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Ionic inhibition of formation of RecA nucleoprotein networks blocks homologous pairing

(DNA aggregation/recombination)

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ABSTRACT **Conditions that favor the complete coating of single-stranded DNA by RecA protein promote the association of these presynaptic filaments with naked double-stranded DNA to form large nucleoprotein networks before homologous pairing occurs. These RecA nucleoprotein networks sequester virtually all of the DNA in the reaction mixture. Conditions that are suboptimal for the formation of the RecA presynaptic filament rendered both the formation of RecA–DNA networks and the subsequent formation of joint molecules sensitive to inhibition by excess ATP or by pyrophosphate when these were added during synapsis. The rate of homologous pairing was directly related to the degree of inhibition of network formation. Various multivalent cations added during synapsis restored both the formation of networks and the pairing of homologous molecules. These observations support the view that the nucleoprotein network is a synaptic intermediate by means of which RecA protein facilitates the conjunction of DNA molecules and the subsequent processive search for homology. Inhibition by multivalent anions and restoration by multivalent cations suggests in addition, that negative charge repulsion inhibits the binding of naked duplex DNA to presynaptic filaments.**

Escherichia coli RecA protein promotes two kinds of homologous pairing of DNA molecules *in vitro*, each of which may play a direct role in genetic recombination—namely, the renaturation of complementary single strands (1) and the formation of joint molecules from single-stranded or partially single-stranded DNA plus duplex DNA, which is called strand invasion (2–4). Strand invasion is an ordered process in which at least three sequential phases can be distinguished. (*i*) A slow *presynaptic phase* consists of the polymerization of RecA protein on single-stranded DNA (5–9), a reaction that is hindered by secondary structure in the single strands (10). (*ii*) A rapid *synaptic phase* can be subdivided into two sequential steps—namely, *conjunction*, the coming together of single-stranded and double-stranded DNA that is mediated by RecA protein independent of homology, and *homologous alignment*, which occurs at least in part by facilitated diffusion within the complex of DNA and protein that results from conjunction (11). (*iii*) A slow *postsynaptic phase* of strand exchange produces heteroduplex DNA and correspondingly displaces an old strand (7, 12, 13).

Some of the earliest observations on homologous pairing promoted by RecA protein indicated that single-stranded DNA plays a special role in the interaction of RecA protein with duplex DNA. Single-stranded DNA was seen to stimulate the partial unwinding of duplex DNA without strand separation in the presence of adenosine 5′-[γ-thio]triphosphate (ATP[γS]) (14), the protection of double-stranded DNA from nuclease digestion (15), and the binding of duplex DNA in a form that was trapped by nitrocellulose filters (16–18). Correspondingly, kinetic studies showed that the pairing of a single strand with duplex DNA by RecA protein was not second order like renaturation but rather behaved like an enzymatic process in which the further reaction of an enzyme–DNA complex limited the rate at saturation (11, 19, 20). We have reported that presynaptic complexes, which are polyvalent with regard to the binding of duplex DNA, cause the formation of rapidly sedimenting coaggregates or networks containing RecA protein, single-stranded DNA, and double-stranded DNA (8). Recently, Rusche *et al.* (21) also observed that single strands stimulate the association of double-stranded DNA with RecA protein in complexes that are trapped by nitrocellulose filters and that sediment rapidly.

In the absence of single strands, RecA protein does not cause the rapid aggregation of duplex DNA; but under one set of conditions, it causes the rapid aggregation of single strands in the absence of duplex DNA, and under another set of conditions, it causes rapid mutually dependent coaggregation of single- and double-stranded DNA (22). Furthermore, these two sets of conditions correspond respectively to those that are optimal for the pairing by RecA protein of complementary single strands—i.e., renaturation—and those that are optimal for the pairing by RecA protein of single strands with duplex DNA—i.e., strand invasion (1, 23, 24). The characterization of RecA nucleoprotein networks (22) together with the experiments reported here support the conclusion that these large networks are dynamic synaptic intermediates in both renaturation and strand invasion.

METHODS

Enzymes and DNA. RecA protein was purified as described by Shibata *et al.* (25). Single-stranded DNA binding protein (SSB) was kindly provided by John Chase of the Albert Einstein College of Medicine, New York. Restriction endonucleases *Hin*cII, *Pst* I, and *Sau*96 I were purchased from New England Biolabs. Creatine phosphokinase was obtained from Sigma. Disodium ATP was purchased from Pharmacia P-L Biochemicals.

Circular single-stranded and circular duplex DNA from phages φX174 and M13 were prepared as described (12, 26). The preparations of circular single-stranded DNA contained less than 5% linear molecules as determined by gel electrophoresis in 1.8% agarose. The method of Kuhnlein *et al.* (27) was used to determine that the percentage of nicked molecules in the superhelical DNA (form I) preparation was less than 10%. This circular duplex DNA was linearized by respective restriction endonucleases under standard conditions specified by the supplier. Concentrations of DNA are expressed in moles of nucleotide residues.

Standard Reaction Conditions. Reactions were performed in 0.5-ml Eppendorf tubes set in a 37°C water bath. Unless

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Abbreviation: SSB, single-stranded DNA binding protein.

otherwise indicated, the preincubation mixture contained 3.0 μM circular single strands in 33 mM Tris·HCl, pH 7.5/1.3 mM ATP/3 mM phosphocreatine/10 units of phosphocreatine kinase per ml/88 μg of bovine serum albumin (nuclease free, Bethesda Research Laboratories) per ml/1.8 mM dithiothreitol/3 mM $MgCl_2$. After 2 min of incubation to bring the reaction mixture up to 37°C, 2.1 μM RecA protein was added, and the mixture was incubated for an additional 10 min at 37°C. In experiments where SSB was present, 0.3 μM SSB was introduced to the incubation mixture 30 sec after RecA protein. After the 10-min preincubation, 6.0 μM linear duplex DNA and 10.3 mM $MgCl_2$ were added to start the formation of coaggregates and joint molecules.

Assay for Joint Molecules. This assay measures the retention by nitrocellulose filters of duplex DNA that has attached to single-stranded or partially single-stranded DNA (28). At the specified times after the addition of linear duplex [^{3}H]DNA to the reaction mixture, an aliquot of 10 μl was taken and placed directly into 12 × 75 mm culture tubes containing 200 μl of ice-cold 25 mM EDTA (pH 7.5). This was followed 2 min later with 4.0 ml of ice cold 1.5 M NaCl/0.15 M sodium citrate. The solution was immediately filtered through a nitrocellulose filter (Sartorius, type SM11306; 0.65 μm) that had been soaked in 1.5 M NaCl/0.15 M sodium citrate. The filters were then rinsed with 6 ml of 1.5 M NaCl/0.15 M sodium citrate, dried under a heat lamp, and assayed by scintillation counting for bound radioactivity.

Assay for Coaggregation of DNA. This assay measures homology-independent conjunction of single- and double-stranded DNA in complexes that sediment at more than 10,000 S (22). At the appropriate times after the addition of duplex [^{3}H]DNA, a 30-μl aliquot of the reaction mixture was centrifuged at room temperature in a 0.5-ml Eppendorf tube at 15,000 × *g* for 2 min (Brinkmann model 5414). From the supernatant fraction, three sequential 9-μl aliquots were taken. From the average concentration of radioactivity in the first two fractions, we estimated the total radioactivity in 30 μl of supernatant. The remaining 3 μl of supernatant and the pellet were resuspended in 200 μl of distilled water, and the radioactivity was determined. A correction was made for radioactivity attributable to the 3 μl of supernatant that was not separated from the pellet. In every experiment, the recovery of labeled DNA in the supernatant and pellet was 95% or greater, and residual radioactivity on the wall of the tube was less than 5%. To eliminate any contribution of homologous pairing to the formation or properties of coaggregates, we used only heterologous combinations of single- and double-stranded DNA to study coaggregation.

RESULTS

Experimental Design. Earlier results indicated that RecA protein promotes homologous pairing by binding first to single-stranded DNA to form presynaptic nucleoprotein filaments (5, 16, 29). However, secondary structure in single-stranded DNA markedly impedes the binding of RecA protein (10); even in 3 mM $MgCl_2$, half as much RecA protein was found in isolated presynaptic filaments compared with filaments formed in 1 mM $MgCl_2$ (ref. 29; Table 1). We observed that the subsequent pairing of such unsaturated filaments with duplex DNA is susceptible to inhibitory and counterinhibitory effects of various small molecules that depended upon the time of addition. Exploration of these effects provided an opportunity to alter widely the rate of homologous pairing by several distinctly different mechanisms and to correlate these changes in rate with the ability of RecA protein to bring together single-stranded and double-stranded DNA in large coaggregates or nucleoprotein networks. In the experiments described below, we assayed the formation of joint molecules by the D-loop assay, which measures homology-dependent pairing, and we assayed coaggregation by centrifugation, which measures the homology-independent conjunction of single-stranded and double-stranded DNA.

Table 1. Countervailing effects of ATP and $MgCl_2$ on the binding of RecA protein to single-stranded DNA

ATP, mM	$MgCl_2$, mM	Binding ratio, RecA protein/nucleotide residues
1.3	1.0	1:4.1
1.3	3.0	1:10.4
1.3	13.3	1:14.6
3.0	3.0	1:6.7
5.0	3.0	1:4.0

Presynaptic complexes were formed by incubating circular single-stranded M13 [^{3}H]DNA with ^{35}S-labeled RecA protein for 10 min at 37°C in the reaction mixture containing the indicated concentrations of ATP and $MgCl_2$. The presynaptic complexes were then isolated by filtration through a Sepharose 2B-300 column (1.7 ml, 0.35 × 8.5 cm) at room temperature as described (29). Twenty-five fractions of 150 μl each were collected at a rate of about 1 min per fraction. The recoveries of single-stranded DNA and RecA protein in the eluate for all experiments were 91.7 ± 1.4% and 90.5 ± 1.3%, respectively (mean ± SEM, n = 10).

Inhibition of Coaggregation and Homologous Pairing by ATP or Pyrophosphate Added During Synapsis. Single-stranded DNA was preincubated with RecA protein in the presence of 1.3 mM ATP and 3 mM $MgCl_2$. The pairing reaction was initiated by the addition of duplex DNA and more $MgCl_2$. In reactions where the addition of duplex DNA was accompanied simultaneously by raising the final ATP concentration from 1.3 to 5–10 mM, both the rate and yield of D-loop formation were drastically reduced (Fig. 1*A*). As demonstrated previously (8, 22), the formation of joint molecules is preceded by the formation of coaggregates that contain RecA protein and virtually all of the DNA in the reaction mixture (Fig. 1). Coaggregation was abolished by extra ATP or by pyrophosphate added at the beginning of synapsis, which we attribute to an effect of multivalent anions (Fig. 1*B*; unpublished observations). Moreover, extra ATP, added 2 min after the formation of joint molecules had been started, immedi-

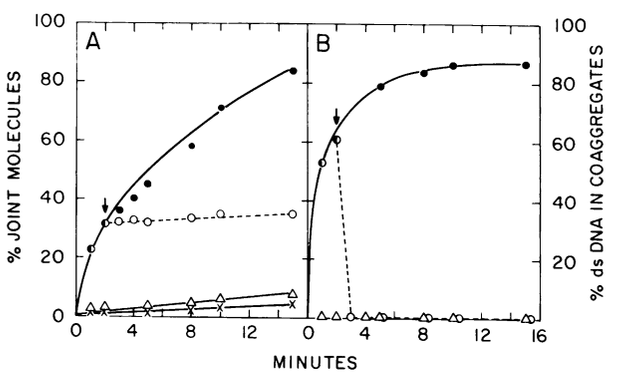

FIG. 1. Inhibition of both coaggregation and homologous pairing by the addition of extra ATP during synapsis. (*A*) Joint-molecule formation. Circular single-stranded M13 DNA was preincubated for 10 min at 37°C in 1.3 mM ATP and 3 mM $MgCl_2$ as described. After the preincubation, we initiated the pairing reaction by adding linear duplex M13 [^{3}H]DNA (M13 form I DNA cleaved with restriction endonuclease *Hin*cII) and 10.3 mM $MgCl_2$ and adjusting the final concentration of ATP to 1.3 mM (●), 5.0 mM (△), or 10.0 mM (×). In one experiment (○), the concentration of ATP was raised to 5.0 mM at 2 min (indicated by arrow) after the pairing reaction had begun. (*B*) Coaggregate formation. Conditions were identical to those described in *A* except that circular single-stranded M13 DNA was used to form coaggregates with linear duplex φX174 [^{3}H]DNA (φX174 form I DNA linearized with restriction endonuclease *Pst* I). Symbols have the same significance as in *A*. ds, Double-stranded.

ately dissolved already existing coaggregates and stopped the further formation of joint molecules (Fig. 1).

The degree of inhibition of the formation of both coaggregates and joint molecules depended on the concentration of ATP added during synapsis. The initial rates of D-loop formation, when calculated from the results shown in Fig. 2*A*, decreased from 13.8 to 1.1 nM/sec when the final concentration of ATP in the reaction mixture was increased from 1.3 to 5 mM. Both the yield and sedimentation rate of coaggregates fell at increasing concentrations of ATP (Fig. 2*B*). The slower rate of sedimentation of coaggregates seen with increasing concentrations of ATP is consistent with a proportional decrease in the steady-state concentration of nonspecific contacts between presynaptic filaments and duplex DNA (Fig. 2*B*).

Joint Restoration of Coaggregation and Homologous Pairing by Conditions That Optimize the Formation of Presynaptic Filaments. Addition of SSB, or preincubation of single-stranded DNA with RecA protein in 1 mM $MgCl_2$ has been shown to remove secondary structure from single strands and to promote complete coating of single-stranded DNA with RecA protein (refs. 9, 10, and 29; Table 1). The formation of presynaptic complexes under these conditions protected both coaggregation and the subsequent formation of joint molecules from the inhibitory action of extra ATP added during synapsis (Fig. 3 *A* and *B*). Similar counterinhibition by SSB or by preincubation of single-stranded DNA in 1 mM $MgCl_2$ was observed when 2 mM pyrophosphate was used as the inhibitory agent instead of 5 mM ATP (data not shown).

When single-stranded DNA was preincubated with RecA protein in 3 mM $MgCl_2$ but the concentration of ATP was raised to 5 mM, the additional ATP promoted the formation of saturated presynaptic filaments (Table 1), and there was no subsequent inhibitory effect of the high concentration of ATP

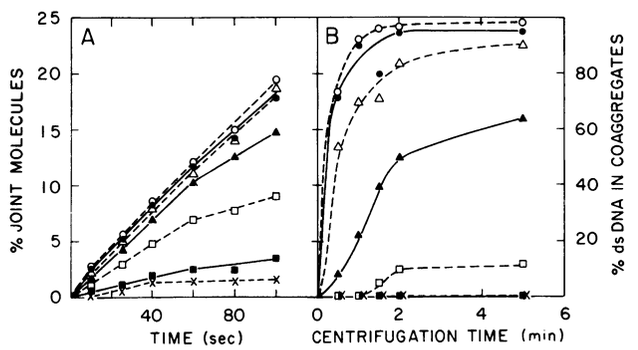

FIG. 2. Correlation of the rate of homologous pairing with the yield and sedimentation rate of coaggregates. (*A*) Presynaptic filaments containing ϕX174 single strands were formed in 1.3 mM ATP and 3 mM $MgCl_2$ as described, and the pairing reaction was started by adding linear duplex ϕX174 [^{3}H]DNA (ϕX174 form I DNA cleaved with restriction endonuclease *Pst* I) and 10.3 mM $MgCl_2$. Immediately after the addition of duplex DNA, the final ATP concentration was adjusted to 1.3 (○), 2.0 (●), 2.5 (△), 3.0 (▲), 3.5 (□), 4.0 (■), or 5.0 (×) mM. The time plotted on the abscissa is elapsed time during the pairing reaction. The slope of each curve is the rate of homologous pairing at the indicated concentration of ATP. (*B*) Coaggregates were formed by mixing linear duplex ϕX174 [^{3}H]DNA (ϕX174 form I DNA linearized with restriction endonuclease *Pst* I) with presynaptic filaments containing circular single-stranded M13 DNA, and the final concentration of ATP was adjusted to various levels as described in *A* immediately after the addition of duplex DNA. Five minutes after initiation of the reaction, we assayed coaggregates by centrifuging aliquots of the reaction mixture for various lengths of time as indicated in the figure. Unlike in *A*, the time shown is the sedimentation time, and the data show that coaggregates that were formed at higher concentrations of ATP sedimented more slowly. ds, Double-stranded.

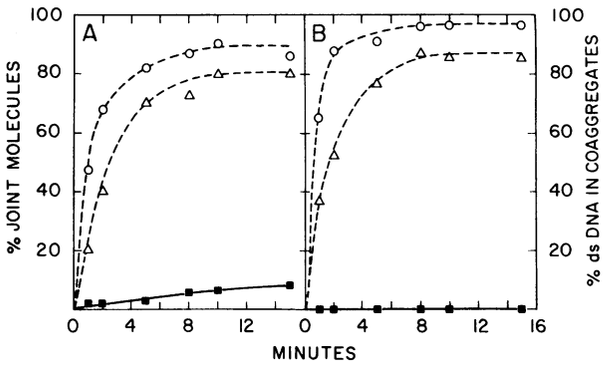

FIG. 3. Joint restoration of coaggregation and homologous pairing by conditions that optimize the formation of presynaptic filaments. (*A*) Circular single-stranded M13 DNA was preincubated in 1.3 mM ATP and 1 (△) or 3 (■) mM $MgCl_2$ in the absence of SSB or in 3 mM $MgCl_2$ in the presence of SSB (○) for 10 min at 37°C. The formation of joint molecules was initiated by adding linear duplex M13 [^{3}H]DNA (M13 form I DNA cleaved with restriction endonuclease *Sau*96 I) and adjusting the final $MgCl_2$ and ATP concentrations to 13.3 and 5.0 mM, respectively. (*B*) Identical procedure and legend as described in *A* except that coaggregates were formed using circular single-stranded M13 DNA and linear duplex ϕX174 [^{3}H]DNA (ϕX174 form I DNA linearized with restriction endonuclease *Pst* I). ds, Double-stranded.

on either coaggregation or homologous pairing (data not shown).

Joint Restoration of Coaggregation and Homologous Pairing by Ionic Conditions That Are Effective Only During Synapsis. In addition to SSB and preincubation of single-stranded DNA in 1 mM $MgCl_2$, two other means were shown to be effective in overcoming the inhibitory action of extra ATP or pyrophosphate on the formation of coaggregates and joint molecules. These were addition of spermidine or high concentrations of $MgCl_2$ at the time of addition of duplex DNA (Fig. 4 *A* and *B*; unpublished observations). The concentrations and the time of addition of cations were critical. At 5 mM ATP,

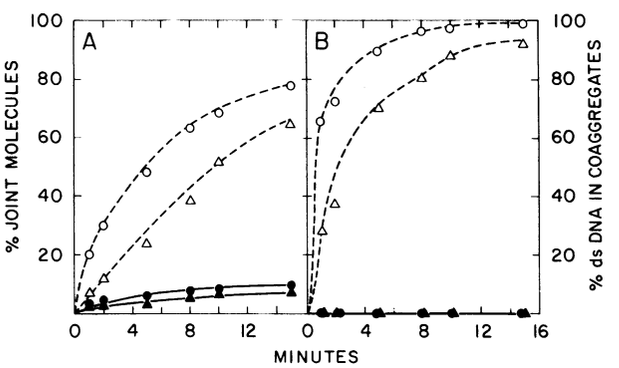

FIG. 4. Joint restoration of coaggregation and homologous pairing by ionic conditions that are effective only during synapsis. (*A*) Joint-molecule formation was initiated by adding linear duplex M13 [^{3}H]DNA (M13 form I DNA cleaved with restriction endonuclease *Sau*96 I) to an incubation mixture containing circular single-stranded M13 DNA, 1.3 mM ATP, and 3 mM $MgCl_2$. Immediately after the addition of duplex DNA, the final concentrations of $MgCl_2$ and ATP were adjusted to 13.3 and 5.0 mM, respectively, and 2 mM spermidine (○) or an equal volume of H_2O (●) was added to the reaction mixture. In another experiment, after the addition of duplex DNA, the final concentration of ATP was raised to 5.0 mM and the final concentration of $MgCl_2$ was adjusted to 25 (△) or 13.3 (▲) mM, and no spermidine was added. (*B*) Conditions and symbols are identical to those in *A* except that coaggregates were formed using circular single-stranded M13 DNA and linear duplex ϕX174 [^{3}H]DNA (ϕX174 form I DNA linearized with restriction endonuclease *Pst* I). ds, Double-stranded.

the optimal concentrations for spermidine and $MgCl_2$ to block the inhibitory action of ATP were 2 and 25 mM, respectively. The counterinhibitory effects of these cations were effective only when they were introduced during the synapsis between RecA nucleoprotein filaments and duplex DNA. Addition of 2 mM spermidine or 25 mM $MgCl_2$ during the formation of presynaptic complexes actually decreased the rate and yield of D-loop formation, presumably by favoring secondary structures in single-stranded DNA and decreasing the amount of bound RecA protein (ref. 10; unpublished observations). As a control, in the presence of 1.3 mM ATP, introduction of spermidine or high concentration of $MgCl_2$ alone during synapsis had no effect on coaggregate or joint-molecule formation (data not shown). Finally, other divalent cations (Mn^{2+} and Co^{2+}) as well as another polyamine (spermine) were effective in alleviating the inhibition produced by ATP whereas NaCl was ineffective (data not shown). Thus, inhibition of synapsis appears to be attributable to multivalent anions, and counterinhibition, to multivalent cations.

DISCUSSION

Previous observations showed that RecA protein causes single-stranded DNA to aggregate when the DNA is incompletely coated with RecA protein. More fully coated single strands no longer aggregate by themselves, but since they have many molecules of RecA protein and a corresponding multiplicity of binding sites for duplex DNA, these nucleoprotein filaments are polyvalent reagents that form large coaggregates with duplex DNA (refs. 8, 21, and 22; see Fig. 5). We found that filaments formed in 3 mM $MgCl_2$ and 1.3 mM ATP are in an intermediate state that forms coaggregates and joint molecules but contains less RecA protein than do apparently saturated complexes (Table 1). Unsaturated filaments formed joint molecules more slowly than filament formed under conditions that favor saturation, and unsaturated filaments were especially sensitive to inhibition by pyrophosphate or by more ATP when either of these was added together with the duplex DNA. Such observations formed the basis for a number of tests that in every case showed a positive correlation between the ability to form coaggregates and the ability to form joint molecules.

Three different treatments applied during the formation of presynaptic complexes alleviated the inhibition of both coaggregation and homologous pairing: these treatments were addition of more ATP itself, reduction of $MgCl_2$ to 1 mM, or addition of SSB. All of these treatments cause more complete coating of single-stranded DNA (ref. 29; Table 1 and Fig. 5). Thus, ATP added early had the opposite effect of ATP added late. Two different treatments applied at the time of addition of duplex DNA also relieved the inhibition of both coaggregation and homologous pairing: these were the addition of polyamines or high concentrations of various divalent cations, including Mg^{2+}. By contrast, a high concentration of $MgCl_2$ (29) or 2 mM spermidine (data not shown) added during the formation of presynaptic complexes inhibited the subsequent formation of joint molecules. Thus addition of each of $MgCl_2$, spermidine, and ATP during presynapsis had opposite effects to addition during synapsis, which suggests that in each case the mechanism of inhibition was different from the mechanism of counterinhibition. We conclude that the inhibitory and counterinhibitory effects can be divided into two classes, those that operate directly on the formation of presynaptic complexes and those that involve ionic interactions that are important for synapsis (Fig. 5).

As a working hypothesis, we propose that the inhibition caused by increased ATP or by pyrophosphate added during synapsis is an ionic effect of multivalent anions and that the counterinhibition exerted by polyamines or by divalent cations is a shielding effect. Presumably, there is a negative charge repulsion in the approach of naked duplex DNA to RecA presynaptic filaments. This same charge repulsion

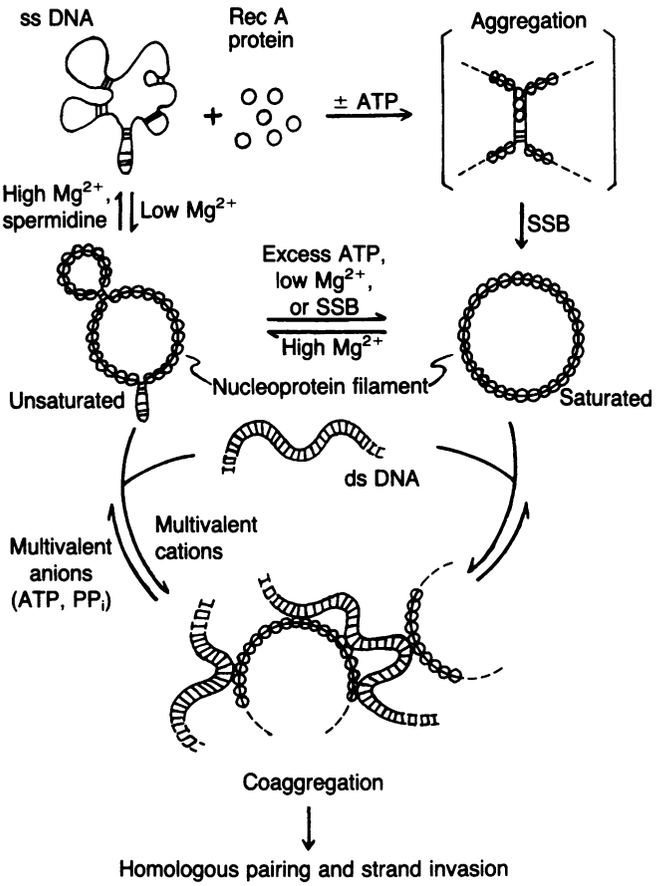

FIG. 5. Interpretation of ionic effects on coaggregation and homologous pairing. RecA protein, by two distinct pathways, promotes, respectively, the aggregation of single strands and the coaggregation of single-stranded (ss) plus double-stranded (ds) DNA (8, 22). Conditions that are optimal for the aggregation of single strands by RecA protein are correlated with those that are optimal for renaturation of complementary single strands (8, 17, 22, 24). By contrast, conditions that suppress the independent aggregation of single strands favor instead the mutually dependent coaggregation of single-stranded and double-stranded DNA and correlate with the conditions that favor strand invasion (8, 22). In the presynaptic reaction, RecA protein polymerizes on single-stranded DNA to form a nucleoprotein filament (5–9). Factors that stabilize secondary structure in single-stranded DNA—e.g., Mg^{2+} and spermidine—hinder the binding of RecA protein (10, 23). Under the conditions described in this report—i.e., 1.3 mM ATP and 3 mM Mg^{2+}—single strands are incompletely coated by RecA protein (Table 1). Factors that destabilize secondary structure, such as a low concentration of Mg^{2+} (1 mM) or addition of SSB, favor saturation of single strands by RecA protein (10). Excess ATP (5 mM), added during the presynaptic phase, also favors saturation (Table 1), either by reducing the concentration of free Mg^{2+} ions or by driving the binding of RecA protein. To initiate synapsis, duplex DNA is added to nucleoprotein filaments, which results in the rapid formation of coaggregates and, subsequently, homologous pairing and strand invasion. Addition of multivalent anions, including ATP (5 mM) and pyrophosphate (PP_i), during the synaptic phase inhibits both coaggregation and homologous pairing, whereas multivalent cations, including Mg^{2+}, Co^{2+}, and Mn^{2+}, spermidine, and spermine, simultaneously restore coaggregation and homologous pairing. At higher concentrations of multivalent ions, saturated RecA nucleoprotein filaments are susceptible to similar effects as unsaturated filaments. Inhibition of coaggregation and homologous pairing between saturated nucleoprotein filaments and duplex DNA has been observed by adding 10 mM ATP during synapsis, and the inhibition was similarly countered by the addition of Mg^{2+} and spermidine (unpublished data).

probably occurs but is less significant quantitatively when presynaptic filaments are formed under more ideal conditions (see the legend to Fig. 5). Experiments related to the mechanisms of inhibition and counterinhibition will be described elsewhere.

By varying the concentration of ATP added as an inhibitor, we explored further the relation between coaggregation and homologous pairing. We observed a direct correlation between the sedimentation rate and yield of coaggregates on the one hand and the rate of homologous pairing on the other (Fig. 2). At concentrations of ATP that completely eliminated the formation of rapidly sedimenting coaggregates, the initial rate of formation of joint molecules was reduced 10-fold below the optimal rate (Fig. 2).

Elsewhere we have described observations that characterize the formation and properties of aggregates containing single-stranded DNA and of coaggregates that also contain duplex DNA (22). The conditions that promote aggregation of single strands by RecA protein correspond to those that favor the pairing of complementary single strands. Incomplete coating of single strands by RecA protein favors aggregation and renaturation; both processes are inhibited by SSB (17, 22) but are relatively insensitive in inhibition by ADP, and neither has an absolute requirement for ATP (22, 24). By contrast, complete coating of single strands by RecA protein blocks aggregation but favors instead the mutually dependent coaggregation of single strands and duplex DNA. Both coaggregation and the pairing of single strands with duplex DNA require ATP, both are promoted by SSB, and both are sensitive to inhibition by ADP and salt.

Coaggregates, which sequester all of the DNA in solution, constitute domains that exchange DNA more slowly than they give rise to joint molecules. Using genetically and radioactively labeled DNA, we found that molecules that had to exchange between separately formed coaggregates in order to pair with their genetic complements were incorporated into joint molecules at an initial rate that was nearly 1 order of magnitude less than the incorporation of molecules that were not required to exchange (22). These kinetic observations, taken together with the experiments described here, support the view that coaggregates are not side products but rather are intermediates on the path of formation of joint molecules. Preliminary results (unpublished data) indicate that the positive effect of molecular length on the rate of homologous pairing (11) correlates with effects of molecular length on the formation of coaggregates and the exchange of duplex DNA, which further suggests that coaggregates play a direct role in accelerating the search for homology.

The correlations between the properties of aggregation and renaturation, on the one hand, and between the properties of coaggregation and strand invasion on the other (see above), enable one to understand the basis for the observed differences between renaturation and strand invasion as well as their similar first-order kinetics (11, 24). Both processes can be seen to depend upon conditions that are optimal for bringing the respective DNA molecules together. More significantly, however, the observed correlations support the concept that, in both renaturation and strand invasion, RecA protein promotes homologous pairing by a novel synaptic process in which DNA molecules are first brought together in dynamic nucleoprotein networks that rapidly rearrange to search for homology. Similar interpretations have been made by Bryant and Lehman (24) with regard to the renaturation of complementary single strands by RecA protein and by Kmiec and Holloman (30) with regard to their observations on strand invasion promoted by the *rec1* gene product of *Ustilago maydis*, a eukaryotic enzyme that has many similarities to RecA protein. Thus RecA protein, its prokaryotic homologs (31–33) and *rec1* gene product belong to a class of DNA binding proteins that is distinguished from others, such as the helix-destabilizing proteins, by its synaptic mechanism.

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