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RecA Filament Dynamics during DNA Strand Exchange Reactions*

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The role of ATP hydrolysis in RecA protein-mediated DNA strand exchange reactions remains controversial. Competing models suggest that ATP hydrolysis is coupled either to a simple redistribution of RecA monomers within a filament to repair filament discontinuities, or more directly to rotation of the DNA substrates to drive branch movement unidirectionally. Here, we test key predictions of the RecA redistribution idea. When ATP is hydrolyzed, DNA strand exchange is accompanied by a RecA exchange reaction, between free and bound RecA protomers in the interior of RecA filaments, that meets a central prediction of the model. The RecA protomer exchange is not required for, and does not occur during, the "search for homology" in which the single-stranded DNA within a RecA-ssDNA nucleoprotein filament is homologously aligned with the duplex DNA. Instead, the RecA exchange is triggered by the completion of strand exchange (a strand switch to generate a hybrid DNA product) in any given segment of the filament. In effect, formation of hybrid DNA leads to a change in filament conformation to one with properties approximating those of RecA filaments bound to double-stranded DNA. Addition of the RecA K72R mutant protein to a reaction with the wild type protein leads to the formation of mixed filaments and a poisoning of the DNA strand exchange reaction. Under some conditions, a facile RecA protomer exchange is observed, and significant ATP is hydrolyzed, even though DNA strand exchange is entirely blocked by the mutant protein. A redistribution of RecA protomers coupled to ATP hydrolysis is not sufficient in itself to explain how ATP hydrolysis facilitates DNA strand exchange. A RecA protomer exchange may nevertheless play an important role in the DNA strand exchange process.

RecA protein from *Escherichia coli* (352 amino acid residues; M_r 37,842) plays a central role in the processes of recombinational DNA repair, homologous recombination, induction of the SOS response to DNA damage, and the partitioning of chromosomes at cell division (1–8). RecA is an ancient protein present in all bacteria, with structural and functional homologues in eukaryotes ranging from yeast to humans (4, 9, 10).

In vitro, RecA protein promotes a set of DNA strand exchange reactions that mimic its presumed roles *in vivo*. In a typical DNA strand exchange reaction (Fig. 1), RecA protein first forms a nucleoprotein filament on circular ssDNA[1] with a stoichiometry of one RecA monomer per three nucleotides. The ssDNA within the filament is then paired with a linear duplex DNA and aligns homologous sequences between these two DNAs. A strand switch then occurs within the filament, producing a nascent region of hybrid DNA. Subsequently, the hybrid DNA is extended unidirectionally, 5′ to 3′ relative to the ssDNA in the original filament, until strand exchange is completed. At the end of the reaction, RecA protein remains bound to the hybrid duplex product DNA (11, 12). The fact that RecA protein is bound to ssDNA at the onset of DNA strand exchange and dsDNA at the end is of central importance in the present study.

RecA protein is a DNA-dependent ATPase, with a monomer k_{cat} of 30 min^{-1} when bound to ssDNA (dATP is hydrolyzed at rates about 20% higher). ATP is hydrolyzed uniformly throughout the nucleoprotein filament (13). When a homologous duplex DNA is added to the reaction, the k_{cat} drops abruptly to about 20 min^{-1} and remains at that level throughout the ensuing DNA strand exchange reaction (14). A significant DNA strand exchange reaction, often producing a thousand base pairs or more of hybrid DNA, can occur within RecA filaments that are not hydrolyzing ATP (15–18). This demonstrates that the filament itself is set up to incorporate up to three DNA strands and promote a DNA strand switch using nothing more than the binding energy available inside the filament groove. However, when ATP is not hydrolyzed, DNA strand exchange typically halts well before the reaction reaches completion. ATP hydrolysis is coupled in some manner to the final stage of DNA strand exchange (19), conferring on the reaction unidirectionality and the capacity to bypass barriers and accommodate 4 DNA strands (18–23). The rate of ATP hydrolysis during DNA strand exchange is similar to that observed in RecA filaments bound directly to dsDNA (5, 6). The actual molecular function of this ATP hydrolysis has been controversial, with many proposals focusing on RecA filament assembly and disassembly processes.

RecA nucleoprotein filaments rapidly assemble on ssDNA in the 5′ to 3′ direction (24) at a rate in excess of 1000 RecA monomers min^{-1} $filament^{-1}$. RecA protein does not readily bind to dsDNA at neutral pH due to a very slow nucleation step (25, 26). RecA filaments also disassemble in an end-dependent manner from the filament end opposite to that at which assembly occurs (27–29). On double-stranded DNA, this process is highly pH-dependent, being absent at pH 6.0 and occurring at the maximal rate (about 3 monomers s^{-1}) above pH 8.0 (27). A similar end-dependent disassembly of RecA filaments has recently been observed and characterized on ssDNA, occurring from the filament end nearest the 5′ end of a linear ssDNA molecule (29). The RecA filaments are replaced by SSB protein as disassembly proceeds. This disassembly process is suppressed when pH is lowered to about 6, when dATP replaces

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[1] The abbreviations used are: ss, single-stranded; ds, double-stranded; RecA, the wild type RecA protein; OAc, acetate ion; bp, base pair(s); kb, kilobase pair(s); ATPγS, adenosine 5′-*O*-(3-thio)triphosphate; SSB, the single-stranded DNA binding protein of *E. coli*; wt, wild type.

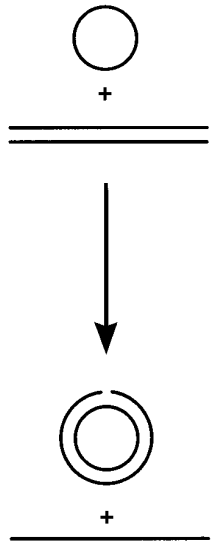

FIG. 1. **RecA protein-mediated DNA strand exchange reactions.**

ATP, or when RecO and RecR proteins are included (29).

In the filament interior, RecA protein undergoes little exchange with RecA protein in the free pool when the filament is formed on ssDNA, with no protomer exchange at all observed in the presence of dATP (30, 31). However, on dsDNA, an exchange between RecA monomers in the free and bound forms in the filament interior has been documented. This exchange process is independent of the number of dsDNA ends, the length of dsDNA, and reaction pH (31). In effect, a change occurs when a second complementary DNA strand is present within the RecA filament, producing a filament that is more dynamic with respect to exchange between free and bound forms.

The characterization of the RecA protomer exchange reactions has made extensive use of a RecA mutant protein, RecA K72R, in which the lysine to arginine substitution occurs within a highly conserved ATP binding motif (16, 18). This mutant protein binds but does not hydrolyze ATP or dATP. In the presence of dATP, RecA K72R promotes a limited degree of DNA strand exchange. This mutant protein also forms mixed filaments with wild type RecA on either ssDNA or dsDNA (31) and has been proven to be a very useful tool for exploring the properties of RecA nucleoprotein filaments (16, 18, 31).

The stability of RecA protein filaments is a crucial parameter in evaluating models for the roles of ATP hydrolysis in the final stages of DNA strand exchange. Two models are broadly consistent with observations published to date. Both explain the limited extent of DNA strand exchange observed under most conditions when ATP is not hydrolyzed. The RecA monomer redistribution model proposed by Kowalczykowski and coworkers (15–17) suggests that DNA strand exchange without ATP hydrolysis is limited by discontinuities in the RecA filaments, with ATP hydrolysis coupled only to a redistribution of RecA monomers to repair the discontinuities. The alternative facilitated DNA rotation models suggest that any discontinuities involve the DNA pairing intermediate rather than the RecA filaments. ATP hydrolysis is instead directly coupled to coordinated rotation of the two DNA substrates (2, 18, 19, 32). Efficient DNA strand exchange takes place under conditions in which RecA protein remains quantitatively bound to the hybrid DNA product (12), showing that a net disassembly of the RecA filament is not a mechanistic requirement. However, a redistribution of RecA monomers within the filament remains a formal possibility. In the continuing discussion, we define filament dynamics in terms of the association and/or dissociation of RecA protomers, acknowledging that the actual species that bind or dissociate (monomers, dimers, other?) have not been defined (5, 33, 34).

The various models make a number of predictions with respect to RecA filament dynamics. If the effects of ATP on DNA strand exchange reactions are to be explained solely by redistribution of RecA monomers within the filaments, then an exchange of RecA monomers between free and bound forms must occur during DNA strand exchange. Further, the RecA monomer exchange must be sufficient in itself to overcome all limitations observed in the absence of ATP hydrolysis and permit a complete DNA strand exchange to occur. These fundamental requirements of the RecA redistribution model have never been addressed experimentally.

As already noted, RecA filaments begin DNA strand exchange bound to ssDNA and end the reaction bound to dsDNA. Since there is a documented RecA protomer exchange in filaments formed on dsDNA but not those formed on ssDNA (31), one might expect that the RecA protomer exchange required by the RecA redistribution model might be activated as DNA strand exchange took place. The present experiments were carried out to test this prediction and also to determine whether a protomer exchange, if observed, might adequately explain the effects of ATP hydrolysis on DNA strand exchange.

MATERIALS AND METHODS

Enzymes and Biochemicals—*E. coli* RecA protein (wtRecA) and RecA K72R mutant were purified by a procedure developed for the RecA K72R mutant protein (18). RecA protein was stored in R buffer (20 mM Tris-OAc 80%+, pH 7.5, 1 mM dithiothreitol, 0.1 mM EDTA, and 10% (w/v) glycerol). All RecA protein preparations were more than 95% pure and free of detectable nucleases. The concentration of RecA protein preparations were determined by absorbance at 280 nm using an extinction coefficient of $\epsilon_{280} = 0.59\ A_{280}\ mg^{-1}$ ml (35). *E. coli* single-stranded DNA binding protein (SSB) was purified as described elsewhere (36) with the minor modification that a DEAE-Sepharose column was added to ensure removal of single-stranded exonucleases. The concentration of SSB protein was determined by absorbance at 280 nm using an extinction coefficient of $\epsilon_{280} = 1.5\ A_{280}\ mg^{-1}$ ml (37). Tris buffer was from Fisher. Proteinase K, lactic dehydrogenase, pyruvate kinase, phosphoenolpyruvate, and nicotinamide adenine dinucleotide (reduced form, $NADH^{+}$) were purchased from Sigma. Ultrapure dATP, ATP, and DEAE-Sepharose resin were from Pharmacia Biotech Inc. Hydroxylapatite resin was from Bio-Rad.

DNA—Supercoiled duplex DNA and circular single-stranded DNA from bacteriophage M13mp8 (7229 bp) and M13mp8.52 (7251 bp) (20) were prepared as described previously (30, 38, 39). The concentrations of dsDNA and ssDNA stock solutions were determined by absorbance at 260 nm, using 50 and 36 $\mu g\ ml^{-1}\ A_{260}^{-1}$, respectively, as conversion factors. DNA concentrations are expressed in terms of total nucleotides. Linear duplex DNA substrates were generated by complete digestion of supercoiled DNA by appropriate restriction endonucleases. The protein was removed by extraction with phenol/chloroform/isoamyl alcohol (25:24:1) and chloroform/isoamyl alcohol (24:1). The DNA fragments were finally concentrated by ethanol precipitation.

DNA Strand Exchange Reaction Conditions—Unless otherwise specified, all reactions (500 μl) were performed at 37 °C in a strand exchange reaction buffer containing 25 mM Tris-OAc (80% cation, pH 7.5), 10 mM magnesium acetate, 3 mM potassium glutamate, 1 mM dithiothreitol, 3 mM dATP, 5% (w/v) glycerol, a dATP regeneration system (8 mM phosphoenolpyruvate, 20 units ml^{-1} pyruvate kinase), and a coupling system (4 mM NADH and 10 units ml^{-1} lactic dehydrogenase). The final pH after the addition of all reaction components was 7.4. Circular single-stranded DNA (3 μM) was preincubated with either wtRecA protein or a mixture of wtRecA and RecA K72R mutant protein (2 μM) for 10 min before SSB protein (0.3 μM) was added to form the presynaptic ssDNA filaments. The dATP hydrolytic activity of RecA protein was monitored by a coupled spectrophotometric assay (see below) for at least 20 min to ensure reaching a steady state rate. Linear duplex dsDNA was added in 33% excess (8 μM) to initiate the strand exchange reaction. The reactions continued to be monitored by the coupled dATPase assay. At the same time, the reactions were also monitored by agarose gel assays (see below).

RecA protein challenge experiments were carried out as follows. Reactions were assembled as described above with all reaction components but the linear dsDNA. RecA K72R mutant was added to preformed wtRecA-ssDNA filaments. The added mutant protein changed the composition of the RecA protein in the overall reaction mixture without affecting the dATP hydrolysis rate of wtRecA filaments, since exchange of mutant protein into filaments preformed on ssDNA does not occur (31). The change in dATP hydrolysis was then monitored upon addition of duplex DNA to determine if exchange into the filaments was induced by the strand exchange process. The same strand exchange reactions were also followed by agarose-gel assays. Some challenge reactions started out with mixed filaments containing both the wt and mutant RecA protein, and the mutant content (percent of mutant relative to total RecA) was then shifted up by addition of RecA K72R mutant, or shifted down by addition of wtRecA protein.

Coupled dATPase Assays—The rate of dATP hydrolysis was measured spectrophotometrically with a coupled enzyme protocol (28, 40). A Perkin-Elmer Lambda 7 double-beam recording spectrophotometer equipped with two thermojacketed cuvette holders, each capable of holding six cuvettes, was used for absorbance measurements. The cell path length and band pass were 0.5 cm and 2 nm, respectively. The regeneration of dATP from dADP and phosphoenolpyruvate with the oxidation of NADH can be followed by the decrease in the absorbance at 380 nm. Absorbances at 380 nm were recorded every 60 s. The measurements were done at 380 nm rather than 340 nm (the absorbance maximum for NADH), to remain within the linear range of the spectrophotometer over extended time courses. An increase in the concentration of ATP or dATP or any of the coupling system components did not change the observed ATP or dATPase rate; the data obtained reflect the initial velocity of ATP hydrolysis at all times (28, 40).

Agarose Gel Assays—Aliquots (18 μl) were taken from the strand exchange reactions at 0, 10, 20, 40, 60, and 80 min. The zero time point was taken immediately after the dsDNA was added and the reaction mixtures were mixed in the cuvette. The reactions were stopped by the addition of 0.25 volume of gel loading buffer (60 mM EDTA, 5% SDS, 25% (w/v) glycerol, 0.2% bromphenol blue) and left on ice until the reaction was complete. Samples were electrophoresed overnight in a 0.8% agarose gel at 2 V cm^{-1}. After the gel was stained with ethidium bromide (1 mg/ml) for at least 30 min and destained for at least 2 h, the gel was then photographed over an ultraviolet transilluminator and/or scanned by FluoroImaginer SI (Molecular Dynamics). The intensities of DNA bands were quantified with ImageQuant software (version 4.2). To correct for variability in sample loading onto the agarose gel, bands corresponding to full-length hybrid duplex product and linear dsDNA substrate were quantified as the fraction of the total fluorescing DNA in a given gel lane. DNA strand exchange product formation is expressed as the fraction of the total dsDNA present as nicked circular product. There was no effort to correct the results for background fluorescence in the gel lanes, and this quantitation method therefore tends to underestimate the generation of product DNA. Since the linear dsDNA substrate is added in excess, the maximum possible conversion to product is 75%.

RESULTS

Experimental Design—This study was designed to document changes in the properties of RecA filaments during DNA strand exchange reactions expected on the basis of differences previously noted for RecA filaments formed on dsDNA relative to those formed on ssDNA. An increased apparent cooperativity and an internal exchange of RecA monomers between free and bound forms is observed in the filaments formed on dsDNA (31). These changes are more distinct and readily interpreted when dATP replaces ATP as the nucleotide cofactor in RecA reactions (31). Also, the RecA K72R mutant protein functions only in the presence of dATP (16, 18). Hence, the work described below makes use of dATP exclusively.

When added RecA K72R mutant protein is exchanged into a RecA filament, the exchange is manifested by increases in the content of mutant *versus* wild type protein in the filaments and an associated decline in the rate of dATP hydrolysis. The presence of any given amount of RecA K72R in mixed filaments has a much greater inhibitory effect on dATP hydrolysis by the remaining wtRecA monomers when the filaments are formed on dsDNA, and this is the effect we attribute to an increase in apparent cooperativity (31).

Changes in the rates of dATP hydrolysis in the filaments is the most convenient method to monitor the exchange of RecA monomers. Use of the coupled spectrophotometric dATPase assay permits the observation of changes in real time. This assay is indirect, and in principle a decrease in dATPase rate could be interpreted as either an exchange that increases the mutant content of filaments or a net decrease in total RecA protein bound to the DNA. In these experiments, we interpret all changes in dATPase rate to reflect changes in the mutant protein content of the filaments. This can be justified in several ways. First, we have previously demonstrated that there is no net dissociation of RecA protein from RecA filaments during DNA strand exchange under the conditions used in these experiments. RecA protein remains quantitatively bound to the hybrid DNA product of strand exchange as long as ATP is regenerated (11, 12). In addition, we have calibrated the rates of dATP hydrolysis in mixed filaments formed on ssDNA and dsDNA to the actual amount of mutant protein present in the filaments measured with more direct methods (31). When exchange occurs, the content of mutant protein in the mixed filaments generally approaches the overall mutant *versus* wild type protein content of the reaction mixture over the course of many minutes, with corresponding and predictable changes in the rates of dATP hydrolysis which can be checked against the rates observed in the earlier work (31). Under the conditions we are using for DNA strand exchange (pH 7.5), very little RecA protein binds directly to dsDNA due to the very slow nucleation step (25, 26).[2] Thus, any effect of direct RecA protein binding to the linear dsDNA substrate (prior to its involvement in DNA strand exchange) can be neglected on the time scale used in these reactions.

In the present study, filament states were compared before and during DNA strand exchange reactions using the dATPase assay. The formation of DNA intermediates and hybrid DNA products in the same DNA strand exchange reactions was usually monitored concurrently by an agarose gel assay. To further simplify the interpretations of these results, excess dsDNA was used to convert most ssDNA into pairing intermediates. Therefore, the measured dATP hydrolysis during strand exchange mainly reflects the amount of dATP hydrolyzed by RecA filaments promoting DNA strand exchange.

RecA Protein Filaments Undergo a Change in State during a DNA Strand Exchange Reaction—The wild type and K72R mutant RecA proteins compete for binding sites on ssDNA on an equal basis, and form mixed filaments (31). When the total RecA protein is in large excess relative to DNA binding sites, the decline in dATPase activity by the wild type protein is exactly proportional to the fraction of total protein represented by the mutant (31). If 20% of the added protein is mutant, then the dATPase activity declines by 20%. In contrast, when the mixed filaments are formed on dsDNA, the inhibitory effect of the mutant protein is greatly in excess of its fractional representation in the mixed filaments, reflecting the enhanced cooperativity already described (31). If DNA strand exchange is initiated with a mixed filament bound to ssDNA and containing some mutant RecA K72R protein, and a change in filament state to that typical of filaments bound to dsDNA occurs over the course of the reaction, it would be manifested by a substantial decline in the rate of dATP hydrolysis even without a change in the fraction of mutant RecA protein in the filaments.

Filaments were first formed on ssDNA with wild type RecA protein alone or with mixtures of wild type and mutant pro-

[2] Q. Shan and M. M. Cox, unpublished data.

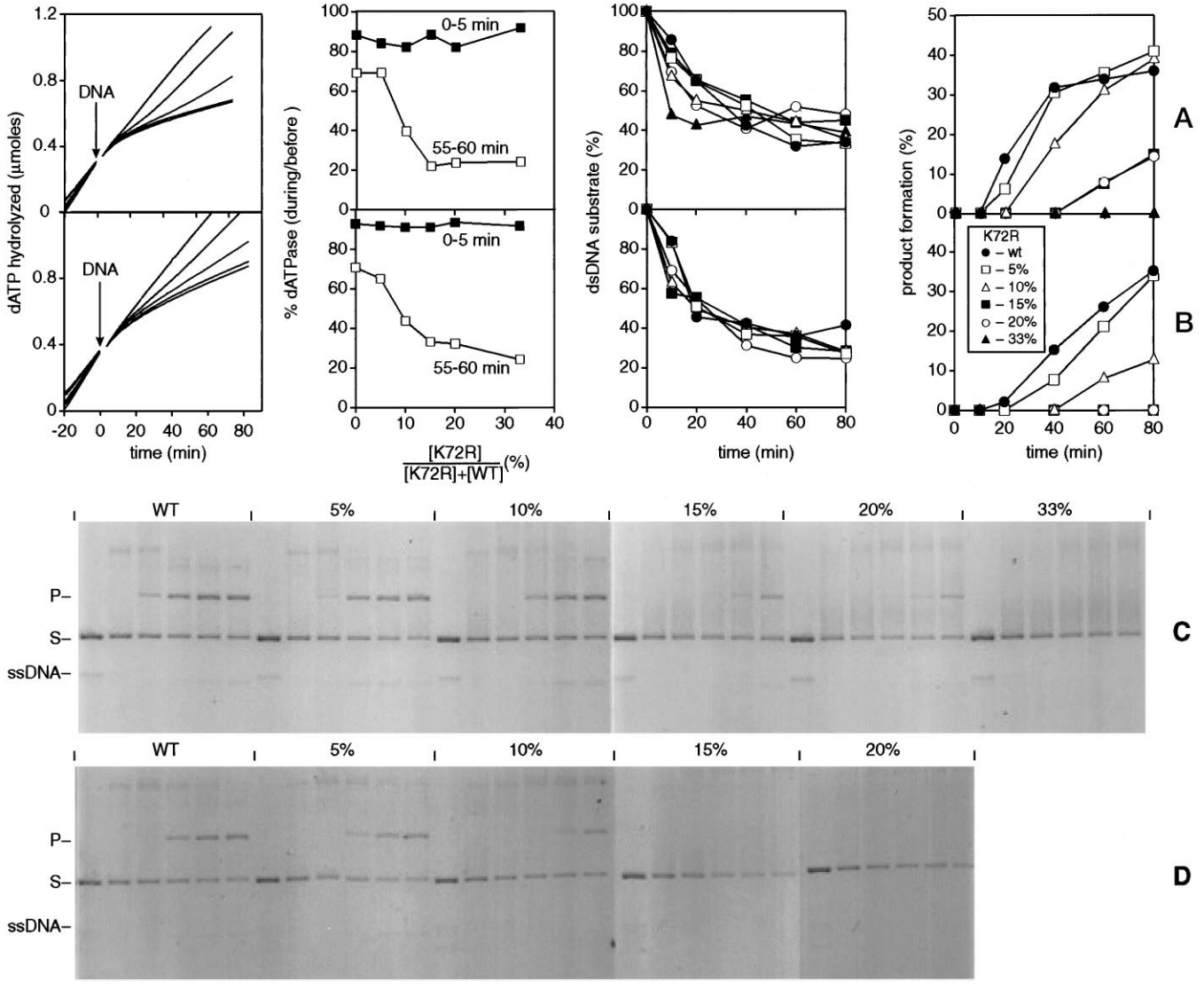

FIG. 2. **Characterization of DNA strand exchange reactions carried out by RecA filaments containing a mixture of wild type and K72R mutant proteins.** Reactions (500 μl) were carried out as described under "Materials and Methods," and contained 3 μM M13mp8 circular ssDNA, 8 μM M13mp8 (cleaved by *Sma*I) or M13mp8.52 (cleaved by AlwNI) linear dsDNA, 0.3 μM SSB protein, and 3 mM dATP. Reactions also contained mixtures of wtRecA and RecA K72R protein with a total RecA protein concentration kept constant at 2 μM. Panels are arranged in four rows. *Row A* involves reactions involving homologous M13mp8 DNA substrates. *Row B* involves reactions with a 52-bp insertion in the linear dsDNA substrate (M13mp8.52). Six reactions were carried out with RecA filaments containing 0–33% K72R mutant protein (0–20% for the reactions with M13mp8.52 dsDNA). The wild type and mutant proteins were added together to form mixed filaments on ssDNA. After 30 min of incubation at 37 °C, with continuous monitoring of ATP hydrolysis, strand exchange was initiated by addition of the linear dsDNA substrate (*arrow*). In each row, the *left panel* shows some or all of the dATPase data. In each case, the fastest rates observed are those with wild type RecA protein alone, and the presence of mutant protein generates a decrease in dATPase rates over time. The derived rates are displayed in the *second panel*. Rates were calculated in the first 5 min, and between 55–60 min into the reaction. The *third* and *fourth panels* in each row are derived from the strand exchange assays in *rows C* and *D*. *Row C* shows the completely homologous DNA strand exchange reactions, and *row D* shows the reactions when the dsDNA is M13mp8.52. In *rows A* and *B*, the uptake of dsDNA into joint molecules and the generation of fully exchanged DNA products are summarized in the *third* and *fourth panels*, respectively. As noted under "Materials and Methods," the procedure used to quantify the results from the agarose gel assays minimizes variability between lanes but tends to underestimate the production of nicked circular DNA products. The maximum theoretical conversion of linear duplex DNA nicked circles in these experiments is 75%. The key given in one panel in *row B* applies to symbols used in all strand exchange data panels. In the *left panel* of *row A*, the second line from the left reflects the hydrolysis of dATP by filaments containing 5% mutant protein. In this experiment, there is a slow decrease in the rate of dATP hydrolysis, followed by a partial recovery. This effect was noted only with RecA filaments containing relatively low levels (5–10%) of mutant protein.

teins. In the reactions with mutant/wild type protein mixtures, filaments were formed so that the mutant content of the filaments conformed to the fractional representation of mutant protein in the overall reaction mixture (31). Rates of dATP hydrolysis were monitored for at least 20 min before addition of homologous linear dsDNA to initiate strand exchange. The dATPase activity continued to be monitored during DNA strand exchange (Fig. 2, *A* and *B*, *left panels*), with the rates summarized in Table I.

The presence of RecA K72R mutant protein in the filaments produced slow but large decreases in dATPase rates during DNA strand exchange. There were two phases to the decline. Initially, the dATP hydrolysis rates decreased rapidly by about 15% in all reactions (see rates calculated between 0 and 5 min after addition of dsDNA). This rapid decline reflects a change associated with the initial homologous alignment of the two DNA substrates, and occurs within the first few minutes, even in the reaction with no mutant protein. This phenomenon has been characterized previously (14). With ATP, the declines in ATP hydrolysis in this rapid phase are greater (~30% total)

TABLE I

Rates of dATP hydrolysis before and during RecA-mediated strand exchange reactions

Reactions are those shown in Fig. 2, *panels A*. dATPase rates were calculated before and during DNA strand exchange reactions, and are expressed as μM min^{-1}. Rates during strand exchange were calculated from both 0–5-min and 55–60-min regions, respectively. The percent decrease refers to the decline in dATP hydrolytic activity resulting from strand exchange.

[K72R]		During strand exchange		Percent decrease	
[K72R]+[wt]	Before	0–5 min	55–60 min	0–5 min	50–55 min
%					
0	38.2	33.7	26.4	11.8	31.0
5	36.6	30.8	25.3	15.9	30.8
10	34.6	28.5	13.7	17.8	60.3
15	31.0	24.9	6.9	11.6	77.9
20	30.3	24.9	7.2	17.9	76.2
33.3	23.7	21.8	5.8	8.2	75.7

than observed with dATP. A second phase in the decline in dATPase activity, slower but much greater in extent, became evident as reactions proceeded. The extent of the decline in the second phase was highly dependent on the RecA K72R content in the mixed filaments. When 15% of total RecA was K72R mutant, the dATPase rate decreased by 78%, which was substantially higher than that observed in the wtRecA control reaction. Little further decrease in dATP hydrolysis was observed when K72R was more than 15% of the RecA mixture. The second phase of the decline occurred over a period of 20–40 min. This is comparable to the period of time over which DNA strand exchange occurs with these DNA substrates, and suggests that the second phase of the decline is associated with the formation of hybrid product DNA as strand exchange proceeds. The final rates of dATP hydrolysis were comparable to those observed previously for mixed filaments formed directly on dsDNA at lower pH values (31). When the filaments contained only wild type protein, little decrease in the rate of dATP hydrolysis was observed after the rapid initial decline, consistent with continued binding of RecA protein to the hybrid DNA product of strand exchange as observed previously (11, 12).

The strand exchange reaction was monitored independently by the agarose gel assay (Fig. 2, *A*, *two right panels*, and *C*). Increasing the K72R mutant content of the filaments tended to poison the overall DNA strand exchange reaction. No nicked circular DNA products were observed when the mutant content reached 33%, although the formation of strand exchange intermediates (joint molecules) was essentially unaffected (Fig. 2*A*).

RecA protein-mediated DNA strand exchange can proceed past short heterologous sequence insertions in the dsDNA substrate, but the bypass requires ATP hydrolysis (18, 20). We therefore examined the effects of the presence of the mutant protein in mixed filaments on the bypass of a 52 bp insertion in the linear dsDNA. The patterns observed for dATP hydrolysis were similar to those seen with completely homologous DNA substrates (Fig. 2*B*, *left panels*). However, much less mutant protein was required to eliminate the complete strand exchange reaction. When only 15% of the RecA was mutant, the bypass reaction generated no detectable products (Fig. 2, *B*, *right panels*, and *D*).

Both phases of the decline in dATPase activity depended on homology in the dsDNA substrate. When the dsDNA was heterologous, the rate of dATP hydrolysis was essentially unaffected regardless of the content of mutant protein in the RecA nucleoprotein filaments (Fig. 3 and data not shown). This indicates that the change in filament state during strand exchange reflected in the decline in dATPase rates is not associated with the "search for homology" phase of the DNA strand exchange reaction. RecA filaments can bind to a heterologous duplex DNA in the course of the search for homology (6, 7), but their dATPase properties are not affected.

An unusual effect on the dATPase activity was observed

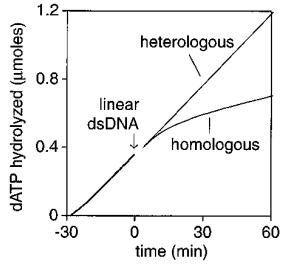

FIG. 3. **Changes in RecA filament state during DNA strand exchange are homology-dependent.** Reactions were carried out as described under "Materials and Methods," and contained 3 μM M13mp8 circular ssDNA, 8 μM M13mp8 linear dsDNA (homologous DNA cleaved by *Sma*I) or 8 μM ϕX174 linear dsDNA (heterologous DNA cleaved by *Pst*I), and 0.3 μM SSB protein. The RecA filaments contained 15% RecA K72R protein. Protocols were otherwise identical to those described in the legend to Fig. 2

when the content of mutant protein was relatively low (5–10%). Upon addition of the dsDNA, the rate of dATPase would decrease over 30 min or so, then recover to nearly the rate observed before the dsDNA addition (Fig. 2 and data not shown). We do not have an explanation for this effect.

The Effects of Mg^{2+} on the DNA Strand Exchange Reactions Promoted by Mixed RecA Filaments—The concentration of Mg^{2+} can be limited so that Mg^{2+}·dATP complexes are formed but little free Mg^{2+} is present. Since DNA-DNA interactions are facilitated by free Mg^{2+}, these conditions will limit homologous DNA pairing interactions, but will permit DNA branch migration in any joint molecules that do form. Under these conditions, the RecA K72R mutant protein will produce, over a period of 6–8 h, a low level of completely exchanged hybrid DNA products greater than 7 kb in length (18). Higher Mg^{2+} concentrations facilitate DNA pairing (joint molecule formation) in RecA-mediated DNA strand exchange reactions (18, 41). However, high Mg^{2+} blocks the formation of the completely exchanged hybrid DNA products with RecA K72R mutant protein alone. We wished to determine if the effects shown in Fig. 2, in particular the poisoning of the strand exchange reaction by mutant protein, were affected by the Mg^{2+} concentration in any way that might alter the central conclusions of this study.

The time courses of dATP hydrolysis of wtRecA-mediated strand exchange reactions at different Mg^{2+} concentrations are shown in Fig. 4 (*leftmost panels*, *rows A–C*), and dATP hydrolysis rates before and during DNA strand exchange reactions are summarized in Table II. All reactions contained 3 mM dATP. The ssDNA-dependent dATP hydrolytic activity observed prior to the initiation of DNA strand exchange shows little dependence on the Mg^{2+} concentrations above 2 mM in all reactions. There was also little effect of dsDNA on the rate of dATP hydrolysis when the RecA filaments were entirely wild type (except for the small rapid decline seen immediately after

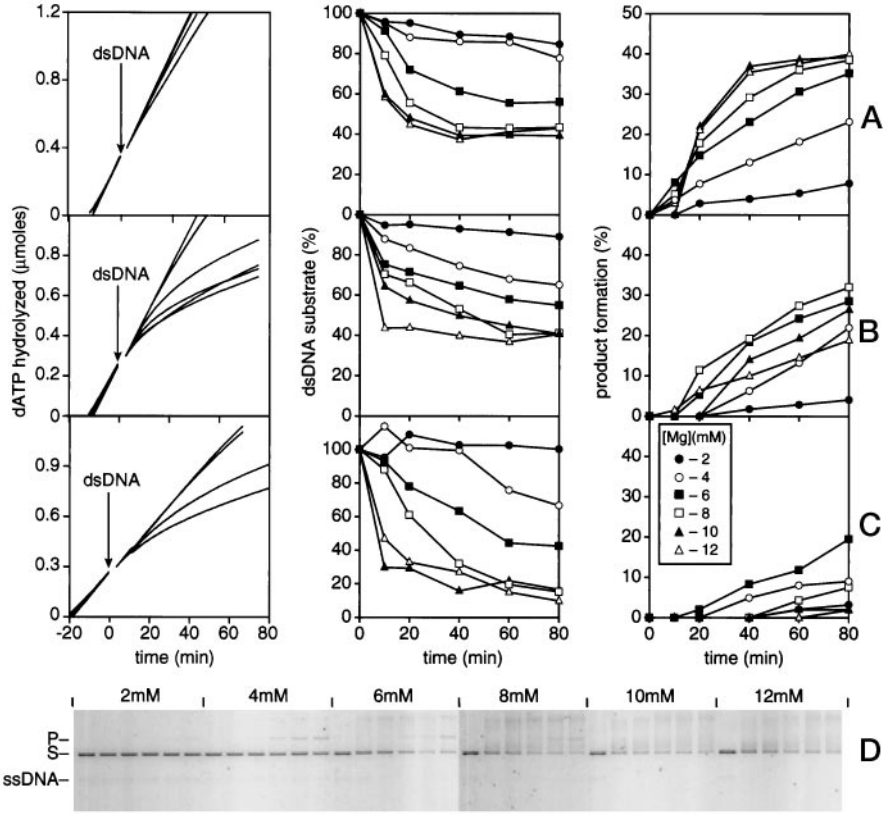

FIG. 4. **Effects of Mg^{2+} ion on RecA protein-mediated DNA strand exchange reactions with mixed filaments.** Reaction conditions were the same as in Fig. 2, except that the Mg^{2+} concentration was varied from 2 to 12 mM. Sets of reactions are again arranged in horizontal rows. *Rows A* and *B* show reactions involving filaments with wild type RecA protein or 20% RecA K72R, respectively. *Rows C* and *D* show reactions with filaments containing 33% RecA K72R. *Panels left* to *right* in *rows A–C* show dATPase activities, uptake of dsDNA into joint molecules, and formation of completely exchanged DNA products. The strand exchange assays for the filaments containing 33% RecA K72R protein are shown in *row D*. The symbol key shown in *row C* applies to all panels.

TABLE II

Rates of dATP hydrolysis before and during RecA protein-mediated DNA strand exchange reactions; Mg effects

Reactions are those shown in Fig. 4, *panels A* and *B*. dATPase rates were calculated before and during DNA strand exchange reactions, and are expressed as $\mu M\ min^{-1}$. The dATPase rates were calculated at the indicated times after addition of the linear dsDNA substrate to initiate DNA strand exchange. The percent decrease refers to the decline in dATP hydrolytic activity resulting from strand exchange.

[Mg2+]	Before	During (40–45 min)	Percent decrease
mM			
Filaments with wild type RecA protein alone			
2	40.3	40.3	NC
4	43.6	39.8	8.7
6	42.4	35.4	16.6
8	41.4	31.8	23.2
10	39.7	29.8	25.1
12	38.2	28.3	25.9

[Mg2+]	Before	During strand exchange		Percent decrease	
		0–5 min	55–60 min	0–5 min	55–60 min
mM					
Filaments containing 20% RecA K72R					
2	32.9	33.7	32.6	NC	NC
4	35.3	35.1	24.0	0.4	31.8
6	34.5	33.4	9.9	3.0	71.2
8	33.9	29.5	7.5	13.2	77.9
10	32.2	25.6	7.3	20.6	77.4
12	31.1	23.7	10.0	23.8	68.0

[a] NC, no change.

dsDNA addition; Fig. 4, *row A*). The DNA strand exchange reaction promoted by the wild type protein produced completely exchanged products optimally at 10 mM Mg^{2+}, consistent with results obtained in many studies over the last 15 years. The higher levels of Mg^{2+} are needed to facilitate the DNA-DNA interactions needed for DNA pairing to form joint molecules, as is evident in the *middle panel* of *row A*.

When the filaments contained either 20 or 33% mutant protein, larger effects on dATP hydrolysis were seen. The drop in dATPase rate during strand exchange increased as a function of Mg^{2+} concentration from 2 to 12 mM, approaching a maximum with Mg^{2+} concentrations of about 10 mM. Agarose gel assays show that duplex substrate uptake (Fig. 4, *middle panels* in *rows B* and *C*) also increased as a function of Mg ion concentration up to 12 mM. However, the production of strand exchange products by the mixed filaments was generally optimal at Mg^{2+} concentrations of about 6 mM. (Fig. 4, *right panels* in *B* and *C*). Poisoning of the overall DNA strand exchange was somewhat less evident at Mg^{2+} concentrations of 4 or 6 mM. With a mutant protein content of 33%, some fully exchanged products were observed at these Mg^{2+} concentrations, although they were largely absent at 10 mM Mg^{2+}.

The presence of the mutant protein in mixed filaments still poisoned the overall strand exchange reaction even at 4 or 6 mM

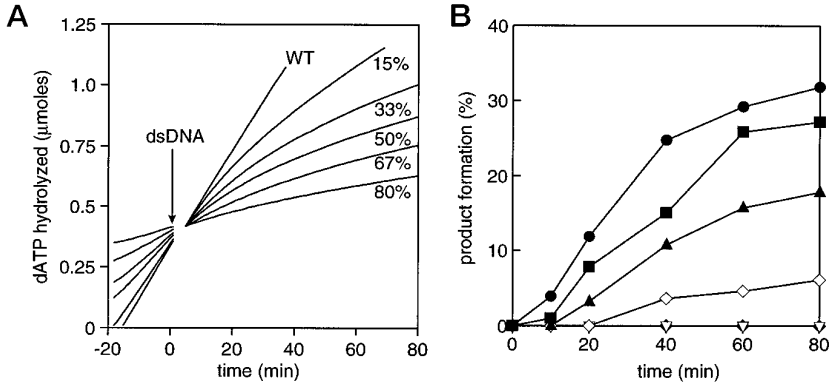

FIG. 5. **DNA strand exchange reactions carried out by RecA filaments containing a mixture of wild type and K72R mutant proteins in the presence of 6 mM Mg^{2+} ion.** Reactions were carried out as in Fig. 2. *Panel A* shows rates of dATP hydrolysis, with filaments at the indicated content of RecA K72R protein. *Panel B* shows the generation of completely exchanged DNA products in the same reactions. The curves reflect reactions with 0 (●), 15 (■), 33 (▲), 50 (◇), 67 (♦), and 80% (▽) RecA K72R protein.

Mg^{2+}, but higher levels of the mutant protein were required. At 6 mM Mg^{2+}, the mutant protein content of the mixed filaments had to exceed 50% before the formation of completely exchanged hybrid DNA products was effectively shut down (Fig. 5). In the reactions in which the mutant protein in the mixed filaments blocks the formation of DNA strand exchange products, there is still a slow decline in the rate of dATP hydrolysis that correlates well to the course of a normal strand exchange reaction (Fig. 5 and Table III). We attempt to explain this phenomenon below.

Exchange of RecA Protein between Free and Bound Pools Occurs during DNA Strand Exchange Reactions—We next explored whether the changes in dATP hydrolysis rates observed in Figs. 2–5 reflected a progressive change to the filament state characterized by RecA protomer exchange in the filament interior. This required the kinds of challenge experiments used in an earlier study to detect RecA protomer exchange (31). Filaments were formed on ssDNA with wild type RecA protein, and the rates of dATP hydrolysis monitored (Fig. 6*A*). Addition of RecA K72R protein at any level did not affect the rate of dATP hydrolysis (Fig. 6*A*), reflecting the lack of RecA protomer exchange between free and bound forms when the filaments are formed on ssDNA (31). When homologous dsDNA was added, however, the rate of dATP hydrolysis slowly declined when the mutant protein was present. This is characteristic of an exchange reaction in which the wild type protein is replaced by mutant protein. The decline begins slowly and proceeds over 30–40 min, again correlating well to observed rates of DNA strand exchange (Fig. 6, *B–D*). This provides evidence that the onset of DNA strand exchange gradually brings about a change in filament state to that in which RecA protomer exchange occurs.

The RecA protomer exchange between free and bound forms occurs slowly, suggesting it is associated with the formation of hybrid DNA product. No exchange was observed (there was no observable change in the rate of dATP hydrolysis) when heterologous dsDNA was added to the reaction (data not shown). Therefore, the RecA protomer exchange does not facilitate the "search for homology" required to align homologous sequences in the two DNA substrates, and in fact does not occur during this process. Even though it depended on homology in the duplex DNA substrate, the RecA exchange process did not correlate well with the formation of completely exchanged hybrid DNA products. In fact, higher amounts of mutant protein tended to block the formation of strand exchange products, even though the mutant protein could not exchange into the RecA filaments until strand exchange was initiated. Note that the formation of strand exchange intermediates (incorporation of dsDNA into joint molecules) was not affected by the mutant protein or its exchange into the filaments (Fig. 6*C*). Effects were observed only on the formation of the completed products (Fig. 6*D*).

We examined the effects of Mg ion concentration on the RecA protomer exchange process. These effects generally followed patterns outlined in Figs. 4 and 5. Results obtained after the addition of mutant protein corresponding to 20% of the total protein are shown in Fig. 7 and Table IV. The RecA exchange generally increased as a function of Mg^{2+} concentration, as did the uptake of dsDNA substrate to generate joint molecules. The generation of completely exchanged hybrid DNA products was optimal at about 6 mM Mg^{2+}.

The RecA protomer exchange process could produce both increases and decreases in the mutant content of the filaments. A series of mixed mutant/wild type RecA filaments were formed on ssDNA with mutant contents of 5, 15, or 50%. When additional K72R mutant or wild type protein was added to the filaments with 15% mutant protein (intended to produce a final of 5 or 50% mutant content), no change in the rate of dATP hydrolysis was observed, again consistent with the lack of RecA exchange on ssDNA. Upon addition of dsDNA, changes in dATPase activity similar to those characterized in Figs. 2 and 3 were observed (Fig. 8). The experiments previously challenged with additional wild type or mutant protein tended to follow paths consistent with a shift in mutant to the new level. The change is especially evident in the reaction challenged with wild type protein to bring the total back to 5% (Fig. 8*A*). These results demonstrate that a facile exchange of RecA protomers occurs regardless of the starting content of mutant protein in the filaments.

The generation of fully exchanged hybrid DNA products in the strand exchange reactions also tended to reflect the final concentration of mutant protein present after the challenge. If the mutant content were lowered by addition of wild type protein, the reaction improved substantially (Fig. 8*B*). If the mutant content was increase from 15 to 50%, the generation of products was blocked (Fig. 8*B*).

DISCUSSION

There are three major conclusions to this study. First, an exchange between free and bound forms of RecA protein occurs during DNA strand exchange. Second, the RecA exchange does not occur during or in any way facilitate the "search for homology" that proceeds during the earliest stages of DNA strand exchange. Instead, the RecA exchange depends upon the presence of homologous sequences in the duplex DNA substrate and is triggered slowly over the time course of strand exchange, suggesting it is somehow connected to the progressive formation of the hybrid DNA product. Third, the RecA exchange reaction, in and of itself, is insufficient to overcome the limited extent of DNA strand exchange observed when ATP is not hydrolyzed.

The second conclusion should not be surprising, even though a RecA protomer exchange is sometimes postulated to play a role in the search for homology (*e.g.* Ref. 42). DNA pairing to

TABLE III

Rates of dATP hydrolysis before and during RecA protein-mediated DNA strand exchange reactions; challenge experiments

Reactions are those shown in Fig. 5, *panel A*. dATPase rates were calculated before and during DNA strand exchange reactions, and are expressed as μM min^{-1}. The amounts of challenging RecA K72R protein were expressed as percent of total RecA protein after challenge. The dATPase rates during strand exchange were calculated from both 0–5-min and 40–50-min periods. The percent decrease refers to the decline in dATP hydrolytic activity resulting from strand exchange.

[K72R]		During strand exchange		Percent decrease	
[K72R]+[wt]	Before	0–5 min	40–50 min	0–5 min	40–50 min
%					
0	40.5	33.7	26.7	16.5	33.7
10	39.7	32.4	14.9	18.3	63.9
20	40.4	34.1	9.8	14.9	75.5
33.3	40.2	33.1	8.4	18.0	79.2
50	41.1	33.9	7.6	14.9	81

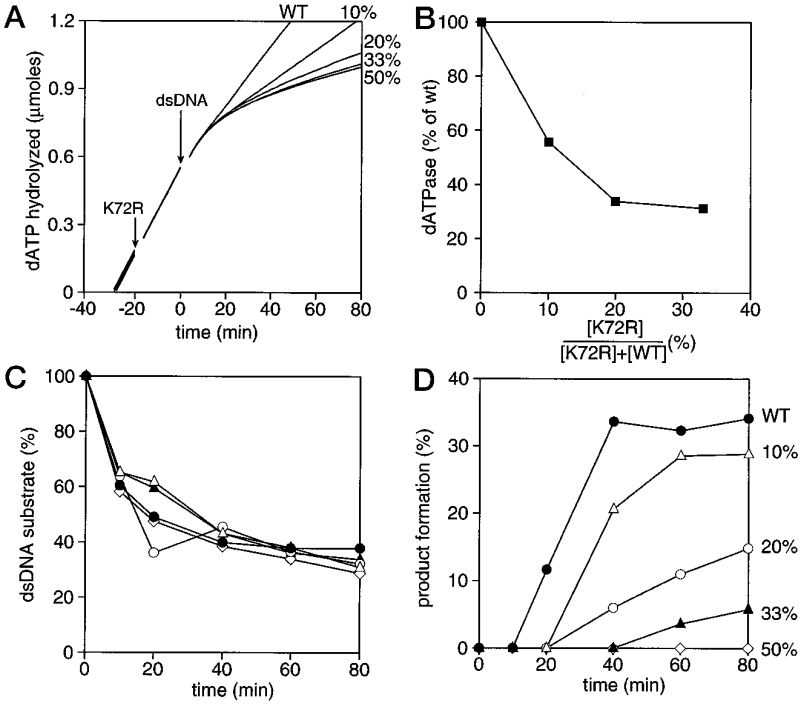

FIG. 6. **Exchange of RecA protein between free and bound forms during RecA protein-mediated DNA strand exchange reactions.** Reactions were carried out as described under "Materials and Methods." First, wtRecA-ssDNA filaments were formed by using 2 μM wtRecA, 0.3 μM SSB protein, and 3 μM M13mp8 circular ssDNA. The dATP hydrolytic activity of these preformed ssDNA filament was monitored for at least 20 min before different amounts of RecA K72R mutant were added such that K72R represented 0–50% of the total RecA after the challenge. The dATP hydrolysis was monitored for another 20 min. M13mp8 linear dsDNA substrate (8 μM) was then added to initiate the DNA strand exchange reactions. The final volume of these reactions after dsDNA substrate addition was 500 μl. The strand exchange reactions were monitored by the coupled dATPase assay and agarose gel assay simultaneously. *Panel A* shows the time courses of dATP hydrolysis. *Panel B* shows the decrease of final dATP hydrolysis rates relative to the rate in the wtRecA control reaction (in which no K72R was added) as a function of RecA K72R protein content in the filaments. The dATP hydrolysis rates were calculated from data obtained 40–50 min after addition of dsDNA in *panel A*. *Panel C* shows dsDNA substrate uptake, and *panel D* shows the generation of fully exchanged DNA products. The symbols used in *panels C* and *D* reflect the RecA K72R content of filaments indicated in *panel D*.

form joint molecules clearly occurs efficiently in the absence of any ATP hydrolysis (15–18). These published results demonstrate that the RecA filament can align a ssDNA with a homologous dsDNA using only the binding energy available within the filament groove.

The first and third conclusions are most relevant to an improved understanding of the role of ATP hydrolysis in the later stages of DNA strand exchange. The RecA redistribution hypothesis (15–17) is predicated on the fact that RecA protein filaments, even those formed with the mutant RecA K72R, can promote DNA strand exchange without ATP hydrolysis. Strand exchange in these filaments would be limited only by discontinuities in the filaments. The only postulated role of ATP hydrolysis is to redistribute RecA monomers to eliminate filament discontinuities (15–17). The key testable predictions of this hypothesis are that the postulated filament discontinuities exist, that ATP hydrolysis facilitates a RecA protomer exchange between free and bound forms as necessary to redistribute the RecA, and that this exchange is sufficient to explain all of the observed effects of ATP hydrolysis on DNA strand exchange (*e.g.* unidirectionality and the bypass of heterologous DNA insertions).

The results of the present study provide the first demonstration that the necessary RecA protomer exchange actually occurs. The time course of the exchange reactions, especially when examined in the light of results of an earlier study (31), strongly suggest that RecA protomer exchange is triggered by the formation of hybrid dsDNA within the filaments as strand exchange proceeds. The simplest interpretation of the results is that a change in filament state is achieved over the course of DNA strand exchange to one with RecA protomer exchange properties previously assigned to RecA complexes bound to dsDNA (31), even though the initial complex bound to ssDNA is refractory to exchange.

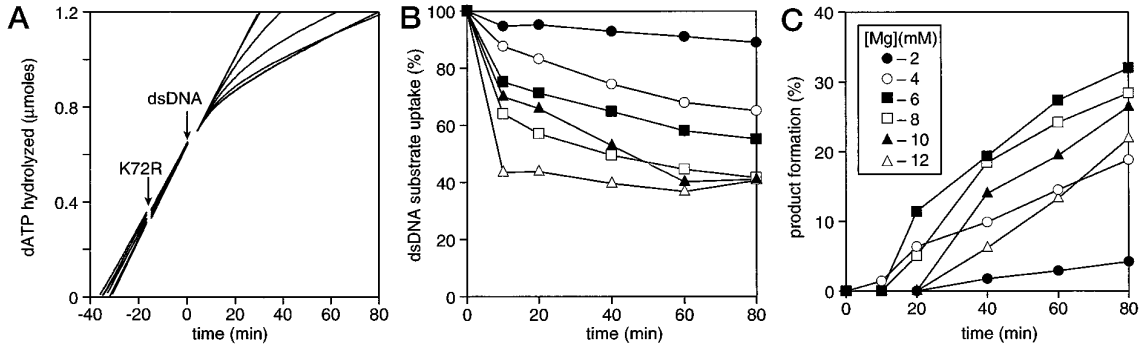

FIG. 7. **Effects of Mg^{2+} ion on RecA protein-mediated DNA strand exchange reactions with mixed filaments.** Reactions and symbols are identical to those used in *rows A–C* of Fig. 4. The only difference is that the filaments were formed and incubated with 2 mM wild type RecA protein, as in Fig. 6, and then challenged with sufficient RecA K72R to bring the overall mutant content in the reaction to 20% (indicated by an *arrow*). Reactions were initiated with linear dsDNA (M13mp8) 20 min later, and the dATPase (*left panel*) and DNA strand exchange reactions (*middle* and *right panels*) were monitored simultaneously.

TABLE IV

Rates of dATP hydrolysis before and during RecA protein-mediated DNA strand exchange reactions; Mg effects

Reactions are those shown in Fig. 7. dATPase rates were calculated before and during DNA strand exchange reactions, and are expressed as μM min^{-1}. dATPase rates before strand exchange were calculated from regions before and after K72R mutant addition. dATPase rates during strand exchange were calculated from both 0–5-min and 30–40-min regions in Fig. 7*A*, respectively. The percent decrease refers to the decline in dATP hydrolytic activity resulting from strand exchange.

[Mg2+]	Before strand exchange		During strand exchange		Percent decrease	
	Before K72R	After K72R	0–5 min	30–40 min	0–5 min	55–60 min
mM						
2	39.0	41.5	41.1	38.8[a]	1	6.5[a]
4	43.9	43.0	42.5	33.3[a]	1.2	22.4[a]
6	43.3	42.2	40.2	17.1	4.8	59.5
8	40.7	40.7	37.0	11.4	9.0	71.9
10	39.8	40.0	33.4	10.2	16.6	74.5
12	38.5	39.5	31.9	11.4	19.2	71.2

[a] Rate calculated from 25–30-min region in Fig. 6*A*.

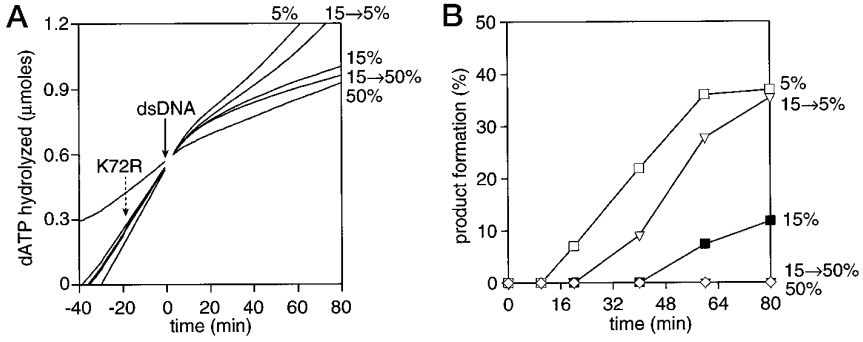

FIG. 8. **Effects of RecA K72R protein content shifts in mixed filaments.** RecA K72R protein content shift experiments are similar to the protein challenge experiments in Fig. 6, except that the preformed RecA filaments on ssDNA contained 5% (one reaction), 15% (three reactions), or 50% (one reaction) RecA K72R protein in a total of 2 μM RecA protein. Reactions also contained 0.3 μM SSB protein and 3 μM M13mp8 circular ssDNA. Reactions (dATP hydrolysis) were monitored for 20 min. In two of the reactions containing 15% mutant protein, the overall RecA K72R content was then shifted to 5 or 50% by adding wtRecA or K72R mutant, respectively (at the *arrow* labeled *K72R*). The dsDNA substrate (8 μM M13mp8 linear dsDNA) was then added to initiate DNA strand exchange reactions. during these reactions. *Panel A* shows the time courses for dATP hydrolysis. *Panel B* shows the quantified product formation of the DNA strand exchange reactions in *panel A*.

However, the RecA protomer exchange we observe is not sufficient to explain the effects of ATP hydrolysis on DNA strand exchange. The RecA exchange occurs under almost all conditions, and can produce both increases and decreases in the content of the mutant K72R RecA protein in mixed filaments. The generation of completely exchanged hybrid DNA products of strand exchange can be decreased or eliminated when the mixed filaments contain relatively modest amounts of the mutant protein, even though a RecA exchange continues. The decline in the completion of the DNA strand exchange reaction is especially evident when the dsDNA substrate contains a 52 bp heterologous insertion that must be bypassed. Here, the reaction is halted when the mutant content of the filaments reaches 15% (Fig. 2), even though filaments with this mutant content exhibit a facile RecA exchange process (Fig. 8).

The postulated effects of RecA filament discontinuities and the bypass of heterologous DNA insertions during DNA strand exchange have been addressed in other studies. We have shown elsewhere that the addition of excess RecA K72R mutant protein to a strand exchange reaction, sufficient to fill in any filament discontinuities that might exist, does not relieve the barrier to completion of DNA strand exchange by the mutant protein once the initial joint molecules have been formed (18). This observation tends to call into question the idea that the reactions carried out in the absence of ATP hydrolysis are limited only by filament discontinuities. There is, in fact, no evidence that the postulated discontinuities in either the RecA K72R filaments or wild type RecA filaments formed in the

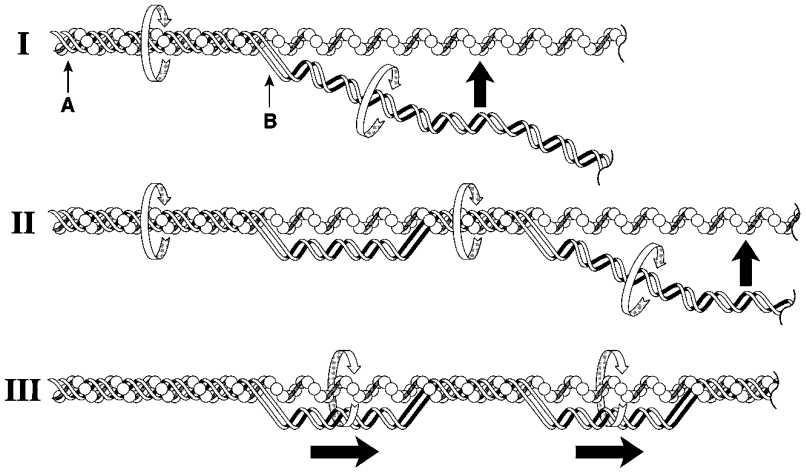

FIG. 9. **Proposed formation of discontinuous DNA pairing intermediates during RecA protein-mediated DNA strand exchange.** *I*, duplex DNA is drawn into the helical groove of a RecA protein filament formed on ssDNA. The paired region is defined as that between points *A* and *B*. Further incorporation of the dsDNA into the filament requires filament and DNA rotation as indicated. As the paired region is extended, additional pairing interactions at any point along the filament are intramolecular. If pairing occurs at the point indicated by the *heavy arrow*, a loop of DNA outside the filament between point *B* and the *heavy arrow* is created as shown in *II*. Additional pairing interactions (*heavy black arrow*) may occur to create any number of external loops. Resolution of the loops requires the rotation of the loop DNA about the outside of the filament as shown in *III*. This will spool DNA into the filament groove at one end of the loop, and out of the groove at the other end. The direction of rotation determines the direction of loop movement along the helix axis. A model for how such DNA rotation might be coupled to ATP hydrolysis has been proposed elsewhere (2, 18, 19).

presence of ATPγS (15–17) actually exist, much less play a role in the observed limitations to strand exchange. In another recent study,[3] we also demonstrate that a RecA protomer exchange process coupled to ATP hydrolysis cannot explain the bypass of heterologous DNA insertions.

The results of these and other studies are better accommodated by the formation of a discontinuous DNA pairing intermediate (as opposed to the formation of discontinuous filaments), which must be resolved by a DNA rotation process coupled to ATP hydrolysis (2, 18–22). To introduce this alternative, we must point out another apparent experimental paradox evident in this and earlier studies. Strand exchange slowly converts a filament on ssDNA, which does not undergo a RecA protomer exchange process, to a filament on dsDNA which does undergo protomer exchange. The extent of the change is consistent with an altered state spanning the entire length of the filament or most of it, even when there is enough mutant protein in the filament to block the completion of strand exchange. In earlier work, rapid changes in rates of ATP hydrolysis (<2 min) were noted upon the addition of homologous dsDNA, the extent of which depended on the length of homology in the duplex DNA substrate (14). This indicated that homologous interactions between the DNA substrates occurred over their entire length at early times, even though DNA strand exchange was not completed until many minutes later. How can homology be sensed all along the duplex DNA substrate by the RecA filament, even when DNA strand exchange is not complete?

To explain these and all of the other results in the present and earlier studies, we suggest that a discontinuous DNA pairing intermediate is formed as proposed based on an earlier study (18) and outlined in Fig. 9. DNA pairing would be facilitated by binding energy in the RecA filament, drawing homologous duplex DNA into the filament even in the absence of ATP hydrolysis as proposed by Kowalczykowski and colleagues (15–17). As the pairing proceeded, drawing additional DNA into the filament would require rotation of the filament and the DNA as shown in Fig. 9 (*I*). As this process extends the segment of paired DNA drawn into the filament, pairing at other points along the RecA filament becomes intramolecular and more probable. If pairing occurs in any other location, a loop of DNA is trapped topologically on the exterior of the filament between the paired regions. As pairing continues, a number of such loops might be generated. Although pairing is illustrated as though it were initiated at an end, it could in principle be initiated anywhere and generate the same intermediate. The discontinuous intermediate would allow the formation of hybrid DNA at many sites all along the paired DNAs at an early stage of DNA strand exchange. However, in the absence of ATP hydrolysis, the loops would be static, and the extent of stable strand exchange that persists after removal of RecA protein would be defined by the distance between the end of the paired linear duplex and the first loop. The pairing would also occur at either end of the linear dsDNA substrate with equal probability. This would explain the limited extent of DNA strand exchange observed when ATP is not hydrolyzed (15–18, 22) as well as the lack of unidirectionality (22).

When ATP is hydrolyzed, we propose that the hydrolysis is coupled to rotation of the DNA in the loop, such that it migrates in one direction and is ultimately resolved (2, 6, 19). In this process, the loop DNA would be bound to every sixth RecA monomer along the filament in the loop region. The loop DNA would be passed around the outside of the filament, from one set of monomers to the next, coupled to ATP hydrolysis. The rates of DNA branch movement and ATP hydrolysis during DNA strand exchange are related quantitatively in a manner predicted by this facilitated DNA rotation process (19).

The present results are also readily explained by the model in Fig. 9. The RecA filament is designed to stabilize the hybrid DNA product of strand exchange. As the discontinuous pairing intermediate was formed, a strand switch might occur in all of the paired regions to form regions of hybrid DNA throughout the length of the filament, and triggering a more or less general change in RecA filament state to the form where RecA protomer exchange occurs. If the formation of the discontinuously paired regions occurred slowly over a period of many minutes, the RecA filament transition would follow suit, potentially explaining the slow kinetics of the transition evident in Figs. 2–8. In addition, the presence of mutant protein in the filaments

[3] K. J. MacFarland and M. M. Cox, manuscript submitted for publication.

could readily poison a facilitated DNA rotation that relied on ATP hydrolysis in all RecA monomers. The rotation would be halted once the content of mutant RecA K72R protein was sufficient to produce tracts of mutant protein incapable of hydrolyzing ATP and passing the DNA around the filament exterior, explaining the block to the completion of strand exchange in mixed filaments where the initial formation of paired joint molecules proceeds with normal kinetics. The block to the completion of DNA strand exchange is much harder to explain in the context of the RecA redistribution model, since the presence of mutant protein in the filaments seems to have little effect on the RecA exchange between free and bound forms.

ATP hydrolysis confers several properties on RecA-mediated DNA strand exchange reactions. It renders the reaction unidirectional, allows the bypass of heterologous DNA insertions, and accommodates 4-strand exchange reactions (6, 18, 20–23). Although we argue that RecA protomer exchange coupled to ATP hydrolysis does not explain these effects of ATP hydrolysis, a facile RecA protomer exchange reaction accompanies DNA strand exchange under conditions commonly used to examine the reaction *in vitro*. We do not dismiss the possibility that this may play a role in DNA strand exchange yet undefined, or that the mechanism by which ATP hydrolysis facilitates DNA strand exchange may be some hybrid of the DNA rotation and RecA redistribution ideas. An alternative is that the RecA protomer exchange has no mechanistic role in DNA strand exchange at all. Instead, the onset of protomer exchange may simply reflect a change in RecA filament state to a form that is more readily recycled following DNA strand exchange.

Finally, recent studies suggest that a filament containing only RecA protein may never exist *in vivo*. RecA filament assembly and disassembly are modulated by the RecO and RecR proteins (29, 43), and perhaps by additional proteins such as RecF, RuvA, or RuvB. The effects of other proteins on the RecA protomer exchange have not yet been evaluated.

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