Translocation of Escherichia coli recA Protein from a Single-stranded Tail to Contiguous Duplex DNA*

Sandra L. Shaner and Charles M. Radding

From the Departments of Human Genetics and Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut 06510

Duplex DNA with a contiguous single-stranded tail was nearly as effective as single-stranded DNA in acting as a cofactor for the ATPase activity of recA protein at neutral pH and concentrations of MgCl₂ that support homologous pairing. The ATP hydrolysis reached a steady state rate that was proportional to the length of the duplex DNA attached to a short 5′ single-stranded tail after a lag. Separation of the single-stranded tail from most of the duplex portion of the molecule by restriction enzyme cleavage led to a gradual decline in ATP hydrolysis. Measurement of the rate of hydrolysis as a function of DNA concentration for both tailed duplex DNA and single-stranded DNA cofactors indicated that the binding site size of recA protein on a duplex DNA lattice, about 4 base pairs, is similar to that on a single-stranded DNA lattice, about four nucleotides. The length of the lag phase preceding steady state hydrolysis depended on the DNA concentration, length of the duplex region, and the polarity of the single-stranded tail, but was comparatively independent of tail length for tails over 70 nucleotides in length. The lag was 5–10 times longer for 3′ than for 5′ single-stranded tailed duplex DNA molecules, whereas the steady state rates of hydrolysis were lower. These observations show that, after nucleation of a recA protein complex on the single-stranded tail, the protein samples the entire duplex region via an interaction that is labile and not strongly polarized.

The binding affinity of recA protein for both single-stranded and double-stranded DNA (McEntee et al., 1981; Cotterill et al., 1982; Menetski and Kowalczykowski, 1985; Weinstock et al., 1981a, 1981b) and its affinity for other molecules of recA protein (Kuramitsu et al., 1981; Flory and Radding, 1982; Cotterill and Fersht, 1983; Morrical and Cox, 1985) are modulated by the binding of mononucleotide substrates. Binding of mononucleotides appears to induce allosteric transitions in the protein as indicated by the disruption of crystals of recA protein during attempts to diffuse ATP into crystals grown in the absence of nucleotide (McKay et al., 1980) and by the difference in the repeat distance of the nucleoprotein complex in the presence or absence of bound nucleotide (Flory and Radding, 1982; Flory et al., 1984; Stasiak and Egelman, 1986). The modulation of these binding affinities of recA protein for DNA through the operation of the ATP hydrolysis cycle is apparently essential for strand exchange (Cox and Lehman, 1981; Kahn and Radding, 1984), whereas the preceding steps in the homologous pairing reaction, conjunction, and homologous alignment of DNA molecules, require only the binding of ATP (McEntee et al., 1979; Cox and Lehman, 1981; Riddles and Lehman, 1985; Hongberg et al., 1985).

In addition, in the presence of ATP at neutral pH, the affinity of recA protein for purely duplex DNA is enhanced by previous binding of single-stranded DNA to the protein. Observations on homologous pairing promoted by recA protein first showed that one of the two DNA substrates must be single-stranded or partially single-stranded (Shibata et al., 1979; McEntee et al., 1979; DasGupta et al., 1980; Cassuto et al., 1980; Cunningham et al., 1980). When recA protein binds to single-stranded DNA, it forms a helical nucleoprotein filament (Koller et al., 1983; Flory et al., 1984; Williams and Spengler, 1986) with many weak binding sites for duplex DNA (Tsang et al., 1985a). Subsequent addition of free duplex DNA to these polyvalent filaments creates large networks that are instrumental in concentrating the DNA and rapidly sampling many sequences (Chow and Radding, 1985; Tsang et al., 1985a; Gonda and Radding, 1986).

A small single-stranded region on an otherwise duplex DNA molecule also affects the interaction of the recA protein with the double-stranded portion of the molecule. At neutral pH and concentrations of MgCl₂ that support strand exchange, purely duplex DNA is a poor cofactor for the ATPase activity of recA protein (Shibata et al., 1981; Weinstock et al., 1981a, 1981b). At a concentration of MgCl₂ where strand exchange cannot occur, double-stranded DNA with short contiguous regions of single-stranded DNA supported a rate of ATP hydrolysis much greater than expected based on the amount of single-stranded DNA present (West et al., 1980). Furthermore, when introduced to superhelical DNA by either low MgCl₂ concentrations or transient joint molecule formation, recA protein unwound the DNA extensively (Ohtani et al., 1982; Shibata et al., 1982; Iwabuchi et al., 1983). Bianchi et al. (1985) observed a limited helicase (strand-separating) activity of recA protein requiring single-stranded sites adjacent to the duplex region, a requirement common to other helicases (Geider and Hoffmann-Berling, 1981). Cassuto et al. (1980) observed evidence of a low level of homologous pairing far downstream from single-stranded sites that provided access for recA protein.

All of the observations cited above indicate that recA protein gains access to duplex DNA via single-stranded regions. To get more information about that process and the possible role played by recA protein while bound to duplex DNA, we

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have studied the ATPase activity in the presence of duplex molecules with single-stranded tails.

**EXPERIMENTAL PROCEDURES**

**Materials—**Escherichia coli recA protein was purified as previously described (Shibata et al., 1981) and stored at 4 °C in 50 mM Tris-HCl (pH 7.5 at 22 °C), 1 mM dithiothreitol, 0.3 mM EDTA, 10% (v/v) glycerol. The concentration of recA protein in stock solutions was determined by absorbance at 260 nm using the extinction coefficient of 8.5 × 10^13 M^-1 cm^-1 (Tsang et al., 1985b).

Circular single-stranded and closed circular duplex DNA from phage M13 were prepared as described (Cunningham et al., 1980). All DNA solutions were stored at 4 °C in 10 mM Tris-HCl (pH 8.0, 22 °C), 1 mM EDTA. Concentrations of single-stranded DNA were determined by absorbance at 260 nm using an extinction coefficient of 66.5 × 10^13 M^-1 cm^-1. Concentrations of double-stranded DNA were also determined by absorbance at 260 nm. An extinction coefficient of 66.5 × 10^13 M^-1 cm^-1 was used.

Restriction enzymes and exonuclease III were purchased from New England Biolabs. λ-Exonuclease was purchased either from New England Biolabs or Bethesda Research Laboratories, or was the gift of K. Muniyappa (Yale University). S1 nuclease was purchased from Sigma. Pancreatic DNase I was from Boehringer Mannheim and proteinase K was from EM Reagents. All radiocinuclease were from Amersham Corp.

**Preparation of Duplex DNA with Single-Stranded Ends—**Supercoiled duplex M13 DNA was linearized with HpaI or Hinfl following the procedure recommended by the supplier. Digestion of the linear duplex M13 DNA with exonuclease III to form 5' single-stranded ends was performed in 10 mM Tris-HCl (pH 7.5), 2 mM MgCl₂, 120 mM NaCl at temperatures ranging from 18 to 26 °C (Wu et al., 1976; Guo and Wu, 1982). Digestion of the linear duplex M13 DNA with λ-exonuclease to form 3' single-stranded ends was performed in 5 mM Tris-HCl (pH 7.5), 1 mM MgCl₂, 60 mM NaCl at 18–20 °C. A similar procedure was followed with both enzymes to ensure that all linear duplexes were tailed and to minimize the heterogeneity of the tail lengths. The linear duplex DNA was first titrated with the exonuclease to determine the number of units needed to ensure that all ends were digested. These reactions were run for 20–40 min. The correct amount of enzyme was judged to be that which produced a digest in which all the input linear duplex was quantitatively retained by nitrocellulose filters under conditions specific for the retention of duplex DNA. A nuclease, the amount of released nucleotide was determined by quan-

**Digestion of ATP by recA Protein**—Digestion of ATP by recA protein with M13 viral single-stranded DNA as cofactor was monitored as described previously (Kim et al., 1981; Shibata et al., 1981). Ten-minute time courses were usually adequate to determine accurately the steady state rate of ATP hydrolysis from the slope of the linear region of the time course. For duplex M13 DNA with 3' single-stranded ends, time courses of 30–60 min were performed to determine the steady state rates of hydrolysis. The slope of the linear region of all time courses was determined by linear least squares regression after exclusion of time points during the lag phase. The lag times were then calculated using the least squares equation determined in the regression analysis. To avoid possible variations in assay activity due to aging of the recA protein preparations, we collected all data shown in any given figure or table over a short period of time using the same preparation of recA protein. We have found that, over a 60-min time course, duplex DNA cut with restriction enzymes producing a four-nucleotide overhang is as poor a cofactor as DNA cut with restriction enzymes producing flush ends. We have therefore referred to all DNA as blunt ended if it has not been treated with λ-exonuclease or exonuclease III.

**RESULTS**

**recA Protein Can Use Linear Duplex DNA with Single-Stranded Tails as a Cofactor in ATP Hydrolysis—**The hydrolysis of ATP by recA protein with M13 viral single-stranded DNA as cofactor was monitored as described above with linear DNA to that observed with linear duplex M13 DNA with two single-stranded 5' tails, 135 nucleotide residues in length (Fig. 1 and Table I). When the 5' tailed duplex DNA was used as cofactor, the time course of ATP hydrolysis displayed a lag of about 1 min prior to achieving the steady state rate (Fig. 1B). By contrast, there was no discernable lag when single-stranded DNA was the cofactor (Fig. 1A), in agreement with previous studies (Weinstock et al., 1981, 1981b; Tsang et al., 1985b).

The hydrolysis activity of recA protein when tailed duplex was used as the DNA cofactor was not attributable solely to protein bound to the single-stranded regions. The measured rate of ATP hydrolysis far exceeded that expected for a concentration of single-stranded DNA equal to the concentration of nucleotide residues contributed by the single-stranded tails. As seen in Fig. 1, comparable rates of ATP hydrolysis were obtained for single-stranded DNA and tailed duplex DNA with a single-stranded nucleotide concentration of only 0.9 μM. For
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FIG. 1. Comparison of ATP hydrolysis at 37°C by recA protein as a function of time when the DNA cofactor was (A) circular single-stranded M13 DNA or (B) linear duplex M13 DNA with 5' single-stranded ends 135 nucleotides long. Reaction components were 10 mM MOPS, 14 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 2% glycerol, 0.5% ethanol, 2.0 μM recA protein, and 1.5 mM ATP. In A, the concentrations of single-stranded nucleotides were 0.2 μM (•), 0.5 μM (△), 0.9 μM (○), or 5.0 μM (□). In B, the concentrations of single-stranded nucleotides were 0.9 μM (○), 0.5 μM (△), or 0.2 μM (□).

TABLE I
Comparison of parameters for ATP hydrolysis for single-stranded and linear duplex M13 DNA

<table>
<thead>
<tr>
<th>[DNA]</th>
<th>Rate [μM ADP produced/min]</th>
<th>Lag [min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Single-stranded residues] [Molecule]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single-stranded M13 DNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>0.031</td>
<td>1.0</td>
</tr>
<tr>
<td>0.5</td>
<td>0.078</td>
<td>2.2</td>
</tr>
<tr>
<td>0.9</td>
<td>0.14</td>
<td>3.7</td>
</tr>
<tr>
<td>5.0</td>
<td>0.78</td>
<td>19.6</td>
</tr>
<tr>
<td>20.0</td>
<td>3.1</td>
<td>61.3</td>
</tr>
<tr>
<td>50.0</td>
<td>7.8</td>
<td>69.4</td>
</tr>
<tr>
<td>Linear duplex M13 DNA with 5' single-stranded tails</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.11</td>
<td>0.41</td>
<td>10.5</td>
</tr>
<tr>
<td>0.14</td>
<td>0.50</td>
<td>13.2</td>
</tr>
<tr>
<td>0.20</td>
<td>0.75</td>
<td>25.5</td>
</tr>
<tr>
<td>0.50</td>
<td>1.86</td>
<td>49.4</td>
</tr>
<tr>
<td>0.90</td>
<td>3.33</td>
<td>64.9</td>
</tr>
</tbody>
</table>

FIG. 2. A, the steady state rate of ATP hydrolysis versus length of the DNA, when the cofactors were linear duplex M13 DNA molecules of varying duplex length with a 5' single-stranded end 220 nucleotides long. Standard hydrolysis assay conditions were used: 0.50 nM (molecule) DNA cofactor, 2.0 μM recA protein, 1.5 mM ATP, 10 mM MOPS, 14 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 2% glycerol, 0.5% ethanol, 37°C. Points shown are the average of two to six determinations, with the exception of the points at 1270 and 2529 nucleotides, which were not duplicated. Error bars represent the standard deviation. B, the lag preceding the steady state rate versus the total length of the molecule.
blunt-ended φX174 DNA to the tailed AcoI fragment did not enhance the rate of hydrolysis (1.6 μM ADP/min) above that shown by the tailed duplex alone (data not shown).

Since the single-stranded DNA must be attached to duplex DNA in order for the rate of ATP hydrolysis to be proportional to the length of the duplex DNA, we examined the stability of the association of recA protein with the duplex part of the molecule. We used the 5827-bp AcoI fragment of M13 duplex DNA with a 5' single-stranded tail 220 nucleotides long as cofactor. After 5 min, we added SnaBI to cleave the DNA, forming a 4557-bp fragment with blunt ends and a 1270-bp fragment with one 220 residue single-stranded tail. Ten minutes after addition of the restriction enzyme, the rate of ATP hydrolysis fell to a level that was much below the uncleaved control and that was much closer to the rate supported by DNA that was cleaved before the reaction was started (Fig. 3). Complete cleavage of the DNA by SnaBI under these conditions within 10 min after its addition was confirmed by gel electrophoresis (data not shown).

The direct relation between the steady state level of ATP hydrolysis and the length of the duplex portion of molecules with a single-stranded tail demonstrates that recA protein samples the entire length of the duplex region of DNA. The inhibition of the steady state rate of hydrolysis by restriction cleavage of the duplex DNA during the reaction shows both that the SnaBI site was accessible, although recA protein sampled the whole molecule, and that the attachment of recA protein to the duplex portion was transitory. These conclusions are further supported by experiments described in the accompanying paper (Shaner et al., 1987b).

Duplex DNA molecules with single-stranded tails are capable of binding recA protein and interacting with homologous duplexes to form joint molecules that have the classical Holliday structure and that undergo reciprocal strand exchange (DasGupta et al., 1981; West et al., 1981). To test whether the lag and the observed rates of ATP hydrolysis might arise from the formation of joint molecules, we examined the effects of two cations known to affect this process. Variations from the standard conditions included reducing the [MgCl₂] used to 2 mM and adding 2 mM spermidine. Triplicate measurements were made at the standard condition. The mean value and standard deviations are reported for both parameters. For the latter two conditions, duplicate experiments were performed with each DNA.

![Fig. 3. ATP hydrolysis as a function of time at standard hydrolysis conditions (see Fig. 2) for the following forms of DNA cofactor. ○, the 5827-bp AcoI fragment of M13 DNA with one 5' single-stranded tail 220 nucleotides long; ○, the same singly tailed AcoI fragment cleaved with SnaBI prior to the hydrolysis reaction to form a tailed 1270-bp fragment and a blunt-ended 4557-bp fragment; ○, the same singly tailed 5827 AcoI fragment to which SnaBI was added after the steady state rate of hydrolysis had been reached; ○, the blunt-ended 5827-bp AcoI fragment cut with SnaBI prior to the hydrolysis reaction to form two blunt-ended fragments 1270 or 4557 bp in length.](image)
explained in terms of a change in available numbers of single-stranded residues with ionic strength-dependent changes in the DNA secondary structure (Tsang et al., 1985b). This effect should not be observed with a cofactor which is mostly duplex. From data at 2 mM MgCl$_2$ for the tailed duplex M13 DNA, we estimated the binding site size to be 3.1 ± 0.7 bp per bound protein (Fig. 4). From four titrations with tailed duplex DNA at 10 mM MgCl$_2$ (Fig. 4), we found a mean site size of 3.8 ± 0.8 bp per bound protein. This value is reasonably consistent with most other estimates in the literature (Volodin et al., 1982; Dombroski et al., 1983; DiCapua et al., 1982). Within the precision of our measurements, the binding site size on duplex DNA does not change with MgCl$_2$ concentration and is similar to that on single-stranded DNA.

The Rates of ATP Hydrolysis Are Smaller and the Lags Are Longer for 3’ Single-stranded Tailed Duplexes than for 5’ Tailed Duplexes—The foregoing experiments involved only duplex molecules with 5’ single-stranded tails. However, duplex DNA with 3’ single-stranded tails also supported ATP hydrolysis by recA protein. Time courses of ATP hydrolysis for reactions in which the DNA cofactor was either a 3’ or 5’ tailed duplex M13 are compared in Fig. 5A. Although the lag for the 3’ tailed duplex was about 5 times longer than that observed for a molecule with a 5’ tail of comparable length, the steady state rate rose to over half of the cooperative value (8.4 versus 15 μM ADP/min). These findings suggest that the association of recA protein with the duplex part of a partially single-stranded molecule can extend bidirectionally from the single-stranded region, a conclusion supported by other data (Cassuto and Howard-Flanders, 1986; Shaner et al., 1987a). The Length of the Single-stranded Tail Has Little Effect—The binding of recA protein to single-stranded DNA has been shown to have moderate cooperativity (Menetski and Kowalczykowski, 1985). We examined the effect of the length of the single-stranded tail on the ATP hydrolysis reactions in order to test the importance of these cooperative interactions to the formation of interactions with the duplex regions of these DNA molecules.

The lag measured during hydrolysis reactions with both 3’ and 5’ tailed duplexes showed little dependence on the length of the single-stranded tail for tails more than 70 nucleotides in length (Fig. 5B). The lag was at least 5-fold greater for DNA molecules with 3’ tails than for 5’ tails throughout the range of tail lengths studied. This effect will be discussed further below. With a 6770-bp fragment isolated from one of the ends of λ DNA with a 12-nucleotide 5’ tail, the reaction failed to reach a steady state rate of hydrolysis during a 30-min time course (not shown). This suggests that a single-stranded region between 12 and 70 nucleotides is required for efficient use of the duplex DNA in the hydrolysis reaction, but that little enhancement of the ability of the protein to interact with the duplex is gained by lengthening the tail further. This observation agrees with an earlier one that showed that a single-stranded region of 30–40 nucleotides permitted ATP hydrolysis with an otherwise duplex cofactor (West et al., 1980).

**DISCUSSION**

**Nucleation, Translocation, and Dissociation of Binding of recA Protein to Tailed Duplex DNA**—DNA molecules with a stretch of single-stranded DNA contiguous to a region of duplex DNA functioned well as a cofactor for ATP hydrolysis by recA protein under conditions at which homologous pairing and strand exchange occur. The reaction displayed an initial lag phase followed by a steady state rate of ATP hydrolysis that was proportional to the length of the duplex DNA. This direct proportionality between the steady state rate of hydrolysis and the length of contiguous duplex DNA when the concentration of single-stranded ends was held constant showed that the reaction is supported by tailed molecules. Results from interaction of recA protein with the entire molecule, rather than from stalled growth of the recA protein complex at the junction between single-stranded and duplex DNA.

The proportionality between the steady state level of ATP hydrolysis and DNA length implies that hydrolysis did not occur only at the ends of a single linear polymer of recA protein on DNA. Thus, translocation of protein from single-stranded tails to duplex DNA either does not involve treading-milling (Wegner, 1976) or does not involve a single continuous filament. Observations described in Shaner et al. (1987a) and the following paper (Shaner et al., 1987b) show that the recA filament is indeed discontinuous.

The interaction of recA protein with the duplex region required the presence of adjacent single-stranded DNA. Negligible ATP hydrolysis was observed with purely duplex DNA under these conditions. With tailed duplex DNA, the rate of hydrolysis of ATP decayed when the tail was detached by
nuclease action, indicating that recA protein dissociated from the duplex portion and was unable to rebind in the absence of a single-stranded tail. Thus, the steady state hydrolysis of ATP with tailed duplex DNA clearly reflects a cycle of nucleation of binding of recA protein in the single-stranded region, translocation or polymerization into the duplex, and dissociation.

**Mechanism of the Hydrolysis Reaction**—On the basis of our observations and consideration of the structure of tailed duplex DNA, we would propose the following mechanism for the ATP hydrolysis reaction:

\[
\text{nA} + \text{tailed duplex} \rightarrow I \rightarrow I_1 \rightarrow H.
\]

The first step involves the initial binding equilibrium of recA protein (A) with the tailed duplex to form a complex with only the single-stranded tail (I). In the second step, complex formation between recA protein and the DNA extends beyond the single-stranded tail into the region involved in complementary base pairing (I1). After formation of this complex, an isomerization occurs resulting in formation of a hydrolyzing complex (H) within the duplex region.

The need for a single-stranded tail to obtain significant amounts of hydrolysis under these conditions provides experimental evidence that the intermediate I1 is necessary for the pathway to the final hydrolyzing complex. The data in the accompanying paper (Shamer et al., 1987b) show that the large increase in the lag observed when the polarity of the single-stranded tail is changed from 5' to 3' (Fig. 5B) occurs because extension of recA protein complex formation into the duplex region (I1) can be rate-limiting.

At 10 mM MgCl2 and a constant concentration of recA protein, the lag decreased with increasing concentrations of DNA molecules (Table I). According to the mechanism proposed, an increase in the concentration of DNA molecules of fixed duplex length might decrease the lag by increasing the number of single-stranded nucleation sites and thus increasing the concentration of I1 or I2.

The postulated isomerization step enables one to rationalize the increase in the lag with increasing length of duplex DNA up to about 2500 bp (Fig. 2B). If complex formation throughout the duplex region were rate-limiting, then the lag would be expected to increase with increasing length of the duplex. Since the lag did not increase for lengths above 2500 bp, we interpret this to imply a need to form a protein complex of a critical length with the duplex next to the single-stranded region before ATP hydrolysis can proceed readily. This critical length may be similar to the size of nascent heteroduplex joints, 200-600 bp (Wu et al., 1982; Bianchi et al., 1983; Kahn and Radding, 1984; Christiansen and Griffith, 1986). Duplex length is known to affect the kinetics of helix denaturation (Record and Zimm, 1972; Cantor and Schimmel, 1980). Also, although enhancement of helix stability with increasing duplex length occurred below 3000 bp, none was observed for DNA between 3000 and 1.5 x 10^6 bp in length (Crothers et al., 1965). If the protein forces a change in the local twist of the DNA within this complex, increasing the total length of duplex might not increase the difficulty of unwinding the DNA within this complex for total lengths longer than 2500 bp. For binding of the E. coli RNA polymerase at promoters, topological unwinding of the duplex was observed in the earliest detectable intermediate (Buc and McClure, 1985; Spassky et al., 1985), but formation of a transcribing complex required a major protein conformational change (Roe et al., 1985) before actual strand separation was observed (Spassky et al., 1985). The data presented here and by other labs (Kowalczykowski, 1985) do not identify whether the major barrier to formation of the catalytically active recA protein complex is the helix denaturation or the protein conformational change inducing that denaturation. For instance, the absence of a lag when single-stranded M13 DNA is used as cofactor may be due to the ability of recA protein to hydrate ATP without the need to denature regions of secondary structure, but could also be due to the ability of the protein to undergo any necessary conformational changes more readily as a result of the greater ease of extending and compressing a single-stranded DNA backbone.

**Models for the Interaction with Tailed Duplex DNA**—The binding of ATP (Kowalczykowski, 1986) in the catalytic site of recA protein increases the binding affinity for single-stranded DNA and decreases the affinity for double-stranded DNA, whereas binding of ADP to recA protein decreases the affinity for single-stranded DNA and increases the affinity for duplex DNA (Cotterill et al., 1982). These observations form the basis for two alternative hypotheses, a translocation model and a polymerization model, to rationalize interaction between recA protein and tailed duplex DNA. A cluster of cooperatively interacting protein molecules is apparently required for ATP hydrolysis (Ogawa et al., 1978; Weinstock et al., 1981b; Kowalczykowski, 1986), but in the translocation model diagrammed in Fig. 6, we have pictured only one protein molecule for simplicity. Initially, the cluster of recA protein molecules is bound to the single-stranded region immediately adjacent to a duplex region. In the absence of any bound nucleotide in the catalytic site, this recA protein complex displays a helical pitch of about 6.5 nm and a DNA axial rise per residue of 0.2 nm (Koller et al., 1983; Flory et al., 1984; Stasiak and Egelman, 1986; Williams and Spengler, 1986). Upon binding ATP, the recA protein complex undergoes a conformational change that is characterized by a helical pitch of 9.5 nm and extends the DNA backbone to an axial repeat of about 0.4 nm/nucleotide (Koller et al., 1983; Flory et al., 1984; Williams and Spengler, 1986; Stasiak and Egelman, 1986). ATP is then hydrolyzed. With ADP now in the catalytic site, the recA protein locally dissociates from the single-stranded site and preferentially binds the duplex DNA ahead of it, with a simultaneous conformational change. This change, inferred from Cotterill et al. (1982), may encourage dissociation of ADP from the catalytic site. After dissociation of the ADP, the protein complex again assumes the 6.5-nm helical pitch conformation which contracts the DNA backbone. The compression of the DNA backbone to optimize protein-DNA contacts within this complex may be a source of backbone strain in duplex regions. Alleviation of this strain could be related to the limited helicase activity of recA protein (Bianchi et al., 1985) and the mechanism of strand exchange. This less extended configuration may be characteristic of the single-stranded binding mode of the protein in which ATP can rebind, with subsequent increase in the helical pitch. The protein is now primed for another cycle.

Yet another possible model for how a stable recA protein complex on the single-stranded DNA might result in binding to the duplex is one which need not include translocation of individual monomers. The complex might permit polymerization of recA protein monomers with bound ATP on the filament beyond the duplex junction in the 0.4 nm/nucleotide conformation. Individual molecules or clusters of molecules bound to the duplex might be induced to hydrolyze the ATP as a result of the enhanced affinity of recA protein for duplex when ADP is bound to the catalytic site. The cyclic extension and recontraction of the conformation experienced by the
polymerizes unidirectionally on single-stranded DNA with an apparent 5' to 3' polarity in the presence of ATP. If the movement of the recA protein complex from a single-stranded tail into contiguous duplex DNA were also strictly unidirectional, then one would expect that either the 5' or 3' single-stranded tail, but not both, would permit recA protein to use the interior duplex region as a cofactor in ATP hydrolysis. The substantial rates of ATP hydrolysis observed with either tailed duplex DNA suggested that extension of the complex formation into the duplex by translocation or polymerization can occur with either a 5' to 3' or a 3' to 5' polarity from the single-stranded end. Data in the accompanying paper (Shaner et al., 1987b) confirm this conclusion. The significantly longer lag times displayed by reactions with 3' tailed duplexes indicate that extension in the 5' to 3' direction is kinetically favored under these conditions. The preferred direction of extension is the same as that identified by Register and Griffith (1985) for unidirectional elongation of the recA protein polymer binding to single-stranded DNA. Their analysis would not have differentiated unidirectional growth from bidirectional growth with unequal rates of elongation at each end. It is also possible that only the interaction with double-stranded DNA is bidirectional.

In addition, the smaller rates of hydrolysis measured with the 3' tailed duplexes indicate that less recA protein was bound at steady state in these reactions in the kinetically less favored direction. This latter observation seems in agreement with other data which showed that, in the presence of ATP·P·γ·S, equal inhibition of site-specific cleavage of 3' or 5' tailed duplex DNA molecules by a restriction endonuclease required a 4-fold higher input concentration of recA protein in reactions with a 3' tailed duplex than with a 5' tailed duplex (Cassuto and Howard-Flanders, 1986).

Estimate of Efficiency of ATP Hydrolysis and the Size of the recA Oligomeric Unit—We have used our ATP hydrolysis data to estimate the maximal efficiency of ATP hydrolysis per recA protein monomer per base pair of strand exchange. We assume that the observed hydrolysis reflects localized strand separation of the duplex cofactor and that the rate of this process is identical to the rate of strand separation which accompanies strand exchange. The maximal rate of ATP hydrolysis with an excess of 5' tailed duplex DNA as cofactor corresponds to hydrolysis of one ATP molecule by each recA protein monomer about every 2 s (Table I) if all input recA protein molecules are assumed to be bound and hydrolyzing. The rate of strand exchange under similar reaction conditions, in the absence of single-strand binding protein, is 1 to 2 bp/s (Kahn and Radding, 1984). If these assumptions hold, then the hydrolysis of one ATP molecule by any individual monomer of recA protein would correspond to a 2-4-bp movement of the heteroduplex joint during strand exchange. Ronan and Kowalczykowski (1986) have calculated a similar value by relating the amount of product formed during strand exchange to the amount of ATP hydrolyzed in an analogous reaction with only duplex DNA as cofactor.

This normalized estimate of the efficiency of ATP hydrolysis per base pair of heteroduplex formed can be used to estimate the minimal size of the oligomeric recA protein structure involved in moving the branch point when combined with measurements of the overall number of ATP molecules hydrolyzed per base pair exchanged. One difficulty in performing this calculation is that the ATP hydrolysis measured with the typical strand exchange reaction system, a circular single-stranded DNA pairing with homologous linear duplex DNA (DasGupta et al., 1980; Cox et al., 1983), is a composite of ATP hydrolysis directly involved in movement of the hetero-

FIG. 6. Model for translocation of recA protein from single-stranded DNA into adjacent duplex DNA. For simplicity in this schematic representation, we show only one molecule in a cluster of recA protein molecules bound to the DNA. In the first step, recA protein is bound to the 4 single-stranded nucleotide residues immediately adjacent to the region of duplex DNA. The catalytic site is empty and therefore the complex is in the conformation possessing a 6.5-nm helical pitch and an axial rise per residue of about 0.2 nm (Flory et al., 1984; Stasiak and Egelman, 1986; Williams and Spengler, 1986). In the second step, ATP binds to the catalytic site, inducing a conformational change in the protein which results in a different orientation between individual monomers and displays a helical pitch of 9.5 nm (0.4 nm/residue) (Flory et al., 1984; Stasiak and Egelman, 1986; Williams and Spengler, 1986). After hydrolysis, ADP is bound within the catalytic site which produces local dissociation of the recA protein from the single-stranded region and permits the protein to move into the duplex region. No data are available on the structure of the complex formed between DNA and recA protein with bound ADP, so we have arbitrarily shown no change in the orientation of the complex. We have shown the recA protein translocating 2 bp on the basis of the speculative calculation given under "Discussion." Upon dissociation of the ADP in the final step shown, the protein again assumes the 6.5-nm helical pitch conformation. The constricted axial rise per residue required of the DNA for formation of optimal protein-DNA contacts within this complex may be a source of backbone strain in the duplex region and may be related to the limited helicase activity of recA protein (Bianchi et al., 1985) and the mechanism of strand exchange.

A cluster of monomers may permit propagation of polymerization far from the single-stranded tail. Movement of recA protein binding into the duplex region by treadmilling of the cluster might proceed by a variant of this basic polymerization scheme.

Polarity of recA Protein Interaction with Single- and Double-stranded DNA—Under various conditions, recA protein polymerizes by itself or on DNA (Flory and Radding, 1982; Cetterill and Fersht, 1983; Morrical and Cox, 1985; Koller et al., 1983; Dunn et al., 1982). Register and Griffith (1985) inferred from electron microscopic studies that recA protein
odsilon sheet and ATP hydrolyzed by recA protein on any single-stranded DNA sites not involved in pairing. Reaction conditions which minimize or eliminate the binding of recA protein to single-stranded DNA after formation of a stable heteroduplex will minimize gratuitous ATP hydrolysis of the latter type. The presence of single-strand binding protein in a reaction in which every step is performed at 37 °C in 12 mM MgCl₂ competes with recA protein for binding sites on the single-stranded DNA (Tsang et al., 1985b), especially for binding sites on the displaced strand (Soltis and Lehman, 1983). Binding of ADP to recA protein also decreases the binding affinity to single-stranded DNA (Menetski and Kowalczykowski, 1985), but does not as readily inhibit the ability of recA protein to continue to perform strand exchange on stable joint molecules (Wu et al., 1982; Cox et al., 1983; Kahn and Radding, 1984). Therefore, after joint molecule formation at 37 °C at 12 mM MgCl₂ in the presence of appropriate amounts of single-strand binding protein and ADP, little recA protein should be bound to single-stranded DNA and therefore little ATP hydrolysis unrelated to movement of the heteroduplex should occur. A value of 16 ATP molecules hydrolyzed per base pair formed has been measured under such experimental conditions (Cox et al., 1983). If the difference between this number and our estimate of one ATP hydrolyzed to move one recA protein monomer 2-4 bp reflects such experimental conditions (Cox et al., 1986).

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