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# Genetic recombination in *Escherichia coli*: Holliday junctions made by RecA protein are resolved by fractionated cell-free extracts

(nucleoprotein filaments/*recB sbcB(C)*/resolution/nuclease)

BERNADETTE CONNOLLY AND STEPHEN C. WEST*

Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Herts, EN6 3LD, United Kingdom

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ABSTRACT **_Escherichia coli_ RecA protein catalyzes reciprocal strand-exchange reactions between duplex DNA molecules, provided that one contains a single-stranded gap or tail, to form recombination intermediates containing Holliday junctions. Recombination reactions are thought to occur within helical RecA–nucleoprotein filaments in which DNA molecules are interwound. Structures generated *in vitro* by RecA protein have been used to detect an activity from fractionated *E. coli* extracts that resolves the intermediates into heteroduplex recombinant products. Resolution occurs by specific endonucleolytic cleavage at the Holliday junction. The products of cleavage are characteristic of patch and splice recombinants.**

General genetic recombination in wild-type *Escherichia coli* is dependent upon the product of the *recA* gene (1). *In vitro*, RecA protein catalyzes homologous pairing and strand-exchange reactions leading to the formation of heteroduplex DNA (for review, see refs. 2–4). An intermediate in the reaction is a Holliday junction in which two homologous duplex molecules are linked by a crossover point (5). Although RecA protein is required for the formation of Holliday junctions, it plays no part in the process by which junctions are resolved to give recombinant products.

Attempts to identify a junction-specific nuclease from *E. coli* have concentrated on *in vitro* studies using synthetic DNA substrates. So far these efforts have failed to detect such a protein from *E. coli*, although similar studies have led to the identification of proteins from bacteriophage T4 (6, 7), bacteriophage T7 (8), *Saccharomyces cerevisiae* (9–11), and mammalian cells (12). While synthetic junctions are useful for the study of junction geometry and the characterization of reactions carried out by junction-specific nucleases (for review, see ref. 13), they lack several features that are essential to the structure of a true Holliday junction. Most importantly, synthetic junctions lack homology between arm sequences, and branch migration is unable to occur. Moreover, they are protein-free and may not be representative of recombination structures found *in vivo*.

The ability of RecA protein to generate Holliday junctions *in vitro* (14–16) led us to an alternative approach for the detection of a Holliday junction-specific nuclease from *E. coli*. In RecA-promoted strand-exchange reactions between gapped circular and linear duplex DNA, Holliday junctions are produced in an efficient manner (17). In these reactions, strand exchange occurs within a RecA–nucleoprotein filament (for review, see ref. 3) in which the Holliday junctions are likely to be of unique structure. This structure has been used to screen *E. coli* extracts for a Holliday junction-resolving protein. The detection and preliminary characterization of an activity that acts upon such structures are described.

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## MATERIALS AND METHODS

**Enzymes and Proteins.** Purified phage T4 endonuclease VII (60,000 units per μl) was a gift of Börries Kemper (University of Cologne, Cologne, F.R.G.). Phage T7 endonuclease I (0.3 mg/ml) was purified by a modification of a published procedure from strain BL21 (DE3) carrying pLysS and the T7 endonuclease I overexpression plasmid pAR2471 (8, 18). *E. coli* RecA protein was prepared as described (19). Concentrations of RecA in the text refer to moles of monomeric protein.

**DNA Substrates.** φX174 gapped circular duplex DNA (gDNA) was prepared with a 162-nucleotide single-stranded gap between the *Pst* I and *Ava* I restriction sites (20). Linearized φX174 replicative form I DNA was 3′-end-labeled by using dideoxy[α-$^{32}$P]ATP and terminal transferase (Amersham). DNA with a unique 3′-end-label was made by *Ava* II restriction digestion of 3′-end-labeled *Pst* I-linearized ΦX174 duplex DNA, and the large fragment was purified as described (17). Linearized pCJ10 DNA (17) was 3′-end-labeled by using dideoxy[α-$^{32}$P]ATP or 5′-end-labeled by using [γ-$^{32}$P]ATP (Amersham) and T4 DNA polynucleotide kinase. All DNA concentrations refer to moles of nucleotide residues.

**Assay for Resolution.** Reactions were performed at 37°C. Complexes between RecA and gDNA were formed in 20 mM Tris·HCl, pH 7.5/25 mM $MgCl_2$/2 mM dithiothreitol/2 mM ATP/100 μg of bovine serum albumin per ml by incubation for 5 min. Strand exchange was initiated by addition of linear duplex DNA, and incubation was continued for 10 min. *E. coli* extracts or T4 endonuclease VII were then added, and reactions were incubated for 10 min. Reactions were stopped and deproteinized by addition of 0.1 vol of stop buffer [20 mg of proteinase K per ml/5% (wt/vol) SDS/100 mM Tris·HCl, pH 7.5/50 mM EDTA] followed by a 10-min incubation.

**Analysis of DNA.** Electrophoresis of DNA was carried out in 0.7% agarose gels with 40 mM Tris·HCl, pH 7.9/5 mM sodium acetate/1 mM EDTA or under denaturing conditions in 5% polyacrylamide gels with 89 mM Tris borate, pH 8.3/2 mM EDTA/7 M urea. Denaturing gels were fixed in 10% (vol/vol) methanol/10% (vol/vol) acetic acid. Gels were dried, and DNA was visualized by autoradiography on Kodak XAR film. Densitometry was performed with an LKB Ultroscan XL laser densitometer.

**Cell Growth.** *E. coli* strain NH5033 *recB21 sbcB15* (*sbcC*) *endA1* (21) was grown in broth (32 g of tryptone, 20 g of yeast extract, 5 g of NaCl, 10 g of $K_2HPO_4$, 1.85 g of $KH_2PO_4$, and 10 mg of vitamin $B_1$ per liter) in a 12-liter fermenter at 37°C to an $OD_{650}$ of 5.5. Cells (172 g) were harvested and resuspended at 3 ml/g in ice-cold 50 mM Tris·HCl, pH 7.5/25% (wt/vol) sucrose. Aliquots were frozen and stored at −70°C.

**Preparation of Cell Extracts.** Cell paste (43 g) was thawed at room temperature, and all further steps were performed at

Abbreviation: gDNA, gapped circular duplex DNA.

*To whom reprint requests should be addressed.

4°C. Lysozyme [5 mg/ml in 0.25 M Tris·HCl (pH 8)] was added to 0.83 mg/ml, and the mixture was incubated for 15 min. EDTA (0.5 M) was added to 6.6 mM, and incubation was continued for 10 min. To lyse the cells, Brij 58 [1% (wt/vol) in 50 mM Tris·HCl, pH 8/2 mM dithiothreitol] was added to 0.5%, and incubation was continued for 30 min with gentle stirring. Cell debris was removed by centrifugation for 1 hr at 35,000 rpm in a Beckman Ti 45 rotor. Protein was precipitated from the supernatant (fraction I: 420 ml at 40 mg of protein per ml) by addition of 0.35 g of ammonium sulfate per ml and recovered by centrifugation. The pellet was resuspended in 156 ml of R buffer [50 mM Tris·HCl, pH 7.5/1 mM EDTA/1 mM dithiothreitol/10% (vol/vol) glycerol] and dialyzed overnight against 4 liters of R buffer containing 50 mM KCl (fraction II: 170 ml at 61 mg of protein per ml).

Fraction II was loaded onto a 125-ml DEAE Bio-Gel A column equilibrated with R buffer containing 50 mM KCl. The column was eluted with a 50–400 mM KCl gradient in R buffer (800 ml), and 2-μl aliquots of each fraction were assayed for cleavage of strand-exchange intermediates as described above. Peak activity was eluted at 170–210 mM KCl. Active fractions were pooled (fraction III: 80 ml at 5.6 mg/ml), diluted with an equal volume of R buffer, and applied to an 8-ml phosphocellulose column equilibrated with R buffer containing 50 mM KCl. The phosphocellulose column was eluted with a 50–650 mM KCl gradient in R buffer (100 ml). Peak activity was eluted at 390–440 mM KCl. Active fractions were pooled and dialyzed against 2 liters of R buffer containing 50 mM KCl. Proteins were concentrated by readsorption onto a 2-ml phosphocellulose column and were eluted with 500 mM KCl in R buffer. The protein eluate was dialyzed for 1 hr against R buffer containing 50 mM KCl (fraction IV: 2.5 ml at 2 mg/ml) and stored at −70°C. Working stocks were stable at −20°C.

## RESULTS

**Assay for Resolution.** RecA protein promotes strand exchange between gDNA and homologous linear duplex DNA to generate intermediates that contain Holliday junctions (15) (reaction A in Fig. 1). Previous results have shown that addition of T4 endonuclease VII leads to resolution of the Holliday junction (17). The products of resolution are linear dimer (cleavage at b and d in Fig. 1) and nicked circular and nicked linear (cleavage at a and c) DNA molecules. The linear dimer has a unique electrophoretic mobility in agarose gels with respect to both the DNA substrates and products of complete strand exchange. To detect Holliday junction-resolving activity in fractionated *E. coli* extracts, we used the formation of linear dimer DNA as an assay.

To overcome the problem of degradation of the DNA substrates, extracts of strain NH5033 deficient in exonuclease V (*recBCD*), exonuclease I (*sbcB*), and endonuclease I (*endA*) were used (21).

**Fractionation of Cell Extracts.** Cell-free extracts were prepared and fractionated by DEAE and phosphocellulose chromatography as described. The elution profile of a representative phosphocellulose column is shown in Fig. 2. An aliquot of each fraction was assayed by addition to an on-going RecA-promoted strand-exchange reaction between gDNA and uniformly $^{32}$P-labeled linear duplex DNA (reaction A in Fig. 1). The products of the reaction were visualized by agarose gel electrophoresis. The action of RecA protein alone on these substrates led to complete strand exchange as seen by the formation of nicked circular duplex DNA (Fig. 2, lane n). In contrast, resolution of the reaction intermediates by T4 endonuclease VII produced a linear dimer (Fig. 2, lane m). When aliquots of the phosphocellulose fractions (Fig. 2, lanes a–l) were included in the reaction, we observed the formation of a band that comigrated with the T4 endonuclease VII-generated linear dimer. Since the activity was weak, peak fractions (18–20) were concentrated 10-fold by readsorption and elution from a small phosphocellulose column. After concentration, inclusion of fraction IV in the strand-

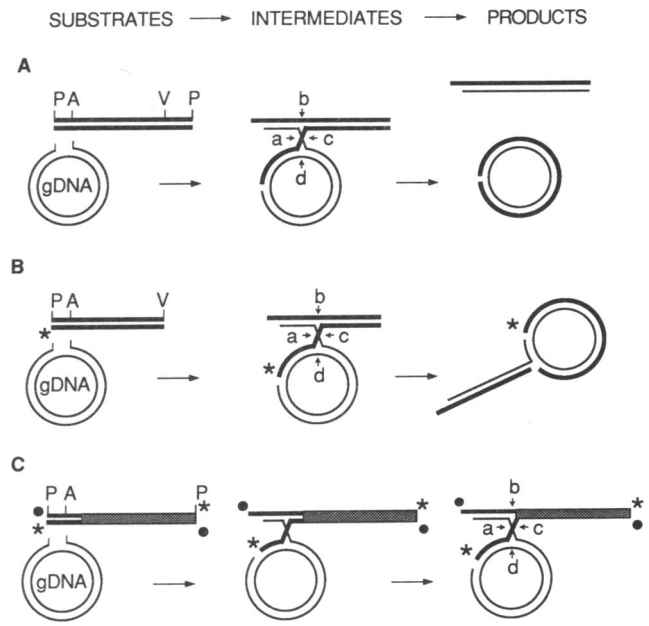


FIG. 1. Schematic diagram of strand-exchange reactions. Reaction **A**: strand exchange between φX174 gDNA and *Pst* I-linearized φX174 duplex DNA. The products of complete strand exchange are gapped linear and nicked circular duplex DNA. Cleavage of the Holliday junction intermediate at a and c generates nicked linear monomer and nicked circular duplex DNA (patch recombinants); cleavage at b and d generates a nicked linear dimer (splice recombinant) (see figure 2 of ref. 17). Reaction **B**: strand exchange between gDNA and duplex DNA linearized with *Pst* I and *Ava* II. The product of strand exchange is a σ structure. Resolution of the intermediate is as for reaction **A**. Reaction **C**: strand exchange between gDNA and *Pst* I-linearized chimeric duplex (pCJ10). The product of strand exchange is an α structure. The shaded region of the chimeric duplex represents nonhomologous DNA. P, *Pst* I; A, *Ava* I; V, *Ava* II; *, 3′-end labels; ●, 5′-end labels.

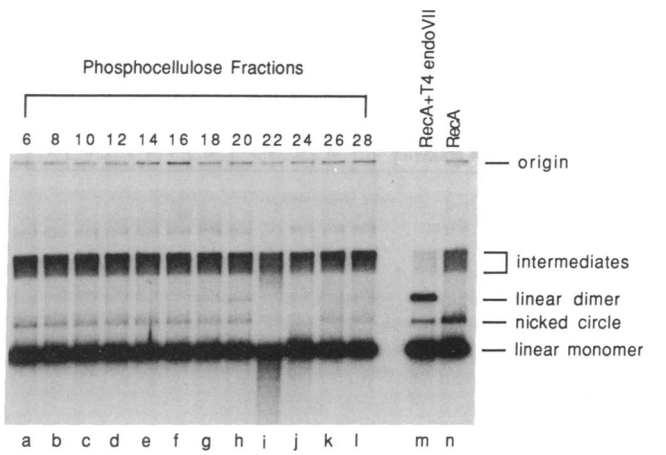


FIG. 2. Cleavage of recombination intermediates by fractionated *E. coli* extracts. Reaction mixtures (10 μl) containing gDNA (5 μM), RecA (1.3 μM), and uniformly $^{32}$P-labeled *Pst* I-linearized φX174 duplex DNA (5.3 μM) (reaction A in Fig. 1) were incubated as described. After a 10-min incubation, reaction mixtures were supplemented with: 2 μl of phosphocellulose fractions 6–28 (lanes a–l), T4 endonuclease VII (25 units) (lane m), and no additions (lane n). Incubation was continued for 10 min (lanes a–m) or 20 min (lane n). After deproteinization, reaction products were analyzed by 0.7% agarose gel electrophoresis.

exchange reaction led to the efficient production of dimer-sized DNA (Fig. 3, lane c).

Although purified through two column steps, at this stage of purity fraction IV contained many proteins as observed by SDS/polyacrylamide electrophoresis. At protein–DNA concentrations comparable to those used in the resolution assays, fraction IV removed ≈20% of 5′- or 3′-$^{32}$P-end label from linear duplex DNA. Similarly, the fraction contained a small amount of single-stranded endonuclease activity. However, we were unable to detect any nicking of supercoiled covalently closed duplex DNA, as determined by the conversion of replicative form I DNA to nicked circular DNA by agarose gel electrophoresis (data not shown).

**Reaction Requirements.** To investigate the requirements for the formation of the dimer-sized DNA species, reactions were performed in which various components were omitted. When gDNA (Fig. 3, lane e), RecA protein (Fig. 3, lane g), or ATP (Fig. 3, lane i) were omitted from the reaction, inclusion of fraction IV failed to produce dimer-sized DNA. Formation of this DNA by fraction IV was observed only in the complete strand-exchange reaction (Fig. 3, lane c).

**Characterization of the Dimer.** The formation of a dimer-sized molecule could occur by resolution of recombination intermediates or by other means, such as the ligation of linear duplex DNA. Ligation of the *Pst* I-linearized DNA would be expected to produce dimeric forms *i*, *ii*, and *iii* in Fig. 4*A*. In contrast, resolution of the recombination intermediates would produce dimeric form *i* exclusively (17). To distinguish between these possibilities, the dimer-sized band produced by fraction IV was purified from an agarose gel and subjected to restriction analysis. Because of poor separation, some reaction intermediates were excised with the dimer-sized band. During isolation, these intermediates underwent a process of branch migration taking them back to the starting substrates and leading to some contamination of the dimer-sized DNA with linear monomer DNA (Fig. 4*B*, lane a). Restriction enzyme digestion of the isolated DNA with *Pst* I generated a band of 5.4 kilobases (kb) (Fig. 4*B*, lane b). Digestion with *Stu* I generated three bands of 5.4, 4.5, and 0.9 kb (Fig. 4*B*, lane c). Other DNA bands visible in lanes b and c were produced by incomplete restriction digestion. These

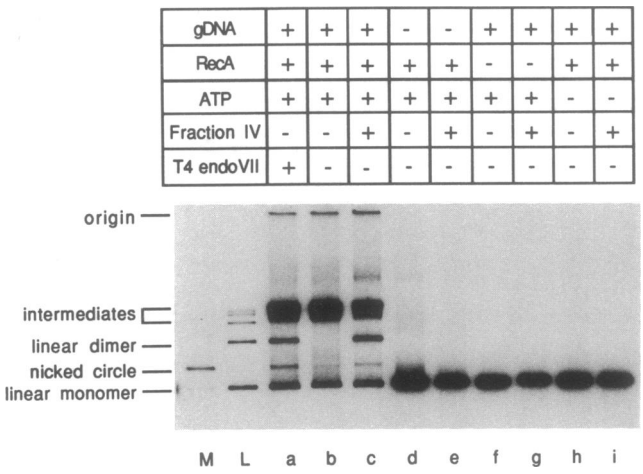


FIG. 3. Requirements for the formation of the dimer-sized product. Reaction mixtures (10 μl) contained linear duplex DNA (3.8 μM) (reaction A in Fig. 1) and gDNA (5 μM) and RecA (2.6 μM) where indicated. After a 10-min incubation, fraction IV (4 μg) or T4 endonuclease VII (20 units) was added as indicated, and the mixtures were incubated for 10 min. The *Pst* I-linearized duplex DNA was 3′-end-labeled. DNA products were analyzed as for Fig. 2. Lanes: a–i, components as indicated; M, φX174 nicked circular and linear duplex DNA markers; L, ligation ladder of linear duplex φX174 DNA.

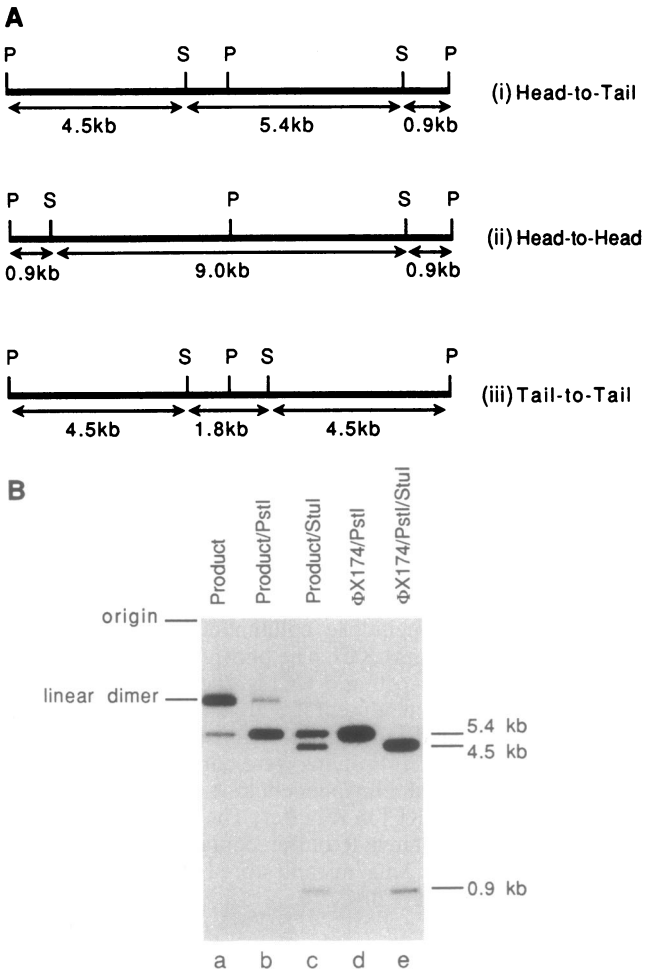


FIG. 4. Characterization of the dimer product. (*A*) Arrangement of *Pst* I and *Stu* I restriction sites in linear dimers formed by end-to-end joining of monomers. (*B*) Reaction A in Fig. 1 was performed as described by using a reaction mixture (100 μl) containing gDNA (10 μM), RecA (2.6 μM), and uniformly $^{32}$P-labeled *Pst* I-linearized φX174 duplex DNA (17 μM). After a 10-min incubation, fraction IV (40 μg) was added, and incubation was continued for 10 min. After deproteinization the dimer product was purified by electrophoresis and elution from a 0.7% agarose gel. Analysis of the dimer product was by restriction digestion. Lanes: a, dimer product; b, product cut by *Pst* I; c, product cut by *Stu* I; d, marker of φX174 cut by *Pst* I; e, marker of φX174 cut by *Pst* I and *Stu* I. P, *Pst* I; S, *Stu* I.

results indicated that the isolated dimer-length DNA contained two monomer-length molecules aligned in a head-to-tail fashion (dimer *i* in Fig. 4*A*). Since the formation of a head-to-tail linear dimer is characteristic of resolution of the recombination intermediate by T4 endonuclease VII (17), these results indicate that fraction IV resolves Holliday junctions made by RecA protein.

**Resolution Occurs in Two Directions.** Enzymatic resolution of the Holliday junction formed in reaction A (Fig. 1) produces linear dimer DNA (cleavage at b and d) or nicked circular and nicked linear DNA (cleavage at a and c). In the above reaction, the latter resolution products were indistinguishable from the $^{32}$P-labeled nicked circular product of strand exchange and the $^{32}$P-labeled linear duplex substrate. To determine whether resolution occurs in both directions, reactions were carried out with a truncated linear duplex substrate that was 3′-end-labeled at one terminus only (reaction B in Fig. 1).

Addition of fraction IV to a strand exchange reaction between gDNA and the truncated linear duplex produced

linear dimer and nicked circular DNA (Fig. 5, lane c). These results show that resolution of the recombination intermediate by fraction IV occurs in either of two directions, although the linear dimer was produced in a 3:1 ratio over the nicked circle DNA. On disruption of the RecA–nucleoprotein filament by cooling and addition of ADP prior to fraction IV, this bias was removed (Fig. 5, lane d). A similar bias and its removal have been observed in reactions with T4 endonuclease VII (17).

**Specific Endonucleolytic Cleavage of the Holliday Junction.** To determine whether the linear dimer and nicked circular molecules were the products of cleavage at the Holliday junction, gDNA was allowed to react with *Pst* I-linearized pCJ10 (reaction C in Fig. 1). This chimeric DNA contains 329 base pairs (bp) of $\phi$X174 DNA and 4149 bp of pBR322 DNA (17). By using these substrates, strand exchange is driven by RecA protein through the $\phi$X174 sequences until the Holliday junction is blocked by the heterologous pBR322 sequences. To detect specific cleavage at the site of the stalled Holliday junction, reactions between gDNA and 3′-end-labeled pCJ10 were supplemented with fraction IV. The reaction products were analyzed on a denaturing polyacrylamide gel, which allowed single-base resolution. As markers for cleavage at the junction point, we used fragments generated by T4 endonuclease VII and T7 endonuclease I; both resolve RecA-generated Holliday junctions *in vitro* (ref. 17; B. M. Müller, C. A. Jones, and S.C.W., unpublished observations). In reactions with fraction IV, >90% of the cleavage occurred at a specific site (Fig. 6, lane c). Comparison with fragments produced by T7 endonuclease I (Fig. 6, lane a) and T4 endonuclease VII (Fig. 6, lane b) located the cleavage site to the position of the stalled Holliday junction. Cleavage of the Holliday junction was not observed in the absence of fraction IV (Fig. 6, lane d) or in reactions from which RecA was omitted (Fig. 6, lane f) or when $\phi$X174 gDNA was replaced with gDNA made from the plasmid pACYC184 (Fig. 6, lane e).

Using 5′-end-labeled pCJ10 DNA, we determined that cleavage in the opposite direction also occurred at the junction point. In this case, specific cleavage was again seen close to the site of incision by T4 endonuclease VII (compare lanes g and h in Fig. 6) at the site of the Holliday junction.

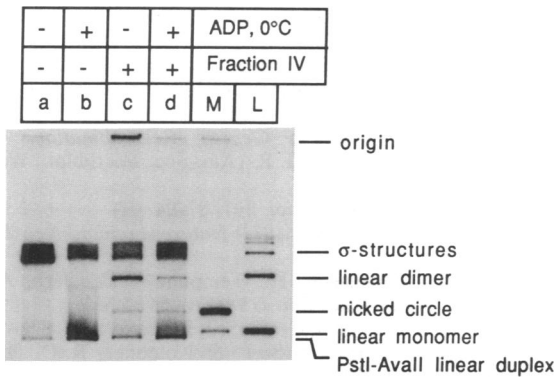


FIG. 5. Bidirectional resolution in the presence and absence of the RecA filament. Reaction mixtures (40 $\mu$l) containing gDNA (5 $\mu$M), RecA (1.3 $\mu$M), and $\phi$X174 linear duplex DNA (7.6 $\mu$M) were incubated as described. The *Pst* I–*Ava* II linear duplex DNA was 3′-end-labeled at the *Pst* I terminus (reaction **B** in Fig. 1). After a 10-min incubation, the reaction mixture was divided into 10-$\mu$l aliquots. Lanes: a, no additions; b, ADP was added to 3 mM, and the reaction mixture after 10 min on ice was returned to 37°C; c, fraction IV (4 $\mu$g) was added; d, as in lane b but with addition of fraction IV (4 $\mu$g); M, $\phi$X174 nicked circular and linear duplex DNA markers; L, ligation ladder of linear duplex $\phi$X174 DNA. Reaction mixtures were incubated for a further 10 min at 37°C. DNA products were analyzed as for Fig. 2.

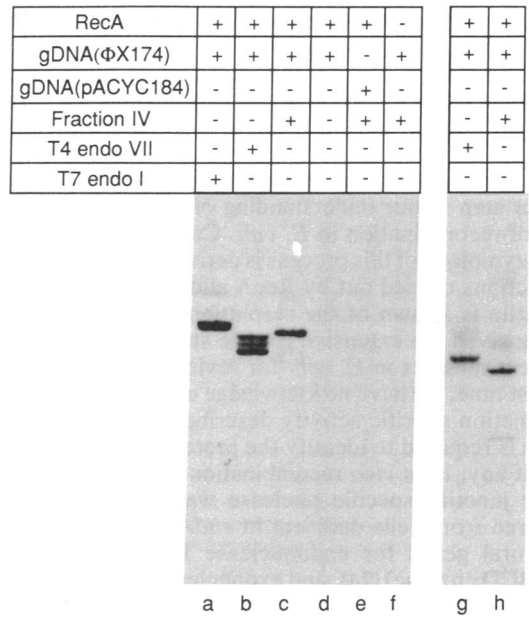


FIG. 6. Resolution occurs by endonucleolytic cleavage of the Holliday junction. All reaction mixtures contained *Pst* I-linearized pCJ10 DNA (reaction C in Fig. 1). RecA (1.8 $\mu$M) and gapped $\phi$X174 (5.2 $\mu$M) or gapped pACYC184 (5.2 $\mu$M) DNA were included where indicated, and incubation was for 10 min as described. Lanes: a–f, 3′-end-labeled linear DNA (5.1 $\mu$M); g and h, 5′-end-labeled linear DNA (6.3 $\mu$M). Control reaction mixtures (50 $\mu$l) were supplemented with 0.6 ng of T7 endonuclease I (lane a) or 100 units of T4 endonuclease VII (lanes b and g). Other reaction mixtures (100 $\mu$l) were supplemented with 40 $\mu$g of fraction IV (lanes c, e, f, and h), or there were no additions (lane d). Reaction mixtures were then incubated for 30 min. Reaction products were deproteinized and analyzed by denaturing gel electrophoresis.

These results show that the formation of linear dimer and nicked circular products by fraction IV result from specific endonucleolytic cleavage of the Holliday junction.

To further investigate the specificity of cleavage, resolution reactions containing gapped and linear duplex $\phi$X DNA (reaction **A** in Fig. 1) were supplemented with pACYC184 form I DNA as a nonspecific competitor. Using a 5-fold excess of competitor, we observed that the level of resolution was reduced to 60% of that observed in the absence of competitor (data not shown).

## DISCUSSION

In this paper, we describe the detection of an activity from fractionated *E. coli* extracts that resolves Holliday junctions. Using gapped and linear duplex DNA molecules, we show that recombination intermediates produced in RecA-mediated strand-exchange reactions are processed into recombinant DNA products. Several lines of evidence suggest the presence of a nucleolytic activity that is specific for Holliday junctions. (*i*) The activity is specific for intermediates generated by strand exchange; in the absence of RecA protein or its cofactor ATP, or in otherwise complete reactions from which one DNA substrate was omitted, we were unable to detect the formation of recombinant products. (*ii*) The products of resolution by the *E. coli* activity are identical to those produced by T4 endonuclease VII, a known Holliday junction-resolving protein. (*iii*) The linear dimer and nicked circular duplex products are characteristic of resolution of the junction in either of two directions by the pairwise cleavage of DNA strands of like polarity. They are therefore representative of "splice" and "patch" recombinants, respectively. (*iv*) In experiments in which strand exchanges

were driven against a heterologous block, specific endonucleolytic cleavage was observed at the site of the stalled Holliday junction. (*v*) Resolution reactions were relatively insensitive to the inclusion of competitor DNA. (*vi*) Fraction IV did not contain nonspecific double-stranded endonuclease activity.

The demonstration of this activity may represent an important step in our understanding of the molecular mechanism of recombination in *E. coli*. Current understanding of the enzymology of this process is derived from *in vitro* studies of reactions carried out by RecA and RecBCD proteins. As yet, little is known of the resolution reaction. This is surprising given the extensive genetic studies of recombination deficient mutants in *E. coli* (for review, see ref. 22). At the present time, we have no knowledge of the gene that encodes the junction-specific activity described here. Further purification is required to identify the protein and to determine its role, if any, in *in vivo* recombination.

The junction-specific nuclease was detected in extracts prepared from cells deficient in *endA*, *recB*, and *sbcB*, the structural genes for endonuclease I (23), exonuclease V (RecBCD enzyme) (24), and exonuclease I (25), respectively. The cells are also thought to carry *sbcC*, a mutation that arose spontaneously in *recB sbcB* mutants (26). The use of the *endA recB sbcB* mutant was necessary to minimize degradation of the DNA substrates in the strand-exchange reaction. However, this choice may have been fortuitous, since plasmid recombination in *recBC sbcBC* cells is increased $10^3$-fold relative to that of wild-type cells (27). Whether resolution proteins are induced by this or other genetic backgrounds or by conditions that generate DNA damage remains to be determined.

It has been hypothesized that the *recBCD* gene product may be involved in the resolution of Holliday junctions (28, 29). However, a role for RecBCD enzyme in resolution is unlikely because (*i*) figure-eight DNA molecules containing Holliday junctions are resolved with similar frequencies after transformation into wild-type or *recB* cells (16), (*ii*) RecBCD enzyme is incapable of cleaving synthetic Holliday junctions by a specific resolution reaction about the junction point (30), (*iii*) neither the purified RecBCD holoenzyme nor the individual subunits resolve Holliday structures made *in vitro* by RecA protein (B. M. Müller, P. Boehmer, P. T. Emmerson, and S.C.W., unpublished results), (*iv*) the junction-specific nuclease reported here was detected in fractionated recB extracts, and (*v*) an activity that cleaves cruciform structures has been observed in *recB sbcB* extracts (30). Any relationship between this latter activity and the one described here is unknown.

Although the DNA substrate used in our experiments mimics a true recombination intermediate, it is not clear whether the activity described is confined to the resolution of junctions formed by RecA protein. At the present time the activity on synthetic model Holliday junctions has not been determined. However, it is clear that helical RecA-nucleoprotein filaments formed on gDNA provide the structural framework for subsequent recombination reactions (3), and the present experiments indicate that resolution may occur while DNA molecules are aligned within the filament. Molecular models for the action of RecA protein involve the formation of three- or four-stranded DNA helices (3, 17), and recent observations indicate that three-stranded regions of DNA remain stable after removal of RecA protein (31). Since we find that cleavage occurs in the presence of RecA or after its dissociation by ADP, it is possible that interwound DNA structures of this type provide the structural basis for enzymatic recognition.

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