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.

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. Since even a single unrepaired DSB can lead to death or harmful chromosomal re-arrangements, 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). NHEJ is the predominant DSB repair mechanism in the G1 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/Lig1 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/G2 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,
involved in yeast HR having functional with most of the known gene products highly conserved throughout eukaryotes.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 G2, 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.1

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, TG1-3 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 groups20,21 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 Drosophila, 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

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**RAD57, RAD59, MRE11 and XRS2—mediated HR.** 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.10
In mitotic cells, Mps3 localizes to telomeres that are clustered into 6–8 foci at the nuclear periphery. Likewise, in meiotic cells Mps3 is required for formation of well-defined chromosomal bouquets where telomeres transiently localize into a single focus, and Mps3 is required for rapid telomere-limited chromosome movements during meiotic prophase. Likewise, meiotic bouquet formation also requires Sun1 and Sun2 in mammalian cells.

The interactions between yeast Mps3 and telomeres is likely to be mediated by a direct interaction with the Est1 component of the telomerase machinery. 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. 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. 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. 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. To test whether Est1 plays a central role in anchoring DSB chromatin to Mps3, we monitored association of Mps3 with a persistent DSB in isogenic cells lacking Est1. Est1 functions downstream of Rad51 in this pathway. 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. 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. 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. Furthermore, Shore and colleagues have demonstrated that Cdc13 and Ku70/Ku80 cooperate in the recruitment of Mps3 to a persistent DSB in isogenic wildtype and ku70Δ strains. 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. 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. 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%). 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. In a recent study from Gasser and colleagues, live cell imaging was used to monitor the subnuclear location of a single, unrepairred DSB. 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. In these studies, cells that lack telomerase (est2Δ) 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. In both cases, 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...
SUMOylation, peripheral localization, and GCRs. Potential roles for the NPC in recombinational repair appear 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. 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. Further, the recombination factor, Rad52, accumulates in foci that co-localize with eroded telomeres, suggesting that NPC association may also promote recombinational repair events that rescue critically shortened telomeres.

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Genetic studies have shown that slx5Δ and slx8Δ 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. Moreover, slx5/8Δ strains show increased spontaneous DNA damage in the form of Rad52 foci and increased numbers of GCRs. Interestingly, the form of Rad52 foci and increased spontaneous DNA damage in human cells, although further analysis has revealed a closer homologue in RNFL4, which can substitute for the function of both proteins in yeast.

In a recent study, Burgess et al. demonstrated that Slx8 localizes to replication centers marked by Pol30 foci. 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 MP3S, and previous studies from Nagai and colleagues, 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 (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, although 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 MP3S, and previous studies from Nagai and colleagues, 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 (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.

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 ku70Δ MPS3-13Myc strains using polyclonal anti-myc antiserum (9E10, SantaCruz). A single DSB was induced at the MAT locus in derivatives of the MATa 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.

References
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 Sir5/Sir 8 respectively. These modifications then guide the healing and repair events of the DSB at the periphery.