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On the Role of ATP Hydrolysis in RecA Protein-mediated DNA Strand Exchange

I. BYPASSING A SHORT HETEROLOGOUS INSERT IN ONE DNA SUBSTRATE*

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Jong-Il Kim, Michael M. Cox‡, and Ross B. Inman

From the Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin, Madison, Wisconsin 53706

RecA protein promotes a substantial DNA strand exchange reaction in the presence of adenosine 5′-*O*-3-(thio)triphosphate (ATPγS) (Menetski, J. P., Bear, D. G., and Kowalczykowski, S. C. (1990) *Proc. Natl. Acad. Sci. U. S. A.* 87, 21–25), calling into question the role of ATP hydrolysis in the strand exchange reaction. Here, we demonstrate that the ATPγS-mediated reaction can go to completion when the duplex DNA substrate is only 1.3 kilobase pairs in length. The ATPγS-mediated reaction, however, is completely blocked by a 52-base pair heterologous insertion in either DNA substrate. This same barrier is readily bypassed when ATP replaces ATPγS. This indicates that at least one function of recA-mediated ATP hydrolysis is to bypass structural barriers in one or both DNA substrates during strand exchange. This suggests that ATP hydrolysis is directly coupled to the branch migration phase of strand exchange, not to promote strand exchange between homologous DNA substrates during recombination, but instead to facilitate the bypass of structural barriers likely to be encountered during recombinational DNA repair.

The first catalytic activity reported to be associated with the purified recA protein of *Escherichia coli* was a DNA-dependent ATPase (Ogawa *et al.*, 1979; Roberts *et al.*, 1979). While many other activities have since been attributed to recA protein and characterized extensively, the role of ATP hydrolysis has become one of the most controversial mechanistic issues concerning recA action.

In vitro, recA protein promotes a set of DNA strand exchange reactions that closely mimic the putative central steps in homologous genetic recombination *in vivo* (for reviews, see Roca and Cox, 1990; Radding, 1991; Kowalczykowski, 1991; West, 1992). The most common model reactions involve substrates derived from bacteriophage M13 or φX174, and the active species in strand exchange reactions is a recA nucleoprotein filament that forms on ssDNA[1] or a gapped duplex. A second DNA molecule, typically a homologous linear duplex, is then aligned with the first to form a pairing intermediate. An exchange of strands ensues, which can be described as a facilitated unidirectional branch migration reaction.

ATP is hydrolyzed during strand exchange, but its function is not clear. In a typical three-strand exchange reaction with substrates about 6,000 bp in length, there are about 100 ATPs hydrolyzed for every base pair of heteroduplex DNA formed. This expenditure of chemical energy is hard to rationalize, since the reactions are isoenergetic and branch migration itself is a spontaneous reaction. In addition, Menetski *et al.* (1990) have demonstrated that substantial strand exchange (up to 3.4 kbp) can occur under some conditions when ATPγS (which is essentially not hydrolyzed by recA protein) is substituted for ATP. A similar result was obtained by Rosselli and Stasiak (1990) using very short DNA substrates. These workers have suggested that a major function of ATP hydrolysis is to facilitate recA dissociation from the products of DNA strand exchange and recycle recA protein.

RecA protein dissociates in a polar fashion from the filament end opposite to that where assembly takes place (Lindsley and Cox, 1990), and this must be coupled in some manner to ATP hydrolysis. Furthermore, recA protein must logically dissociate from the DNA at some point during or following strand exchange. However, ATP hydrolysis occurs throughout the recA filament with an optimal monomer k_{cat} approaching 30 min^{-1} (Brenner *et al.*, 1987; Schutte and Cox, 1987; Kowalczykowski and Krupp, 1987), and dissociation rarely accounts for more than a very small fraction of the ATP hydrolytic events. Furthermore, under some conditions ATP hydrolysis proceeds with no detectable dissociation (Neuendorf and Cox, 1986). When the *E. coli* single-strand DNA binding protein (SSB) is included in the reaction, essentially all of the recA protein bound initially to the ssDNA is found associated with the heteroduplex DNA product (Pugh and Cox, 1987; Roca and Cox, 1990).[2] There is no mechanistic requirement for the dissociation of recA protein from the product duplex DNA during strand exchange and no evidence that it occurs with SSB present under the conditions generally found to be optimal for the reaction (Roca and Cox, 1990). Dissociation of recA protein therefore does not provide a complete explanation for the ATP hydrolytic activity.

A more complete rationale for ATP hydrolysis in this system can be arrived at by considering the cellular function of recA protein. In addition to homologous genetic recombination, recA protein is also a key component in SOS induction, SOS mutagenesis, and recombinational repair (Roca and Cox, 1990; Witkin, 1991; Cox, 1991). While all of these functions are important to the cell, the recombination function has received most of the attention.

We have recently argued that the structure and mechanistic

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‡ To whom correspondence should be addressed: Tel.: 608-262-1181; Fax: 608-265-2603.

[1] The abbreviations used are: ssDNA, single-stranded DNA; ATPγS, adenosine 5′-*O*-(thio)triphosphate; SSB, single-stranded DNA binding protein of *E. coli*; SDS, sodium dodecyl sulfate; AMT, 4′-amino-4,5′,8-trimethylpsoralen; bp, base pair(s); kbp, kilobase pair(s).

[2] C. Ullsperger and M. M. Cox, unpublished results.

features of the recA system can be rationalized only if recombinational repair, rather than recombination, is viewed as its primary function (Roca and Cox, 1990; Cox, 1991). While the strand exchange reactions that must take place during recombination are quite similar to those that occur during recombinational repair processes such as postreplication repair (West *et al.*, 1981), their energetic and mechanistic requirements are quite different. The requirements of maintaining genetic diversity through an occasional isoenergetic strand exchange during recombination at random locations in the genome could be met with a much simpler system than recA. During postreplication repair, however, the reaction is no longer isoenergetic. Strand exchange must occur in a particular location. It must bypass DNA lesions and other barriers that can vary greatly in size and structure, and the outcome cannot be left to chance.

In this context, the recA system begins to make molecular sense. The filamentous form of the active species and ATP hydrolysis would both make important contributions during repair processes in which strand exchange must bypass DNA lesions with close to 100% efficiency. The filament excludes other DNA binding proteins from the DNA, and ATP hydrolysis renders the reaction unidirectional and provides the energy required to bypass whatever structural barriers are encountered. *In vitro*, recA protein-mediated strand exchange readily bypasses pyrimidine dimers, mismatches, and heterologous inserts in one DNA up to several hundred base pairs in length (Livneh and Lehman, 1982; DasGupta and Radding, 1982; Bianchi and Radding, 1983). The requirements of DNA repair provide a good explanation for the maintenance of this complex system over 1.5 billion years of evolution.

In this view, the recA system evolved as a repair system, and recombination is simply a molecular byproduct (Roca and Cox, 1990; Cox, 1991).

The interior of the recA filament accommodates three DNA strands (Takahashi *et al.*, 1989, 1991; Kubista *et al.*, 1990; Müller *et al.*, 1990), and binding energy within the filament groove may facilitate a strand switch between homologous strands and explain the observations of Menetski *et al.* (1990) and Rosselli and Stasiak (1990) that some strand exchange proceeds with ATPγS. If ATP hydrolysis is viewed as an augmentation of this process that forces the branch migration in one direction and past structural barriers, then the ATPγS-mediated reaction should be blocked by relatively modest structural barriers. In this report, we demonstrate that the ATPγS-mediated reaction is blocked by a 52-base pair heterologous insert in either of the DNA substrates, an insert that is readily bypassed when ATP is hydrolyzed. This provides evidence that lesion bypass during recombinational repair is one of the functions of ATP hydrolysis in the recA system.

EXPERIMENTAL PROCEDURES

Enzymes and Biochemicals—*E. coli* recA protein was purified and stored as described previously (Cox *et al.*, 1981). The recA protein concentration was determined by absorbance at 280 nm using an extinction coefficient of $\epsilon_{280} = 0.58\ A_{280}$ mg/ml (Craig and Roberts, 1981). *E. coli* SSB protein was purified as described (Lohman *et al.*, 1986), and was stored frozen at −70 °C in a buffer containing 20 mM Tris-HCl (40% cation, pH 8.4), 0.15 M NaCl, 1 mM EDTA, 1 mM β-mercaptoethanol, and 50% glycerol. The concentration of the SSB protein stock solutions was determined by absorbance at 280 nm using an extinction coefficient of $\epsilon_{280} = 1.5\ A_{280}$ mg/ml (Lohman and Overman, 1985). Restriction endonucleases and T4 polynucleotide kinase were purchased from New England Biolabs. Calf intestinal alkaline phosphatase, ATPγS, and Tris buffer were purchased from Boehringer Mannheim. Proteinase K, formamide, pyruvate kinase, phosphoenolpyruvate, creatine kinase, phosphocreatine, and ATP were obtained from Sigma. Radionucleotides were purchased from Amersham Corp., and 4′-amino-4,5′,8-trimethylpsoralen (AMT) was purchased from Calbiochem.

DNA—Duplex and ssDNA substrates were derived from bacteriophage M13mp8 (Messing and Vieira, 1982). Circular duplex and single-stranded DNA from M13mp8 and its derivatives were prepared using methods described previously (Davis *et al.*, 1980). The bacteriophage M13mp8.52 was constructed by replacing the 30-bp *Eco*RI-*Hin*dIII fragment of bacteriophage M13mp8 with a 52-bp *Eco*RI-*Hin*dIII fragment derived from the plasmid pJFS36 (Senecoff *et al.*, 1985). The sequence of M13mp8.52 was verified using the Sequenase version 2.0 sequencing kit from United States Biochemical Corp. (Bedale *et al.*, 1991). The concentrations of ssDNA and double-stranded DNA stock solutions were determined by absorbance at 260 nm using 36 and 50 μg/ml A_{260}, respectively, as conversion factors. DNA concentrations are expressed in terms of total nucleotides.

Complete digestion of FI M13mp8 with the appropriate restriction enzyme (*Eco*RI or *Pst*I) yielded linear duplex DNA substrates. To generate shorter (1.3 kbp) fragments of M13mp8 and M13mp8.52, the DNA was digested with the restriction enzymes *Nae*I and *Bgl*II using conditions recommended by the enzyme supplier. The purified DNA fragments (1301 and 1323 bp from M13mp8 and M13mp8.52, respectively) were obtained by electroelution of DNA bands from agarose gels. Elution of DNA from agarose gels was performed using a unidirectional electroeluter (International Biotechnologies, Inc.) or by electroelution into dialysis membranes (Sambrook *et al.*, 1989).

Reaction Conditions—Unless stated elsewhere, all reactions were carried out at 37 °C in solutions containing 25 mM Tris-acetate (80% cation, pH 7.5), 1 mM dithiothreitol, 5% glycerol, 3 mM potassium glutamate, 10 mM (or indicated concentration) magnesium acetate, and an ATP-regenerating system (5 units/ml pyruvate kinase and 3 mM phosphoenolpyruvate or 10 units/ml creatine kinase and 12 mM phosphocreatine). Reactions with ATPγS included 5 mM magnesium acetate (rather than 10) unless indicated and 1 mM ATPγS. DNA, SSB, and recA protein concentrations are indicated for each experiment. ATP (1 mM) and SSB were added to start strand exchange reactions after the incubation of duplex and circular ssDNA with recA protein at 37 °C for 10 min. The order of addition is described in figure legends for experiments in which it was changed.

Agarose Gel Assays—Aliquots (20 μl) of strand exchange reactions described above were removed at each time point, and the reactions were stopped by addition of 5 μl of gel loading buffer (0.125% bromphenol blue, 25 mM EDTA, 25% glycerol, and 5% SDS). These aliquots were stored on ice until after the last time point was taken. Samples were electrophoresed overnight in a 0.8% agarose gel. Some samples were cross-linked with AMT before loading agarose gel electrophoresis.

Cross-linking Conditions—In order to block spontaneous branch migration, some strand exchange reaction mixtures were cross-linked with AMT prior to deproteinization. The psoralen derivative (AMT) was added to a final concentration of 30 μg/ml, and the samples were incubated for 3 min at room temperature followed by a 5-min exposure to long-wave UV light on ice. Intensity of UV light ranged between 2 and 4 milliwatts/cm^2 at 365 nm. This AMT cross-linking treatment resulted in at least one cross-link per 220 bp (Bedale *et al.*, 1991). Deproteinization of the samples was then carried out by treatment with SDS and proteinase K as described below.

Electron Microscopy—Visualization of reactions by electron microscopy was carried out by spreading the entire strand exchange reaction mixture or purified product DNAs electroeluted from agarose gels as described in the text. DNAs were briefly cross-linked with AMT prior to removal of recA protein and SSB to prevent changes in the DNA species due to spontaneous branch migration. RecA and SSB proteins were removed by treatment with 1% SDS and 1 mg/ml (final concentration) proteinase K for 30 min at 37 °C. All samples were then either dialyzed against 20 mM NaCl and 5 mM EDTA overnight at room temperature or loaded onto small spun columns (Umlauf *et al.*, 1990), and the column flow-through was dialyzed against 20 mM NaCl and 5 mM EDTA. The dialyzed samples were spread as described previously (Bedale *et al.*, 1991). Intermediates formed in the strand exchange reactions were verified by partially denaturing the samples (Inman and Schnös, 1970). Photography and computer-assisted length measurement of DNA molecules were performed as described previously (Littlewood and Inman, 1982).

RESULTS

Experimental Design—The purpose of this work is to determine whether ATP hydrolysis is required for recA protein-mediated DNA strand exchange past heterologous insertions

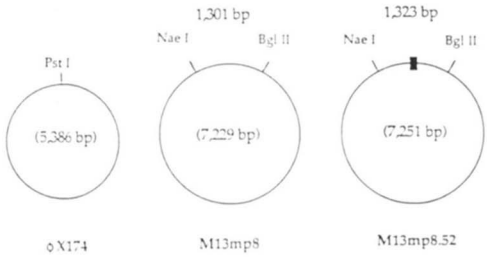

FIG. 1. **DNA substrates used in this study.**

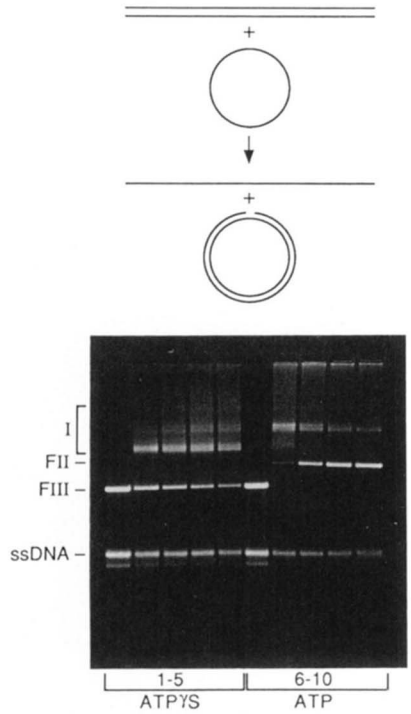

FIG. 2. **RecA protein-mediated DNA strand exchange in the presence of ATPγS.** Reactions were carried out as described under "Experimental Procedures." Reactions included 5 μM M13mp8 circular ssDNA, 8 μM M13mp8 linear duplex DNA, 3 μM recA protein, 0.5 μM SSB, and (in the ATP reaction) the phosphocreatine and creatine phosphokinase ATP regenerating system. Time points in each series (*lanes 1–5* or *6–10*) were 0, 10, 30, 60, and 90 min, respectively. In the ATPγS experiment, SSB was preincubated with the DNA substrates for 5 min before the addition of ATPγS and recA protein to initiate the reaction. The order of addition for the ATP experiment and other reaction conditions are described under "Experimental Procedures."

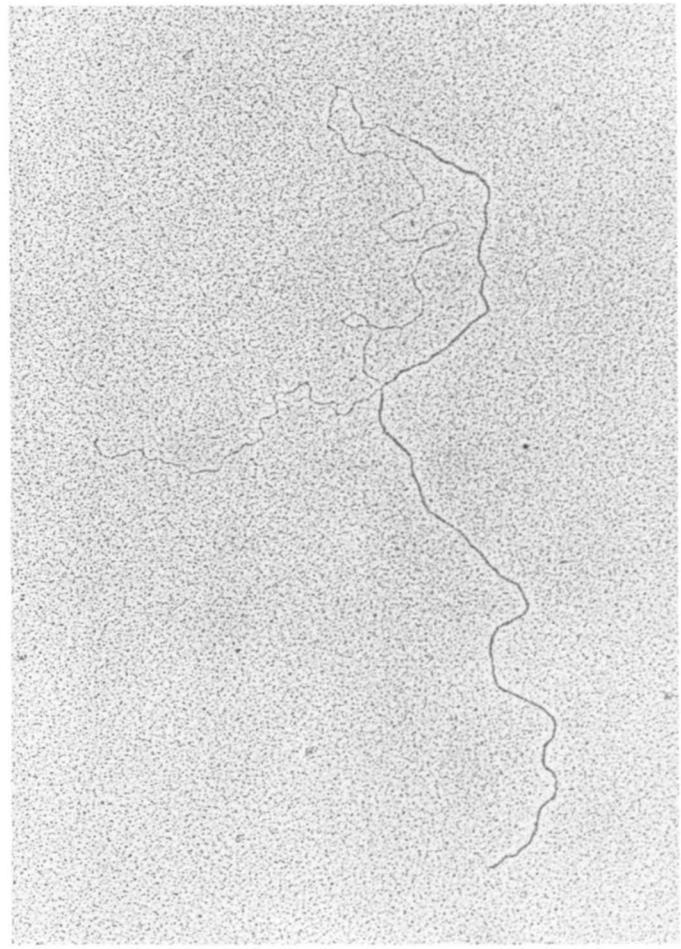

FIG. 3. **Electron microscopy of recA-mediated DNA strand exchange in the presence of ATPγS.** An intermediate generated in the ATPγS reaction with circular single strands and linear duplex DNA substrates derived from bacteriophage φX174. Reaction conditions are described in the legend to Fig. 2 and under "Experimental Procedures." The intermediate has the form expected for invasion and displacement of one strand of the linear duplex by the single-stranded circle.

in one of the two DNA substrates. The criteria is based on the observation of Menetski *et al.* (1990) that substantial strand exchange can occur in the presence of ATPγS. Here, we seek to determine if the reaction with ATPγS can bypass heterologous inserts that are readily bypassed in the ATP reaction.

The system we used was designed to allow us to readily observe and quantify strand exchange past a heterologous insertion, if it occurred. With DNA substrates derived from bacteriophage M13 (6407 bp) the strand exchange reaction with ATPγS generates joint molecules with heteroduplex DNA segments that can average as much as 3.4 kbp in length, but complete products of strand exchange are not observed (Menetski *et al.*, 1990). For this study, we chose to use a duplex DNA fragment 1.3 kbp in length in order to observe a complete strand exchange reaction with ATPγS that would be efficient enough that any change in the reaction due to the presence of a heterologous insert would be readily apparent. Reactions were monitored both by agarose gel electrophoresis and electron microscopy. The latter method allowed us to measure the progress of strand exchange directly in individual DNA molecules. The heterologous insert, where present, was placed at the center of the 1.3-kbp region undergoing strand exchange; this enabled us to determine where the insert was in each molecule on the electron microscope grid without having to distinguish one end of the duplex DNA substrate from the other.

The substrates are illustrated in Fig. 1. The 52-bp heterologous insertion in M13mp8.52 replaces the 30-bp sequence between the *Eco*RI and *Hin*dIII restriction sites in M13mp8. M13mp8.52 is therefore only 22 bp longer than M13mp8. The study proceeded in two parts. First, we repeated the results of Menetski *et al.* (1990) with ATPγS to establish a point of reference and to further characterize that reaction. We then examined the effects of the heterologous inserts.

RecA Protein-mediated DNA Strand Exchange in the Presence of ATPγS—In this series of experiments we used reaction conditions very similar to those used by Menetski *et al.* (1990), with the major difference being that we used DNA substrates derived from bacteriophage M13mp8 (7,229 bp) instead of substrates derived from M13 (6,407 bp).

RecA protein-mediated strand exchange in the presence of ATPγS is illustrated in Figs. 2 and 3. In Fig. 2, a timecourse of strand exchange in the presence of ATPγS is compared

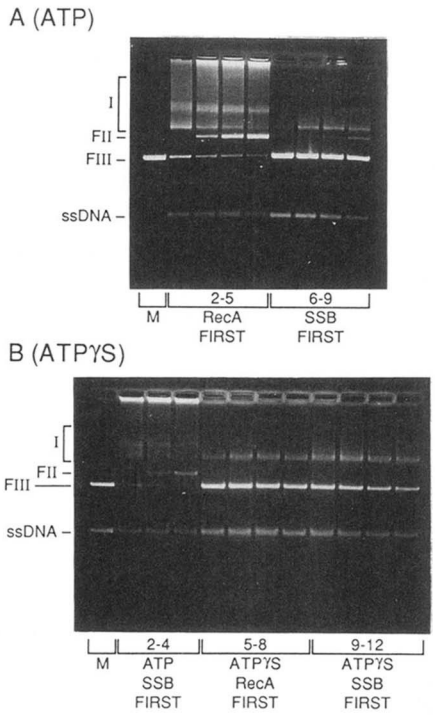

FIG. 4. **The effect of order of addition of recA protein and SSB on the DNA strand exchange reaction.** DNA substrates and reaction conditions are as described in Fig. 2. Where recA protein was added first, the protocol follows that described under "Experimental Procedures." When SSB was added first, SSB was preincubated with DNA substrates at 37 °C for 5 min before addition of recA protein and ATP or ATPγS.

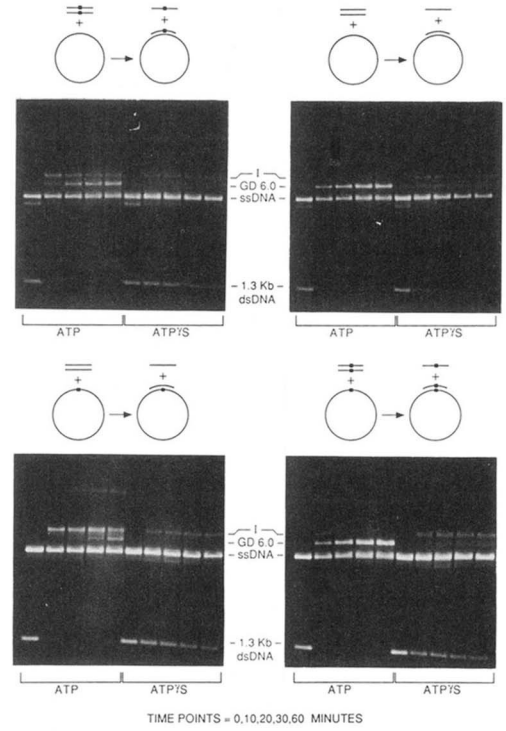

FIG. 5. **RecA protein-mediated DNA strand exchange past a 52-bp heterologous insert.** Reactions were carried out as described under "Experimental Procedures" under conditions individually optimized for the ATP and ATPγS reactions (in the ATPγS reactions, 5 mM magnesium acetate was used and SSB was added prior to recA protein). Reactants, reaction intermediates (*I*), and gapped duplex products ($GD_{6.0}$) are marked. The reaction monitored by each gel is described by the reaction schematic immediately above it. The 52-bp insert is denoted by ■ in some substrates shown in the reaction schematics. Each reaction is shown in five lanes, representing time points of 0, 10, 30, 60, and 90 min, left to right.

with an ATP-mediated reaction. In the ATP reaction, linear duplex substrate (FIII) quickly disappears and is largely converted to the circular nicked duplex (FII) product by the end of the reaction. The ATPγS reaction generates large amounts of reaction intermediates but no detectable FII products.

These reactions were further examined by electron microscopy. In the ATP reaction, 66% of the duplex DNA (in an examination of 82 duplex or partially duplex molecules) was converted to circular duplex products after 60 min. In the ATPγS reaction, circular duplex products were not observed; most of the DNA was present in complexes involving three or more DNA substrate molecules. Simple intermediates (involving one circular single strand and one linear duplex) were observed but probably represented no more than 1% of the DNA present. Fourteen of the simpler molecules were chosen at random and photographed. Of these, eight had the expected intermediate structure (see Fig. 3). The other six either were missing a displaced single-strand tail (1), had one or more strand breaks that precluded analysis (3), or involved a reaction between a circular single strand and two duplexes (2). The heteroduplex regions in the eight standard intermediates were measured; they ranged from 190 to 7020 base pairs, with the average being 3350 bp. Although these molecules are not typical in this sample, the more complex molecules had generally undergone extensive strand exchange (albeit with multiple partners) and we use these measurements simply to indicate that substantial strand exchange occurred in this sample in the presence of ATPγS. In another series of experiments a similar ATPγS-mediated reaction was carried out with substrates derived from bacteriophage φX174 (5386 bp). The results were similar except that in this case some double-stranded circular products were observed (data not shown), indicating that complete strand exchange can occur with quite long DNA substrates in the presence of ATPγS. Simple intermediates (Fig. 3) were also more common with the φX174 substrates.

We also explored the effects of the order of addition of recA protein and SSB in these reactions (Fig. 4). The ATP reaction is greatly inhibited under otherwise optimal reaction conditions when SSB is added prior to recA protein, as reported previously. The ATPγS reaction, under its optimal reaction conditions, proceeds somewhat better when SSB is added prior to recA protein.

ATPγS-mediated DNA Strand Exchange Is Blocked by a 52-bp Heterologous Insert in One DNA Substrate—The reactions of circular ssDNA (either M13mp8 or M13mp8.52) with 1.3-kbp duplex DNA fragments (either with or without a 52-bp heterologous insert) were monitored with the agarose gel assay (Fig. 5). The expected product of a complete strand exchange reaction is a gapped duplex DNA with a 1.3-kbp region of heteroduplex DNA and a 6.0-kbp region of ssDNA, denoted $GD_{6.0}$. All four possible substrate combinations were tested, and in each case the ATP and ATPγS reactions (each with its optimal reaction conditions) were compared side by side. Production of $GD_{6.0}$ was observed in every reaction containing ATP, although the 52-bp insert appeared to lower the efficiency of the reaction somewhat. The insert had the greatest effect on the ATP reaction when it was in the ssDNA substrate, but significant amounts of $GD_{6.0}$ product were formed at late time points. In the experiments with completely homologous substrates, the linear 1.3-kbp duplexes were converted quantitatively to $GD_{6.0}$ in the ATP reactions (the ssDNA is present in excess in these experiments). In the reactions with ATPγS, some $GD_{6.0}$ was generated when completely homologous DNAs were used, although the amounts

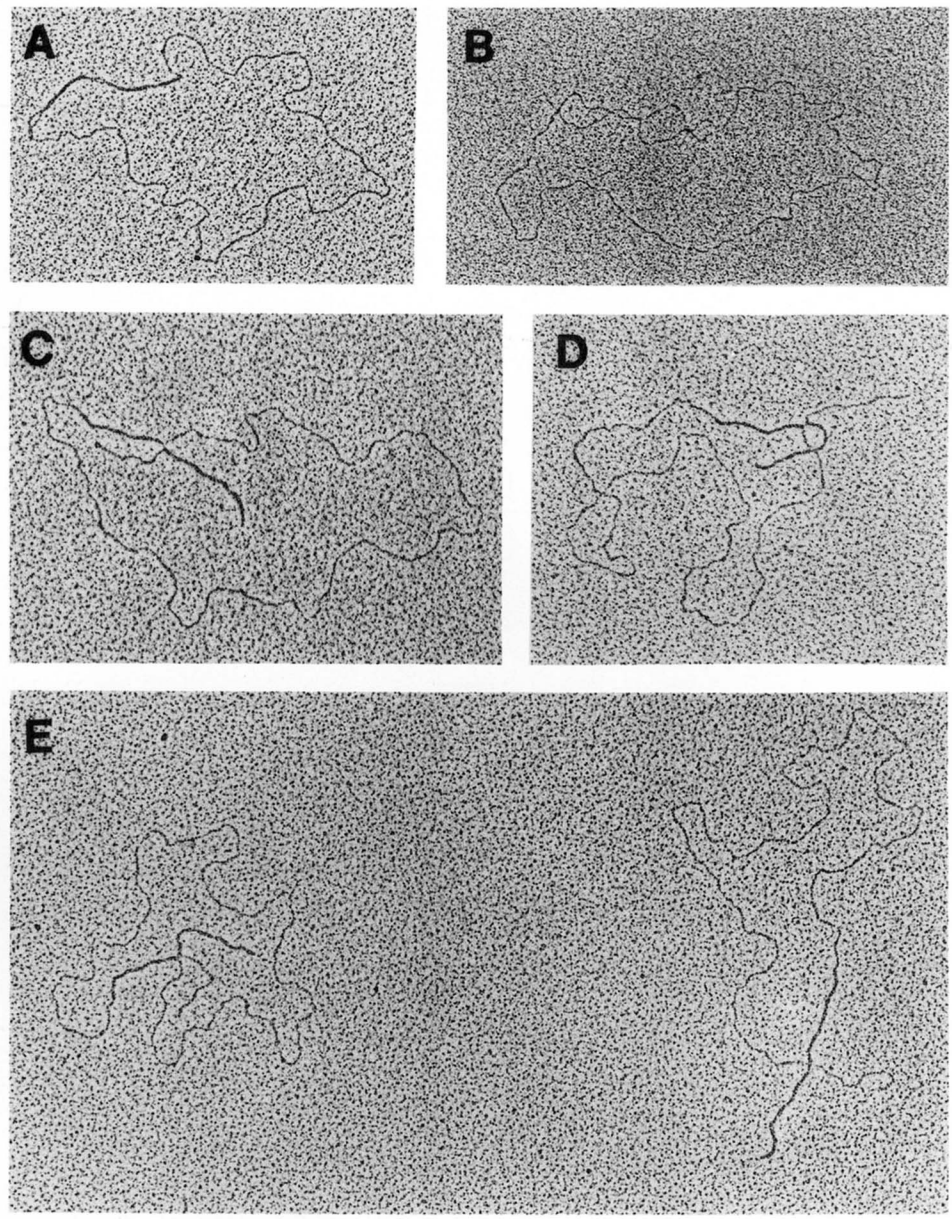

FIG. 6. **Electron microscopy of recA protein-mediated DNA strand exchange through a heterologous insert.** *A*, the $GD_{6.0}$ product formed in the presence of ATP; *B*, same as *A*, but the double-stranded region has been denatured to demonstrate strand continuity. *C–E*, intermediates generated in the ATPγS reaction between M13mp8 ssDNAs and the 1.3-kbp fragment from M13mp8.52.

were reduced relative to the ATP reaction (Fig. 5). *No* $GD_{6.0}$ product was detected by gel assay, however, when the 52-bp insert was present in only one of the two substrates in an ATPγS reaction. Substantial amounts of reaction intermediates were evident on the gels in these experiments.

To determine if the ATPγS reaction was blocked by the heterologous insert, the reactions of M13mp8 ssDNA with duplex fragments (with or without the insert) were further investigated by electron microscopy. For the completely homologous substrates, 80% of the duplex fragments were converted to products ($GD_{6.0}$) after 30 min in the ATP reaction. When ATPγS was used, the efficiency was lower, but even here 18% of the duplex DNA on the grid was in product form; 70% of the duplex DNA in the ATPγS reaction was unreacted and the rest was present as molecules with unusual forms.

When the heterologous insert was present in the duplex substrate, 50% or more of the duplex DNA was still converted to product in the ATP reaction (Fig. 6*A*). A randomly chosen sample of 41 of these product molecules was examined after AMT cross-linking and denaturation; strand breaks were found in only one of these molecules, indicating that strand exchange through these short heterologus inserts can proceed without the strand breaks observed by Bedale *et al.* (1991). Length measurements of the duplex segments confirmed that these molecules were $GD_{6.0}$ products in which strand exchange had proceeded past the heterologous insert.

In the ATPγS reaction, 334 duplex or partially duplex molecules in a 30-min reaction were chosen at random. Of these, 23% were reaction intermediates of the standard type, 73% were unreacted linear duplexes, and 4% were classified as unusual forms (generally broken molecules with one or more DNA arm missing). No molecules were found that could be identified as reaction products in which strand exchange had gone to completion. In a second experiment, 25 intermediate molecules were selected at random from this grid and photographed (examples are shown in Fig. 6, *C–E*). The heteroduplex regions in these molecules were measured, and the results are shown in Fig. 7. The distribution of heteroduplex lengths halts abruptly in the middle of the linear duplex fragment, indicating that the 52-bp insert is virtually an impenetrable barrier to the ATPγS reaction.

DISCUSSION

Our primary conclusion is that ATP hydrolysis is required for recA protein-mediated DNA strand exchange past heter-

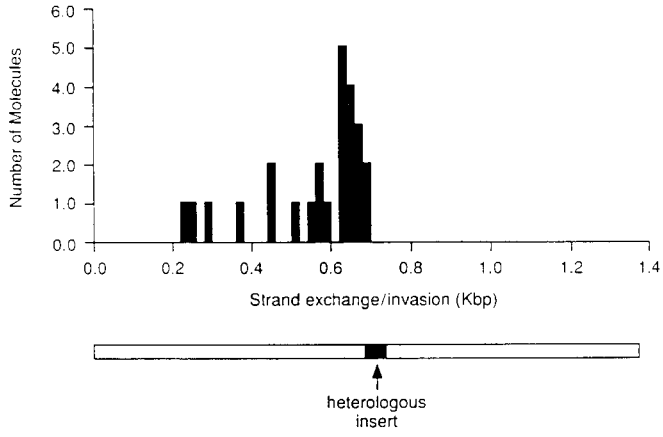

FIG. 7. **Distribution of the lengths of heteroduplex DNA in the ATPγS reaction between M13mp8 ssDNA and the 1.3-kbp fragment from M13mp8.52.**

ologous inserts in one of the DNA substrates. The results of Menetski *et al.* (1990) that efficient recA-mediated strand exchange can occur under specified conditions provide a good criterion for testing candidate functions for the ATP hydrolytic activity of recA protein. The present experiments show that strand exchange with completely homologous substrates is substantially more efficient when ATP is hydrolyzed; strand exchange through a heterologous insert is *completely* dependent on ATP hydrolysis. Using short oligonucleotides for substrates, Rosselli and Stasiak have recently observed that pairing and/or strand exchange in the ATPγS reaction can be blocked with inserts as small as 6 bp.[3]

These results bring us back to a consideration of the primary function of recA protein. Strand exchange between homologous DNA substrates as might occur during genetic recombination is isoenergetic and can clearly occur without ATP hydrolysis. Strand exchange involving a substrate with a heterologous insert or other barrier, as might be required during recombinational DNA repair, requires ATP hydrolysis. We argue that ATP hydrolysis is coupled in some manner to strand exchange, precisely because the primary function of the reaction is recombinational DNA repair. In the accompanying paper (Kim *et al.*, 1992), we demonstrate that ATP hydrolysis is required for recA protein-mediated strand exchange involving four strands. This observation may be a manifestation of the same coupling mechanism.

At a molecular level, to what could ATP hydrolysis be coupled in order to facilitate strand exchange past heterologous inserts and other barriers? Most of the speculation concerning the role of ATP hydrolysis has centered on dissociation of recA monomers from the filament and, to a lesser extent, the strand switch itself (Howard-Flanders *et al.*, 1984; Menetski *et al.*, 1990; Rosselli and Stasiak, 1990; West, 1992). As outlined in the Introduction, however, dissociation does not begin to account for the amounts of ATP hydrolyzed or for hydrolysis in the interior of the filament where dissociation does not occur. In addition, when SSB is present along with an ATP regeneration system, strand exchange proceeds efficiently without significant dissociation of recA protein. It is particularly difficult to see how ATP hydrolysis could facilitate strand exchange past barriers in the DNA if its primary molecular consequence is *dissociation* of recA monomers. Elsewhere, we have suggested a model in which ATP hydrolysis is coupled directly to a coordinated rotation of the two DNA substrates (Cox, 1989). The model provides a rationale for strand exchange past barriers and accounts for all of the ATP hydrolyzed during strand exchange (Cox, 1989, 1990). As indicated in the accompanying paper (Kim *et al.*, 1992), the model also provides a mechanism that helps explain the requirement for ATP hydrolysis with four DNA strands.

One aspect of these experiments deserves some further comment. Bedale *et al.* (1990) observed that strand exchange past large heterologous sequence barriers at one end of the duplex DNA was facilitated by DNA strand breaks generated by an undefined mechanism. The efficient strand exchange through short heterologous inserts observed here in the ATP reaction did not involve strand breakage. An evaluation of mechanisms for the bypass of heterologous inserts is continuing.

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