Rad51 Uses One Mechanism to Drive DNA Strand Exchange in Both Directions*

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Eugeni A. Namsaraev and Paul Berg‡

From the Department of Biochemistry, Beckman Center for Molecular and Genetic Medicine, Stanford University School of Medicine, Stanford, California 94305-4825

The Rad51 protein of Saccharomyces cerevisiae, like its bacterial counterpart RecA, promotes strand exchange between circular single-stranded DNA (ssDNA) and linear double-stranded DNA (dsDNA) in vitro. However, the two proteins differ in the requirement for initiating joint molecules and in the polarity of branch migration. Whereas RecA initiates joint molecules from any type of ends on the dsDNA and branch migration proceeds exclusively in the 5'- to 3'-direction with respect to the single strand DNA substrate, initiation mediated by Rad51 requires a complementary 3'- or 5'-overhanging end of the linear dsDNA and branch migration proceeds in either direction. Here we report that the rates of Rad51-mediated branch migration in either the 5'- to 3'- or 3'- to 5'-directions are affected to the same extent by temperature and MgCl₂. Furthermore, branch migration in both directions is equally impeded by insertions of non-homologous sequences in the dsDNA, insertions of 6 base pairs or more being completely inhibitory. We have also found that the preference of strand exchange in the 5'- to 3'-direction does not change if RPA is replaced by Escherichia coli SSB or T4 gene 32 proteins, suggesting that the preference for the direction of strand exchange is intrinsic to Rad51. Based on these results, we conclude that Rad51-promoted branch migration in either direction occurs fundamentally by the same mechanism, quite probably by stabilizing successively formed heteroduplex base pair.

Rad51 of Saccharomyces cerevisiae shares structural and functional homology with the bacterial recombination protein RecA and, like RecA, is involved in various recombination and DNA repair processes in eukaryotes (1–3). Both proteins form structurally similar nucleoprotein filaments with single-stranded DNA (ssDNA) and dsDNA (4, 5). Furthermore, both proteins catalyze joint molecule formation and complete strand exchange between circular ssDNA and linear dsDNA in the presence of ATP (6–8).

Rad51-mediated strand exchange can be divided into three distinct phases. First, Rad51 binds to ssDNA with a stoichiometry of one monomer per three nucleotides. Unlike RecA, however, binding of Rad51 to DNA requires ATP, and neither ATPγS nor AMP-PNP (nonhydrolyzable analogues) can substitute (9). ATP stabilizes Rad51 and promotes a structural alteration that is necessary for efficient binding of Rad51 to DNA (10). In the second phase, homologous sequences in the ssDNA and the dsDNA are paired and, as a result, a new duplex DNA (hereafter referred to as the heteroduplex DNA) is formed by a switch of complementary strands between the two DNA substrates. However, unlike RecA, Rad51 requires an overhanging complementary 3’ or 5’ end on the dsDNA to initiate the strand exchange (8). In the third and final phase of the reaction, the relatively short region of heteroduplex DNA is extended by branch migration until complete exchange occurs. In the RecA-mediated strand exchange, the ensuing branch migration requires ATP hydrolysis and is unidirectional in the 5’- to 3’-direction with respect to the ssDNA (11–13). By contrast, branch migration in the Rad51-promoted strand exchange proceeds in either direction depending on whether a 5’ or 3’ end initiates the joint molecule; strand exchange is about 3–5-fold faster in the 5’- to 3’-direction than in the opposite direction (8, 14).

Here, we address the issue of the mechanism of Rad51-promoted branch migration and aim to understand the molecular mechanism, which could account for the difference in rate in the two directions. One way to reconcile the difference in the rate of branch migrations in the 5’- to 3’- and 3’- to 5’-directions is to propose that they are driven by two different mechanisms. For example, Rad51 could use a “RecA-like” mechanism to drive branch migration in the 5’- to 3’-direction and a “RecA-unlike” process in the 3’- to 5’-direction. We presumed that the two processes might be affected differently in response to different conditions or insertions of non-homologous stretches in the dsDNA substrate. Accordingly, we measured the rates of branch migration in the two directions at different temperatures, with different levels of Mg²⁺, with insertions of 2–16 bp of heterologous DNA near the middle of the dsDNA, and with different single-stranded DNA-binding proteins. Our results show that the rate of branch migration in either the 5’- to 3’- or 3’- to 5’-directions is affected identically over the temperature range 25–37 °C and with 2.5–24 mm MgCl₂. Furthermore, branch migration in both directions is equally impeded by insertions of non-homologous sequences in the dsDNA, insertions of 6 bp or more being completely inhibitory. These results indicate that Rad51-promoted branch migration is fundamentally the same in either direction. Moreover, the preference of strand exchange in the 5’- to 3’-direction is unchanged if Escherichia coli SSB or T4 gene 32 proteins replace RPA in the strand exchange reaction, suggesting that the difference in the rate of strand exchange in the two directions is intrinsic to Rad51.

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‡ To whom correspondence should be addressed: Beckman Center, B062, Stanford University School of Medicine, Stanford, CA 94305-5425. Tel.: 650-723-6170; Fax: 650-725-4951; E-mail: pberg@cmgm.stanford.edu.
1 The abbreviations used are: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; SSB, E. coli single-stranded DNA binding protein; RPA, yeast single-stranded DNA-binding protein and replication protein A; Mes, 2-(N-morpholino)ethanesulfonic acid; ATPγS, adenosine 5’-O-thiotriphosphate; AMP-PNP, adenylyl imidodiphosphate; bp, base pair(s).

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Mechanism for Rad51-promoted Branch Migration

FIG. 1. Rad51 is required for branch migration. Reactions were carried out as described under “Experimental Procedures,” with 5.3 μM Rad51, 1 μM RPA, 20 μM 32P-labeled pBluescriptSK(+) circular ssDNA, and 77 μM linear pBluescriptSK(+) dsDNA cleaved with PstI. Joint molecules were formed initially, and after 30 min the proteins were removed by adding SDS and proteinase K; the incubation was continued for the indicated times. jm, joint molecules; nc, nicked circular dsDNA; ss, single-stranded.

EXPERIMENTAL PROCEDURES

Proteins and DNA—Yeast Rad51 was expressed in insect cells and purified as described previously (8). Yeast replication protein A (RPA) was expressed in E. coli using the pJM126 plasmid (15) and purified as described (16). E. coli ssDNA-binding protein SSB and T4 gene 32 protein were obtained from Amersham Pharmacia Biotech. The concentrations of Rad51 and RPA were determined using extinction coefficients of 1.26 × 104 and 8.8 × 104 at 280 nm, respectively. The concentration of the DNA substrates is expressed as nucleotide equivalents. Circular (+) strand ssDNA, 32P-labeled (+) strand pBluescriptSK(+) ssDNA, and pBluescriptSK(+) dsDNA (Stratagene) and its derivatives were prepared as described (14). Plasmids with short heterologous insertions were prepared by inserting of 2 bp (GA), 4 bp (CATG), 5 bp (GATGGA), 6 bp (CATGGA), 10 bp (TGAGCCATGG), or 16 bp (TGAGATCCCATGGACG) into the BbsHI site (2881 position) of the pBluescriptSK(+) plasmid. Linear pBluescriptSK(+) dsDNA and its derivatives with different types of termini were prepared by cleavage with appropriate restriction endonucleases; linear dsDNA with 3’ overhanging termini were made with ApoI or PstI endonucleases; linear dsDNA with 5’ overhanging termini were generated with EcoRI or BamHI endonucleases.

Strand Exchange Assay—Strand exchange was measured using the modified agarose gel assay described previously (14). In the standard reaction, 32P-labeled circular (+) strand pBluescriptSK(+) ssDNA (20 μM) was preincubated with Rad51 (5.3 μM) in buffer containing K-Mes, pH 6.5 (40 mM), dithiothreitol (1 mM), glycerol (5%), MgCl2 (2 mM) and ATP (0.5 mM) for 7 min at 37 °C. RPA (1 μM) was then added; 7 min later, linear dsDNA (77 μM), MgCl2 (12 mM), and ATP (3 mM) were added, and the reaction was incubated at 25, 30, or 37 °C. The reaction was stopped by the addition of SDS (to 0.5%) and proteinase K (to 0.8 mg/ml), and the mixture was incubated at 37 °C for 20 min. The concentrations given are final. The products of the reaction were analyzed by gel electrophoresis on 1% agarose gel in TAE buffer containing 40 mM Tris acetate (pH 7.5), 0.5 mM EDTA, visualized by autoradiography, and quantitated using a PhosphorImager (Molecular Dynamics). To determine the effect of Mg2⁺ on branch migration, strand exchange was initiated under standard conditions at 30 °C for 40 min, and then the reaction mixture was diluted with strand exchange buffer containing different concentrations of MgCl2 and incubation was continued for additional time.

ATP Hydrolysis Assay—ATP hydrolysis was measured as described previously (9). The effect of Mg2⁺ on ATPase activity of Rad51 bound to ssDNA or dsDNA was determined as follows. After preincubation of Rad51 (5.3 and 8 μM) with ssDNA (20 μM) or dsDNA (77 μM), respectively, under strand exchange conditions, the reaction mixture was diluted 4 times with the same buffer containing 10 units/ml pyruvate kinase, 10 units/ml lactate dehydrogenase, 0.3 mM phosphoenolpyruvate, 0.15 mM NADH, and different concentrations of MgCl2. Oxidation of NADH, which measures ATP formation, was followed at 30 °C. The effect of temperature on ATP hydrolysis was determined as described

FIG. 2. Kinetics of Rad51-promoted strand exchange between circular ssDNA and homologous linear dsDNA. After preincubation of circular ssDNA with Rad51 and RPA for 10 min at 37 °C, the reaction was started by the addition of homologous linear dsDNA with either 3’ (A) or 5’ (B) complementary overhanging ends (prepared by cleavage of the pBluescriptSK(+) DNA with either PstI or EcoRI restriction endonucleases) and incubated at 37, 30, or 25 °C. At the indicated times, samples (6 μl) were removed and the DNA products were analyzed by agarose gel electrophoresis followed by autoradiography and quantitation. C, comparison of the rates of branch migrations in the two possible directions at different temperatures. The rates of branch migration were calculated from the amount of final product (nicked circular dsDNA) that accumulated during the interval between the time when the amount of joint molecules reached 60% and the amount of nicked circular dsDNA was below 20%. jm, joint molecules; nc, nicked circular dsDNA; ovh, overhanging end.
Fig. 3. Rad51-promoted strand exchange is blocked by a heterologous insert longer than 6 bp into the dsDNA. A, the reactions were performed as described in Fig. 1 except that the linear dsDNA contained insertions of 2, 4, 5, 6, 10, or 16 bp of heterologous sequence located near
above, except that the reaction was diluted with the buffer containing 10 mM MgCl₂, and incubation were carried at 25, 30, or 37 °C.

RESULTS

Branch Migration Depends on Rad51—Rad51 promotes DNA strand exchange between 32P-labeled circular (+) pBlue-ScriptSK (+) ssDNA and linear pBlue-ScriptSK (+) dsDNA with either a 3'- or 5'-overhanging complementary end. The reaction consists of two distinct steps: rapid formation of joint molecules and a slow lengthening of the heteroduplex DNA by branch migration (8, 14). Previously, we reported (8), as have others (5–7), that the initial DNA pairing is Rad51-dependent. Fig. 1 shows that the subsequent (slow) branch migration is also dependent on functional Rad51. Removal of Rad51 from the joint molecules by the addition of SDS and proteinase K at 30 min after the initiation of strand exchange halts the accumulation of the fully exchanged product, nicked circular dsDNA, over the next 4 h. Once formed, however, joint molecules are not disrupted by removal of Rad51 from the complex. We conclude that branch migration following joint molecule formation requires Rad51.

Branch Migration Is More Sensitive to Temperature Than Joint Molecule Formation—Although yeast Rad51 and E. coli RecA are structurally and functionally similar, they differ in their mode of branch migration. During strand exchange, RecA promotes branch migration exclusively in the 5'- to 3'-direction with respect to the ssDNA substrate (11, 12), while Rad51 promotes branch migration in either the 5'- to 3'- or 3'- to 5'-direction depending on which end of the complementary strand initiates the joint molecule (8, 14). Although the formation of joint molecules is equally efficient irrespective of which type of end initiates the strand exchange, the ensuing branch migration is more rapid in the 5'- to 3'-direction with respect to the ssDNA (8, 14). One way to reconcile the difference in the rates of branch migration in opposite directions is to suppose that they are driven by two different mechanisms. A "RecA-like" mechanism drives branch migration in the 5'- to 3'-direction, and a "RecA-unlike" process is responsible for branch migration in the opposite direction. Consequently, we tested for whether the two processes could be affected differentially.

First, we compared the rate of strand exchange reaction between circular ssDNA and linear dsDNA with either a 3'- or 5'-overhanging complementary end at different temperatures. After preincubation of Rad51, RPA, and ssDNA for 10 min at 37 °C and following the addition of dsDNA, incubations were carried out at 25, 30, or 37 °C, and the amount of joint molecules and fully exchanged product were measured (14). Fig. 2 (A and B) shows that the overall reaction has two phases: rapid formation of joint molecules and slow accumulation of the fully exchanged product. After a 40-min incubation at 30 or 37 °C, about 60–70% of the circular ssDNA is converted to joint molecules, irrespective of whether the linear dsDNA had 3' or 5' overhanging ends. Joint molecule formation is somewhat slower at 25 °C; however, the reaction reaches about the same level after 80 min of incubation. By contrast, branch migration to form the fully exchanged product is notably more affected by temperature (Fig. 2, A and B). Although the accumulation of fully exchanged product is more than 4 times greater when the strand exchange is initiated by a 3'- overhanging end compared with a 5'- overhanging end, the effect of temperature on the rate of branch migration is about the same: a 2.2 times decrease of the amount of nicked circular dsDNA between 37 and 30 °C and 2.4 times between 30 and 25 °C (Fig. 2C). Note, however, that strand exchange initiated by a 3'- overhanging end is about 4.5 times greater than when initiated by a 5'- overhanging end (Fig. 2C). Thus, temperature affects the rate of strand exchange to the same extent irrespective of whether branch migration proceeds in the 5'- or 3'- or 3'- to 5'-direction with respect to the ssDNA.

Branch Migration Is Blocked by 6-bp or Longer Heterologous Insertions in Linear dsDNA—RecA has the remarkable ability to bypass insertions of up to 180 bp of heterologous sequence in the dsDNA substrate during strand exchange. This activity is completely dependent on ATP hydrolysis (17–19). By contrast, short heterologous insertions into the dsDNA substrate (Fig. 3A) impede the completion of the Rad51-promoted strand exchange (Fig. 3B). The amount of fully exchanged product decreases irrespective of the direction of branch migration as the length of the heterologous insertion increases; no fully exchanged product is formed at 30 °C when the length of the insertion is greater than 6 bp (Fig. 3B).

To determine if Rad51’s ability to promote branch migration through the heterologous insertion is influenced by temperature, the rates of accumulation of fully exchanged product were measured at 25, 30, and 37 °C. Under these circumstances, as well, branch migration in either direction is increasingly inhibited as the size of the non-homologous insertion increases. Although the absolute values of the rates of branch migration in the two directions are different, the extent of the inhibition is similar at 25–37 °C (Fig. 3C). The significance of the small effect of the insertion on branch migration initiated at the 3'-overhanging end compared with the 5'-overhanging end, although consistent, is unclear.

To separate the effect of temperature on the rate of branch migration from the effect of temperature on bypassing the heterology alone, the results of strand exchange with linear dsDNA having heterologous insertions at 25 and 37 °C were normalized to the results obtained at 30 °C. Fig. 3D shows that incubation at 37° or 25 °C not only changes the rate of branch migration but also affects the ability of Rad51 to bypass the heterologous insertions. The ability to bypass 2-bp heterologous insertion is unaffected by temperature, but higher temperature increases the efficiency of bypassing the longer heterologous insertions, while the lower temperature decreases the capacity to bypass the insertions. Interestingly, the rate of branch migration increases about 5-fold between 25 and 37 °C, but the efficiency of bypassing the 5-bp insertion increases only 3 times over the same temperature range.

Mg²⁺ Affects Branch Migration but Does Not Alter the Efficiency of Bypassing Heterologous Insertions—To determine the effect of Mg²⁺ on branch migration, strand exchange was initiated under standard conditions, and after 40 min of incubation, the reactions were diluted with the same buffer containing different Mg²⁺ concentrations. The samples were then incubated further, and the amount of completely exchanged product formed after the dilution was determined. Fig. 4A shows that branch migrations in both the 5'- to 3'- and 3'- to 5'-directions are equally influenced by Mg²⁺ concentration and that branch migration is most efficient at 2.5 mM Mg²⁺ decreas-
ing about 4-fold as the Mg$^{2+}$ concentration increases from 2.5 mM to 24 mM. Rad51’s ability to bypass a 4- or 5-bp heterologous insertion is also diminished as the Mg$^{2+}$ concentration increases (Fig. 4, B and C). Curiously, however, the Mg$^{2+}$ concentration affects branch migration to the same extent irrespective of whether the linear dsDNA lacks or contains a 4- or 5-bp heterologous insertion (Fig. 4, B and C). There results, as well as those with variable temperature, show that increasing the rate of branch migration does not affect the efficiency of bypassing a heterologous insert suggesting that the process of branch migration and bypassing a heterology are independent.

**ATP Hydrolysis Is Not Correlated with the Rate of Branch—**

ATP hydrolysis is required for the RecA-promoted strand exchange reaction to proceed in a unidirectional manner and to bypass heterologous insertions (20). Moreover, a correlation between rates of strand exchange and ATP hydrolysis for RecA has been documented (21, 22). Rad51’s considerably lower ATPase, as compared with that for RecA (6, 7), could account for its lower rate of strand exchange.

To explore this parameter, we determined the effects of temperature and Mg$^{2+}$ on the rate of ATP hydrolysis in the presence of ssDNA or dsDNA. Fig. 5A shows that the rate of either ssDNA or dsDNA-dependent ATP hydrolysis is increased about 2.5-fold with increasing temperature from 25 to 37 °C, while
the rate of branch migration increases more than 4.5-fold over the same range (Fig. 3C). Furthermore, while branch migration increases about 3-fold as the Mg$^{2+}$ concentration decreases from 24 mM to 2.5 mM, there is no effect on the rate of ATP hydrolysis (Fig. 5B). These results suggest that there is no correlation between the rate of ATP hydrolysis and branch migration in Rad51-promoted strand exchange reaction.

The Preference for Branch Migration in the 5'-to-3', 3'-to-5' Direction Is Not Influenced by the Single Strand DNA-binding Protein—RPA is required for Rad51 promoted strand exchange (6–8). Quite possibly, progressive preferential binding of RPA in the 5'-to-3' direction on ssDNA contributes to the preference in the directionality of the strand exchange (23). We tested that possibility by substituting other single-stranded DNA-binding proteins, E. coli SSB and T4 gene 32 for RPA. Both SSB and gene 32 protein stimulate Rad51-promoted strand, but as with RPA joint molecules initiated with linear dsDNA having 3' overhanging ends are converted to fully exchanged product about 3 times faster than ones initiated by linear dsDNA with 5' overhanging ends (Fig. 6). Our data suggest that the preference in strand exchange in the 5'- to 3'-direction on ssDNA is most likely an intrinsic property of Rad51.

Although Rad51 promotes ATP-dependent strand exchange in vitro, there are similarities and striking differences in the way the two proteins carry out the reaction. Both joint molecule formation and the ensuing branch migration require the respective proteins; once joint molecules are formed, they are stable in the absence of the proteins but branch migration fails to follow. The requirement for joint molecule formation is, however, different. Rad51 initiates joint molecule formation only from overhanging complementary ends of linear dsDNA, while RecA utilizes linear dsDNA with any type of ends. Consequently, unlike RecA, the Rad51 promotes branch migration in either direction depending on whether the 3' end of linear dsDNA initiates the joint molecule (8, 14).

In this work, we attempted to characterize several features of the branch migration with a view to determining the basis for the different rates in the two directions. With respect to the temperature of the reaction, joint molecule formation occurs rapidly and is only slightly influenced over the temperature range 25–37 °C; however, the formation of fully exchanged product is slow and more strongly influenced by temperature. Nevertheless, although the rate of branch migration in the 5'-to-3' direction with regard to the ssDNA is about 4 times faster than in the opposite direction, both are equally affected over this temperature range. Next, we determined if the rate of branch migration in each direction is affected by having to traverse various lengths of heterologous sequences in the linear dsDNA. Our data show that heterologous insertions of 6 bp or more near the middle of the linear dsDNA does not influence joint molecule formation initiated by either 5' or 3' overhanging ends, but formation of the fully exchanged product is blocked equally in each case. Moreover, the rate of branch migration in each direction is decreased about 3-fold as the Mg$^{2+}$ concentration increases from 2.5 mM to 24 mM. The results of the three types of experiments, the effect of temperature, non-homologous insertions in the dsDNA and of Mg$^{2+}$ suggest that, even though branch migration in the 5'-to-3' direction proceeds faster than in the 3'-to-5' direction with respect to the ssDNA, both processes are probably driven by the same mechanism.

In a recent study, de Laat and co-workers (23) showed that human RPA binds to ssDNA in the 5'-to-3' direction. If yeast RPA polymerizes in the same way on the replaced single strand DNA, that could explain the preferred directionality of the strand exchange. However, when E. coli SSB and T4 gene 32 proteins, neither of which are known to have a preferential polarity of ssDNA binding, were substituted for yeast RPA in the Rad51-promoted strand exchange reaction, there was no change in the preferential direction of the Rad51-promoted strand exchange; joint molecules initiated by linear dsDNA with 3' overhanging ends are converted to fully exchanged product faster than those initiated by 5' overhanging ends. This indicates that the preference in the direction of strand exchange is most likely a property of Rad51 itself.

The resemblance of Rad51 with RecA ends when the role of ATP in strand exchange is compared. Rad51 hydrolyzes ATP much more slowly than does RecA (6, 7, 10). Although the role of ATP hydrolysis in RecA-mediated strand exchange is unclear, it is believed to be important for promoting unidirectional branch migration and to account for its ability to bypass insertions of heterologous sequences (13, 17–19, 24). Furthermore, several groups have shown that there is a direct coupling between NTP hydrolysis and RecA-mediated DNA strand exchange (21, 22). However, we have found no indication of coupling between the rate of ATP hydrolysis and branch migration.

**DISCUSSION**

**Fig. 6. Effect of E. coli SSB or T4 gene 32 protein on polarity of Rad51-promoted Branch Migration**
with Rad51. When ATPγS, a nonhydrolyzable analogue, is used in place of ATP, RecA promotes branch migration in either direction and fails to bypass heterologous insertions as short as 6 bp in length (13, 17). Moreover, mutant RecA K72R, which is unable to hydrolyze ATP, behaves like RecA with ATPγS (25, 26). Because Rad51 behaves in strand exchange, in much the same way as RecA that cannot hydrolyze ATP, we surmise it likely that Rad51 drives branch migration without the need for ATP hydrolysis.

What then is responsible for driving branch migration by Rad51 and why the preference in the 5'-to-3' direction with respect to the ssDNA? The branch migration promoted by Rad51 has very distinctive properties; the rate of branch migration increases with increasing temperature and by lowering Mg?+ concentration. Branch migration is blocked by heterologous insertions of greater than 6 bp in dsDNA. Although the rate of branch migration and the efficiency of bypassing heterology increase with increasing temperature, there is no correlation between the rate of branch migration and ability to bypass heterologous insertions with different Mg?+ concentrations. This suggests that the two are independent. These features are characteristic of spontaneous “thermal” branch migration (27, 28). Considering that Rad51 does not utilize ATP hydrolysis for branch migration, we suspect that Rad51 does not act like a helicase by denaturing the dsDNA at the point of strand exchange. Rather, we consider it more likely that Rad51 modulates spontaneous branch migration, possibly, by stabilizing newly formed heteroduplex dsDNA as do E. coli RecT or β protein of phage λ (29, 30). As for the preference in the direction of strand exchange, Rad51 forms a highly regular, right-handed helical filament with ssDNA and dsDNA similar to RecA (4, 5), so it is likely that there is a polar orientation of Rad51 monomers in the Rad51-ssDNA filament. This polar orientation of the Rad51 monomer or cluster of monomers in the region at or before the branch point could favor a formation of new heteroduplex DNA preferably in one direction over another.

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REFERENCES
1. Aboussekhra, A., Chanet, R., Adjiri, A., and Fabre, F. (1992) Mol. Cell. Biol. 12, 3224–3234
2. Basile, G., Aker, M., and Mortimer, R. K. (1992) Mol. Cell. Biol. 12, 3235–3246
3. Shinohara, A., Ogawa, H., and Ogawa, T. (1992) Cell 69, 457–470
4. Ogawa, T., Yu, X., Shinohara, A., and Egelman, E. H. (1993) Science 259, 1896–1899
5. Sung, P., and Robberson, D. L. (1995) Cell 83, 453–461
6. Sung, P. (1994) Science 265, 1241–1243
7. Sugiyama, T., Zaitseva, E. M., and Kowalczykowski, S. C. (1997) J. Biol. Chem. 272, 7840–7845
8. Namsaraev, E. A., and Berg, P. (1997) Mol. Cell. Biol. 17, 5359–5368
9. Namsaraev, E. A., and Berg, P. (1998) J. Biol. Chem. 273, 6177–6182
10. Namsaraev, E. A., and Berg, P. (1998) Biochemistry 37, 11932–11939
11. Cox, M. M., and Lehman, I. R. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 6018–6022
12. West, S. C., Cassuto, E., and Howard-Flanders, P. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 6149–6153
13. Jain, S. K., Cox, M. M., and Inman, R. B. (1994) J. Biol. Chem. 269, 20653–20661
14. Namsaraev, E. A., and Berg, P. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 10477–10481
15. He, Z., Wong, J. M. S., Maniar, H. S., Brill, S. J., and Ingles, C. J. (1996) J. Biol. Chem. 271, 28243–28249
16. Brill, S. J., and Stillman, B. (1989) Nature 342, 92–95
17. Rosselli, W., and Stasiak, A. (1991) EMBO J. 10, 4391–4396
18. Kim, J. I., Cox, M. M., and Inman, R. B. (1992) J. Biol. Chem. 267, 16438–16443
19. MacFarland, K. J., Shan, Q., Inman, R. B., and Cox, M. M. (1997) J. Biol. Chem. 272, 17675–17685
20. Cox, M. M. (1994) Trends Biochem. Sci. 19, 217–222
21. Bedale, W. A., and Cox, M. M. (1996) J. Biol. Chem. 271, 5725–5732
22. Nayak, S., and Bryant, F. R. (1999) J. Biol. Chem. 274, 25979–25982
23. de Laat, W. L., Appeldoorn, E., Sugasawa, K., Weterings, E., Jaspers, N. G., and Hoeijmakers, J. H. (1998) Genes Dev. 12, 2398–2409
24. Konforti, B. B., and Davis, R. W. (1992) J. Mol. Biol. 227, 38–53
25. Rehruer, W. M., and Kowalczykowski, S. C. (1993) J. Biol. Chem. 268, 1292–1297
26. Shao, Q., Cox, M. M., and Inman, R. B. (1996) J. Biol. Chem. 271, 5712–5724
27. Panyutin, I. G., and Hsieh, P. (1993) J. Mol. Biol. 230, 418–424
28. Panyutin, I. G., Biwas, I., and Hsieh, P. (1995) EMBO J. 14, 1819–1826
29. Hall, S. D., and Kolodner, R. D. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3205–3209
30. Li, Z., Karakousis, G., Chiu, S. K., Reddy, G., and Radding, C. M. (1998) J. Mol. Biol. 276, 733–744