Sumoylation: A new wrestler in the DNA repair ring

Luis Aragón*

Cell Cycle Group, MRC Clinical Sciences Centre, Imperial College London, Du Cane Road, London W12 0NN, United Kingdom

Eukaryotic cells contain three essential complexes with heterodimers of Smc (structural maintenance of chromosomes) proteins at their core, namely cohesin, condensin, and the Smc5–6 complex. These complexes perform structural roles on chromosomes related to different DNA metabolic processes, in the case of Smc5–6 damage repair. However, two groups working independently, Zhao and Blobel (1) as described in this issue of PNAS, and Watts and colleagues (2), have demonstrated that, unlike in cohesin and condensin complexes, one of the non-Smc subunits of the Smc5–6 complex also possesses an enzymatic activity. Mms21/Nse2 is the third SUMO E3 ligase in the yeast genome besides the known ligases Siz1 and Siz2. SUMO conjugate targets for Mms21/Nse2 include other subunits of the Smc5–6 complex as well as repair proteins like Ku70. The implication of these findings is that the Smc5–6 complex not only has a structural function, but is also capable of handling biochemical activities, perhaps reflecting an overall function in recognition of DNA structures and activation of repair pathways.

SUMO Ligases in Budding Yeast

Eukaryotic proteins are subjected to a wide range of posttranslational modifications including the covalent attachment of proteins. Ubiquitin is the most familiar of the proteinaceus modifiers, and the enzymology of its activation and transfer has been extensively studied. Recently, several ubiquitin-related proteins have been identified and shown to form covalent attachments to proteins. One of the most intriguing of these is SUMO (small ubiquitin-related modifier). In vertebrates, there are several variants of SUMO (SUMO-1, -2, and -3), whereas only one (Smt3p) has been found in the budding yeast *Saccharomyces cerevisiae*. Smt3p becomes attached to targets through a multistep process that requires an activating (E1-activating enzyme), a conjugating (E2-conjugating enzyme), and a ligating (E3-ligase) enzyme (3). So far, two E3 SUMO ligases have been described in *S. cerevisiae*, namely Siz1 and Siz2 (4, 5). One of the standing questions in the field is how many SUMO E3 ligases exist, because yeast appears to have no further potential SUMO E3s other than Siz1 and Siz2, yet sumoylation is still detected *in vivo* in the absence of these two enzymes. Unlike ubiquitylation, sumoylation is not known to target proteins for degradation, but rather, Smt3p modification is thought to antagonize ubiquitin-dependent degradation, regulate protein–protein interactions, and alter the subcellular localization of conjugates (3).

Zhao and Blobel (1) demonstrate that, in addition to Siz1 and Siz2, the genome of budding yeast contains at least one more SUMO E3 ligase. The authors isolate Mms21 through a clever genetic screen designed to identify proteins involved in sumoylation. The screen is built upon their previous work demonstrating that the budding yeast myosin-like proteins (Mlp1 and -2) are functionally related to sumoylation through a role in anchoring the desumoylation enzyme Ulp1 to a subset of nuclear pore complexes (NPCs) (6). Deletion of Mlps resulted in the delocalization of Ulp1 to the nuclear interior and the consequent desumoylation of SUMO (Smt3p) conjugates (6). Under such conditions, i.e., the altered localization of Ulp1 to the nucleoplasm, the activity of proteins that conjugate Smt3p (sumoylation) is likely to be critical. Zhao and Blobel used this rationale to screen for genes synthetically lethal with the simultaneous deletion of Mlp1 and Mlp2; Mms21 was pulled in this screen. Zhao and Blobel realized that their newly identified gene *MMS21* is part of the Smc5–6 complex, a multisubunit complex involved in DNA repair (7). Biochemical and cytological analysis with different subunits of the Smc5–6 complex revealed that, in *S. cerevisiae*, the complex contains eight subunits in total and it localizes throughout the nucleoplasm and at specific perinuclear foci (1). Not surprisingly, given the screen, Mms21 was found to contain a putative SP-RING domain similar to that found in SUMO E3 ligases. This domain was truncated in the mutant isolated, *mms21-11*. Furthermore, Zhao and Blobel identify two Mms21 SUMO-conjugate targets, Smc5 and Ku70, and confirm that these proteins are indeed sumoylated in an Mms21-dependent manner *in vitro* and *in vivo*. The presence of a putative SP-RING domain with potential SUMO E3 ligase activity had also been noticed for the fission yeast ortholog of Mms21, Nse2 (8). In a parallel study, Watts and colleagues (2) show that purified *Schizosaccharomyces pombe* Nse2 is indeed a SUMO E3 ligase capable of conjugating SUMO to several of the subunits of Smc5–6 complex (2). Furthermore, these authors demonstrate that mutagenesis of the ring motif in Nse2 abolishes its sumoylating activity, preventing sumoylation of Smc5–6 components *in vivo* (2).

Abolition of sumoylation activity sensitizes cells to DNA damaging agents.

Both studies conclude that the SP-RING domain (and consequently the sumoylation activity) is not required for cell viability in either yeast (1, 2); however, abolition of this activity sensitizes cells to DNA damaging agents (1, 2) and, in *S. cerevisiae*, causes a range of seemingly unrelated phenotypes, including nucleolar fragmentation, loss of telomere clustering at the nuclear periphery, misregulation of normal telomere length, and enhancement of telomeric silencing (1). Zhao and Blobel show that both Smc5 and yKu70 are polysumoylated in response to methyl methanesulfonate (MMS) and that this becomes abolished in the *mms21-11* mutant, thus demonstrating that Mms21 mediated sumoylation is an integral part in the DNA damage response. The sumoylation of Smc5–6 subunits suggests that the function of the complex is itself regulated through this modification (1, 2). Eukaryotic cells contain three multiprotein complexes with heterodimers of Smc proteins, namely cohesin, condensin, and the Smc5–6 complex. Interestingly, Smc5–6 is not the first Smc complex to be regulated by SUMO, as recent work has shown that one of condensin's subunits, Ycs4, is regulated by sumoylation during anaphase (9).

Zhao and Blobel (1) identify Ku70 as a target of Mms21 activity. The Ku70/80 complex is known to be recruited to double strand breaks to perform a bridging role in the end-to-end

See companion article on page 4777.

*E-mail: luis.aragon@csc.mrc.ac.uk.

fusion process during nonhomologous end joining (10) and deletion of *KU70* renders cells sensitive to MMS (11). Therefore, the identification of Ku70 as a target of Mms21 sumoylation in response to MMS is particularly revealing because it shows that the localization and/or interactions of Ku70 are at least partly modulated by the activity of the Smc5–6 complex (1).

The Smc5–6 Complex

The Smc5–6 complex is one of the three Smc complexes found in eukaryotic genomes (12, 13). The first one, cohesin, holds sister chromatids together after DNA replication. The second, condensin, is required to compact chromosomes during mitosis. Finally, the exact role of Smc5–6 is presently unclear, although the complex seems to be important for the cellular responses to DNA damage as well as having an essential function.

The first report regarding the Smc5–6 complex was the identification of a radiation sensitive mutant of the *Schizosaccharomyces pombe rad18* gene (*SMC6*) named *rad18-X* (14–16). Through epistasis analysis, *rad18* was then placed in a postreplicative repair pathway independent of nucleotide-excision repair (16). An interacting protein, *Spr18*, also found initially in *Schizosaccharomyces pombe*, turn out to be the heterodimeric partner, Smc5p (17). Since then, the Non-Smc Elements in the complex has grown to four in *Schizosaccharomyces pombe* (Nse1–4) (8, 18–20) and six in *S. cerevisiae* (Nse1–6) (1, 21, 22). The Smc5–6 complex is involved in damage repair because all mutants identified to date exhibit sensitivities to a variety of DNA damaging agents. However, the complex is also thought to carry out an essential function, and presently it is unclear to what extent the DNA repair and essential functions overlap. Temperature-sensitive mutants of the *Schizosaccharomyces pombe rad18* (*SMC6*) gene exhibit a nonspecific terminal phenotype where cells are able to go through a few rounds of division before arresting growth (19). As mutant cells divide, the appearance of a "cut" phenotype, where incompletely separated nuclei are cut by the formation of a new septum, suggests a requirement for the accurate transmission of chromosomes. In *S. cerevisiae*, the Smc5/6 heterodimer is necessary for the disjunction of the repetitive ribosomal DNA (23). A temperature-sensitive mutant of *SMC6*, *smc6-9*, undergoes catastrophic mitosis, where missegregation of rRNA-encoding DNA (rDNA) repeats is observed (23). *smc6-9* cells accumulate X-shaped intermediates in rDNA at the time of segregation and these are the likely cause for the failure in the disjunction of the rDNA repeats (23). The viable sumoylation inactive mutant *mms21-11* (5) exhibits phenotypes related (although not as severe) to those observed for the inviable *smc6-9* mutant (23), namely nucleolar fragmentation and telomere misregulation. Therefore, the essential and damage-repair roles of the Smc5–6 complex might be related to each other.

Sumoylation is emerging as a critical factor in cellular processes ranging from cell cycle regulation and chromosome metabolism to transcriptional control. One more function related to this fascinating posttranslational modification in DNA repair has now been added by the independent work of Zhao and Blobel (1) and Watts and colleagues (2). Although the precise mechanisms by which Mms21/Nse2-dependent sumoylation regulates the DNA remains mysterious, the work presented in this issue of PNAS (1) provides an intriguing start to understanding the unique properties of the enigmatic Smc5–6 complex in budding yeast and possibly all eukaryotes.

1. Zhao, X. & Blobel, G. (2005) *Proc. Natl. Acad. Sci. USA* **102,** 4777–4782.
2. Andrews, E. A., Palecek, J., Sergeant, J., Taylor, E., Lehmann, A. R. & Watts, F. Z. (2005) *Mol. Cell. Biol.* **25,** 185–196.
3. Melchior, F. (2000) *Annu. Rev. Cell Dev. Biol.* **16,** 591–626.
4. Johnson, E. S. & Gupta, A. A. (2001) *Cell* **106,** 735–744.
5. Takahashi, Y., Kahyo, T., Toh, E. A., Yasuda, H. & Kikuchi, Y. (2001) *J. Biol. Chem.* **276,** 48973–48977.
6. Zhao, X., Wu, C. Y. & Blobel, G. (2004) *J. Cell Biol.* **167,** 605–611.
7. Lehmann, A. R. (2005) *DNA Rep.* **4,** 309–314.
8. McDonald, W. H., Pavlova, Y., Yates, J. R., III, & Boddy, M. N. (2003) *J. Biol. Chem.* **278,** 45460–45467.
9. D'Amours, D., Stegmeier, F. & Amon, A. (2004) *Cell* **117,** 455–469.
10. Ramsden, D. A. & Gellert, M. (1998) *EMBO J.* **17,** 609–614.
11. Feldmann, H., Driller, L., Meier, B., Mages, G., Kellermann, J. & Winnacker, E. L. (1996) *J. Biol. Chem.* **271,** 27765–27769.
12. Cobbe, N. & Heck, M. M. (2004) *Mol. Biol. Evol.* **21,** 332–347.
13. Hirano, T. (2002) *Genes Dev.* **16,** 399–414.
14. Nasim, A. & Smith, B. P. (1975) *Genetics* **79,** 573–582.
15. Phipps, J., Nasim, A. & Miller, D. R. (1985) *Adv. Genet.* **23,** 1–72.
16. Lehmann, A. R., Walicka, M., Griffiths, D. J., Murray, J. M., Watts, F. Z., McCready, S. & Carr, A. M. (1995) *Mol. Cell. Biol.* **15,** 7067–7080.
17. Fousteri, M. I. & Lehmann, A. R. (2000) *EMBO J.* **19,** 1691–1702.
18. Harvey, S. H., Sheedy, D. M., Cuddihy, A. R. & O'Connell, M. J. (2004) *Mol. Cell. Biol.* **24,** 662–674.
19. Pebernard, S., McDonald, W. H., Pavlova, Y., Yates, I. J. & Boddy, M. N. (2004) *Mol. Biol. Cell.* **15,** 4866–4876.
20. Morikawa, H., Morishita, T., Kawane, S., Iwasaki, H., Carr, A. M. & Shinagawa, H. (2004) *Mol. Cell. Biol.* **24,** 9401–9413.
21. Fujioka, Y., Kimata, Y., Nomaguchi, K., Watanabe, K. & Kohno, K. (2002) *J. Biol. Chem.* **277,** 21585–21591.
22. Hazbun, T. R., Malmstrom, L., Anderson, S., Graczyk, B. J., Fox, B., Riffle, M., Sundin, B. A., Aranda, J. D., McDonald, W. H., Chiu, C. H., *et al.* (2003) *Mol. Cell* **12,** 1353–1365.
23. Torres-Rosell, J., Machin, F., Farmer, S., Jarmuz, A., Eydmann, T., Dalgaard, J. & Aragon, L. (2005) *Nat. Cell Biol.* **7,** 412–419.