SUMOylation

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SUMOylation is a dynamic and essential Post-Translation Modification (PTM) consisting on the conjugation of Small Ubiquitin-like Modifiers (SUMOs) to an acceptor lysine of a substrate protein. SUMOylation predominantly regulates nuclear processes and its dysregulation is associated to diseases including cancer. SUMOs share a similar three-dimensional structure with other Ubiquitin-Like Modifiers (UBLs). However, SUMOs differ due to their flexible N-terminus, which also contains the site for SUMO chain formation. All eukaryotes express at least one SUMO paralogue. Five SUMO family members have been identified in humans (SUMO1, SUMO2, SUMO3, SUMO4, and SUMO5. However, SUMO1, SUMO2, and SUMO3 are the main family members where they are commonly classified as SUMO1 and SUMO2/3 because of the high similarity between mature SUMO2 and SUMO3. All SUMO paralogues are similar in structure but differ in expression levels. SUMO2 is the most abundant family member in mammalian cells. Studies in mice show that the knockout of SUMO2 is embryonic lethal, while SUMO1 and SUMO3 knockout mice were associated to mild phenotypes, possibly because SUMO2 might compensate the loss of either SUMO1 or SUMO3. Similarly to ubiquitination, SUMO is conjugated in a in a 3-step enzymatic cascade that involves a dimeric E1 activating enzyme (SAE1 and SAE2), an E2 conjugating enzyme (Ubc9), and several SUMO E3 enzymes.

Keywords: ubiquitin ; SUMO ; E3 enzymes

1. SUMO E3 enzymes

The major class of SUMO E3 enzymes is the PIAS family composed of five members. This family is characterized by its Siz/Pias Really Interesting New Gene (SP-RING) domain that binds to the SUMO E2 enzyme Ubc9. The five members share structural similarity and act in a similar manner as RING E3 ubiquitin ligases [1]. However, in contrast to Ub RING E3 enzymes, knockout studies in mice showed that PIAS SUMO E3 enzymes seem to be redundant and not essential. Mice displayed mild phenotypes, indicating that the lack of one member of the PIAS family is either dispensable or compensated for other members of the PIAS family [2][3]. The substrate specificity that Ub RING E3 enzymes exhibit is yet to be questioned in the PIAS family.

On the other side, the nucleoporin RanBP2/Nup358 does not contain the SP-RING domain, but instead can form a complex with RanGAP1, Ubc9, and SUMO1 to enable E3 ligase activity [4]. This component of the NPC appears to have different roles during the cell cycle. RanBP2 enriches at kinetochores and the mitotic spindle having essential functions in nucleoplasmic transport during interphase [5] and chromosome segregation mitosis [6]. In addition, new functions are emerging in DNA damage, as recent research has reported the E3 activity of RanBP2 in DNA polymerase lambda SUMOylation [7]. All these functions match with knockout mice studies showing that RanBP2$^{-/-}$ mice were unviable, highlighting the essential role of RanBP2 [8].

Another class of SUMO E3 enzymes was discovered recently. The zinc finger ZNF451 family is composed of ZNF451 isoform 1 (ZNF451-1), isoform 2 (ZNF451-2), isoform 3 (ZNF451-3), and the primate-specific KIAA1586. ZNF451-1 and ZNF451-2 are very similar in contrast to the distant members ZNF451 isoform 3 (ZNF451-3) and KIAA1586. All members share a practically identical N-terminus that includes catalytic tandem SUMO interactive motifs (SIMs) which are necessary for SUMO conjugation. Both SIMs work together, the first SIM places the donor SUMO, while a second SIM binds SUMO on the back side of the E2 enzyme for subsequent SUMO conjugation to the substrate protein [9][10]. In contrast to the N-terminus, the C-terminus differs between family members. ZNF451-1 contains C_2H_2-Zinc finger domains, whereas ZNF451-2 lacks residues 870-917 due to alternative splicing. ZNF451-3 encodes a C-terminal deletion of 933-1061 and holds a LAP2alpha domain which is not present in the other family members [11]. The ZNF451 class seems to be inefficient in the initial conjugation of SUMO, although this tandem-SIMs region is sufficient to extend a SUMO chain and form a SUMO polymer. This activity is referred to as E4 elongase. In addition, the ZNF451 class has been implicated in SUMO2/3 polymers formation during both proteasome inhibition and DNA damage stresses [9]. Years later, due to its

role in DNA-Protein crosslink repair by stalled TOP2 SUMOylation, it was suggested to re-name this ZNF451 class “ZATT” (zinc finger protein associated with TDP2 and TOP2) although these are probably not the only substrates [12].

SLX4 contains a BTB domain and three putative SIMs essential for SUMO binding and SUMOylation. The BTB domain seems to be important for protein–protein interaction and oligomerization necessary for the formation of the SLX4 complex. Pull-down experiments employing SLX4 SIMs mutants show the capacity of SLX4 to SUMOylate xeroderma pigmentosum group-F (XPF). Interestingly, SLX4 can SUMOylate itself with both SUMO1 and SUMO3. However, SLX4 seems to preferentially use SUMO3 for XPF SUMOylation. SLX4 SUMO E3 activity plays a role in response to global and local replication stress [13].

Additional E3 enzymes have been identified in other organisms, such as herpesvirus where they have a possible role during infection. Examples are SM, UL69, and UL54. Interestingly, the SM and UL69 show preference for SUMO1 and UL54 for SUMO2 [14]. There is still a lot to discover and research to be done, but a complex network could be emerging where SUMO E3 enzymes use different SUMO modifiers to form different chains, in order to lead a substrate protein to a particular function in the cell, which seems to be tightly regulated.

2. SUMO Polymers

The discovery of the ZNF451 class and its E4 elongase activity gave rise to the study of a possible physiological role of poly-SUMOylation, although the knowledge about SUMO chains signaling remains limited compared to the ubiquitin chain field. A notable difference between SUMO1 and SUMO2/3 polymers resides in the ability to form SUMO chains.

In contrast to SUMO1, SUMO2 and SUMO3 possess a K11 in their flexible N-terminus, which is located in a sequence motif, ψKXE, where ψ represents a large hydrophobic amino acid and X any amino acid. This sequence is referred to as SUMO consensus motif. The consensus motif is preferentially targeted for SUMOylation and it is also present in other potential SUMOylation target proteins [15]. This K11 in the SUMO consensus motif allows SUMO2/3 to form K11 SUMO chains [16]. Although this K11 seems to be the main site for SUMO chains, site-specific mass spectrometry approaches have revealed several other SUMO acceptor lysines within SUMO1, SUMO2, and SUMO3 [17]. For example, SUMO1 contains an inverted SUMO consensus site, ExK [18], and harbors an N-terminal K7, which tolerates low efficient SUMO1 chains formation as demonstrated in vitro and in vivo by site-specific mass spectrometry [17][19]. However, it seems that SUMO1 works as a capping factor, terminating SUMO2/3 chain formation [16][20].

The main consequence of SUMO conjugation seems to be the alteration of binding surfaces of the substrate protein, which can either hinder or promote intra- or intermolecular interactions. Additionally, SUMO is able to promote molecular interaction due to its affinity to SIMs. SIMs are short peptide sequences mostly located in unstructured regions of the modified protein or interacting proteins [21]. These SIMs allow non-covalent interaction between SUMOylated proteins and effector proteins which contain SIMs [22]. Given the fact that SUMOylation occurs predominantly in the nucleus and nuclear bodies, its role varies from transcription regulation and chromatin remodeling to DNA repair and cell cycle progression [23]. Although SUMO polymers have been previously reviewed [24][25], to date there is no indication that different SUMO chain linkages fulfil distinct roles within human cells. However, work in *S. pombe* revealed the possible role of two different SUMO chain linkages (K14 and K30) in response to replication arrest [26].

3. SUMO and Ubiquitin Crosstalk

SUMO chains on target substrates can be a signal for the recruitment of SUMO-targeted ubiquitin ligases (STUbLs), leading to a crosstalk between SUMOylation and Ubiquitination. STUbLs, such as the RING-finger protein 4 (RNF4), contain a RING domain that binds to an E2 ubiquitin enzyme, and SIMs that allow the interaction with SUMOylated substrates and increase the preference for SUMO modified targets. RNF4 possesses at least three closely spaced SIMs and shows a clear preference for substrates that are modified by a SUMO chain or at least three SUMO moieties [27]. RNF4-mediated ubiquitination results in either K48 or K63 ubiquitin linkages, which, respectively, label the substrate protein for proteasomal degradation or for the recruitment of ubiquitin-binding motif containing proteins. This mechanism has been implicated in a variety of cellular processes, including promyelocytic leukemia (PML) nuclear body (NB) integrity, mitosis, and DNA Damage Response (DDR) [27][28][29][30]. RNF4 regulates DNA damage signaling by controlling the lifetime of proteins involved in DNA repair such as the check point mediator MDC1. It also regulates the whole SUMOylation machinery, E1, E2, and E3s, by labeling the members for proteasomal degradation [31][29][32][33]. However, it is yet to be discovered how only some poly-SUMOylated proteins are targeted by STUbLs and how different STUbLs can bind selective SUMOylated targets.

The SUMO and Ubiquitin interplay can also be modified by SUMO-targeted ubiquitin proteases (STUPs). STUPs can recognize poly-SUMOylated proteins and are able to modify the ubiquitin chains on SUMOylated targets by their ability to remove Ub. To date, three STUPs have been identified. USP7 seems to remove ubiquitin from SUMO targets with a role in DNA replication [34]. USP11 might have a role regulating nuclear bodies by limiting RNF4 activity [35]. The last STUP is Ataxin-3 (ATX3), which seems to participate in the regulation of MDC1 counteracting the RNF4 activity [36]. Finally, there is not only crosstalk with ubiquitination but also with other PTMs. This was observed in a very deep profiling of the SUMO proteome [37].

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