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Unidirectional Branch Migration Promoted by Nucleoprotein Filaments of RecA Protein and DNA

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RecA Protein Promotes Branch Migration

Most of the models proposed to explain homologous genetic recombination have at least a few common features. A step is usually included in which one strand of a duplex DNA molecule is paired with complementary sequences in another duplex. This results in a branched DNA molecule equivalent or related to the intermediate originally proposed by Holliday (Holliday 1964). This pairing or "synapsis" is usually followed by branch migration to extend the heteroduplex regions (Radding 1978; Stahl 1979).

The RecA protein of *Escherichia coli* plays a central role in homologous genetic recombination in vivo (Dressler and Potter 1982; Radding 1982). In vitro, purified RecA protein promotes the two processes described above—synapsis and branch migration. Of the many model reactions set up to study these processes, perhaps the most informative has been the RecA protein-promoted exchange of strands between circular (+) single-strands and linear duplexes derived from simple bacteriophages (Cox and Lehman 1981a). This reaction occurs in two readily distinguished phases corresponding to synapsis and branch migration, as illustrated in Figure 1. For a summary of current experiments designed to elucidate the mechanism of the synapsis phase of the reaction, the reader is referred elsewhere (Gonda and Radding 1983; Bianchi et al. 1983; Soltis and Lehman 1983b). The mechanism by which RecA protein promotes branch migration has received significantly less attention and will be considered below.

Branch migration refers to the exchange of like strands at a branch point in DNA. First recognized by Lee et al. (1970), the process results in the movement of the branch point with no net loss or gain of paired bases. This concept was first incorporated into a model for homologous genetic recombination by Broker and Lehman (1971). The incorporation of a branch migration step in recombination models reflects a constraint imposed by genetic evidence that has shown that the heteroduplex regions formed during recombination can be thousands of base pairs in length (Broker 1973; Tsujimoto and Ogawa 1977; Sodergren and Fox 1979).

Branch migration involving the Holliday intermediate is illustrated in Figure 2. Studies employing branched DNA molecules constructed in vitro have demonstrated that this is a spontaneous reaction under physiological conditions of pH and ionic strength (Thompson et al. 1976; Radding et al. 1977; Warner and Tessman 1978). Estimates of the rate of spontaneous branch migration of up to 6000 steps/sec have been reported under conditions commonly employed in RecA protein-promoted reactions in vitro (Warner and Tessman 1978). Some recent measurements have suggested that spontaneous branch migration is somewhat slower (Gellert et al. 1983; Courey and Wang 1983). Some of these estimates, however, approached rates that might account for the extensive branch migration believed to occur during homologous genetic recombination in vivo. It therefore seemed likely that branch migration was uncatalyzed or came about as a result of asymmetric replication during recombination (Radding 1978).

A possible role for RecA protein in promoting branch migration was suggested by the observation that extensive regions of heteroduplex DNA were sometimes formed during RecA protein-promoted pairing reactions (DasGupta et al. 1980). The branch migration that occurs during RecA protein-promoted strand exchange has two additional properties that unambiguously implicate RecA protein in this process and dis-

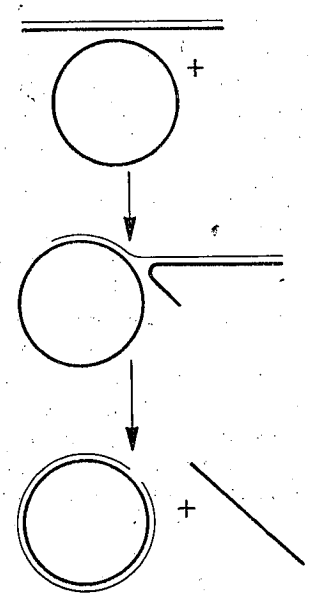

Figure 1. RecA protein-promoted DNA strand exchange. The substrates represented are a circular (+) strand derived from a bacteriophage (e.g., ϕX174) and a homologous linear duplex obtained by restriction endonuclease cleavage of the supercoiled, circular replicative form (RFI) of the same bacteriophage DNA.

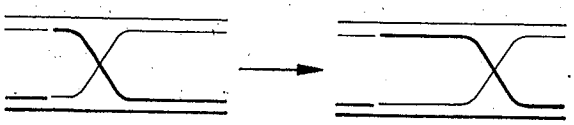

Figure 2. Spontaneous branch migration within a Holliday intermediate.

tinguish it from spontaneous branch migration. These are (1) a continuous requirement for ATP hydrolysis (Cox and Lehman 1981a), and (2) an absolute polarity (Cox and Lehman 1981b; Kahn et al. 1981; West et al. 1981b). The polarity is especially important. RecA protein-promoted branch migration is relatively slow. The rate appears to be no higher than 20 bp/sec. This is more than an order of magnitude slower than the spontaneous process. The spontaneous reaction is random, however, and the extent of branch migration observed is limited by the laws of probability. Given a rate of 6000 steps/sec and assuming a random walk, a branch point has a 1 in 3 chance of migrating 850 bp in 8 min. The slower but unidirectional RecA protein-promoted reaction ensures that a branch point will migrate up to 9600 bp in one direction in the same amount of time with probabilities approaching 100%.

The significance of this reaction extends beyond its role as a step in homologous genetic recombination. RecA protein-promoted branch migration has been demonstrated to proceed past DNA lesions, including thymine dimers (Livneh and Lehman 1982), short mismatches (DasGupta and Radding 1982), and to some degree even extensive mismatches (Bianchi et al. 1983). This capability may be especially important in postreplication repair (West et al. 1981c). In addition, the coupling of ATP hydrolysis to a unidirectional branch migration provides another opportunity to examine the mechanism by which chemical energy is used to drive unidirectional processes in living systems. The problem is analogous in some respects to muscle contraction and the movement of ions across a membrane against a gradient.

Two more properties of RecA protein and the reactions it promotes must be described before addressing the mechanism by which RecA protein promotes branch migration. First, RecA protein-promoted DNA strand exchange is apparently inefficient in terms of the amount of ATP hydrolyzed per base pair of heteroduplex DNA formed (Cox et al. 1983b). This will be described in greater detail below. Second, RecA protein forms filaments. Electron microscopy has revealed that RecA protein:DNA complexes take the form of highly structured filaments that often cover the entire length of DNA molecules many thousands of base pairs long (McEntee et al. 1981a,b; DiCapua et al. 1982; Dunn et al. 1982; Flory and Radding 1982). Under some conditions, RecA protein will form filaments even in the absence of DNA (McEntee et al. 1981a; Cotterill and Fersht 1983). The structures formed on DNA are 10–12 nm in diameter and exhibit a regular helical repeat. Details of the structures as revealed by both electron microscopy and X-ray crystallography are reviewed elsewhere (Howard-Flanders et al. 1984). We note here one feature of these structures that is relevant to the present discussion: Many published electron micrographs of RecA protein bound to a circular DNA molecule show a continuous, circular RecA filament with no apparent ends.

RecA Nucleoprotein Filaments Represent the Active Species in RecA Protein-promoted DNA Strand Exchange

In the discussion that follows, the phrase "RecA protein:ssDNA complex" refers to a RecA nucleoprotein filament in which stoichiometric amounts of RecA protein are bound to single-stranded DNA (ssDNA). Several pieces of evidence indicate that such complexes represent the active species in RecA protein-promoted DNA strand exchange. First, strand exchange and most other RecA protein-promoted reactions exhibit a requirement for stoichiometric concentrations of RecA protein (Shibata et al. 1979; Cox and Lehman 1981a; Weinstock et al. 1981a,b; West et al. 1981a; Cox et al. 1983a). A number of measurements of the binding of RecA protein to ssDNA have yielded stoichiometries of 3–8 nucleotides per bound RecA protein monomer (McEntee et al. 1981b; Silver and Fersht 1982; Dombroski et al. 1983; S.W. Morrical and M.M. Cox, in prep.; Bryant et al., this volume). The DNA strand exchange reaction depicted in Figure 1 does not proceed optimally unless the RecA protein concentration exceeds that required to saturate the ssDNA present at a level of 1 RecA monomer per 4 nucleotides (Cox et al. 1983a). Second, a high degree of cooperativity is evident in many RecA protein-promoted reactions. RecA protein binds cooperatively to ssDNA (McEntee et al. 1981a,b; Roberts et al. 1979). Strong cooperativity is also observed for ATP binding in RecA protein-catalyzed, DNA-dependent ATP hydrolysis (Weinstock et al. 1981a,b). RecA protein monomers are believed to bind ATP at only one site. Finally, there is a direct correlation between the stability of RecA protein:ssDNA complexes and the rate of the subsequent strand exchange reactions (Cox and Lehman 1982; Cox et al. 1983a,b; Soltis and Lehman 1983a).

Evidence has been presented that formation of a stoichiometric RecA protein:ssDNA complex is one of the first steps in RecA protein-promoted strand exchange, a prerequisite for synapsis and branch migration (Cox and Lehman 1982; Flory and Radding 1982). These complexes can thus be described as "initiation complexes" (Howard-Flanders et al. 1984), a term that we adopt here.

The Role of Single-stranded DNA-binding Protein

The single-stranded DNA-binding protein of *E. coli* (SSB) stimulates the RecA protein-promoted DNA

strand exchange reaction illustrated in Figure 1 (Cox and Lehman 1981a,b; West et al. 1982; Cox et al. 1983a). Evidence has been presented that this stimulation is a result of a stabilization of RecA protein:ssDNA complexes by SSB. This observation provides part of the evidence indicating that a correlation exists between complex stability and the overall rate of strand exchange noted above. In the absence of SSB, dissociation of RecA protein from initiation complexes is accelerated by ATP (R. Bryant and I.R. Lehman, pers. comm.). If a second population of ssDNA molecules is added as a challenge after formation of initiation complexes in the absence of SSB (at minimally saturating levels of RecA protein), it is rapidly bound by RecA protein that has dissociated from initiation complexes or that was not bound to DNA at the time of the challenge. If SSB is present, however, no significant migration of RecA protein to the challenge ssDNA is observed. Instead, the RecA protein incorporated into initiation complexes in the presence of SSB remains in those complexes throughout the subsequent strand exchange reaction. The mechanism by which this effect of SSB occurs is unclear.

The kinetic consequences of the addition of SSB to RecA protein–promoted strand exchange reactions are complex. The overall strand exchange reaction is stimulated 5-fold to 10-fold by SSB at RecA protein concentrations minimally adequate to saturate the ssDNA. Additional RecA protein stimulates the SSB-independent reaction but does not affect the reaction in the presence of SSB (Cox et al. 1983a). The effect of SSB is therefore decreased somewhat when supersaturating concentrations of RecA protein are employed. Stimulation of strand exchange by SSB is most apparent in the synapsis phase of the reaction (Cox et al. 1983a). If net formation of heteroduplex base pairs is measured and the data is corrected for the different rates of synapsis observed in the presence and absence of SSB, it can be demonstrated that the effect of SSB on branch migration is less than 2-fold (Bianchi and Radding 1983). It is likely that any observed stimulation of net heteroduplex formation by SSB does not reflect an acceleration of the rate of branch migration in individual branched DNA molecules undergoing strand exchange. Instead, this stimulation can probably be accounted for by the relative instability of RecA protein:ssDNA complexes in the absence of SSB. This is simply a restatement of the conclusion that the primary effect of SSB is on the stability of these complexes. There is no evidence that the inherent rate of RecA protein–promoted branch migration is affected by SSB. Branch migration in the absence of SSB, however, is limited somewhat by a tendency for RecA protein:ssDNA complexes to dissociate before strand exchange is completed. The SSB-stabilized complexes of RecA protein with ssDNA, which initiate strand exchange, remain intact throughout the reaction (Cox and Lehman 1982). Any treatment that causes dissociation of these filamentous RecA protein:ssDNA complexes (e.g., high concentrations of ADP; Cox et al. 1983b) will bring branch migration to a halt. These complexes therefore represent the active form of RecA protein during branch migration as well as during the other phases of strand exchange.

The Efficiency of ATP Hydrolysis

The effect of SSB cannot be measured in terms of the effect on the overall rate of strand exchange alone. SSB also enhances the *efficiency* of RecA protein–promoted DNA strand exchange. In the absence of SSB, more than 1000 ATP molecules are hydrolyzed by RecA protein for every base pair of heteroduplex DNA created. SSB improves this efficiency by nearly an order of magnitude, to 100–200 ATP molecules consumed per base pair of heteroduplex DNA created (Cox et al. 1983b). SSB may thus be considered a "coupling factor," although the reaction is still apparently quite inefficient.

This efficiency can be improved still further by the addition of low concentrations of ADP. ADP affects RecA protein–promoted DNA strand exchange in several ways. Under optimal conditions for strand exchange, ATP hydrolysis does not proceed to completion but ceases after 40–60% of the added ATP has been converted to ADP. It has been shown that this cessation of ATP hydrolysis reflects a dissociation of RecA protein:ssDNA complexes formed in the presence of SSB. This dissociation is mediated by the ADP/ATP ratio. Lower concentrations of ADP exhibit a different effect. ADP is a competitive inhibitor of RecA protein–promoted ATP hydrolysis and the expected decrease in ATP hydrolysis is observed as ADP is titrated into the reaction mixture. In contrast, if the rate of strand exchange is measured as a function of ADP concentration, an enhancement is observed at low ADP concentrations. The enhancement is reversed as the ADP concentration approaches the point at which the RecA protein complexes dissociate (Cox et al. 1983b). At the point of greatest enhancement, the rate of ATP hydrolysis has been decreased so that the net effect is a gain in the apparent efficiency of the reaction. The best efficiency observed under these conditions is 16 ATP molecules consumed for each base pair of heteroduplex DNA created (Cox et al. 1983b). This data appears to indicate that much of the ATP hydrolysis observed during RecA protein–promoted DNA strand exchange is uncoupled. Alternatively, this apparent inefficiency may be an inherent part of the mechanism of branch migration.

Constraints on Models for RecA Protein–promoted Branch Migration

The question under consideration can be stated as follows: What is the mechanism by which an extended nucleoprotein filament of RecA protein promotes a unidirectional branch migration reaction coupled to ATP hydrolysis? It can be argued that much of the

ATP hydrolysis described above is simply uncoupled, and thus irrelevant, or that the structures observed in published electron micrographs may exhibit some features that are artifacts of the methods used to fix and photograph them. It is important, however, to determine if models can be generated that explain all of the data described above. We wish to consider here models that will (1) provide a role for a stable nucleoprotein filament of RecA protein, (2) provide an explanation for the inefficiency of ATP hydrolysis and the effects of ADP, and (3) result in a unidirectional branch migration reaction. The mechanism by which an initiation complex is formed or synapsis occurs will not be considered.

A consideration of points (1) and (2) suggests a relationship between the two. If a role can be found for an extended filament of RecA protein in this reaction, then the apparent inefficiency in ATP hydrolysis becomes more reasonable. Without considering a specific mechanism, if cooperative ATP hydrolysis by all of the RecA monomers in a RecA filament is required to bring about branch migration at a single point, then the reaction *must* be inefficient. The increased efficiency observed when low levels of ADP are present might be explained by the destabilization of RecA protein complexes by ADP. If high concentrations of ADP result in complete dissociation of RecA protein:ssDNA complexes, it is possible that lower levels of ADP simply shorten the complexes. The apparent efficiency of the reaction should increase as the average length of the filamentous complexes is decreased until a size is reached that is inadequate to carry out the reaction.

Data presently available in the literature that is relevant to the mechanism of RecA protein–promoted branch migration have a number of shortcomings. Some of these have recently been listed (Howard-Flanders et al. 1984). It is not clear, for example, whether all of the RecA monomers in a filament are equivalent with respect to ATP hydrolysis and dissociation from the filament, or whether these processes can occur only at the ends of a filament. We have no method at present that can distinguish one end of a filament from another for kinetic experiments. More information is required with regard to the general properties of DNA binding, stoichiometries, ATP hydrolysis, filament structure, monomer structure, and complex structure. With this in mind, two models will be presented below in very general terms. In both cases the molecular details may take a variety of forms. Although in both cases the models are consistent with available data, the information required to test each rigorously is largely unavailable. They are not presented, therefore, as a solution to the problem described above. Instead, it is hoped that they will make a contribution in defining areas where more information is required. We should emphasize here that we have no strong reason to prefer either of these models to the excellent and detailed model for RecA protein–promoted DNA strand exchange recently described by Howard-Flanders et al. (1984).

MODELS

I. Treadmilling

Evidence has accumulated that translocation of tubulin and actin filaments in the cell comes about via a head-to-tail polymerization reaction or treadmilling (Cleveland 1982). There is, under appropriate conditions, a net polymerization at one end of the filament with a net depolymerization at the other. This results in a directional movement of the filament. Originally proposed by Wegner (1976), this phenomenon can occur only if it is coupled to an irreversible step, such as hydrolysis of a nucleoside triphosphate. At steady state in these systems, there is a flux of monomers through the filaments. The efficiency is determined by the ratio, s, of the subunit flux through the polymers to the total number of association and dissociation events at both ends per unit time. If association occurs exclusively at one end and dissociation at the other, $s = 1$. Under most conditions, the subunit flux is considerably less efficient than this in these systems. Values of s between 0.002 and 0.04 have been determined for tubulin (Bergen and Borisy 1980; Zeeberg et al. 1980; Cote and Borisy 1981; Caplow et al. 1982). These values can be increased to 0.26 by adding microtubule-associated accessory proteins (Cote and Borisy 1981). Values of s less than 1 have also been reported for actin. As the s value decreases, the number of nucleoside triphosphates hydrolyzed for every net addition of one subunit at the growing end increases (Pollard and Mosseker 1981).

Such a mechanism could therefore explain the apparent inefficiency of RecA protein–promoted branch migration. RecA protein filaments could treadmill, with movement of the branch point and thus extension of the heteroduplex coupled to association or dissociation of RecA protein monomers at one end of an asymmetric filament. The efficiency of the reaction would be determined by the efficiency of the flux of RecA protein monomers through the filaments. This model is not inconsistent with the observation that RecA protein does not move from stable complexes to challenging DNA molecules (Cox and Lehman 1982). It must be assumed simply that RecA protein monomers that dissociate from filaments will bind other filaments in preference to a new and uncoated molecule.

This model is somewhat difficult to reconcile with a continuous, circular filament of RecA protein that has no ends. It is not clear, however, that this type of structure is maintained throughout the reaction of Figure 1. A treadmilling model that incorporates some of the features of tubulin and actin treadmilling is illustrated in Figure 3. This is only one of many possible forms a treadmilling reaction could take in this system. The model proposed by Howard-Flanders et al. (1984)

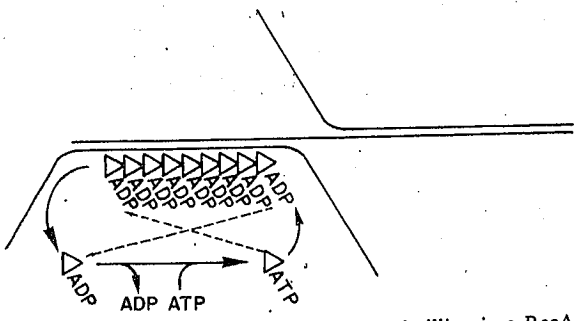

Figure 3. A hypothetical scheme for treadmilling in a RecA nucleoprotein filament, coupled to and driving directional branch migration.

incorporates some of the features of a treadmilling model.

II. The Rotation Model

A branch point between two DNA molecules will be moved uniquely in one direction if the DNA molecules are rotated with respect to each other as illustrated in Figure 4. This could be accomplished by rotation of two RecA protein filaments bound to the DNA molecules in the manner shown. The filaments would rotate with respect to one another, using a ratchetlike mechanism mediated by transient contacts between RecA monomers within each filament. The same thing could be accomplished in principle by a single filament bound to one DNA molecule and ratcheting around the other. This model is compatible with a circular filament, which could be rotated as shown in Figure 5. The apparent inefficiency of ATP hydrolysis is explained by the need to hydrolyze ATP throughout the filament to bring about branch movement at a single point. As described above, the enhancement of the efficiency by ADP could be explained by a shortening of the filaments. Shorter filaments could carry out the reaction more efficiently. The interactions between filaments might be coupled to ATP hydrolysis in a manner analogous in some respects to the actin-myosin interaction during muscle contraction (Jencks 1982a,b). Rotation of the filaments by 360° would result in a branch migration of 10.4 bp in relaxed DNA, and up to 16 bp if the DNA is extensively unwound by RecA

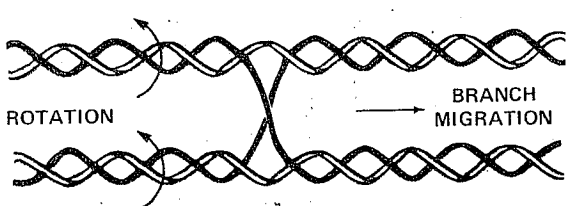

Figure 4. Directional branch migration driven by concerted rotation of homologous strands in a Holliday intermediate. RecA protein polymerized on one or both strands promotes the rotation through transient interstrand contacts, coupled to ATP hydrolysis (not shown).

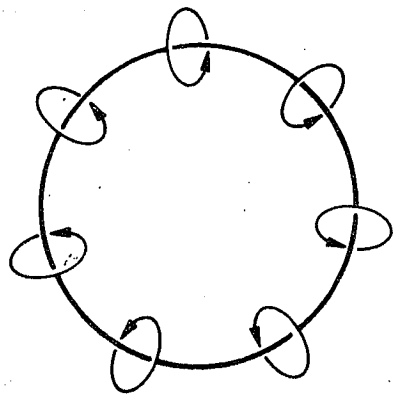

Figure 5. Possible rotation of a circular RecA nucleoprotein complex.

protein, as indicated by results from a number of laboratories (Cunningham et al. 1979; McEntee et al. 1981b; DiCapua et al. 1982; Flory and Radding 1982). If one ATP is hydrolyzed by every RecA monomer to bring about this rotation, then branch migration will consume 84–129 molecules of ATP for every base pair of heteroduplex DNA created. This calculation is for strand exchange involving substrates derived from bacteriophage ϕX174 (5386 bp) and 1 RecA protein monomer per 4 bp. This is very consistent with the efficiency observed with these substrates in the presence of SSB (Cox et al. 1983b). This efficiency should change as the length of the substrate (and the RecA protein complex) changes, a prediction that has not yet been tested. The helical nature of the filaments would permit only a fraction of the monomers in each filament to be in contact with the other filament at any moment. The others would thus be free to rotate to some extent. This model has the unique feature that rotation will lead to movement of the branch whether or not RecA protein is bound at the branch point. RecA protein may thus act at a distance. As with the previous model, the structural and mechanistic details that could bring about this rotation could take a variety of forms.

It should be pointed out that RecA protein catalyzes the hydrolysis of ATP in the presence of ssDNA whether or not a strand exchange reaction is taking place. ATP hydrolysis is clearly uncoupled from branch migration under these conditions. It is possible, however, that this ATP hydrolysis is coupled to a RecA protein reaction, such as treadmilling or rotation of adjacent filaments, which would result in branch migration if the appropriate substrates were provided. It is also possible that even if the general features of one of these models is correct, that much of the observed ATP hydrolysis is truly uncoupled.

We have initiated a number of experiments to examine properties of RecA protein filaments in an attempt to begin to rigorously test these and other models for RecA protein–promoted branch migration. These have included light scattering studies designed to elu-

Table 1. ssDNA Binding Data

	Tris (mM)	$MgCl_2$ (mM)	Stoichiometry[a]	Apparent K_d (nM)
Filament formation blocked	20	0	3.7 ± 0.3	7 ± 3
	40	10	9 ± 1	5 ± 1
Filament forming conditions	10	10	4.0 ± 0.6	600 ± 200

All reactions were carried out at 37°C and at pH 7.5.
[a]Nucleotide residues per RecA protein monomer at saturation point.

cidate the relationship between RecA protein filaments formed in the presence and absence of DNA and experiments designed to detect exchange of RecA monomers between different RecA protein:ssDNA complexes. Results are summarized below.

MATERIALS AND METHODS

RecA protein, SSB, and φX174 and M13*ori*C26 (Kaguni et al. 1979) circular ssDNAs were prepared as described previously (Cox and Lehman 1981a, 1982; Cox et al. 1983a,b). Experiments employing light scattering, turbidity measurements, and sucrose density gradient analysis will be described in detail elsewhere (S.W. Morrical and M.M. Cox; S.K. Neuendorf and M.M. Cox; both in prep.).

RESULTS AND DISCUSSION

ssDNA Binding in the Presence and Absence of RecA Protein Filaments

Light scattering has been employed to monitor the formation of RecA protein filaments in the absence of DNA, and the binding of RecA protein to ssDNA to form the extended nucleoprotein complexes described above. Free filament formation requires critical amounts of Mg^{++} and is very sensitive to pH, salt, and buffer concentrations. Since RecA protein filament formation occurs only within a relatively narrow range of conditions, it is possible to examine the effects of this reaction on the RecA protein–ssDNA equilibrium by comparing the apparent affinities of RecA protein for ssDNA under conditions that stimulate or inhibit filament formation. If RecA protein binds to ssDNA in the form of preformed filaments, then binding to ssDNA should be enhanced under conditions that are optimal for free filament formation. If, on the other hand, the RecA protein:ssDNA complexes that participate in strand exchange are assembled by the successive and cooperative binding of smaller units (monomers, dimers, tetramers, etc.), then formation of free filaments might inhibit binding of RecA protein to ssDNA by competing for free RecA protein monomers or oligomers. A determination of apparent dissociation constants for RecA protein:ssDNA complexes formed under filament-promoting and filament-inhibiting conditions indicates that the latter case is true (S.W. Morrical and M.M. Cox, in prep.).

Under conditions that block free filament formation, apparent dissociation constants (K_d) of 5–10 nM are obtained for the RecA protein:ssDNA complex (Table 1). The magnitude of this constant is not affected appreciably by either Mg^{++} ions or by Tris buffer concentration. Under near-optimal conditions for free filament formation, however, the apparent affinity of RecA protein for ssDNA decreases by 2 orders of magnitude (Table 1). The simplest explanation for this observation is that free filament formation and ssDNA binding by the RecA protein are *competing* reactions. The apparent decrease in the affinity of RecA protein for ssDNA is not due to a change in intrinsic affinity caused by different reaction conditions but is instead an equilibrium effect brought about by the sequestering of RecA protein monomers in a form that cannot interact with ssDNA. These results indicate that free RecA protein filaments do not bind ssDNA. RecA protein monomers are exchanged between free filaments and RecA protein:ssDNA complexes via a dissociative mechanism. These results also indicate that RecA protein filaments that form in the absence of DNA are structurally and functionally distinct from the high-molecular-weight RecA protein:ssDNA complexes that are active intermediates in RecA protein–promoted DNA strand exchange. Therefore, free RecA protein filaments do not appear to participate directly in the DNA strand exchange pathway.

Exchange of RecA Protein Monomers between "Stable" RecA Protein:ssDNA Complexes

Evidence has been presented that RecA protein:ssDNA complexes are stabilized in the presence of ATP and SSB. Under these conditions, RecA protein does not migrate to uncoated ssDNA molecules added in a challenge experiment subsequent to the formation of the stable RecA protein:ssDNA complexes. It is possible, however, that RecA protein does dissociate from these complexes. In this case the lack of RecA protein on the challenge DNA would reflect a bias for cooperative binding of free RecA protein to a new RecA protein:ssDNA complex rather than to an unbound ssDNA molecule. It is possible, therefore, that RecA protein may exchange between RecA protein:ssDNA complexes even in the presence of SSB. To determine whether such an exchange occurs, RecA protein:ssDNA complexes were formed on two circular ssDNA molecules of different sizes in the presence of ATP and SSB. The ssDNA molecules were either

12173 (M13*ori*C26) or 5386 (ϕX174) nucleotides in length. These result in RecA protein:ssDNA complexes sufficiently different in size to be separated in a sucrose density gradient. By forming one RecA protein:ssDNA complex with ^{35}S-labeled RecA protein and the other complex with unlabeled protein, mixing the two complexes, and then separating them by sucrose density gradient sedimentation, movement of RecA protein between complexes can be detected. Results are summarized in Figure 6. All reactions contain an ATP regenerating system, ^{35}S-labeled or unlabeled RecA protein present at a stoichiometry of 1 RecA monomer per 4 nucleotides of ssDNA, and SSB at 1 monomer per 10 nucleotides. Reactions were stopped by addition of adenosine-5′-*O*-(3-thiotriphosphate) (ATPγS), a treatment that prevents dissociation of RecA protein from DNA. If complexes are formed in the absence of SSB and the resulting reaction mixture is analyzed in a sucrose density gradient, most of the RecA protein is found in a peak corresponding to free RecA protein that migrates at the top of the gradient (Fig. 6A). In the presence of SSB, all of this RecA protein is found in a peak corresponding to RecA protein:ssDNA complexes (Fig. 6B). In Figure 6C the experiment of Figure 6B has been repeated, except that a second population of unlabeled RecA protein:ssDNA complexes formed on M13*ori*C26 ssDNA has been added after addition of ATPγS. No migration of RecA protein between complexes is observed in the presence of ATPγS. If the two populations of complexes ([^{35}S]RecA protein:ϕX174 ssDNA and unlabeled RecA protein:M13*ori*C26 ssDNA) are again assembled separately, then mixed and incubated with ATP for 30 min before addition of ATPγS, a different result is observed. A significant fraction of the labeled RecA protein (>30%) has migrated from the ϕX174 complexes to the M13*ori*C26 complexes. The result is the same if labeled RecA protein is used in the complexes with M13*ori*C26 ssDNA and the RecA protein in the ϕX174 ssDNA complexes is unlabeled (S.K. Neuendorf and M.M. Cox, in prep.). These results show that RecA protein preferentially migrates between existing RecA nucleoprotein complexes under conditions in which no transfer of protein to free ssDNA is observed. The exchange could occur through the addition of RecA monomers to the growing end of a nucleoprotein filament; therefore, this result is consistent with a treadmilling model for RecA protein–promoted branch migration but does not demonstrate that the reaction proceeds by this type of mechanism. It has not yet been determined whether the exchange occurs between adjacent complexes or involves a free RecA protein intermediate. It is also unclear whether exchange involves the ends of an extended RecA nucleoprotein filament or whether all RecA protein monomers in the filament are equivalent with respect to the exchange reaction.

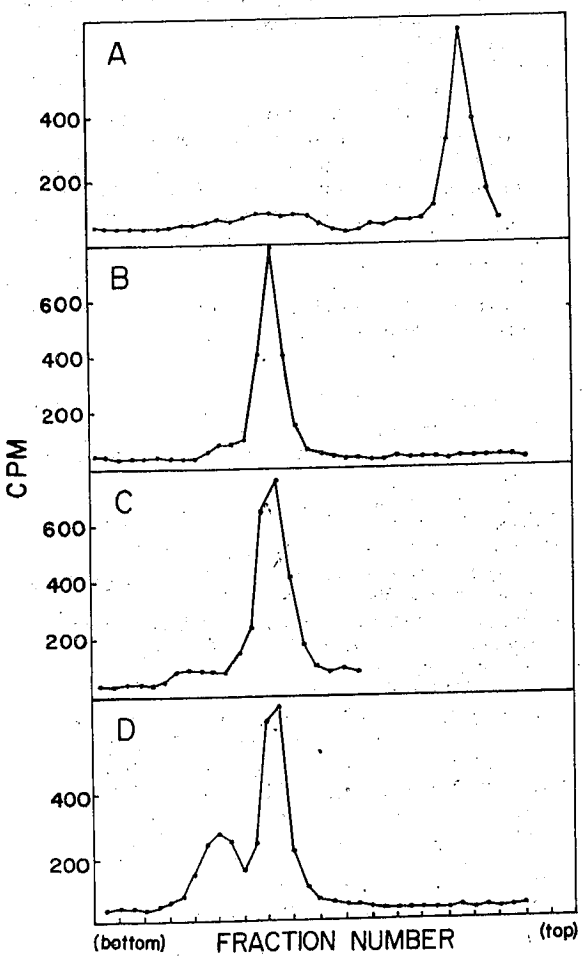

Figure 6. Exchange of RecA protein between RecA nucleoprotein complexes. RecA protein:ssDNA complexes (1 RecA protein monomer/4 nucleotide residues) were formed in the presence of an ATP regenerating system and ATP, with or without SSB. ATPγS was added to stabilize the complexes prior to sedimentation in sucrose density gradients containing $MgCl_2$ and ATPγS. (*A*) [^{35}S]RecA protein:ϕX174 ssDNA (−SSB). (*B*) [^{35}S]RecA protein:ϕX174 ssDNA (+SSB). (*C*) [^{35}S]RecA protein:ϕX174 ssDNA (+SSB) stabilized with ATPγS prior to mixing with RecA protein:M13*ori*C26 ssDNA (+SSB), similarly stabilized; the mixture was incubated for 30 min at 37°C. (*D*) [^{35}S]RecA protein:ϕX174 ssDNA (+SSB) mixed with RecA protein:M13*ori*C26 ssDNA (+SSB) and incubated for 30 min at 37°C prior to addition of ATPγS.

SUMMARY

Described above are the initial results from a series of experiments designed to more carefully define the mechanism of RecA protein–promoted DNA strand exchange, and in particular, the branch migration phase of this reaction. We have also presented two general models for RecA protein–promoted branch migration. Although the data required to demonstrate a particular mechanism are presently unavailable, we believe these models will provide a useful framework to suggest future experiments. The models also suggest possible explanations for observations that are presently unexplained. In particular, it is possible to explain the observed inefficiency of ATP hydrolysis in this system without invoking the notion that much of the ATP hydrolysis observed is simply uncoupled.

If the utilization of extensive filaments of RecA protein to carry out branch migration is inherently inefficient, why might such a mechanism be employed by the cell? The answer to this question may lie in the role of RecA protein–promoted branch migration in postreplication repair (West et al. 1981c). It might be expected that the branch points in a RecA protein–promoted branch migration reaction are susceptible to nuclease degradation. Such degradation would halt branch migration and prevent postreplication repair. One of the roles of the RecA nucleoprotein filament may be to protect the branch point from nuclease degradation and ensure formation of an extensive region of heteroduplex DNA. The apparent energetic cost of this reaction may be a relativley small price to pay to ensure the repair of a potentially lethal DNA lesion.

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