SUMO under stress

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Abstract
During the last decade, SUMOylation has emerged as a central regulatory post-translational modification in the control of the fate and function of proteins. However, how SUMOylation is regulated itself has just started to be delineated. It appears now that SUMO (small ubiquitin-related modifier) conjugation/deconjugation equilibrium is affected by various environmental stresses, including osmotic, hypoxic, heat, oxidative and genotoxic stresses. This regulation occurs either at the level of individual targets, through an interplay between stress-induced phosphorylation and SUMOylation, or via modulation of the conjugation/deconjugation machinery abundance or activity. The present review gives an overview of the connections between stress and SUMOylation, the underlying molecular mechanisms and their effects on cellular functions.

Introduction
SUMOylation is a post-translational modification consisting of covalent conjugation of ubiquitin-like proteins called SUMO (small ubiquitin-related modifier)-1, SUMO-2 and SUMO-3 (referred to as SUMO-2/3 because of their high similarity) to target proteins. SUMO conjugation necessitates an enzymatic cascade resembling that of ubiquitination. Thus it involves a single SUMO-activating enzyme (Uba2–Aos1), a SUMO-conjugating enzyme (Ubc9) and, in most cases, a SUMO-E3 facilitating component such as PIAS [protein inhibitor of activated STAT (signal transducer and activator of transcription)] family, RanBP2 (Ran binding protein 2) or Pc2. SUMO is conjugated via its C-terminal glycine residue to the ε-NH₂ group of lysine residues, usually, but not always, located within consensus sites, ψKXE, where ψ is a large hydrophobic amino acid, K the modified lysine, X any amino acid and E a glutamic acid residue. SUMOylation is a highly dynamic modification thanks to the deSUMOylases of the SENP (sentrin-specific protease) family that cleave the isopeptide bond between SUMO and its target protein [1].

SUMOylation is a highly regulated post-translational modification, and recent work by different laboratories has revealed multiple cross-talk between SUMOylation and other post-translational modifications (ubiquitination, acetylation and phosphorylation). In particular, an extended consensus motif has been defined, the PDSM (phospho-dependent SUMO motif), ψKXEXXSP [3].

What stresses SUMO and how?
The initial observation suggesting modulation of SUMO conjugation by stresses came from Saitoh and Hinchey [5], who reported that various environmental stresses (osmotic and oxidative stress, heat shock) increase global SUMOylation by the SUMO-2/3 isoforms but have little effect on SUMO-1 conjugation. They suggested that the difference between the isoforms is due to the low abundance of non-conjugated SUMO-1 compared with SUMO-2/3, which is not limiting in the cell. Along the same line, oxidative and ethanol stresses were shown to increase global SUMOylation in yeast [6]. Further work revealed that the situation is not that simple at the level of individual targets, as it depends on the stress intensity or duration. The effects of specific stresses on particular targets, the molecular mechanisms involved as well as the consequences on targets functions are reviewed below and summarized in Figure 1.
Various environmental stresses can regulate SUMOylation, either at the level of the conjugation machinery or through target protein phosphorylation. Regulation of the conjugation machinery is either transcriptional (broken arrows) or post-translational (plain lines). Phosphorylation of the targets by stress-activated kinases either increases SUMOylation (1), in particular when taking place within PDSM motifs, or decreases it (2), depending on the target.

Osmotic stress was shown to have little effect on global SUMOylation by SUMO-2/3 [5] compared with heat shock and oxidative stress. At the level of individual targets, osmotic stress induced by sorbitol increases SUMOylation of the STAT1 transcription factor via Ser-727 phosphorylation by the p38 MAPK (mitogen-activated protein kinase) pathway, although this serine residue is not within a PDSM [7]. The effects of this increase in SUMOylation on STAT1 activity have not been elucidated. Osmotic stress induced by high salt leads to a dramatic increase in c-Myb SUMOylation by the SUMO-2/3 isoforms [8]. The underlying mechanisms, although yet unidentified, are expected to be identical with those taking place under heat shock (see below).

Heat shock has more severe effects on SUMOylation. Globally, it enhances conjugation by the SUMO-2/3 isoforms [5]. At the level of specific substrates, this is more contrasted. HSF1 (heat-shock factor-1) SUMOylation is strongly induced by heat shock [9], which results in increased DNA binding and activity. This heat-shock-induced SUMOylation on Lys-298 requires Ser-303 phosphorylation, located within a PDSM [10]. As mentioned above, c-Myb SUMOylation is also strongly enhanced upon heat shock. The mechanisms involved have not been clarified since this increased SUMOylation is not due to activation of the p38 and JNK (c-Jun N-terminal kinase) pathway, and phosphorylation within c-Myb PDSM has only minor effects [8]. Contrasting with these examples, c-Fos SUMOylation on Lys-265 disappears rapidly after heat shock, which is correlated with an increased Thr-232 phosphorylation (D. Tempé and G. Bossis, unpublished work), known to inhibit c-Fos SUMOylation [11]. Therefore activation of c-Fos by Thr-232 phosphorylation [12] is probably linked to inhibition of Lys-265 SUMOylation, which represses its transcriptional activity [11]. SUMOylation of topoisomerase I [13] and PML (promyelocytic leukaemia) [14] are also strongly reduced by heat shock. Besides its effect via target phosphorylation, recent evidence suggests that heat shock could activate specific elements of the SUMO conjugation machinery. RSUME (RWD-containing sumoylation enhancer), a newly identified SUMO-E3, is transcriptionally induced by heat shock [15]. Although it cannot explain short-term increase in SUMOylation observed after heat shock [5], this could concern RSUME substrates, such as IκB (inhibitory κB) and HIF1 (hypoxia-inducible factor 1), during recovery.

Oxidative stress has been studied in more detail with regard to its relationship to SUMOylation. Initially shown to increase SUMOylation by SUMO-2/3 at a high H₂O₂ concentration (100 mM) [5], the situation appeared more complex when the strength of the stress was taken into consideration. Indeed, low to moderate oxidative stress (below 1 mM H₂O₂) inhibits global SUMOylation by inducing the formation of a reversible disulfide bridge between the catalytic cysteine residues of the E1 and E2 enzymes. At 1 mM H₂O₂, most cellular proteins are deSUMOylated within 1 h [16]. At higher doses, which probably never occur in living organisms, isopeptidases are also inactivated by creation of an intra- or inter-molecular disulfide bridge [17], which counteracts deSUMOylation associated with inactivation of the conjugating machinery. At the level of individual target proteins, oxidative stress induced by H₂O₂ was indeed shown to increase SUMOylation of ERM (100 mM, 20 min) [18] and NEMO (NF-κB essential modulator) (2 mM, 40 min) [19,20], which is not contradictory to the results presented above considering the H₂O₂ doses used. On the contrary, very low H₂O₂ doses (<10 μM) have minimal effects on the conjugation machinery [16]. In this range, the regulation of SUMOylation is probably due to the activation of specific signalling cascades. Accordingly, enhanced phosphorylation of p53 probably explains the increase in its SUMOylation observed on treatment with 5 μM H₂O₂ [21]. Nitric oxide (NO), which also induces oxidative stress, has been shown to induce global deSUMOylation of cellular proteins, as...
shown for H$_2$O$_2$. The mechanism is nevertheless different since NO does not affect E1 and E2 activities. Instead NO is targeting Pias3 SUMO-E3 through S-nitrosation of its Cys-459, which facilitates its interaction with Trim32 (a ubiquitin ligase) and its degradation [22].

Hypoxic stress was shown to induce global protein SUMOylation. This is correlated with increased transcription of the SUMO-1 gene, which starts within 4–8 h after hypoxia induction [23,24]. At the level of individual substrates, HIF1, the SUMOylation of which is also increased during hypoxia, has been particularly documented. As mentioned above for heat shock, the RSUME SUMO-E3 expression is increased during hypoxia, which enhances HIF1 SUMOylation. The consequences of this increase in HIF1 SUMOylation are a matter of debate [25]. In some reports, this leads to its stabilization and increased transcriptional activation of target genes [15,26], whereas in one study, HIF1 SUMOylation, which is maintained low by SENP-1 isopeptidase, is proposed to target the protein for degradation via the VHL (von Hippel–Lindau protein) ubiquitin E3 [27].

Genotoxic stress comprises stresses generating DNA double-strand break. They are induced by chemotherapeutic agents (etoposide, doxorubicin, cisplatin, camptothecin etc.), ionizing radiations and UV exposure. In the yeast Saccharomyces pombe, SUMOylation of Rad52, which plays key roles in recombination pathways, is strongly increased by various genotoxics [28], although the underlying mechanisms are not known. In mammalian cells, UV has been shown to increase the SUMOylation of DJ1, a multifunctional protein regulating transcription [29], XPC, which is involved in DNA repair [30], and the histone acetyltransferase Tip60 [31]. On the contrary, UV exposure decreases the SUMOylation of hRIPβ [human RPA (replication protein A)-interacting protein β], a protein involved in DNA repair [32]. Topoisomerase I SUMOylation is greatly enhanced by its specific poison, camptothecin, in both yeast and mammalian cells [13,33]. Doxorubicin, which is widely used in chemotherapy, was shown to decrease KAP-1 (kinase-associated protein-1) SUMOylation via its ATM (ataxia telangectasia mutated; DNA-damage-activated kinase)-mediated phosphorylation on Ser-824 [34,35]. SUMOylation of the transcriptional co-repressor KAP-1 is required for recruiting the SETDB1 (SET domain, bifurcated 1) histone methyltransferase and one component of the NuRD repressor complex to the chromatin [36]. This probably explains why its deSUMOylation by doxorubicin relieves its transcriptional repression [34]. Similarly, doxorubicin decreases p53 SUMOylation via its increased phosphorylation on Ser-20, which reduces binding to Ubc9 [37]. The best-characterized regulation of SUMOylation by genotoxics is probably that of NEMO, one of the subunits of the IKK (inhibitory κB kinase) kinase complex, which plays key roles in NF-κB activation. SUMOylation of NEMO leads to its translocation to the nucleus and is required for IKK activation and subsequent NF-κB signalling in response to genotoxic stress [38]. Its SUMOylation is enhanced by etoposide (VP16) in an ATM-independent manner [38], which depends on PIDD (p53-induced protein with death domain) [39]. Mechanistically, etoposide increases NEMO interaction with the PIASy SUMO-E3, responsible for its SUMOylation, in particular through reactive oxygen species generation [19].

Which mechanisms for stress-induced (de-)SUMOylation?

We have seen from the above-cited work that SUMOylation is regulated by many stresses although the underlying mechanisms have just started to be delineated. So far, two types of mechanisms can explain stress-induced regulation of SUMOylation: (i) the interplay between phosphorylation and SUMOylation at the level of the target proteins and (ii) the regulation of the conjugating or deconjugating machinery (SUMO, E1, E2, E3, isopeptidases). Concerning the first point, a number of SUMOylation reactions are regulated by phosphorylation, probably induced by stress-activated kinases such as p38 and JNK, or ATM in the case of genotoxic stress. When phosphorylation occurs within a PDSM, it leads to increased SUMOylation, probably through increased negative charge around the Ubc9-binding site. Accordingly, stretches of negatively charged amino acids adjacent to the consensus ψKXE motif increase Ubc9 binding [40]. In other cases, phosphorylation of sites that are not necessarily close to the modified lysine residue in the primary sequence can impair SUMOylation. In such cases, phosphorylation could lead to structural changes, which could result in hiding the Ubc9 or SUMO E3-binding site. Alternatively, stress-induced regulation of SUMOylation could be linked to lysine availability, as SUMOylation can be prevented by competitive ubiquitination or acetylation [4]. The second way to regulate SUMOylation is by directly targeting the conjugation/deconjugation machinery. Global effects are achieved by targeting E1 or E2 enzymes, since they are both unique and required for SUMOylation of all substrates. More restricted effects are seen by regulating specific SUMO-E3, which are involved in the SUMOylation of a limited number of proteins. Regulation of the conjugation machinery involves both (i) transcriptional activation, as for SUMO-1 [23,24] and RSUME [18] after hypoxia, and (ii) post-translational regulations such as oxidation of E1, E2 [16] and SENP-1 [17], nitrosation of Pias3 [22] and phosphorylation of PIASxα [41]. It is likely that other levels of regulation will soon be discovered to fully explain SUMOylation variations under stress conditions.

Is stress-regulated SUMO conjugation instrumental for stress response?

This question is far from being understood. For example, the SUMOylation pathway is critical for cells to survive genotoxic stress. In yeast, inhibition of the SUMO-conjugating pathway leads to increased sensitivity to a wide range of genotoxics [42]. In human cells, inhibition of the SUMO pathways, either by a dominant-negative Ubc9 [43] or by the viral protein Gam-1, which targets E1 for degradation [44], sensitizes...
cells to genotoxic-induced apoptosis. Nevertheless, although global, these effects are probably due to the deSUMOylation of particular subsets of SUMO targets involved in DNA repair, cell cycle arrest or apoptosis. Concerning the regulation at the level of individual targets, variations of their SUMOylation are expected to modulate the effect of this modification on their activity. In particular, considering the number of transcription factors, associated activators and repressor complexes which are SUMOylated, it is likely that their stress-regulated SUMOylation is involved in gene activation, allowing further appropriate cell response. More generally, understanding how stress-induced regulation of SUMOylation, whether global and/or target-specific, participates in cell responses to stress is a challenging question. Beyond reinforcing the central role of SUMOylation in the cell, answering this paramount question will undoubtedly bring new perspectives in the treatment of the numerous pathological disorders associated with environmental stresses.

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References


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