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3′ homologous free ends are required for stable joint molecule formation by the RecA and single-stranded binding proteins of *Escherichia coli*

(plectonemic joint molecules/gel assay/electron microscopy)

BOYANA B. KONFORTI* AND RONALD W. DAVIS

Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305

Contributed by Ronald W. Davis, October 8, 1986

ABSTRACT The RecA protein of *Escherichia coli* is important for genetic recombination *in vivo* and can promote synapsis and strand exchange *in vitro*. The DNA pairing and strand exchange reactions have been well characterized in reactions with circular single strands and linear duplexes, but little is known about these two processes using substrates more characteristic of those likely to exist in the cell. Single-stranded linear DNAs were prepared by separating strands of duplex molecules or by cleaving single-stranded circles at a unique restriction site created by annealing a short defined oligonucleotide to the circle. Analysis by gel electrophoresis and electron microscopy revealed that, in the presence of RecA and single-stranded binding proteins, a free 3′ homologous end is essential for stable joint molecule formation between linear single-stranded and circular duplex DNA.

The *Escherichia coli* protein RecA promotes homologous pairing and strand exchange *in vitro*. One simple combination of DNA molecules studied extensively is circular single-stranded (ss DNA) and homologous linear duplex DNA. In the presence of single-stranded binding protein (SSB), RecA promotes exchange of the single-stranded circle with its linear homolog in the duplex in three distinct steps: presynapsis, synapsis, and branch migration [for a review of all steps in the pairing process, see Cox and Lehman (1)]. In the presynaptic step, RecA binds cooperatively and stoichiometrically to ss DNA, forming filaments. These nucleoprotein filaments or initiation complexes then pair with duplex DNA. The synaptic complex formed is a structure in which the ss DNA contained in the nucleoprotein filament is aligned with complementary sequences in the duplex DNA. The formation of this joint molecule is accompanied by a limited unwinding of the duplex DNA. Two types of synaptic structures have been observed and distinguished by their different topological requirements and stabilities. Paranemic joints, in which the incoming strand is paired with its complement in the duplex but is not interwound, appear early in the reaction, can occur in the absence of a free end, and are unstable upon deproteinization or at elevated temperatures. Plectonemic joints, in which the DNA strands are interwound, are formed later in the reaction, require that the incoming single strand and its complement from the original duplex be free to rotate around each other, and are stable in the presence of protein denaturants or at elevated temperatures. Paranemic joints form rapidly and are presumed to be precursors of the plectonemic joints. Conversion of a paranemic joint into a plectonemic joint followed by extension of the heteroduplex region through the process of branch migration results in the net exchange of DNA strands. Branch migration requires ATP hydrolysis, and it exhibits a unique polarity.

Kahn *et al.* (2) have shown that the formation of joint molecules occurs in such a fashion that only the 5′ end of the viral (+)-strand is displaced from the duplex DNA (Fig. 1*B*). Using the same substrates, Cox and Lehman (3) confirmed this polarity by restriction endonuclease analysis of RecA-promoted branch migration. West *et al.* (4) concluded that the polarity was the same using linearized duplex DNA and homologous circular ss DNA that carried a short hybridized fragment. These data indicate that, in reactions involving linear duplex and ss circular DNA substrates, the polarity of RecA-catalyzed strand exchange *in vitro* is 5′-to-3′ relative to the ss DNA.

While the pairing of circular ss DNA with linear duplex DNA is a rapid and efficient reaction whose substrates and products are well characterized, it is not clear what relationship exists between these substrates and those found *in vivo*. It would be of interest to examine the formation of stable joint molecules in reactions involving a ss DNA substrate possessing a free end because such a substrate is likely to be more representative of recombinogenic DNA existing in the cell than the ss circular DNAs used in previous *in vitro* studies (2–6). In this study, the formation of joint molecules between linear ss DNA and circular duplex DNA substrates has been analyzed. These studies reveal that homology at the 3′ end of the linear ss DNA is essential for stable RecA-catalyzed joint molecule formation. This observation is consistent with some existing biochemical data (4, 7), which indicate an important role for free homologous 3′ ends in recombination. However, it apparently contradicts predictions based on the polarity of strand exchange involving circular ss and linear duplex DNA substrates (2–4): if RecA-catalyzed strand exchange proceeds 5′-to-3′ relative to the ss DNA, then linear ss DNAs in which homology to the duplex circle is restricted to the 3′ end would form joint molecules that dissociate with time, while those in which homology is present at the 5′ end would grow and become more stable. Two possible explanations to resolve this apparent paradox and their relevance to our understanding of the strand exchange process are discussed.

MATERIALS AND METHODS

Enzymes and Proteins. RecA was a gift of D. Soltis and R. Bryant (this department). SSB was purchased from Pharmacia P-L Biochemicals; restriction endonucleases *Bam*HI, *Cla* I, and *Eco*RI, from New England Biolabs; and *Hin*cII, from Boehringer Mannheim.

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Abbreviations: ss DNA, single-stranded DNA; SSB, ss DNA binding protein.

*To whom reprint requests should be addressed.

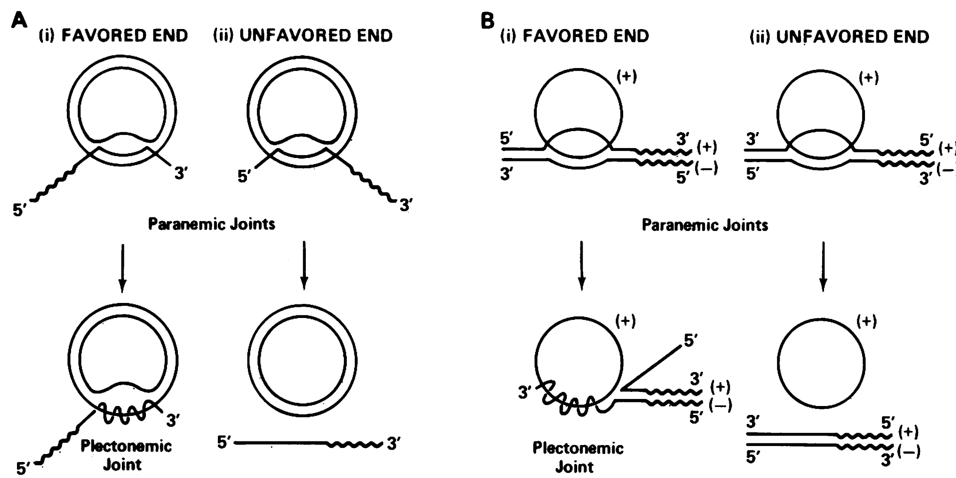

FIG. 1. Comparison of substrates used to study homologous pairing and strand exchange promoted by RecA *in vitro*. (*A*) Linear single strands and superhelical DNA. (*B*) Circular single strands and linear duplex DNA. Wavy lines represent nonhomologous DNA sequences.

Closed Circular Duplex DNAs. pMC874 contains 6.2 kilobases (kb) of the *lac* operon [including 3085 base pairs (bp) of *lacZ*] inserted into pACYC177 (8). pBR322-*lacZ*Δ was constructed by exchanging the *Bam*HI/*Eco*RI fragment of pBR322 (9) with the *Bam*HI/*Eco*RI fragment of pMC874 (8). Supercoiled pMC874, pBR322, and pBR322-*lacZ*Δ DNAs were isolated from a saturated culture and purified by CsCl/ethidium bromide equilibrium centrifugation. M13-mp18 (10) replicative form DNA was prepared by infecting cells, grown to OD_{600} = 0.5 in YT (8.0 g of tryptone/5 g of yeast extract/2.5 g of NaCl per liter) medium, with phage (multiplicity of infection = 20) (11). Cells were harvested after 1.5 hr and lysed, and the supercoiled DNA was isolated as above. All DNA concentrations are expressed in moles of nucleotide residues.

Linear ss DNAs. Three methods were used to obtain linear ss DNAs in which homology to the duplex DNA was restricted to either the 5′ or 3′ end.

(*i*) pBR322-*lacZ*Δ DNA (5 μg) was cut at the unique *Eco*RI restriction site and labeled at the 5′ end with polynucleotide kinase and [γ-^{32}P]ATP or at the 3′ end with DNA polymerase I large fragment and [α-^{32}P]dATP. The DNA was precipitated with ethanol and resuspended in 0.05 M NaOH and allowed to stand on ice for 15 min. The sample (50 μl) was adjusted to 10% glycerol and 0.01% bromophenol blue and electrophoresed through 1% low-gelling-temperature SeaPlaque (FMC, Rockland, ME) agarose in TAE buffer (40 mM Tris, pH 8.1/20 mM acetic acid/2 mM Na_2EDTA) at 1.5 V/cm for 12 hr at room temperature. DNA was visualized by ethidium bromide staining. The two bands of ss DNA were cut out of the gel, and the DNA was isolated by extraction with phenol.

(*ii*) pBR322-*lacZ*Δ DNA (10 μg) was linearized with *Eco*RI and labeled at one end or the other as above. The labeled linear duplex was cleaved into large and small fragments by *Cla* I. The large fragment was purified by agarose gel electrophoresis and denatured with alkali, and the strands were separated exactly as described above. Only one of the strands was labeled, and this one was used in subsequent joint molecule assays.

(*iii*) Circular ss DNA from bacteriophage M13mp18-*lacZ*Δ was annealed with a specific oligonucleotide to generate a unique restriction site. M13mp18-*lacZ*Δ was constructed by replacing the *Eco*RI/*Hin*cII fragment of M13mp18 with a *Eco*RI/*Hpa* I fragment of pMC874 comprising 2 kb of *lacZ* sequences. Viral (+)-strand ss DNA was prepared by concentration of phage by precipitation with PEG and removal of the viral coat protein by extraction with phenol (11). The circular ss DNA was annealed with a specific 16-mer or 18-mer, which generated a *Eco*RI or *Hin*cII site, respectively. After cleavage with the appropriate enzyme, the resultant linear ss DNAs were purified by gel electrophoresis and labeled at the 5′ end with polynucleotide kinase.

Assay for Joint Molecules. Standard reaction mixtures (total volume, 20 μl) contained 5 μM linear ss DNA, 10 μM circular duplex DNA, 33 mM Tris·HCl (pH 7.5), 13 mM $MgCl_2$, 1 mM dithiothreitol, and 1 mM ATP. RecA, one monomer per three nucleotides of ss DNA, was added and incubated for 5 min at 37°C. SSB, one monomer per eight nucleotides of ss DNA, was then added, and the incubation was continued for 20 min. The reactions were stopped by the addition of Na_2EDTA to 20 mM and $NaDodSO_4$ to 1%. Joint molecule formation was monitored by agarose gel electrophoresis of reaction mixtures, followed by autoradiography. Samples were adjusted to 10% glycerol and 0.01% bromophenol blue and electrophoresed through 0.7% agarose with the TAE buffer system. Gels were dried onto DE 81 paper (Whatman) and exposed to Kodak X-Omat AR-5 film for ≈1 hr.

Southern Analysis. DNA was electrophoresed through a 0.7% agarose gel in TAE buffer and transferred directly to Genetran (Plasco, Woburn, MA) without prior denaturation or hydrolysis. Hybridization was carried out with ^{32}P-labeled M13mp18 prepared by annealing the M13 17-mer sequencing primer (New England Biolabs) and extending with DNA polymerase I large fragment in the presence of [α-^{32}P]dCTP. Synthesis was directed away from *lacZ′* sequences, making a complementary copy of M13mp18 vector DNA. Synthesis was stopped early enough to leave the *lacZ* sequences predominantly ss. The probe was not denatured prior to hybridization. Hybridization was performed at 42°C in 50% formamide (vol/vol)/3× SSPE/5% sodium dextran sulfate (wt/vol)/1% $NaDodSO_4$ (wt/vol, where 1× SSPE = 150 mM NaCl/10 mM $NaH_2PO_4 \cdot H_2O$/1 mM Na_2EDTA, pH 7.4). The filter was washed twice for 15 min each in 50%/formamide/5× SSPE/0.2% $NaDodSO_4$ at room temperature and twice for 30 min each in 0.1× SSPE/0.1% $NaDodSO_4$ at 60°C.

Electron Microscopy. The spreading solution contained: 10 mM Na_2CO_3, 3.4 mM Na_2EDTA, 7 mM NaCl, 2.5% (vol/vol) formaldehyde, 10% (vol/vol) formamide, 0.01% cytochrome *c*, and 0.1–1 μg of DNA per ml. Spreadings were performed as described by Schnös and Inman (12), and samples were examined in a Philips EM300 at 40 kV.

RESULTS

The formation of joint molecules was studied by using closed circular duplex DNA and linear ss DNA in which homology to the duplex was limited to one end. A schematic diagram of the substrates used and the products formed is shown in Fig. 1*A*. In these experiments, the substrates share 3085 bp of homology to the *lacZ* region. Linear ss DNA was generated

as follows. pBR322-*lacZ*Δ DNA was linearized and labeled at the ends with ^{32}P, and the single strands were separated after denaturing the DNA and electrophoresing through neutral agarose gels. The two linear ss DNAs (fast- and slow-migrating) generated in this way were distinguished with respect to the end (5′ or 3′) carrying *lacZ* sequences by Southern analysis using M13mp18 as probe (Fig. 2*A*). The closed circular duplex DNA substrate was pMC874, which contains 6.2 kb of the *lac* operon (including 3085 bp of *lacZ*) inserted into pACYC177 (8). Except for the *lacZ* region, no homology is shared between the parental plasmids pBR322-*lacZ*Δ and pMC874.

Strand exchange reaction mixtures included one of the ^{32}P-labeled linear single strands, circular duplex DNA, RecA, SSB, ATP, and Mg^{2+}. After incubation for 20 min at 37°C, the reactants and products were separated by electrophoresis through a 0.7% agarose gel and visualized by autoradiography. Three species were detectable in the complete reaction mixture containing linear ss DNA in which homology to the duplex circle was limited to the 3′ end (Fig. 2*B*, lane 1): a faster migrating band, which moved with a mobility corresponding to linear ss pBR322-*lacZ*Δ DNA; a middle band corresponding to duplex linear pBR322-*lacZ*Δ DNA, which probably arose from contamination of ss DNA with its complement; and a slower migrating band, which corresponded to joint molecules. The third (slower migrating) band was observed only in complete reaction mixtures containing RecA and duplex DNA. No band was observed in the absence of RecA (Fig. 2*B*, lane 2) or duplex DNA (Fig. 2*B*, lane 3). Only linear ss DNAs in which *lacZ* sequences were located at the 3′ end formed joint molecules (Fig. 2*B*, lane 1); those in which *lacZ* sequences were limited to the 5′ end did not (Fig. 2*B*, lane 4).

To verify that joint molecule formation depends on a free homologous end and to eliminate the possibility of joint molecule formation by contaminating complementary ss DNA with a 5′ homologous end, the following two experiments were performed. In both sets of experiments standard reactions were carried out and analyzed as above. The important difference between the two experiments was the way in which the potential problem of contaminating complementary ss DNA was circumvented.

In the first experiment, pBR322-*lacZ*Δ circular duplex DNA cut to a linear form and labeled at either the 5′ or 3′ end was cleaved into large and small fragments by *Cla* I. The large fragments were purified and denatured with alkali, and the strands were separated as described above. Only one of the strands was labeled, and this one was used in subsequent joint molecule assays. The complementary strand was unlabeled and would not be detected in the autoradiograph. The 5′-end-labeled ss DNA carried 3′ *lacZ* homology and 5′ pBR322 homology, whereas the 3′-end-labeled ss DNA carried 5′ *lacZ* homology and 3′ pBR322 homology. Each of these ss DNA substrates was incubated with closed circular duplex DNA sharing homology with *lacZ* (pMC874) or pBR322 (pBR322). The reaction products were analyzed as before. Joint molecules were observed only in reactions in which homology with the duplex DNA was restricted to the 3′ end of the linear ss DNA (Fig. 3, lanes 3 and 6).

In the second experiment, linear ss DNA was generated by annealing short, defined oligonucleotides to viral (+)-strand circular DNA, thereby creating a unique restriction site and cleaving the duplex region with the appropriate restriction endonuclease. In this method there was no contaminating complementary ss DNA present in the reaction mixture. Linear ss DNAs so generated carrying 3′ *lacZ* homology and

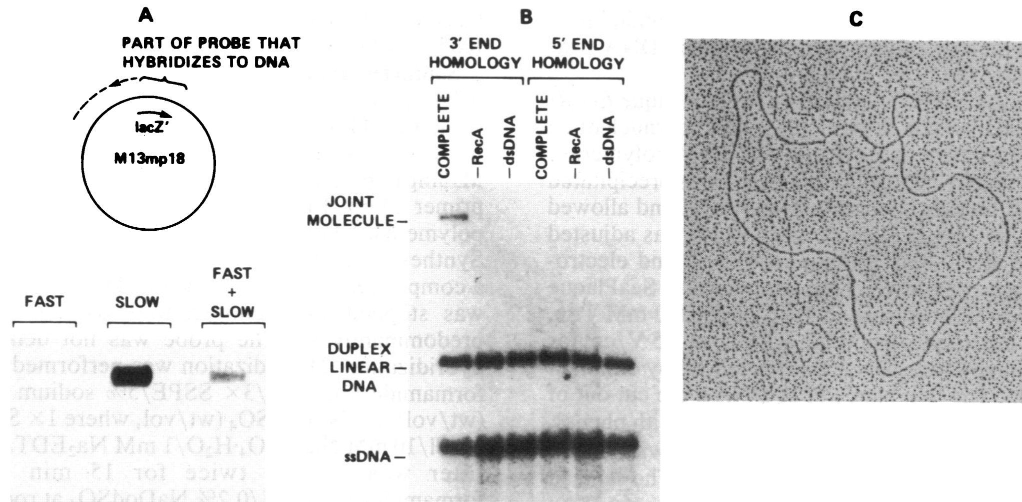

FIG. 2. (*A*) Southern analysis to determine whether the 3′ or 5′ end of the linear ss substrates carries *lacZ* sequences. The DNA samples were as follows: fast-migrating pBR322-*lacZ*Δ (0.2 μg), slow-migrating pBR322-*lacZ*Δ (0.2 μg), and a 1:1 mixture of fast- and slow-migrating pBR322-*lacZ*Δ (0.2 μg total). (Fast- and slow-migrating ss DNA refers to the relative mobility of the two strands of pBR322-*lacZ*Δ under strand-denaturing conditions.) Since the DNA was transferred directly from the gel to the filter without prior denaturation or hydrolysis, only linear ss DNA would be detected in the autoradiograph. Therefore, linear duplex DNA that resulted from complementary linear ss DNA would not be detected. The weak signal in lane 3 was probably due to excess fast or slow linear ss DNA that did not react with its complement to form linear duplex DNA and, thus, remained available for hybridization. An arrow below the *lacZ* sequence shows the direction of transcription. A broken arrow represents the M13 17-mer sequencing primer, and a broken line shows the direction of DNA synthesis. (*B*) Stable joint molecule formation between ^{32}P-labeled linear ss pBR322-*lacZ*Δ and supercoiled pMC874 in the presence of RecA and SSB as measured by autoradiography. Linear ss DNAs were generated by the first method described in *Materials and Methods*. The two linear ss substrates were complementary strands: homology with the *lacZ* portion of the circular duplex was limited to the 3′ end in the first three reactions and the 5′ end in the next three reactions. The reaction mixtures were complete or lacked either RecA or duplex DNA (dsDNA). (*C*) Electron micrograph of a joint molecule formed by RecA in the presence of SSB, ATP, and Mg^{2+} from linear ss pBR322-*lacZ*Δ carrying 3′ *lacZ* homology and supercoiled pMC874. After incubation for 20 min at 37°C, a complete standard reaction mixture (20 μl) was diluted 1:4 in 1× reaction buffer and loaded onto a 10–30% sucrose gradient in 0.05 M Tris·HCl, pH 8.0/0.025 M NaCl/0.5 mM Na_3EDTA. Gradients were centrifuged at 55,000 rpm for 1.5 hr at 4°C in a Beckman TSL55 rotor in a Beckman TL100 centrifuge. Fractions (28 × 50 μl) were collected from the bottom of the tube, and aliquots (25 μl) were analyzed by agarose gel electrophoresis followed by autoradiography. Fractions enriched for joint molecules (>90%) were examined by electron microscopy.

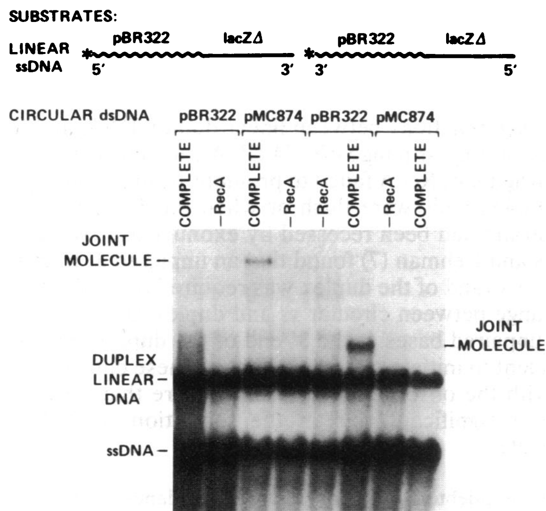

FIG. 3. Detection of stable joint molecules formed between ^{32}P-labeled linear ss DNA and circular duplex DNA in the presence of RecA and SSB by autoradiography. Linear ss DNAs were generated by the second method described in *Materials and Methods*. Standard reactions were performed by using circular duplex molecules that share homology with either the 3' or 5' end of the labeled linear ss DNA. In the first set of four reactions, 5' end-labeled ss DNA carrying *lacZ* sequences at the 3' end and pBR322 sequences at the 5' end was incubated with either pBR322 or pMC874 in complete and RecA-depleted reaction mixtures. In the next four reactions, 3'-end-labeled ss DNA carrying *lacZ* sequences at the 5' end and pBR322 sequences at the 3' end was incubated with either pBR322 or pMC874 in complete and RecA-depleted reaction mixtures.

5' M13mp18 homology reacted with duplex circular DNA carrying *lacZ* homology (pMC874) to form stable joint molecules (Fig. 4, lane 4) but failed to react with M13mp18 (Fig. 4, lane 10). Conversely, linear ss DNAs carrying 3' M13mp18 homology and 5' *lacZ* homology did not react with pMC874 (Fig. 4, lane 1) but did form joint molecules with M13mp18 (Fig. 4, lane 7). Taken together, these data demonstrate that homology at the 3' end of linear ss DNA is essential for stable joint molecule formation. Moreover, these experiments suggest that joint molecule formation is not sequence specific.

To confirm that the RecA protein and duplex DNA-dependent species observed in the previous experiments were joint molecules, the structure of the slower migrating species in Fig. 2*B* (lane 1) was characterized by electron microscopy. The products of a standard complete reaction were sedimented through a sucrose density gradient, and aliquots were analyzed by gel electrophoresis. A fraction enriched for the putative joint molecules and lacking any detectable ss DNA was examined by electron microscopy. In >40 molecules examined, all ss DNA was found complexed with duplex circles forming joint molecules, an example of which is shown in Fig. 2*C*. When the above analysis was repeated with linear ss DNA carrying 5' *lacZ* homology, no such structures were visible in >25 molecules examined. These observations confirm that, under optimal strand-exchange conditions [in the presence of SSB protein (13)], 3' homologous ends are required for stable joint molecule formation by RecA protein.

DISCUSSION

The results presented here demonstrate that in the presence of ATP, Mg^{2+}, and SSB, the formation of stable joint molecules between linear ss DNA and circular duplex DNA by RecA requires a free homologous 3' end. While existing biochemical studies (4, 7) on RecA protein point to the importance of free homologous 3' ends in strand exchange, it is not clear whether this polarity is a consequence of the unidirectional assembly of RecA along ss DNA and/or the polarity of branch migration. The two models, described in detail below, are proposed to account for the observed polarity of stable joint molecule formation. The first is a modification of existing models of RecA-catalyzed strand exchange in which branch migration proceeds 5'-to-3' with respect to the initiation complex (2–4). In the second, branch migration advances 3'-to-5' relative to the 3' homologous free end available for pairing.

The first model is as follows. Initially, RecA assembles onto ss DNA in the 5'-to-3' direction (14). These nucleoprotein filaments (or initiation complexes) interact with circular duplex DNA to form paranemic joints in which homologous sequences are aligned. Movement and/or extension of these paranemic joints continues until an end is reached, whereupon the free strand intertwines to form a stable plectonemic joint. Finally, branch migration proceeds in the 5'-to-3' direction with respect to the initiation complex. As a consequence of this polarity, linear ss DNAs in which homology is restricted to the 3' end would be expected to form joint molecules with duplex circles that dissociate preferentially, while those in which homology is limited to the 5' end would grow and form stable joint molecules that would not dissociate with time. The experiments described here, showing that only those linear ss DNAs with a free homologous 3' end form stable joint molecules with circular duplex DNAs, contradict this prediction.

In order to reconcile these apparently anomalous results with the existing model of RecA-promoted recombination, it is necessary to invoke an additional property of RecA action

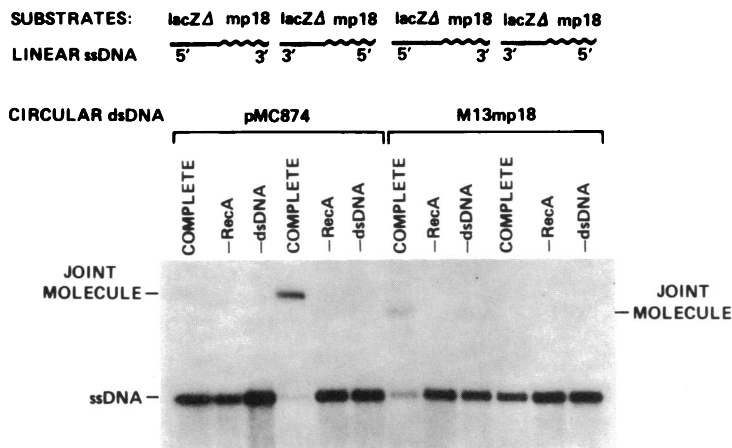

FIG. 4. Formation of stable joint molecules between ^{32}P-labeled linear ss DNA and supercoiled DNA in the presence of RecA and SSB. Standard reactions were performed by using linear ss DNA in which homology to the duplex circle was restricted to either the 3' or 5' end of the linear ss DNA. Linear ss DNAs were generated by the third method described in *Materials and Methods*. In the first set of six reactions, labeled linear ss DNAs carrying *lacZ* sequences at the 5' end (first three lanes) or 3' end (next three lanes) were incubated with pMC874. In the next set of six reactions, labeled linear ss DNAs carrying M13mp18 sequences at the 3' end (first three lanes) or 5' end (next three lanes) were incubated with M13mp18. The reaction mixtures were complete or lacked either RecA or duplex DNA (dsDNA).

in the presence of SSB. Under these conditions, ss DNA is initially covered by SSB (13). Nucleation and cooperative polymerization of RecA onto ss DNA displaces the SSB, resulting in the formation of RecA–ss DNA filaments or initiation complexes (13). Using duplex DNA templates with either 3′ or 5′ ss DNA tails, Register and Griffith (14) showed that RecA polymerized in the 5′-to-3′ direction on ss DNA and that 3′ ends were at least 10 times more likely to be covered by RecA as 5′ ends. If SSB were to act in a similar manner under the reaction conditions used in this study, 5′ homologous free ends would be prevented from forming plectonemic joints because of the presence of undisplaced SSB remaining at the 5′ ends of such nucleoprotein complexes. After $NaDodSO_4$ treatment, these paranemic joints would be lost. In contrast, 3′ homologous free ends could form stable joint molecules, since plectonemic joints formed at the time of protein removal (by the addition of $NaDodSO_4$) no longer would be constrained to move in the 5′-to-3′ direction but, instead, would be free to branch migrate spontaneously.

By this model, the polarity of stable joint molecule formation observed here is a consequence of the polarity of RecA polymerization. On the other hand, the polarity of strand exchange observed previously (2–4) with linear duplex DNA and ss circles would depend only on the polarity of RecA-catalyzed branch migration because ss circles would be uniformly coated by RecA, and the 5′ and 3′ ends of the (−)-strand of the linear duplex DNA would be equally available for pairing. One further prediction of this model is that, in the absence of SSB, stable joint molecules would preferentially form between duplex circles and linear ss DNAs in which homology was restricted to the 5′ end, assuming that RecA could completely coat even the very 5′ end. In fact, data obtained by Radding and co-workers (15, 16) using tailed duplex molecules and supercoiled DNA are consistent with this prediction.

A second equally plausible model is that branch migration proceeds in the 3′-to-5′ direction with respect to the linear ss DNA. However, this polarity is opposite to that obtained with linear duplex DNA and circular ss DNA (2–4). How can this paradox be resolved? If the polarity of strand exchange catalyzed by RecA is defined relative to the available homologous 3′ end rather than the initiation complex (RecA-coated ss DNA), then the two sets of reactions schematically diagrammed in Fig. 1 *A* and *B* proceed with the same polarity. According to this unifying view, the free homologous 3′ end, whether present on a ss DNA substrate or at the end of a duplex linear DNA, forms stable joint molecules with superhelical DNA (Fig. 1*A*) and circular single strands (Fig. 1*B*), respectively. In this model, the polarity of branch migration, 3′ to 5′ with regard to the homologous 3′ end available, determines the overall polarity of strand exchange.

Indeed, data concerning the effects of duplex DNA termini on strand exchange indicate an important role for an unpaired 3′ end in strand exchange reactions involving duplex linear and ss circular DNAs. West *et al.* (4) and Soltis and Lehman (7) demonstrated that the 3′ end of the linear duplex must extend beyond the 5′ end for strand exchange to occur efficiently. West *et al.* (4) showed that, in reciprocal strand exchange reactions between linear duplex DNA and homologous circular ss phage ϕX174 DNA carrying a short hybridized fragment, RecA failed to promote strand exchange when the duplex ends were flush or when the 3′ terminus of the (−)-strand had been recessed by exonuclease III treatment. Soltis and Lehman (7) found that an unpaired 3′ terminus on the (−)-strand of the duplex was required for efficient strand exchange between circular ss and duplex DNAs. As few as four unpaired bases at the 3′ end of the duplex appear to be sufficient to initiate strand exchange. These data are consistent with the observations presented here that a free 3′ end plays a significant role in the formation of stable joint molecules.

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