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Promotion and Regulation of Homologous Recombination by DNA Helicases

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Abstract

In eukaryotes, homologous recombination (HR) provides an important means to eliminate DNA double-stranded breaks and other chromosomal lesions. Accordingly, failure in HR leads to genomic instability and a predisposition to various cancer types. While HR is clearly beneficial for genome maintenance, inappropriate or untimely events can be harmful. For this reason, HR must be tightly regulated. Several DNA helicases contribute to HR regulation, by way of mechanisms that are conserved from yeast to humans. Mutations in several HR-specific helicases e.g. BLM and RECQ5, are either associated with cancer-prone human syndromes or engender the cancer phenotype in animal models. Therefore, delineating the role of DNA helicases in HR regulation has direct relevance to cancer etiology. Genetic, cytological, biochemical, and other analyses have shown that DNA helicases participate in early or late stages of HR, to disrupt nucleoprotein filaments that harbor the Rad51 recombinase or dissociate the D-loop intermediate made by Rad51, or to prevent undesirable events and/or minimize potentially deleterious crossover products. Moreover, the ensemble that harbors BLM and topoisomerase III α can dissolve the double Holliday junction, a complex DNA intermediate generated during HR, to produce non-crossover products. These regulatory pathways function in parallel to promote the usage of the genome-preserving synthesis dependent strand annealing HR pathway or otherwise suppress crossover formation.

Keywords

helicase; homologous recombination; DNA repair; Rad51

1. Introduction

DNA double strand breaks (DSBs) occur frequently in cells as the result of replication fork collapse, endogenous chromosome damage, or upon exposure to ionizing radiation and mutagenic chemicals. Unrepaired or misrepaired DSBs result in genomic instability and genetic alterations which can lead to cell death or transformation [1]. Cells have developed two distinct mechanisms for the repair of gratuitous DSBs. The non-homologous end joining (NHEJ) pathway is particularly important during the G1 and early S phases of the cell-cycle, and repair by this pathway usually involves only a limited amount of DNA end processing. Since homologous recombination (HR) typically utilizes the intact sister chromatid to guide the repair process, it is active mostly during the S and G2 phases of the cell cycle. HR is dependent upon extensive processing of the DSB ends in a manner that yields 3' ssDNA tails (see below). These DSB repair pathways also differ in the extent to which they are conservative. NHEJ often entails the gain or loss of nucleotides and is thus an error-prone pathway. Whereas,

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especially when the sister chromatid is used as the information donor, HR is largely an error-free means of repair. Herein, we focus on the helicases that regulate HR to ensure that the desirable outcome of genome preservation is achieved.

After DSB formation, nucleolytic processing of the ends results in a pair of 3' ssDNA tails, which attract the recombinase protein Rad51, leading to the assembly of an extended, right-handed helical Rad51 filament, commonly referred to as the presynaptic filament [2] (Fig. 1). With the aid of one of several accessory factors, such as the Swi2/Snf2-related DNA motor protein Rad54 [3], the presynaptic filament conducts a search for a homologous DNA sequence, then invades the latter to form a DNA joint called the displacement loop, or D-loop. The D-loop can be resolved by one of several means, with different consequences. In the canonical double-strand break repair (DSBR) pathway, DNA synthesis initiated from the primer terminus of the D-loop serves to enlarge the structure, allowing capture of the second end of the break, resulting in a DNA intermediate that harbors a double Holliday junction (dHJ) (Fig. 2A). The dHJ is cleaved by a specialized endonuclease called HJ resolvase, to yield a mixture of crossover and non-crossover products (Fig. 2A, [4] reviewed in [2,5]). Alternatively, the synthesis-dependent strand annealing (SDSA) pathway, through the employment of a specialized DNA helicase, resolves the D-loop structure to generate exclusively non-crossover recombinants (Fig. 2B). In the third pathway, the dHJ intermediate is "dissolved" via the combined action of a DNA helicase and topoisomerase to yield exclusively non-crossover recombinants (Fig. 2C). Since crossover HR is prone to producing chromosome aberrations, such as arm translocations, mitotic cells mainly employ the latter two non-crossover pathways in processing HR intermediates. The DNA helicases and helicase complexes that function to regulate HR are listed in Table 1, and assays for the salient features germane for understanding their mechanism of action are described below.

2. Determination of helicase activity

DNA helicases are motor proteins that use the energy derived from ATP hydrolysis to translocate on DNA, ssDNA in particular. The majority of DNA helicases are able to separate the strands in dsDNA, some are capable of dissociating specific DNA structures, and yet a selected few are endowed with the ability to remove proteins from either ssDNA or dsDNA. In general, all of these functions are coupled to ATP hydrolysis. The studies of DNA helicases typically start with an examination of their ability to unwind duplex DNA or DNA structures, and the results can provide important clues as to their biological function.

2.1 Generation of DNA helicase substrates

A variety of DNA substrates for examining helicase activity can be conveniently generated from oligonucleotides. The most basic substrate consists of radiolabeled DNA that harbors a duplex region and an adjoining 3' or 5' ssDNA tail. More intricate structures that resemble HR intermediates, such as the D-loop and Holliday junction, can also be easily prepared. As a general practice, a selected oligonucleotide is 5' end-labeled with [γ - 32 P] ATP using T4 polynucleotide kinase. The unincorporated nucleotides are removed by passage through a Biospin column (Bio-Rad). The DNA substrates are constructed by hybridizing equimolar amounts of the radiolabeled oligonucleotide with its companion, unlabelled oligonucleotide (s). Hybridization of the constituent oligonucleotides is conducted by heating equimolar amounts of the oligonucleotides at 95°C for 10 min in buffer H (50 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 100 mM NaCl), followed by slow cooling to room temperature over the course of several hours. Hybridized DNA substrates are separated from un-annealed oligonucleotides in 10% nondenaturing polyacrylamide gels run in TAE buffer (40 mM Tris acetate, pH 7.4, 0.5 mM EDTA) at 4°C, to prevent dissociation of the substrate. The substrate has a slower mobility than unannealed oligonucleotides and can be visualized using a UV lamp. The region of the gel that contains the substrate is excised with a razor blade and its recovery is achieved

by electroelution (typically with an electrical current of 30 mA) in dialysis tubing in TAE buffer at 4°C overnight. Substrates that are commonly used in our studies and by colleagues in the field are shown in Fig. 3A.

2.2 Helicase polarity determination

DNA helicases translocate on ssDNA with a unique polarity, i.e. either in a 3' to 5' or 5' to 3' direction. The duplex DNA substrates with a defined ssDNA overhang (Fig. 3B) are useful for checking helicase polarity. Typically, varying amounts of the helicase of interest are incubated at 37°C with the substrates (300 nM nucleotides) in 10 µl of buffer D (30 mM Tris-HCl, pH 7.5, 2.5 mM MgCl₂, 2 mM ATP, 50 mM KCl, 1 mM dithiothreitol, and 100 µg/ml bovine serum albumin). The experimental variables that one would normally test are the pH, KCl or MgCl₂ concentration, and incubation temperature. An aliquot of the reaction mixtures is drawn at various timepoints up to 1 h, and treated with SDS (0.2% final) and proteinase K (0.5 mg/ml) at 37°C for 1 min to digest away the helicase protein. The ³²P-labeled DNA fragment generated as a result of substrate unwinding by the helicase protein is separated from the substrate in a 10% nondenaturing polyacrylamide gel in TAE buffer at 4°C. The gel is then laid on top of a sheet of Whatman DE81 paper (Whatman International Limited), and placed in a gel drier operated at 80°C for 1h, or until completely dry. Analysis is by phosphorimaging or autoradiography. Helicases with a 3'-5' polarity will only unwind the substrate with a 3' ssDNA overhang, and those with the opposite polarity will only be active on the substrate with a 5' overhang. (Fig. 3B).

2.3 Verification of ATP requirement

A simple test for ATP requirement will be to omit ATP from the DNA unwinding reaction, or to substitute ATP with a non-hydrolyzable analogue, such as ATP-γ-S or AMP-PNP. Ideally, mutants of the helicase protein that lack the ability to hydrolyze ATP would be constructed, purified, and tested as well. In our laboratory, when constructing mutants, we have mostly focused on the Walker type A or type B motif needed for ATP binding and hydrolysis as a mutagenesis target [6,7]. For the assay, a varying amount (typically 5 to 100 nM final concentration) of the purified helicase protein is incubated in 10 µl of buffer A (30 mM Tris-HCl, pH 7.5, 2.5 mM MgCl₂, 0.1 to 1.0 mM [γ -³²P] ATP, 1 mM dithiothreitol, and 100 µg/ml bovine serum albumin) without any DNA or with a ssDNA or dsDNA cofactor (25 µM nucleotides or base pairs). The reaction mixtures are incubated at 37°C, and a small aliquot (1 or 2 µl) is removed and mixed with an equal volume of 500 mM EDTA to halt the reaction. The released radiolabeled inorganic phosphate (Pi) is separated from the ATP by thin layer chromatography in polyethyleneimine cellulose sheets [3], and the level of ATP hydrolysis is determined by phosphorimaging or autoradiographic analysis. Again, various experimental variables - the pH, KCl or MgCl₂ concentration, and incubation temperature - are advisable.

3. Regulatory actions of HR by helicases

As discussed earlier, specialized DNA helicases have been found to regulate HR proficiency or outcome at various stages of the HR reaction. Methods for determining at what stage and through what mechanism a given helicase protein functions in HR regulation are described below.

3.1 Testing for disruption of the nucleoprotein filament

As discussed earlier, the Rad51 recombinase protein mediates HR within the context of a helical protein filament assembled on ssDNA. It is perhaps not surprising that this presynaptic filament represents an important target for HR regulation via the action of specialized DNA helicases. Specifically, the Srs2 helicase in budding yeast and the RECQ5 helicase in humans have been shown to possess the ability to disrupt the presynaptic filament, in a manner that is potentiated

by the single-strand DNA binding protein RPA [8-10]. Through their presynaptic filament disruptive action, these helicases effectively prevent undesirable HR events and may also help avoid the capture of the second DNA end during HR (Fig. 2A) to result in the suppression of crossover formation. Biochemical tests have been devised to assay for the anti-recombinase activity of the Srs2/RECQ5 class of anti-recombinases, as below. Moreover, electron microscopy represents a convenient means for visualizing the Rad51 presynaptic filament disruptive function of these DNA helicases [8-11].

3.1.1 Topoisomerase I-linked Rad51 presynaptic filament disruptive assay—The basis of this assay is explained in Fig. 4. Briefly, pre-assembled presynaptic filaments (i.e. Rad51-ssDNA nucleoprotein complexes) are incubated with the helicase protein in question. The presynaptic filament disruptive action of the helicase protein can be enhanced by RPA, which prevents the reformation of the presynaptic filament. Then, topologically relaxed duplex DNA is added to trap the Rad51 molecules freed from ssDNA as a result of anti-recombinase function. Since Rad51 binding induces lengthening of the DNA trap, the level of anti-recombination function can be conveniently monitored as a DNA linking number change upon treatment of the duplex with topoisomerase I (Fig. 4; [9]). The product of this reaction, an underwound DNA species called form U, is resolved from other DNA species by agarose gel electrophoresis and then revealed by ethidium bromide staining. The reaction protocol is detailed below:

1. For the preparation of topologically relaxed DNA, supercoiled ϕ X174 DNA (New England Biolabs; 80 μ g) is generated by its incubation with calf thymus topoisomerase I (Invitrogen; 20 U) for 90 min at 37°C in 500 μ l of buffer containing 50 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 100 mM NaCl, and 1 mM DTT. Reaction mixtures are checked for complete relaxation of the DNA by agarose gel electrophoresis and ethidium bromide staining. Relaxed DNA has a slower mobility than supercoiled dsDNA. The relaxed DNA is purified by use of a PCR clean-up kit (Qiagen) and stored in TE buffer (20 mM Tris-HCl, pH 7.5, with 0.2 mM EDTA). Note that other supercoiled DNA types can be used in place of ϕ X174 DNA.
2. To assemble presynaptic filaments, Rad51 (4 μ M) is incubated at 37°C for 5 min with ϕ X174 circular (+) strand DNA (20 μ M nucleotides) in 10 μ l of buffer R (35 mM Tris-HCl pH 7.4, 2.0 mM ATP, 2.5 mM MgCl₂, 50 mM KCl, 1 mM DTT, containing an ATP-regenerating system consisting of 20 mM creatine phosphate and 20 μ g/ml creatine kinase). We use ϕ X174 ssDNA as it can be purchased from a commercial source (New England Biolabs), but it can substituted by other ssDNA species, such as pBluescript or M13 ssDNA. The plasmids used for the expression of yeast and human Rad51 proteins and the protocols used for their purification have been described [12,13].
3. The helicase protein (typically 20-100 nM) in 0.5 μ l and RPA (1 μ M) in 0.5 μ l are added, followed by a 4-10 min incubation at 37°C.
4. Topologically relaxed ϕ X174 DNA (12.5 μ M nucleotides) in 0.5 μ l and 2.5 U calf thymus topoisomerase I (Invitrogen) are incorporated to complete the reaction, which is incubated for 5-10 min at 37°C.
5. The reaction mixtures are deproteinized by the addition of SDS (0.5%) and proteinase K (0.5 mg/ml) at 37°C for 3 min.
6. The deproteinized reaction mixtures are subject to electrophoresis in 0.9% agarose gels, for 1.5 h at 130 mA, in TAE buffer (40 mM Tris-acetate, pH 7.5, 0.5 mM EDTA).
7. The DNA species are stained with ethidium bromide. If a helicase possesses Rad51 presynaptic filament dissociative activity, then form U DNA will be evident.

8. To verify ATP requirement for helicase's action, one can use helicase defective variants, such as those that harbor a mutation in the Walker type A or type B motif, in the above assay.

3.1.2 Magnetic bead-based Rad51 presynaptic filament disruptive assay—The basis of this assay is explained in Fig. 5. Rad51 presynaptic filaments are assembled and then incubated with the helicase with and without RPA, as before. The Rad51 molecules that have been displaced from the ssDNA are trapped on dsDNA bound to magnetic beads through a biotin-streptavidin linkage (Fig. 5; Roche Applied Science). The magnetic beads are captured using a magnetic separation rack (New England Biolabs), and the supernatant removed. Rad51 is eluted from the bead-bound dsDNA by treatment with SDS. Both the bead and supernatant fractions are then analyzed by denaturing polyacrylamide gel electrophoresis and Coomassie Blue staining to reveal the level of Rad51 presynaptic filament dissociation [9]. The reaction protocol is detailed below:

1. The 600 bp dsDNA used in the experiment is prepared by PCR amplification of pBluescript SK DNA using a biotinylated primer and a non-biotinylated primer, as described [14]. The 5'-biotinylated primer 1 (5'-AAATCAATCTAAAGTATATATGAG-3') and non-biotinylated primer 2 (5'-TGAGTACTCACCAGTCACAG-3') are used. The amplified DNA is deproteinized by use of a PCR-clean up kit (Qiagen), and dissolved in TE buffer. To immobilize the biotinylated dsDNA on streptavidin magnetic beads (Roche Applied Science), 30 µg of the DNA is mixed with 400 µl of beads in 800 µl of buffer A (10 mM Tris-HCl, pH 7.5, 100 mM NaCl, and 1 mM EDTA) for 4 h at 25°C. The beads are washed twice with 800 µl of buffer A containing 1M NaCl and stored in 400 µl of buffer A at 4°C. The beads contained 50 ng of the biotinylated DNA per µl of suspended volume. Note that another biotinylated DNA fragment should suffice.
2. Rad51 presynaptic filaments are assembled in 15 µl of buffer and incubated with the helicase protein, as before.
3. 5 µl of the streptavidin magnetic beads containing the biotinylated dsDNA is added to the reaction, followed by constant mixing for 5 min at 23°C.
4. The beads are captured with the magnetic separation rack (New England Biolabs), and the supernatant reserved.
5. The beads are washed twice with 50 µl buffer, and the bound Rad51 is eluted by treatment with 20 µl 1% SDS.
6. The supernatant, which contains unbound Rad51, and the SDS eluate (10 µl each) are analyzed by SDS polyacrylamide-gel electrophoresis (SDS-PAGE). If the helicase is able to disrupt the nucleoprotein filament, then Rad51 will be trapped on the dsDNA, and thus appear in the bead fraction when the helicase is present.
7. One would want to include helicase defective variants in the above assay to verify the requirement of ATP for the activity of the helicase.

3.2 Testing for a role in SDSA promotion

In the SDSA pathway that generates exclusively non-crossover recombinants, the newly synthesized strand is dissociated from the D-loop to allow its annealing to the other end of the break (Fig. 2B). Several DNA helicases, including BLM, Mph1, FANCM, and RTEL have been found to disrupt the D-loop structure [8,11,15], and, consistent with this attribute, genetic evidence has shown a role of these helicases in the promotion of SDSA type of HR events [11,15-20]. Below, we provide a protocol for testing the D-loop dissociative function of DNA helicases, based on our studies of the Mph1 helicase (Fig. 6; [11]).

3.2.1 Assay to test D-loop dissociation

1. The oligonucleotide 5'- AAATC AATCT AAAGT ATATA TGAGT AAAC TGGTC TGACA GTTAC CAATG CTAA TCAGT GAGGC ACCTA TCTCA GCGAT CTGTC TATTT -3' that is complementary to positions 1932 to 2021 of pBluescript SK+ DNA (Stratagene), is 5' end-labeled with [γ - 32 P] ATP using T4 polynucleotide kinase. The unincorporated nucleotides are removed by passage through a Biospin column (Bio-Rad).
2. The 32 P-labeled oligonucleotide (3 μ M nucleotides) is incubated with Rad51 (1 μ M) for 5 min at 37°C in 12.5 μ l of buffer D (35 mM Tris-HCl, pH 7.5, 50 mM KCl, 2 mM ATP, 2.5 mM MgCl₂, 1 mM DTT, including an ATP-regenerating system consisting of 20 mM creatine phosphate and 20 μ g/ml creatine kinase), to assemble the presynaptic filaments [9].
3. Next, purified Rad54 protein is added in 0.5 μ l to 150 nM, and the reaction incubated for 3 min at 23°C. Rad54 enhances the ability of Rad51 to mediate the D-loop reaction [21] and is therefore critical for the sensitivity of the assay,
4. The reaction is initiated by adding supercoiled pBluescript DNA (50 μ M base pairs) and the helicase protein (typically 20 to 100 nM), followed by a 1-10 min incubation at 30°C.
5. The reaction mixtures are deproteinized as before, and then resolved in a 1% agarose gel in TAE buffer. The gel is dried and subjected to phosphorimaging analysis. The D-loop has a slower mobility than the labeled oligonucleotide.
6. One would want to include helicase defective variants in the above assay to verify the ATP requirement for the activity of the helicase.

The inclusion of a helicase, such as Mph1 [11] capable of unwinding the D-loop, will cause a decrease in the amount of D-loop formation. To ensure that the DNA helicase does not act by dissociating the Rad51 presynaptic filaments, the biochemical assays described earlier for the Srs2 and RECQ5 helicases can be applied, as can electron microscopy to quantify the level of presynaptic filaments [11].

3.3 Double-Holliday junction (dHJ) dissolution assay

As discussed earlier and summarized in Fig. 2A, capture of the second DNA end by the enlarged D-loop leads to the formation of a double Holliday junction, or dHJ, which can be cleaved by a resolvase protein to yield crossover and non-crossover products. Alternatively, the dHJ can be “dissolved” via the concerted action of the BLM helicase together with topoisomerase III α (Topo III α) to yield exclusively non-crossover products (Fig. 2C, [22]). The activity of the BLM-Topo III α pair is enhanced by the BLAP75/Rmi1 protein [23]. Thus far, no other helicase has been shown to catalyze dHJ dissolution in conjunction with TOPIII α , though it is expected that the yeast Sgs1-Top3-Rmi1 complex, the orthologue of BLM-Topo III α -BLAP75, possesses such an attribute as well. The dHJ dissolution reaction protocol is detailed below:

1. For substrate construction [22,24,25], 10 pmol of oligonucleotide 5'- CGTTA GTGGA TTCGT GCGTT TTCGC ACGAA TCCTG ATTAC CATGA GTGGT ACACT GGCTT TTGCC AGTGT ACCTG TCGAG -3' is 5' end-labeled with 32 P. This is annealed to 30 pmol basepairs of oligonucleotide 5'- GTAAT CACCG ACCAA TGCTT TTGCA TTGGT CGGAC TAACG CTCGA CACCA GCGCC ACGTT TTCGT GGCGC TGGAC TCATG -3' as before, followed by ligation to create the dHJ substrate (Fig. 7A [22,25,26]). Annealing of the oligonucleotides is achieved by heating to 95°C for 5 min in buffer containing 50 mM Tris-HCl, pH 7.5 and 10mM MgCl₂, followed by slow cooling to room temperature over the course of several

hours. T4 polynucleotide ligase (10U) is then added, and ligation proceeded at 4 to 8°C for 16 h [24,25].

2. The ligation reaction is mixed with an equal volume of 90% formamide in TAE buffer, heated at 95°C for 5 min to dissociate the unligated substrate, and then resolved in an 8% polyacrylamide gel containing 20% formamide and 8 M urea in TAE buffer at 55°C. The portion of the gel containing the dHJ substrate is identified with the use of a UV lamp and then excised, and the substrate was electroeluted into TAE buffer in dialysis tubing, as before. The purified dHJ substrate is concentrated in a Centricon YM-30 device (Millipore) and then filter dialyzed into TE buffer [22,25,27]. Quality test of the dHJ substrate is by digestion with the *HhaI* and *RsaI* restriction enzymes, as described [22,25]. The procedure for the creation of the dHJ substrate has recently been summarized in detail [25].
3. BLM (typically 2-20 nM) is incubated with Topo III α (100 nM) and BLAP75 (5-30 nM) for 10 min on ice in 11.5 μ l of reaction buffer (50 mM Tris-HCl, pH 7.8, 2 mM ATP, 0.8 mM MgCl₂, 50 mM KCl, 1 mM DTT, 100 μ g/ml bovine serum albumin, and an ATP-regenerating system).
4. The dHJ substrate is added in 1 μ l to 1.2 nM, followed by a 1-10 min incubation at 37°C.
5. The reaction mixtures are deproteinized and mixed with an equal volume of 10 μ l of loading buffer (20 mM Tris-HCl, pH 7.5, 50% glycerol, and 0.08% Orange G) containing 50% urea, incubated at 95°C for 3 min and resolved in an 8% polyacrylamide gel containing 20% formamide and 8 M urea in TAE buffer at 55°C.
6. The gels are dried and examined by phosphorimager analysis.
7. If the dHJ is dissolved by the activity of a helicase, then the radiolabelled oligonucleotide will be observed to migrate separately (and below) the dHJ substrate. Cleavage of the substrate with *HhaI* will result in release of the circular radiolabelled product, which when run independently will serve as a marker for the dissolution product. Digestion of the substrate with *RsaI* results in a linearized labeled 64-mer oligonucleotide, which will run faster through the gel than the closed circular plasmid. A summary showing the expected products of resolvase action versus dissolution products is shown in Fig. 7B. The methods for creating and detecting these substrates have recently been described in detail [25].
8. Ideally, one would include helicase and topoisomerase defective variants in the above assay.

4. Concluding remarks

Through the aid of genetic and biochemical methods, novel DNA helicases that are able to regulate HR frequency and outcome have been identified in different eukaryotic organisms. These DNA helicases are in general conserved in their structure and function, acting in separate pathways to help avoid undesirable HR events or to achieve a high degree of fidelity during the HR reaction [28,29]. The activity of the above-mentioned helicases in HR is summarized in Fig. 8. Importantly, these DNA helicases are critical for genome maintenance, such that their mutational inactivation can lead to chromosome instability and the cancer phenotype. The continuing functional dissection of these DNA helicases and their companion factors therefore has human health relevance.

References

1. Vamvakas S, Vock EH, Lutz WK. Crit Rev Toxicol 1997;27:155–174. [PubMed: 9099517]

2. Sung P, Krejci L, Van Komen S, Sehorn MG. J Biol Chem 2003;278:42729–42732. [PubMed: 12912992]
3. Petukhova G, Stratton S, Sung P. Nature 1998;393:91–94. [PubMed: 9590697]
4. Szostak JW. Methods Enzymol 1983;101:245–252. [PubMed: 6310327]
5. Paques F, Haber JE. Microbiol Mol Biol Rev 1999;63:349–404. [PubMed: 10357855]
6. Story RM, Weber IT, Steitz TA. Nature 1992;355:318–325. [PubMed: 1731246]
7. Walker JE, Eberle A, Gay NJ, Runswick MJ, Saraste M. Biochem Soc Trans 1982;10:203–206. [PubMed: 6216131]
8. Hu Y, Raynard S, Sehorn MG, Lu X, Bussen W, Zheng L, Stark JM, Barnes EL, Chi P, Janscak P, Jasin M, Vogel H, Sung P, Luo G. Genes Dev 2007;21:3073–3084. [PubMed: 18003859]
9. Krejci L, Van Komen S, Li Y, Villemain J, Reddy MS, Klein H, Ellenberger T, Sung P. Nature 2003;423:305–309. [PubMed: 12748644]
10. Veaute X, Jeusset J, Soustelle C, Kowalczykowski SC, Le Cam E, Fabre F. Nature 2003;423:309–312. [PubMed: 12748645]
11. Prakash R, Satory D, Dray E, Papusha A, Scheller J, Kramer W, Krejci L, Klein H, Haber JE, Sung P, Ira G. Genes Dev 2009;23:67–79. [PubMed: 19136626]
12. Sigurdsson S, Trujillo K, Song B, Stratton S, Sung P. J Biol Chem 2001;276:8798–8806. [PubMed: 11124265]
13. Sung P. Genes Dev 1997;11:1111–1121. [PubMed: 9159392]
14. Chi P, Kwon Y, Seong C, Epshtein A, Lam I, Sung P, Klein HL. J Biol Chem 2006;281:26268–26279. [PubMed: 16831867]
15. Barber LJ, Youds JL, Ward JD, McIlwraith MJ, O'Neil NJ, Petalcorin MI, Martin JS, Collis SJ, Cantor SB, Auclair M, Tissenbaum H, West SC, Rose AM, Boulton SJ. Cell 2008;135:261–271. [PubMed: 18957201]
16. Aylon Y, Liefshitz B, Bitan-Banin G, Kupiec M. Mol Cell Biol 2003;23:1403–1417. [PubMed: 12556499]
17. Ira G, Haber JE. Mol Cell Biol 2002;22:6384–6392. [PubMed: 12192038]
18. Ira G, Malkova A, Liberi G, Foiani M, Haber JE. Cell 2003;115:401–411. [PubMed: 14622595]
19. McVey M, Adams M, Staeva-Vieira E, Sekelsky JJ. Genetics 2004;167:699–705. [PubMed: 15238522]
20. Weinert BT, Rio DC. Nucleic Acids Res 2007;35:1367–1376. [PubMed: 17272294]
21. San Filippo J, Sung P, Klein H. Annu Rev Biochem 2008;77:229–257. [PubMed: 18275380]
22. Wu L, Hickson ID. Nature 2003;426:870–874. [PubMed: 14685245]
23. Raynard S, Zhao W, Bussen W, Lu L, Ding YY, Busygina V, Meetei AR, Sung P. J Biol Chem 2008;283:15701–15708. [PubMed: 18390547]
24. Fu TJ, Tse-Dinh YC, Seeman NC. J Mol Biol 1994;236:91–105. [PubMed: 8107128]
25. Bachrati CZ, Hickson ID. Methods Mol Biol 2009;582:91–102. [PubMed: 19763944]
26. Raynard S, Bussen W, Sung P. J Biol Chem 2006;281:13861–13864. [PubMed: 16595695]
27. Bussen W, Raynard S, Busygina V, Singh AK, Sung P. J Biol Chem 2007;282:31484–31492. [PubMed: 17728255]
28. Singh DK, Ahn B, Bohr VA. Biogerontology 2009;10:235–252. [PubMed: 19083132]
29. Sung P, Klein H. Nat Rev Mol Cell Biol 2006;7:739–750. [PubMed: 16926856]
30. Gari K, Decaillet C, Delannoy M, Wu L, Constantinou A. Proc Natl Acad Sci U S A 2008;105:16107–16112. [PubMed: 18843105]
31. Kohzaki M, Hatanaka A, Sonoda E, Yamazoe M, Kikuchi K, Vu Trung N, Szuts D, Sale JE, Shinagawa H, Watanabe M, Takeda S. Mol Cell Biol 2007;27:2812–2820. [PubMed: 17283053]
32. Osman F, Dixon J, Barr AR, Whitby MC. Mol Cell Biol 2005;25:8084–8096. [PubMed: 16135800]
33. Lorenz A, Osman F, Folkte V, Sofueva S, Whitby MC. Mol Cell Biol 2009;29:4742–4756. [PubMed: 19546232]
34. Yu X, Jacobs SA, West SC, Ogawa T, Egelman EH. Proc Natl Acad Sci U S A 2001;98:8419–8424. [PubMed: 11459984]

35. Menetski JP, Bear DG, Kowalczykowski SC. Proc Natl Acad Sci U S A 1990;87:21–25. [PubMed: 2404275]

Fig. 1. The Rad51 nucleoprotein filament. (A) Rad51 exists as a seven subunit ring when in solution, but assembles into a right-handed extended helical filament on ssDNA and dsDNA [34]. Formation of the filament requires ATP binding by Rad51, but not ATP hydrolysis [35]. (B) Electron micrograph of the *S. cerevisiae* Rad51-ssDNA filament. Scale bar is equal to 200 nm.

Fig. 2.

Pathways of DSB repair. After the DSB occurs, 5' ends are resected to yield 3' ssDNA. This serves to recruit repair factors resulting in formation of a nucleoprotein filament which is competent to invade duplex DNA, forming a D-loop structure. (A) After DNA synthesis and D-loop extension, capture of the other side of the break leads to formation of a double Holliday junction (dHJ) structure. This can be resolved to yield either crossover or non-crossover products. (B) In the SDSA pathway, the invading strand of the D-loop is displaced, and anneals to the other side of the break. DNA synthesis and ligation occur, resulting in the formation of non-crossover products. (C) Certain helicases can migrate the dHJ. The resulting hemicatenane structure can be resolved by a topoisomerase, resulting in non-crossover repair products.

Fig. 3. Examples of helicase assay substrates. (A) A variety of helicase substrates can be created, shown here are a few examples of relevant helicase substrates that mimic HR intermediates. Grey numbers indicate the length of the arms of each substrate. (B) Examples of helicase substrates that can be used to determine the directionality of the helicase.

Fig. 4.

Topoisomerase I-linked assay. Reaction scheme for detecting Rad51 presynaptic filament disruption. “Hel.” stands for the helicase of interest. Topo I stands for calf-thymus topoisomerase I. PK stands for proteinase K.

Fig. 5. Bead-based turnover assay. The reaction scheme for detecting Rad51 presynaptic filament disruption. The supernatant and eluate from the bead fractions are analyzed by SDS-PAGE. Hel. stands for the helicase of interest.

Fig. 6. D-loop assay. The reaction scheme for Rad51-mediated D-loop formation. Certain helicases, such as Mph1, can dissociate Rad51-made D-loops.

Fig 7. Double Holliday junction dissolution assay. (A) A schematic representation of the oligonucleotides used in the construction of the dHJ [22]. (B) A schematic detailing the expected products if the dHJ is dissolved by a helicase (panel I) or resolved by a resolvase (panel II). Only the radio-labeled products are shown.

Fig. 8.

Summary figure showing HR regulation by helicases. Helicases such as Srs2 and RecQ5 work at an early stage of HR and disrupt the Rad51 presynaptic filament. Helicases such as Mph1 or FANCM can unwind the invading strand of the D-loop, resulting in the formation of entirely noncrossover products. At later stages helicases can work with topoisomerases to resolve the dHJ structure, to result in noncrossover product formation. An unknown resolvase resolves the dHJ into noncrossover and crossover products.

Table 1

Helicases involved in HR. A listing of the DNA helicases and helicase complexes that function to regulate HR. The yeast and human orthologues are listed.

Yeast	Human	Biochemical Function
Srs2 [9,10]	RecQ5 [8]	Disrupts the Rad51 presynaptic filament
Mph1 [11]	FANCM [30]	Dissociates Rad51-made D-loops
Sgs1-Top3	BLM-TopoIII α [22,23]	Catalyzes dHJ dissolution
	RTEL [14]	Dissociates Rad51-made D-loops

*Even though the WRN helicase has been implicated in HR regulation, its precise role is unknown (reviewed in [28]). Moreover, the *S. pombe* Fbh1 helicase and its mammalian orthologue have been shown to regulate HR [31,32] and this has been speculated to stem from an ability of Fbh1 to disrupt the Rad51 presynaptic filament [33].