The combined action of exonuclease I and recA protein leads to a kind of reverse DNA strand exchange in which joint molecules formed on the “wrong” or distal end of a linear duplex in the presence of ATP are stabilized by exonuclease I degradation of the displaced (+) strand. Continued pairing and degradation of the displaced strand leads to strand exchange that appears to progress with a polarity opposite that of the normal recA protein-promoted reaction (i.e. 3'→5' with respect to the (+) strand). However, in contrast to the normal 5'→3' strand exchange, the displaced strand is completely degraded in the process. When the linear duplex DNA substrate has a heterologous region at the 5' (proximal) end, the major product (described in a previous study (Bedale, W. A., Inman, R. B., and Cox, M. M. (1991) J. Biol. Chem. 266, 6499–6510)) is a circular duplex DNA molecule with a double-stranded tail whose length corresponds closely to the heterologous segment of the substrate. The origin of this product is here shown to be the result of the exonuclease activity of exonuclease I (either added exogenously or present as a trace contaminant of recA protein or SSB protein preparations), as opposed to endonucleolytic or mechanical breakage. The levels of exonuclease I required to generate these products are sufficiently low that they are undetected by assays for exonuclease contamination in recA protein preparations. These results demonstrate that the interplay of recA protein with other enzymes can have a profound effect on both the mechanism and outcome of recA protein-promoted DNA strand exchange. They also demonstrate that the (+) strand of the duplex DNA substrate is at least transiently displaced in recA protein-mediated pairing even when joint molecules are limited to the distal end.

The recA protein of *Escherichia coli* is required for homologous recombination in *vivo* and is also to promote DNA strand exchange reactions in *vitro* (Cox et al., 1987; Radding, 1989; Roca and Cox, 1990). Probably the most common in *vitro* assay used to study recA protein-promoted DNA strand exchange is the three-strand exchange reaction depicted in Fig. 1A. RecA protein forms a filament on the circular single-stranded DNA substrate in the first step of this reaction. SSB protein of *E. coli* is also included in the in *vitro* reactions and probably facilitates formation of a uniform and stable filament of recA protein on the ssDNA (Cox et al., 1987; Kowalczykowski, 1991; Radding, 1989). Homologous linear duplex DNA can then pair with the nucleoprotein filament. These pairing intermediates may involve regions of triple-helical DNA (Hsieh et al., 1990; Jain et al., 1992; Rao et al., 1993). In reactions between completely homologous DNA substrates, strand exchange begins at the 5' (proximal) end of the (+) strand of the duplex and branch migration continues in the 5'-3' direction (Cox and Lehman, 1981a; Kahn et al., 1981; West et al., 1981).

Joint molecules are also formed efficiently on the opposite (distal) end of the linear duplex but are generally not converted to strand exchange products because of the polarity of the branch migration phase of the reaction (Cox and Lehman, 1981a). Upon removal of recA protein, the joint molecules formed at the distal end of the duplex in the presence of ATP yield triplex DNA structures which are resistant to nuclease degradation and thermal denaturation (Hsieh et al., 1990; Rao et al., 1991a, 1991b). It has been postulated (Hsieh et al., 1990; Rao et al., 1991a; Stasiak, 1992) that the stability of these triplex joints reflects an inability to displace the (+) strand from the joint at the 3' end.

RecA protein is a DNA-dependent ATPase, and ATP hydrolysis is required during the branch migration phase of the reaction for efficient DNA strand exchange (Cox and Lehman, 1981b). While the molecular role of this ATP hydrolysis is not entirely clear, it has recently been demonstrated that ATP hydrolysis is required to drive strand exchange through short heterologous insertions (Kim et al., 1992b; Rosselli and Stasiak, 1991), to render the reaction unidirectional2 and to carry out strand exchange reactions involving four DNA strands (Kim et al., 1992a).

The ability of recA protein-promoted DNA branch migration to overcome structural barriers in the DNA is critical to the cell for processes such as recombinational DNA repair. The mechanism by which one type of barrier to recombination (short heterologous insertions located near the center of an otherwise homologous duplex) is overcome has been addressed in a number of studies (Bianchi and Radding, 1983; Jwang and Radding, 1992; Kim et al., 1992b; Rosselli and Stasiak, 1991). In a previous paper (Bedale et al., 1991), we investigated the generation of apparent DNA strand breaks by recA protein is explained*.

(Received for publication, November 5, 1992, and in revised form March 23, 1993)

Wendy A. Bedale‡, Ross B. Inman, and Michael M. Cox‡
From the Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin, Madison, Wisconsin 53706

---

*This work was supported by National Institutes of Health Grants GM32336 (to M. M. C.) and GM14711 (to R. B. I.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡Supported by National Institutes of Health Predoctoral Training Grant T32-GM7215 and a Procter and Gamble Predoctoral Fellowship.

§To whom correspondence should be addressed. Tel.: 608-262-1181.

---

1. The abbreviations used are: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; FII DNA, supercoiled circular form of DNA as isolated from *E. coli* cells; FII DNA, the nicked circular form of the same molecule; FIII DNA, the linear form of the same molecule; ATP-yS, adenosine-5'-O-(3-thiotriphosphate); PAGE, polyacrylamide gel electrophoresis; bp, base pairs.

2. S. Jain, C. Cowan, and J. Kim, unpublished results.
the effect of heterologous sequences located at the ends of linear duplex DNA on recA protein-promoted DNA strand exchange. We found in the case of 3′ (distal) heterology (with respect to the (+) strand) that strand exchange results in branched products (Fig. 1F), indicating that normal strand exchange was halted by the heterologous regions. In the case of 5′ (proximal) heterology, we predicted strand exchange would be blocked. Instead, these reactions were surprisingly efficient in the formation of joint molecules, and many of the products had undergone complete strand exchange. Interestingly, these products had double-stranded tails that corresponded to the length of heterology present in the original duplex DNA (Fig. 1C). Since recA protein–promoted branch migration proceeds 5′→3′ with respect to the (+) strand in completely homologous strand exchange reactions, we proposed that these unusual 5′→3′ products resulted from a break or nick in the (+) strand of the duplex at the junction of the heterology and the homology. We have used the term “breakage” to refer to the production of these circular duplex molecules with short dsDNA tails (or 5′→3′ molecules) since their formation requires at least one scission in the (+) strand of the duplex DNA. However, the exact process by which these products were generated was unclear (Bedale et al., 1991). In this paper we present results that indicate that the term breakage is a misnomer. The 5′→3′ products are formed as a result of the presence of exonuclease I acting processively from the 3′ end of the (+) strand of the duplex. Furthermore, the combination of exonuclease I (which is a trace contaminant of some recA and SSB protein preparations generated by a variety of published procedures) and recA protein leads to a kind of reverse strand exchange reaction which mimics the action of some eukaryotic strand exchange proteins and may have significance in vivo. This result also provides evidence that the (+) strand of the duplex DNA substrate is at least transiently displaced when pairing occurs at the distal end of the duplex and is accessible to exonuclease I.

While this paper was in preparation, Konforti and Davis (1992) reported that the presence of exonuclease I recA protein-mediated DNA strand exchange reactions facilitates the formation of joint molecules containing short regions of homology at the 3′ end of the (+) strand and presented evidence suggesting that the 3′ end of the (+) strand of the duplex had been displaced to some degree. The results presented here confirm and greatly expand on these observations.

### Experimental Procedures

#### Enzymes and Biochemicals

- E. coli recA protein was purified as described (Cox et al., 1981). Other preparations of recA protein were generously provided by Dr. Stephen Brenner (E. I. Du Pont de Nemours & Co, Wilmington, DE), Dr. Stephen Kowalczykowski (University of California, Davis, CA), and Dr. Charles Radding (Yale University, New Haven, CT). Additional recA protein was overproduced and purified using plasmid pGE226 in E. coli strain SK4642, which was the generous gift of Dr. Jack Griffith (University of North Carolina, Chapel Hill, NC). This host strain is a bacteriophage P2 host strain that is a bacteriophage P2 DNA as a substrate (Lehman and Nussbaum, 1964). RecA protein concentrations were determined using a procedure provided by Dr. Paul Modrich and Dr. Deani Cooper (Duke University, Durham, NC). Rabbit anti-exonuclease I antiserum was the generous gift of Dr. Paul Modrich (Duke University, Durham, NC). Rabbit anti-exonuclease I antiserum was the generous gift of Dr. Stephen Modrich (Duke University, Durham, NC). RecA protein that was purified from a strain of E. coli SSB protein was purified as described (Cox et al., 1981). Other preparations of recA protein were generously provided by Dr. Stephen Brenner (E. I. Du Pont de Nemours & Co, Wilmington, DE), Dr. Stephen Kowalczykowski (University of California, Davis, CA), and Dr. Charles Radding (Yale University, New Haven, CT).

- Calf intestinal alkaline phosphatase, T4 polynucleotide kinase, and T7 endonuclease I were purchased from New England Biolabs. Exonuclease I (1 mg/ml and 131 units/μg; 1 unit releases 10 mononucleotides in 30 min at 37°C using bovine plasma DNA as a substrate (Lehman and Nussbaum, 1986)) was the generous gift of Dr. Sidney Kushner (University of Georgia, Athens, GA). Additional exonuclease I and Microcosmus luteus ATP-dependent DNase were purchased from United States Biochemical Corp. Exonuclease VII (0.225 mg/ml and 6.3 units/μg; 1 unit releases 1 nmol of ssDNA to a trichloroacetic acid-soluble form in 30 min at 37°C (Vales et al., 1982)) was the generous gift of Dr. John Chase (U. S. Biochemical Corporation). Additional exonuclease VII was generously provided by Dr. Stephen Modrich (University of North Carolina, Chapel Hill, NC). E. coli RecBCD (46 mg/ml and 2.4 × 10^6 units/mg; 1 unit releases 1 nmol of nucleotides from dsDNA in 20 min at 37°C (Eichler et al., 1991) was the generous gift of Dr. Gerald Smith (Fred Hutchinson Cancer Center, Seattle, WA). Rabbit anti-exonuclease VII antiserum was the generous gift of Dr. Stephen Modrich (Duke University, Durham, NC). Rabbit anti-exonuclease I antiserum was obtained by Hazleton Research Products (Denver, PA) using conventional procedures. RecA protein or purified exonuclease I antiserum were purified by protein A column chromatography (Pierce Scientific). This procedure eliminated all detectable contaminating nuclease activities (data not shown). Nitrocellulose (0.1-μm pore size) was from Schleicher and Schuell. Proteinase K, creatine kinase, phosphocreatine, ATP, goat anti-rabbit IgG (coupled to alkaline phosphatase), Protein A, and 5-bromo-4-chloro-3-indolyl phosphate were purchased from Sigma. Protein molecular weight markers were purchased from Bio-Rad. Radionucleotides were purchased from Amersham Corp.

- DNA—Duplex and ssDNA were derived from bacteriophage M13 (Messing and Vieira, 1982). The bacteriophage M13mp18 (7427 bp total length) contains a 198-bp heterologous insertion at the SmaI restriction site in the polylinker of M13mp8 (Bedale et al., 1991). Duplex and ssDNA were prepared using previously described methods (Davis et al., 1980; Messing, 1983; Neuwald and Cox, 1986). Oligonucleotides were synthesized and gel-purified. Circular M13mp8 ssDNA (present at 500 μM) was added to the oligonucleotide (present at 50 μM) in standard restriction enzyme buffer. The mixture was incubated at 37°C for 2 h. It was then cooled slowly to room temperature. EcoRI restriction enzyme was added (2 units/μg of DNA) and the reaction was incubated at 37°C. The extent of digestion was monitored by electrophoresis on 1.4% agarose minigels. After digestion was completed, residual protein was removed by 1:1 extraction of the reaction with phenol/chloroform/isooamy alcohol (25:24:1) and chloroform/isooamy alcohol (24:1), followed by ethanol precipitation. The resuspended DNA was incubated at 65°C for 10 min to restore normal DNA secondary structure and stored on ice.

Preparation of “Endless” DNA—DNA with hairpin oligonucleotides annealed at the ends was prepared by a procedure provided by Dr. Paul Modrich and Dr. Deani Cooper (Duke University, Durham, NC). Endless DNA was synthesized using purified recA protein, ssDNA, and E. coli RecBCD enzyme or M. luteus ATP-dependent DNase. The final ligation mixture was then treated with recBCD enzyme or M. luteus ATP-dependent DNase. The reaction mixture was monitored by electrophoresis on 1.4% agarose minigels. After digestion was completed, residual protein was removed by 1:1 extraction of the reaction with phenol/chloroform/isooamy alcohol (25:24:1) and chloroform/isooamy alcohol (24:1). The reaction volume was reduced by repeated precipitation with α-chymotrypsin and dialyzed extensively against TE (25 mM Tris HCl, pH 7.5, 1 mM EDTA).

#### Single-stranded Exonuclease Assay—Unlabeled linear M13mp8 ssDNA was diluted to 30 μM in the same reaction buffer used for strand exchanges (25 mM Tris acetate (80% cation, pH 7.5, 10 mM potassium acetate, 3 mM magnesium acetate, 500 mM potassium glutamate, 5% glycerol). In some reactions, SSB protein (3 μM) was also present. RecA protein or purified exonuclease was added, and the mixture was...
incubated at 37 °C for 90 min. An aliquot of each reaction (10 μl) was mixed with 2.5 μl of gel loading buffer (Type II, (Sambrook et al., 1989)) and 10% SDS and subjected to agarose gel electrophoresis as described (Bedale et al., 1991).

Strand Exchange Reaction Conditions—Reactions contained 25 mM Tris acetate (80% cation, pH 7.5), 10 mM magnesium acetate, 3 mM potassium glutamate, 1 mM dithiothreitol, 5% glycerol, and an ATP regenerating system (12 mM phosphocreatine, and 10 units/ml creatine kinase). Duplex DNA and ssDNA, both at 20 μm, were preincubated with 6.7 μM recA protein for 10 min before ATP (3 mM), and SSB (2 μM) were added to start the reaction.

Immunoblots—Proteins were separated by SDS-PAGE (9% acrylamide). Immunoblots were performed by the procedure of Walczak et al. (1993).

Denaturing Gel Assays—Strand exchange reactions were performed with 5' end-labeled dsDNA that contained 186 bp of 5' heterology. To determine whether breakage of the (+) strand of the duplex occurs during strand exchange, the labeled products were denatured and electrophoresed on denaturing 6% acrylamide gels (Bedale et al., 1991). Breakage at the heterology/homology junction results in the production of discrete bands at about 200 bases on such gels (Bedale et al., 1991).

Other Methods—Agarose gel electrophoresis and electron microscopy were performed as described previously (Bedale et al., 1991).

RESULTS

Experimental Strategy—This project was an outgrowth of our attempts to determine the origin of the σ-like products we had observed previously in reactions where the dsDNA substrate contained 5' (proximal) heterology (Fig. 1C) (Bedale et al., 1991). This σ-like product could only be generated by cleavage or breakage of the (+) strand of the duplex DNA substrate near the heterology/homology junction. There were two major possibilities; the activity responsible for the production of the σ-like molecules was either intrinsic to recA protein itself or involved a contaminating nuclease. Since the recA protein preparations that we used lacked detectable nuclease activity against both linear and circular ss or dsDNA, one possibility was a nuclease that was specific for interme-

![Fig. 1. RecA protein-promoted three strand exchange reactions. A, a three strand exchange reaction between completely homologous circular ssDNA and linear duplex DNA, leading to the production of a nicked circular duplex and the displaced linear (+) strand; B, a short heterology (200 bp) at the 3' end of the (+) strand of the duplex (distal heterology) results in the formation of a circular duplex molecule with a short double-stranded tail and a long single-stranded tail (Bedale et al., 1991). C, a short heterology (200 bp) at the 5' end of the (+) strand of the duplex (proximal heterology) results in the production of a circular duplex with a short double-stranded tail corresponding to the length of heterology with certain recA protein preparation. The homologous part of the (+) strand of the duplex is absent in this product molecule, as though it were derived from the molecule shown in parenthesis.](image-url)

This was an unintended oversight by another researcher in this laboratory. It was not picked up by MMC, with whom the responsibility lies. We do not know what error occurred in the generation of the preparation that resulted in the observed contamination. It is not due to the procedure used (that of Lohman, et al., 1986). A total of four other SSB protein preparations generated in this laboratory by the same procedure before and since the preparation in question have exhibited no exonuclease I contamination.
Effects of Exonuclease I on RecA-mediated Strand Exchange 15007

FIG. 2. Denaturing gel assay to screen different recA protein preparations. Strand exchange reactions and electrophoresis were performed as described (Bedale et al., 1991) with 5' end-labeled DNA containing 198 bp of 5' heterology. Denaturation of the σ-like products shown in Fig. 1C results in the production of a labeled DNA fragment ~200 bases long, corresponding to the 5' end of the (±) strand of the original dsDNA. The DNA size markers (lane not shown) were derived from a 32P end-labeled MspI digest of pBR322 DNA. Lane 1 is a 0 min time point. All other lanes are 90 min time points corresponding to different reactions. The reactions shown in lanes 2-11 were performed with recA protein preparations 1-10, respectively, described in Table I. The reactions in lanes 12, 13, and 15 were all performed with recA protein preparation 1, which was purified from an exonuclease I-free strain of E. coli. Lane 12 corresponds to a reaction that was performed with an SSB protein preparation known to be contaminated with a low level of exonuclease I. Lane 13 corresponds to a 20-μl reaction to which 0.13 units of exonuclease I were added. Lane 14 corresponds to a reaction in which partially purified recA protein (Fraction III) was substituted for purified recA protein (Cox et al., 1981). Lane 15 corresponds to a 20-μl reaction to which 0.14 units of exonuclease VII were added.

aration with the original SSB protein preparation, generation of the σ-like products was observed (data not shown). In addition, the original SSB protein was able to restore generation of the σ-like products to other recA protein preparations that lacked this activity (Fig. 2, lane 12). We previously had examined several different recA protein preparations and several SSB preparations, but we did not try every combination of recA protein preparation with SSB protein preparation. It appears, therefore that the generation of the σ-like products was due to a contaminating enzymatic activity that can be present in some recA protein preparations and was present in at least one SSB preparation.

RecA Protein Preparations That Promote the Generation of σ-like Products Possess Single-stranded Exonuclease Activity—A clue to determining how the generation of σ-like products occurs came from completely homologous strand exchange reactions where 5’ end-labeled linear duplex DNA was used. Two labeled products are usually generated in this reaction: a nicked circular duplex and the displaced linear ssDNA. However, in reactions involving recA protein preparations that promoted the formation of σ-like molecules, we found that the labeled linear ssDNA product was not observed (data not shown). Circular ssDNA in these reactions was not degraded (data not shown). This observation suggested that a contaminating single-stranded exonuclease may either remove the 5’ label or completely degrade the displaced ssDNA.

This exonuclease contaminant is not readily detected in the nuclease screens routinely performed on all of our recA protein preparations. When the recA preparations that promote the generation of σ-like molecules were screened for single-stranded exonuclease activity under conditions similar to those used in DNA strand exchange, no degradation of unlabeled linear ssDNA was observed (data not shown). The SSB protein present in normal strand exchange experiments is not normally added to these nuclease assays, however. When the assay for single-stranded exonuclease activity was repeated in the presence of SSB protein, single-stranded exonuclease activity was observed for each of the recA protein preparations that promoted the generation of σ-like products (Fig. 3, lanes 5, 6, and 8). RecA protein preparations that did not promote the generation of σ-like products did not possess single-stranded exonuclease activity even in the presence of SSB (Fig. 3, lanes 3–4, 7, and 9–12). In addition, the SSB protein preparation that promoted the generation of σ-like products possessed single-stranded exonuclease activity (in the absence of recA protein) while other SSB preparations did not (Fig. 3, lanes 2 and 13). The very strong correlation between single-stranded exonuclease activity and the ability to generate σ-like molecules suggested that the two activities might be related. However, it was not obvious how a single-stranded exonuclease might be responsible for what appeared to be endonucleolytic cleavage of double-stranded DNA.

Addition of Exonuclease I or Exonuclease VII Can Restore Formation of σ-like Molecules to RecA Protein Preparations That Lack This Activity—The inability of certain recA protein preparations to promote the formation of σ-like molecules provided a way to identify the contaminating activity responsible for this activity. We screened a number of purified DNA recombination and repair enzymes for the ability to generate σ-like products. Of all the purified enzymes tested, only exonuclease I and VII were capable of restoring this activity to recA protein preparations that lacked this activity. Fig. 4, lane 6, demonstrates that 0.1 units of exonuclease VII in a 20-μl reaction is sufficient to restore the ability to generate σ-like products to the levels seen with the recA protein preparations that had this activity. Maximum product formation (conversion of about 15% of substrate dsDNA into σ-like products in this experiment) is observed at about 1 unit of exonuclease VII/20-μl reaction. Additional exonuclease VII does not increase the amount of σ-like products observed (data not shown). Comparatively less exonuclease I is needed to restore the formation of σ-like products. Generation of σ-like products occurs with the addition of 0.01 units of exonuclease I in a 20-μl reaction (Fig. 5, lane 6). This amount of exonuclease I is equivalent to approximately 1 molecule of exonuclease I/20,000 recA protein molecules. We note that this level of exonuclease I contamination in recA protein preparations is undetectable unless SSB is added to standard single-stranded exonuclease assays. In contrast, the amount
Effects of Exonuclease I on RecA-mediated Strand Exchange

Table I

RecA protein preparations used in this study

| RecA protein preparation | Breakage | ss exonuclease activity | ss exo inhibited by anti-ExoI | ExoI immunoblot | Method of purification |
|--------------------------|----------|-------------------------|-------------------------------|-----------------|-----------------------|
| 1                        | –        | –                       | NA*                           | –               | Cox et al., 1981      |
| 2                        | –        | –                       | NA                            | –               | Purchased from USB    |
| 3                        | +        | –                       | +                              | –               | A                     |
| 4                        | +        | +                       | –                              | +               | A                     |
| 5                        | –        | –                       | –                              | –               | Unpublished procedure |
| 6                        | +        | +                       | –                              | +               | B                     |
| 7                        | –        | –                       | NA                            | –               | C                     |
| 8                        | +        | +                       | –                              | –               | C                     |
| 9                        | –        | –                       | NA                            | –               | Kowalczykowski*       |
| 10                       | –        | –                       | NA                            | –               | NA*                   |

*NA, not applicable.

Effects of Exonuclease I on RecA-mediated Strand Exchange

Ten different recA protein preparations were screened for the ability to generate σ-like products as discussed in the Introduction (see also Fig. 1C). These preparations were also screened for single-stranded exonuclease activity and the presence of exonuclease I by immunoblotting. In addition, those recA protein preparations that exhibited single-stranded exonuclease activity were incubated with anti-exonuclease I antibodies to determine whether all of the single-stranded exonuclease activity present could be attributed to exonuclease I (see “Experimental Procedures”). Purification protocols used to obtain the recA preparations were: A, Griffith and Shores (1985); B, Shibata et al. (1981); and C, Cox et al. (1981). The results described here should not be taken to indicate the presence of exonuclease I in the recA preparations used to generate published data from any laboratory. Note that in many cases the recA protein preparations were not obtained from the laboratory whose purification procedure is cited. The preparation used in line 6 was a preparation known to contain exonuclease I and provided by C. Radding at our request.

Exonuclease I or VII contaminates those recA protein preparations that promote formation of σ-like products and if either of these enzymes is in some way responsible for this activity. Several experimental results provide evidence that exonuclease VII does not contaminate any of these recA protein preparations. First, exonuclease VII possesses a 5'-3' as well as a 3'-5' single-stranded exonuclease activity. In recA protein-promoted DNA strand exchange reactions where the duplex DNA has 3' heterology with respect to the (+) strand, the major product has a single-stranded tail with a 5' end (Bedale et al., 1991), (Figs. 1B and 6, reaction A). The addition of purified exonuclease I, a 3'-5' single-stranded exonuclease, has no effect on the mobility of this product band (Fig. 6, reaction C). The addition of purified exonuclease VII (at the minimal level required to restore formation of σ-like molecules) results in an increase in mobility of this product band (Fig. 6, reaction D). This change in mobility is consistent with degradation of the long single-stranded tail by 5'-3' exonuclease activity. Since no such mobility change is noticed in those reactions with recA protein preparations that can promote formation of 3'-5' products (Fig. 6, reaction B), this result suggests exonuclease VII does not contaminate those recA protein preparations that have this activity.

In addition to this biochemical evidence, immunological evidence also argues against exonuclease VII being the enzyme responsible for the production of σ-like molecules. First, Western analysis with anti-exonuclease VII antibodies did not detect exonuclease VII in any of the recA protein preparations tested (data not shown). Second, anti-exonuclease VII antibodies (at concentrations which can inhibit exonuclease VII activity, data not shown) do not block the generation of σ-like products that is observed with those recA preparations that have this activity intrinsically (Fig. 7, lanes 3-5). These antibodies are, however, capable of blocking the generation of σ-like molecules that result from the addition of purified exonuclease VII (Fig. 7, lanes 10-12).

Exonuclease I, in contrast to exonuclease VII, is present in those recA protein preparations that promote the formation of σ-like products. RecA protein purified from an E. coli host strain in which the chromosomal region surrounding the sbcB gene had been deleted does not promote the formation of σ-like products (Fig. 2, lanes 2 and 3), yet the addition of purified exonuclease I is sufficient to restore this activity to these recA protein preparations (Fig. 2, lane 13). Western analysis using

Fig. 3. Agarose gel assay of single-stranded exonuclease activity found in different recA protein preparations. Single-stranded DNA was incubated with recA protein and other proteins as described under “Experimental Procedures.” All reactions contain SSB protein at 3 μg. Lane 1 is a 0 min time point. All other lanes are 90 min time points. No additional proteins were added to the reaction shown in lane 2. The conditions and protein preparations for each reaction shown in lanes 3-12 were identical to those shown in lanes 2-11, respectively, in Fig. 2 (preparations 1-10, respectively, in Table I). Lane 13 corresponds to a reaction that was performed with an SSB protein preparation known to be contaminated with a low level of exonuclease I. Lane 14 corresponds to a 10-μl reaction to which 0.13 units of exonuclease I were added. Lane 15 corresponds to a reaction in which partially purified recA protein (Fraction III) was substituted for purified recA protein (Fraction IV) (Cox et al., 1981). Lane 16 corresponds to a 10-μl reaction to which 0.14 units of exonuclease VII were added.

Table I

RecA protein preparations used in this study

| RecA protein preparation | Breakage | ss exonuclease activity | ss exo inhibited by anti-ExoI | ExoI immunoblot | Method of purification |
|--------------------------|----------|-------------------------|-------------------------------|-----------------|-----------------------|
| 1                        | –        | –                       | NA*                           | –               | Cox et al., 1981      |
| 2                        | –        | –                       | NA                            | –               | Purchased from USB    |
| 3                        | +        | –                       | +                              | –               | A                     |
| 4                        | +        | +                       | –                              | +               | A                     |
| 5                        | –        | –                       | –                              | –               | Unpublished procedure |
| 6                        | +        | +                       | –                              | +               | B                     |
| 7                        | –        | –                       | NA                            | –               | C                     |
| 8                        | +        | +                       | –                              | –               | C                     |
| 9                        | –        | –                       | NA                            | –               | Kowalczykowski*       |
| 10                       | –        | –                       | NA                            | –               | NA*                   |

*NA, not applicable.

Effects of Exonuclease I on RecA-mediated Strand Exchange

Table I

RecA protein preparations used in this study

| RecA protein preparation | Breakage | ss exonuclease activity | ss exo inhibited by anti-ExoI | ExoI immunoblot | Method of purification |
|--------------------------|----------|-------------------------|-------------------------------|-----------------|-----------------------|
| 1                        | –        | –                       | NA*                           | –               | Cox et al., 1981      |
| 2                        | –        | –                       | NA                            | –               | Purchased from USB    |
| 3                        | +        | –                       | +                              | –               | A                     |
| 4                        | +        | +                       | –                              | +               | A                     |
| 5                        | –        | –                       | –                              | –               | Unpublished procedure |
| 6                        | +        | +                       | –                              | +               | B                     |
| 7                        | –        | –                       | NA                            | –               | C                     |
| 8                        | +        | +                       | –                              | –               | C                     |
| 9                        | –        | –                       | NA                            | –               | Kowalczykowski*       |
| 10                       | –        | –                       | NA                            | –               | NA*                   |

*NA, not applicable.
Effects of Exonuclease I on RecA-mediated Strand Exchange

FIG. 4. Exonuclease VII can restore the ability to generate σ-like molecules to recA protein preparations that lack this activity. Strand exchange reactions and electrophoresis were carried out with 5′ end-labeled DNA containing 198 bp of 5′ heterology as described under “Experimental Procedures.” The markers (lane 1) are derived from a 32P end-labeled MspI digest of pBR322 DNA. Lane 2 is a 0 min time point. All other lanes are 90 min time points. Lane 3 corresponds to a reaction that was carried out with recA protein preparation 4 (Table I). Lane 4 corresponds to a reaction that was performed with recA protein preparation 8 (Table I). Lane 5 corresponds to a 20-μl reaction containing recA protein preparation 8 (Table I) to which 1.2 units of exonuclease VII were added.

anti-exonuclease I antibodies demonstrated that those recA protein preparations that promote the generation of σ-like products contain contaminating exonuclease I (Fig. 8, lanes 3, 4, and 6). The amounts of contaminating exonuclease I are very low, on the order of one molecule of exonuclease I for every 7000–30,000 recA protein molecules. This amount of contaminating enzyme is similar to the amount of pure exonuclease I that is needed to restore the production of σ-like molecules to those recA protein preparations that lack this activity. In addition, the amounts of contaminating exonuclease I present in recA protein preparations correlates very well with the level of σ-like products observed with each protein preparation (compare Fig. 8 with Fig. 2) and with the presence of single-stranded exonuclease activity (Fig. 3). Purified anti-exonuclease I antibodies can inhibit purified exonuclease I, although relatively large amounts of the antibodies are required (data not shown). Preimmune serum does not have any effect on the single-stranded exonuclease activity (data not shown). The antibodies that inhibit purified exonuclease I can also inhibit the single-stranded exonuclease activity found in all but one of the recA protein preparations that promote the formation of σ-like molecules, but have no effect on the single-stranded exonuclease activity of exonuclease VII (data not shown). Exonuclease I also appears to be a contaminant of the original SSB protein preparation that had the ability to generate σ-like molecules (Fig. 8, lane 11), and the single-stranded exonuclease activity in the SSB protein preparation can also be inhibited by the anti-exonuclease I antibodies (data not shown).

FIG. 5. Exonuclease I can restore the ability to generate σ-like molecules to recA protein preparations that this activity. Strand exchange reactions and electrophoresis were carried out with 5′ end-labeled DNA containing 198 bp of 5′ heterology as described under “Experimental Procedures.” The DNA size markers (lane not shown) were derived from a 32P end-labeled MspI digest of pBR322 DNA. Lane 1 is a 0 min time point. All other lanes correspond to 90 min time points. All reaction volumes were 20 μl. Lane 2 corresponds to a reaction that was performed with recA protein preparation 8. Lane 3 corresponds to a reaction that was performed with recA protein preparation 4. Lane 4 corresponds to a reaction from which recA protein was omitted to which 1 unit of exonuclease I was added. Lanes 5–8 correspond to reactions containing recA protein preparation 8 to which 0.001, 0.01, 0.1, and 1 units of exonuclease I were added, respectively.

Exonuclease I Allows RecA Protein-promoted DNA Strand Exchange to Proceed with a Reverse Polarity—How could a
Effects of Exonuclease I on RecA-mediated Strand Exchange

**Fig. 6.** Effects of the addition of exonucleases on the mobility of branched products from reactions with 3' (distal) heterology. Strand exchange reactions were carried out with single-stranded M13mp8 and linear dsDNA containing 198 bp of 3' heterology as described under “Experimental Procedures.” Such reactions result in the efficient formation of the product shown in Fig. 1B, which migrates on agarose gels at the position labeled X. For each reaction A-D, the time points shown correspond to 0, 10, 20, 40, and 60 min of reaction. The marker (lane M) is M13mp8 (FI and FII) DNA. Reactions A, C, and D were performed using recA protein preparation 8 (Table I). Reaction B was performed using recA protein preparation 4. Exonuclease I (1 unit in an 80-μl reaction) was added to reaction C at the same time as the recA protein. Exonuclease VII (0.1 unit in an 80-μl reaction) was added to reaction D at the same time as the recA protein.

Single-stranded exonuclease generate an apparently internal scission in duplex DNA? One possibility is that the single-stranded exonuclease has a latent endonuclease activity (perhaps specific for recombination intermediates) in addition to its well characterized single-stranded exonuclease activity. Another possibility is shown in Fig. 9B. Homologous sequences at the 3' (distal) end of the duplex DNA with respect to the (+) strand first could pair with the recA protein-coated ssDNA (Fig. 9B, i). If the 3' end of the (+) strand of the duplex is displaced, exonuclease I could catalyze the degradation of this strand and stabilize the nascent joint (Fig. 9B, ii). ssDNA protein may be present on this displaced single strand, which would stimulate the action of exonuclease I (Molineux and Gefter, 1975). Pairing adjacent to the stabilized joint would displace more of the (+) strand of the original duplex, making it progressively accessible to exonuclease I (Fig. 9B, iii). Pairing and strand displacement must precede exonuclease I action; the (-) strand is already annealed when exonuclease I acts. Exonucleolytic degradation of the (+) strand of the original duplex would continue (Fig. 9B, iv) until the paired region reached the heterology/homology junction and no further displacement of the (+) strand could occur (Fig. 9B, v).

To distinguish between these two possible mechanisms for exonuclease I action, we first decided to examine early reaction intermediates by electron microscopy. We reasoned that if the exonuclease model was correct, products with two double-stranded tails should be present at early time points. One of the tails would be short and would correspond to the length of heterology, while the other tail should begin long and would shorten with time (Fig. 9A). In this endonuclease model, the displaced ssDNA should still be present at early time points. Its 3' end is still base paired to the (-) strand and should therefore remain resistant to exonuclease I until complete strand displacement has occurred. In the case of the exonuclease model, only one double-stranded tail should be present at any time point, and its length should be long at early time points but very short (corresponding to the length of the heterology) at later time points (Fig. 9B). According to this model, the displaced ssDNA should be immediately degraded by exonuclease I and therefore should not be observed. Strand exchange reactions were performed with duplex DNA that had 198 bp of 5' heterology with respect to the (+) strand. Aliquots were withdrawn at early time points of re-
Another set of experiments also supports the exonuclease model as opposed to the endonuclease model. In these experiments, a short oligonucleotide hairpin was ligated to the ends of the duplex DNA, generating in effect an "endless" DNA molecule (Fig. 13A). If an endonucleolytic nick in the duplex DNA was responsible for the initiation of strand exchange, then the absence of a free end on the duplex DNA would not be expected to affect the reaction. If, however, the end of the DNA is important, then the presence of the hairpin would be expected to block the reaction. Strand exchange reactions with duplex DNA (with or without hairpins) were performed using recA protein (preparation 4 in Table I), which promoted the generation of \( \sigma \)-like molecules and was found by immunobLOTS to be contaminated with exonuclease I. The products of the reaction were analyzed by agarose gel electrophoresis and by electron microscopy. The presence of hairpins greatly decreased the amount of duplex DNA that formed joint molecules in the presence of exonuclease I (Table III, "blocked types") could have arisen from strand exchange initiated at nicks located at random sites in the duplex DNA.

The Presence of Exonuclease I Increases the Efficiency of Joint Molecule Formation by RecA Protein—The results presented here and in a previous paper (Bedale et al., 1991) show that the presence of very small amounts of single-stranded exonuclease allows recA protein to promote strand exchange with DNA substrates that are normally poor substrates for \textit{in vitro} recombination. This idea is substantiated in Table III and in Fig. 13B. In reactions with linear duplex DNA that contains 198 bp of \( 5' \) heterology (no hairpins), the presence of exonuclease I allows apparently all of the linear duplex DNA substrate to be incorporated into joint molecules at 90 min. The limited strand exchange in the absence of exonuclease I that occurs with these substrates appears to be largely limited to substrate molecules containing nicks.

**DISCUSSION**

Our major conclusion is that the combination of a \( 3'-5' \) single-stranded exonuclease and recA protein leads to a kind of reverse DNA strand exchange that allows recombination to occur much more efficiently in reactions where the \( 5' \) (proximal) end of the DNA is blocked from initiating strand exchange by the presence of a heterologous sequence. Previous work from this laboratory (Bedale et al., 1991), which demonstrated that strand exchange between circular single-stranded DNA and dsDNA containing short heterologous sequences at the \( 5' \) (initiating) end were surprisingly efficient, can be explained by this exonuclease-assisted strand transfer. A model illustrating how an exonuclease could facilitate this reaction is shown in Fig. 9B. The exonuclease-enhanced reaction begins at the \( 3' \) end of the (+) strand of the incoming duplex, which is the opposite end of the duplex DNA from which strand exchange between completely homologous substrates normally proceeds (Cox and Lehman, 1981a; Kahn et al., 1981; West et al., 1981). The strand transfer in the presence of single-stranded exonuclease may occur in this "backwards" direction (\( 3'-5' \) with respect to the (+) strand) by the combined (and possibly synergistic) actions of recA protein and exonuclease. RecA protein would pair the homologous regions of the dsDNA to the ssDNA. If some displacement of the \( 3' \) end of the dsDNA occurs at this time, a \( 3'-5' \) exonuclease could attack this displaced end. Pairing must be a prerequisite for exonuclease I action, since no exonucleaseolytic degradation occurs with completely heterologous duplex DNA (Bedale et al., 1991, data not shown). Additional cycles of
Fig. 9. Models for the role of exonuclease I in facilitating recA protein-promoted DNA strand exchange in reactions involving 5’ (proximal) heterology. A, original breakage or endonucleolytic model. i, pairing of homologous sequences and strand breakage at the heterology/homology junction in the (+) strand of the duplex DNA permits strand exchange to occur. ii and iii, DNA strand exchange continues 5’-3’ with respect to the (+) strand. iv, final products are a double-stranded circular molecule with a double-stranded tail (corresponding to the heterology in the original duplex substrate) and the displaced linear ssDNA. B, alternative exonucleolytic model. i, pairing of homologous sequences results in the displacement of the 3’ end of the dsDNA. Exonuclease I can then attack this strand (ii). iii and iv, continued transfer of the (−) strand to the circular ssDNA accompanied by exonucleytic digestion of the displaced (+) strand of the original duplex. v, when transfer of the homologous region of the (−) strand is complete, no more displacement of the (+) strand of the original duplex can occur and exonuclease I digestion halts. The final product is a double-stranded circular molecule with a double-stranded tail (corresponding to the heterology in the original duplex substrate).

pairing, displacement, and exonucleytic degradation could occur and would effectively transfer all of the homologous portion of the (−) strand of the dsDNA to the ssDNAs. The final product of this reaction is a double-stranded circle with a short double-stranded tail corresponding to the length of heterology present at the 5’ end of the original dsDNA substrate. With our best DNA preparations, 60% or more of the input duplex DNA can be converted to scorable o-like molecules by the combined action of recA protein and exonuclease I. These experiments confirm and extend the observations of Konforti and Davis (1992) that exonuclease I can facilitate formation of joint molecules on the 3’ end of the (+) strand of the duplex. When much longer homologous regions are provided in the duplex DNA and the reactions then analyzed by electron microscopy, a dynamic interplay of recA protein and exonuclease I is revealed that leads to the formation of discrete and predictable products.

In an earlier paper (Bedale et al., 1991), the production of this unusual DNA species was attributed to either an endonucleolytic or mechanical break in the dsDNA at the heterology/homology junction that would allow strand exchange to initiate and proceed in the normal 5’-3’ direction from this break (Fig. 9A). Biochemical evidence presented here, however, indicates that the presence of a single-stranded exonuclease is required for the production of this species in a recA protein-promoted reaction. In addition, the electron microscopy and agarose gel results described here support the idea that strand exchange begins at an end of the original linear DNA substrate rather than at an internal nick or break generated specifically at the heterology/homology junction.

The apparent ability of exonuclease I to act on joint molecules formed when homology is limited to the distal end of the DNA is relevant to the current discussion of the proposed three-stranded structure of such distal joint molecules (Hsieh et al., 1990a; Rao et al., 1991a; Stasiak, 1992). Whereas joint molecules formed at the proximal end of the duplex DNA participate in strand exchange with displacement of the (+) strand of the original dsDNA, distal joints can be isolated (after recA removal) in the form of stable DNA triplexes as determined by their thermal stability and sensitivity to strand-specific nucleases (Hsieh et al., 1990a; Rao et al., 1991a).

A major rationale for the formation of stable triplexes exclusively at the distal end is that the (+) strand of the duplex, lacking a free 5’ end here, is not readily displaced (Hsieh et al., 1990; Rao et al., 1991a; Stasiak, 1992). It would seem unlikely that such distal joint molecules would be sus-
Effects of Exonuclease I on RecA-mediated Strand Exchange

Electron microscopy of reactions with 5' (proximal heterology) as a function of time

A strand exchange reaction was performed with daDNA containing 198 bp of 5' heterology as described (Bedale et al., 1991). Aliquots were withdrawn at 0, 3, 8, 15, 24, and 50 min of reaction, and prepared for microscopy as described (Bedale et al., 1991). Complex species (those involving >2 substrate DNA molecules) were not included in the counts.

| DNA species* | 0 | 3 | 8 | 15 | 24 | 50 | Total no. counted |
|--------------|---|---|---|----|----|----|------------------|
| 1. ds linear (substrate) | 99 | 83 | 65 | 21 | 16 | 20 | 447 |
| 2. ds circle with short ds tail (product)' | 0 | 0 | 0 | 2 | 14 | 14 | 52 |
| 3. Gapped circle with long ds tail' (presumed intermediate) | 0 | 10 | 27 | 15 | 14 | 7 | 89 |
| 4. Gapped circle with and without short ds tail' | 0 | 1 | 0 | 2 | 4 | 1 | 13 |
| 5. Gapped circle with long ds tail' and short ss tail' | 0 | 1 | 2 | 3 | 3 | 4 | 2 |
| 6. Gapped circle with long ds tail' and short ss tail' | 1 | 3 | 0 | 0 | 0 | 1 | 9 |
| 7. Gapped circle with two ds tails# | 0 | 1 | 4 | 4 | 5 | 5 | 27 |

Total molecules counted: 121, 239, 49, 126, 56, 301, 892

* Complex species (containing >2 substrate molecules), broken intermediates or products, and ss circles were not counted; ss circles were in excess and represented >50% of all molecules.

# ds tail corresponds roughly to the length of heterology, about 200 bp.

% ds tail is significantly longer than 200 bp; tail lengths are not uniform.

These products presumably arose as a result of nicks in the duplex substrate.

Tail lengths are not uniform.

None of the miscellaneous species had 2 ds tails; none were observed more than 5 times for all the time points combined.

If this interpretation is correct, the rationale is that the stability of triplexes at the distal end reflects an inability to displace the (+) strand needs to be reexamined. Other work has also hinted that the 3' end of the (+) strand in distal joints is displaced at least some of the time (Adzuma, 1992; Stasiak, 1992). ATP hydrolysis appears to be required for the sensitivity of distal joint molecules to exonuclease I; ρ-like products are not generated in the presence of ATPγS (Bedale et al., 1991). The (+) strand of the original duplex substrate in these experiments is sensitive to exonuclease I in the presence of ATP but not in the presence of ATPγS, providing new evidence that the displacement of this strand is one function of ATP hydrolysis. Clearly, the structure and characteristics of distal joints need to be elucidated before a resolution to these issues can be obtained.

The evident sensitivity of the duplex (+) strand to exonuclease I does not preclude the current interpretation of several studies (Hsieh et al., 1990; Rao et al., 1991a) that provide evidence for the formation of stable triplex structures in distal joints. For example, there could be a dynamic equilibrium between a triplex intermediate and products (with the (+)

1. S. Jain and M. Cox, unpublished results.
2. W. Bedale and M. Cox, unpublished results.
Effects of Exonuclease I on RecA-mediated Strand Exchange

Fig. 11. Electron microscopy of double-stranded tailed species at 15 and 50 min of reaction. Strand exchange reactions between single-stranded M13 mp8 (+) and M13mp8.198 (EcoRI) were performed as described under "Experimental Procedures." The reaction was stopped at various times and prepared for electron microscopy as described (Bedale et al., 1991). Electron micrographs of representative molecules after 15 min (left panels) and 50 min (right panels) are shown.

Fig. 12. Time course of formation of double-stranded tailed products and intermediates. Strand exchange reactions between single-stranded M13 mp8 (+) and M13mp8.198 (EcoRI) were performed as described under "Experimental Procedures" and prepared for electron microscopy as previously described (Bedale et al., 1991). Total numbers of molecules counted are shown in Table II.

strand displaced) in these joints that permits the isolation of triplexes when recA protein is removed. The apparent displacement of the (+) strand in joints formed at both ends of the duplex, however, makes it much harder to explain why joint molecules (and triplexes) appear to be more stable when formed at the distal end.

An additional conclusion of our work is that recA protein preparations can often be contaminated with exonuclease I, and that trace, almost undetectable amounts of exonuclease I present in recA protein preparations can have dramatic effects on certain recA protein-promoted reactions. Three of 10 recA protein preparations examined by immunoblots contained exonuclease I (Fig. 8). The contaminating exonuclease I was present at very low levels, on the order of one molecule exonuclease I for every 20,000 recA protein molecules. When these recA protein preparations were screened for single-stranded exonuclease activity, none was detected unless SSB protein was also present. SSB protein has been shown to interact physically with exonuclease I and to stimulate its activity (Molineux and Gefter, 1975). The amount of single-stranded exonuclease activity present in recA protein preparations (as judged by the amount of purified exonuclease I that is needed to restore production of σ-like molecules to those preparations that lack this activity, Fig. 5) is comparable to the amount of exonuclease I present in those preparations (as judged by immunoblots, Fig. 8), suggesting that exonuclease I is the enzyme actually responsible for the formation of σ-like molecules.

Exonuclease I was not present in any recA protein preparations tested that were purified by the method of Cox et al. (1981) (Table I). This purification procedure consists of polymin P precipitation, phosphocellulose chromatography, and ssDNA cellulose chromatography. The ssDNA cellulose col-
Effects of Exonuclease I on RecA-mediated Strand Exchange

Table III

| DNA species*     | Closed, +exo I | Closed, −exo I | Open, (normal) ends, +exo I | Open, (normal) ends, −exo I |
|------------------|----------------|----------------|-----------------------------|-----------------------------|
| ds linear (substrate) | 68             | 73             | <1                          | 50                          |
| Gapped circle    | 6              | 9              | 25                          | 20                          |
| Gapped circle with short ds tail | 4              | <1             | 5                           | 2                           |
| ds circles with short ss tail | 0              | <1             | 1                           | 4                           |
| ds circle with long ss tail | 3              | 6              | 7                           | 18                          |
| Blocked type     | 10             | 0              | 0                           | 0                           |
| Miscellaneous    | 5              | 6              | 1                           | 2                           |

Total molecules counted | 273 | 135 | 246 | 144

*Reactions were spread for microscopy after 90 min of reaction.

Electron microscopy of strand exchange reactions in the presence of exonuclease I with endless DNA

Strand exchange reactions were performed with DNA containing 198 bp of 5' heterology or with endless DNA as described under "Experimental Procedures." Reactions were used as described using recA protein preparations that contained exonuclease I or that were free of exonuclease I. After 90 min of reaction, products were prepared for electron microscopy as described (Bedale et al., 1991). Single-stranded circles and complex species (those involving >2 substrate molecules) were not included in the counts.

Figure 13: Strand exchange reactions in the presence of exonuclease I with endless DNA. A, scheme for making endless DNA. Hairpin oligos were ligated to M13mp8.198 (EcoRI) DNA as described under "Experimental Procedures." B, strand exchange reactions and agarose gel electrophoreses were performed as described under "Experimental Procedures." The marker (lane M) is M13mp8 DNA (FI-III). For each reaction A-D, the time points shown correspond to 0, 45, and 90 min of reaction. Reactions A and B contain the endless DNA shown in A and produced as described under "Experimental Procedures." Reactions C and D contain ssM13mp8 and M13mp8.198 (EcoRI); the latter has 198 bp of 5' (proximal) heterology. Reactions A and C were performed with recA protein preparation 4, which is contaminated with exonuclease I. Reactions B and C were performed with recA protein preparation 1, which was purified from an E. coli strain that is exonuclease I-free.

The observation that exonuclease I can increase the efficiency of recA protein-mediated DNA strand exchange when a heterologous sequence is present at the 5' end of the duplex DNA in vitro suggests a possible role for exonuclease I in vivo recombination. Exonuclease I is the primary single-stranded exonuclease in E. coli (Chase and Richardson, 1974) and is specific for ssDNA, which it degrades processively in the 3'-5' direction (Lehman and Nussbaurn, 1964; Weiss, 1981). The enzyme will degrade single-stranded tails on dsDNA molecules to within six to eight nucleotides of the base-paired region (Brutlag and Kornberg, 1972). Exonuclease I is the product of the sbcB gene, and sbcB mutations suppress the recombination deficiency and UV light and mitomycin C sensitivity of recbc and recC mutations (Kushner et al., 1971), suggesting a possible role for exonuclease I in DNA recombination or repair (Kushner et al., 1971). Mutants which lack exonuclease I show no detectable defects in DNA metabolism, however, so its precise role remains unclear (Weiss, 1981). Interestingly, properties of another class of mutations in the gene for exonuclease I suggest that the enzyme may have an additional activity. This class of mutations, xonA, suppresses the DNA repair but not the recombinational deficiencies in recbc mutants. Phillips et al. (1988) suggested that the xonA gene product might block the ability of enzymes from an alternative recombination pathway to recognize the DNA substrate. This might occur if exonuclease I interacted with other ssDNA binding proteins, such as SSB protein and recA protein (Phillips et al., 1988). An interaction of exonuclease I and recA may be especially important in the process of recombinational repair. Interestingly, it was also our attempts to understand how recA protein facilitated bypass of structural barriers in the DNA during strand exchange (an activity important for repair rather than recombination) that initially led to these studies.

*S. Kowalczykowski, personal communication.
The proposed action of an exonuclease in homologous recombination has precedent in eukaryotic DNA strand transferases. Sep1 protein is a strand transferase from yeast which possesses an intrinsic 5'-3' exonuclease activity (Johnson and Kolodner, 1991). The action of this nuclease is necessary for the initiation of strand exchange. The exonuclease degrades the ends of the dsDNA substrate so that 3' single-stranded tails are generated. These single-stranded tails can pair with complementary ssDNA; branch migration can then occur. Under conditions that prevent the nuclease activity, strand exchange can occur if the ends of the DNA are resected by an exogenous exonuclease (Johnson and Kolodner, 1991). The polarity of Sep1 protein promoted DNA strand exchange is a consequence of polarity of the nuclease activity itself and not of branch migration. If the exogenous exonuclease leaves 3' single-stranded tails, strand exchange proceeds 5'-3' (with respect to the (+) strand), while if it leaves 5' single-stranded tails, strand exchange proceeds 3'-5' (Johnson and Kolodner, 1991).

The ability of recA protein, SSB protein, and exonuclease I to function together in vitro during homologous recombination makes it tempting to speculate that they may cooperate similarly in vivo. Exonuclease I can greatly improve the efficiency of in vitro DNA strand exchange reactions in situations where the 5' (proximal) end is blocked by heterology. SSB protein stimulates recA protein-promoted strand exchange dramatically (Cox and Lehman, 1981b, 1982; Cox et al., 1983) and probably enhances the activity of exonuclease I by binding to the strand that exonuclease I degrades. Although exonuclease I was not observed to bind directly to a recA protein affinity column (Freitag and McEntee, 1988), the observation that exonuclease I and recA protein copurify through many purification steps suggests that the two proteins may interact physically. Exonuclease I and SSB are also known to interact and copurify under certain conditions (Molineux et al., 1974). Many important questions still remain, such as whether or not exonuclease I plays a similar role with recA protein and SSB protein in vivo as it appears to play in vitro, and if it is the only single-stranded exonuclease that affects recA protein-promoted strand exchange. The ability of at least one single-stranded exonuclease, exonuclease VII, to promote this reaction makes the assigning of specific roles difficult. It is at least possible that one of several functions of exonuclease I in vivo is to facilitate homologous recombination in situations where recA protein does not work efficiently, and that other 3'-5' single-stranded exonucleases can promote similar reactions.

Acknowledgments—We thank the many people who kindly provided us with proteins and expertise, especially Sidney Kushner, John Chase, Jack Griffith, Stephen Brenner, Charles Radding, Stephen Kowalczykowski, Paul Modrich, Gerald Smith, Andrew Taylor, Deani Cooper, Dan Diaz, Maria Schnos, David Inman, and Claire Walczak. We also thank Charles Radding and B. J. Rao for a careful reading and critique of the manuscript.

REFERENCES

Aduama, K. (1992) Genes & Dev. 6, 1679-1694
Bedale, W. A., Inman, R. B., and Cox, M. M. (1991) J. Biol. Chem. 266, 6499-6510
Bedwell, M. E., and Radding, C. M. (1983) Cell 35, 511-520
Bretlau, D., and Kornberg, A. (1972) J. Biol. Chem. 247, 241-248
Chase, J. W., and Vales, L. D. (1981) Gene Amplification and Analysis, pp. 147-156, Elsevier/North Holland, New York
Cox, M. M., and Lehman, R. R. (1981a) Proc. Natl. Acad. Sci. U. S. A. 78, 6015-6022
Cox, M. M., and Lehman, L. R. (1981b) Proc. Natl. Acad. Sci. U. S. A. 78, 3433-3437
Cox, M. M., and Lehman, L. R. (1982) J. Biol. Chem. 257, 8522-8532
Cox, M. M., McEntee, K., and Lehman, L. R. (1981) J. Biol. Chem. 256, 4676-4678
Cox, M. M., Soltis, D. A., Livneh, Z., and Lehman, L. R. (1983) Cold Spring Harb. Symp. Quant. Biol. 48, 101-203
Cox, M. M., Fugh, B. C., Schutte, B. C., Lindsay, J. E., Lee, J., and Morrical, S. W. (1987) DNA Replication and Recombination, pp. 597-607, Alan R. Liss, New York
Craig, N. L., and Roberts, J. W. (1981) J. Biol. Chem. 256, 8039-8044
Davis, R. W., Bothean, D., and Roth, J. B. (1985) in Advanced Bacterial Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
Eichler, D. C., and Lehman, T. M. (1977) J. Biol. Chem. 252, 499-503
Freitag, N. N., and McEntee, K. E. M. (1988) J. Biol. Chem. 263, 1852-1858
Griffith, J., and Shores, C. G. (1985) Biochemistry 24, 158-162
Hsieh, P., Camerini-Otero, C. S., and Camerini-Otero, R. D. (1990) Genes & Dev. 4, 1961-1963
Jain, S. K., Inman, R. B., and Cox, M. M. (1992) J. Biol. Chem. 267, 4215-4222
Johnson, A. W., and Kolodner, R. D. (1991) J. Biol. Chem. 266, 14046-14054
Jawang, B., and Radding, C. M. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 7690-7694
Kahn, R., Cunningham, B. P., Das Gupta, C., and Radding, C. M. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 4786-4790
Kim, J., Cox, M. M., and Inman, R. B. (1992a) J. Biol. Chem. 267, 16444-16448
Kim, J. J., Cox, M. M., and Inman, R. B. (1992b) J. Biol. Chem. 267, 16438-16443
Kowalczykowski, S. C. (1991) Annu. Rev. Biophys. Biophys. Chem. 20, 539-575
Kowalczykowski, S., Nagaishi, H., Tempelin, A., and Clark, A. J. (1971) Proc. Natl. Acad. Sci. U. S. A. 68, 824-827
Lohman, T. M., and Overman, L. (1983) J. Biol. Chem. 259, 3620-3626
Lohman, T. M., and Overman, L. B. (1983) J. Biol. Chem. 260, 3594-3605
Lohman, T. M., Green, J. M., and Beyer, R. S. (1986) Biochemistry 25, 21-25
Messing, J. (1982) Methods Enzymol. 83, 201-275
Messing, J., and Vieira, J. (1982) Gene (Amst.) 19, 269-276
Messing, J., and Vieira, J. (1982) Gene (Amst.) 19, 269-276
Molineux, I. J., and Gefter, M. L. (1975) J. Mol. Biol. 98, 811-825
Molineux, I. J., Friedman, S., and Gefter, M. L. (1974) J. Biol. Chem. 249, 6990-6998
Mori, S. K., and Cox, M. M. (1980) J. Biol. Chem. 261, 8276-8282
Phillips, G. J., Prasher, D. C., and Kushner, S. R. (1988) J. Bacteriol. 170, 2389-2394
Prasher, D. C., Conarro, L., and Kushner, S. R. (1983) J. Biol. Chem. 258, 6340-6345
Radding, C. M. (1989) Biochim. Biophys. Acta 1008, 131-145
Rao, B. J., Dutreix, M., and Radding, C. M. (1991a) Proc. Natl. Acad. Sci. U. S. A. 88, 2986-2988
Rao, B. J., Jawang, B., and Dutreix, M. (1991b) Biochemistry 30, 363-370
Rao, B. J., Chiu, S. K., and Radding, C. M. (1993) J. Mol. Biol., in press
Roca, A. I., and Cox, M. M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 415-419
Rosellini, W., and Stasiak, A. (1993) EMBO J. 10, 4391-4396
Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) in Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
Shibuta, T., Cunningham, R. R., and Radding, C. M. (1981) J. Biol. Chem. 256, 7557-7564
Stasiak, A. (1992) Mol. Microbiol. 6, 3267-3276
Vales, L. D., Rahin, B. A., and Chase, J. W. (1982) J. Biol. Chem. 257, 8799-8808
Walczak, C. E., Marchese-Ragona, S. P., and Nelson, D. L. (1993) Cell Motil. Cytoskel. 24, 17-28
Wetts, B. (1983) The Enzyme 14, 203-231
West, S. C., Cassuto, E., and Howard-Flanders, P. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 6149-6155