

Sussex Research

The role of *Schizosaccharomyces pombe* SUMO ligases in genome stability

Felicity Watts, Andrew Skilton, J C Ho, Lara Katrina Boyd, M A Trickey, Lisa Gardner, Francois-Xavier Ogi

Publication date

01-12-2007

Licence

This work is made available under the **Copyright not evaluated** licence and should only be used in accordance with that licence. For more information on the specific terms, consult the repository record for this item.

Citation for this work (American Psychological Association 7th edition)

Watts, F., Skilton, A., Ho, J. C., Boyd, L. K., Trickey, M. A., Gardner, L., & Ogi, F.-X. (2007). *The role of Schizosaccharomyces pombe SUMO ligases in genome stability* (Version 1). University of Sussex.
<https://hdl.handle.net/10779/uos.23313434.v1>

Published in

Biochemical Society Transactions

Link to external publisher version

<https://doi.org/10.1042/BST0351379>

Copyright and reuse:

This work was downloaded from Sussex Research Open (SRO). This document is made available in line with publisher policy and may differ from the published version. Please cite the published version where possible. Copyright and all moral rights to the version of the paper presented here belong to the individual author(s) and/or other copyright owners unless otherwise stated. For more information on this work, SRO or to report an issue, you can contact the repository administrators at sro@sussex.ac.uk. Discover more of the University's research at <https://sussex.figshare.com/>

The role of Schizosaccharomyces pombe SUMO ligases in genome stability

Article (Unspecified)

Citation:

Watts, Felicity, Skilton, Andrew, Ho, J C, Boyd, Lara Katrina, Trickey, M A, Gardner, Lisa and Ogi, Francois-Xavier (2007) The role of Schizosaccharomyces pombe SUMO ligases in genome stability. Biochemical Society Transactions, 35 (6). pp. 1379-1384. ISSN 0300-5127

This version is available from Sussex Research Online: <http://sro.sussex.ac.uk/2052/>

This document is made available in accordance with publisher policies and may differ from the published version or from the version of record. If you wish to cite this item you are advised to consult the publisher's version. Please see the URL above for details on accessing the published version.

Copyright and reuse:

Sussex Research Online is a digital repository of the research output of the University.

Copyright and all moral rights to the version of the paper presented here belong to the individual author(s) and/or other copyright owners. To the extent reasonable and practicable, the material made available in SRO has been checked for eligibility before being made available.

Copies of full text items generally can be reproduced, displayed or performed and given to third parties in any format or medium for personal research or study, educational, or not-for-profit purposes without prior permission or charge, provided that the authors, title and full bibliographic details are credited, a hyperlink and/or URL is given for the original metadata page and the content is not changed in any way.

Regulation of Protein Function by SUMO Modification

A Biochemical Society Focussed Meeting held at Manchester Conference Centre, Manchester, U.K., 25–27 June 2007. Organized and Edited by R. Hay (Dundee, U.K.) and A. Sharrocks (Manchester, U.K.).

The role of *Schizosaccharomyces pombe* SUMO ligases in genome stability

F.Z. Watts¹, A. Skilton, J.C.-Y. Ho², L.K. Boyd, M.A.M. Trickey³, L. Gardner, F.-X. Ogi and E.A. Outwin⁴

Genome Damage and Stability Centre, School of Life Sciences, University of Sussex, Falmer, Brighton BN1 9QG, U.K.

Abstract

SUMOylation is a post-translational modification that affects a large number of proteins, many of which are nuclear. While the role of SUMOylation is beginning to be elucidated, it is clear that understanding the mechanisms that regulate the process is likely to be important. Control of the levels of SUMOylation is brought about through a balance of conjugating and deconjugating activities, i.e. of SUMO (small ubiquitin-related modifier) conjugators and ligases versus SUMO proteases. Although conjugation of SUMO to proteins can occur in the absence of a SUMO ligase, it is apparent that SUMO ligases facilitate the SUMOylation of specific subsets of proteins. Two SUMO ligases in *Schizosaccharomyces pombe*, Pli1 and Nse2, have been identified, both of which have roles in genome stability. We report here on a comparison between the properties of the two proteins and discuss potential roles for the proteins.

Introduction

The mechanisms involved in the post-translational modification of proteins by SUMOylation resemble those associated with ubiquitination. However, there are a number of significant differences in the two processes. SUMO (small ubiquitin-related modifier), like ubiquitin, is produced as a precursor protein that is processed to the mature form to reveal a double glycine (–GG) motif. It is then activated by a SUMO activator (which is heterodimeric, unlike the ubiquitin activator that consists of a single protein), and then passed to a SUMO conjugator (E2), of which there is only one, unlike the case with ubiquitination, where there are several E2s. However, perhaps the most striking difference is in the requirement for a SUMO ligase. While there are

dozens, or perhaps even hundreds, of ubiquitin ligases, far fewer SUMO ligases have so far been characterized.

SUMO ligases fall into a number of classes. The first is the PIAS [protein inhibitor of activated STAT (signal transducer and activator of transcription)] family: members of this class contain an SP-RING [Siz-PIASRING (really interesting new gene)] related to RING zinc finger domains (Figure 1A). As well as the mammalian PIAS proteins (e.g. [1–3]), this group includes the *Saccharomyces cerevisiae* Siz1, Siz2, Mms21 and Zip3 proteins [4–6], the *Schizosaccharomyces pombe* Pli1 and Nse2 proteins [7,8] and human Mms21 [9]. Other SUMO ligases include RanBP2 (Ran-binding protein 2) [10], Pc2 (polycomb 2) protein [11] and HDAC2 [11a].

The PIAS family of SUMO ligases

The presence of a RING zinc finger domain in the PIAS family of SUMO ligases is reminiscent of the largest family of ubiquitin E3 ligases. However, whether this domain folds similarly to that in the ubiquitin E3s is unknown, since the RING domains in the SUMO ligases lack two of the conserved cysteine residues that are present in the ubiquitin E3s. Immediately upstream of the SP-RING motif, the Siz and PIAS proteins, but not the Nse2/Mms21 SUMO ligases contain a SAP (Scaffold attachment factor, *A*cinus and *P*IAS) domain at the N-terminus. SAP regions in these and other

Key words: genome stability, Nse2, meiosis, *Schizosaccharomyces pombe*, small ubiquitin-related modifier (SUMO) ligase, SUMOylation.

Abbreviations used: ChIP, chromatin immunoprecipitation; 5-FOA, 5-fluoro-orotic acid; HU, hydroxyurea; STAT, signal transducer and activator of transcription; PIAS, protein inhibitor of activated STAT; SAP, Scaffold attachment factor, *A*cinus and *P*IAS; RING, really interesting new gene; SP-RING, Siz-PIAS RING; SUMO, small ubiquitin-related modifier; TBZ, thiabendazole.

¹To whom correspondence should be addressed (email f.z.watts@sussex.ac.uk).

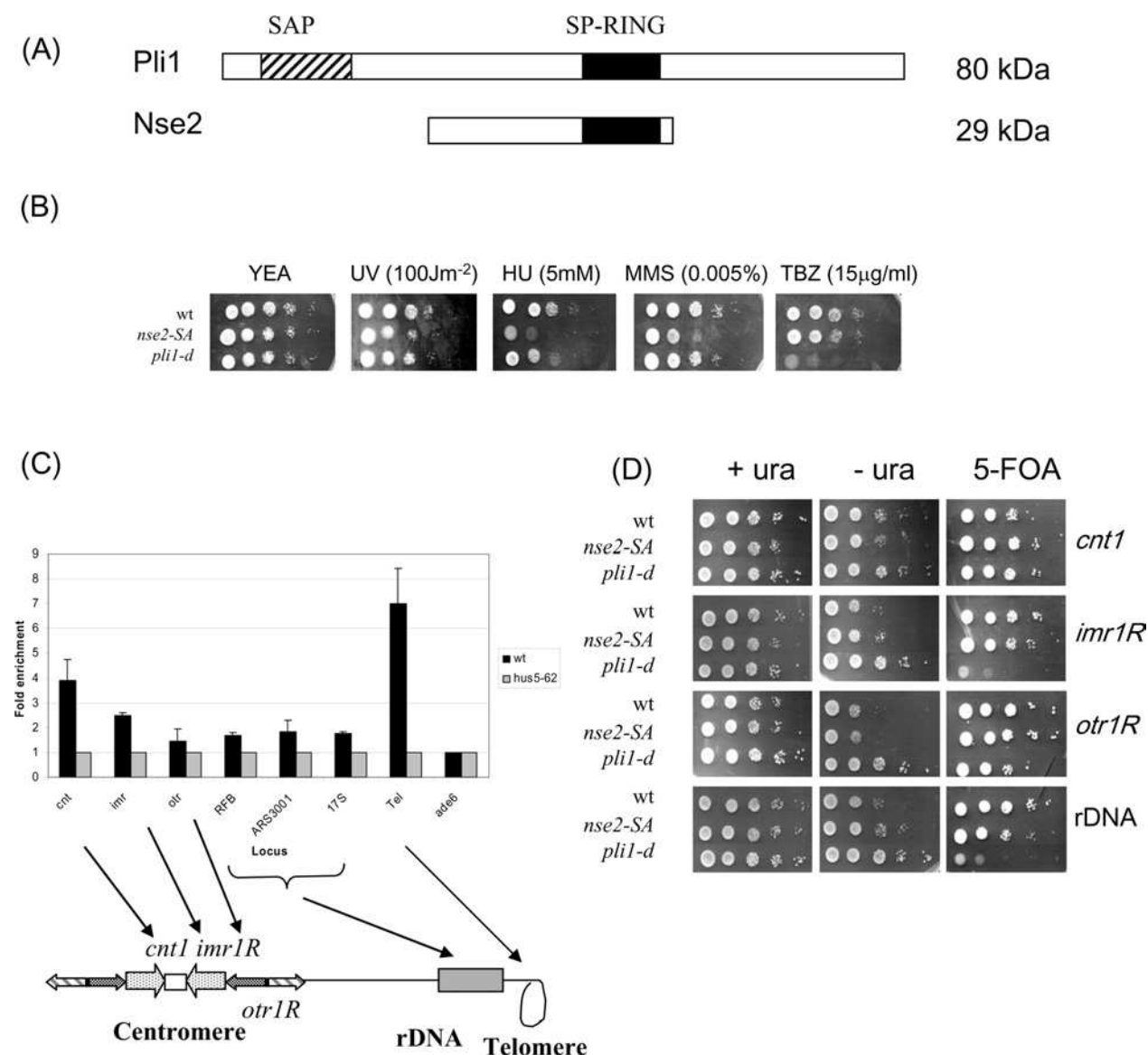
²Present address: Centre for the Study of Liver Diseases, Department of Surgery, The University of Hong Kong, Pokfulam, Hong Kong.

³Present address: Marie Curie Research Institute, The Chart, Oxted, Surrey RH8 0TL, U.K.

⁴Present address: Department of Oncological Sciences, Mount Sinai School of Medicine, New York, NY 10029, U.S.A.

Figure 1 | Comparison of the phenotypes of *pli1-d* and *nse2-SA* mutants

(A) Comparison of the organization of the Pli1 and Nse2 proteins. (B) Comparison of the sensitivities of *nse2-SA* and *pli1-d* to DNA-damaging agents, the DNA synthesis inhibitor, HU, and the microtubule inhibitor, TBZ. Aliquots (7 μ l) of 10-fold serial dilutions were spotted on to plates as indicated. (C) ChIP using anti-SUMO sera. ChIP was undertaken on asynchronous cultures according to the method of Strahl-Bolsinger et al. [30] using anti-SUMO serum [31]. QPCR (quantitative PCR) was undertaken using QuantiTect SYBR Green PCR master mix (Qiagen). Fold enrichments were calculated as described in [32]. (D) Silencing assays. Aliquots (7 μ l) of 10-fold dilutions of wild-type, *nse2-SA* and *pli1-d* cells containing the *ura4* gene inserted at *cnt1*, *imr1R*, *otr1R* [33,34] or at the *rDNA* [35] were plated on to media as indicated.



proteins are capable of binding DNA (e.g. [12]). Although the Nse2/Mms21 proteins do not contain a SAP domain, they are associated with DNA, being components of the chromatin-associated Smc5/6 complex [13,14].

Evidence of a role for SUMO and SUMO ligases in genome stability

Evidence of a role for SUMO in genome stability came from early studies on mutants defective in SUMO conjugation that displayed nuclear abnormalities as well as sensitivities

to a range of DNA-damaging agents, such as UV, IR, methyl methanesulfonate and the DNA synthesis inhibitor, HU (hydroxyurea) [15–18].

Following the first identification of the Siz1 and Siz2 SUMO ligases in *S. cerevisiae* [4], two related SUMO ligases, Pli1 and Nse2, were identified in *S. pombe* [7,8]. Surprisingly, unlike mutations in previously identified components of the SUMOylation pathway, deletion of *pli1* results in a rather subtle phenotype, despite the fact that the level of SUMOylation in these cells is dramatically reduced [7]. In this respect, it

resembles the *S. cerevisiae* *siz1* and *siz2* genes [4]. In contrast, Nse2 is essential for cell viability [8]. However, the essential function of Nse2 is likely to be due to the fact that the protein is a component of the essential Smc5/6 complex rather than to its SUMO ligase function, since mutation of the RING zinc finger-like domain to produce the *nse2-SA* strain, causing loss of SUMO ligase activity, results in viable cells [8]. Unlike the situation with *pli1-d*, the level of high- M_r SUMO-containing species in *nse2-SA* cells resembles that in wild-type cells.

Comparison of the phenotypes of *pli1-d* and *nse2-SA* cells indicates that the mutants respond quite differently to DNA-damaging agents (Figure 1B). *nse2-SA* cells are sensitive to HU, methyl methanesulfonate and IR, while *pli1-d* cells are not, but instead are sensitive to the microtubule inhibitor, TBZ (thiabendazole). These results imply that the two SUMO ligases have at least some non-overlapping functions in maintaining genome stability.

Comparison of the properties of *S. pombe* SUMO ligase mutants

ChIP (chromatin immunoprecipitation) using anti-SUMO serum indicates that, in *S. pombe*, SUMO is enriched at regions of complex chromatin structure (Figure 1C). This enrichment is dependent on a functional Hus5 (SUMO conjugator), indicating that the SUMO present at these loci is likely to be in the form of SUMO conjugates, rather than free SUMO. These results are consistent with the recent observation that Hus5 is enriched at regions of heterochromatin [19]. In particular, there is substantial enrichment of SUMO at telomeres and at the central regions of *S. pombe* centromeres. These results imply that SUMOylation has role(s) at these regions. In contrast with what we observe with SUMO, ChIP of Pli1 and Smc6 (as a marker for Nse2) indicates that, while Pli1 and Smc6 are associated with the chromatin, neither of the proteins is specifically enriched at telomeres or centromeres in asynchronously dividing cultures with a slight enrichment of Smc6 at telomeres (F.Z. Watts and A. Irmisch, unpublished work).

If SUMO ligases have roles at telomeres and centromeres, it might be expected that *pli1-d* or *nse2-SA* mutants would display mini-chromosome instability. This is indeed the case: *pli1-d* and *nse2-SA* cells show an increase in mini-chromosome loss of 10- and 15-fold respectively over that observed in wild-type cells [7] (F.Z. Watts, unpublished work).

The loss of mini-chromosome stability in the SUMO ligase mutants could arise through defects in a number of different processes. One possibility might be due to problems associated with telomere maintenance. Early experiments demonstrated that the *S. pombe* SUMO-null mutant has elongated telomeres compared with those in wild-type cells [17]. Longer telomeres have also been detected in *pli1-d* cells, but not in *nse2-SA* [20]. These results add to the notion that Pli1 and Nse2 contribute differently to genome stability.

Other regions important for chromosome segregation are the centromeres. Unlike the centromeres in *S. cerevisiae* that are of the order of 100 bp in length, those in *S. pombe* more closely resemble the centromeres in higher eukaryotes

and are approx. 40–50 kb in length. Owing to their complex chromatin structure, genes inserted into these regions are subjected to transcriptional silencing (e.g. [21]). To assay for silencing in *S. pombe* the *ura4* gene is frequently used since both expression and lack of expression can be tested, using –ura and 5-FOA (5-fluoro-orotic acid)-containing media respectively (5-FOA kills cells that are ura+). In *pli1-d* cells, this silencing ability is lost at *imr1R*, the right-hand side inner repeat region of the centromere on chromosome I, as observed by the growth on –ura medium and the absolute loss of growth on 5-FOA plates [7] (Figure 1D). Interestingly, loss of silencing is not observed at any of the centromeric loci in the *nse2-SA* mutant (Figure 1D). In addition to the silencing defect at *imr1R*, *pli1-d* cells undergo increased gene conversion at this locus [7]. Loss of silencing in *pli1-d* cells is also observed at the *rDNA* locus (which consists of hundreds of tandemly repeated 10.9 kb copies of the 17, 5.8 and 25 S sequences) on chromosome III (Figure 1D).

The role of SUMO and SUMO ligases in meiosis

In addition to their roles in genome stability during the mitotic cell cycle and in response to DNA damage, SUMO and SUMO ligases also have role(s) in meiosis [6,22,23]. Early studies in *Drosophila* on a SUMO conjugator mutant implicated SUMO as being required for dissociation of heterochromatic regions of homologues at the end of meiotic prophase I [22]. More recently, it has been demonstrated that SUMO is associated with pachytene chromosomes in *S. cerevisiae* [6,23] and that mutants in the SUMO conjugator, Ubc9, are defective in synapsis. We have analysed meiosis (more specifically, the ability to form spores) in *nse2-SA/nse2-SA* and *pli1-d/pli1-d* homozygotes and compared it with meiosis in wild-type and *hus5-62/hus5-62* (the *hus5-62* mutation allows interaction of Hus5 with SUMO, but not the conjugation of SUMO on to target proteins [23a]) homozygotes. Figure 2(A) indicates that unlike the case with wild-type cells where regular four-spore asci are observed, the *hus5-62* diploids form aberrant asci. This is also the case with *pli1-d* diploids, but not with *nse2-SA* diploids. Spore viability is dramatically reduced in *hus5-62* and *pli1-d* diploids (23 and 32% respectively) compared with that observed in wild-type and *nse2-SA* diploids (95 and 92% respectively).

One of the processes associated with meiosis is recombination. We were therefore interested in determining whether Pli1 is required for genetic crossing over during meiosis. One of the intervals that we investigated was between *ade7* and *arg6* (approx. 72 kb apart) on chromosome II (Figure 2B). The frequency of crossing over in *pli1-d* cells was reduced 1.9-fold compared with that in the wild-type cross; this compares with a reduction of cross-over frequency for *rhp51* (defective in the *S. pombe* homologue of Rad51) of 2.3-fold [24].

Targets

While many proteins can be SUMOylated without the aid of a ligase *in vitro* if the SUMO conjugator is present in excess, it is likely that SUMO ligases facilitate the SUMOylation

Figure 2 | *pli1-d* but not *nse2-SA* is defective in meiosis

(A) Strains were crossed for 3 days on sporulation medium and then photographed using a Zeiss AxioPlan 2 microscope. (B) Frequency of crossing over between the *arg6-1* and *ade7-152* mutations on chromosome II. Spores were plated on to rich medium and then replica plated separately on to plates lacking arginine or adenine.

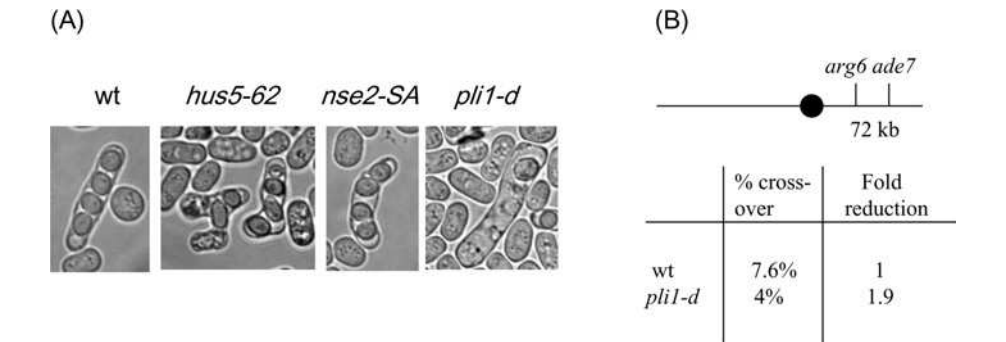
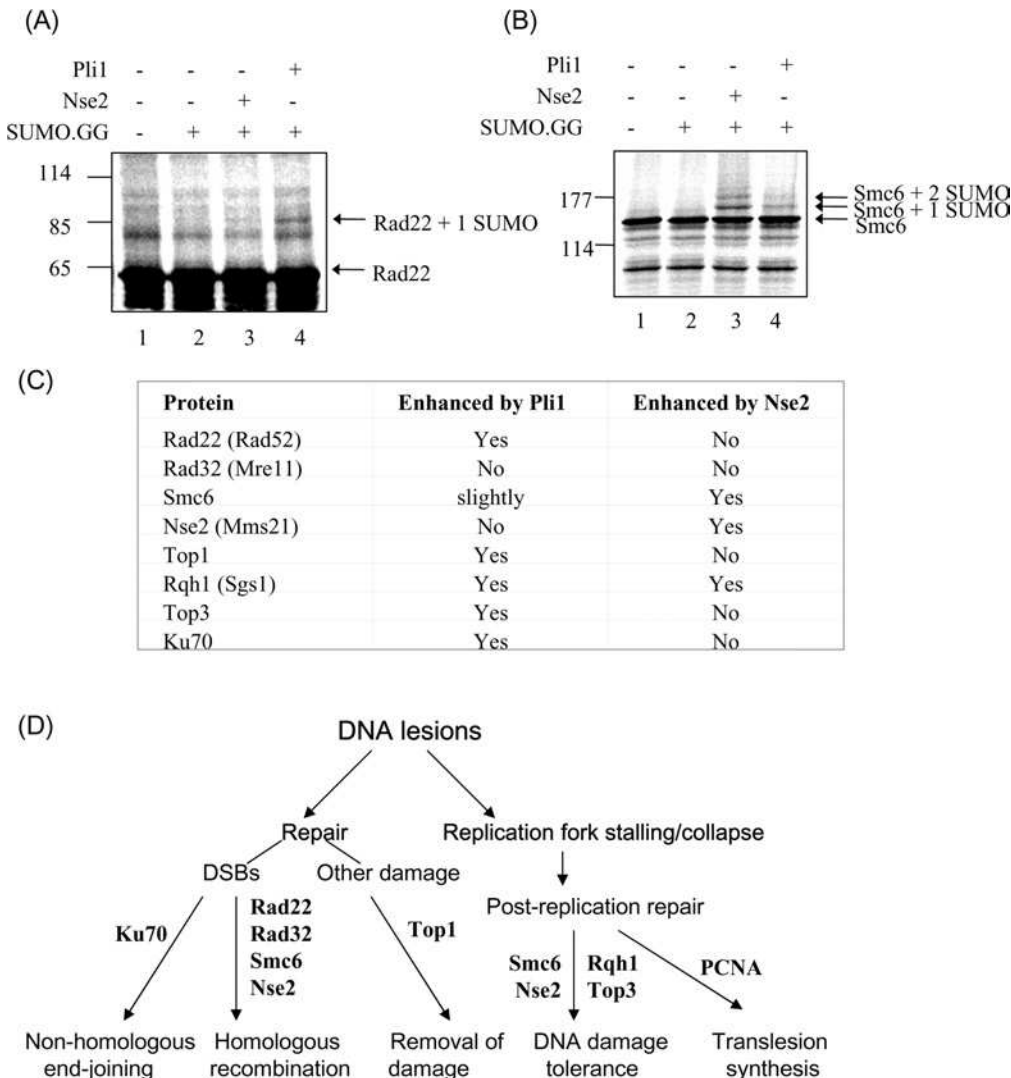


Figure 3 | Nse2 and Pli1 act as SUMO ligases on different targets

In vitro SUMOylation assays were undertaken on ³⁵S-labelled proteins as indicated using the methods of Ho et al. [31] and Andrews et al. [8]. (A) Rad22; and (B) Smc6; molecular names are in kDa. (C) Summary of dependency of SUMOylation of selected targets on Pli1 and Nse2. (D) Scheme showing the involvement of SUMOylated proteins in DNA-damage-response pathways.



of specific subsets of proteins *in vivo*. To date, Pli1 has been shown to act as a SUMO ligase for the recombination protein Rad22 (the homologue of Rad52) [7] (Figure 3A). In contrast, Nse2 does not affect the SUMOylation of Rad22, but facilitates the SUMOylation of some of the members of the Smc5/6 complex, namely Smc6, Nse3 and itself [8] (Figure 3B). In addition to these substrates, we have tested a number of other proteins in *in vitro* SUMOylation assays to determine whether they are dependent on either Pli1 or Nse2 for SUMOylation (Figure 3C). From these results, it is clear that some proteins require either Pli1 or Nse2 for SUMOylation, while SUMOylation of other proteins can be facilitated by both Pli1 and Nse2 or are not affected by incubation with either of the two ligases. For example, SUMOylation of two topoisomerases, Top1 and Top3, and the non-homologous end-joining protein, Ku70, is facilitated by Pli1, but not by Nse2 (Figure 3C). Unlike Rad22, where SUMOylation is enhanced by Pli1, SUMOylation of another recombination protein Rad32 (the homologue of Mre11) is not enhanced by either Pli1 or Nse2. In contrast, SUMOylation of the RecQ-like helicase Rqh1 can be facilitated by both Pli1 and Nse2. Of these proteins tested *in vitro*, the dependency on a specific ligase *in vivo* has been demonstrated for Smc6 and Rad22 [7,8]. It will now be necessary to analyse SUMOylation of the remaining proteins *in vivo* to confirm these results.

Substrate specificity has also been observed for SUMO ligases in other organisms (e.g. [25]). However, it appears that the requirement for specific ligases for SUMOylation of individual proteins may differ between different organisms. For example, SUMOylation of Ku70 is facilitated by Mms21 (the homologue of Nse2) in *S. cerevisiae*, but by Pli1 in *S. pombe*.

Summary

When compared with the number of ubiquitin ligases, relatively few SUMO ligases have been characterized to date. Since genomes contain large numbers of genes encoding RING zinc finger proteins, it is quite possible that more SUMO ligases will be identified.

Figure 3(D) shows that SUMOylation is likely to contribute to genome stability through the modification of proteins in many of the DNA-damage-response pathways. While a number of these proteins have been shown to be modified in other organisms, it appears that SUMOylation, and the regulation of SUMOylation, of individual proteins may differ between organisms. For example, PCNA (proliferating-cell nuclear antigen) is clearly SUMOylated during S-phase in *S. cerevisiae* [26,27] but this is not the case in *S. pombe* or mammals [28].

Comparison of the phenotypes of *pli1-d* and *nse2-SA* indicates that while both proteins have roles in genome stability, they have at least some non-overlapping roles. Pli1 appears to be required for maintaining the stability of regions of complex chromatin structure, whereas Nse2 is likely to have a more global role, possibly in processes required for repair or for recovery from replication fork collapse, e.g. as described in [29]. For Pli1, it might be expected that some of its key substrates are associated with telomeres or centromeres. In this respect,

it is interesting to note that Rad22, a Pli1-dependent target, is enriched at telomeres, implying that Rad22 might have a role in the elongated telomere phenotype in *pli1-d* cells. However, this does not appear to be the case, as the elongated telomeres have been shown to be due to increased telomerase activity [20]. Thus, while it is clear that Pli1 and Nse2 have roles in genome stability and that each of them has specific targets, the identities of the key substrates responsible for the various aspects of SUMO function remain to be determined.

We thank the BBSRC (Biotechnology and Biological Sciences Research Council), MRC, CRUK (Cancer Research UK) and the Leverhulme Trust for support (S03/G152, G0500308, C1206/A5451 and RFG/108390).

References

- Kahyo, T., Nishida, T. and Yasuda, H. (2001) *Mol. Cell* **8**, 713–718
- Sachdev, S., Bruhn, L., Sieber, H., Pichler, A., Melchior, F. and Grosschedl, R. (2001) *Genes Dev.* **15**, 3088–3103
- Schmidt, D. and Muller, S. (2002) *Proc. Natl. Acad. Sci. U.S.A.* **99**, 2872–2877
- Johnson, E.S. and Gupta, A.A. (2001) *Cell* **106**, 735–744
- Zhao, X. and Blobel, G. (2005) *Proc. Natl. Acad. Sci. U.S.A.* **102**, 4777–4782
- Cheng, C.H., Lo, Y.H., Liang, S.S., Ti, S.C., Lin, F.M., Yeh, C.H., Huang, H.Y. and Wang, T.F. (2006) *Genes Dev.* **20**, 2067–2081
- Xhemalce, B., Seeler, J.S., Thon, G., Dejean, A. and Arcangioli, B. (2004) *EMBO J.* **23**, 3844–3853
- Andrews, E.A., Palecek, J., Sergeant, J., Taylor, E., Lehmann, A.R. and Watts, F.Z. (2005) *Mol. Cell. Biol.* **25**, 185–196
- Potts, P.R. and Yu, H. (2005) *Mol. Cell. Biol.* **25**, 7021–7032
- Pichler, A., Gast, A., Seeler, J.S., Dejean, A. and Melchior, F. (2002) *Cell* **108**, 109–120
- Kagey, M.H., Melhuish, T.A. and Wotton, D. (2003) *Cell* **113**, 127–137
- Zhao, X., Sternsdorf, T., Bolger, T.A., Evans, R.M. and Yao, T.P. (2005) *Mol. Cell. Biol.* **25**, 8456–8464
- Okubo, S., Hara, F., Tsuchida, Y., Shimotakahara, S., Suzuki, S., Hatanaka, H., Yokoyama, S., Tanaka, H., Yasuda, H. and Shindo, H. (2004) *J. Biol. Chem.* **279**, 31455–31461
- McDonald, W.H., Pavlova, Y., Yates, III, J.R. and Boddy, M.N. (2003) *J. Biol. Chem.* **278**, 45460–45467
- Sergeant, J., Taylor, E., Palecek, J., Foustier, M., Andrews, E.A., Sweeney, S., Shinagawa, H., Watts, F.Z. and Lehmann, A.R. (2005) *Mol. Cell. Biol.* **25**, 172–184
- al-Khodairy, F., Enoch, T., Hagan, I.M. and Carr, A.M. (1995) *J. Cell Sci.* **108**, 475–486
- Shayeghi, M., Doe, C.L., Tavassoli, M. and Watts, F.Z. (1997) *Nucleic Acids Res.* **25**, 1162–1169
- Tanaka, K., Nishide, J., Okazaki, K., Kato, H., Niwa, O., Nakagawa, T., Matsuda, H., Kawamukai, M. and Murakami, Y. (1999) *Mol. Cell. Biol.* **19**, 8660–8672
- Taylor, D.L., Ho, J.C., Oliver, A. and Watts, F.Z. (2002) *J. Cell Sci.* **115**, 1113–1122
- Shin, J.A., Choi, E.S., Kim, H.S., Ho, J.C., Watts, F.Z., Park, S.D. and Jang, Y.K. (2005) *Mol. Cell* **19**, 817–828
- Xhemalce, B., Riising, E.M., Baumann, P., Dejean, A., Arcangioli, B. and Seeler, J.S. (2007) *Proc. Natl. Acad. Sci. U.S.A.* **104**, 893–898
- Ekwall, K., Cranston, G. and Allshire, R.C. (1999) *Genetics* **153**, 1153–1169
- Apionishev, S., Malhotra, D., Raghavachari, S., Tanda, S. and Rasooly, R.S. (2001) *Genes Cells* **6**, 215–224
- Hooker, G.W. and Roeder, G.S. (2006) *Curr. Biol.* **16**, 1238–1243
- Ho, J.C. and Watts, F.Z. (2003) *Biochem. J.* **372**, 97–104
- Grishchuk, A.L., Kraehenbuehl, R., Molnar, M., Fleck, O. and Kohli, J. (2004) *Curr. Genet.* **44**, 317–328
- Reindle, A., Belichenko, I., Bylebyl, G.R., Chen, X.L., Gandhi, N. and Johnson, E.S. (2006) *J. Cell Sci.* **119**, 4749–4757

-
- 26 Hoege, C., Pfander, B., Moldovan, G.L., Pyrowolakis, G. and Jentsch, S. (2002) *Nature* **419**, 135–141
- 27 Stelter, P. and Ulrich, H.D. (2003) *Nature* **425**, 188–191
- 28 Watts, F.Z. (2006) *DNA Repair* **5**, 399–403
- 29 Branzei, D., Sollier, J., Liberi, G., Zhao, X., Maeda, D., Seki, M., Enomoto, T., Ohta, K. and Foiani, M. (2006) *Cell* **127**, 509–522
- 30 Strahl-Bolsinger, S., Hecht, A., Luo, K. and Grunstein, M. (1997) *Genes Dev.* **11**, 83–93
- 31 Ho, J.C., Warr, N.J., Shimizu, H. and Watts, F.Z. (2001) *Nucleic Acids Res.* **29**, 4179–4186
- 32 Chakrabarti, S.K., James, J.C. and Mirmira, R.G. (2002) *J. Biol. Chem.* **277**, 13286–13293
- 33 Allshire, R.C., Javerzat, J.P., Redhead, N.J. and Cranston, G. (1994) *Cell* **76**, 157–169
- 34 Allshire, R.C., Nimmo, E.R., Ekwall, K., Javerzat, J.P. and Cranston, G. (1995) *Genes Dev.* **9**, 218–233
- 35 Thon, G. and Verhein-Hansen, J. (2000) *Genetics* **155**, 551–568
-

Received 2 July 2007
doi:10.1042/BST0351379