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recA Protein-promoted ATP Hydrolysis Occurs throughout recA Nucleoprotein Filaments*

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Stephen L. Brenner‡§, Richard S. Mitchell‡, Scott W. Morrical¶‖, Sandra K. Neuendorf¶, Brian C. Schutte¶‖, and Michael M. Cox¶**

From ‡E. I. du Pont de Nemours, Central Research and Development Department, Experimental Station, E328, Wilmington, Delaware 19898 and the ¶Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, Wisconsin 53706

When recA protein binds cooperatively to single-stranded DNA to form filamentous nucleoprotein complexes, it becomes competent to hydrolyze ATP. No correlation exists between the ends of such complexes and the rate of ATP hydrolysis. ATP hydrolysis is not, therefore, restricted to the terminal subunits on cooperatively bound recA oligomers, but occurs throughout the complex. Similarly, during recA protein-promoted branch migration (during DNA strand exchange), ATP hydrolysis is not restricted to recA protein monomers at the branch point. DNA cofactors of lengths varying from 16 bases to over 12,000 bases support ATP hydrolysis. The maximum value of k_{cat} at infinite DNA concentration is about 29/min independent of the length of the DNA cofactor. The apparent dissociation constant, however, is a strong function of DNA length, providing evidence for a minimum site size of 30–50 bases for efficient binding of recA protein.

recA protein promotes the complete exchange of strands between circular single strands and homologous linear duplex DNAs derived from bacteriophages. This reaction mimics the activity of recA protein in homologous genetic recombination in *Escherichia coli*. At least three phases of the reaction are easily distinguished (1–4): (i) the formation of a filamentous nucleoprotein complex containing ssDNA[1] and stoichiometric amounts of recA protein; (ii) synapsis or pairing, in which sequences in the ssDNA are aligned and paired with homologous sequences in the duplex; and (iii) branch migration, in which one strand of the duplex DNA is gradually and completely displaced by the incoming circular single strand. The third phase of this reaction is generally rate-limiting and has several important properties that are the focus of this report; *i.e.* recA protein-promoted branch migration exhibits a unique polarity (5–7) and it is the only phase of the reaction that requires ATP hydrolysis (1, 8).

Spontaneous branch migration *in vitro* is a completely random process (9), *i.e.* for a single branch point with two possible directions of branch migration, neither direction will be favored over the other. In principle, the intrinsic binding energy of a protein might be used to accelerate the rate at which such a process approaches equilibrium, but it cannot alter the equilibrium by forcing the reaction in one direction at the expense of the other (10–12). Providing a direction requires the investment of chemical energy. The link between ATP hydrolysis and the unidirectional branch migration promoted by recA protein is thus of fundamental interest. This appears to constitute a classical coupled vectorial system (10, 11), biochemically analogous to muscle contraction, membrane ion pumps, and treadmilling reactions in actin and tubulin filaments.

The active species in this system is the filamentous complex of recA protein and ssDNA formed in the first phase of the reaction. The question we wish to address is: What is the mechanism by which a recA nucleoprotein complex promotes a unidirectional branch migration coupled to ATP hydrolysis? The limited speculation that has appeared to date has drawn heavily on two well-studied filamentous systems, tubulin and actin. These proteins carry out a treadmilling reaction in which there is a net dissociation of monomers from one end of the filament and net association at the other end. This behavior, which is thermodynamically disallowed for an equilibrium system, is driven by the hydrolysis of nucleoside triphosphates (12, 13–16). Similarly, unidirectional branch migration could be due to association and dissociation of recA monomers at the ends of a recA nucleoprotein filament coupled to ATP hydrolysis. A detailed model linking branch migration to dissociation of recA protein from a filament has been proposed (17).

The dependence of recA protein-promoted branch migration on ATP hydrolysis suggests that a demonstrable link should exist between ATP hydrolysis and the critical events that permit branch movement. If association and dissociation of recA protein are critical events for unidirectional branch migration, ATP hydrolysis should be associated with this process. The experiments described here were designed to determine whether ATP hydrolysis was restricted to recA monomers at or near the ends of these filamentous nucleoprotein complexes, where association and dissociation would be most likely to occur. These experiments represent one of the first quantitative tests of models for branch migration in which the driving force is ATP hydrolysis coupled to recA monomer on-off reactions at the ends of cooperatively bound recA spiral filaments (17).

EXPERIMENTAL PROCEDURES

Materials—*E. coli* recA protein was purified as previously described (18) or by the procedure of Griffith and Shores (19). The concentra-

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§ To whom correspondence should be addressed.

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[1] The abbreviations used are: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; oligo(dT), oligomers of deoxythymidine; poly(dT), extended polymers of deoxythymidine; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; ATPγS, adenosine 5′-*O*-(thio)triphosphate.

tion of recA protein in stock solutions was determined by absorbance at 280 nm, using an extinction coefficient of 2.23×10^4 M^{-1} cm^{-1} (20). *E. coli* SSB protein was purified by a published procedure (21). SSB stock concentrations were determined by absorbance at 280 nm, using an extinction coefficient of 2.83×10^4 M^{-1} cm^{-1} (22). Bacteriophage single-stranded and duplex DNAs (ϕX174, M13mp8, M13oriC26) were purified as described previously (23, 24). Mixed oligonucleotides were synthesized at the University of Wisconsin Biotechnology Center. These oligonucleotides were synthesized to support a separate project. Their sequences contain no significant bias favoring one nucleotide over another and are considered random for purposes of this study. Concentrations of ssDNA and mixed oligonucleotides were determined by absorbance at 260 nm, using an extinction coefficient of 9350 M^{-1} cm^{-1}. Oligo(dT) polymers of specific length were synthesized at du Pont. Poly(dT) was purchased from Pharmacia and had an average length of about 600 nucleotides. Concentration of oligo(dT)s were determined by using an extinction coefficient of 8520 M^{-1} cm^{-1}. All reported DNA concentrations represent total nucleotides. Restriction enzymes were purchased from New England Biolabs. All other biochemicals and enzymes were purchased from Sigma.

Instrumentation—Absorbance measurements were obtained from a Perkin-Elmer Lambda 7 double-beam recording spectrophotometer equipped with a thermojacketed cuvette holder attached to a constant-temperature water circulator or a Hewlett-Packard 8450A diode array spectrophotometer equipped with a solid-state temperature controlled cell.

Procedure—ATP hydrolysis was monitored either by a previously described assay employing ^{3}H-labeled ATP (25), by an assay which measures the formation of inorganic phosphate from [γ-^{32}P]ATP (26), or by a spectrophotometric coupled assay (23) as indicated. Identical results were obtained using all three assay systems. The S1 nuclease assay for heteroduplex formation during DNA strand exchange has also been described (4).

Reaction Condition—Unless noted, reactions were carried out at 37 °C in buffer A (25 mM Tris (80% cation, final pH = 7.2), 10 mM $MgCl_2$, 0.4 mM KCl, 1 mM dithiothreitol, 5% glycerol) or buffer B (10 mM Hepes, pH 7.5, 1 mM dithiothreitol, 30 mM KCl, 12 mM $MgCl_2$, 50 μg/ml bovine serum albumin) and the indicated concentrations of DNA, ATP, recA protein, and SSB. Where indicated, an ATP regeneration system was included consisting of 2 mM phosphoenolpyruvate and 2.5 units of pyruvate kinase per ml of reaction mixture. Both buffer systems were found to be equally competent for DNA strand exchange reactions and for ssDNA-dependent ATP hydrolysis.

RESULTS

ATP Hydrolysis Is Not Restricted to the Ends of recA Nucleoprotein Filaments—ATP hydrolysis by recA nucleoprotein filaments was examined in a series of experiments in which the primary variable was the size of the ssDNA cofactor. Molecules of ssDNA varying in size from 16 to 12,173 nucleotides were tested, and all were cofactors in recA protein-promoted ATP hydrolysis (Figs. 1–4, Tables I and II). The ssDNA-binding site for recA protein is approximately four nucleotides; therefore, the range of DNA cofactors examined would be expected to bind from about 4 to over 3,000 recA monomers.

In one set of experiments a series of homopolymers was examined, thus avoiding the possible complication of regions of secondary structure in the ssDNA. recA titrations were performed with oligo(dT) cofactors of varying length at a fixed nucleotide concentration of 2.5 μM. The concentration of complete DNA molecules (and thus the potential concentration of nucleoprotein filament ends) therefore decreased as the oligomer size was increased. As shown in Fig. 1, the ATPase rate observed with poly(dT) was never exceeded with any of the shorter oligonucleotides. On the contrary, shorter oligonucleotides appeared less effective as cofactors than poly(dT). Observed rates of ATP hydrolysis declined further when a series of random oligonucleotides were substituted for the oligo(dT) homopolymers as cofactors for ATP hydrolysis (Table I). This could reflect either the presence of regions of secondary structure which would impede recA protein binding, or could be due to a higher intrinsic affinity of the recA protein for (dT)-oligomers relative to a random sequence, or to a combination of these effects. In reactions carried out using closed bacteriophage ssDNAs (Fig. 2 and Table I) and in the presence of SSB, ATP hydrolysis occurred with maximum velocities and recA titration curves similar to those observed for poly(dT) (compare Figs. 1 and 2 and Table I). A 2-fold increase in the size of the circular DNA had no effect on the rate of ATP hydrolysis at saturating levels of recA, as long as the concentration of DNA bases remained constant. In the absence of SSB, however, ATPase rates were much lower, and a gradual increase in the rate of ATP hydrolysis was observed with increasing recA protein concentration, although the rate never approached that seen in the presence of SSB at any of the recA concentrations examined. These results are likely due to the progressive binding of recA protein to regions of secondary structure in the bacteriophage ssDNA (27).

With poly(dT), or the bacteriophage DNAs in the presence of SSB, the ATP hydrolysis rate was unaffected by recA

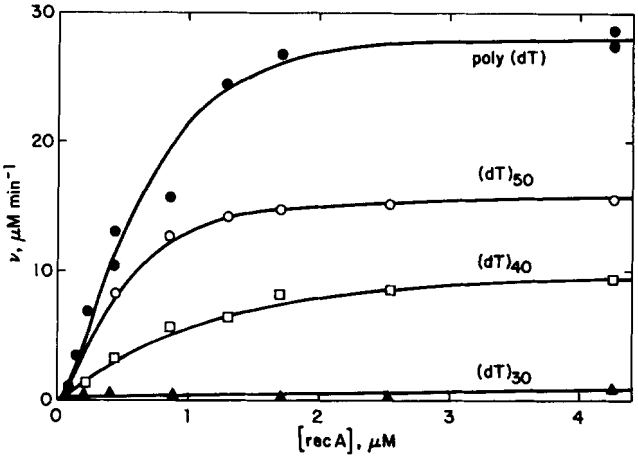

FIG. 1. **ATP hydrolysis by recA protein: dependence on recA protein concentration with (dT)-oligomers as cofactors.** ATP hydrolysis rates were determined using a coupled-enzyme assay containing an ATP-regenerating system (see "Experimental Procedures"). Reactions contained 1.0 mM ATP, 2.5 μM of the (dT)-oligomer, and the indicated concentrations of recA protein. Each recA concentration point was a separate experiment. Reactions were carried out at 37 °C in buffer B.

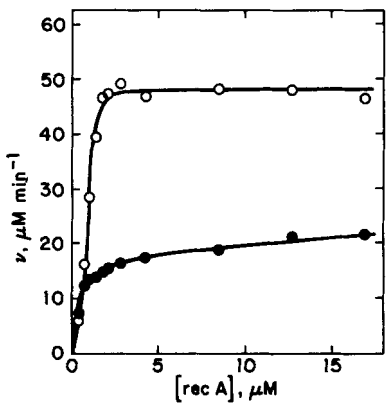

FIG. 2. **ATP hydrolysis by recA protein: dependence on recA protein concentration with ssDNA (M13mp8) as a cofactor.** ATP hydrolysis rates were determined as described in Fig. 1 with the exception that the ssDNA (M13mp8) concentration was 5.0 μM and the reaction was carried out in buffer A. O—O, reactions carried out with 0.33 μM SSB included; ●—●; reactions with SSB omitted.

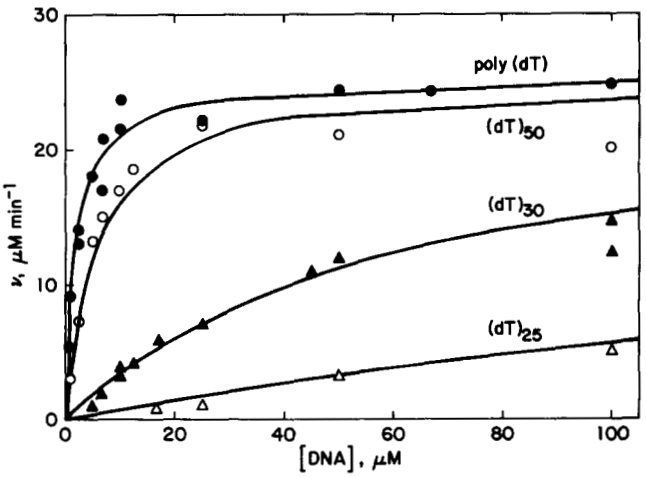

FIG. 3. **ATP hydrolysis by recA protein: dependence on DNA concentration with oligo(dT) cofactors.** Reactions were carried out under the conditions specified in Fig. 1 except that the recA protein concentration was fixed at 0.85 μM and the concentration of the (dT)-oligomers was varied. Each DNA concentration was a separate experiment. The lines were drawn using the equation $v = v_{max}[DNA]/(K_D + [DNA])$ where v_{max} and the apparent dissociation constants K_D, as determined from the double-reciprocal plots in Fig. 4, are listed in Table II.

protein concentrations in excess of those required to saturate the lattice (see below for discussion of recA affinities to oligonucleotides). This is consistent with the observation that only DNA-bound recA can hydrolyze ATP. recA protein forms filaments on circular ssDNA that exhibit no obvious interruptions in the electron microscope (28, 29). Such filaments may have no free recA protein filament ends. If ATP hydrolysis occurred only at free recA filament ends, one might then expect a decrease in the ATP hydrolysis rate when the lattice of a circular ssDNA molecule is saturated with recA protein. A decrease in the rate of ATP hydrolysis with increasing recA protein concentration was never observed in experiments with three different circular ssDNA cofactors, even when the recA concentration was more than 10-fold higher than necessary to occupy all available DNA-binding sites. Correspondingly, if ATP hydrolysis is end-dependent, we might expect a higher ATPase rate per bound recA at recA concentrations insufficient to saturate the DNA lattice. Under these conditions, the recA would be bound in clusters, and the number of protein oligomers (and therefore ends) would be higher than for a fully saturated lattice, which would have only two ends (or possibly no ends for a closed circular ssDNA). Under no conditions did we observe an increase in the ATP hydrolysis rate per recA as the recA concentration was decreased below saturation. In addition, if the bacteriophage cofactors in the absence of SSB were punctuated with regions of secondary structure to which the recA protein could not bind, one might expect the number of free recA filament ends to be higher than for the fully covered substrates (+SSB). The ATPase rate was always higher, however, in the presence of SSB than in its absence, again arguing against localization of ATPase activity at nucleoprotein filament ends.

The Apparent Affinity of recA Protein for Oligonucleotides Is a Strong Function of the Oligonucleotide Length—The observation that short DNA oligomers are less effective as cofactors for ATP hydrolysis than longer oligomers (Fig. 1, Table I) could indicate weaker binding to recA protein. Alternatively, monomers at the ends of filaments could actually be less able to hydrolyze ATP than internal ones. To decide between these possibilities, we examined the DNA concentra-

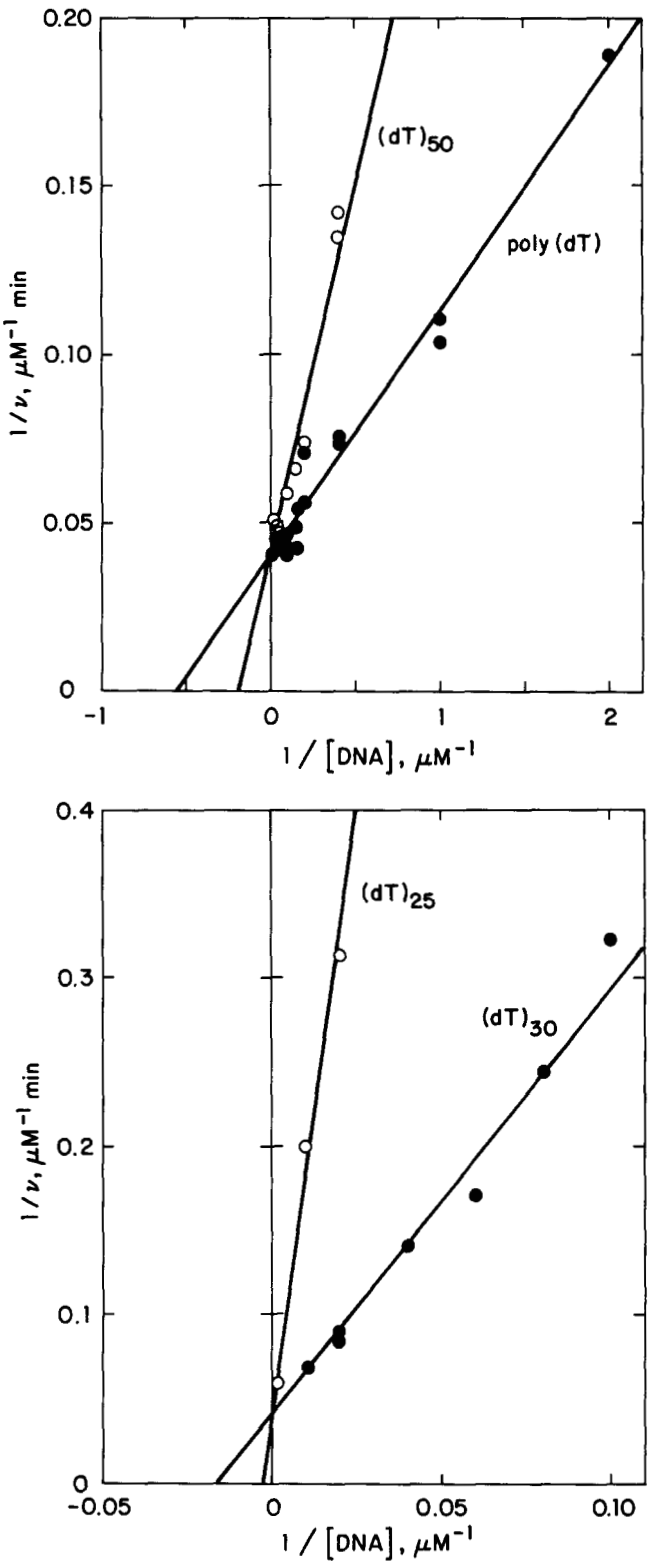

FIG. 4. **Double-reciprocal plots of recA ATPase as a function of the concentration of various (dT)-oligomers.** The data in Fig. 3, as well as some data points at higher DNA concentrations, are replotted in double-reciprocal form. Note that the scale on the abscissa for poly(dT) and $(dT)_{50}$ (*top*) is different from that for the shorter oligomers (*bottom*). The *y*-intercept gives the value of $1/v_{max}$ and the *x*-intercept is $-1/K_D$ (apparent) (see "Discussion").

TABLE I

recA protein-promoted ATP hydrolysis: effect of the size of the DNA cofactor

ssDNA cofactor	[ssDNA]	SSB (+/−)[a]	[recA protein][b]	k_{cat}(max)[c]	ν_{max}[c]
	μM		μM	min^{-1}	$\mu M\ min^{-1}$
Poly(dT)	2.5	−	0–5	24	29
$(dT)_{50}$	2.8	−	0–5	17	16
$(dT)_{40}$	2.7	−	0–5	6	10
$(dT)_{30}$	9.0	−	0–5	2	3
$(dT)_{16}$	100.0	−	1		1–2
M13mp8	5.0	−	0–18	17	22
M13mp8	5.0	+	0–18	31	49
M13oriC26	5.0	−	0–18	17	21
M13oriC26	5.0	+	0–18	28	58
77-mer[d]	5.0	−	0–2	5.4	4.9
39-mer	5.0	−	0–2	4.8	5.5
18-mer	5.0	−	0–2	1.8	0.9

[a] Where indicated, SSB concentration was maintained at 0.33 μM in M13mp8 experiments and 1.0 μM M13oriC26 experiments.

[b] Range of recA protein concentrations employed.

[c] Maximum value obtained over the range of recA protein concentration employed, in those cases where recA protein concentration was varied.

[d] Random sequence oligonucleotides of the length indicated.

TABLE II

Kinetic parameters for oligo(dT)-dependent hydrolysis of ATP by recA protein

ν_{max} and K_D(app) values obtained from the double-reciprocal plots in Fig. 3 are summarized below. The rate in the absence of added oligonucleotide was less than 0.1 μM ATP hydrolyzed per min. The [recA] = 0.85 μM in all experiments.

Oligonucleotide	K_D(app)	ν_{max}
	μM	μM *ATP hydrolyzed/min*
Poly(dT)	1.8	25
$(dT)_{50}$	5.6	25
$(dT)_{30}$	63	25
$(dT)_{25}$	333	25

tion dependence of the ATPase for a series of DNA homopolymer cofactors of varying length (Figs. 3 and 4, Table II) at a fixed recA concentration of 0.85 μM. As shown in Fig. 3, although $(dT)_{30}$ appeared ineffective as a cofactor when present at 2.5 μM in the recA titration (Fig. 1), much higher concentration of this oligonucleotide activated the ATPase efficiently. Oligo(dT) 50 bases long was nearly as effective as poly(dT), while even $(dT)_{16}$ activated the ATPase marginally at concentrations of 100 μM (Table I). The data in Fig. 3 are replotted in double-reciprocal form in Fig. 4. For all (dT)-oligomers examined, the double-reciprocal plots were linear and extrapolated to indistinguishable values of ν_{max} (see Table II) of about 25 μM ATP hydrolyzed per min, or a maximum k_{cat} of about 29 ATP hydrolyzed per recA per min. The apparent dissociation constants were, however, a strong function of the oligonucleotide length, varying from a low of about 1.8 μM for poly(dT) to a high of over 300 μM for $(dT)_{25}$ (see Table II). Decreasing the oligonucleotide size from poly(dT) to $(dT)_{50}$ only increased the apparent dissociation constant 3-fold. Decreasing the oligonucleotide size from $(dT)_{50}$ to $(dT)_{25}$, however, increased the apparent dissociation constant an additional 60-fold. Thus, efficient binding of recA seems to require an oligonucleotide of 30–50 bases, a potential binding site for 8–12 recA monomers.

In all of these experiments the rate of ATP hydrolysis appears to reflect the *total* amount of recA protein in nucleoprotein filaments rather than the potential concentration of filament ends. This tight coupling between recA protein binding to DNA and ATP hydrolysis allows a mechanistic interpretation of the k_{cat} values determined from the recA titration curves in Figs. 1 and 2 and from the extrapolated values of ν_{max}/[recA] from the DNA titrations in Figs. 3 and 4. For a stoichiometric recA titration, the maximum value of k_{cat} represents the intrinsic rate of hydrolysis, on average, per bound recA monomer. Where binding to ssDNA or oligonucleotides is weaker, k_{cat} would have a maximum at that recA concentration where the largest fraction of the added recA protein is bound to DNA. Thus, k_{cat}(max) represents a lower limit on the rate of ATP hydrolysis per bound recA monomer.[2] Our best estimate of this value from the recA titrations under the conditions used in these studies is about 30 ATPs hydrolyzed per min per recA monomer. The k_{cat} value obtained from the DNA titrations, where a fixed amount of recA is saturated with DNA cofactor, was about 29/min, in excellent agreement with the value obtained from recA titrations.

TABLE III

recA protein-promoted ATP hydrolysis during DNA strand exchange

Reactions were carried out as described under "Experimental Procedures" and contained 2.8 μM recA protein, 0.7 μM SSB, 7 μM ssDNA, and (where indicated) 15 μM duplex DNA.

Substrate DNA	Size	Homologous duplex DNA[a]	k_{cat}(app)[b]	ν
	base pairs		min^{-1}	$\mu M\ min^{-1}$
ϕX174	5,386	+	20	57
		−	27	76
M13mp8	7,229	+	20	57
		−	26	72
M3oriC26	12,173	+	21	58
		−	24	69

[a] Full-length linear duplex DNA derived from the indicated bacteriophage.

[b] Calculated by assuming 1 recA monomer is bound (and competent to hydrolyze ATP) for every four nucleotides of ssDNA present at the beginning of the reaction.

ATP Hydrolysis during DNA Strand Exchange—ATP hydrolysis was also followed in complete DNA strand exchange reactions. Three reactions were analyzed in which the only variable was the size of the DNA undergoing exchange (Table III). Substrates varied in size from 5386 (ϕX174) to 12,173 (M13oriC26) nucleotides. Concentrations of DNA (in nucleotides) were held constant as were concentrations of all other components. The concentration of *complete* DNA molecules in the reaction mixtures therefore decreased in inverse proportion to the increase in size of the DNA. The number of branch points which must have migrated to provide a given extent of strand exchange also decreased correspondingly with the increase in DNA size. The extent of the DNA strand exchange reaction (in terms of the percentage of input DNA converted to products) was comparable in all these reactions. The addition of homologous duplex DNA resulted in an immediate 20–30% drop in the rate of ATP hydrolysis relative to the rate measured in the presence of ssDNA alone. This decrease in rate is evident in the data in Table III and was found to be completely dependent on homology in the duplex DNA substrate. The subsequent rate of ATP hydrolysis remained constant throughout DNA strand exchange and for 10–30 min after the reaction was complete.[3] This pattern for ATP hydrolysis observed during DNA strand exchange was nearly identical for each of the sets of DNA substrates em-

[2] The recA protein-DNA-binding site size can be derived from the recA protein concentration at which the maximum value of k_{cat} occurs when binding is stoichiometric. If binding is weaker, a lower limit for the binding site size is obtained. The assumption must be made that all of the DNA is accessible to recA protein binding (see Ref. 5).

[3] B. Schutte, unpublished observations.

ployed in these experiments, regardless of size. We were therefore unable to detect a correlation between the rate of ATP hydrolysis and the number of DNA branch points undergoing migration. This again is consistent with the idea that ATP hydrolysis is not restricted to recA monomers at the branch point, but occurs throughout the filamentous complex within which the branch is moving.

DISCUSSION

Our principal conclusion is that recA protein-promoted ATP hydrolysis occurs throughout filamentous nucleoprotein complexes of recA and ssDNA. This conclusion is based on a wide variety of related observations which demonstrate that there is no correlation between the concentration of nucleoprotein filament ends and the steady-state rate of ATP hydrolysis. The observations include: 1) The rate of ATP hydrolysis per DNA-bound recA monomer is the same whether the DNA cofactor is $(dT)_{50}$ or a large circular ssDNA derived from a bacteriophage. The potential concentration of filament ends varies by more than 2 orders of magnitude in this comparison, and saturation of binding by recA protein can be clearly demonstrated in both cases. 2) For (dT)-oligomers, ν_{max} remains constant as the oligomer length is varied. 3) Addition of a large excess of recA protein to a circular ssDNA molecule, which might be expected to produce a contiguous circular filament without ends, has no effect on the rate of ATP hydrolysis. 4) Decreasing the concentration of recA protein to subsaturating levels with respect to large DNA cofactors, which might be expected to lead to shorter clusters of recA protein and an increase in the number of ends, never causes an increase in the amount of ATP hydrolyzed per recA monomer. 5) When recA protein is bound to native ssDNA in the absence of SSB, where recA protein binding should be restricted by DNA secondary structure and ATP hydrolysis should reflect a number of short filaments rather than a single large one, ATP hydrolysis by each monomer decreases rather than increases. These observations are completely consistent with all data currently available in the literature.

This result extends to the complete DNA strand exchange reaction in which the theoretical concentration of branch points was varied by a factor of 2, and no correlation was observed between the rate of ATP hydrolysis and the number of DNA branch points undergoing migration. This demonstrates that ATP hydrolysis occurs throughout the filamentous recA complex during strand exchange rather than at the branch point.

Two potential complications exist with regard to the data interpretation presented above. First, it has been shown that recA-DNA complexes can anneal end-to-end by noncovalent recA-recA association (30). The number of filament ends might therefore be smaller than expected from a straightforward consideration of the number of separate DNA molecules present in a given experiment. The end-to-end association, however, appears to be slow (occurring on a time scale of minutes). If ATP hydrolysis were dependent on the concentration of filament ends, we would expect to observe a time-dependent decrease in the rate of ATP hydrolysis as the number of available ends decreased. In no case examined to date, with any of the ssDNA substrates employed in this study, has the rate of ATP hydrolysis been observed to slow down on a time scale of several hours (when an ATP regeneration system was provided). The second potential complication involves the DNA-independent formation of recA protein filaments which is observed under some conditions (31, 32). If preformed recA protein filaments were intermediates in DNA binding, the template for the formation of the nucleoprotein complexes could be the recA filament rather than the DNA. In this case, a single recA filament might bind several short oligonucleotides. To explain our data, such filaments would have to be long enough to bind circular ssDNA molecules 5,386 and 12,173 bases in size. At the same time, an explanation would be required for the inefficiency in binding oligo(dT) 30-mers relative to 50-mers. We therefore argue that our data are most easily explained by the polymerization of recA protein on the DNA template and the subsequent hydrolysis of ATP by recA monomers throughout the resulting complex.

These results demonstrate that ATP hydrolysis by recA nucleoprotein filaments is not restricted to the ends of the complexes, but do not prove that all recA monomers in such a filament hydrolyze ATP. The limited structural analysis based on Fourier transform enhancement of electron microscopic images supports the idea of equivalent monomers in a spiral filament (33). However, since it is known that recA filaments formed in the absence of DNA do not hydrolyze ATP (31, 32)[4] and since the precise path of the DNA in the nucleoprotein filament is not known, we cannot rule out the possibility that only every nth recA monomer is in contact with the DNA and is hydrolyzing ATP. Inasmuch as ν_{max} remains constant for oligomer cofactors as short as $(dT)_{25}$, we estimate that n must be ≤ 6.

ATP hydrolysis has been used as an indirect probe in this study to extract parameters for recA protein binding to a series of (dT)-oligomers. The only information in this analysis that is essential to our basic conclusion is the demonstration that ν_{max} for ATP hydrolysis does not vary for the oligomers tested. The interpretation of the individual apparent dissociation constants is subject to some limitations. A basic assumption of the analysis used to derive K_D is that the rate constant k_{cat} of the catalytic step is small compared to the rate constants for all binding reactions in the system, *i.e.* that the enzymatic reaction rate is proportional to the "equilibrium" concentration of recA-ATP complexes that are bound to the DNA. The values of k_{cat} for all DNA substrates have been interpreted in terms of an intrinsic "monomeric" recA ATPase, with no consideration of the possible effects of cooperativity (in which neighboring recA monomers might have bound ATP, ADP, or ADP-P_i) on the hydrolysis rate. As discussed by Chen and co-workers (34), a more general treatment would explicitly take into account both cooperativity and the nature of the "piggyback" nucleotide ligand carried by the recA subunit.

Quantitative analysis of recA-ssDNA binding isotherms has been carried out by Menetski and Kowalczykowski (35) using the n-mer binding to an infinite lattice model of McGhee and von Hippel (36). The use of this model to extract thermodynamic parameters from binding isotherms of protein ligands to nucleic acid lattices has been reviewed by Kowalczykowski *et al.* (37). For shorter oligomers, the more general method of Epstein (37, 38), which considers finite length lattices, can be applied. We have chosen not to analyze our data at present in the context of these models for several reasons. 1) Much of the information to be derived from such an analysis depends on an accurate determination of the oligomeric species which actually binds (39). The recA oligomer that binds to the DNA lattice may well contain more than one monomer (31, 32),[5,6] and thus the curve-fitting exercise would yield ambiguous results. 2) These analyses do not take into account the possible effects of ATP hydrolysis. As pointed out by Chen *et al.*

[4] S. L. Brenner and J. Griffith, unpublished observations.
[5] J. Griffith and J. Heuser, unpublished observations.
[6] S. L. Brenner and W. Stafford, unpublished observations.

(34), the McGhee and von Hippel (36) isotherms represent only a limiting case of the more general "piggyback" activity isotherms for a DNA-activated ATPase such as recA protein. 3) The current data base for thermodynamic parameters for recA protein binding to ssDNA (35) was obtained entirely with chemically modified fluorescent DNA. The affinity of recA protein for this modified substrate has been reported to be several orders of magnitude greater than for native ssDNA (40), limiting the value of this data for general comparisons.

The surprising linearity of the $1/\nu$ *versus* 1/[DNA] plots (Fig. 4) has been seen for several other cofactor-activated ATPase activities (see, *e.g.*, Refs. 41, 42) and may provide important mechanistic clues. One possible interpretation is that the rate-determining step for the ATPase occurs with the recA not bound to the DNA cofactor, as in the case of the actomyosin ATPase (41). It is also true, however, that the McGhee and von Hippel (36) binding isotherms as a function of lattice concentration give remarkably linear double-reciprocal plots when the degree of cooperativity is low.[7] The precise interpretation of the empirical K_D values determined from the double-reciprocal plots must await future mechanistic studies.

The apparent K_D is a strong function of ssDNA length below about 50 bases, with a dramatic decrease in affinity between 30 and 25 bases. Thus, efficient DNA binding requires a site that can hold about 8–12 recA monomers. Given the tendency of recA protein to aggregate (31, 32), a substantial amount of the recA protein in solution might be present in oligomers in that size range which then represent the basic binding units. In agreement with our general observations, Leahy and Radding (43) have observed a trend of decreased binding affinity with decreasing oligonucleotide length using a nitrocellulose filter binding assay. They were able to detect binding with oligonucleotides as short as 9 bases, but retention of complexes with cofactors shorter than 20 residues required the presence of the nonhydrolyzable analog ATPγS instead of ATP. We have not observed activation of the recA ATPase by oligomers as short as 9-mers, even at millimolar concentrations, consistent with their observations.

A variety of models have been proposed for recA protein-promoted branch migration which differ in the roles envisioned for the recA spiral filament and the possible molecular consequences of ATP hydrolysis (17, 44). The results presented here, in addition to the observation that ATP hydrolysis appears not to be tightly coupled to association or dissociation of recA from nucleoprotein filaments (24), effectively eliminate a tubulin-like or actin-like treadmilling mechanism in this system. The results also suggest that the important molecular events in recA protein-promoted branch migration do not occur exclusively at filament ends. Much more experimental information is required to assess alternative models, including strand rotation (44) or an actomyosin-like cross-bridge cycling fueled by ATP hydrolysis (45). The results reported in this study and other available evidence, however, indicate that it is now important to consider and test models in which the critical events in the mechanism of recA protein-promoted branch migration occur throughout the recA-DNA complex, rather than uniquely at the branch point.

[7] S. L. Brenner, unpublished observations.

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