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Regulation of the SUMOylation System in Gene Expression

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Summary

Protein sumoylation has emerged as an important regulatory mechanism for the transcriptional machinery. Sumoylation is a highly dynamic process that is regulated in response to cellular stimuli or pathogenic challenges. Altered activity of the SUMO conjugation system is associated with human cancers and inflammation. Thus, understanding the regulation of protein sumoylation is important for the design of SUMO-based therapeutic strategies for the treatment of human diseases. Recent studies indicate that the sumoylation system can be regulated through multiple mechanisms, including the regulation of the expression of various components of the sumoylation pathway, and the modulation of the activity of SUMO enzymes. In addition, extracellular stimuli can signal to the nucleus to trigger the rapid promoter recruitment of SUMO E3 ligases, resulting in the immediate repression of transcription. Finally, the sumoylation system can also be regulated through crosstalk with other posttranslational modifications, including phosphorylation, ubiquitination, and acetylation.

Introduction

The covalent attachment of small ubiquitin-like modifier (SUMO) to protein substrates is defined as SUMOylation [1–3]. The mammalian SUMO family consists of four members: SUMO-1, -2, -3, and -4. Protein sumoylation proceeds in three steps that resemble ubiquitination, but the enzymes involved in sumoylation and ubiquitination are different. SUMO is first activated in an ATP-dependent process by forming a thioester bond with the catalytic cysteine of the E1-activating enzyme, which is a heterodimer consisting of two proteins SAE1 and SAE2 (also known as Aos1 and Uba2, respectively). Next, SUMO is transferred to the catalytic cysteine of the E2-conjugating enzyme Ubc9, which directly binds to substrates. Subsequently, SUMO is conjugated to the protein substrate by forming an isopeptide bond between SUMO and the ϵ -amino group of a lysine side chain on the substrate. Unlike ubiquitination, sumoylation can occur without E3 ligases *in vitro*. However, SUMO E3 ligases can enhance the efficiency and provide substrate specificity to the sumoylation process. SUMO E3 ligases include the protein inhibitor of activated STAT family (PIAS), the polycomb group protein Pc2, and Ran binding protein 2 (RanBP2) [4,5]. The list of SUMO E3 ligases is growing as additional proteins with SUMO E3 ligase activity have been recently identified. For example, histone deacetylase 4 (HDAC4) can promote the sumoylation of MEF2, independent of its deacetylase activity [6]. The human topoisomerase I- and p53-binding

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protein Topors can function as both a ubiquitin and a SUMO E3 ligase for p53 [7,8]. Interestingly, a recent study indicates that the PHD domain of the KAP1 corepressor functions as an intramolecular E3 ligase that promotes the sumoylation of its adjacent bromodomain, which is required for KAP1-mediated gene silencing [9]. SUMO conjugation is reversible, and the SUMO-specific protease (SENPs) family is responsible for rapid removal of SUMO from sumoylated protein substrates [10].

SUMO conjugation participates in various cellular processes, and the most recognized function of sumoylation is to regulate transcription. In fact, most sumoylation substrates identified so far are involved in transcriptional regulation. Sumoylation can either positively or negatively regulate transcription, although a majority of studies has identified a functional role of sumoylation in transcriptional repression.

Sumoylation can regulate transcription through multiple mechanisms, a topic which has been extensively reviewed [1,11,12]. Various components of the transcriptional machinery can be subjected to SUMO modification, such as transcription factors themselves, transcriptional co-regulators, and chromatin-remodeling proteins. Sumoylation can regulate transcription at multiple steps, including the DNA binding activity of transcription factors, the subcellular localization of transcription factors, the interaction between transcription factors and co-regulators, and the chromatin structure.

Sumoylation is a dynamic process that is regulated during cellular processes or under pathogenic conditions. Although sumoylation plays an important role in the regulation of transcription, how sumoylation itself is regulated has been poorly understood. This review will highlight the most recent progress made on the understanding of the molecular mechanisms involved in the regulation of the SUMO conjugation process.

Regulation of the expression of the Sumoylation System

The sumoylation system can be regulated at the level of expression. It has been shown that the expression of various components of the sumoylation system is regulated under certain physiological or pathogenic conditions.

During keratinocyte differentiation, the sumoylation system was transiently upregulated by Ca^{2+} signaling [13]. Ca^{2+} induced the transcriptional activation of the genes encoding several components of the sumoylation system, including SAE1/SAE2, Ubc9, SUMO2/3, and PIASx. In human endometrial stromal cells, cAMP and progesterone signaling induced significant changes in the expression of PIAS SUMO E3 ligases and SENPs [14]. In addition, hypoxia can induce the expression of SUMO-1 [15] and an RWD-containing sumoylation enhancer (RSUME) that functions to promote protein sumoylation (see the next section for details) [16]. The elevated expression of Ubc9 has been observed in a number of human malignancies, such as lung adenocarcinoma, melanoma, breast and ovarian carcinoma [17]. Despite these interesting findings, little is known about the molecular mechanisms responsible for the regulation of the expression of the sumoylation system.

Recently, it has been reported that the increased expression of a 30-kDa dominant-negative isoform (C/EBP α 30) of CCAAT/enhancer-binding protein α (C/EBP α), which is present in acute myeloid leukemia (AML) patients with mutations in *Cebpa* gene, can induce the transcriptional induction of Ubc9 [18]. But initial analysis failed to detect the direct association of C/EBP α 30 with a promoter region of Ubc9. How C/EBP α 30 regulates the transcription of Ubc9 remains to be understood.

Recent studies suggest that the stability of SUMO enzymes can be regulated by ubiquitin E3 ligases. It was shown that human homologues of seven in absentia 2 (hSiah2), a known

ubiquitin E3 ligase, targeted PIAS1 for degradation through the ubiquitination-proteasome pathway [19]. The ubiquitin E3 ligase Trim32, which is mutated in human limb-girdle muscular dystrophy type 2H and Bardet-Biedl syndrome, can promote the ubiquitination and degradation of PIASy [20]. In addition, the adenoviral protein Gam1 recruits two cellular cullin RING ubiquitin ligases to target the degradation of the SUMO E1 enzyme [21]. These studies suggest the involvement of the ubiquitination system in the regulation of protein sumoylation.

Regulation of the Activity of the SUMO Enzymes

In addition to regulate the expression levels of the components of the SUMO conjugation system, the intrinsic activity of the enzymes in the SUMO pathway can also be modulated by cellular stimuli (Fig. 1). Bossis and Melchior showed that lower concentrations of reactive oxygen species (ROS) caused the formation of a disulfide bond involving the catalytic cysteines of the SUMO E1 subunit Uba2 and the E2 enzyme Ubc9, resulting in the prevention of Ubc9-SUMO thioester formation, and thus the inhibition of SUMO conjugation [22]. ROS such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\cdot OH$), can be produced in response to a variety of stimuli, including DNA-damaging agents, cellular stresses, cytokines and growth factors. Therefore, the inhibition of the activity of SUMO conjugating enzymes by ROS may represent a commonly utilized mechanism to regulate global sumoylation by multiple stimuli.

Most recently, a protein named RSUME (RWD-containing sumoylation enhancer) has been reported that can enhance overall SUMO-1, -2, and -3 conjugation [16]. RSUME binds to the E2 enzyme Ubc9 and increases the noncovalent association of Ubc9 with SUMO, which leads to the enhanced Ubc9-SUMO thioester formation and SUMO conjugation. Interestingly, RSUME expression is induced by hypoxia, which leads to the enhanced sumoylation of HIF-1 α that promotes the stabilization and transcriptional activity of HIF-1 α during hypoxia. It should be pointed out that the role of sumoylation in the regulation of HIF-1 α stability is controversial. A recent study indicates that the hypoxia-induced sumoylation of HIF-1 α targets HIF-1 α for degradation through the von Hippel-Lindau (VHL) protein-mediated ubiquitin-proteasome pathway [23]. SENP1 functions to deconjugate sumoylated HIF-1 α and prevents the degradation of HIF-1 α during hypoxia. Consistently, *Senp1* disruption leads to the degradation of HIF-1 α , resulting in decreased erythropoietin (Epo) production and severe fetal anemia. Thus, sumoylation of HIF-1 α triggers the ubiquitination and the subsequent degradation of HIF-1 α . Clearly, further studies are needed to clarify these controversial findings on the role of sumoylation in the regulation of HIF-1 α stability during hypoxia. Nevertheless, the studies by Carbia-Nagashima et al. strongly suggest that RSUME is a novel regulator of the sumoylation system.

The activity of a SUMO E3 ligase can be regulated by its sumoylation substrate. For example, the polycomb group protein Pc2 binds to homeodomain interacting protein kinase 2 (HIPK2), and acts as a SUMO E3 ligase for HIPK2 [24]. Upon DNA damage, HIPK2 is activated and then phosphorylates Pc2 on Thr495 as well as several other sites. The phosphorylation of Pc2 enhances its SUMO ligase activity toward HIPK2, resulting in the enhanced sumoylation and thus increased activity of HIPK2 in gene repression. These studies identify an interesting mechanism for the regulation of a SUMO E3 ligase activity by its sumoylation target.

A Signaling Pathway to Recruit SUMO E3 Ligase for Gene Repression

SUMO E3 ligases function to promote the efficiency and substrate specificity of sumoylation. Recent studies indicate that SUMO conjugation can be fine-tuned by regulating the subcellular localization of SUMO E3 ligases (Fig. 2). It has been well documented that extracellular stimuli such as cytokines and growth factors induce rapid changes in gene expression to modulate

various cellular functions. One important question in the SUMO research field is to understand how extracellular signals can rapidly mobilize the sumoylation system to regulate specific gene expression in the nucleus. PIAS1 E3 ligase is localized in the nucleus and can regulate the activity of a number of transcription factors such as NF- κ B, STAT1, and PPAR γ upon ligand stimulation [25–27]. Gene targeting studies indicate that the removal of PIAS1 does not affect the global sumoylation, and PIAS1 negatively regulates the transcriptional induction of a subset of NF- κ B or STAT1-target genes, with a notable preference for cytokines and chemokines [25,26]. Recently, it has been shown that PIAS1 becomes rapidly phosphorylated on Ser90 residue in response to proinflammatory stimuli such as TNF α and LPS [28]. The Ser90 phosphorylation of PIAS1 does not affect its intrinsic SUMO ligase activity, but is required for targeting PIAS1 to the promoters of PIAS1-responsive genes, resulting in the immediate inhibition of inflammatory gene induction. The kinase that mediates PIAS1 Ser90 phosphorylation in response to TNF α and LPS has been identified as IKK α , which is largely localized in the cytoplasm but can rapidly translocate into the nucleus upon ligand stimulation [29]. Interestingly, the ability of IKK α to phosphorylate PIAS1 on Ser90 requires the SUMO ligase activity of PIAS1. Consistently, elevated sumoylation can enhance the IKK α -mediated PIAS1 phosphorylation [28]. It is possible that the sumoylation of IKK α and/or PIAS1 promotes the kinase activity of IKK α toward PIAS1, a hypothesis that can be tested by further experiments. Nevertheless, these studies identify an interesting regulatory pathway in which extracellular stimuli signal to the nucleus to regulate transcription by targeting a SUMO E3 ligase to gene promoters (Fig. 2).

Crosstalk between Sumoylation and Other Posttranslational Modifications

Sumoylation can be regulated through crosstalk with other posttranslational modification events. Phosphorylation has been shown to regulate SUMO conjugation through a highly conserved motif named PDSM (phosphorylation-dependent sumoylation motif) [30]. The PDSM motif, which contains a SUMO consensus site and an adjacent proline-directed phosphorylation site (ψ KxExxSP, where ψ represents large hydrophobic residue and x is any amino acid), regulates phosphorylation-dependent sumoylation of multiple transcription factors, including heat-shock factors (HSFs), myocyte enhancer factor 2 (MEF2), and estrogen-related receptors (ERR) α and γ [31–34]. This phosphorylation-dependent regulation of sumoylation has been referred to as a phospho-sumoyl switch [35]. Similarly, an extended sumoylation motif that contains clusters of acidic residues located downstream of the core SUMO modification sites has been identified in proteins such as the transcription factor Elk-1 [36].

SUMO is conjugated to lysine residues of the substrates. The lysine residue is also a target of several other posttranslational modifications, including ubiquitination, acetylation, and methylation. It has been documented that SUMO conjugation can occur on the same lysine residue as ubiquitination or acetylation in some proteins. For example, the competition between sumoylation and ubiquitination on the same lysine residue regulates the stability of I κ B α [37]. It should be noted that sumoylation and ubiquitination do not necessarily compete with each other. In some cases, sumoylation actually acts as a recognition signal for a ubiquitin ligase [23]. The interplay between sumoylation and acetylation has been observed in the regulation of proteins such as MEF2, histone, and hypermethylated in cancer 1 (HIC1) [38–41]. In the case of MEF2, the sumoylation-acetylation switch is regulated by phosphorylation [39]. These studies demonstrate the importance of signaling crosstalk in the regulation of protein sumoylation.

Conclusion

The field of protein sumoylation has advanced rapidly, especially in the area of understanding the molecular mechanisms of sumoylation in transcriptional regulation. Sumoylation is a dynamic process that is regulated during normal cellular processes and under pathogenic conditions. Recent studies have uncovered several mechanisms for the regulation of the sumoylation system. However, many important questions remain to be answered. For example, little is known about how the expression levels of various components of the SUMO system are regulated. In addition, the signaling pathways that regulate the activity of SUMO enzymes in response to various stimuli need to be characterized. Finally, the abnormal regulation of the sumoylation pathway in human diseases such as cancers should be explored. Clearly, the research field on the regulation of sumoylation is exciting and challenging.

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Figure 1. The regulation of the SUMOylation system

A schematic representation of the regulation of the SUMO conjugation system at multiple steps. Protein sumoylation is accomplished in three steps involving three groups of enzymes: E1 (SAE1/SAE2), E2 (UBC9), and E3 ligases such as protein inhibitors of activated STAT (PIAS), Ran binding protein 2 (RanBP2), and the polycomb group protein Pc2. The SUMO-specific protease (SENPs) family is responsible for the removal of SUMO from substrates. Hypoxia can induce the expression of SUMO-1 and an RWD-containing sumoylation enhancer (RSUME). Viral proteins such as Gam1 can target SAE1 for degradation through the ubiquitin-proteasome pathway. DNA damage can induce the SUMO ligase activity of Pc2. Cytokines such as TNF α signal to the nucleus to target PIAS1 E3 ligase for gene repression (See Figure 2 for details). Reactive oxygen species (ROS) can cause the formation of UBC9-SAE2 complex, which inhibits UBC9-SUMO thioester formation. RSUME binds to UBC9 to promote UBC9-SUMO thioester formation.

Figure 2. A signaling pathway to recruit PIAS1 SUMO E3 ligase to gene promoters for transcriptional repression

Inflammatory stimuli such as **TNF α** and **LPS** activates the kinase **IKK α** , which then translocates into the nucleus where it binds to **PIAS1** and then phosphorylates **PIAS1** on Ser90 residue. The **IKK α** -mediated **PIAS1** phosphorylation is dependent on the SUMO E3 ligase activity of **PIAS1**, suggesting the possible sumoylation (**Su?**) of **PIAS1** and/or **IKK α** during this process. Phosphorylated **PIAS1** disassociates from **IKK α** , and is then recruited to the promoters of **PIAS1**-responsive genes for transcriptional repression.