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Asymmetry in the recA Protein-DNA Filament*

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The apparent DNA site size obtained from an assay monitoring the ATPase activity of *Escherichia coli* recA protein (n = 3.5) differs from that determined from a direct DNA binding assay (n = 7) done under identical conditions. Investigation of this discrepancy indicates that at a DNA:protein ratio of 3.5:1, one-half of the recA protein population is less sensitive to ATPase activity inhibition by the nonhydrolyzable ATP analogue adenosine 5′-*O*-(3-thiotriphosphate) (ATPγS), suggesting that the recA protein filament is asymmetric with respect to NTP affinity. This asymmetry does not depend on the presence of ATPγS since the apparent K_m for ATP derived from single-stranded DNA-dependent ATP hydrolysis activity is dependent on the DNA:protein ratio. Three models are proposed to account for the observed site size discrepancy and the NTP binding affinity asymmetry. They differ mainly in the intrinsic site size for each recA protein monomer and in the number of DNA-binding sites/recA molecule. Gel filtration of recA-single-stranded DNA complexes at different DNA:protein ratios complements the enzymological data and provides an additional method of distinguishing among the proposed models. The phenomenon of subunit nonequivalence within the recA protein presynaptic filament may provide a molecular basis for understanding how recA protein can discriminate between different DNA molecules during homologous pairing.

The biochemical properties of the *Escherichia coli* recA protein have been studied in considerable detail. The protein binds to both single- and double-stranded DNA (McEntee *et al.*, 1981). It binds ATP and catalyzes its hydrolysis to ADP and inorganic phosphate (Weinstock *et al.*, 1981). At low salt concentrations, DNA binding by recA protein greatly stimulates the rate at which ATP is hydrolyzed (Pugh and Cox, 1988). The protein possesses an *in vitro* DNA strand exchange activity that is thought to be a good model for its action *in vivo* (West *et al.*, 1981; Cox and Lehman, 1982). recA protein can self-assemble to form fairly regular helical filamentous structures in the presence of DNA, using the DNA as a scaffold for filament assembly (Register and Griffith, 1985; Flory *et al.*, 1984). In the absence of DNA, recA protein aggregation is more sensitive to the ionic conditions and generally forms less ordered aggregates (Cotterill and Fersht, 1983; Brenner *et al.*, 1988; DiCapua *et al.*, 1982). Recently, it was shown that there may be two types of recA protein-recA protein interaction: an end-to-end interaction important for the development of cooperativity, and a lateral interaction which may be important for interfilament contacts (Freitag and McEntee, 1988).

A parameter that is frequently reported in conjunction with many of the biochemical studies on recA protein is the apparent DNA binding stoichiometry. Over the years, no consensus has been reached concerning this stoichiometry. Values ranging from 3–10 nucleotides/recA protein monomer have been reported (Shibata *et al.*, 1981; Bryant *et al.*, 1985; Shaner and Radding, 1987; Morrical *et al.*, 1986). This discrepancy may be a consequence of calculating the stoichiometry from assays that measure an activity dependent upon recA binding to DNA but do not measure DNA binding directly.

In our laboratory, we find a discrepancy in the values obtained for the apparent DNA binding stoichiometry depending on the assay employed (Menetski and Kowalczykowski, 1989). In the DNA binding assay, which directly monitors ssDNA[1] binding by recA protein via a fluorescent signal from etheno M13 DNA, the value obtained is 7 ± 1 nucleotides/monomer. However, when the ssDNA-dependent ATP hydrolysis activity is monitored, under identical conditions, the hydrolysis activity of recA protein saturates at a stoichiometry of 3 ± 1 nucleotides/monomer. When poly(dA), a polynucleotide to which recA protein binds weakly, is used as a ssDNA cofactor for the ATP hydrolysis activity of recA protein, the stoichiometry observed depends on the type of nucleotide cofactor present (Menetski and Kowalczykowski, 1989); with rATP present, the activity saturates at a value of 7 nucleotides/recA monomer, but with dATP, a cofactor that increases the affinity with which recA protein binds to DNA, the ATPase activity saturates at a value of 3 nucleotides/monomer. Since the stoichiometry of ATP binding has been shown to be 1 ATP molecule/recA monomer (Cotterill *et al.*, 1982) these data suggest that there may be two biochemically distinct species of recA protein that comprise the recA protein-ssDNA filament.

In Fig. 1, we illustrate three models that may potentially explain this observed site size discrepancy. The models attempt to reconcile how direct DNA binding studies indicate an apparent stoichiometry of 1 recA protein/7 nucleotides, whereas under identical conditions the ATPase assay yields an apparent stoichiometry of 1 recA protein/3–4 nucleotides. The feature common to all three models is that the maximum fluorescent signal is observed at a DNA:protein ratio of 7:1, but additional protein is able to bind to this complex that is not detected by the DNA binding assay but does result in an increase in the observed ATPase rate until saturation occurs

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[1] The abbreviations used are: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; etheno M13 DNA, modified single-stranded M13 DNA containing 1,N^6-ethenoadenosine and 3,N^4-ethenocytidine; ATPγS, adenosine 5′-*O*-(3-thiotriphosphate); poly(dT), polydeoxythymidylic acid; poly(dA), polydeoxyadenylic acid; SSB protein, single-stranded DNA-binding protein.

at a DNA:protein ratio of 3.5:1. In Fig. 1*A*, a second set of recA protein is shown binding to the 7:1 complex. The binding of this second set of recA protein does not result in a fluorescence increase in the DNA binding assay but is able to hydrolyze ATP, increasing the rate of ATP hydrolysis until saturation at 3.5:1. Thus, the final complex is asymmetric with respect to DNA binding. In Fig. 1*B*, recA protein is shown accommodating a maximum of 7 nucleotides/monomer via two DNA-binding sites. Occupancy of only one DNA-binding site is sufficient for ATP hydrolysis, resulting in the observed stoichiometry for ATPase activity. In this model, all the recA molecules in any given complex are equivalent in their interaction with the DNA, though the "$n = 7$" complex may behave differently than the "$n = 3.5$" complex. A model like this has been advanced by Takahashi *et al.* (1989), based on fluorescent DNA binding and flow linear dichroism studies of recA protein-DNA complexes. In Fig. 1*C*, the recA protein molecule is shown occluding 7 nucleotides/monomer but only directly interacting with 3.5 nucleotides. However, binding of a single protomer is enough to result in the maximum fluorescent signal at that DNA-binding site (perhaps due to extension and unstacking of all 7 occluded nucleotide residues). The binding of additional recA protein beyond the 7:1 ratio is consequently undetected by the fluorescent assay but does result in additional ATP hydrolysis. In the 3.5:1 complex, the contacts made by recA protein with the DNA appear to be equivalent; however, recA protein binding in the "overlapping" mode may be sterically limited in its interaction with the DNA. Certainly, "end-to-end" binding must be favored over overlapping binding during initial recA association with the single-stranded DNA to result in apparent saturation at 7 nucleotides/monomer in the DNA binding assay. As a result, the protein-protein interactions may not be equivalent in the final 3.5:1 complex and could display functional asymmetry.

Since the interaction of recA protein with DNA has substantial effects on the affinity displayed for ATP and vice versa (Kowalczykowski, 1986; Menetski and Kowalczykowski, 1985), one may hypothesize that functional asymmetry with respect to either ATP affinity or DNA affinity, or both, may be displayed by the complexes in Fig. 1 and this asymmetry would be manifest when the DNA:protein ratio exceeds 7:1. The experiments described here are designed to ascertain whether such nonequivalence exists and to permit discrimination among the models presented.

MATERIALS AND METHODS

recA Protein—recA protein was purified from *E. coli* strain JC12772 (Uhlin and Clark, 1981) by using a preparative protocol[2] based on spermidine precipitation (Griffith and Shores, 1985). Protein concentrations were determined by using an extinction coefficient of 2.7×10^4 M^{-1} cm^{-1} at 280 nm.

Chemicals—ATP and ATPγS were purchased from Pharmacia LKB Biotechnology Inc. and Boehringer Mannheim, respectively, and were dissolved as concentrated stock solutions at pH 7.5. Concentrations of adenine nucleotides were determined spectrophotometrically by using an extinction coefficient of 1.54×10^4 M^{-1} cm^{-1} at 260 nm.

Etheno M13 ssDNA—Etheno M13 DNA was made as described by Menetski and Kowalczykowski (1985). The concentration of etheno M13 DNA was determined using an extinction coefficient of 7000 M^{-1} cm^{-1} at 260 nm (Menetski and Kowalczykowski, 1987).

M13 DNA—M13 ssDNA and the replicative form dsDNA were isolated as described by Messing (1983). The replicative form was linearized using *Eco*RI restriction endonuclease. The concentrations of ssDNA and dsDNA were determined using extinction coefficients at 260 nm of 8784 and 6500 M^{-1} cm^{-1}, respectively.

NTP Hydrolysis Assay—The rate of ATP hydrolysis was measured by following the oxidation of NADH spectrophotometrically at 340 nm as described by Kowalczykowski and Krupp (1987). The buffer employed was 25 mM Tris acetate (pH 7.5), 10 mM magnesium acetate, 0.1 mM dithiothreitol, 1.5 mM phosphoenolpyruvate, and 12.5 units/ml each of lactate dehydrogenase and pyruvate kinase. Unless otherwise specified, the order of addition of reaction components was recA protein, etheno M13 DNA, and ATPγS. This was allowed to equilibrate to the assay temperature of 37 °C. The reaction was then started by the addition of ATP. The rate was monitored until steady-state hydrolysis was observed, and the rate was calculated from the corresponding linear portion.

Gel Filtration Chromatography—Gel filtration was carried out on a 1 × 27-cm S-1000 (Pharmacia) column. The elution buffer was the same as the buffer in which the complexes were prepared (25 mM Tris acetate (pH 7.5), 1 mM magnesium acetate, 0.1 mM dithiothreitol, and 50 μM ATPγS). The concentration of etheno M13 DNA was held constant at 40 μM, and the recA protein concentration was changed to achieve the desired ratios. The sample was applied in a 200-μl volume and eluted with a linear flow rate of 15 cm h^{-1}.

Joint Molecule Assay—The joint molecule formation reaction was monitored using an agarose gel assay without the addition of ethidium bromide (Menetski and Kowalczykowski, 1989). Reaction buffer contained 25 mM Tris acetate (pH 7.5), 0.1 mM dithiothreitol, and initially, 1 mM magnesium acetate. The concentrations of M13 ssDNA and linear dsDNA were 10 and 20 μM, respectively. An ATP-regenerating system consisting of 4 mM phosphoenolpyruvate and 25 units of pyruvate kinase/ml was included in all reactions. The order of addition of reaction components was as follows. To reaction buffer, recA protein was added at the desired concentration followed by 10 μM ATPγS and ssDNA. This was allowed to thermally equilibrate to 37 °C. The ATP-regenerating system was added, followed by 3 mM ATP. Magnesium acetate was added to a final concentration of 10 mM. Linear dsDNA was then added to begin the reaction. Time points were taken, and sodium dodecyl sulfate and EDTA were added to 0.5% and 25 mM, respectively, and run on a 0.8% agarose gel at 2 V/cm for 7 h. The gel was stained in a solution containing 2 μg/ml of ethidium bromide. Photographs were taken and the negatives scanned on a Zeineh soft laser scanning densitometer interfaced to a HP3390A integrator. The disappearance of substrate molecules (linear dsDNA) was monitored and expressed as the percentage of substrate molecules utilized.

Curve Fitting—The data from the ATPγS titrations were fit to one of two equations using Minsquare (Micromath, Salt Lake City), a nonlinear least squares regression fitting program. The data were fit to either a single hyperbola of the form

$$\% \text{ inhibition} = \frac{M[\text{I}]}{K_{i,\text{app}} + [\text{I}]} \tag{1}$$

or to a double hyperbola of the form

$$\% \text{ inhibition} = \frac{M1[\text{I}]}{K_{i1,\text{app}} + [\text{I}]} + \frac{M2[\text{I}]}{K_{i2,\text{app}} + [\text{I}]} \tag{2}$$

and

$$M1 + M2 = 100$$

where M = the maximum percent inhibition, [I] = the concentration of ATPγS, and $K_{i,\text{app}}$ = the apparent K_i for ATPγS.

The equations given above are the simplest that could be fit to the data obtained from the experiments. When fitting the data from etheno M13 DNA and poly(dT) at 3.5:1, no global minimum was found by the fitting program if $M1$ and $M2$ were not equal; thus the contribution of each hyperbola was weighted equally ($M1 = M2 = M_{\text{tot}}/2$). The use of these equations is justified by the following consideration. Equation 3 describes a velocity equation for competitive inhibition (Segel, 1975).

$$\frac{v}{V_{\max}} = \frac{[\text{S}]}{K_m(1 + [\text{I}]/K_i) + [\text{S}]} \tag{3}$$

K_m is the Michaelis constant of the enzyme for the substrate S, and $V_{\max}$ is the maximum velocity of enzyme. We can rearrange the equation and obtain Equation 4.

$$\% \text{ inhibition} = 100(1 - v/V_{\max}) = \frac{100[\text{I}]}{K_i(1 + [\text{S}]/K_m)} + [\text{I}] \tag{4}$$

Thus, it can be seen that the apparent K_i described in Equations 1 and 2 is equal to the intrinsic K_i multiplied by the term $(1 + [\text{S}]/K_m)$.

[2] S. C. Kowalczykowski, manuscript in preparation.

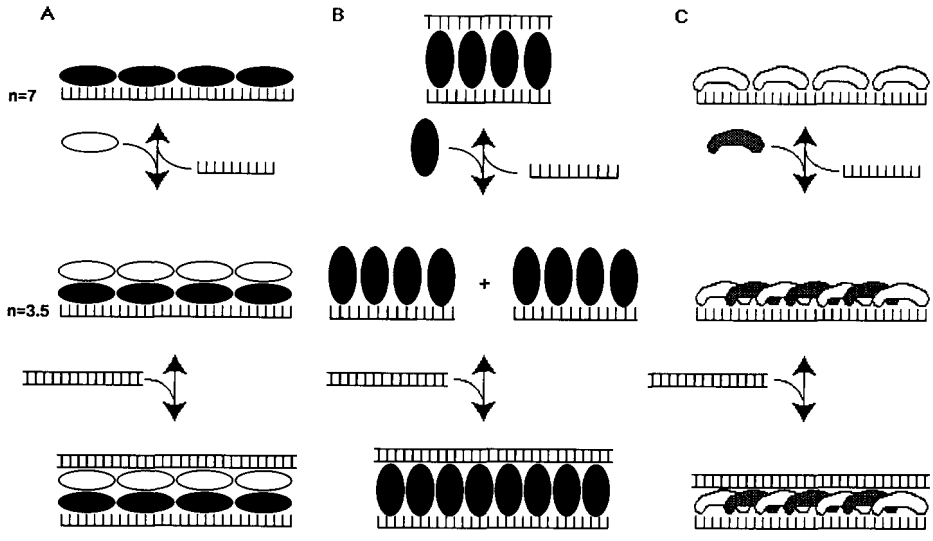

FIG. 1. **Potential models to account for the site size discrepancy.** Each recA protein has one site for ATP binding and hydrolysis. Every recA protein monomer associated with the ssDNA, either directly or indirectly, can hydrolyze ATP at the same rate. The complexes at the 7:1 ratio give the maximum fluorescent signal in the DNA binding assay. The complexes at the 3.5:1 ratio are fully saturated with respect to ATPase activity. In *A* and *C*, at the 3.5:1 ratio, the different shading patterns are intended to illustrate the potential asymmetry of the recA protein filament.

Therefore, the apparent K_i will be affected by the affinity of recA protein for ATP as would be expected.

Several criteria were used to determine the degree to which the appropriate equation fit a particular data set. These include the estimation of *M*, *i.e.* how close the value is to the theoretical value of 100%; evidence of any systematic deviation in the curve fit to the observed data; and the value for the sum of the squares from the fitting procedure. It was also observed that attempts to fit data using the equation for a double hyperbola when a single hyperbola was sufficient resulted in the two apparent K_i values converging to the value subsequently obtained from the single hyperbola fit. This result gave us further confidence in the validity of the data fit to the double hyperbola equation.

RESULTS

Experiments were designed to determine whether any functional asymmetry could be detected, particularly with regard to ATP binding affinity, at the diagnostic stoichiometries depicted in Fig. 1. These experiments exploit the fact that recA protein binds the ATP analogue, ATPγS, with very high affinity ($K_d < 0.4$ μM) (Kowalczykowski, 1986) and is essentially unable to hydrolyze this molecule (Weinstock *et al.*, 1981). When ATPγS binds to recA protein, it induces a very high affinity for DNA, resulting in an extremely stable complex (Menetski and Kowalczykowski, 1985). Previous experiments have shown that ATPγS acts as a competitive inhibitor of ATP hydrolysis, with an apparent K_i of 0.6 μM (Weinstock *et al.*, 1981).

ATPase Activity in the Presence of ATPγS—The first experiment involves forming a recA protein-ssDNA complex at a DNA(nucleotides):protein molar ratio of 7:1 (to form the complexes depicted in Fig. 1, *top line*) in the presence of a low concentration of ATPγS (10 μM). In these initial experiments, etheno M13 DNA is used as the DNA substrate since it lacks secondary structure and makes the interpretation of the results less complicated. After allowing this complex to form, 3 mM ATP is added and the rate of ATP hydrolysis is measured. A second aliquot of protein is then added while the rate of ATP hydrolysis is still being measured. This addition of protein brings the DNA:protein ratio to 3.5:1. Table I summarizes the results from such an experiment. As expected, the rate from the first addition of protein is very low (≃15% of the rate observed in the absence of ATPγS), indicating that the ATPγS is inhibiting ATP hydrolysis. Upon the second addition of protein, the rate of hydrolysis observed is one-half the rate expected if all the protein present were hydrolyzing ATP (Table I). A third addition of protein does not increase the rate significantly (not shown), indicating that the complex is saturable and that the second addition of protein had not displaced the recA protein initially bound to the ssDNA. Since the ATP hydrolysis observed is completely dependent on the presence of DNA (not shown), ATPγS is not directing the formation of DNA-free protein filaments that are able to hydrolyze ATP. Monitoring the ATPase rate for 2–3 times longer than the usual assay length of 20 min showed that the rate achieved is steady-state, indicating there is no slow inhibition by ATPγS occurring. Finally, reversing the above mentioned order of ATP and ATPγS addition does not change the results obtained. However, since the kinetics of inhibition are quite slow,[3] this order of addition is not employed.

TABLE I

Rates of ATP hydrolysis by recA protein in the presence and absence of ATPγS as a function of the DNA:protein ratio

Reaction conditions are as described under "Materials and Methods." At the 7:1 ratio, the initial recA concentration is 0.45 μM. The recA protein concentration at the 3.5:1 ratio is 0.9 μM. The ssDNA concentration is 3 μM, and the ATP-γ-S concentration is 10 μM.

Initial DNA:protein ratio	Rate of ATP hydrolysis			
	Initial rate		Rate after second recA addition	
	−ATPγS	+ATPγS	−ATPγS	+ATPγS
	μM min^{-1}		μM min^{-1}	
7:1	12	2.1	25	12.0
3.5:1	25	11.8	25	12.5

The second experiment is similar to the first except that the initial concentration of protein (before ATP addition) is higher, resulting in a DNA:protein ratio of 3.5:1. The intent was to form a stable recA protein-ssDNA complex in the presence of ATPγS that would not accommodate any further recA protein binding (as depicted in the *lower line* of Fig. 1). Since it was expected that all the recA protein would be complexed with ATPγS, initially no significant rate of ATP hydrolysis upon addition of ATP is expected. Instead, when ATP is added to the reaction, the rate of hydrolysis observed is identical with that seen in the previous experiment after the second addition of protein (Table I). This complex is indeed saturated, since further recA protein addition has no

[3] S. D. Lauder and S. C. Kowalczykowski, unpublished observations.

effect on the rate of ATP hydrolysis (Table I). This result indicates that ATP is able to displace (or replace) ATPγS from a segment of the population of recA protein molecules in the recA protein-ssDNA filament.

ATPase Activity in the Presence of ATPγS Is Dependent on the recA Protein-ssDNA Stoichiometry—To determine the stoichiometry of this complex formation more precisely, and to establish whether ATPγS is inhibiting a specific fraction of the recA protein population, experiments like those described previously were performed, while the amount of recA protein in the reaction was varied (Fig. 2). In the absence of ATPγS, the rate of ATP hydrolysis by recA protein increases as the recA protein concentration is increased, until the rate reaches a maximum upon DNA saturation. The apparent DNA binding stoichiometry derived from this data is approximately 3 nucleotides/recA monomer. When 10 μM ATPγS is present in an otherwise identical reaction mixture, little ATP hydrolysis is observed until a certain recA protein concentration is exceeded. The amount of protein initially required before hydrolysis is observed is dependent upon the DNA concentration, since twice the amount of protein is required when the DNA concentration is doubled (Fig. 2). The DNA:protein ratio after which the ATPase rate begins to increase is 7:1 in the presence of ATPγS. The observed rates saturate at a DNA:protein ratio of 3:1. This is identical with the value obtained in the absence of ATPγS. Finally, in the presence of ATPγS, the observed ATP hydrolysis rate saturates at roughly one-half that observed when all the recA protein present is hydrolyzing ATP (*i.e.* in the absence of ATPγS). These experiments confirm that ATPγS is not simply inhibiting ATP hydrolysis of all the recA protein by 50% but is preferentially inhibiting one-half the recA population 100%. Since recA protein has only one ATP-binding site (Cotterill *et al.*, 1982), at the 3:1 DNA:protein ratio there must be two species of recA protein molecules present that differ in their sensitivities to inhibition by ATPγS.

ATPγS Inhibition of recA Protein ATPase Activity—The previous results strongly suggest that two classes of recA protein constitute the nucleoprotein filament, distinguished by different affinities for NTP. To determine whether this affinity difference could be demonstrated more directly, the inhibition of ATPase activity was assayed as a function of ATPγS concentration. This was done at three different DNA:protein ratios, changing the DNA concentration to achieve the desired ratio (Fig. 3). At the lower DNA:protein ratio of 3:1, there is sufficient recA protein to fully saturate the DNA. The models depicted in Fig. 1, *A* and *C*, now have

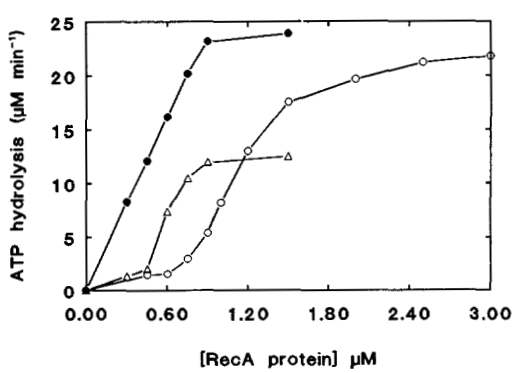

FIG. 2. **A recA protein titration in the presence and absence of ATPγS.** The *filled circles* and *open triangles* are data obtained at 3 μM etheno M13 ssDNA in the absence and presence of 10 μM ATPγS, respectively. The *open circles* are data in the presence of 10 μM ATPγS and 6 μM etheno M13 ssDNA. The concentration of ATP in these reactions is 3 mM.

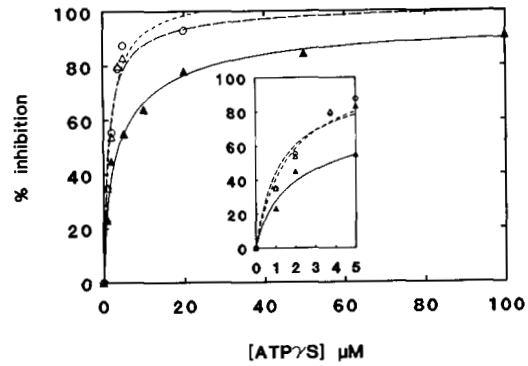

FIG. 3. **ATPγS titrations of recA protein ATPase activity at various DNA:protein ratios.** Reaction conditions are identical with those in Fig. 2, except that the protein concentration was held constant at 0.5 μM and the concentration of etheno M13 ssDNA was varied to obtain the desired DNA:protein ratio. The data at the 3:1 ratio are represented by the *filled triangles*. The data at the DNA:protein ratios of 16:1 and 8:1 are represented by the *open circles* and *triangles*, respectively. The equation of the *solid line*, fit to the 3:1 data, is $y = 47.5x/(x + 1.02) + 47.5x/(x + 10.33)$. The equation of the *long dashed line*, fit to the 16.1 data, is $y = 95x/(x + 1.18) + 7.07x/(x + 18)$. For comparison, the *short dashed line* is the equation of a single hyperbola fit to the 16:1 data where $y = 106.7x/(x + 1.65)$. The *inset* shows detail at low ATPγS concentrations.

TABLE II

Summary of parameters derived from ATPγS inhibition curves

The DNA concentration in these experiments is 3.5 μM. The recA protein concentration is 1 mM. The experimental conditions are as described under "Materials and Methods."

Polynucleotide	Inhibition	K_i[a]	K_{i1}	K_{i2}
	%	μM	μM	μM
Poly(dT)	100[b]		3.9 ± 0.7	32.9 ± 6
Etheno M13	95 ± 5		1.0 ± 0.3	10.3 ± 5
Poly(dA) + dATP	107 ± 4	4.4 ± 0.5		
Poly(dA) + ATP	111 ± 5	6.4 ± 0.9		
M13 ssDNA without SSB	101 ± 3	12.1 ± 1		
M13 ssDNA + SSB	105 ± 11	6.7 ± 2		

[a] This parameter is the apparent K_i derived from a single hyberbola fit. The parameters $K_{i1,app}$ and $K_{i2,app}$ are the apparent K_i values derived from the double hyperbola fit. The indicated errors are the standard deviations obtained from the fitting procedure.

[b] This parameter was held constant in the fitting procedure.

recA molecules which are potentially nonequivalent in their association with DNA; this is not the case with the model shown in Fig. 1*B*. The data obtained at the 3:1 ratio (Fig. 3, *filled triangles*) cannot be fit to a single hyperbola, but they can be fit by an equation for the sum of two hyperbolas, each weighted equally. This equation describes the behavior of two recA protein species with differing sensitivities to inhibition by ATPγS. The calculated apparent K_i values are approximately 1 and 10 μM (Table II). We interpret this as an indication of two species of recA protein present at this ratio: a species that binds NTP strongly, termed the "high NTP affinity" form, and a species that binds NTP more weakly, termed the "low NTP affinity" form. In contrast, at the 8:1 and 16:1 DNA:protein ratios (Fig. 3, *open triangles* and *circles*, respectively), there should be only one type of recA protein-ssDNA interaction, regardless of the model considered. The data show an approximately hyperbolic inhibition of the rate of ATP hydrolysis, consistent with the expectation that only one recA protein species is present. While the data at the 16:1 and 8:1 ratios can be fit to a single hyperbola fairly well, slightly better (as judged by the values obtained for the sum of the squares) results are obtained if the data are fit to an equation similar to the one described above. In this case,

however, the contribution of the two hyperbola was *not* weighted equally. The results from these fits indicate that the predominant species (95%) is the high NTP affinity form (apparent K_i = 1.2 μM) and there is only a minor component (7%) corresponding to the low NTP affinity form (apparent K_i = 18 μM) of recA protein.

Different Polynucleotide Substrates Result in Different Apparent K_i Values for ATPγS Inhibition of ATPase Activity—All previous experiments employed etheno M13 as the polynucleotide cofactor. Since recA protein does not bind to all polynucleotides with equal affinity and the affinity for the polynucleotide affects the affinity for the NTP, ATPγS titrations were performed using other polynucleotides. Poly(dT), a polynucleotide that recA protein binds more strongly than it binds etheno M13 DNA, and poly(dA), a polynucleotide that recA protein binds more weakly, were employed.

Fig. 4 shows that a higher concentration of ATPγS is required to achieve complete inhibition of the poly(dT)-dependent ATPase activity. This is reflected in the higher apparent K_i values (Table II) obtained from the double hyperbola fit. This result is not unexpected since an increase in the affinity for both ATP and ATPγS will result in less inhibition at any given ATPγS concentration (see Equation 4). It is also apparent from Fig. 4 that the poly(dA)-dependent ATPase activity is most easily inhibited by ATPγS; in contrast to the poly(dT) data, these data are well fit by a single hyperbola. This is not unexpected given that the apparent stoichiometry derived from recA protein titrations on poly(dA) is 7 nucleotides/recA protein monomer when ATP is the nucleotide cofactor and the ATP hydrolysis rate in the absence of ATPγS is one-half that observed with poly(dT) or etheno M13 DNA at the same DNA:protein ratio (Menetski and Kowalczykowski, 1989). When dATP is present as a cofactor, the apparent stoichiometry for poly(dA) is 3.5 nucleotides/recA protein monomer and the rate in the absence of ATPγS is twice that observed when ATP is used as the cofactor (Menetski and Kowalczykowski, 1989). However, the resulting ATPγS titration curve is still well fit by a single hyperbola with a slightly lower K_i.

The behavior of unmodified M13 ssDNA was also examined at a constant DNA:protein ratio but in either the presence or absence of SSB protein. It was previously shown that the rate of ATP hydrolysis in the absence of SSB protein is roughly half that observed when SSB protein is present, due to the inability of recA protein to access DNA involved in secondary structure; when SSB protein is present, the rate increases to that expected if all the protein were hydrolyzing ATP (Kowalczykowski and Krupp, 1987). The effect of ATPγS on the ATPase activity under these conditions is summarized in Table II. In the presence of SSB protein, the apparent K_i is lower. The difference in the apparent K_i values is probably due to the presence of excess recA protein that is not bound to the ssDNA when SSB protein is absent from the reaction mix. This is based on the observation that, when an ATPγS titration is done using etheno M13 DNA as the DNA cofactor at a DNA:protein ratio of 1.75:1 (*i.e.* at excess recA protein), the apparent K_i for ATPγS increases 3-fold over that observed at the 3.5:1 ratio (not shown), suggesting that excess recA protein may reduce the effectiveness of inhibition by ATPγS. It appears that the polynucleotides to which recA binds more weakly (*i.e.* poly(dA) and ssM13 DNA) give inhibition curves that do not display experimentally detectable two-component behavior. However, as shown in the next section, ssM13 DNA displays behavior that is indicative of the presence of nonidentical protein-DNA complexes.

ATP Concentration Dependence of recA Protein ATPase Activity at Different DNA:Protein Ratios—If this asymmetry is a general property of the recA protein-ssDNA filament and not dependent upon the presence of ATPγS, then recA protein-catalyzed ATP hydrolysis might be expected to exhibit different apparent K_m values at different protein:DNA ratios (more accurately described as $S_{0.5}$, since the curves are sigmoidal (Neet, 1983)). Fig. 5*a* shows that, at the 16:1 DNA:protein ratio, the ATP hydrolysis data show a very cooperative transition with an $S_{0.5}$ value of 43 μM ATP. The data at the 3:1 ratio do not show this sharp transition and the shape of the curve is not symmetric about the midpoint,

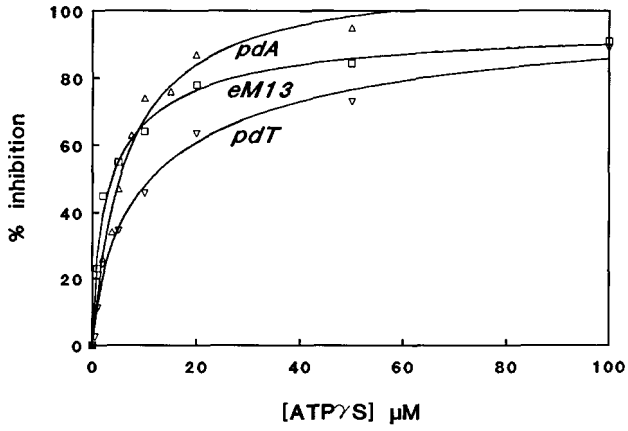

FIG. 4. **ATPγS concentration dependence of recA protein ssDNA-dependent ATP hydrolysis activity using various polynucleotides.** recA protein concentration was 1 μM, and the concentration of the various polynucleotides was 3.5 μM. The concentration of ATP is 3 mM. The experiments done with poly(dT) (*pdT*) are represented by the *inverted triangles*; poly(dA) (*pdA*), by *triangles*; and etheno M13 (*eM13*), by *squares*.

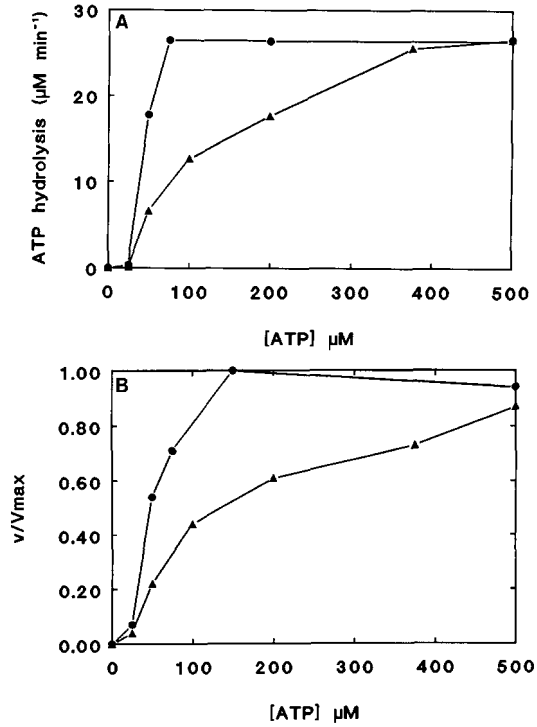

FIG. 5. **ATP concentration dependence of recA protein ssDNA-dependent ATP hydrolysis activity using etheno M13 (*A*) and native ssM13 (*B*).** recA protein concentration was held constant at 1 μM, and the ssDNA concentration was changed to achieve the desired ratio. The *filled circles* are data at a 16:1 ratio; the *filled triangles* are data at a 3:1 ratio.

suggesting multiple forms of recA protein. The resulting $S_{0.5}$ value is increased to 109 μM.

Native M13 ssDNA was also used as a polynucleotide cofactor to ensure that this behavior was not limited to etheno M13 DNA and also to see whether asymmetry could be detected using this approach. As can be seen in Fig. 5*B*, the behavior of recA protein with M13 ssDNA is essentially identical with that observed when etheno M13 is employed. The data is presented as fractional V_{max} because the rate at the ratio of 3.5:1 is roughly half that observed at the ratio of 16:1 due to the absence of SSB protein. These results are consistent with the existence of two enzymatically distinct forms of recA protein at the different DNA:protein ratios and also demonstrate that this phenomenon is a general feature of recA protein-NTP interaction and not one unique to ATPγS or to a particular polynucleotide.

Gel Filtration of recA-DNA-ATPγS Complexes—The models as illustrated in Fig. 1, *A* and *C*, predict that there should be significant differences in the molecular weights of the 7:1 and 3.5:1 complexes. Gel filtration was selected as a method for determining the apparent molecular weights of the various complexes. The results from these experiments are shown in Fig. 6. All the nucleoprotein complexes elute with an apparent molecular weight that is higher than that observed for either DNA or recA protein alone, showing that these complexes are chromatographically stable in the presence of ATPγS. The elution behavior of the 3.5:1 and 7:1 complexes reproducibly show that the 3.5:1 complex appears to be larger than the 7:1 complex. To show that these complexes were discrete species and the differences observed were not simply a result of large protein-DNA aggregates forming as the amount of recA protein was increased, a complex was formed at a ratio of 1.5:1 and chromatographed. At this ratio, the recA protein concentration is well in excess of that required for complete saturation of the available DNA. The elution profile of this complex shows a species that elutes at the same position as the 3.5:1 complex as well as a large peak corresponding to free recA protein. One would also predict that a 3.5:1 complex should be convertible to a 7:1 complex simply by adding more DNA. Indeed, an experiment of this nature results in a complex that elutes at a position indistinguishable from a directly prepared 7:1 complex (not shown). Thus, these experiments support the results from the enzymological studies, further showing that these complexes are a discrete, saturable, and interconvertible species.

Joint Molecule Formation in the Presence of ATPγS—Given the relevance of the DNA strand exchange reaction to recA protein function *in vivo*, it was important to evaluate what role the two recA protein species play in promoting pairing between homologous single-stranded and double-stranded DNA. In the presence of ATPγS, a heterogeneous population of joint molecules is produced (Menetski *et al.*, 1990). Thus, the disappearance of input linear dsDNA in the agarose gel assay was used to quantitate the extent of joint molecule formation using the experimental conditions defined here. In Fig. 7, the percentage of substrate DNA molecules utilized is plotted as a function of the recA protein concentration. The shape of the graph resembles the protein titration shown in Fig. 2, suggesting that the nucleoprotein structure present at the higher ratio of DNA to protein may not promote efficient pairing with dsDNA. However, homologous pairing begins to increase at a recA protein concentration that apparently is insufficient for formation of the 3.5:1 complexes depicted in Fig. 1. This apparent discrepancy may result from the fact that the M13 ssDNA contains secondary structure that recA protein cannot access, even at a low magnesium ion concentration (1 mM). As a result, the effective ssDNA concentration is lower than the actual input DNA concentration, and consequently, formation of the 3.5:1 complexes would occur at lower recA protein concentrations. To see if this was the case, the ATPase activity was monitored under the same conditions as the strand exchange assay (*i.e.* in the presence of ATPγS) except that dsDNA was not added. Based on the results obtained with ssDNA devoid of secondary structure, the recA protein concentration at which the ATPase activity begins to increase defines the amount of recA protein required to form a saturated 7:1 complex (*i.e.* the presence of the high affinity NTP form only). Any observed ATPase activity results from the low NTP affinity protein molecules that comprise one-half the total recA protein present in the recA protein-ssDNA filament at a ratio of 3.5:1 (DNA:protein). Fig. 7 shows that, as expected, the protein concentration dependence observed using M13 ssDNA resembles that seen in Fig. 2; however, the actual protein concentration required for formation of each characteristic complex is lower due to the presence of DNA secondary structure. Also consistent with the data in Fig. 2, the observed ATP hydrolysis rate at saturation in Fig. 7 is 43% of that observed when ATPγS is omitted (not shown). As can be seen from the data in Fig. 7, the observed ATPase activity and the amount of substrate molecule utilization correspond very well. This would seem to

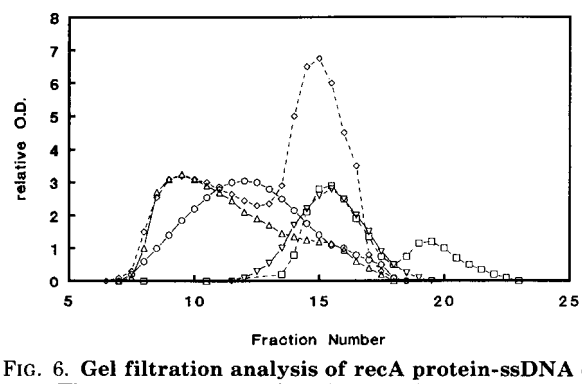

FIG. 6. **Gel filtration analysis of recA protein-ssDNA complexes.** The concentration of etheno M13 DNA is 40 μM. recA protein was added to achieve the desired ratios. The elution buffer (25 mM Tris acetate, 1 mM magnesium acetate, 0.1 mM dithiothreitol, and 50 μM ATPγS, (pH 7.5)) is the same as the buffer in which the complexes are prepared. The data for the 3:1 ratio are represented by *triangles*; 7:1 ratio, by *circles*; 1.5:1 ratio, by *diamonds*; recA protein alone, by *squares*; and etheno M13 DNA, by *inverted triangles*.

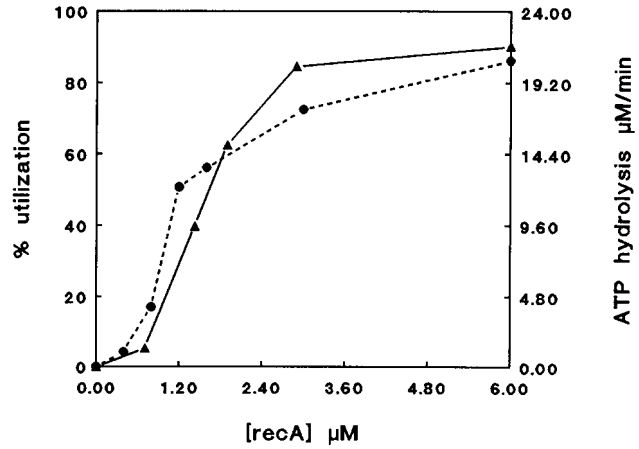

FIG. 7. **Comparison of DNA strand exchange and ATPase activities as a function of recA protein concentrations.** Reaction conditions are as described under "Materials and Methods." The data from the strand exchange experiments are represented by *triangles*. The ATPase data are represented by *circles*.

indicate that the presence of the low NTP affinity form of recA protein (as well as the high NTP affinity form) is necessary for efficient pairing of homologous DNA molecules. It has recently been shown that recA protein can produce joint molecules with large regions of heteroduplex DNA in the absence of ATP hydrolysis (Menetski *et al.*, 1990). Therefore, in Fig. 7, although the ATPase activity and substrate utilization are coincident, the ATP hydrolysis activity is not the important feature and is merely serving to quantitate the amount of low NTP affinity recA protein present.

The Addition of Homologous dsDNA Decreases the ssDNA-dependent ATPase Rate—Schutte and Cox (1987) observed that, when homologous linear dsDNA was added to an ongoing ssDNA-dependent ATPase assay, a 33% reduction in the rate resulted. They showed further that this decrease was completely dependent on the added dsDNA being homologous to the ssDNA and was the result of long paranemic joint molecule formation. If the recA protein hydrolyzing ATP in our joint molecule assay described above (*i.e.* in the presence of added ATPγS) is responsible for homologous pairing of dsDNA with the ssDNA, then this ATPase activity should decrease when homologous dsDNA is added. Linear dsDNA, both homologous M13 and nonhomologous plasmid DNA, was added to an ongoing ATPase assay identical with the conditions used in the joint molecule assay (not shown). A 25% decrease in the ATPase rate is observed when homologous dsDNA is added, suggesting the formation of paranemic joint molecules. This decrease is not observed when nonhomologous dsDNA is added. This result further implicates the low NTP affinity form of recA protein in the pairing process since this is the form which is hydrolyzing ATP in this assay due to the presence of ATPγS.

DISCUSSION

We have shown that a saturated recA protein-ssDNA filament shows nonequivalence with respect to NTP binding affinity. The primary means for detecting this nonequivalence has been the differential inhibition of the ATP hydrolysis activity of recA protein by ATPγS. However, the $S_{0.5}$ for ATP is dependent on the protein:DNA ratio demonstrating that this phenomenon does not require the presence of ATPγS. Nonequivalence is not limited to a particular polynucleotide either, as demonstrated by both the ATPγS concentration-dependent inhibition of poly(dT)- and etheno M13 ssDNA-dependent ATP hydrolysis and the ATP concentration dependence of etheno M13 ssDNA- and native M13 ssDNA-dependent ATP hydrolysis.

Our experiments show that, at saturating concentrations of recA protein (DNA:protein ratios of 3.5:1 and lower), 50% of the DNA-bound recA protein population has a lower apparent affinity for NTP. This population must be located in a unique environment within the recA protein-DNA filament; thus, the structure of the complex must be asymmetric. Since the binding of ssDNA by recA protein increases the affinity for NTP, this asymmetry most likely arises through nonequivalent recA protein-DNA contacts in the fully saturated 3.5:1 complex. Nonequivalence is intrinsic to two of the models proposed (see Fig. 1, *A* and *C*). The other model (see Fig. 1*B*) depicts all of the recA protein protomers in the 3.5:1 complex as being identical with regard to the contacts made with the DNA. Since recA protein has only one ATP-binding site per monomer, this model is unable to account for the NTP affinity asymmetry observed with the 3.5:1 complexes, unless negative cooperativity involving every other monomer is invoked.

The model proposed in Fig. 1*B* (model B) is consistent with stoichiometric data (Bryant *et al.*, 1985; Menetski and Kowalczykowski, 1989; Takahashi *et al.*, 1989; Zlotnick *et al.*, 1990) but it predicts only a 3% difference in the theoretical molecular weights of the complexes at the two ratios. It would seem unlikely that these complexes would be resolved as different species using gel filtration chromatography if the model in Fig. 1*B* is correct. Even if they could be resolved, the relative order of elution that we observe is reversed from what this model predicts. In contrast, the models in Fig. 1, *A* and *C*, predict that the 3.5:1 complex will have an apparent molecular weight almost twice that of the 7:1 complex. The gel filtration experiments provide physical evidence for the existence of discrete species that display relative molecular weights that agree with the models proposed in Fig. 1, *A* and *C*, and consequently support the enzymological experiments. Model B, as we have depicted it, does not predict the behavior observed here. However filament-filament interaction is an aspect we have not detailed in our models and thus makes complete elimination of model B difficult based solely on the physical characteristics that are available from gel filtration chromatography.

A recent paper by Zlotnick *et al.* (1990) offers evidence in support of a recA protein filament that possesses two DNA-binding sites, each monomer binding to 6 nucleotides (3 nucleotides/site), as in our model B (see Fig. 1). Though some of the data they present cannot be easily reconciled with models A and C, as noted previously, model B does not predict subunit nonequivalence unless negative cooperativity is invoked.

The model described in Fig. 1*A* readily accounts for the observations from this paper; however, this model is at variance with the 6-fold screw axis of the recA protein DNA complex derived from image reconstruction studies (Egelman and Stasiak, 1986). We have termed the recA protein molecules that are shown binding directly to the ssDNA in Fig. 1*A* "primary protomers," and the molecules that are not associated directly with the ssDNA are termed "secondary protomers." The primary protomers are proposed to bind NTP with higher affinity than do the secondary protomers, by virtue of their direct contact with the ssDNA. Although the secondary protomers exhibit lower apparent affinity for NTP, they are still able to bind both single- and double-stranded DNA. In addition, this model requires that the second set of bound recA protein (secondary protomers) is activated for ATP hydrolysis in the absence of a direct interaction with DNA. This requirement is not unreasonable given the observation by Pugh and Cox (1988) that recA protein ATPase activity can be stimulated by high salt concentrations in the absence of DNA; we envision a similar form of activation due to assembly of free recA protein onto the ssDNA-bound recA protein.

The model depicted in Fig. 1*C* also offers a way to satisfy the site size discrepancy. In addition, the resulting filament at the 3.5:1 ratio would most likely have (within experimental error) the correct helical pitch and symmetry, as well as the correct number of recA molecules/base pair as determined from electron micrographs. This model requires that a second "cryptic" DNA-binding site is developed upon formation of the saturated complex or, alternatively, the second set of bound recA protein monomers do not fully articulate with the ssDNA if binding to homologous dsDNA molecules is to be accommodated. Asymmetry results from the presumption that both the protein-protein and protein-DNA contacts are not equivalent; there may be some steric considerations that limit the strength of the DNA-protein interaction made by protein binding in the "overlapping" mode. Though this model is distinct from that depicted in Fig. 1*A*, we consider the recA

protein molecules that initially bind to the ssDNA (*top part* of figure) as primary protomers, whereas the second set of bound recA protein represents the secondary protomers.

To discuss model B in the context of the above terminology, it is necessary to define primary and secondary DNA-binding sites. The 3.5:1 complex illustrated in Fig. 1*B* has only the primary DNA site occupied. This occupancy somehow results in asymmetry in the ssDNA-recA protein filament. However, in the 7:1 complex, both the primary and secondary sites are occupied. One must then postulate that the binding of the second DNA strand by the complex results in the loss of experimentally detectable asymmetry.

The models proposed in Fig. 1, *A* and *C*, would require at least two types of recA protein-protein interaction. Recently, Freitag and McEntee (1988) examined recA protein-protein interaction using an affinity chromatography approach. They showed that there are two types of interaction. An end-to-end interaction, hydrophobic in nature, was hypothesized to be important for the development of DNA binding cooperativity, and another lateral interaction, ionic in nature, was proposed for interactions between two filaments of recA protein. This interfilament interaction has been observed in electron micrographs as "bundling" of recA protein-DNA complexes (Egelman and Stasiak, 1986).

There are a significant number of results, and observations from a variety of experimental approaches can either be accommodated by the models presented here or used to further support the concept of a dimeric protomer as the functional unit of recA protein activity. Studies examining the oligomeric state of recA protein have found that the smallest and often most predominant species is a dimer of recA protein (McEntee *et al.*, 1981; Cotterill and Fersht, 1983). Menge and Bryant (1988) have proposed that a recA protein dimer is the smallest functional unit that will satisfy their kinetic observations of ATP-stimulated recA protein GTPase activity. The recA protein transfer reaction, as described by Menetski and Kowalczykowski (1987), is a bimolecular reaction composed of two components, a fast transferring and a slow transferring component. They noted that at a DNA to recA protein molar ratio below 14:1 (*i.e.* DNA in excess), a single kinetic species was present. At all higher ratios, two kinetic species are present, consistent with models A and C. These examples lend support to the idea of a recA protein dimer (minimally) as a functional unit. In the future, any model proposed to describe the behavior of the recA protein-ssDNA complex will need to incorporate both the stoichiometry data as well as the phenomenon of asymmetry.

One of the least well understood aspects of recA protein function is the pairing and alignment of homologous DNA molecules. The models presented offer simple ways to envision the pairing of homologous DNA molecules. The structures themselves may play a role in pairing two DNA molecules, but the phenomenon of asymmetry could also potentially play a role in the homology search step of recA protein-catalyzed joint molecule formation. This suggestion follows from our observation that joint molecule formation and secondary protomer appearance are coincident (Fig. 7). This leads us to conclude that both high and low NTP affinity species are important for efficient joint molecule formation and that the primary protomer, alone, is insufficient for homologous pairing. The asymmetry described here with regard to NTP affinity should also extend to DNA binding affinity, since there is a close thermodynamic link between these two properties, *i.e.* the binding of ssDNA by recA increases the affinity of the protein for ATP and vice versa (Kowalczykowski, 1986; Menetski and Kowalczykowski, 1985). A role for this DNA binding affinity asymmetry might be to provide a means for rapid sampling of homology between two DNA molecules held together in the recA-DNA filament. The reduced affinity of the secondary protomers for DNA may result in more transient DNA binding. Sampling of homology between adjacent molecules could continue until homologous complementary DNA-DNA interactions stabilized the otherwise transitory contacts.

There are a number of enzymes that exhibit asymmetry with respect to substrate binding affinity, *i.e.* the binding of substrate or an effector molecule (ATP and ssDNA, respectively, in this case) reduces the affinity for the substrate at another site(s). This has been referred to as negative cooperativity and "half-the-sites" reactivity. CTP synthase, glyceraldehyde-3-phosphate dehydrogenase, and mitochondrial ATPase are frequently cited examples (Conway and Koshland, 1972; Huang *et al.*, 1982). In the case of the enzymes mentioned above, only a fraction of the total binding sites can ultimately be occupied. For the binding of ATP to recA protein, this is not true since the observed stoichiometry of binding is 1 ATP molecule/recA protein molecule (Cotterill *et al.*, 1982). However, under some conditions with ADP, recA protein behaves much like the above cited enzymes, exhibiting a stoichiometry of 0.3–0.5 ADP molecules/recA protein monomer (Cotterill *et al.*, 1982). In addition, although these enzymes are multisubunit enzymes, the size of the recA protein-ssDNA filaments is much greater (*e.g.* 1000–2000 protein monomers/M13 ssDNA molecule). Thus, whereas recA shares some of the characteristics associated with the "classic" examples of enzyme asymmetry, the concept of asymmetry in such an extensive protein complex does appear to be a novel one.

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Note Added in Proof—Examination of the magnesium ion dependence of asymmetry as manifest by the ATP concentration dependence of ATPase activity shows that, at 1 mM magnesium ion concentration, formation of asymmetric complexes is significantly diminished. This suggests that there is a magnesium ion-dependent association that is essential to the phenomenon of asymmetry.

REFERENCES

Brenner, S. L., Zlotnick, A. & Griffith, J. D. (1988) *J. Mol. Biol.* **20,** 959

Bryant, F. R. & Lehman, I. R. (1985) *Proc. Natl. Acad. Sci. U. S. A.* **82,** 297

Conway, A. & Koshland, D. E., Jr. (1972) *Biochemistry* **11,** 241

Cotterill, S. M. & Fersht, A. R. (1983) *Biochemistry* **22,** 3525

Cotterill, S. M., Satterthwait, A. C. & Fersht, A. R. (1982) *Biochemistry* **21,** 4332

Cox, M. M. & Lehman, I. R. (1982) *J. Biol. Chem.* **257,** 8523

DiCapua, E., Engel, A., Stasiak, A. & Koller, T. (1982) *J. Mol. Biol.* **157,** 87

Egelman, E. H. & Stasiak, A. (1986) *J. Mol. Biol.* **191,** 677

Flory, J., Tsang, S. S. & Muniyappa, K. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81,** 7026

Freitag, N. & McEntee, K. (1988) *J. Biol. Chem.* **263,** 19525

Griffith, J. & Shores, C. G. (1985) *Biochemistry* **24,** 158

Huang, C. Y., Rhee, S. G. & Chock, P. B. (1982) *Annu. Rev. Biochem.* **51,** 935

Kowalczykowski, S. C. (1986) *Biochemistry* **25,** 5872

Kowalczykowski, S. C. & Krupp, R. A. (1987) *J. Mol. Biol.* **193,** 97

McEntee, K., Weinstock, G. M. & Lehman, I. R. (1981) *J. Biol. Chem.* **256,** 8835

Menetski, J. P. & Kowalczykowski, S. C. (1985) *J. Mol. Biol.* **181,** 281

Menetski, J. P. & Kowalczykowski, S. C. (1987) *J. Biol. Chem.* **262,** 2085

Menetski, J. P. & Kowalczykowski, S. C. (1989) *Biochemistry* **28,** 5871
Menetski, J. P., Bear, D. G. & Kowalczykowski, S. C. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 21
Menge, K. L. & Bryant, F. R. (1988) *Biochemistry* **27,** 2635
Messing, J. (1983) *Methods Enzymol.* **101,** 20
Morrical, S. W., Lee, J. W. & Cox, M. M. (1986) *Biochemistry* **25,** 1482
Neet, K. E. (1983) in *Contemporary Enzyme Kinetics and Mechanism* (Purich, D. L., ed) pp. 267–320, Academic Press, New York
Pugh, B. F. & Cox, M. M. (1988) *J. Biol. Chem.* **263,** 76
Register, J. C., III & Griffith, J. (1985) *J. Biol. Chem.* **260,** 12308
Schutte, B. C. & Cox, M. M. (1987) *Biochemistry* **26,** 5616
Segel, I. H. (1975) *Biochemical Calculations,* 2nd Ed, pp. 246–252, John Wiley & Sons, Inc., New York
Shaner, S. L. & Radding, C. M. (1987) *J. Biol. Chem.* **262,** 9211
Shibata, T., DasGupta, C., Cunningham, R. P., Williams, J. G. K., Osber, J. & Radding, C. M. (1981) *J. Biol. Chem.* **256,** 7565
Takahashi, M., Kubista, M. & Norden, B. (1989) *J. Mol. Biol.* **205,** 137
Uhlin, B. E. & Clark, A. J. (1981) *J. Bacteriol.* **148,** 386
Weinstock, G. M., McEntee, K. & Lehman, I. R. (1981) *J. Biol. Chem.* **256,** 8829
West, S. C., Cassuto, E. & Howard-Flanders, P. (1981) *Proc. Natl. Acad. Sci. U. S. A.* **78,** 2100
Zlotnick, A., Mitchell, R. S. & Brenner, S. L. (1990) *J. Biol. Chem.* **265,** 17050