SUMO chains: polymeric signals

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Abstract
Ubiquitin and ubiquitin-like proteins are conjugated to a wide variety of target proteins that play roles in all biological processes. Target proteins are conjugated to ubiquitin monomers or to ubiquitin polymers that form via all seven internal lysine residues of ubiquitin. The fate of these target proteins is controlled in a chain architecture-dependent manner. SUMO (small ubiquitin-related modifier) shares the ability of ubiquitin to form chains via internal SUMOylation sites. Interestingly, a SUMO-binding site in Ubc9 is important for SUMO chain synthesis. Similar to ubiquitin–polymer cleavage by USPs (ubiquitin-specific proteases), SUMO chain formation is reversible. SUMO polymers are cleaved by the SUMO proteases SENP6 [SUMO/sentrin/SMT3 (suppressor of mif two 3)-specific peptidase 6], SENP7 and Ulp2 (ubiquitin-like protease 2). SUMO chain-binding proteins including ZIP1, SLX5/8 (synthetic lethal of unknown function 5/8), RNF4 (RING finger protein 4) and CENP-E (centromere-associated protein E) have been identified that interact non-covalently with SUMO chains, thereby regulating target proteins that are conjugated to SUMO multimers. SUMO chains play roles in replication, in the turnover of SUMO targets by the proteasome and during mitosis and meiosis. Thus signalling via polymers is an exciting feature of the SUMO family.

Introduction
SUMOs (small ubiquitin-related modifiers) regulate the activity of target proteins via covalent modification [1]. SUMOylation can alter the function of target proteins by regulating protein–protein interactions, which can result in altered subcellular localization and activity. Vertebrates express three SUMO family members, SUMO-1, SUMO-2 and SUMO-3. Extensive SUMO families have been uncovered in plants. In contrast, lower eukaryotes express a single SUMO family member. SUMOylation is a reversible process; specific proteases that are known as SENPs [SUMO/sentrin/SMT3 (suppressor of mif two 3)-specific peptidases] remove SUMOs from target proteins. Genetic studies have established that reversible SUMOylation is critical for the viability of eukaryotes [1,2]. Extensive sets of target proteins have been identified for SUMOs including transcription factors, replication factors, translation factors, DNA repair factors, cytoskeleton components and metabolic enzymes [3–5]. While target proteins are predominantly conjugated to SUMO monomers, SUMOs are also able to form multimers [6,7]. Several proteins interact with SUMOylated proteins in a non-covalent manner via a SIM (SUMO interaction motif).

SUMO-2 and SUMO-3 contain an internal SUMOylation site
The mammalian SUMO family members SUMO-2 and SUMO-3 contain an internal consensus site for SUMOylation that is missing in SUMO-1 [8]. Internal SUMOylation sites are also present in the single SUMO family members that are expressed by lower eukaryotes including Smt3 (SUMO in Saccharomyces cerevisiae) and Pmt3 (SUMO in Schizosaccharomyces pombe) [9–11]. These sites enable polymerization and are located in the flexible N-terminal parts of SUMOs. Previously, it was found that SUMO-2 and SUMO-3 polymerize efficiently in vitro in unanchored forms and also anchored to recombinant PML (promyelocytic leukaemia protein) [8].

Whether SUMOs are able to multimerize in cells remained an open question in the field for several years. PML, HDAC4 (histone deacetylase 4) and PCNA (proliferating-cell nuclear antigen) were indirectly identified as targets for SUMO chains [8,12,13], and SUMO-1 and SUMO-3 were co-purified with SUMO-2 under denaturing conditions, possibly via SUMO polymer formation [14]. Recently, direct and conclusive evidence for the existence of endogenous SUMO chains in cells was provided by MS employing an in vitro to in vivo strategy to assign complex fragmentation spectra of the relevant tryptic peptides that were derived from SUMOylated SUMOs [15]. SUMO chain formation was shown to occur via the consensus SUMOylation site Lys11 in SUMO-2 and SUMO-3 in vivo and in vitro, but also via the non-consensus site Lys4 in vitro. Interestingly, SUMO-1 is able to SUMOylate SUMO-2 and SUMO-3 in vitro and in vivo, thereby limiting chain extension in vitro. Other laboratories have found that SUMO-1 can form multimers in vitro via non-consensus SUMOylation sites, but the

Key words: centromere-associated protein E (CENP-E), small ubiquitin-related modifier (SUMO), Ubch9, ubiquitin-like protease 2 (Ulp2).
Abbreviations used: CENP-E, centromere-associated protein E; PML, promyelocytic leukaemia protein; E3kA, retinoblastoma protein Phosphatase; RNF, RING finger protein; SUMO, small ubiquitin-related modifier; SENP, SUMO/sentrin/SMT3 (suppressor of mif two 3)-specific peptidase; SIM, SUMO interaction motif; SRT, synthetic lethal of unknown function; Smt3, SUMO in Saccharomyces cerevisiae; Ulp2, ubiquitin-like protease 2.

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physiological relevance of these findings is unclear [16]. Interestingly, SUMO-2/3 chains were found to accumulate to very high levels during cellular stress such as acute heat shock [3], suggesting that they are involved in stress responses.

SUMO chain assembly by Ubc9 and SUMO E3 ligases

Interestingly, the SUMO E2 enzyme Ubc9 interacts with SUMO in two different manners, via a thioester bond and in a non-covalent manner [17,18]. Non-covalent interaction enhances SUMO chain formation, and mutations in Ubc9 that disrupt this interaction strongly reduce SUMO multimerization in vitro, indicating that SUMO chains are efficiently pre-assembled by Ubc9 and could be transferred en bloc to a target protein. E3 ligases further enhance SUMO polymerization in vitro [16,19–21].

It is still unclear how the SUMOylation machinery discriminates between targets that are conjugated to SUMO monomers and targets that are conjugated to SUMO multimers.

SUMO proteases disassemble SUMO chains

SUMO chains are generally very short lived due to rapid processing by SUMO proteases. The SUMO protease SENP6 contains four SIMs and SENP7 contains two SIMs that might be important for preferential interaction and processing of SUMO chains compared with SUMO monomers [22,23]. In vitro, the catalytic domains of SENP6 and SENP7 are sufficient for the processing of SUMO chains [24,25]. In time course assays, SENP6 cleaved SUMO-2 and SUMO-3 chains more efficiently than SENP7, whereas SENP7 was more efficient than SENP2 [24]. Knockdown of SENP6 results in the accumulation of SUMO-2/3 in PML bodies [22].

In yeast, the SUMO protease Ulp2 (ubiquitin-like protease 2) plays an important role in disassembling SUMO chains. Smt3 was shown to accumulate in chains in S. cerevisiae strains that were deficient in the SUMO protease Ulp2 [10]. The accumulation of SUMO chains might explain the severe phenotypic defects in the Ulp2Δ mutant, including poor growth, sensitivity to a variety of stress conditions, defects in chromosome segregation and recovery from cell cycle checkpoint arrest [10,26–28]. However, Smt3 chain formation is not required for viability of S. cerevisiae since a mutant Smt3, which lacked all internal lysine residues, could functionally replace the wild-type protein under normal conditions [10].

SUMO polymers play a role during meiosis

Ubiquitin multimerizes via all internal lysine residues [29,30]. Different ubiquitin chain-binding proteins have been uncovered that interact non-covalently with these chains in a linkage-dependent or linkage-independent manner [31,32]. Similarly, SUMO chain-binding proteins have been identified that interact non-covalently with SUMO chains via SIMs, thereby regulating the fate of target proteins that are conjugated to SUMO multimers (Figure 1). The first protein that was shown to bind SUMO chains was ZIP1, a protein that interacts preferentially with unanchored Smt3 chains in S. cerevisiae, but not with the Smt3 monomer [33]. ZIP1 is an integral component of the synaptonemal complex, a proteinaceous complex that mediates synopsis between homologous chromosomes during meiotic prophase. Smt3 chains accumulate during meiosis and are required for proper assembly of the synaptonemal complex. Importantly, S. cerevisiae strains deficient in Smt3 chain formation showed a strong reduction in sporulation, indicating that Smt3 chains are required for meiosis. Interestingly, a single SIM was mapped in ZIP1, which is surprising since it would be expected that multiple closely spaced SIMs would be required for SUMO chain binding.

SUMO polymers play a role during mitosis

The microtubule motor protein CENP-E (centromere-associated protein E) is another very interesting SUMO-2/3 chain-binding protein [34]. This protein has a strong preference for SUMO-2 chains compared with SUMO-1 chains or SUMO-1 or SUMO-2 monomers. Similar to ZIP1, a single SIM was mapped in CENP-E. Mutating this SIM was sufficient to abolish SUMO chain binding and kinetochore localization of CENP-E. The homodimeric state of CENP-E [35] is a potential explanation for the interaction of CENP-E with SUMO chains, since the homodimer might contain two closely spaced SIMs that interact preferentially with SUMO chains. BubR1 and Nuf2 are two other SUMO-2 conjugated kinetochore components that could provide docking sites for CENP-E. It remains to be established whether BubR1 and Nuf2 are indeed conjugated to SUMO-2 chains.
SUMO polymers regulate the response to replication arrest

The flexible N-terminus of Pmt3, the single SUMO family member that is expressed in *S. pombe*, is significantly longer than those of SUMO in *S. cerevisiae* and human SUMOs and contains two lysine residues that are acceptor sites for SUMO polymer formation *in vitro* [11]. *S. pombe* strains that are deficient in Pmt3 chain formation show aberrant cell and nuclear morphologies, including elongated cells and chromatin alterations. Furthermore, these strains are sensitive to the DNA synthesis inhibitor hydroxyurea, but not to other DNA damaging agents including camptothecin, MMS (methyl methanesulfonate) or UV light. This indicates that SUMO chains are required for a proper response to replication arrest. The target proteins that are conjugated to Pmt3 chains during replication arrest and the proteins that interact with these Pmt3 chain conjugates remain to be identified.

SUMO polymers might regulate cross-talk with the ubiquitin–proteasome system

SUMO-2/3 chains accumulate on inhibition of the proteasome by MG-132 (the proteasome inhibitor carbobenzoxy-α-leucyl-α-leucyl-leucinal), suggesting that these chains could play a role during proteasomal targeting of proteins [4]. Interestingly, the RNFs (RING finger proteins) Slx5 (synthetic lethal of unknown function 5) and Slx8 in *S. cerevisiae* and RNF4 in mammals have been identified as SUMO chain binders that ubiquitinate SUMOylated proteins to mediate subsequent proteasomal degradation [36–38]. RNF4 contains four closely spaced SIMs that mediate SUMO chain binding [37]. PML was the first RNF4 target protein that has been identified and RNF4 was shown to be required for PML degradation in response to arsenic trioxide. RNF4 is also critical for PML-RARα (retinoic acid receptor α) degradation in response to arsenic trioxide in promyelocytic leukemia [39]. It remains to be established whether SUMO chains are essential for PML and PML-RARα degradation in cells. The significance of SUMO chains for proteasomal degradation of target proteins is currently unclear, since a lysine-deficient SUMO-2 mutant behaves identically with wild-type SUMO-2 on proteasome inhibition [4]. Equal amounts of ubiquitin copurified with wild-type and lysine-deficient SUMO-2 on proteasome inhibition. Nevertheless, a restricted number of SUMO target proteins could exist that are conjugated to SUMO chains and to uncover interactions between these SUMO chain conjugates and SUMO chain binders.

**Future directions**

SUMO chains are unfolding as important regulators of diverse cellular processes. The identification of novel SUMO chain-binding proteins will increase our understanding of SUMO polymer signalling. Database searches yield large numbers of proteins that contain closely spaced SIMs; therefore it is likely that many other SUMO chain-binding proteins will be identified in the future. Furthermore, it will be important to identify novel target proteins that are conjugated to SUMO chains and to uncover interactions between these SUMO chain conjugates and SUMO chain binders.

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**References**