Cell, Vol. 117, 47–56, April 2, 2004, Copyright ©2004 by Cell Press

Saccharomyces cerevisiae Mer3 Helicase Stimulates 3′–5′ Heteroduplex Extension by Rad51: Implications for Crossover Control in Meiotic Recombination

Olga M. Mazina,[1,2] Alexander V. Mazin,[1,2] Takuro Nakagawa,[3,4] Richard D. Kolodner,[3] and Stephen C. Kowalczykowski[1,*]

[1]Division of Biological Sciences
Sections of Microbiology and of Molecular and Cellular Biology
Center for Genetics and Development
University of California
Davis, California 95616

[2]Department of Biochemistry
Drexel University College of Medicine
Philadelphia, Pennsylvania 19102

[3]Ludwig Institute for Cancer Research
Cancer Center
and Department of Medicine
University of California San Diego School of Medicine
La Jolla, California 92093

Summary

Crossover and noncrossover recombinants can form by two different pathways during meiotic recombination in *Saccharomyces cerevisiae*. The *MER3* gene is known to affect selectively crossover, but not noncrossover, recombination. The Mer3 protein is a DNA helicase that unwinds duplex DNA in the 3′ to 5′ direction. To define the underlying molecular steps of meiotic recombination, we investigated the role of Mer3 helicase in DNA strand exchange promoted by Rad51 protein. We found that Mer3 helicase does not function as an initiator of DNA pairing events but, rather, it stimulates DNA heteroduplex extension in the 3′ → 5′ direction relative to the incoming (or displaced) single-stranded DNA. Conversely, Mer3 helicase blocks DNA heteroduplex extension in the 5′ → 3′ direction. Our results support the idea that Mer3 helicase stabilizes nascent joint molecules via DNA heteroduplex extension to permit capture of the second processed end of a double-stranded DNA break, a step which is required for crossover recombinant product formation.

Introduction

Meiotic recombination is required for the faithful segregation of homologous chromosomes in most organisms (Kleckner, 1996; Pâques and Haber, 1999; Roeder, 1997). In *Saccharomyces cerevisiae,* meiotic recombination is initiated by DNA double-stranded breaks (DSB) introduced in specific regions of chromosomes by a specialized enzyme, Spo11 (Keeney et al., 1997). Subsequently, the DSB is resected to produce an intermediate with a 3′-overhanging ssDNA tail in a process that involves the action of the Mre11/Rad50/Xrs2 heterotrimer (Lee et al., 1998; Sun et al., 1991). The ensuing steps of meiotic recombination require Rad51 and Dmc1 proteins, and other members of the Rad52 epistasis group (Bishop et al., 1992; Shinohara et al., 1997). The biochemical properties of these proteins indicate that Rad51 and Dmc1 proteins bind to the ssDNA tails to form filamentous nucleoprotein structures that promote a search for homologous DNA (Hong et al., 2001; Ogawa et al., 1993; Sung, 1994). When DNA homology is found, DNA strand invasion by one processed end produces a single-ended invasion product, the joint molecule (Hunter and Kleckner, 2001). Afterward, it is currently thought that joint molecules continue down one of two different pathways to produce crossover or noncrossover recombinant molecules (Allers and Lichten, 2001; Hunter and Kleckner, 2001). Synthesis-dependent strand annealing (SDSA) produces noncrossover products primarily (Allers and Lichten, 2001), whereas capture of the second processed DNA end produces Holliday junctions (Schwacha and Kleckner, 1994) that can be resolved to produce either of the recombinant products (Szostak et al., 1983). While *RAD51* and *DMC1* genes are required for the generation of both types of meiotic recombinants (Bishop et al., 1992; Shinohara et al., 1992, 1997), a number of genes including *ZIP1*, *ZIP2*, *ZIP3*, *MSH4*, *MSH5*, *MLH1*, *MLH3*, *EXO1*, and *MER3* are specifically required to produce the normal level of crossover products (Agarwal and Roeder, 2000; Börner at al., 2004; Chua and Roeder, 1998; Hollingsworth et al., 1995; Hunter and Borts, 1997; Khazanehdari and Borts, 2000; Kirkpatrick et al., 2000; Nakagawa and Ogawa, 1999; Ross-Macdonald and Roeder, 1994; Sym et al., 1993; Tsubouchi and Ogawa, 2000; Wang et al., 1999). Under appropriate conditions, mutations in these genes greatly reduce the frequency of crossover formation, without affecting the frequency of noncrossovers.

Among the proteins encoded by these genes, Mer3 is of particular interest because it has a distinct helicase activity and can use Holliday junctions as a substrate (Nakagawa and Kolodner, 2002b). The *MER3* gene of *S. cerevisiae* was identified first as a meiosis-specific gene that is important for the regulation of crossing over and accurate chromosome disjunction (Nakagawa and Ogawa, 1999). A *mer3* mutation causes hyper-resection of dsDNA breaks during meiosis, indicating that Mer3 protein is required for the transition of the DSB into later intermediates. Also, this mutation produces a random distribution of the remaining crossovers along the chromosome, apparently undermining the crossover-interference mechanism (Nakagawa and Ogawa, 1997, 1999). Mer3 protein is a helicase that unwinds 50, 100, and 631 dsDNA regions in an ATP-dependent manner. Mer3 helicase hydrolyzes ATP in the absence of DNA, but hydrolysis is stimulated 5- to 6-fold by either single- or double-stranded DNA (Nakagawa et al., 2001). Unwinding of the longer, 631 base pair DNA duplex is aided by the ssDNA binding protein of *S. cerevisiae*, RPA, or of *E. coli*, SSB. Mer3 helicase unwinds DNA molecules with a substrate preference of 3′-overhang > Holliday junction >> blunt end > 5′-overhang, implying that the

*Correspondence: sckowalczykowski@ucdavis.edu
[4]Present address: Department of Biology, Osaka University School of Science, Toyonaka, Osaka 560-0043, Japan.

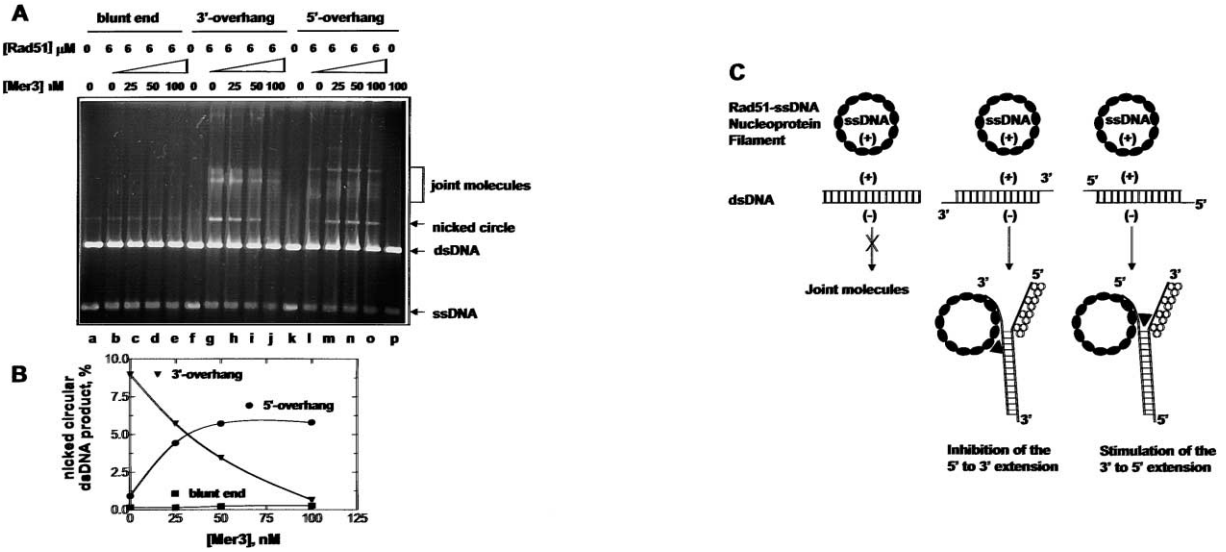

Figure 1. Mer3 Helicase Stimulates Rad51-Mediated DNA Strand Exchange that Produces Displaced ssDNA with a 3′ Terminus

(A) After preincubation of ssDNA with Rad51 and RPA, various amounts of Mer3 protein (0, 25, 50, and 100 nM) were added to Rad51 nucleoprotein filaments, and DNA strand exchange was initiated by addition of homologous linear DNA. The reaction was incubated for 3 hr at 37°C whereupon aliquots (10 μl) were withdrawn from the reaction mixture, deproteinized, mixed with 1/10 volume of loading buffer, and loaded onto a 1% agarose gel. The DNA products were analyzed after electrophoresis followed by ethidium bromide staining.

(B) The yield of nicked circular dsDNA product was quantified for each of the substrate sets in panel A and plotted *versus* the Mer3 protein concentration.

(C) The direction of DNA heteroduplex extension depends on which end of the complementary strand in the linear dsDNA substrate is used to initiate joint molecule formation. Rad51 does not form joint molecules when the dsDNA has blunt ends (Namsaraev and Berg, 1998). The complementary DNA strands are indicated as positive (+) and negative (−) DNA strands. Rad51 protein is depicted as an oval, RPA as a trimer of circles, and Mer3 helicase as a triangle whose direction shows the polarity of enzymatic action.

polarity of Mer3 helicase translocation is 3′ to 5′ with regard to the ssDNA tail (Nakagawa et al., 2001).

To better understand the role of the Mer3 protein in the DNA recombination process, we studied the effect of Mer3 helicase on DNA strand exchange promoted by Rad51 protein in vitro. Our data demonstrate that Mer3 helicase stimulates DNA heteroduplex extension in the 3′ to 5′ direction relative to the displaced DNA strand. Conversely, Mer3 helicase inhibits DNA strand exchange, if DNA heteroduplex extension were to proceed in the 5′ → 3′ direction (i.e., when the invading or displaced DNA has a 5′-ssDNA end). Since DNA heteroduplex extension in the proper direction stabilizes nascent joint molecules that are kinetic precursors to Holliday junctions, our results provide a link between the enzymatic activity of Mer3 protein and its function in regulating crossing over during meiotic recombination.

Results

Mer3 Helicase Stimulates DNA Heteroduplex Extension Only in the 3′ → 5′ Direction

We first investigated the effect of Mer3 helicase on DNA strand exchange promoted by Rad51 protein. Rad51 protein promotes DNA strand exchange between linear dsDNA and circular ssDNA in vitro. However, unlike RecA protein, Rad51 protein requires complementary overhanging ssDNA, at either the 3′ or 5′ end, on the dsDNA substrate for stimulation of DNA strand exchange; it cannot promote this reaction if the dsDNA is blunt ended (Namsaraev and Berg, 1997). DNA heteroduplex extension can proceed in either direction, depending on the nature of the ssDNA end that was used to initiate joint molecule formation (Namsaraev and Berg, 1998, 2000). Furthermore, extension is about 3- to 5-fold faster in the 5′ → 3′ direction, relative to the displaced ssDNA, than in the opposite direction. The reaction is strongly stimulated by RPA: in the absence of RPA, nicked circular dsDNA is not detected and joint molecule formed is reduced from 40% to about 5% (Namsaraev and Berg, 1997). The principal contribution of RPA in promoting DNA strand exchange is to eliminate secondary structure in the ssDNA (Sugiyama et al., 1997) and to bind the displaced linear ssDNA produced upon joint molecule formation (Eggler et al., 2002).

Because of the different DNA pairing and heteroduplex extension polarities displayed, we examined the effect of Mer3 helicase on Rad51-mediated DNA strand exchange using blunt, 5′-, or 3′-overhanging DNA substrates. After preincubation of ssDNA with Rad51 and RPA, various amounts of Mer3 protein (0, 25, 50, and 100 nM) were added to nucleoprotein filaments, and then DNA strand exchange was initiated by addition of homologous linear dsDNA (with either blunt, 5′-, or 3′-overhanging ends). As expected, in the absence of Mer3 helicase, Rad51 protein does not promote DNA strand exchange when the dsDNA has blunt ends (Figure 1A, lane b), but both joint molecules and fully exchanged nicked circular DNA product form when the duplex has an overhang (Figure 1A, lanes g and l). The yield of both types of products is greater for the 3′-overhang substrate (Figure 1A, lane g) than for the 5′-overhang substrate (Figure 1A, lane l).

In the presence of Mer3 helicase, DNA strand ex-

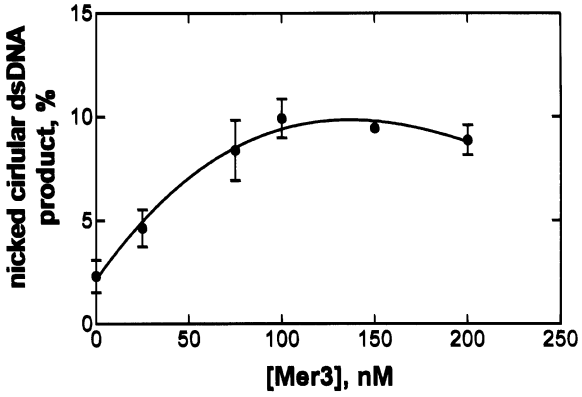

Figure 2. Mer3 Helicase Stimulates DNA Strand Exchange over a Broad Range of Concentration

After preincubation of ssDNA with Rad51 and RPA, various amounts of Mer3 protein were added to Rad51 nucleoprotein filaments, and DNA strand exchange was initiated by addition of homologous linear DNA in which 5′-overhanging ends were labeled with ^{32}P. The reaction was incubated for 3 hr at 37°C whereupon aliquots (10 μl) were withdrawn from the reaction mixture, deproteinized, mixed with 1/10 volume of loading buffer, and loaded onto a 1% agarose gel. The yield of nicked circular dsDNA product was quantified using a Storm 840 Phosphor Imager (Molecular Dynamics).

change with the blunt-ended DNA did not occur at any concentration tested (Figure 1A, lanes c–e, and 1B). This negative result demonstrates that Mer3 cannot serve to initiate recombination by unwinding the ends of the dsDNA; this behavior is in distinct contrast to that of another DNA helicase that is important to recombination, the RecQ protein of *E. coli*, which can initiate recombination by unwinding blunt-ended dsDNA (Harmon and Kowalczykowski, 1998). However, Mer3 helicase did stimulate DNA strand exchange when the dsDNA had a 5′-ssDNA overhang (Figure 1A, lanes m–o, and 1B), producing a greater amount of fully exchanged nicked circular DNA products. Mer3 helicase, without Rad51 (but in the presence of RPA protein), cannot promote DNA strand exchange (Figure 1A, lane p). Thus, Mer3 protein is stimulating branch migration in the 3′ to 5′ direction with respect to the displaced ssDNA strand (Figure 1C), a direction that is consistent with its known translocation polarity of 3′ to 5′ (Nakagawa et al., 2001). In contrast, the opposite result was obtained with dsDNA possessing the 3′-overhanging ssDNA: Mer3 helicase blocked product formation in a concentration-dependent manner (Figure 1A, lanes h–j, and 1B). This result is consistent with Mer3 translocating on the circular ssDNA in the 3′ to 5′ direction, resulting in disruption of the nascent joint molecules (Figure 1C). Thus, Mer3 helicase stimulates DNA heteroduplex extension in the 3′ to 5′ direction, relative to the displaced ssDNA, and inhibits extension in the 5′ to 3′ direction (Figure 1C).

The stimulatory effect of Mer3 helicase on DNA strand exchange was quantified in other experiments by using a DNA substrate with a 5′-overhang that was 5′-end labeled. The yield of nicked circular dsDNA product increased with increasing Mer3 helicase concentration up to 100 nM, after which there was a slight decline in product formation (Figure 2). At the optimum, 100 nM, Mer3 helicase stimulated the yield of nicked circular DNA product by 3-fold. The kinetics of DNA strand exchange promoted by Rad51 in the presence of Mer3 helicase were also examined. At a Mer3 helicase concentration of 100 nM, stimulation by Mer3 helicase was noticeable within 30 min and continued for up to 2 hr (Figure 3). Mer3 protein increased the yield of fully exchanged DNA product (Figure 3B), but not the rate of dsDNA substrate utilization (Figure 3D). At the same time, Mer3 helicase reduced the steady state level of joint molecules by converting them more rapidly into the fully exchanged DNA product (Figures 3B and 3C). Hence, Mer3 protein exerts its effect at the level of DNA heteroduplex extension and not joint molecule forma-

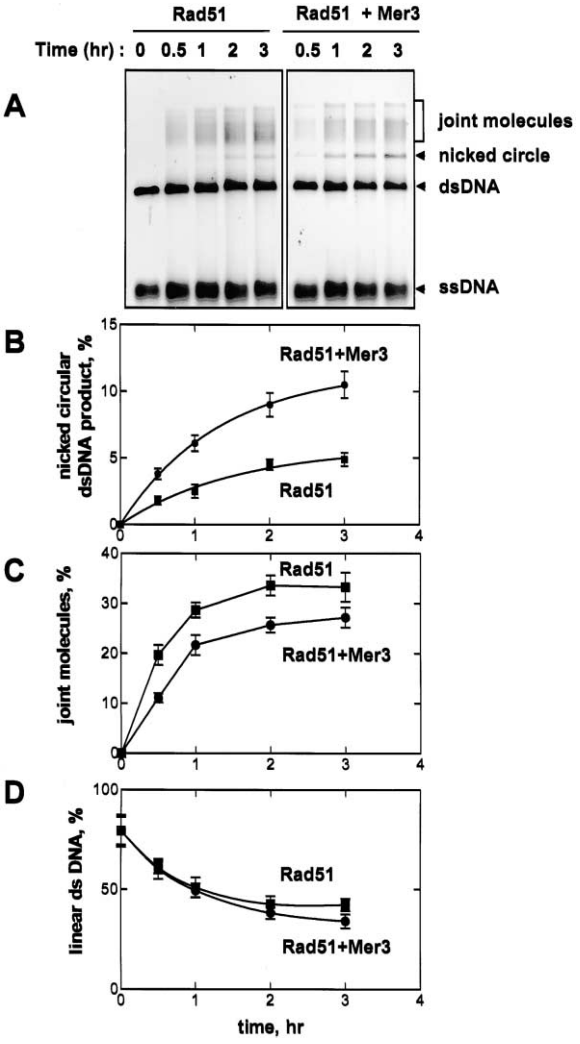

Figure 3. The Kinetics of DNA Strand Exchange Show that DNA Heteroduplex Extension, but Not Joint Molecule Formation, Is Stimulated by Mer3 Helicase

(A) After preincubation of ssDNA with Rad51 and RPA, Mer3 protein at the optimum concentration (100 nM) was added to Rad51 nucleoprotein filaments and DNA strand exchange was initiated by addition of homologous linear DNA in which the 5′-overhang ends were labeled with ^{32}P. The reaction was incubated at 37°C. Aliquots (10 μl) were withdrawn from the reaction mixture at the indicated times, deproteinized, mixed with 1/10 volume of loading buffer, and loaded onto a 1% agarose gel.

(B, C, and D) The amounts of nicked circular dsDNA product, joint molecule, and dsDNA substrate utilization, respectively, were quantified using a Storm 840 Phosphor Imager (Molecular Dynamics).

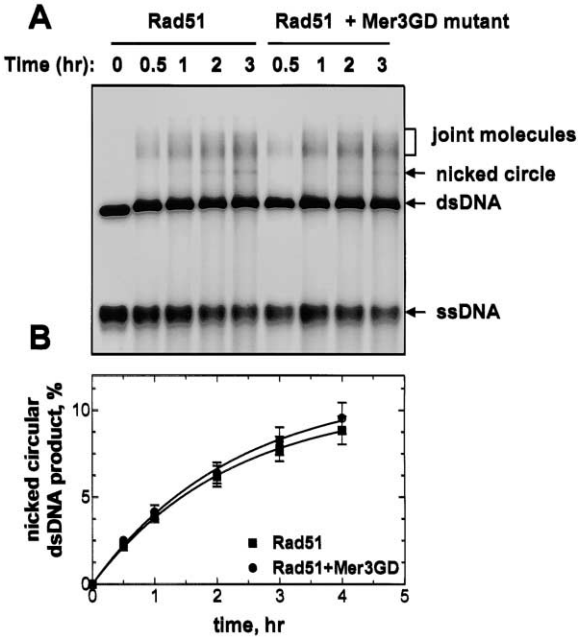

Figure 4. The Mutant Mer3 G166D Protein, which Is Defective for DNA Helicase Activity, Does Not Stimulate DNA Strand Exchange

The experiment was performed as described in Figure 3, but the mutant Mer3 G166D protein (100 nM) was substituted for wild-type Mer3 helicase.

tion. To exclude the possibility that Mer3 helicase stimulation is an artifactual consequence of degradation of the displaced ssDNA strand, we analyzed the DNA strand exchange products using linear dsDNA in which both 3′-ends were labeled with $^{[32]}$P. In such reactions, we could monitor and quantify the amount of fully exchanged product and the amount of displaced ssDNA. Our results showed that Mer3 stimulated the production of displaced ssDNA at the same rate as formation of the nicked circular dsDNA product (data not shown), verifying that degradation of the displaced DNA strand was not the basis for stimulation.

A Mer3 Helicase Mutant Does Not Stimulate DNA Heteroduplex Extension

The *mer3 G166D* mutation, in which the invariant glycine of the Walker motif A consensus sequence, GKT, is changed to an aspartic acid, abolishes the DNA helicase activity of Mer3 protein, decreases crossing over, and impairs crossover interference in vivo (Nakagawa et al., 2001). The spore viability of the *mer3 G166D* mutant is also decreased, which is likely to be due to a high incidence of nondisjunction of homologous chromosomes at the first meiotic division. The mutant Mer3 G166D protein is defective in DNA helicase activity and ATP hydrolysis; however, it retains DNA binding activity that is indistinguishable from that of wild-type protein (Nakagawa et al., 2001). When we substituted the wild-type Mer3 helicase with the Mer3 G166D mutant, there was no change in the rate of DNA strand exchange (Figure 4). These data demonstrate that the DNA helicase activity of Mer3 protein is required for the stimulatory effects observed.

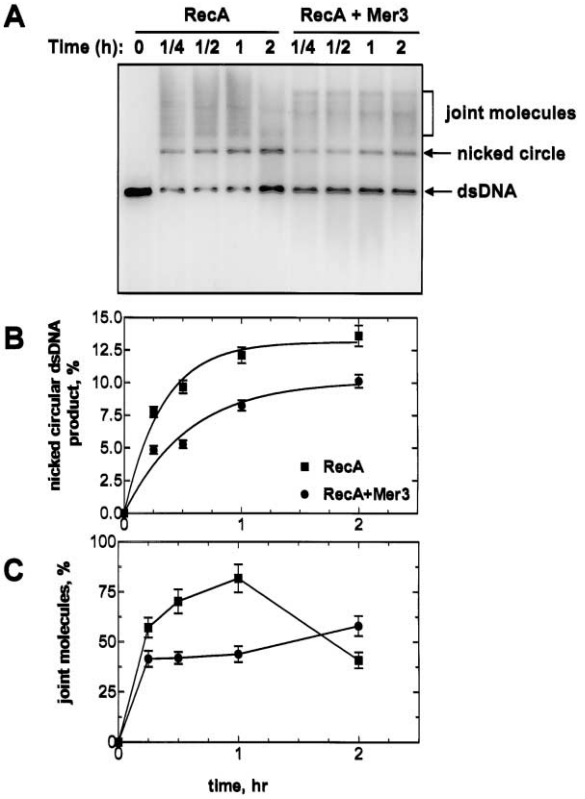

Figure 5. Mer3 Helicase Inhibits DNA Strand Exchange Promoted by RecA Protein

(A) RecA nucleoprotein filaments were assembled as described in Experimental Procedures. Mer3 protein at the optimum concentration (100 nM) was added to RecA nucleoprotein filaments and DNA strand exchange was initiated by addition of homologous linear DNA in which 5′-overhanging ends were labeled with ^{32}P. The reaction was incubated at 37°C. Aliquots (10 μl) were withdrawn from the reaction mixture at the indicated times, deproteinized, mixed with 1/10 volume of loading buffer, and loaded onto a 1% agarose gel. RecA protein promotes branch migration exclusively in the 5′ to 3′ direction relative to the displaced ssDNA.

(B and C) The yield of both nicked circular dsDNA product and joint molecules was quantified using a Storm 840 Phosphor Imager (Molecular Dynamics).

Mer3 Helicase Inhibits DNA Strand Exchange Promoted by RecA Protein

Rad51 protein of *S. cerevisiae* shares structural and functional homology with the bacterial recombinational protein, RecA. However, DNA strand exchange promoted by RecA protein is insensitive to the structure of the dsDNA end, and branch migration proceeds exclusively in the 5′ to 3′ direction with respect to the displaced ssDNA. The effect of Mer3 helicase on DNA strand exchange promoted by RecA protein in the presence of SSB protein was also examined. Mer3 inhibited nicked circle product formation promoted by RecA protein by approximately 1.5- to 2-fold (Figure 5). In the absence of Mer3 helicase, the yield of joint molecules increases and then drops as the joint molecules are chased into the final nicked circular dsDNA products, a common kinetic pattern for RecA protein-mediated DNA strand exchange. Mer3 protein limits the amount of joint molecules formed and slows their conversion into final

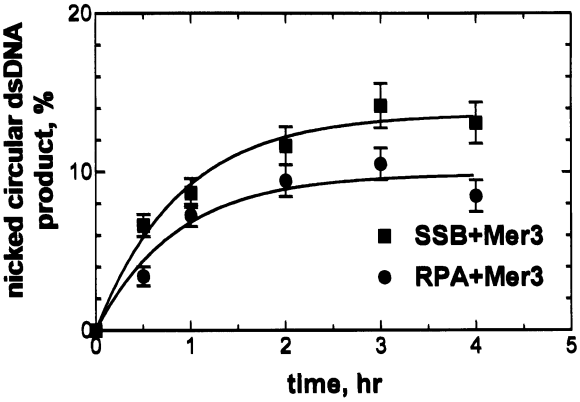

Figure 6. Mer3 Helicase Can Stimulate DNA Strand Exchanged Promoted by Yeast Rad51 Protein in the Presence of Bacterial SSB Protein

This experiment was performed as described in Figure 3, except that RPA protein was replaced by SSB protein. The yield of nicked circular dsDNA product was quantified using a Storm 840 Phosphor Imager (Molecular Dynamics).

products, consistent with a steady state inhibition of joint molecule formation likely due to disruption of the nascent joint molecule. This result is consistent with the inhibition observed for Rad51 protein in the DNA strand exchange assay that results in a displaced 5′-ssDNA tail (Figure 1). We also attempted to use human Rad51 protein in place of the yeast Rad51 protein to determine whether the stimulation by Mer3 helicase displayed a species-specific preference for one of the eukaryotic counterparts. Unfortunately, those experiments could not be unambiguously interpreted because the reaction conditions for these two proteins were not fully compatible: under conditions that are optimal for human Rad51 (Sigurdsson et al., 2001), Mer3 protein retained only about 13% of its activity (our unpublished observations).

Mer3 Helicase Stimulates DNA Strand Exchange Promoted by Rad51 Protein in the Presence of SSB Protein

In the T4 phage recombination system, a DNA helicase, gene 41 protein, is targeted to the displaced strand of joint molecules by a specific interaction with the ssDNA binding protein, gene 32 protein (Salinas and Kodadek, 1995). To determine whether the stimulatory effect of Mer3 helicase is specific for its cognate ssDNA binding protein, RPA was replaced by *E. coli* SSB protein in the reaction promoted by yeast Rad51 protein. We observed stimulation of DNA strand exchange by Mer3 helicase that was approximately the same, within experimental error, in the presence of SSB protein compared to RPA (Figure 6). Thus, it does not appear that Mer3 helicase is being targeted to the displaced ssDNA by a specific interaction with RPA; however, as will be discussed below, Mer3 helicase may instead be recognizing a structural feature of the D loop.

Discussion

Rad51 protein plays a major role in homologous recombination and in DSB repair in eukaryotes (Bianco et al., 1998; Sung et al., 2000; West, 2003). Yeast Rad51 protein shares homology with *E. coli* RecA protein and, like RecA protein, promotes ATP-dependent DNA strand exchange in vitro. However, there are differences in the way that the two proteins carry out the reaction. In DNA strand exchange between circular ssDNA and linear dsDNA, RecA protein utilizes linear dsDNA regardless of the structure of its ends and promotes DNA branch migration unidirectionally in the 5′ to 3′ direction relative to the invading ssDNA (Cox and Lehman, 1981; Kahn et al., 1981). In contrast, Rad51 protein forms joint molecules only if a plasmid-sized dsDNA substrate has either 3′- or 5′- short overhanging ends that are complementary to the ssDNA; joint molecules are not formed with linear DNA having blunt ends (Namsaraev and Berg, 1997). Consequently, DNA heteroduplex extension occurs in either direction, depending on whether the 3′- or 5′-end of linear dsDNA initiates the joint molecule formation and is apparently Rad51 protein dependent (Namsaraev and Berg, 1998). In addition, DNA strand exchange mediated by Rad51 protein is greatly stimulated by an array of auxiliary proteins, such as Rad52, Rad54, Rad55, and Rad57 proteins, and RPA (Alexeev et al., 2003; Clever et al., 1997; Hays et al., 1995; Jiang et al., 1996; Mazin et al., 2000a, 2003; New and Kowalczykowski, 2002; New et al., 1998; Petukhova et al., 1998, 1999; Shinohara and Ogawa, 1998; Shinohara et al., 1998; Solinger and Heyer, 2001; Solinger et al., 2001, 2002; Sugiyama et al., 1998; Sung, 1997; Van Komen et al., 2002). Our experiments demonstrate that Mer3 helicase also plays an important role in the DNA strand exchange process by extending the DNA heteroduplex in the 3′ to 5′ direction relative to the invading DNA strand.

The Mer3 protein is the only known DNA helicase that is involved in crossover control in meiosis (Nakagawa and Ogawa, 1999). A *mer3* mutation reduces crossover frequencies without affecting the yield of noncrossovers. Furthermore, the remaining crossovers show a random distribution along a chromosome. Although Mer3 protein is not required for DSB formation, it was observed that in a *mer3* deletion mutant, a fraction of DSBs became hyper-resected. In immunostaining experiments with anti-Rad51 and anti-Dmc1 antibodies, Rad51 and Dmc1 foci accumulate in a *mer3* deletion mutant. These results are consistent with the possibility that Mer3 protein functions in the step that involved conversion from a processed DSB that is bound with Rad51 and Dmc1 proteins to formation of a stable recombination intermediate. It was suggested that Mer3 protein and other proteins involved in the regulation of crossing over may act either early in recombination to produce or stabilize intermediates that lead to initiation of crossing over or late in recombination to modulate the yield of crossovers during the resolution of Holliday junctions intermediates (Nakagawa et al., 1999; Nakagawa and Kolodner, 2002b). Thus, these genetic experiments clearly define the possible physiological roles of Mer3 protein.

Our in vitro results demonstrate that Mer3 helicase stimulates DNA heteroduplex extension in Rad51-promoted DNA strand exchange and thus strongly support the conclusion that at least one role of Mer3 protein is stabilization of joint molecules that are on the pathway

to Holliday junction formation and, hence, crossover formation. The helicase activity of Mer3 protein is essential for this stimulation; the Mer3G166D mutant protein, which is devoid of this activity, fails to stimulate DNA heteroduplex extension. When pairing by Rad51 protein was blocked by using dsDNA with blunt ends, initiation of DNA strand exchange was not observed in the presence of Mer3 helicase. Therefore, we conclude that Mer3 helicase does not act to process a DSB to produce ssDNA that can be utilized by Rad51 protein; rather it acts afterwards to extend the nascent joint molecules formed by Rad51-promoted pairing. In this regard, Mer3 helicase is clearly distinct from other biochemically defined recombination helicases, such as RecQ and RecBCD helicases, which act to process DNA breaks into suitable initial substrates for DNA pairing.

Mer3 helicase shows a strong polarity bias with respect to stimulation of DNA strand exchange. Mer3 stimulates DNA heteroduplex extension only when DNA strand exchange occurs between circular ssDNA (+) and dsDNA with a complementary (−) 5′-ssDNA overhang that produces a displaced DNA (+) strand starting at the 3′-end. In this case, the 3′ to 5′ direction of DNA heteroduplex extension coincides with the 3′ to 5′ direction that Mer3 helicase unwinds dsDNA and with its inferred direction of translocation (Figure 1C, right). Conversely, Mer3 helicase inhibits the DNA strand exchange that starts with dsDNA containing a 3′-overhang, and that would proceed in the 5′ to 3′ direction. In this case Mer3 protein unwinds the intermediate joint molecules and works against Rad51 protein (Figure1C, middle). A consequence of this biased stimulation is that Mer3 helicase enforces a 3′–5′ polarity for DNA heteroduplex extension onto Rad51 protein, which, on it own, is capable of pairing tailed dsDNA with 3′- or 5′-ending ssDNA (Mazin et al., 2000b); in fact, because Rad51 protein actually shows a bias for pairing 5′-terminated DNA, Mer3 helicase essentially reverses the natural bias of Rad51 protein to one that is more effective for recombination of 3′-ended ssDNA.

In addition, an interesting feature of Mer3 helicase behavior in the pairing reaction with the 5′-overhanging substrate (Figure 1C, right) is the observation that nascent joint molecules are not disrupted (Figure 1A), even though Mer3 helicase could potentially disrupt the joint molecule by translocating in the 3′ to 5′ direction on the circular ssDNA and unwinding the paired 5′-end of the linear dsDNA. The fact that the circular ssDNA is coated by Rad51 protein does not afford an explanation for this bias because Mer3 helicase can clearly bind to this same ssDNA and unwind the joint molecule formed using the 3′-overhanging dsDNA substrate (Figure 1C, middle). However, careful consideration of the joint molecule intermediate formed with the 5′-overhanging substrate reveals that the DNA structure presented to a Mer3 helicase that would translocate on the circular ssDNA is different from that presented to a Mer3 helicase that would act on the displaced ssDNA: in the former case, a simple DNA duplex is the helicase substrate whereas, in the latter case, dsDNA with a ssDNA tail is the substrate. Thus, our work would suggest that Mer3 helicase has a preference for dsDNA with a 3′-ssDNA tail extending from the middle of the duplex region. This structure is, in fact, "half" of a D loop structure and would suggest that Mer3 helicase would migrate D loops in the direction of heteroduplex extension, a view that is consistent with our results and interpretations. Consequently, Mer3 helicase may have a previously undisclosed preferential affinity or activity on D loop structures, a preference that could explain the specificity observed here.

Mer3 helicase is a eukaryotic helicase for which a role in DNA heteroduplex extension is established by both genetic and, now, biochemical criteria. Although many helicases have been implicated in meiotic and mitotic recombination processes, the clearly unique phenotypes associated with mutation of each of these various helicases indicates that each has a distinct function in the biochemical steps of recombination. In fact, the only other yeast helicase that is known to be involved in recombination and that has been biochemically characterized with respect to its behavior in recombination reactions is the Srs2 protein, another helicase with 3′–5′ unwinding polarity (Krejci et al., 2003; Veaute et al., 2003). However, in marked contrast to Mer3 protein, Srs2 helicase specifically interacts with and dissociates Rad51 nucleoprotein filaments, thereby blocking DNA strand exchange. This behavior is fully consistent with the phenotypic behavior of Srs2 helicase and serves to control unwanted recombination at an early step in the process. Yet another helicase involved in recombination, the RecQ protein of *E. coli*, behaves distinctly differently from both Mer3 and Srs2 helicases. In experiments similar to those used here, RecQ helicase was found to initiate recombination by unwinding dsDNA and to create a substrate for RecA protein function, but not interact directly with RecA protein; furthermore, at higher concentrations, RecQ helicase could also disrupt all joint molecules (Harmon and Kowalczykowski, 1998). In contrast, Mer3 helicase could not initiate pairing reactions, and it showed selectivity for the joint molecules that were extended versus those that were disrupted (Figure 1). Thus, despite the abundance of DNA helicases, it is clear that the different helicases involved in genetic recombination have unique functions. These functions, in turn, are revealed from a consideration of both genetic and biochemical analysis; in the latter case, the function is inferred from either the substrate specificity or the protein-protein interactions of the particular helicase.

How is the ability of Mer3 helicase to promote DNA heteroduplex extension in Rad51 protein-promoted DNA strand exchange related to its role in regulating crossovers in vivo? A possible relationship is depicted in Figure 7. It is known that in meiosis, the DSB that initiates recombination is processed exonucleolytically into duplex DNA with a 3′-ssDNA tail (Sun et al., 1991). Rad51 and/or Dmc1 proteins bind to the ssDNA tails, search for DNA homology, and then invade the dsDNA homolog to form a joint molecule. Our results are consistent with a role for Mer3 helicase in binding the displaced strand of the joint molecule, at the point of the strand crossover, to drive DNA heteroduplex extension in the 3′ to 5′ direction. This heteroduplex extension would stabilize the joint molecule, thereby facilitating both replication and capture of the second processed DNA end that would lead to generation of the double Holliday junction intermediate (Figure 7, left). Resolution of the Holliday junctions by two alternative cuttings would pro-

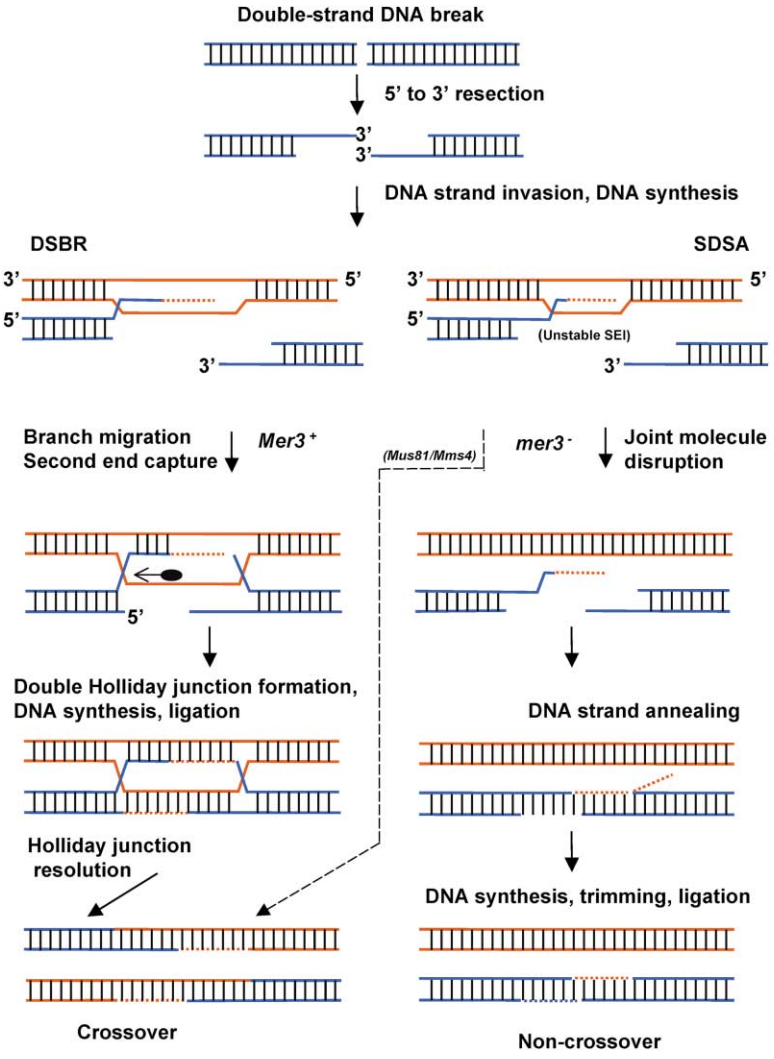

Figure 7. Role of Mer3 Helicase in Meiotic Recombination

Top: DSBs are processed exonucleolytically to form tailed dsDNA with 3′-ends. Rad51 and DMC1 proteins bind to these tails and promote invasion of ssDNA into the homologous dsDNA forming joint molecules. Left: As proposed in the original double-strand break repair (DSBR) model (Szostak et al., 1983), Holliday junction formation involves DNA replication and heteroduplex extension. Mer3 helicase catalyzes 3′ → 5′ DNA heteroduplex extension to stabilize the nascent joint molecule, thereby favoring the DSBR pathway (left side of illustration). DNA replication facilitates capture of second DNA end by Rad52-mediated annealing to the displaced strand of the joint molecule, to initiate formation of a second Holliday junction. Alternative resolution of Holliday junction generates crossovers and noncrossovers. When Mer3 helicase is absent (right side of illustration), the likelihood of stabilizing the nascent D loop structures is decreased, fewer Holliday junction are formed, and the frequency of crossover formation is decreased. However, DSB repair by the parallel pathway of synthesis-dependent strand annealing (SDSA) (Allers and Lichten, 2001; Pâques and Haber, 1999) would be unaffected by the *mer3* mutation. In SDSA, the newly synthesized DNA strand within the joint molecule is displaced and then anneals with the other processed DNA end. Break repair is completed by further DNA synthesis and ligation, and only noncrossover products are formed.

duce either crossover or noncrossover recombinants. In the absence of Mer3 helicase, the nascent DNA heteroduplex would not extend sufficiently and, therefore, would be unstable; in fact, recent physical analysis demonstrates that the stable single-end invasion recombination intermediate, which is the obligate precursor for both Holliday junction and crossover recombinant formation, is not detected in a *mer3* strain (Börner et al., 2004 [this issue of *Cell*]). Instead of forming the Holliday junction, joint molecules could dissociate (or be dissociated by another helicase) after limited DNA synthesis and then would anneal with the other processed DNA end as proposed in the synthesis-dependent strand annealing (SDSA) model (Allers and Lichten, 2001; Nakagawa et al., 1999; Pâques and Haber, 1999) (Figure 7, right). In this case, all recombinants will be noncrossovers.

The biochemical properties of Mer3 helicase substantiate the hypothesis that differentiation between crossover and noncrossover recombinants is established early in meiosis. Mer3 helicase-promoted DNA heteroduplex extension plays an important role in this differentiation. The results described, combined with the genetic data, provide a link between enzymatic activity of Mer3 helicase and its role in crossing over during meiosis.

Experimental Procedures

Proteins and DNA

Escherichia coli RecA protein was purified using a protocol based on spermidine acetate precipitation (Griffith and Shores, 1985; Kowalczykowski et al., 1987; Mirshad and Kowalczykowski, 2003). Single-stranded DNA binding (SSB) protein was purified as described (LeBowitz, 1985), as were *S. cerevisiae* Rad51 protein (New et al., 1998; Zaitseva et al., 1999), RPA (Sugiyama et al., 1997), and both wild-type and mutant Mer3 helicase (Nakagawa and Kolodner, 2002a).

Single- and double-stranded ϕX174 DNA was purchased from New England Biolabs. Cleavage of ϕX174 dsDNA with appropriate restriction endonucleases resulted in linear dsDNA with different termini: dsDNA with 3′-overhanging termini (4 nt) was made with PstI endonuclease; linear dsDNA with 5′-overhanging termini (4 nt) was made with XhoI endonuclease; and linear dsDNA with blunt ends was made with StuI endonuclease. The XhoI-cleaved DNA was labeled at the 5′-end by treating with shrimp alkaline phosphatase, followed by end labeling with [γ-^{32}P] ATP using T4 polynucleotide kinase (New England Biolabs). The XhoI-cleaved DNA was labeled at the 3′-end by adding only one nucleotide to the recessed 3′-ends using the Klenow fragment of *E. coli* PolI (New England Biolabs)

and [α-^{32}P] dTTP. Unincorporated nucleotides were removed using MicroSpin G-25 columns (Amersham Pharmacia Biotech Inc.). The commercial ϕX174 dsDNA was sometime contaminated with trace amounts of nicked ϕX174 ssDNA that could be detected after labeling, but this low level amount of ssDNA had no effect on any of the experiments reported. DNA concentration was measured spectrophotometrically (1 A_{260} unit of dsDNA = 50 μg/ml) and is provided as the molar nucleotide concentration.

DNA Strand Exchange

Rad51 nucleoprotein filaments were formed by incubation of Rad51 protein (6 μM) with ssDNA (20 μM) in standard buffer, containing 33 mM HEPES (pH 7.0), 2 mM DTT, 100 μg/ml bovine serum albumin, 2 mM ATP, 3 mM phosphoenolpyruvate, 20 U/ml pyruvate kinase, 1.2 mM magnesium acetate at 37°C for 3 min, followed by an increase in the magnesium acetate concentration to 6.2 mM. Incubation was then continued for another 10 min. Either RPA (1 μM) or SSB (1.8 μM) protein was added to the nucleoprotein filaments, and the magnesium acetate concentration was increased one more time to 16.2 mM. Incubation was then continued for another 5 min. RecA nucleoprotein filaments were assembled at slightly different conditions by incubating RecA protein (12 μM) with ssDNA (20 μM) in standard buffer containing 25 mM Tris acetate (pH 7.5), 6 mM magnesium acetate, 1 mM DTT, 100 μg/ml bovine serum albumin, 1 mM ATP, 3 mM phosphoenolpyruvate, 20 U/ml pyruvate kinase at 37°C for 10 min. SSB (1.8 μM) protein was added to the nucleoprotein filaments and incubation was continued for another 5 min. Mer3 helicase (100 nM) was then added, and DNA strand exchange was initiated by addition of homologous dsDNA (40 μM) to the nucleoprotein filaments. Aliquots were withdrawn from the reaction mixture, deproteinized by the addition of SDS to 1% and proteinase K to 500 μg/ml, followed by incubation for 5 min at 37°C; mixed with 1/10 volume of loading buffer (1 mM EDTA pH 8.0, 50% glycerol and 0.1% bromophenol blue); and loaded onto a 1% agarose gel. Products of DNA strand exchange were visualized by staining with ethidium bromide and were quantified using a Storm 840 Phosphor Imager (Molecular Dynamics).

Acknowledgments

We are grateful to Andrei Alexeev, Mark Dillingham, Naofumi Handa, Cynthia Haseltine, Xuan Li, James New, Zeynep Ozsoy, Maria Spies, Tomohiko Sugiyama, Edgar Velencia-Morales, and Yun Wu for their comments on the manuscript. This work was supported by the following grants: NIH grant GM62653 to S.C.K., NIH grant GM26017 to R.D.K., Human Frontier Science Program to T.N., Drexel University College of Medicine Startup funds, and Pennsylvania Health Research Formula Funds from the Tobacco Settlement Act to A.M.

Received: August 21, 2003
Revised: March 10, 2004
Accepted: March 17, 2004
Published: April 1, 2004

References

Agarwal, S., and Roeder, G.S. (2000). Zip3 provides a link between recombination enzymes and synaptonemal complex proteins. Cell *102*, 245–255.

Alexeev, A., Mazin, A., and Kowalczykowski, S.C. (2003). Rad54 protein possesses chromatin-remodeling activity stimulated by the Rad51-ssDNA nucleoprotein filament. Nat. Struct. Biol. *10*, 182–186.

Allers, T., and Lichten, M. (2001). Differential timing and control of noncrossover and crossover recombination during meiosis. Cell *106*, 47–57.

Bianco, P.R., Tracy, R.B., and Kowalczykowski, S.C. (1998). DNA strand exchange proteins: A biochemical and physical comparison. Front. Biosci. *3*, D570–D603.

Bishop, D.K., Park, D., Xu, L., and Kleckner, N. (1992). *DMC1*: a meiosis-specific yeast homolog of E. coli *recA* required for recombination, synaptonemal complex formation, and cell cycle progression. Cell *69*, 439–456.

Börner, G.V., Kleckner, N., and Hunter, N. (2004). Crossover/noncrossover differentiation, synaptonemal complex formation and regulatory surveillance at the leptotene/zygotene transition of meiosis. Cell *117*, this issue, 29–45.

Chua, P.R., and Roeder, G.S. (1998). Zip2, a meiosis-specific protein required for the initiation of chromosome synapsis. Cell *93*, 349–359.

Clever, B., Interthal, H., Schmuckli-Maurer, J., King, J., Sigrist, M., and Heyer, W.D. (1997). Recombinational repair in yeast: functional interactions between Rad51 and Rad54 proteins. EMBO J. *16*, 2535–2544.

Cox, M.M., and Lehman, I.R. (1981). Directionality and polarity in recA protein-promoted branch migration. Proc. Natl. Acad. Sci. USA *78*, 6018–6022.

Eggler, A.L., Inman, R.B., and Cox, M.M. (2002). The Rad51-dependent pairing of long DNA substrates is stabilized by replication protein A. J. Biol. Chem. *277*, 39280–39288.

Griffith, J., and Shores, C.G. (1985). RecA protein rapidly crystallizes in the presence of spermidine: A valuable step in its purification and physical characterization. Biochemistry *24*, 158–162.

Harmon, F.G., and Kowalczykowski, S.C. (1998). RecQ helicase, in concert with RecA and SSB proteins, initiates and disrupts DNA recombination. Genes Dev. *12*, 1134–1144.

Hays, S.L., Firmenich, A.A., and Berg, P. (1995). Complex formation in yeast double-strand break repair: participation of Rad51, Rad52, Rad55, and Rad57 proteins. Proc. Natl. Acad. Sci. USA *92*, 6925–6929.

Hollingsworth, N.M., Ponte, L., and Halsey, C. (1995). MSH5, a novel MutS homolog, facilitates meiotic reciprocal recombination between homologs in Saccharomyces cerevisiae but not mismatch repair. Genes Dev. *9*, 1728–1739.

Hong, E.L., Shinohara, A., and Bishop, D.K. (2001). Saccharomyces cerevisiae Dmc1 protein promotes renaturation of single-strand DNA (ssDNA) and assimilation of ssDNA into homologous supercoiled duplex DNA. J. Biol. Chem. *276*, 41906–41912.

Hunter, N., and Borts, R.H. (1997). Mlh1 is unique among mismatch repair proteins in its ability to promote crossing-over during meiosis. Genes Dev. *11*, 1573–1582.

Hunter, N., and Kleckner, N. (2001). The single-end invasion: an asymmetric intermediate at the double-strand break to double-holliday junction transition of meiotic recombination. Cell *106*, 59–70.

Jiang, H., Xie, Y., Houston, P., Stemke-Hale, K., Mortensen, U.H., Rothstein, R., and Kodadek, T. (1996). Direct association between the yeast Rad51 and Rad54 recombination proteins. J. Biol. Chem. *271*, 33181–33186.

Kahn, R., Cunningham, R.P., DasGupta, C., and Radding, C.M. (1981). Polarity of heteroduplex formation promoted by Escherichia coli recA protein. Proc. Natl. Acad. Sci. USA *78*, 4786–4790.

Keeney, S., Giroux, C.N., and Kleckner, N. (1997). Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. Cell *88*, 375–384.

Khazanehdari, K.A., and Borts, R.H. (2000). EXO1 and MSH4 differentially affect crossing-over and segregation. Chromosoma *109*, 94–102.

Kirkpatrick, D.T., Ferguson, J.R., Petes, T.D., and Symington, L.S. (2000). Decreased meiotic intergenic recombination and increased meiosis I nondisjunction in exo1 mutants of Saccharomyces cerevisiae. Genetics *156*, 1549–1557.

Kleckner, N. (1996). Meiosis: how could it work? Proc. Natl. Acad. Sci. USA *93*, 8167–8174.

Kowalczykowski, S.C., Clow, J.C., Somani, R., and Varghese, A. (1987). Effects of the Escherichia coli SSB protein on the binding of *Escherichia coli* RecA protein to single-stranded DNA: Demonstration of competitive binding and the lack of a specific protein-protein interaction. J. Mol. Biol. *193*, 81–95.

Krejci, L., Van Komen, S., Li, Y., Villemain, J., Reddy, M.S., Klein, H., Ellenberger, T., and Sung, P. (2003). DNA helicase Srs2 disrupts the Rad51 presynaptic filament. Nature *423*, 305–309.

LeBowitz, J. (1985). Biochemical mechanism of strand initiation in

bacteriophage lambda DNA replication, PhD thesis, Johns Hopkins University, Baltimore, Maryland.

Lee, S.E., Moore, J.K., Holmes, A., Umezu, K., Kolodner, R.D., and Haber, J.E. (1998). Saccharomyces Ku70, mre11/rad50 and RPA proteins regulate adaptation to G2/M arrest after DNA damage. Cell *94*, 399–409.

Mazin, A.V., Bornarth, C.J., Solinger, J.A., Heyer, W.D., and Kowalczykowski, S.C. (2000a). Rad54 protein is targeted to pairing loci by the Rad51 nucleoprotein filament. Mol. Cell *6*, 583–592.

Mazin, A.V., Zaitseva, E., Sung, P., and Kowalczykowski, S.C. (2000b). Tailed duplex DNA is the preferred substrate for Rad51 protein-mediated homologous pairing. EMBO J. *19*, 1148–1156.

Mazin, A.V., Alexeev, A.A., and Kowalczykowski, S.C. (2003). A novel function of Rad54 protein. Stabilization of the Rad51 nucleoprotein filament. J. Biol. Chem. *278*, 14029–14036.

Mirshad, J.K., and Kowalczykowski, S.C. (2003). Biochemical basis of the constitutive coprotease activity of RecA P67W protein. Biochemistry *42*, 5937–5944.

Nakagawa, T., and Ogawa, H. (1997). Involvement of the MRE2 gene of yeast in formation of meiosis-specific double-strand breaks and crossover recombination through RNA splicing. Genes Cells *2*, 65–79.

Nakagawa, T., and Ogawa, H. (1999). The Saccharomyces cerevisiae MER3 gene, encoding a novel helicase-like protein, is required for crossover control in meiosis. EMBO J. *18*, 5714–5723.

Nakagawa, T., and Kolodner, R.D. (2002a). The MER3 DNA helicase catalyzes the unwinding of Holliday junctions. J. Biol. Chem. *277*, 28019–28024.

Nakagawa, T., and Kolodner, R.D. (2002b). Saccharomyces cerevisiae Mer3 is a DNA helicase involved in meiotic crossing over. Mol. Cell. Biol. *22*, 3281–3291.

Nakagawa, T., Datta, A., and Kolodner, R.D. (1999). Multiple functions of MutS- and MutL-related heterocomplexes. Proc. Natl. Acad. Sci. USA *96*, 14186–14188.

Nakagawa, T., Flores-Rozas, H., and Kolodner, R.D. (2001). The MER3 helicase involved in meiotic crossing over is stimulated by single-stranded DNA-binding proteins and unwinds DNA in the 3′ to 5′ direction. J. Biol. Chem. *276*, 31487–31493.

Namsaraev, E., and Berg, P. (1997). Characterization of strand exchange activity of yeast Rad51 protein. Mol. Cell. Biol. *17*, 5359–5368.

Namsaraev, E.A., and Berg, P. (1998). Branch migration during Rad51-promoted strand exchange proceeds in either direction. Proc. Natl. Acad. Sci. USA *95*, 10477–10481.

Namsaraev, E.A., and Berg, P. (2000). Rad51 uses one mechanism to drive DNA strand exchange in both directions. J. Biol. Chem. *275*, 3970–3976.

New, J.H., and Kowalczykowski, S.C. (2002). Rad52 protein has a second stimulatory role in DNA strand exchange that complements replication protein-A function. J. Biol. Chem. *277*, 26171–26176.

New, J.H., Sugiyama, T., Zaitseva, E., and Kowalczykowski, S.C. (1998). Rad52 protein stimulates DNA strand exchange by Rad51 and replication protein A. Nature *391*, 407–410.

Ogawa, T., Shinohara, A., Nabetani, A., Ikeya, T., Yu, X., Egelman, E.H., and Ogawa, H. (1993). RecA-like recombination proteins in eukaryotes: functions and structures of *RAD51* genes. Cold Spring Harb. Symp. Quant. Biol. *58*, 567–576.

Pâques, F., and Haber, J.E. (1999). Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. Microbiol. Mol. Biol. Rev. *63*, 349–404.

Petukhova, G., Stratton, S., and Sung, P. (1998). Catalysis of homologous DNA pairing by yeast Rad51 and Rad54 proteins. Nature *393*, 91–94.

Petukhova, G., Van Komen, S., Vergano, S., Klein, H., and Sung, P. (1999). Yeast Rad54 promotes Rad51-dependent homologous DNA pairing via ATP hydrolysis-driven change in DNA double helix conformation. J. Biol. Chem. *274*, 29453–29462.

Roeder, G.S. (1997). Meiotic chromosomes: it takes two to tango. Genes Dev. *11*, 2600–2621.

Ross-Macdonald, P., and Roeder, G.S. (1994). Mutation of a meiosis-specific MutS homolog decreases crossing over but not mismatch correction. Cell *79*, 1069–1080.

Salinas, F., and Kodadek, T. (1995). Phage T4 homologous strand exchange: a DNA helicase, not the strand transferase, drives polar branch migration. Cell *82*, 111–119.

Schwacha, A., and Kleckner, N. (1994). Identification of joint molecules that form frequently between homologs but rarely between sister chromatids during yeast meiosis. Cell *76*, 51–63.

Shinohara, A., and Ogawa, T. (1998). Stimulation by Rad52 of yeast Rad51-mediated recombination. Nature *391*, 404–407.

Shinohara, A., Ogawa, H., and Ogawa, T. (1992). Rad51 protein involved in repair and recombination in S. cerevisiae is a RecA-like protein. Cell *69*, 457–470.

Shinohara, A., Gasior, S., Ogawa, T., Kleckner, N., and Bishop, D.K. (1997). Saccharomyces cerevisiae recA homologues RAD51 and DMC1 have both distinct and overlapping roles in meiotic recombination. Genes Cells *2*, 615–629.

Shinohara, A., Shinohara, M., Ohta, T., Matsuda, S., and Ogawa, T. (1998). Rad52 forms ring structures and co-operates with RPA in single-strand DNA annealing. Genes Cells *3*, 145–156.

Sigurdsson, S., Trujillo, K., Song, B., Stratton, S., and Sung, P. (2001). Basis for avid homologous DNA strand exchange by human Rad51 and RPA. J. Biol. Chem. *276*, 8798–8806.

Solinger, J.A., and Heyer, W.D. (2001). Rad54 protein stimulates the postsynaptic phase of Rad51 protein-mediated DNA strand exchange. Proc. Natl. Acad. Sci. USA *98*, 8447–8453.

Solinger, J.A., Lutz, G., Sugiyama, T., Kowalczykowski, S.C., and Heyer, W.D. (2001). Rad54 protein stimulates heteroduplex DNA formation in the synaptic phase of DNA strand exchange via specific interactions with the presynaptic Rad51 nucleoprotein filament. J. Mol. Biol. *307*, 1207–1221.

Solinger, J.A., Kiianitsa, K., and Heyer, W.D. (2002). Rad54, a Swi2/Snf2-like recombinational repair protein, disassembles Rad51:dsDNA filaments. Mol. Cell *10*, 1175–1188.

Sugiyama, T., Zaitseva, E.M., and Kowalczykowski, S.C. (1997). A single-stranded DNA-binding protein is needed for efficient presynaptic complex formation by the *Saccharomyces cerevisiae* Rad51 protein. J. Biol. Chem. *272*, 7940–7945.

Sugiyama, T., New, J.H., and Kowalczykowski, S.C. (1998). DNA annealing by RAD52 protein is stimulated by specific interaction with the complex of replication protein A and single-stranded DNA. Proc. Natl. Acad. Sci. USA *95*, 6049–6054.

Sun, H., Treco, D., and Szostak, J.W. (1991). Extensive 3′-overhanging, single-stranded DNA associated with the meiosis-specific double-strand breaks at the *ARG4* recombination initiation site. Cell *64*, 1155–1161.

Sung, P. (1994). Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast RAD51 protein. Science *265*, 1241–1243.

Sung, P. (1997). Yeast Rad55 and Rad57 proteins form a heterodimer that functions with replication protein A to promote DNA strand exchange by Rad51 recombinase. Genes Dev. *11*, 1111–1121.

Sung, P., Trujillo, K.M., and Van Komen, S. (2000). Recombination factors of Saccharomyces cerevisiae. Mutat. Res. *451*, 257–275.

Sym, M., Engebrecht, J.A., and Roeder, G.S. (1993). ZIP1 is a synaptonemal complex protein required for meiotic chromosome synapsis. Cell *72*, 365–378.

Szostak, J.W., Orr-Weaver, T.L., Rothstein, R.J., and Stahl, F.W. (1983). The double-strand break repair model for recombination. Cell *33*, 25–35.

Tsubouchi, H., and Ogawa, H. (2000). Exo1 roles for repair of DNA double-strand breaks and meiotic crossing over in Saccharomyces cerevisiae. Mol. Biol. Cell *11*, 2221–2233.

Van Komen, S., Petukhova, G., Sigurdsson, S., and Sung, P. (2002). Functional cross-talk among Rad51, Rad54, and replication protein A in heteroduplex DNA joint formation. J. Biol. Chem. *277*, 43578–43587.

Veaute, X., Jeusset, J., Soustelle, C., Kowalczykowski, S.C., Le Cam, E., and Fabre, F. (2003). The Srs2 helicase prevents recombination by disrupting Rad51 nucleoprotein filaments. Nature *423*, 309–312.

Wang, T.F., Kleckner, N., and Hunter, N. (1999). Functional specificity of MutL homologs in yeast: evidence for three Mlh1-based heterocomplexes with distinct roles during meiosis in recombination and mismatch correction. Proc. Natl. Acad. Sci. USA *96*, 13914–13919.

West, S.C. (2003). Molecular views of recombination proteins and their control. Nat. Rev. Mol. Cell Biol. *4*, 435–445.

Zaitseva, E.M., Zaitsev, E.N., and Kowalczykowski, S.C. (1999). The DNA binding properties of *Saccharomyces cerevisiae* Rad51 protein. J. Biol. Chem. *274*, 2907–2915.