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## II. Co-dominant Effects of RecA142 Protein on Wild-type RecA Protein Function

**Stephen C. Kowalczykowski and Renee A. Krupp**

*Department of Molecular Biology*
*Northwestern University Medical School*
*Chicago, IL 60611, U.S.A.*

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In the accompanying paper, RecA142 protein was found to be completely defective in DNA heteroduplex formation. Here, we show that RecA142 protein not only is defective in this activity but also is inhibitory for certain activities of wild-type RecA protein. Under appropriate conditions, RecA142 protein substantially inhibits the DNA strand exchange reaction catalyzed by wild-type RecA protein; at equimolar concentrations of each protein, formation of full-length gapped duplex DNA product molecules is less than 7% of the amount produced by wild-type protein alone. Inhibition by RecA142 protein is also evident in $S_1$ nuclease assays of DNA heteroduplex formation, although the extent of inhibition is less than is observed for the complete DNA strand exchange process; at equimolar concentrations of wild-type and mutant proteins, the extent of DNA heteroduplex formation is 36% of the wild-type protein level. This difference implies that RecA142 protein prevents, at minimum, the branch migration normally observed during DNA strand exchange. RecA142 protein does not inhibit either the single-strand (ss) DNA-dependent ATPase activity or the coaggregation activities of wild-type RecA protein. This suggests that these reactions are not responsible for the inhibition of wild-type protein DNA strand exchange activity by RecA142 protein. However, under conditions where RecA142 protein inhibits DNA strand exchange activity, RecA142 protein renders the M13 ssDNA-dependent ATPase activity of wild-type protein sensitive to inhibition by single-strand DNA-binding protein, and it inhibits the double-strand DNA-dependent ATPase activity of wild-type RecA protein. These results imply that these two activities are important components of the overall DNA strand exchange process. These experiments also demonstrate the applicability of using defective mutant RecA proteins as specific codominant inhibitors of wild-type protein activities *in vitro* and should be of general utility for mechanistic analysis of RecA protein function both *in vitro* and *in vivo*.

### 1. Introduction

In the accompanying paper (Kowalczykowski *et al.*, 1989), we characterized the biochemical properties of RecA142 protein, a mutant RecA protein that is defective in genetic recombination *in vivo*. We focused on enzymatic activities that might be important for homologous pairing function *in vitro* and assumed that defects in these activities were responsible for the defect *in vivo*. The RecA142 protein cannot catalyze formation of heteroduplex DNA as detected by three different assays. The biochemical defects assumed to be responsible for the failure in heteroduplex DNA formation are: (1) RecA142 protein is unable to form a characteristic ATP-induced high-affinity single-strand (ss†) DNA-binding state; (2) because of the first defect, RecA142 protein fails to compete effectively with SSB protein for limited ssDNA-binding sites, thus preventing presynaptic complex formation; (3) RecA142 protein cannot promote coaggregation between ssDNA and dsDNA molecules, which could result in defective synapsis; and (4) presumably because of the first defect, RecA142

† Abbreviations used: ssDNA, single-strand DNA; SSB protein, *E. coli* single-strand DNA-binding protein; dsDNA, double-strand DNA; etheno M13 ssDNA, M13 single-strand DNA containing 1,$N^6$-etheno-adenosine and 3,$N^4$-etheno-cytidine residues; ATP-$\gamma$-S, adenosine-5′-*O*′-(3-thiophosphate); PEP, phosphoenolpyruvate.



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protein is defective in dsDNA-dependent ATPase activity, which could inhibit joint molecule formation and branch migration.

A defect in presynapsis can be readily identified, since presynaptic complex formation is the first step in the DNA strand exchange reaction sequence. The defect in presynaptic complex formation by RecA142 protein is due to the displacement of RecA142 protein from the ssDNA by SSB protein, thus blocking DNA strand exchange at its earliest point. This impediment also blocks all subsequent steps of DNA strand exchange, thereby limiting conclusions that can be drawn regarding the function of coaggregation and dsDNA-dependent ATPase activity to events beyond presynapsis.

The inhibitory effect of SSB protein on presynaptic complex formation by RecA142 protein can be avoided by omitting SSB protein from the *in vitro* reactions, so that defects which occur subsequent to presynapsis might become detectable. We observed that, without SSB protein, joint molecule formation is still inhibited (Kowalczykowski *et al.*, 1989), even though RecA142 protein can bind to ssDNA and hydrolyze ATP (events that indicate formation of a presynaptic nucleoprotein complex capable of ATP hydrolysis). The failure to form joint molecules in the absence of SSB protein might imply that RecA142 protein was defective in either synapsis or nascent exchange of DNA strands, or both. Consistent with the synaptic defect explanation is the lack of coaggregation activity by RecA142 protein. Because of this coaggregation (synaptic) defect, it is difficult to ascertain whether the impaired dsDNA-dependent ATPase activity contributes to the failure in joint molecule formation. To reveal such a contribution, it would be necessary to find conditions where coaggregation is either unimpaired or unnecessary.

Some *recA* alleles (e.g. *recA1*) display a phenotype that is codominant to the wild-type allele (Sedgwick & Yarranton, 1982; Yarranton & Sedgwick, 1982; Yancey & Porter, 1984). *In vitro*, purified RecA1 protein inhibits both the ssDNA-dependent ATPase activity (Ogawa *et al.*, 1978) and the strand exchange activity of wild-type RecA protein (Rusche *et al.*, 1985; Kowalczykowski, unpublished results). In addition, complementation of lambda repressor cleavage activity occurs between RecA441 and RecA430 both *in vivo* and *in vitro* (Rebollo *et al.*, 1984; Moreau & Roberts, 1984). These results suggested that further insight into RecA protein function could be derived from biochemical studies of mixtures containing both RecA142 and wild-type proteins. If RecA142 protein could uniquely inhibit an activity of wild-type RecA142 protein or, alternatively, if wild-type protein could complement a defect of RecA142 protein, then additional mechanistic information could be derived.

Here we describe the effects of RecA142 protein on the *in vitro* activities of wild-type RecA protein. We show that RecA142 protein has profound effects on the ability of wild-type RecA protein to catalyze DNA heteroduplex formation. Inhibition of DNA heteroduplex formation is a consequence of the inhibitory effects of RecA142 protein on both the SSB protein-stimulated ssDNA-dependent ATPase activity and the dsDNA-dependent ATPase activity of the wild-type protein. We find that in mixtures of RecA142 and wild-type proteins, coaggregation function is not impaired. This complementation of RecA142 protein coaggregation function by wild-type protein permits interpretation of the RecA142 protein inhibition results without the complications imposed by coaggregation defects. Thus, we show that RecA142 protein is a specific codominant inhibitor of certain *in vitro* reactions catalyzed by wild-type protein. The significance and possible mechanism of inhibition are discussed. The use of mutant RecA proteins to inhibit wild-type protein functions both *in vivo* and *in vitro* may be a very useful approach for dissecting the mechanism of RecA protein action.

## 2. Materials and Methods

The materials and experimental methods employed are identical with those described in the accompanying paper (Kowalczykowski *et al.*, 1989), unless indicated otherwise. The one exception is that the products of the DNA strand exchange reaction were analyzed on agarose gels run in the absence of ethidium bromide and later stained to visualize the DNA bands. By running the agarose gels in this manner, both reaction intermediates (i.e. plectonemic joint molecules) and products (i.e. gapped circular dsDNA) can be resolved (Menetski, 1988).

For all studies involving mixtures of wild-type and mutant RecA proteins, both proteins were always mixed together in the assay buffer prior to the addition of any DNA. If SSB protein was present, it was always added 1 to 2 min following addition of the ssDNA. The DNA strand exchange (agarose gel), DNA heteroduplex ($S_1$ nuclease), or joint molecule (nitrocellulose filter) assays were all initiated by the addition of dsDNA as the final constituent.

## 3. Results

### (a) *Effects of RecA142 protein on the ssDNA-dependent ATPase activity of wild-type RecA protein*

During characterization of RecA142 protein, we observed that it altered the enzymatic properties of wild-type RecA protein. Since the ssDNA-dependent ATPase activity of RecA protein is easily assayed and is related to presynaptic complex formation, this activity was used to survey the effects of RecA142 protein on wild-type RecA protein.

In the absence of SSB protein, RecA142 protein has slight, but reproducible, effects on wild-type RecA protein ssDNA-dependent ATPase activity (Fig. 1). Either slight stimulation or inhibition is observed, depending on RecA protein concentration

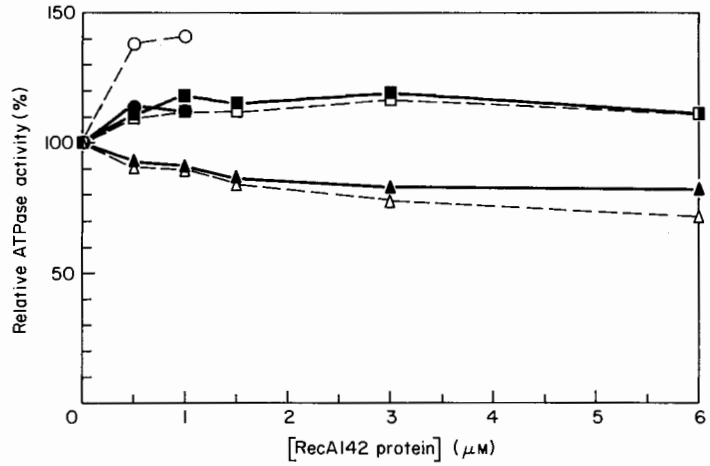


**Figure 1.** Effect of RecA142 protein on the ssDNA-dependent ATPase activity of wild-type RecA protein. ATPase assays were carried out using standard ATPase assay conditions as described in Materials and Methods. RecA142 protein was present at the concentration indicated in the Figure; the following DNA substrates (all at 3 μM) were used with the indicated wild-type RecA protein and salt concentrations: (▲) M13 ssDNA at 1·5 μM-RecA protein and 0 mM-NaCl; (△) M13 ssDNA at 1·5 μM-RecA protein and 200 mM-NaCl; (●) M13 ssDNA at 0·5 μM-RecA protein and 0 mM-NaCl; (○) M13 ssDNA at 0·5 μM-RecA protein and 200 mM-NaCl; (■) etheno M13 DNA at 1·5 μM-RecA protein and 0 mM-NaCl; (□) etheno M13 DNA at 1·5 μM-RecA protein and 300 mM-NaCl. The ATPase activity is expressed relative to the absolute rate of ATP hydrolysis observed in the absence of added RecA142 protein. The absolute rates of ATP hydrolysis, given in the order presented above are: 13·4, 12·4, 11·9, 5·5, 26·4 and 23·6 μM/min, respectively.

and on the type of DNA substrate used. When the concentration of wild-type RecA protein is sufficient to saturate all potential M13 ssDNA binding sites (e.g. 1·5 μM-wild-type protein, triangles), addition of up to fourfold molar excess of RecA142 protein results in an 18% inhibition of observed ATPase activity at 0 mM-NaCl and 28% inhibition at 200 mM-NaCl. In contrast, if etheno M13 ssDNA is employed as the DNA substrate (squares), RecA142 protein increases the observed ATP hydrolysis rate by as much as 19% at these protein and DNA concentrations†.

Since the inhibition of wild-type RecA protein ATPase activity by RecA142 protein observed at saturating ratios of protein to M13 ssDNA binding sites can be explained by competitive effects, lower protein concentrations were examined. At lower ratios of protein to ssDNA, addition of RecA142 protein always increases the observed rate of ATP hydrolysis; e.g. at 0·5 μM-wild-type RecA protein, RecA142 protein increases the rate when M13 ssDNA is used (Fig. 1, filled circles). Surprisingly, this is true even at 200 mM-NaCl, where the M13 ssDNA-dependent ATPase activity of RecA142 protein is negligible (only 4% of the activity observed without NaCl; see Fig. 6 of Kowalczykowski *et al.*, 1989). This suggests that wild-type protein can stabilize the RecA142 protein–ssDNA complex against disruption by NaCl.

This stabilizing effect is particularly pronounced at a lower protein concentration (0·2 μM, Fig. 2) that is subsaturating relative to the ssDNA concentration. With no added NaCl, a mixture of RecA142 and wild-type proteins displays the ATP hydrolysis rate expected from the sum of their individual ATP hydrolysis rates (Fig. 2, filled bars). However, at 300 mM-NaCl the ATPase activity of a mixture of the two proteins is 1·7-fold *greater* than the sum of their individual activities (cross-hatched bars), suggesting that RecA142 protein is contributing to the observed ATP hydrolysis rate in these mixtures. The NaCl sensitivity of the equimolar mixture of proteins is identical with that of wild-type protein alone (i.e. a 65% decrease in ATP hydrolysis rate at 300 mM-NaCl). This sensitivity is substantially lower than the 99% inhibition observed for RecA142 protein alone at this salt concentration. Effects of comparable magnitude are observed with M13 ssDNA at 200 mM-NaCl (not shown). These non-additive observations suggest that the wild-type protein can stabilize RecA142 protein against inhibition by NaCl at subsaturating ratios of protein to ssDNA. This, in turn, implies that both proteins are physically interacting with one another.

### (b) *Effect of SSB protein on ssDNA-dependent ATPase activity*

In the previous paper (Kowalczykowski *et al.*, 1989), we reported that SSB protein inhibited the

† Although identical protein and ssDNA concentrations are used in the M13 ssDNA and etheno M13 ssDNA experiments, the concentration of *available* ssDNA binding sites is higher in the etheno M13 ssDNA experiments due to the absence of DNA secondary structure in etheno M13 DNA (Kowalczykowski & Krupp, 1987).

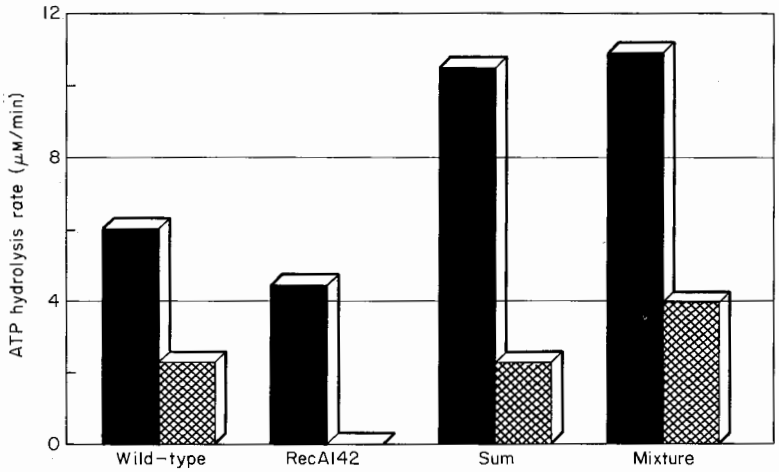


**Figure 2.** Comparison of the ssDNA-dependent ATPase activity of mixtures of RecA142 and wild-type proteins to the activity of the individual proteins. The concentration of each RecA protein is 0·2 μM and the etheno M13 ssDNA concentration is 3·0 μM. Wild-type, wild-type RecA protein alone; RecA142, RecA142 protein alone; Sum, the calculated sum of the "Wild-type" and "RecA142" columns; Mixture, the experimentally observed rate for a mixture containing 0·2 μM of each RecA protein. Filled bars are data at 0 M-NaCl; cross-hatched bars are data at 300 mM-NaCl.

formation of a presynaptic complex between RecA142 protein and M13 ssDNA, as measured by ATPase activity; i.e. ATP hydrolysis by RecA142 protein is completely inhibited by SSB protein. Figure 3 summarizes the effect of SSB protein on the ssDNA-dependent ATPase activity of wild-type RecA protein in the presence of increasing concentrations of RecA142 protein. Upon addition of SSB protein, wild-type RecA protein alone displays a 2·3 to 2·5-fold increase in ATP hydrolysis rate due to binding of additional RecA protein to the M13 ssDNA (Kowalczykowski & Krupp, 1987).

The behavior of the RecA protein mixtures is dependent on concentrations of each protein present and on whether SSB protein is added (Fig. 3). RecA142 protein has a relatively small effect on the observed rate of ATP hydrolysis in the absence of SSB protein. However, in the presence of SSB protein, addition of RecA142 protein progressively reduces the observed rate; complete inhibition occurs when the concentration of RecA142 protein is approximately twofold higher than the concentration of wild-type RecA142 protein. Thus, RecA142 protein diminishes the ability of wild-type protein to compete with SSB protein for ssDNA.

When the effect of SSB protein on the ssDNA-dependent ATPase activity of mixtures of wild-type and of RecA142 protein was examined using the

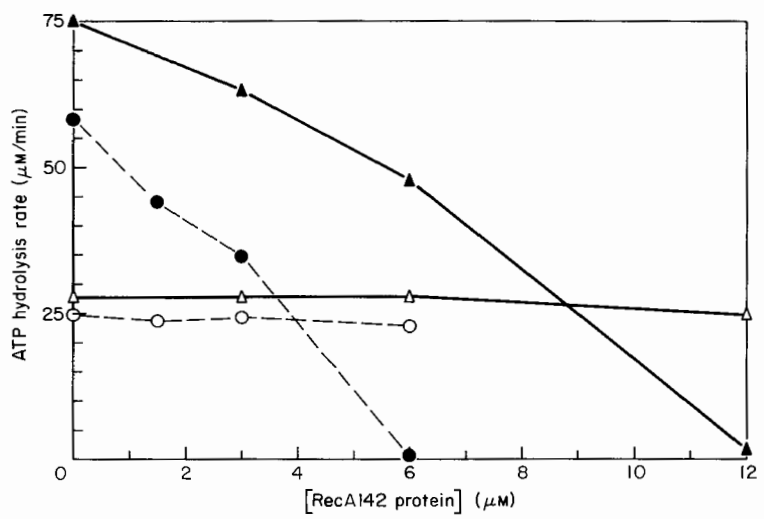


**Figure 3.** Effect of RecA142 protein on the M13 ssDNA-dependent ATPase activity of wild-type RecA protein in the presence of SSB protein. The buffer employed for DNA strand exchange experiments was used for these ATPase assays (i.e. the concentration of magnesium acetate was 10 mM and that of PEP was 7·5 mM). The concentrations employed were 9·9 μM-M13 ssDNA, 0·9 μM-SSB protein (when present), and either 6 μM-wild-type RecA protein (△, ▲) or 3 μM-wild-type RecA protein (○, ●); RecA142 protein was present at the concentration indicated. The open symbols are results obtained in the absence of SSB protein; the filled symbols are in the presence of SSB protein.

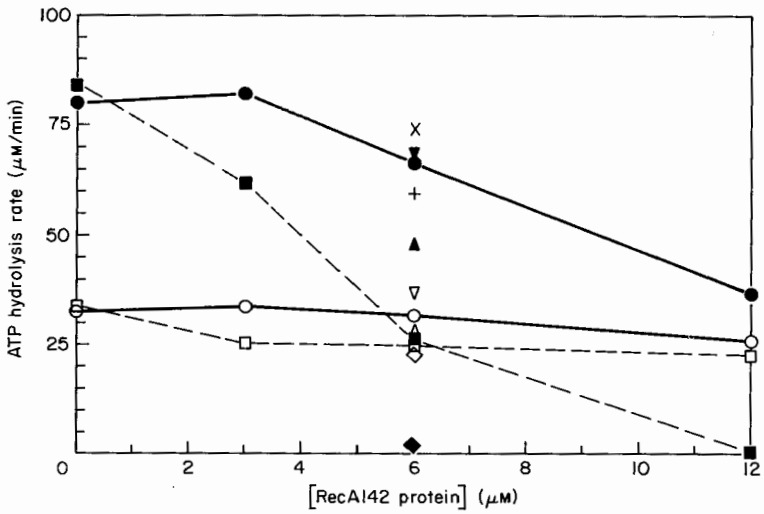


**Figure 4.** Effect of PEP concentration on the ATPase activity of mixtures of wild-type and RecA142 proteins in the presence of SSB protein. The buffer employed for DNA strand exchange experiments was used for these ATPase assays (i.e. the concentration of magnesium acetate was 10 mM), except that the PEP concentration was varied as indicated. The concentrations employed were 9·9 μM-M13 ssDNA, 0·9 μM-SSB protein (when present), 6 μM-wild-type RecA protein, and RecA142 protein at the concentrations indicated. The concentrations of PEP employed were 1·5 mM (▽, ▼); 3 mM (×); 4·5 mM (○, ●); 6 mM (+); 7·5 mM (△, ▲); 9 mM (□, ■); 13·5 mM (◇, ◆). The open symbols are in the absence of SSB protein; the filled symbols are in the presence of SSB protein.

slightly different buffer conditions normally employed for the ATPase and DNA strand exchange assays, an unexpected effect of phosphoenolpyruvate (PEP) concentration was observed. Figure 4 shows that increasing concentrations of PEP *increased* the sensitivity of these RecA protein mixtures to inhibition by SSB protein; this effect is more pronounced at the higher RecA142 protein concentrations. This result was surprising, since neither RecA protein alone displayed any significant alteration in ATPase activity, either with or without SSB protein present, when the PEP concentration was varied from 0·075 to 7·5 mM (not shown). Though the magnitude of this PEP effect was surprising, a slight effect of PEP concentration was observed previously on the ssDNA binding properties of wild-type RecA protein (Menetski *et al.*, 1988).

Since PEP is an essential ingredient of the ATPase assay and is also used in the DNA strand exchange assay to prevent inhibition due to accumulation of ADP, the properties of wild-type and RecA142 protein mixtures were examined at the lowest concentration of PEP that could be judiciously employed in DNA strand exchange experiments (3 mM-PEP; since the DNA strand exchange assays are typically run for 60 min, a greater concentration of PEP is required than in the ssDNA-dependent ATPase assays, which are run for only 5 to 10 min). Figure 5 shows the effect of increasing concentrations of wild-type RecA protein on the M13 ssDNA-dependent ATPase activity of RecA142 protein. (To permit comparison with DNA strand exchange results described below, these experiments were carried out in the presence of 6 mM-magnesium acetate and 3 mM-PEP, and are presented as a function of wild-type protein concentration.) Consistent with Figure 3, the activity of RecA142 and wild-type protein mixtures is relatively unaltered when SSB protein is absent. Also, as shown in the previous paper, SSB protein completely inhibits the activity of RecA142 protein alone. The addition of wild-type RecA protein can restore ATPase activity in the presence of SSB protein, but only at elevated (>1 μM) wild-type protein concentrations. Restoration of activity by wild-type protein is apparently competitive with RecA142 protein concentration. Presumably the effects of RecA142 protein on the ssDNA-dependent ATPase activity of wild-type RecA protein in the presence of SSB protein represent alterations in the stability or integrity of the presynaptic filament. Thus, variations in both RecA142 protein and PEP concentration can be exploited to examine their effects on the biochemical activities of wild-type RecA protein.

### (c) *RecA142 inhibits the double-stranded DNA-dependent ATPase activity of wild-type RecA protein*

Since RecA142 protein shows relatively little M13 dsDNA-dependent ATPase activity (Kowalczykowski *et al.*, 1989), the effect of RecA142 protein on the dsDNA-dependent ATPase activity of the wild-type protein was assessed. Figure 6 shows that the activity of mixtures of these two proteins is substantially diminished relative to the wild-type protein alone; the addition of RecA142 protein increases the length of the lag phase and decreases the final steady-state ATP hydrolysis rate. These results, together with results from

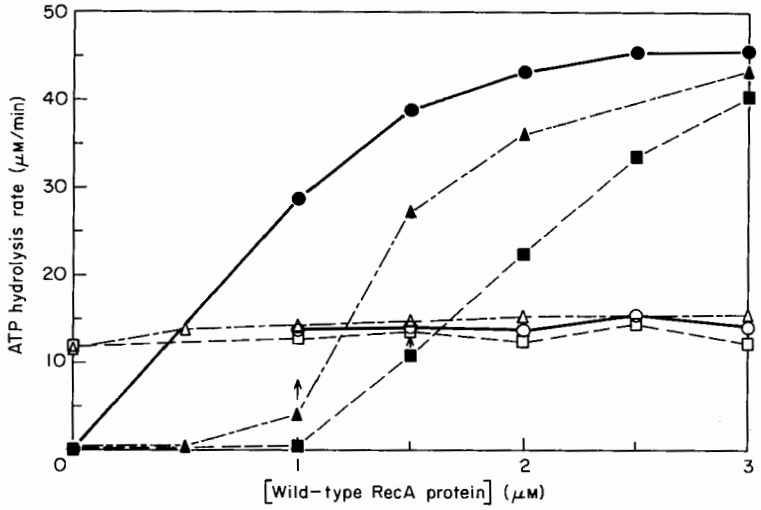


**Figure 5.** Effect of wild-type RecA protein on the M13 ssDNA-dependent ATPase activity of RecA142 protein in the presence of SSB protein. The buffer employed for DNA strand exchange experiments was used for these ATPase assays except that the concentration of magnesium acetate was 6 mM and the PEP concentration was 3 mM. The concentrations employed were 5·0 μM-M13 ssDNA and 0·45 μM-SSB protein (when present), with the wild-type RecA and RecA142 protein at the concentrations indicated. The concentrations of RecA142 protein employed were 0 μM (○, ●); 1·5 μM (△, ▲); and 3 μM (□, ■). The open symbols are in the absence of SSB protein, the filled symbols are in the presence of SSB protein. The 2 points marked with arrows displayed a rate that was still slowly increasing at the end of the reaction.

assays conducted at a lower concentration of RecA protein, are summarized in Table 1.

To permit a comparison with DNA strand exchange reaction results (below), dsDNA-dependent ATPase assays were also conducted under the conditions employed for the DNA strand exchange reaction (either 10 mM-magnesium acetate and 7·5 mM-PEP, or 6 mM-magnesium acetate and 3 mM-PEP). Similar inhibitory effects of RecA142 protein on wild-type protein activity are obtained (data not shown) and the data are summarized in Table 1. The data show that RecA142 protein has a significant impact on the dsDNA-dependent ATPase activity of wild-type protein, increasing the lag phase by as much as 300% and decreasing the steady-state rate by as much as 90%. No lag phase

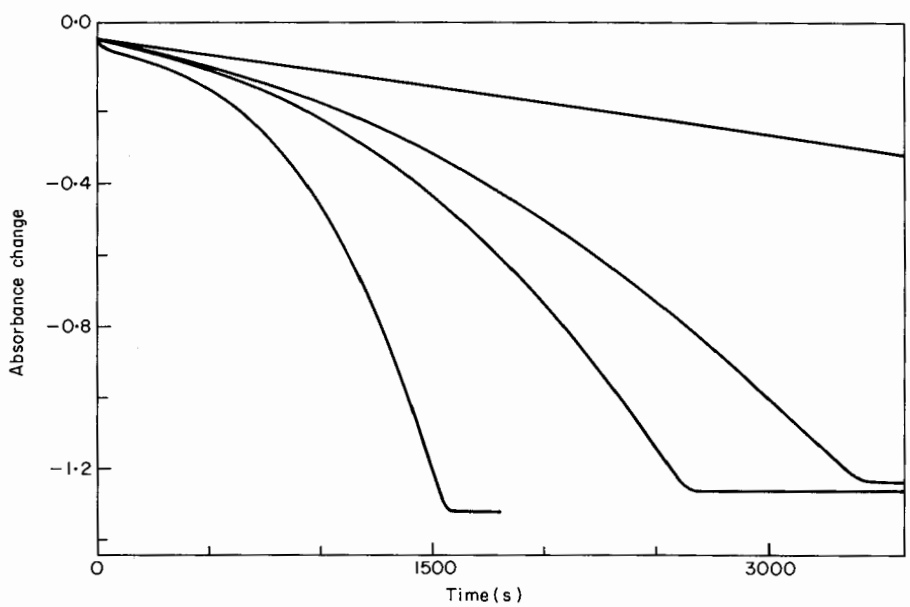


**Figure 6.** Double-strand DNA-dependent ATP hydrolysis activity of RecA protein. ATPase assays were carried out in standard buffer (i.e. containing 10 mM-magnesium acetate and 7·5 mM-PEP), as described in Materials and Methods, using linear M13 dsDNA. The traces represent (from left to right): 3 μM-wild-type RecA protein; a mixture of 3 μM each of wild-type and RecA proteins; a mixture of 3 μM-wild-type and 6 μM-RecA142 proteins; and 3 μM-RecA142 protein alone. A decrease of 1 absorbance unit corresponds to the hydrolysis of 0·16 mM-ATP.

**Table 1**
*Effect of RecA142 protein on the dsDNA-dependent ATPase activity of wild-type RecA protein*

| | | % of wild-type activity | | | | | |
|---|---|---|---|---|---|---|---|
| [$RecA_{wt}$] ($\mu$M) | [RecA142] ($\mu$M) | Lag time A | Rate A | Lag time B | Rate B | Lag time C | Rate C |
| 3·0 | 0 | 100 | 100 | 100 | 100 | 100 | 100 |
| 3·0 | 1·5 | 114 | 82 | 112 | 84 | 130 | 33 |
| 3·0 | 3·0 | 146 | 47 | 132 | 53 | 150 | 17 |
| 3·0 | 6·0 | 153 | 30 | 199 | 20 | Ind | 9 |
| 0 | 3·0 | None | 6 | None | 2 | None | 2 |
| 1·5 | 0 | 100 | 100 | 100 | 100 | 100 | 100 |
| 1·5 | 1·5 | 141 | 49 | 157 | 42 | Ind | 9 |
| 1·5 | 3·0 | n.d. | n.d. | 309 | 20 | Ind | 8 |
| 0 | 1·5 | None | 4 | None | 2 | None | 3 |

The percentage of wild-type RecA protein dsDNA-dependent ATPase activity is reported. The calculation is based either on the length of the observed lag phase ("Lag time" columns) or on the observed steady-state rate of ATP hydrolysis ("Rate" columns). The concentration of M13 dsDNA is 8·4 $\mu$M; "None" indicates there is no lag phase (i.e. the ATP hydrolysis rate is linear); "n.d." indicates not determined; "Ind" indicates that the lag time was indeterminate due to the poor distinction between the lag and steady-state phases; wt, wild-type. The capital letters refer to the experimental conditions of the assay: A, standard ATPase assay reaction buffer was employed (i.e. the magnesium acetate concentration was 10 mM and the PEP concentration was 1·5 mM); B, standard DNA strand exchange reaction buffer was employed except that the magnesium acetate concentration was 6 mM and the PEP concentration was 3 mM; and C, standard DNA strand exchange reaction buffer was employed, (i.e. the magnesium acetate concentration was 10 mM and the PEP concentration was 7·5 mM). The relative variability in the lag time and the rate values is approximately 20 and 10 percentage units, respectively. The lag time is defined by extrapolating the steady-state rate back to zero ATP hydrolysis. For condition A, the average lag times obtained at 3·0 $\mu$M and 1·5 $\mu$M-wild-type RecA protein are 910($\pm$20%) s and 930 s, respectively; the steady-state ATP hydrolysis rates are 14($\pm$20%) and 13 $\mu$M/min, respectively; for B, the average lag times obtained at 3·0 $\mu$M and 1·5 $\mu$M-wild-type RecA protein are 920($\pm$15%) s and 940 s, respectively; the average steady-state ATP hydrolysis rates are 9·2($\pm$10%) and 7·7 $\mu$M/min, respectively; and for C, the average lag times obtained at 3·0 $\mu$M-wild-type RecA protein are 1360($\pm$15%) s and 1460 s, respectively; the steady-state ATP hydrolysis rate are 5·7($\pm$10%) and 4·8 $\mu$M/min.

is observed for RecA142 protein alone; instead, a linear but slow rate of ATP hydrolysis is observed for as long as 2·5 hours (data not shown). As mentioned in the accompanying paper (Kowalczykowski *et al.*, 1989), this low but real ATP hydrolysis rate may reflect limited utilization of A+T-rich regions within the M13 dsDNA. Thus, as with the ssDNA-dependent ATPase activity in the *presence* of SSB protein, the dsDNA-dependent ATPase activity of wild-type RecA protein displays a sensitivity to the presence of RecA142 protein.

### (a) *ATP hydrolysis in the presence of both single and double-stranded DNA*

Under appropriate conditions, addition of dsDNA to a complex of RecA protein and ssDNA decreases the observed ATP hydrolysis rate by up to 33% (Schutte & Cox, 1987). The reason for the decrease is unclear, but it is dependent upon association of the RecA protein–ssDNA complex with homologous dsDNA in a paranemic complex. Since RecA142 protein is proficient in ssDNA-dependent ATPase activity but is deficient in dsDNA-dependent ATPase activity, the effect of RecA142 protein on this homology-dependent change in ATP hydrolysis may disclose whether the observed ATP hydrolysis decrease reflects ssDNA-dependent or dsDNA-dependent processes. Table 2 shows the effect of RecA142 protein on the behavior of wild-type protein in such an experiment. Upon addition of dsDNA, the ATP hydrolysis rate for wild-type protein alone decreases by 19%. If an equimolar amount of RecA142 protein is present in addition to the wild-type protein, the ssDNA-dependent ATP hydrolysis rate is unaffected; however, upon addition of dsDNA, a 46% decrease in the ATP hydrolysis rate is observed. This decrease is 2·5-fold

**Table 2**
*Effect of RecA142 protein on the ssDNA and dsDNA-dependent ATP hydrolysis rate of wild-type protein*

| | ATP hydrolysis rate ($\mu$M/min) | | |
|---|---|---|---|
| | Before dsDNA | After dsDNA | % decrease |
| Wild-type RecA protein alone | 58 (100%) | 47 (100%) | 19 |
| RecA142 and wild-type protein mixture | 59 (102%) | 32 (68%) | 46 |

ATPase assays were carried out in standard assay buffer (i.e. containing 10 mM-magnesium acetate and 1·5 mM-PEP) as described in Materials and Methods. The concentrations employed were 8 $\mu$M-M13 ssDNA, 16 $\mu$M-M13 dsDNA and 0·8 $\mu$M-SSB protein. The concentration of wild-type RecA protein was 2 $\mu$M and RecA142 protein, when present, was also 2 $\mu$M. The ATP hydrolysis rate reported is the steady-state value observed either before addition of dsDNA (as the final component) or the rate observed after addition of dsDNA. The numbers in parentheses represent the percentage of ATP hydrolysis relative to wild-type protein alone. The "% decrease" column shows the decrease in ATP hydrolysis after addition of dsDNA.

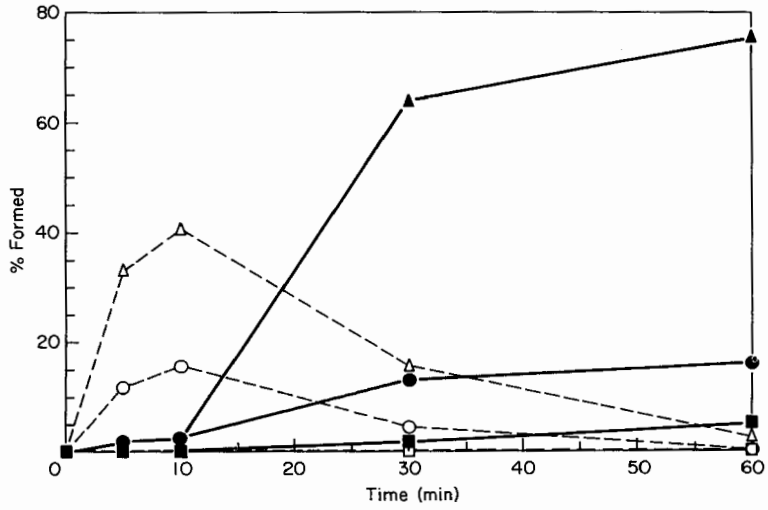


**Figure 7.** Effect of RecA142 protein on the DNA strand exchange activity of wild-type RecA protein. Agarose gel assays were carried out as described in Materials and Methods. Standard DNA strand exchange buffer was used (i.e. containing 10 mM-magnesium acetate and 7·5 mM-PEP). The concentrations employed were 9·9 μM-M13 ssDNA, 16·8 μM-M13 dsDNA, 0·9 μM-SSB protein, 6 μM-wild-type RecA protein, and the following amounts of RecA142 protein; 0 μM (△, ▲); 3·0 μM (○, ●); 6·0 μM (□, ■). Filled symbols represent final product and open symbols represent reaction intermediates.

greater than that observed upon addition of dsDNA to wild-type protein alone. (Upon addition of an equimolar concentration of wild-type protein, no decrease in ATP hydrolysis is observed, since the final RecA protein concentration is in stoichiometric excess relative to the total DNA concentration (Roman & Kowalczykowski, 1986).) These results demonstrate that, whereas RecA142 protein has no effect on the ssDNA-dependent ATPase activity of wild-type RecA protein under these conditions (consistent with data in Fig. 4), it reduces the ssDNA and dsDNA-dependent rate of wild-type protein by an additional 32%. Under these buffer conditions, the dsDNA-dependent ATPase activity of wild-type protein is reduced by 51% to 53% by an equimolar concentration of RecA142 protein (see Table 1). Taken together, these data suggest that the reduction in ATP hydrolysis observed upon addition of dsDNA to a RecA protein presynaptic complex is probably due to dsDNA-dependent ATP hydrolysis events. This is likely to be a result of the fact that dsDNA is a poorer substrate for ATP hydrolysis than ssDNA (Kowalczykowski *et al.*, 1987; Menetski & Kowalczykowski, 1989).

### (e) *RecA142 protein inhibits the DNA strand exchange activity of wild-type RecA protein*

Figure 7 demonstrates the effect of RecA142 protein on the DNA strand exchange activity of wild-type protein under typical reaction conditions (i.e. 10 mM-magnesium acetate and 7·5 mM-PEP). It shows that wild-type RecA protein can convert approximately 75% (±15%) of the linear dsDNA into product (gapped dsDNA molecules) in 60 minutes. The steady-state amount of intermediate (plectonemic joint molecules) formed is a maximum (42% ± 15%) at about 10 minutes. Addition of RecA142 protein to the reaction substantially decreases both product and intermediate formation. At 3 μM-RecA142 protein, the rate and extent of both intermediate and product formation are reduced by 60% to 70%. An equimolar amount of RecA142 protein (6 μM) almost completely inhibits catalysis of DNA strand exchange by wild-type RecA protein.

Table 3 reveals that RecA142 protein inhibits the

**Table 3**
*Effect of RecA142 protein on the DNA strand exchange activity of wild-type RecA protein*

| | Product yield (%)† [Wild-type RecA protein] (μM) | | |
|---|---|---|---|
| [RecA142 protein] (μM) | 6·0 | 3·0 | 1·5 |
| 0 | 100 | 100 | 100 |
| 1·5 | n.d. | n.d. | < 1 |
| 3·0 | 21 | 4 | n.d. |
| 6·0 | 7 | 7 | n.d. |
| 12·0 | 3 | n.d. | n.d. |

DNA strand exchange was measured using the agarose gel assay described in Materials and Methods. The concentrations employed were 9·9 μM-M13 ssDNA, 16·8 μM-M13 dsDNA, 0·9 μM-SSB protein, and RecA protein at the concentrations indicated. The concentrations of magnesium acetate and PEP were 10 mM and 7·5 mM, respectively.

† Product yield was determined after 60 min of reaction and is expressed as the percentage yield relative to the wild-type RecA protein alone control reaction. The absolute amounts of product formation are 75%, 57% and 28% for the reactions containing 6, 3 and 1·5 μM-wild-type RecA protein, respectively. The experimental uncertainty of the values reported for the mixture experiments is approximately 5 to 10 percentage units. "n.d." indicates "not done".

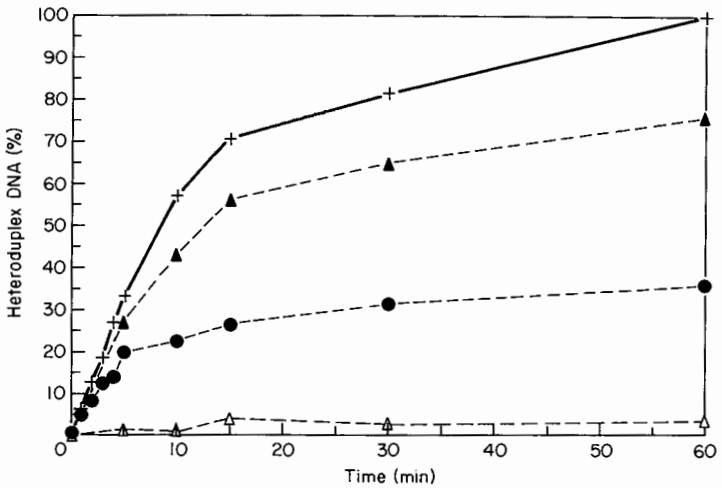


**Figure 8.** Heteroduplex DNA formation catalyzed by wild-type and RecA142 proteins. $S_1$ nuclease assays were carried out as described in Materials and Methods. Standard buffer was used (i.e containing 10 mM-magnesium acetate and 7·5 mM-PEP). The concentrations employed were 9·9 μM-M13 ssDNA, 16·8 μM-M13 dsDNA, 0·9 μM-SSB protein, and either: (+), 6 μM-wild-type RecA protein; (△), 6 μM-RecA142 protein; (▲) a mixture containing 6 μM-wild-type and 3 μM-RecA142 proteins; (●) a mixture containing 6 μM each of wild-type and RecA142 proteins.

DNA strand exchange activity of wild-type protein over a wide range of protein concentrations. This is true whether the wild-type RecA protein concentration (6 μM) either exceeds the amount required to saturate the DNA substrates or is limiting (1·5 μM). However, the relative amount of inhibition appears to be greater at the lower concentrations of wild-type RecA protein. Since RecA142 protein is inhibiting wild-type protein DNA strand exchange function at the subsaturating concentration (1·5 μM), competition for limiting DNA-binding sites cannot explain the observed inhibition. Thus, although the RecA142 protein itself is unable to catalyze DNA strand exchange, it must still retain the ability to interact (negatively) with the wild-type protein. This interaction with wild-type protein significantly interferes with a step (or steps) of the DNA strand exchange process. The proposed nature of these steps will be addressed in Discussion.

When the effect of RecA142 protein on DNA heteroduplex formation by wild-type protein is monitored *via* the $S_1$ nuclease assay, results similar to the gel assay are obtained (Fig. 8). RecA142 protein substantially reduces the final extent of heteroduplex DNA formation. At equimolar concentrations of the two proteins (6 μM each), the yield of heteroduplex DNA produced is 36%(±4%); at a molar ratio of 2 to 1 (wild-type : RecA142 protein), the yield is 76%(±4%). When the concentration of wild-type protein is reduced to either 3·0 μM or 1·5 μM, the presence of an equimolar amount of RecA142 protein results in the formation of only 13%(±4%) or 7%(±5%), respectively, of the amount of heteroduplex DNA obtained with wild-type protein alone (not shown). These values are higher than those obtained in the agarose gel assay (Table 3), because product formation in the gel assay measures only those molecules that have undergone complete exchange of DNA strands, whereas the $S_1$ nuclease assay measures total heteroduplex DNA formation. These results imply that some of the DNA molecules have undergone only partial strand exchange in the mixed RecA protein reactions. This quantitative difference suggests that RecA142 protein prevents the extension of DNA heteroduplex joints necessary for complete exchange of DNA strands.

The rates of DNA heteroduplex formation are more precisely determined in the $S_1$ nuclease assay than in the gel assay. For 6 μM-wild-type protein alone, the initial rate of DNA heteroduplex formation derived from the data in Figure 8 is 0·55(±0·09) μM-(nucleotides) per minute, which is in agreement with a previous estimate in acetate-based buffer (Roman & Kowalczykowski, 1986). This value corresponds to an average rate of DNA strand exchange of approximately 8 nucleotides per second. For the reaction carried out in the presence of 3 μM-RecA142 protein, the initial rate of DNA heteroduplex formation is 0·46 μM per minute, which is nearly the same within experimental error. The rate of heteroduplex DNA formation in a reaction containing an equimolar mixture of wild-type and mutant proteins is 0·36 μM per minute. Thus, the rate of heteroduplex formation displays a 40% inhibition, whereas the extent of reaction is inhibited by 65%. This demonstrates that RecA142 protein inhibits the extent of DNA heteroduplex formation to a greater degree than it affects the rate of heteroduplex formation.

The effect of RecA142 protein on the ability of wild-type RecA protein to promote joint molecule formation was also examined under conditions identical with those used in Figures 7 and 8. The extent of joint molecule formation as measured by nitrocellulose filter assay was approximately 35%(±10%) of the wild-type level, when an

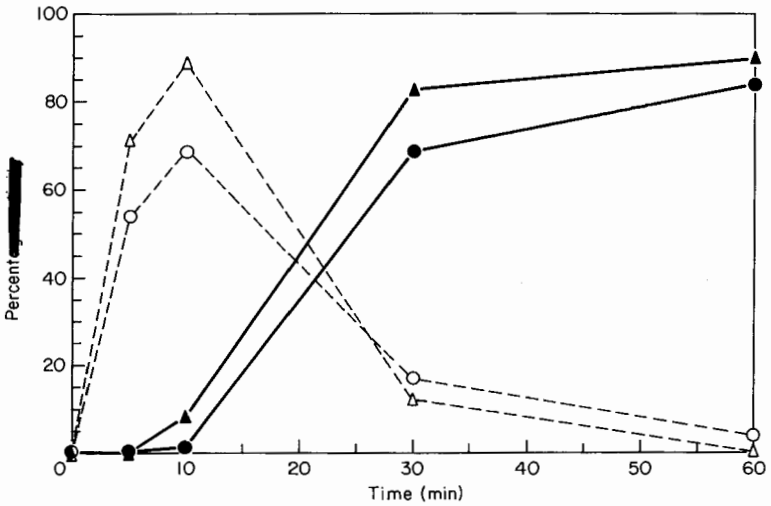


**Figure 9.** Effect of RecA142 protein on the DNA strand exchange activity of wild-type RecA protein. Agarose gel assays were carried out as described in Materials and Methods. The DNA strand exchange buffer contained 6 mM-magnesium acetate and 3 mM-PEP. The concentrations employed were 5·0 μM-M13 ssDNA, 8·4 μM-M13 dsDNA, 0·45 μM-SSB protein and the following amounts of RecA protein; 3 μM-wild-type RecA protein, (△, ▲); 3·0 μM each of wild-type RecA and RecA142 proteins (○, ●). Filled symbols represent final product and open symbols represent reaction intermediates.

equimolar amount of each RecA protein (6 μM each) was present (not shown). This agrees with the inhibition observed for intermediate formation in the agarose gel assay (Fig. 7) and confirms that RecA142 protein inhibits joint molecule formation as well.

Since PEP concentration affects the ATPase activity of mixtures of mutant and wild-type RecA proteins, its effect on the DNA strand exchange activity of the RecA protein mixtures was examined using the agarose gel assay (Fig. 9). When the PEP concentration is reduced to 3 mM (and magnesium acetate concentration to 6 mM†), a slight but experimentally reproducible inhibition (10 to 20%) of wild-type protein activity by an equimolar concentration of RecA142 protein is observed. A similar experimental time-course (with a 25 to 35% inhibition relative to wild-type alone) is observed when the concentration of each RecA protein is reduced to 1·5 μM (not shown). However, in contrast to the behavior indicated in Figure 7 and Table 1, there is little effect on the final extent of product formation.

### (f) *Coaggregation properties of mixtures of RecA142 and wild-type proteins*

Another characteristic of the RecA142 protein is its inability to promote the formation of coaggregates (Kowalczykowski *et al.*, 1989). An inhibition of wild-type coaggregation activity by RecA142 protein could also contribute to the inhibitory effects observed in the DNA strand exchange reaction. Therefore, the ability of mixtures of wild-type and mutant proteins to carry out coaggregation was also examined. As can be seen in Table 4, RecA142 protein does not inhibit the coaggregation activity of the wild-type protein; in fact, a slight enhancement is observed. Therefore, although the defect in coaggregation can be of significance to strand exchange reactions catalyzed

† When the PEP concentration was reduced while $Mg^{2+}$ concentration was maintained at 10 mM, reaction intermediates formed that failed to enter the agarose gel (not shown). Formation of these intermediates was time-dependent and required RecA protein and DNA homology. These intermediates were eventually converted into gapped duplex DNA product molecules but at a slow rate (requiring longer than 60 min). Formation of these intermediates was likely due to the reinitiation of strand exchange by the originally displaced DNA single-strand. Reduction of the magnesium ion concentration eliminated formation of these intermediates. Since, in the presence of these intermediates, branch migration was no longer rate limiting and quantitative analysis was more difficult, all experiments conducted at lower PEP concentrations used a lower magnesium acetate concentration as well.

**Table 4**
*Coaggregation properties of mixtures of wild-type and mutant RecA proteins*

| | | % in pellet | |
|---|---|---|---|
| [Wild-type] | [RecA142] | ssDNA | dsDNA |
| 2 | 0 | 88 | 90 |
| 2 | 2 | 97 | 100 |
| 0 | 2 | 15 | 2 |
| 1 | 0 | 91 | 78 |
| 1 | 1 | 100 | 98 |

Coaggregation was assayed as described in Materials and Methods; the reactions were carried out using a 10 mM-magnesium acetate preincubation step in the presence of SSB protein.

by RecA142 protein alone, a defect in coaggregation is *not* the cause of the significant inhibition observed for mixtures of the two proteins in the strand exchange reaction (Table 3).

When the effect of PEP concentration on coaggregation was examined, a surprising result was obtained. At either 10 mM-magnesium acetate and 7·5 mM-PEP or 6 mM-magnesium acetate and 3 mM-PEP, no coaggregation by wild-type RecA protein is detected. This result was unexpected since, under these conditions, DNA strand exchange readily occurs (Figs 7 and 9). Failure to detect coaggregates may indicate a lack of sensitivity of the coaggregation assay, but it also demonstrates that gross coaggregation as defined by this assay is not essential for efficient DNA strand exchange.

## 4. Discussion

In the accompanying paper (Kowalczykowski *et al.*, 1989), we showed that RecA142 protein is defective in DNA strand exchange activity. This inability is due to flaws in properties that are thought to underlie the presynaptic, synaptic, and postsynaptic steps of the DNA strand exchange mechanism. Since it was difficult to ascertain the importance of any steps that follow the presynaptic defect, we have subsequently examined the effect of RecA142 protein on the biochemical activities of wild-type RecA protein with the hope of further establishing their function in DNA heteroduplex formation. We find that RecA142 protein can interact with wild-type RecA protein to form a mixed complex, or filament, which displays altered biochemical properties. The evidence for protein–protein interactions between these two RecA proteins (rather than the alternative explanation of competitive binding for DNA-binding sites) is particularly apparent at protein concentrations that are subsaturating relative to the DNA concentration; under these conditions, competitive effects do not come into play. Depending on the activity monitored, either wild-type protein can enhance the activity of RecA142 protein, as seen in the ssDNA-dependent ATPase activity at elevated NaCl concentrations, or RecA142 protein can diminish the activity of wild-type protein, as seen in the DNA strand exchange activity. Thus, varied co-dominant effects on biochemical properties are observed.

Under appropriate conditions, RecA142 protein has a substantial inhibitory effect on the DNA strand exchange activity of wild-type RecA protein. Under these conditions, RecA142 protein also inhibits both the ssDNA-dependent ATPase activity of wild-type protein when SSB protein is present, and the dsDNA-dependent ATPase activity. These activities are associated with the presynaptic and postsynaptic steps of DNA strand exchange, respectively. In contrast, the coaggregation activity of mixtures of RecA142 and wild-type protein is unaltered, demonstrating that RecA142 protein does not inhibit this function of wild-type protein and that wild-type protein can complement this biochemical defect of RecA142 protein. This result is significant, since coaggregation activity is thought to function in the synaptic phase of DNA strand exchange. Consequently, the inhibition of DNA strand exchange in mixtures of RecA142 and wild-type proteins cannot be ascribed to blockage at the synaptic (coaggregation) step. Thus, as hoped, differential inhibitory effects of RecA142 protein on wild-type protein properties are observed and can be exploited to assess the role of events related to ssDNA and dsDNA-dependent ATP hydrolysis in the DNA strand exchange mechanism.

The inhibitory effects of RecA142 protein on both the DNA strand exchange and the DNA heteroduplex formation activities of wild-type protein can be substantial. At an equimolar concentration of the two proteins (e.g. 6 $\mu$M), both the modified gel assay and the nitrocellulose filter assay show that RecA142 protein inhibits the steady-state amount of joint molecules formed by approximately 50%; of these, only 7% undergo complete exchange of DNA strands as measured by the gel assay. Under identical conditions, the $S_1$ nuclease assay indicates that 36% of the dsDNA is heteroduplex. Together, these results demonstrate that most of the joint molecules have undergone only partial exchange of DNA strands. Consequently, RecA142 protein can inhibit all aspects of DNA heteroduplex formation, including the nascent exchange of DNA strands (joint molecule formation) and the subsequent extension (branch migration) of the joint molecules into products having completely exchanged DNA strands.

The inhibition of DNA heteroduplex formation by RecA142 protein must be related to the observed inhibition of either the ssDNA-dependent ATPase activity of wild-type RecA protein when SSB protein is present or the dsDNA-dependent ATPase activity, or both. The ssDNA-dependent ATPase activity in the presence of SSB protein is indicative of active presynaptic complex formation (Kowalczykowski & Krupp, 1987). Conditions that permit maximal ATPase activity coincide with those that permit DNA strand exchange to occur; conversely, conditions that permit complete inhibition of ssDNA-dependent ATPase activity by SSB protein correspond to those that prevent DNA strand exchange (Roman & Kowalczykowski, 1986). Though not proven, we assume that the partial inhibition of wild-type RecA protein ssDNA-dependent ATPase activity by RecA142 protein in the presence of SSB protein indicates formation of only partially active presynaptic complexes. The exact nature of these partially active complexes is uncertain, but it appears that the incorporation of RecA142 protein into a presynaptic filament with wild-type RecA protein results in the formation of a mixed filament that is unable to compete effectively with SSB protein. Consequently, SSB protein can displace some of the RecA protein from the ssDNA,

resulting in a partially functional presynaptic filament. The interspersed binding of SSB protein within the filament prevents participation in DNA strand exchange unless the SSB protein is displaced.

The effects of PEP concentration on the sensitivity of the mixed RecA protein–ssDNA complexes to inhibition by SSB protein were unexpected. Previously, PEP was observed to reduce the stability of wild-type RecA protein–etheno M13 ssDNA complexes, as measured by the salt concentration required to half-dissociate this complex (Menetski *et al.*, 1988). Higher PEP concentrations lowered the salt concentration. This effect was observed to be competitive with ATP concentration and not due to potential chelation of $Mg^{2+}$ by PEP. Consistent with this observation, we find that the inhibitory effects of PEP on the ssDNA-dependent ATPase activity of the RecA protein mixtures, in the presence of SSB protein, are more pronounced at a lower ATP concentration (Kowalczykowski & Krupp, unpublished results). Thus, it appears that PEP is a competitive inhibitor of ATP binding. Though we had observed a slight effect of PEP concentration on the etheno M13 ssDNA binding properties, no effect on the enzymatic activities of wild-type protein was noted. However, we can now detect effects of PEP concentration on certain RecA protein-dependent properties; e.g. the rate of SSB protein dissplacement from ssDNA is somewhat PEP concentration-sensitive (Lavery & Kowalczykowski, unpublished results); and the properties of the mutant RecA430 protein are sensitive to PEP concentration (Menetski & Kowalczykowski, unpublished results). The one characteristic common to the experimental conditions which exhibit sensitivity to PEP concentration is that they are suboptimal for RecA protein function. Therefore, if a RecA protein-dependent activity is near a threshold for proper function, then a higher concentration of PEP may exert an inhibitory effect that is competitive with ATP concentration. Unfortunately, these results demonstrate that PEP is not simply a passive component of the ATP-regenerating system (preliminary etheno M13 ssDNA binding studies indicate that the substrate used in an alternative ATP-regenerating system, phosphocreatine, does not affect the salt titration midpoint; Menetski & Kowalczykowski, unpublished results). However, with the appropriate controls, PEP concentration can be utilized as an experimental variable in much the same manner as NaCl or magnesium chloride concentration is used.

Though the inhibitory effects of SSB protein on the mixed RecA protein presynaptic filaments may be sufficient to explain the failure to observe complete exchange of DNA strands, the dsDNA-dependent ATPase activity of mixed protein complexes is also reduced and may contribute to the strand exchange defect at a postsynaptic stage. RecA142 protein affects both the lag time and the steady-state rate of dsDNA-dependent ATP hydrolysis. Previously, it was noted that a correlation existed between the *amount* of product formed in a strand exchange reaction and the *amount* of ATP hydrolyzed in the dsDNA-dependent hydrolysis reaction (Roman & Kowalczykowski, 1986). Since the rate of branch migration is the rate-limiting step in the DNA strand exchange reaction, it was suggested that the dsDNA-dependent ATPase activity and branch migration were related. A model for that relationship was presented (Kowalczykowski, 1987). Though the initial correlation was between the amount of ATP hydrolyzed (in the dsDNA-dependent reaction) and the amount of strand exchange product formed, it could be inferred that a similar parallel might exist between the rates of these two processes. However, the data presented here demonstrate that, although the rate of DNA heteroduplex formation by the wild-type protein is reduced when RecA142 protein is present, the reduction is not as great as that observed for the rate of dsDNA-dependent ATP hydrolysis (though the effects on the lag phase are less). This result means that the rate of DNA heteroduplex formation (i.e. branch migration) does *not* correlate with the rate of dsDNA-dependent ATPase activity. How is dsDNA-dependent ATPase activity related to DNA heteroduplex formation? A molecular view of this relationship has already been presented (Kowalczykowski & Krupp, 1987; Kowalczykowski, 1987) and was evolved from the following logic. Initially, RecA protein is bound to ssDNA in the presynaptic complex, and all of the ATP hydrolysis is ssDNA-dependent; however, after exchange of DNA strands, RecA protein is now associated with the newly formed heteroduplex dsDNA. At this point, the observed dsDNA-dependent ATP hydrolysis is a reflection of the *amount* of heteroduplex DNA formed in the DNA strand exchange reaction; the proportionality constant relating these two activities is a function of the experimental conditions used. Thus, it appears that the dsDNA-dependent ATP hydrolysis reaction serves to monitor (in an after-the-fact manner) the extent of DNA heteroduplex formation.

Elsewhere, we have proposed that dsDNA-dependent ATP hydrolysis occurs *after* a dsDNA opening event that is crucial to joint molecule formation and DNA strand exchange (Kowalczykowski *et al.*, 1987; Kowalczykowski, 1987). We proposed that the ATP-bound form of RecA protein, which has a high affinity for ssDNA (Menetski & Kowalczykowski, 1985), is responsible for a dsDNA opening event. In a limiting case, ATP hydrolysis is *not* required for the dsDNA opening event but is merely a consequence of that opening event. Recent data suggest that this limiting case is, in fact, correct (Menetski, 1988; Menetski & Kowalczykowski, unpublished results). This view is also consistent with the biochemical properties of RecA142 protein. RecA142 protein cannot catalyze joint molecule formation or branch migration by itself, and it inhibits catalysis of both of these

activities by the wild-type protein. Since it is defective in the ATP-dependent induction of the characteristic high-affinity ssDNA binding state (Menetski *et al.*, 1988), RecA142 protein is unable to promote the ATP-dependent opening of dsDNA necessary for dsDNA-dependent ATPase activity. Because joint molecule formation, branch migration and dsDNA-dependent ATPase activity require a dsDNA opening step, it is likely that all of these RecA142 protein defects result from the same root cause, namely the failure to promote an ATP binding-induced opening of dsDNA. The observation that RecA142 protein alone cannot promote DNA strand exchange in the absence of SSB protein (Kowalczykowski *et al.*, 1989) is consistent with this interpretation, especially in light of the observation here that coaggregation is not essential for DNA strand exchange. Since, in the absence of SSB protein, RecA142 protein exhibits normal ssDNA-dependent ATPase activity, it is reasonable to conclude that failure to promote DNA strand exchange is due to defective interactions with dsDNA, which are manifest as a reduction in the dsDNA-dependent ATPase activity.

A surprising effect of RecA142 protein on the strand exchange activity of the wild-type protein is the observation that the mutant protein exerts an effect on the final yield of DNA molecules which have undergone complete strand exchange. This effect is evident in Figures 7 and 8, which show that the endpoint of the reaction is dramatically affected; longer incubation times (90 to 120 min) have little effect on the yield of the reaction. In this regard, the RecA142 protein is acting formally as an irreversible inhibitor of RecA protein-catalyzed DNA heteroduplex formation rather than as a simple competitive inhibitor. This result is even more curious because not all of the enzymatic activities of the wild-type protein are inhibited in a comparable irreversible manner (e.g. ssDNA-dependent ATPase activity in the absence of SSB protein or coaggregation activity). However, both the ssDNA-dependent ATPase activity in the presence of SSB protein and the dsDNA-dependent ATPase activity are inhibited by RecA142 protein. This parallel in inhibition behavior further implies that the events that underlie these ATP hydrolytic activities are important to DNA heteroduplex formation.

A possible molecular explanation for this unexpected inhibition pattern relies on the filamentous nature of RecA protein–DNA complexes. Since RecA142 protein inhibits DNA strand exchange promoted by wild-type RecA protein, even under conditions where the number of DNA-binding sites is in excess over the total RecA protein concentration, incorporation of the mutant protein within the wild-type protein filament must be occurring. This mixed RecA protein filament, in turn, must be defective in growth of the DNA heteroduplex joint. On the basis of the data presented here, irreversible inhibition of DNA heteroduplex extension could result from either defective presynaptic complex formation or defective postsynaptic (filament-dependent) branch migration. In either case, the presence of a RecA142 protein (or some contiguous cluster of RecA142 proteins) may prevent further elongation of the filament (for either kinetic or equilibrium reasons), resulting in a physical discontinuity in the filament; a second possibility is that the filament is physically continuous but is functionally discontinuous due to incorporation of non-functional RecA142 protein molecules. The net effect is that DNA heteroduplex formation is prevented when the RecA142 protein molecule(s) is encountered. For unknown reasons, reinitiation beyond the discontinuity is prevented. Further studies of the structure, composition and enzymatic behavior of mixed mutant and wild-type filaments of RecA protein should provide considerable insight into the functioning of this protein.

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## References

Kowalczykowski, S. C. (1987). *Trends Biochem. Sci.* **12**, 141–145.

Kowalczykowski, S. C. & Krupp, R. A. (1987). *J. Mol. Biol.* **193**, 97–113.

Kowalczykowski, S. C., Clow, J. & Krupp, R. A. (1987). *Proc. Nat. Acad. Sci., U.S.A.* **84**, 3127–3131.

Kowalczykowski, S. C., Burk, D. L. & Krupp, R. A. (1989). *J. Mol. Biol.* **207**, 719–733.

Menetski, J. P. (1988) Ph.D. thesis, Northwestern University.

Menetski, J. P. & Kowalczykowski, S. C. (1985). *J. Mol. Biol.* **181**, 281–295.

Menetski, J. P. & Kowalczykowski, S. C. (1989). *Biochemistry,* in the press.

Menetski, J. P., Varghese, A. & Kowalczykowski, S. C. (1988). *Biochemistry,* **27**, 1205–1212.

Moreau, P. L. & Roberts, J. W. (1984). *Mol. Gen. Genet.* **198**, 25–34.

Ogawa, T., Wabiko, H., Tsurimoto, T., Horii, T., Masukata, H. & Ogawa, H. (1978). *Cold Spring Harbor Symp. Quant. Biol.* **43**, 909–916.

Rebollo, J. E., Moreau, P. L., Blanco, M. & Devoret, R. (1984). *Mol. Gen. Genet.* **195**, 83–89.

Roman, L. J. & Kowalczykowski, S. C. (1986). *Biochemistry,* **25**, 7375–7385.

Rusche, J. R., Konigsberg, W. & Howard-Flanders, P. (1985). *J. Biol. Chem.* **260**, 949–955.

Schutte, B. C. & Cox, M. M. (1987). *Biochemistry,* **26**, 5616–5625.

Sedgwick, S. G. & Yarranton, G. T. (1982). *Mol. Gen. Genet.* **185**, 93–98.

Yancey, S. D. & Porter, R. D. (1984). *Mol. Gen. Genet.* **193**, 53–57.

Yarranton, G. T. & Sedgwick, S. G. (1982). *Mol. Gen. Genet.* **185**, 99–104.

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