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Rad52 Protein Has a Second Stimulatory Role in DNA Strand Exchange That Complements Replication Protein-A Function*

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Rad52 protein plays a central role in double strand break repair and homologous recombination in *Saccharomyces cerevisiae*. We have identified a new mechanism by which Rad52 protein stimulates Rad51 protein-promoted DNA strand exchange. This function of Rad52 protein is revealed when subsaturating amounts (relative to the single-stranded DNA concentration) of replication protein-A (RPA) are used. Under these conditions, Rad52 protein is needed for extensive DNA strand exchange. Interestingly, in this new role, Rad52 protein neither acts simply as a single strand DNA-binding protein *per se* nor, in contrast to its previously identified stimulatory roles, does it require physical interaction with RPA because it can be substituted by the *Escherichia coli* single strand DNA-binding protein. We propose that Rad52 protein acts by stabilizing the Rad51 presynaptic filament.

Homologous recombination and double strand break (DSB)[1] repair in the yeast *Saccharomyces cerevisiae* are under the control of the *RAD52* epistasis group of genes, which includes *RAD51*, *RAD52*, *RAD54*, *RAD55*, *RAD57*, *RAD59*, *RDH54/TID1*, *RAD50*, *MRE11*, and *XRS2* (1, 2). For these processes, there exist multiple pathways that can be distinguished according to their need for Rad51 protein, the structural and functional homologue of the *Escherichia coli* DNA strand exchange protein, RecA. Both *RAD51*-dependent and *RAD51*-independent pathways exist, and they share a common requirement for *RAD52* (1). Several proteins of the *RAD52* epistasis group interact directly with one or more other proteins of the group. Rad52 protein physically interacts with Rad51 protein (3) and with replication protein-A (RPA) (4), the yeast functional homologue of the *E. coli* single strand DNA-binding protein SSB.

Rad51 protein promotes DNA strand exchange *in vitro* (5). Using the single strand circular and homologous linear duplex DNA substrate system, complete heteroduplex formation by Rad51 protein was strongly dependent on the presence of RPA. RPA maximizes heteroduplex product yield by increasing the amount of ssDNA that can be incorporated into the active complex, which is composed of Rad51 protein and ssDNA and known as the presynaptic filament. In this respect, the RecA protein- and Rad51 protein-promoted reactions are different. Although SSB stimulates the activity of RecA protein, RecA protein can promote a significant level of DNA strand exchange in the absence of SSB (6). The most effective level of RPA was found to be that which could completely saturate the ssDNA (7), and complete product formation was extremely sensitive to the order of protein addition to ssDNA (5). When Rad51 protein is introduced to the ssDNA before RPA, DNA strand exchange occurs efficiently. However, RPA and Rad51 protein compete for binding to the same ssDNA binding sites. Because RPA binds more rapidly to ssDNA, when RPA is bound to ssDNA prior to or simultaneous with Rad51 protein, formation of the Rad51 nucleoprotein filament is inhibited and DNA strand exchange is blocked. Consequently, while RPA is an essential cofactor for DNA strand exchange, it is also an inhibitor because of its ability to outcompete Rad51 protein for binding to ssDNA. Although dsDNA is a substrate in the reaction, it too can inhibit the reaction by tightly binding and sequestering Rad51 protein (8, 9) when present prior to the formation of a functional Rad51 protein-ssDNA complex.

We and others (9–11) have shown that Rad52 protein can surmount these inhibitory effects to increase DNA strand exchange. Using a mechanism that is dependent on specific Rad52-RPA and Rad52-Rad51 protein-protein interactions, Rad52 protein allows Rad51 to gain access to the ssDNA by facilitating the displacement of RPA. In targeting Rad51 protein to ssDNA, Rad52 protein also reduces the sequestration of Rad51 protein by dsDNA. The effect of Rad52 protein on DNA strand exchange can be summarized by its ability to increase the assembly of a critical component of the reaction, the presynaptic filament. Here we have demonstrated that Rad52 protein can stimulate DNA strand exchange by acting in a different manner, one that does not require RPA as an intermediary.

EXPERIMENTAL PROCEDURES

Proteins and DNA—Rad52 protein was purified as described (9). Rad51 protein was purified as described (5, 9) with the following modifications. A cleared lysate was prepared in 500 mM KCl and fractionated over Q-Sepharose. Flow-through fractions were pooled, reduced to 200 mM KCl by dialysis, and applied to a Cibacron blue column from which Rad51 protein eluted at 1 M KCl. The sizing chromatography step was omitted. RPA was purified as described (7), as were SSB (12) and RecA (13). ϕX174 ss- and dsDNA were purchased from New England Biolabs. The duplex linear substrate was produced by *Pst*I digestion of ϕX174 dsDNA followed by phenol/chloroform/isoamyl alcohol (25:24:1) extraction, ether extraction, ethanol precipitation, and resuspension in TE (10 mM Tris-Cl, 1 mM EDTA).

DNA Strand Exchange—DNA strand exchange reactions were performed essentially as described (9). Briefly, reactions (12.5 μl) were assembled with 42.5 mM MOPS (pH 7.4), 20 mM NaCl, 3 mM magnesium

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[1] The abbreviations used are: DSB, double strand break; ss, single strand; ds, double strand; nt, nucleotide(s); RPA, replication protein-A; MOPS, 4-morpholinepropanesulfonic acid.

acetate, 1 mM dithiothreitol, 25 μg/ml bovine serum albumin, 2.5 mM ATP, and 33 μM (nucleotides) φX174 ssDNA (New England Biolabs). Saturating levels of Rad51 protein (11 μM) were used in all experiments. RPA concentration was as indicated with the stoichiometric (1×) concentration equal to 1 μM (1 RPA per 30 nucleotides). Unless noted otherwise, a 1× concentration of Rad52 protein (0.77 μM) was used where 1× equals one Rad52 protein per 37.5 nt. In experiments using *E. coli* SSB to replace RPA, it was added at the concentrations specified; a 1× concentration corresponds to the level needed to saturate the ssDNA, which is 1 SSB monomer per 15 nt. When RecA protein was substituted for Rad51 protein, it was present in stoichiometric amounts equal to one RecA protein per 3 nt; the magnesium ion concentration was 4 mM, the ATP concentration was 1 mM, and an ATP regeneration system was included (13). Except where specified, the order of protein addition was Rad51 protein, RPA, and Rad52 protein. Where used, *E. coli* proteins (RecA protein and SSB) replaced their yeast counterparts (Rad51 protein and RPA, respectively) using the same order of addition. The incubation time between the addition of each protein was kept constant at 5 min. Five minutes following the addition of all proteins (except where noted), the reaction was initiated by the addition of *Pst*I-linearized φX174 dsDNA to a final concentration of 33 μM (base pairs). Spermidine acetate was added to a final concentration of 4 mM. Reactions were terminated after 90 min, or at the indicated times, by addition of SDS (0.5% final concentration) and proteinase K (1 mg/ml final concentration) followed by incubation at 37° for 15 min prior to addition of gel loading buffer and application onto 1% agarose gels. After electrophoresis at 40 V for 17 h in TAE (40 mM Tris acetate, 2 mM EDTA) buffer, the gels were stained in ethidium bromide, rinsed, and photographed using a Gel Print 2000i (Biophotonics) gel documentation system. The resulting image was analyzed using ImageQuant 5.0 software.

ATPase Hydrolysis Assays—ATPase activity was measured at 37 °C as described (7). In reactions examining the effect of salt, NaCl was added immediately following the addition of dsDNA.

RESULTS

We addressed the possibility that Rad52 protein might function to stimulate DSB repair and homologous recombination in ways not previously identified. Our approach was to use reaction conditions that were suboptimal for DNA strand exchange. Optimal conditions require concentrations of Rad51 protein capable of saturating all sites on the ssDNA so that assembly of a full-length, uninterrupted Rad51-ssDNA filament is possible (and preventable only by the presence of a secondary structure). These conditions require that RPA, which is responsible for melting these stably paired regions of ssDNA, also be present in stoichiometric amounts. Saturating levels correspond to ratios of 1:3 nt for Rad51 protein and 1:30 nt for RPA (defined as 1×) (7). As noted earlier, when both proteins are used at these levels, prior formation of a RPA-ssDNA complex inhibits DNA strand exchange (Fig. 1*A*, *lane 3*) by blocking formation of the Rad51 protein-ssDNA presynaptic filament. Addition of Rad52 protein antagonizes this inhibition and restores formation of the nicked circle product (Fig. 1*A*, *lane 4*).

We anticipated that when RPA is allowed to associate with ssDNA prior to Rad51 protein ("RPA-first" reactions) reducing RPA to subsaturating levels should permit access by Rad51 protein to the ssDNA. If nucleation of the Rad51 presynaptic filament were the limiting step in RPA displacement, then RPA displacement would be rapid and little stimulation by Rad52 protein should be expected. Instead, stimulation by Rad52 protein is seen at all subsaturating RPA levels tested (Fig. 1*A*, *lanes 6*, *8*, *10*, *12*). Strikingly, the RPA concentration can be reduced 67% without reducing product yield (*lane 6*) because complete DNA strand exchange, as signified by nicked circular DNA formation, is equal to or even slightly exceeds that observed with saturating RPA (*lane 4*). These results suggest that Rad52 protein may complement RPA function.

Because interpretation of this effect is complicated by the possibility that some of the stimulation may yet be attributable to eviction of RPA by Rad52 protein, reactions were repeated with Rad51 protein added prior to RPA. Rad52 protein has only a minor effect when saturating RPA is present (Fig. 1*B*, *lanes 4* and *5*) but clearly stimulates product formation at all subsaturating levels (*lanes 7*, *9*, *11*, *13*, *15*), indicating unambiguously that Rad52 protein can complement RPA function.

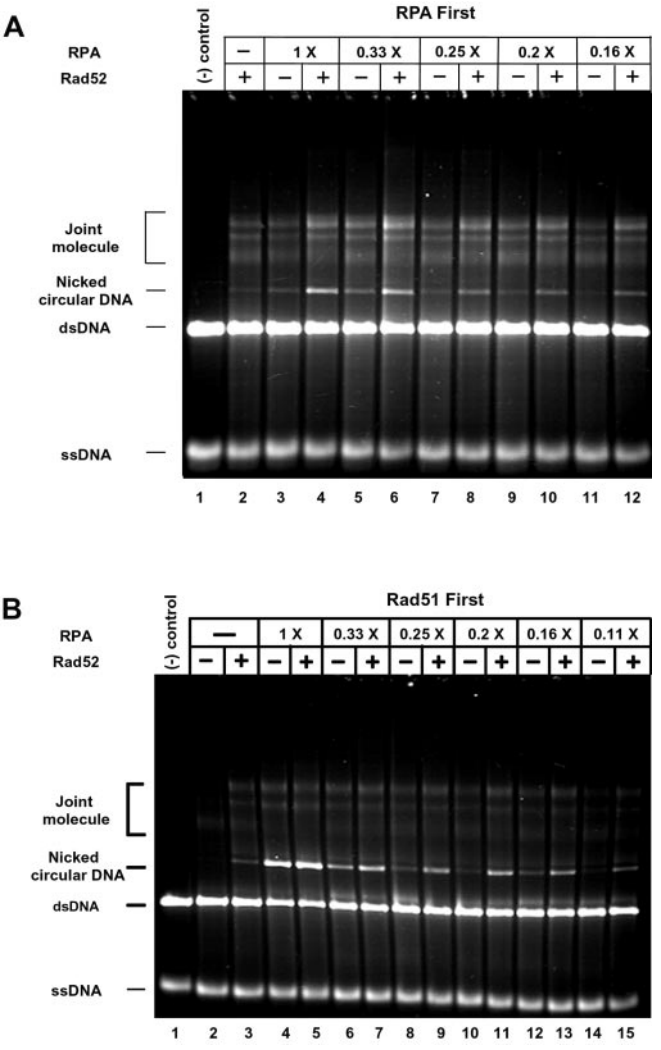

FIG. 1. **Rad52 stimulates the extent of fully exchanged DNA strands at suboptimal RPA concentrations.** *A*, RPA, at the indicated fractional saturation, is complexed with ssDNA 5 min prior to Rad51 addition (*RPA First* protocol). Ethidium bromide-stained gels of standard reactions are shown. Starting substrates (*ssDNA* and *dsDNA*), intermediates (*joint molecule*), and products (*Nicked circular DNA*) are identified. When Rad52 was present, it was the last protein added. *B*, same as *A*, except that Rad51 is assembled on ssDNA 5 min before addition of the indicated amounts of RPA (*Rad51 First* protocol). *Lane 2* contains only Rad51.

An additional effect of Rad52 protein is also evident in these experiments. Although a stoichiometric level of Rad51 protein is required for efficient DNA strand exchange, Rad51 protein alone is not sufficient to produce stably paired molecules because only negligible levels of joint species are formed when RPA is omitted (5, 7). However, when Rad52 protein is included in the absence of RPA, joint molecules are formed at levels comparable to that produced when RPA is present but no Rad52 protein is added (Fig. 1*A*, *lanes 2* and *3*). The ability of Rad52 protein to substitute for RPA was unexpected because these two proteins mediate apparently antagonistic reactions: RPA denatures secondary structure (14) whereas the robust annealing activity of Rad52 protein promotes duplex DNA formation (15–17).

Stimulation may result from an increase in both reaction rate and extent. Time courses performed at optimal RPA levels

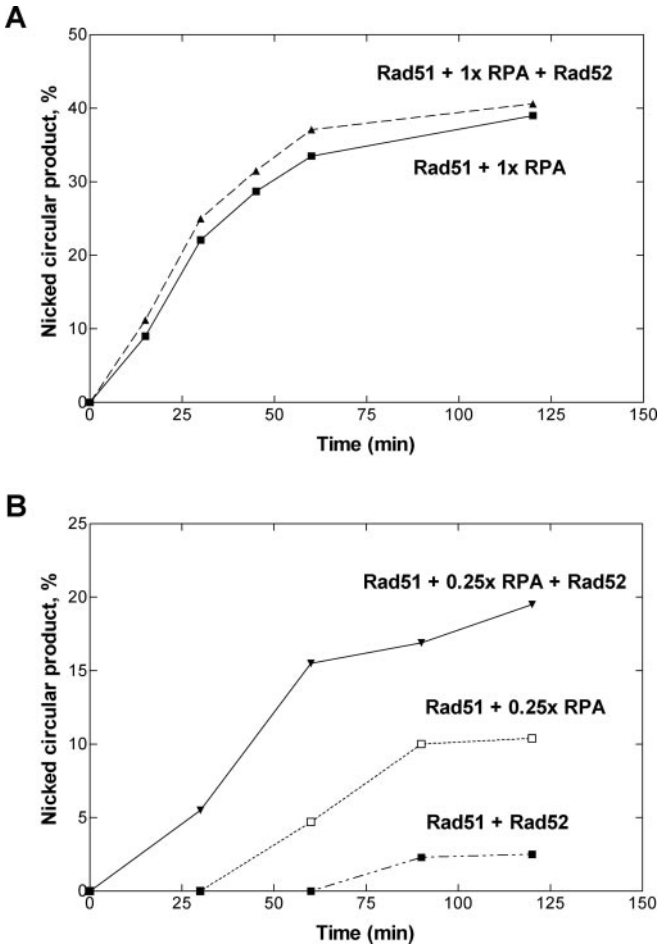

FIG. 2. **Time courses of Rad51 First DNA strand exchange reactions.** *A*, RPA was present at 1× stoichiometric level and added subsequent to Rad51 presynaptic complex formation. *Filled squares*, Rad51 + 1× RPA; *filled triangles*, Rad51 + 1× RPA + Rad52. *B*, same as *A*, except RPA was present at 0.25× stoichiometric level. *Open squares*, Rad51 + 0.25× RPA + Rad52; *filled inverted triangles*, Rad51 + 0.25× RPA; *filled squares*, Rad51 + Rad52.

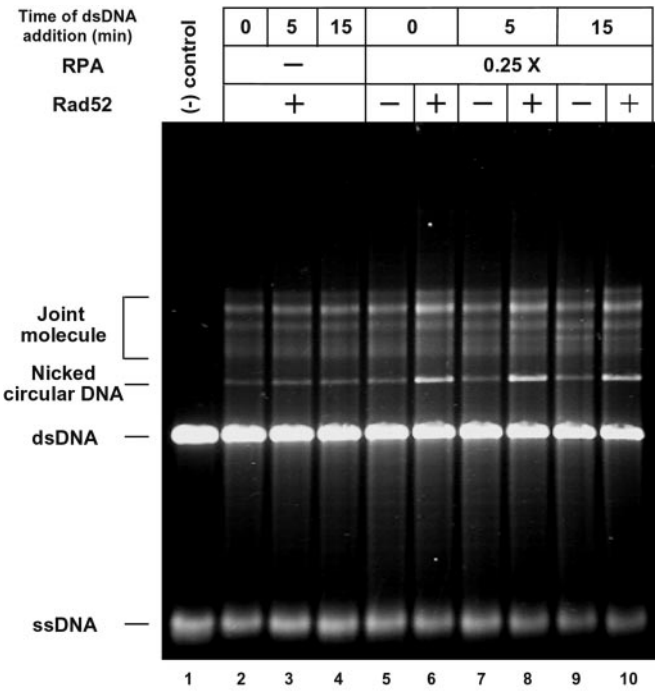

FIG. 3. **Stimulation of full DNA strand exchange does not depend on the length of time needed for presynaptic assembly.** Rad51 was added prior to RPA, and the length of time between the addition of Rad52 and dsDNA was as indicated. Reactions were terminated 90 min after DNA strand exchange was initiated by addition of dsDNA.

show that neither the rate nor the extent of nicked circular DNA formation is dependent on Rad52 protein, and each reaction is 50% complete by 25 min (Fig. 2*A*). However, at 0.25× RPA, the result is significantly different: primarily by eliminating a lag phase of about 30 min, Rad52 protein reduces the time for 50% completion from 65 to 35 min (Fig. 2*B*), which is much closer to the time needed with 1× RPA.

If dsDNA is introduced prior to the formation of a Rad51-ssDNA complex, the Rad51 protein (which binds rapidly and tightly to dsDNA) becomes sequestered and DNA strand exchange is inhibited (8, 9). In RPA-first reactions, we showed previously that stimulation by Rad52 protein increased directly with the length of time between the additions of Rad52 protein and dsDNA (9). This time dependence reflects the kinetics of RPA displacement and incorporation of Rad51 protein into the nucleoprotein filament, which prevents sequestration of Rad51 protein by dsDNA.[2] We performed comparable Rad51 protein-first experiments and varied the interval from 0 to 15 min (Fig. 3, *lanes 6*, *8*, *10*). No time-dependent difference was observed. This suggests that Rad52 protein either alters the filament quickly in such a way that Rad51 protein cannot be sequestered by dsDNA or, alternatively, that it acts later in DNA strand exchange.

If Rad52 protein simply functioned as an alternative SSB, a reduction in the amount of RPA should require a compensatory increase in the level of Rad52 protein. To test this expectation, the concentration of Rad52 protein was varied at three different levels of RPA. As seen in Fig. 4, there was no systematic increase in the amount of Rad52 protein needed as the RPA concentration was reduced. These results suggest instead that, because the optimal level of Rad52 protein remains approximately constant, the concentration at which its effect is strongest is determined by a fixed level of some other species, possibly the presynaptic filament. Also supportive of the hypothesis that Rad52 protein does not act as an SSB *per se* is the fact that it cannot completely replace RPA. We note, however, that in reactions containing Rad51 and Rad52 proteins (but no RPA) a small amount of product formation (~3–4%) is reproducibly observed (Fig. 1*A*, *lane 2*; Fig. 1*B*, *lane 3*; Fig. 3, *lanes 2–4*; Fig. 5, *lanes 2* and *3*) that is never seen in reactions containing only Rad51 protein (Fig. 1*B*, *lane 2*).

Prior assembly of either a Rad51 or *E. coli* RecA protein presynaptic complex permits the strand transfer activity of either protein to be stimulated by either RPA or SSB (5–7). We exploited this fact to test whether specific protein interactions were required for complementation of RPA function by Rad52 protein. At a 1× concentration of SSB, no stimulation by Rad52 protein is detected (data not shown). However, at all lower levels tested, although product formation was proportional to the level of SSB, as expected and as seen also with RPA, we found that Rad52 protein stimulated complete DNA strand transfer (Fig. 5). This indicates that stimulation is not mediated by an interaction of Rad52 protein with RPA and, moreover, does not even require RPA. Substitution of RecA protein for Rad51 protein in reactions containing various concentrations of either RPA (Fig. 6*A*) or SSB protein (Fig. 6*B*) failed to support stimulation, suggesting that Rad52 protein exerts its effect through specific interaction with Rad51 protein.

How this interaction might affect the presynaptic filament and how it might affect the response of the filament to chal-

[2] T. Sugiyama and S. Kowalczykowski, submitted for publication.

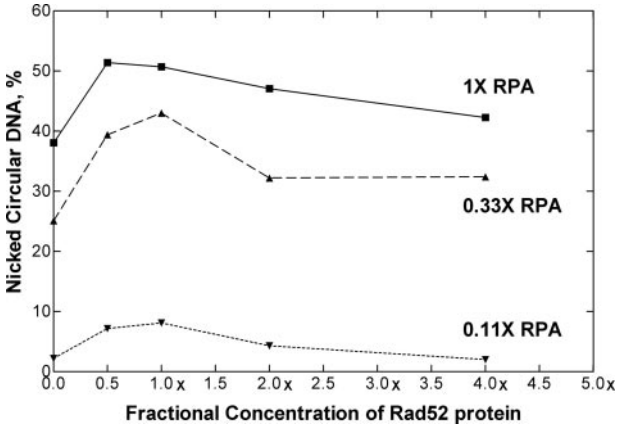

FIG. 4. **Optimal stimulation of complete DNA strand exchange by Rad52 is independent of RPA concentration.** Rad51 was added prior to RPA. *Squares*, 1× RPA; *triangles*, 0.33× RPA; *inverted triangles*, 0.11× RPA.

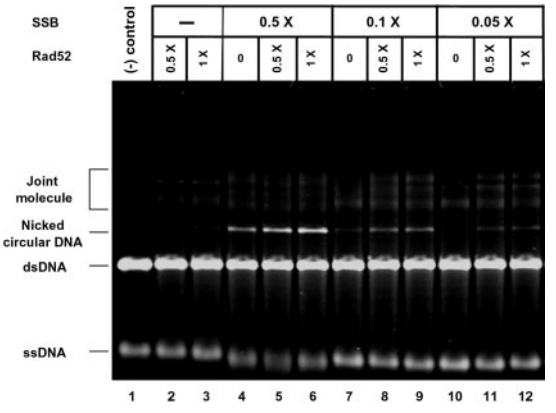

FIG. 5. **Stimulation of DNA strand exchange does not require a specific interaction with RPA.** Ethidium bromide-stained gel of standard reactions in which SSB, at the indicated stoichiometry relative to ssDNA, was substituted for RPA and added 5 min after Rad51. 1× stoichiometries for all proteins are defined under "Experimental Procedures."

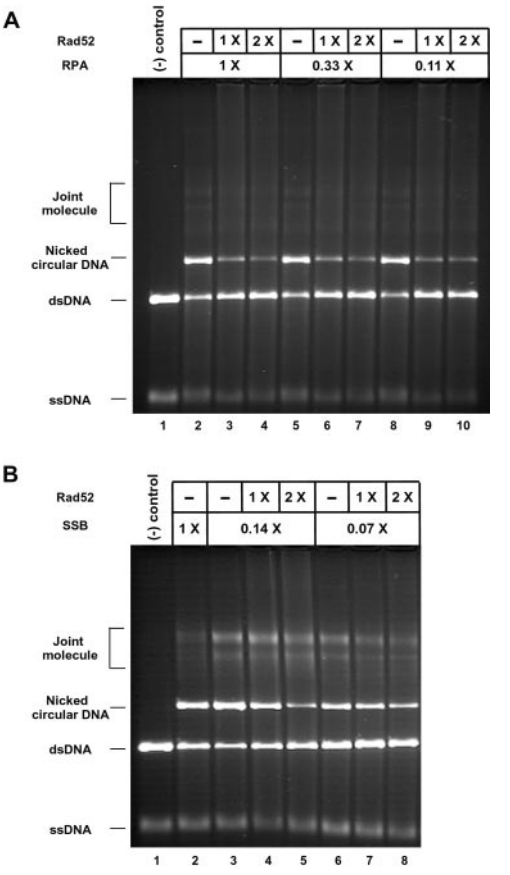

FIG. 6. **Rad52 does not stimulate DNA strand exchange when Rad51 is replaced by RecA.** Ethidium bromide-stained gels of DNA strand exchange reactions with RecA present at a stoichiometry relative to ssDNA of 1:3 nt. In all cases where it was included, Rad52 was the last protein added. *A*, RecA-promoted DNA strand exchange in the presence of the heterologous single strand DNA-binding protein RPA. RPA was added at the indicated concentrations 5 min after the addition of RecA. *B*, RecA-promoted DNA strand exchange reactions in which SSB has replaced RPA.

lenge by salt was examined using the ssDNA-dependent ATPase activity of Rad51 as a measure of Rad51 protein-ssDNA complex formation. The results presented in Fig. 7 show that in the absence of added NaCl, Rad51 protein ATPase activity was increased by about 20% upon addition of Rad52 protein, in line with the level of stimulation previously reported using the same substrate (11). When NaCl was present, up to a concentration of 500 mM, including Rad52 protein resulted in an enhancement of activity by an extent similar in magnitude to that seen in reactions devoid of salt.

Our observation that Rad52 protein can compensate for a reduction in RPA concentration appears to contradict the findings of Song and Sung (18), who concluded that Rad52 protein had no effect. Their reaction conditions, which included 50 mM KCl as well as a change in the order of protein addition such that RPA was added after Rad51 and Rad52 proteins were simultaneously allowed to bind ssDNA, differed from ours. These variations were tested individually to determine which, if either, could account for the observed discrepancy. Although altering the staging of protein addition did have a modest effect in our hands, the presence of 50 mM KCl was sufficient to abrogate stimulation by Rad52 protein (Fig. 8*A*). Because the yield of nicked circular DNA product was less than that seen in Fig. 2, most likely due to the use of a somewhat less active preparation of Rad52 protein, a time course of DNA strand exchange was also performed to confirm the effect of the addition of KCl. The results (Fig. 8*B*) are completely consistent with those in Fig. 8*A*, and they show more specifically that 50 mM KCl restores the lag phase eliminated by Rad52 protein in the absence of salt (Fig. 2). The kinetics of product formation in the reaction containing both KCl and Rad52 protein are identical to those of reactions, with or without added salt, where Rad52

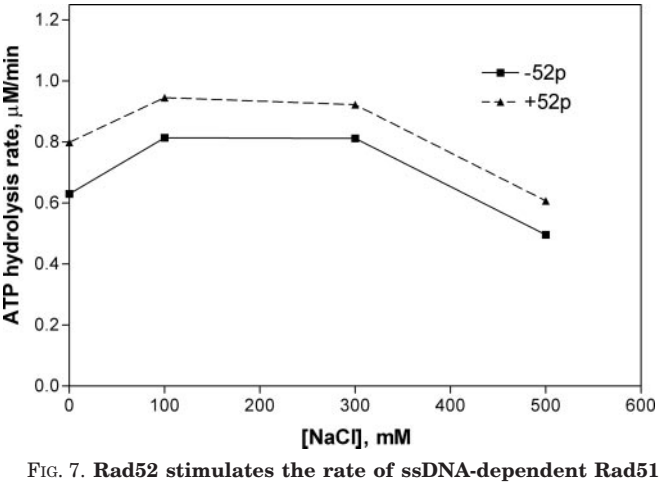

FIG. 7. **Rad52 stimulates the rate of ssDNA-dependent Rad51 ATP hydrolysis.** ATPase assays were performed at 37 °C as described under "Experimental Procedures," and where present, Rad52 was added 5 min after addition of Rad51. *Filled squares*, Rad51; *filled triangles*, Rad51 + Rad52 (1:7.5 nt).

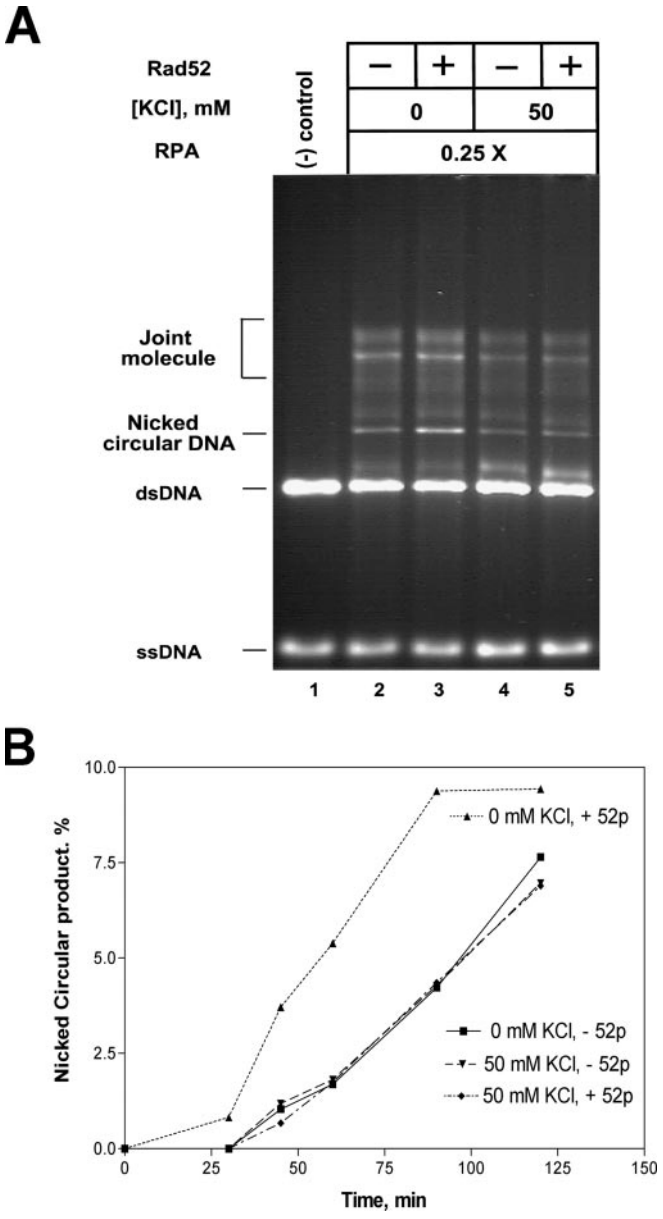

FIG. 8. **Rad52 stimulation of DNA strand exchange is defeated by 50 mM KCl.** *A*, ethidium-stained gel of DNA strand exchange reactions, terminated after 90 min, containing a subsaturating concentration (0.25×) of RPA and Rad52 and 50 mM KCl as indicated. The order of protein addition was Rad51, RPA, and Rad52. *B*, time courses of DNA strand exchange reactions shown in *A*. *Filled squares*, Rad51 + 0.25× RPA, 0 mM KCl; *filled triangles*, Rad51 + 0.25× RPA + Rad52, 0 mM KCl; *filled inverted triangles*, Rad51 + 0.25× RPA, 50 mM KCl; *filled diamonds*, Rad51 + 0.25× RPA + Rad52, 50 mM KCl.

protein is omitted. The difference in our observations can, therefore, be fully explained by this effect.

DISCUSSION

Rad52 protein interacts physically and in a species-specific manner with Rad51 protein and RPA. An important conclusion drawn from *in vitro* analysis is that this property is critical, under conditions expected to reflect those present in the cell, for Rad52 protein to properly coordinate the series of steps which lead to the renaturation of complementary ssDNA or the exchange of DNA strands over several kilobases. For the DNA strand exchange reaction examined in this work, previous studies demonstrated that under optimal conditions where Rad51 protein and RPA are at saturating levels relative to ssDNA, the severe inhibition of product formation that occurs when RPA is allowed to bind ssDNA prior to Rad51 protein can be reversed by Rad52 protein. To examine other possible functions of Rad52 protein in DNA strand exchange, we investigated its effect when conditions were rendered suboptimal by limiting the amount of RPA.

Our results demonstrate that Rad52 protein can stimulate the formation of completely exchanged DNA strands, thereby compensating for a reduction in RPA, and that this stimulation is seen irrespective of the order of RPA or Rad51 protein binding to ssDNA relative to the binding of Rad52 protein. This effect is particularly evident in an RPA-first reaction in which addition of Rad52 protein restores product yield to that seen with optimal conditions even though the concentration of RPA was reduced by two-thirds (Fig. 1*A*, *lanes 4* and *6*). Joint molecule production is also affected by Rad52 protein. Formation of these partially exchanged intermediates is normally dependent on the presence of RPA because they are not readily formed by Rad51 protein alone. In this case as well, Rad52 protein can substitute for RPA (Fig. 1*A*, *lanes 2* and *3*; Fig. 1*B*, *lanes 3* and *4*; Fig. 5, *lanes 2* and *3*). The enhancement of both joint molecule intermediates and nicked circle products by Rad52 protein seen here differs significantly from the behavior of HsRad52 protein. In a reaction that showed no stimulation by RPA, HsRad51 protein-mediated joint molecule formation could be stimulated by HsRad52 protein when HsRad51 protein was reduced 2-fold to a suboptimal level (19). However, at a saturating level (such as that used in this work) of HsRad51 protein, HsRad52 protein actually inhibited DNA strand exchange (20).

To eliminate a lengthy lag phase such that the rate as well as the extent of DNA strand exchange is increased, Rad52 protein must accelerate a rate-limiting step, and formation of the presynaptic filament is a good candidate for this step. Consistent with a role in filament assembly is the fact that the addition of dsDNA, which essentially quenches filament formation by its ability to bind Rad51 protein, is not inhibitory when added immediately after the addition of Rad52 protein (Fig. 3). This result suggests that all of the Rad51 protein is engaged quickly in the presynaptic filament. The reduction in the amount of time required for Rad52 protein to act on the filament via its effect on Rad51 protein presumably reflects the fact that the need to first displace RPA while simultaneously promoting the nucleation/binding of Rad51 protein has been greatly reduced or eliminated when Rad51 is allowed to bind ssDNA before RPA. This rapid effect contrasts with the situation in RPA-first reactions where the yield of complete products is proportional to the amount of time between addition of Rad52 protein and dsDNA (9).

The amount of Rad52 protein needed to elicit a maximal stimulatory response remained almost invariant over a 9-fold range of RPA concentrations, indicating that Rad52 protein is not acting as an SSB *per se* (Fig. 4). If it were, RPA and Rad52 protein levels should be inversely related because less ssDNA is bound when RPA is decreased. This observation indicated that the concentration of Rad52 protein is set by a constant level of some other component of the reaction, such as the presynaptic complex. An inference from this result is that Rad52 protein acts through Rad51 protein but independently of RPA. This hypothesis was tested in the following two ways. 1) When RecA was substituted for Rad51 protein, no stimulation was detected when either RPA (Fig. 6*A*) or SSB (Fig. 6*B*) was present. 2) When Rad51 protein was used and RPA was replaced by SSB, Rad52 protein stimulated nicked circle production at all subsaturating levels examined (Fig. 5).

We conclude that although DNA strand exchange requires ssDNA binding activity, the stimulatory effect of RAD52 pro-

tein is a consequence of specific interactions with Rad51 protein and does not demand direct interaction with RPA, which is, in fact, completely dispensable.

In attempting to understand how Rad52 protein might function, some models can be eliminated from consideration. One possibility is that Rad52 protein acts postsynaptically in a manner analogous to SSB. SSB increases the yield of strand exchange product by binding the displaced strand and inhibiting the binding of RecA protein that could lead to strand re-invasion and a return to starting substrates (21). If Rad52 protein were to bind to the displaced strand, however, it could recruit any available Rad51 protein, as has been shown for the presynaptic phase of DNA strand exchange (9, 11). It might then actually promote re-invasion which would, consequently, reduce product yield. Therefore, a postsynaptic role seems unlikely. Another possibility is that Rad52 protein, via specific interaction with RPA, increases the RPA concentration for a subpopulation of presynaptic complexes to achieve levels closer to saturation. This model is untenable because SSB, which can substitute for RPA, is not known to interact with Rad52 protein. In a third model, Rad52 protein can participate as an SSB protein. This scenario predicts that the optimal level of Rad52 protein would be directly proportional to the amount of ssDNA left unbound by RPA. This model is inadequate because the data in Fig. 4 (showing that the optimal Rad52 protein concentration is essentially unchanged as RPA concentration is varied) are not congruent with this prediction.

Instead we favor a role for Rad52 protein during presynapsis, where it stabilizes the presynaptic filament and/or potentiates the ability of Rad51 protein to penetrate regions of weaker secondary structure. Results published by Shinohara and Ogawa (11), showing that Rad52 protein stimulates the rate of Rad51 protein-dependent ATP hydrolysis, support such a filament stabilizing role. Our work here has confirmed this result and extended it by demonstrating a Rad52 protein-dependent enhancement of ATPase activity in the presence of NaCl concentrations up to 500 mM (Fig. 7). Because this stimulation is relatively constant over the range tested, a formal possibility is that Rad52 protein directly affects the ssDNA-dependent Rad51 protein ATP turnover number. Evidence from the same set of experiments (Fig. 3*a* of Ref. 11) suggests that this is not the case. The rate of ATP hydrolysis in reactions containing stoichiometric amounts of Rad51 protein and RPA (conditions which promote complete filament formation) is identical to the rate in reactions that also contain Rad52 protein. The simplest interpretation of this result is that the mere presence of Rad52 protein is not sufficient to increase the ATP turnover rate by Rad51 protein. Additionally, we are unaware of any precedent for this type of effect on the extensively characterized *E. coli* RecA protein. In a second experiment, Shinohara and Ogawa (11) observed that the ATPase activity of Rad51 protein could be inhibited by SSB protein, which occurs as a consequence of the competition between these two proteins for binding to ssDNA. Addition of Rad52 protein to reactions containing both Rad51 protein and SSB, however, resulted in a severalfold increase in ATPase activity over the SSB-depressed level.

When the findings presented here are taken together with earlier results (9–11,19), a more unified picture of Rad52 protein activity emerges in which it plays two separate, successive roles to stimulate both the extent and the rate of DNA strand exchange. In the first role, Rad52 protein facilitates the displacement of RPA and assists, along with the Rad55/Rad57 heterodimer (22), the loading of Rad51 protein onto ssDNA. Rad52 protein in its second role promotes formation of a single contiguous filament by contributing additional stability through its physical interaction with Rad51 protein, resulting in the reduced RPA requirement seen here.

The description of a new stimulatory role for Rad52 protein in recombination and DSB repair provides additional evidence for and insight into the complex interplay in these processes between Rad52 protein, Rad51 protein, and RPA. RPA, besides its role in homologous recombination, is required for DNA replication and nucleotide excision repair and in yeast also acts as a transcription regulatory factor (for review, see Ref. 14). An implication of this work is that Rad52 protein may buffer DNA strand exchange from the competing demands of other RPA-requiring processes, especially under conditions of severe genotoxic stress, by substantially expanding the range of RPA concentrations over which DSB repair can efficiently occur.

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