Homologous DNA Pairing by Human Recombination Factors Rad51 and Rad54*

Human Rad51 (hRad51) and Rad54 proteins are key members of the RAD52 group required for homologous recombination. We show an ability of hRad54 to promote transient separation of the strands in duplex DNA via its ATP hydrolysis-driven DNA supercoiling function. The ATPase, DNA supercoiling, and DNA strand opening activities of hRad54 are greatly stimulated through an interaction with hRad51. Importantly, we demonstrate that hRad51 and hRad54 functionally cooperate in the homologous DNA pairing reaction that forms recombination DNA intermediates. Our results should provide a biochemical model for dissecting the role of hRad51 and hRad54 in recombination reactions in human cells.

In eukaryotic organisms, the repair of DNA double-stranded breaks by homologous recombination is mediated by a group of evolutionarily conserved genes known as the RAD52 epistasis group. Members of the RAD52 group (RAD51, RAD52, RAD54, RAD55, RAD57, RAD59, and RDH54/TID1) were first uncovered in genetic screens in the budding yeast Saccharomyces cerevisiae (1, 2). In mammals, the efficiency of homology-directed recombinational DNA repair is modulated by the tumor suppressors BRCA1 and BRCA2 (3), providing a compelling link between this DNA repair pathway and the suppression of tumor formation. The involvement of the homologous recombination machinery in the maintenance of genome stability and tumor suppression underscores the need for deciphering the action mechanism of this machinery.

During the recombinational repair of DNA double-stranded breaks, a single-stranded DNA intermediate is utilized by the recombination machinery to invade a DNA homolog, most often the sister chromatid, to form a DNA joint molecule referred to as a D-loop (2). D-Loop formation is critical for subsequent steps in the recombination reaction, which include repair DNA synthesis and resolution of recombination intermediates (1, 2), that lead to the restoration of the integrity of the injured chromosome.

In the past several years, biochemical studies have begun to shed light on the functions of the human RAD52 group proteins in DNA joint formation. Much of the published work has centered on the human Rad51 (hRad51) protein, which is structurally related to the Escherichia coli recombinase enzyme RecA (4). Like RecA, hRad51 assembles into a right-handed filament on single-stranded (ss) DNA in a reaction that is dependent on ATP binding (reviewed in Ref. 5). Importantly, hRad51 protein has been shown to have DNA pairing and strand exchange activities that yield DNA joints between homologous ssDNA and double-stranded DNA substrates (6–8). The homologous pairing and strand exchange function of hRad51 is augmented by replication protein A (RPA), a heterotrimeric single-stranded DNA binding factor (6, 8), by hRad52 protein (9), and by the Rad51B-Rad51C heterodimeric complex (10), which is the functional equivalent of the yeast Rad55-Rad57 complex (11).

The RAD54 encoded product belongs to the Swi2/Snf2 protein family (12). Purified hRad54 exhibits DNA-dependent ATPase and DNA supercoiling activities (13–15). However, the manner in which hRad54 influences the hRad51-mediated recombination reaction has remained mysterious. Here we report our biochemical studies that show functional interactions between hRad51 and hRad54 in DNA supercoiling and homologous DNA pairing reactions. We discuss how hRad51 and hRad54 cooperate to make DNA joints during recombination processes.

**EXPERIMENTAL PROCEDURES**

**Anti-Rad54 Antibodies**—The first 238 amino acid residues of the human Rad54 protein were fused to glutathione S-transferase in the vector pGEX-3X. The fusion protein was expressed in E. coli strain BL21 (DE3) and purified from inclusion bodies by preparative denaturing polyacrylamide gel electrophoresis and used as antigen for raising polyclonal antibodies in rabbits. The same antigen was covalently conjugated to cyanoargent bromide-activated Sepharose 4B and used as affinity matrix to purify the antibodies from rabbit antisera, as described (16).

**Rad54 Expression and Purification**—A recombinant baculovirus containing the cloned hRad54 cDNA with an added FLAG epitope at the C terminus was generated. HighFive insect cells were infected with the recombinant baculovirus at a multiplicity of infection of 10 and harvested after 48 h of incubation. An extract was prepared from 500 ml of insect cell culture (5 × 10⁶ cells) using a French Press in 60 ml of cell breakage buffer (50 mM Tris-HCl, pH 7.5, 2 mM EDTA, 10% sucrose, 200 mM KCl, 1 mM dithiothreitol, 1 mM phenylmethylsulfonfluoride, and the following protease inhibitors at 3 μg/ml each: apotinin, chymostatin, leupeptin, and pepstatin). After centrifugation (100,000 × g for 60 min), the clarified extract was loaded onto a Q-Sepharose column (10-ml matrix). The flow-through fraction from the Q column was fractionated in a sulfo-propyl-Sepharose column (10-ml matrix) with a 50-ml, 0–700

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* The abbreviations used are: hRad51, human Rad51; yRad51, yeast Rad51; ss, single-stranded; BSA, bovine serum albumin; AMP-PNP, adenosine 5′-β,γ-iminonitrophosphate; ATP-γ-S, adenosine 5′-3-O-(thio)triphosphate.
mM KCl gradient in K buffer (20 mM KH$_2$PO$_4$ at pH 7.4, 0.5 mM EDTA, 1 mM dithiothreitol, and 10% glycerol). Fractions containing the peak of hRad54 were pooled and loaded onto a 1-ml Macro-hydroxyapatite (Bio-Rad) column, which was eluted with 30 ml of 0–300 mM KH$_2$PO$_4$ in K buffer. The peak fractions were pooled and mixed with 1.5 ml of Antiserum hRad54 (Sigma) and rocked for 3 h at 4°C. The FLAG agarose was washed three times with 3 ml of 150 mM KCl in buffer K before eluting hRad54 using the same buffer containing 1 mg/ml of the FLAG peptide (Sigma). hRad54 (~1 mg) eluted from the FLAG matrix was concentrated in a Centricon-30 microconcentrator to 5 mg/ml and stored in small aliquots at –70°C.

**Topoisomerase I—E. coli topoisomerase I** was purified to near homogeneity from the *E. coli* strain JM101 with plasmid pJW312-sal containing the topA gene under the Lac promoter, as described (17).

**Binding of hRad54 to Affi-hRad51 Beads**—Purified hRad51 and bovine serum albumin (BSA) were coupled to Affi-Gel 15 beads at 4°C following the instructions of the manufacturer (Bio-Rad). The resulting matrices contained 4 and 12 mg/ml hRad51 and BSA, respectively. Purified hRad54 (1.2 μg) was mixed with 5 μl of Affi-Rad51 or Affi-BSA at 4°C for 30 min in 30 μl of buffer containing 100 mM KCl and 0.1% Triton X-100 by constant tapping. The beads were washed twice with 50 μl of the same buffer before being treated with 30 μl of 2% SDS at 37°C for 5 min to elute the bound hRad54. The various fractions (4 μl each) were analyzed by immunoblotting to determine their content of hRad54.

**DNA Substrates**—Topologically relaxed 4X174 DNA was prepared as described (18), and pBluescript SK DNA was made in *E. coli* DH5α and purified as described (19). The oligonucleotide used in the n-loop reaction is complementary to positions 1932–2022 of the pBluescript SK DNA and had the sequence 5'-AAATCAATCTAAAGTATATATGAGT-

**Fig. 1.** Purification of hRad54. A, expression of hRad54 in insect cells. Extracts from uninfected insect cells (lane 1) and from insect cells infected with the recombinant hRad54 baculovirus (lane 2) were probed with affinity-purified anti-hRad54 antibodies. In lane 3, 100 ng of purified hRad54 was also subjected to immunoblotting. B, purified hRad54 protein (1 μg) was run in an 8% denaturing polyacrylamide gel and stained with Coomassie Blue.

**RESULTS**

**Human Recombination Factors—**Human Rad51 was expressed in a recA*-E. coli* strain and purified to near homogeneity as described previously (8). The hrad51 K133R mutant, which harbors the change of the conserved lysine residue in the Walker type A nucleotide binding motif to arginine, was also similarly expressed and purified. In agreement with previously published results (20), hrad51 K133R has negligible ATPase activity compared with wild type hRad51 (data not shown). We cloned the human RAD54 cDNA from a testis cDNA library using the polymerase chain reaction. The entire cloned *hrad54* cDNA was sequenced to ensure that it agreed with the published sequence (21). We obtained ~1 mg of nearly homogeneous hRad54 (Fig. 1B) from 500 ml of insect cell culture by a combination of conventional column chromatography and affinity binding to an antibody specific for the DNA strand opening activity of hRad54.

**DNA Supercoiling and DNA Strand-opening Reactions**—Increasing amounts of hRad54 were incubated with 80 ng of relaxed 4X174 DNA (12 μM nucleotides) for 2 min at 23°C in 12 μl of reaction buffer (20 mM Tris-HCl, pH 7.4, 5 mM MgCl$_2$, 1 mM dithiothreitol, 100 μM ATP, and an ATP-regenerating system consisting of 10 mM creatine phosphate and 28 μg/ml creatine kinase). Following the addition of 100 ng of E. coli topoisomerase I in 0.5 μl, the reactions were incubated for 10 min at 23°C and then deproteinized with treatment of 0.5% SDS and proteinase K (0.5 mg/ml) for 10 min at 37°C. Samples were run on 1% agarose gels in TAE buffer (35 mM Tris acetate, pH 7.4, 0.5 mM EDTA) at 23°C and then stained with ethidium bromide. In the experiment in Fig. 4B, the relaxed DNA was incubated with the indicated amounts of hRad51 and hRad54 for 2 min at 23°C, followed by the addition of topoisomerase and a 10-min incubation at 23°C. For the P1 sensitivity experiments in Figs. 3C and 4C, the reactions were assembled in the same manner except that 0.4 unit of P1 nuclease (Roche) was used instead of hRad51. The DNA species were resolved in a 1% agarose gel containing 10 μg ethidium bromide in TAE buffer.

**Loop Reaction**—For the time course reactions (25 μl, final volume) in Fig. 5, hRad51 or hrad51 K133R (800 nM) was incubated with the 5'-labeled ss oligonucleotide (2.5 μM nucleotides) for 3 min at 37°C in 22 μl of reaction buffer (20 mM Tris-HCl, pH 7.4, 100 μg/ml BSA, 1.5 mM MgCl$_2$, 2 mM ATP, and the ATP-regenerating system described above). This was followed by the addition of hRad54 (120 nm) in 1 μl and incubation at 23°C for 2 min. The reaction was completed by adding the pBluescript SK replicative form DNA (0.1 μg base pairs) in 2 μl. The reaction mixtures were incubated at 30°C, and 3.8-μl aliquots were withdrawn at the indicated times, deproteinized, and run in 1% agarose gels in TAE buffer. The gels were dried and the levels of n-loop were quantified by phosphorimage analysis. The reactions in which ATP, hRad51, or hRad54 was omitted or ATP was replaced by ATP-S or AMP-PNP were scaled down 2-fold to a 12.5-μl final volume, but they were otherwise assembled and processed in exactly the same manner.

**DNA Supercoiling and DNA Strand Opening by hRad54—**Tan et al. (14) showed an ability of hRad54 to alter the DNA linking number of a nicked plasmid in the presence of DNA ligase. The induction of DNA linking number change was dependent on ATP hydrolysis by hRad54 (14). The same group also used scanning force microscopy to provide evidence that hRad54 tracks on DNA when ATP is hydrolyzed (15). A schematic depicting the basis for tracking-induced DNA supercoiling by hRad54 is given in Fig. 3A.

The yRad54 protein also tracks on DNA and, as a result,
Figure 2. hRad54 interacts with hRad51. Purified hRad54 (1.2 μg) was mixed with Affi-beads containing either BSA (Affi-BSA) or hRad51 (Affi-Rad51) in 30 μl and washed twice with 50 μl buffer, followed by treatment of the beads with 30 μl of SDS to elute bound hRad54. The starting material (I), supernatant (S), the two washes (W1 and W2), and the SDS eluate (E). 4 μl each, were subjected to immunoblotting to determine their hRad54 content.

Figure 3. hRad54 supercoils DNA and promotes DNA strand opening. A, basis for hRad54-induced supercoiling, as per Ristic et al. (15) and Van Komen et al. (18). The free energy from ATP hydrolysis fuels the tracking of a hRad54 oligomer on DNA, producing a positively supercoiled domain ahead of protein movement and a negatively supercoiled domain behind. B, increasing amounts of hRad54 (20, 400, and 750 nM in lanes 3–5, respectively) were incubated with topologically relaxed DNA (20 μM nucleotides) and E. coli topoisomerase I in buffer that contained ATP. The highest amount of hRad54 (750 nM) was also incubated with the DNA substrate in the absence of topoisomerase (lane 6) and in the presence of topoisomerase but with the omission of ATP (lane 7) or the substitution of ATP by ATPγS (γ-S; lane 8) and AMP-PNP (PNP; lane 9). DNA alone (lane 1) or DNA incubated with topoisomerase (lane 2) was also included. The reaction mixtures were run in a 1% agarose gel, which was treated with ethidium bromide to reveal the DNA species. C, increasing amounts of hRad54 (200, 400, and 750 nM in lanes 3–5, respectively) were incubated with topologically relaxed DNA (20 μM nucleotides) and P1 nuclease in buffer that contained ATP. The highest amount of hRad54 (750 nM) was also incubated with the DNA substrate in the absence of P1 (lane 6) and in the presence of P1 but with the omission of ATP (lanes 7 and 8) or the substitution of ATP by ATPγS (γ-S; lane 9). DNA alone (lane 1) or DNA incubated with P1 in the absence of hRad54 (lane 2) were also included. The reaction mixtures were run in a 1% agarose gel containing 10 μM ethidium bromide.

generates positive and negative supercoils in the DNA substrate. Removal of the negative supercoils by treatment with E. coli topoisomerase I leads to the accumulation of positive supercoils and the formation of an overwound DNA species called Form OW (18). Here we used the same strategy to examine the ability of hRad54 to supercoil DNA. As shown in Fig. 3B, in the presence of topoisomerase, purified hRad54 protein readily induces a linking number change in the DNA (18). The DNA supercoiling reaction is dependent on ATP hydrolysis, as revealed by its omission or substitution with a nonhydrolyzable analogue (ATPγS or AMP-PNP) (Fig. 3B).

We asked whether the negative supercoils generated as a result of hRad54 tracking on the DNA substrate (15) (Fig. 3, A and B) leads to transient DNA strand opening by examining the sensitivity of a relaxed DNA template to the single-strand specific nuclease P1, as per Van Komen et al. (18). Fig. 3C shows that incubation of topologically relaxed DNA with hRad54 rendered the relaxed DNA substrate sensitive to P1 nuclease, as indicated by the accumulation of nicked circular and linear DNA forms. The DNA strand opening reaction is also completely dependent on ATP hydrolysis (Fig. 3C).

Activities of hRad54 Are Stimulated by hRad51—The results presented here (Fig. 2) and elsewhere (22) have unveiled a specific interaction between hRad51 and hRad54. We examined whether the hRad54 ATPase would be enhanced upon interaction with hRad51. As shown in Fig. 4A, a much higher rate of ATP hydrolysis was seen when hRad54 was combined with hRad51. The fact that yRad51 was ineffective in this reaction (Fig. 4A) indicates that the action of hRad51 is specific. Although hRad51 is known to have a weak ATPase activity (23), the fact that the hrad51 K133R mutant protein, which binds but does not hydrolyze ATP (20), was just as effective in
promoting ATP hydrolysis (data not shown) strongly indicated that the increase in ATP hydrolysis was because of enhancement of the hRad54 ATPase function.

We next asked whether the DNA supercoiling activity of hRad54 would also be up-regulated by hRad51. The results showed that hRad51 stimulates the supercoiling reaction, as indicated by a much higher level of Form OW DNA (Fig. 4B). Because negative supercoiling generated by hRad54 leads to DNA strand opening (Fig. 3C), we thought that hRad51 might also promote this activity. Indeed, the inclusion of hRad51 greatly elevated the nicking of the relaxed DNA substrate by P1 nuclease (Fig. 4C). Even with the inclusion of hRad51, no Form OW DNA or nicking of DNA was seen when ATP was omitted or substituted by the nonhydrolyzable analogues ATPγS and AMP-PNP (Fig. 4, B and C). Thus, the results revealed that hRad51 markedly stimulates the ability of Rad54 to supercoil DNA and unwind DNA strands. The hrad51 K133R protein was just as effective as wild type hRad51 in enhancing the DNA supercoiling and strand opening activities of hRad54 (data not shown). Furthermore, we found that yRad51 does not stimulate the hRad54 activities (data not shown), thus indicating a high degree of specificity in the hRad51 action.

hRad51 and hRad54 Cooperate in Homologous DNA Pairing—The RecA/Rad51 class of general recombinases is central to recombination processes by virtue of their ability to catalyze the homologous DNA pairing reaction that yields heteroduplex DNA joints (2, 24). Because hRad51 and hRad54 physically interact (22) (Fig. 2) and hRad51 enhances the various activities of hRad54 (Fig. 4), it was of considerable interest to examine the influence of hRad54 on hRad51-mediated homologous DNA pairing.

The homologous pairing assay monitors the incorporation of a 32P-labeled single-stranded oligonucleotide into a homologous supercoiled target (pBluescript) to give a D-loop structure (Fig. 5A). As reported before (25) and reiterated here (Fig. 5B), hRad51 by itself is not particularly adept at forming D-loop. Importantly, the inclusion of hRad54 rendered D-loop formation possible. D-Loop formation by the combination of hRad51 and hRad54 requires ATP hydrolysis, because no D-loop was seen when ATP was omitted or when it was replaced by either ATPγS or AMP-PNP (Fig. 5B). Significantly, the time course revealed a cycle of rapid formation and disruption of D-loop, such that the D-loop level reached its maximum by 1 min but declined rapidly thereafter (Fig. 5, B and D). In fact, by the reaction end point of 6 min, little or no D-loop remained (Fig. 5, B and D). Such a cycle of D-loop synthesis and reversal seems to be a general characteristic for the RecA/Rad51 class of recombinases (26, 27). Because the RecA-ssDNA nucleoprotein filament disassembles upon ATP hydrolysis (24), we considered the possibility that the dissociation of D-loop seen here (Fig. 5B) could be related to ATP hydrolysis-mediated turnover of hRad51. To test this premise, we used the hrad51 K133R mutant protein, which binds but does not hydrolyze ATP (20), with hRad54 in the D-loop assay. True to prediction, with hrad51 K133R, the D-loop amount increased with time, reaching a much higher final level than when hRad51 was used (Fig. 5, B–D); by 4 min, 23% of the input ssDNA or 55% of the pBluescript plasmid DNA had been incorporated into the D-loop structure. As expected, with both hRad51/hRad54 and hrad51 K133R/hRad54, formation of D-loop required both the 90-mer substrate and the pBluescript target, and substitution of the pBluescript DNA with the heterologous dX174 DNA completely abolished D-loop formation (data not shown).

DISCUSSION

It has been deduced from biochemical and scanning force microscopy analyses that Rad54 tracks on DNA, producing positive supercoils ahead of the protein movement and negative supercoils tailing it (15, 18). As a result of interaction with hRad51, the ATPase, DNA supercoiling, and DNA strand opening activities of Rad54 are greatly enhanced (this work). Petukhova et al. (19) first reported that yRad51 enhances homologous DNA pairing by yRad51. Here we have presented biochemical evidence that hRad51 and hRad54 also work in concert to make DNA joints. Interestingly, the hRad51/hRad54-mediated D-loop reaction undergoes a rapid cycle of joint formation and dissociation. We have speculated that ATP hydrolysis by hRad51 could have resulted in its turnover from the bound ssDNA. This might have led to the transfer of hRad51/hRad54 to the displaced strand in the D-loop to initiate a second round of homologous pairing with the newly formed DNA joint. The presumed secondary pairing reaction could have accounted for the dissociation of the initial D-loop. Consistent with this hypothesis, the ATP hydrolysis-defective hrad51 K133R mutant is much more adept at forming D-loop than the wild type protein. Previously, studies in yeast and chicken DT40 cells with the same Rad51 ATPase mutant have
shown that it is biologically active but that an increased level of this mutant is needed for full complementation of the various phenotypes of Rad51-deficient cells (20, 28). The fact that the hrad51 K133R mutant is even more effective than the wild type protein in the D-loop reaction strongly suggests that the slighted biological efficacy (20, 28) and observed dominance (29) of this protein are because of a reason other than a diminished ability to mediate homologous pairing. The hrad51 K133R mutant may form a highly stable complex with DNA, thus reducing the effective concentration of free protein available for recombination reactions. Importantly, our biochemical results predict that other members of the RAD52 group may function to prevent reversal of the D-loop reaction catalyzed by hrad51/hRad54.

Inactivation of the hRad54 ATPase activity impairs the ability to carry out recombination in vivo (14), consistent with the premise that the ATP hydrolysis-dependent DNA supercoiling and DNA strand opening activities of hRad54 are germane for recombination. As discussed here and elsewhere (15, 18, 30), it is likely that the DNA strand opening activity of hRad54 promotes the acquisition of an unwound DNA structure conducive for the formation of the nascent DNA joint that links recombining chromosomes. The ability of hRad54 to pull the incoming duplex molecule through its fold (i.e. tracking) is also expected to enhance the rate at which the duplex can be sampled by the hRad51-ssDNA nucleoprotein filament for homology. Finally, it remains a distinct possibility that the dynamic DNA topological changes induced by the combination of hRad51 and hRad54 are critical for the remodeling of chromatin during recombination.

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REFERENCES

1. Paques, F., and Haber, J. E. (1999) Microbiol. Mol. Biol. Rev. 63, 349–404
2. Sung, P., Trujillo, K., and Van Komen, S. (2000) Mutat. Res. 451, 257–275
3. Pierce, A. J., Stark, J. M., Araujo, F. D., Moynahan, M. E., Berwick, M., and Jasin, M. (2001) Trends Cell Biol. 11, 552–59
4. Shinohara, A., Ogawa, H., Matsumoto, Y., Ushio, N., Ikeo, K., and Ogawa, T. (1993) Nat. Genet. 4, 239–243
5. Yu, X., Jacobs, S. A., West, S. C., Ogawa, T., and Egeland, E. H. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 8419–8424
6. Baumann, P., Benson, F. E., and West, S. C. (1996) Cell 87, 757–766
7. Gupta, R. C., and MacLennan, J. H., Gold, K. L., and Radding, C. M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 463–468
8. Sigurdsson, S., Trujillo, K., Song, B.-W., Stratton, S., and Sung P. (2001) J. Biol. Chem. 276, 8798–8806
9. Benson, F. E., Baumann, P., and West, S. C. (1998) Nature 391, 401–404
10. Sigurdsson, S., Van Komen, S., Bussen, W., Schild, D., Albala, J. S., and Sung, P. (2001) Genes Dev. 15, 3308–3318
11. Sung, P. (1997) Genes Dev. 11, 1111–1121
12. Eisen, J. A., Sweder, K. S., and Hanawalt, P. C. (1995) Nucleic Acids Res. 23, 2715–2723
13. Swagemakers, S. M. A., Essers, J., de Wit, J., Hoeijmakers, J. H. J., and Kanaar, R. (1996) J. Biol. Chem. 271, 28292–28297
14. Tan, T. L., Essers, J., Citterio, E., Swagemakers, S. M. A., de Wit, J., Benson, F. E., Hoeijmakers, J. H., and Kanaar, R. (1999) Curr. Biol. 9, 325–328
15. Riste, D., Wyman, C., Paulusma, C., and Kanaar, R. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 8454–8460
16. Sung, P., Prakash, L., Matson, S. W., and Prakash, S. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 8951–8955
17. Lynn, R. M., and Wang, J.-C. (1989) Proteins 6, 231–239
18. Van Komen, S., Petukhova, G., Sigurdsson, S., Stratton, S., and Sung, P. (2000) Mol. Cell 6, 563–572
19. Petukhova, G., Stratton, S., and Sung, P. (1998) Nature 393, 91–94
20. Morrison, C., Shinohara, A., Ogawa, H., Matsuda, Y., Ushio, N., Ikeo, K., and Ogawa, T. (1993) Nat. Genet. 4, 239–243
21. Weichselbaum, R. R., and Takeda, S. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 8460–8465
22. Kanaar, R., Truelstr, C., Swagemakers, S. M. A., Essers, J., Smit, B., Franssen, J. H., Pastink, A., Bezzubova, O. Y., Buerstedde, J. M., Clever, B., Heyer, W. D., and MacLennan, J. H., Pastink, A., Bezzubova, O. Y., Buerstedde, J. M., Clever, B., Heyer, W. D., and MacLennan, J. H. (1997) Nucleic Acids Res. 25, 4106–4110
23. Benson, F. E., Stasiak, A., and West, S. C. (1994) EMBO J. 13, 5764–5771
24. Bianco, P. R., Tracy, R. B., and Kowalczykowski, S. C. (1998) Front. Biosci. 3, D580–603
25. Mazin, A. V., Zaitseva, E., Sung, P., and Kowalczykowski, S. C. (2000) EMBO J. 19, 1148–1156
26. Shibata, T., Obi, T., Iwabuchi, M., and Ando, T. (1982) J. Biol. Chem. 257, 13981–13986
27. McIlwraith, M. J., Van Dyck, E., Masson, J. Y., Stasiak, A. Z., Stasiak, A., and West, S. C. (2000) J. Mol. Biol. 304, 151–164
28. Sung, P., and Stratton, S. A. (1996) J. Biol. Chem. 271, 27983–27986
29. Stark, J. M., Hu, P., Pierce, A. J., Moynahan, M. E., Ellis, N., and Jasin, M. (2002) J. Biol. Chem. 277, 20185–20194
30. Mazin, A. V., Bornath, C. J., Solinger, J. A., Heyer, W. D., and Kowalczykowski, S. C. (2000) Mol. Cell 6, 583–592
