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Rad54, the Motor of Homologous Recombination

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Abstract

Homologous recombination (HR) performs crucial functions including DNA repair, segregation of homologous chromosomes, propagation of genetic diversity, and maintenance of telomeres. HR is responsible for the repair of DNA double-strand breaks and DNA interstrand cross-links. The process of HR is initiated at the site of DNA breaks and gaps and involves a search for homologous sequences promoted by Rad51 and auxiliary proteins followed by the subsequent invasion of broken DNA ends into the homologous duplex DNA that then serves as a template for repair. The invasion produces a cross-stranded structure, known as the Holliday junction. Here, we describe the properties of Rad54, an important and versatile HR protein that is evolutionarily conserved in eukaryotes. Rad54 is a motor protein that translocates along dsDNA and performs several important functions in HR. The current review focuses on the recently identified Rad54 activities which contribute to the late phase of HR, especially the branch migration of Holliday junctions.

Keywords

homologous recombination; Holliday junctions; branch migration; Snf2 proteins; RecQ helicases; Rad54 protein; ATP-dependent DNA translocation; DNA double-strand break repair

1. Introduction

HR is critical for maintaining genome stability in all living organisms [1,2]. HR is responsible for the repair of DNA double-strand breaks, the most harmful type of DNA damage, and for accurate chromosome segregation [3–6]. Malfunction of HR causes genome instability leading to cancer and various chromosomal abnormalities such as Down's and other syndromes [7–10]. The salient feature of the HR mechanism is the use of homologous DNA sequences as a template to achieve high fidelity repair of DNA double-strand breaks. The process of HR involves enzymatic processing of the broken dsDNA into a resected DNA duplex with protruding 3'-ssDNA tails (Fig. 1) [11–14]. Following resection, a recombinase, Rad51 (or its prokaryotic homolog RecA), is loaded onto the ssDNA to form a contiguous helical nucleoprotein filament, which searches for an intact homologous dsDNA template [15–19]. Once the homologous sequence is found, the recombinase protein promotes the exchange of DNA strands that leads to formation of joint molecules (D-loops) [20–22]. Further steps along

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this pathway involve the recovery of lost information by DNA polymerase using intact DNA as a template, capture of the second end of broken chromosome, formation and branch migration of Holliday junctions, and resolution of the recombination intermediates to complete the process of DNA double-strand break repair and genetic exchange between homologous DNA molecules [3].

Rad54 protein belongs to the core of the enzymatic machinery that carries out HR in eukaryotes [23]. Rad54 is a motor protein that translocates along dsDNA in an ATP hydrolysis dependent manner [24,25]. It promotes chromatin remodeling and protein displacement from dsDNA. Rad54 interacts physically and functionally with Rad51 protein [26,27] and strongly stimulates the Rad51 DNA strand exchange activity [28]. Rad54 binds Holliday junctions and drives their branch migration [29]. It interacts with Mus81-Eme1(Mms4), a structure-specific endonuclease, stimulating its DNA cleavage activity [30–32]. Thus, it has become increasingly clear that Rad54 is not merely a multifunctional protein; by acting through virtually all steps of HR and interacting with different protein partners Rad54 helps to link the entire HR process together. Several excellent review articles covering various activities and functions of Rad54 have been published recently [2,33–35]. The current paper places an emphasis on the recently identified Rad54 activities, especially the branch migration of Holliday junctions which contributes to the late phase of HR.

2. Functions of Rad54 in Recombination and DNA repair

The *RAD54* gene was discovered in genetic screens for *Saccharomyces cerevisiae* mutants that would confer strong ionizing radiation (IR) sensitivity, but only moderate ultra violet (UV) light sensitivity [36–38]. Based primarily on complementation analysis, the *RAD54* gene was classified as a member of the *RAD52* epistasis group that constitutes the core of the enzymatic machinery responsible for HR and DNA double-strand break repair [23]. Together with *rad51* and *rad52*, *rad54* is one of the three most IR-sensitive single mutants in *S. cerevisiae* [39,40].

After Rad51, Rad54 is the second best conserved protein of the Rad52 group, a generally well conserved group in eukaryotes. For instance, *S. cerevisiae* Rad54 (ScRad54) shares 66% similarity and 48% identity with its human homologue [24,41,42]. Rad54 homologues have been identified in a wide range of eukaryotes including *S. pombe* [43], *Arabidopsis* [44], *Drosophila* [45], chicken [46], zebrafish [24], mouse, humans [41], and, at least, in one archaean genus, *Sulfolobus* [47,48].

In spite of the structural conservation, functions of the Rad52 group members in recombination and DNA repair across different organisms are not fully conserved. In *S.cerevisiae and S. pombe*, *rad51 and rad54* mutants (*rhp51* and *rhp54*, in *S. pombe*) display severe and almost indistinguishable phenotypes with respect to their damage sensitivities (e.g., IR, UV-light), recombination, and chromosome loss in mitotic cells [43,49]. In contrast, the phenotypes of *RAD51*$^{-/-}$ and *RAD54*$^{-/-}$ mutants are markedly different in mice, as well as in chicken cells. Disruption of the *RAD51* gene causes early embryonic lethality of animals; Rad51 knockouts fail to proliferate even in cell culture [50–52]. On the other hand, disruption of the *RAD54* gene results in viable mice. Still there is heightened sensitivity of mouse embryonic stem cells and chicken DT-40 cells to IR and methyl methanesulfonate (MMS) [46,53,54].

In mammals, the *RAD54* gene plays the most important role during early developmental stages. Whereas mouse *RAD54*$^{-/-}$ embryonic stem cells are sensitive to IR, the adult *RAD54*$^{-/-}$ mice are no more IR-sensitive than the wild type animals, because their IR-sensitivity is rescued by the non-homologous end-joining (NHEJ) repair pathway [54]. However, regardless of the developmental stage, *RAD54*$^{-/-}$ mice are hypersensitive to DNA damage caused by interstrand cross-linking agents (ICL), e.g., mitomycin C, which cannot be repaired by the NHEJ [55].

Rad54 plays an important role in reducing the rate of spontaneous chromosome loss. Rad54 inactivation causes increased rates of spontaneous chromosome loss in *S. cerevisiae* diploid cells [56], spontaneous minichrosome loss in *S. pombe* cells [43], and various spontaneous chromosome aberrations in DT-40 cells [57]. Consistent with the role of Rad54 in the maintenance of genome stability, several missense mutations of the human *RAD54* gene were linked to breast, colon and lymphoma cancers [58].

In addition to recombinational DNA repair, Rad54 also plays an important, but less critical, role in other classes of HR events. In *S. cerevisiae*, *rad54* mutations cause a relatively mild defect in mating-type switching, whereas *rad51* and *rad52* mutations completely abolish this recombination event [59]. Also, in *S. cerevisiae*, Rad54 plays a relatively minor role in meiotic recombination, due to the presence of a meiosis-specific homologue, Rdh54/Tid1 [60]. Consequently, the *rad54* mutant forms viable spores, albeit at reduced frequency (25–65%), while the *rad51* mutant is fully defective in viable spore formation [61,62]. However, in the *rad54 rdh54* double mutant, spore viability is reduced to the level of the *rad51* mutant, indicating that the activities collectively possessed by these two homologous proteins are important for meiotic recombination [63]. In mice, Rad54 plays a minor role in meiotic recombination as well, since $RAD54^{-/-}$ mutants are fertile [53]. In higher eukaryotes, meiosis-specific Rad54 homologues remain to be identified. Mammalian Rad54B, which was initially thought to be the Rad54 meiosis-specific homolog, does not appear to have an important function in meiosis; as both $RAD54B^{-/-}$ and $RAD54^{-/-}$ $RAD54B^{-/-}$ mice are fertile [64].

3. Expression of the *RAD54* gene

In vivo, Rad54 is a moderately abundant nuclear protein. It was estimated that there are 7×10^3 and 2.4×10^5 Rad54 molecules per cell in exponentially growing diploid yeast cells and in an unsynchronized population of mouse embryonic stem (ES) cells, respectively [65,66]. In comparison, there are 6.7×10^5 Rad51 molecules in mouse ES cells [66]. In both yeast and mammals, *RAD54* expression shows a cell cycle dependence; *RAD54* transcription increases during late G1 phase [66–69]. The increased level of Rad54 and other recombination proteins in the S and G2 phases corresponds to the important role of HR during DNA replication and may account for the increased resistance of cells to DSB-inducing agents in G2 [70]. In mammals, *RAD54* gene expression is controlled by E2F family transcription factors; during G_o and early G_1 *RAD54* gene expression is repressed by E2F4, and subsequently activated in G1/S phase by E2F1 [71]. Recently, it was found that the level of Rad54 protein during the cell cycle can also be regulated post-translationally. In *S. pombe*, Rhp54 (a Rad54 homologue) is ubiquitylated and degraded in G1 phase [72]. In *S. cerevisiae*, expression of *RAD54* and *RAD51*, but not *RAD52*, is damage inducible [61,73]. In contrast, mammalian cells show no increase in the Rad54 protein level in response to DNA damage. However, treatment of mammalian cells with IR causes Rad54 and other proteins of the Rad52 group to accumulate in foci at sites of DNA damage in the nucleus [74,75].

4. Rad54 protein structure

The *S. cerevisiae RAD54* gene was first cloned and sequenced in Mortimer's laboratory [76, 77]. Sequencing identified Rad54 as a member of the group of proteins involved in translocation along nucleic acids, which are broadly defined as helicases [78,79]. The common feature of these proteins is that they couple nucleotide triphosphate hydrolysis to directional movement along nucleic acids, which may or may not result in strand separation of nucleic acids. Members of this group have a role in almost every cellular process from DNA replication and repair, transcription, translation, splicing, and nuclear transport [80].

Of the six helicase superfamilies [80], Rad54 is a member of Superfamily (SF) 2 [78]. Like all other SF2 and SF1 (Supefamily 1 helicases) proteins, Rad54 possesses seven classical

"signature" motifs: I, Ia, II, III, IV, V, and VI [79] plus several more recently identified motifs, like TxGx (Fig. 2A). These motifs define the two tandem RecA-like folds (RecA-like Lobes 1 and 2), which constitute the "core" or the minimal translocation motor that is responsible for conversion of chemical energy of ATP into protein conformational changes and, consequently, for the mechanical motion [80].

The conservation of these signature motifs can clearly be seen by comparing the structures of Rad54 and the SF1 DNA helicase protein PcrA [24,81]. Based on a series of structures solved with the PcrA protein, a mechanism has been proposed to explain how ATP hydrolysis by helicases is linked to DNA translocation. In the apoenzyme, the lysine residue of motif I occupies the magnesium ion binding site. Binding of the ATP-Mg^{2+} complex displaces the lysine, which in turn, forms a hydrogen bond with the ATP β-phosphate. The threonine of motif I and aspartate of motif II coordinate the magnesium ion. This alignment allows the glutamate of motif II to activate a water molecule that hydrolyzes the bond between the β and γ-phosphates, which is coordinated with a glutamate in motif III, and arginines in motifs IV and VI [81]. The arginine of motif VI, also known as the arginine finger, senses the hydrolysis and transmits the hydrolysis-induced conformation changes from the ATPase active site to the DNA binding site, resulting in DNA translocation. Mutational analysis of PcrA found that mutation of the arginine finger has the most deleterious effect on the helicase activity of any single mutation on this protein [82]. All of these amino acid residues are conserved between PcrA and Rad54, with the only exception being the PcrA arginine of motif IV, which is instead found in motif VI of Rad54 [24].

SF1 and SF2 proteins were segregated based on the sequence divergence of motifs III, IV, V, and VI and spacing between the seven conserved motifs [79,83,84]. Moreover, motif III is located in different regions of SF1 and SF2 proteins.

Specifically, Rad54 belongs to the Snf2 (also known as SWI2/SNF2) protein family of dsDNA-dependent ATPases. In contrast to canonical DNA helicases, none of the Snf2 family members was shown so far to promote strand separation of duplex DNA. Instead, the Snf2 proteins are capable of chromatin remodeling, DNA topology alterations, and displacement of proteins from DNA [85]. All of these activities are presumed to require translocation of the proteins on DNA [86]. Several structural characteristics distinguish the Snf2 proteins from proteins of other SF2 families, e.g., DEAD-box RNA helicases, the RecQ helicase family, the DEAH helicase family, and others [79]. Each RecA-like lobe contains one of the Snf2-specific insertions HD1 and HD2 (helical domains 1 and 2) (Fig. 2B). In the primary sequence, these protrusions are inserted between motifs III and IV, making the spacing between these two motifs much larger in Snf2 proteins as compared to the rest of the SF2 proteins; there are more than 160 residues in this region in Snf2 proteins compared to 38 and 78 for the typical SF2 helicases NS3 and RecG, respectively [87]. Besides these protrusions, the Snf2 family is defined by 15 conserved blocks designated Snf-A to N (block A is also known as the TxGx motif) (Fig. 2B) [88]. The N-terminal domain (NTD) is unique to Rad54 orthologs (Fig. 2C). Proteolysis studies have shown the Rad54 NTD is relatively unstructured [27], and has been shown to contain the Rad51 binding site [26,27,89–91].

X-ray crystallographic structures of large regions of two Rad54 orthologs, from zebrafish and *Sulfolobus solfataricus*, have been solved [24,47,92]. The zebrafish Rad54 structure includes a part of the N-terminal domain, the two RecA-like α/β-domains (lobe 1 and 2) found in all SF1 and SF2 helicases, and the C-terminal domain (CTD) [24]. Each RecA-like lobe contains one insertion HD1 and HD2, respectively, which appear as protrusions [24,47]. The CTD appears to be the only element unique to Rad54 that is present in the truncated structures. The CTD contains a tentative Zn-coordinating motif that may stabilize its entire assembly (Fig. 2D).

A crystal structure of the catalytic domain of the *S. solfataricus* Rad54 homolog bound to a dsDNA substrate has given an insight into the mechanism of Snf2 ATPases translocation along dsDNA. The crystal structure indicates that the Rad54 RecA-like bi-lobal core requires at least 12–15 base pairs to grab DNA. *S. solfataricus* Rad54 binds both strands of the dsDNA through mainly phosphodiester backbone contacts, along the DNA minor groove. However the most extensive protein:DNA contacts are made with one strand of the duplex, indicating the particular importance of one DNA strand in the translocation mechanism. Rad54 binding does not cause strand separation; and the bound dsDNA retains a B-form conformation [47]. Overall, the Rad54 structural data are consistent with the inchworm translocation mechanism, similar to that described for other helicases [80], however important details of this mechanism remain to be clarified.

5. The Rad54 biochemical activities

5.1. Rad54 is a dsDNA-dependent ATPase

The biochemical properties of Rad54, especially the yeast and human orthologues, have been extensively characterized. As expected for an Snf2 protein, Rad54 was found to be an ATPase with a strict dependence on dsDNA [28,93,94]. Both yeast and human Rad54 are robust ATPases that hydrolyze ATP with a k_{cat} ~ 3000–6000 min^{-1} [95]; our unpublished observations); earlier k_{cat} estimates gave lower k_{cat} values (1000–2000 min^{-1}) probably due to suboptimal assay conditions. The $K_M{}^{ATP}$ of the HsRad54 ATPase activity is approximately 400–500 μM ([96]; our unpublished observations).

Rad54 binds ssDNA and dsDNA with similar affinities, although binding to ssDNA does not stimulate ATP hydrolysis [28,94,96]. Compared to linear ssDNA or dsDNA, Rad54 shows significantly higher binding affinity for branched DNA structures, with the strongest binding preference for the partial Holliday junction, in which one of the four arms is represented by ssDNA [29,95]. The affinity of HsRad54 for the PX junction is, approximately, 200-fold higher than that for ssDNA or dsDNA fragments of the same length [29].

5.2. Rad54 translocates along dsDNA

Like other members of the Snf2 family, but unlike canonical DNA helicases, Rad54 does not display DNA strand separation activity, at least, in conventional DNA helicase assays [28]. However, like canonical helicases, Rad54 can translocate on DNA. DNA translocation of Rad54 was first inferred from biochemical data. It was found that Rad54 produces topological changes in DNA structure, introducing equivalent positive and negative supercoiled domains into closed circular dsDNA in an ATP hydrolysis dependent manner [75,93,97,98]. It is known that DNA translocating proteins generate positive supercoils in the DNA ahead and negative supercoils behind the protein [99,100]. Although on covalently closed circular plasmid the positively and negatively supercoiled regions annihilate each other, their mutual annihilation may be prevented by the presence of two (or more) oppositely oriented translocation complexes (Fig. 3). Consistent with Rad54's ability to translocate on DNA, the ATP hydrolysis rate of Rad54 shows an increase with the increase of dsDNA length [101], a property shared with other DNA translocating ATPases [102,103]. Also, as expected for DNA translocating protein, Rad54 can dissociate a DNA triple-helix [104] in the assay that was originally developed to follow the translocation of a type I restriction endonuclease along DNA [105]. Finally, translocation of ScRad54 and ScRdh54 (Tid1), a Rad54 meiosis-specific homologue, along single molecules of dsDNA was visualized directly, demonstrating rapid and highly processive movement at ~300 bp/s for ScRad54 and 80–120 bp/s for ScRdh54 [25,86,106].

The precise structure of the active Rad54 complex that tranlocates on DNA remains unknown. Generation of supercoils in covalently closed DNA by a translocating protein requires a very

large resistance to the rotational motion of the protein around the DNA, which can be achieved, if a translocating enzyme interacts with two or more sites on DNA, e.g., within a multimeric complex formed by the enzyme [97,99]. Indeed, while both yeast and human Rad54 protein exists as a monomer in solution [93] (Mazina and Mazin, unpublished results), Rad54 binding to DNA causes oligomerization of the protein [93]; the cross-linking experiments indicated formation of a dimer, as the principal oligomeric species, and larger oligomers [93,95]. Formation of oligomers by HsRad54 and ScRad54 in complexes with DNA was also detected using atomic force microscopy [97] and electron microscopy [107].

5.3. Rad54 promotes chromatin remodeling

Like other members of the Snf2 family, Rad54 promotes chromatin remodeling, i.e., nucleosome redistribution along DNA, in an ATP hydrolysis dependent manner [104,108–112]. This Rad54 activity likely depends on its dsDNA translocation activity. Chromatin remodeling may also involve specific interactions of Rad54 with histones, as the N-terminus of ScRad54 interacts specifically with the N-terminal tail of histone H3 [111]. It was suggested that chromatin remodeling activity of Rad54 may serve at least two functions in HR. First, Rad54 can start to clear nucleosomes and other chromatin-bound proteins at the break site prior to end-processing. This hypothesis is supported by *in vivo* studies showing an effect of Rad54 on the accessibility of the *HML* donor site for the HO endonuclease [110]. Second, Rad54 might also facilitate the search for homology during synapsis by contending with chromatin structure of the target DNA. Indeed, *in vitro* studies show that Rad54 stimulates DNA strand exchange of Rad51 on chromatin-loaded DNA substrates to a greater extent than on naked DNA substrates, [108]. More work, however, is needed to fully appreciate the role of the Rad54 chromatin remodeling activity *in vivo*.

6. Interactions between Rad54 and Rad51

6.1. Functional and physical interactions between Rad54 and Rad51

Rad54 performs its functions in an association with Rad51, a protein which promotes DNA strand exchange, a basic step of HR. Interactions between these two proteins are extensive and critical to the function of HR in eukaryotes [1,26,113]. It was found that Rad54 and Rad51 interact functionally and physically. The molecular mechanisms of these interactions were the subject of extensive biochemical studies over last decade. In *S. cerevisiae*, over-expression of Rad54 can suppress certain repair phenotypes of *rad51* mutants [26,114]; and the rate and extent of Rad51 recruitment to the HO-induced DSB is significantly reduced in the absence of Rad54 [115]. In mouse ES cells, IR-induced Rad54 foci co-localize with Rad51 foci [75]. Moreover, Rad51 foci formation shows dependence on Rad54. Physical interactions between Rad51 and Rad54 proteins are species-specific and conserved from archea to humans [26,48, 90,91,114]. Experiments with truncated Rad54 mutants show that the Rad54 N-terminal domain is primarily responsible for interactions with Rad51 [27,89]. Importantly, Rad54 physically interacts both with free Rad51 protein and with Rad51, when it is a component of the Rad51-nucleoprotein filament, which is the active species in DNA strand exchange [116].

6.2. Rad54 stimulates DNA strand exchange activity of Rad51

The role of Rad54 in HR was significantly clarified by the key discovery made by Galina Petukhova and Patrick Sung, who found that *S. cerevisiae* Rad54 strongly stimulates the DNA strand exchange activity of Rad51 [28].This stimulation is evolutionarily conserved: arheal, *Drosophila*, and human Rad54 orthologs stimulate DNA strand exchange activity of their cognate Rad51 [48,101,108,117]. It was found that the stimulation depends on the ATPase activity of Rad54 [28,93], indicating that Rad54 translocation on dsDNA plays a role in stimulation of DNA strand exchange activity. The mechanism of DNA strand exchange stimulation involves formation of a complex between Rad54 and the Rad51 nucleoprotein

filament [98,118,119]. In this complex, translocation of potential target DNA by Rad54 is linked to the DNA homology search process promoted by the Rad51-nucleoprotein filament. It was proposed that the translocation activity of Rad54 may both provide a more efficient delivery of dsDNA to the site of the homology search within the filament and cause transient disruption of dsDNA base pairs as a consequence of DNA translocation. Such disruption of normally stable base pairs may make them more available for interaction with the ssDNA bound within the Rad51 nucleoprotein filament.

6.3. Reciprocal stimulation of Rad54 activities by the Rad51 nucleoprotein filament

Formation of a tripartite complex of Rad51-Rad54-ssDNA has significant and synergistic effect on the activities of both Rad51 and Rad54. While Rad54 stimulates the DNA strand exchange activity of Rad51, in its turn, Rad51 stimulates the biochemical activities of Rad54, the dsDNA-dependent ATP hydrolysis and DNA topology modification activity (generation of positive and negative supercoils) [98,117,118]. Also, Rad51 increases the processivity of Rad54 DNA translocation along DNA [101], though the effect of Rad51 on the rate of Rad54 translocation remains to be determined. Finally, Rad51 stimulates chromatin remodeling [104,108,109,111,112] and DNA branch migration activity of Rad54 protein [120].

6.4. Rad54 can both stabilize and disrupt the Rad51 nucleoprotein filament

While ATPase-dependent dsDNA translocation by Rad54 may play a critical role in stimulation of DNA strand exchange, Rad54 employs additional mechanisms of Rad51 stimulation. By forming a complex with the Rad51 nucleoprotein filament, Rad54 stabilizes the filament; Rad54 was shown to increase *i)* the filament resistance against dissociation at elevated salt concentrations, *ii)* the Rad51-dependent protection of dsDNA against cleavages by restriction endonucleases, and *iii)* the Rad51 ability to compete with RPA for ssDNA binding [115, 116]. Thus, Rad54 may play a role of a mediator protein that specifically stimulates Rad51 by increasing its competiveness with RPA for ssDNA binding during the filament formation [121]. The filament stabilization function of Rad54 does not depend on its ATPase activity, the Rad54 ATPase-deficient mutant is still proficient in Rad51 filament stabilization [116]. The fact, that the ATPase activity of Rad54 is required for its biological function [65,93] indicates that the ATPase activity of Rad54 is essential for the steps of HR which occur after filament formation, but it does not rule out the biological significance of the Rad51-DNA filament stabilization by Rad54. Indeed, it was shown that the *S. cerevisiae* strain expressing the ATPase-deficient *rad54K341R*, but not *rad54*Δ allele, is proficient in recruitment of Rad51 to the site of DNA double-strand breaks, consistent with the stabilization of the Rad51-DNA filament by Rad54 [110].

While Rad54 can stabilize the Rad51 filament formed on both ssDNA and dsDNA, paradoxically, Rad54 was also shown to disrupt the Rad51-dsDNA filament [122,123]. The active form of Rad51 is the filament formed on ssDNA. However, by analogy with *E. coli* RecA protein [124], it is likely that Rad51 remains associated with the dsDNA heteroduplex, the product of DNA strand exchange. Therefore, dissociation from dsDNA would help recycling Rad51 for new rounds of recombination.

Co-existence of two apparently opposing Rad54 activities, filament stabilization and dissociation, may seem difficult to reconcile. However, as it was discussed above, Rad54 has two distinct binding sites, the N-terminal Rad51 binding site, and the motor core dsDNA binding site. It is therefore likely that Rad51 displacement from dsDNA and stabilization of the Rad51-DNA filament is mediated by interaction with two different Rad54 sites. The displacement of Rad51 protein from dsDNA occurs at the Rad54 dsDNA binding site during protein translocation along dsDNA, whereas Rad51 filament stabilization is mediated by interaction of Rad51 protein monomers with the Rad54 specialized binding site (Fig. 4). This

model agrees with previous observations that Rad54 can stabilize Rad51 filaments on both ssDNA and dsDNA, but can displace Rad51 only from dsDNA [116,122]. It also agrees with the observation that Rad54 can disrupt only partially saturated Rad51-dsDNA complexes, in which protein-free dsDNA regions are available for initiation of Rad54 translocation [122, 123]. Finally, the model is supported by the fact that both displacement of Rad51 from dsDNA and DNA translocation require ATP hydrolysis by Rad54 (and by Rad51) [123], whereas stabilization of the Rad51-ssDNA filament is ATPase independent [110,116].

7. Branch migration activity of Rad54 protein

7.1. The function(s) of Rad54 at the late stage of homologous recombination

Although Rad54 protein is likely to participate in HR at different stages [2,34], genetic data indicate the significance of the post-synaptic function of Rad54, downstream of Rad51 [1,35, 113]. Critical evidence for the post-synaptic function of Rad54 was obtained from the analysis of *rad54 srs2* double mutants. Thus, while the *rad51 srs2* double mutant is viable, the *rad54 srs2* double mutant is not [125]. Importantly, the *rad51* mutation suppresses the synthetic lethality of *rad54 srs2* [126], indicating that Rad51-generated recombination intermediates are normally resolved by Rad54 and Srs2, but became lethal in the absence of these two proteins.

Post-synapsis comprises the steps after D-loop formation and includes 1) heteroduplex extension and formation of Holliday junctions, 2) priming of DNA synthesis from the invading 3'-OH end, 3) branch migration of Holliday junctions, 4) resolution of Holliday junction intermediates, and 5) sealing of the strands to restore two intact and contiguous duplex DNAs (Fig. 1). Current data indicate that Rad54, a truly versatile protein, may contribute to several post-synaptic steps. *In vitro*, it was shown that ScRad54 stimulates heteroduplex extension during DNA exchange promoted by ScRad51 [127]. *In vitro*, both ScRad54 and its meiosis-specific homologue ScRdh54 catalyze ScRad51 removal from dsDNA in an ATP-dependent fashion [122,128], presumably to provide an access of DNA polymerases to the invading 3'-OH end for DNA synthesis [129]. Chromatin immunoprecipitation (ChIP) analysis that monitored the recruitment of ScRad54 protein to an HO endonuclease-induced DSB demonstrated that ScRad54 is required for the creation of a mature strand invasion structure after synapsis. This result is consistent with a role of Rad54 in stimulation of DNA synthesis [110,130]. Finally, it was recently found that Rad54 binds efficiently to Holliday junctions and promote their branch migration with high efficiency in an ATP hydrolysis dependent manner [29,95,120]. Also, Rad54 stimulates the cleavage activity of Mus81-Eme1(Mms4), a structure-specific endonuclease, that cleaves Holliday junction-like structures [31,32]. In this section we focus on the DNA branch migration activity of Rad54.

7.2. The properties of the Holliday junction

The structure formed in the process of HR, when the strands are exchanged between two different DNA molecules is known as a Holliday junction (also called X-junction or 4-way junction) (Fig. 1). Formation of this structure was first predicted by Robin Holliday [131], whose model for HR provided molecular basis for both gene conversion and crossingover. The remarkable feature of the Holliday junction is the ability to branch migrate along the DNA axis. Branch migration of a Holliday junction is a process in which one DNA strand is progressively exchanged for another (Fig. 5). Branch migration of Holliday junctions may serve several important functions. It may extend or shorten the heteroduplex DNA, formed after DNA strand invasion, affecting the length of conversion tracks and thereby the amount of genetic information transferred between the two DNA molecules [3]. Branch migration may cause dissociation of recombination intermediates and thereby affect the choice between a crossover and non-crossover pathway by which recombination will proceed [132]. An increasing number of genetic and biochemical data indicate that formation and branch

migration of Holliday junctions may also play a critical role in re-starting stalled replication forks during cell recovery after DNA damage[133–137].

The structure of the Holliday junction was analyzed by different methods including comparative gel electrophoresis, molecular modeling, fluorescence resonance energy transfer (FRET) assay, NMR and by X-ray studies [138]. Surprising discoveries on the Holliday junction conformation have been made pertinent to the branch migration mechanism. Thus, early models of HR assumed a parallel orientation of DNA strands of the Holliday junction, because it was expected that the homologous duplexes would be aligned side by side during DNA strand exchange [138,139]. However, the antiparallel conformation of the Holliday junction has been firmly established by X-ray studies [140–142] (Fig. 6A). These results mean that at least one homologous chromosome must locally rotate 180° to keep the global side by side chromosome orientation and to maintain the antiparallel conformation of the Holliday junction at the same time.

The Holliday junction may exist in the stacked or unstacked (extended) conformation. In the stacked conformation, pairs of helical arms stack coaxially to form nearly continuous criss-crossed duplexes (Fig. 6B). In the unstacked one, the Holliday junction forms a four-fold symmetric nearly planar structure (Fig. 6C). X-ray studies show that the conformation of both duplexes composing the Holliday junction is indistinguishable from standard B-form DNA, with the exception of the crossovers at the junction; the Holliday junction has all standard Watson-Crick base pairs [140–142] (Fig. 6B). The compactness of the junction results in a sterical clash of four phosphate groups (within 6.5 Å of each other) in the DNA backbone at the point of the crossover. In the presence of metal ions such as magnesium or calcium (at 100 μM and greater) that neutralize electrostatic repulsions caused by the phosphates, Holliday junctions exist in the stacked conformation (Fig. 6B), whereas in the absence of metal ions, the DNA junction is fully extended (Fig. 6C). The switch between the extended and stacked conformations is important for proteins acting on Holliday junctions. Some of them, such as the RuvA protein that promotes loading of the RuvB branch migration protein on DNA, shows a binding preference to the Holliday junctions in the extended conformation [143], whereas other proteins, such as the hMSH4–hMSH5 complex, preferentially bind the Holliday junction in the stacked conformation [144].

Branch migration occurs while the Holliday junction is in an extended state, and it stops when the molecule adopts a stacked conformation (Fig. 6D) [145]. A single step of branch migration requires the breakage of two base pairs at the point of strand exchange located on diametrically opposite arms, rotation around, and formation of the terminal base pairs on the remaining two arms. Rotation of DNA strands during branch migration promoted by RuvAB was observed in real time using single-molecule techniques [146]. In the absence of bivalent ions, when the Holliday junction exists in the open conformation, preferable for branch migration (Fig. 6D), the rate of spontaneous branch migration is extremely fast with a step time of 300–400 μs [147], which corresponds to a migration rate of 2500–3300 bp/sec. In the presence of magnesium (>100 μM), branch migration is quite slow with an estimated rate of about 3.3–3.7 bp/sec [147]. Because spontaneous branch migration proceeds bi-directionally as a random walk process, under physiological conditions spontaneous branch migration would be inefficient and therefore requires the assistance of specialized enzymes, which increase the overall rate of branch migration by providing directionality and processivity to the process.

7.3. Structural and biochemical properties of branch migration proteins

The Holliday junction is a substrate for structure-specific nucleases which are responsible for its cleavage and formation of crossover products, and for proteins which promote its branch migration [148,149]. In prokaryotes, RuvAB and RecG enzymes promote branch migration of Holliday junctions [148,150–153]. More recently, several eukaryotic branch migration

proteins have been discovered. Known branch migration enzymes belong to the ATPases associated with diverse cellular activities (AAA+) family (RuvB) and the SF2 helicase superfamily (all other proteins) (Table 1). These SF2 proteins include (1) RecG, a member of the RecG family; (2) several members of the RecQ helicase family; (3) FANCM, a member of the DEAH helicase family; and (4) Rad54, a member of the Snf2 family of DNA translocases. In humans, members of the evolutionarily conserved RecQ family include BLM, WRN, RECQ1, and RECQ5 [154–158]. The RecA family recombinases can also promote branch migration of Holliday junctions [159,160], however because of their unique nature and specific branch migration mechanisms these proteins need to be discussed separately.

Despite structural diversity among SF2 and AAA+ branch migration proteins, all of these proteins *i)* are capable of translocation on DNA in an ATPase-dependent manner, and *ii)* show high binding affinity to the Holliday junction (or other cruciform DNA structures that are capable to branch migrate). In the RuvAB protein complex, these two functions are segregated between RuvA and RuvB proteins, which function as the structure-specific DNA-binding protein and the motor protein, respectively [150]. In other branch migration proteins these two functions are performed by the same polypeptide. In RecQ helicases, the structure-specific DNA-binding region is likely associated with the HRDC domain located in the C-terminus of these proteins, separately from the motor domain [161]. In Rad54 and FANCM, the domains responsible for binding to the Holliday junction remain to be identified. In addition to branch migration activity, all branch migration enzymes, except Rad54, possess DNA helicase activity, albeit weak, with either the 3'→5' or the 5'→3' (RuvAB only) polarity.

7.4. Rad54 is an efficient branch migration protein

Like other branch migration proteins, Rad54 can translocate on dsDNA in an ATP hydrolysis dependent manner [25] and bind efficiently to synthetic Holliday junctions [29,95]. HsRad54 shows an approximately 30-fold and 200-fold higher affinity for the X-junction and the partial X-junction (PX-junction), respectively, comparing with dsDNA. The PX-junction structurally resembles one end of a D-loop, the product of DNA strand invasion – an appropriate substrate for DNA branch migration proteins. Despite extensive biochemical studies, the branch migration activity of Rad54 eluded detection for a long time. Conventional DNA substrates (Fig. 7A) that were developed previously for enzymes that combine branch migration and helicase activity are unsuitable for Rad54, which does not have canonical helicase activity [28]. To demonstrate the branch migration activity of Rad54 a fully movable X-junction has been constructed (Fig. 7B) [29,162]. This substrate can branch migrate until complete DNA strand separation without a need for a helicase activity. In addition to 4-stranded branch migration, Rad54 catalyzes 3-stranded branch migration of PX-junction (Fig. 5B). As expected, the branch migration activity of Rad54 depends on ATP hydrolysis [29]. The branch migration activity of Rad54 was also demonstrated by using plasmid-size DNA substrates. It was found that HsRad54 protein efficiently promotes branch migration Holliday junctions through DNA regions of several thousand base pairs (Fig. 8A) [29]. On similar DNA substrates, the rate of branch migration promoted by HsRad54 is comparable with that of RuvAB, a classical branch migration protein from *E.coli* (Fig. 8). Although HsRad54 does not have canonical helicase activity, it can promote branch migration of Holliday junctions through regions heterology up to six bases on short synthetic DNA substrates, or even longer stretches of heterology on plasmid DNA (M. Rossi and A. Mazin, unpublished data) (Table 1).

7.5. Stimulation of the Rad51-dependent heteroduplex extension by Rad54

As described above, Rad54 stimulates the DNA pairing activity of Rad51. Interestingly, it was shown that ScRad54 can specifically stimulate the heteroduplex extension step in the 3-strand DNA exchange reaction promoted by ScRad51 [127]. The mechanism of this stimulation remains controversial, primarily because the mechanism of heteroduplex extension promoted

by Rad51 is not itself well understood. For instance, it was suggested that heteroduplex extension could be viewed as a succession of individual DNA strand invasion steps promoted by the Rad51 nucleoprotein filament [163]. In this case, stimulation of heteroduplex extension may be mechanistically similar to stimulation of Rad51 DNA strand exchange activity, which involves formation of a Rad54-Rad51-DNA complex (see the previous section). Alternatively, Rad54 can stimulate heteroduplex extension due to its inherent DNA 3-strand branch migration activity [29]. The former mechanism is supported by the observation that ScRad54 shows species-specificity in stimulation of the heteroduplex extension, i.e., it stimulates heteroduplex extension promoted by ScRad51, but not by EcRecA, indicating an important role of Rad54-Rad51 protein-protein interactions [127]. Conversely, it was shown that the 3-strand branch migration activity of HsRad54 is inhibited on substrates that are fully covered by HsRad51 [120], arguing against the latter mechnaism. Further work is needed to determine whether the observed stimulation is due to an ability of Rad54 to enhance Rad51 DNA pairing through protein-protein interactions or due to the Rad54 DNA branch migration activity. It is also possible that both mechanisms contribute to the stimulation of heteroduplex extension to a certain degree.

7.6. Rad54 forms a multimeric complex, an active species that promotes branch migration

The smallest branched DNA substrate that efficiently stimulates ATP hydrolysis consists of three DNA arms, two short dsDNA arms, 15 bp each, and an ssDNA arm of 45 nt [95]. This result is consistent with the crystallographic data showing that the *S. solfataricus* Rad54 catalytic core domain can accommodate a 15 bp dsDNA fragment [47]. A stiochiometric titration of HsRad54 with this “minimal” DNA substrate revealed that only two HsRad54 monomers were needed to achieve the maximal rate of ATP hydrolysis [95]. In contrast, a significantly higher HsRad54 stoichiometry, 10±2 Rad54 monomers per junction, was required to achieve the maximal rate of branch migration. Formation of these larger complexes probably involves Rad54 monomer-monomer interactions, because the ATPase activity that depends on DNA binding did not increase in these complexes, compared to dimers. These data indicate that the HsRad54 dimer is responsible for initial specific recognition of the Holliday junction, whereas a large10±2 complex is required for branch migration *per se*. Previously, it was shown that different multimeric forms of RECQ1 are also associated with different enzymatic activities of the protein [164].

7.7. A tentative role of the Rad54 Branch Migration activity in HR

At the initial steps of HR, Rad51 protein promotes formation of joint molecules (D-loops) (Fig. 1). Following D-loop formation, the 3’ end of the invading ssDNA is extended by DNA polymerase, retrieving the information lost at the site of the break. Afterward, the extended joint molecules formed during the initial steps of HR may continue down one of two pathways (Fig. 1) [165,166]. Either they dissociate, leading to rejoining of the broken chromosome through synthesis-dependent strand annealing (SDSA) (Fig. 1A) [165], or they proceed through capture of the second processed DNA end, producing single or double Holliday junctions (Fig. 1B) [167,168], which are later resolved by structure-specific endonucleases [3]. Dissociation of joint molecules (D-loops) after the completion of template-dependent DNA synthesis represents an essential step of DSB repair via the SDSA mechanism [3]. Recent data show that HsRad54 can indeed promote dissociation of D-loops formed by HsRad51 protein owing to its ATP-dependent branch-migration activity (Fig. 9A). Importantly, HsRad54 can dissociate native (non-deproteinized) D-loops containing HsRad51 protein, which mimic *in vivo* recombination intermediates. HsRad54 can also dissociate double D-loops [132], which can be generated by Rad52 through annealing of the second end of broken DNA to the single D-loops [132,169–171]. It was demonstrated that dissociation of double D-loops by HsRad54 occurs through a mechanism, in which D-loop dissociation is directly associated to rejoining of the broken DNA ends without a reannealing step (Fig. 9B) [132]. Double D-loops can lead

to formation of double Holliday junctions and then crossovers, which in somatic cells might lead to detrimental loss of heterozygosity and lead to genome instability. Therefore, double D-loop dissociation by Rad54 may play an important role by promoting HR via non-crossover pathways (Fig. 1); see discussion in [172].

Genetic data support the hypothesis that its D-loop dissociation activity may contribute to the function of Rad54 during the late stages of HR. Specifically, the ability of Rad54 to dissociate D-loops *in vitro* agrees with the reduced length of gene conversion tracts in yeast strains that overexpress Rad54 protein [173].

In vitro, DNA intermediates containing a double Holliday junction can also be dissolved by BLM in a complex with topoisomerase IIIα and BLAP75 [174,175]. This activity implies a possible backup role of BLM during HR in somatic cells. Failure of Rad54 to dissociate D-loops, particularly double D-loops, may lead to generation of double Holliday junctions that can still be resolved by BLM-topoisomerase IIIα-BLAP75. Consistent with this view, *RAD54*$^{-/-}$ *BLM*$^{-/-}$ double knockouts in chicken DT-40 cells increase chromosomal instability to a level that is much greater than that observed for single knockouts of each gene [57].

Thus, DNA branch migration activity of HsRad54 protein may play a part in the double-stranded break repair mechanism postulated by the SDSA model [3] (Fig. 1A). According to this model, the invading ssDNA strand, after its extension by DNA polymerase, is displaced from the template DNA strand and then re-anneals with the second end of the broken DNA molecule. Further steps of double-stranded break repair via the SDSA mechanism lead predominantly to the formation of non-crossover recombinants (Fig. 1A).

7.8. Factors that affect interactions between Rad51 and Rad54

Since interaction between Rad51 and Rad54 plays such a significant role in HR, some of the factors that affect this interaction may have an important regulatory effect on HR. *In vitro*, HsRad51 requires Ca^{2+} to form an active, stabile nucleoprotein filament capable of performing the strand exchange reaction [176]. Under these conditions, HsRad54 stimulates the DNA strand exchange activity of HsRad51 [101]. However, when Ca^{2+} is depleted, e.g., by adding EGTA, the HsRad51-DNA filament adopts an inactive conformation, and HsRad54 then dissociates D-loops structures containing HsRad51 [132]. Thus, the conformational state of the HsRad51-ssDNA filament may regulate the outcome of the filament's interaction with HsRad54.

It was recently discovered that *S. cerevisiae* Hed1 protein may affect interactions between Rad51 and Rad54 [177]. Hed1 specifically disrupts interactions between ScRad51 and ScRad54, thereby downregulating ScRad51 activity. At the same time, Hed1 leaves the interaction between ScRad54 and ScDmc1 intact. It was proposed that preferential association of ScRad54 with ScDmc1 facilitates formation of crossovers, which are required for correct segregation of homologous chromosomes during meiosis.

8. Interaction between Rad54 and Mus81

Rad54 protein was shown to interact physically with Mus81, a structure-specific endonuclease; and this physical interaction between Rad54 and Mus81 proteins is evolutionarily conserved in eukaryotes [30–32,178,179]. Mus81 is a member of the XPF/MUS81 family of nucleases, which share a highly conserved motif (V/IERKX3D) that constitutes an integral part of the endonuclease catalytic site. Mus81 functions as a heterodimer with a non-catalytic partner protein known as Eme1 in fission yeast and humans or as Mms4 in budding yeast and *Drosophila* [180]. Mus81 was first proposed to have a role in the resolution of Holliday junctions in studies on *S. pombe*, where Mus81 was identified as a critical factor for the

production of viable spores, for survival under conditions that lead to stalling of replication fork progression, and for viability in the absence of RecQ helicase, Rqh1 [181,182]. All of the defects in *S. pombe mus81* mutants were rescued by expression of RusA, a bacteriophage resolvase that is highly specific for Holliday junctions [181,183]. Mus81 protein is widely conserved among eukaryotes, including *S. cerevisiae* [30], *S. pombe* [182], *Arabidopsis thaliana* [184], mice [178,185], and humans [186]. The *mus81* and *eme1* (*mms4*) mutants show meiotic defects in *S. cerevisiae* and mice, although not as severe as in *S. pombe* [187,188, 189]. The role of Mus81-Eme1 as a component of an authentic nuclear Holliday junction resolvase remains controversial, because *in vitro* ScMus81 and HsMus81 exhibit very little cleavage activity on Holliday junctions, while delivering robust cleavage of a variety of other branched DNA structures, including D-loops, nicked Holliday junctions, forks and 3'-flaps [190–195]. Recently, another eukaryotic nuclear Holliday junction resolvase Gen1/Yen1, which has a strong preference for intact Holliday junctions, was identified [196]. The specific contributions made by Mus81 and Gen1/Yen1 in resolving Holliday junctions remain to be elucidated.

The functional significance of Rad54 interaction with Mus81-Eme1(Mms4) was confirmed in the experiments demonstrating that HsRad54 and ScRad54 stimulate DNA cleavage activity of HsMus81-Eme1 and ScMus81-Mms, respectively [31,32]. Stimulation of DNA cleavage activity of Mus81-Eme1(Mms4) by Rad54 has important biological implications because it shows a functional link between Rad54, a branch migration protein, and Mus81–Eme1(Mms4), a structure-specific endonuclease. Interestingly, Bloom's syndrome helicase (BLM), another human protein that promotes branch migration of Holliday junctions, can also stimulate the DNA cleavage activity of HsMus81-Eme1 [197]. However, stimulation of HsMus81-Eme1 cleavage activity is specific for HsRad54 and BLM, and is not a generic property of all branch migration proteins, as ScRad54 and RECQ1 do not stimulate HsMus81-Eme1 [31].

The fact that Rad54 shows a high affinity for branched DNA[29] and that Mus81-Eme1(Mms4) stimulation requires species-specific interactions with the cognate Rad54 are consistent with a model in which Rad54 targets Mus81 to DNA junctions via protein-protein interactions [31,32]. This model is also supported by the observation that the stimulation of HsMus81-Eme1(Mms4) activity occurs only if Rad54 is added to DNA-junctions first or, at least, concurrently with Mus81-Eme1(Mms4), indicating that stimulation occurs *in cis* and that formation of Rad54 complexes on DNA-junctions is important for Mus81-Eme1 stimulation. No stimulation of the HsMus81-Eme1 cleavage activity by HsRad54 was observed in the absence of a nucleotide cofactor; ADP and ATPγS support stimulation although at a smaller degree than ATP [31]. The process of branch migration promoted by HsRad54 did not appear to be essential for stimulation of the HsMus81-Eme1 cleavage activity. Stoichiometric titration data indicate that formation of a multimeric hRad54 complex is required for stimulation of HsMus81-Eme1 [31]. Although Rad54 binding to DNA and multimerization occurs in the absence of nucleotide cofactors [93], HsRad54 forms multimers of different size in the presence and in the absence of ATP [97]. It therefore seems possible that in the presence of nucleotide cofactors, HsRad54 may form nucleoprotein complexes that either more efficiently stimulate loading of HsMus81 on DNA junctions or facilitate HsRad54 dissociation from DNA junctions, thereby providing HsMus81 with an access for DNA junction. Surprisingly, the stimulation of ScMus81-Mms4 by ScRad54 does not require a nucleotide cofactor [32]. This result may indicate important differences between the mechanisms of Mus81 stimulation by human and by yeast Rad54 orthologs, which remain to be further explored.

Stimulation of Mus81-Eme1 activity by Rad54 *in vitro* is consistent with genetic data, indicating that Rad54 and Mus81 act in the same DNA repair pathway in response to UV-damage, to replication-blocking chemicals (camptothecin and hydroxyurea) in *S. cerevisiae* [30,32] and in response to ICL-inducing agents (mitomycin C and cisplatin) in mouse ES cells

[178]. However, Mus81 and Rad54 are not obligatory functional partners: whereas Mus81$^{-/-}$ ES cells are hypersensitive to hydroxyurea, Rad54$^{-/-}$ ES cells are not [178]. Also, in S. *pombe* Mus81 and Rad54 likely act in parallel but distinct pathways of DNA repair, as *mus81rad54* double mutants have severely reduced viability [198]. However, poor growth and hypersensitivity of *S. pombe mus81 rad54* double mutants to genotoxic agents is suppressed by loss of upstream proteins, such as Rad51 and Rad55 [199], demonstrating a link between the function of these two proteins and HR. Thus, genetic data together with the results on Rad54 stimulation of Mus81 endonuclease activity suggest that Rad54 and Mus81 may cooperate in processing of Holliday junctions intermediates which are formed during the repair of DSB or stalled replication forks.

9. Conclusions

Rad54 is one of the key proteins of HR that plays a crucial role in maintaining genome integrity. Rad54 has an important function in DNA repair and recombination (Fig. 10). It is a motor protein that translocates on duplex DNA in an ATPase-dependent manner. Through the network of interacting protein partners and unique set of biochemical activities, Rad54 helps to link together early and late steps of HR. At early steps, Rad54 may facilitate HR events by helping Rad51 filament formation on ssDNA through specific protein-protein interactions with Rad51. It may promote HR by strongly stimulating the DNA strand exchange activity of Rad51 protein and catalyzing chromatin remodeling. At later steps, Rad54 may promote an ATP dependent branch migration of Holliday junctions. This is a remarkably complex activity, in which Rad54 binds specifically to the Holliday junction, forms a multimeric active complex, and drives branch migration by coupling chemical energy consumption with mechanical motion. The transition from the Rad54 early function in stimulating Rad51 to branch migration of Holliday junctions remains to be understood. Rad51 stimulates Rad54 branch migration activity likely by promoting loading of Rad54 on the Holliday junction. However, Rad54 branch migration does not require the presence of Rad51, indicating that different types of Rad54-Rad51 nucleoprotein complexes are responsible for DNA strand exchange and branch migration of Holliday junctions. Also, Rad54 physically interacts and stimulates the DNA cleavage activity of the structure-specific endonuclease Mus81-Eme1(Mms4), which may play an important role in resolution of HR intermediates. The mechanism of this stimulation likely involves recruitment of the Mus81 endunuclease to the Holliday junction-like structure by Rad54. The wealth of knowledge accumulated on the Rad54 functions and activities through extensive genetic, biochemical, and structural studies provides a strong momentum for the outgoing research, which will undoubtedly deepen our understanding of the mechanisms of the Rad54 activities and their regulation and will help to better define the function of Rad54 in HR.

Abbreviations

D-loop	displaced loop
SDSA	synthesis dependent strand annealing
DSBR	double-stranded break repair
BLM	Bloom’s syndrome helicase
WRN	Werner’s syndrome helicase
PX-junction	partial X-junction
Ec	*Escherichia coli*
Sc	*Saccharomyces cerevisiae*

Dm	*Drosophila melanogaster*
Hs	*Homo sapiens*

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References

1. Krogh BO, Symington LS. Recombination proteins in yeast. Annu Rev Genet 2004;38:233–271. [PubMed: 15568977]
2. San Filippo J, Sung P, Klein H. Mechanism of eukaryotic homologous recombination. Annu Rev Biochem 2008;77:229–257. [PubMed: 18275380]
3. Pâques F, Haber JE. Multiple pathways of recombination induced by double-strand breaks in Saccharomyces cerevisiae. Microbiology and Molecular Biology Reviews 1999;63:349–404. [PubMed: 10357855]
4. Neale MJ, Keeney S. Clarifying the mechanics of DNA strand exchange in meiotic recombination. Nature 2006;442:153–158. [PubMed: 16838012]
5. Bishop DK. Multiple mechanisms of meiotic recombination. Cell 2006;127:1095–1097. [PubMed: 17174887]
6. Stark JM, Jasin M. Extensive loss of heterozygosity is suppressed during homologous repair of chromosomal breaks. Mol Cell Biol 2003;23:733–743. [PubMed: 12509470]
7. Jasin M. Homologous repair of DNA damage and tumorigenesis: the BRCA connection. Oncogene 2002;21:8981–8993. [PubMed: 12483514]
8. Agarwal S, Tafel AA, Kanaar R. DNA double-strand break repair and chromosome translocations. DNA Repair (Amst) 2006;5:1075–1081. [PubMed: 16798112]
9. Whitby MC. Junctions on the road to cancer. Nat Struct Mol Biol 2004;11:693–695. [PubMed: 15280881]
10. Venkitaraman AR. Cancer susceptibility and the functions of BRCA1 and BRCA2. Cell 2002;108:171–182. [PubMed: 11832208]
11. Gravel S, Chapman JR, Magill C, Jackson SP. DNA helicases Sgs1 and BLM promote DNA double-strand break resection. Genes Dev 2008;22:2767–2772. [PubMed: 18923075]
12. Mimitou EP, Symington LS. Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. Nature 2008;455:770–774. [PubMed: 18806779]
13. Zhu Z, Chung WH, Shim EY, Lee SE, Ira G. Sgs1 helicase and two nucleases Dna2 and Exo1 resect DNA double-strand break ends. Cell 2008;134:981–994. [PubMed: 18805091]
14. Nimonkar AV, Ozsoy AZ, Genschel J, Modrich P, Kowalczykowski SC. Human exonuclease 1 and BLM helicase interact to resect DNA and initiate DNA repair. Proc Natl Acad Sci U S A 2008;105:16906–16911. [PubMed: 18971343]
15. Kowalczykowski SC. Structural biology: snapshots of DNA repair. Nature 2008;453:463–466. [PubMed: 18497811]
16. Chen Z, Yang H, Pavletich NP. Mechanism of homologous recombination from the RecA-ssDNA/dsDNA structures. Nature 2008;453 489-484.
17. Stasiak A, Di Capua E. The helicity of DNA in complexes with recA protein. Nature 1982;299:185–186. [PubMed: 7050731]
18. Ogawa T, Yu X. A. Shinohara and E.H. Egelman Similarity of the yeast RAD51 filament to the bacterial RecA filament. Science 1993;259:1896–1899. [PubMed: 8456314]
19. Mazin AV, Kowalczykowski SC. The specificity of the secondary DNA binding site of RecA protein defines its role in DNA strand exchange. Proceedings of the National Academy of Sciences of the United States of America 1996;93:10673–10678. [PubMed: 8855238]

20. Sung P. Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast RAD51 protein. Science 1994;265:1241–1243. [PubMed: 8066464]
21. Holloman WK, Radding CM. Recombination promoted by superhelical DNA and the recA gene of Escherichia coli. Proc Natl Acad Sci U S A 1976;73:3910–3914. [PubMed: 792876]
22. McEntee K, Weinstock GM, Lehman IR. Initiation of general recombination catalyzed in vitro by the recA protein of Escherichia coli. Proc. Natl. Acad. Sci. USA 1979;76:2615–2619. [PubMed: 379861]
23. Symington LS. Role of RAD52 epistasis group genes in homologous recombination and double-strand break repair. Microbiol Mol Biol Rev 2002;66:630–670. table of contents. [PubMed: 12456786]
24. Thoma NH, Czyzewski BK, Alexeev AA, Mazin AV, Kowalczykowski SC, Pavletich NP. Structure of the SWI2/SNF2 chromatin-remodeling domain of eukaryotic Rad54. Nat Struct Mol Biol 2005;12:350–356. [PubMed: 15806108]
25. Amitani I, Baskin RJ, Kowalczykowski SC. Visualization of Rad54, a chromatin remodeling protein, translocating on single DNA molecules. Mol Cell 2006;23:143–148. [PubMed: 16818238]
26. Clever B, Interthal H, Schmuckli-Maurer J, King J, Sigrist M, Heyer WD. Recombinational repair in yeast: functional interactions between Rad51 and Rad54 proteins. Embo J 1997;16:2535–2544. [PubMed: 9171366]
27. Raschle M, Van Komen S, Chi P, Ellenberger T, Sung P. Multiple interactions with the Rad51 recombinase govern the homologous recombination function of Rad54. J Biol Chem 2004;279:51973–51980. [PubMed: 15465810]
28. Petukhova G, Stratton S, Sung P. Catalysis of homologous DNA pairing by yeast Rad51 and Rad54 proteins. Nature 1998;393:91–94. [PubMed: 9590697]
29. Bugreev DV, Mazina OM, Mazin AV. Rad54 protein promotes branch migration of Holliday junctions. Nature 2006;442:590–593. [PubMed: 16862129]
30. Interthal H, Heyer WD. MUS81 encodes a novel helix-hairpin-helix protein involved in the response to UV- and methylation-induced DNA damage in Saccharomyces cerevisiae. Mol Gen Genet 2000;263:812–827. [PubMed: 10905349]
31. Mazina OM, Mazin AV. Human Rad54 protein stimulates human Mus81-Eme1 endonuclease. Proc Natl Acad Sci U S A 2008;105:18249–18254. [PubMed: 19017809]
32. Matulova P, Marini V, Burgess RC, Sisakova A, Kwon Y, Rothstein R, Sung P, Krejci L. Cooperativity of Mus81.Mms4 with Rad54 in the resolution of recombination and replication intermediates. J Biol Chem 2009;284:7733–7745. [PubMed: 19129197]
33. Tan TL, Kanaar R, Wyman C. Rad54, a Jack of all trades in homologous recombination. DNA Repair (Amst) 2003;2:787–794. [PubMed: 12826279]
34. Heyer WD, Li X, Rolfsmeier M, Zhang XP. Rad54: the Swiss Army knife of homologous recombination? Nucleic Acids Res 2006;34:4115–4125. [PubMed: 16935872]
35. Symington LS, Heyer WD. Some disassembly required: role of DNA translocases in the disruption of recombination intermediates and dead-end complexes. Genes Dev 2006;20:2479–2486. [PubMed: 16980577]
36. Game JC, Mortimer RK. A genetic study of X-ray sensitive mutants in yeast. Mutation Research 1974;24:281. [PubMed: 4606119]
37. Suslova NG. Isolation of Saccharomyces cerevisiae mutants sensitive to ionizing radiation. Radiobiologiya 1969;9:933–936.
38. Zacharov IA, Suslova NG, Fedorova IV. The genetic basis of radiosensetivity in yeast. Genetika 1970;6:76–83.
39. Game JC. DNA double-strand breaks and the RAD50-RAD57 genes in Saccharomyces. Semin Cancer Biol 1993;4:73–83. [PubMed: 8513150]
40. Friedberg, EC.; Walker, GC.; Siede, W. DNA Repair and Mutagenesis. Washington, D.C: ASM Press; 1995.
41. Kanaar R, Troelstra C, Swagemakers SM, Essers J, Smit B, Franssen JH, Pastink A, Bezzubova OY, Buerstedde JM, Clever B, Heyer WD, Hoeijmakers JH. Human and mouse homologs of the Saccharomyces cerevisiae RAD54 DNA repair gene: evidence for functional conservation. Current Biology 1996;6:828–838. [PubMed: 8805304]

42. Petrini JH, Bressan DA, Yao MS. The RAD52 epistasis group in mammalian double strand break repair. Semin Immunol 1997;9:181–188. [PubMed: 9200329]
43. Muris DF, Vreeken K, Carr AM, Murray JM, Smit C, Lohman PH, Pastink A. Isolation of the Schizosaccharomyces pombe RAD54 homologue, rhp54+, a gene involved in the repair of radiation damage and replication fidelity. J Cell Sci 1996;109(Pt 1):73–81. [PubMed: 8834792]
44. Shaked H, Avivi-Ragolsky N, Levy AA. Involvement of the Arabidopsis SWI2/SNF2 chromatin remodeling gene family in DNA damage response and recombination. Genetics 2006;173:985–994. [PubMed: 16547115]
45. Kooistra R, Vreeken K, Zonneveld JB, de Jong A, Eeken JC, Osgood CJ, Buerstedde JM, Lohman PH, Pastink A. The Drosophila melanogaster RAD54 homolog, DmRAD54, is involved in the repair of radiation damage and recombination. Mol Cell Biol 1997;17:6097–6104. [PubMed: 9315669]
46. Bezzubova O, Silbergleit A, Yamaguchi-Iwai Y, Takeda S, Buerstedde JM. Reduced X-ray resistance and homologous recombination frequencies in a RAD54−/− mutant of the chicken DT40 cell line. Cell 1997;89:185–193. [PubMed: 9108474]
47. Durr H, Korner C, Muller M, Hickmann V, Hopfner KP. X-ray structures of the Sulfolobus solfataricus SWI2/SNF2 ATPase core and its complex with DNA. Cell 2005;121:363–373. [PubMed: 15882619]
48. Haseltine CA, Kowalczykowski SC. An archaeal Rad54 protein remodels DNA and stimulates DNA strand exchange by RadA. Nucleic Acids Res. 2009
49. Muris DF, Vreeken K, Carr AM, Broughton BC, Lehmann AR, Lohman PH, Pastink A. Cloning the RAD51 homologue of Schizosaccharomyces pombe. Nucleic Acids Res 1993;21:4586–4591. [PubMed: 8233794]
50. Lim DS, Hasty P. A mutation in mouse rad51 results in an early embryonic lethal that is suppressed by a mutation in p53. Mol Cell Biol 1996;16:7133–7143. [PubMed: 8943369]
51. Tsuzuki T, Fujii Y, Sakumi K, Tominaga Y, Nakao K, Sekiguchi M, Matsushiro A, Yoshimura Y, Morita T. Targeted disruption of the Rad51 gene leads to lethality in embryonic mice. Proc Natl Acad Sci U S A 1996;93:6236–6240. [PubMed: 8692798]
52. Sonoda E, Sasaki MS, Buerstedde JM, Bezzubova O, Shinohara A, Ogawa H, Takata M, Yamaguchi-Iwai Y, Takeda S. Rad51-deficient vertebrate cells accumulate chromosomal breaks prior to cell death. Embo J 1998;17:598–608. [PubMed: 9430650]
53. Essers J, Hendriks RW, Swagemakers SM, Troelstra C, de Wit J, Bootsma D, Hoeijmakers JH, Kanaar R. Disruption of mouse RAD54 reduces ionizing radiation resistance and homologous recombination. Cell 1997;89:195–204. [PubMed: 9108475]
54. Essers J, van Steeg H, de Wit J, Swagemakers SM, Vermeij M, Hoeijmakers JH, Kanaar R. Homologous and non-homologous recombination differentially affect DNA damage repair in mice. Embo J 2000;19:1703–1710. [PubMed: 10747037]
55. De Silva IU, McHugh PJ, Clingen PH, Hartley JA. Defining the roles of nucleotide excision repair and recombination in the repair of DNA interstrand cross-links in mammalian cells. Mol Cell Biol 2000;20:7980–7990. [PubMed: 11027268]
56. Schmuckli-Maurer J, Rolfsmeier M, Nguyen H, Heyer WD. Genome instability in rad54 mutants of Saccharomyces cerevisiae. Nucleic Acids Res 2003;31:1013–1023. [PubMed: 12560498]
57. Wang W, Seki M, Narita Y, Sonoda E, Takeda S, Yamada K, Masuko T, Katada T, Enomoto T. Possible association of BLM in decreasing DNA double strand breaks during DNA replication. Embo J 2000;19:3428–3435. [PubMed: 10880455]
58. Matsuda M, Miyagawa K, Takahashi M, Fukuda T, Kataoka T, Asahara T, Inui H, Watatani M, Yasutomi M, Kamada N, Dohi K, Kamiya K. Mutations in the RAD54 recombination gene in primary cancers. Oncogene 1999;18:3427–3430. [PubMed: 10362365]
59. Schmuckli-Maurer J, Heyer WD. The Saccharomyces cerevisiae RAD54 gene is important but not essential for natural homothallic mating-type switching. Mol Gen Genet 1999;260:551–558. [PubMed: 9928934]
60. Klein HL. RDH54, a RAD54 homologue in Saccharomyces cerevisiae, is required for mitotic diploid-specific recombination and repair and for meiosis. Genetics 1997;147:1533–1543. [PubMed: 9409819]

61. Shinohara A, Ogawa H, Ogawa T. Rad51 protein involved in repair and recombination in S. cerevisiae is a RecA-like protein. Cell 1992;69:457–470. [PubMed: 1581961]
62. Schmuckli-Maurer J, Heyer WD. Meiotic recombination in RAD54 mutants of Saccharomyces cerevisiae. Chromosoma 2000;109:86–93. [PubMed: 10855498]
63. Shinohara M, Shita-Yamaguchi E, Buerstedde JM, Shinagawa H, Ogawa H, Shinohara A. Characterization of the roles of the Saccharomyces cerevisiae RAD54 gene and a homologue of RAD54, RDH54/TID1, in mitosis and meiosis. Genetics 1997;147:1545–1556. [PubMed: 9409820]
64. Wesoly J, Agarwal S, Sigurdsson S, Bussen W, Van Komen S, Qin J, van Steeg H, van Benthem J, Wassenaar E, Baarends WM, Ghazvini M, Tafel AA, Heath H, Galjart N, Essers J, Grootegoed JA, Arnheim N, Bezzubova O, Buerstedde JM, Sung P, Kanaar R. Differential contributions of mammalian Rad54 paralogs to recombination, DNA damage repair, and meiosis. Mol Cell Biol 2006;26:976–989. [PubMed: 16428451]
65. Clever B, Schmuckli-Maurer J, Sigrist M, Glassner BJ, Heyer WD. Specific negative effects resulting from elevated levels of the recombinational repair protein Rad54p in Saccharomyces cerevisiae. Yeast 1999;15:721–740. [PubMed: 10398342]
66. Essers J, Hendriks RW, Wesoly J, Beerens CETM, Smit B, Hoeijmakers JHJ, Wyman C, Dronkert MLG, R K. Analysis of mouse Rad54 expression and its implications for homologous recombination. DNA Repair 2002;1:779–793. [PubMed: 12531026]
67. Cole GM, Schild D, Mortimer RK. Two DNA repair and recombination genes in Saccharomyces cerevisiae, RAD52 and RAD54, are induced during meiosis. Mol Cell Biol 1989;9:3101–3104. [PubMed: 2506437]
68. Cole GM, Mortimer RK. Failure to induce a DNA repair gene, RAD54, in Saccharomyces cerevisiae does not affect DNA repair or recombination phenotypes. Mol Cell Biol 1989;9:3314–3322. [PubMed: 2552291]
69. Johnston LH, Johnson AL. The DNA repair genes RAD54 and UNG1 are cell cycle regulated in budding yeast but MCB promoter elements have no essential role in the DNA damage response. Nucleic Acids Research 1995;23:2147–2152. [PubMed: 7610042]
70. Takata M, Sasaki MS, Sonoda E, Morrison C, Hashimoto M, Utsumi H, Yamaguchi-Iwai Y, Shinohara A, Takeda S. Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. Embo J 1998;17:5497–5508. [PubMed: 9736627]
71. Ren B, Cam H, Takahashi Y, Volkert T, Terragni J, Young RA, Dynlacht BD. E2F integrates cell cycle progression with DNA repair, replication, and G(2)/M checkpoints. Genes Dev 2002;16:245–256. [PubMed: 11799067]
72. Trickey M, Grimaldi M, Yamano H. The anaphase-promoting complex/cyclosome controls repair and recombination by ubiquitylating Rhp54 in fission yeast. Mol Cell Biol 2008;28:3905–3916. [PubMed: 18426916]
73. Cole GM, Schild D, Lovett ST, Mortimer RK. Regulation of RAD54- and RAD52-lacZ gene fusions in Saccharomyces cerevisiae in response to DNA damage. Molecular & Cellular Biology 1987;7:1078–1084. [PubMed: 3550429]
74. Essers J, Houtsmuller AB, van Veelen L, Paulusma C, Nigg AL, Pastink A, Vermeulen W, Hoeijmakers JH, Kanaar R. Nuclear dynamics of RAD52 group homologous recombination proteins in response to DNA damage. Embo J 2002;21:2030–2037. [PubMed: 11953322]
75. Tan TL, Essers J, Citterio E, Swagemakers SM, de Wit J, Benson FE, Hoeijmakers JH, Kanaar R. Mouse Rad54 affects DNA conformation and DNA-damage-induced Rad51 foci formation. Curr Biol 1999;9:325–328. [PubMed: 10209103]
76. Calderon IL, Contopoulou CR, Mortimer RK. Isolation and characterization of yeast DNA repair genes. Current Genetics 1983;7:93–100.
77. Emery HS, Schild D, Kellogg DE, Mortimer RK. Sequence of RAD54, a Saccharomyces cerevisiae gene involved in recombination and repair. Gene 1991;104:103–106. [PubMed: 1916269]
78. Troelstra C, van Gool A, de Wit J, Vermeulen W, Bootsma D, Hoeijmakers JH. ERCC6, a member of a subfamily of putative helicases, is involved in Cockayne's syndrome and preferential repair of active genes. Cell 1992;71:939–953. [PubMed: 1339317]

79. Gorbalenya AE, Koonin EV. Helicases: amino-acid sequence comparisons and structure-function relationships. Curr Opin in Struct Biol 1993;3:419–429.
80. Singleton MR, Dillingham MS, Wigley DB. Structure and mechanism of helicases and nucleic acid translocases. Annu Rev Biochem 2007;76:23–50. [PubMed: 17506634]
81. Velankar SS, Soultanas P, Dillingham MS, Subramanya HS, Wigley DB. Crystal structures of complexes of PcrA DNA helicase with a DNA substrate indicate an inchworm mechanism. Cell 1999;97:75–84. [PubMed: 10199404]
82. Soultanas P, Dillingham MS, Velankar SS, Wigley DB. DNA binding mediates conformational changes and metal ion coordination in the active site of PcrA helicase. J Mol Biol 1999;290:137–148. [PubMed: 10388562]
83. Walker JE, Saraste M, Runswick MJ, Gay NJ. Distantly related sequences in the alpha- and beta-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. EMBO J 1982;1:945–951. [PubMed: 6329717]
84. Bird LE, Subramanya HS, Wigley DB. Helicases: a unifying structural theme? Curr Opin Struct Biol 1998;8:14–18. [PubMed: 9519291]
85. Haushalter KA, Kadonaga JT. Chromatin assembly by DNA-translocating motors. Nat Rev Mol Cell Biol 2003;4:613–620. [PubMed: 12923523]
86. Nimonkar AV, Amitani I, Baskin RJ, Kowalczykowski SC. Single molecule imaging of Tid1/Rdh54, a Rad54 homolog that translocates on duplex DNA and can disrupt joint molecules. J Biol Chem 2007;282:30776–30784. [PubMed: 17704061]
87. Flaus A, Owen-Hughes T. Mechanisms for ATP-dependent chromatin remodelling. Curr Opin Genet Dev 2001;11:148–154. [PubMed: 11250137]
88. Flaus A, Martin DM, Barton GJ, Owen-Hughes T. Identification of multiple distinct Snf2 subfamilies with conserved structural motifs. Nucleic Acids Res 2006;34:2887–2905. [PubMed: 16738128]
89. Alexiadis V, Lusser A, Kadonaga JT. A conserved N-terminal motif in Rad54 is important for chromatin remodeling and homologous strand pairing. J Biol Chem 2004;279:27824–27829. [PubMed: 15105430]
90. Golub EI, Kovalenko OV, Gupta RC, Ward DC, Radding CM. Interaction of human recombination proteins Rad51 and Rad54. Nucleic Acids Res 1997;25:4106–4110. [PubMed: 9321665]
91. Jiang H, Xie Y, Houston P, Stemke-Hale K, Mortensen UH, Rothstein R, Kodadek T. Direct association between the yeast Rad51 and Rad54 recombination proteins. J Biol Chem 1996;271:33181–33186. [PubMed: 8969173]
92. Durr H, Flaus A, Owen-Hughes T, Hopfner KP. Snf2 family ATPases and DExx box helicases: differences and unifying concepts from high-resolution crystal structures. Nucleic Acids Res 2006;34:4160–4167. [PubMed: 16935875]
93. Petukhova G, Van Komen S, Vergano S, Klein H, Sung P. Yeast Rad54 promotes Rad51-dependent homologous DNA pairing via ATP hydrolysis-driven change in DNA double helix conformation. J Biol Chem 1999;274:29453–29462. [PubMed: 10506208]
94. Swagemakers SM, Essers J, de Wit J, Hoeijmakers JH, Kanaar R. The human RAD54 recombinational DNA repair protein is a double-stranded DNA-dependent ATPase. J Biol Chem 1998;273:28292–28297. [PubMed: 9774452]
95. Mazina OM, Rossi MJ, Thomaa NH, Mazin AV. Interactions of human rad54 protein with branched DNA molecules. J Biol Chem 2007;282:21068–21080. [PubMed: 17545145]
96. Tanaka K, Kagawa W, Kinebuchi T, Kurumizaka H, Miyagawa K. Human Rad54B is a double-stranded DNA-dependent ATPase and has biochemical properties different from its structural homolog in yeast, Tid1/Rdh54. Nucleic Acids Res 2002;30:1346–1353. [PubMed: 11884632]
97. Ristic D, Wyman C, Paulusma C, Kanaar R. The architecture of the human Rad54-DNA complex provides evidence for protein translocation along DNA. Proc Natl Acad Sci U S A 2001;98:8454–8460. [PubMed: 11459989]
98. Van Komen S, Petukhova G, Sigurdsson S, Stratton S, Sung P. Superhelicity-driven homologous DNA pairing by yeast recombination factors Rad51 and Rad54. Mol Cell 2000;6:563–572. [PubMed: 11030336]
99. Liu LF, Wang JC. Supercoiling of the DNA template during transcription. Proc Natl Acad Sci U S A 1987;84:7024–7027. [PubMed: 2823250]

100. Wu HY, Shyy SH, Wang JC, Liu LF. Transcription generates positively and negatively supercoiled domains in the template. Cell 1988;53:433–440. [PubMed: 2835168]
101. Mazina OM, Mazin AV. Human Rad54 protein stimulates DNA strand exchange activity of hRad51 protein in the presence of Ca2+ J Biol Chem 2004;279:52042–52051. [PubMed: 15466868]
102. Young MC, Schultz DE, Ring D, von Hippel PH. Kinetic parameters of the translocation of bacteriophage T4 gene 41 protein helicase on single-stranded DNA. J Mol Biol 1994;235:1447–1458. [PubMed: 8107085]
103. Saha A, Wittmeyer J, Cairns BR. Chromatin remodeling by RSC involves ATP-dependent DNA translocation. Genes Dev 2002;16:2120–2134. [PubMed: 12183366]
104. Jaskelioff M, Van Komen S, Krebs JE, Sung P, Peterson CL. Rad54p is a chromatin remodeling enzyme required for heteroduplex DNA joint formation with chromatin. J Biol Chem 2003;278:9212–9218. [PubMed: 12514177]
105. Firman K, Szczelkun MD. Measuring motion on DNA by the type I restriction endonuclease EcoR124I using triplex displacement. Embo J 2000;19:2094–2102. [PubMed: 10790375]
106. Prasad TK, Robertson RB, Visnapuu ML, Chi P, Sung P, Greene EC. A DNA-translocating Snf2 molecular motor: Saccharomyces cerevisiae Rdh54 displays processive translocation and extrudes DNA loops. J Mol Biol 2007;369:940–953. [PubMed: 17467735]
107. Kiianitsa K, Solinger JA, Heyer WD. Terminal association of Rad54 protein with the Rad51-dsDNA filament. Proc Natl Acad Sci U S A 2006;103:9767–9772. [PubMed: 16785421]
108. Alexiadis V, Kadonaga JT. Strand pairing by Rad54 and Rad51 is enhanced by chromatin. Genes Dev 2002;16:2767–2771. [PubMed: 12414729]
109. Alexeev A, Mazin A, Kowalczykowski SC. Rad54 protein possesses chromatin-remodeling activity stimulated by the Rad51-ssDNA nucleoprotein filament. Nat Struct Biol 2003;10:182–186. [PubMed: 12577053]
110. Wolner B, Peterson CL. ATP-dependent and ATP-independent roles for the Rad54 chromatin remodeling enzyme during recombinational repair of a DNA double strand break. J Biol Chem 2005;280:10855–10860. [PubMed: 15653683]
111. Kwon Y, Chi P, Roh DH, Klein H, Sung P. Synergistic action of the Saccharomyces cerevisiae homologous recombination factors Rad54 and Rad51 in chromatin remodeling. DNA Repair (Amst) 2007;6:1496–1506. [PubMed: 17544928]
112. Zhang Z, Fan HY, Goldman JA, Kingston RE. Homology-driven chromatin remodeling by human RAD54. Nat Struct Mol Biol 2007;14:397–405. [PubMed: 17417655]
113. Rattray AJ, Symington LS. Multiple pathways for homologous recombination in Saccharomyces cerevisiae. Genetics 1995;139:45–56. [PubMed: 7705645]
114. Morgan EA, Shah N, Symington LS. The requirement for ATP hydrolysis by Saccharomyces cerevisiae Rad51 is bypassed by mating-type heterozygosity or RAD54 in high copy. Mol Cell Biol 2002;22:6336–6343. [PubMed: 12192033]
115. Wolner B, van Komen S, Sung P, Peterson CL. Recruitment of the recombinational repair machinery to a DNA double-strand break in yeast. Mol Cell 2003;12:221–232. [PubMed: 12887907]
116. Mazin AV, Alexeev AA, Kowalczykowski SC. A novel function of Rad54 protein. Stabilization of the Rad51 nucleoprotein filament. J Biol Chem 2003;278:14029–14036. [PubMed: 12566442]
117. Sigurdsson S, Van Komen S, Petukhova G, Sung P. Homologous DNA pairing by human recombination factors Rad51 and Rad54. J Biol Chem 2002;277:42790–42794. [PubMed: 12205100]
118. Mazin AV, Bornarth CJ, Solinger JA, Heyer WD, Kowalczykowski SC. Rad54 protein is targeted to pairing loci by the Rad51 nucleoprotein filament. Molecular Cell 2000;6:583–592. [PubMed: 11030338]
119. Solinger JA, Lutz G, Sugiyama T, Kowalczykowski SC, Heyer WD. 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 2001;307:1207–1221. [PubMed: 11292336]
120. Rossi MJ, Mazin AV. Rad51 protein stimulates the branch migration activity of Rad54 protein. J Biol Chem 2008;283:24698–24706. [PubMed: 18617519]

121. Sung P, Krejci L, Van Komen S, Sehorn MG. Rad51 recombinase and recombination mediators. J Biol Chem 2003;278:42729–42732. [PubMed: 12912992]
122. Solinger JA, Kiianitsa K, Heyer WD. Rad54, a Swi2/Snf2-like Recombinational Repair Protein, Disassembles Rad51:dsDNA Filaments. Mol Cell 2002;10:1175–1188. [PubMed: 12453424]
123. Li X, Zhang XP, Solinger JA, Kiianitsa K, Yu X, Egelman EH, Heyer WD. Rad51 and Rad54 ATPase activities are both required to modulate Rad51-dsDNA filament dynamics. Nucleic Acids Res 2007;35:4124–4140. [PubMed: 17567608]
124. Pugh BF, Cox MM. recA protein binding to the heteroduplex product of DNA strand exchange. J. Biol. Chem 1987;262:1337–1343. [PubMed: 3543003]
125. Palladino F, Klein HL. Analysis of mitotic and meiotic defects in Saccharomyces cerevisiae SRS2 DNA helicase mutants. Genetics 1992;132:23–37. [PubMed: 1327956]
126. Schild D. Suppression of a new allele of the yeast RAD52 gene by overexpression of RAD51, mutations in srs2 and ccr4, or mating-type heterozygosity. Genetics 1995;140:115–127. [PubMed: 7635279]
127. Solinger JA, Heyer WD. Rad54 protein stimulates the postsynaptic phase of Rad51 protein-mediated DNA strand exchange. Proc Natl Acad Sci U S A 2001;98:8447–8453. [PubMed: 11459988]
128. Chi P, Kwon Y, Seong C, Epshtein A, Lam I, Sung P, Klein HL. Yeast recombination factor Rdh54 functionally interacts with the Rad51 recombinase and catalyzes Rad51 removal from DNA. J Biol Chem 2006;281:26268–26279. [PubMed: 16831867]
129. Li X, Heyer WD. RAD54 controls access to the invading 3'-OH end after RAD51-mediated DNA strand invasion in homologous recombination in Saccharomyces cerevisiae. Nucleic Acids Res 2009;37:638–646. [PubMed: 19074197]
130. Sugawara N, Wang X, Haber JE. In vivo roles of Rad52, Rad54, and Rad55 proteins in Rad51-mediated recombination. Mol Cell 2003;12:209–219. [PubMed: 12887906]
131. Holliday R. A mechanism for gene conversion in fungi. Genet Res 1964;5:282–304.
132. Bugreev DV, Hanaoka F, Mazin AV. Rad54 dissociates homologous recombination intermediates by branch migration. Nat Struct Mol Biol 2007;14:746–753. [PubMed: 17660833]
133. McGlynn P, Lloyd RG, Marians KJ. Formation of Holliday junctions by regression of nascent DNA in intermediates containing stalled replication forks: RecG stimulates regression even when the DNA is negatively supercoiled. Proc Natl Acad Sci U S A 2001;98:8235–8240. [PubMed: 11459958]
134. Postow L, Ullsperger C, Keller RW, Bustamante C, Vologodskii AV, Cozzarelli NR. Positive torsional strain causes the formation of a four-way junction at replication forks. J Biol Chem 2001:2790–2796. [PubMed: 11056156]
135. Michel B, Grompone G, Flores MJ, Bidnenko V. Multiple pathways process stalled replication forks. Proc Natl Acad Sci U S A 2004;101:12783–12788. [PubMed: 15328417]
136. Rothstein R, Michel B, Gangloff S. Replication fork pausing and recombination or "gimme a break". Genes Dev 2000;14:1–10. [PubMed: 10640269]
137. Seigneur M, Bidnenko V, Ehrlich SD, Michel B. RuvAB acts at arrested replication forks. Cell 1998;95:419–430. [PubMed: 9814711]
138. Lilley DM. Structures of helical junctions in nucleic acids. Q Rev Biophys 2000;33:109–159. [PubMed: 11131562]
139. Sha R, Liu F, Seeman NC. Direct evidence for spontaneous branch migration in antiparallel DNA Holliday junctions. Biochemistry 2000;39:11514–11522. [PubMed: 10985798]
140. Nowakowski J, Shim PJ, Prasad GS, Stout CD, Joyce GF. Crystal structure of an 82-nucleotide RNA-DNA complex formed by the 10–23 DNA enzyme. Nat Struct Biol 1999;6:151–156. [PubMed: 10048927]
141. Ortiz-Lombardia M, Gonzalez A, Eritja R, Aymami J, Azorin F, Coll M. Crystal structure of a DNA Holliday junction. Nat Struct Biol 1999;6:913–917. [PubMed: 10504723]
142. Eichman BF, Vargason JM, Mooers BH, Ho PS. The Holliday junction in an inverted repeat DNA sequence: sequence effects on the structure of four-way junctions. Proc Natl Acad Sci U S A 2000;97:3971–3976. [PubMed: 10760268]

143. Ariyoshi M, Nishino T, Iwasaki H, Shinagawa H, Morikawa K. Crystal structure of the holliday junction DNA in complex with a single RuvA tetramer. Proc Natl Acad Sci U S A 2000;97:8257–8262. [PubMed: 10890893]
144. Snowden T, Acharya S, Butz C, Berardini M, Fishel R. hMSH4-hMSH5 recognizes Holliday Junctions and forms a meiosis-specific sliding clamp that embraces homologous chromosomes. Mol Cell 2004;15:437–451. [PubMed: 15304223]
145. Karymov M, Daniel D, Sankey OF, Lyubchenko YL. Holliday junction dynamics and branch migration: single-molecule analysis. Proc Natl Acad Sci U S A 2005;102:8186–8191. [PubMed: 15917329]
146. Han YW, Tani T, Hayashi M, Hishida T, Iwasaki H, Shinagawa H, Harada Y. Direct observation of DNA rotation during branch migration of Holliday junction DNA by Escherichia coli RuvA-RuvB protein complex. Proc Natl Acad Sci U S A 2006;103:11544–11548. [PubMed: 16864792]
147. Panyutin IG, Hsieh P. The kinetics of spontaneous DNA branch migration. Proc Natl Acad Sci U S A 1994;91:2021–2025. [PubMed: 8134343]
148. Liu Y, West SC. Happy Hollidays: 40th anniversary of the Holliday junction. Nat Rev Mol Cell Biol 2004;5:937–944. [PubMed: 15520813]
149. Heyer WD. Recombination: Holliday junction resolution and crossover formation. Curr Biol 2004;14:R56–R58. [PubMed: 14738748]
150. West SC. Processing of recombination intermediates by the RuvABC proteins. Annual Review of Genetics 1997;31:213–244.
151. Sharples GJ, Ingleston SM, Lloyd RG. Holliday junction processing in bacteria: insights from the evolutionary conservation of RuvABC, RecG, and RusA. J Bacteriol 1999;181:5543–5550. [PubMed: 10482492]
152. Lloyd RG, Buckman C. Genetic analysis of the recG locus of Escherichia coli K-12 and of its role in recombination and DNA repair. J. Bacteriol 1991;173:1004–1011. [PubMed: 1846849]
153. Lloyd RG, Sharples GJ. Dissociation of synthetic Holliday junctions by *E. coli* RecG protein. EMBO J 1993;12:17–22. [PubMed: 8428576]
154. Karow JK, Constantinou A, Li JL, West SC, Hickson ID. The Bloom's syndrome gene product promotes branch migration of holliday junctions. Proc Natl Acad Sci U S A 2000;97:6504–6508. [PubMed: 10823897]
155. Constantinou A, Tarsounas M, Karow JK, Brosh RM, Bohr VA, Hickson ID, West SC. Werner's syndrome protein (WRN) migrates Holliday junctions and co-localizes with RPA upon replication arrest. EMBO Rep 2000;1:80–84. [PubMed: 11256630]
156. Kanagaraj R, Saydam N, Garcia PL, Zheng L, Janscak P. Human RECQ5beta helicase promotes strand exchange on synthetic DNA structures resembling a stalled replication fork. Nucleic Acids Res 2006;34:5217–5231. [PubMed: 17003056]
157. Bugreev DV, Brosh RM Jr, Mazin AV. RECQ1 possesses DNA branch migration activity. J Biol Chem 2008;283:20231–20242. [PubMed: 18495662]
158. Wu L, Bachrati CZ, Ou J, Xu C, Yin J, Chang M, Wang W, Li L, Brown GW, Hickson ID. BLAP75/RMI1 promotes the BLM-dependent dissolution of homologous recombination intermediates. Proc Natl Acad Sci U S A 2006;103:4068–4073. [PubMed: 16537486]
159. Murayama Y, Kurokawa Y, Mayanagi K, Iwasaki H. Formation and branch migration of Holliday junctions mediated by eukaryotic recombinases. Nature 2008;451:1018–1021. [PubMed: 18256600]
160. Conley EC, West SC. Homologous pairing and the formation of nascent synaptic intermediates between regions of duplex DNA by RecA protein. Cell 1989;56:987–995. [PubMed: 2647306]
161. Hickson ID. RecQ helicases: caretakers of the genome. Nat Rev Cancer 2003;3:169–178. [PubMed: 12612652]
162. Bugreev DV, Mazina OM, Mazin AV. Analysis of branch migration activities of proteins using synthetic DNA substrates. Nature Protocols (2006) published online. 2006 September; (doi: 2010.1038/nprot.2006.2217).
163. Bianco PR, Tracy RB, Kowalczykowski SC. DNA strand exchange proteins: a biochemical and physical comparison. Front Biosci 1998;3:D570–D603. [PubMed: 9632377]

164. Muzzolini L, Beuron F, Patwardhan A, Popuri V, Cui S, Niccolini B, Rappas M, Freemont PS, Vindigni A. Different quaternary structures of human RECQ1 are associated with its dual enzymatic activity. PLoS Biol 2007;5:e20. [PubMed: 17227144]

165. Allers T, Lichten M. Differential timing and control of noncrossover and crossover recombination during meiosis. Cell 2001;106:47–57. [PubMed: 11461701]

166. Hunter N, Kleckner N. The single-end invasion: an asymmetric intermediate at the double-strand break to double-holliday junction transition of meiotic recombination. Cell 2001;106:59–70. [PubMed: 11461702]

167. Cromie GA, Hyppa RW, Taylor AF, Zakharyevich K, Hunter N, Smith GR. Single Holliday junctions are intermediates of meiotic recombination. Cell 2006;127:1167–1178. [PubMed: 17174892]

168. Schwacha A, Kleckner N. Identification of double Holliday junctions as intermediates in meiotic recombination. Cell 1995;83:783–791. [PubMed: 8521495]

169. Nimonkar AV, Sica RA, Kowalczykowski SC. Rad52 promotes second-end DNA capture in double-stranded break repair to form complement-stabilized joint molecules. Proc Natl Acad Sci U S A 2009;106:3077–3082. [PubMed: 19204284]

170. Sugiyama T, Kantake N, Wu Y, Kowalczykowski SC. Rad52-mediated DNA annealing after Rad51-mediated DNA strand exchange promotes second ssDNA capture. Embo J 2006;25:5539–5548. [PubMed: 17093500]

171. McIlwraith MJ, West SC. DNA repair synthesis facilitates RAD52-mediated second-end capture during DSB repair. Mol Cell 2008;29:510–516. [PubMed: 18313388]

172. Mazina OM, Mazin AV, Nakagawa T, Kolodner RD, Kowalczykowski SC. Saccharomyces cerevisiae Mer3 helicase stimulates 3'-5' heteroduplex extension by Rad51; implications for crossover control in meiotic recombination. Cell 2004;117:47–56. [PubMed: 15066281]

173. Kim PM, Paffett KS, Solinger JA, Heyer WD, Nickoloff JA. Spontaneous and double-strand break-induced recombination, and gene conversion tract lengths, are differentially affected by overexpression of wild-type or ATPase-defective yeast Rad54. Nucleic Acids Res 2002;30:2727–2735. [PubMed: 12087154]

174. Wu L, Hickson ID. The Bloom's syndrome helicase suppresses crossing over during homologous recombination. Nature 2003;426:870–874. [PubMed: 14685245]

175. Raynard S, Bussen W, Sung P. A double Holliday junction dissolvasome comprising BLM, topoisomerase IIIalpha, and BLAP75. J Biol Chem 2006;281:13861–13864. [PubMed: 16595695]

176. Bugreev DV, Mazin AV. Ca2+ activates human homologous recombination protein Rad51 by modulating its ATPase activity. Proc Natl Acad Sci U S A 2004;101:9988–9993. [PubMed: 15226506]

177. Busygina V, Sehorn MG, Shi IY, Tsubouchi H, Roeder GS, Sung P. Hed1 regulates Rad51-mediated recombination via a novel mechanism. Genes Dev 2008;22:786–795. [PubMed: 18347097]

178. Hanada K, Budzowska M, Modesti M, Maas A, Wyman C, Essers J, Kanaar R. The structure-specific endonuclease Mus81-Eme1 promotes conversion of interstrand DNA crosslinks into double-strands breaks. Embo J 2006;25:4921–4932. [PubMed: 17036055]

179. Mimida N, Kitamoto H, Osakabe K, Nakashima M, Ito Y, Heyer WD, Toki S, Ichikawa H. Two Alternatively Spliced Transcripts Generated from OsMUS81, a Rice Homolog of Yeast MUS81, Are Up-Regulated by DNA-Damaging Treatments. Plant Cell Physiol 2007;48:648–654. [PubMed: 17327258]

180. Ciccia A, McDonald N, West SC. Structural and Functional Relationships of the XPF/MUS81 Family of Proteins. Annu Rev Biochem 2008;77:259–287. [PubMed: 18518821]

181. Boddy MN, Gaillard PH, McDonald WH, Shanahan P, Yates JR 3rd, Russell P. Mus81-Eme1 are essential components of a Holliday junction resolvase. Cell 2001;107:537–548. [PubMed: 11719193]

182. Boddy MN, Lopez-Girona A, Shanahan P, Interthal H, Heyer WD, Russell P. Damage tolerance protein Mus81 associates with the FHA1 domain of checkpoint kinase Cds1. Mol Cell Biol 2000;20:8758–8766. [PubMed: 11073977]

183. Blais V, Gao H, Elwell CA, Boddy MN, Gaillard PH, Russell P, McGowan CH. RNA interference inhibition of Mus81 reduces mitotic recombination in human cells. Mol Biol Cell 2004;15:552–562. [PubMed: 14617801]

184. Hartung F, Suer S, Bergmann T, Puchta H. The role of AtMUS81 in DNA repair and its genetic interaction with the helicase AtRecQ4A. Nucleic Acids Res 2006;34:4438–4448. [PubMed: 16945961]

185. McPherson JP, Lemmers B, Chahwan R, Pamidi A, Migon E, Matysiak-Zablocki E, Moynahan ME, Essers J, Hanada K, Poonepalli A, Sanchez-Sweatman O, Khokha R, Kanaar R, Jasin M, Hande MP, Hakem R. Involvement of mammalian Mus81 in genome integrity and tumor suppression. Science 2004;304:1822–1826. [PubMed: 15205536]

186. Chen XB, Melchionna R, Denis CM, Gaillard PH, Blasina A, Van de Weyer I, Boddy MN, Russell P, Vialard J, McGowan CH. Human Mus81-associated endonuclease cleaves Holliday junctions in vitro. Mol Cell 2001;8:1117–1127. [PubMed: 11741546]

187. Osman F, Dixon J, Doe CL, Whitby MC. Generating crossovers by resolution of nicked Holliday junctions: a role for Mus81-Eme1 in meiosis. Mol Cell 2003;12:761–774. [PubMed: 14527420]

188. de los Santos T, Loidl J, Larkin B, Hollingsworth NM. A role for MMS4 in the processing of recombination intermediates during meiosis in Saccharomyces cerevisiae. Genetics 2001;159:1511–1525. [PubMed: 11779793]

189. Holloway JK, Booth J, Edelmann W, McGowan CH, Cohen PE. MUS81 generates a subset of MLH1-MLH3-independent crossovers in mammalian meiosis. PLoS Genet 2008;4:e1000186. [PubMed: 18787696]

190. Kaliraman V, Mullen JR, Fricke WM, Bastin-Shanower SA, Brill SJ. Functional overlap between Sgs1-Top3 and the Mms4-Mus81 endonuclease. Genes Dev 2001;15:2730–2740. [PubMed: 11641278]

191. Doe CL, Ahn JS, Dixon J, Whitby MC. Mus81-Eme1 and Rqh1 involvement in processing stalled and collapsed replication forks. J Biol Chem 2002;277:32753–32759. [PubMed: 12084712]

192. Gaillard PH, Noguchi E, Shanahan P, Russell P. The endogenous Mus81-Eme1 complex resolves Holliday junctions by a nick and counternick mechanism. Mol Cell 2003;12:747–759. [PubMed: 14527419]

193. Whitby MC, Osman F, Dixon J. Cleavage of model replication forks by fission yeast Mus81-Eme1 and budding yeast Mus81-Mms4. J Biol Chem 2003;278:6928–6935. [PubMed: 12473680]

194. Ciccia A, Constantinou A, West SC. Identification and characterization of the human mus81-eme1 endonuclease. J Biol Chem 2003;278:25172–25178. [PubMed: 12721304]

195. Fricke WM, Bastin-Shanower SA, Brill SJ. Substrate specificity of the Saccharomyces cerevisiae Mus81-Mms4 endonuclease. DNA Repair (Amst) 2005;4:243–251. [PubMed: 15590332]

196. Ip SC, Rass U, Blanco MG, Flynn HR, Skehel JM, West SC. Identification of Holliday junction resolvases from humans and yeast. Nature 2008;456:357–361. [PubMed: 19020614]

197. Zhang R, Sengupta S, Yang Q, Linke SP, Yanaihara N, Bradsher J, Blais V, McGowan CH, Harris CC. BLM helicase facilitates Mus81 endonuclease activity in human cells. Cancer Res 2005;65:2526–2531. [PubMed: 15805243]

198. Doe CL, Whitby MC. The involvement of Srs2 in post-replication repair and homologous recombination in fission yeast. Nucleic Acids Res 2004;32:1480–1491. [PubMed: 14993467]

199. Osman F, Whitby MC. Exploring the roles of Mus81-Eme1/Mms4 at perturbed replication forks. DNA Repair (Amst) 2007;6:1004–1017. [PubMed: 17409028]

200. Kim JI, Cox MM, Inman RB. On the role of ATP hydrolysis in RecA protein-mediated DNA strand exchange. II. Four-strand exchanges. J Biol Chem 1992;267:16444–16449. [PubMed: 1644828]

201. Muller B, Burdett I, West SC. Unusual stability of recombination intermediates made by Escherichia coli RecA protein. Embo J 1992;11:2685–2693. [PubMed: 1628627]

202. Marrione PE, Cox MM. Allosteric effects of RuvA protein, ATP, and DNA on RuvB protein-mediated ATP hydrolysis. Biochemistry 1996;35:11228–11238. [PubMed: 8780528]

203. Parsons CA, Tsaneva I, Lloyd RG, West SC. Interaction of Escherichia coli RuvA and RuvB proteins with synthetic Holliday junctions. Proc Natl Acad Sci U S A 1992;89:5452–5456. [PubMed: 1608954]

204. Tsaneva IR, Muller B, West SC. RuvA and RuvB proteins of Escherichia coli exhibit DNA helicase activity in vitro. Proc Natl Acad Sci U S A 1993;90:1315–1319. [PubMed: 8433990]

205. Dawid A, Croquette V, Grigoriev M, Heslot F. Single-molecule study of RuvAB-mediated Holliday-junction migration. Proc Natl Acad Sci U S A 2004;101:11611–11616. [PubMed: 15292508]

206. Amit R, Gileadi O, Stavans J. Direct observation of RuvAB-catalyzed branch migration of single Holliday junctions. Proc Natl Acad Sci U S A 2004;101:11605–11610. [PubMed: 15292509]

207. Adams DE, West SC. Bypass of DNA heterologies during RuvAB-mediated three-and four-strand branch migration. J Mol Biol 1996;263:582–596. [PubMed: 8918940]

208. Martinez-Senac MM, Webb MR. Mechanism of translocation and kinetics of DNA unwinding by the helicase RecG. Biochemistry 2005;44:16967–16976. [PubMed: 16363810]

209. Singleton MR, Scaife S, Wigley DB. Structural analysis of DNA replication fork reversal by RecG. Cell 2001;107:79–89. [PubMed: 11595187]

210. Lloyd RG, Sharples GJ. Processing of recombination intermediates by the RecG and RuvAB proteins of Escherichia coli. Nucleic Acids Res 1993;21:1719–1725. [PubMed: 8388095]

211. Whitby MC, Vincent SD, Lloyd RG. Branch migration of Holliday junctions: identification of RecG protein as a junction specific DNA helicase. Embo J 1994;13:5220–5228. [PubMed: 7957087]

212. van Brabant AJ, Ye T, Sanz M, German IJ, Ellis NA, Holloman WK. Binding and melting of D-loops by the Bloom syndrome helicase. Biochemistry 2000;39:14617–14625. [PubMed: 11087418]

213. Karow JK, Chakraverty RK, Hickson ID. The Bloom's syndrome gene product is a 3'-5' DNA helicase. J Biol Chem 1997;272:30611–30614. [PubMed: 9388193]

214. Bachrati CZ, Hickson ID. RecQ helicases: suppressors of tumorigenesis and premature aging. Biochem J 2003;374:577–606. [PubMed: 12803543]

215. Wu L, Hickson ID. DNA helicases required for homologous recombination and repair of damaged replication forks. Annu Rev Genet 2006;40:279–306. [PubMed: 16856806]

216. Ozgenc A, Loeb LA. Current advances in unraveling the function of the Werner syndrome protein. Mutat Res 2005;577:237–251. [PubMed: 15946710]

217. Orren DK, Brosh R, Nehlin JO, Machwe A, Gray MD, Bohr VA. Enzymatic and DNA binding properties of purified WRN protein: high affinity binding to single-stranded DNA but not to DNA damage induced by 4NQO. Nucleic Acids Res 1999;27:3557–3566. [PubMed: 10446247]

218. Opresko PL, Cheng WH, Bohr VA. Junction of RecQ helicase biochemistry and human disease. J Biol Chem 2004;279:18099–18102. [PubMed: 15023996]

219. Gray MD, Shen JC, Kamath-Loeb AS, Blank A, Sopher BL, Martin GM, Oshima J, Loeb LA. The Werner syndrome protein is a DNA helicase. Nat Genet 1997;17:100–103. [PubMed: 9288107]

220. Shen JC, Gray MD, Oshima J, Loeb LA. Characterization of Werner syndrome protein DNA helicase activity: directionality, substrate dependence and stimulation by replication protein A. Nucleic Acids Res 1998;26:2879–2885. [PubMed: 9611231]

221. Brosh RM Jr, Orren DK, Nehlin JO, Ravn PH, Kenny MK, Machwe A, Bohr VA. Functional and physical interaction between WRN helicase and human replication protein A. J Biol Chem 1999;274:18341–18350. [PubMed: 10373438]

222. Cui S, Arosio D, Doherty KM, Brosh RM Jr, Falaschi A, Vindigni A. Analysis of the unwinding activity of the dimeric RECQ1 helicase in the presence of human replication protein A. Nucleic Acids Res 2004;32:2158–2170. [PubMed: 15096578]

223. Sharma S, Sommers JA, Choudhary S, Faulkner JK, Cui S, Andreoli L, Muzzolini L, Vindigni A, Brosh RM Jr. Biochemical analysis of the DNA unwinding and strand annealing activities catalyzed by human RECQ1. J Biol Chem 2005;280:28072–28084. [PubMed: 15899892]

224. Ozsoy AZ, Sekelsky JJ, Matson SW. Biochemical characterization of the small isoform of Drosophila melanogaster RECQ5 helicase. Nucleic Acids Res 2001;29:2986–2993. [PubMed: 11452023]

225. Prakash R, Krejci L, Van Komen S, Anke Schurer K, Kramer W, Sung P. Saccharomyces cerevisiae MPH1 gene, required for homologous recombination-mediated mutation avoidance, encodes a 3' to 5' DNA helicase. J Biol Chem 2005;280:7854–7860. [PubMed: 15634678]

226. Gari K, Decaillet C, Stasiak AZ, Stasiak A, Constantinou A. The Fanconi anemia protein FANCM can promote branch migration of Holliday junctions and replication forks. Mol Cell 2008;29:141–148. [PubMed: 18206976]

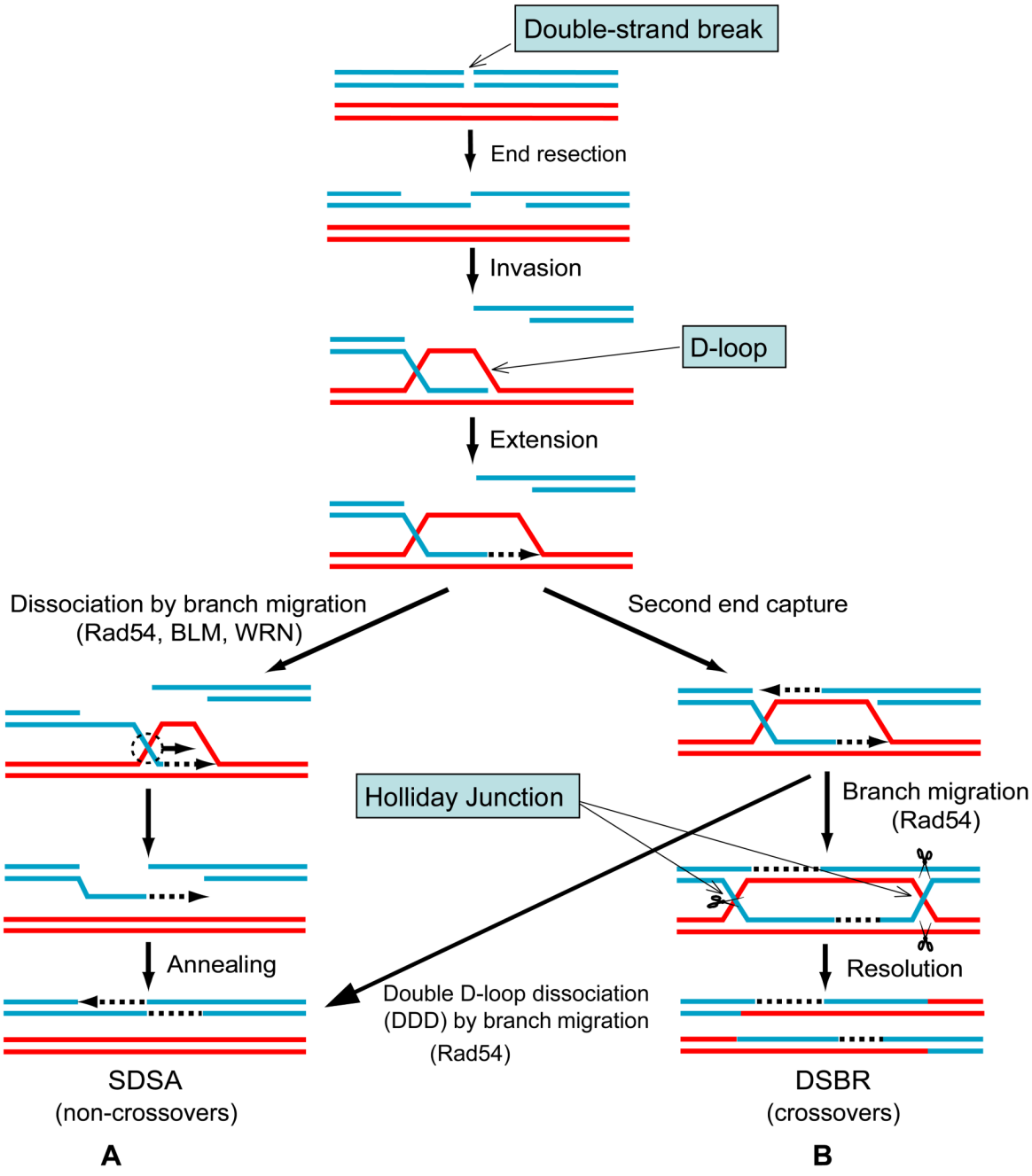

Fig. 1. DNA double strand break (DSB) repair by HR

Initial steps of HR (see explanations in the text) result in formation of Joint molecules (D-loops) that are further extended by DNA polymerase and processed afterward through the two major pathways, SDSA or DSBR. (A) Repair of DSB through the **s**ynthesis **d**ependent **s**trand **a**nnealing (SDSA) mechanism resuls in non-crossover recombinants. Dissociation of extended D-loop by branch migration is a crucial step of the SDSA mechanism. (B) The **d**ouble-**s**tranded **b**reak **r**epair (DSBR) mechanism, which is more frequent during meiosis, results in crossover recombinants. DSB repair by HR generates Holliday junctions, which may branch migrate along the DNA axis. After second end capture, branch migration would either stabilize double D-loops by increasing the length of DNA heteroduplex or cause their dissociation channeling

recombination intermediates through the **d**ouble **D**-loop **d**issociation (DDD) mechanism into the SDSA pathway [132].

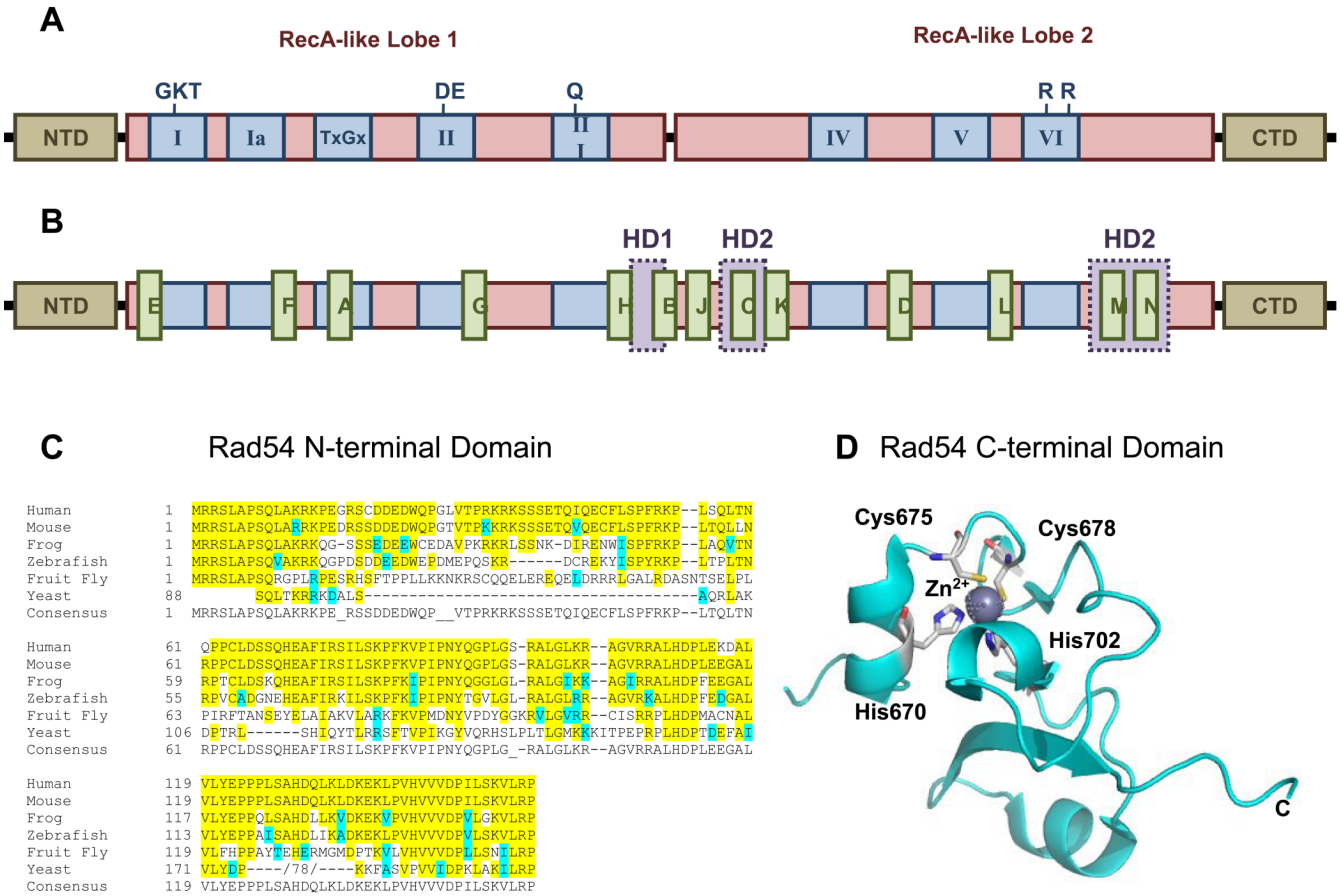

Fig. 2. Structural domains of Rad54

(A) Schematic diagram highlighting the conserved SF2 motifs of Rad54. The RecA-like domains are shown in red with the seven “signature” motifs (along with the TxGx domain) displayed in blue. The single letter abbreviations above the motifs blocks emphasize the location of conserved amino acid residues, whose role in ATP hydrolysis is well-defined for SF2 family proteins. The NTD (the N-terminal domain) and the CTD (the C-terminal domain) are displayed in tan. (B) Schematic diagram highlighting the conserved motifs of Rad54 that define the Snf2 family of proteins; with the Snf blocks shown in green (designated from “A” to “N” by the order of identification), and the HD (helical domain) motifs in purple. The TxGx motif was first designated as the Snf2 family specific motif Snf-A [24], but was later reclassified as TxGx when this motif was found to be conserved throughout the SF2 family [88]. Motifs are shown in relative position to one another, but are not drawn to scale. (C) Sequence alignment of the N-terminal domain of Rad54 from *Homo sapiens* (Human), *Mus musculus* (Mouse), *Xenopus tropicalis* (Frog), *Danio rerio* (Zebrafish), *Drosophila melanogaster* (Fruit Fly), and *Saccharomyces cerevisiae* (Yeast). Residues that match the consensus sequence are highlighted in yellow, and the residues that are similar to the consensus are highlighted in blue. (D) The X-ray crystal structure of C-terminal domain of zebrafish Rad54 (PDB code: 1Z3I) [24]. This domain is specific to the Rad54 protein and contains a zinc-coordinating motif. The residues involved in zinc ion coordination are labeled and represented as sticks in the model.

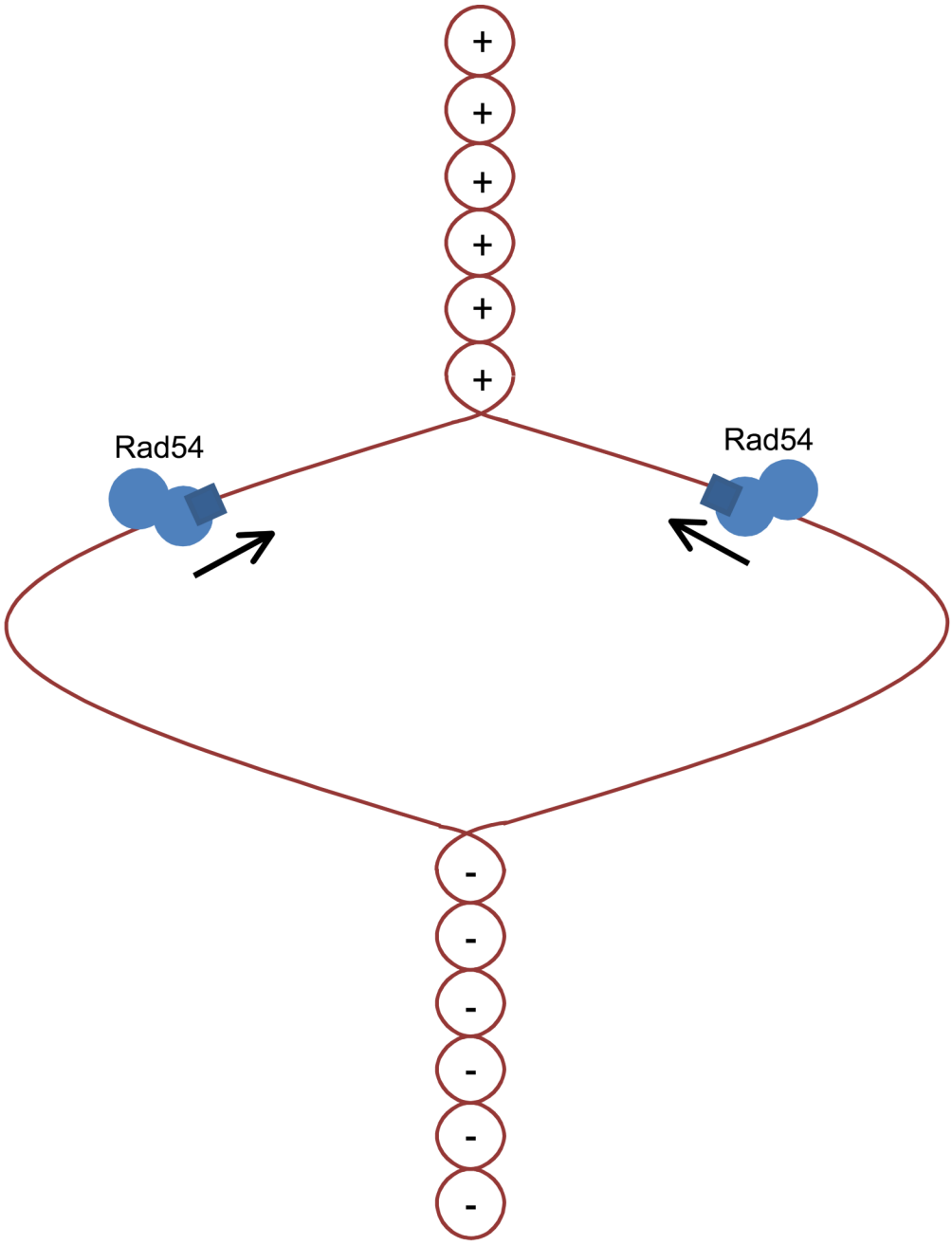

Fig. 3.

Model of Rad54-induced supercoiling. Rad54 (represented as the blue shape; figure-eight represents the dual RecA-like domains, and the square represents the N-terminal domain) is shown bound to covalently closed circular plasmid DNA. Supercoils may accumulate on this type of DNA substrate if two (or more) active Rad54 complexes are bound to the same DNA molecule and move in opposing directions. As the Rad54 complexes translocate (direction of translocation is indicated with arrows), positive supercoils (+) would accumulate in front of complexes and negative supercoils (−) would accumulate behind.

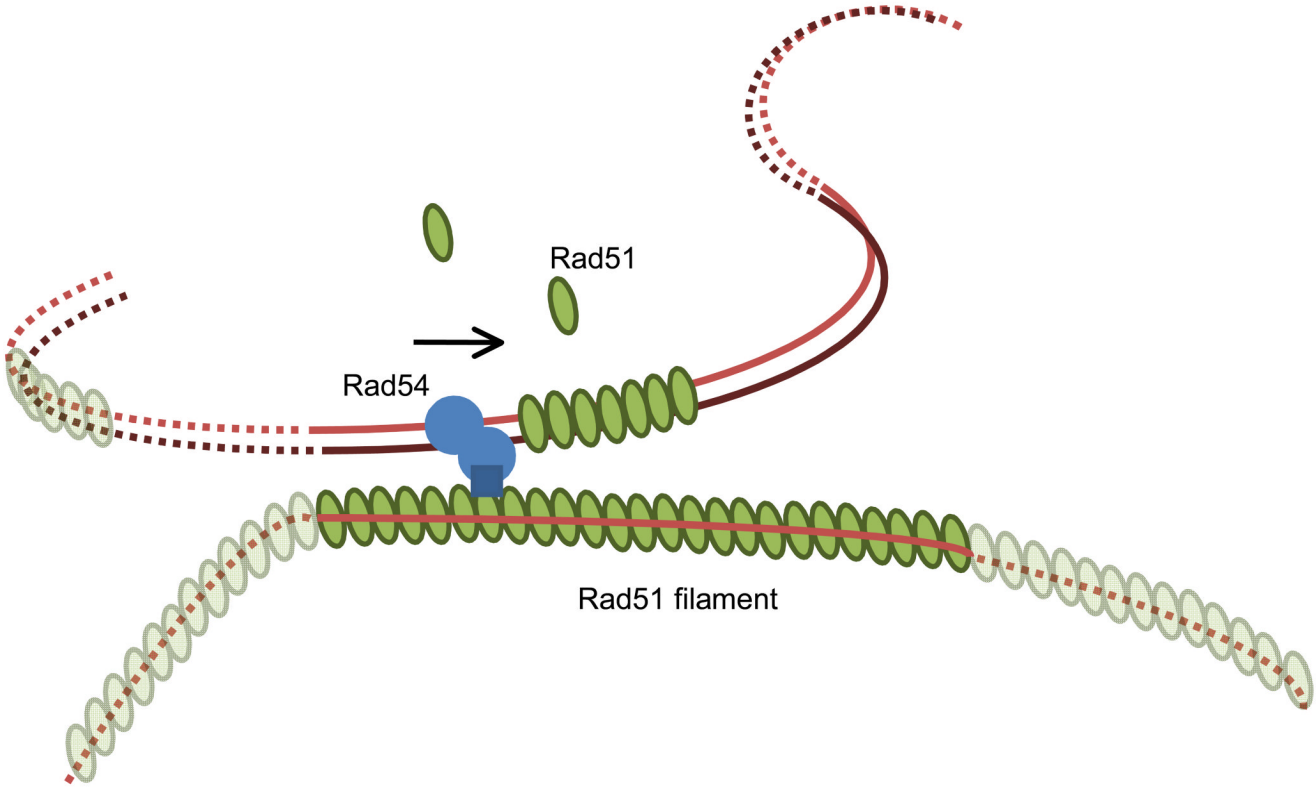

Fig. 4.

Rationalizing the ability of Rad54 to both stabilize and disrupt the Rad51 filament. The Rad54 protein (blue shape) contains two distinct binding sites. The N-terminal domain of Rad54 (square) specifically interacts with Rad51 (green oval). This interaction is thought to stabilize individual Rad51 monomers, which adds to the overall stability of the filament. On the other hand, the dual RecA-like domains of Rad54 (figure eight) form the binding site for dsDNA. As Rad54 translocates on the dsDNA (direction of translocation is indicated with arrows), it will displace the Rad51 monomers, thereby disrupting the filament.

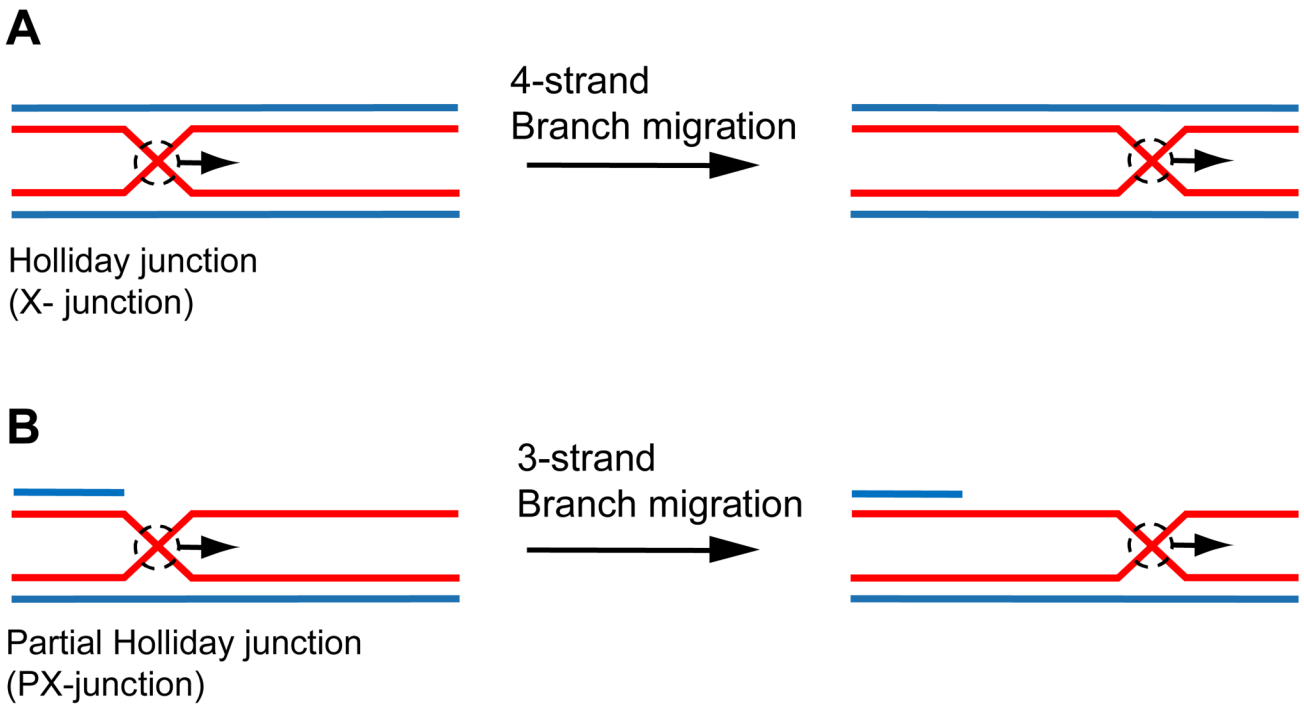

Fig. 5. Schematic representation of the 4-stranded (A) and 3-stranded (B) branch migration
X-junctions and PX-junctions were used as substrates in these two reactions, respectively.

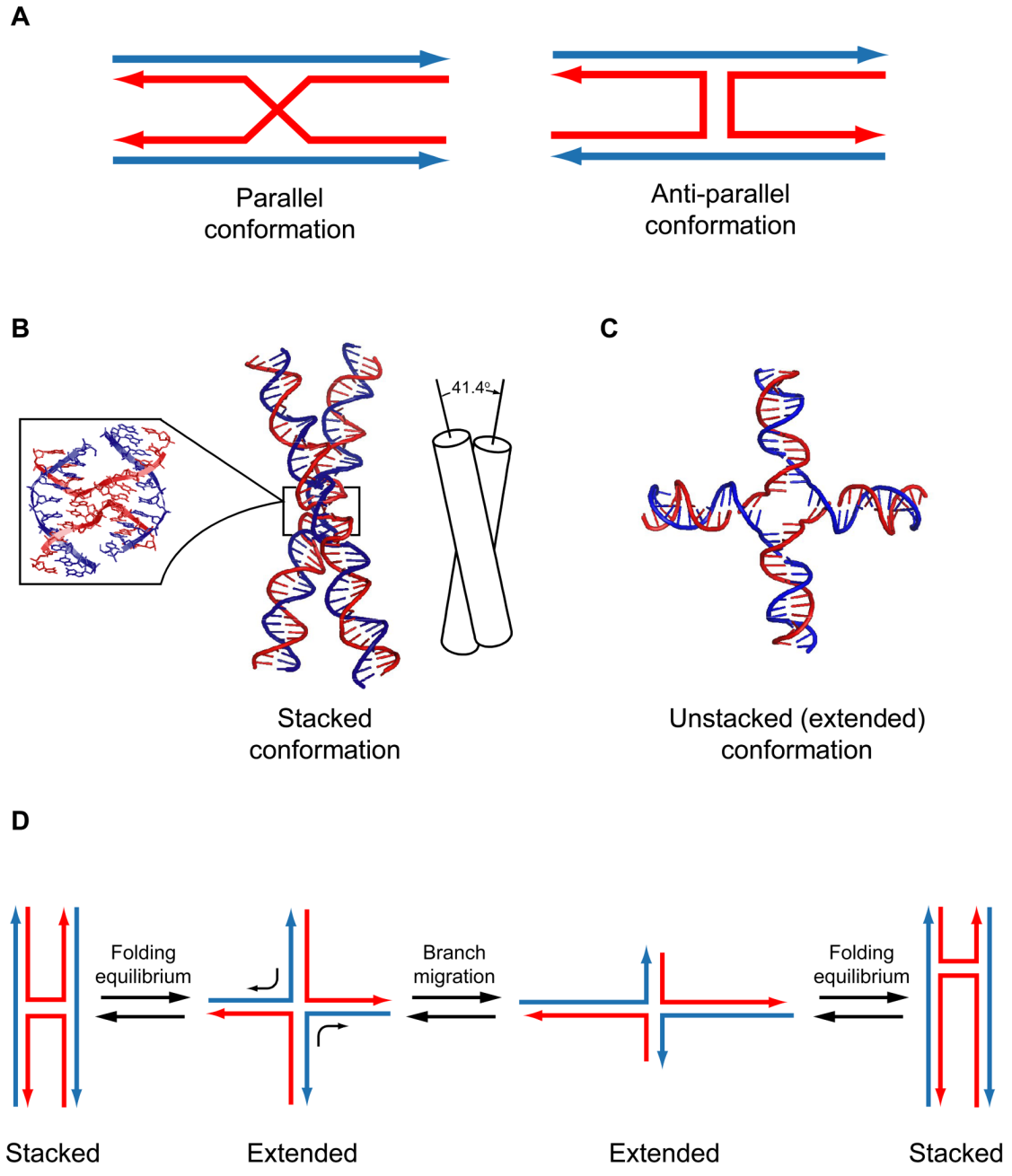

Fig. 6. Conformational forms of the Holliday junction

(A) The parallel and antiparallel conformation of the Holliday junction. The antiparallel conformation differs from the parallel one by a 180° rotation of one of the exchanging dsDNA molecule around the junction. **(B)** Holliday junctions were crystallized in the stacked anti-parallel conformation [142]. For clarity, the insert (left) contains a close up view of the crossover point of the Holliday junction. Two stacked duplexes form a right-handed twist with an angle of 41.4° (right). **(C)** A Holliday junction in the unstacked (extended) conformation with four arms directed toward the corners of the square. **(D)** The scheme shows folding equilibrium between the stacked and extended conformations of Holliday junction, which depends on the concentration of divalent metal ions [138]. Branch migration occurs while the

Holliday junction is in the extended state and stops when the molecule adopts the staked conformation.

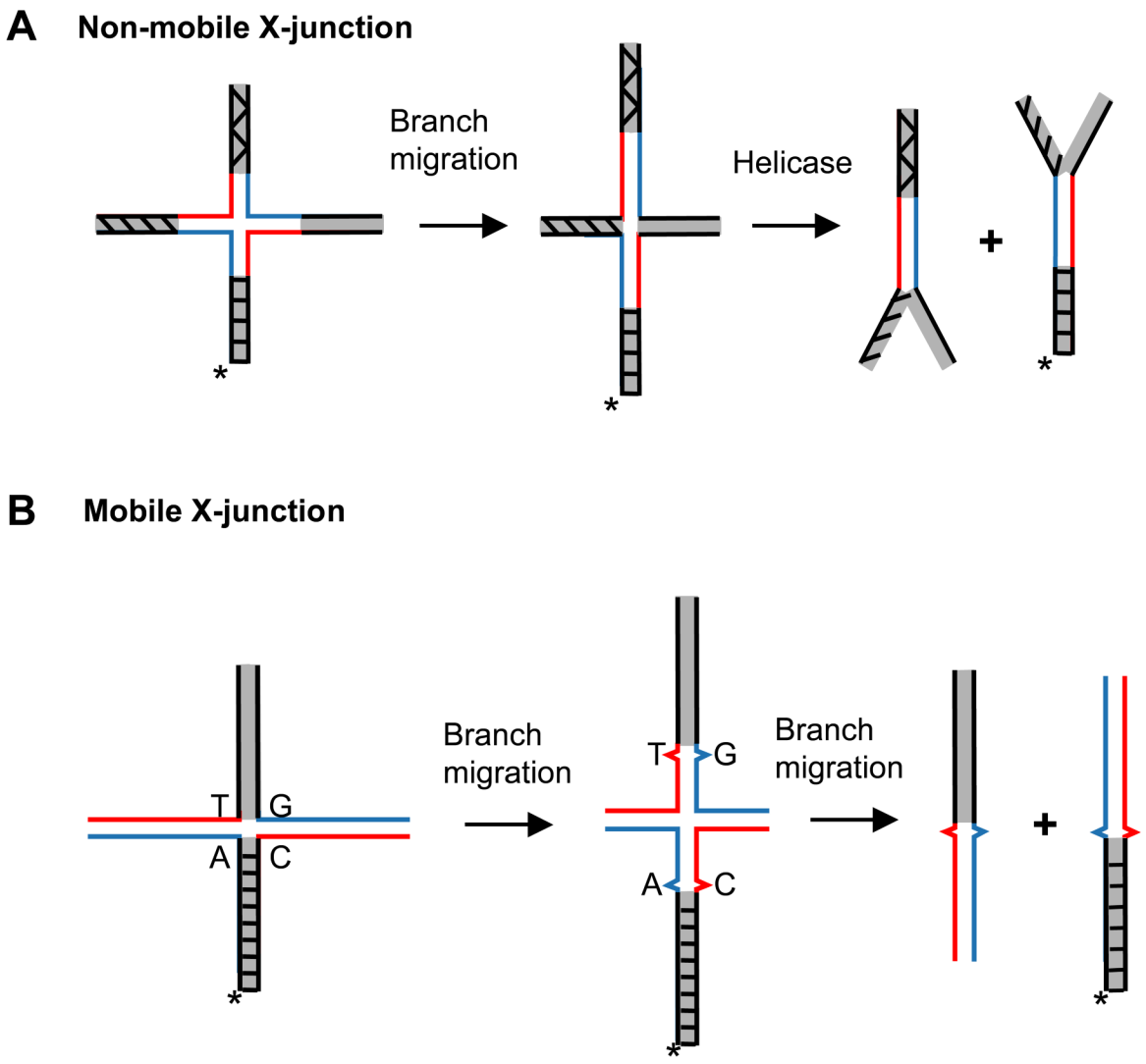

Fig. 7. Holliday junction substrates for branch migration proteins

(A) Non-movable or partially movable X-junctions require DNA helicase activity for their dissociation. These substrates have been previously used for analysis of branch migration activity of prokaryotic branch migration proteins, RuvAB and RecG, and for RecQ helicases, which combine branch migration with conventional DNA helicase activity. (B) Movable X-junctions (can branch migrate in only one direction) were designed for Rad54 that does not possess DNA helicase activity. Movable junctions contain a mismatch to block spontaneous branch migration. Shaded regions denote heterologous DNA terminal branches. ^{32}P-label at the DNA 5'-end (indicated by the asterisk) is required for visualization of the products of branch migration after gel-electrophoresis in polyacrylamide gels.

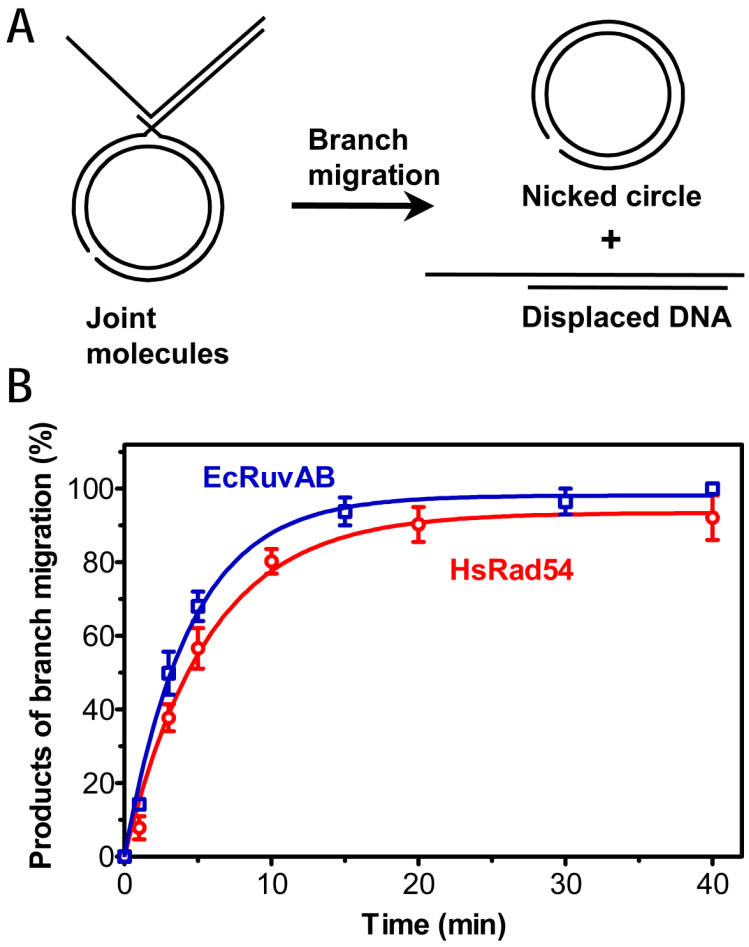

Fig. 8. The kinetics of branch migration by HsRad54 and E. coli RuvAB (Mazina and Mazin, unpublished results)

(A) Experimental scheme. (B) Joint molecules were produced by using either RecA or HsRad51 recombinases in 4-strand exchange reactions and then deproteinized, as described [29,200]. The joint molecules (0. 3 nM) made by RecA or HsRad51 were used for branch migration by RuvAB (140 nM RuvA and 360 nM RuvB) at 37 °C or HsRad54 (200 nM) at 30 °C, respectively. The reaction conditions were the same, as described previously [29,201] The DNA products were analyzed by electrophoresis in agarose gels and quantified using a Storm 840 Phosphor Imager. Data are the mean of three experiments. Error bars represent standard error of the mean. RuvAB was purchased from BioAcademia Inc., Japan.

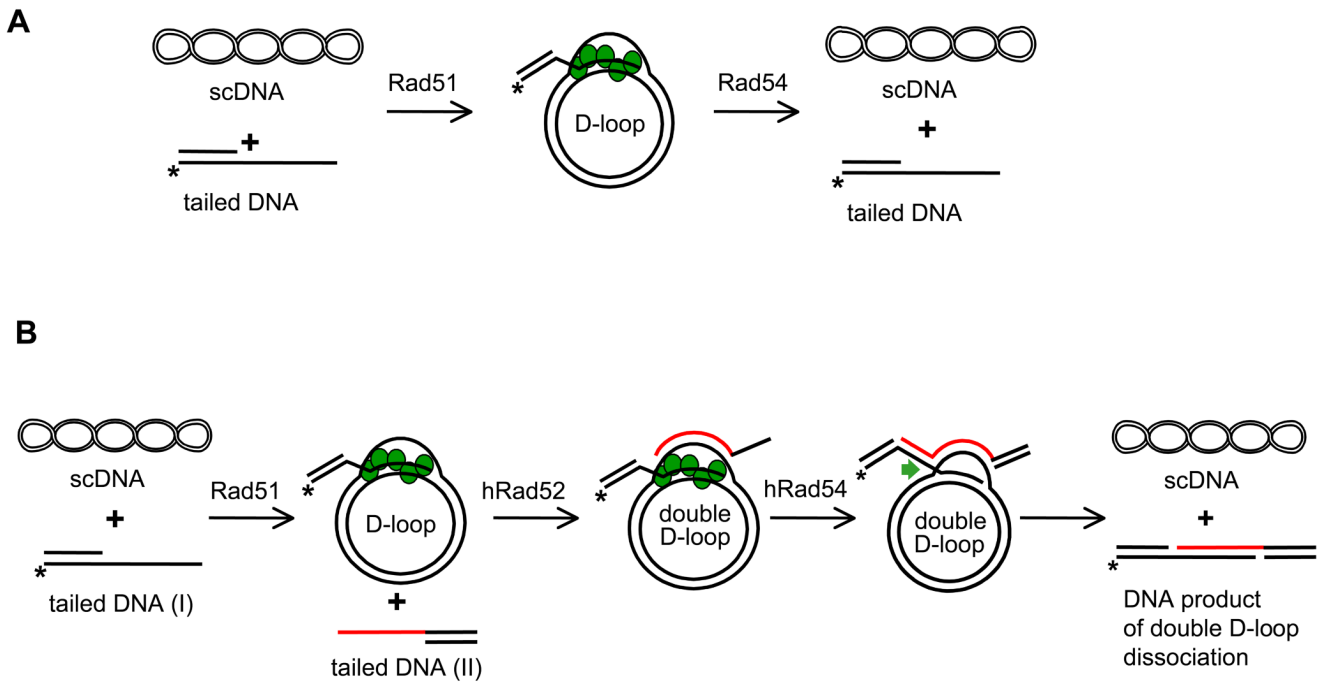

Fig. 9. HsRad54 promotes dissociation of joint molecules (D-loops)

HsRad54 promotes dissociation of native (non-deproteinized) single D-loops produced by HsRad51 (denoted by green circles) (A) or native double D-loops produced by RAD51 and RAD52 proteins (B). The green arrow marks formation of the Holliday junction during branch migration. The asterisk indicates ^{32}P label.

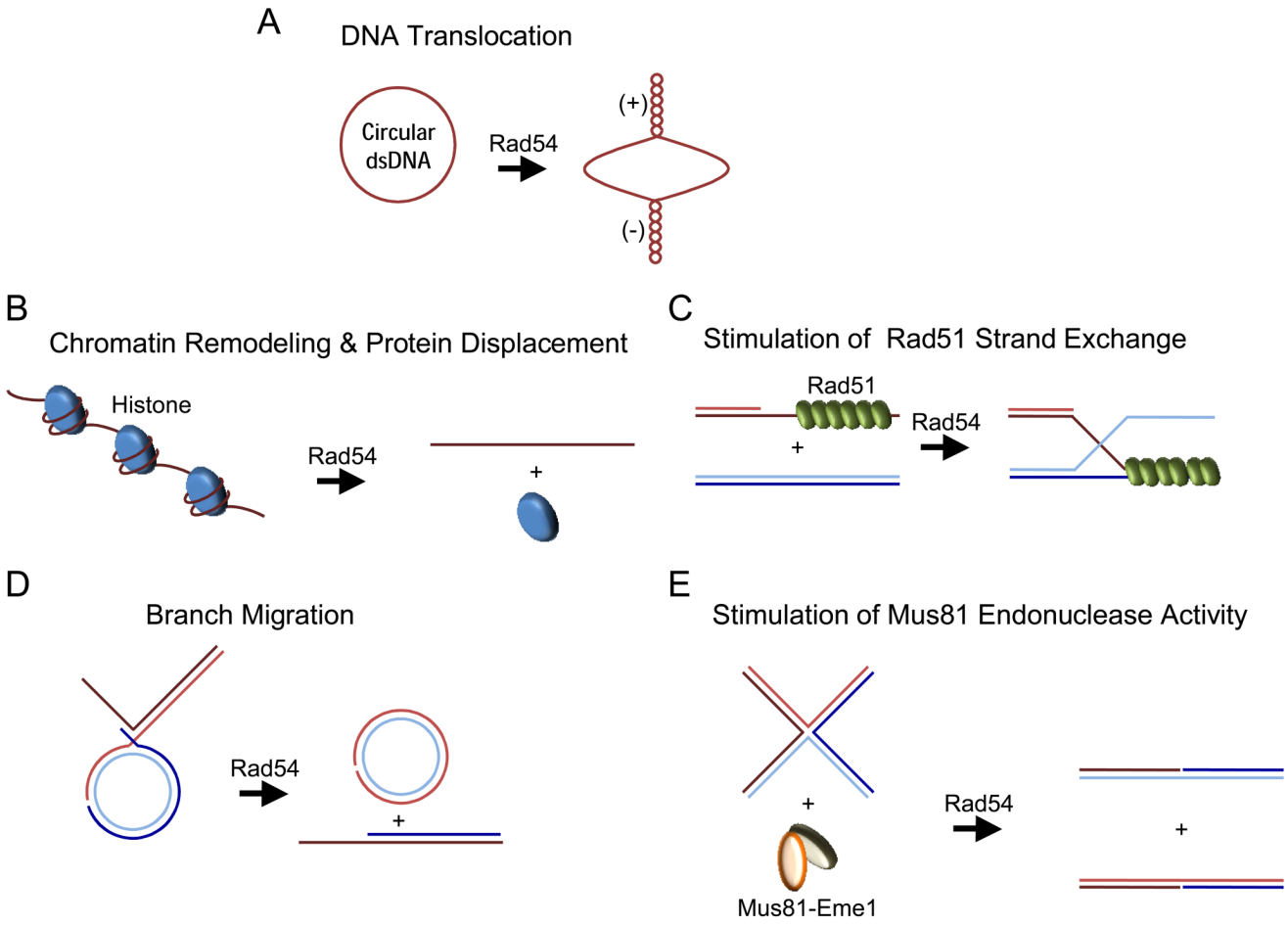

Fig. 10. Activities of Rad54

Rad54 possesses a unique set of biochemical activities that allow it to function at every stage along the HR pathway. **(A)** The dsDNA translocation activity (shown by the accumulation of supercoiling in DNA) is thought to be required for most, if not all other Rad54 activities. **(B)** The chromatin remodeling and protein displacement activities free DNA for processing by other proteins. **(C)** Rad54 stimulates the DNA strand exchange activity of Rad51 by forming a specific complex with this protein. **(D)** Through its branch migration activity, Rad54 processes HR intermediates. **(E)** Rad54 contributes to the resolution of these intermediates by stimulating the endonuclease activity of Mus81-Eme1.

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Table 1

The biochemical properties of branch migration proteins

Branch migration protein	Biochemical properties				
	ATPase activity rate	**DNA binding preference**	**Helicase activity*, (polarity, extent)**	**Branch migration /translocation rate**	**Heterology Bypass**
RuvAB	dsDNA, 140 min^{-1} ssDNA, 110 min^{-1} [202]	X-junction [203]	5'→3; < 200 bp; up to 560 bp with SSB [204]	branch migration ~98 bp/sec [205,206]	~1 kbp in 3-strand; ~0.3 kbp in 4-strand [207]
RecG	dsDNA, 460 min^{-1} [208]	Branched, X-junction, Y-junction, forked DNA [209,210]	3'→5' 26–52 bp [211].	translocation 26 bp/sec [208]	not available
BLM	ssDNA, dsDNA, ~3200 min^{-1} [212,213]	X-junction, G-quadruplex, D-loops, [154,214]	3'→5' [213]; < 100 bp; up to 500 bp with RPA [215]	not available	not available
WRN	dsDNA, 30 min^{-1} ssDNA, 200 min^{-1} [216,217]	G-quadruplex, D-loops, bubbles, branched DNA [214,216,218]	3'→5'; 40–53 bp [216,219,220] ~ 850 bp with RPA [216,221]	not available	not available
Hs / Sc RAD54	dsDNA, 6000 min^{-1} ([28]; Mazina and Mazin, unpublished data)	PX-junction, X-junction, branched DNA [29]	not detectable	translocation 300 bp/sec [25]	~6 bp on synthetic DNA substrates; ~ 48 bp on plasmid-based α-structures ([29]; Rossi and Mazin, unpublished data)
RECQ1	ssDNA, 30 min^{-1} [222]	X-junction, flap, D-loop, forked DNA [223]	3'→5'; < 110 bp; ~ 501 bp with RPA [222]	not available	not available
Dm RECQ5	ssDNA, 1380 min^{-1} [224]	3'-tailed DNA[224]	3'→5'; < 98 bp [224]	not available	not available
FANCM / ScMph1	ssDNA, 1500 min^{-1} [225]	X-junction, replication fork, flap, forked DNA[226]	3'→5'; 40 bp; ~100 bp with RPA [225]	not available	not available

*Helicase activities of the listed enzymes were measured on either ssDNA tailed or partial duplex substrates. The 5'-ssDNA tailed substrates were required for RuvAB and the 3'-ssDNA tailed substrates for other helicaeses. No helicase activity was detected on blunt-end dsDNA.