Analysis of Two Distinct Single-stranded DNA Binding Sites on the recA Nucleoprotein Filament*

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The binding stoichiometry of Escherichia coli recA protein to single-stranded DNA (ssDNA) determined by two separate assays differs by a factor of 2.2--2.4. Using the fluorescence of etheno-DNA (€DNA), a chemically modified ssDNA, the stoichiometry was found to be 7.0 ± 0.6 bases/recA protein monomer in a nucleoprotein filament. Using a competition assay, a similar stoichiometry, 7.5 bases/recA, is found for unmodified poly(dT). Using the DNA-dependent ATPase of recA, which monitors bound protein rather than bound DNA, we find that each recA monomer needs to bind only 3.1 ± 0.5 bases to fully activate the ATPase. The difference in site size determined by the two assays indicates that there are two DNA binding sites with differential effects on ATPase activation. When recA protein is mixed with ssDNA at a ratio of 7 bases/recA or greater, the complex that forms contains 7 bases/recA and acts as a kinetic trap for the ssDNA. Upon further addition of recA protein, no additional ATPase activity is observed. If, on the other hand, the ssDNA is initially mixed with excess recA (at a ratio of 3--3.5 bases/recA or less) the ATPase activity is twice as high. Analysis of the binding curves suggests that the first DNA strand binds recA to form a filament with a stoichiometry of 3--3.5 bases/protein monomer. The ATPase activity of recA is completely active in this complex. A second strand of DNA can then be bound to this filament yielding a final stoichiometry of 7 bases/protein monomer. The presence of this second strand neither enhances nor inhibits ATP hydrolysis. This ternary complex may mimic the structures formed by recA in searching for homologous DNA sequences and/or in the strand exchange reaction.

The recA protein is essential for DNA repair and homologous recombination in Escherichia coli. It is one of the most studied recombinases and serves as a paradigm for understanding recombination mechanisms in both prokaryotes and eukaryotes (for reviews, see Cox and Lehman (1987), Griffith and Harris (1988), and Kowalczykowski (1991). Unlike most proteins of DNA replication and site-specific recombination, the active form of recA is oligomeric, composed of a helical protein filament that binds single- or double-stranded DNA. Each recA monomer also binds ATP and has a DNA-dependent ATPase activity. All of the recA monomers bound to DNA appear to be capable of hydrolyzing ATP (Brenner et al., 1987).

Double-stranded DNA binds to recA with a stoichiometry of 3 base pairs/recA monomer as shown by electron microscopy (Di Capua et al., 1982), by filter binding studies in the presence or absence of ATP (Bryant et al., 1985), and by the displacement of the intercalator ethidium bromide from the DNA upon recA binding (Dombroski et al., 1983). Using the dsDNA-dependent ATPase to monitor bound protein also yields a 3-base pair to one protein stoichiometry (Mitchell et al., 1988; Menetski and Kowalczykowski, 1989). The stoichiometry of ssDNA binding to recA is less certain. Filter binding experiments designed to trap recA-ssDNA complexes generally give a site size of 3--4 bases/protein monomer. However, when the filter binding assay is carried out in the presence of ATPγS (a very slowly hydrolyzed analog of ATP), twice the usual stoichiometry is observed (Bryant et al., 1985). In solution studies, the 7-base/recA stoichiometry has also been found in the presence of ATPγS (Takahashi et al., 1989). Using etheno-DNA (€DNA, a chemically modified ssDNA) whose fluorescence changes upon recA binding, this higher stoichiometry is also observed (Menetski and Kowalczykowski, 1985; Mitchell et al., 1988).

Lauder and Kowalczykowski (1991) have proposed three possible explanations for the apparently inconsistent 3--3.5- and 7-base/recA stoichiometries as follows: a recA-ssDNA filament at a 7:1 stoichiometry may form a complex with DNA-free recA, reducing the measured stoichiometry to 3.5; recA may bind a single strand of DNA with either a 3:5:1 or 7:1 stoichiometry; or two strands of DNA may bind to a single filament of recA, each at a stoichiometry of 3--3.5 bases/recA. There is a good deal of circumstantial evidence to support the latter model. Such structures have been advanced previously by others as a possible intermediate in the strand exchange reaction carried out by recA during homologous recombination (Howard-Flanders et al., 1984; Bryant et al., 1985; Mitchell et al., 1988; Takahashi et al., 1989; Müller et al., 1990; and Zlotnick et al., 1990). Intermediates in the strand exchange reaction containing two DNA molecules have been observed in electron micrographs (Register et al., 1987; Register and Griffith, 1988). Biochemical studies of such joint molecules show that the initially bound ssDNA substrate and the homologous strand of the dsDNA molecule are protected from nuclease digestion, while the complementary strand, which is exchanged in the reaction, is susceptible (Chow et al., 1986). These observations indicate that recA filaments can include more than one strand of ssDNA, though the precise stoichiometry of the DNA to protein in the different stages of recA activity is not easy to define.

In this paper, we provide direct evidence that the recA filament has two distinct DNA binding sites, which differentially regulate the DNA-dependent ATPase activity, thereby...
rationalizing the apparent discrepancies in the literature. Alternative models that have been proposed to explain the differences between the ATPase and fluorescence assays (Lauder and Kowalczykowski, 1991) are inconsistent with our observations. We observe that the two sites are nonequivalent and may provide a pathway for formation of important intermediates in the homologous recombination pathway.

EXPERIMENTAL PROCEDURES

Materials—Polynucleotides and ATP were from PL Biochemicals. NADH, phosphoenolpyruvate, and the enzymes of the ATP regenerating system, lactate dehydrogenase and pyruvate kinase, were from Sigma. The enzymes were dialyzed to remove ammonium sulfate and were used without further purification. E. coli single-stranded binding protein (SSB) was purchased from U.S. Biochemical Corp. ATPyS was from Boehringer Mannheim. Chloroacetaldehyde from Pfaltz and Bauer was distilled immediately prior to use; the fractions distilling from 82–88 °C were used for subsequent DNA modification.

DNA—Wild type M13 phage DNA was purified by published procedures (Register et al., 1987) and the concentration measured using an \( A_{280} \) of 8000 \( M^{-1} \ cm^{-1} \) as determined by a phosphate assay (Bartlett, 1959). poly(dT) concentration was determined using an \( A_{260} \) of 8540 \( M^{-1} \ cm^{-1} \) (Ts'o et al., 1966).

We have observed that literature procedures for the synthesis of etheno-modified ssDNA, cDNA, often cause degradation of the ssDNA to fragments less than 200 bases long. To minimize this fragmentation of the DNA, we prepared cDNA by incubating 0.3–0.6 mM M13 DNA, 50 mM NaOAc, pH 5.0, 1 mM EDTA, with one-sixth volume freshly distilled chloroacetaldehyde at 45 °C for 90 min. The reaction was quenched with one-third volume of cold 1.5 mM Tris, pH 8.8, and then concentrated by centrifugation in a Centricon 30 (Amicon). Washing the sample with 20 mM Tris-HCl, pH 7.0, four times in a Centricon 30 was sufficient to remove all of the chloroacetaldehyde. An \( A_{260} \) of 7000 \( M^{-1} \ cm^{-1} \) was determined for cDNA by a phosphate assay (Bartlett, 1959). Typical cDNA spectra prepared by this method were characterized by absorbance ratios of \( A_{260}/A_{280} = 1.3 \), \( A_{260}/A_{280} = 1.2 \), and \( A_{260}/A_{280} = 2.0 \). Samples of cDNA that diverged from this ideal were typically contaminated by chloroacetaldehyde. The integrity of the resultant modified DNA was assessed by electrophoresis on 1% alkaline agarose gels followed by visualization using Kodak Stains-All. Yields were typically greater than 90% and degradation of the DNA minimal.

RecA Preparation and Extinction Coefficient Determination—RecA protein was purified to greater than 95% homogeneity from the GE645 strain of E. coli, the gift of Dr. George Weinstock (University of Texas Medical School at Houston, and M. D. Anderson Cancer Center), by the procedure of Griffith and Shores (1985) as modified in Izawa et al. (1987). An extinction coefficient of \( \epsilon_{280}^{cm} = 6.1 \pm 0.5 \) \((23,200 \ M^{-1} \ cm^{-1}) \) was determined by amino acid analysis. This extinction coefficient is within 5% of the value of 6.33 of Tsang et al. (1985) and 5.9 of Craig and Roberts (1981). Absorptivities were corrected for light scattering by subtracting the light scattering at 320 nm from the absorbance at 280 nm. For low levels of light scattering, where the absorbance at 320 nm is less than 15% of the absorbance at 280 nm, we have found this simple correction to be adequate.

Reaction Conditions—All reactions were carried out at 37 °C in HMK buffer (10 mM HEPES, 12 mM MgCl2, 30 mM KCl, 1 mM dithiothreitol, 50 µg/ml bovine serum albumin) or HMM buffer (10 mM HEPES, 12 mM MgCl2, 30 mM NaCl, 1 mM dithiothreitol) at pH 7.2. Where noted, an ATP regenerating system was included consisting of 1 mM ATP, 2 mM phosphoenolpyruvate, 0.75 mM NADH, and 2.24 units each of pyruvate kinase and lactate dehydrogenase. For experiments comparing binding observed by DNA fluorescence and inferred from the ATP hydrolysis rate, the buffers and reagents used for the two assays are the same except that 0.75 mM NADH was present in reactions assayed for ATP hydrolysis; this component of the ATP regeneration system interferes with fluorescence measurements and can be omitted since fluorescence assays are always conducted under conditions where only a small fraction (<20%) of the ATP is consumed during the assay. The hydrolysis of ATP is assayed either by quantitation of \( 32P \) inorganic phosphate (Pollard and Korn, 1973) or by a spectrophotometric, enzyme-coupled assay (Morrical et al., 1986). Unless noted, each point on a graph represents an individual sample rather than a point in a titration of a single sample.

ATPase activity determined using hydrolysis of [\( \gamma-32P \)]ATP was compared with that determined using the enzyme-linked assay. Results were in quantitative agreement (data not shown), though the \( 32P \) assay shows greater variability, and the enzyme-linked assay has a lag time of up to several minutes (not seen with the \( 32P \) assay) before reaching steady state.

Results

RecA–DNA Binding Isotherms—The interaction of RecA protein with DNA was characterized using signals both from the bound protein (the DNA-dependent ATPase activity of RecA) and from the bound nucleic acid (the observed change in the fluorescence of DNA that occurs upon binding to RecA). As shown in Fig. 1, the binding curves generated by

![Fig. 1](image-url)
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monitoring these two signals are significantly different. When the concentration of recA is increased at a fixed DNA concentration (Fig. 1A), the fluorescence increases at a greater rate than the ATPase. At a recA concentration where the fluorescence has reached 50% of its maximal value, the ATPase is only 25% of its maximum. Conversely, when the recA concentration is fixed and DNA concentration is increased (Fig. 1B), the ATPase activity saturates before the DNA fluorescence; at 50% of maximal ATPase, the fluorescence is only about 25% saturated.

Clearly, a direct analysis of the binding curves using traditional binding analysis is not possible due to the cooperative nature of the binding, but even a more rigorous treatment taking into account cooperativity and overlapping binding sites (McGhee and Von Hippel, 1974) is inappropriate, since the two binding signals give different isotherms. It is, however, possible to deduce a stoichiometry for each assay independently, which depends only on the saturating values of the fluorescence and ATPase and not the shape of the binding curve. These site sizes give insight into the reasons why the assays appear to give irreconcilable results.

The detailed analysis for the ATPase assay is shown in Table I. In the top part of the table, it is shown that when a fixed amount of ssDNA is saturated with recA, the maximum ATPase increases linearly with the fixed ssDNA concentration in the assay at a rate of 9.7 (±2.0) μM ATP hydrolyzed/min/μM DNA. The bottom part of the table shows a similar linear increase in maximum ATPase as a function of the fixed recA concentration in a DNA titration, with a K_a of 28.3 (±5.0) μM ATP hydrolyzed/μM recA. Therefore, at saturation, the number of bases bound per recA is simply 28.3/9.7 or 3.1 (±0.6). This site size of ~3 bases/recA is in agreement with a large body of literature.

As seen in Fig. 1A, the fluorescence saturates before the ATPase when the recA concentration is varied, indicating that less recA is required to saturate the fluorescence of a fixed amount of DNA, i.e. that the apparent site size is larger. This intuitive analysis is confirmed by a more detailed analysis of paired DNA and recA titrations such as those shown in Fig. 1. In these experiments, the site sizes were calculated for each fixed pair of experiments to allow for small differences in the degree of etheno-modification (and thus fluorescence intensity) between different preparations. The results of a number of such paired experiments resulted in an average site size of 7.0 (±0.6) bases/recA.

RecA-poly(dT) Binding—RecA binds poly(dT) with higher affinity than ssDNA (Menetski and Kowalczykowski, 1987). This is demonstrated in Fig. 2A, where, at relatively low concentrations of recA, most of the recA added to a mixture of DNA and poly(dT) is bound by the nonfluorescent poly(dT) (compare open and closed circles). It is possible to take advantage of the difference in affinity to deduce the amount of poly(dT) bound per recA monomer. A rough estimate of the binding site size in these experiments can be obtained by extrapolation of the linear portions of the binding curves. For 4.7 μM ssDNA, without any poly(dT), the initial slope of the binding curves extrapolates to saturation at 0.64 μM, giving a stoichiometry of 7.4 bases/recA, in excellent agreement to the values obtained by analysis of saturation titrations given above. For 4.7 μM ssDNA and 5.0 μM poly(dT), the linear portion extrapolates to 1.37 μM recA, and the stoichiometry is (4.7 + 5.0)/1.37 = 7.1 bases/recA. A difference binding curve (Fig. 2, inset) extrapolates to 0.76 μM recA for 5.0 μM poly(dT), giving a stoichiometry of 6.5 bases/recA monomer, and saturates at 0.65 μM recA, giving a stoichiometry of 5.65 = 7 bases/recA. The stoichiometry of recA for poly(dT) determined via this indirect method is the same as the stoichiometry of recA for ssDNA providing evidence that the larger site size is not an artifact of the use of ssDNA.

DNA Cofactors and the ATPase Activity—In order to demonstrate that ssDNA stimulates the ATPase activity of recA in the same fashion as unmodified ssDNA cofactors, we compared the ATPase activity of recA protein using ssDNA, poly(dT), M13 ssDNA, and M13 ssDNA + E. coli SSB (Fig. 3). The data for ssDNA, poly(dT), and M13 ssDNA with SSB are superimposable. In the absence of SSB, the same amount of M13 ssDNA supports only about 20–25% of the maximal ATPase activity. The SSB protein has been shown to melt out regions of secondary structure from ssDNA (Muniyappa et al., 1984), thus allowing recA protein to saturate the M13 DNA. Etheno-modification of the DNA alters the adenosine and cytosine residues thereby preventing base pairing. This lack of dsDNA character in ssDNA is further demonstrated by the absence of a melting transition observed by hyperchromicity and by the inability of ssDNA to bind ethidium bromide in agarose gels after electrophoresis (data not shown). Under the low salt conditions in this study, the DNA substitutes lacking secondary structure that we tested all have similar binding characteristics and support the same maximal observed ATPase K_a of ~30 ATP recA^{-1} min^{-1}. Thus, both the high site size observed via fluorescence and the lower site size observed by ATPase are not artifacts due to the use of ssDNA.

Another possible source of artifacts would be generated if the ATPase rate was dependent on the recA binding density on the DNA. An analysis of binding curves by the elegant method of Bujalowski and Lohman (1987) (not shown) indicates that the ATPase rate is in fact independent of binding density on the ssDNA.

Titrations—Each of the points on the binding isotherms discussed above was obtained by monitoring the ATPase and/or fluorescence of a single sample, rather than by multiple additions of recA or DNA to a single reaction as in a normal titration. We adopted this procedure after we observed inconsistent values for the V_m of the ATPase reaction when titrating poly(dT) with recA. A direct comparison of the single sample/point isotherm and a titration is shown in Fig. 4. In

**TABLE I**

*Site size from ATPase experiments*

| [dDNA] V_{max} | K_{a} |
|----------------|------|
| μM | ATP min^{-1} | ATP min^{-1} base^{-1} |
| 1.0 | 9.4 ± 1.3 | 9.4 ± 1.3 |
| 1.6 | 17.7 | 11.1 |
| 2.5 | 25.0 | 10.0 |
| 2.6 | 26.0 ± 5.2 | 10 ± 2.0 |
| 3.2 | 33 | 10.3 |
| 5.0 | 49.5 | 9.9 |
| average 9.7 ± 2.0 (n = 11) | |

| [recA] V_{max} | K_{a} |
|----------------|------|
| μM | ATP min^{-1} | ATP min^{-1} protein^{-1} |
| 0.5 | 13.0 ± 3.5 | 26.8 ± 7.0 |
| 0.6 | 16.9 | 28.2 |
| 1.0 | 28.6 ± 2.4 | 28.6 ± 2.4 |
| 2.0 | 58.8 | 29.4 |
| 3.0 | 113.7 | 37.9 |
| average 28.3 ± 5.0 (n = 11) | |
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**Fig. 2.** Competition of poly(dT) and ssDNA for recA. recA binding isotherms at fixed concentrations of 4.7 μM ssDNA (○) or 4.7 μM cDNA and 5.0 μM poly(dT) (○). Each point represents data for an individual reaction performed in HMN buffer with 1 mM ATP. Inset, a difference binding isotherm calculated from the data in A. Δ[recA] = [recA]F + d(T) - [recA]F. Where [recA]F is the concentration of recA required to produce a given change in the cDNA fluorescence in the presence or absence of poly(dT).

**Fig. 3.** The maximal velocity of the DNA-dependent ATPase is independent of the DNA composition. The ATPase of mixtures of 2.5 μM DNA, either poly(dT) (△), cDNA (○), M13 ssDNA with SSB protein (●), or M13 ssDNA (●), with recA in HMK buffer with an ATP-regenerating system. In the sample with SSB protein, 0.125 μM SSB protein is added to M13 DNA after the addition of the recA protein to give a 20-base/SSB stoichiometry.

In this experiment, the concentration of ssDNA (here poly(dT)) is fixed at 3 μM. Based on the analysis above, we expect that this is sufficient DNA to saturate the ATPase of approximately 1 μM recA. We observe that, at low concentrations of recA, below ~1 μM, the titration versus single sample/point curves coincide, but when recA is titrated into samples at higher concentrations, there is no additional increase in ATPase. The final ATPase seen in a titration is about 50% that seen when the isotherm is obtained by the use of individual samples for each point rather than by titration. From this experiment, it appears that 3 μM poly(dT) can activate the ATPase of only about 50% of the recA when the sample is prepared by titration; this implies a site size twice as large, ~6-7 bases/recA. The latter value is consistent with the site size determined by fluorescence, whether fluorescence binding curves are measured using individual samples for each point or by titration (data not shown). No difference is observed between the ATPase curves generated by titrating a fixed amount of recA with poly(dT) whether the experiments are done via direct titration or by the preparation of individual samples for each point.
samples for each DNA concentration. The model we propose (see "Discussion" and Fig. 6) accounts for the two different site sizes and reconciles these apparently contradictory observations.

DISCUSSION

We have investigated recA binding to dDNA by the DNA fluorescence and by the DNA-dependent ATPase activity of the recA protein itself. The difference in the shape of ssDNA binding curves for these two assays is the fundamental observation described in this paper. We have shown that the ATPase activity of recA protein is maximal when recA binds to 3.1 ± 0.6 DNA bases, determined from the ATPase rate of saturated DNA and saturated recA. Conversely, the site size determined for recA by dDNA fluorescence is 7.0 ± 0.6, approximately twice the size determined from the ATPase data. Finally, the recA filament can kinetically trap two ssDNA molecules at a stoichiometry of 7 bases/recA moner (3–3.5 from each strand), although the second strand neither enhances nor inhibits the ATPase of the nucleoprotein filament, which is fully activated when the first strand of ssDNA binds.

There have been two limiting models discussed in the literature to explain the discrepancy between the ATPase and fluorescence experiments (Lauder and Kowalczykowski, 1991). In one model, each recA monomer can bind 6–7 bases, but a filament so assembled is capable of binding a second layer of recA monomers and activating their ATPase without any further DNA binding. In the second model, a single recA protein filament containing a single strand of DNA is capable of binding a second strand of DNA with no effect on the ATPase of the nucleoprotein complex. The experiment in Fig. 4 clearly shows that the second model is correct; recA nucleoprotein filaments can contain two molecules of ssDNA (Howard-Flanders et al., 1984; Bryant et al., 1985; Mitchell et al., 1988; Takahashi et al., 1989; Zlotnick et al., 1990). The recA ATPase is fully activated by one strand of DNA; the binding of a second strand of DNA does not allosterically effect the rate of ATP hydrolysis. We have verified that carefully prepared, intact dDNA is a good analog of natural ssDNA for recA in terms of activating the ATPase, binding, and site size, and that both dDNA fluorescence and ATPase activity correlate directly with binding.

These observations suggest a direct dissection of binding to each site (Fig. 5). To produce this figure, the fluorescence and ATPase binding curves are normalized to two and one DNA site filled, respectively. A third isotherm is generated by subtraction. We find that the first DNA site is nearly filled before any DNA binds to the second site. The formation of the nucleoprotein complex appears to be driven by recA binding the first DNA strand. Previously, we found that the second DNA strand has a greater off rate from the recA filament than the first strand (Zlotnick et al., 1990), and, in Fig. 5, we observe that the second strand also has a lower affinity for the recA-DNA complex. This analysis of DNA binding by recA emphasizes the difference between the two sites. The high affinity binding of the first strand has a role in the assembly of the nucleoprotein filament and activates the DNA-dependent ATPase. The second strand, which must bind assembled recA-DNA filaments, has a lower affinity for recA and does not affect ATPase activity. We speculate that formation of a ternary complex of recA with two DNA molecules may have a role in searching for DNA homology to the initially bound strand, stabilizing parameiotic joints and/or the strand exchange reaction. Although we have relied on ssDNA substrates, one or both of these DNA binding sites may be suitable for binding dsDNA (Pugh and Cox, 1987).

The affinity of recA for ssDNA is dependent on base content with poly(dT) > (dDNA > poly(dA)). When poly(dT) is bound, the nucleoprotein complex does not readily disso-
icate (see Zlotnick et al. (1990)). When dDNA is used, however, the nucleoprotein complexes appear to readily rearrange themselves (see Laudier and Kowalczykowski (1991)). Thus, when recA is added in small amounts to poly(dT) (Fig. 6), a complex containing two strands of ssDNA is formed (stoichiometry of 7 bases/recA). When additional recA is added, the complex does not dissociate, and the second poly(dT) stand remains kinetically trapped in the complex. Alternatively, when recA is mixed with the poly(dT) directly, complexes are formed containing only a single strand of ssDNA; therefore, the same amount of poly(dT) binds up to twice as much recA as seen in Fig. 6.

The addition of aliquots of poly(dT) to a constant concentration of recA produces isotherms identical to those obtained by single mix experiments for each concentration of ssDNA. At saturation, the $K_{sat}$ is $\sim$30 ATP recA$^{-1}$ min$^{-1}$. This result is expected, since the first DNA site, which activates the ATPase, fills first. Since the two DNA binding sites are equivalent by fluorescence, the fluorescence monitored in either a titration of DNA with recA, or a titration of recA with DNA is identical to binding curves generated from individual reactions. Our observations indicate that when excess recA is present, addition of a second strand of ssDNA to a preformed nucleoprotein complex containing one strand of ssDNA is less favorable than the formation of a second such filament (Fig. 5).

In an elegant series of electron microscopy image reconstructions (Egelman and Yu, 1989), the location of the DNA in recA nucleoprotein filaments has been identified at the core of the recA helix, parallel to the axis. The DNA appears to have equivalent contact with each protein monomer. As the equivalent position of each monomer is occupied by one DNA polymer, our data suggest there must be a second DNA binding site on recA protein. Similar conclusions were reached by Müller et al. (1990) based on electron microscopy and electrophoretic experiments performed in the presence of ATP$_2$. Two tentative DNA binding sites have been identified in the crystal structure of recA (Story et al., 1992). The identified sequences of recA are consistent with genetic information and the electron microscopy model. Unfortunately, there is no DNA bound to the crystallized recA, and the loops that form much of the binding site are disordered, leaving some question as to whether they form two DNA binding sites or are simply two parts of a single binding site.

The biochemical evidence for two discrete types of bound DNA in the recA-DNA filament provides some underpinnings for the mechanism of formation of triple-stranded DNA as an intermediate in the strand exchange (Rao et al., 1991). The role of DNA in establishing two classes of recA ATPase activity, as determined by sensitivity to ATP$_2$S inhibition, remains unexplained (Laudier and Kowalczykowski, 1991).

Neither electron microscopy nor x-ray crystal structures have yet revealed the detailed molecular contacts between recA protein in oligomeric filaments and the multiple strands of polynucleotides that must be bound during the strand exchange reaction. Additional structural or mutational analyses should help define the critical residues for binding of the two phosphodiester backbones that position the DNA strands for subsequent recombination events.