In an effort to clarify the requirement for ATP in the recA protein-promoted renaturation of complementary DNA strands, we have analyzed the mutant recA1 protein which lacks single-stranded DNA-dependent ATPase activity at pH 7.5. Like the wild type, the recA1 protein binds to single-stranded DNA with a stoichiometry of one monomer per approximately four nucleotides. However, unlike the wild type, the mutant protein is dissociated from single-stranded DNA in the presence of ATP or ADP. The ATP analogue adenosine 5'-O-3'(thiotriphosphate) appears to stabilize the binding of recA1 protein to single-stranded DNA but does not elicit the stoichiometry of 1 monomer/8 nucleotides or the formation of highly condensed protein-DNA networks that are characteristic of the wild type recA protein in the presence of this analogue. The recA1 protein does not catalyze DNA renaturation in the presence of ATP, consistent with the dissociation of recA1 protein from single-stranded DNA under these conditions. However, it does promote a pattern of Mg"+-dependent renaturation identical to that found for wild type recA protein.

The renaturation of complementary DNA strands catalyzed by the recA protein of *Escherichia coli* is stimulated by ATP (1). However, under certain conditions, renaturation can proceed efficiently in the absence of a nucleotide cofactor (2). In an effort to clarify the role of ATP in the recA protein-catalyzed renaturation of DNA, we have compared the wild type recA protein with the mutant recA1 protein that lacks a ssDNA-dependent ATPase (3). We have found that the recA1 protein does not catalyze renaturation in the presence of ATP. However, it does promote Mg"+-dependent renaturation in a manner identical to that of the wild type recA protein. Thus, only ATP-dependent DNA renaturation is defective in the recA1 mutant.

**EXPERIMENTAL PROCEDURES**

**Materials**

RecA protein was purified to homogeneity as described (4). RecA1 protein was generously provided by Dr. Steve West (Yale University). 32P- or 35S-labeled DNA, calf thymus DNA, and phosphocreatine were from Sigma. DNasel and snake venom phosphodiesterase were from Worthington. ATP-S and creatine phosphokinase were from Boehringer Mannheim. GF/C filters were from Whatman and nitrocellulose filters (HAWP, 0.45-μm pore diameter) were from Millipore. [3P]ATP, [γ-32P]ATP, and [γ-32P]ATP-S were from Amersham. Unlabeled and [3H]-labeled circular αX ssDNA (+ strand) and [3H]-labeled linear duplex αX DNA were prepared as described (5). [3H]-labeled linear duplex αX DNA was denatured as described (2).

**Methods**

**Nuclease Protection Assay**—Nuclease protection assays were carried out as described previously (6). Reaction mixtures (500 μl) contained 25 mM Tris-HCl (pH 7.5), 10 mM MgCl2, 30 μM [3H]-αX ssDNA, 5% glycerol, 1 mM recA or recA1 protein, and the indicated nucleotide cofactors. When an ATP regeneration system was included, it consisted of 8 mM phosphocreatine and 40 μg/ml creatine phosphokinase. After a 5-min incubation at 25°C, DNAsel (10 μg) and venom phosphodiesterase (10 μg) were added, and the amount of [3H]-αX ssDNA remaining resistant to nuclease digestion was determined as previously described (6).

**Nitrocellulose Filter-binding Assay**—All nitrocellulose filter-binding assays were carried out in solutions containing 25 mM Tris-HCl (pH 7.5), 10 mM MgCl2, 5% glycerol, and the indicated amounts of αX ssDNA, nucleotide cofactor, and recA or recA1 protein unless otherwise indicated. Following incubation, the reaction mixtures were filtered on KOH-treated nitrocellulose filters, washed with 1 ml of reaction buffer, dried, and then assayed for the appropriate radioactivity. All procedures were carried out at 25°C.

RecA Protein Transfer Kinetics—In the standard transfer reaction, recA or recA1 protein (1 μM) was preincubated with 30 μM [3H]-αX ssDNA in 25 mM Tris-HCl (pH 7.5), 10 mM MgCl2, and 1 mM dithiothreitol for 5 min at 25°C. The preincubation solution was then mixed with an equal volume of challenge solution containing 25 mM Tris-HCl (pH 7.5), 10 mM MgCl2, 1 mM dithiothreitol, and 150 μg/ml of heat-denatured calf thymus DNA; the mixture was kept at 25°C. At various times, aliquots (75 μl) were removed and assayed by nitrocellulose filter binding as described above. Alternatively, aliquots (75 μl) were quenched with ATP-S (500 μM) and assayed by nuclease digestion as described previously (6). In the nitrocellulose filter assay, zero time points were measured by mixing 37.5 μl of the preincubation solution with 37.5 μl of reaction buffer, followed by filtering. Zero time points for the nuclease protection assay were measured as previously described (6).

**DNA Renaturation Assay**—Renaturation assays were carried out as described (2). Reaction mixtures contained 25 mM Tris-HCl (pH 7.5), 5% glycerol, [3H]-labeled denatured PstI cleaved αX DNA, MgCl2, nucleotides, and recA or recA1 protein at the concentrations given in the figure legends.

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‡The abbreviations used are: ssDNA, single-stranded DNA; αX, bacteriophage αX174; ATP-S, adenosine 5'-O-3'-thiotriphosphate.

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with 1 ml of acid-washed Norit (30 mg/ml in 0.1 N HCl), vortexed, and then centrifuged. The supernatants (1.0 ml) were mixed with 6 ml of aqueous scintillation-counting solution and then assayed for \(^{32}P\). Zero time points were measured by adding 100 \(\mu\)l of the preincubation solution. The supernatants (1.0 ml) were mixed with 6 ml of aqueous scintillation-counting solution and then assayed for \(^{32}P\). The 

**RESULTS**

**Binding of recA and recA1 Proteins to ssDNA: Nuclease Protection**—We have described a nuclease digestion protection method to quantitate the binding of recA monomers to ssDNA (6). The stoichiometries of protection obtained using this assay are summarized in Table I. In the absence of nucleotide, each recA monomer protected approximately four nucleotides of ssDNA from digestion. The same stoichiometry was found for complexes formed in the presence of ATP plus an ATP regeneration system. In the presence of ATP without the ATP regeneration system or in the presence of ADP, all of the DNA remained susceptible to nuclease digestion, presumably because of the weaker binding of recA protein to ssDNA under these conditions. The stoichiometry of protection remained at 1 recA monomer/4.0 nucleotides rather than increasing 2-fold as it did in the case of the wild type recA protein.

**Binding of recA and recA1 Proteins to ssDNA: Nitrocellulose Filter Binding**—The binding of recA and recA1 proteins to ssDNA was also examined by nitrocellulose filter binding. A fixed concentration of \([\text{3H}]\)ssDNA (30 \(\mu\)m) was incubated with increasing concentrations of recA or recA1 protein and the amount of ssDNA retained on nitrocellulose filters was measured. As shown in Fig. 1, the recA and recA1 proteins retained ssDNA with a similar efficiency. For the recA protein, approximately 1 recA monomer/30 nucleotides of ssDNA was required to retain >80% of the ssDNA molecules, corresponding to approximately 200 recA monomers for every ssDNA molecule. Since, in principle, the binding of a single recA monomer to each ssDNA molecule should be sufficient for retention, the value of 200 probably reflects a cooperative mode of binding in which subsaturating levels of recA protein bind to ssDNA as clusters of contiguous monomers. The binding of recA1 protein to ssDNA showed a similar behavior.

The nitrocellulose filter-binding assay was used to evaluate the effects of various nucleotides on the binding of recA and recA1 proteins to ssDNA. [\(\text{3H}\)]ssDNA (30 \(\mu\)m) was incubated with sufficient concentrations of recA or recA1 protein (1 \(\mu\)m) to give approximately 80% retention on nitrocellulose filters as judged by the titration curves above. Various nucleotides were then added to the mixture, and the efficiency of retention of the [\(\text{3H}\)]ssDNA on a nitrocellulose filter was measured (Table II). No ssDNA was retained by either recA or recA1 protein in the presence of MgCl\(_2\). In the presence of 10 mM MgCl\(_2\), both recA and recA1 protein gave the expected retention. Treatment of the recA-ssDNA complex with ATP, ADP, or ATPyS resulted in no loss of efficiency of retention. In contrast, when the recA-ssDNA complex was treated with either ATP or ADP, an almost complete loss of binding was observed; ATPyS did not decrease the filter binding efficiency. These results are consistent with the nuclease protection results in the previous section and indicate that ATP and ADP promote the rapid dissociation of recA1 protein from ssDNA. Interestingly, dTTP caused a complete loss of binding for both the recA and recA1 proteins.

**Transfer of recA and recA1 Proteins between ssDNA Molecules**—The nuclease protection assay can be used to measure the transfer of recA protein from one ssDNA molecule to another and to determine the effects of various nucleotides on this process (6). In a typical experiment (Fig. 2), recA protein (1 \(\mu\)m) was incubated with [\(\text{3H}\)]ssDNA (30 \(\mu\)m) to form a recA-ssDNA complex. The transfer reaction was initiated by the addition of unlabeled challenge DNA (15 eq) to the preformed complexes. The reactions were then quenched after various times intervals by the addition of ATPyS which prevents dissociation of the recA-ssDNA complex. The complexes were then subjected to nuclease digestion. As shown in Fig. 2, there was a first order decay in the amount of [\(\text{3H}\)]ssDNA protected from nuclease digestion as a function of time after the initiation of transfer, reflecting equilibration of recA protein between the labeled and unlabeled DNA populations. The \(t_{1/2}\) for equilibration using 15 eq of challenge DNA was approximately 5 min. A detailed study of recA protein transfer using this assay is found in Ref. 6.

The nuclease protection assay could not be used to measure...
the transfer of recA1 protein because of the relative instability of the recA1 - ssDNA-ATPγS complex.2 Under certain conditions it is, however, possible to measure recA protein transfer kinetics by nitrocellulose filter binding. A fixed concentration of wild type recA protein (1 μM) was incubated with [3H]φX ssDNA (30 μM) to form a recA - ssDNA complex. As determined above, this level of recA protein is sufficient to cause retention of approximately 80% of the [3H]ssDNA molecules. Transfer was initiated by the addition of unlabeled challenge DNA (15 eq) to the preformed complexes. At various times, aliquots were removed and filtered on nitrocellulose. As shown in Fig. 2, there was a first order decay in the retention of the [3H] φX ssDNA as a function of time after the initiation of transfer. The time course of recA protein transfer obtained by the nitrocellulose filter-binding method was very similar to that obtained by nuclease protection and gave a t_{1/2} for equilibrium of approximately 5 min.

The nitrocellulose filter-binding assay was also used to measure the transfer of recA1 protein. As shown in Fig. 3, the t_{1/2} for equilibrium of recA1 protein with 15 eq of challenge DNA was approximately 1 min, about five times faster than for the wild type protein. Inclusion of ATPγS in the reaction mixture substantially reduced the rate of recA1 protein transfer (t_{1/2} > 30 min). ATPγS has a similar effect on the rate of transfer of the wild type recA protein (6).

Formation of recA1 -ssDNA-ATPγS Complexes—The observation that ATPγS caused a substantial reduction in the rate of recA1 protein transfer suggests that ATPγS does interact with the recA1 protein, increasing its affinity for ssDNA. However, recA1 - ssDNA-ATPγS complexes are different from recA - ssDNA-ATPγS complexes in several ways. First, as described above, ATPγS did not cause an increase in protection stoichiometry of ssDNA binding to the recA

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**TABLE II**

Retention of φX ssDNA on nitrocellulose filters by recA and recA1 proteins

<table>
<thead>
<tr>
<th>Condition</th>
<th>DNA retained</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>recA</td>
</tr>
<tr>
<td>No nucleotide</td>
<td>100%</td>
</tr>
<tr>
<td>ATP</td>
<td>106</td>
</tr>
<tr>
<td>ADP</td>
<td>87</td>
</tr>
<tr>
<td>ATPγS</td>
<td>100</td>
</tr>
<tr>
<td>dTTP</td>
<td>2</td>
</tr>
<tr>
<td>EDTA</td>
<td>1</td>
</tr>
<tr>
<td>ATPγS + EDTA</td>
<td>100</td>
</tr>
<tr>
<td>MgCl2</td>
<td>1</td>
</tr>
</tbody>
</table>

* The reaction mixture (+ATPγS) was incubated for 5 min in the absence of EDTA. EDTA was then added to a concentration of 0.5 mM, and the incubation was continued for 5 min more before filtering.

protein. Second, electron microscopic studies showed that the addition of ATPγS to φX ssDNA (30 μM) complexed with subsaturating levels of recA1 protein (1 μM) did not produce the highly condensed networks of protein and DNA, as it did in the case of the wild type recA protein.2 Third, recA1 - ssDNA-ATPγS complexes were stable to the addition of EDTA whereas recA1 ssDNA-ATPγS complexes were completely disrupted, as judged by nitrocellulose filter binding (Table II).

In order to clarify the effect of ATPγS on the recA1 protein, we examined directly the binding of ATPγS to recA1 ssDNA and recA1 ssDNA complexes. φX ssDNA (30 μM) was incubated with either recA or recA1 protein (1 μM) to form the desired protein-DNA complexes. [35S]ATPγS (200 μM) was then added to the preformed complexes. The mixtures were filtered through nitrocellulose and the 35S retained on the filter measured. 1.0 (±0.05) molecule of [35S]ATPγS was

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1 F. R. Bryant and I. R. Lehman, unpublished observations.
bound for every recA monomer in the recA-ssDNA complex. A similar experiment with recA1 protein showed that approximately 0.8 molecule of [35S]ATPyS was retained per monomer, indicating that the recA1 protein also binds a single equivalent of ATPγS. However, the value for recA protein decreased with successive washings of the filter, suggesting that the recA1-ssDNA-ATPγS complexes are subject to dissociation. In contrast, the recA-ssDNA-ATPγS complexes were stable to multiple washings. Neither the recA nor recA1 protein retained [35S]ATPyS on the nitrocellulose filters in the absence of ssDNA.

Kinetics of ATP Hydrolysis—Under standard conditions (6 μM recA protein and 50 μM φX ssDNA), recA protein catalyzed ATP hydrolysis with a steady-state rate of 5 min⁻¹. Under the same conditions, but with recA1 protein, there was no detectable hydrolysis of ATP (Fig. 4). Although it is not hydrolyzed, ATP does cause the dissociation of recA1 protein from ssDNA. Since ADP also causes the dissociation of recA1 protein from ssDNA, it was conceivable that recA1 protein catalyzed the hydrolysis of a single molecule of ATP to ADP, as a result it dissociated from ssDNA and was somehow inactivated with respect to further ATPase activity. The hydrolysis of a single ATP molecule per recA1 monomer would not be readily detected using the standard TLC-based ATPase assay. Therefore, a more sensitive assay was employed. Reactions were carried out at 50 μM φX ssDNA and 6 μM recA protein using [γ-32P]ATP as substrate. At various times, aliquots were quenched with 0.5 N HCl and analyzed for [32P]P, using the Norit absorption assay.

The pre-steady-state time course for ATP hydrolysis by the wild type recA protein is shown in Fig. 5. Following a lag of approximately 4 s, recA protein catalyzed the hydrolysis of ATP at a rate of 4.8 s⁻¹ in excellent agreement with the steady-state rate of 5.0 s⁻¹ obtained using the TLC assay. The lag in the start of ATP hydrolysis was observed under a variety of conditions: (i) when recA protein was added last, (ii) when [γ-32P]ATP was added last, and (iii) when [γ-32P]ATP was added to an ongoing reaction containing recA1 protein, ssDNA, and unlabeled ATP. The mechanistic significance of this kinetic lag is under investigation.

The same measurements were made for reactions containing recA1 protein. As shown in Fig. 5, the recA1 protein exhibited no ATPase activity, even when measured at a sensitivity sufficient to easily detect the hydrolysis of a single equivalent of ATP per recA1 monomer.

The possibility still remained that recA1 protein interacts with ATP to form a covalent phosphoryl-enzyme intermediate that is unable to break down properly, resulting in a modified protein that is inactive with respect to further ATPase activity. If such an intermediate were stable to acid treatment, this event may not have been detected by the Norit assay which detects only 32P not absorbed to the charcoal. To test for this possibility, ATPase reactions were carried out as described above. After 50 s, the reactions were mixed with trichloroacetic acid to precipitate the protein. The quenched reactions were then filtered on GF/C filters which were then assayed for [32P]P. No radioactivity above background (minus protein) was found for either the recA or recA1 proteins (data not shown). Thus, the dissociative effect of ATP on the recA1 protein does not appear to be due to the turnover of a single equivalent of ATP.

Renaturation of Complementary Single Strands—The separated (+) and (−) strands of PstI-cleaved [3H]φX DNA serve as substrates for the recA protein-promoted renaturation of complementary DNA strands. A typical time course carried out at the optimal ratio (2) of one recA monomer (1 μM) per 30 nucleotides of φX ssDNA (30 μM) is shown in Fig. 6. There was no renaturation during the time course of the experiment in the absence of recA protein. In the presence of recA protein and ATP, rapid renaturation occurred with approximately 75% of the ssDNA becoming S1 nuclease-resistant within 10 min. In the absence of ATP, a burst of renaturation was observed (approximately 30%) followed by a slow second phase (2 > 5 min). When the MgCl₂ concentration was increased from 10 to 30 mM, the ATP-independent reaction proceeded at the same rate as the ATP-stimulated reaction.

The renaturation activity of the recA1 protein was determined under the same conditions used for the wild type protein (Fig. 6). In 10 mM MgCl₂, the recA1 protein promoted an ATP-independent renaturation reaction identical to that obtained with recA protein. Furthermore, increasing the MgCl₂ concentration from 10 to 30 mM resulted in a stimulation of the ATP-independent renaturation reaction exactly as observed with the wild type protein. However, when the MgCl₂ concentration was kept at 10 mM and ATP (500 μM);
Fig. 6. Renaturation of alkali-denatured [³H]DNA by recA and recA1 proteins. Reactions were carried out as described under "Experimental Procedures." Reaction solutions contained 30 μM denatured [³H]DNA, 1 μM recA protein (left) or 1 μM recA1 protein (right), and the indicated concentrations of MgCl₂ and ATP.

was added, the renaturation activity of the recA1 protein was completely eliminated. This finding is consistent with dissociation of recA1 protein from ssDNA in the presence of ATP (ADP also eliminated the renaturation activity of recA1 protein). Thus the recA1 mutation clearly separates the ATP-dependent and ATP-independent pathways by which the recA protein catalyzes the renaturation of complementary DNA strands.

DISCUSSION

RecA1 mutants of E. coli are defective in all known functions of the recA gene (8). The recA1 mutation has been identified as a missense mutation in which a glycine at position 160 is replaced by an aspartic acid residue (9). The purified recA1 protein does not function as a ssDNA-dependent ATPase and is unable to promote DNA strand exchange (3, 10). Nevertheless, it is able to catalyze the ATP-independent renaturation of complementary DNA strands.

The recA1 protein binds ssDNA with a stoichiometry of 1 monomer/3.5 nucleotides, a value that is very close to 1 monomer/4 nucleotides found for the wild-type protein using nuclease protection (6) as well as other methods (3, 11). The binding of both recA and recA1 proteins to ssDNA appears to be cooperative based on nitrocellulose filter-binding measurements. These results support earlier reports that recA1 protein forms a complex with ssDNA in the absence of ATP (3, 12).

The affinity of the recA and recA1 proteins for ssDNA were compared by analyzing the kinetics of protein transfer between ssDNA populations. In control reactions with the recA protein, transfer was measured by both nuclease protection and nitrocellulose filter binding. Under conditions of excess challenge DNA, the two assays gave similar time courses of equilibrium with a t₁/₂ of about 5 min in the absence of nucleotide. Agreement between the two assays was not necessarily expected. The nuclease protection assay measures total recA protein bound to the initial ³H-strand; a transfer of 50% of the recA monomers results directly in 50% decrease in nuclease protection. In the nitrocellulose filter-binding assay, however, a transfer of 50% of the recA monomers from the initial ³H-strand could conceivably leave each ³H-labeled DNA molecule with half its recA protein; full nitrocellulose filter binding might still occur and the transfer would go undetected. The finding that the nuclease protection and the filter binding assays do give the same time course suggests that transfer of recA protein is cooperative with many recA monomers (clusters) transferring from one DNA molecule to another in a single event. In this case, at 50% transfer, 50% of the ³H-labeled DNA molecules would have lost all of their recA protein, resulting in a 50% decrease in nuclease protection and a corresponding 50% decrease in filter binding.

Cooperative transfer of the recA protein may proceed by the interaction of a recA-ssDNA complex with a second ssDNA molecule, resulting in the formation of a transiently two-stranded intermediate. This intermediate could then undergo a "switching" event leading to the transfer of the recA cluster from the initial ssDNA molecule to the second ssDNA molecule. Alternatively, transfer may occur by the cooperative dissociation of a cluster of recA monomers followed by the cooperative rebinding of the monomers to the second strand.

We were unable to use the nuclease protection assay to measure the transfer of recA1 protein because of the inability of ATPγS to function as an effective quench for the transfer process. However, transfer of recA1 protein could be measured by filter binding. The half-time for equilibration using 15 eq of challenge DNA was approximately 1 min, about 5-fold faster than that found for the wild type recA protein, indicating that the mutant protein may have a reduced affinity for ssDNA.

Although the recA and recA1 proteins interact similarly with ssDNA in the absence of nucleotide, they exhibit very different properties in the presence of nucleotide cofactors. ATP causes an increase in the rate of transfer of wild type recA protein as a result of its hydrolysis to ADP and Pᵢ (6). Despite the increase in rate of transfer, nuclease protection experiments indicate that most of the recA protein is still bound to ssDNA at any given time; even in the presence of ATP (and an ATP regeneration system), the protection stoichiometry remains at 1 recA monomer/4 nucleotides. As noted above, the transfer may occur by a cooperative two-strand switching mechanism that does not necessarily require dissociation of recA protein.

We showed previously that ATPγS causes a 2-fold increase in the stoichiometry of nuclease protection by the recA protein when ssDNA is in excess relative to recA protein. We also
found that addition of ATPγS to ssDNA complexed with subsaturating levels of recA protein results in the formation of highly condensed networks of DNA and protein. One interpretation of these results is that ATPγS traps recA protein in a conformation in which it is tightly bound to two strands of ssDNA (6). Formation of such networks and the increase in binding stoichiometry necessarily require an excess of ssDNA over recA protein. When recA protein is in excess over ssDNA, and ATPγS is added, the stoichiometry remains at 1 recA monomer/4 nucleotides. In this case there would only be enough ssDNA to occupy a single binding site.

RecA1 protein also interacts with ATPγS by the following two criteria: (i) direct binding of recA1-ssDNA-ATPγS complexes to nitrocellulose filters and (ii) reduction in the rate of protein in a conformation in which it is tightly bound to two strands of ssDNA. Despite this apparent interaction, ATPγS does not cause the 2-fold increase in the stoichiometry of nuclease protection by the recA1 protein, nor does it produce the highly condensed recA1-ssDNA networks typical of wild type recA protein. Thus, the doubled stoichiometry and the network formation found for wild type recA protein may be related phenomena.

The pattern of renaturation catalyzed by the recA1 and recA proteins is consistent with these proposals. The wild type recA protein catalyzes renaturation in the presence of 10 mM MgCl2, even in the absence of ATP. We previously suggested that this reaction proceeds via the formation of aggregates of recA-ssDNA complexes which serve to bring DNA sequences into a higher effective concentration, thereby increasing the rate of renaturation (2). Such aggregates may form via the interaction of recA-ssDNA complexes with a second strand of ssDNA as was suggested above for the recA transfer reaction. ATP independent renaturation in the presence of 10 mM Mg2+ shows a burst of renaturation (approximately 30% of maximum in 1 min) followed by a very slow second phase. The slow phase may be due to the initial formation of a fraction of nonproductive aggregates in which the DNA strands are not in position to renature. ATP may stimulate renaturation by stimulating the movement of recA protein to new strands, thereby facilitating the formation of new productive aggregates. Alternatively ATP may stimulate renaturation by facilitating the dissociation of recA protein from ssDNA, thereby allowing the zipperping of helices to occur unimpeded.

The recA1 protein shows the same pattern of interaction with ssDNA in the absence of ATP as the wild type protein, and it also shows a similar pattern of ATP-independent renaturation. The recA1 protein-promoted renaturation reactions are thus also accommodated by a model in which recA1-ssDNA complexes interact with a second strand of ssDNA to form renaturation intermediates. Unlike the wild type protein, inclusion of ATP causes dissociation of recA protein from ssDNA, thereby preventing aggregation and eliminating renaturation activity.

It has recently been reported that the incubation of recA protein with ssDNA results in the formation of protein-DNA aggregates that are large enough to be sedimented out of solution by low speed centrifugation (13). These aggregates may correspond to the intermediates in recA-promoted renaturation proposed above. These intermediates occur only with subsaturating levels of recA protein, consistent with the proposal that they form via ssDNA-recA-ssDNA interactions and not ssDNA-recA-recA-ssDNA interactions. It has also been reported that the recA1 protein is incapable of forming aggregates with ssDNA (12). This observation was made in the presence of ATP, which we show here causes the dissociation of recA1 protein from ssDNA and the elimination of renaturation activity. Since recA1 protein does promote the normal pattern of ATP-independent renaturation, we tested the ability of recA1 protein to promote the aggregation of ssDNA in the absence of ATP. Our results show that recA1 protein does indeed cause aggregation of ssDNA in absence of ATP under the same conditions that it promotes renaturation. In agreement with the reported results (12), no aggregation by recA1 protein was found in the presence of ATP. Thus, the patterns of recA and recA1 protein-induced aggregation of ssDNA are consistent with the patterns of recA- and recA1-promoted renaturation.

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