A Conserved N-terminal Motif in Rad54 Is Important for Chromatin Remodeling and Homologous Strand Pairing*

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The Swi2/Snf2-related protein Rad54 is a chromatin remodeling enzyme that is important for homologous strand pairing catalyzed by the eukaryotic recombinase Rad51. The chromatin remodeling and DNA-stimulated ATPase activities of Rad54 are significantly enhanced by Rad51. To investigate the functions of Rad54, we generated and analyzed a series of mutant Rad54 proteins. Notably, the deletion of an N-terminal motif (amino acid residues 2–9), which is identical in Rad54 in Drosophila, mice, and humans, results in a complete loss of chromatin remodeling and strand pairing activities, and partial inhibition of the ATPase activity. In contrast, this conserved N-terminal motif has no apparent effect on the ability of DNA to stimulate the ATPase activity or of Rad51 to enhance the DNA-stimulated ATPase activity. Unexpectedly, as the N terminus of Rad54 is progressively truncated, the mutant proteins regain partial chromatin remodeling activity as well as essentially complete DNA-stimulated ATPase activity, both of which are no longer responsive to Rad51. These findings suggest that the N-terminal region of Rad54 contains an autoinhibitory activity that is relieved by Rad51.

Chromatin is the natural form of the DNA substrate in a variety of processes that include transcription, replication, repair, and recombination. The participation of chromatin in these processes involves dynamic changes in its structure. There are two key strategies by which the structure of chromatin is altered. First, the components of chromatin are co-valently modified. For instance, the DNA is methylated, such as at CpG residues, and the histones are acetylated, methylated, phosphorylated, ubiquitylated, and ADP-ribosylated (for reviews, see Refs. 1–4). Second, the structure of chromatin is reconfigured by ATP-utilizing chromatin remodeling factors, which disrupt histone-DNA contacts in the nucleosome and catalyze nucleosome mobility (for reviews, see Refs. 5–11).

Homologous strand pairing is a key step in the repair of DNA double-strand breaks by homologous recombination (for reviews, see Refs. 12–19). In this reaction, the eukaryotic recombinase Rad51 assembles onto single-stranded DNA (which is generated at the site of a double-strand break) to give a nucleoprotein filament that, upon invasion and annealing to homologous double-stranded DNA, yields a structure termed a D-loop. Rad51 belongs to the Rad52 epistasis group of genes (including Rad50, Rad51, Rad52, Rad54, Rad55, Rad57, Rad59, MRE11, and XRCC2). These genes, which are conserved from yeast to humans, have been shown to be important for double-strand break repair by homologous recombination.

In this study, we focus upon the Rad54 protein. Rad54 is a member of the Rad52 group of genes. In addition, the Rad54 protein is in the Snf2-like family of DNA-dependent ATPases (20), which includes the ATPase subunits of a variety of ATP-dependent chromatin remodeling factors such as SWI/SNF1,1 RSC, NURF, ACF, CHRAC, INO80.COM, SWR1, NuRD, RSF, and others. Rad54 has been found to stimulate homologous strand pairing by Rad51 with naked DNA (21–25) or chromatin (26, 27). In addition, Rad54 has been found to mediate chromatin remodeling in a reaction that is strongly enhanced by Rad51 (26–28). Moreover, in the absence of superhelical tension, strand pairing by Rad51 and Rad54 occurs with much higher efficiency with chromatin templates than with naked DNA templates (26).

To study the biochemical function of Rad54, we have performed a systematic deletion analysis of the Drosophila Rad54 protein. We were particularly interested in the regulation of Rad54-dependent chromatin remodeling by Rad51. We found that an N-terminal nine-amino acid motif, which is conserved in Drosophila, mouse, and human Rad54, plays a key role in the function of the protein. The analysis of a series of mutant Rad54 proteins further suggests that Rad51 may function in part to relieve autoinhibition of chromatin remodeling by Rad54.

EXPERIMENTAL PROCEDURES

Plasmids and Proteins—Full-length Drosophila Rad51 and Rad54 proteins with C-terminal FLAG tags were synthesized in Sf9 cells by using a baculovirus expression system as described previously (26). The deletion and ATPase mutant versions of Rad54 were also synthesized as C-terminally FLAG-tagged proteins. The mutant constructs were prepared by using mutagenesis kits from Clontech and Amersham Biosciences. The complete sequences of all constructs were verified by DNA sequencing. Recombinant ACF, recombinant nucleosome assembly protein-1, and core histones from Drosophila embryos were purified as described previously (29, 30).

D Loop Reactions—D loop reactions were performed as described previously (26) with plasmid pU6LNS and the DL2 153-mer oligonucleotide, which is complementary to pU6LNS. Briefly, radiolabeled DL2 oligonucleotide was incubated with Rad51 in buffered medium (25 mM Tris acetate at pH 7.5, 10 mM magnesium acetate, 100 μg/ml bovine serum albumin, 1 mM dithiothreitol, 2 mM ATP, 3 mM phosphoenolpyruvate, 20 units/ml pyruvate kinase) at 27 °C for 20 min. Then, Rad54 protein and pU6LNS (as plasmid DNA or chromatin) were added, and

1 The abbreviations used are: SWI/SNF, switch/sucrose non-fermenting; RSC, remodel the structure of chromatin; NURF, nucleosome remodeling factor; ACF, ATP-utilizing chromatin assembly and remodeling factor; CHRAC, chromatin accessibility complex; INO80.COM, Ino80 chromatin remodeling complex; NuRD, nucleosome remodeling histone deacetylase complex; RSF, remodeling and spacing factor.
the reaction was incubated at 27 °C for 4 min. The reaction was terminated by the addition of EDTA to 50 mM and SDS to 1% (w/v). The sample was treated with proteinase K (to 500 μg/ml) at 37 °C for 20 min and then 0.10 volume of 20% Ficoll, 0.1% bromphenol blue was added. The resulting DNA species were resolved by 1% agarose gel electrophoresis. The gel was dried on anion exchange chromatography paper (DE51, Whatman) and subjected to autoradiography. In a standard reaction, the final concentrations of the components were as follows: Rad51 (200 nM), Rad54 proteins (46 nM), DL2 oligonucleotide (1 nM), Pu6LNS (2 nM).

ATPase Assays—ATPase assays were performed under D loop reaction conditions. In a total volume of 30 μl, wild-type or mutant Rad54 protein (23 nM) was incubated in buffered medium (25 mM Tris acetate at pH 7.5, 10 mM magnesium acetate, 100 μg/ml bovine serum albumin, 1 mM dithiothreitol, 2 mM ATP) with 0.5 μM of [γ-32P]ATP (Amersham Biosciences) at 27 °C for the indicated times. Where noted, Rad51 (100 nM) and Pu6LNS (2 nM) were included. The reactions were terminated by the addition of EDTA to 50 mM and SDS to 1% (w/v), and 2 μl of the reaction mixtures were subjected to thin layer chromatography on a polyethylenimine cellulose plate (SelectoScientific). Prior to use, the chromatography plate was prerun in water and dried at room temperature for 60 min. The ATP hydrolysis products were resolved in 0.8 M LiCl, 0.8 M acetic acid. The percentage of hydrolyzed ATP was quantitated with a PhosphorImager.

Chromatin Remodeling Assays—Restriction enzyme accessibility assays for chromatin remodeling were performed essentially as described previously (26). Wild-type or deletion mutant Rad54 proteins were incubated with salt dialysis chromatin (reconstituted with Pu6LNS) in the presence of the HaeIII restriction enzyme (15 units, Invitrogen) at 27 °C for 1 h in D loop reaction buffer medium. Where indicated, Rad51 and/or ATP was included in the reactions. The samples were deproteinized and subjected to ethidium bromide. The final concentrations of the reaction components were as follows: Rad51, 200 nM; Rad54 proteins, 83 nM; chromatin, 2 nM; ATP, 2 mM.

RESULTS

Synthesis and Purification of Rad54 Deletion Mutant Proteins—To identify specific functional subregions of Drosophila Rad54, we constructed a series of N- and C-terminal deletion mutant versions of the protein. Rad54 is a member of the Snf2-like family of DNA-dependent ATPases, which share seven conserved motifs (20). As shown in Fig. 1A, Rad54 consists of a central region with homology to DNA-dependent ATPases along with N- and C-terminal extensions. The mutant proteins were constructed with C-terminal FLAG tags, synthesized in Sf9 cells by using a baculovirus expression system, and purified by FLAG-affinity chromatography (Fig. 1B). Some of the mutant proteins, such as Rad54Δ2–75 and Rad54Δ2–100, appear as double bands. Both of these bands were detected with a polyclonal antibody that was raised against a C-terminal fragment of Drosophila Rad54 (amino acid residues 684–784), and thus, the faster migrating species might be N-terminally truncated versions of the mutant proteins or alternatively modified forms of the protein. In the following experiments, we compared the activities of equimolar amounts of the “full-length” mutant Rad54 proteins as estimated by quantitative Western blot analyses with antibodies against Rad54 and/or FLAG peptide.

The N Terminus of Rad54 Is Required for Stimulation of Chromatin Remodeling by Rad51—First we sought to test the chromatin remodeling activity of the mutant Rad54 proteins. Rad54 is an ATP-dependent nucleosome remodeling factor with activity that is strongly stimulated by Rad51 (26–28). To analyze chromatin remodeling by the deletion mutant versions of Rad54, we used the restriction enzyme accessibility assay in which the ATP-dependent remodeling of nucleosomes facilitates the access of a restriction enzyme (HaeIII) to DNA packaged into chromatin.

We thus tested the Rad54 proteins for chromatin remodeling activity (Fig. 2). With wild-type Rad54, we observed chromatin remodeling that is strongly enhanced by Rad51 as seen previously (26–28). The deletion of the N-terminal 50 amino acid residues results in a loss of detectable remodeling activity in the absence or presence of Rad51 (Fig. 2, Δ2–50). Somewhat unexpectedly, there is a partial restoration of chromatin re-
modeling activity upon deletion of an additional 25 or 50 amino acid residues from the N terminus (Fig. 2A, δ2–75 and δ2–100). The stimulation of remodeling by Rad51 appears to be significantly reduced in the δ2–75 protein and absent in the δ2–100 protein. Then, upon further deletion of another 25 or 51 amino acid residues, there is no detectable remodeling activity (Fig. 2A, δ2–125 and δ2–151). The analysis of C-terminally deleted proteins revealed that the removal of the C-terminal 38 residues results in a protein with chromatin remodeling activity that is comparable with that of wild-type Rad54 (Fig. 2B, Δ747–784), whereas the removal of the C-terminal 112 residues results in a loss of remodeling activity (Fig. 2B, Δ673–784). We further confirmed that chromatin remodeling by the wild-type, δ2–75, δ2–100, and Δ747–784 proteins is dependent upon ATP (data not shown). These results indicate that the enhancement of Rad54-mediated chromatin remodeling by Rad51 requires the N terminus of Rad54.

The Rad54 Deletion Mutant Proteins Are Mostly Inactive for D Loop Formation—Next we examined whether the Rad54 deletion mutant proteins are active in homologous strand pairing reactions. We and others have seen that Rad54 and Rad51 are able to catalyze D loop formation with a chromatin template and homologous oligonucleotide (26, 27). We observed further that the packaging of DNA into chromatin significantly enhances the efficiency of D loop formation in the absence of superhelical tension (26). To determine the importance of the N and C termini of Rad54 in strand pairing, we carried out D loop assays with the wild-type and mutant Rad54 proteins.

First, we carried out reactions in which we used either supercoiled naked DNA (Fig. 3, DNA) or chromatin prepared by salt dialysis methodology (Fig. 3, Chromatin) along with a homologous oligonucleotide. These experiments revealed that all of the deletion mutant proteins were inactive for D loop formation, except for the Δ747–784 protein, which possesses activity that is comparable with that of wild-type Rad54, and Δ2–75, which exhibits ∼5–10% of the activity of wild-type Rad54 with chromatin and DNA, respectively (Fig. 3A).

We also carried out strand pairing reactions with “relaxed” chromatin that was prepared by the assembly of relaxed DNA into chromatin with the ACF and nucleosome assembly protein-1 assembly factors in the presence of topoisomerase I (Fig. 3B). In these experiments, either chromatin or naked DNA control samples were obtained from parallel reactions in which core histones were present or absent. Then we used these DNA templates in strand pairing reactions with the Rad54 proteins and found that the assembly of DNA into chromatin significantly stimulates homologous strand pairing with the wild-type and Δ747–784 Rad54 proteins (Fig. 3C). We did not observe any detectable D loop formation with the other mutant Rad54 proteins.

These results indicate that the deletion of the C-terminal 38 amino acid residues of Drosophila Rad54 has little effect on the ability of Rad54 to catalyze chromatin remodeling and homologous strand pairing. On the other hand, the deletion of 50, 100, 125, or 151 amino acid residues from the N terminus or 112 residues from the C terminus results in a loss of strand pairing activity with either supercoiled naked DNA or chromatin. The Δ2–75 mutant protein exhibits a low level of activity for chromatin remodeling and strand pairing. These findings suggest that deletion of residues 51 through 75 results in a partial relief of an inhibitory activity.

ATPase Activity of the Rad54 Deletion Proteins—Then we determined the effect of the deletion mutations upon the ATPase activity of Rad54. Rad54 has a DNA-stimulated ATPase activity that is enhanced by Rad51 (25, 27, 31) but not further increased by the presence of nucleosomes (27). In these experiments, we measured ATP hydrolysis by the Rad54 proteins in the absence or presence of DNA and the further en-
enhancement of the DNA-stimulated ATPase activities by Rad51 (Fig. 4). Wild-type Drosophila Rad54 possesses a DNA-stimulated ATPase activity that is enhanced by Rad51. (We observed the same amount of stimulation of Rad54 DNA-stimulated ATPase activity with either free Rad51 or Rad51 in a nucleo-protein complex with single-stranded DNA [data not shown].) In the absence of DNA, however, Rad51 does not appear to stimulate the ATPase activity of wild-type or mutant Rad54 proteins (Fig. 4B). (With wild-type Rad54 in the absence of DNA, there is a suggestive increase in the level of ATPase activity upon addition of Rad51. Analysis of the results revealed, however, that this difference is not statistically significant.) The ΔN-50 protein has a DNA-stimulated ATPase activity that is further enhanced by Rad51, and the DNA + Rad51-stimulated activity of ΔN-50 is about 25% of that of wild-type Rad54. The Δ2–75 protein has a DNA-stimulated ATPase activity that is comparable with that of wild-type Rad54, but the DNA-stimulated ATPase activities of the Δ2–75 and Δ2–100 proteins are not significantly enhanced by Rad51. The impaired Rad51 enhancement of the DNA-stimulated ATPase activities of the Δ2–75 and Δ2–100 proteins correlates with the reduction of Rad51 stimulation of chromatin remodeling by Δ2–75 and Δ2–100 (Fig. 2A). The Δ2–125, Δ2–151, and Δ673–784 proteins are essentially inactive for ATPase activity. The Δ747–784 protein has an ATPase activity that is similar to that of wild-type Rad54.

The N-terminal Nine Amino Acid Residues of Rad54 Are Critical for Chromatin Remodeling and Strand Pairing—The higher chromatin remodeling activities of the Δ2–75 and Δ2–100 proteins relative to the Δ2–50 protein (Fig. 2A) and the insensitivity of the DNA-stimulated ATPase activities of the Δ2–75 and Δ2–100 proteins to enhancement by Rad51 (Fig. 4) prompted us to examine the N-terminal region of Rad54 in greater detail. Perhaps the most notable feature of this region is that the N-terminal nine amino acid residues of human, mouse, and Drosophila Rad54 are identical (Fig. 5A). To test whether these nine residues are important for Rad54 function, we synthesized and purified a mutant Rad54 protein, termed Rad54Δ2–9, in which the conserved N-terminal motif is deleted (Fig. 5B). The DNA + Rad51-stimulated ATPase activity of Δ2–9 protein is about 40% of that of wild-type Rad54 (Fig. 5C). As seen with wild-type Rad54, the ATPase activity of Δ2–9 is responsive to enhancement by Rad51 in the presence but not in the absence of DNA (Fig. 5C). In contrast, the Δ2–9 protein exhibits no detectable activity for either chromatin remodeling (Fig. 5D) or homologous strand pairing in DNA or chromatin (Fig. 5E).

These results reveal the importance of the N-terminal motif of Drosophila Rad54 in chromatin remodeling and strand pairing. In addition, the Δ2–9 protein has substantial ATPase activity, and thus, the deletion of the conserved N-terminal motif results in an uncoupling of the ATPase activity from the chromatin remodeling and strand pairing activities. The stimulation of the ATPase activity of Δ2–9 by DNA indicates that it can interact with DNA, and the enhancement of the DNA-stimulated ATPase activity of Δ2–9 by Rad51 further suggests that it can interact productively with Rad51 in the presence of DNA. The properties of the Δ2–50 protein are nearly identical to those of the Δ2–9 protein. Thus it seems likely that the properties of the Δ2–50 protein are mostly caused by the loss of amino acid residues 2–9. Upon deletion of 75–100 N-terminal residues of Rad54 (Δ2–75 and Δ2–100), there is a partial recovery of chromatin remodeling activity (Fig. 2) and, to a lesser extent, strand pairing activity (Fig. 3A), and interactions with Rad51 appear to decrease (Figs. 2 and 4). The deletion of residues 51–75 also results in an essentially complete restoration of DNA-stimulated ATPase activity that is insensitive to enhancement by Rad51. Then, upon further deletion of the N terminus of Rad54 (Δ2–125 and Δ2–151), there is a loss of ATPase activity (Fig. 4). Thus, these findings suggest that mutation of the conserved N-terminal motif of Rad54 results in the unmasking of an inhibitory region that blocks chromatin remodeling and strand pairing and partially inhibits ATP hydrolysis but does not affect interactions with DNA or Rad51.

Chromatin Remodeling and D Loop Formation Require the ATPase Activity of Drosophila Rad54—Because the Δ2–9 protein is active for ATPase activity and inactive for chromatin remodeling and strand pairing, we tested whether the ATPase activity...
of Drosophila. Rad54 is important for chromatin remodeling and strand pairing. It has been shown that ATPase-defective mutant versions of yeast Rad54 are inactive for D loop formation in strand pairing assays with either naked supercoiled DNA or salt dialysis chromatin as described in the legend to Fig. 3. Wild-type Rad54 protein or the deletion mutant Rad54Δ2–9 were used – Wild-type Rad54 protein or the deletion mutant Rad54Δ2–9/H9004 acid residues of Rad54 nor Rad51 was added. The black dots in Fig. 2, except that the final concentration of Rad54 proteins was 46 nM. Restriction enzyme accessibility assays were performed as described in the legend to Fig. 4. The rate of ATP hydrolysis was normalized to that observed with wild-type Rad54 in the presence of DNA and Rad51. The error bars indicate standard deviations. The final concentrations of the components were as follows: Rad54 proteins, 23 nM; Rad51, 100 nM; supercoiled plasmid DNA (pU6LNS), 2 nM. The error bars indicate the standard deviations. Coomassie Brilliant Blue R-250. The Rad54 protein, as detected by Coomassie staining or by Western blot, appears as a doublet, as is sometimes seen with full-length Drosophila Rad54 (cf. Fig. 1B). C, the DNA + Rad51-stimulated ATPase activity of Rad54Δ2–9 is ~40% of that of wild-type Rad54. ATPase assays were performed as described in the legend to Fig. 4. The rate of ATP hydrolysis was normalized to that observed with wild-type Rad54 in the presence of DNA and Rad51. The error bars indicate standard deviations. The final concentrations of the components were as follows: Rad54 proteins, 23 nM; Rad51, 100 nM; supercoiled plasmid DNA (pU6LNS), 2 nM. D, the N-terminal nine amino acid residues of Rad54 are important for chromatin remodeling. Restriction enzyme accessibility assays were performed with Rad51 and Rad54 proteins as described in the legend to Fig. 2, except that the final concentration of Rad54 proteins was 46 nM. The black dots denote reference chromatin samples to which neither Rad54 nor Rad51 was added. E, deletion of the N-terminal nine amino acid residues of Drosophila Rad54 results in a loss of D loop formation. Wild-type Rad54 protein or the deletion mutant Rad54Δ2–9 were used in strand pairing assays with either naked supercoiled DNA or salt dialysis chromatin as described in the legend to Fig. 3A. chromatin (27) and nucleosome remodeling (28). However, yeast Rad54 does not appear to possess the N-terminal motif that is present in the Drosophila and mammalian Rad54 proteins, and thus, it was possible that there would be some differences in the properties of the yeast and Drosophila proteins.

In these experiments, we mutated lysine residue 191 (Lys-191) of Drosophila Rad54 to either arginine or alanine. Rad54 Lys-191 is a residue in the putative nucleotide binding motif and is analogous to yeast Rad54 residue Lys-341, which is important for strand pairing catalyzed by Rad51 and Rad54 (22, 27) and nucleosome remodeling (28). Thus, Drosophila Rad54(K191R) and Rad54(K191A) proteins were synthesized and purified (Fig. 6A). In ATPase assays, Rad54(K191R) exhibited partial activity, whereas Rad54(K191A) was found to be essentially inactive (Fig. 6B). Next, we carried out chromatin remodeling assays with the mutant proteins and observed that Rad54(K191R) possesses low but detectable activity, whereas
Rad54(K191A) is inactive (Fig. 6C). We then performed D loop assays and found that both mutant proteins were essentially inactive with either naked DNA or chromatin substrates (Fig. 6D and E).

Thus, the ATPase activity is important for chromatin remodeling and strand pairing by *Drosophila* Rad54. These results are generally consistent with those obtained with the analogous mutant versions of yeast Rad54. One slight difference is that we observed partial chromatin remodeling activity with the *Drosophila* K191R mutant protein, whereas the yeast K341R mutant protein does not exhibit any detectable remodeling activity (28). The difference may be, however, a matter of the sensitivity of the assays rather than a significant distinction in the intrinsic properties of the proteins.

**DISCUSSION**

In this work, we examined the effect of subregions of *Drosophila* Rad54 upon ATPase activity, chromatin remodeling, and D loop formation. We identified regions that have both positive and negative effects on chromatin remodeling and D loop formation. In addition, an N-terminal nine-amino acid motif, which is identical in Rad54 in *Drosophila*, mice, and humans, is important for the function of the protein.

It is interesting to consider how Rad51 might affect the activities of Rad54. From previous work, it is known that Rad54 interacts with Rad51 (21, 32–34) as well as a Rad51 single-stranded DNA nucleoprotein filament (35). In the presence of DNA, Rad51 enhances the ATPase activity of Rad54 (Fig. 4). This ability of Rad51 to enhance the DNA-stimulated ATPase activity of Rad54 reflects a productive interaction between Rad51 and Rad54. Thus, by comparison of the ratio of the rates of ATP hydrolysis by Rad54+Rad51+DNA versus Rad54+DNA (Figs. 4B and 5C), we observed that Rad51 enhances the DNA-stimulated ATPase activity of the wild-type, Δ2–9, and Δ2–50 proteins but has a reduced or negligible effect on the Δ2–75 and Δ2–100 proteins (Fig. 4). Also, Rad51 enhances chromatin remodeling by the wild-type protein but has a reduced or negligible effect on remodeling by the Δ2–75 or Δ2–100 proteins (Fig. 2A). These results suggest that a subregion of Rad54 that includes residues 50–75 is important for the stimulation of Rad54 activities by Rad51.

We also found that the chromatin remodeling activity of Rad54 is lost upon deletion of the N-terminal 9 or 50 residues (with the Δ2–9 and Δ2–50 proteins) but is then partially regained upon the further removal of another 25–50 residues (with the Δ2–75 and Δ2–100 proteins). In addition, the chromatin remodeling activity does not correlate with the ATPase activity; the Δ2–9 protein is inactive for chromatin remodeling but has substantial ATPase activity. These findings suggest that deletion of a small region of the N terminus of Rad54 results in the masking of a motif that inhibits chromatin remodeling activity, and then, upon further deletion to residue 75, the inhibitory motif is removed, and the chromatin remodeling activity is partially restored.

In the analysis of the N-terminal deletion series, it is notable that chromatin remodeling activity is regained as the responsiveness of Rad54 to Rad51 is lost. In fact, Rad51 might stimulate chromatin remodeling, at least in part, by counteracting autoinhibition of remodeling by Rad54. In this hypothetical model, the conserved N-terminal nine amino acid residues of Rad54 are required for Rad51 to act as an anti-inhibitor. In this manner, deletion of the N-terminal motif of Rad54 results in constitutive autoinhibition of chromatin remodeling (as seen in Fig. 5). Then, deletion of the Rad54 autoinhibitory region, which appears to be in the inhibitory residues 50 and 75, results in a partial restoration of remodeling activity that is not enhanced by Rad51 (as seen in Fig. 2).

From the chromatin perspective, Rad54 is an interesting remodeling protein because it is regulated by Rad51. It is our hope that continued analyses of Rad54 will provide new insights into not only homologous recombination but also the basic mechanisms by which ATP-utilizing factors can alter chromatin structure.

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