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Advanced article

Article Contents

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Eukaryotic cells utilise the dynamic addition and removal of SUMO, a small ubiquitin-like modifier (UBL), to modulate protein functions, interactions and localisation. Protein SUMOylation involves a cascade of dedicated enzymes that facilitate the covalent modification of specific lysine residues on target proteins with monomers or polymers of SUMO. The cellular homeostasis of SUMOylated proteins is also regulated by SUMO proteases and SUMO-targeted ubiquitin ligase (STUbLs). SUMO proteases cleave SUMO from modified proteins. In contrast, STUbLs ubiquitinate proteins modified with SUMO chains. Recent data suggests that ubiquitination via STUbLs effects the turnover of SUMOylated proteins as well as the spatio-temporal composition of complexes that contain SUMO-modified proteins. Defects in the controlled addition, removal and turnover of SUMO-modified proteins greatly affect cellular fitness and contribute to developmental defects, cancer and protein aggregation disorders.

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Introduction

The functional spectrum of any organism's proteome is vastly increased by post-translational modification of individual gene products. A single protein may be subject to modification with small functional groups (e.g. phosphate, acetate, methyl), large molecules (e.g. sugars and lipids) or entire proteins (e.g. ubiquitin, SUMO (small ubiquitin-like modifier), and Nedd8). The first post-translation modifier protein, ubiquitin, was discovered in 1975 as a lymphocyte differentiation factor, and by the 1980s it was established that ubiquitin can be covalently linked to other proteins in an adenosine triphosphate (ATP)-dependent manner and plays an important role in protein turnover and many other cellular processes (Varshavsky, 2006). It took another 15 years before a SUMO was identified. SUMO was initially cloned as a suppressor of a Mif2 centromere mutant (Smt3) in yeast and showed considerable homology (18%) to ubiquitin. By 1996, several groups had identified proteins that either interacted or were covalently modified with mammalian SUMO (reviewed in Wilson, 2009).

Components of the protein cascade involved in ubiquitylation had been worked out before SUMO was discovered. After processing, the di-glycine carboxy terminal end of ubiquitin forms a covalent thioester bond intermediate with an ubiquitin-E1 activating enzyme in an ATP-dependent reaction. Next, ubiquitin is transferred to one of several E2 conjugating enzymes and then to a protein target, either directly from the E2 or in a process facilitated by E3 ligases (for review of relevant references, see Kerscher *et al*. (2006). Modification of proteins with SUMO and its variants was found to follow an analogous process, albeit with

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SUMO-specific E1, E2 and E3 enzymes and SUMO-specific proteases (see details below).

What is the role that SUMO plays in the cell? Exhaustive mass spectrometric analyses have revealed that hundreds (if not thousands) of proteins are SUMOylated, and at this point it is difficult to name a cellular pathway that is not somehow affected by SUMOylation. Nevertheless, deducing the role SUMO modification imparts on a particular protein remains surprisingly difficult. To date, SUMO modification and de-modification of cellular proteins have been shown to play a regulatory role in processes including nuclear transport, signal transduction, stress response and cell-cycle progression, just to name a few (reviewed in Varshavsky, 2006; Wilson, 2009; Kerscher *et al*., 2006; Jentsch and Psakhye, 2013; Hickey *et al*., 2012). SUMO also plays a role in protein homeostasis but, unlike poly-ubiquitinated proteins, poly-SUMOylated proteins are not directly targeted to the proteasome for degradation. Therefore, one of the most exciting findings of late is that a specific class of SUMO-targeted ubiquitin ligases (STUbLs) ubiquitinate SUMOylated proteins to be degraded or disassembled, providing a new paradigm for the versatility of the SUMO and ubiquitin modification system and the cross-talk between different protein modifiers (Sriramachandran and Dohmen, 2014). Several recent examples of how SUMO and STUbLs regulate SUMO homeostasis, protein quality control, protein aggregation and DNA (deoxyribonucleic acid) damage repair are explained in detail below.

SUMO Variants

Mammalian cells encode three highly conserved paralogs of SUMO (SUMO1, SUMO2 and SUMO3), while yeast and several other small eukaryotes only encode one. A fourth mammalian paralogue, SUMO4, is encoded in the human genome but its role remains enigmatic. SUMO2 and SUMO3 can form polymeric chains and are so similar (95%) that they are often detected by the same antibody and hence reported as SUMO2/3. SUMO1, in contrast, shares only about 50% identity with the other SUMO isoforms, lacks internal lysines for conjugation and forms polymeric chains inefficiently. Therefore, SUMO1 mono-SUMOylates and its substrates may also act as a chain terminator for polymeric chains of SUMO2/3. Budding yeast SUMO, Smt3, is most similar to SUMO1 (50%) but carries internal lysines, which allow it to form chains. Smt3 and its conjugates are enriched in the nucleus and, outside of the nucleus, on septin proteins that form a ring at the bud neck of dividing cells (Elmore *et al*., 2011 and references therein). The sub-cellular localisation of vertebrate SUMO1 and SUMO2/3 differs because of the protein targets to which they are conjugated and is to some extent cell-type-specific. For example, SUMO1 resides in in the nucleus, in distinct nuclear foci, and is enriched at the nuclear pore complex because it modifies the nuclear transport factor RanGAP1. SUMOylation of RanGAP1 promotes its association with the nuclear pore complex protein RanBP2 to form a multi-subunit SUMO E3 ligase (Werner *et al*., 2012). In contrast, SUMO2/3 are also enriched in the nucleus and nuclear bodies but also associate with mitotic chromosomes because they modify topisomerase-2 and other chromosomal proteins. Unless otherwise specified, gene and protein names in the following discussion refer to those of budding yeast *Saccharomyces cerevisiae*.

The SUMOylation Machinery

The mechanism of SUMOylation parallels the process of ubiquitination but employs a cascade of SUMO-specific enzymes that ultimately facilitate the conjugation of SUMO to its protein target through an isopeptide bond (Figure **1b**). The E1 and E2 enzymes of this SUMO modification cascade were identified because they both bound to a yeast SUMO affinity column. The E1 enzyme consists of Uba2 and Aos1, two proteins that are similar to each other and also to Uba1, the monomeric ubiquitin E1 enzyme (Johnson *et al*., 1997). Subsequently, the SUMO E2 conjugating enzyme Ubc9 was identified because it bound a column containing the SUMO/Aos1/Uba2 complex (Johnson *et al*., 1997). Purified Ubc9 is able to form a thioester bond with SUMO but not ubiquitin, and combining Ubc9, SUMO, ATP and cell extracts resulted in the formation of high molecular weight SUMO conjugates. Mammalian paralogues of SUMO E1 and E2 enzymes are called Sae1/Sae2 and Ubc9, respectively, and are involved in the conjugation of all three SUMO isoforms described above.

Protein SUMOylation and Consensus Sites

Proteins are SUMOylated in an ATP-dependent process that involves the formation of a thioester bond between Uba2 and conjugation-competent SUMO (reviewed in Kerscher *et al*., 2006 and see Figure **1**). Subsequently, Ubc9 binds to Aos1/Uba2 and the thioester bond is transferred to Ubc9. In the final step, SUMO-charged Ubc9 binds a canonical SUMOylation consensus motif and catalyses the formation of an isopeptide bond between SUMO and the substrate protein (Figure **1b**). The SUMOylation consensus motifs can be represented as ψKxE/D, with ψ being a large hydrophobic amino acid and x any residue. SUMOylation consensus motifs are present in many proteins and conveniently identified using programs such as SUMOplot and others (http://sumosp.biocuckoo.org, http://www.abgent.com/sumoplot). However, predicted SUMOylation sites must be confirmed experimentally, which is often a difficult undertaking because of the SUMOylation of non-consensus lysines and the often transient (or limited) modification with SUMO. In the end, only about half of all SUMOylated proteins expressed in the cell contain a recognisable SUMOylation motif and require alternative means for efficient modification (Wilson, 2009).

SUMO E3 Ligases

SUMOylation via Aos1/Uba2 and Ubc9 alone is inefficient and, to enhance SUMOylation, all eukaryotic cells utilise SUMO-specific E3 enzymes. SUMO E3 ligases either stimulate Ubc9-mediated SUMOylation or facilitate the interaction of Ubc9 with its substrates including those that lack SUMOylation

consensus sites. Most SUMO E3 ligases are conserved members of the Siz/PIAS-type family (protein inhibitor of activated STAT) and interact with Ubc9 by means of a hallmark Siz1/PIAS RING domain (SP-RING). Yeast cells lacking Siz1 and Siz2 are greatly reduced in SUMOylated proteins, underlining the importance of SUMO E3 ligases for overall SUMOylation. There is strong evidence that Siz/PIAS-type SUMO E3 ligases play important roles in cellular processes, which ensure proper chromosome segregation and genome maintenance (Nie and Boddy, 2015 and references therein). Two additional SUMO ligases in yeast, Mms21 and ZIP3, also contain SP-RINGs but lack conserved domains for DNA-binding (SAP) and nuclear retention (PINIT) that are present in the other Siz/PIAS-type enzymes (reviewed by Jentsch and Psakhye, 2013). Mms21 and ZIP3 are required for recombinational DNA repair and synapse formation, respectively.

SUMO E3 Ligases have Unique and Overlapping Substrates

Inside the nucleus, Siz1 and Siz2 cooperate in pathways that suppress excessive homologous recombination and prevent gross chromosomal rearrangements. Specifically, Siz1 and Siz2 can both SUMOylate the DNA polymerase processivity factor PCNA to recruit the helicase Srs2 and restrict inappropriate recombination (Burkovics *et al*., 2013 and references therein). Furthermore, Siz1 and Siz2 also regulate chromosome segregation via SUMOylation of the kinetochore protein Ndc10 and transcriptional regulation via modification of histone H2B and H4 (Montpetit *et al*., 2006; Nathan *et al*., 2006). However, there are also several examples which reveal that the functions of Siz1 and Siz2 are not entirely redundant. For example, Siz1 is the only SUMO E3 ligase that is transported out of the nucleus to SUMOylate septins, which form a ring-shaped scaffold at the bud neck of dividing cells (Westerbeck *et al*., 2013 and references therein). SUMOylated septins are believed to recruit important cell-cycle regulatory proteins to the bud neck and also play a role in septin ring disassembly. One of the functions uniquely attributed to Siz2 is the anchoring of telomeres to the nuclear envelope (NE). Only deletion of Siz2, but not Siz1 or Mms21, resulted in the loss of telomeres from the nuclear periphery (see references in Further Readings section). Several proteomics studies using mass spectrometry have helped to clarify the unique and overlapping targets of the various SUMO E3 ligases in yeast (Albuquerque *et al*., 2013; Srikumar *et al*., 2013). Probably the most recent addition among the SUMO ligases is Wss1. Wss1 belongs to a new family of metalloproteases that also possess SUMO ligase activity and is involved in resolving DNA repair complexes. A recent study by Balakirev *et al*. (2015) shows that the SUMO ligase activity of Wss1 enhances SUMO chains on protein complexes associated with DNA breaks and this activates the protease activity of Wss1. Since Wss1 also associates with Cdc48, a protein complex disassemblase that also interacts with STUbLs, it is ideally suited to extract SUMOylated proteins from chromatin, especially when DNA–protein cross-links are present.

There are a handful of mammalian SUMO E3 ligases with SP-RINGs, including PIAS1, PIAS3, PIASx and its splice variants alpha and beta, PIASy and the Mms21 homologue Nse2. In addition, mammalian cells express SUMO ligases such as RanBP2, an NPC-associated protein involved in nucleocytoplasmic trafficking and chromosome segregation, and several CBX proteins that are related to the polycomb protein Pc in *Drosophila melanogaster* and play a role in transcriptional repression (reviewed in Wang and Dasso, 2009). Both RanBP2 and CBX proteins do not contain an SP-RING and use mechanisms distinct from PIAS-type enzymes for SUMO ligation. Proteins of the SprT family that are similar to yeast Wss1 play a role in protecting stalled replication forks in mammalian cells. However, as yet there is no evidence that they possess the ability to form SUMO chains or exhibit protease activity (Balakirev *et al*., 2015).

As the name indicates, PIAS proteins were originally identified due to their ability to bind and modulate the activity of STAT transcription factors. Indeed, PIAS proteins bind and even SUMOylate STATs but this modification may not play a role in transcriptional repression. On the other hand, PIAS1 and PIAS4 are critically important for double-strand breaks (DSB)-repair signalling involving SUMO and ubiquitin at sites of DSB repair (Galanty *et al*., 2009). Briefly, SUMOylation by PIAS1 and PIAS4 leads to recruitment of the STUbL RNF4 (discussed below) and promotes DSB repair by remodelling complexes that accumulate at DSB repairs (Galanty *et al*., 2012). Another recent example underscores the function of PIAS proteins in cancer-related signalling pathways and involves PIASxα. PTEN, a tumor suppressor and negative regulator of the PI3K-Akt signalling pathway, was shown to be SUMOylated by PIASxα; when two SUMO-acceptor lysines on PTEN were mutated, the protein was ubiquitinated and degraded. Correlatively, overexpression of PIASxα in nude mice reduced tumor size, but only when PTEN was also expressed. These findings suggest that SUMO E3 ligases may be considered tumor suppressors (Wang *et al*., 2014).

Dual SUMO and Ubiquitin Ligase

Interestingly, some mammalian E3 ligases are reported to have dual SUMO and ubiquitin ligase activities. These include topoisomerase I binding, arginine/serine-rich E3 ligases (TOPORS) that SUMOylate p53, TNF receptor-associated factor 7 (TRAF7) involved in SUMOylation of c-Myb, the tripartite motif containing 27 (TRIM27) that SUMOylates Mdm2 and the ubiquitin E3 ligase UHRF2 that SUMOylates ZNF131 (Oh and Chung, 2013). It is telling that mutating the RING domain in these proteins ablates either the SUMO ligase activity (TRIM27) or the ubiquitin ligase activity (TOPORS), but not both. Therefore, it is apparent that distinct mechanisms for SUMO and ubiquitin ligation are at work.

DeSUMOylation

Protein SUMOylation is reversible, and SUMO conjugates can be cleaved by SUMO-specific proteases. The first SUMO-specific

protease, Ulp1p, was identified in a biochemical screen for Smt3/SUMO-cleaving enzymes among yeast proteins expressed in *Escherichia coli* (Hochstrasser and Li, 1999). Subsequently, a second SUMO protease, Ulp2p/Smt4p, was identified as a SUMO-cleaving enzyme because of its homology to Ulp1p's cysteine protease (Li and Hochstrasser, 2000). The two yeast SUMO proteases, Ulp1 and Ulp2, contain a conserved cysteine protease domain that can remove the SUMO moiety from modified proteins.

Specifically, Ulp2 plays a role in the removal of SUMO and SUMO chains from nuclear proteins. Ulp1, as mentioned above, has two contrasting cellular functions. Ulp1 first facilitates SUMOylation by processing precursor SUMO into its conjugation-competent form. Additionally, Ulp1 also facilitates deSUMOylation by removing SUMO from nuclear and cytosolic proteins after conjugation. Therefore, impairment of Ulp1 results both in the accumulation of SUMO conjugates and the inability to carry out de novo SUMOylation. The resulting lack of mature SUMO has been shown to adversely affect cellular DNA repair processes, the processing and export of the 60S pre-ribosomal particle, nucleus–cytoplasm trafficking and cell viability (reviewed in Hickey *et al*., 2012). The best evidence that Ulp1 plays a unique role in cell cycle progression comes from a temperature-sensitive ulp1 mutant (ulp1ts), which arrests in the G2/M phase of the cell cycle. This mutant accumulates cell-cycle-stage-specific SUMO conjugates at permissive and non-permissive temperatures. However, expression of mature SUMO in the ulp1ts mutant neither suppresses the temperature-sensitive phenotype nor the lethality of a ULP1 deletion (Hochstrasser and Li, 1999). This finding suggests that the lethality of Ulp1 mutants may be due to the failure to remove SUMO from important cell-cycle regulators. The proteins that Ulp1 must deSUMOylate to ensure proper cell cycle progression have not yet been identified, but a recent study in fission yeast suggests that the SUMO E3 ligase Siz1/Pli1 may be one of them (Nie *et al*., 2012).

The SUMO proteases Ulp1 and Ulp2 are exquisitely conserved and have been found in yeasts, flies, fish, mice and humans. The catalytic domain of Ulp1 is most similar to mammalian SENP1 and SENP2, while Ulp2 is more similar to SENP6 and SENP7. SENP proteins differ in intracellular localisation, SUMO isotype preference, ability to process SUMO precursors and substrate specificity. Functionally, the different SENPs are involved in various processes such as ribosome biogenesis (SENP3 and SENP5), kinetochore assembly (SENP6), transcriptional regulation (SENP1 and SENP2), cell division and mitochondrial inheritance (SENP5), just to name a few (Hickey *et al*., 2012; Wang and Dasso, 2009). Clinically, SUMO homeostasis has been linked to cancer progression (prostate, liver, colon and adenocarcinoma) and in at least a few of these cases the alteration in SUMO homeostasis has been linked to the dysregulation of the SUMO protease SENP1 (Zhang *et al*., 2013; Wang *et al*., 2013). It remains unknown how many SENP proteins are involved in disease progression and exactly which SUMOylated substrates are involved. It is, however, clear that SUMO and SUMO pathway proteins have taken a place as important biomarkers in research and disease diagnosis.

How do SUMO Proteases Target their SUMOylated Substrates?

The substrate specificity of SUMO proteases is, at least in part, regulated through their localisation. For example, yeast Ulp2 and vertebrate SENP6 and SENP7 SUMO proteases localise within the nucleus. In contrast, both Ulp1 and vertebrate SENP1 and SENP2 are enriched at the NE through their interactions with the nuclear pore complex (Elmore *et al*., 2011 and references therein). The sub-cellular localisation of SUMO proteases appears to be regulated by their amino-terminal extensions, limiting potential substrates that are accessible to SUMO proteases. Unfortunately, there is still limited information about how SUMO proteases target their respective nuclear and cytosolic substrates *in vivo* (Elmore *et al*., 2011). One possibility is that SUMO proteases may contain structural features that allow for non-covalent interactions with SUMO and SUMO-modified proteins as they enter the nucleus. Indeed, conserved SUMO-interacting motifs (SIMs) (detailed below), have been predicted in the non-catalytic domains of yeast SUMO protease Ulp2, as well as mammalian SENP1,2,6,7 and DESI, a recently discovered new SUMO protease (reviewed in Hickey *et al*., 2012; Jentsch and Psakhye, 2013). However, the functional relevance of these predicted SIMs is unclear. For example, mutating SIMs in the carboxy-terminal domain of Ulp2 caused only a mild accumulation of poly-SUMO chains, while the ability to bind SUMO chains *in vitro* was not affected (Hickey *et al*., 2012 and references therein). SIMs have also not been experimentally confirmed in Ulp1; rather, the crystal structure of the catalytic domain bound to yeast SUMO reveals that both proteins interact through multiple residues that are distributed across a SUMO-binding surface and form salt bridges with SUMO (Mossessova and Lima, 2000). Only the carboxy terminus of bound SUMO is inserted into a hydrophobic tunnel that leads towards Ulp1's active site. SUMO processing and de-conjugation require an active-site cysteine residue that resides at the end of this tunnel. It has been suggested that this configuration is conserved in the SENPs and may allow for the accommodation of many different SUMOylated proteins, as well as unprocessed SUMO precursors (Mossessova and Lima, 2000). If the catalytic cysteine of some SUMO proteases, for example, Ulp1 and SENP1, is replaced with a non-catalytic serine or alanine, these proteins trap their SUMOylated substrates. A substrate-trapping Ulp1 mutant has been exploited to study how this SUMO protease is targeted to septins and other SUMOylated targets *in vivo* and *in vitro* (Elmore *et al*., 2011).

SUMO as a Building Block for Macromolecular Assemblies

Our understanding of SUMO biology and function has been significantly advanced by the discovery of proteins and protein domains that contain SIMs, which interact non-covalently with SUMO. A canonical SIM sequence consists of a hydrophobic core (e.g. V/I-X-V/I-V/I) that is often juxtaposed with a stretch of acidic and/or phosphorylated amino acids (Kerscher *et al*., 2006). Only one type of SIM has been identified for SUMO with

additional reports of specific zinc finger proteins being able to bind SUMO (Guzzo *et al*., 2014). In contrast, there are close to 20 structurally diverse families of ubiquitin-binding domains (UBDs) for ubiquitin (Rahighi and Dikic, 2012). Additional SUMO-binding and SIM-like domains are predicted to exist but have yet to be found or confirmed. Proteins that otherwise lack sufficient affinity for each other may interact via SUMO and SIMs. This can be useful for subcellular targeting and the formation of large protein complexes, for example, during the biogenesis of nuclear bodies (e.g. PML bodies) (Kerscher, 2007). Additionally, some proteins contain both SIMs and ubiquitin-interacting motifs, and it is believed that these proteins may be able to interact with hybrid SUMO–ubiquitin chains formed by STUbLs. For example, hybrid SUMO–ubiquitin chains formed by the STUbLs Slx5/Slx8 and RNF4 are recognised by yeast Ufd1 (the substrate-recruiting cofactor of the Cdc48p-Npl4p-Ufd1p complex) and mammalian Rap80 (a ubiquitin-interacting motif containing protein with a role in the DNA damage response), respectively. These hybrid SUMO–ubiquitin chains represent SUMO-dependent signals that have been shown to orchestrate DNA repair functions (Guzzo and Matunis, 2013; Nie *et al*., 2012b).

STUbLs

STUbLs, the first functionally identified in yeasts, have given credence to a proteolytic role of SUMO. STUbLs are ubiquitin E3 ligases that can specifically target and bind SUMO chains or proteins modified with SUMO chains and facilitate their ubiquitination (Figures **2** and **3**). Members of this unusual family of ubiquitin ligases are well conserved, contain a RING domain required for their ubiquitylation activity and use multiple SIMs to target SUMOylated substrates. There are at least four STUbL proteins in the budding yeast *S. cerevisiae* (Slx5, Slx8, Uls1/Ris1 and Rad18), three in fission yeast S. pombe (Rfp1, Rfp2, and Slx8) and at least two in multicellular eukaryotes including humans (RNF4 and Arcadia/RNF111) (Sriramachandran and Dohmen, 2014). Budding yeast Slx5 and Slx8 form a STUbL complex, which plays an important role in the DNA damage response, genome maintenance and the proteasome-mediated degradation of specific transcriptional regulators. Included in a growing list of experimentally confirmed ubiquitylation targets of Slx5/Slx8 are the transcriptional regulators Mot1 and Matalpha2 (*in vivo*), the homologous recombination protein Rad52 (*in vitro*) and SUMOylated Siz1 (*in vitro* and *in vivo*). In contrast, Uls1/Ris1 plays a role in counteracting replicative stress and telomere maintenance. Two interesting targets of the Uls1 STUbL are SUMOylated Rap1, a transcriptional regulator that has been implicated in preventing telomere fusions, and the cytosolic nuclear migration protein Pac1 that targets dynein to the ends of microtubules. Rad18, a ubiquitin ligase that is stimulated by SUMOylation of its substrate, the sliding clamp protein PCNA, is likely the newest member of a growing family of STUbLs in yeast and beyond (STUbLs and STUbL substrates are excellently reviewed in Sriramachandran and Dohmen, 2014).

STUbL-mediated ubiquitylation does not always result in the immediate proteasome-mediated degradation of SUMOylated target proteins. For example, there is now good evidence that Cdc48-Ufd1-Npl4, an ATPase complex that dislodges proteins from chromatin, is targeted to SUMO and ubiquitin co-modified substrates to protect cells from genome instability (Nie *et al*., 2012b; Bergink *et al*., 2013). It is, therefore, likely that STUbLs and Cdc48-Ufd1-Npl4 cooperate to disrupt DNA-associated protein complexes and target them for subsequent degradation. Taken together with the observation that the Wss1 SUMO-activated protease also interacts with Cdc48, a theme emerges as to how SUMOylation can lead to the remodelling and disassembly of protein complexes.

Regulation of SUMO Pathway Components by STUbLs

SUMOylation is a highly dynamic process, and SUMOylation patterns in the cell change rapidly in response to cell-cycle stage or cell stress. Several interesting studies have emerged that detail how SUMO pathway components are regulated to control SUMO homeostasis in the cell. First, oxidative stress rapidly (and reversibly) disables SUMO E1 (Uba2) and E2 (Ubc9) enzymes via the formation of a disulfide bond between their catalytic cysteines (Bossis and Melchior, 2006). Second, in yeast, increased auto-SUMOylation of the SUMO conjugating enzyme Ubc9 was found to reduce its ability to SUMOylate septins in mitosis (Ho *et al*., 2011). Third, several human SENPs (1,2,3,6,7) are rapidly disabled by heat stress, thus allowing for the rapid accumulation of SUMO conjugates and SUMO chains when cells are subjected to heat stress (Pinto *et al*., 2012).

More recently, two studies provide a glimpse into the role that auto-SUMOylation plays in the regulation of SUMO E3 ligases. Westerbeck *et al*. (2013) identified Siz1 as an interactor with the STUbL subunit Slx5 and found that deletion of Slx5 increases the steady-state level of SUMOylated Siz1. Normally, Siz1 is exported from the nucleus via the karyopherin Msn5 to SUMOylate bud-neck-localised septins. However, the authors of this study found that, if its nuclear export in mitosis is prevented by deletion of Msn5, auto-SUMOylated Siz1 accumulates and is rapidly degraded by the Slx5/Slx8 STUbL (Figure **4**). A similar observation was made by Nie *et al*. (2012a), who found that levels of the SUMOylated Siz1 orthologue Pli1 in fission yeast is rapidly degraded in a nup132Δ mutant leading to its STUbL-dependent degradation. The authors found that SUMOylated Pli1 in the nup132Δ cells increased because the levels and activity of the SUMO protease Ulp1 were reduced in this mutant. In summary, both findings suggest that STUbLs play an important role in the cell-cycle-specific degradation of the SUMOylated Siz1/Pli E3 ligase and this raises the possibility that other SUMO-E3 ligases (e.g. Siz2 and Mms21, PIAS) and SUMOylated Ubc9 are regulated in a similar manner.

STUbLs have their RING Fingers in DNA Repair

STUbLs, reviewed above, are also intricately involved in DSB-repair-related processes. For example, yeast cells harboring

a deletion of SLX5 or SLX8 are hyper-sensitive to genotoxic insults. Furthermore, cells lacking these STUbLs accumulate gross chromosomal rearrangements, show enhanced foci of the DNA repair protein Rad52, associate with dsDNA breaks and ubiquitinate Rad52 *in vitro*. Attesting to the high degree of structural and functional conservation of STUbLs, RNF4 can suppress a variety of DNA damage phenotypes associated with deletions of SLX8 and SLX5, or both (Mullen *et al*., 2011). One important aspect of the initial characterisation of human RNF4 focused on its interaction with PML, a tumour suppressor and main constituent of PML nuclear bodies that plays a critical role in the response to genotoxic insults (reviewed in Reineke and Kao, 2009). RNF4 can be recruited to PML bodies and this recruitment is enhanced by SUMO, leading under some conditions to the degradation of the PML protein. As detailed below, our functional understanding of the relevance of the interaction between PML and RNF4 is still far from complete.

Recently, several studies have focused on the question of how RNF4 plays into the choreography of DSB repair. These studies show that RNF4 is involved in recruiting and remodelling proteins at sites of dsDNA breaks (Luo *et al*., 2012; Galanty *et al*., 2012; Yin *et al*., 2012). Using laser micro-irradiation assays, it was shown that RNF4 visibly accumulates at dsDNA breaks within 15 min of DNA damage and that its SIMs are involved in this localisation (Yin *et al*., 2012; Luo *et al*., 2012). RNF4 appears to be recruited to the dsDNA break site because of SUMOylated Mdc1. Mdc1, a DNA damage checkpoint mediator protein, is recruited early to the dsDNA break site and is then SUMOylated by PIAS1/4. The authors of two studies also show that recruitment of 53BP1, a DNA damage response factor that recognises altered chromatin at dsDNA breaks, is dependent on RNF4. As the dsDNA break repair response unfolds, Mdc1 and 53BP1 disappear as Rad51 accumulates on single-stranded resected DNA. This exchange for Rad51 requires the removal of RPA1 that coats single-stranded DNA at resected DNA breaks. However, this exchange does not take place when RNF4 is depleted or the SUMO-dependent interaction with Mdc1 (and RPA1) is prevented. One simplified model is, then, that these early responders (especially SUMOylated Mdc1 and RPA1) must be removed from dsDNA breaks via RNF4-mediated ubiquitination so that Rad51-mediated homologous recombination can take place. Finally, RNF4 is also involved in recruiting the 19S proteasome regulator subunit PSMD4 to dsDNA breaks, linking RNF4s function in remodelling the dsDNA break site to protein turnover (Luo *et al*., 2012). The model painted by these studies is by no means complete, and there remains the pressing question of why several additional ubiquitin ligases, such as RNF8, RNF168 and BRCA1, can also be found at dsDNA break sites and whether they cooperate with RNF4. One possible answer may be found in the observation that ligases can produce distinct kinds of ubiquitin chains. For example, RNF4 produces hybrid SUMO–ubiquitin chains (K-63-linked ubiquitin chains on SUMO) that are recognised by the adapter protein Rap80 and help to recruit BRCA1, which is known to form ubiquitin chains linked via lysine 6 on ubiquitin (Guzzo *et al*., 2012). The various SUMO and ubiquitin chains therefore have the potential to signal the processing stage of dsDNA breaks, ensuring that the appropriate DNA repair factors are recruited, assembled, removed or degraded at the appropriate time (Figure **5**). See also: **DNA Strand Break Repair and Human Genetic Disease** a0021478

RNF4 Takes on Cystic Fibrosis

The observation that Slx5/Slx5 plays a role in the proteolytic turnover of a mutant transcriptional regulator in yeast, Mot1-103, provided the first indication that STUbLs are involved in protein quality control (Wang and Prelich, 2009). Recent work by Ahner *et al*. (2013) extends on this finding by showing that a mutant form of the cystic fibrosis transmembrane conductance regulator (CFTR), the protein responsible for the debilitating respiratory disease cystic fibrosis, is regulated by SUMO and RNF4. One mutation in CFTR that results in cystic fibrosis is due to the loss of a phenylalanine residue at position 508 of the CFTR protein (CFTRΔF508), which prevents the protein from leaving the endoplasmic reticulum (ER) to assume its normal function in transporting chloride ions across the plasma membrane. Ahner *et al*. determined that overexpression of a small heat shock protein, Hsp27, resulted in the increased degradation of CFTRΔF508 but not wild-type CFTR in a cell culture model. Since Hsp27 is predicted to interact with Ubc9, the authors investigated whether SUMOylation was involved in Hsp27-mediated degradation of CTFR. Indeed, overexpression of Ubc9 resulted in reduced steady-state levels of CFTRΔF508 while having no effect on wild-type CTFR. Correspondingly, overexpression of Senp1 (a SUMO protease) increased the levels of both CFTRΔF508 and wild-type CTFR. *In vitro*, CFTRΔF508, but not wild-type CTFR, was SUMOylated by Ubc9. This increased SUMOylation was correlated to the association of Hsp27 with CFTRΔF508, resulting in the increased recruitment of Ubc9. In addition, the authors propose that the misfolding caused by the mutation exposes additional SUMOylation sites, an interesting hypothesis that remains to be tested.

Although the effect of SUMOylation on the CFTRΔF508 mutant is interesting in itself, it did not explain the degradation of CTFR. Therefore, the authors examined RNF4 as a potential ubiquitin ligase for SUMOylated CTFR. Overexpression of RNF4, but not a dominant-negative RNF4 RING mutant, preferentially reduced CFTRΔF508 levels.

In summary, this work provides important evidence for the critical role of SUMO and RNF4 in protein quality control functions. In this particular case, RNF4 engages a non-nuclear substrate that is also well-studied in endoplasmic reticulum associated degradation (ERAD). There is currently no evidence that RNF4 leaves the nucleus to interact with its substrates. However, a previous study revealed that an ER membrane-embedded ubiquitin ligase (Doa10) gains access to the inner nuclear membrane to interact with a nuclear substrate (Deng and Hochstrasser, 2006). Therefore, it will be interesting to determine whether the RNF4-mediated ubiquitylation of CFTRΔF508 takes place in the cytosolic compartment as proposed, or at the inner nuclear membrane that is contiguous with the ER. See also: **Degradation of Misfolded Secretory and Membrane Proteins and Associated Diseases** a0022577

STUbLs Take on Aggregation-Prone Proteins

Spinocerebellar ataxia type 1 (SCA1) is an untreatable neurodegenerative disorder resulting a progressive loss of physical control over muscle movements and speech of the patients afflicted. The causative agent behind SCA1 is the expression of a polyglutamine-expanded variant of the Ataxin protein (Atxn1). Atxn1 with less than 35 glutamine residues is considered normal, while variants of Atxn1 with more than 39 are increasingly pathogenic (Pagon *et al*., 1993). Both normal and pathogenic Atxn1 are enriched in the nucleus and co-localise, at least in part, with PML nuclear bodies. However, expression of the pathogenic Atxn1 (e.g. Atxn1 80Q) is believed to interfere with critical nuclear functions, especially transcription, and results in nuclear Atxn1 inclusions that affect the size, distribution and contents of PML nuclear bodies.

Both normal and pathogenic Atxn1 are SUMOylated, and it was found that an increase in poly-Q length negatively affected the levels of SUMO1-modified Atxn1 (Riley *et al*., 2005). More recently, Guo *et al*. (2014) functionally connected these observations. They found that co-overexpression of PML and Atxn1 dramatically reduced the levels of polyglutamine-expanded, aggregated Atxn1 80Q but not soluble Atxn1 80Q or normal Atxn1 30Q, suggesting that PML specifically targets the pathogenic variant for degradation. The authors tested several conditions and found that the effect of PML was negated when proteasome function was inhibited, when SUMO2 was absent or unable to form chains and when a mutant of RNF4 was expressed. In summary, the authors proposed that PML acts as a SUMO E3 ligase that interacts specifically with insoluble Atxn1 80Q, causing its SUMOylation and subsequent RNF4-mediated degradation.

Indeed, has been shown previously that PML can SUMOylate proteins in a RING-dependent manner (Quimby *et al*., 2006). Even though this study primarily focused on Atxn1, it also investigated whether another poly-Q expanded protein, huntingtin Htt 97Q, the causative agent of Huntington's disease, could be targeted by PML and RNF4. Unlike Atxn1, Htt 97Q forms both cytosolic and nuclear aggregates. Overexpression of RNF4 dramatically reduced the steady-state levels of insoluble huntingtin, while PML overexpression was less effective and only affected nuclear Htt aggregates. It is noteworthy that Htt 97Q is also SUMOylated by other SUMO E3 ligases such as PIAS1, as was shown by O'Rourke *et al*. (2013). Ultimately, it appears clear at this point that STUbLs cooperate with several SUMO E3 ligases to manage aggregation-prone proteins in eukaryotic cells.

See also: **Huntington Disease**

a0005150

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Key Concepts

- SUMO encodes a small ubiquitin-like modifier that is covalently attached to lysines in target proteins.
- SUMOylation, the process of SUMO conjugation to a target protein, frequently occurs on a lysine situated in a SUMOylation consensus site.
- SUMO conjugates can be removed from target proteins by SUMO-specific proteases.
- Vertebrates express three different SUMO isoforms: SUMO1, SUMO2 and SUMO3, encoded by three different genes.
- SUMOs can form polymers via internal SUMOylation sites in SUMO2 and SUMO3.
- Several proteins can bind non-covalently to SUMOs via SUMO interaction motifs (SIMs).
- A finely balanced SUMOylation/de-SUMOylation system is required for eukaryotic life.
- Cross-talk between SUMOylation and ubiquitination plays a critical in the turnover of some SUMOylated proteins as well as the spatiotemporal composition of complexes that contain SUMO-modified proteins.

Glossary

Consensus SUMOylation site# A target protein site that is frequently used for SUMO conjugation and that is defined as ψKxE/D, where ψ represents a large hydrophobic amino acid and x can be any amino acid.

E1# The enzyme that activates SUMO for conjugation.

E2# The enzyme that conjugates SUMO to target proteins.

E3# A SUMO ligase (e.g. Siz/PIAS) that catalyses SUMOylation.

Ulp/SENP# A SUMO-specific protease that removes SUMO from target proteins.

SUMO (small ubiquitin-like modifier)# Small proteins belonging to the ubiquitin family that are covalently attached to target proteins in the process referred to as SUMOylation.

STUbL# A SUMO-targeted ubiquitin ligase (e.g. Slx5/Slx8 or RNF4) that uses SUMO-interacting motifs to interact with SUMO chain-modified proteins. RING domains are a hallmark of STUbls and are required for ubiquitylation.

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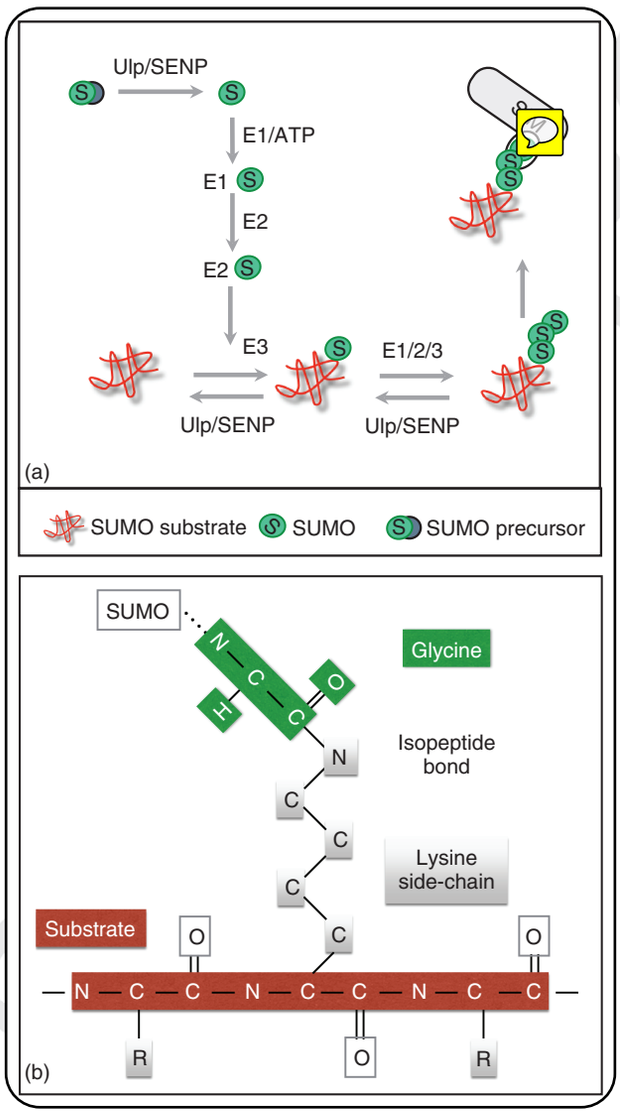

Figure 1 SUMOylation. (a) The SUMO precursor is rendered conjugation-competent by processing through a SUMO protease (Ulp/SENP). In an ATP-dependent reaction, the conjugation-competent SUMO is transferred to an activating enzyme (E1), handed off to an conjugating enzyme (E2) and finally transferred to the substrate in a reaction that frequently requires a SUMO ligase (E3). SUMO chains on substrates are generated by multiple E1/E2/E3 cycles and depend on internal lysines in SUMO. SUMO and SUMO chains can be pruned by SUMO proteases. SUMO chains play an important role in the interaction with other proteins that contain SIMs to interact with SUMOylated proteins. (b) Depiction of an isopeptide bond through a lysine side chain of the substrate and the carboxy-terminal glycine of the processed SUMO protein.

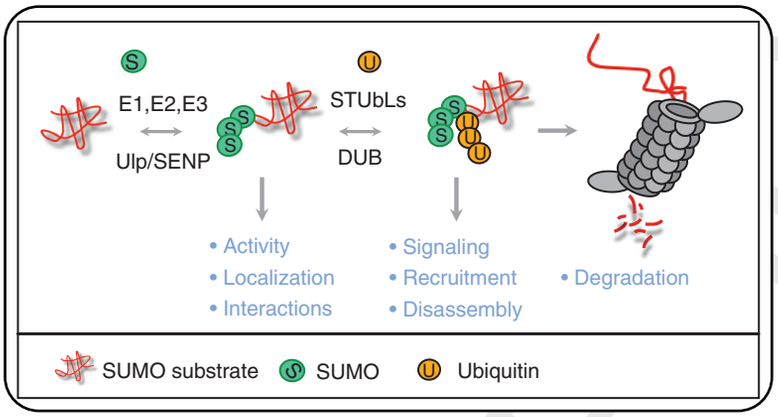

Figure 2 Consequences of SUMOylation. SUMO E1, E2 and E3 enzymes are involved in the covalent modification of proteins with monomeric SUMO or polymeric SUMO chains. SUMOylation can modulate the activity, localisation and interactions of a modified protein. STUbLs target and ubiquitinate proteins modified with SUMO chains, resulting in the formation of hybrid SUMO/ubiquitin chains. These modifications can be reversed by the activity of SUMO proteases (Ulp/SENP) or deubiquitinating enzymes (DUB). Ultimately, hybrid SUMO/ubiquitin chains lead to proteasome-mediated degradation, either directly or after recruitment of other factors that disassemble and remodelling protein complexes.

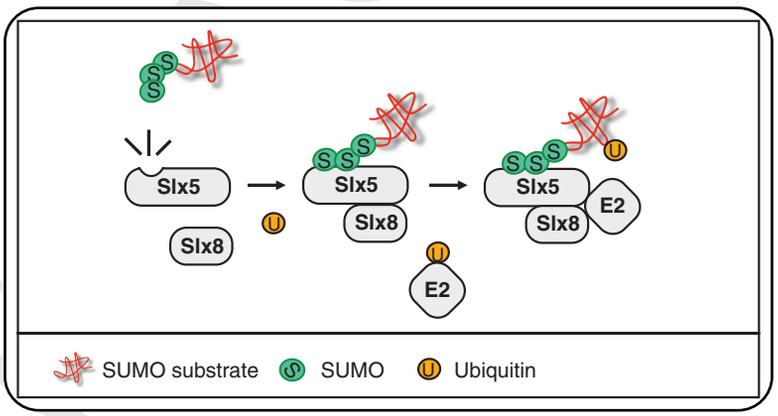

Figure 3 STUbL-mediated ubiquitylation of SUMOylated proteins. Slx5 and Slx8 are RING-domain proteins that heterodimerise to form a STUbL complex (mammalian RNF4 forms a homodimer). Slx5 is the targeting subunit of this complex and contains at least four SIMs (\|/) that facilitate its binding to SUMOylated substrates (red protein with a chain of green SUMO monomers). A ubiquitin E2, Ubc4 or Ubc6, is required to ubiquitylate SUMOylated proteins in a reaction dependent on RING domains in both Slx5 and Slx8.

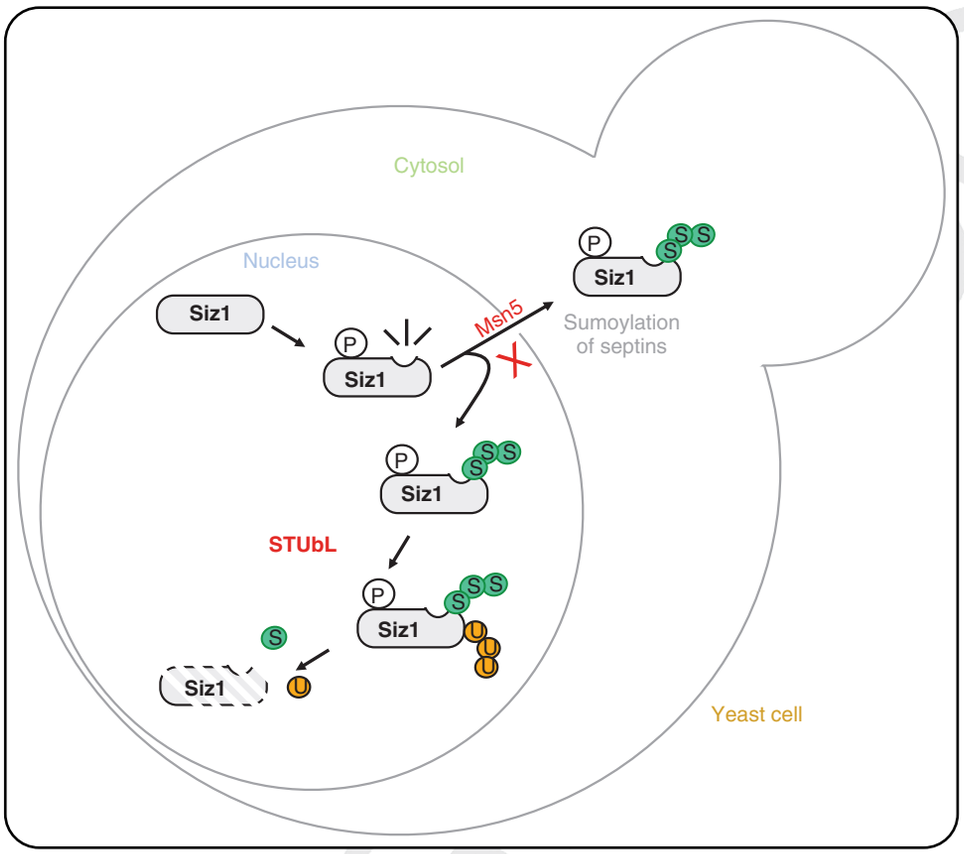

Figure 4 STUbL-mediated degradation of the nuclear pool of SUMOylated Siz1. At the onset of mitosis, the SUMO ligase Siz1 becomes phophorylated (p) via an unknown kinase and exported to the cytosol via the karyopherin Msn5. In *msn5*Δ mutants (x), SUMOylated Siz1 acumulates in the nucleus, becomes ubiquitylated by the STUbL Slx5/Slx8 and is degraded.

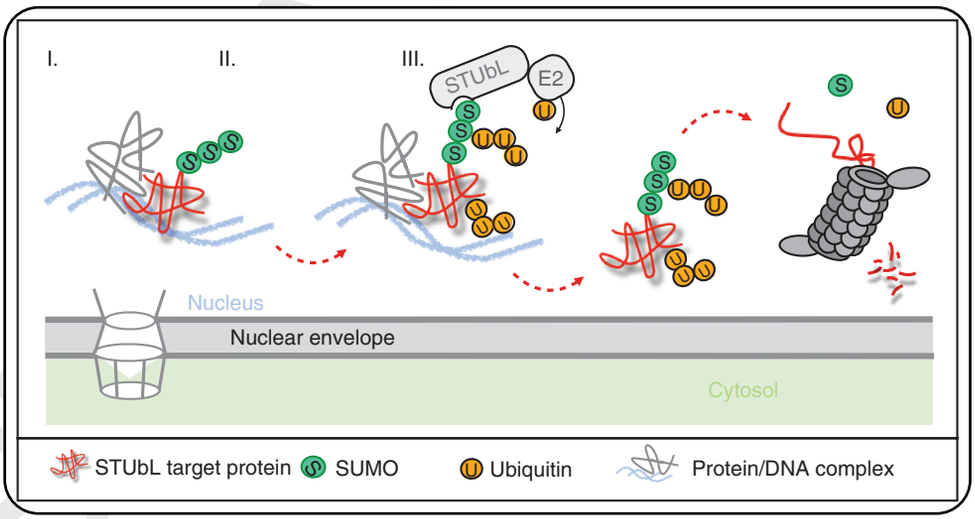

Figure 5 STUBL-mediated rearrangement of a protein complex in the nucleus. (I) SUMOylated proteins can accumulate in the nucleus, possibly as part of a protein/DNA complex. Examples described in this review include SUMOylated Siz1, Ataxin and several DNA repair proteins. (II) STUbls are recruited to proteins modified with SUMO chains resulting in the formation of hybrid SUMO/ubiquitin chains. (III) The formation of hybrid SUMO/ubiquitin chains on the STUbL target proteins may lead to targeting, extraction and disassembly of the associated protein/DNA complex, possibly via the disassemblase Cdc48. The extracted ubiquitylated protein may be subject to proteasome-mediated degradation, while SUMO and ubiquitin chains are cleaved and degraded.

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AU:3	Please check and confirm if the head levels are correctly identified.
AU:4	Please confirm if these abbreviations 'PIAS', 'RING' and 'STAT' need to be spelt out. If yes, please provide the expansions.
AU:5	Please confirm if this abbreviation 'SAP' need to be spelt out. If yes, please provide the expansion.
AU:6	Please confirm if this abbreviation 'PINIT' need to be spelt out. If yes, please provide the expansion.
AU:7	Please confirm if this abbreviation 'PCNA' need to be spelt out. If yes, please provide the expansion.
AU:8	Please provide journal title, volume number and page range for reference Pagon et al. (1993).
AU:9	Please provide publisher name and location for reference Wilson (2009).