Cell Cycle 12:7, 1015–1017; April 1, 2013; © 2013 Landes Bioscience

Expanding SUMO and ubiquitin-mediated signaling through hybrid SUMO-ubiquitin chains and their receptors

Catherine M. Guzzo and Michael J. Matunis*

Department of Biochemistry and Molecular Biology; Johns Hopkins University; Bloomberg School of Public Health; Baltimore MD USA

Monomeric and polymeric forms of SUMO and ubiquitin are covalently attached to substrates and recognized by effector proteins containing SUMO-interacting motifs (SIMs) or ubiquitin-interacting motifs (UIMs), thereby triggering a wide range of biological responses. SUMO and ubiquitin were thought to represent distinct homotypic signals, until recent studies revealed the presence of hybrid SUMO-Ub chains. Synthesis of SUMO-Ub chains is dependent on the activity of SUMO-targeted ubiquitin ligases (STUbLs) that specifically recognize and ubiquitinate SUMO chains on substrates (**Fig. 1A**). SUMO-Ub chains were originally identified on proteins destined for proteasomal degradation. As exemplified in humans, SUMO-Ub chains synthesized by RNF4 target PML (promyelocytic leukemia protein) for proteasomal degradation.[1,2] However, it remained unclear whether SUMO-Ub chains are recognized as distinct signals by hybrid chain-specific receptors or by receptors recognizing ubiquitin alone.

Recently, we provided two important insights into SUMO-Ub chain signaling. We revealed that hybrid chains are recognized as distinct entities by receptor proteins containing tandem SUMO- and ubiquitin-interacting motifs (tSIM-UIMs), and that hybrid chains mediate recruitment of DNA repair factors to damage sites. RAP80, a subunit of the BRCA1-A complex, contains tandem UIMs that mediate high affinity interactions with K63-linked ubiquitin chains formed at DNA damage sites.[3] We characterized RAP80 as the first SUMO-Ub chain receptor, demonstrating that the tSIM-UIMs in RAP80 enable it to interact with ~80-fold greater affinity to hybrid SUMO-Ub chains compared with homotypic SUMO or ubiquitin chains.[4] Moreover, SUMO-Ub chains are synthesized at DNA damage sites by RNF4 and recognized by RAP80 to mediate BRCA1 recruitment.[4]

Identification of RAP80 as a SUMO-Ub chain receptor led us to search for SIMs in other UIM-containing proteins, leading to identification of SIMs in ataxin-3, S5a and STAM (**Fig. 1B**). Given the occurrence of tSIM-UIMs in multiple proteins, we propose that tSIM-UIMs may function as a general class of SUMO-Ub chain receptor. In addition to UIMs, approximately 20 different types of ubiquitin-binding domains (UBDs) have been characterized. Thus, proteins containing a SIM in close proximity to a variety of UBDs could function as SUMO-Ub chain receptors. Our studies of the four types of UBDs found in four distinct subunits of the BRCA1-A complex have provided preliminary evidence for the existence of tandem SIM-UBDs. We predicted SIMs adjacent to each type of UBD and demonstrated that these subunits have SUMO-binding activity.[4] Thus, a wide variety of receptors with specificity for SUMO-Ub chains may exist.

The deubiquitinating enzyme Usp25 was the first UIM-containing protein identified to contain a SIM, yet it was not characterized as a SUMO-Ub chain receptor.[5] Instead, the SIM in Usp25 was shown to bind SUMO and promote covalent SUMO modification between tandem UIMs, thus inhibiting deubiquitinating activity.[5] It is intriguing to speculate that the tSIM-UIMs in Usp25 may engage in high-affinity interactions with SUMO-Ub chains, thereby acting as a hybrid chain isopeptidase that would regulate chain levels. Like Usp25, the deubiquitinating enzyme ataxin-3 has tSIM-UIMs. The UIMs in ataxin-3 preferentially bind to K48-linked ubiquitin chains, and the presence of a SIM suggests that ataxin-3 could bind to SUMO-Ub chains containing K48-linked ubiquitin. It can be predicted that SUMO-Ub chains containing K48-linked ubiquitin function as signals for degradation, whereas hybrid chains containing K63-linked ubiquitin function as signals for DNA damage repair.

Recently, it was reported that IκBα is more efficiently degraded when modified by SUMO-Ub chains compared with homotypic chains of SUMO or ubiquitin.[6] However, it remains unclear whether proteins modified by hybrid chains and targeted for degradation are recognized by tSIM-UIM receptors. We identified a potential SIM adjacent to the UIMs in the proteasome subunit S5a, raising the possibility that S5a functions as a hybrid chain receptor that promotes efficient degradation of proteins modified with SUMO-Ub chains. It is also intriguing that multiple proteins of the 19S proteasome share structural and functional similarities to the proteins of the BRCA1-A complex.[7] Our findings that multiple proteins of the BRCA1-A complex may act as hybrid chain receptors suggests that multiple proteasome subunits may also have similar activity.[4] In addition to proteasomal degradation, SUMO-Ub chains may also function as signals that affect lysosomal

*Correspondence to: Michael J. Matunis; Email: mmatunis@jhsph.edu
Submitted: 03/05/13; Accepted: 03/14/13
http://dx.doi.org/10.4161/cc.24332
Guzzo CM, et al. Sci Signal 2012; 5:ra88; PMID:23211528; http://dx.doi.org/10.1126/scisignal.2003485

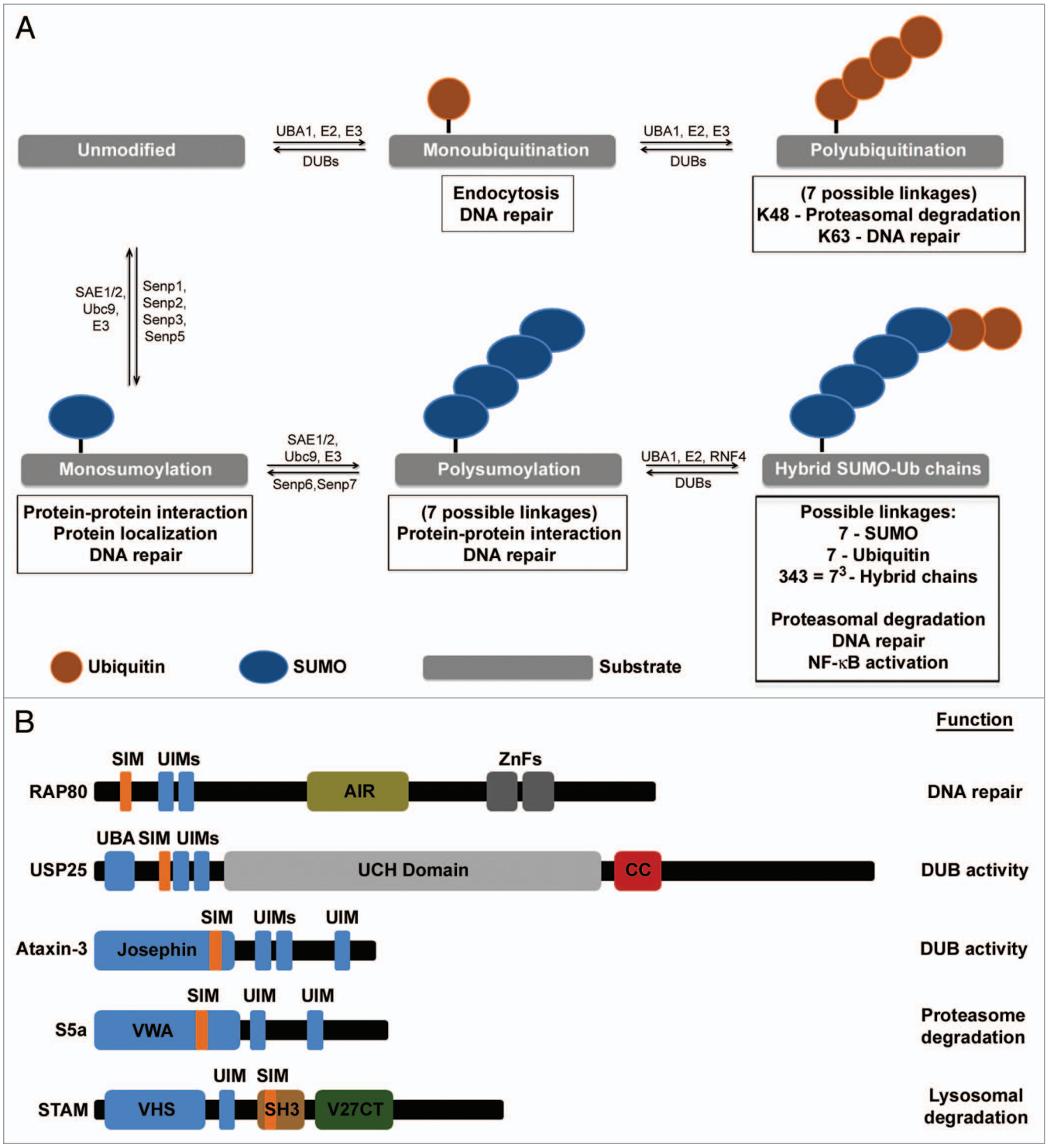

Figure 1. SUMO and ubiquitin-mediated signaling. (**A**) Substrate conjugation and functional consequences. SUMO and ubiquitin are enzymatically conjugated to substrates by E1 (activation), E2 (conjugation) and E3 (ligation) enzymes. RNF4 covalently attaches ubiquitin to SUMO, forming hybrid SUMO-Ub chains. Functional consequences of modifications are shown in boxes. (**B**) Hybrid SUMO-Ub chain receptors. Tandem SIM-UIMs in RAP80 and Usp25 were previously characterized. Predicted SIMs in close proximity to UIMs in ataxin-3, S5a and STAM are shown. Proteins containing tSIM-UIM have various functions, suggesting that their interaction with hybrid SUMO-Ub chains will mediate a wide range of biological responses. AIR, abraxas-interacting region; ZnFs, zinc fingers; UBA, ubiquitin associated domain; UCH, ubiquitin c-terminal hydrolase; CC, coiled coil; VWA, von Willebrand factor type A domain; VHS, domain found in Vps27, Hrs and STAM; SH3, src homology 3; V27CT, Vps27 C-terminal domain.

targeting, as UIMs are found in several components of the lysosomal degradation pathway.[8] We predicted SIMs in several of these proteins, including STAM (signal-transducing adaptor molecule), a protein involved in sorting of substrates for lysosomal degradation.

In summary, we predict that SUMO-Ub chains will be recognized by a variety of receptors containing tandem SIMs and UBDs to mediate a wide range of biological functions. Signaling through hybrid chains provides advantages of specificity, as the coordinated and sequential action of both SUMO and ubiquitin-conjugating enzymes is required for their synthesis, and increased affinity. In addition, because chain linkage and biological outcome are functionally connected, hybrid SUMO-Ub chains expand the potential for distinct signaling by SUMO and ubiquitin by the increasing repertoire of structurally distinct chains (**Fig. 1B**).

References

1. Lallemand-Breitenbach V, et al. Nat Cell Biol 2008; 10:547-55; PMID:18408733; http://dx.doi.org/10.1038/ncb1717
2. Tatham MH, et al. Nat Cell Biol 2008; 10:538-46; PMID:18408734; http://dx.doi.org/10.1038/ncb1716
3. Sims JJ, et al. Mol Cell 2009; 33:775-83; PMID:19328070; http://dx.doi.org/10.1016/j.molcel.2009.02.011
4. Guzzo CM, et al. Sci Signal 2012; 5:ra88; PMID:23211528; http://dx.doi.org/10.1126/scisignal.2003485
5. Meulmeester E, et al. Mol Cell 2008; 30:610-9; PMID:18538659; http://dx.doi.org/10.1016/j.molcel.2008.03.021
6. Aillet F, et al. PLoS One 2012; 7:e51672; PMID:23284737; http://dx.doi.org/10.1371/journal.pone.0051672
7. Wang B, et al. Genes Dev 2009; 23:729-39; PMID:19261749; http://dx.doi.org/10.1101/gad.1770309
8. Hofmann K, et al. Trends Biochem Sci 2001; 26:347-50; PMID:11406394; http://dx.doi.org/10.1016/S0968-0004(01)01835-7v