Supporting Information

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SI Materials and Methods

Proteins. RecQ (1), SSB (2), RecA (3), RecF (4), and RecO and RecR (5) were purified as described. Klenow fragment (3′→5′ exo⁻) and restriction endonucleases were purchased from New England Biolabs.

RecJ was purified as described previously (6) with some modifications as follows. *Escherichia coli* BL21 (DE3) containing pLysS (7) and pRDK115 (6) was used for the overexpression of RecJ. Overexpression of soluble RecJ nuclease in cell extracts required using a fresh transformant and as well as a temperature for expression lower than 25 °C. A fresh transformant of BL21 (DE3) (containing pLysS) with plasmid pRDK115 was plated onto LB containing 100 μg/mL ampicillin and 25 μg/mL chloramphenicol, and was incubated at 37 °C overnight. To two plates (200–500 colonies on each), several milliliters of LB medium were added and the all colonies were scraped off. This suspension was added to 1.2 L of LB medium with 100 μg/mL ampicillin. The culture was grown at 30 °C until OD_{600} reached 0.5. Isopropyl β-D-1-thiogalactopyranoside was added at final concentration of 100 μg/mL and incubation was continued for 24 h at 20 °C. About 50% of RecJ was found in the supernatant fraction after cell lysis and ultracentrifugation. Generally, the centrifugation was done at 7,000 × *g* for 10 min in the following steps. To the 17 mL of the crude extract (A_{260} = 40) in TEMG buffer [20 mM Tris·HCl (pH 7.5), 0.1 mM EDTA, 5 mM 2-mercaptoethanol, and 10% (vol/vol) glycerol], polyethylenimine was added to a final concentration of 0.5%. After stirring at 4 °C for 30 min, the sample was centrifuged. The precipitate was suspended in 20 mL of TEMG buffer containing 80 mM ammonium sulfate and stirred at 4 °C for 30 min. After centrifugation, solid ammonium sulfate was added to the supernatant at a final concentration of 50% saturation, and stirred at 4 °C for 30 min. After centrifugation, the precipitate was suspended in 5 mL of TEMG buffer containing 0.1 M NaCl. To adjust the sample's conductivity to the same value as that of 0.1 M NaCl, 10.8 mL of TEMG buffer was added to the sample. Then, RecJ was purified by using PBE94 (Amersham) and hydroxyapatite (Bio-Rad) columns as described previously (6), but without the Cibacron Blue column. The specific activity of the final fraction was determined to 2.4×10^5 U/mg, which is similar to the previously reported value (6). About 1.3 mg of RecJ nuclease was obtained from 1.2 L culture by this method.

The overexpression vector for SSB lacking eight amino acids from the C terminus was constructed by PCR followed by insertion of the NdeI-BamHI fragment into pET-3a (7) and named pT7-SSB-ΔC8. The sequences of primers used for PCR are 5′-CGGAATTCCATATGGCCAGCAGAGGCG-3′ and 5′-CGGATCCGGATTACATCGGCGGCTCGTTAGACG-3′. The protein was expressed in *E. coli* BL21 (DE3) carrying pLysS and pT7-SSB-ΔC8 at 37 °C and purified as reported previously (2), but with the following modifications. HiTrapQ HP (Amersham) was used instead of ssDNA cellulose. The precipitate after ammonium sulfate was suspended in TEMG buffer containing 0.2 M NaCl, and the conductivity of sample was adjusted to that of TEMG buffer with 0.2 M NaCl by adding TEMG buffer. The sample was then loaded onto a HiTrapQ HP column (5 mL) previously equilibrated with TEMG buffer containing 0.2 M NaCl. The flow-through fraction was pooled; the pH was adjusted to 8.5 by adding Tris-base; and the sample was again loaded onto a HiTrapQ HP column previously equilibrated with TEMG (pH 8.5) buffer with 0.2 M NaCl. The flow-through fraction was pooled and dialyzed against stock buffer containing 50 mM Tris·HCl (pH 7.5), 1 mM EDTA, 200 mM NaCl, 2 mM DTT, and 50% (vol/vol) glycerol.

DNA Substrates. Plasmid pUC19 DNA (8) was prepared by alkaline lysis followed by centrifugation in ethidium bromide and cesium chloride. Synthetic DNA oligonucleotides, KAT-11, KAT-12, KAT-21, and KAT-22 were purchased from OPERON and purified by PAGE. The sequences of KAT-11 (80 nt) and KAT-21 (40 nt) were nucleotides 640–719, and 640–679 from pUC19 (KAT-11: 5′-TGAATCGGCCAACGCGCGGGGAGAGGCG-GTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCAC-TGACTCGCTGCGCTC-3′; KAT-21: the same as 5′-half of KAT-11). KAT-12 (80 nt) and KAT-22 (40 nt) are complimentary to KAT-11 and KAT-21.

Linear dsDNA with different end structures used for joint molecule formation was prepared as follows. DNA with a 4-nt overhang was prepared by digestion of pUC19 supercoiled DNA with EcoRI or HindIII. DNA with a blunt-end was prepared by end-filling using Klenow fragment (exo⁻) with a mixture of dGTP, dATP, dCTP, and dTTP (dNTP). For the preparation of 3-, 2-, and 1-nt overhangs at the EcoRI site, only ddATP, dATP, and dATP/ddTTP were used in the end-filling reaction, respectively. For the preparation of 3-, 2-, and 1-nt overhangs at the HindIII site, only ddATP, dATP/ddGTP, and dNTP/ddCTP were used, respectively. The concentrations of deoxy- and dideoxynucleoside triphosphates were 0.1 and 16.7 μM, respectively. The end-filling reaction was done at room temperature for 30 min, followed by inactivation of enzyme at 65 °C for 30 min.

^{32}P-labeled linear dsDNA was prepared by mixing 1.6 pmol (molecule) of NdeI-digested pUC19, 3.3 pmol [α-^{32}P]dATP, 25 pmol dTTP, and 5 U Klenow-fragment (exo⁻) in 59 μL of buffer containing 10 mM Tris·HCl (pH7.5), 5 mM $MgCl_2$, and 7.5 mM DTT. After incubation at room temperature for 30 min, 5 pmol of cold dATP was added and incubation was continued for 5 min; the enzyme was denatured by incubation at 65 °C for 30 min. Finally, unincorporated nucleotides were eliminated using a Microspin G-25 (GE Healthcare) spin column.

The labeled linear dsDNA was dissolved at final concentration of 20 μM (nt) in buffer containing 40 mM Tris-acetate (pH 7.5), 20 mM magnesium acetate, 0.2 mg/mL BSA, 2 mM DTT, and 10% (vol/vol) glycerol. Eight different restriction enzymes were used to generate the 4-nt 5′-overhangs (EcoRI, SalI, and HindIII), 2-nt 5′-overhang (AccI), blunt-ends (SmaI and HincII), and 4-nt 3′-overhangs (KpnI and PstI). To cleave the dsDNA at its multicloning site, the indicated restriction enzyme was added at a final concentration of 0.4 U/μL and the sample was incubated at 37 °C for 90 min. After the inactivation of restriction enzyme at 65 °C for 30 min, the DNA was used in the resection assay.

Preparation of the Maxam–Gilbert Ladder. Markers for the DNA sequencing gel were prepared by cleaving 1.6 pmol (molecule) of NdeI-cleaved pUC19 (^{32}P-labeled, as described above), with EcoO109I. After inactivating the enzyme at 65 °C for 30 min, the products were separated by electrophoresis using a low-melting agarose gel (0.8%) in 1× TAE buffer. The larger band was cut out, diluted 10× by TE buffer [10 mM Tris·HCl (pH 7.5) and 1 mM EDTA], melted by incubation at 65 °C, and frozen at −20 °C overnight. After centrifugation of the thawed sample at 7,000 × *g* for 5 min, the supernatant was taken and divided into 10 tubes (each contained 450 μL). The aliquots were kept at −20 °C until used. To precipitate the DNA, 50 μL of 3 M sodium acetate (pH 7.5) and 1 mL of ethanol were added to 450 μL of the DNA

solution. After the incubation on ice for 30 min, DNA was collected by centrifugation at 7,000 × *g* for 5 min, rinsed with 95% (vol/vol) ethanol, dried, and suspended in 20 μL of water. To 10 μL of the DNA solution, 25 μL of formic acid was added, and the sample was incubated for 2 min at room temperature. Alternatively, to the other 10 μL of DNA solution, 30 μL of hydrazine was added and sample was incubated for 4 min. The formic acid and hydrazine reactions were stopped by adding 200 μL of hydrazine stop buffer [0.3 M sodium-acetate (pH 7.5), 0.1 mM EDTA, and 25 μg/mL calf-thymus DNA] and 750 μL ethanol, then chilled in an ice-KCl bath (−7 °C). The following reactions were done for both the samples simultaneously. After centrifugation at 7,000 × *g* for 5 min, the supernatant was removed and the precipitate was washed with 95% (vol/vol) ethanol twice and then suspended in 200 μL of water. After the reprecipitation by addition of 20 μL of 3 M sodium acetate and 500 μL ethanol, followed by incubation at −20 °C for 1 h and centrifugation at 7,000 × *g* 5 min, the precipitate was rinsed with 95% (vol/vol) ethanol twice, dried, and then suspended in 70 μL of 10% (vol/vol) piperidine. After incubation at 90 °C for 30 min, the sample was dried, suspended in 30 μL of H_2O, dried, suspended in 20 μL of H_2O, and then dried again. The sample was finally suspended in 10 μL of formamide mix and incubated at 90 °C for 10 min before loading onto the sequencing gel.

DNA Unwinding Assays. To prepare ^{32}P-labeled dsDNA, KAT-11 and KAT-21 were end-labeled by using [γ-^{32}P]ATP and T4-polynucleotide kinase (New England Biolabs). The 80-bp blunt-ended dsDNA, 40-bp blunt-ended dsDNA, and dsDNA with a 3′-ssDNA overhang were prepared by annealing cold KAT-12, KAT-22, and KAT-22 to ^{32}P-KAT-11, ^{32}P-KAT-21, and ^{32}P-KAT-11, respectively. The sample contained 1 nM (molecule concentration) ^{32}P-DNA and 1-μM SSB protein in 15 μL of buffer [20 mM Tris-acetate (pH 7.5), the indicated concentration of magnesium acetate, 1 mM ATP, 1 mM PEP, 40 U/mL pyruvate kinase, 1 mM DTT, and 0.1 mg/mL BSA]. After preincubation at 37 °C for 5 min, RecQ (final concentration of 10 nM) was added and the samples were further incubated for 5 min. To the 15-μL sample at 0 or 5 min after adding RecQ, 1.5 μL of solution containing 5% SDS and 250 mM EDTA was added. The samples were kept on ice until all were collected, then treated by Proteinase K by adding 3 μL of 1 mg/mL Proteinase K solution containing 0.05% bromophenol blue and 50% (vol/vol) glycerol, and incubated at 37 °C for 30 min. The samples were analyzed by 12% (wt/vol) polyacrylamide gel in 1× TAE buffer and autoradiography. The amount of product at 5 min was quantified as a measure of unwinding.

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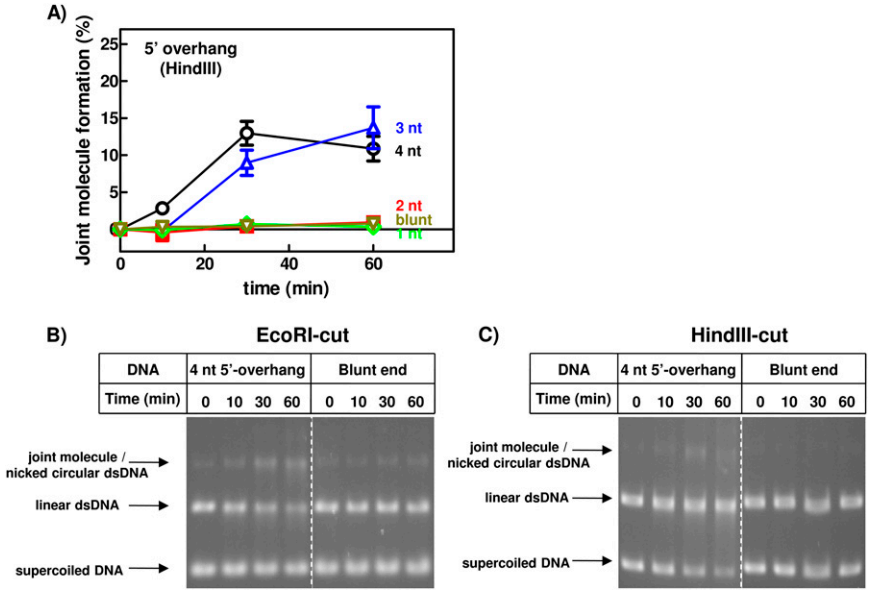

Fig. S1. The reaction conditions are the same as those in Fig. 1*C*, except that HindIII-cut pUC19 DNA with different end structures was used. (*A*) Effect of linear DNA-end structure on joint molecule formation was investigated using HindIII-cut pUC19 DNA that was filled-in to produce the overhangs indicated. Joint molecule assays, such as those shown in Fig. 1*B*, were quantified to determine the percentage of the limiting linear dsDNA that was converted into joint molecules. Each experiment was performed twice; the error bars show SE, unless smaller than the symbol. (*B*) The reaction conditions are the same as those in Fig. 1*B*, except that EcoRI-cut pUC19 (*Left*) and both EcoRI-cut and Klenow-filled pUC19 (*Right*) were used. (*C*) The reaction conditions are the same as those in Fig. 1*B*, except that HindIII-cut pUC19 (*Left*) and both HindIII-cut and Klenow-filled pUC19 (*Right*) were used.

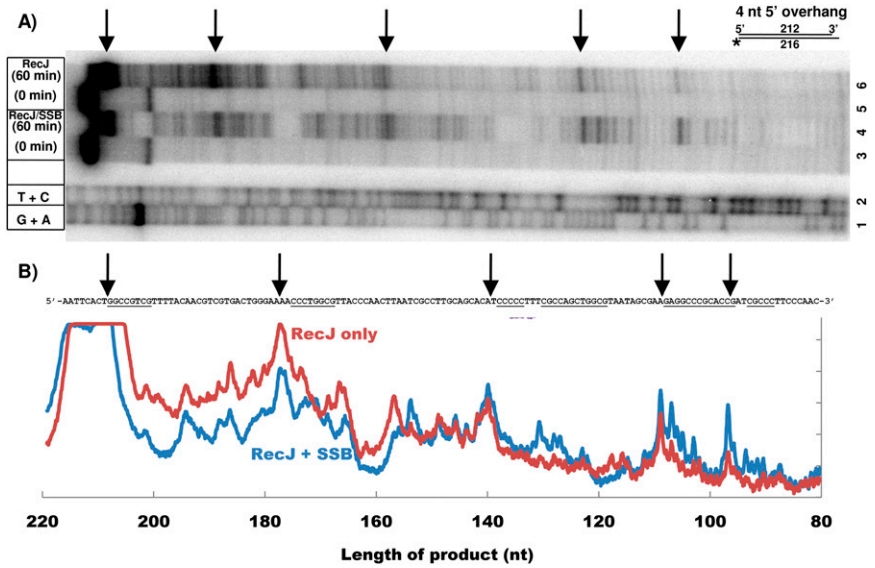

Fig. S2. RecJ nuclease frequently stops near GC-rich sequences during resection of dsDNA. (*A*) ^{32}P-labeled EcoRI-cut pUC19 dsDNA was treated with RecJ and SSB (lanes 3 and 4) or RecJ alone (lanes 5 and 6). Analysis was at 0 and 60 min by PAGE assay. Maxam–Gilbert sequencing samples were loaded in lanes 1 and 2. Electrophoresis direction is left to right. Note that the DNA was 3′-end–labeled, so the Maxam–Gilbert sequencing ladder is 5′ to 3′ from left to right (*SI Materials and Methods*). Black arrows show the five major products observed both in the presence and absence of SSB. (*B*) Scan of band intensities from lanes 4 and 6 in *A* (blue and red lines, respectively). Horizontal scale is calculated from the Maxam–Gilbert sequencing ladder (lanes 1 and 2 in *A*). Black arrows were as in *A*. The underlined sequence shows regions with five or more guanosine/cytidine nucleotides in a 6-nt span.

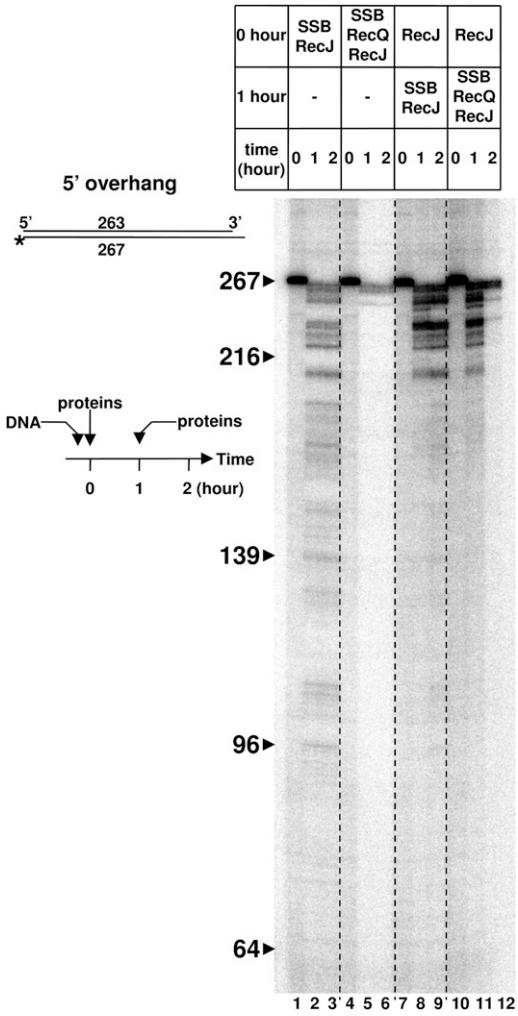

Fig. S3. RecJ nuclease does not reinitiate degradation on resection intermediates. The resection of ^{32}P-labeled, HindIII-cut pUC19 DNA was performed in two steps and analyzed by PAGE. The order of addition of each component is shown at the left side of figure. At 0 or 1 h, the proteins indicated at the top of figure were added. When the SSB and RecJ were added at 0 h and nothing was added at 1 h (lanes 1–3), the partially degraded intermediates accumulated at 1 h (lane 2) and remained after a 2-h incubation (lane 3). When RecQ helicase was also added at 0 h (lanes 4–6), most of substrate was almost completely degraded, except for the products resected a few nucleotides both at 1 and 2 h (lanes 5 and 6). When only RecJ was added at 0 h (lanes 7–9), the resection products after 1 h were longer than in the presence of SSB (compare lanes 2 and 8). Most of these intermediates could not be degraded further after subsequent addition of RecJ and SSB and further incubation (lane 9). This observation demonstrates that the RecJ cannot reinitiate resection on the accumulated intermediates. However, when RecQ was also added (lanes 10–12), the intermediate molecules were degraded upon subsequent addition of RecJ and SSB, showing that RecQ is required for reinitiation of resection from DNA intermediates with 3′-ssDNA tails.

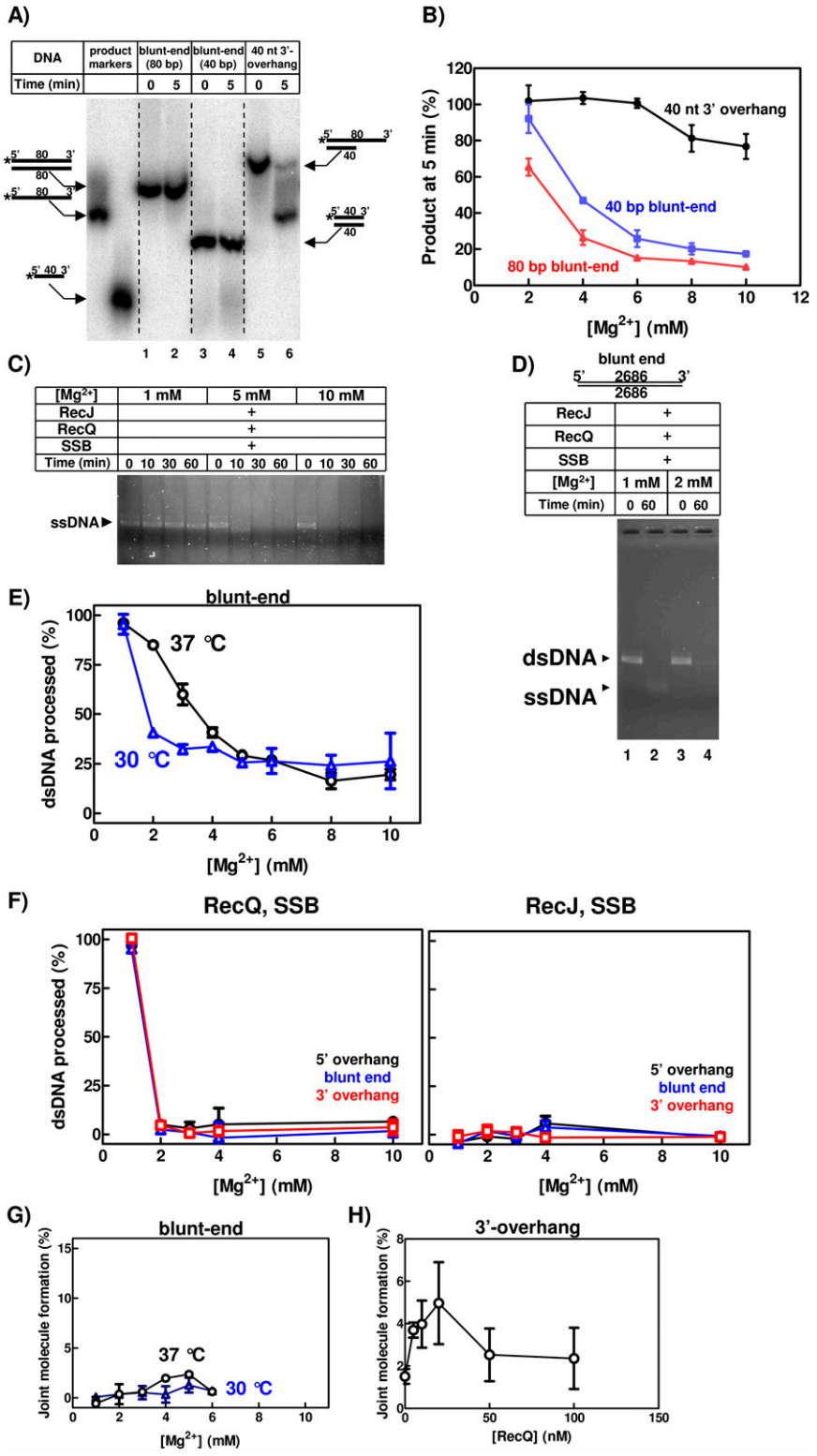

Fig. S4. (*A*) Unwinding of ^{32}P-labeled DNA with blunt-ends or a 3′-ssDNA overhang by RecQ helicase in 10 mM magnesium acetate with SSB protein assayed by native PAGE (*SI Materials and Methods*). DNA substrates (80-bp dsDNA, 40-bp dsDNA, and 40-bp dsDNA with 40-nt 3′-ssDNA overhang) are depicted on both sides of the gel image. (*B*) Quantification and plot of DNA unwinding product at 5 min as a function of Mg^{2+} concentration. The experiments were performed twice and error bars show SE, unless smaller than the symbol. (*C*) Resection of ssDNA by RecJ nuclease is enhanced at increasing concentrations of Mg^{2+}. HincII-cut pUC19 DNA was first unwound by RecQ in the presence of 1 mM Mg^{2+}, then treated with RecJ nuclease in the presence of the indicated concentration of magnesium acetate. (*D*) Extensive resection of blunt-ended DNA by RecQ, RecJ, and SSB at 37 °C was measured using the agarose gel assay. The concentration of magnesium acetate was either 1 or 2 mM. In 1 mM, the reaction was mostly unwinding by RecQ (lane 2); in 2 mM, a smear below the dsDNA appeared (lane 4), indicating that resection occurred. (*E*) Quantification of dsDNA processed in 60 min in *D* is plotted as a function of time for reactions at 30 °C (blue triangles)

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vs. 37 °C (black circles); the data at 30 °C are the same as those in Fig. 4*B* and are included here to permit comparison with the 37 °C data. The experiments were performed twice and error bars show SEs, unless smaller than the symbol. "dsDNA processed" represents the loss of full-length dsDNA substrate at 60 min. Note that at 1 mM Mg^{2+}, this value represents mainly unwinding by RecQ helicase. (*F*) Quantification and plot of DNA processed in 60 min as a function of Mg^{2+} concentration using the agarose gel assay as in Fig. 4, in the presence of RecQ and SSB but absence of RecJ (*Left*), and in the presence of RecJ and SSB but absence of RecQ (*Right*). The experiments were performed twice and error bars show SEs, unless smaller than the symbol. (*G*) Plot of joint molecule formation in 60 min as a function of Mg^{2+} concentration using the SmaI-cut pUC19 as the linear dsDNA substrate. The reaction was performed at either 30 °C or 37 °C. The experiments were performed twice, and error bars show SEs, unless smaller than the symbol. (*H*) Joint molecule formation as a function of RecQ helicase concentration. DNA with a 3′-ssDNA overhang (PstI-cut pUC19) and supercoiled dsDNA were used as substrates; the assay time is 60 min. The magnesium acetate concentration was 5 mM. Other conditions were the same as the experiments in Fig. 1. The experiments were performed twice, and error bars show SEs.