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RecA Protein Promoted Homologous Pairing *in Vitro*

PAIRING BETWEEN LINEAR DUPLEX DNA BOUND TO HU PROTEIN (NUCLEOSOME CORES) AND NUCLEOPROTEIN FILAMENTS OF RecA PROTEIN-SINGLE-STRANDED DNA*

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RecA protein promotes two distinct types of synaptic structures between circular single strands and duplex DNA; paranemic joints, where true intertwining of paired strands is prohibited and the classically intertwined plectonemic form of heteroduplex DNA. Paranemic joints are less stable than plectonemic joints and are believed to be the precursors for the formation of plectonemic joints. We present evidence that under strand exchange conditions the binding of HU protein, from *Escherichia coli*, to duplex DNA differentially affects homologous pairing *in vitro*. This conclusion is based on the observation that the formation of paranemic joint molecules was not affected, whereas the formation of plectonemic joint molecules was inhibited from the start of the reaction. Furthermore, introduction of HU protein into an ongoing reaction stalls further increase in the rate of the reaction. By contrast, binding of HU protein to circular single strands has neither stimulatory nor inhibitory effect. Since the formation of paranemic joint molecules is believed to generate positive supercoiling in the duplex DNA, we have examined the ability of positive superhelical DNA to serve as a template in the formation of paranemic joint molecules. The inert positively supercoiled DNA could be converted into an active substrate, *in situ*, by the action of wheat germ topoisomerase I. Taken collectively, these results indicate that the structural features of the bacterial chromosome which include DNA supercoiling and organization of DNA into nucleosome-like structures by HU protein modulate homologous pairing promoted by the nucleoprotein filaments of recA protein single-stranded DNA.

Escherichia coli recA protein promotes homologous pairing, *in vitro*, between a variety of DNA substrates. The simplest is the annealing of complementary single strands: more complex reactions, which require the hydrolysis of ATP, are collectively termed strand transfer reactions (Radding, 1982; Howard-Flanders *et al.*, 1984; Cox and Lehman, 1987; Griffith and Harris, 1988). The DNA from the filamentous phages have been the source of a pair of useful substrates, duplex DNA and circular single-stranded DNA (ssDNA).[1] Work done in various laboratories to understand homologous pairing between linear duplex DNA and ssDNA promoted by recA protein has been highly informative in defining the major phases of the reaction: presynapsis, synapsis, and strand exchange (Radding 1982; Cox and Lehman, 1987). The presynaptic polymerization of recA protein on ssDNA is rather a slow reaction which could be accelerated by the action of single strand binding protein (SSB) from a variety of sources (Cox and Lehman, 1982; Cox *et al.*, 1983; Muniyappa *et al.*, 1984; Kahn and Radding, 1984). The nucleoprotein filament of recA protein-ssDNA, which is the obligatory intermediate, acquires the ability to search for homology and subsequently establishes synapsis with the duplex DNA (Flory *et al.*, 1984; Tsang *et al.*, 1985; Morrical *et al.*, 1986; Julin *et al.*, 1986; Gonda and Radding, 1986; Kowalczykowski *et al.*, 1987).

All of these studies have been done with naked duplex DNA as the substrate. Several lines of evidence indicate that in bacteria there exist two distinct levels of DNA condensation: large superhelical domains and organization of DNA into shorter domains with DNA-binding proteins (Stoningen and Pettijohn, 1971; Rouviere-Yaniv and Gross, 1975; Griffith, 1976; Varshavsky *et al.*, 1977). The best studied of these are the histone-like proteins or HU protein, a major chromosomal protein, that shares sequence homology with eukaryotic histones. HU protein is an heterodimer composed of two subunits, HU-1 and HU-2, and binds to DNA as a tetramer covering about 60 bp of DNA (Pettijohn, 1988; Drilica and Rouviere-Yaniv, 1987). Recent studies of Broyles and Pettijohn (1986) have shown a reduction in helical periodicity of DNA, from 10.5 to 8.5 bp/turn, bound to HU protein. Thus, wrapping of DNA around HU protein is partially offset by tightening the DNA helix and such a reduction in helical periodicity should have a major effect in recognition of specific sequences in DNA, in processes such as recombination, replication, and transcription.

We have been concerned with how the presynaptic nucleoprotein complex of recA protein-ssDNA search for homology and subsequently pair with the duplex DNA that is complexed with proteins. In this paper, we ask directly if nucleosome-like structures formed with *E. coli* HU protein and DNA have an effect on homologous pairing promoted by recA protein. Since the binding of HU protein causes tightening of the DNA duplex, we investigated the effect of positive superhelicity on homologous pairing promoted by recA protein.

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[1] The abbreviations used are: ssDNA, circular single-stranded DNA; SSB, *E. coli* single strand binding protein; bp, base pairs; HEPES, 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid; Form I DNA, closed circular form with negative linking difference; form IV DNA, relaxed closed circular DNA.

MATERIALS AND METHODS

Enzymes and Proteins—RecA protein was purified essentially according to the method of Shibata *et al.*, (1981), and its concentration was determined as described (Tsang *et al.*, 1985). *E. coli* SSB was prepared by the method of Lohman *et al.* (1986). HU protein was purified by the method of Broyles and Pettijohn (1986) and protein concentration was determined by the method of Bradford (1976) using bovine serum albumin as a standard. Wheat germ topoisomerase I was from Promega-Biotech. Restriction endonucleases were from New England Biolabs. All other chemicals were of analytical grade.

Preparation of DNA Substrates—M13 Gori1 circular duplex [^{3}H]DNA and M13 ssDNA were prepared, and their concentration was determined as described by Cunningham *et al.* (1980). M13 Gori1 linear duplex DNA with flanking G4 sequences and G4 sequences buried in the molecule was prepared by cleaving Form I DNA with restriction endonucleases *Xho*I and *Bam*HI, respectively. M13 Gori1 positively superhelical DNA was prepared by treating with reverse gyrase from *Sulfolobus acidocaldarius* (a generous gift of A. Kikuchi, University of Tokyo, Japan) as described (Nakasu and Kikuchi, 1985). The reactions were terminated with excess of EDTA, phenol-extracted, and dialyzed.

Standard Reaction Conditions—Unless otherwise indicated, the reaction mixtures contained 33 mM Tris-HCl (pH 7.5), 1 mM dithiothreitol, 1.5 mM ATP, 3 mM phosphocreatine, 12 mM $MgCl_2$, 10 units of phosphocreatine kinase/ml, and 0.1 mg/ml of bovine serum albumin. Eight μM M13 ssDNA was incubated with 0.5 μM SSB in the above reaction mixture for 5 min at 37 °C, followed by 2.7 μM recA protein for 10 min to form presynaptic complexes.

M13 Gori1 linear duplex [^{3}H]DNA was incubated with appropriate concentration of HU protein in a 10-μl reaction mixture containing 10 mM HEPES buffer (pH 7.8) and 1 mM EDTA or standard recA protein assay buffer (above) for 30 min at 37 °C. The reaction for the formation of joint molecules was initiated by adding HU protein-DNA complexes to the standard reaction mixtures containing presynaptic nucleoprotein complexes.

Nitrocellulose Filter Binding Assay for Joint Molecules—This assay is based on the method of Beattie *et al.* (1977) measures the retention by nitrocellulose filters of duplex DNA attached to ssDNA. The formation of plectonemic joint molecules was measured by taking 10-μl aliquots from the reaction mixtures and adding them into 200 μl of 25 mM EDTA (pH 9.0) at 4 °C. After brief mixing and taking an aliquot for the determination of total input radioactivity, we added 5 ml of cold 0.15 M sodium citrate and 1.5 M NaCl and filtered through a nitrocellulose filter (Millipore: HAWP, 0.45 μm). The paranemic joint molecules were measured by taking aliquots and adding them into 5 ml of 0.15 M sodium citrate, 1.5 M NaCl at 4 °C and filtered as described above. Nitrocellulose filters were washed with 5 ml of cold 0.15 M sodium citrate and 1.5 M NaCl and dried under a heat lamp. The filters were put into vials with toluene containing 0.5% diphenyloxazole, and radioactivity was measured in a liquid scintillation counter. The yield of joint molecules was expressed as a percentage of the total counts retained on the filter.

Agarose Gel Electrophoresis—The conversion of M13 Gori1 positively superhelical DNA to Form I DNA was analyzed on 0.8% agarose gels in 89 mM Tris borate, 2 mM EDTA (pH 8.3). The electrophoresis was done at 40 V for 18 h. Gels were stained with 0.5 μg/ml ethidium bromide, destained, and photographed.

RESULTS

Experimental Design—RecA protein promotes homologous pairing of ssDNA with the duplex DNA resulting in the formation of two distinct types of synaptic structures. In the absence of a free end at the region of homology recA protein forms paranemic joints in which the two strands are "not truly" intertwined and in the presence of a free end it readily promotes the formation of intertwined plectonemic joints (Bianchi *et al.*, 1983; Riddles and Lehman, 1985; Christiansen and Griffith, 1986). To investigate the role of HU protein, we used duplex DNA from the chimeric phage M13 Gori1 and ssDNA from phage M13, since we can readily produce suitable linear duplex molecules having homology either at the ends or buried in the molecule as illustrated in Fig. 1. We have approached these problems by measuring the ability of these templates, complexed with HU protein, to support the formation of plectonemic as well as paranemic joints.

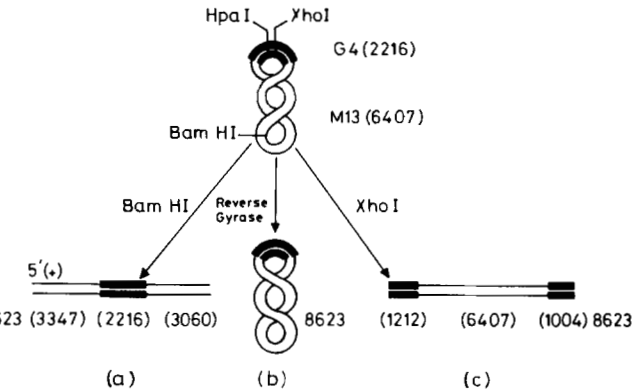

FIG. 1. **DNA substrates.** Map of chimeric bacteriophage M13 Gori1 duplex DNA. Restriction digestion and preparation of substrates is described under "Materials and Methods." *Thick* and *thin lines* represent sequences derived from bacteriophages G4 and M13, respectively. The *numbers* in *parentheses* indicate the length in base pairs of that region, and *numbers without parentheses* represent the total length of the DNA molecule. *a*, linear duplex DNA prepared by digesting negatively superhelical DNA with *Bam*HI. *b*, positively superhelical DNA prepared by incubating negatively superhelical DNA with reverse gyrase. *c*, linear duplex DNA prepared by cleavage of negatively superhelical DNA with *Xho*I.

Construction of Nucleosome-like Templates with HU Protein—Nucleosome-like structures were assembled by incubating appropriate concentrations of HU protein with M13 Gori1 linear [^{3}H]DNA (Fig. 1, *substrate a* or *c*) in a buffer containing 10 mM HEPES and 1 mM EDTA (pH 7.8) at 37 °C for 30 min (Broyles and Pettijohn, 1986) or in a standard recA protein assay buffer (see "Materials and Methods"). The formation of nucleosome-like structures was ascertained by two methods: gel electrophoresis and susceptibility to DNase I digestion. When stoichiometric amounts of HU protein and linear duplex DNA were used, the complex of HU protein-DNA showed retarded migration on agarose gels (data not shown). Since gel electrophoresis is inherently laborious, we routinely used susceptibility to DNase I digestion as the criteria for the formation of nucleosome-like structures. DNase I cleaves naked ssDNA and duplex DNA to a mixture of oligo- and mononucleotides, but the DNA is protected once it is bound by protein(s). In the experiments described here we examined the ability of HU protein to protect M13 Gori1 linear duplex [^{3}H]DNA (Fig. 1, *substrate a* or *c*) incubated under conditions suitable for the formation of joint molecules. As illustrated in Fig. 2, in the absence of HU protein linear duplex [^{3}H]DNA was digested by DNase I. Greater than 90% of the [^{3}H]DNA became acid soluble within 2 min of incubation with 2 μg/ml of DNase I. However, under identical conditions in the presence of HU protein, [^{3}H]DNA exhibited resistance to digestion even at 10 μg/ml of DNase I (Fig. 2, closed *versus* open symbols). More importantly, both the linear duplex [^{3}H]DNA molecules showed similar kinetics indicating that the binding of HU protein to these DNA fragments was uniform and does not show any regard to nucleotide sequence location on DNA.

Formation of Joint Molecules with Chromatin Templates—We next examined the ability of M13 Gori1 linear duplex DNA complexed with HU protein to form joint molecules with nucleoprotein filaments of recA protein-M13 ssDNA. The nucleoprotein filaments of recA protein-M13 ssDNA were formed in the presence of SSB to avoid complications that might arise by the presence of secondary structure which impedes the formation of joint molecules (Muniyappa *et al.*, 1984). We formed nucleoprotein filaments of recA protein-

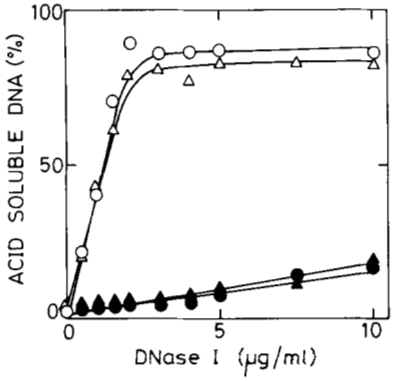

FIG. 2. **Protection of M13 Gori1 linear duplex [^{3}H]DNA from digestion by pancreatic DNase I in the presence or absence of *E. coli* HU protein.** M13 Gori1 duplex [^{3}H]DNA (9 μm) (Fig. 1, *substrate a* or *c*) was incubated either in a standard recA protein assay buffer or in a buffer (20 μl) containing 10 mM HEPES (pH 7.8) and 1 mM EDTA with or without 1 μM HU protein at 37 °C for 30 min. $MgCl_2$ was added to 12 mM, and DNA was digested with the indicated concentrations of DNase I for 2 min at 37 °C. DNase I digestion was terminated by the addition of EDTA to 25 mM, 20 μl of cold 1.75% perchloric acid, and 20 μl of 0.88 μg/ml denatured calf thymus DNA. The tubes were kept on ice for 30 min and centrifuged in an Eppendorf microfuge for 10 min. The amount of DNA was determined by measuring the radioactivity present in the acid soluble supernatant fraction. △ and ○, M13 Gori1 Form I DNA cleaved with *Xho*I and *Bam*HI, respectively; ▲ and ●, above fragments in the presence of HU protein.

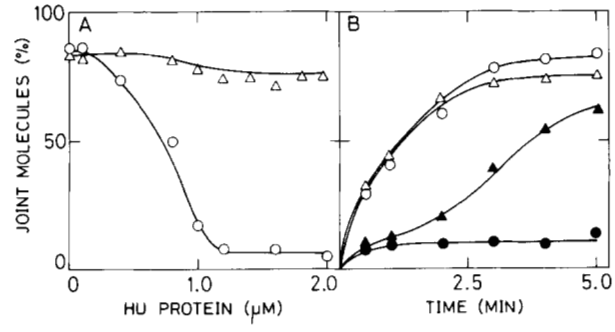

FIG. 3. **Formation of paranemic and plectonemic joint molecules in the presence of HU protein.** *A*, effect of increasing amounts of HU protein. Reaction mixtures (30 μl) in a standard assay buffer containing 8 μM M13 ssDNA, 0.5 μM SSB, 12 mM $MgCl_2$, and an ATP regeneration system were preincubated for 5 min at 37 °C, after which we added 2.8 μM recA protein and incubated for 10 min. The formation of joint molecules was initiated by the addition of linear duplex [^{3}H]DNA complexed with HU protein. The reaction was terminated 5 min later as described under "Materials and Methods." △, paranemic joint molecules formed with M13 Gori1 linear duplex [^{3}H]DNA (Form I DNA cleaved with *Xho*I). ○, plectonemic joint molecules obtained with M13 Gori1 linear duplex [^{3}H]DNA (Form I DNA cleaved with *Bam*HI). *B*, time course of formation of joint molecules. The concentration of reactants and incubations were done in a 90-μl reaction mixture essentially as described above. Aliquots (10 μl) were taken at the indicated time intervals and processed as described under "Materials and Methods." Plectonemic joint molecules: ○, in the absence of HU protein; ●, in the presence of HU protein. Paranemic joint molecules: △, in the absence of HU protein; ▲, in the presence of HU protein.

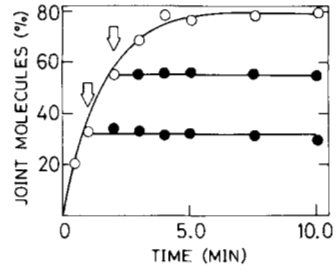

FIG. 4. **Effect of addition of HU protein to an ongoing reaction on the formation of plectonemic joint molecules between M13 Gori1 linear duplex [^{3}H]DNA and M13 ssDNA.** The concentration of reactants and incubations were done as in Fig. 3. HU protein 1 μM was added at the times indicated by the *arrow*. Aliquots were taken and processed as described under "Materials and Methods." ○, in the absence of HU protein; ●, in the presence of HU protein.

ssDNA in the presence of SSB by two methods: one was essentially as described by Tsang *et al.*, (1985) and the other involved preincubation of ssDNA with SSB prior to recA protein as described under "Materials and Methods." The nucleoprotein filaments obtained by both the procedures were indistinguishable in their ability to support the formation of joint molecules. In one set of experiments we incubated full-length M13 Gori1 linear duplex [^{3}H]DNA (Fig. 1, *substrate a* or *c*) with varying amounts of HU protein. After appropriate times of incubation, we mixed M13 Gori1 linear duplex [^{3}H]DNA (Fig. 1, *substrate a* or *c*) complexed with HU protein with nucleoprotein filaments to initiate the formation of joint molecules. Five minutes later we terminated the reaction by adding an equal volume of cold 0.15 M sodium citrate and 1.5 M NaCl and 25 mM EDTA where necessary. After taking an aliquot for the determination of total radioactivity, we diluted the reaction mixture with 5 ml of cold 1.5 M NaCl and 0.15 M sodium citrate and assayed for joint molecules as described under "Materials and Methods." As shown in Fig. 3*A*, in the absence of HU protein the yield of plectonemic and paranemic joint molecules was indistinguishable. However, in the presence of increasing concentrations of HU protein there was a gradual decline in the yield of plectonemic joint molecules, and at 1.2 μM HU protein concentration, their formation was completely inhibited. By striking contrast the formation of paranemic joint molecules was not affected (Fig. 3*A*, *open circles versus open triangles*).

Since the extent of paranemic as well as plectonemic joint molecules were measured 5 min after the initiation of the reaction, we wished to gain some insight into the initial phase of the reaction. To investigate the effect of HU protein on the initial kinetics of the reaction, we incubated full-length M13 Gori1 linear duplex [^{3}H]DNA (Fig. 1, *substrate a* or *c*) with 2 μM HU protein as described above. Similarly, we incubated M13 ssDNA with SSB and recA protein and combined them to initiate the formation of joint molecules. At the indicated time intervals we took aliquots and assayed for joint molecules. As shown in Fig. 3*B*, the kinetics of formation showed a lag period of 2 min before paranemic joint molecules were detected (Fig. 3*B*, *closed triangles*). By contrast, the formation of plectonemic joint molecules was completely inhibited (Fig. 3*B*, *closed circles*). In the absence of HU protein the formation of plectonemic as well as paranemic joint molecules was indistinguishable, however (Fig. 3*B*, *open circles* and *triangles*). These results indicate that the formation of plectonemic and paranemic joints were similarly inhibited during the initial phase of the reaction, but the block was somehow overcome in the formation of paranemic joint molecules. The effect(s) of HU protein persisted even when the nucleoprotein filaments used were formed in the absence of SSB.

We investigated next the effect of addition of HU protein on the formation of plectonemic joint molecules in an ongoing reaction (Fig. 1, *substrate a* or *c*). As shown in Fig. 4, when 2 μM HU protein was added 1 and 2.0 min after the initiation

of the reaction, there was a rapid decline in the rate of the formation of plectonemic joint molecules; however, HU protein did not cause their dissociation. Ionic strength has been believed to exert a marked effect on stabilizing the interaction of HU protein with duplex DNA. It has been shown by Broyles and Pettijohn (1986) that the dissociation half-life of complexes of HU protein-DNA in 50 mM NaCl was about 0.6 min. Addition of varying amounts of NaCl to 150 mM either during the formation of HU protein-DNA complexes or during formation of plectonemic joint molecules did not have any discernible effect. Other related experiments showed that HU protein did not promote the retention of M13 Gori1 linear duplex [^{3}H]DNA (Fig. 1, *substrate a* or *c*) on nitrocellulose filters either in the absence or in the presence of recA protein, SSB or both under these assay conditions.

Effect of Binding of HU Protein to Single-stranded DNA on the Formation of Plectonemic Joint Molecules—HU protein has been shown to bind to ssDNA as well as duplex DNA and thereby stabilize the DNA against thermal denaturation (Pettijohn, 1988; Drilica and Rouviere-Yaniv, 1987). SSB, yet another protein from *E. coli*, genetically shown to be involved in homologous recombination, potentiates both the formation of joint molecules and strand exchange, *in vitro*, acting primarily via ssDNA (Glassberg *et al.*, 1979; Cox *et al.*, 1983; Muniyappa *et al.*, 1984). To measure their effect on pairing, we preincubated M13 ssDNA with HU protein and SSB with M13 ssDNA separately followed by recA protein in a standard reaction mixture. The control reaction consisted of recA protein alone. The reaction for joint molecules was started by adding M13 Gori1 naked duplex [^{3}H]DNA (Fig. 1, *substrate a* or *c*). As shown in Fig. 5, the formation of plectonemic joint molecules was maximal in the presence of SSB, whereas in the presence of HU protein or with recA protein alone the reaction was half-maximal. We conclude from these results that the binding of HU protein to ssDNA has neither stimulatory nor inhibitory effect.

Role of Positive Superhelicity in the Formation of Paranemic Joint Molecules—Broyles and Pettijohn (1986) showed that the helical periodicity of DNA when complexed with HU protein is reduced from 10.5 to 8.5 bp, indicating that the DNA is more tightly wound. Several years ago, Holloman and Radding (1976) suggested that superhelicity may play a role in homologous recombination. Since unwinding of a closed duplex DNA by the nucleoprotein filaments of recA protein-ssDNA necessarily induces a compensating overwinding of the DNA elsewhere in the molecule, we wished to examine the ability of tightly wound DNA, *per se*, to serve as a template in the formation of paranemic joint molecules. For this purpose, we prepared positively supercoiled M13 Gori1 duplex DNA by using "reverse gyrase" from *S. acidocaldarius*. When we incubated presynaptic nucleoprotein filaments of recA protein-M13 ssDNA with positively superhelical M13 Gori1 [^{3}H]DNA (Fig. 1, *substrate b*), the formation of joint molecules was not detectable over a period of 60 min (data not shown). It has been shown that topoisomerase I from eukaryotes is capable of relaxing positively superhelical DNA by introducing "swivel" into the DNA (Wang, 1985). To test directly whether we can activate the inert positively superhelical M13 Gori1 [^{3}H]DNA to serve as substrate (Fig. 1, *substrate b*) in the formation of joint molecules, we incubated nucleoprotein filaments of M13 ssDNA-recA protein with varying concentrations of topoisomerase I. As illustrated in Fig. 6*A*, in the absence of topoisomerase I, the extent of formation of joint molecules was negligible, and the residual activity obtained was directly correlated to the presence of small amounts of nicked duplex molecules in the preparation. However, with the addition of increasing amounts of topoisomerase I there was corresponding increase in the formation of joint molecules (Fig. 6*A*, *triangles*). When we examined the products of the reaction on an agarose gel, most of the input positively super-

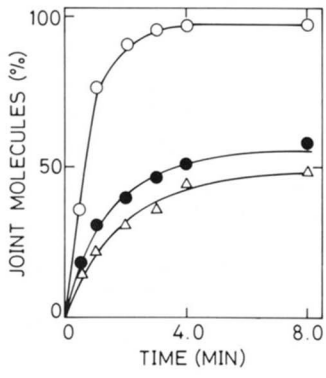

FIG. 5. **Effect of binding of HU protein to M13 single-stranded DNA on the formation of plectonemic joint molecules.** Reactions were formed as described under "Materials and Methods." The reaction for joint molecules was initiated by the addition of 5 μM naked linear duplex [^{3}H]DNA (Fig. 1, *substrate a*) to presynaptic complexes formed in the presence and absence of HU protein. ○, in the presence of SSB; ●, in the presence of HU protein; and △, in the absence of HU protein and SSB.

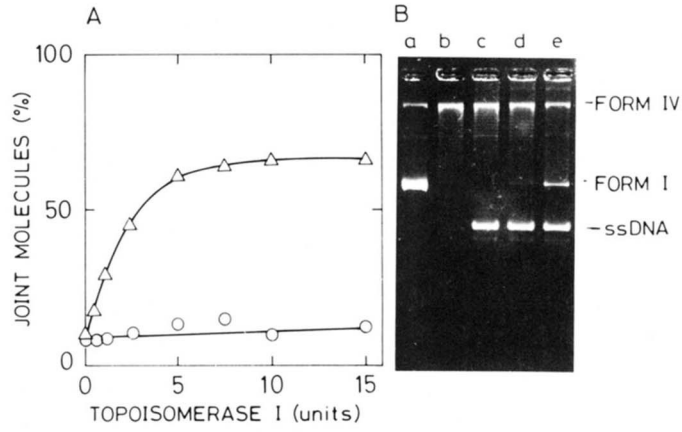

FIG. 6. **Stimulation of paranemic joint molecule formation between recA protein-M13 ssDNA and M13 Gori1 positively superhelical duplex [^{3}H]DNA by wheat germ topoisomerase I.** *A*, the concentration of substrates and reaction conditions is described under the legend to Fig. 3. Eight μM M13 ssDNA was preincubated (30 μl) first with SSB and then with recA protein as described under "Materials and Methods." The reaction for joint molecules was initiated by the addition of 5 μM naked M13 Gori1 positively superhelical [^{3}H]DNA (Fig. 1, *substrate b*). We immediately added the indicated amounts of topoisomerase I and an equal volume of buffer to the control reactions. The reaction was terminated by the addition of 30 μl of 5 M NaCl. An aliquot was taken for the determination of total radioactivity, and the remaining sample was assayed for paranemic joint molecules. ○, positively superhelical DNA alone; △, positively superhelical DNA with the indicated amounts of topoisomerase I. *B*, unwinding of M13 Gori1 positively superhelical DNA (Fig. 1, *substrate a*) by the presynaptic complexes of recA protein-M13 ssDNA. Reaction mixtures containing topoisomerase I were formed as described above. The reaction was terminated by the addition of sodium dodecylsulfate to 0.1%, EDTA to 25 mM, and proteinase K to 0.2 mg/ml and incubated for 15 min at 37 °C. The individual samples, after the addition of tracking dye, were loaded on a agarose gel, and electrophoresis was done as described under "Materials and Methods." *Lane a*, markers; *lane b*, M13 Gori1 positively superhelical DNA; *lane c*, M13 Gori1 positively superhelical DNA plus M13 ssDNA in the absence of recA protein but in the presence of 5 units of topoisomerase I; *lane d*, same as *c* but in the presence of nucleoprotein filaments of recA protein-M13 ssDNA and 2.5 units of topoisomerase I; *lane e*, same as *d* but in the presence of 5 units of topoisomerase I.

helical DNA was converted to Form I DNA, indicating that the formation of joint molecules was dependent on the action of topoisomerase I (Fig. 6*B*).

DISCUSSION

In eukaryotes, DNA and histones constitute a repetitive structure called nucleosome, and the possibility that DNA in prokaryotes is organized into nucleosome-like structure although remains uncertain, there is compelling evidence supporting the existence of nucleosome-like structures (Pettijohn, 1988). HU protein from *E. coli*, a major chromosomal protein, binds to DNA with no sequence specificity but plays an important role in DNA processes such as replication (Dixon and Kornberg, 1984; Ogawa *et al.*, 1985), site-specific recombination (Craigie *et al.*, 1985; Johnson *et al.*, 1986; Surette *et al.*, 1987), and differential activation of recognition of specific DNA sequences (Flashner and Gralla, 1988). It is pertinent here to mention that a recent genetic study involving mutations in hupB and hupA genes, which code for Hu-1 and Hu-2 proteins, respectively, has indicated the importance of HU proteins in cell viability and survival (Wada *et al.*, 1988). In the present study we have investigated the role of HU protein on homologous pairing between M13 Gori1 linear duplex DNA and M13 ssDNA, *in vitro*, promoted by recA protein. The binding of HU protein to linear M13 Gori1 duplex DNA under strand exchange conditions was confirmed qualitatively by gel electrophoresis and quantitatively by nuclease protection studies.

The principal conclusion of the present study is that under strand exchange conditions, binding of HU protein to linear duplex DNA differentially affects homologous pairing *in vitro*. This conclusion is based on the relatively simple observation that the formation of paranemic joint molecules was not affected, whereas the formation of plectonemic joint molecules was sharply inhibited. RecA protein promotes two distinct types of synaptic structures between duplex DNA and circular single-stranded DNA; paranemic joints, where true intertwining of paired strands is prohibited, and the classically intertwined plectonemic structure of duplex DNA. Paranemic joints are less stable than plectonemic joints and are believed to be the intermediates in strand exchange reactions between linear duplex and ssDNA (Bianchi *et al.*, 1983; Riddles and Lehman, 1985). Among the structures considered previously for paranemic joints is a three-stranded structure in which the strands are believed to be aligned in a side-by-side fashion (Bianchi *et al.*, 1983; Riddles and Lehman, 1985; Christiansen and Griffith, 1986). An alternate structure was proposed by Cox and his colleagues (Schutte and Cox, 1987) where homologous contacts are periodic rather than continuous occurring once per helical turn of the duplex DNA. The length of the paranemic joint was estimated to vary from 400 bp to several kilobases (Bianchi *et al.*, 1983; Christiansen and Griffith, 1986; Schutte and Cox, 1987).

The effect of HU protein on the formation of joint molecules was unique in some respects. Although the formation of plectonemic joint molecules was inhibited from the start of the reaction, the formation of paranemic joint molecules was inhibited at earlier times which was overcome during later times in the reaction. These results suggest that in the presence of HU protein there is a major topological restraint to the formation of both the types of joint molecules. The apparent lack of initiation of plectonemic joint molecules, which require a free end in the region of homology (Bianchi *et al.*, 1983; Riddles and Lehman, 1985), is particularly striking. The inhibition may be due to the inaccessibility of the end in the region of homology to the incoming presynaptic complex or the difficulty encountered by the nucleoprotein filament in unwinding the double helical DNA bound to HU protein. The later possibility is unlikely, since the nucleoprotein filaments of recA protein-ssDNA were able to form paranemic joint molecules. These results suggest that in the case of paranemic joints the presynaptic complexes of recA protein-ssDNA establish synapsis with the naked duplex DNA located between the two nucleosome core particles. It is likely that the available internucleosome distance for pairing is limited, and to expand the synaptic joint by branch migration, the incoming presynaptic complex would have to displace the bound HU protein. An alternate explanation may be related to the nature of the search for homology. The search for homology in a duplex molecule covered with HU protein would require more time and hence may decelerate the rate of the reaction; however, our data do not distinguish between these models.

Unlike the DNA in eukaryotic chromatin, prokaryotic DNA is torsionally strained (Pettijohn, 1982). Numerous studies have shown that negative supercoiling facilitates strand opening and can influence promoter strength in both positive and negative directions thus increasing the structural repertoire of DNA (Wang, 1985). Conversely, positive supercoiling is presumed to antagonize the same functions of DNA, although, to our knowledge, evidence is lacking. In this context, the absence of an end either in the duplex DNA or in ssDNA formation of paranemic joint molecules promoted by recA protein is believed to generate positive superhelical turns, since synapsis requires unwinding of the duplex DNA. It is important to note that the duplex DNA complexed with HU protein shows a reduction in helical periodicity (Broyles and Pettijohn, 1986). Consistent with these observations, positively superhelical DNA turned out to be inert in the formation of joint molecules; however, in the presence of wheat germ topoisomerase I, which removes positive superhelical turns, the inert substrate was converted into a good substrate. The lack of formation of plectonemic joint molecules in the presence of HU protein may not be due to the accumulation of positive superhelical turns, since the substrate was linear duplex DNA and the torsional stress might get relieved from the free ends. An alternative explanation is that the presynaptic complexes are unable to displace, simultaneously, HU protein from the duplex ends and plectonemic form joint molecules.

In summary, the presynaptic nucleoprotein filaments of recA protein-ssDNA are unable to initiate the formation of plectonemic joint molecules with duplex DNA complexed with HU protein but are able to form paranemic joint molecules, albeit with a lag. We speculate that the occurrence of a lag in the reaction may be attributed to the time required either to search for homology or for the displacement of HU protein from the duplex DNA, which are not mutually exclusive. A secondary aspect of the present study is the observation that the positively superhelical DNA exerts a negative effect on the formation of joint molecules which could be relieved by the action of topoisomerase I, suggesting a prominent role for these enzymes in homologous recombination. Although the detailed molecular events underlying homologous recombination, *in vivo*, remains to be elucidated, any attempt towards that direction should consider the organization of duplex DNA in the context of supercoiling and nucleosome structures.

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REFERENCES

Beattie, K. L., Wiegand, R. C., and Radding, C. M. (1977) *J. Mol. Biol.* **116,** 783–803

Bianchi, M., Das Gupta, C., and Radding, C. (1983) *Cell* **34,** 931–939

Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254

Broyles, S. S., and Pettijohn, D. E. (1986) *J. Mol. Biol.* **187,** 47–60

Christiansen, G., and Griffith, J. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83,** 2066–2070

Cox, M. M., and Lehman, I. R. (1982) *J. Biol. Chem.* **257,** 8523–8532

Cox, M., and Lehman, I. R. (1987) *Annu. Rev. Biochem.* **56,** 229–262

Cox, M. M., Soltis, D. A., Lehman, I. R., DeBrosse, C., and Benkovic, S. J. (1983) *J. Biol. Chem.* **258,** 2586–2592

Craigie, R., Aarndt-Jovin, D. J., and Mizuuchi, K. (1985) *Proc. Natl. Acad. Sci. U. S. A.* **82,** 7570–7574

Cunningham, R. P., Das Gupta, C., Shibata, T., and Radding, C. (1980) *Cell* **20,** 223–235

Dixon, N. E., and Kornberg, A. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81,** 424–428

Drilica, K., and Rouviere-Yaniv, J. (1987) *Microbiol. Rev.* **51,** 301–319

Flashner, Y., and Gralla, J. D. (1988) *Cell* **54,** 713–721

Flory, J., Tsang, S. S., and Muniyappa, K. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81,** 7026–7030

Griffith, J. (1976) *Proc. Natl. Acad. Sci. U. S. A.* **73,** 563–567

Griffith, J., and Harris, L. (1988) *CRC Crit. Rev. Biochem.* **23,** S53–S86

Gonda, D. K., and Radding, C. M. (1986) *J. Biol. Chem.* **261,** 13087–13096

Holloman, W. K., and Radding, C. (1976) *Proc. Natl. Acad. Sci. U. S. A.* **73,** 3910–3914

Howard-Flanders, P., West, S. C., and Stasiak, A. (1984) *Nature* **309,** 215–220

Johnson, R. C., Bruist, M. F., and Simon, M. I. (1986) *Cell* **46,** 531–539

Julin, D. A., Riddles, P. W., and Lehman, I. R. (1986) *J. Biol. Chem.* **261,** 1025–1030

Kahn, R., and Radding, C. M. (1984) *J. Biol. Chem.* **259,** 7495–7503

Kowalczykowski, S., Clow, J., Somani, R., and Varghese, A. (1987) *J. Mol. Biol.* **193,** 81–95

Lohman, T., Green, J. M., and Beyer, R. S. (1986) *Biochemistry* **25,** 21–25

Morrical, S. W., Lee, J., and Cox, M. (1986) *Biochemistry* **25,** 1482–1494

Muniyappa, K., Shaner, S., Tsang, S. S., and Radding, C. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81,** 2757–2761

Nakasu, S., and Kikuchi, A. (1985) *EMBO J.* **4,** 2705–2710

Ogawa, T., Baker, T. A., van der Ende, A., and Kornberg, A. (1985) *Proc. Natl. Acad. Sci. U. S. A.* **82,** 3562–3566

Pettijohn, D. E. (1982) *Cell* **30,** 667–669

Pettijohn, D. E. (1988) *J. Biol. Chem.* **263,** 12793–12796

Radding, C. M. (1982) *Annu. Rev. Genet.* **16,** 405–437

Riddles, P. W., and Lehman, I. R. (1985) *J. Biol. Chem.* **260,** 165–169

Rouviere-Yaniv, J., and Gross. F. (1975) *Proc. Natl. Acad. Sci. U. S. A.* **72,** 3428–3452

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

Shibata, T., Cunningham, R. P., and Radding, C. M. (1981) *J. Biol. Chem.* **256,** 7557–7564

Stoningen, O. G., and Pettijohn, D. (1971) *Proc. Natl. Acad. Sci. U. S. A.* **68,** 6–9

Surette, M., Buch, S., and Chaconas, G. (1987) *Cell* **49,** 253–262

Tsang, S. S., Muniyappa, K., Azhderian, E., Gonda, D. K., Radding, C. M., Flory, J., and Chase, J. W. (1985) *J. Mol. Biol.* **185,** 295–309

Varshavsky, A. J., Nedoospasov, S. A., and Bakayer, V. V. (1977) *Nucleic Acids Res.* **4,** 2725–2745

Wada, M., Kano, Y., Ogawa, T., Okazaki, T., and Imamoto, F. (1988) *J. Mol. Biol.* **204,** 581–591

Wang, J. C. (1985) *Annu. Rev. Biochem.* **54,** 665–697