A guide for targeted SUMO removal

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SUMO homeostasis is important for many cellular processes. In the current issue of *Genes & Development*, Liang and colleagues (pp. 802–815) demonstrate how a desumoylation enzyme is targeted to the nucleolus for removing SUMO from specific substrates and how curtailing sumoylation levels can regulate transcription in this nuclear compartment.

SUMO is emerging as an important protein modifier in diverse cellular processes. Much progress has been made in understanding SUMO conjugation to various substrates via the SUMO E1, E2, and E3 enzymes. Equally important is the reverse process mediated by the desumoylation enzymes, including Ulps in yeast and the homologous SENPs in mammals. Lack of these enzymes leads to genome instability, transcriptional aberrations, and proteomic alterations, all of which can contribute to the developmental deficiencies seen in animals with reduced desumoylation (Hickey et al. 2012). Biochemical and proteomic studies have suggested that various desumoylation enzymes target different substrates. How each desumoylation enzyme is guided toward specific substrates remains poorly understood.

Several recent studies address the above question using budding yeast as a model system. An earlier study links the Ulp2 desumoylation enzyme to the management of ribosomal DNA (rDNA), which harbors 100–200 copies of the rDNA repeat (Srikumar et al. 2013). Ulp2 was found to localize to rDNA, promote rDNA stability, and interact with a nucleolar resident protein, Csm1 (Srikumar et al. 2013). Csm1 and its partner, Lrs4, form the Cohibin complex that bridges the nuclear envelope and rDNA (Huang et al. 2006). Cohibin connects to rDNA through the Tof2 scaffold protein, which itself is anchored to rDNA by binding to Fob1, which decorates the replication fork barrier (RFB) at each rDNA repeat (Fig. 1). This chain of protein interactions helps to maintain rDNA localization near the nuclear envelope and suppress RNA polymerase II (Pol II)-mediated transcription (Huang et al. 2006). The latter effect, termed rDNA silencing, also requires the histone deacetylase Sir2, which establishes histone marks disfavoring Pol II transcription. Sir2 and the Tof2 paralog Net1 are also tethered to Fob1 (Huang et al. 2006). Conversely, Sir2, Net1, and Tof2 help to stabilize the Fob1–RFB association. As such, the protein network involving Cohibin, Tof2, Sir2, Net1, and Fob1 helps to both establish Pol II transcription suppression and block replication forks approaching the 35S transcription unit, avoiding transcription and replication collision (Fig. 1).

Why is Ulp2 needed at rDNA? It turns out that the aforementioned protein network is a sumoylation hot spot and that Ulp2 is responsible for keeping the sumoylation levels of this network in check (Cremona et al. 2012; de Albuquerque et al. 2016; Gillies et al. 2016). Gillies et al. (2016) demonstrated that maintaining balanced sumoylation levels of Tof2, Net1, and Fob1 is important for their association with rDNA. The present study by Liang et al. (2017) further addresses how Ulp2 is brought close to its substrates in rDNA. The investigators mapped the Csm1 and Ulp2 interaction regions and determined their structure. They further showed that Csm1 can interact simultaneously with Ulp2 and Tof2 and solved the structure of the fusion peptides from the three proteins. Structure-guided mutagenesis then identified *ulp2* mutations that reduce Csm1 binding and a *tof2* mutant that reduces Csm1 interaction. Importantly, both mutants increase Tof2 sumoylation and lower rDNA silencing (Fig. 1). These data suggest a model in which Csm1 guides Ulp2 to its target proteins by simultaneous interaction with both the enzyme and the target.

The implications of this model likely go beyond just one substrate. Liang et al. (2017) showed that deleting the Csm1-binding region of Ulp2 also increases kinetochore protein sumoylation. This fits with previous findings that Csm1 is localized at kinetochores and that kinetochore protein sumoylation has important effects on chromosomal segregation (Yong-Gonzales et al. 2012; Alonso et al. 2015). Thus, an intriguing possibility suggested by Liang et al. (2017) is that Csm1 is a protein guide for Ulp2 at both rDNA and kinetochores. Testing this model and examining Ulp2 targeting mechanisms in a broader context will expand our understanding on multiple SUMO removal processes.
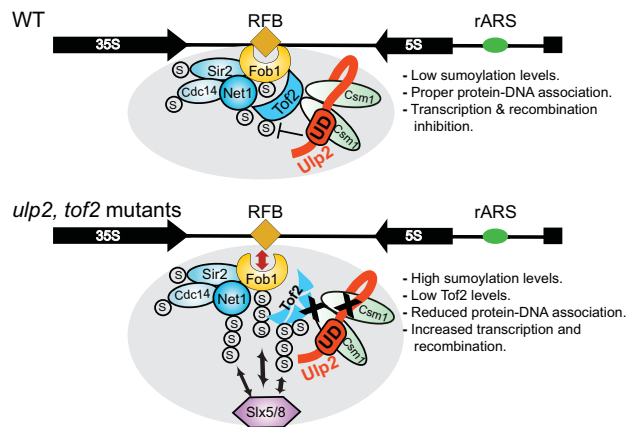
Figure 1. Ulp2 and Slx5/8 regulate nucleolar proteins and rDNA functions. The *top* panel depicts rDNA maintenance in wild-type (WT) cells. One rDNA repeat is shown with RFB located approximately a few hundred base pairs from the 35S and 5S transcription units. RFB is bound by Fob1, which can anchor the Cdc14–Sir2–Net1 complex (also known as RENT) and the Tof2–Csm1 complex to this locus. Ulp2, which is tethered to Csm1, maintains low sumoylation levels of Fob1 and its associated proteins. This function helps to inhibit RNA Pol II-mediated transcription and homologous recombination around the RFB site. (*Bottom*) When the Ulp2–Csm1 or Tof2–Csm1 interactions are disrupted, Ulp2 loses its proximity to Tof2 and, consequently, its ability to curtail the sumoylation of proteins present at the RFB. This results in the formation of poly-SUMO chains on Tof2, Net1, and Fob1 that can be acted on by STUbLs (SUMO targeted ubiquitin ligase; Slx5–Slx8), leading to reduced Tof2 levels and protein binding to rDNA. Consequently, this can interfere with transcriptional silencing and rDNA instability. (rARS) rDNA replication origin; (UD) Ulp2 catalytic domain.

The studies by Liang et al. (2017) and Gillies et al. (2016) also address the deleterious consequences of not curtailing the sumoylation levels of rDNA proteins. Their findings implicate the STUbL (SUMO targeted ubiquitin ligase) enzyme, called Slx5/8 in budding yeast. STUbLs recognize SUMOylated proteins via arrays of SUMO interaction motifs (SIMs) and then conjugate ubiquitin to these proteins or SUMO (Nie and Boddy 2016). This can lead to either protein degradation or protein stripping from DNA by segregase (Nie and Boddy 2016). Slx5/8 has been implicated previously in rDNA functions. Importantly, Gillies et al. (2016) showed that STUbL loss rescues several *ulp2* defects, including reduced rDNA association of Tof2, Net1, and Fob1 (Fig. 1). Liang et al. (2017) further revealed that removing STUbL or mutating its SIMs suppresses the rDNA silencing defects of *ulp2* mutants. This rescue is partly due to the restoration of Tof2 levels in *ulp2* and *tof2* mutants that are defective in Csm1 binding (Fig. 1). Taken together, their data suggest a model in which excessive Tof2 sumoylation leads to STUbL-mediated Tof2 removal from rDNA, which subsequently dampens rDNA silencing. Further testing of this model awaits biochemical analysis of how STUbL and sumoylation directly affect Tof2 protein turnover. In addition, it is important to understand whether the antagonistic actions between Ulp2 and STUbL apply to other sumoylated proteins.

The role of Ulp2 in rDNA regulation can have a domino effect, leading to further physiological changes. For example, misregulation of Net1, which functions together with the multifunctional Cdc14 phosphatase, can influence a range of nuclear functions, such as mitotic exit and chromosomal segregation (Gillies et al. 2016). Also, Ulp2 substrates that affect rDNA replication and recombination can exert indirect effects on overall genome fitness and aneuploidy formation (Gillies et al. 2016; Ryu et al. 2016). As the mammalian Ulp2 homolog SENP6 also resides in the nucleolus (Hickey et al. 2012), findings in budding yeast can stimulate the studies of how SENP6 functions, how human repetitive sequences are maintained through modulating sumoylation, and how such functions influence human health.

Acknowledgments

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