RESEARCH COMMUNICATION

Distinct roles for the RSC and Swi/Snf ATP-dependent chromatin remodelers in DNA double-strand break repair

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The failure of cells to repair damaged DNA can result in genomic instability and cancer. To efficiently repair chromosomal DNA lesions, the repair machinery must gain access to the damaged DNA in the context of chromatin. Here we report that both the RSC and Swi/Snf ATP-dependent chromatin-remodeling complexes play key roles in double-strand break (DSB) repair, specifically by homologous recombination (HR). RSC and Swi/Snf are each recruited to an in vivo DSB site but with distinct kinetics. We show that Swi/Snf is required earlier, at or preceding the strand invasion step of HR, while RSC is required following synapsis for completion of the recombinational repair event.

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The high-fidelity transmission of genetic information is essential for proper cell growth and viability. Chromosomal DNA lesions resulting from replication errors or from a variety of environmental and endogenous insults such as radiation, chemicals, or free radicals must be promptly and accurately repaired in order to maintain genomic integrity. Errors in any of the steps involved in the recognition or repair of damaged DNA may result in the rearrangement or gain or loss of chromosomes or tumorigenesis (Stewart et al. 1999; Pierce et al. 2001).

Double-strand breaks (DSBs), the most serious of eukaryotic DNA lesions, are repaired by two distinct and complementary pathways: nonhomologous end joining (NHEJ) and homologous recombination (HR). In NHEJ, the two ends of a DSB are reunited in the absence of sequences homologous to the break site by the double-stranded DNA (dsDNA) end-binding heterodimer KU (Yku70p/Yku80p in budding yeast) and DNA ligase IV and its cofactor XRCC4 (Dnl4p and Lif1p in budding yeast) (Critchlow and Jackson 1998; Valerie and Povirk 2003). In HR, the ends of the broken chromosome must locate an intact undamaged homologous template whose DNA sequences are then copied to repair the DSB. HR is mediated by the evolutionarily conserved *RAD52* epistasis group proteins, which include budding yeast Rad51p, Rad52p, Rad54p, Rad55p, Rad57p, Rad59p, Mre11p, Rad50p, and Xrs2p (Symington 2002). Approximately 90% of the DSBs in *Saccharomyces cerevisiae* and 50% of those in mammalian mitotic cells are repaired by HR (Paques and Haber 1999; Johnson and Jasin 2000). Recombinational repair in yeast involves the initial resection of the 5′ ends of DNA flanking the break by an exonuclease, followed by binding of the replication protein A (RPA) single-stranded DNA (ssDNA)-binding protein to the exposed 3′ ssDNA. The Rad51p strand exchange protein then displaces RPA to form the presynaptic filament (New et al. 1998), a process mediated by Rad52p (Sung 1997; Shinohara and Ogawa 1998), the Rad55p/Rad54p heterodimer (Sung et al. 2000; Symington 2002), and Rad54p (Wolner et al. 2003). Following its successful search for a homologous sequence, Rad51p facilitates the presynaptic filament's invasion of the homologous template. DNA replication of the homologous template is then followed by ligation to the distal broken DNA end (Paques and Haber 1999). Recently, studies demonstrating the interdependent recruitment of checkpoint and recombinational repair proteins to DSBs in vivo have provided a molecular time-frame of DSB repair events (Sugawara et al. 2003; Wolner et al. 2003; Lisby et al. 2004).

These two highly conserved pathways must repair DSBs in the context of chromatin, implicating enzymes that modulate chromatin structure in DNA repair (see Peterson and Cote 2004). Indeed, histone acetyltransferase and histone deacetylase complexes are implicated in the repair of DSBs by either NHEJ or HR (Bird et al. 2002; Qin and Parthun 2002; Downs et al. 2004). ATP-dependent nucleosome-remodeling factors are also implicated in repair: The Rad54p enzyme functions in multiple steps of DSB repair by HR (Alexiadis and Kadonaga 2002; Alexeev et al. 2003; Wolner and Peterson 2005), INO80 has recently been linked to DSB repair (Morrison et al. 2004; van Attikum et al. 2004), and mutants of SWR1 are sensitive to DNA damaging agents (Mizuguchi et al. 2004).

Despite emerging roles for chromatin-remodeling enzymes in DSB repair, the paucity of mechanistic data demonstrating how and in which steps of the DSB repair pathways ATP-dependent chromatin-remodeling enzymes function prompted us to examine the roles of the yeast RSC and related Swi/Snf complexes in repair of DSBs. RSC is essential for cell viability and has been implicated in transcriptional regulation (Angus-Hill et al. 2001) and chromosome transmission (Tsuchiya et al. 1998; Hsu et al. 2003; Baetz et al. 2004), and more recently in sister chromatid cohesion (Baetz et al. 2004; Huang et al. 2004). Swi/Snf functions in regulating transcription. Although in vitro experiments have linked Swi/Snf to the nucleotide excision repair pathway (Hara and Sancar 2002), its in vivo function in DNA repair, and in particular DSB repair, is unknown.

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Here we present evidence that both RSC and Swi/Snf complexes play key roles in DNA DSB repair by HR. RSC and Swi/Snf associate with a DSB site with different kinetics, suggesting functional differences between the remodelers. Indeed, following a DSB, Swi/Snf is recruited to the donor sequence and is essential for repair at a step during or prior to synapsis, whereas RSC function is required for HR repair at a step post-synapsis.

Results and Discussion

To investigate the role of the RSC ATP-dependent remodeling complex in DNA repair, we first compared the growth of several temperature-sensitive *rsc* mutants to wild-type cells on rich medium in the presence of the DNA damaging agents bleomycin, hydroxyurea, methyl methane sulfonate, or ultraviolet (UV) radiation, each of which can directly or indirectly induce DSBs. Bleomycin generates free radicals that can cause DSBs. Hydroxyurea lowers the dNTP pool, thereby collapsing replication forks, and activates the intra-S checkpoint. Methylmethane sulfonate (MMS) alkylates purines, which, when left unrepaired, can result in DSBs after replication. UV irradiation primarily causes pyrimidine dimers, which, when left unrepaired, can occasionally create DSBs following replication. *rsc* mutants were hypersensitive to all three of the DNA damaging agents and to UV exposure when grown at their respective semipermissive temperatures (Fig. 1A). An earlier study also reported enhanced sensitivity of *rsc1* and *rsc2* mutants to γ irradiation (Bennett et al. 2001), suggesting further that RSC functions directly in DNA DSB repair.

Interestingly, mutants of the related Swi/Snf chromatin-remodeling complex, *snf5*Δ and *snf2*Δ, were also hypersensitive to bleomycin and hydroxyurea and only moderately sensitive to MMS and UV irradiation at 25°C and 35°C (Fig. 1B). As expected, the *rad52*Δ recombination mutant, but not the *yku70*Δ end-joining mutant, also showed decreased viability in the presence of bleomycin, hydroxyurea, MMS, or UV light. The variability in sensitivities among mutants could reflect the different types of mutations or the inherent functional differences of subunits (Angus-Hill et al. 2001). Together, these results are consistent with roles for both RSC and Swi/Snf in the repair of DSBs.

Because eukaryotic DSBs can be repaired by NHEJ or HR, we evaluated the roles of *rsc* or *swi/snf* mutants in either repair pathway using epistasis analysis and plasmid-based repair assays. The enhanced hypersensitivity of *rsc rad52* and *rsc yku70* double mutants to bleomycin, hydroxyurea, and MMS suggests that RSC may function both in NHEJ and HR (Supplementary Fig. 1). We also assayed plasmid end joining in five *rsc* and two *swi/snf* mutants using a standard plasmid recircularization assay (Erdemir et al. 2002). The *sth1*, *sfh1*, *rsc1*, and *rsc2* mutants of RSC, as well as the *snf2* and *snf5* mutants of Swi/Snf, were able to religate the linearized plasmid in vivo, inexplicably better than or as well as wild-type cells. As expected, the control *yku70*Δ cells could not rejoin a linearized plasmid, while *rad52*Δ cells recircularized linearized plasmids as efficiently as wild-type cells (Fig. 2A; Boulton and Jackson 1996; data not shown). Thus, these *rsc* and *swi/snf* mutants were not defective in end joining of linearized plasmids, although it is still possible that RSC and Swi/Snf might be important in the context of chromosomal NHEJ.

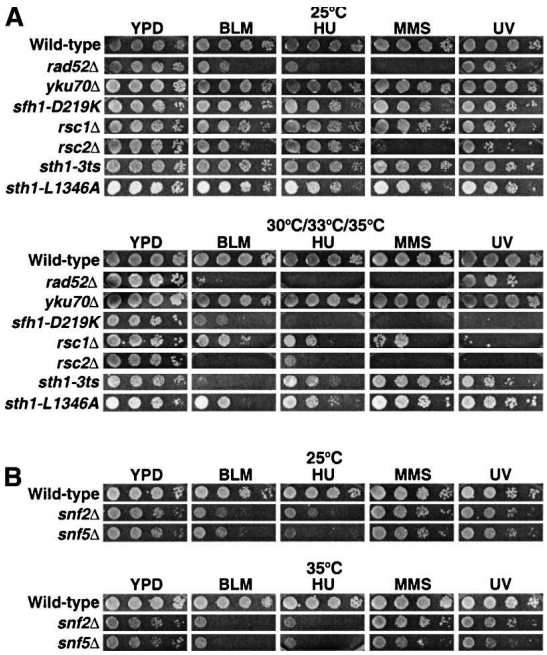

Figure 1. *rsc* and *swi/snf* mutants are hypersensitive to genotoxic agents. (*A*) Tenfold serial dilutions of wild-type (BLY76), *rad52*Δ (BLY772), *yku70*Δ (BLY773), *sfh1-D219K* (BLY650), *rsc1*Δ (BLY788), *rsc2*Δ (BLY662), *sth1-3ts* (BLY49), and *sth1-L1346A* (BLY491) strains were spotted onto rich YPD medium in the absence or presence of 3 mU/mL bleomycin, 50 mM hydroxyurea, 0.01% MMS, or 75 J/m^2 UV light. Cells were incubated for 3–4 d at permissive (25°C) or at each of their respective semipermissive (33°C for *rad52*Δ, *yku70*Δ and *sfh1-D219K*; 35°C for *rsc1*Δ, *rsc2*Δ, *sth1-3ts*, and *sth1-L1346A*) temperatures; wild-type cells were grown at 30°C. (*B*) Tenfold serial dilutions of wild-type (BLY1), *snf2*Δ (BLY35), and *snf5*Δ (BLY3) strains were spotted onto YPD medium in the absence or presence of 3 mU/mL bleomycin, 50 mM hydroxyurea, 0.01% MMS, or 75 J/m^2 UV radiation and incubated at 25°C and 35°C for 3–4 d.

HR was first examined using a plasmid-based repair assay in which successful recombination or single-strand annealing (SSA) with an 800-bp PCR fragment produces full-length functional β-galactosidase (Erdemir et al. 2002). We found that each of the *rsc* and *swi/snf* mutants tested achieved only 10%–50% of the repair of wild-type cells, suggesting that RSC and Swi/Snf play important roles in repair of DSBs by either HR or SSA. As expected, *rad52*Δ cells were completely unable to carry out repair, while *yku70*Δ cells were proficient in repair (Fig. 2B; Paques and Haber 1999; data not shown). Thus, analysis of the *sth1*, *sfh1*, *rsc1*, and *rsc2* alleles uncovered defects in the repair of DSBs by either HR or SSA.

To gain further evidence for a role for RSC in DSB repair, we examined whether overexpression of a protein involved in HR could rescue the hypersensitivity of *rsc* mutants to genotoxic agents. Remarkably, we found that *rsc1*Δ, *rsc2*Δ, and *sfh1-219* cells transformed with high-copy plasmids expressing *RAD52* exhibited partial to nearly wild-type resistance to MMS (Fig. 2C).

We predicted that if RSC and Swi/Snf function directly in DSB repair by HR, then they should be recruited to DSB sites. To test this, we exploited mating-type switching in yeast in which the HO endonuclease creates a site-specific DSB at the mating-type (*MAT*) locus to induce HR from the silent *HML* and *HMR* mating loci. Initially, we examined the kinetics of Sth1p, Snf5p, and

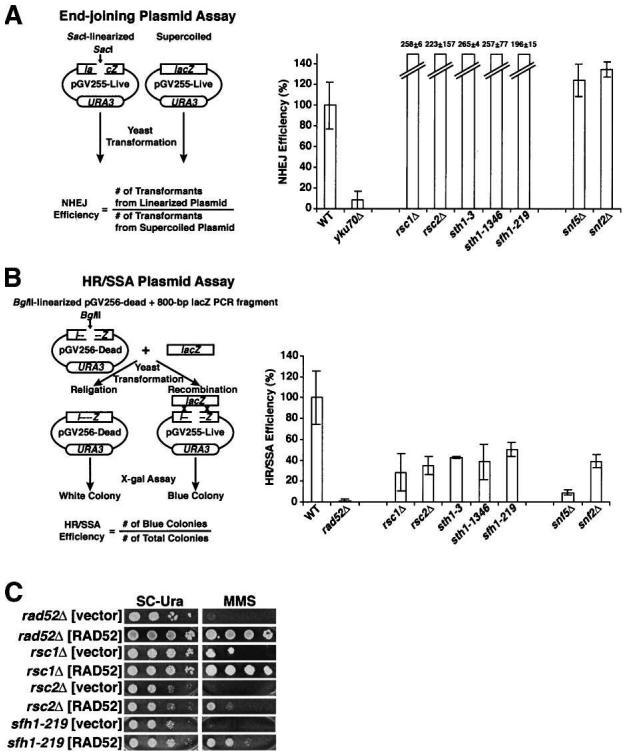

Figure 2. *rsc* and *swi/snf* mutants are competent for plasmid end joining but defective in repair by HR/SSA. (*A*) *rsc* and *swi/snf* mutants are capable of repair by plasmid end joining. Wild-type (BLY76), *yku70*Δ (BLY773), *rsc1*Δ (BLY788), *rsc2*Δ (BLY662), *sth1-3ts* (BLY49), *sth1-L1346A* (BLY491), *sfh1-219* (BLY650), *snf5*Δ (BLY3), and *snf2*Δ (BLY16) strains transformed in parallel with the SacI-linearized or supercoiled pGV255-live plasmid were plated onto selective SC-Ura medium at 30°C for 4–5 d. Values represent the ratios of the number of transformants from linearized plasmid to the number of transformants from supercoiled plasmid, normalized to values obtained from wild-type strains. The means and standard deviations of at least three independent experiments are plotted. (*B*) *rsc* and *swi/snf* mutants are defective for HR/SSA. The same strains as in *A* and *rad52*Δ (BLY772) were cotransformed with the BglII-linearized pGV256-dead plasmid and an 800-bp *lacZ* PCR fragment amplified from pGV255-live, plated onto SC-Ura medium at 30°C for 4–5 d, and assayed for β-galactosidase activity. Values represent the ratios of blue colonies to total colonies, normalized to values obtained from wild-type strains. The means and standard deviations of at least three independent experiments are plotted. (*C*) *RAD52* overexpression partially rescues the MMS hypersensitivity of *rsc* mutants. *rad52*Δ (BLY772), *rsc1*Δ (BLY788), *rsc2*Δ (BLY662), and *sfh1-219* (BLY650) strains transformed with either pRS426:RAD52 or pRS426 alone were 10-fold serially diluted and spotted onto SC-Ura medium in the absence or presence of 0.01% MMS at 35°C (or at 33°C for *sfh1-219*) for 3–4 d.

Rad52p recruitment to both sides of the DSB site in strains lacking both the *HML* and *HMR* homologous donor sequences (Fig. 3) so that the initial binding of proteins to the DSB site would be more readily observed. Remarkably, Sth1p was rapidly recruited to both sides of the DSB site within 10 min of DSB induction and reached maximal levels by 20 min (Fig. 3B), suggesting that RSC function is required early in DSB repair. Snf5p was also recruited to the same *MAT* Y and *MAT* Z sequences, but in contrast to RSC, appeared only after 40 min of DSB induction (Fig. 3D). Recruitment of both Snf5p and Snf2p, the ATPase subunit of the Swi/Snf complex, to the DSB site increased steadily over the next 4 h, whereas Sth1p's presence at the DSB site increased only slightly during the same period (Fig. 3C,E; data not shown), suggesting that Swi/Snf plays a relatively later role in DSB repair. Similar to Swi/Snf, Rad52-myc13p was recruited to the DSB site within 40 min of HO induction and its association with the broken DNA ends increased steadily for at least 4 h (Fig. 3F,G), as shown previously (Wolner et al. 2003; Miyazaki et al. 2004).

To investigate repair of the DSB by gene conversion in the wild-type and remodeling mutants, we used strains containing *HML*α as the donor template. This would permit us to examine repair of the HO-induced DSB at *MAT*a by recombination with the homologous *HML*α locus. The HR repair of chromosomal DNA DSBs is a multiple-step process that includes recruitment of DNA repair enzymes to a DSB site, strand invasion of a homologous template, and ligation. RSC's presence at a DSB site prior to the DNA repair enzymes suggested that it might remodel chromatin to facilitate recruitment of the DNA repair machinery. However, we found that in

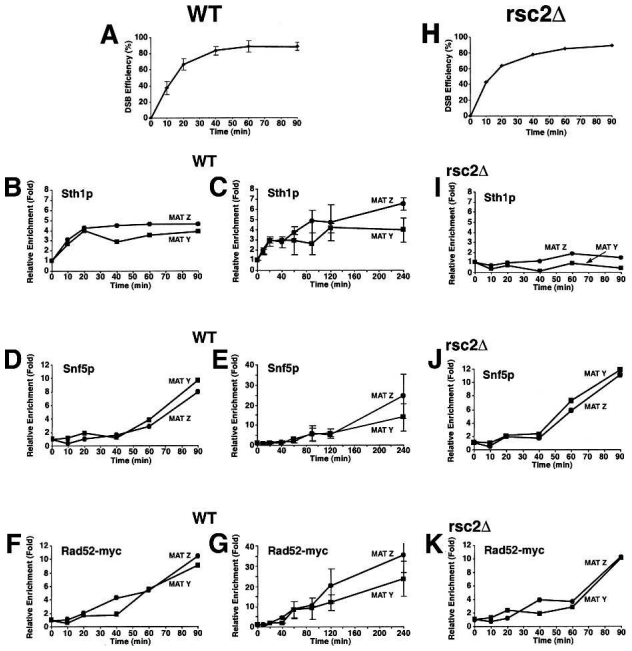

Figure 3. RSC and Swi/Snf are recruited to an in vivo DSB but neither is required for Rad52p's recruitment to a DSB. (*A*,*H*) Kinetics of DSB induction. Wild-type (BLY755) or *rsc2*Δ (BLY780) cells grown to mid-logarithmic phase in 2% raffinose at 30°C were shifted to 2% galactose to induce DSBs and genomic DNA harvested at the indicated times. The averages and standard deviations of five wild-type or three *rsc2*Δ mutant time-course experiments are plotted. (*B*,*D*,*F*) Aliquots of wild-type cells from *A* were processed for ChIP using anti-Sth1p, anti-Snf5p, or anti-Rad52p-13myc antibodies at the indicated times. All ChIPs were quantified by real-time PCR analysis and normalized to the time 0 values and to input DNA. Levels of *CEN*-proximal (*MAT Y*) or *CEN*-distal (*MAT Z*) DNAs immunoprecipitated by anti-Sth1p and anti-Snf5p were additionally normalized to those at the *PRE1* locus. (*C*,*E*,*G*) Long time courses of anti-Sth1p, anti-Snf5p, or anti-Rad52-myc13p ChIP analysis show continuous recruitment of Snf5p and Rad52-myc13p to a DSB. (*I*–*K*) ChIP assays using anti-Sth1p, anti-Snf5p, or anti-Rad52-myc13p antibodies were performed on *rsc2*Δ (BLY780) cells as described for wild-type cells. All ChIP data are representative of at least three independent time-course experiments except for anti-Rad52-myc ChIPs, which were done twice. Error bars represent standard deviations.

the absence of *RSC2* (which prevented the recruitment of Sth1p to the DSB site), Rad52-myc13p, one of the first HR repair enzymes to be recruited to a DSB site (Sugawara et al. 2003), was still recruited to both sides of the DSB with wild-type kinetics and at wild-type levels, suggesting that Rad52p recruitment is independent of RSC function (Fig. 3I,J). Rad52p's recruitment to the DSB also occurred in the absence of Swi/Snf (Supplementary Fig. 2). Similarly, we found that Swi/Snf's association with the DSB was also independent of RSC (Fig. 3K).

Because recruitment of Rad52p to a site-specific DSB was unimpaired in the chromatin-remodeling mutants, we next examined whether synapsis between *MAT* and *HML* occurs in *rsc* or *swi/snf* mutants by comparing the association of Rad51p and Rad52p proteins with *HML*α in *rsc2*Δ, *snf5*Δ, and wild-type cells by chromatin immunoprecipitation (ChIP) (Fig. 4). In *rsc2*Δ mutants, the levels of Rad51p and Rad52p proteins at both *MAT* Z and *HML*α sites were comparable to those in wild-type cells (Fig. 4C,D). In sharp contrast, in *snf5*Δ mutants, although wild-type or slightly higher levels of Rad51p and Rad52p were recruited to *MAT* Z, levels of these proteins at *HML*α were reduced at least fivefold (Fig. 4E,F). Importantly, recruitment of Rad51p to *HML*α can be restored in *snf5*Δ mutants carrying a CEN-*SNF5* plasmid but not a CEN plasmid alone (Supplementary Fig. 3). These results suggest an important role for Swi/Snf at or just preceding synapsis formation. Interestingly, we found that the *snf5*Δ cells that survive following 16 h of DSB induction (survival was comparable to wild-type cells) cannot switch mating type (Supplementary Fig. 4), consistent with the lack of synapsis in these cells.

We next asked whether either remodeling complex is necessary for extension of the invading *MAT* DNA strand on the homologous *HML*α donor site by new DNA polymerization (Fig. 5). Notably, in the *rsc2*Δ mutant, extension of the invading strand occurred with wild-type kinetics (Fig. 5C). As predicted, *snf5*Δ mutants

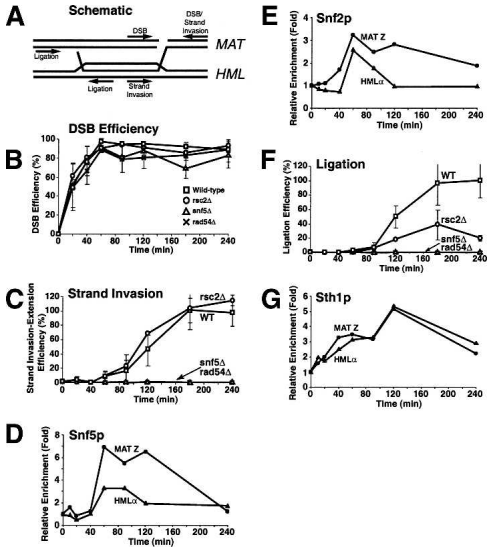

Figure 5. *rsc* and *swi/snf* are defective at distinct steps of HR repair. (*A*) Schematic diagram of an HR intermediate during mating-type switching. Primers used to monitor DSB, strand invasion-extension, and completion of repair are indicated. (*B*,*C*,*F*) DSBs were induced in wild-type (BLY747), *rsc2*Δ (BLY784), *snf5*Δ (BLY786), and *rad54*Δ (BLY804) strains as described in Figure 4. Genomic DNA was isolated at the indicated time points and monitored by quantitative real-time PCR analysis for efficiency of DSB formation (*B*), primer extension (*C*), and completion of repair (*F*) (see Supplemental Material for details). Primer extension and completion of repair were arbitrarily set at 100% for the highest wild-type level. All values were normalized to an *ACT1* internal control. The values are the averages of three independent experiments except for experiments carried out in *snf5*Δ and *rad54*Δ strains, which were each done twice. Error bars indicate one standard deviation. (*D*,*E*,*G*) Chromatin was also isolated from wild-type (BLY747) strains at the indicated time points and immunoprecipitated with anti-Snf5p, anti-Snf2p, or anti-Sth1p antibodies, followed by quantitative real-time PCR analysis as described in Figure 3.

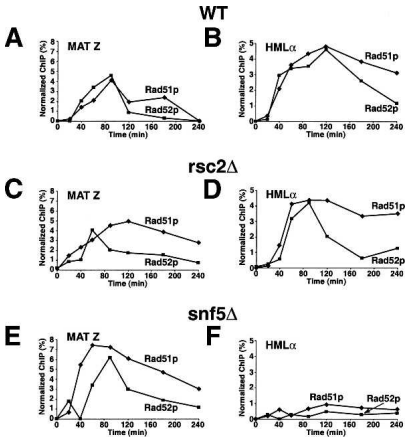

Figure 4. Synapsis formation between *MAT* and *HML* is defective in *snf5* mutants but not *rsc2* mutants. Wild-type (BLY747), *rsc2*Δ (BLY784), or *snf5*Δ (BLY786) strains carrying the *HML*α donor sequence were treated with 2% galactose to induce expression of *HO* endonuclease, and then with 2% dextrose after 1 h to repress *HO*. Chromatin prepared from wild-type (*A*,*B*), *rsc2*Δ (*C*,*D*), or *snf5*Δ (*E*,*F*) cells at the indicated time points was immunoprecipitated with anti-Rad51p or anti-Rad52p antibodies. The immunoprecipitated DNAs were quantified by real-time PCR amplification using primers specific to *MAT* Z or *HML*α regions and normalized to *ACT1* promoter sequences.

failed to extend invading strands, as these mutants failed to establish synapsis and in this regard, were indistinguishable from *rad54*Δ mutants (Fig. 5C). We also found that both RSC and Swi/Snf were recruited to the homologous donor sequence by 40 min and 1 h, respectively, preceding the appearance of the primer extension product. Binding of both RSC and Swi/Snf remained high for ~1 h and then decreased to nearly baseline levels once primer extension products appeared (Fig. 5, cf. D,E,G and C). These data are consistent with roles for both remodelers in recombinational repair, and further suggest that Swi/Snf might clear nucleosomes on the *HML* donor in order to expose nucleosomal DNA to the homology-searching complex.

Following strand invasion and resolution of the Holliday intermediates, DNA ligation completes the recombinational repair of the DNA DSB. Importantly, in *rsc2*Δ mutants, this latter post-synaptic DNA repair step was defective, achieving only 20% of wild-type cells, suggesting that RSC plays a role in HR repair at a step(s) following the initiation of new DNA synthesis by the invading 3′ single-stranded tail. *snf5*Δ mutants were entirely defective in post-synaptic repair since these cells could not perform strand invasion (Fig. 4F). Thus, RSC and Swi/Snf are both required for HR repair of DSBs, but function at different stages: Swi/Snf is required at or just preceding

strand invasion, while RSC is required following extension of the invading strand.

Although we found that *rsc2*Δ mutants were defective in a post-synaptic step of HR, RSC's rapid association with both sides of a DSB site suggests that RSC also plays an early role during HR. Interestingly, RSC and the Mre11/Rad50/Xrs2 complex, ATM-related Tel1p kinase, and ATR-related Mec1p kinase are recruited to DSB sites with similar kinetics (this study; Lisby et al. 2004), raising the possibility that an early MRX-, Tel1p-, or Mec1p-mediated modification of chromatin is a prerequisite for RSC's association with DSBs; a comparable chromatin modification could be responsible for Swi/Snf's later recruitment. Recent studies implicating RSC in the cohesion of sister chromatids (Baetz et al. 2004; Huang et al. 2004) and those demonstrating a requirement of cohesion for DSB repair (Strom et al. 2004; Unal et al. 2004) suggest that RSC might be necessary for DNA damage-induced cohesion. One model to explain RSC's later role in completing HR repair, which is supported by its association with *HML*α donor sites, is that RSC's remodeling activity is required for the post-synapsis dissociation of the invading DNA from donor DNA prior to ligation.

Interestingly, *rsc30* and *rsc8* were recently identified in a genetic screen for mutants that are defective in NHEJ (Shim et al. 2005). Therefore, RSC appears to play a broad role in the repair of DSBs, facilitating repair by both HR and NHEJ; individual subunits might differentially contribute to HR or NHEJ, or distinct RSC isoforms might be involved in distinct repair pathways. Future experiments will elucidate the mechanism(s) by which RSC is directed to repair DSBs by HR or NHEJ.

Swi/Snf binds to a DSB site later than RSC at about the time that Rad52p and Rad54p HR repair enzymes bind (Sugawara et al. 2003; Wolner et al. 2003; Miyazaki et al. 2004) and is required to achieve synapsis between *MAT* and *HML*α loci. Therefore, the very modest *swi/snf* mutant sensitivity to MMS and insensitivity to GAL-HO expression (Supplementary Fig. 4) were surprising; one possible explanation is that the relative balance of DSB repair is upset in *swi/snf* mutants to favor end joining.

Both Swi/Snf and RSC also function in transcription. Although *swi/snf* and *rsc* mutants do not affect expression of DNA repair enzymes under the conditions assayed (Sudarsanam et al. 2000; Angus-Hill et al. 2001), Swi/Snf is required for the DNA damage transcriptional response (DUN response) (see Sharma et al. 2003). Thus, while the results presented here in both nonswitching and switching strains strongly suggest that Swi/Snf and RSC participate directly in early and late steps in HR repair, global changes in transcription factor or repair factor levels mediated by these remodeling complexes likely contribute at least partly to the overall efficiency of DSB repair.

The requirement of two distinct ATP-dependent remodelers in HR repair emphasizes the crucial role of chromatin remodelers in repairing DNA in the context of chromatin. In addition, the separate roles of RSC and Swi/Snf demonstrate the specificity with which two ATP-dependent chromatin remodelers can function in the same process, providing new insight into both the mechanism of action of chromatin-remodeling complexes in DNA damage repair and the cellular coordination necessary to maintain genome integrity.

Materials and methods

Strains, media, and plasmids

S. cerevisiae strains used in the study are listed in Supplementary Table 1. Mutant and plasmid construction and additional methods are described in the Supplemental Material.

Plasmid-based DNA end-joining and HR assays

In vivo plasmid-based end-joining assays and in vivo plasmid-based HR/SSA assays were performed as previously described (Erdemir et al. 2002).

Chromatin immunoprecipitation

Asynchronous cultures were grown overnight at 30°C in YEP media containing 2% raffinose. When cultures reached mid-logarithmic phase, expression of the *HO* endonuclease was induced by the addition of 2% galactose. Chromatin was prepared at the indicated time points as previously described (Geng and Laurent 2004) and immunoprecipitated with anti-Sth1, anti-Snf5, anti-Snf2, anti-myc, affinity-purified anti-Rad51 (generously provided by L. Symington, Columbia University, New York, NY), or anti-Rad52 (Santa Cruz Biotechnology, Inc.) antibodies. The levels of immunoprecipitated DNAs were measured by quantitative real-time PCR as described (Geng and Laurent 2004). The relative enrichment was determined by the fold increase of ChIPed DNA relative to that at time 0. IP efficiencies of *MAT* Z and *HML*α sequences using anti-Rad51 and anti-Rad52 antibodies are shown relative to those of an *ACT1* promoter sequence. For each time course, *HO*-induced DSBs were verified by quantitative real-time PCR of genomic DNA. Primers used for DSB analyses and ChIPs are listed in Supplementary Table 2.

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