Supporting Information

Rossi et al. 10.1073/pnas.1016072108

SI Text

SI Materials and Methods. ***Restriction endonuclease protection assay on oligonucleotide substrates.*** The RecA protection assay was performed by incubating RecA (1 μM) with ^{32}P-labeled tailed DNA (A4 or A5) (33 nM) in buffer containing 35 mM Tris HCl, pH 7.5, 15 mM $MgCl_2$, 2 mM DTT, 3 mM ATP, and 100 μg/mL BSA for 2 min at 37 °C. The RecA filament was then "frozen" by the addition of 10 μM ATPγS and incubated for an additional 2 min at 37 °C. Digestion was initiated by the addition of BamHI (0.5 U/μL) and incubated for 20 min at 37 °C. The samples were deproteinized by treatment with 1.6 mg/mL proteinase K, 0.8% SDS, 6% glycerol, 0.01% bromphenol blue and analyzed in 10% (17:1) polyacrylamide gels.

The RAD51 protection assay was performed by incubating RAD51 (1.5 μM) with ^{32}P-labeled tailed DNA (A4 or A5) (33 nM) in buffer containing 25 mM Tris-acetate (pH 7.5), 10 mM magnesium acetate, 2 mM DTT, 200 mM NaCl, 2 mM ATP, 15 mM phosphocreatine, 10 units/mL creatine phosphokinase, and 100 μg/mL BSA for 10 min at 37 °C. Digestion was initiated by the addition of BamHI (1.5 U/μL) and incubated for 30 min at 37 °C. The samples were deproteinized by treatment with 1.6 mg/mL proteinase K, 0.8% SDS, 6% glycerol, 0.01% bromphenol blue and analyzed in 10% (17:1) polyacrylamide gels.

DNA strand-exchange assay with oligonucleotide-based substrates. The nucleoprotein filaments were formed by incubating RecA (2 μM) with ^{32}P-labeled ssDNA (A7) (6 μM, nucleotides) in buffer containing 35 mM Tris-HCl, pH 7.5, 15 mM $MgCl_2$, 2 mM DTT, 3 mM ATP, 10 mM creatine phosphate, 10 units/mL creatine phosphokinase, and 100 μg/mL BSA for 10 min at 37 °C. The reactions were initiated by the addition of complementary dsDNA (B6) (2 μM, base pairs). Aliquots were withdrawn from the reaction mixture at the indicated time points and deproteinized by treatment with 0.96 mg/mL proteinase K, 1.4% SDS, 7.5% glycerol, 0.015% bromphenol blue for 5 min at 22 °C. The DNA products were analyzed by electrophoresis in 10% polyacrylamide gels (17:1) in 1X TBE buffer (89 mM Tris-borate, pH 8.3, and 1 mM EDTA).

Branch migration (BM) assay on oligonucleotide-based substrates. The assay was performed as described in main text. The DNA products were analyzed by electrophoresis 10% polyacrylamide gels (29:1) in 1X TBE buffer (89 mM Tris-borate, pH 8.3, and 1 mM EDTA) at 4 °C. Gels were dried on DE81 chromatography paper (Whatman) and quantified using a Storm 840 PhosphorImager (Amersham Biosciences).

Spectrophotometric assay to measure the RecA ATPase. RecA was incubated with 5′-tail HJs (B5, 7.5 μM base pairs) in buffer containing 35 mM Tris-HCl, pH 7.5, 3 mM $MgCl_2$, 1 mM DTT, 3 mM ATP, 3 mM phosphoenolpyruvate, pyruvate kinase (20 units/mL), lactate dehydrogenase (20 units/mL), and NADH (200 μg/mL) at 37 °C. The oxidation of NADH, coupled to ADP phosphorylation, resulted in a decrease in absorbance at 340 nm, which was continuously monitored by a Hewlett-Packard 8453 diode array spectrophotometer using UV-Visible ChemStation software. The rate of ATP hydrolysis was calculated from the rate of change in absorbance using the following formula: rate of ATP hydrolysis $(\mu M/\min)$ = rate of A_{340} decrease (s^{-1}) x 9,880.

Measuring of the RAD51 ATPase by TLC. Reactions were carried out in buffer containing 25 mM Tris acetate (pH 7.5), 2 mM ATP, 1 μCi [γ-^{32}P] ATP, 50 μM [nt (nucleotides)] DNA (circular ϕX174 ssDNA or supercoiled ϕX174 DNA), 2 mM DTT, 10 mM magnesium acetate, and RAD51 (5 μM) for 2 h at 37 °C. The extent of ATP hydrolysis was determined by thin layer chromatography (TLC) on PEI-cellulose plates in 1 M formic acid with 0.5 M LiCl, and the products were quantified by using a Storm 840 PhosphorImager (Molecular Dynamics).

BM on plasmid-based DNA substrates by RecA. RecA nucleoprotein filaments were assembled by incubation of RecA protein (4 μM) with gapped pBSK(+) DNA (20 μM, nucleotides) in buffer containing 35 mM Tris-HCl, pH 7.5, 15 mM $MgCl_2$, 2 mM DTT, 3 mM ATP, 10 mM phosphocreatine, 10 units/mL creatine phosphokinase, and 100 μg/mL BSA for 5 min at 37 °C. SSB (ssDNA-binding protein) (0.33 μM) was added to the nucleoprotein filaments and incubation was continued for another 1 min. DNA exchange reactions were initiated by the addition of homologous dsDNA (20 μM, nucleotides) to the nucleoprotein filaments. After 10 min, the reaction was stopped to produce deproteinized joint molecule (JMs) by addition of proteinase K to 1.6 mg/mL and SDS to 0.8%, followed by incubation for a further 15 min at 37 °C.

Deproteinized JMs were supplemented with EDTA to 2 mM and passed twice through S-400 Spin columns (GE Healthcare) equilibrated with 30 mM Tris-HCl, pH 7.5 (1, 2). After addition of $MgCl_2$ to 10 mM the JMs were kept at -20 °C. BM of deproteinized JM (0.32 nM, molecules) was carried out in buffer containing 30 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 2 mM DTT, 2 mM ATP, 10 mM phosphocreatine, and 10 units/mL creatine phosphokinase at 37 °C. RecA (4 μM) was added to the reaction mixtures. When indicated, SSB (0.4 μM or 55 nM) was added to the reaction. The reaction mixtures were incubated for the indicated periods of time. The samples were analyzed as described for RAD51 reaction.

BM on plasmid-based DNA substrates by RAD51. Nucleoprotein filaments were formed by incubating RAD51 protein (5 μM) with pBSK (+) gapped DNA (20 μM, nt) (4-stranded reaction) in buffer containing 25 mM Tris acetate, pH 7.5, 2 mM ATP, 275 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM DTT, and 100 μg/mL BSA for 10 min 37 °C. RPA (Replication protein A) (0.4 μM) was added to the nucleoprotein filaments followed by a 10-min incubation. DNA strand-exchange reaction was initiated by addition of 5′-labeled linear pBSK (+) dsDNA (20 μM, nt). BM of nondeproteinized JMs (σ and α-structures) was initiated by Ca^{2+} depletion with 1.2 mM EGTA.

BM of deproteinized JMs (0.32 nM, molecules) was carried out in buffer containing 30 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 350 mM NaCl, 2 mM DTT, 2 mM ATP, 8 mM phosphocreatine, and 8 units/mL creatine phosphokinase at 37 °C. RAD51 (10 μM) was added to the reaction mixtures. When indicated, RPA (0.4 μM or 55 nM) was added to the reaction. The DNA products were deproteinized by treatment with stop buffer for 15 min at 37 °C and analyzed in 1.5% agarose gels in 1× TAE buffer (40 mM Tris-acetate, pH 8.0 and 1 mM EDTA).

Digestion of the displaced ssDNA of JMs with an ssDNA-specific nucleases. To remove 5′-ssDNA tail from JMs made by RecA, ^{32}P-labeled JMs were treated with Exonuclease VII (0.03 U/μL) in buffer containing 35 mM Tris-HCl, pH 7.5, 15 mM $MgCl_2$, 2 mM

DTT, 3 mM ATP, 10 mM phosphocreatine, 10 units/mL creatine phosphokinase, and 100 μg/mL BSA for 10 min at 37 °C. BM was initiated by addition of RecA (10 μM) and carried out for 30 min. In control experiments, nonradioactive RecA-generated JMs containing 5′-ssDNA tails were incubated with Exonuclease I as described below. BM was initiated by addition of RecA (4 μM) in buffer containing 2 mM ATP, 2 mM DTT, 10 mM phosphocreatine, and 10 units/mL creatine phosphokinase and carried out for 1 h at 37 °C.

To remove 3′-ssDNA tail from JM made by RAD51, ^{32}P-labeled JM were treated with Exonuclease I (0.5 U/μL) in buffer, containing 30 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 2 mM DTT for 20 h at 37 °C. Then, JMs were supplemented with 350 mM NaCl, 2 mM ATP, 8 mM phosphocreatine, and 8 units/mL creatine phosphokinase. BM was initiated by addition of RAD51 (10 μM) and carried out for 20 h.

The samples were deproteinized and analyzed in 1.5% agarose gels. Gels with radioactive DNA were dried on DE81 chromatography paper (Whatman) and visualized using a Storm 840 PhosphorImager (GE Healthcare). Nonradioactive gels were stained in ethidium bromide solution (2 μg/mL in 1× TAE) and visualized using an AlphaImager 3400 (Alpha Innotech).

1. Bugreev DV, Mazina OM, Mazin AV (2006) Rad54 protein promotes branch migration of Holliday junctions. *Nature* 442:590–593.

2. Rossi MJ, Mazina OM, Bugreev DV, Mazin AV (2010) Analyzing the branch migration activities of eukaryotic proteins. *Methods* 51:336–346.

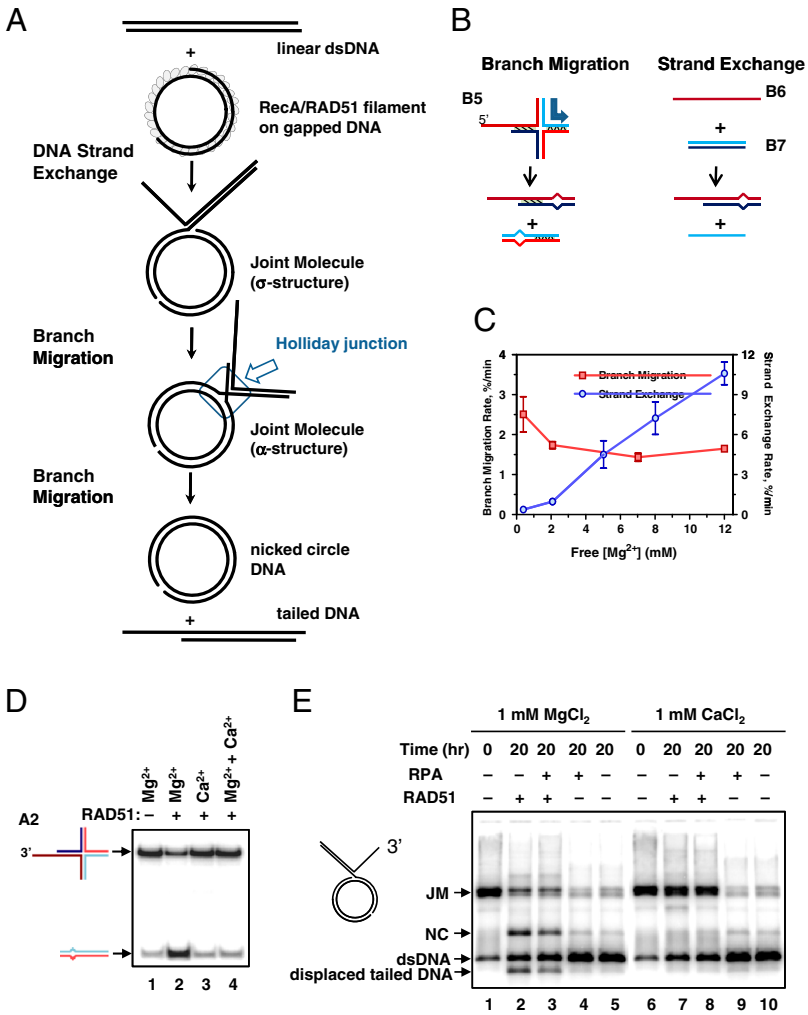


Fig. S1. RecA/Rad51 BM activity is mechanistically distinct from its DNA strand-exchange activity. (*A*) Steps of JM formation and the 4-strand DNA exchange reaction. (*B*) Outline of the BM and DNA strand exchange on synthetic substrates. (*C*) Effect of Mg^{2+} on RecA-promoted DNA strand exchange and BM. DNA strand exchange between ssDNA (B6) and dsDNA (B7) was performed at indicated Mg^{2+} concentrations. BM reactions were performed using a 5′-tail HJ (B5; 32 nM). For each condition, the initial rate of DNA strand exchange and BM were determined using the linear part of the kinetic curve during the first 5 and 10 min of the reactions, respectively. Data are the mean of at least three experiments. Error bars represent SEM. (*D*) Ca^{2+} inhibits BM activity of RAD51. BM initiated on 3′- tail HJs (A2; 32 nM) by RAD51 (2.6 μM) was carried out in the presence of either 10 mM magnesium acetate (Mg^{2+}), 10 mM calcium chloride (Ca^{2+}), or 5 mM magnesium acetate and 5 mM calcium chloride (Mg^{2+} + Ca^{2+}) for 45 min. The DNA products were deproteinized and analyzed by electrophoresis in 8% (29:1) polyacrylamide gels. (*E*) RAD51 (10 μM) and/or RPA (55 nM) were incubated with deproteinized JMs (0.32 nM) in the presence of 1 mM $MgCl_2$ (lanes 2 to 4) or 1 mM $CaCl_2$ (lanes 7 to 9) for 20 h in the presence of 2 mM ATP. Spontaneous BM after 20 h of incubation is shown in lanes 5 and 10. The DNA substrates are shown in lanes 1 and 6. The loss of JMs observed in the absence of RAD51 is due to spontaneous BM.

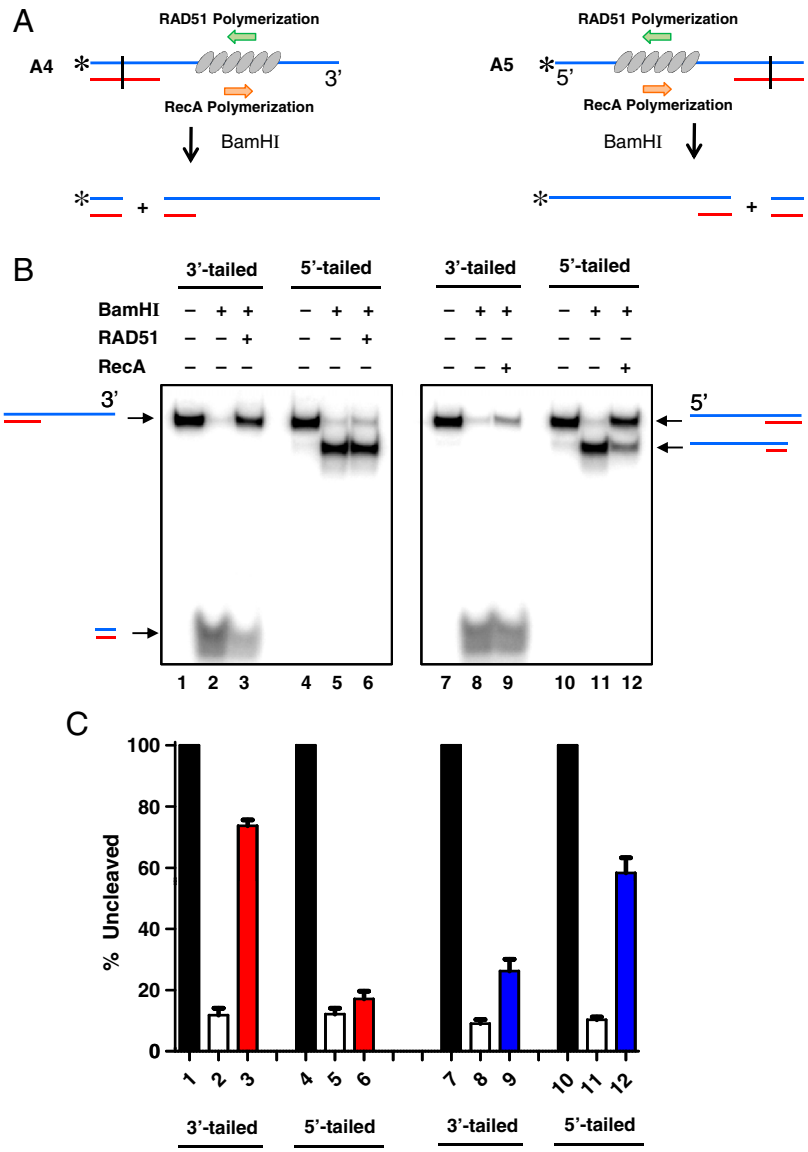


Fig. S2. RAD51 polymerizes on ssDNA with a 3′-5′ polarity, which is opposite to the polarity of RecA polymerization. (*A*) Tailed DNA molecules were designed to contain either a 3′ ssDNA tail (A4) or a 5′ ssDNA tail (A5), with the dsDNA portion of each containing a BamHI site (vertical black line). The tailed DNA molecules were incubated with RAD51 or RecA, and then digested with BamHI. BamHI will only cleave DNA molecules if the dsDNA portion is free of recombinase protein. If RAD51 possesses a 3′-5′ polarity, which is opposite the 5′-3′ polarity of RecA; then in our experimental design, RAD51 would preferentially protect the 3′-tailed substrate, whereas RecA would preferentially protect the 5′-tailed substrate; asterisk denotes ^{32}P label. (*B*) BamHI digest of tailed DNA molecules protected with RAD51 or RecA. 3′-tailed DNA (lanes 1 and 7) was incubated with BamHI in the absence of recombinase (lanes 2 and 8), in the presence of RAD51 (lane 3), or in the presence of RecA (lane 9). 5′-tailed DNA (lanes 4 and 10) was incubated with BamHI in the absence of recombinase (lanes 5 and 11), in the presence of RAD51 (lane 6), or in the presence of RecA (lane 12). (*C*) Quantification of the data presented in (*B*). Negative (−BamHI,−RecA/RAD51) and positive (+BamHI, −RecA/Rad51) controls are shown with black and white bars, respectively. Test conditions with RAD51 and RecA are shown with red and blue bars, respectively. Data are the mean of at least three experiments. Error bars represent SEM.

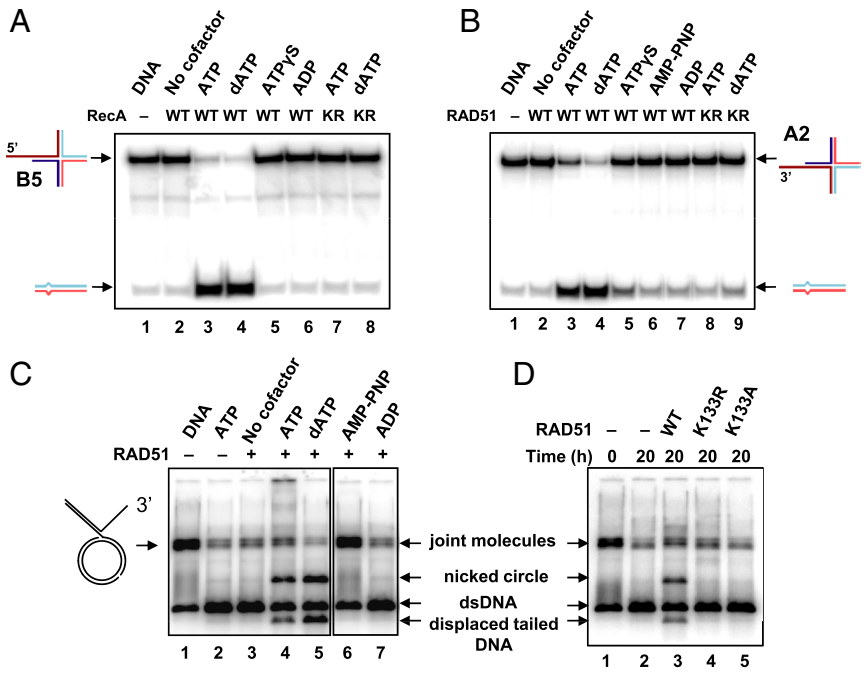


Fig. S3. BM by RecA/RAD51 requires ATP or dATP hydrolysis. (*A*) Effect of nucleotide cofactors on BM by RecA. RecA (3 μM) was incubated with 5′-tail HJs (B1; 32 nM) for 45 min in the presence of 3 mM ATP (lane 3), dATP (lane 4), ATPγS (lane 5), ADP (lane 6), or in the absence of a nucleotide cofactor (lane 2). RecA-independent BM in the presence of 3 mM ATP after a 45 min incubation is shown in lane 1. Wild type RecA was replaced with the ATPase-deficient K72R mutant (3 μM) in lanes 7 and 8. (*B*) Effect of nucleotide cofactors on BM by RAD51. RAD51 (2.6 μM) was incubated with 3′-tail HJs (A2; 32 nM) for 45 min in the presence of 2 mM ATP (lane 3), dATP (lane 4), ATPγS (lane 5), AMP-PNP (lane 6), ADP (lane 7), or in the absence of a nucleotide cofactor (lane 2). RAD51-independent BM in the presence of 2 mM ATP after a 45 min incubation is shown in lane 1. BM of the ATPase-deficient K133R mutant (2.6 μM) was analyzed in the presence of 2 mM ATP or dATP (lanes 8 and 9, respectively). The ATP regeneration system was included only in the reactions containing ATP and dATP. (*C*) BM by RAD51 on plasmid-based DNA substrates requires ATP or dATP hydrolysis. RAD51 (10 μM) was incubated with deproteinized JMs (0.32 nM) (lane 1) for 20 h in the presence of 2 mM ATP (lane 4), dATP (lane 5), AMP-PNP (lane 6) or ADP (lane 7), or in the absence of a nucleotide cofactor (lane 3). Protein-free BM in the presence of 2 mM ATP after 20 h of incubation is shown in lane 2. (*D*) The RAD51 ATPase mutants do not promote BM. Wild type RAD51 (10 μM) (lane 3) or its ATPase mutants K133R (lane 4) and K133A (lane 5) were incubated with deproteinized JMs (0.32 nM) (lane 1) for 20 h in the presence of 2 mM ATP. Spontaneous BM in the presence of ATP after 20 h of incubation is shown in lane 2. Note, loss of JMs observed in the presence of inactive RAD51 or in the absence of RAD51 is due to spontaneous BM.

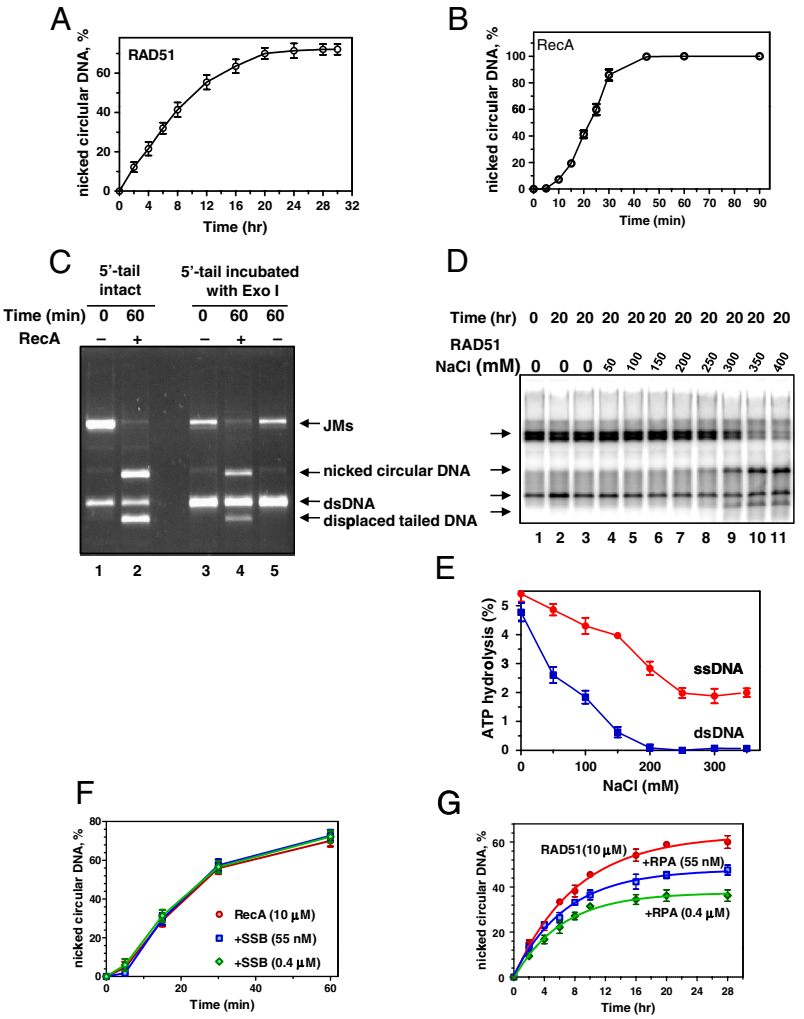


Fig. S4. The displaced ssDNA strand is essential for BM. (*A*) and (*B*) The kinetics of BM by RAD51 and RecA. BM was initiated by adding RAD51or RecA (10 μM) to deproteinized and purified JMs containing the 3′ or 5′-ssDNA displaced strand (0.32 nM) (lane 1) that were generated in RAD51-promoted DNA strand exchange between ^{32}P-labeled pBSK (+) linear dsDNA and gapped DNA. The DNA products were analyzed by electrophoresis on 1.5% agarose gels. Data are the mean of at least three experiments. Error bars represent SEM. (*C*) Exonuclease I is a 3′-5′ specific exonuclease that does not inhibit the BM of RecA-generated JMs containing 5′ ssDNA tails. Incubation of RecA with these substrates (lane 4) leads to formation of nicked circle products as seen in the absence of Exonuclease I treatment (lane 2). Note, when RecA-generated JMs are incubated with Exonuclease I there is a decrease in JMs due to spontaneous BM during the 20 h incubation (compare lane 3 to lane 1). A similar control could not be performed with RAD51-generated JMs because Exonuclease VII has both 5′ to 3′ and 3′ to 5′ exonuclease activities. (*D*) BM of deproteinized JMs (0.32 nM, molecules) by RAD51 (10 μM) was carried out at 37 °C for 20 h as described in *SI Text: Materials and Methods* in the absence of salt (lane 3) or in the presence of increasing NaCl concentrations (lanes 4–11). To monitor spontaneous BM, JMs (lane 1) were incubated in the absence of Rad51 for 20 h (lane 2). The DNA products were deproteinized and analyzed in 1.5% agarose gels. (*E*) Modulation of the RAD51 ATPase activity by salt. ATP hydrolysis by RAD51 (5 μM) was carried out at indicated NaCl concentrations in the absence of DNA or in the presence of either ϕX174 ssDNA (50 μM, nt) or dsDNA (50 μM, nt) as described in Experimental Procedures. RAD51-mediated DNA-independent ATP hydrolysis was subtracted at each salt concentration tested. Error bars represent SEM. (*F*) RecA (10 μM) was incubated with deproteinized JMs (0.32 nM) (lane 1) for 2 min and then the reaction was continued in the presence of SSB (55 nM and 0.4 μM, respectively). (*G*) RAD51 (10 μM) was incubated with deproteinized JMs (0.32 nM) for 15 min (lane 1) and then the reaction was continued in the presence of RPA (55 nM and 0.4 μM, respectively). The DNA products were analyzed by electrophoresis in a 1% agarose gel. Data are the mean of at least three experiments. Error bars represent SEM. The DNA substrates are shown in lanes 1 and 6.

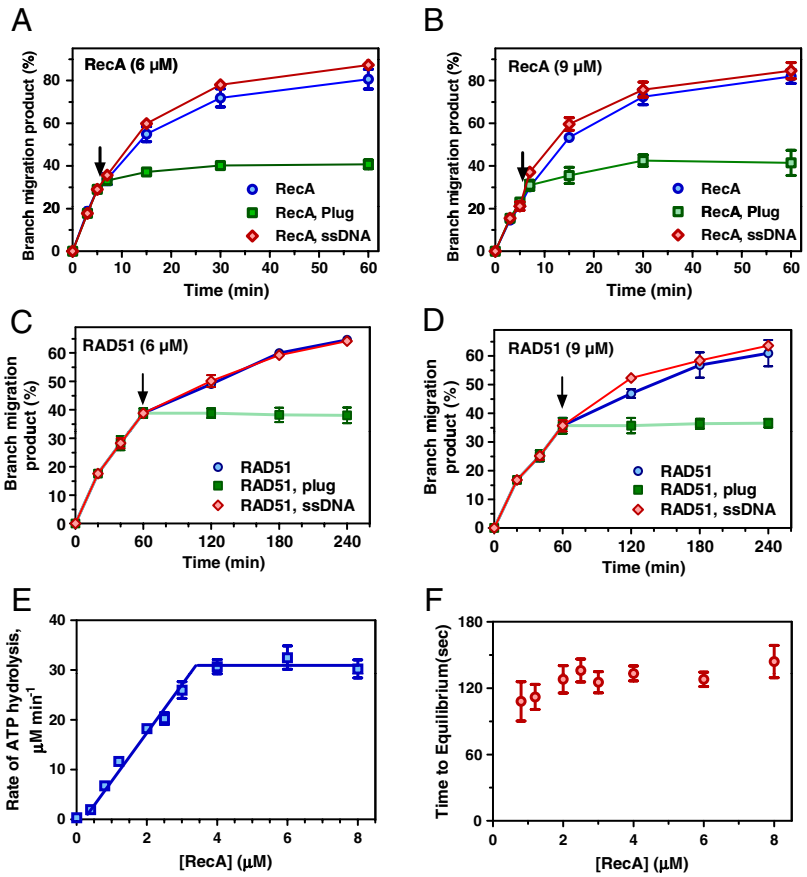


Fig. S5. An ongoing BM can be halted by an ssDNA-plug that blocks RecA/RAD51 binding to ssDNA. (*A*) and (*B*) BM was initiated on 5′-tail HJs (B1; 32 nM) by the addition of RecA (6 μM and 9 μM) and continued for 5 min. Then, the reaction mixture was divided between three test-tubes. In the first, the incubation was continued for another 55 min. In the second and third ssDNA (48 nM), which is complementary (B3) (ssDNA-plug) or noncomplementary (B4) to the 5′-tail ssDNA, respectively, were added followed by a 55 min incubation. Data are the mean of at least three experiments. Error bars represent SEM. (*C*) and (*D*) BM was initiated on 3′-tail HJs (A2; 32 nM) by the addition of RAD51 (6 μM and 9 μM) and continued for 1 h. Then, the reaction mixture was divided between three test-tubes. In the first, the incubation was continued for another 3 h. In the second and third ssDNA (48 nM), which is complementary (C2) (ssDNA-plug) or noncomplementary (C3) to the 3′-tail ssDNA, respectively, were added followed by a 3 h incubation. Data are the mean of at least three experiments. Error bars represent SEM. (*E*) The effect of RecA concentration on the rate of ATP hydrolysis. ATPase assays were carried using 5′-tailed HJs (B5; 7.5 μM, base pairs) as a DNA cofactor. (*F*) The amount of time required for the RecA ATPase reaction to reach the steady state. For each RecA concentration, we determined the period of time between the addition of RecA and the point at which the rate of ATP hydrolysis reached its maximum. This value was reported as the "time to equilibrium." Data are the mean of at least three experiments. Error bars represent SEM.

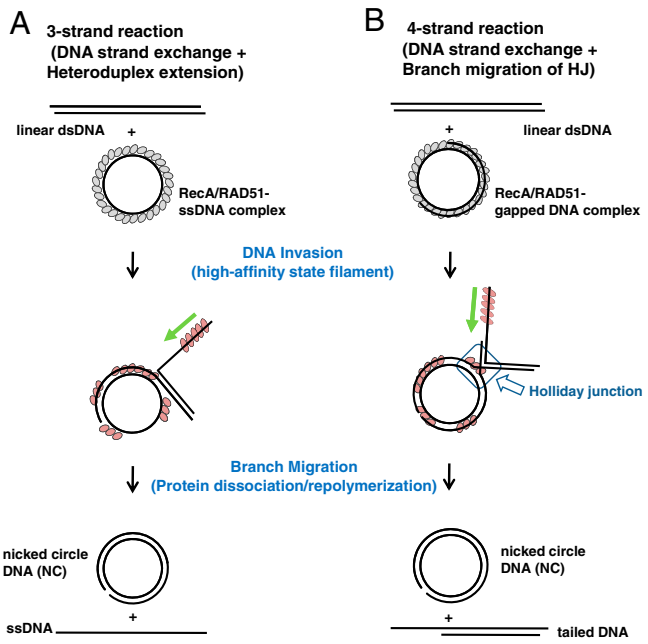


Fig. S6. RecA/RAD51 possesses two mechanistically distinct activities: DNA strand exchange and BM. (*A*) and (*B*) At the first step, both the 3-strand and 4-strand reactions are initiated by the invasion of the high-affinity state RecA/RAD51-ssDNA filament (gray ovals) into homologous duplex DNA. This step, at which one DNA strand of the duplex DNA is transferred to the complementary ssDNA residing within the filament to produce JMs, is known as DNA strand exchange. At the second step, RecA/RAD51 polymerization/dissociations (red ovals) on the displaced ssDNA strand of JMs promote BM in both the 3-strand and 4-strand reactions. This step requires a dynamic filament, in which RecA/RAD51 is prone to DNA dissociation and reassociation. There is a distinction in behavior of RecA and RAD51 nucleoprotein filaments during BM. The RecA filament remains in the high-affinity ATP-bound state in the presence of ATP, but still able to dissociate from DNA during ATP hydrolysis. In contrast, the RAD51 filament by hydrolyzing ATP converts into the low-affinity state that contains ~70% of ADP (1). We show in the current paper that this dissociation-prone form of the RAD51 filament is the most active in BM. One could envision a model in which the transition between the high-affinity and low-affinity filament states can be modulated by RAD51 mediator proteins.

1 Bugreev DV, Mazin AV (2004) Ca^{2+} activates human homologous recombination protein Rad51 by modulating its ATPase activity. *Proc Natl Acad Sci USA* 101:9988–9993.

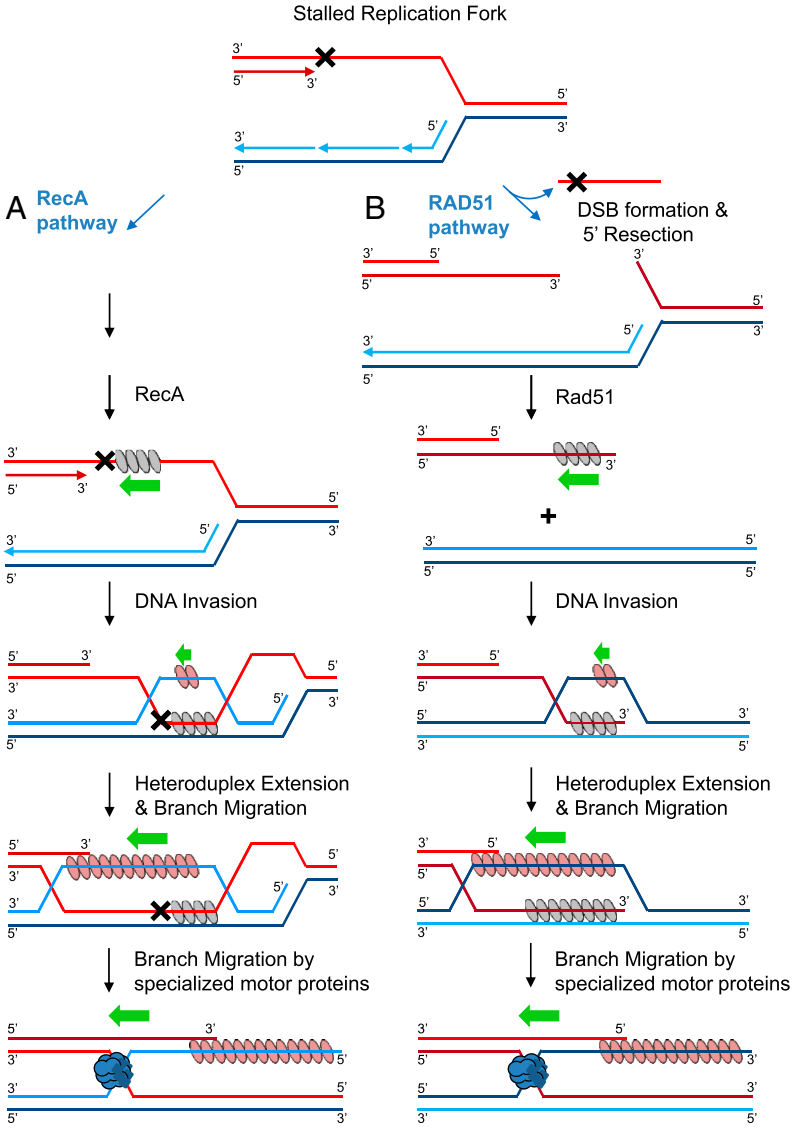
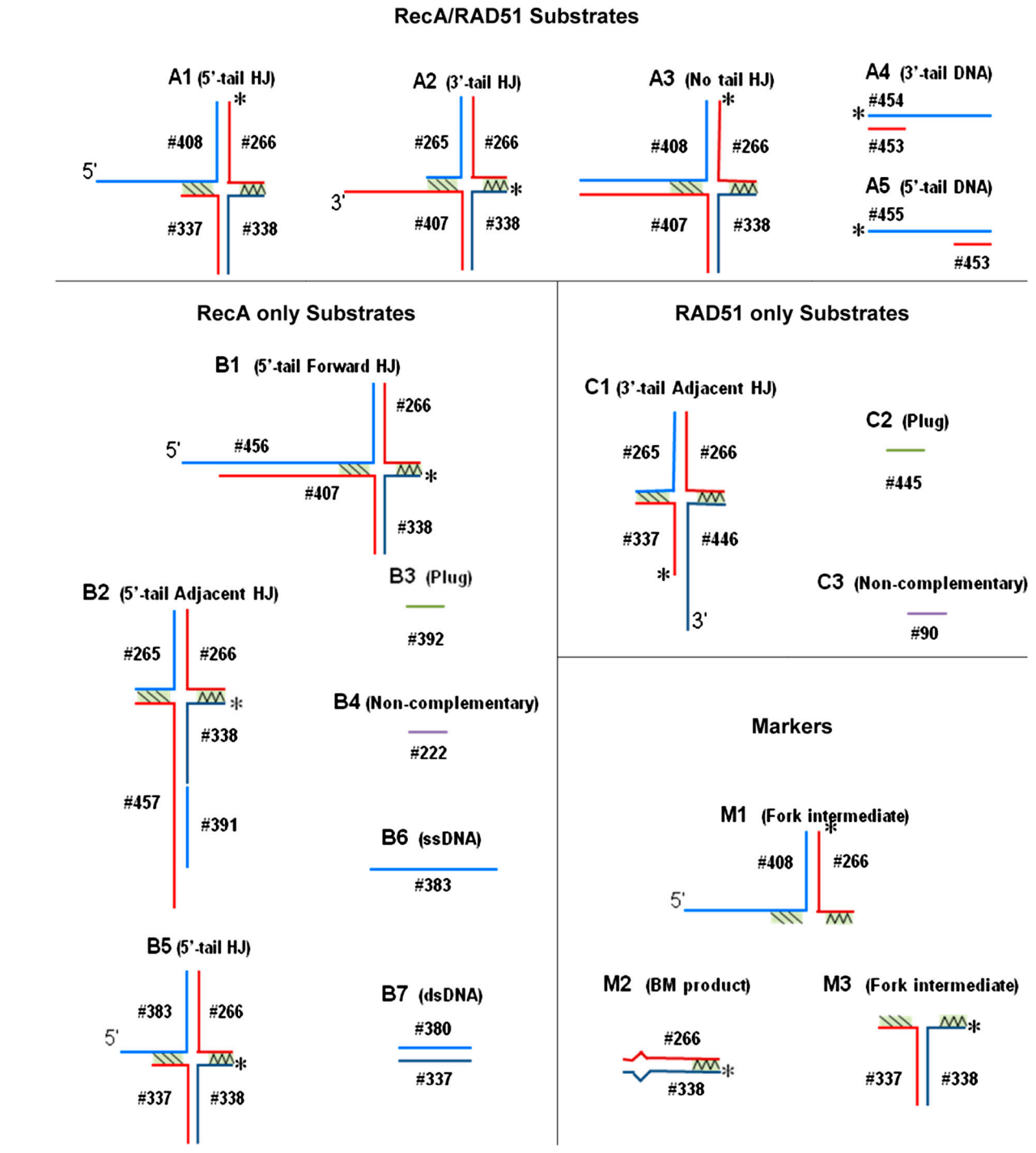


Fig. S7. A hypothetical role of RecA/RAD51 BM activity during stalled replication fork restart. A lesion (**x**) in the leading strand of DNA replication results in a stalled replication fork, exposing a region of ssDNA. (*A*) In the RecA pathway, the RecA filament (gray ovals) will form on the exposed ssDNA and promote invasion with the lagging strand template, forming a D-loop. Then cycles of RecA polymerization/dissociation on the displaced ssDNA lagging strand (red ovals) will stabilize the D-loop structure through BM to reach the 3′-OH end that can be used as a primer for DNA repair synthesis. Following the initial stabilization by RecA, specialized DNA translocating BM proteins (blue shape) take over and continue the BM reaction. (*B*) In the RAD51 pathway, the replication fork is initially cleaved creating a DNA double strand break (DSB), and then the lesion is excised by 5′ end resection to produce DNA with a 3′ ssDNA tail. With an exposed 3′ tail, RAD51 can promote DNA invasion to form a D-loop and then extend and stabilize this D-loop through BM. BM is then continued by specialized enzymes (blue shape).

Table S1. Oligonucleotide Substrates used in this study

Substrate structures *



Substrate sequences [†]

Number	Length, nt	Sequence, 5′ → 3′
#90	90	CGG GTG TCG GGG CTG GCT TAA CTA TGC GGC ATC AGA GCA GAT TGT ACT GAG AGT GCA CCA TAT GCG GTG TGA AAT ACC GCA CAG ATG CGT
#222	32	TC GTG GTG AGG AGA GGT CAG TGC TGC GGC
#265	60	CCT GCA TAC AGA TGT TGA CCC AGC ACT GAC TAC TGT CGT C**A**A TCA TCG TGC ATC ACA GTG
#266	60	CAC TGT GAT GCA CGA TGA T**T**G ACG ACA GTA GTC AGT GCT GCA GTG GTC AGG TGT CAT CAC
#337	60	CAC TGT GAT GCA CGA TGA T**C**G ACG ACA GTA GTC AGT GCT GGG TCA ACA TCT GTA TGC AGG
#338	60	GTG ATG ACA CCT GAC CAC TGC AGC ACT GAC TAC TGT CGT C**G**A TCA TCG TGC ATC ACA GTG
#380	60	CCT GCA TAC AGA TGT TGA CGC AGC ACT GAC TAC TGT CGT CGA TCA TCG TGC ATC ACA GTG
#383	90	ACA GTT AAA TTG CTA ACG CAG TCA GGC ACC CCT GCA TAC AGA TGT TGA CCC AGC ACT GAC TAC TGTCGT C**A**A TCA TCG TGC ATC ACA GTG
#391	90	GGT GCC TGA CTG CGT TAG CAA TTT AAC TGT GTA CAA TCT GCT CTG ATG CCG CAT AGT TAA GCC AGC CCC GAC ACC CAC AAT TAA GCT CTA
#392	34	AGC CAT CCG CAA AAA TGA CCT CTT ATC AAA AGG A
#407	150	CAC TGT GAT GCA CGA TGA T**C**G ACG ACA GTA GTC AGT GCT GGG TCA ACA TCT GTA TGC AGG GGT GCC TGA CTG CGT TAG CAA TTT AAC TGT GTA CAA TCT GCT CTG ATG CCG CAT AGT TAA GCC AGC CCC GAC ACC CAC AAT TAA GCT CTA
#408	150	TAG AGC TTA ATT GTG GGT GTC GGG GCT GGC TTA ACT ATG CGG CAT CAG AGC AGA TTG TAC ACA GTT AAA TTG CTA ACG CAG TCA GGC ACC CCT GCA TAC AGA TGT TGA CCC AGC ACT GAC TAC TGT CGT C**A**A TCA TCG TGC ATC ACA GTG
#445	90	TAG AGC TTA ATT GTG GGT GTC GGG GCT GGC TTA ACT ATG CGG CAT CAG AGC AGA TTG TAC ACA GTT AAA TTG CTA ACG CAG TCA GGC ACC
#446	150	GTG ATG ACA CCT GAC CAC TGC AGC ACT GAC TAC TGT CGT C**G**A TCA TCG TGC ATC ACA GTG GGT GCC TGA CTG CGT TAG CAA TTT AAC TGT GTA CAA TCT GCT CTG ATG CCG CAT AGT TAA GCC AGC CCC GAC ACC CAC AAT TAA GCT CTA
#453	30	CTG CTC AAT TCG GAT CCC TAC CAC TTG TCC
#454	90	GGA CAA GTG GTA GGG ATC CGA ATT GAG CAG TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT
#455	90	TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT GGA CAA GTG GTA GGG ATC CGA ATT GAG CAG
#456	184	TCC TTT TGA TAA GAG GTC ATT TTT GCG GAT GGC TTA GAG CTT AAT TGT GGG TGT CGG GGC TGG CTT AAC TAT GCG GCA TCA GAG CAG ATT GTA C ACA GTT AAA TTG CTA ACG CAG TCA GGC ACC CCT GCA TAC AGA TGT TGA CCC AGC ACT GAC TAC TGT CGT C**A**A TCA TCG TGC ATC ACA GTG
#457	184	TCC TTT TGA TAA GAG GTC ATT TTT GCG GAT GGC TTA GAG CTT AAT TGT GGG TGT CGG GGC TGG CTT AAC TAT GCG GCA TCA GAG CAG ATT GTA C ACA GTT AAA TTG CTA ACG CAG TCA GGC ACC CAC TGT GAT GCA CGA TGA T**C**G ACG ACA GTA GTC AGT GCT GGG TCA ACA TCT GTA TGC AGG

*The asterisk denotes the ^{32}P label; and hatched shading denotes regions () of heterology. Preparation of these substrates is described in Experimental Procedures.

For RecA/RAD51 substrates, the following forked DNA intermediates were annealed #408/266* and #337/338 (A1, 5′-tail HJ), #407/338* and #265/266 (A2, 3′-tail HJ), and #408/266* and #407/338 (A3, no tail HJ).

For RecA only substrates, the following forked DNA intermediates were annealed #456/266 and #407/338* (B1, 5′-tail Forward HJ), #265/266 and #457/391/338* (B2, 5′-tail Adjacent HJ), and #383/266 and #337/338* (B5, 5′-tail HJ).

For RAD51 only substrates, the following forked DNA intermediates were annealed #446/337* and #265/266 (C1, 3′-tail Adjacent HJ) were annealed.

[†] The **single bold letters** indicate the nucleotides that form mismatched pairs in the BM products as shown in Table S1.

Table S2. BM rates on plasmid-based Holliday Junction substrates

BM rate	References
RuvAB	
Biochemical (bulk) BM assays *	
10–20 bp/s	(1)
34 bp/s ‡	(2)
Single molecule method	
49 ± 1.5 bp/s	(3), corrected (10)
43 ± 4.3 bp/s	(4)
RecA *(Biochemical BM assay)* *	
3 ± 4 bp/s	(5, 6)
3.4 bp/s ‡	this study
RecG *(Biochemical BM assay)**	
8 ± 40 bp/s †	(7)
Human RAD54 *(Biochemical BM assay)* *	
34 bp/s ‡	(8)
Human RAD51 *(Biochemical BM assay)* *	
0.3 bp/s ‡	this study

*The rate was determined by the earliest appearance of the BM product in an agarose gel.
†245 bp/s rate of replication fork regression was reported for RecG (9).
‡The same DNA substrate were used in these experiments.

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