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Opening the DNA repair toolbox

Localization of DNA double strand breaks to the nuclear periphery

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Efficient repair of DNA double strand breaks is essential for cells to avoid increased mutation rates, genomic instability, and even cell death. Consequently, cells have evolved multiple mechanisms for rapidly repairing these DNA lesions, including error-free homologous recombination as well as error-prone pathways such as nonhomologous end joining. What happens to DSBs that are repaired inefficiently or not at all? Recently, several studies in budding yeast have shown that these more recalcitrant DSBs are localized to the nuclear periphery through interactions between the nuclear envelope protein, Mps3, and proteins associated with DSB chromatin. Why these DSBs are tethered to the nuclear periphery is still not clear, though the current view is that alternative repair pathways may be activated at the periphery in a final attempt to repair the lesion. In this Extra View, we discuss these recent reports, and we show that the Est1 component of the telomerase machinery plays an essential role in anchoring DSB chromatin to the nuclear envelope protein, Mps3.

Key words: Mps3, DNA repair, telomerase, nuclear periphery, Est1

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Introduction

DNA double-strand breaks (DSBs) arise as a consequence of external factors, such as gamma irradiation, or endogenous cellular metabolism. Indeed, one major drawback of respiration is that the byproducts of this metabolic process, oxygen free radicals, are potent sources of DNA strand breaks and base modifications. Remarkable, the genome of a proliferating human cell will suffer ~10–50 DSBs within each cell cycle.[1] Since even a single unrepaired DSB can lead to death or harmful chromosomal re-arrangements,[2] it is imperative that the cell develops a very efficient and sophisticated mechanism to detect and repair every DSB.

Repair of DSBs is carried out by two major mechanisms—non-homologous end joining (NHEJ) and homologous recombination (HR).[3] NHEJ is the predominant DSB repair mechanism in the G_1 phase of the cell cycle, although NHEJ can take place throughout the cycle. In this pathway, the broken DNA ends are recognized and bound by the Ku70/Ku80 heterodimer, a complex that is conserved throughout eukaryotes. In *S. cerevisiae*, the Mre11/Rad50/Xrs2 complex is also involved in the processing of DSBs for NHEJ, whereas in mammals NHEJ is associated with a complex of DNA-dependent protein kinase (DNA-PK) and the nuclease, Artemis.[4] Ligation of the broken DNA ends is accomplished by the Lig4/Lif1 complex in *S. cerevisiae* and Lig4/XRCC4 and XLF/Cernunnos factors in mammals.[5-7] Due to nucleolytic processing of DNA ends to make them compatible for subsequent ligation, NHEJ can result in short deletions, and thus this process is error-prone.[4]

Homologous recombination (HR) functions primarily in the S/G_2 phase of the cell cycle, and it relies on sequence homology from an undamaged sister chromatid or a homologous DNA sequence to use as a template for copying the missing information. Consequently, the HR pathway is essentially error-free.[8] Genetic analyses in *S. cerevisiae* reveal that the proteins coded by the *RAD52* epistasis group—*RAD50, RAD51, RAD52, RAD54, RAD55,*

RAD57, RAD59, MRE11 and *XRS2*—mediate HR.[3] The basic HR pathway is highly conserved throughout eukaryotes, with most of the known gene products involved in yeast HR having functional homologs in higher eukaryotes.[9,10]

The first step of HR involves processing of the DSB such that the 5'-ends of the DNA duplex that flank the DSB are nucleolytically processed to generate long, 3' single-stranded tails. The processed DSB ends are initially bound by the single strand DNA binding protein, RPA. Long stretches of ssDNA that are generated by resection, as well as the binding of RPA, provide a critical signal for a damage repair signaling cascade.[11] The end result of this signaling cascade is the activation of checkpoint effector kinases that promote arrest of the cell cycle in late G_2, ensuring that every effort to repair the lesion is made prior to entry into mitosis. RPA is subsequently replaced by the key recombinase, Rad51. This Rad51-ssDNA nucleoprotein filament performs a search for a homologous DNA duplex and catalyzes formation of a DNA joint that can be subsequently extended by DNA polymerases, ultimately resulting in repair of the DSB.[3]

In addition to HR and NHEJ, cells can employ inefficient, alternative repair pathways to heal a chromosome, often leading to gross chromosomal rearrangements (GCRs). GCRs include non-reciprocal translocations, chromosome fusions, isoduplications and de novo addition of telomeres.[12] Translocations constitute a minor percentage of GCR events, whereas deletion and loss of genetic material distal to the DSB and subsequent de novo telomere addition is a major contributor to GCRs. In yeast, the telomerase complex consists of Est1p, Est2p (the catalytic component), Est3p, Sm proteins, and the *TLC1* RNA.[13] At natural telomeric ends, long tracts of single stranded, $TG_{1\text{-}3}$ telomeric repeats provide high affinity binding sites for Cdc13p, a single-stranded DNA binding protein which recruits the telomerase machinery by direct interactions with the Est1p subunit.[14] A second pathway for telomerase recruitment also exists which involves interactions between *TLC1*, Est1/Est2/Est3 and the Ku70/80 heterodimer.[15,16] This latter mechanism appears to play a key role in de novo telomere formation at DSBs that lack extensive TG repeats.[16,17]

Persistent DSBs are Targeted to the Nuclear Periphery

How does the cell respond when a DSB remains un-repaired for a long period of time? In yeast, cells can eventually inactivate the cell cycle checkpoint response (a process termed checkpoint adaptation) and proceed through mitosis with a broken chromosome, but adaptation occurs only after ~15 hours of failed DSB repair.[18] Recently, we used a combination of live cell imaging, chromatin immunoprecipitation analyses, and chromosome conformation capture (3C) to demonstrate that a persistent DSB is re-localized within 1–2 hours to the nuclear periphery.[19] This re-localization process requires the Rad51 recombinase, the ssDNA binding protein Cdc13, DSB processing, and the nuclear envelope protein, Mps3. Indeed, the Mps3 protein can be formaldehyde-crosslinked to chromatin adjacent to a persistent DSB, reflecting the anchoring of the DSB to the nuclear periphery. Likewise, studies from two other groups[20,21] have reported similar phenomena, and these studies demonstrated that peripheral localization of an un-repaired DSB requires an intact cell cycle checkpoint and sumoylation of the histone variant, H2A.Z.

Does peripheral localization of a DSB only occur for lesions that cannot be repaired by NHEJ or HR? We found that DSBs that are rapidly repaired (<2 hours) do not localize to the nuclear periphery,[19] consistent with results from both the Gasser and Jentsch groups.[20,21] Notably, these efficiently repaired DSBs do not lead to robust activation of the cell cycle checkpoint. In contrast, we found that DSBs that are repaired slowly (>3 hours) undergo localization to the nuclear periphery.[19] Thus, localization of DSBs to the nuclear periphery is not restricted to un-repairable DSBs, but correlates more generally with slowly repaired DSBs that robustly activate the cell cycle checkpoint. These studies also indicate that the periphery is not generally inhibitory to recombinational repair, although we found that early steps of the HR process do occur with slower kinetics if a DSB is allowed to anchor at the periphery. This is consistent with a recent report that Mps3-dependent tethering of telomeres to the nuclear periphery inhibits inter-telomere recombination.[22]

What Anchors DSB Chromatin to the Nuclear Periphery?

Chromatin immunoprecipitation analyses have shown that several nucleoporins (Nic96, Nup133, Nup84), and two different inner nuclear membrane proteins (Heh2 and Mps3) associate with chromatin surrounding DSBs that are localized to the nuclear periphery.[19-21] Strikingly, and in contrast to alterations in nucleoporins, deletions of an N-terminal domain of Mps3 eliminates peripheral localization of persistent DSBs.[19] This indicates that Mps3 plays a central role in peripheral localization, and that Mps3 may directly interact with one or more proteins at the DSB.

Mps3/Nep98p (Mono-polar spindle/Nuclear envelope protein) is an essential spindle pole body (SPB) protein which was first identified in two independent screens, one for mutants defective in SPB assembly,[23] and another for proteins interacting with Jem1p and required for nuclear fusion during mating.[24] Mps3 is a 79 kDa integral membrane protein with a predicted inter-membrane region from residues 155–170, and an N-terminal domain (residues 1–155) that is predicted to protrude into the nucleoplasm. The C-terminus of Mps3 is believed to localize to the inter-membranous space of the nuclear membrane. Mps3 contains a SUN domain (Sad1-Unc84) within its inter-membrane region which allows it to connect the SPB with the nuclear envelope.[25,26] Mps3 is the only SUN domain protein in budding yeast, while at least two SUN domain proteins exist in *C. elegans* and Drosophlila, and mammalian cells have four SUN domain proteins. In the latter case, SUN domains are known to interact with the KASH domain of outer nuclear membrane proteins, such as Nesprin, bridging the nuclear envelope to the actin cytoskeleton.[27] In yeast, Mps3 localizes to the SPB, but it is also localized throughout the length of the inner nuclear membrane.[28]

In mitotic cells, Mps3 localizes to telomeres that are clustered into 6–8 foci at the nuclear periphery,[29-31] and this clustering requires the N-terminal domain of Mps3.[28] Likewise, in meiotic cells Mps3 is required for formation of well-defined chromosomal bouquets where telomeres transiently localize into a single focus,[32,33] and Mps3 is required for rapid telomere-led chromosome movements during meiotic prophase.[34] Likewise, meiotic bouquet formation also requires Sun1 and Sun2 in mammalian cells.[35,36]

The interactions between yeast Mps3 and telomeres is likely to be mediated by a direct interaction with the Est1 component of the telomerase machinery.[37] Similarly, interactions between Mps3 and the telomerase machinery appear likely to regulate DSB localization to the periphery. Components of the telomerase machinery, Cdc13, Est1 and Est2, are recruited to un-repairable or slowly repaired DSBs.[19] Furthermore, inactivation of Cdc13 cripples the recruitment of Mps3 to DSB chromatin, suggesting that one or more components of the telomerase machinery may provide the "bridge" between the DSB and Mps3.[19] Recruitment of Cdc13 to the ssDNA of a processed DSB was unexpected, as previous data had suggested that Cdc13 only binds with high affinity to ssDNA that contains telomeric TG_{1-3} repeats. Cdc13 does bind to single stranded TG_{1-3} repeats with a picomolar dissociation constant, but Cdc13 also binds to ssDNA that lacks intact TG_{1-3} repeats with a nanomolar dissociation constant.[38] Thus, Cdc13 has an affinity for random ssDNA that is on the same order of magnitude as other ssDNA binding proteins, like RPA.

Previous two-hybrid and co-immunoprecipitation studies have demonstrated that Mps3 interacts with the Est1 component of the telomerase complex.[37,39] To test whether Est1 plays a central role in anchoring DSB chromatin to Mps3 at the nuclear periphery, we used chromatin immmunoprecipitation assays (ChIP) to monitor association of Mps3 with a persistent or a slowly repaired DSB in wildtype and *est1*Δ cells (**Fig. 1**). When a persistent DSB is induced in a wildtype strain that lacks homologous donor sequences, Mps3 is recruited within 2 hours to DNA proximal to the DSB, reflecting localization of the DSB to the nuclear periphery (**Fig. 1A**). In contrast, Mps3 was not recruited to this DSB in the absence of Est1 (**Fig. 1A**). Likewise, Mps3 was recruited within 2- to 4-hours to a DSB that is slowly repaired by single strand annealing, but recruitment to this DSB also required *EST1* (**Fig. 1B**). Notably, recruitment of Rad51 was not diminished in the absence of Est1, indicating that Est1 functions downstream of Rad51 in this pathway (**Fig. 1C**). These data are consistent with a simple model in which Cdc13 binds to the ssDNA of a processed DSB and recruits Est1 by a direct interaction.[14] Recruitment of Est1 to the DSB would then facilitate subsequent interactions between Est1 and Mps3, resulting in an anchoring of the DSB at the nuclear periphery.

In addition to Cdc13, the Ku70/80 heterodimer binds to DSBs and has been implicated in recruitment of the Est1/Est2/TLC1/Esc3 telomerase complex.[16,40] Ku70 interacts directly with the *TLC1* component of the telomerase complex, and inactivation of Ku70 has a dramatic effect on the efficiency of de novo telomere addition.[16] Furthermore, Shore and colleagues have demonstrated that Cdc13 and Ku70/Ku80 cooperate in the recruitment of Est2 to an HO-induced DSB.[14] To test the possibility that Ku70-dependent recruitment of the telomerase machinery may also contribute to peripheral localization of a persistent DSB, we monitored recruitment of Mps3 to a persistent DSB in isogenic wildtype and *ku70*Δ strains (**Fig. 2**). In the absence of Ku70, recruitment of Mps3 to DSB chromatin is severely decreased, with only a 4- to 5-fold recruitment after 4 hours of DSB formation. Thus, inactivation of Cdc13, Est1 or Ku70 disrupts peripheral localization of a persistent DSB, further emphasizing the key role of the telomerase machinery in this process.

De novo telomere addition at the nuclear periphery? Our results indicate that the telomerase machinery is recruited to a slowly repaired DSB, and that interactions between subunits of the telomerase complex (e.g., Est1) and the nuclear envelope protein, Mps3, lead to a sequestering of these DSBs at the nuclear periphery. Do these events then lead to obligatory de novo telomere formation? A previous study from Kramer and Haber demonstrated that de novo telomere formation is an extremely rare event at a persistent, HO-induced DSB.[41] In contrast, de novo telomere formation becomes efficient if a tract of 81 telomeric TG_{1-3} repeats is inserted adjacent to the HO recognition site.[42] These sequences provide a "seed" for subsequent extension of the telomeric end by the telomerase enzyme. Insertion of a small number of TG-rich sequences at 1.6 kb or 10 kb from the HO-induced DSB can also enhance de novo telomere formation, but the frequency of such events remains very low (~1%).[41] Thus, although slowly repaired DSBs are recognized as potential substrates for de novo telomere addition by the telomerase machinery, de novo telomere formation is not efficient in the absence of suitable TG_{1-3} repeats.

Nuclear pore complexes and Mps3: two distinct repair environments at the periphery? What if telomere addition fails? Several studies suggest that persistent, un-repaired DSBs may be shuttled from their initial, Mps3 interaction sites on the nuclear envelope to nuclear pore complexes (NPCs). This two-step model for DSB localization was recently discussed by Gartenberg.[43] In a recent study from Gasser and colleagues, live cell imaging was used to monitor the subnuclear location of a single, unrepaired DSB.[20] They observed that the DSB became immobilized at the nuclear periphery two hours following DSB induction, and several components of the NPC could be detected by ChIP at the DSB. Furthermore, evidence was presented suggesting that a replication fork that stalls at a DSB is also localized to NPCs.

Recently, Lisby and colleagues have reported that eroded yeast telomeres also co-localize with NPCs.[44] In these studies, cells that lack telomerase (*est2*Δ) were grown for 20–50 generations to induce erosion of telomeres. Alternatively, a single, short telomere was created in telomerase deficient cells using a novel Flp recombinase-based system.[45] In both cases, these critically shortened telomeres recruited components of the recombination (e.g., Rad52) and cell cycle checkpoint (e.g., Mre11, Ddc2) machinery, and the eroded telomeres became NPC-associated. In contrast, unperturbed

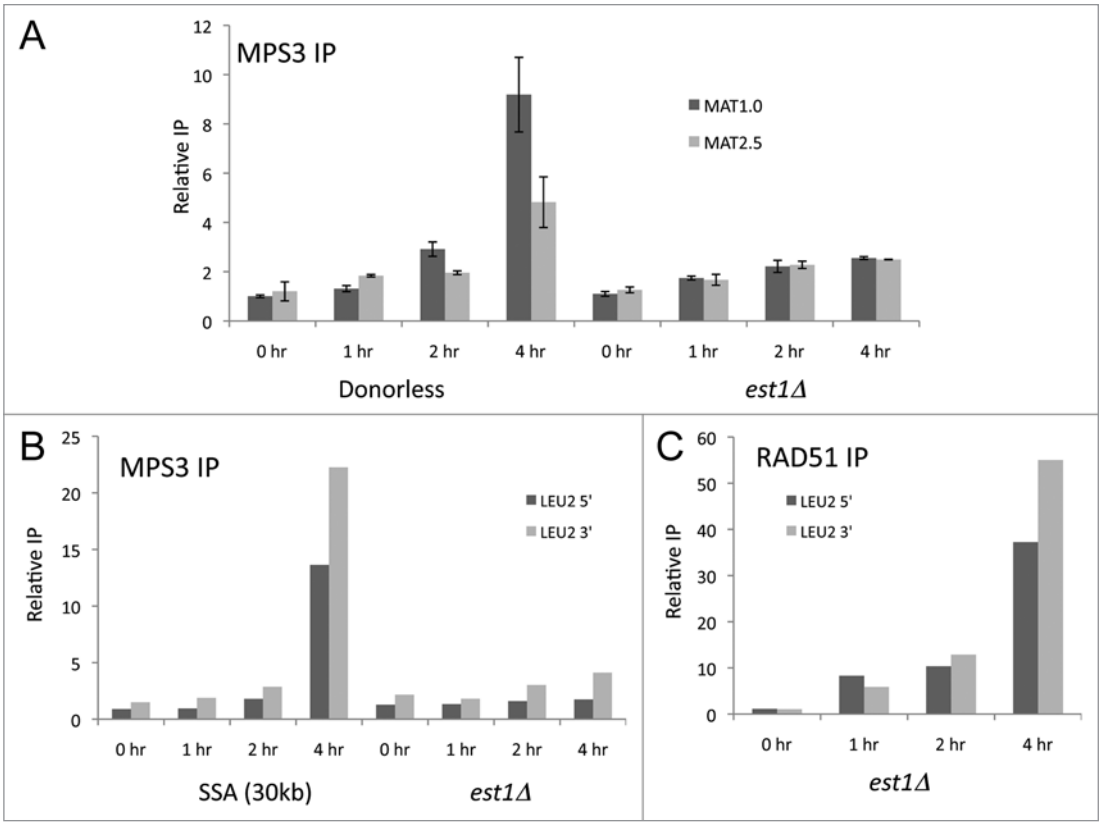

Figure 1. Est1 is essential for recruitment Mps3 to persistent and slowly repaired DSBs. (A) ChIP analysis of Mps3p was conducted in isogenic *MPS3-13Myc* and *est1Δ MPS3-13Myc* strains using polyclonal anti-myc antiserum (9E10, SantaCruz). Strains also contain a *GAL10-HO* gene, allowing for induction of the HO endonuclease by growth in galactose media. A single DSB was induced at the *MAT* locus in derivatives of the *MAT***a** donorless strain (CY915) in which the homologous donors, *HML* and *HMR*, have been deleted. Unique primers were designed 1.0 and 2.5 kb distal to the DSB, and ChIP was carried out and analyzed as described.[18] The %IP (IP/Input) values were normalized to the %IP for the control *ACT1* ORF. To compare between each of the two independent experiments shown, the %IP values were normalized to the time zero samples to yield the fold IP values plotted on the y-axis. All ChIPs plotted on the same panel were always carried out simultaneously. Error bars represent one standard error of the mean for three independent experiments. Primer sequences are available upon request. (B) ChIP analysis of Mps3 in an SSA strain (YMV45) where the HO-cs is present within the *LEU2* gene on chromosome III. This DSB is repaired by single-strand annealing after ~30 kb of resection. Assays performed as in (A). (C) Recruitment of Rad51 to a persistent DSB does not require Est1. ChIP was carried out using Rad51p antibodies (Santa Cruz Biochem.), and immunoprecipitated DNAs were analyzed as in (A).

telomeres normally localize to 3–6 foci at the nuclear periphery in S/G_2 cells, and this clustering requires Mps3.[28] Indeed, a recent study suggests that Mps3 provides the primary anchor that tethers telomeres to the nuclear periphery.[46] Thus these data suggest that eroded telomeres, much like DSBs that fail to form a de novo telomere, may re-localize from Mps3 sites on the nuclear membrane to NPCs.

Whereas Mps3-containing regions of the nuclear envelope may promote telomere addition, the NPC may represent a site at the nuclear periphery that promotes recombinational repair. Mutations that disrupt the Nup84 subcomplex of the NPC are synthetically lethal in combination with mutations in the *RAD52* group of genes, and more recent E-MAP data (epistatic miniarray profiling) place components of the Nup84 subcomplex and *RAD52* gene products together within the same group.[20] Gasser and colleagues have demonstrated that tethering a chromosomal locus to sites near or at NPCs can enhance spontaneous gene conversion, and this enhanced rate of recombinational repair requires the Nup84 component of the NPC.[20] Furthermore, the recombination factor, Rad52, accumulates in foci that co-localize with eroded telomeres, suggesting that NPC association may also promote recombination events that rescue critically shortened telomeres.[44]

SUMOylation, peripheral localization and GCRs. Potential roles for the NPC in recombinational repair appears to be tightly linked to SUMOylation pathways. For instance, the essential yeast SUMO peptidase, Ulp1, is associated with the NPC, and loss of its peripheral localization leads to DNA damage phenotypes reminiscent of *nup84* mutants.[47-49] Likewise, the Slx5 and Slx8 SUMO-targeted ubiquitin ligases map to the same E-MAP group as Nup84 and *RAD52* group members, and Slx5 and Slx8 associate with NPCs and can be detected by ChIP at a persistent DSB.[20] *SLX5* and *SLX8* were first identified in a screen for mutants that show synthetic lethality with mutations in the *SGS1* gene that encodes a homolog of the RecQ helicase.[50] It has been suggested that the activity of the Slx5/8 complex in yeast may reflect that of the BRCA1-BARD1 complex in

human cells,[51] although further analysis has revealed a closer homologue in *RNF4*, which can substitute for the function of both proteins in yeast.[52]

Genetic studies have shown that s*lx5*Δ and *slx8*Δ mutations are synthetically lethal with nearly every component of the SUMO pathway, and these mutants show a huge increase in the level of SUMOylated proteins.[53] Moreover, *slx5/8*Δ strains show increased spontaneous DNA damage in the form of Rad52 foci and increased numbers of GCRs.[20,54] Interestingly, the nucleoplasmic domain of Mps3 is required for the enhanced level of genome instability observed in the absence of Slx5.[19] This provides the strongest evidence supporting the view that unrepaired DSBs must first transit through Mps3 sites at the periphery prior to re-localizing to NPCs. Furthermore, these genetic data indicate that GCR events that occur in the absence of Slx5 require peripheral association of DSBs.

In a recent study, Burgess et al. demonstrated that Slx8 localizes to replication centers marked by Pol30 foci.[55] This is associated with an increase in the number and duration of Rad52 foci during S-phase, suggesting that Slx5/8 may act to suppress damage that occurs during replication and to channel that damage into faster repair pathways. The relevant substrates for Slx5/8 are not currently known, though it has been suggested that they may target the degradation of proteins involved in Rad51-independent recombination pathways. Slx5/8 may also target components of the telomerase machinery since spontaneous de novo telomere events are enhanced in *slx5* mutants. Given the genetic interactions between *SLX5* and *MPS3*,[19] and previous studies from Nagai and colleagues,[20] collapsed replication forks may localize to NPCs where Slx5/8 acts on SUMOylated proteins to inhibit de novo telomere addition and Rad51-independent repair events. In this model, the collapsed fork may initially interact with the NPC, and de novo telomere addition would only occur in the absence of Slx5 activity. These latter events would require re-localization from the NPC to Mps3 sites at the periphery. Alternatively, collapsed forks might transit Mps3 sites prior to NPC association

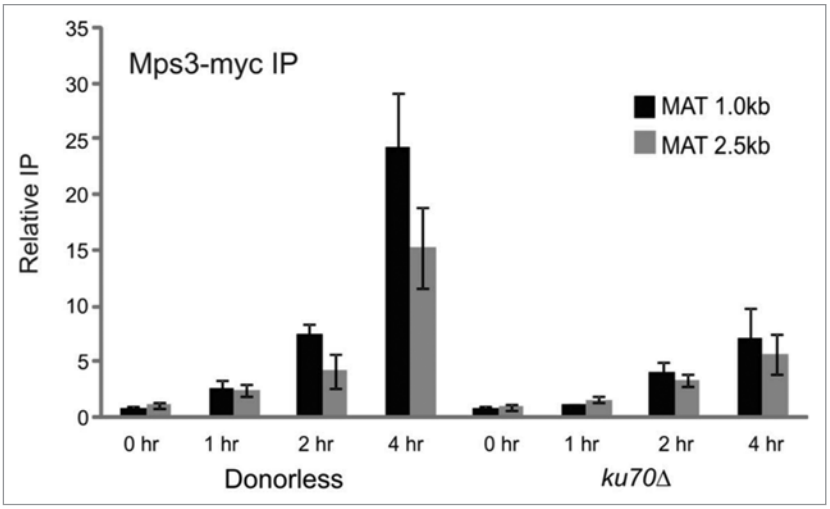

Figure 2. Ku70 is required for optimal recruitment of Mps3 to a persistent DSB. ChIP analysis of Mps3p was conducted in isogenic *MPS3-13Myc* and *ku70*Δ *MPS3-13Myc* strains using polyclonal anti-myc antiserum (9E10, SantaCruz). A single DSB was induced at the *MAT* locus in derivatives of the *MAT***a** donorless strain (CY915) in which the homologous donors, *HML* and *HMR*, have been deleted. Assays were performed as described in the legend to Figure 1.

(**Fig. 3**). Although such models are clearly speculative, it is clear that the nuclear periphery is a complex environment in which many different repair pathways may be regulated.

Studies in yeast indicate that slowly repaired DSBs that have been processed for HR are localized to the nuclear periphery. It is currently unclear if a similar pathway exists in mammalian cells. Live cell imaging data has suggested that DSBs are immobile in the mammalian nucleus,[56] but the majority of such DSBs are likely to be substrates for NHEJ and thus would not be expected to enter into the pathway described in yeast. One possibility is that DSBs that are induced within mammalian cells that cannot undergo NHEJ, such as cells defective in the Artemis ligase complex, may show DSB movements to the periphery. Given the large size of the mammalian nucleus (>50 microns), such movements may parallel the dramatic chromosome movements that occur during meiosis. Notably, these meiotic movements require SUN domain proteins in both yeast and mammalian cells, indicating that the machinery for large-scale localization to the nuclear periphery may be conserved. Whether the nuclear periphery of mammalian cells harbors an environment that is permissive for alternative repair pathways remains to be directly investigated.

References

1. Vilenchik MM, Knudson AG. Endogenous DNA double-strand breaks: Production, fidelity of repair and induction of cancer. Proc Natl Acad Sci USA 2003; 100:19-21.
2. Downs JA, Nussenzweig MC, Nussenzweig A. Chromatin dynamics and the preservation of genetic information. Nature 2007; 447:951-8.
3. Paques F, Haber JE. Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. Microbiol Mol Biol Rev 1999; 63:349-404.
4. Lieber MR, Ma Y, Pannicke U, Schwarz K. Mechanism and regulation of human non-homologous DNA end-joining. Nat Rev Mol Cell Biol 2003; 4:712-20.
5. Cahill D, Connor B, Carney JP. Mechanisms of eukaryotic DNA double strand break repair. Front Biosci 2006; 11:1958-76.
6. Daley JM, Palmbos PL, Wu D, Wilson TE. Nonhomologous end joining in yeast. Annu Rev Genet 2005; 39:431-51.
7. van Gent DC, van der Burg M. Non-homologous end-joining, a sticky affair. Oncogene 2007; 26:7731-40.
8. Jackson SP. Sensing and repairing DNA double-strand breaks. Carcinogenesis 2002; 23:687-96.
9. Sonoda E, Takata M, Yamashita YM, Morrison C, Takeda S. Homologous DNA recombination in vertebrate cells. Proc Natl Acad Sci USA 2001; 98:8388-94.
10. Thompson LH, Schild D. Homologous recombinational repair of DNA ensures mammalian chromosome stability. Mutat Res 2001; 477:131-53.
11. Harper JW, Elledge SJ. The DNA damage response: ten years after. Mol Cell 2007; 28:739-45.
12. Pennaneach V, Putnam CD, Kolodner RD. Chromosome healing by de novo telomere addition in *Saccharomyces cerevisiae*. Mol Microbiol 2006; 59:1357-68.
13. Seto AG, Zaug AJ, Sobel SG, Wolin SL, Cech TR. *Saccharomyces cerevisiae* telomerase is an Sm small nuclear ribonucleoprotein particle. Nature 1999; 401:177-80.
14. Bianchi A, Negrini S, Shore D. Delivery of yeast telomerase to a DNA break depends on the recruitment functions of Cdc13 and Est1. Mol Cell 2004; 16:139-46.

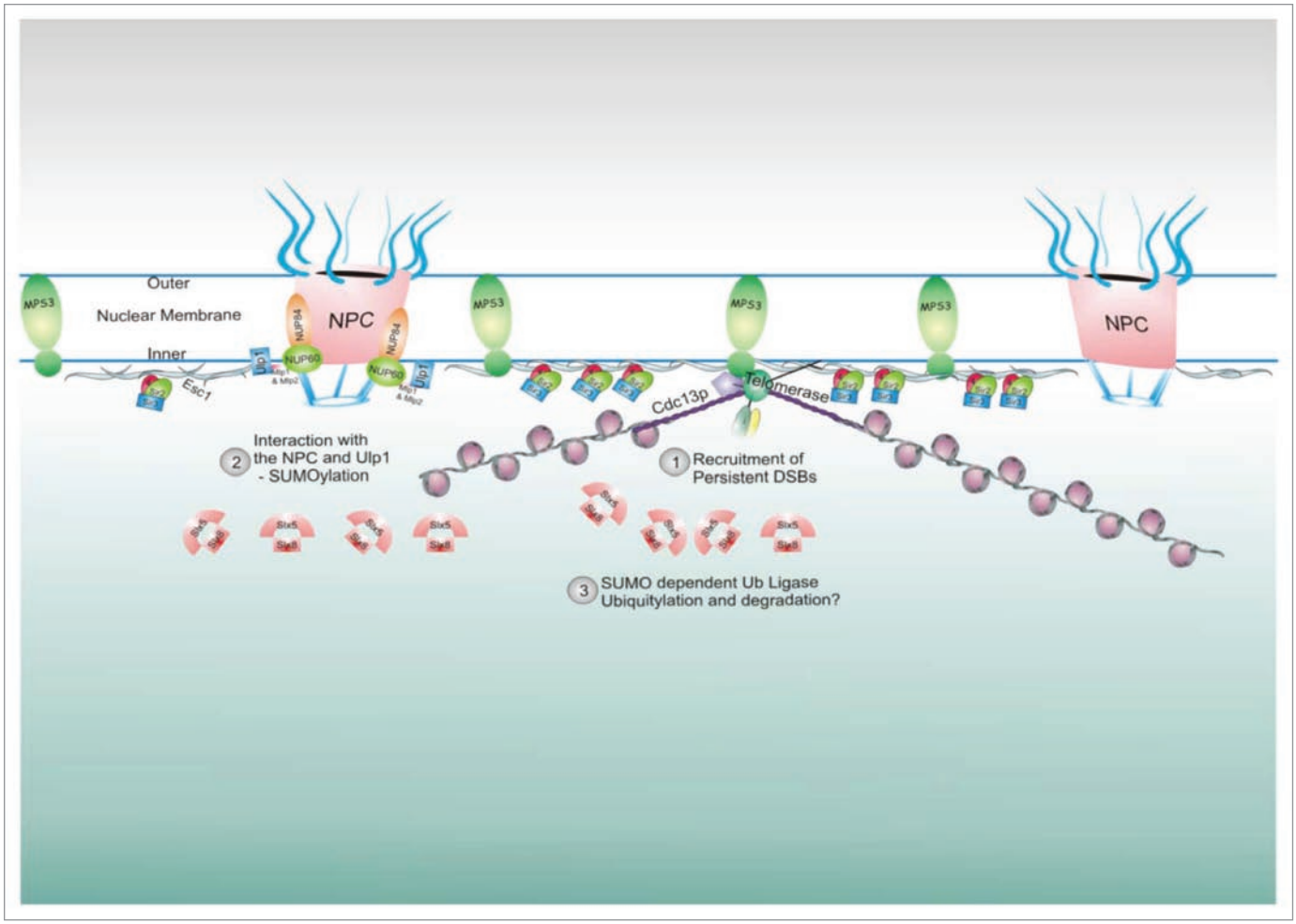

Figure 3. Model for localization of a persistent or slowly repaired DSB to the nuclear periphery. A persistent or slowly repaired DSB promotes the recruitment of Cdc13 and the Est1/Est2/Est3 telomerase complex. Interactions between the Est1 subunit and the integral membrane protein, Mps3 leads to localization of the DSB to the nuclear envelope. Once at the envelope, the DSB may subsequently interact with the NPC and undergo SUMOylation/de-SUMOylation as well as later ubiquitylation reactions by the SUMO protease Ulp1 and Slx5/8 respectively. These modifications then guide the healing and repair events of the DSB at the periphery.

15. Chan A, Boule J-B, Zakian VA. Two pathways recruit telomerase to *Saccharomyces cerevisiae* telomeres. PLoS Genet 2008; 4:1000236.
16. Stellwagen AE, Haimberger ZW, Veatch JR, Gottschling DE. Ku interacts with telomerase RNA to promote telomere addition at native and broken chromosome ends. Genes Dev 2003; 17:2384-95.
17. Negrini S, Ribaud V, Bianchi A, Shore D. DNA breaks are masked by multiple Rap1 binding in yeast: implications for telomere capping and telomerase regulation. Genes Dev 2007; 21:292-302.
18. Toczyski DP, Galgoczy DJ, Hartwell LH. CDC5 and CKII control adaptation to the yeast DNA damage checkpoint. Cell 1997; 90:1097-106.
19. Oza P, Jaspersen SL, Miele A, Dekker J, Peterson CL. Mechanisms that regulate localization of a DNA double-strand break to the nuclear periphery. Genes Dev 2009; 23:912-27.
20. Nagai S, Dubrana K, Tsai-Pflugfelder M, Davidson MB, Roberts TM, Brown GW, et al. Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase. Science 2008; 322:597-602.
21. Kalocsay M, Hiller NJ, Jentsch S. Chromosome-wide Rad51 spreading and SUMO-H2A.Z-dependent chromosome fixation in response to a persistent DNA double-strand break. Mol Cell 2009; 33:335-43.
22. Schober H, Ferreira H, Kalck V, Gehlen LR, Gasser SM. Telomerase and SUN domain protein Mps3 anchors and protects telomeres in budding yeast. Genes Dev 2009; In press.
23. Jaspersen SL, Giddings TH Jr, Winey M. Mps3p is a novel component of the yeast spindle pole body that interacts with the yeast centrin homologue Cdc31p. J Cell Biol 2002; 159:945-56.
24. Nishikawa S, Terazawa Y, Nakayama T, Hirata A, Makio T, Endo T. Nep98p is a component of the yeast spindle pole body and essential for nuclear division and fusion. J Biol Chem 2003; 278:9938-43.
25. Jaspersen SL, Martin AE, Glazko G, Giddings TH Jr, Morgan G, Mushegian A, et al. The Sad1-UNC-84 homology domain in Mps3 interacts with Mps2 to connect the spindle pole body with the nuclear envelope. J Cell Biol 2006; 174:665-75.
26. Tzur YB, Wilson KL, Gruenbaum Y. SUN-domain proteins: 'Velcro' that links the nucleoskeleton to the cytoskeleton. Nat Rev Mol Cell Biol 2006; 7:782-8.
27. Starr DA, Han M. Role of ANC-1 in tethering nuclei to the actin cytoskeleton. Science 2002; 298:406-9.
28. Bupp JM, Martin AE, Stensrud ES, Jaspersen SL. Telomere anchoring at the nuclear periphery requires the budding yeast Sad1-UNC-84 domain protein Mps3. J Cell Biol 2007; 179:845-54.
29. Gotta M, Laroche T, Formenton A, Maillet L, Scherthan H, Gasser SM. The clustering of telomeres and colocalization with Rap1, Sir3 and Sir4 proteins in wild-type *Saccharomyces cerevisiae*. J Cell Biol 1996; 134:1349-63.
30. Klein F, Laroche T, Cardenas ME, Hofmann JF, Schweizer D, Gasser SM. Localization of RAP1 and topoisomerase II in nuclei and meiotic chromosomes of yeast. J Cell Biol 1992; 117:935-48.
31. Palladino F, Laroche T, Gilson E, Axelrod A, Pillus L, Gasser SM. SIR3 and SIR4 proteins are required for the positioning and integrity of yeast telomeres. Cell 1993; 75:543-55.
32. de La Roche Saint-Andre C. Alternative ends: Telomeres and meiosis. Biochimie 2008; 90:181-9.
33. Scherthan H. Telomeres and meiosis in health and disease. Cellular and Molecular Life Sciences (CMLS) 2007; 64:117-24.
34. Conrad MN, Lee CY, Chao G, Shinohara M, Kosaka H, Shinohara A, et al. Rapid telomere movement in meiotic prophase is promoted by NDJ1, MPS3, and CSM4 and is modulated by recombination. Cell 2008; 133:1175-87.

35. Schmitt J, Benavente R, Hodzic D, Stewart CL, Alsheimer M. Transmembrane protein Sun2 is involved in tethering mammalian meiotic telomeres to the nuclear envelope. Proc Natl Acad Sci USA 2007; 104:7426-31.
36. Ding X, Xu R, Yu J, Xu T, Zhuang Y, Han M. SUN1 Is required for telomere attachment to nuclear envelope and gametogenesis in mice. Dev Cell 2007; 12:863-72.
37. Antoniacci LM, Kenna MA, Skibbens RV. The nuclear envelope and spindle pole body-associated Mps3 protein bind telomere regulators and function in telomere clustering. Cell Cycle 2007; 6:75-9.
38. Eldridge AM, Halsey WA, Wuttke DS. Identification of the determinants for the specific recognition of single-strand telomeric DNA by Cdc13&#x2020. Biochemistry 2006; 45:871-9.
39. Uetz P, Giot L, Cagney G, Mansfield TA, Judson RS, Knight JR, et al. A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae*. Nature 2000; 403:623-7.
40. Grandin N, Damon C, Charbonneau M. Cdc13 cooperates with the yeast Ku proteins and Stn1 to regulate telomerase recruitment. Mol Cell Biol 2000; 20:8397-408.
41. Kramer KM, Haber JE. New telomeres in yeast are initiated with a highly selected subset of TG_{1-3} repeats. Genes Dev 1993; 7:2345-56.
42. Diede SJ, Gottschling DE. Telomerase-mediated telomere addition in vivo requires DNA primase and DNA polymerases ± and '. Cell 1999; 99:723-33.
43. Gartenberg MR. Life on the edge: telomeres and persistent DNA breaks converge at the nuclear periphery. Genes Dev 2009; 23:1027-31.
44. Khadaroo B, Teixeira MT, Luciano P, Eckert-Boulet N, Germann SM, Simon MN, et al. The DNA damage response at eroded telomeres and tethering to the nuclear pore complex. Nat Cell Biol 2009; 11:980-7.
45. Abdallah P, Luciano P, Runge KW, Lisby M, Geli V, Gilson E, et al. A two-step model for senescence triggered by a single critically short telomere. Nat Cell Biol 2009; 11:988-93.
46. Schober H, Ferreira H, Kalck V, Gehlen LR, Gasser SM. Yeast telomerase and the SUN domain protein Mps3 anchor telomeres and repress subtelomeric recombination. Genes Dev 2009; 23:928-38.
47. Loeillet S, Palancade B, Cartron M, Thierry A, Richard G-F, Dujon B, et al. Genetic network interactions among replication, repair and nuclear pore deficiencies in yeast. DNA Repair 2005; 4:459-68.
48. Palancade B, Doye V. Sumoylating and desumoylating enzymes at nuclear pores: underpinning their unexpected duties? Trends Cell Biol 2008; 18:174-83.
49. Palancade B, Liu X, Garcia-Rubio M, Aguilera A, Zhao X, Doye V. Nucleoporins prevent DNA damage accumulation by modulating Ulp1-dependent sumoylation processes. Mol Biol Cell 2007; 18:2912-23.
50. Mullen JR, Kaliraman V, Ibrahim SS, Brill SJ. Requirement for three novel protein complexes in the absence of the Sgs1 DNA helicase in *Saccharomyces cerevisiae*. Genetics 2001; 157:103-18.
51. Mullen JR, Brill SJ. Activation of the Slx5-Slx8 ubiquitin ligase by poly-small ubiquitin-like modifier conjugates. J Biol Chem 2008; 283:19912-21.
52. Sun H, Leverson JD, Hunter T. Conserved function of RNF4 family proteins in eukaryotes: targeting a ubiquitin ligase to SUMOylated proteins. EMBO J 2007; 26:4102-12.
53. Wang Z, Jones GM, Prelich G. Genetic analysis connects SLX5 and SLX8 to the SUMO pathway in *Saccharomyces cerevisiae*. Genetics 2006; 172:1499-509.
54. Zhang C, Roberts TM, Yang J, Desai R, Brown GW. Suppression of genomic instability by SLX5 and SLX8 in *Saccharomyces cerevisiae*. DNA Repair 2006; 5:336-46.
55. Burgess RC, Rahman S, Lisby M, Rothstein R, Zhao X. The Slx5-Slx8 complex affects sumoylation of DNA repair proteins and negatively regulates recombination. Mol Cell Biol 2007; 27:6153-62.
56. Soutoglou E, Dorn JF, Sengupta K, Jasin M, Nussenzweig A, Ried T, et al. Positional stability of single double-strand breaks in mammalian cells. Nat Cell Biol 2007; 9:675-82.