THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 283, NO. 16, pp. 10445–10452, April 18, 2008

ATP-dependent Chromatin Remodeling by the *Saccharomyces cerevisiae* Homologous Recombination Factor Rdh54*

Received for publication, January 4, 2008, and in revised form, February 15, 2008 Published, JBC Papers in Press, February 20, 2008, DOI 10.1074/jbc.M800082200

YoungHo Kwon[‡], Changhyun Seong[‡], Peter Chi[‡], Eric C. Greene[§], Hannah Klein[¶], and Patrick Sung[‡1]

From the [‡]Department of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut 06520, the [§]Department of Biochemistry and Molecular Biophysics, Columbia University, New York, New York 10032, and the [¶]Department of Biochemistry and Kaplan Cancer Center, School of Medicine, New York University, New York, New York 10016

Saccharomyces cerevisiae RDH54 **is a key member of the evolutionarily conserved *RAD52* epistasis group of genes needed for homologous recombination and DNA double strand break repair. The *RDH54*-encoded protein possesses a DNA translocase activity and functions together with the Rad51 recombinase in the D-loop reaction. By chromatin immunoprecipitation (ChIP), we show that Rdh54 is recruited, in a manner that is dependent on Rad51 and Rad52, to a site-specific DNA double strand break induced by the HO endonuclease. Because of its relatedness to Swi2/Snf2 chromatin remodelers, we have asked whether highly purified Rdh54 possesses chromatin-remodeling activity. Importantly, our results show that Rdh54 can mobilize a mononucleosome along DNA and render nucleosomal DNA accessible to a restriction enzyme, indicative of a chromatin-remodeling function. Moreover, Rdh54 co-operates with Rad51 in the utilization of naked or chromatinized DNA as template for D-loop formation. We also provide evidence for a strict dependence of the chromatin-remodeling attributes of Rdh54 on its ATPase activity and N-terminal domain. Interestingly, an N-terminal deletion mutant (rdh54Δ102) is unable to promote Rad51-mediated D-loop formation with a chromatinized template, while retaining substantial activity with naked DNA. These features of Rdh54 suggest a role of this protein factor in chromatin rearrangement during DNA recombination and repair.**

The genomic DNA in eukaryotic cells is organized into chromatin, which harbors repeated nucleosome units each comprising an octamer of four histone proteins, H2A, H2B, H3, and H4, and 147 bp of DNA (1, 2). The folding of DNA on the surface of histone proteins within the nucleosome inevitably poses an accessibility problem during various DNA transactions, including DNA replication, recombination, and repair. One mechanism to efficiently overcome this structural hindrance is by an ATP hydrolysis-dependent chromatin-remodeling process, such that remodeling proteins render DNA more accessible by weakening DNA:histone contacts, sliding nucleosomes along DNA, or removing H2A-H2B dimers from the nucleosome (3–5).

We are interested in delineating the mechanism of homologous recombination (HR)[2] that is mediated by proteins of the *RAD52* epistasis group, including how the HR machinery negotiates the constraints posed by chromatin structure. Regarding the latter, of special interest are two key members of the *RAD52* epistasis group, Rad54 and Rdh54 proteins. These HR factors play multifaceted roles in HR (6–8), and they belong to the Swi2/Snf2 protein superfamily, members of which are well known ATP hydrolysis-dependent chromatin remodelers (9, 10). Rad54 and Rdh54 both possess a DNA-dependent ATPase activity and a dsDNA translocase function that is fueled by ATP hydrolysis (8, 11–15). The DNA translocase activity of these Swi2/Snf2-like HR factors can modify DNA topology (11–13), enhance D-loop formation by the Rad51 recombinase (11, 16), accelerate the rate at which DNA strands are exchanged during the HR reaction (17, 18), and process branched DNA intermediates, including the Holliday structure, that are formed during HR (15, 17). Remarkably, Rad54 and Rdh54 also catalyze the removal of Rad51 from dsDNA, an attribute that is likely important for the intracellular recycling of Rad51 and for freeing the primer end in the nascent D-loop structure from Rad51 to facilitate the initiation of repair DNA synthesis (8, 19, 20). Based on cytological and chromatin immunoprecipitation analyses, it has been suggested that Rdh54 and Rad54 also function to release the meiosis-specific recombinase Dmc1 from bulk chromatin (21). Importantly, and consistent with its relatedness to the Swi2/Snf2 family of chromatin remodelers, Rad54 protein has a chromatin-remodeling function that is fueled by its ATPase activity (22–24) and is greatly enhanced via its association with Rad51 (25). Rad54 also works with Rad51 to promote the formation of D-loops with a chromatinized template (23, 24, 26).

Here, we provide evidence that, like Rad54 and several other Swi2/Snf2 family members, Rdh54 possesses a chromatin-re-

* This work was supported by National Institutes of Health research grants ES07061, GM57814, GM53738 (to H. L. K.) and GM074739 (to E. C. G.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s).

[1] To whom correspondence should be addressed: Dept. of Molecular Biophysics and Biochemistry, Yale University School of Medicine, 333 Cedar St., SHM-C130, New Haven, CT 06520-8024. Tel.: 203-785-4552; Fax: 203-785-6404; E-mail: Patrick.Sung@yale.edu.

[2] The abbreviations used are: HR, homologous recombination; ss, single-stranded; ds, double-stranded; ChIP, chromatin immunoprecipitation; DSB, double strand break; RSC, remodel the structure of chromatin.

modeling activity whose functionality is reliant on ATP hydrolysis. We show that Rdh54 works in conjunction with Rad51 to mediate D-loop formation with chromatinized DNA. Interestingly, in addition to its involvement in complex formation with Rad51, the conserved N-terminal region of Rdh54 is needed for maximal efficiency in chromatin remodeling as well. We have also employed chromatin immunoprecipitation (ChIP) to demonstrate the Rad51/Rad52-dependent delivery of Rdh54 protein to a site-specific DSB. Our results contribute toward dissecting the multifaceted role of Rdh54 in HR and DNA repair.

EXPERIMENTAL PROCEDURES

ChIP Assay—The ChIP procedure was carried out essentially as described (27). All the yeast strains used are isogenic to JKM179 strain (Δ*ho* Δ*hml*::*ADE1 MATα* Δ*hmr*::*ADE1 ade1–110 leu2,3–112 lys5 trp1*::*hisG ura3–52 ade3*::*GAL10:HO*), which harbors the *HO* gene under the control of the galactose-inducible *GAL10* promoter. A sequence that codes for a triple FLAG tag was attached to the 3′-end of the chromosomal *RDH54* gene to facilitate the immunoprecipitation of Rdh54 by commercially available anti-FLAG antibodies. NatMX was used as the selectable marker in the deletion of *RAD51*, *RAD52*, and *RDH54*. Cells were grown in YP containing 3% glycerol to reach mid-log phase, and then galactose (2%) was added to induce the HO endonuclease. At the designated times, an aliquot (45 ml) of the cell culture was treated with formaldehyde (1%) for 20 min and then quenched with 125 mM glycine for 5 min. Cell lysates were prepared and incubated with anti-Rad51 or anti-Rad52 antibodies (our own laboratory stocks) and protein G magnetic beads (Invitrogen) or with anti-FLAG affinity gel (Sigma). The immunoprecipitates were washed extensively and then incubated at 65 °C to reverse protein-DNA cross-links. Radioactive semiquantitative PCR was performed to amplify the *MAT Z* locus and the *PHO5* sequence, which was included as the internal control (27). After electrophoresis in a polyacrylamide gel, the PCR products were quantified in a Personal FX phosphorimager (Bio-Rad). The *MAT Z* signal at each time point was divided by the corresponding *PHO5* signal and normalized to the 0-h signal.

Protein Purification—Rad51 was purified from *Saccharomyces cerevisiae* cells tailored to overexpress the recombinase, as described (28). Rdh54 and its mutant variants were expressed in and purified from *Escherichia coli* Rosetta cells (Novagen), as described (19). RSC was purified from extracts of yeast cells that harbor chromosomally tandem affinity purification (TAP)-tagged *RSC2* (which codes for a subunit of RSC), as described (29).

Preparation of Chromatinized DNA—Nucleosomal arrays were assembled with pBluescript SK II DNA, and the histone octamer was purified from the nuclei of chicken erythrocytes (30) by the salt dialysis method, as described previously (24, 25). For the construction of the mononucleosome substrates, a 314-bp DNA fragment derived from the *RAD51* gene promoter was generated by PCR using plasmid pN124 as template (22). This DNA fragment was purified with the MinElute PCR purification kit (Qiagen) and 5′-end-labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase (New England Biolabs). The mononucleosome was assembled using the purified chicken histone octamer and the 314-bp DNA by salt dialysis, and the N1 and N3 species were isolated from preparative polyacrylamide gels, as described (22).

D-loop Formation on Naked and Chromatinized DNA Templates—D-loop reactions were performed essentially as described in our published work (19, 25, 31). Briefly, the Rad51 presynaptic filament was assembled by incubating a ^{32}P-labeled 90-mer oligonucleotide (2.4 μM nucleotides) and Rad51 (0.8 μM) in buffer D (35 mM Tris-HCl, pH 7.5, 1 mM dithiothreitol, 5 mM $MgCl_2$, 60 mM KCl, 100 ng/μl bovine serum albumin, 2 mM ATP, and an ATP-regenerating system comprising 20 μg/ml creatine kinase and 20 mM creatine phosphate) for 5 min at 37 °C. The indicated amounts of Rdh54 protein, one of the rdh54 mutant proteins, or RSC and free or the chromatinized form of pBluescript (35 μM base pairs) were then added to the reaction, which was incubated for 10 min at 30 °C. The reaction mixtures were deproteinized with SDS (0.5%) and proteinase K (0.5 mg/ml) for 5 min at 37 °C, followed by electrophoresis in 0.9% agarose gels in TAE buffer (40 mM Tris acetate, pH 7.4, 0.5 mM EDTA). The gels were dried onto DE81 paper (Whatman), and the radiolabeled DNA species were visualized and quantified in a phosphorimaging apparatus (Personal Molecular Imager FX, Bio-Rad) with the aid of Quantity One software (Bio-Rad). The reactions with RecA used 1 μM of the recombinase and were assembled, processed, and analyzed as above. The percentage of D-loop refers to the fraction of the ^{32}P-labeled 90-mer oligonucleotide substrate being converted into the D-loop.

HaeIII Accessibility Test for Chromatin Remodeling—The chromatinized pBluescript SK II DNA (35 μM base pairs) was mixed with the indicated amounts of Rdh54, one of the rdh54 mutant proteins, or RSC and HaeIII (10 units) in 12.5 μl of buffer D, followed by a 30-min incubation at 30 °C. Where indicated, Rad51 and the 90-mer DNA (at a ratio of 3 nucleotides/Rad51 monomer) used in the D-loop reaction were preincubated at 37 °C for 5 min in the reaction buffer prior to incorporating the chromatinized DNA, test protein, and HaeIII. After deproteinizing treatment, as above, the reaction mixtures were resolved in a 1.5% agarose gel, and the DNA species were stained with ethidium bromide.

Nucleosome Mobilization Test—The mononucleosome substrates (0.8 nM) were incubated with the indicated amounts of Rdh54 or one of the rdh54 mutant proteins in 12.5 μl of buffer D at 30 °C for 1 h. Where indicated, Rad51 and the 90-mer DNA (at a ratio of 3 nucleotides/Rad51 monomer) used in the D-loop reaction were preincubated at 37 °C for 5 min in the reaction buffer prior to incorporating the substrate and test protein. Following the addition of φX174 replicative form DNA (121 μM base pairs, ~90% supercoiled form), the reaction mixtures were resolved in 4% polyacrylamide gels in TB buffer (45 mM Tris-borate, pH 8.3). The gels were dried and subject to phosphorimaging analysis, as above.

RESULTS

Rad51/Rad52-dependent DSB Recruitment of Rdh54—ChIP has been employed to demonstrate the recruitment of various HR factors, including Rad51, Rad52, and Rad54, to a site-specific DSB that is induced by the HO endonuclease at the MAT locus located on chromosome 3 (27, 32). ChIP was

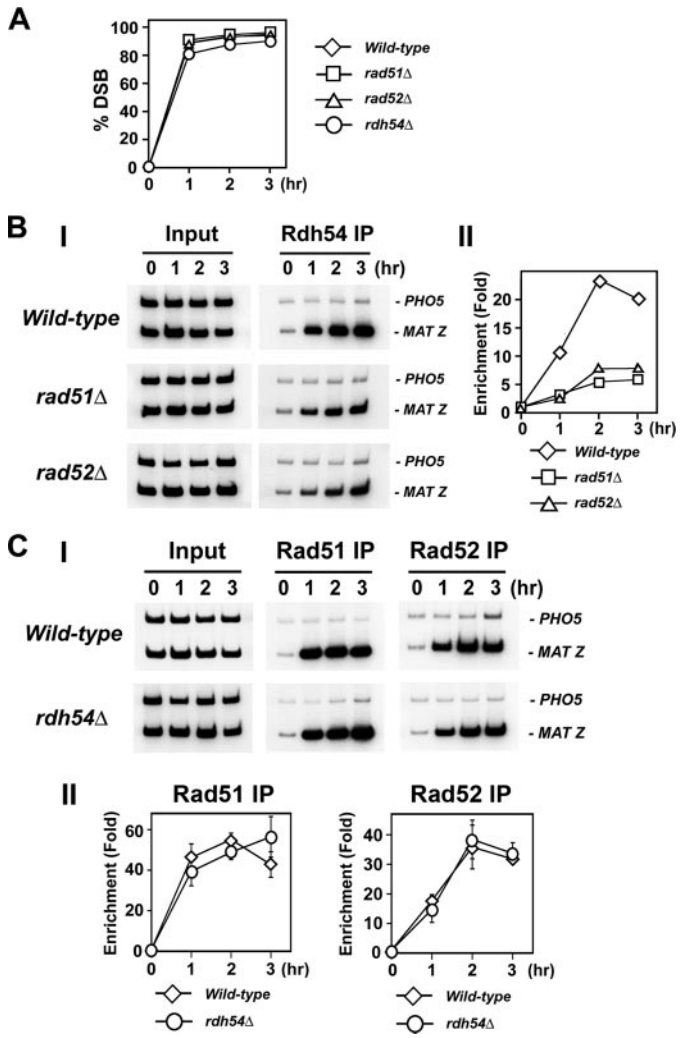

FIGURE 1. **Recruitment of Rdh54, Rad51, and Rad52 to a site-specific DSB.** *A*, kinetics of DSB induction in isogenic yeast strains. Wild-type, *rad51*Δ, *rad52*Δ, and *rdh54*Δ cells were grown in mid-log phase, and galactose (2%) was added to induce HO expression. DNA was isolated from the cells at the indicated time and DSB formation at the *MAT* locus was monitored by PCR using primers that flank the DSB sites. The PCR product was separated, quantified, normalized, and plotted as described under "Experimental Procedures" and in a previous study (27). *B*, recruitment of Rdh54 to the HO-induced DSB in wild-type, *rad51*Δ, or *rad52*Δ cells. The *MAT Z* target sequence and control *PHO5* sequence were PCR-amplified. *Panel I* and *panel II* show the PCR products and the quantification of the results, respectively. *C*, recruitment of Rad51 and Rad52 to the HO-induced DSB in wild-type or *rdh54*Δ cells. The *MAT Z* target sequence and control *PHO5* sequence were PCR-amplified. *Panel I* and *panel II* show the PCR products and the quantification of the results, respectively.

used in our study to test whether Rdh54 is similarly targeted to the HO-made DSB. The ChIP experiments involved the use of isogenic wild-type, rad51Δ, rad52Δ, and rdh54Δ yeast strains that harbor the HO gene whose expression is inducible by the addition of galactose to the growth medium. For the immunoprecipitation of HR proteins, we used affinity-purified polyclonal anti-Rad51 and anti-Rad52 antibodies that we had raised and attached a triple FLAG tag to the C terminus of the chromosomally expressed Rdh54 protein to allow for the use of commercially available anti-FLAG antibody in Rdh54 immunoprecipitation. We first verified that the kinetics and extent of DSB induction were the same in all the yeast strains (Fig. 1*A*). We then carried out the requisite

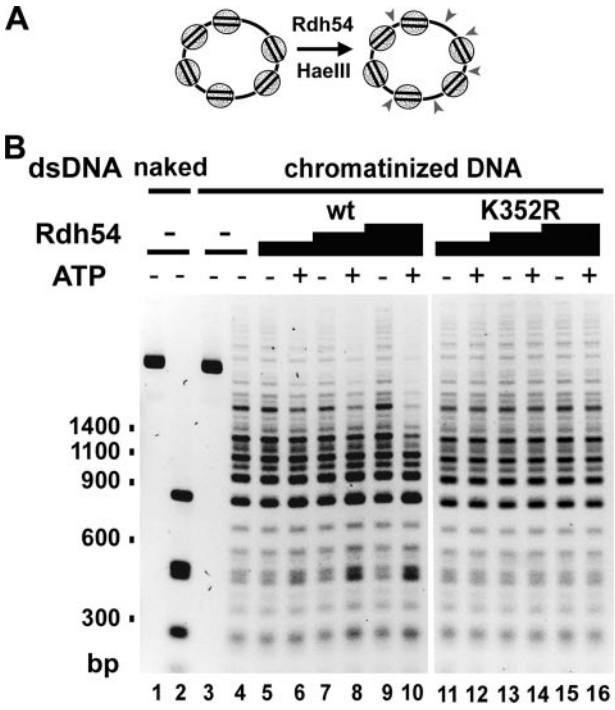

FIGURE 2. **Enhancement of chromatin accessibility by Rdh54.** *A*, schematic for the HaeIII accessibility assay. The HaeIII sites (14 in all) on the plasmid molecules are shielded by the nucleosomes but become accessible upon the remodeling of the nucleosome array by Rdh54. *B*, the HaeIII accessibility assay was conducted with wild-type Rdh54 or the ATP hydrolysis defective rdh54 K352R mutant (0.125, 0.25, and 0.5 μM) in the presence or absence of ATP, as indicated. Naked DNA was used in *lanes 1* and *2*, and HaeIII was omitted in *lanes 1* and *3*.

ChIP experiments to examine the targeting of the aforementioned HR proteins to the MAT Z sequence that is proximal to the HO break and also to ask whether the DSB targeting of Rad51 and Rad52 are in any way affected by Rdh54, and *vice versa*.

As shown in Fig. 1*B*, Rdh54 became highly enriched at the HO break, by 10 fold one hour after HO endonuclease induction, reaching the maximum of 20 fold enrichment two to three hours after HO induction. The DSB targeting of Rdh54 was greatly diminished, but not completely abolished, in the isogenic *rad51*Δ and *rad52*Δ strains. In agreement with the published results (27, 32), both Rad51 and Rad52 proteins were recruited to the DSB (Fig. 1*C*). The recruitment of Rad51 or Rad52 to the HO break was not significantly altered in the absence of Rdh54. Thus, the ChIP data indicate that Rdh54 is recruited to DSBs in a Rad51/Rad52-dependent manner and suggest that the timely DSB recruitment of Rad51 and Rad52 does not rely upon Rdh54.

Rdh54 Enhances the Restriction Enzyme Accessibility of Nucleosomal DNA—We first employed a restriction endonuclease accessibility test to ask whether Rdh54 can remodel chromatin structure. For this, we assembled a nucleosomal array on plasmid DNA to use as substrate. Owing to the presence of phased nucleosomes, the DNA in the substrate is rather resistant to the restriction enzyme HaeIII, and restructuring of the array by chromatin remodelers is conveniently revealed by an increased HaeIII accessibility (Fig. 2*A*) (23, 25). As shown in Fig. 2*B*, Rdh54 enhanced the HaeIII accessibility of the nucleo-

somal array, with the degree of accessibility being dependent on the concentration of Rdh54 (Fig. 2*B*). To determine whether the ability of Rdh54 to remodel the nucleosomal array is dependent upon ATP hydrolysis, we either omitted ATP from the reaction or substituted the wild-type protein with the ATP hydrolysis-defective K352R variant. In either case, there was no change in the accessibility of the array DNA to HaeIII (Fig. 2*B*). Taken together, the results provide the first evidence for an ATP hydrolysis-dependent chromatin-remodeling activity in Rdh54.

Nucleosome Sliding by Rdh54—A number of chromatin remodelers have the ability to reposition a mononucleosome (22, 33–35). To examine whether Rdh54 possesses such activity, we utilized a nucleosome sliding assay with substrates that harbor a mononucleosome positioned either near one of the ends (N1) or the center (N3) of a ^{32}P-labeled 314-bp DNA fragment. N1 and N3 have very different mobilities in a native polyacrylamide gel, with the former being the faster migrating species (Fig. 3*A*). Two types of restructuring of the mononucleosome substrates by chromatin remodelers are possible: 1) simple repositioning of the nucleosome in the substrates will generate novel species that have a gel mobility different than that of either N1 or N3, and 2) eviction of the nucleosome will produce free DNA that migrates at the gel front. As shown in Fig. 3 (*B* and *C*), incubation of the N1 and N3 mononucleosome substrates with Rdh54 in the presence of ATP led to the generation of multiple novel mononucleosome species (designated, collectively, as N2) and a significant amount of free DNA, providing evidence for a nucleosome sliding activity in Rdh54. However, given the inherent limitations of the experimental design, we might have underestimated the level of nucleosome removal from the substrate DNA.

Consistent with the results from the restriction enzyme accessibility assay (Fig. 2), Rdh54-mediated nucleosome repositioning is strictly dependent on ATP hydrolysis, because 1) the omission of ATP inactivated the efficacy of Rdh54 (data not shown) and 2) the ATPase-defective rdh54 K352R mutant protein was inactive in the reaction (Figs. 2*C* and 3*B*). The above results allowed us to conclude that Rdh54 catalyzes ATP hydrolysis-dependent nucleosome sliding.

Rdh54 Co-operates with Rad51 in D-loop Formation with Chromatinized DNA—Having demonstrated a chromatin-remodeling activity in Rdh54 (Figs. 2 and 3), we asked whether Rad51 and Rdh54 would catalyze D-loop formation with a chromatinized DNA template. To do this, the Rad51 presynaptic filament was incubated, with or without Rdh54, with homologous dsDNA that was either naked or pre-assembled into a nucleosomal array. As reported before (11, 19) and reiterated here, with naked dsDNA, Rdh54 greatly enhanced the ability of the Rad51 presynaptic filament to form D-loops (Fig. 4, *B–D* and *F*). Importantly, Rdh54 also rendered D-loop formation with the chromatinized DNA robust, such that the yield of D-loops with the nucleosomal substrate was comparable to that obtained with naked DNA (Fig. 4, *B–D* and *F*). Control experiments showed that Rdh54 alone is devoid of D-loop forming ability (Fig. 4*B*) (11). As expected, the *E. coli* RecA protein was adept at D-loop formation with naked DNA but not chromatinized DNA (Fig. 4, *E* and *F*), and the inclusion of Rdh54 had no effect on the ability of RecA to make D-loops with either naked or chromatinized DNA (11) (data not shown). Thus, the Rad51-Rdh54 pair can catalyze efficient D-loop formation even when the dsDNA partner is chromatinized. Other results confirmed that the D-loop reaction catalyzed by Rad51 and Rdh54 requires ATP hydrolysis by Rdh54, because the rdh54 K352R protein was inactive in this regard (data not shown).

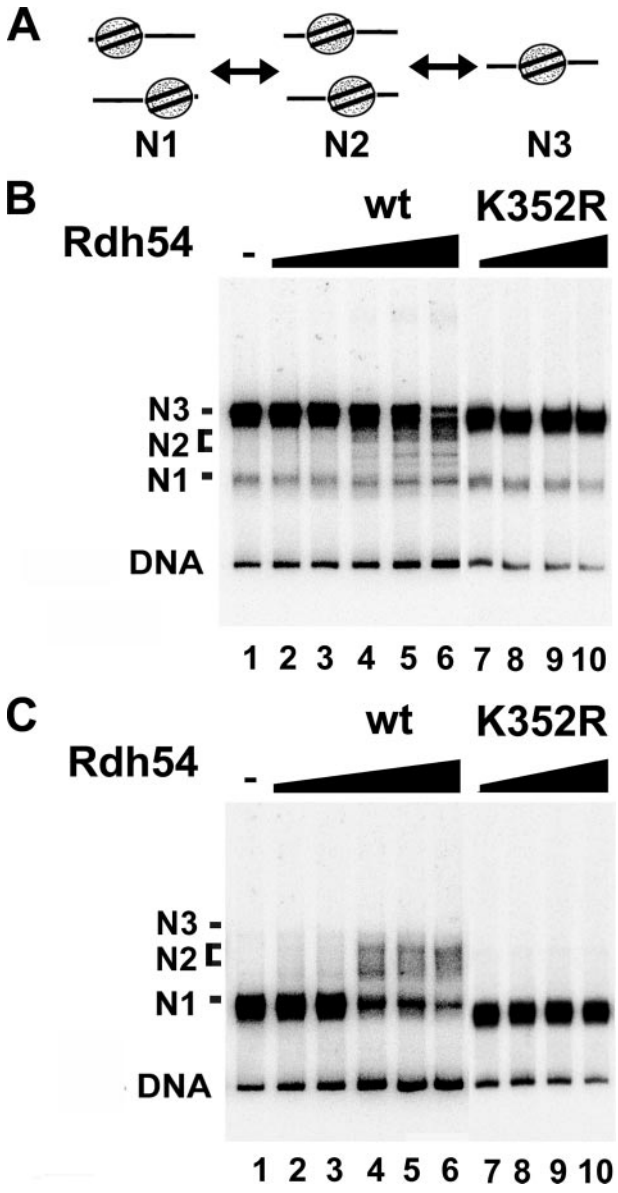

FIGURE 3. **Nucleosome mobilization by Rdh54.** *A*, the mononucleosome substrates and their remodeling. N1 harbors a mononucleosome near either end of the 314-bp DNA fragment and N3 species has a mononucleosome near the center of the DNA (22, 43). Sliding of the mononucleosome generate novel species, collectively identified as N2. *B*, N3 was incubated with either with Rdh54 (4, 8, 16, 24, or 32 nM) or rdh54 K352R (8, 16, 24, or 32 nM) and then analyzed. *C*, same as *B*, except that N1 was used as the substrate.

The Chromatin Remodeler RSC Does Not Promote the D-loop Reaction—The above results have provided clear evidence for a chromatin-remodeling activity in Rdh54 (Figs. 2 and 3) and for functional synergy between Rad51 and Rdh54

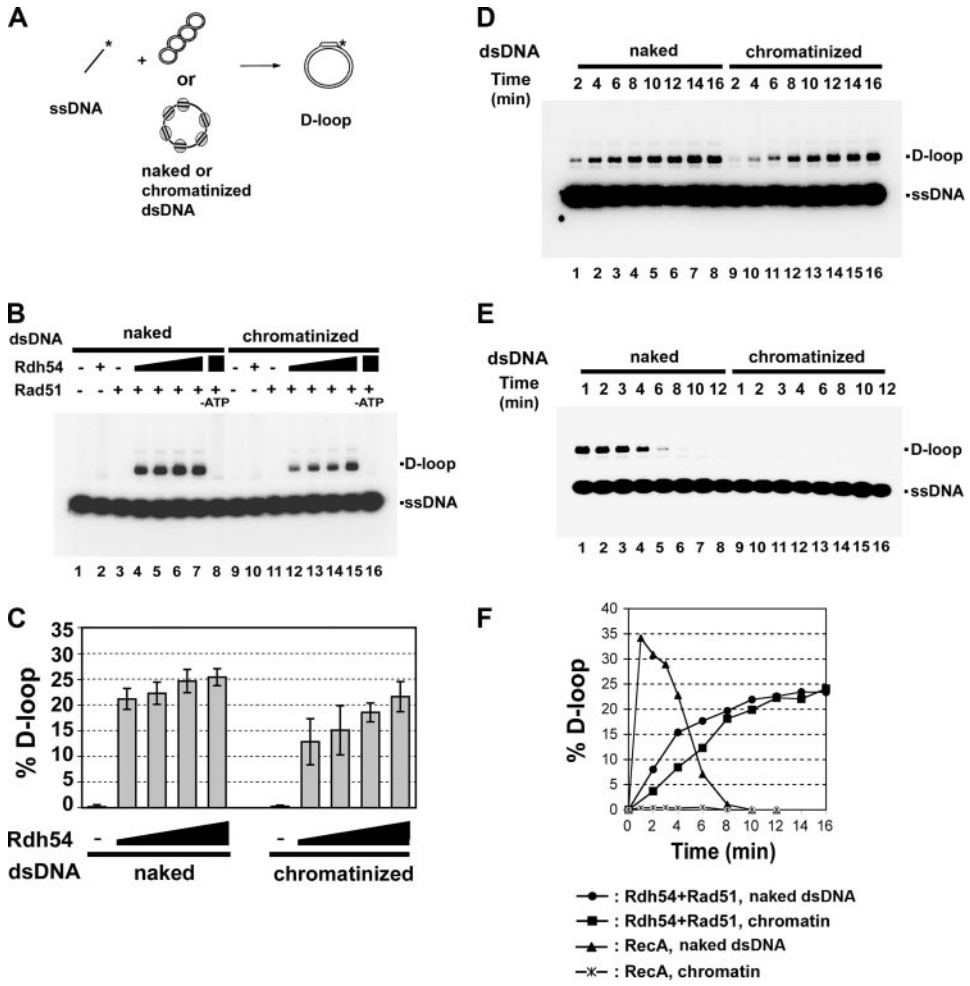

FIGURE 4. **D-loop formation with chromatinized DNA by Rad51 and Rdh54.** *A*, schematic of the D-loop reaction. *B*, D-loop reactions mediated by Rad51 (0.8 μM) and Rdh54 (0.2, 0.3, 0.4, or 0.5 μM) with either naked or chromatinized dsDNA. *C*, quantification of the results in *lanes 3–7* and *11–15* of *B*. *D*, time course of D-loop reactions (Rad51, 0.8 μM; Rdh54, 0.5 μM) with either naked or chromatinized dsDNA. *E*, time course of D-loop reactions mediated by RecA with either naked or chromatinized dsDNA. *F*, quantification of the results in *D* and *E*.

in D-loop formation with chromatinized DNA (Fig. 4). The fact that Rdh54 does not enable RecA to catalyze D-loop formation with chromatinized DNA (data not shown) suggests that the stimulatory effect of Rdh54 is specific to Rad51. To further ascertain the specificity of the observed functional synergy between Rad51 and Rdh54, we purified RSC2 complex, a known chromatin remodeler (36) (Fig. 5*A*), and used it together with Rad51 in D-loop reactions that employed either naked or chromatinized DNA as template. As shown in Fig. 5*B*, the purified complex was able to remodel chromatinized DNA and enhance HaeIII accessibility (Fig. 5*B*). However, RSC was unable to substitute for Rdh54 in D-loop reactions, regardless of whether naked or chromatinized DNA was used (Fig. 5*C*). Thus, the ability of Rad51 and Rdh54 to mediate D-loop formation with chromatinized DNA is in all likelihood contingent upon not only the chromatin-remodeling activity of Rdh54, but also on specific complex formation of Rdh54 with Rad51.

Role of the Conserved Rdh54 N Terminus in Chromatin Remodeling—In Rdh54, the Swi2/Snf2 core concerned with DNA binding and ATP hydrolysis is separate from the N-terminal 280 or so residues of the protein (37, 38). The N-terminal extension appears to be conserved among Rdh54 orthologues (39). To test the idea that the Rdh54 N-terminal domain might be involved in the interaction with factors important for HR, we previously constructed N-terminally truncated variants (Δ34, Δ102, and Δ133) of this protein and provided evidence that 1) all three truncation mutants retain the biochemical attributes (DNA binding, ATPase, and DNA translocase activities) of the full-length protein, 2) the rdh54 Δ34 mutant protein retains the ability to interact and functionally synergize with Rad51, and 3) the rdh54 Δ102 mutant protein is slightly compromised for Rad51 binding and functional synergy with Rad51, but the rdh54 Δ133 mutant is severely impaired in these regards (19). We used the HaeIII accessibility and the mononucleosome repositioning assays described earlier to test whether the three N-terminal truncation mutations affect the chromatin-remodeling activity of Rdh54. In the HaeIII accessibility test, rdh54 Δ34 showed a slightly diminished level of activity compared with full-length Rdh54, whereas rdh54 Δ102 and rdh54 Δ133 appeared to be defective (Fig. 6*A*). Interestingly, all three N-terminally truncated rdh54 mutant proteins displayed little or no activity in the mononucleosome repositioning assay (Fig. 6*B*). Thus, the results reveal an involvement of the conserved N terminus of Rdh54 in the chromatin remodeling function of this HR factor.

The Rdh54 N Terminus Is Indispensable for D-loop Formation with Chromatinized DNA—We next ascertained the impact that the N-terminal truncation mutations have on the ability of Rdh54 to promote Rad51-mediated D-loop formation with chromatinized DNA. Consistent with previously published results (19), when naked dsDNA was used as substrate, we saw a slightly attenuated ability of the rdh54 Δ34 and rdh54 Δ102 proteins but a complete defect in the rdh54 Δ133 mutant to promote D-loop formation (Fig. 7). Interestingly, with chromatinized dsDNA, although the rdh54 Δ34 mutant protein retained a significant level of activity, neither the Δ102 nor the Δ133 variant was efficacious (Fig. 7).

Rad51 Does Not Enhance Chromatin Remodeling by Rdh54—Published results have shown that Rad51 to stimulates ATPase, DNA supercoiling, and DNA strand opening activities of Rdh54 (19). We asked whether Rad51 similarly enhances the chromatin-remodeling function of Rdh54. However, we could find lit-

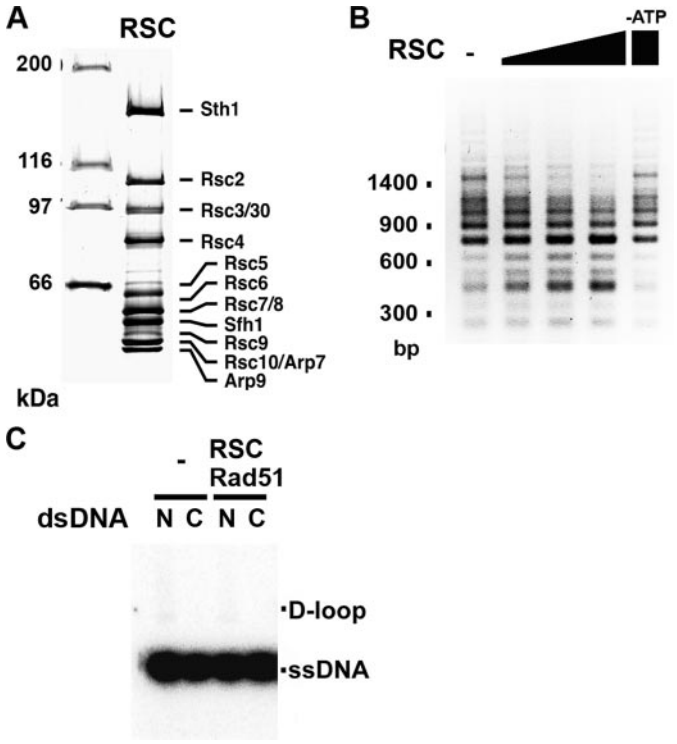

FIGURE 5. **Lack of functional synergy between Rad51 and RSC.** *A*, purified RSC was analyzed by SDS-PAGE and Coomassie Blue staining. *B*, the chromatin-remodeling activity of purified RSC (5, 10, and 20 nM) was examined using the HaeIII accessibility assay, as in Fig. 2. *C*, RSC (20 nM) was tested with Rad51 (0.8 μM) for the ability to promote D-loop formation with either naked (*N*) or chromatinized (*C*) DNA.

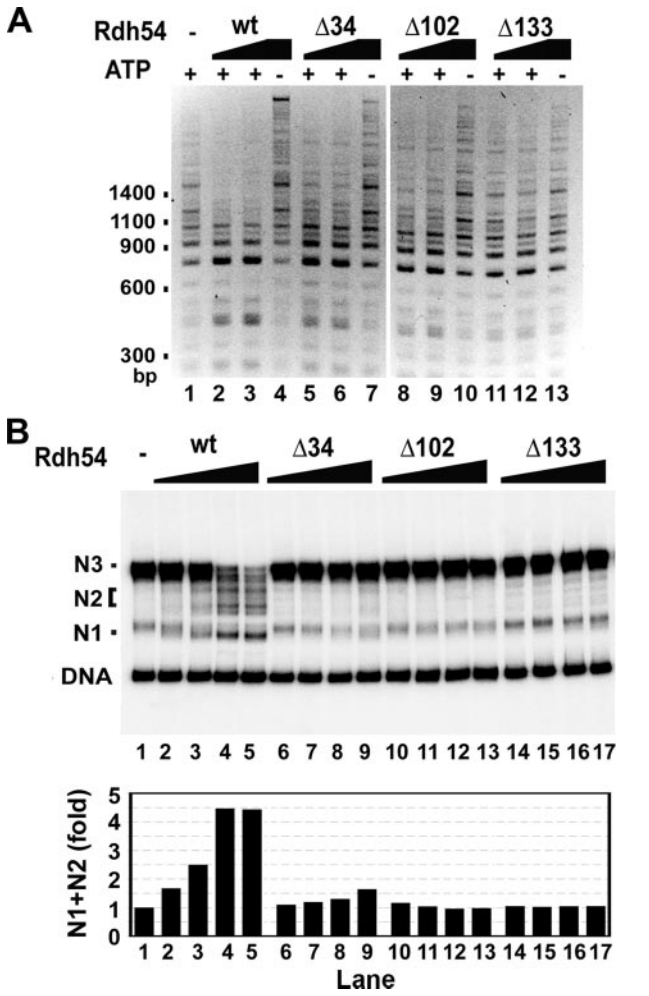

FIGURE 6. **Dependence of chromatin remodeling on the N terminus of Rdh54.** *A*, the chromatin-remodeling activity of 0.3 or 0.5 μM of Rdh54 and of its N-terminally truncated variants (rdh54 Δ34, Δ102, and Δ133 that lack the N-terminal 34, 102, and 133 amino acid residues, respectively) was examined using the HaeIII accessibility assay, as in Fig. 2. *B*, nucleosome sliding by Rdh54 and its N-terminal truncation variants (8, 16, 32, or 48 nM) was examined with the substrate N3, as in Fig. 3. Quantification of the data, presented in the histogram, was done by measuring N1 and N2 species generated after the remodeling reaction.

tle or no such enhancement in either the HaeIII accessibility test (Fig. 8, *A* and *B*) or the mononucleosome repositioning assay (Fig. 8, *C* and *D*).

DISCUSSION

Studies by several laboratories, including ours, have found a DNA translocase activity in Rdh54 protein (11, 14, 15, 19). This activity is utilized to alter DNA topology (11), facilitate DNA strand invasion by Rad51 (11, 19), dissociate branched DNA intermediates (15), and release Rad51 and likely the related meiotic recombinase Dmc1 from dsDNA (19, 21). Consistent with its relatedness to the Swi2/Snf2 family of chromatin remodelers, including the HR protein Rad54 (22–24), the use of two well established assay systems in this study have allowed us to conclude that Rdh54 possesses a chromatin-remodeling activity as well. As in the case of other Swi2/Snf2-like remodelers, chromatin remodeling by Rdh54 is fueled by ATP hydrolysis, such that the rdh54 K352R variant, lacking ATPase activity, is completely defective in this regard. Previous work by us has demonstrated an involvement of the N terminus of Rdh54 in mediating complex formation with Rad51 (19). The data presented here reveal that this conserved N-terminal region is also indispensable for chromatin remodeling. Specifically, deletion of the first 34 residues in Rdh54, while having no noticeable effect on Rad51 binding (19), ablates the ability of Rdh54 to reposition a mononucleosome. Moreover, even though the rdh54 Δ102 mutant is only slightly impaired for Rad51 binding (19), it is functionally defective in either the nucleosome array accessibility assay or the mononucleosome repositioning test. We note that deletion of as few as the first nine residues from the N terminus of the *Drosophila melanogaster* Rad54 protein adversely affects its chromatin-remodeling function (40), whereas a much larger N-terminal truncation (Δ129) in the *S. cerevisiae* Rad54 protein does not seem to have a significant impact in this regard (31). It remains to be established whether the N terminus of *Sc*Rdh54 and *Dm*Rad54 share a common attribute that is critical for their chromatin remodeling function.

Aside from demonstrating a chromatin-remodeling activity in Rdh54, we have furnished evidence that this Swi2/Snf2-like factor functionally synergizes with Rad51 in the formation of D-loops with chromatinized DNA. Importantly, the noted synergy between Rad51 and Rdh54 is highly specific, because Rdh54 neither enhances the D-forming ability of the heterologous recombinase RecA (11) nor enables RecA to make D-loops

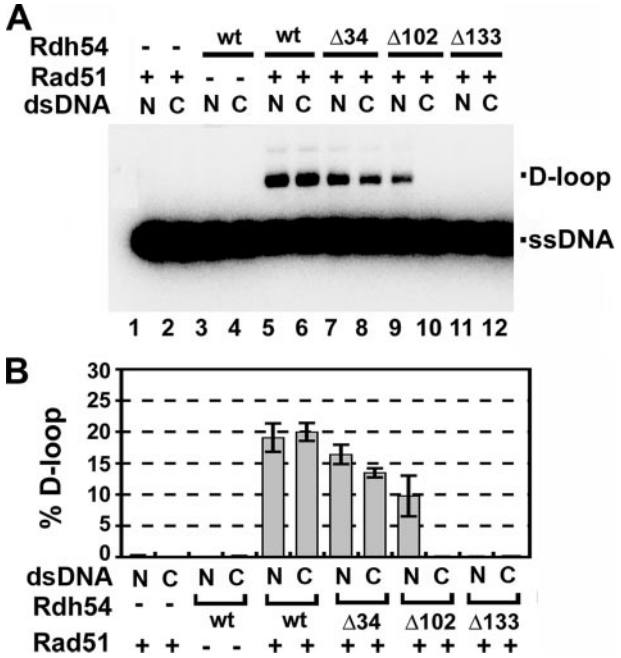

FIGURE 7. **Requirement for the Rdh54 N terminus of in D-loop formation with chromatinized DNA.** *A*, Rdh54 and its N-terminal truncation variants (0.5 μM) were examined with Rad51 (0.8 μM) for the ability to promote the D-loop reaction with either naked (*N*) or chromatinized (*C*) DNA. *B*, quantification of the results in *A*.

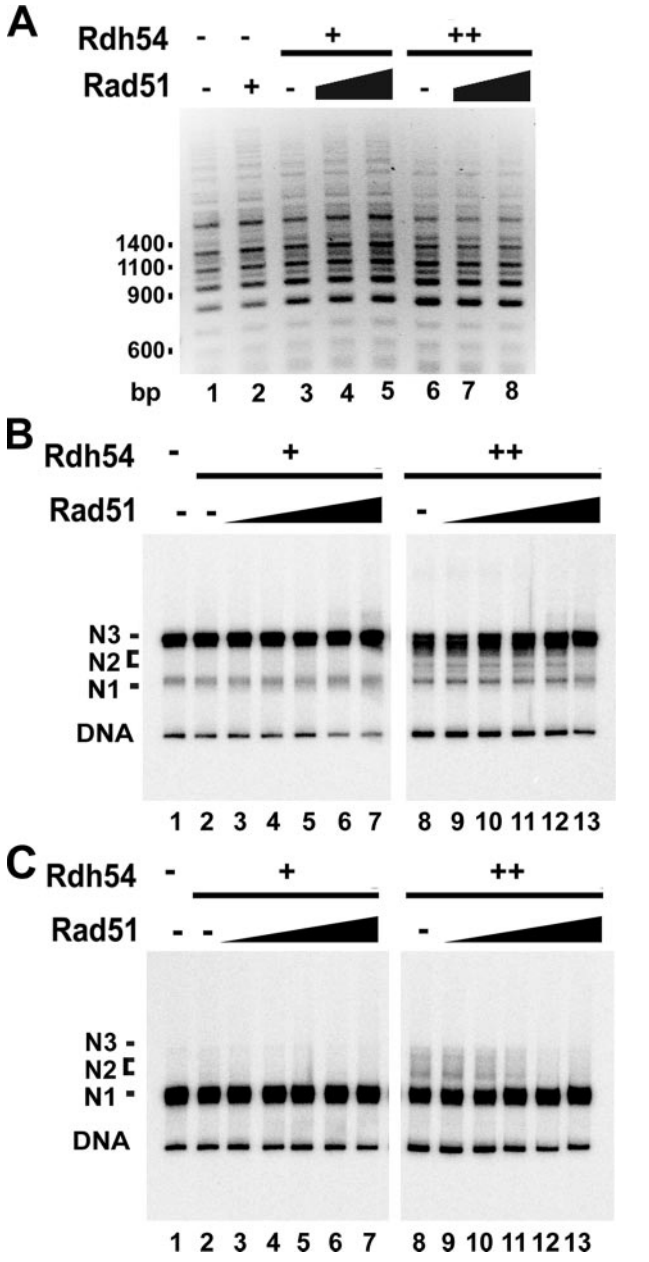

FIGURE 8. **Effect of Rad51 on the chromatin-remodeling activity of Rdh54.** *A*, HaeIII accessibility test was carried out with Rdh54 (0.06 or 0.125 μM) and Rad51 (0.1, 0.3, or 0.5 μM). *B* and *C*, nucleosome sliding test using either the N3 or N1 substrate (*B* and *C*, respectively) was carried out with Rdh54 (8 or 16 nM) and Rad51 (25, 50, 100, 200, or 400 nM).

with chromatinized DNA. Furthermore, the *S. cerevisiae* chromatin remodeler RSC, a multisubunit complex that harbors the Swi2/Snf2-like motor protein Sth1, is ineffective in the D-loop reaction with Rad51, regardless of whether naked or chromatinized DNA is employed. According to our published work (19), we would like to suggest that the functional synergy of Rad51 and Rdh54 in the DNA strand invasion reaction that involves chromatin stems from a specific interaction between the two HR factors.

By cytology, Lisby *et al.* (41) have found co-localization of Rdh54 with a variety of HR proteins in nuclear foci upon treatment of yeast cells with ionizing radiation. The targeting of Rdh54 to these DNA damage-induced foci is dependent upon Rad51 and Rad52 (41). Consistent with these cytological data, we have shown by ChIP that Rdh54 is recruited to the HO-made DSB at the *MAT* locus. Importantly, DSB targeting of Rdh54 is strongly attenuated in *rad51*Δ and *rad52*Δ cells. These results, together with the cytological data of Lisby *et al.* (41), help establish that Rdh54 is recruited to DNA lesions and that the recruitment process is largely dependent on Rad51 and Rad52.

Aside from its involvement in HR and DNA repair reactions, Rdh54 is important for chromosome segregation in meiosis I. In its meiotic role, it is thought that Rdh54 cooperates with the meiosis-specific recombinase Dmc1 in the mediation of inter-homologue recombination that links the chromosome homologues to allow for the proper alignment of the homologues on the spindle apparatus and their faithful disjunction in the first meiotic division (38, 42). The research material and methodology that we and others have described (11, 19, 21 and this work) should prove valuable for future studies directed at defining the likely functional synergy between Rdh54 and Dmc1.

Acknowledgment—We thank Stephen Kowalczykowski for providing plasmid pN124.

REFERENCES

1. Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F., and Richmond, T. J. (1997) *Nature* **389,** 251–260
2. Richmond, T. J., and Davey, C. A. (2003) *Nature* **423,** 145–150
3. Becker, P. B., and Horz, W. (2002) *Annu. Rev. Biochem.* **71,** 247–273
4. Flaus, A., and Owen-Hughes, T. (2004) *Curr. Opin. Genet. Dev.* **14,** 165–173

5. Saha, A., Wittmeyer, J., and Cairns, B. R. (2006) *Nat. Rev. Mol. Cell. Biol.* **7,** 437–447
6. Sung, P., Trujillo, K. M., and Van Komen, S. (2000) *Mutat. Res.* **451,** 257–275
7. Krogh, B. O., and Symington, L. S. (2004) *Annu. Rev. Genet.* **38,** 233–271
8. Heyer, W. D., Li, X., Rolfsmeier, M., and Zhang, X. P. (2006) *Nucleic Acids Res.* **34,** 4115–4125
9. Eisen, J. A., Sweder, K. S., and Hanawalt, P. C. (1995) *Nucleic Acids Res.* **23,** 2715–2723
10. Flaus, A., Martin, D. M., Barton, G. J., and Owen-Hughes, T. (2006) *Nucleic Acids Res.* **34,** 2887–2905
11. Petukhova, G., Sung, P., and Klein, H. (2000) *Genes Dev.* **14,** 2206–2215
12. Van Komen, S., Petukhova, G., Sigurdsson, S., Stratton, S., and Sung, P. (2000) *Mol. Cell* **6,** 563–572
13. Ristic, D., Wyman, C., Paulusma, C., and Kanaar, R. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98,** 8454–8460
14. Prasad, T. K., Robertson, R. B., Visnapuu, M. L., Chi, P., Sung, P., and Greene, E. C. (2007) *J. Mol. Biol.* **369,** 940–953
15. Nimonkar, A. V., Amitani, I., Baskin, R. J., and Kowalczykowski, S. C. (2007) *J. Biol. Chem.* **282,** 30776–30784
16. Petukhova, G., Stratton, S., and Sung, P. (1998) *Nature* **393,** 91–94
17. Bugreev, D. V., Mazina, O. M., and Mazin, A. V. (2006) *Nature* **442,** 590–593
18. Solinger, J. A., Lutz, G., Sugiyama, T., Kowalczykowski, S. C., and Heyer, W. D. (2001) *J. Mol. Biol.* **307,** 1207–1221
19. Chi, P., Kwon, Y., Seong, C., Epshtein, A., Lam, I., Sung, P., and Klein, H. L. (2006) *J. Biol. Chem.* **281,** 26268–26279
20. Solinger, J. A., Kiianitsa, K., and Heyer, W. D. (2002) *Mol. Cell* **10,** 1175–1188
21. Holzen, T. M., Shah, P. P., Olivares, H. A., and Bishop, D. K. (2006) *Genes Dev.* **20,** 2593–2604
22. Alexeev, A., Mazin, A., and Kowalczykowski, S. C. (2003) *Nat. Struct. Biol.* **10,** 182–186
23. Alexiadis, V., and Kadonaga, J. T. (2002) *Genes Dev.* **16,** 2767–2771
24. Jaskelioff, M., Van Komen, S., Krebs, J. E., Sung, P., and Peterson, C. L. (2003) *J. Biol. Chem.* **278,** 9212–9218
25. Kwon, Y., Chi, P., Roh, D. H., Klein, H., and Sung, P. (2007) *DNA Repair* (*Amst.*) **6,** 1496–1506
26. Zhang, Z., Fan, H. Y., Goldman, J. A., and Kingston, R. E. (2007) *Nat. Struct. Mol. Biol.* **14,** 397–405
27. Wolner, B., van Komen, S., Sung, P., and Peterson, C. L. (2003) *Mol. Cell* **12,** 221–232
28. Sung, P., and Stratton, S. A. (1996) *J. Biol. Chem.* **271,** 27983–27986
29. Wittmeyer, J., Saha, A., and Cairns, B. (2004) *Methods Enzymol.* **377,** 322–343
30. Stein, A. (1989) *Methods Enzymol.* **170,** 585–603
31. Raschle, M., Van Komen, S., Chi, P., Ellenberger, T., and Sung, P. (2004) *J. Biol. Chem.* **279,** 51973–51980
32. Sugawara, N., Wang, X., and Haber, J. E. (2003) *Mol. Cell* **12,** 209–219
33. Flaus, A., and Owen-Hughes, T. (2003) *Biopolymers.* **68,** 563–578
34. Hamiche, A., Sandaltzopoulos, R., Gdula, D. A., and Wu, C. (1999) *Cell* **97,** 833–842
35. Langst, G., Bonte, E. J., Corona, D. F., and Becker, P. B. (1999) *Cell* **97,** 843–852
36. Cairns, B. R., Lorch, Y., Li, Y., Zhang, M., Lacomis, L., Erdjument-Bromage, H., Tempst, P., Du, J., Laurent, B., and Kornberg, R. D. (1996) *Cell* **87,** 1249–1260
37. Durr, H., Flaus, A., Owen-Hughes, T., and Hopfner, K. P. (2006) *Nucleic Acids Res.* **34,** 4160–4167
38. Shinohara, M., Shita-Yamaguchi, E., Buerstedde, J. M., Shinagawa, H., Ogawa, H., and Shinohara, A. (1997) *Genetics* **147,** 1545–1556
39. Tanaka, K., Kagawa, W., Kinebuchi, T., Kurumizaka, H., and Miyagawa, K. (2002) *Nucleic Acids Res.* **30,** 1346–1353
40. Alexiadis, V., Lusser, A., and Kadonaga, J. T. (2004) *J. Biol. Chem.* **279,** 27824–27829
41. Lisby, M., Barlow, J. H., Burgess, R. C., and Rothstein, R. (2004) *Cell* **118,** 699–713
42. Symington, L. S. (2002) *Microbiol. Mol. Biol. Rev.* **66,** 630–670, table of contents
43. Eberharter, A., Langst, G., and Becker, P. B. (2004) *Methods Enzymol.* **377,** 344–353