A STUbL wards off telomere fusions

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Protection of chromosomal ends from erroneous recognition as double-strand breaks (DSBs) is critical for maintaining genome integrity. Unprotected ends can activate DNA repair pathways leading to catastrophic fusions or rearrangements of chromosomes. A study in *The EMBO Journal* identifies Uls1 as a new player in this end-protection process, utilizing SUMO and ubiquitin modifications to prevent chromosomal fusions by clearing a polysumoylated form of Rap1.

Telomeres, the ends of linear chromosomes, pose a tremendous challenge for eukaryotic cells, because their resemblance to DNA DSBs puts them at constant risk of being targeted by repair pathways. Thus, these ends must be kept off the radar of the two major DSB repair pathways, non-homologous end joining (NHEJ) and homologous recombination (HR). A number of studies in the model system budding yeast previously identified a central role for the Rap1 protein in preventing NHEJ at telomeres. Rap1 is an evolutionarily conserved protein that binds to the double-stranded DNA region of telomeres. It inhibits NHEJ via at least three different pathways (Marcand *et al*, 2008). Two of these are mediated by its binding partners Rif2 and Sir4, respectively, with a less well-characterized third pathway independent of either interactor. How these pathways enable NHEJ evasion is not entirely clear, but recent findings of posttranslational modifications of telomere-binding proteins, particularly by SUMO, hinted at a possible role for dynamic protein modifications in regulating telomeric functions.

Attachment of the SUMO protein to a lysine residue in target proteins can change both their interaction profiles, by allowing other proteins to bind or impairing interactions, and their biochemical functions. Additionally, since the SUMO polypeptide itself contains lysines, poly-SUMO chains can be generated, which may recruit SUMO-targeted ubiquitin ligases (STUbLs) via the latter's multiple SUMO-interacting motifs (SIMs). STUbLs further contain a RING ubiquitin ligase domain, enabling them to ubiquitinate the target for proteasomal degradation (Uzunova *et al*, 2007).

Recently, several studies have found sumoylation to be important for telomere regulation in different organisms. In both fission and budding yeasts, disruption of sumoylation dramatically elongates telomeres (Tanaka *et al*, 1999; Zhao and Blobel 2005). Sumoylated proteins at budding yeast telomeres include Rap1, the Ku70/80 complex, Cdc13, Pif1, the Sir2/3/4 complex as well as the RecQ helicase Sgs1 (Figure 1). Cdc13 sumoylation helps to restrict telomerase

function by strengthening Cdc13 binding to the telomerase-inhibitory STN complex, likely in early S phase (Hang *et al*, 2011). Sumoylation of Sir4 and the Ku70/80 complex

Figure 1 Roles of sumoylated telomere proteins in *Saccharomyces cerevisiae*. First, SUMO contributes to telomere length homeostasis by regulating telomerase functions. This is achieved by Cdc13 sumoylation, which enhances telomerase-inhibitory protein interactions, as well as sumoylation of Sir4 and Ku70/80, which mediates telomere anchoring to the nuclear periphery. Second, sumoylated Sgs1 helicase promotes telomeric recombination in telomerase-negative cells. Third, new results from Lescasse *et al* (2013) now reveal that the STUbL Uls1 can remove polysumoylated Rap1, and thereby alleviate the interference with NHEJ inhibition conferred by poly-SUMO-Rap1.

supports telomere anchoring to the nuclear periphery and consequently affects telomere repeat addition (Ferreira *et al*, 2011). In addition to telomerase regulation, sumoylation has also been implicated in telomeric HR processes, for example, Sgs1 sumoylation promotes telomeric recombination in telomerase-deficient yeast cells (Lu *et al*, 2010). In human cells, particularly in a subset of telomerase-negative cancer cells, sumoylation enables the alternative lengthening of telomeres (ALT) pathway that uses HR mechanisms to elongate telomeres. The ALT pathway requires both the SUMO E3 ligase Mms21 to sumoylate telomeric proteins, and PML nuclear bodies, which are enriched for sumoylated proteins (reviewed in Chung *et al* (2012)).

Now, Lescasse *et al* (2013) reveal a novel function for sumoylation at yeast telomeres by elucidating Uls1-mediated NHEJ inhibition. Uls1 has been implicated in diverse processes, including the regulation of mating type switching efficiency, transcriptional silencing, replication stress response and the removal of ssDNA exchange protein Rad51 from undamaged DNA (Zhang and Buchman 1997, Shah *et al*, 2010, Cal-Bakowska *et al*, 2011). It is both a STuBL enzyme and a member of the SWI/SNF family of DNA-dependent ATPases (Zhang and Buchman 1997; Uzunova *et al*, 2007), and likely influences the above processes by both substrate modification and by ATPase-mediated removal of proteins from DNA. This appears to be the case in its newly identified NHEJ-inhibiting function at telomeres, since mutations of key residues affecting either function increase telomere fusion frequencies. Interestingly, Lescasse *et al* (2003) show that the elevated level of telomere fusions due to NHEJ activity in *uls1Δ* cells is suppressed by inhibiting the formation of SUMO chains, and identify Rap1 as the target of poly-SUMO modification. They demonstrate this by both detecting an increase of polysumoylated Rap1 in *uls1Δ* cells, and showing that two point mutations at putative sumoylation sites reduce Rap1 sumoylation and decrease fusions in *uls1Δ* cells. Moreover, genetic analyses suggest that Uls1 functions in the Rif2-dependent and Rif2/Sir4-independent pathways of Rap1-mediated NHEJ inhibition. The authors propose a model in which polysumoylation of Rap1 interferes with its ability to inhibit NHEJ, and removal of this form of Rap1 by Uls1-mediated ubiquitination and translocase-like activity restores the perturbed function, thus preventing NHEJ.

Such a model is supported by nicely executed experiments as described in the paper. The regulatory paradigm invoked here may be broadly applicable to other cellular processes as a general means for preventing the unwarranted persistence of more 'detrimental' forms of proteins. As in the case of other new discoveries, this work raises several questions for future investigation, of which three are particularly intriguing. The first relates to the biological function of Rap1 sumoylation. Does sumoylated Rap1 only serve a negative role or could it also have positive effects? Could the biological consequences of mono- versus polysumoylated Rap1 be

different? Decreasing Rap1 sumoylation by mutating major sumoylation sites does not lead to telomere length changes or other noticeable telomere defects, suggesting that sumoylation of Rap1 may not make positive contributions to telomere maintenance. One could argue that Rap1 could simply be modified as a bystander in an environment favourable to sumoylation. On the other hand, sumoylation of Rap1 increases upon DNA damage (Hang *et al*, 2011). Perhaps, such a situation requires rapid alteration of Rap1 function in favour of DNA lesion repair, consistent with the requirement of sumoylation in the DNA damage response (Cremona *et al*, 2012). Alternatively, as Rap1 also affects transcription, a process well known to be influenced by sumoylation, it is possible that modified Rap1 may contribute to this function. Generation of an unsumoylatable Rap1 mutant and examination of how this affects different aspects of Rap1 function will provide the answer to this question.

The second question is how polysumoylated Rap1 impairs NHEJ inhibition. While SUMO chains can serve as a signal for ubiquitination and degradation, here, they may additionally impair Rap1 interactions, and consequently NHEJ inhibition, via steric hindrance. The third question regards the mechanisms by which Uls1 removes polysumoylated Rap1. Lescasse *et al* (2013) propose a two-step model that entails the sequential action of its STuBL and translocase activities, as both are required for inhibition of telomeric fusions, and mutations affecting only one have partial effects compared to *uls1Δ*. This is an attractive model, although it still needs further testing such as biochemical characterization of these Uls1 mutants. Furthermore, it will be important to discriminate whether the STuBL activity is a prerequisite for the translocase function, or if the two activities are independent. An intriguing possibility is that binding to a SUMO chain and/or ubiquitin conjugation allows the licensing of translocase activity. Such a mechanism could permit specific protein removal from DNA only upon recognition of poly-SUMO chains. This is of great importance considering the need to curb translocases from haphazardly stripping proteins off DNA.

In summary, the findings by Lescasse *et al* (2013) add yet another dimension to the regulation of telomere end protection, and further energize investigations of the emerging function of sumoylation in regulating genome integrity. Identification and characterization of additional Uls1 targets will further uncover the roles of this protein modification in multiple cellular processes.

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Conflict of interest

The authors declare that they have no conflict of interest.

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