A new class of SUMO proteases

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The small ubiquitin-related modifier (SUMO) is a ubiquitin-like (UBL) protein that can be conjugated to hundreds of different proteins. Such 'sumoylation'—which is highly dynamic—alters the stability, localization or functional properties of the modified substrate, most often by altering its interactions with other proteins [1,2]. Enzymes known to remove SUMO from substrates, the SUMO proteases, have important regulatory roles, and until now all those described belong to the same protein superfamily. A study in this issue of *EMBO reports* provides the first unambiguous biochemical identification of a novel type of SUMO protease, which is broadly conserved and important for the regulation of a specific transcription factor [3]. These and other recent results raise the exciting prospect of additional SUMO proteases still to be discovered.

SUMO is covalently attached to substrate lysine side chains through a well-known enzymatic cascade (Fig 1A; [1]). Vertebrates encode at least three functioning SUMO proteins: SUMO1, and the nearly identical SUMO2 and SUMO3. All are synthesized with carboxy-terminal propeptides that must be removed; the mature C-terminus is then coupled by amide or 'isopeptide' linkage to substrate lysines. Only a small fraction of most substrates is modified by SUMO at any given time. This is often due to the high rate of enzymatic cleavage of the SUMO–protein isopeptide bond by SUMO proteases or isopeptidases.

The first identified SUMO protease Ulp1 (ubiquitin-like protein-specific protease 1), was isolated in an activity-based screen of proteins from the yeast *Saccharomyces cerevisiae* [4]. All other known SUMO proteases have been identified by virtue of their sequence similarity to yeast Ulp1. *S. cerevisiae* has a second, related SUMO protease, Ulp2, whereas humans have six, known as SUMO/sentrin-specific proteases (SENPs) [5]. A seventh, more divergent human ULP/SEN enzyme, SENP8, is specific for another UBL, NEDD8. Notably, the ULP/SEN enzymes constitute a branch

within a larger group of proteases, the CE clan of cysteine proteases. Similarly to SENP8, not all CE clan enzymes are SUMO proteases. For example, a bacterial protein, Elad, is a ubiquitin-specific protease, and many clan members from viruses are primarily involved in viral polyprotein processing [6].

The answer to the converse question, whether all SUMO proteases are ULP/SEN

enzymes, has been less clear. A candidate for a novel SUMO protease is Wss1 (weak suppressor of *smt3-1*), an *S. cerevisiae* protein identified originally as a high-copy suppressor of a mutant version of *SMT3*, the yeast SUMO gene [7]. Subsequent bioinformatic analysis predicted that Wss1 was a zinc-dependent protease, the founding member of the WLM (Wss1-like metalloprotease) family [8]. Yeast *wss1* mutations show extensive genetic interactions with other SUMO pathway mutations, including those in the SUMO proteases [9]. Biochemical experiments with engineered substrates suggested that Wss1 could cleave the linkage between SUMO and a polypeptide, but this specificity did not extend to other substrates [9]. Thus, it remains unclear whether Wss1 has a broader specificity,

Fig 1 | SUMO protease dynamics. (A) SUMO maturation and conjugation cycle. (B) Comparison of mouse DeSI-1 and DeSI-2 with the known SUMO protease SENP1. The catalytic domains with histidine (H) and cysteine (C) residues are highlighted. (C) BZEL inhibition of *blimp-1* transcription is enhanced by BZEL sumoylation, which is reversed by DeSI-1. BZEL, BTB-ZF protein expressed in effector lymphocytes; DeSI, desumoylating isopeptidase; SENP1, SUMO/sentrin-specific proteases; SUMO, small ubiquitin-related modifier.

perhaps more akin to viral polyprotein-processing enzymes that cleave at multiple short consensus sites, than to typical UBL-specific proteases.

By contrast, the new study by Shin and colleagues provides compelling evidence of SUMO-specific isopeptide bond cleavage for two mammalian enzymes, desumoylating isopeptidase (DeSI)-1 and DeSI-2 (Fig 1B). Although lacking obvious sequence similarity to ULP/SEN proteases, they are also cysteine proteases. Interestingly, they were suggested to be ubiquitin-system-associated proteases in the same bioinformatic study that uncovered the WLM family [8]. The predicted cysteine proteases were originally named PPPDE peptidases—permuted papain-fold peptidases of dsRNA viruses and eukaryotes.

The papain fold characterizes all ubiquitin-specific and UBL-specific cysteine proteases characterized so far. In most, the catalytic cysteine responsible for peptide-bond attack precedes the histidine that functions as a general base in the primary sequence. In the PPPDE peptidases, this catalytic residue order is reversed (circularly permuted), as is true of the ULP/SEN proteases. Recent structural studies support the inclusion of the PPPDE proteases in the NlpC/P60 superfamily of papain-like enzymes, particularly the circularly permuted subgroup, despite their weak sequence similarity [10].

The Yun group identified mouse DeSI-1—also known as Pppde2—in a yeast two-hybrid screen using the transcription factor BZEL as bait. BZEL (ZBTB46) is a sequence-specific transcriptional repressor, and *blimp-1* is one of its targets. DeSI-1 shares 23% protein identity with DeSI-2, primarily in a catalytic domain of ~140 amino acids (Fig 1B). Likely orthologues of these mouse proteins are found in many species. Interestingly, DeSI-1 seems to be predominantly cytoplasmic with some nuclear staining, which would be consistent with its association with the BZEL repressor, whereas DeSI-2 is almost exclusively cytoplasmic. By contrast, all of the ULP/SENPs are concentrated in the nucleus, sometimes to specific nuclear structures such as nuclear pore complexes or nucleoli [5].

Both ubiquitylated and sumoylated forms of BZEL were detected in transfected cells, but overexpression of DeSI-1 specifically reduced the latter. Mutating the putative active-site cysteine to serine (C108S) abolished this reduction in

SUMO-BZEL, consistent with the hypothesis that DeSI-1 is a SUMO-specific protease. In co-transfection analyses, BZEL could be modified by SUMO1, SUMO2 or SUMO3. DeSI-1 seems to cleave all three conjugates with efficiencies similar to that of the broadly acting SENP1 enzyme.

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The apparent desumoylation of BZEL by DeSI-1 might reflect indirect effects, such as activation of another SUMO protease or inhibition of SUMO conjugation. Therefore, Shin and colleagues showed that recombinant DeSI-1 directly desumoylates purified, *in vitro* sumoylated, recombinant SUMO-BZEL, whereas the DeSI-1-C108S mutant did not. The cleavage probably occurred specifically after the C-terminus of SUMO1, because DeSI-1 could also be modified by SUMO1-vinyl sulphone (VS), a suicide substrate selective for SUMO proteases. DeSI-1-C108S was not modified by SUMO1-VS, and the wild-type enzyme was not modified by ubiquitin-VS. Interestingly, neither DeSI-1 nor DeSI-2 was active toward a fusion of SUMO with a C-terminal His6 sequence, unlike the SENP1 catalytic domain. This suggests that DeSI-1 specifically recognizes the SUMO-lysine side chain linkage, although the His6 tail could also interfere with enzyme activity. DeSI-1 has a weak activity towards free polySUMO2/3 chains, implying an ability to cleave SUMO-SUMO isopeptide linkages. In all, these data strongly support the conclusion that DeSI-1 is a SUMO-specific isopeptidase.

In the ubiquitin system, deubiquitylating enzymes (DUBs) are second only to the ubiquitin ligases in their diversity—humans have almost 100 DUBs. By contrast, despite the identification of many different SUMO-modified proteins, only six ULP/SEN SUMO proteases had been described. Most have a broad range of substrates, whereas initial data from Shin and colleagues suggest a high degree of substrate specificity for DeSI-1. Bulk SUMO conjugates detected by anti-SUMO immunoblotting were not significantly altered upon reduction of DeSI-1 levels. In addition, two previously characterized ULP/SEN substrates—SUMO-PML and SUMO- Δ Np63—were not reduced by DeSI-1 *in vivo* or *in vitro*. Hence,

DeSI-1 might act on a relatively small number of targets.

How does DeSI-1 affect BZEL function? In B-cell lines, co-transfection of DeSI-1 with BZEL led to increased *blimp-1* reporter activity. This was not observed with the inactive DeSI-1-C108S construct. Conversely, after reduction of DeSI-1, sumoylated BZEL increased and the activity of the *blimp-1* reporter decreased. These data are consistent with sumoylation of BZEL enhancing its repressor activity (Fig 1C). Future work with DeSI gene knockouts in mice should help determine the *in vivo* function of DeSI-1 in regulating BZEL, as well as the full range of functions of these proteases.

The discovery of a completely novel class of SUMO proteases raises the question of whether more are yet to be found. Baker's yeast appears to get by with relatively few such enzymes despite the large number of sumoylated yeast proteins. Metazoans, however, might require additional means for fine-tuning the spectrum of SUMO-modified proteins. Six ULP/SEN genes is a modest number, but some are subject to alternative splicing, generating isoforms that might have distinct specificities. As noted above, the DeSI enzymes are part of a much larger superfamily of cysteine proteases. Other members might also be SUMO-specific enzymes. Among the large group of putative DUBs, many remain enzymatically uncharacterized; some might turn out to be SUMO proteases. Future work should unravel how cells exploit this anticipated complexity in the control of SUMO dynamics.

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