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PML, SUMO, and RNF4: Guardians of Nuclear Protein Quality

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In this issue of *Molecular Cell*, Guo et al. (2014) report that misfolded or aggregated nuclear proteins, such as pathogenic polyQ proteins, are cleared by a SUMO-dependent quality control pathway, which involves the E3 SUMO ligase PML and the SUMO-targeted ubiquitin ligase RNF4.

The cellular proteome is continuously exposed to intrinsic and extrinsic hazards that challenge proper folding of proteins (Wolff et al., 2014). The main causes for the generation of aberrant proteins are DNA mutations, transcriptional or translational errors, and exogenous factors, such as heat or oxidative stress. The accumulation of terminally misfolded proteins can trigger the formation of toxic protein aggregates that are associated with human malignancies, such as Alzheimer's, Parkinson's, or Huntington's disease (HD). HD is the prototypic example for a group of neurodegenerative pathologies that is caused by abnormal expansions of CAG DNA triplets. These expansions are translated into a polyglutamine (polyQ) tract that is prone to aggregation once its length reaches a certain threshold. Several types of spinocerebellar ataxias also belong to the group of polyQ diseases. SCA1 (spinocerebellar ataxia type 1) is characterized by the expansion of a polyQ stretch in ataxin-1 (Atxn1). To cope with mutant polyQ proteins or other misfolded proteins, eukaryotes have developed an elaborate network of protein quality control (PQC) systems (Wolff et al., 2014). The first lines of defense are molecular chaperones, which are able to refold aberrant proteins and eventually disassemble protein aggregates. If this repair system fails, cells make use of two major pathways, the ubiquitin-proteasome system and selective macroautophagy, for the removal of terminally misfolded proteins in the cytoplasm. Of note, however, aggregation-prone proteins, including pathogenic polyQ proteins, are also present in the nucleus, necessitating a nuclear quality control machinery (Shibata and Morimoto, 2014). Nuclear PQC is particularly important in terminally differentiated cells, like neurons, because in their postmitotic state no mitotic breakdown of the nuclear envelope takes place. Despite its importance, our understanding of nuclear PQC is limited. In particular, it has largely remained elusive how aberrant nuclear proteins are recognized and eventually cleared.

In the current issue of *Molecular Cell*, Guo et al. (2014) now delineate a pathway for the degradation of misfolded nuclear proteins in mammalian cells. The authors follow the fate of the aggregation-prone mutant polyQ proteins, Atxn1 82Q and Htt 97Q (huntingtin with a 97Q extension) as well as misfolded model substrates that do not belong to the polyQ type. For both types of misfolded proteins, they define a clearance pathway that integrates the promyelocytic leukemia protein PML, the ubiquitin-related modifier SUMO, and the SUMO-targeted E3 ubiquitin ligase (StUbL) RNF4 (Figure 1A). The authors propose that PML recognizes misfolded proteins and acts as a SUMO E3 ligase by promoting the attachment of SUMO to these substrates. In this pathway PML seems to preferentially catalyze modification with the SUMO2/SUMO3 variants that are prone to the formation of polymeric SUMO chains. SUMO2/SUMO3 chains subsequently serve as a docking site for RNF4, which earmarks the misfolded proteins for proteasomal degradation by adding an ubiquitin tag. The physiological significance of this pathway was demonstrated in a genetic mouse model for SCA1, in which loss of PML aggravates the neurodegenerative phenotype.

This work extends previous findings that revealed a general induction of SUMO2/SUMO3 conjugation in response to the accumulation of misfolded proteins under proteotoxic stress (Tatham et al., 2011). Moreover, it provides a molecular explanation for the reported PML-dependent clearance of mutant ataxin-7, a polyQ protein causing spinocerebellar ataxia type 7 (SCA7) (Janer et al., 2006). The identification of PML as an E3 SUMO ligase that appears to preferentially discern misfolded proteins, as shown for mutant Atxn1, is one key finding of the current work. PML, also known as TRIM19, is a member of the TRIM (tripartite motif) family, which is composed of more than 70 members in mammalian cells. TRIM family members are characterized by three common domains in their N-terminal region, which are often referred to as the RBBC motif (Figure 1B). This motif is composed of a RING finger, one or two B-boxes, and a coiled-coil (CC) domain. RING domains typically confer E3-ubiquitin ligase activity by recruiting E2 ubiquitin conjugation enzymes, and accordingly, several TRIMs promote E2-dependent ubiquitin conjugation. Yang and colleagues previously reported that a subgroup of TRIMs, including PML, promotes conjugation of both SUMO1 and SUMO2/SUMO3 to the model substrates p53 and mdm2 in a purified in vitro system as well as in a cellular setting (Chu and Yang, 2011). PML and other TRIM-type E3 SUMO ligases may function in a manner similar to the PIAS/Siz subclass of E3 SUMO ligases, which uses a variant RING domain, known as SP-RING, to promote SUMO transfer to substrates. Notably, recent work has demonstrated that PIAS1 enhances SUMO modification of mutant Htt, raising the possibility that on some substrates PML may cooperate with PIAS family members (O'Rourke

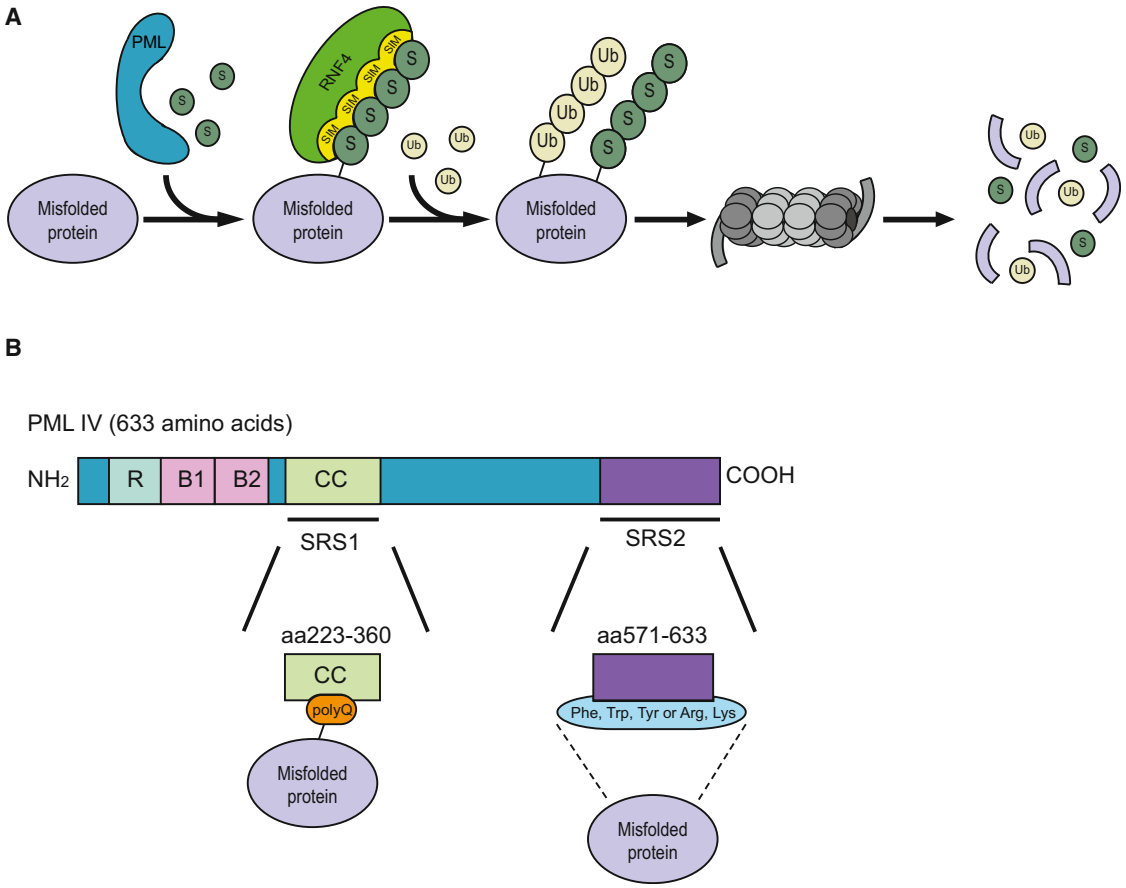

Figure 1. Recognition and Clearance of Misfolded Proteins by the Concerted Action of PML, SUMO, and RNF4

(A) Nuclear misfolded proteins are selectively recognized by PML and marked with poly-SUMO2/SUMO3 chains. RNF4, which is a SUMO-dependent E3 ubiquitin ligase, binds to the poly-SUMO2/SUMO3 chains via tandem SUMO interacting motifs (SIM) and ubiquitylates the protein, which leads to its proteasomal degradation.

(B) Domain structure of PML showing the two different substrate recognition sites, SRS1 and SRS2. SRS1, which preferentially binds to pathogenic variants of polyQ proteins, overlaps with the CC region. The SRS2 at the C-terminal region of PML favors binding to misfolded proteins that display aromatic and positively charged amino acids. R, RING finger; B, B-box; CC, coiled-coil.

et al., 2013). Ultimately, more biochemical and structural studies are needed to understand the molecular details of PML-mediated SUMO transfer and to unveil what determines specificity of distinct TRIMs for ubiquitin or SUMO conjugation. Along this line it will be important to see whether other TRIMs are involved in SUMO-dependent PQC pathways and whether they exert redundant or specific functions. The mode of substrate recognition is another key point in understanding the specificity of PML and eventually other TRIMs for misfolded proteins. Using in vitro binding experiments, two substrate recognition regions in PML, designated SRS1 and SRS2, were pinpointed (Figure 1B). It was demonstrated that both SRS function autonomously and do not require molecular chaperones for substrate recognition. SRS1, which preferentially recognizes the pathogenic variants of Htt, corresponds to the coiled-coil domain of PML. Coiled-coils are α-helical supersecondary structures that mediate protein-protein interactions and oligomerization with other CCs. The CC domain of PML could thus serve as an interaction platform for CC structures typically found in long polyQ stretches. However, future, more quantitative biochemical assays and cell-based experiments with CC mutants of PML and other TRIM proteins need to be done to fully support this concept and to determine specificity of various CCs for distinct pathogenic polyQ variants. While SRS1 copes with polyQ structures, SRS2 specifically binds to short peptides in denatured luciferase, which was used as a misfolded model substrate. Peptide scanning revealed that SRS2 favors binding to peptides that were enriched in aromatic and basic amino acids, suggesting a certain specificity code for substrate selection by SRS2. These data are intriguing, but at this stage do not yet allow estimating whether a specific subset of misfolded substrates might be targeted by PML. With respect to SRS2, it is also important to note that this region is encoded by a part of exon 8a and exon 8b. Exon 8a is present in the PMLI isoform, and exons 8a and 8b together are used by the PMLIV and PMLIVa variants. All other isoforms of PML described so far contain neither exon 8a nor 8b, but use exon 7b, which

is missing in PMLI and PMLIV. Distinct splice variants of PML may thus contain specific SRS regions to discern different types of misfolded proteins.

Conceptually, one may ask why cells use a two-step, SUMO-primed pathway for ubiquitylation and clearance of misfolded proteins rather than triggering their ubiquitylation directly. In the budding yeast *Saccharomyces cerevisiae*, the E3 ubiquitin-protein ligase San1 can indeed directly recognize, ubiquitylate, and remove aberrant nuclear proteins (Gardner et al., 2005). However, StUBL-mediated nuclear quality control is operational in *Saccharomyces cerevisiae* as well, as exemplified by the SUMO-targeted ubiquitylation of a mutant version of the transcriptional regulator Mot1 (Wang and Prelich, 2009). The generation of a SUMO-modified substrate intermediate in the PQC pathway of damaged proteins adds an additional regulatory layer to this process. Conjugation of SUMO may allow a multifaceted response and may not necessarily lead to the removal of the protein. Attachment of SUMO1 and SUMO2 in their monomeric form may recruit factors that facilitate refolding or disassembly of aggregates.

Finally, the additional regulatory layer in this pathway that is provided by the SUMO system may also be considered as an option for therapeutic intervention. One concept might be that stimulating the attachment of SUMO2/SUMO3 to a misfolded protein should foster its removal. Because interferon (IFN) is a well-known inducer of PML expression, IFN treatment might be a way to lower the amount of toxic polyQ proteins. Intriguingly, it was indeed found that IFN-β induces the clearance of mutant ataxin-7 and ameliorates the disease in a SCA7 animal model (Chort et al., 2013). Notably, however, in case of Htt, enhanced modification by SUMO2 causes an increase in insoluble Htt, possibly because the accumulation of SUMO conjugates may exceed the capacity of the downstream clearance machinery (O'Rourke et al., 2013). Therapeutic targeting of this elaborate PQC pathway will therefore remain a challenging task.

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An Ultraconserved lnc to miRNA Processing

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Very few specific functions have been assigned to ultraconserved regions. In this issue of *Molecular Cell*, Liz et al. (2014) describe how a lncRNA transcribed from an ultraconserved region can negatively regulate miRNA maturation.

Conservation of DNA, RNA, or amino acid sequence is generally a good indicator of functional regions and of the relative importance of those regions. So when 481 stretches of DNA longer than 200 bp were found to be 100% identical in humans, mice, and rats, it seemed very likely that these ultraconserved elements (also known as ultraconserved regions, or UCRs) had important functions (Bejerano et al., 2004). Surprisingly, the "ultra-important" functions of these regions have been hard to pin down.

UCRs can be transcribed, and those that are were dubbed T-UCRs. Interestingly, the noncoding RNAs from many T-UCRs appear to be differentially regulated in human cancers (Calin et al., 2007; Lujambio et al., 2010; Mestdagh et al., 2010). Esteller and colleagues previously found that in HCT116 cells, several T-UCRs are not transcribed, but their transcription can be activated by treatment with 5-aza-2′-deoxycytidine, which inactivates DNA methyltransferases. They show that CpG islands associated with these T-UCRs are methylated in HCT116 cells and many other cancer cell lines as well as primary tumors (Lujambio et al., 2010). There