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Genome Stability Roles of SUMO-targeted Ubiquitin Ligases

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Abstract

Post-translational modification of the cell's proteome by ubiquitin and ubiquitin-like proteins provides dynamic functional regulation. Ubiquitin and SUMO are well-studied post-translational modifiers that typically impart distinct effects on their targets. The recent discovery that modification by SUMO can target proteins for ubiquitination and proteasomal degradation sets a new paradigm in the field, and offers insights into the roles of SUMO and ubiquitin in genome stability.

Keywords

ubiquitin; SUMO; STUbL; proteasome; degradation; SUMO interaction motif; ubiquitin ligase; DNA repair; genome stability

1. Introduction

Historically, there have been reports of intimate interactions between the SUMO and ubiquitin pathways, including evidence that modification by SUMO could “prime” protein ubiquitination and proteasomal degradation (e.g. [1,2]). However, ubiquitin E3 ligases able to mediate this phenomenon were unidentified and thus, SUMO-directed ubiquitination remained on the fringe of accepted SUMO functions. In this review we cover the recent identification of SUMO-targeted ubiquitin ligases (STUbLs) and highlight their impact on critical cell regulatory pathways. Despite their novelty, STUbLs are already implicated in fundamental and diverse processes such as chemotaxis and genome stability [3]. Notably, the human STUbL provides a missing piece to the long-standing puzzle of how the leukemia therapeutic agent called arsenic trioxide exerts its effects [4,5]. Recent reviews have covered detailed functions of ubiquitin, SUMO and their various modes of interplay [3,6-9]; so herein, we will mainly focus on the role(s) of STUbLs in the maintenance of genome stability.

2. Novel crosstalk between the ubiquitin and SUMO pathways

In a screen for genes required for cell viability in the absence of the budding yeast RecQ helicase Sgs1 (human BLM homolog), a number of so-called *SLX* genes were identified [10]. Two of these *SLX* genes encode RING domain-containing proteins, Slx5 and Slx8, which were shown to form a complex. Primary sequence homologues of Slx5 were only detectable in

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Saccharomycetes, whereas an Slx8 homolog was identified in fission yeast [10]. This indicated that Slx5-Slx8 complexes were likely to be functionally conserved, albeit with highly diverged Slx5 subunits. In a recent fission yeast study, a RING domain protein called Rfp1 was isolated as an interactor of the Smc5-6 DNA repair complex non-SMC subunit Nse5 [11]. Intriguingly, Slx5 also interacts with the budding yeast Nse5 homologue [12], suggesting that Rfp1 and Slx5 might be functionally related. Further analyses showed that Rfp1 forms a complex with fission yeast Slx8 and confirmed that Rfp1 and Slx5 are functionally homologous [11,13]. Database searches using Rfp1 returned clear homologues in higher eukaryotes including human RNF4, indicating that the Slx5-Slx8 complex is functionally conserved in eukaryotes (see Fig. 1; [11,13,14]).

Until recently, the function of the Slx5-Slx8 complex remained enigmatic. Early insights came from an elegant study in budding yeast that identified genomic mutations able to suppress the phenotypes of a hypomorphic allele of the transcriptional regulator Mot1 [15]. The majority of suppressor mutations were found in multiple components of the SUMO pathway, and intriguingly, in *SLX5* and *SLX8*. Together with the increase in cellular SUMO conjugates observed in *slx5Δ* and *slx8Δ* cells [15], this suggested that Slx5-Slx8 is functionally related to the SUMO pathway. Mechanistic insight came from a number of yeast studies that identified SUMO-binding and ubiquitin E3 ligase activities associated with the Slx5 (Rfp1)-Slx8 heterodimeric complex [3,11,13,16,17]. The combination of these activities and observed deregulation of the SUMO pathway in budding and fission yeast cells lacking Slx5 (Rfp1)-Slx8 activity [3,11,13,14,16,17] suggested that Slx5 (Rfp1)-Slx8 constituted a SUMO-targeted E3 ubiquitin ligase (STUbL). The ability of STUbLs to ubiquitinate both SUMO conjugates and proteins containing SUMO-like domains (SLDs) was confirmed both *in vitro* and *in vivo* [11,13,16-18]. Furthermore, STUbL-mediated ubiquitination was shown to promote the proteasomal degradation of SUMO conjugates (Fig. 2; [11,13,16,17]. The human Rfp1-Slx8 homolog, RNF4, was shown to rescue the phenotypes of yeast STUbL mutants, further supporting the functional conservation of STUbLs [11,13,14,16]. Indeed, the STUbL mechanism was recently confirmed in human cells and will be described in a later section [4, 5].

3. Relationship of STUbLs to the SUMO pathway

To understand the role of STUbLs in genome maintenance it is useful to place STUbLs in context with the canonical SUMO pathway. Current data already reveal important insights into the relationship between STUbLs and the SUMO system. Yeast STUbLs, SUMO conjugation enzymes (E1 and E2) and SUMO proteases (Ulp1 and Ulp2) are all either essential, or their mutation causes pronounced growth defects and genomic instability (see Table 1 and Refs. therein). In hypomorphic E1 and E2 mutant cells, sumoylation is attenuated, whereas many proteins accumulate in their SUMO-conjugated states in STUbL and Ulp mutant cells. Thus, it appears that a sensitive balance between the sumoylated and un-sumoylated forms of the proteome is crucial for genome stability. Interestingly, however, abolishing more than 90% of detectable sumoylation in both fission and budding yeast has relatively minor consequences for cell growth [19-21]. For example, the sole PIAS family SUMO E3 ligase of fission yeast, Pli1, is required for the majority of sumoylation and yet its deletion causes no macroscopic growth defects [20,21]. Closer inspection of *pli1Δ* cells reveals subtle genome instability phenotypes including enhanced minichromosome loss, increased telomere length, heterochromatic silencing defects at the centromere and rDNA, as well as increased sensitivity to the microtubule-destabilizing agent thiabendazole (TBZ; [20,21]). The critical SUMO conjugates remaining in Pli1 mutant cells likely depend on the other SUMO E3 ligase of fission yeast called Nse2 [22,23], and consistently, *pli1Δ nse2-SA* mutant cells appear to phenocopy a deletion of fission yeast SUMO [24]. Based on the above observations, the accumulation of

SUMO conjugates in STUbL and Ulp mutant cells appears more detrimental to cell growth and genomic stability than hyposumoylation.

4. SUMO-directed ubiquitination: a chain reaction?

In addition to the genome stability defects shared by yeast Ulp and STUbL mutants, cells mutant for either factor also exhibit an accumulation of high molecular weight poly-SUMO chains [11,13-17]. It is unknown whether these are mainly unanchored poly-SUMO chains or chains conjugated to target proteins. What is clear, however, is that the accumulation of SUMO chains is a major cause of *ulp2Δ* phenotypes [25]. SUMO contains a number of lysine residues that can be used as “acceptors” for another SUMO moiety in the process of poly-SUMO chain formation [26]. Conceptually, these same lysine residues can also be modified by other post-translational modifications, including ubiquitin. SUMO in which all lysine residues are mutated to arginine (SUMO^{K-R}) prevents SUMO chain formation and notably, supports near normal vegetative growth of both fission and budding yeast [11,25]. Interestingly, the SUMO^{K-R} mutant suppresses the sensitivity of *ulp2Δ* cells to elevated temperature, HU, TBZ, and caffeine, indicating that these *ulp2Δ* phenotypes are the result of compromised SUMO chain processing [25].

In light of the following results, SUMO chains are intuitive STUbL targets: (i) STUbLs contain multiple SUMO-interacting motifs (SIMs), which can support simultaneous non-covalent binding to multiple SUMO moieties (e.g. poly-SUMO; [3,5,11]); (ii) STUbL mutant cells accumulate poly-SUMO conjugates and (iii) STUbL ubiquitin ligase activity and substrate affinity are increased towards poly-SUMO conjugates *in vitro* [3,5,16,18]. However, unlike in *ulp2Δ* cells, expression of SUMO^{K-R} in STUbL mutant fission or budding yeast cells is highly toxic ([11,16]; J.P. & M.N.B. unpublished observations). Thus, unexpectedly, SUMO chains are not a significant toxic species in STUbL mutant cells; rather, SUMO chains and STUbLs may have partially redundant functions. For example, proteins modified by SUMO chains might be sequestered in the absence of STUbL activity, thereby partially “detoxifying” this pool of SUMO conjugates. That the SUMO^{K-R} mutant is viable and supports largely wild-type vegetative growth has other important ramifications for STUbL function [11,16,25]. In fission yeast, the inviability of STUbL mutants is suppressed by concomitant deletion of the SUMO E3 ligase Pli1, which demonstrates the essential role of STUbLs is to regulate SUMO conjugates [11]. Therefore, healthy growth of SUMO^{K-R} fission yeast indicates that at least in this organism, the critical STUbL targets are either mono- or multiply mono-sumoylated. Furthermore, since SUMO^{K-R} lacks acceptor lysine residues, ubiquitination of the SUMO moiety is unlikely to be functionally crucial. The analogous SUMO^{K-R} mutant of budding yeast is also relatively aphenotypic in comparison to STUbL mutant cells (e.g. [17,25]). Thus, in the highly divergent budding and fission yeasts, critical STUbL targets apparently do not need to be marked by SUMO chains. How does this fit with the *in vitro* data and *in vivo* observation of SUMO chains in STUbL mutants? The *in vivo* accumulation of poly-SUMO conjugates in STUbL mutants might represent the engagement of futile cycles of SUMO conjugation on a substrate that was destined for degradation. The apparent *in vitro* stimulation of STUbLs by poly-SUMO conjugates is expected, since such chains present many more docking sites and potential ubiquitin acceptor sites (9 lysine sidechains). Further studies are required to resolve the precise nature of STUbL targets and the signals required to trigger their ubiquitination.

5. DNA repair proteins as potential STUbL targets

The genomic instability and genotoxin sensitivity of STUbL mutant yeast indicates that some STUbL clients might be DNA repair factors [10,11,16,17,27]. In addition, mutations in *slx8* display genetic interactions with hypomorphic alleles of homologous recombination repair (HRR) factors, including mutants of *RAD52*, *RAD51* (*S.p. rhp51*), *SGS1* (*S.p. rqh1*), *mus81*

and *rad60* (see Table 1 and below; [10,11,16,27,28]). Interestingly, Rad52 and Sgs1 homologues are sumoylated in both yeast and humans, and Rad60 contains SUMO-like domains (SLDs), making these factors potential STUbL targets [29-34]. Indeed, STUbLs ubiquitinate the DNA repair proteins Rad52, Rad57 and Rad60 *in vitro*, in a manner depending on the presence of STUbL SIMs and substrate SUMO modification (or presence of SLDs) [11,17,18,35]. Despite the *in vitro* data, confirmation that these proteins are *bona fide* STUbL targets *in vivo* is currently lacking. Yeast Rad52 sumoylation levels increase under conditions of DNA damage and during meiosis [32]. However, whereas *rad52Δ* cells are highly defective in HRR, a sumoylation-deficient Rad52 mutant causes only mild HRR defects and is degraded slightly faster than wild-type Rad52 *in vivo* [32]. These data appear inconsistent with Rad52 being a primary STUbL target.

Rad60 modulates HRR repair at stalled and/or broken replication forks, physically and functionally associates with the Smc5/6 complex, and is regulated by the replication checkpoint kinase Cds1 (the fission yeast homolog of Chk2) [33,36-38]. Interestingly, Rad60 contains two SLDs that are recognized by Rfp1-Slx8 (STUbL) both *in vitro* and *in vivo* [11] and this interaction appears important for cell survival following replicative stress [11]. However, no STUbL-dependent *in vivo* ubiquitination of Rad60 was detected in the presence or absence of replicative stress [11]. Therefore, the STUbL-Rad60 functional relationship remains undefined and Rad60 may be either a STUbL target or could modulate STUbL activity through its association with Rfp1.

Rfp1 (Slx5) interacts with the Nse5 subunit of the Smc5/6 DNA repair complex in both fission and budding yeast, indicating that although functionally enigmatic, this is a physiologically important interaction [11,12]. Notably, the Nse2 (Mms21) subunit is a SUMO E3 ligase that sumoylates multiple components of the Smc5/6 holocomplex, other DNA repair proteins and telomere components (see below and [22,39,40]). Thus, Smc5/6 subunits or other Nse2 targets are attractive candidate STUbL targets in regulating genome stability.

Finally, it has been suggested that budding yeast STUbLs can modulate DNA recombination processes by promoting the *sumoylation* of key HRR factors [27]. STUbL dysfunction causes elevated levels of both Rad51-dependent and Rad51-independent recombination events, indicating an increased burden of DNA lesions and/or a failure to suppress potentially deleterious Rad51-independent events [27]. The sumoylation of replication protein A (RPA) appears attenuated in STUbL mutant cells [27] and consistently; STUbL-mediated conjugation of SUMO to DNA replication/repair factors, including RPA, has been detected *in vitro* [41]. Thus, by regulating the sumoylation status of RPA, amongst other factors, STUbLs may influence recombination repair pathway choice and thereby affect genomic stability. It should be noted that whilst STUbLs clearly promote the ubiquitination and degradation of sumoylated proteins, whether they also act as E3 SUMO ligases *in vivo* is somewhat controversial. In fission yeast, both the essential and DNA repair roles of STUbLs are mediated through the regulation of Pli1-dependent SUMO conjugates [11]. This appears inconsistent with a functionally important E3 SUMO ligase activity associated with STUbLs, at least in this organism. The stimulation of *in vitro* sumoylation by budding yeast Slx5 is RING-independent and instead likely depends on its SIMs [41]. This may reflect a specific activity, or be an artifact of the increased local concentration of SUMO and its conjugation machinery on the multiple SIMs of Slx5 [16,17]. Defective sumoylation of certain repair factors *in vivo* could also be an indirect consequence of globally deregulated sumoylation in STUbL mutant cells. Interestingly, an apparently bi-functional SUMO and ubiquitin E3 ligase is not unprecedented. The p53 and Topoisomerase I-interacting protein Topors is able to stimulate both SUMO and ubiquitin conjugation to p53 [42,43]. As for budding yeast STUbLs, the ubiquitination activity of Topors is RING-dependent, whereas sumoylation activity is not. Further studies are required to

determine if STUbLs and Topors are truly bi-functional, or exclusively act as SUMO-targeted ubiquitin ligases.

6. STUbLs wrestle with SUMO for replication fork stability

As STUbLs regulate the sumoylation status of DNA replication/repair-associated factors, and modes of HRR, a negative impact of STUbL dysfunction on the sensitive process of DNA replication may be anticipated. The following synthetic genetic interactions of STUbL mutations provide strong support for this prediction. The viability of STUbL mutant cells depends on the RecQ helicase homologues Sgs1 and Rqh1 of budding and fission yeast, respectively [10,11]. The Sgs1 and Rqh1 (human BLM) helicases function in multiple aspects of HRR, replication fork stabilization and resumption of replication from stalled forks (reviewed in [44-47]). Sgs1 was recently shown to collaborate with sumoylation and the Smc5/6 subunit Nse2 in counteracting toxic recombination at stalled replication forks [29]. Thus, the synthetic lethality of RecQ helicase and STUbL mutations indicates that stalled/damaged replication forks and other potential HRR substrates accumulate in STUbL mutant cells. This notion is supported by the large increase in spontaneous foci of the HRR protein Rad52-GFP observed in STUbL mutant cells of either fission or budding yeast [11,27,48]. In fission yeast, STUbL mutant viability requires the Holliday junction endonuclease Mus81-Eme1 [11], which plays a critical role in replication fork restart [49-56]. Thus, genetic interaction with *mus81Δ* again indicates a role for STUbLs in preventing spontaneous replication fork collapse. Such replication-associated phenomena likely account for the highly elevated mitotic recombination and mutation rates in STUbL mutant cells, and their hypersensitivity to the fork-stalling agent hydroxyurea (HU) [11,27,28].

7. Potential roles for STUbLs in HR-dependent telomere maintenance

Sgs1 facilitates alternative telomere lengthening (ALT) through Rad52-mediated HR [57-59]. Consequently, deletion of Sgs1 or Rad52 speeds up senescence in cells that lack telomerase (Tlc1) and depend on ALT for telomere maintenance [59]. Rapid senescence is also observed in the budding yeast telomerase and STUbL double mutants, *slx8Δ tlc1Δ* and *slx5Δ tlc1Δ*, indicating that STUbLs might facilitate ALT [59]. Intriguingly, the Smc5/6 complex promotes the recruitment of telomeres to promyelocytic leukemia nuclear bodies (PML-NBs) in tumor cells that rely on ALT to escape senescence [39]. The Smc5/6 subunit Nse2 sumoylates telomere-binding proteins Trf1/2, which are members of the shelterin complex that inhibits inappropriate HR at telomeres [60,61]. Trf1/2 sumoylation is required for both telomere recruitment into PML-NBs and for ALT, as knockdown of Smc5 or Nse2 results in telomere delocalization and erosion [39]. Elements of the ALT pathway and sumoylation of the Trf1 ortholog, Taz1, are conserved in fission yeast [62,63]. Taz1 inhibits HR-dependent telomere elongation in telomerase mutants (*trt1Δ*), since *taz1Δ trt1Δ* double mutants maintain linear chromosomes in an HR-dependent manner, in contrast to the chromosome circularization (end fusion) observed in *trt1Δ* cells [63]. In light of the above data, it is possible that STUbLs facilitate ALT by degrading sumoylated telomere-binding factors, including Trf1/2 (Taz1). Consistently, the human STUbL RNF4 localizes to PML-NBs [64], the sites of HR-dependent telomere maintenance in ALT cells [39].

8. STUbLs, arsenic trioxide and leukemia

The most mechanistically defined and exciting STUbL function to date is that of mediating the major therapeutic effect of arsenic trioxide (ATO) in treatment of acute promyelocytic leukemia (APL) [4,5,65]. In 95% of cases, APL is caused by a reciprocal chromosomal translocation between the retinoic acid receptor-alpha gene on chromosome 17 (RARα), with the PML gene on chromosome 15 (reviewed in [66]). Normally, PML forms dynamic subnuclear structures termed PML-nuclear bodies (PML-NBs), which are implicated in

regulating critical cellular pathways such as apoptosis and those linked to genome stability through DNA repair and transcription [67]. In PML-RAR α expressing APL cells, PML-NBs are disrupted by the formation of heterodimers between PML-RAR α and PML. Strikingly, treatment with ATO restores normal PML-NBs in APL cells, releases the block to cellular differentiation and induces disease remission in patients [2,66,68,69]. Several years ago, an elegant study demonstrated that ATO treatment induces the sumoylation of PML and PML-RAR α at Lys160 of PML, which in turn results in their ubiquitination and proteasomal degradation [2]. Spurred by the recent discovery of STUbLs, human RNF4 was tested and confirmed as the critical mediator of ubiquitination and degradation of sumoylated PML/PML-RAR α following ATO treatment [4,5,65]. Interestingly, all three studies determined that SUMO-2/3 modification of PML is required for RNF4-mediated degradation. Since SUMO-2/3 but not SUMO-1 form chains (see [70]), this observation supports that at least for PML, SUMO chains might be a critical signal for RNF4-dependent ubiquitination (see Section 3). SUMO-2/3 chains can form on PML and PML-RAR α *in vivo*, as indicated by the accumulation of high molecular weight SUMO conjugates in siRNA RNF4 experiments [5].

The importance of STUbL-mediated PML degradation in cancer biology likely extends beyond APL, given the diverse regulatory functions of PML in the nucleus. For example, PML was recently demonstrated to be critical for the maintenance of a rare subpopulation of leukemic cells termed leukemia-initiating cells (LICs) [71]. LICs are quiescent, pluripotent, and self-renewing cells that propagate leukemia [72]. Accordingly, LICs are inherently difficult to eradicate by conventional cancer therapies that are tailored toward rapidly cycling cells. Ito *et al.* demonstrated that PML maintains quiescence of chronic myeloid leukemia (CML) blasts [71]. Intriguingly, ATO treatment combined with the chemotherapeutic agent cytosine arabinoside (Ara-C) leads to LIC exhaustion in mouse transplantation experiments, whereas Ara-C treatment itself does not. LIC exhaustion is likely due to ATO-dependent degradation of PML, which forces LICs out of their quiescent state and allows for Ara-C mediated DNA damage to occur during S-phase, leading to cell death [71]. Based on these findings and recent published studies [4,5], SUMO, ubiquitin and RNF4 are apparently collaborating to promote PML degradation in ATO-treated LICs. Cancer stem cells are critical targets in the treatment of multiple tumors, including those of the breast and brain [72]. Thus, it will be important to determine the generality of PML-dependent cancer stem cell maintenance. Overall, RNF4 and its associated regulatory pathways likely represent both important biomarkers for treatment choice and novel therapy targets for improved cancer treatment.

9. STUbLs in transcriptional regulation

Compelling support for STUbL roles in transcriptional regulation comes from two studies in budding yeast [15,73]. Wang *et al.* discovered Slx5 and Slx8 (STUbLs) as loss-of-function suppressors of the transcriptional defects in *mot1* mutant cells. Mot1 is an essential ATPase that regulates transcription (positively and negatively) via modulating TATA-binding protein (TBP) occupancy at promoters (e.g. [74-76]). The specific target(s) of Slx5-Slx8 in rescuing *mot1* mutant phenotypes was not identified; however, Mot1 contains consensus sumoylation sites and thus, in STUbL mutant cells, any sumoylated forms of Mot1 might be stabilized. Although global Mot1 protein levels were unaffected in *slx5/8* Δ cells [15], it is possible that only the fraction of Mot1 at promoters is sumoylated and thus sensitive to STUbL regulation. In another study, Slx5 was identified as a factor that interacts with the NAD-dependent protein deacetylase Sir2 [73]. Furthermore, *slx5* Δ cells exhibit silencing defects at telomeres and *rDNA*, phenomena also observed in *sir2* Δ cells [73]. Sir2 function, localization and silencing signatures were largely unaffected in *slx5* Δ cells, indicating that STUbLs likely act downstream of Sir2 in an undefined later step of transcriptional silencing.

The human STUbL RNF4 (or SNURF) appeared in the literature a decade ago, identified as a novel RING-finger protein and transcriptional co-activator [77,78]. RNF4 is ubiquitously expressed in adult human and mouse tissue, but predominates in the adult testis [78,79], and similar to yeast STUbLs, RNF4 localizes to the nucleus [77,79]. RNF4 enhances the activity of, and interacts with a variety of steroid receptor and non-steroid transcription factors (TFs), and also relieves Trps1-mediated repression of GATA-driven genes [77,79-83]. How RNF4 promotes these apparently disparate effects is currently unknown; however, the finding that RNF4 is a STUbL suggests plausible roles in transcriptional regulation. A common thread is that many TFs co-regulated by RNF4 are also modified by SUMO and ubiquitin and undergo proteasomal degradation (reviewed in [84]). Notably, where investigated, RNF4 interactions with and co-activation of TFs depend on RNF4 regions encompassing the two SIMs and/or a functional RING domain [77,81-83,85]. Thus, RNF4 might bind to sumoylated TFs and promote their ubiquitination/degradation. Consistently, the ubiquitin-proteasome system can have a positive effect on transcription [84,86]. The ubiquitin E2 UBCH7 stimulates the activity of the androgen, progesterone and glucocorticoid receptors (AR, PR and GR) and the retinoic acid receptor (RAR) [86]. In addition, PR and the estrogen receptor alpha (ER α), TFs that are co-activated by RNF4, undergo dynamic re-cycling by the proteasome. Interruption of this cycle by proteasomal inhibition reduces transcriptional activity, possibly by preventing binding of 'fresh' TF to the promoter [87,88]. RNF4-mediated degradation might also remove transcriptional repressors such as Trps1, whose repressive activity is relieved by RNF4 [81]. The apparent link between RNF4 co-activation and proteasomal degradation is, however, not always positive. Similar to ER α and PR, RNF4 co-activated GR undergoes proteasomal re-cycling [89], yet proteasomal inhibition enhances rather than attenuates GR mediated transcription [89]. Thus, specific effects of potential RNF4-mediated degradation on transcription may vary between TFs and/or be context-dependent.

10. STUbLs directly regulate a MAP kinase signaling cascade

In addition to possibly controlling TFs directly (see above), STUbLs affect transcription via regulation of signaling pathways upstream of TF activity. MAP kinase signaling cascades are evolutionary conserved phosphorelay systems that transmit information from cell surface receptors to the nucleus and function to regulate gene expression in response to extracellular stimuli. Mammalian MAP kinases control cell survival, proliferation, and differentiation [90]. In the slime mold *Dictyostelium*, MAP kinase signaling is essential for chemotaxis toward cAMP and involves the MAP kinase kinase Mek1 and the MAP kinase Erk1 (see [91]). Upon cAMP stimulation, Mek1 becomes rapidly sumoylated, translocates from the nucleus to the cell's leading edge, and is later ubiquitinated. Mek1 sumoylation is required for proper chemotaxis, and correlates with Mek1 translocation from the nucleus to the cytoplasm in response to cAMP [91]. Sobko *et al.* demonstrated that Mip1, a potential STUbL homologue based on sequence similarity [3], negatively regulates Mek1 function and affects its subcellular localization [91]. Mek1 and Mip1 interact *in vivo* [91], via a region in Mip1 that contains putative SIMs [3]. Cells deleted for Mip1 largely phenocopy constitutively active Mek1, whereas cells overexpressing Mip1 resemble *mek1Δ* cells [91]. These results are consistent with Mip1 acting as a STUbL, ubiquitinating and downregulating Mek1 activity/stability. Interestingly, however, Sobko *et al.* reported that non-sumoylated Mek1 was still susceptible to Mip1-dependent ubiquitination. If confirmed, this could modify the gamut of STUbL functions.

11. Concluding remarks

The STUbL discovery has revealed unexpected complexity in the modes of crosstalk between SUMO and ubiquitin. Furthermore, STUbLs are opening up multiple new avenues for determining the roles and regulation of SUMO in transcription, DNA repair, genome stability

and human disease (Fig. 3). Finally, STUbL-focused research promises to yield new approaches towards improved therapies in cancer and perhaps other diseases.

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Figure 1. Schematic representation of STUbL protein architecture

The heterodimeric yeast STUbLs are exemplified by the fission yeast Slx8-Rfp complex. Protein domains in both Slx8 and Rfp that are critical for function, including the canonical RING domain, the two SIMs and a C-terminal stretch of 6 hydrophobic amino acids (Ψ_6) are incorporated into a single polypeptide chain in mammalian STUbLs, as exemplified by human RNF4. Respective protein sizes are given in brackets, and sequences of the conserved SIM are indicated above the SIM protein boxes, where Ψ is a hydrophobic amino acid.

Figure 2. Proposed function of the fission yeast Slx8-Rfp STUbL complex and relationship to the SUMO and ubiquitin systems

A specific cellular protein (protein X) exists in a sensitive balance between its sumoylated and non-sumoylated form, with only a minor fraction of its pool being SUMO-modified. This balance is maintained by the activities of SUMO modifying E1 (Fub2/Rad31), E2 (Ubc9), and E3s (Pli1), and either of two SUMO specific proteases (Ulp1/2). Slx8-Rfp recognizes sumoylated forms of protein X via Rfp-mediated non-covalent SUMO-SIM interactions. Aided by ubiquitin E1 and E2s, Slx8 ubiquitinates the sumoylated protein X, either directly and/or on the SUMO moieties, which targets protein X for degradation. After degradation, SUMO and ubiquitin likely return to their cellular pools to be re-used in a new round of SUMO and/or ubiquitin protein modification. SUMO moieties are denoted by S and ubiquitin groups by Ub.

Figure 3. Overview of STUbL functions in genome stability and transcription

See text for details. The replisome is denoted by REP, and telomere binding proteins are denoted by TEBP.

Table 1
Phenotypic comparisons of SUMO isopeptidases vs. STUbLs

		<i>ulp2Δ (Ulp1 mut)</i>	STUbL ^{ts} /Δ	References
NIH-PA Author Manuscript	SUMO pathway interaction	Accumulation of SUMO chains	Y (<i>ulp2Δ</i>) (<i>S.c.</i> , <i>S.p.</i>)	[11,14,15, 25,92]
		Growth defects suppressed by SUMO mutants defective for chain formation	Y (<i>ulp2Δ</i>) (<i>S.c.</i>)	[16,25]
		Growth phenotype suppressed by deleting SUMO E3 ligase	Y (<i>ulp2Δ</i>) (<i>S.c.</i>) Y (<i>S.p.</i>)	[11,15,25]
		Growth phenotype suppressed by over-expressing SUMO E3 ligase	Y (<i>ulp2Δ2</i>) (<i>S.c.</i>)	[93]
		UV sensitivity suppressed by <i>ULP2</i> overexpression	<i>n.d./n.a.</i>	Y (<i>S.p.</i>) [14]
		Synthetic lethal with STUbL	Y (<i>ulp1ΔN338</i>) (<i>S.c.</i>)	<i>n.a.</i> [27]
		High copy suppressor of <i>ulp1^{ts}</i>	N (<i>S.c.</i>)	Y (<i>S.c.</i>) [17]
NIH-PA Author Manuscript	DNA damage sensitivity	HU Sensitivity	Y (<i>ulp2Δ</i> , <i>ulp1^{ts}</i>) (<i>S.c.</i>)	Y (<i>S.p.</i> , <i>S.c.</i>) [10,11,92, 94]
		MMS sensitivity	Y (<i>ulp2Δ</i> , <i>ulp1^{ts}</i>) (<i>S.c.</i>)	N (<i>S.c.</i>) Y (<i>S.p.</i>) [11,28,92]
		UV sensitivity	Y (<i>ulp2Δ</i> , <i>ulp1^{ts}</i>) (<i>S.c.</i>)	Y (<i>S.c.</i> , <i>S.p.</i>) [11,17,92, 95]
		CPT sensitivity	<i>n.d.</i>	N (<i>S.c.</i>) Y (<i>S.p.</i>) [11,28]
	Genomic instability	Elevated DNA repair foci	Y (<i>ulp1^{ts}</i> , <i>ulp1ΔN338</i>) (<i>S.c.</i>)	Y (<i>S.c.</i> , <i>S.p.</i>) [11,27,28, 95,96]
		Elevated spontaneous GCR	<i>n.d.</i>	Y (mainly telomere addition) (<i>S.c.</i>) [28]
		Elevated 2μm plasmid number	Y (<i>ulp1T490L</i> , <i>ulp1ΔES</i>) (<i>S.c.</i>)	Y (<i>S.c.</i>) [27,97]
		Elevated chromosome & centromeric plasmid loss	Y (<i>ulp2Δ2</i> , <i>ulp2Δ</i>) (<i>S.c.</i>)	<i>n.d.</i> [92,93]
		Hyper-recombination	Y (<i>ulp1ΔN338</i> , <i>ulp1I615N</i>) (<i>S.c.</i>)	Y (<i>S.c.</i>) [27,95,96]
		Elevated mutation rate	Y (<i>ulp1I615N</i>) (<i>S.c.</i>)	Y (<i>S.c.</i>) [28,95]
		Telomeric and rDNA silencing defect	Y and <i>n.d.</i> (<i>ulp2Δ</i>) (<i>S.c.</i>)	Y (<i>S.c.</i>) [73]
		Role in telomere maintenance	Y (<i>ulp1Δ160</i>)	Y (<i>S.c.</i>) [59,98]
	Meiotic defects	Sporulation defect	Y (<i>ulp2Δ</i>) (<i>S.c.</i>)	Y (<i>S.c.</i>) [10,92]
NIH-PA Author Manuscript	Cell cycle progression	Growth reduced with G2/M arrest	Y (<i>ulp1^{ts}</i> , <i>ulp1I615N</i> , <i>ulp1ΔES</i>) (<i>S.c.</i>)	Y (<i>S.c.</i> , <i>S.p.</i>) [11,95,97, 99]
		Checkpoint constitutively activated	<i>n.d.</i>	Y (<i>S.c.</i>) [28]
		Impaired checkpoint recovery/adaptation	Y (<i>S.c.</i>)	<i>n.d.</i> [92,94]
	Key genetic interactions	Suppressor of <i>mot1^{ts}</i>	Y (<i>ulp1</i> , <i>ulp2</i>) (<i>S.c.</i>)	Y (<i>S.c.</i>) [15]
		High-copy suppressor of Smc2-6	Y (<i>ULP2</i>) (<i>S.c.</i>)	N (<i>S.c.</i>) [93]
		High-copy suppressor of <i>pds5-1</i>	Y	N [100]
		Synthetic lethal with <i>srs2Δ</i>	Y (<i>ulp1I615N</i>) (<i>S.c.</i>)	N (<i>S.c.</i>) [95,101]
		Synthetic lethal with <i>sgs1Δ/rqh1Δ</i>	N (<i>S.c.</i>)	Y (<i>S.c.</i> , <i>S.p.</i>) [10,11]
		Synthetic lethal with <i>mus81Δ/eme1Δ</i>	N	N (<i>S.c.</i>) Y (<i>S.p.</i>) [11,28]

		<i>ulp2Δ (Ulp1 mut)</i>	STUbL ^{ts} /Δ	References
NIH-PA Author Manuscript		Synthetic lethal/sick with recombination mutants <i>rad51/52/54/55/50Δ</i> or <i>mre11Δ</i>	Y (<i>S.p.</i> : <i>rad51/55Δ</i> and n.d.; <i>S.c.</i> ¹)	[11,48,95, 101]
		Synthetic sick with <i>sir2/4Δ</i>	Y (<i>ulp2Δ</i>) (<i>S.c.</i>)	[73]
	Other	Microtubule destabilizing agent sensitivity	Y (<i>ulp2Δ</i>) (<i>S.c.</i>)	[92] & our unpublished results
		Aberrant mitotic spindles	Y (<i>ulp2Δ</i>) (<i>S.c.</i>)	[92]
		Temperature sensitivity	Y(<i>ulp2Δ</i> , <i>ulp11615N</i>) (<i>S.c.</i>)	[92,95]
	Localization	Nuclear	Y Nuclear (Ulp2) and Nuclear pores (Ulp1) (<i>S.c.</i>)	Y (<i>S.c.</i> , <i>S.p.</i>) Slx8 foci formation during S/G2 phase, colocalizing with PCNA and nucleolar protein Not1 (<i>S.c.</i>)

¹Burgess *et al.* reported suppression of the nibbled growth phenotype in *slx8Δ* cells by deletion of homologous recombination enzymes Rad50, 52, 59 and Mre11 [27]. (*S.c.*), *S. cerevisiae*; (*S.p.*), *S. pombe*; n.a., not applicable; n.d., not done; Y, yes; N, no.