 3 and 4 are known (Dohmen, 2004) so far. SUMO-1, consists of 100 amino acids, shows 50% homology with SUMO-2 and SUMO-3. Because of the very high sequence homology, SUMO-2 and 3 are also termed as SUMO-2/3 (Johnson et al. 2004). NMR structure shows that SUMO-1 and ubiquitin (18% homology) share similar folding pattern. The extra N-terminal domain of SUMO1 is absent in ubiquitin, which provides these two molecules different and distinct structural and functional specificity (Bayer et al., 1998).

For sumoylation (covalent modification of protein by SUMO), any given target protein should possess a well-characterized SUMO consensus motif, which is ψ-K-X-E, where ψ is a hydrophobic amino acid, mainly L (leucine), I (isoleucine) or V (valine). Lysine is the most crucial; its mutation abolishes sumoylation from its target protein. Under normal cellular conditions, it is the SUMO-1 that modifies most of the protein targets, whereas SUMO2/3 preferentially conjugate their target proteins during cellular stress (Saitoh et al., 2000). Details of the sumoylation machinery are shown in figure 1.

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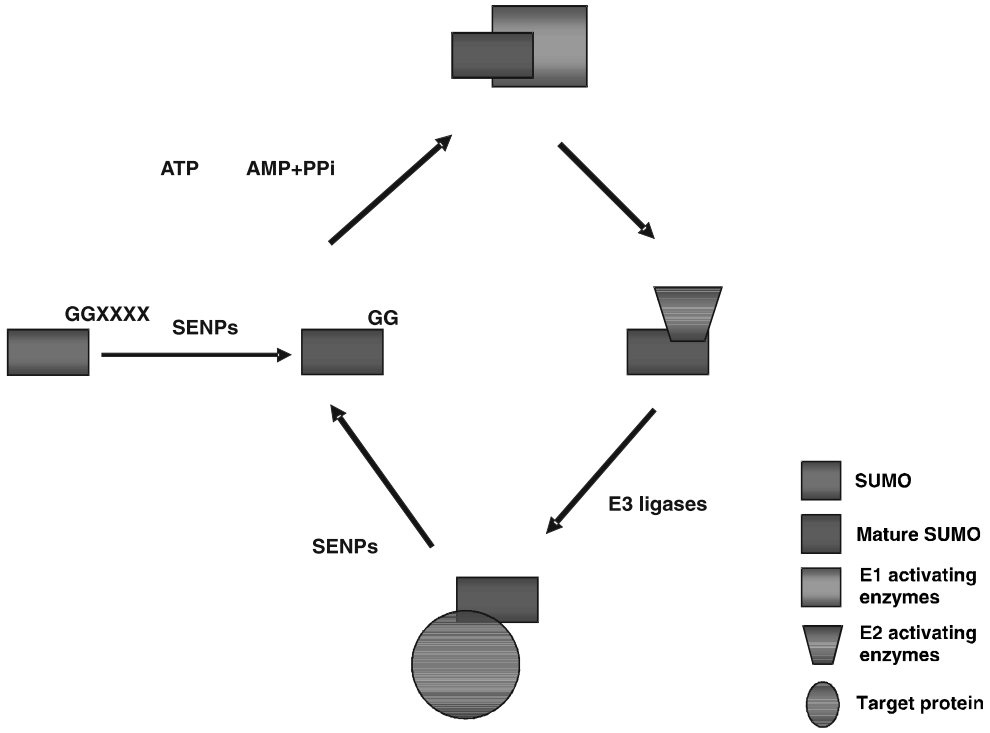

Figure-1: The sumoylation pathway- SENPs (sentrin-specific proteases) removes four C-terminal amino acids from SUMO, thereby exposing the C-terminal Gly-Gly residue of SUMO.

This form of SUMO1 is mature one . E1 activating enzyme (heterodimer of AOS1-UBA2) is required for the activation step, which results in a thioester bond formation between the C-teminal Gly residue and C173 in UBA2. Next step is the transfer of the activated SUMO to the E2 activating enzyme Ubc9. The final step involves in the formation of an isopeptide bond between the C-terminal Gly residue and the Lys residue (third amino acis in a SUMO-consensus motif) of the target protein. SENPs acts on the sumoylated protein to remove SUMO moiety and make this process very dynamic.

In most cases, sumoylation results in the addition of a single SUMO moiety to the individual acceptor lysine residue in the substrate protein. However, there are reports of polySUMO chain formation (like polyubiquitination) *in vitro* as well as *in vivo* (Tatham et al 2001, Mukhopadhyay et al., 2006). Although, the biological relevance of SUMO chain is not clear, Skilton et al. (2009) described the role of SUMO chain formation in response to replication arrest in *S. pombe*, indicating that polySUMOylation might have a more important role than previously thought.

The SUMO substrates and site of sumoylation

Till date, hundreds of proteins have been shown to be modified by SUMO (Table1). As may be noted from Table 1, transcription factors are the most predominantly known SUMO targets, which supports SUMO's global role in regulating chromatin structure and transcription. Although majority of SUMO modifications are reported to be nuclear proteins, the SUMO

machinery is not restricted to the nucleus, rather many protein in the cytosol, plasma membrane, mitochondria and the endoplasmic reticulum are know to be sumoylation targets (Melchior F et al., 2003).

Mechanism of protein regulation by sumoylation

Depending on the status of extra- and intracellular signaling, many substrate proteins can be sumoylated. SUMO brings additional way to modulate protein behaviour. Here we discuss the possible mechanisms by which SUMO modulate its substrate.

Table1 - Various classes of proteins that are modified by SUMO

Transcription factors and chromatin modifiers	**PML-Nbs**	**Nuclear pore complex**	**Cytosolic protein**	**Signal transducer**
P53, Jun, CREB, Lef-1, NFATc1, NFATc2, IRF-1, C-Myb, Pdx1, GATA-2, AP-2, Sp3, C/EBP, STAT1, HDAC1, HDAC4, PIAS1, Pc2, p300, GR, Bright, NcoR, HP1, LRH-1, MEF2, HSF1, Elk-1, PARP-1, SATB2	PML, Sp100, Daxx, TEL	RanGAP1, RanBP2	GLUT1, GLUT4	IκB, Axin, Smad4, Mek1, CamKII

Compartmentalization of the target protein

One of the best characterized features of sumoylated protein is its differential subcellular or subnuclear localisation. Most of the sumoylated protein localize to a specialized subnuclear structure called Promyelocytic Leukaemia Nuclear Bodies (PML-NBs). PML-NBs are large macromolecular structures harboring various kinds of protein and chromatin loops (Boisvert et al., 2000). The prime components of PML-NBs are the sumoylated PML proteins. It also houses transcription factors, chromatin modifiers, DNA repair proteins and most importantly other sumoylated proteins (Dellaire et al., 2004). PML-NBs can also serve as a cellular reservoir for various transcription factors.

Sumoylation can compartmentalize its target protein by sequestration into PML-NBs thereby altering protein's localization and activity. Compartmentalization leads to change of protein's microenvironment, which might be crucial for protein's activity. For instance, the activity of the transcription factor, LEF1 was repressed when it was sequestrated into the PML-NBs upon sumoylation (Sachdev et al., 2001).

Changing protein-protein interaction profile

SUMO can provide a surface for its target proteins to make or break new protein-protein interactions. This has a significant impact on the ongoing protein activity and protein complex assembly. Sumoylation of transcription factors (such as NFATc1, Elk-1) promotes interaction with HDACs (histone deacetylase) thereby modify its target gene promoter from an open to a closed chromatin state, which leads to cessation of ongoing transcription. (Nayak et al., 2009, Yang et al., 2004). Such regulation is critical for the dynamic maintenance of "ON-OFF" switch-like behaviour of a protein.

Sumoylation and cellular differentiation

During the course of development, a less specialized cell becomes more specialized by a process called differentiation. This specifies a particular fate for any given cell. The cell differentiation often characterized by either up/down-regulation of any protein such as transcription factor or fine-tuning a pre-existing protein's activity by adding or removing phosphate group, acetyl group etc. Sumoylation emerged as one of the main regulatory switch which fine-tunes protein activity and results in adopting a different fate by a cell.

NFAT (nuclear factor of activated T cells) is a family of transcription factors, crucial for T cell differentiation. Five different isoform of NFAT (NFATc1, c2, c3, c4 and NFAT5) has been reported so far (Rao et al., 1997). The strong up-regulation of NFAT expression is among one of the key hallmarks of the activated T cells (Serfling et al., 2000). After encountering antigen by T lymphocytes, a cascade of signaling event generates from TCR (T cell receptor), which ultimately culminates into dephosphorylation and activation of NFAT proteins residing in the cytosol. The activated NFAT now translocates to the nucleus and induces the expression of various cytokine genes such as IL2 (Interleukin 2). This event makes the naïve T cell to differentiate into effector T cell, which is characterized by massive expression of NFATc1/A (short isoform of NFATc1) and its target gene IL2. Recently, an isoform-specific NFATc1 sumoylation has been reported (Nayak et al., 2009). While the short isoform of NFAT (NFTAc1/A) can't be sumoylated, the long isoform of NFATc1 (NFATc1/C) is sumoylated and this event suppresses its transcriptional activity by relocating NFATc1/C to PML-nuclear bodies and recruitment of HDAC on its target gene promoter IL2. In naive cell, which shows predominant expression of the long, sumoylatable isoform, the induction level of IL2 is low, whereas it is high in memory and effector T cells (Chuvpilo et al., 1999). Therefore, sumoylation keeps IL2 production at an optimum level required for naïve cells before it differentiate into effector cells, characterized by mass production of IL2 (Nayak et al., 2009).

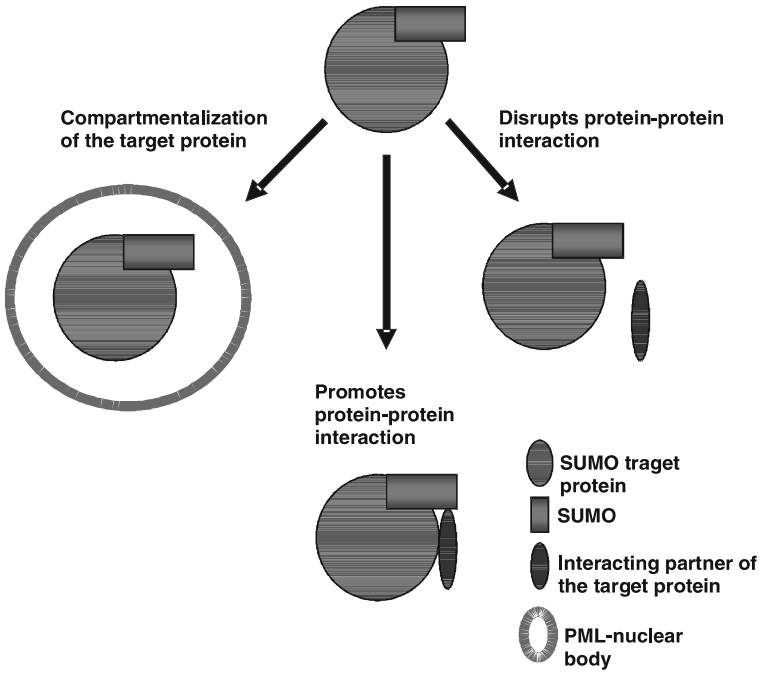

Figure-2 : Mode of SUMO-mediated regulation of substrate protein.

Attaching SUMO moiety with a protein can lead to compartmentalization of the protein. SUMO can also regulates protein function by promoting and/or disrupting interaction with other interaction partners.

Interestingly, in nTregs (which do not express IL-2, synthesize no NFATc1/A after restimulation, unlike conventional CD4+ cells) NFATc1/C expression is predominant and also the level of NFATc1/C sumoylation is enhanced. Hence, NFATc1/C sumoylation might also serve as one of the mechanisms to maintain anergy phenotype (characterized by no IL2 production) of T-cells. By employing isoform-specific sumoylation, activated T cell comes down to a basal level when an antigen is cleared and immune reaction must stop in order to maintain immune homeostasis

Berberich-Siebelt F. et al. (2006), described that sumoylation interferes with the transcription factor C/EBP (CCAAT/Enhancer-Binding Protein)-mediated c-myc repression which dictates a balancing act between proliferation and differentiation in peripheral T cells. In addition, sumoylation of DRIL1 transcription factor has been found to upregulate genes determining leukocyte fate (Prieur et al 2009).

Dictyostelium discoideum (a species of soil-living amoeba) is a classic choice of developmental biologist to study the mechanisms of cell fate determination and differentiation. In response to particular environmental conditions (e.g., starvation) Dictyostelium starts to form aggregate and differentiate into fruit body, which gives rise to single cell offspring. During aggregation, starvation initiates a cascade of biochemical changes, which includes extracellular

cAMP signalling, activation of MAP-kinase pathway etc. (Gilbert 2006). The DdMEK1 (Dictyostelium MAP kinase kinase) is required for proper aggregation in Dictyostelium. Mutation of DdMEK1 has a severe effect on its differentiation, characterized by extremely small aggregate sizes and formation of slugs and terminal fruiting bodies that are significantly smaller than those of wild-type cells (Ma et al., 1997). The Dictyostelium MAP kinase kinase DdMEK1 regulates chemotaxis and is essential for chemoattractant-mediated activation of guanylyl cyclase. DdMEK1 activity is required for the aggregation and chemotaxis, which is followed by differentiation. Sumoylation has emerged as a crucial factor in controlling the fate of Dictyostelium. Sobkho et al (2002) have shown that DdMEK1 is rapidly and transiently sumoylated in response to chemoattractant stimulation. During chemotaxis, sumoylation is essential for proper function of MEK1 and its translocation from the nucleus to the cytosol and cortex, including the leading edge of chemotaxing cells.

Sumoylation and mitosis

Cell differentiation contrasts with cell division in being a quick and dynamic event wherein several post-translational changes orchestrate several mechanical events to divide the cell into two. Many key mitotic players bear potential sumoylation sites but their role in cell division is not known (AN and VMD, unpublished work). First illustration of sumoylation controlling mitosis was reported when Seufert et al (1995) found that the budding yeast Ubc9p (E2 activating enzyme) is required for cyclinB degradation. Also, SMT3 (SUMO paralogues in yeast S. cerevisiae) was found in yeast screen for temperature-sensitive mutants defective in chromosome separation (Biggins et al 2001). More recently, sumoylation is found to be essential for nuclear integrity and chromosome segregation in mice (Nacerddine et al., 2005). In this report the authors found that Ubc9-deficient embryos die at the early post-implantation stage. The same embryos were viable in culture for 2 days, but they failed to expand further and the inner cellular mass cells underwent apoptosis. Loss of Ubc9 resulted in defective chromosome condensation and segregation.

During mitosis, Centromere-associated protein E (CENP-E) plays an important role in maintaining chromosomal stability through efficient stabilization of microtubule capture at kinetochores (Putkey et al., 2002). Sumoylation of this kinetochore bound kinesin-like motor protein is essential for its function during prometaphase when all the kinetochores capture microtubules (Zhang et al., 2008). Cells get arrested in prometaphase when sumoylation of CENP-E is disrupted. Several phosphorylation sites on CENP-E are already known to be critical for its function in both establishing and monitoring kinetochore-microtubule attachment status. However, it is unclear if sumoylation and phosphorylation regulate CENP-E function together. Another possible candidate to study such interdependency between phosphorylation and sumoylation is the centromeric protein Ndc10, which also bears several phosphorylation sites in addition to sumoylation ones. Sumoylation of Ndc10 is required for its spindle localization and regulation of anaphase spindle elongation (Montpetit et al., 2008). In summary, role of sumoylation in chromosome segregation and mitosis is beginning to be unravelled and much is still unknown about the complex regulation of various post-translational modifications.

Regulation of sumoylation by other posttranslational modifications

Molecular regulation of the highly dynamic sumoylation/desumoylation pathway is poorly

understood. There are no established signals, external or internal, which would directly modulate the sumoylation pathway, although the differential and specific choice of SUMO substrates and the time of their modification contribute to the highly selective property of sumoylation pathway. Recent studies have revealed that the phosphorylation status of several substrate proteins, such as c-jun and PML, affects their sumoylation (Müller et al., 2000, Everett et al., 1999). Phosphorylation might negatively influence sumoylation. One classical example of the relationship between phosphorylation with sumoylation has been provided by Yang et al (2003), who found that sumoylation of Elk-1 transcription factor is regulated by MAP kinases. In the absence of MAP kinase pathway activation, Elk-1 sumoylation represses Elk-1-dependent gene expression While upon MAPK-dependent phosphorylation, Elk-1 is de-sumoylated and transcription is activated. However, in case of heat shock transcription factor HSF1, phosphorylation induces its sumoylation (Hilgarth et al 2003). The same lysine residues (apart from SUMO modification) can also be involved for other protein modifications, such as acetylation, methylation and ubiquitination. Therefore, it is possible that these modifications influence each other by simply competing for the same lysine residue. This argument is consistent with the observation that the transcription factor Sp3 contains a lysine that can be acetylated or sumoylated (Sapetschnig et al., 2002).

Conclusion

Twelve years since the discovery of sumoylation, the field has advanced rapidly and has revealed the role of sumoylation in several cellular functions. The central focus, in coming days, should involve studying the regulation of sumoylation as a whole. What are the molecular cues that regulate the delicate balance of highly dynamic SUMO/desumoylation pathway? Are there any post-translational modifications on SUMOs that in turn control its function? Other post-translational modifications including phosphorylation and methylation are already being used as diagnostic and prognostic tools. A systematic study is required to identify global changes in sumoylation/desumoylation during a special cellular condition such as during early developmental stages or diseases. Such studies would be crucial to open avenues for using sumoylation as a therapeutic tool to combat diseases.

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