SWR1 and INO80 Chromatin Remodelers Contribute to DNA Double-Strand Break Perinuclear Anchorage Site Choice

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SUMMARY

Persistent DNA double-strand breaks (DSBs) are recruited to the nuclear periphery in budding yeast. Both the Nup84 pore subcomplex and Mps3, an inner nuclear membrane (INM) SUN domain protein, have been implicated in DSB binding. It was unclear what, if anything, distinguishes the two potential sites of repair. Here, we characterize and distinguish the two binding sites. First, DSB-pore interaction occurs independently of cell-cycle phase and requires neither the chromatin remodeler INO80 nor recombinase Rad51 activity. In contrast, Mps3 binding is S and G2 phase specific and requires both factors. SWR1-dependent incorporation of Htz1 (H2A.Z) is necessary for break relocation to either site in both G1- and S-phase cells. Importantly, functional assays indicate that mutations in the two sites have additive repair defects, arguing that the two perinuclear anchorage sites define distinct survival pathways.

INTRODUCTION

Improperly repaired DNA double-strand breaks (DSBs) can lead to genomic rearrangements and loss of genetic information (Jackson and Bartek, 2009), making them one of the most hazardous forms of genomic damage. DSBs arise both from exogenous agents, such as γ irradiation or chemical insult, and from endogenous events, such as replication fork collapse (Pfeiffer et al., 2000).

DSB repair is generally achieved by two conserved mechanisms: nonhomologous end-joining (NHEJ) or homologous recombination (HR) (Chapman et al., 2012). In haploid yeast, NHEJ is prominent only in G1 phase, whereas, in mammals, it dominates throughout the cell cycle (Smeenk and van Attikum, 2013). Repair by HR requires a homologous donor that serves as a template for DNA synthesis, being most commonly provided by the replicated sister chromatid. The choice of repair by HR over NHEJ is dictated in part by 5’ to 3’ end resection at the break, which requires the activity of the S-phase cyclin-dependent kinase (Ira et al., 2004). The resulting single-stranded DNA (ssDNA) overhang is coated by replication protein A (RPA) and later by the Rad51 recombinase. This ssDNA-Rad51 nucleoprotein filament mediates homology search and strand invasion, enabling error-free, recombination-mediated repair. Other less-precise, recombination-based events can also occur, including break-induced replication or template switching, particularly at damaged replication forks (Aguilera and García-Muse, 2013).

Recent work has highlighted the importance of ATP-dependent chromatin remodelers in DSB repair. In yeast, the remodeler complexes RSC and INO80 and the SWR1 complex (SWR-C) are sequentially recruited to breaks, whereas, in mammalian cells, the SWI/SNF homolog as well as INO80 and SRCAP are implicated in repair pathway choice and outcome (reviewed in Peterson and Almouzni, 2013; Price and D’Andrea, 2013; Seeber et al., 2013b; Smeenk and van Attikum, 2013). The budding yeast complexes INO80 and SWR-C accumulate at breaks at much higher levels in S and G2 than G1, coincident with end resection and Rad51 binding (Bennett et al., 2013). Indeed, the recruitment of INO80 facilitates short-range resection at DSBs and Rad51 binding, possibly because of the preferential eviction of H2A.Z-containing nucleosomes (Papamichos-Chronakis et al., 2011; Tsukuda et al., 2005; van Attikum et al., 2004, 2007). More recent work implicates the FUN30 remodeler in long-range end resection (Chen et al., 2012; Costelloloe et al., 2012). In contrast, SWR-C exchanges H2A-H2B dimers for Htz1-H2B at promoters, telomeres, centromeres, and, in some cases, DSBs, but its loss does not impair end resection (Kobor et al., 2004; Krogan et al., 2003; Luk et al., 2010; Mizuguchi et al., 2004; Papamichos-Chronakis et al., 2006; van Attikum et al., 2007; Wu et al., 2005). Instead, SWR-C appears to promote the association of yeast Ku to broken ends, facilitating error-free NHEJ (van Attikum et al., 2007). INO80, on the other hand, was shown to facilitate replication fork restart after stalling or collapse of replication forks (Papamichos-Chronakis and Peterson, 2008; Shimada et al., 2008).

Another intriguing effect of chromatin remodeler recruitment to DSBs is the enhanced subdiffusive movement scored for
fluorescently tagged DSBs in yeast (Dion et al., 2012; Miné-Hat-tab and Rothstein, 2012; Neumann et al., 2012). Not only the site of damage, but other tagged loci throughout the genome showed a general increase in mobility after DSB induction in a manner dependent on checkpoint response and the INO80 remodeler (Neumann et al., 2012; Seeber et al., 2013a). Other studies established that DSBs, which lack a functional donor for HR shift at least transiently to the nuclear periphery, where they appear to bind either the Nup84 nuclear pore subcomplex or an essential inner nuclear membrane Sad1-Unc-84-related (SUN) domain protein Mps3 (Kalocsay et al., 2009; Nagai et al., 2008; Oza et al., 2009; Oza and Peterson, 2010). Fluorescence microscopy confirmed that critically short telomeres and collapsed replication forks associate with nuclear pores (Khadaroo et al., 2009; Nagai et al., 2008), yet it has remained unclear whether Mps3 and pores constitute independent or interdependent sites of DSB interaction. Moreover, it was unresolved what relationship, if any, exists between the enhanced subdiffusive movement that stems from damage and the localization of DSBs to the inner nuclear membrane (INM).

Previous work from our laboratory has shown that Mps3 and nuclear pores distribute independently around the nuclear rim in vegetatively growing cells (Horigome et al., 2011). Unlike Mps3, nuclear pores harbor the SUMO protease Up1 and the heterodimeric SUMO-dependent ubiquitin ligase Sll5–Sli8 (Nagai et al., 2008; Zhao et al., 2004; Palancade et al., 2007), which is implicated in alternative recombination-mediated pathways of repair (Khadaroo et al., 2009; Nagai et al., 2008). In contrast, Mps3 was shown to sequester DSBs from promiscuous interactions with chromatin and suppress telomere-telomere recombination in mutant strains (Oza et al., 2009; Schober et al., 2009). These results provide indirect arguments that these DSB binding sites have different functions, yet it is unclear what differentiates one binding site from the other.

Here, we combine chromatin immunoprecipitation (ChIP) and fluorescence imaging approaches in appropriate mutant backgrounds in order to distinguish and characterize the two DSB binding sites at the nuclear envelope. We find cell-cycle-dependent binding site selection with differential dependence on the INO80 chromatin remodeler. On the other hand, the related SWR-C and its deposition of Htz1 were required for relocation to both sites. By studying factors that affect DSB mobility, we also distinguish perinuclear binding site choice from DNA-damage-response-enhanced mobility. Finally, we confirm that mutants that ablate one or the other binding site have distinct outcomes on repair, arguing that the spatial segregation of damage participates selectively in pathways of repair.

RESULTS

SWR-C-Dependent H2A.Z Incorporation Is Required to Shift a DSB to the Nuclear Periphery

To study the relocation of damaged DNA to the nuclear periphery, we used a strain in which a unique DSB can be induced at the mating type locus (MAT) by galactose-controlled expression of the homothallic (HO) endonuclease. The donor sequences at HML and HMR are deleted in order to prevent intrachromosomal repair by gene conversion (Figure 1A). To determine the subnuclear localization of the DSB, we inserted an array of lacO sites at 4.4 kb from MAT and expressed a GFP-LacI fusion and either a GFP- or cyan fluorescent protein (CFP)-tagged pore protein (Figures 1A and 1B). In wild-type (WT) cells exposed to galactose for 2 hr, the induced DSB shifts efficiently to the nuclear periphery, and 59% of the cleavage sites mapped to the outermost rim (zone 1; 52% in WT CFP-Nup49-expressing strains; p < 1.0 × 10⁻³⁸ or p = 1.2 × 10⁻¹⁵ versus random; Figures 1C and 1E; Table S3 available online). On the other hand, the uncleaved MAT locus has a random subnuclear localization (Figure 1C) (Nagai et al., 2008).

Histone variant Htz1 is deposited at DSBs, and its loss was shown to abolish the association of the break with Mps3 in S or G2-phase cells (Kalocsay et al., 2009). Although Htz1 physically interacts with Mps3 in vitro, recent work showed that Htz1 also serves as an essential chaperone for the insertion of Mps3 into the INM (Gardner et al., 2011). Thus, the negative effect of htz1 deletion on DSB localization could stem from a failure to integrate Mps3 into the INM and not an absence of Htz1 at the break. To resolve this issue, we made use of a mutant called htz1ΔM6 (Wu et al., 2005), which ensures proper INM localization of Mps3 but fails to bind SWR-C. Therefore, the mutant histone htz1ΔM6 is not incorporated into chromatin by SWR-C (Gardner et al., 2011; Wu et al., 2005). We confirmed that the cleaved MAT locus failed to shift to the nuclear periphery in the htz1Δ strain and that relocation could be faithfully restored by expression of WT Htz1 (Figure 1D). However, the mutant histone htz1ΔM6 failed to support break relocation to the nuclear rim (Figure 1D). This, along with the fact that either loss of Swr1 or the SWR-C component Arab completely eliminated DSB relocation to the nuclear periphery (Figure 1E), argued strongly that the deposition of Htz1 at damage by SWR-C is indeed crucial for DSB relocation. Consistently, complementation of the swr1Δ background with a WT SWR1 gene (+ SWR1; Figure 1F), but not the catalytic site mutation (+ swr1K727G; Figure 1F), restored relocation of the DSB to the nuclear periphery, demonstrating dependence on both SWR-C and Htz1 deposition.

Although SWR-C is implicated in Htz1 incorporation, INO80 has been proposed to remove this histone variant at both damage and other sites (Kobor et al., 2004; Krokan et al., 2003; Luk et al., 2010; Mizuguchi et al., 2004; Papamichos-Chronakis et al., 2006; van Attikum et al., 2007; Wu et al., 2005). To see whether the importance of Htz1 deposition by SWR-C is to recruit INO80 to breaks, we examined whether ablation of the INO80 chromatin remodelling complex would affect DSB relocation. Because ino80Δ itself is lethal in our yeast background, we instead tested the effects of arp5Δ or arp8Δ mutants, which compromise INO80-remodelling activity and reduce INO80 recruitment to breaks (Shen et al., 2000; van Attikum et al., 2004). Surprisingly, there was no effect of arp5 or arp8 deletion on DSB relocation (Figure 1E).

INO80 has been implicated in the removal of nucleosomes to favor resection at DSBs, whereas swr1 mutants showed no defect in resection (van Attikum et al., 2007; Chen et al., 2012). Although resection is not sufficient for relocation, it leads to the binding of Rad51, which, along with Rad52, was shown to be necessary for the detection of damaged DNA at Mps3 by ChIP.
(Kalocsay et al., 2009; Oza et al., 2009). Using our quantitative positioning assay, we tested whether Rad51 or Rad52 was necessary for the perinuclear relocation of the DSB. Surprisingly, and in contrast to Mps3-ChIP results (Kalocsay et al., 2009), we scored a significant enrichment of the DSB at the nuclear periphery in both rad51Δ and rad52Δ mutants (Figures 1G and S1). Altogether, these results led us to propose that in addition to Mps3, a Rad51-independent DSB binding site, should exist at

Figure 1. SWR1-Dependent H2A.Z Incorporation Is Required to Shift DSBs to the Nuclear Periphery
(A) Shown is Chr III in strains GA-1496 and GA-6844 bearing deleted homologous donor loci (hmlΔ/hmrΔ) and a lacO array 4.4 kb from the HO cut site at MAT, which allows visualization by GFP-LacI. Pores are visualized by GFP-Nup49 (GA-1496) or CFP-Nup49 (GA-6844).
(B) Locus position is scored relative to the nuclear diameter in its plane of focus, as described in the Supplemental Information. Distance over diameter ratios are binned into three equal zones.
(C) MAT position in GA-1496 (WT) after 120 min on galactose (Gal) or glucose (Glu). * = significantly nonrandom distribution on the basis of cell number and confidence values from a proportional test between random and experimental distribution (see Table S3).
(D) In strain GA-7095 expressing HTZ1 or htz1ΔM6 from the HTZ1 promoter at URA3, MAT position was scored at 120 min on galactose as in C. Strains: htz1Δ (GA-7095), htz1Δ + HTZ1 (GA-8110), and htz1Δ + htz1ΔM6 (GA-8111).
(E) MAT position relative to CFP-Nup49 in WT (GA-6844), swr1Δ (GA-7003), arp5Δ (GA-7094), arp5Δ (GA-8069), and arp8Δ (GA-7103) as in (C) and (D).
(F) In swr1Δ (GA-7003) strain or same expressing SWR1 or swr1K727G from a TEF promoter, MAT position was scored as in (C) and (D). swr1Δ (GA-7003), swr1Δ + SWR1 (GA-8667), and swr1Δ + swr1K727G (GA-8668).
(G) MAT position scored in WT (GA-6844) and rad51Δ (GA-7099) cells as in (C) and (D) but binned into G1 (un budded) and S (budded) cells. See also Figure S1.
the nuclear periphery. The obvious candidate for this would be the Nup84 subcomplex of the nuclear pore, which was shown by ChIP and fluorescence microscopy to interact with irreparable DSBs in an asynchronous population of cells (Nagai et al., 2008).

Although Kalocsay et al. (2009) claimed that they could not distinguish between pore and Mps3 binding by ChIP, the two INM complexes are indeed distinct by high-resolution fluorescence microscopy (Horigome et al., 2011). In WT cells, endogenously tagged Mps3 (EGFP-Mps3; Figure 2A) shows a bright focus at the spindle pole body (SPB; Figure 2A, arrow) and a weak perinuclear ring. To see whether the weak Mps3 rim staining was pore dependent, we induced the clustering of nuclear pores by deleting a portion of the N-terminal domain of Nup133 (Δ amino acids [aa] 44–236) (Doye et al., 1994). In this mutant, pores cluster without loss of function. However, the EGFP-tagged Mps3 retained its rim staining (Figure 2A, red = pore, green = Mps3), even though the bright SPB was often adjacent to a pore cluster. The independence of the non-SPB Mps3 signals from pores reinforced the hypothesis that the Rad51-independent perinuclear localization of DSBs might reflect their association with pores.

To correlate nuclear pore and/or Mps3 binding with the effects of the mutations described in Figure 1, we performed ChIP assays with Mab414 (an antinuclear pore) and anti-HA (recognizing 3HA-Mps3) in WT and mutant yeast strains (Figure 2B). Consistent with previous work, an induced DSB could be recovered in immunoprecipitates for either nuclear pore or Mps3 in WT cells (Figure 2B). The level of association increased rapidly for 120 min after cut induction before reaching a plateau. In swr1Δ strains, break association with either pores or Mps3 was reduced to a background level, which existed prior to HO induction. Thus, ChIP confirms that the SWR-C is required for DSB relocation.

In contrast, in the INO80-deficient arp8Δ strain, DSB association with the nuclear pore occurred at WT levels, whereas break binding to Mps3 was lost (Figure 2B). Thus, the binding of DSBs to Mps3, but not pores, requires INO80 activity. The fact the breaks bind pores in the absence of INO80 is consistent with the INM-localization of the DSB in arp5Δ and arp8Δ strains, as presented in Figure 1. Given that break association with either the pore or Mps3 required SWR-C, the action of INO80 appears to distinguish damage that is destined for Mps3 from damage that is targeted to pores. This could reflect either the direct binding of INO80 or an alteration of the DSB that is INO80-dependent and renders the DSB able to bind Mps3.

Microscopic Confirmation that INO80 Contributes Only to DSB-Mps3 Association

We sought to confirm this finding with an assay that does not depend on protein-DNA crosslinking, given that formaldehyde crosslinking efficiency varies significantly from protein to protein. To achieve this, we scored for colocalization of a GFP-LacI-tagged DSB and CFP-tagged nuclear pores with high-resolution spinning disk confocal microscopy. To enhance the accuracy of scoring colocalization by microscopy, we used a nup133ΔN background, in which pores form a large, single cluster (Doye et al., 1994). The deletion of the Nup133 N terminus does not affect macromolecular import or export and does not confer sensitivity to DNA-damaging agents, unlike complete loss of Nup133 or Nup84 subcomplex components (Doye et al., 1994; Loeillet et al., 2005). We scored three degrees of colocalization with the pore cluster: fully overlapping, partially overlapping, and juxtaposition (“touching”; Figure 2C). All three degrees of colocalization are consistent with molecular interaction of the break with the pore cluster, given the relative signal sizes of the lacO array and the clustered pore (Schober et al., 2009).

The background level of colocalization was determined with a strain that lacks the gene for the HO endonuclease (hoΔ). In this strain, we found MAT juxtaposed to a pore cluster in 20% of the cells, and this value did not change over time. This background is higher than the computed likelihood of a lacO focus coinciding with the pore cluster (9%) (see Schober et al., 2009). Nonetheless, we use this empirically determined value as the background above which colocalization of the DSB and pore is considered significant (Figures 2C–2E, hoΔ).

In a WT yeast strains, DSB colocalization with pores showed a rapid increase upon induction of cleavage, which was then reduced to a plateau of 30% (Figure 2C). This could indicate that DSB binding at pores is transient for a subpopulation of breaks or else that extensive resection at the break eliminates the lacO signal at later time points (Figure S2). Nonetheless, there was significant colocalization of DSBs with the pore cluster in both G1- and S-phase cells (Figure 2D). Importantly, DSB-pore interaction was diminished in swr1Δ, but not arp8Δ, strains, providing independent confirmation that pore association of a DSB requires SWR-C but not a functional INO80 complex. The slight delay in DSB accumulation at pores in the arp8Δ strain correlates with both reduced chromatin mobility and reduced resection rate at the break in that mutant (Neumann et al., 2012; van Attikum et al., 2007). In conclusion, quantitative microscopy confirms a differential requirement for SWR-C and INO80 in the association of DSBs with pores.

Another variable in break relocation is the stage of the cell cycle at which position is measured. In previous ChIP studies, the Mps3-DSB interaction was detected in asynchronous cultures, yet it was lost when cells were arrested in G1 (Kalocsay et al., 2009). Cell-cycle effects were not examined in the context of DSB association with pores. Taking advantage of the ease with which one can determine cell-cycle stage by yeast cell morphology, we binned the cells scored by microscopy into un budded (G1 phase) and budded cells, counting only those in which the nuclei were still round (early to mid S phase) (Figure 2E). We conclude from this that DSB-pore interaction occurs in both G1- and S-phase cells, reaching 36% and 43% colocalization with pores, respectively. In both sets of cells, pore association depends on SWR-C (Figure 2D) and cleavage (Figure 2E, hoΔ background, ~20%). However, in S phase, DSB association with the pore was independent of INO80 function, and even increased in the absence of Arp8 (Figure 2D). Moreover, whereas Mps3 binding was reported to be sensitive to loss of Rad51 (Kalocsay et al., 2009; Oza et al., 2009), we found that DSB-pore association was intact in the rad51A mutant (Figure 2D). Thus pore-DSB interaction occurs in G1- and S-phase cells and is dependent on SWR-C but independent of Rad51 and INO80. On the other hand, DSB recruitment to Mps3 requires Rad51 and INO80 and is restricted to S phase.
Figure 2. SWR-C Is Required for DSB Binding at Both Nuclear Pores and Mps3, and INO80 Is Only Required for Mps3 Interaction

(A) EGFP-Mps3 and CFP-Nup49 localization in WT (GA-6647) and in pore-clustering cells (nup133ΔN, GA-6650). Images are reproduced from (Horigome et al., 2011) with permission. Arrow = spindle pole body; white bars = 1 μm.

(legend continued on next page)
To make sure that these cell-cycle conclusions were not artifacts of the nup133Δ strain, we confirmed them with ChiP assays for nuclear pore proteins (Mab141, see the Experimental Procedures) and HA-tagged Mps3 in synchronized NUP133+ cells. Cells arrested in G1 showed a cleavage-dependent increase in association with nuclear pores but not Mps3 (Figures 2F and 2G) (Kalocsay et al., 2009; Oza et al., 2009). Both types of association increased in G2- and M-phase cells (Figures 2F and 2G). This contrasts with the fluorescence colocalization analysis, where we scored a drop in DSB-pore interaction in both WT and arp8Δ cells at 120 min after cut induction (Figure S2A). Given that this correlates with a reduced number of bright LacI/lacO foci (<1 per cell), we suggest that the drop in colocalization stems from resection through the lacO repeat sequence at 120 min postcleavage (Figures 2A and 2B). Intriguingly, in both rad51A and arp8Δ cells, DSBs bind more efficiently to pores in S-phase than WT cells, even though the Mps3 interaction drops in these mutants. This is consistent with the two binding sites being competitive, rather than sequential binding sites.

**Htz1 Is Able to Mediate Direct Interaction with Mps3 but Not Nuclear Pores**

The loss of perinuclear interactions in remodeler deletion strains does not necessarily mean that the remodeler itself mediates interaction with pores or Mps3. Rather, the effects could be achieved indirectly by the action of the complex on the substrate; i.e., modification of chromatin or processing of the DSB. However, in the case of Htz1, it was proposed that this histone variant interacts directly either with nuclear pores (Dilworth et al., 2005; Light et al., 2010) or Mps3 (Gardner et al., 2011). To test whether Htz1 incorporation is sufficient to shift chromatin to either nuclear pores, Mps3, or both, we made use of a gain-of-function assay in which LexA fusion proteins are targeted to four double Htz1 incorporation is sufficient to shift chromatin to either nucleoplasmic N-terminal domain of Mps3, which distributes throughout the nucleoplasm (Schober et al., 2009), along with LexA-htz1ΔM6. In this case, LexA-htz1ΔM6 no longer shifted ARS607 to zone 1 in either WT or swr1Δ cells (Figure 3E), suggesting that the soluble Mps3N competes for Htz1-Mps3 inter-

Next, we asked whether the Htz1-mediated interaction with the nuclear periphery reflects binding to nuclear pore complexes, as had been previously shown for LexA-Arp6 (Yoshida et al., 2010). To score this, we targeted LexA-htz1ΔM6 to the LexA/lacO-tagged lys2 in a nup133Δ background expressing CFP-Nup49 and scored colocalization of lys2 with the pore cluster. LexA-htz1ΔM6 was unable to enhance interaction with the nuclear pores above background levels (20%), whereas the targeting of LexA-Swr1 could (Figure 3D). Finally, to see whether Htz1 functions by binding Mps3, we overexpressed the nucleolysing N-terminal domain of Mps3, which distributes throughout the nucleoplasm (Schober et al., 2009), along with LexA-htz1ΔM6. In this case, LexA-htz1ΔM6 no longer shifted ARS607 to zone 1 in either WT or swr1Δ cells (Figure 3E), suggesting that the soluble Mps3N competes for Htz1-Mps3 interaction at the INM. Unfortunately, we were unable to test an mps3Δ mutant in this assay, given that the cells show severely impaired growth (data not shown). In conclusion, the targeting of a LexA-Htz1 fusion is sufficient to shift chromatin to the INM in the absence of damage, probably because of its affinity for Mps3N. Previous work showed that LexA-Arp6 can shift an internal LexA-tagged locus to the nuclear pore cluster (Yoshida et al., 2010) as we show here for LexA-Swr1 (Figure 3D). The significance of this Swr1 interaction for DSB relocation is unclear, given that we showed above that point mutants that eliminate the ATPase activity of SWR-C blocks DSB relocation (Figure 1F). In summary, we suggest that SWR-C functions in DSB relocation in multiple interdependent ways: by depositing Htz1, by serving as a bridge for pore interaction, and possibly by enhancing the subdiffusive mobility of chromatin in response to breaks (Dion et al., 2012; Miné-Hattab and Rothstein, 2012).

**Testing the Role of Remodeler-Enhanced DSB Mobility in DSB Relocalization**

Previous work demonstrated a role forINO80 remodeling activity in chromatin movement both at a DSB and when targeted to undamaged sites (Neumann et al., 2012), whereas the role of SWR-C or Htz1 deposition had not been tested. To examine this, we scored the mobility of a lacO-tagged induced DSB at the ZWF1 locus in the middle of the long left arm of chromosome XIV in swr1Δ and htz1Δ-deficient strains (Figure 4A). The deletion of swr1 did not affect in the mobility of the locus in the absence of damage (Figure 4B); however, after I-SceI-induced cleavage, the dramatic increase in DSB movement that occurs in WT cells was compromised in swr1- and htz1-deficient strains (Figure 4C). The effect was similar in the absence of other SWR-C subunits.
Swc2 and Swc5 (Figure S3), which are necessary for remodeler function (Wu et al., 2005). The reduction in mobility was as strong as, if not stronger than, the reduced mobility of the same DSB in arp8Δ or rad51Δ mutants (Figure 4D) (results from Dion et al., 2012; Neumann et al., 2012). However, in arp8Δ or rad51Δ strains, we still score the shift of DSBs to the INM (Figures 1 and 2), ruling out an absolute requirement of enhanced mobility for relocation to pores.

Having demonstrated that the LexA-mediated targeting of Swr1, Arp6, and Htz1 can mediate relocation to the nuclear periphery, we tested whether they also affect the mobility of the undamaged locus to which they are bound. We find that the expression of LexA-Arp6, LexA-Htz1, or LexA-htz1ΔM6 confers no significant increase in ARS607 mobility (Figures 4E and 4F), yet they can still shift the locus to the INM (Figure 3).

Indeed, the targeting of LexA-Arp6, which can bring an internal locus to pores (Yoshida et al., 2010), reduces ARS607 mobility (Figure 4E). Given that LexA-Arp5, LexA-Arp8, or LexA-Ino80 bound to various undamaged sites enhance locus mobility (Neumann et al., 2012) and do not lead to perinuclear enrichment (Figure S4), we conclude that enhanced random movement is neither sufficient nor necessary for shifting a DSB to the INM.

Crosstalk between Anchorage at Pores and Mps3 Reveals Additive Phenotypes
We have distinguished the two INM binding sites for DSBs with respect to INO80 and cell-cycle dependence, yet it remained possible that the two sites influence each other in some way. For example, pore binding might precede binding to Mps3 or

Figure 3. Htz1 Is Sufficient to Shift an Internal Locus to the Nuclear Periphery
(A) The position of lacO/LexA-tagged ARS607 was visualized by GFP-LacI and scored as in Figure 1B. Cells are binned into G1 or S phase as in Figure 1G. Strains carry either GFP-Nup49 (GA-1461) or CFP-Nup49 (GA-1993). LexA fusion proteins are expressed from plasmids.
(B) WT strain (GA-1993) expresses indicated LexA fusion constructs. ARS607 position was scored as in (A).
(C) Experiments were performed as in (B) with strain bearing swr1Δ (GA-7898).
(D) Pore cluster colocalization for LexA-tagged LYS2 in a strain bearing nup133ΔN (GA-4584) transformed with LexA fusions. Colocalization (pink to red) is presented as in Figure 2C.
(E) In a WT strain (GA-1993) expressing LexA-htz1ΔM6 and Mps3N’, ARS607 position was scored as in (B). Right, same experiments performed with swr1Δ (GA-7898) bearing LexA and either an empty vector or the Mps3N’ construct.
vice versa. This was tested directly by monitoring DSB relocation in mutants lacking one or the other site.

Consistent with earlier findings (Oza et al., 2009), we show through both microscopic analysis and ChIP that DSBs fail to relocate to the nuclear periphery in the mps3Δ65-145 strain (Figures 5A–5C). Interestingly, the relocation defect in the mps3Δ65-145 strain was observed in both G1- and S-phase cells, even though DSB binding to Mps3 is only detected in G1 (Kalocsay et al., 2009). Using the colocalization assay, we confirmed that a GFP-tagged DSB failed to bind the CFP-labeled

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**Figure 4. Enhanced DSB Mobility Depends on SWR-C and H2A.Z but Is Not Required for Relocalization**

(A) Schematic representations of the I-SceI cut site and a lacO array inserted at ZWF1 on chromosome 14L (GA-6208). Strains express CFP-LacI and galactose-induced I-SceI from a 2 μm plasmid.

(B) MSD plots (see the Experimental Procedures) of the ZWF1 locus in WT and swr1Δ cells during S phase in glucose medium show no mobility change for undamaged chromatin in swr1Δ.

(C) MSD plots of I-SceI-induced Rad52-YFP foci in WT (GA-6208), swr1Δ (GA-6335), htz1Δ (GA-6637) strains, and CFP-LacI at uncut site (GA-6215) during S phase. Only the cut WT sample increases mobility.

(D) MSD plots of I-SceI-induced Rad52-YFP foci in WT (GA-6208),arp8Δ (GA-6317), and rad51Δ (GA-6225) strains during S phase reproduced with permission (Dion et al., 2012; Neumann et al., 2012).

(E and F) MSD plots of the undamaged lacO/LexA-tagged ARS607 tracked after binding LexA or the indicated LexA fusion during G1 phase. The reduction in mobility due to Arp6 binding is significant. MSD data are represented as mean ± SEM. See also Figures S3 and S4.
Figure 5. Nonreciprocal Effects of the Loss of Mps3N or of Nup120 on DSB Positioning

(A) Position of cleaved MAT loci in mps3Δ65-145 (GA-7096) scored at 120 min after HO induction as in Figure 1G. The effects were confirmed by nuclear pore ChIP on the cleaved MAT locus: loss of Mps3N reduces break-induced association with pores from 5- to 2-fold (Figure 5C). We could prove that this mutant disrupts interaction with the porin complex, and these are lost upon deletion of any Nup84 subcomplex component (Fernandez-Martinez et al., 2012). Therefore, we used a strain lacking Mps120, which disrupts DSB interaction with the pore (Nagai et al., 2008). Importantly, in this mutant, we found that DSB relocation to the nuclear periphery is lost in G1- phase, but not S-phase, cells (Figure 5D). We could prove that this mutant disrupts interaction with the nuclear pore by scoring colocalization of the tagged DSB with the CFP-labeled pore cluster in mps3Δ65-145 cells. As expected, cleavage-induced association with the nuclear pore cluster was lost upon ablation of Nup120 in both G1- and S-phase cells (Figure 5F). On the other hand, Mps3 ChIP showed WT levels of DSB association with 3HA-Mps3 in the absence of Nup120 (Figure 5F). Thus, Mps3 influences DSB binding to pores but not vice versa.

Repair Defects in mps3 or Pore Mutants Are Additive

To test whether interactions at the two binding sites are functionally epistatic or additive for repair, we constructed double mutants that compromise the Nup84 subcomplex (mps3Δ65-145) and the Mps3 nuclear anchorage domain (mps3Δ65-145). Single and double mutants were challenged with DNA damage. In a simple drop assay that monitors sensitivity to 0.03% methyl methanesulfonate (MMS), we found that the effects of nup120Δ and mps3Δ65-145 on cell viability after plating on MMS were indeed additive (Figure 6A). This suggests that the two anchorage sites mediate different functions under conditions of S phase damage.

To extend this to a more precise pathway of repair, we scored for DSB repair by unequal sister chromatid recombination...
Figure 6. Additive Effects of nup120 and mps3 on Unequal Sister Chromatid Exchange

(A) Serial dilutions of isogenic strains bearing indicated mutations (nup120Δ or nup133Δ and mps3Δ65-145) were spotted onto the YPAD ± 0.03% MMS. Plates are shown after 3 days of growth.

(B) The uSCR frequencies of WT (SCRMTL2), nup120Δ (YH1301), mps3Δ65-145 (YH1302), and nup120Δ mps3Δ65-145 (YH1303) cells were determined by plating on YPAD or SC-His medium. Recombination frequency = number of His+ recombinants per 10^6 colony forming units (CFUs) from eight experiments. Error bars indicate SD.

(C) An I-SceI cut site was placed within the lys5 gene bearing a frameshift mutation on Chr XIV in a strain with a galactose-inducible I-SceI. An induced DSB repaired by gene conversion with the truncated lys5 template at ME10 (Chr VI) allows survival on galactose-Lys plates. Survivors over total plated cells yields the rate of gene conversion. Tested were eight independent colonies of swr1Δ (GA-6386), six colonies of arp8Δ (GA-6382), and 14 colonies of WT (GA-6217). An nsc2Δ strain yielded same as arp8Δ (data not shown). Significance was determined by a Student’s t test (p = 0.0209; WT versus swr1Δ, p = 0.0306; WT versus arp8Δ). Error bars indicate SD.

(uSCR) (González-Barrera et al., 2003; Kadyk and Hartwell, 1992, 1993). It has been proposed that uSCR occurs when the replication fork bypasses lesions that normally obstruct its passage. The frequency of uSCR in the absence of Nup120 did not increase significantly over WT frequencies (Figure 6B). However, in the mps3Δ65-145 mutant, we scored a strong increase in uSCR recombination (Figure 6B). Again, the double mutant showed additive effects, arguing that unequal sister chromatid exchange is indeed repressed by binding to Mps3 (Figure 6B), whereas an alternative pathway of repair appears to be lost by nup120 deletion. If pore and Mps3 anchorage worked on the same pathway to repress recombination, then the two mutations would have shown epistatic effects on uSCR efficiency.

Finally, we scored for rates of homologous recombination with an ectopic donor at an induced DSB (Figure 6C). We found that loss of SWR-C increases HR with an exact ectopic donor, again confirming that sequestration of a DSB at the INM probably disfavors either homology search or the recombination event (Dion et al., 2012; Oza et al., 2009). As expected, the loss of Arp8 had the opposite effect (Figure 6C), confirming earlier results that scored reduced rates of resection and chromatin mobility in this mutant (van Attikum et al., 2007; Neumann et al., 2012).

DISCUSSION

Many nuclear events have been shown to be localized to nuclear subcompartments, although it has remained unclear what establishes the localization of damage and whether different positions impact repair pathways differentially. Here, we exploited both quantitative microscopy and ChIP studies in order to examine the relocation of DSBs to the nuclear envelope in budding yeast. Although each assay has inherent weaknesses, the combination allows us to show conclusively that the two proposed binding sites for DNA damage, the SUN domain protein Mps3, and the Nup84 subcomplex of the nuclear pore, are distinct DSB binding sites. We resolve apparent contradictions in the literature by showing that DSBs bind pores in both G1 and S-phase cells, whereas they associate with Mps3 only in S and G2. A differential requirement for INO80 and SWR-C remodelers for translocation to Mps3 and pores reinforces the argument that the binding sites are distinct. Furthermore, disruption of the two anchors has differential effects on DSB repair: anchor site loss has additive, and not epistatic, impact on the survival of alkylating damage and for uSCR (Figure 6). Finally, we identified a nonreciprocal crosstalk between the Mps3 N terminus and
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previous work has shown that SWR-C is required both for efficient Ku80 recruitment to an induced DSB and for optimal error-free NHEJ (van Attikum et al., 2007). DSB-pore interactions may be stabilized by the affinity of yKu for the pore basket components Mlp1 and Mlp2 (Galy et al., 2000). On the other hand, Arp6 binding to pores is independent of Mlp1 and Mlp2 in G1 phase and is dependent on them in S phase (Yoshida et al., 2010). This may suggest that there is more than one anchorage site for DSBs at pores in G1-phase cells. Pore binding was also implicated in NHEJ-dependent repair of subtelomeric DSBs (Therizols et al., 2006). Finally, and in contrast to swr1Δ, the INO80-deficient mutant arp8Δ did not affect break recruitment to pores in G1, nor did it alter rates of error-free NHEJ (van Attikum et al., 2007).

In S-phase cells, on the other hand, sister chromatid recombination (SCR) becomes the preferred mechanism of DSB repair (González-Barrera et al., 2003; Kadyk and Hartwell, 1992). Although equal SCR is difficult to monitor, unequal exchange (uSCR) can be readily scored. Spontaneous uSCR rates were unaffected in the nup120Δ mutant, whereas rates increased in mps3Δ65-145 (Figure 6). Given that DSB-Mps3 binding is intact in the nup120Δ mutant, Mps3 most likely serves as a repressor of uSCR. This is consistent with previous reports of enhanced recombination between telomeres in mps3ΔN mutants (Schober et al., 2009). Whether Mps3 acts simply by sequestration of the free end or by helping to load Mre11 (González-Barrera et al., 2003) and/or cohesin (Cortés-Ledesma and Aguilera, 2006) is unknown. Consistent with our findings, previous work showed the SWR-C-deficient arp8Δ mutant has an increased level of spontaneous uSCR in this same assay (Kawashima et al., 2007).

In an assay for ectopic HR, we found that loss of INO80 function (arp8Δ) decreased efficiency, whereas swr1 deletion increased the rate of DSB-induced HR (Figure 6). Collectively, these results argue for a recombination-repressive role for Mps3, which is consistent with previous observations on telomere-telomere exchange and the likelihood of DNA-DNA interactions detected in a chromosome conformation capture technology (Oza et al., 2009; Schober et al., 2009). Given that we score a delayed but intact DSB-pore interaction in INO80-deficient strains (Figure 2), we propose that nuclear pore association normally does not repress recombination. However, if Mps3 binding is ablated, then S-phase association with nuclear pores may compensate, as supported by the additive effects of the double nup120Δ mps3Δ65-145 mutant on uSCR. Consistent with this, spontaneous Rad52 foci were found to be more mobile in arp8 and in swr1 mutants, which might favor ectopic repair over SCR (data not shown; Dion et al., 2013). We speculate that the role of the nuclear pore in S-phase DSB repair may reflect SUMO- and/or ubiquitin metabolism because of the nuclear pore-associated SUMO protease Ulp1 and the SUMO-dependent ubiquitin ligase Slx5-Slx8 (Nagai et al., 2008; Zhao et al., 2004). Strains mutant for the Nup84 complex (Nup84, Nup120, and Nup133) are hypersensitive to DNA-damaging agents, synthetic lethal with mutations that impair HR, and accumulate spontaneous damage foci containing Rad52 (Nagai et al., 2008; Palancade et al., 2007).

How do remodelers affect DSB binding site choice? The catalytic effects of the INO80 complex under conditions of DNA

nuclear pores with the use of three independent assays. Collectively, they show that the loss of Mps3 affects damage binding at pores, but the loss of pore binding does not impair association with Mps3. This means that either Mps3 acts on pore organization in a subtle manner that ablates the DSB interaction site or there is a necessary, but very transient, interaction of irreparable breaks with Mps3 prior to binding the pore. This latter is unlikely, given that the remodeler INO80 is selectively required for break association with Mps3 and not nuclear pores. Indeed, our final insight into DSB position stems from the relationship of chromatin remodelers to the subnuclear positioning of damage, as depicted in the model in Figure 7.

DSB recruitment to nuclear pores in G1 phase depends on SWR-C activity. Because there is neither resection nor available homology from the replicated sister in G1-phase cells, the preferred pathway of repair in G1 phase is NHEJ. Consistently,
damage are well documented and include nucleosome eviction, enhanced resection, and enhanced subdiffusive mobility (reviewed in Seeber et al., 2013b). It is likely that INO80 acts on the substrate itself, generating 3' overhang without remaining bound to tether the break to Mps3, given that Ino80, Arp5, or Arp8, when fused to LexA, could not shift a tagged locus to the nuclear periphery (Figure S4). The contribution of SWR-C and Htz1 to DSB relocation and repair may instead reflect their binding at the break site. A role for SWR-C and Htz1 in DSB repair appears to be conserved in mammals, given that the Swr1 homolog p400 ATPase and H2A.Z play critical roles in Rad51-mediated repair (Courilleau et al., 2012; Xu et al., 2012). The striking deposition of H2A.Z at laser-induced damage in mammalian cells correlates with an open conformation of chromatin at DSBs and the loading of the Brca1 complex (Xu et al., 2012). Additionally, H2A.Z exchange appears to restrict formation of ssDNA and favor loading of the Ku70/Ku80 complex. We propose that, in yeast, the equivalent phenomenon is break sequestration by Mps3, which is indeed Htz1 dependent. Intriguingly, SWR-C favors Ku loading in yeast (van Attikum et al., 2007), just as its homolog, SRCAP, does in mammalian cells (Xu et al., 2012), which may suppress recombination by sequestration and thus favor NHEJ. Given that remodelers show conserved functions in surviving DNA damage, it is most likely that a spatial segregation of repair functions, such as that shown here, is a conserved aspect of the cell’s arsenal of defense against genomic insult.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures, five figures, and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.molcel.2014.06.027.

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