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recA protein of *Escherichia coli* promotes branch migration, a kinetically distinct phase of DNA strand exchange

(DNA-binding protein/D loops/heteroduplex DNA/recombination)

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ABSTRACT **The *recA* protein of *Escherichia coli* promotes the complete exchange of strands between full-length linear duplex and single-stranded circular DNA molecules of bacteriophage φX-174, converting more than 50% of the single-stranded DNA into heteroduplex replicative form II-like structures. Kinetically, the reaction can be divided into two phases, formation of short heteroduplex regions (D loops) and extension of the D loops via branch migration. *recA* protein participates directly in both phases. D loops are formed efficiently in the presence of ATP or the nonhydrolyzable ATP analog adenosine 5′-[γ-thio]triphosphate, whereas D-loop extension requires continuous ATP hydrolysis. Complete strand exchange requires a stoichiometric amount of *recA* protein and is strongly stimulated by the single-stranded-DNA-binding protein of *E. coli*.**

Models for homologous genetic recombination generally divide the process into several distinct phases (1–3). The first consists of a pairing reaction between two homologous DNA duplexes, leading to the formation of heteroduplex regions in one or both of the molecules. In the next phase the heteroduplex regions are moved or extended. Such heteroduplex regions may be several thousand nucleotides long (4). Subsequent steps serve to convert the branched structures to recombinant products. A detailed molecular understanding of this complex process has not yet been achieved. However, an important recent advance has been the identification and isolation of the product of the *recA* gene of *Escherichia coli* (5–9), a protein known to be essential at an early stage of homologous recombination (10, 11).

Although it has a molecular weight of only 37,800 (12, 13), the *recA* protein promotes a remarkable variety of reactions *in vitro* (for review see ref. 14). In addition to being a highly specific protease, the *recA* protein is a DNA-dependent ATPase and UTPase, and it promotes the homologous pairing of complementary single strands of DNA and the assimilation of single strands into homologous DNA duplexes to form locally heteroduplex regions termed D loops. Strand assimilation is especially important because, coupled with earlier *in vivo* findings (11), it firmly establishes a direct role for *recA* protein in the first phase of homologous recombination.

Extension of a D loop involves movement of a crossover junction in the DNA, a reaction termed branch migration. This process serves to shorten or extend the heteroduplex region with a reciprocal loss or gain of parental base pairs. Branch migration can be driven enzymatically by the action of polymerases, nucleases, or topoisomerases (3). However, because it may involve little or no net change in free energy, branch migration has also been assumed to be a random and uncatalyzed process. Work with model systems has in fact demonstrated that branch migration occurs spontaneously and, assuming a random walk, proceeds at the rate of 6000 bases sec^{-1} or greater at 37°C (15–19). Significant branch migration would thus be expected to occur subsequent to *recA* protein-promoted strand assimilation. DasGupta *et al.* (20) have recently observed *recA* protein-generated D-loop structures containing heteroduplex regions that extend for several thousand base pairs, implying that branch migration does indeed occur either spontaneously or as a result of the action of *recA* protein (20).

We have found that D loops formed by *recA* protein from full-length linear duplex DNA and circular single-stranded DNA (ssDNA) of bacteriophage φX174 (φX) can be extended by branch migration in a reaction promoted by *recA* protein in the presence of *E. coli* ssDNA-binding protein (SSB). The result is a complete exchange of strands with the efficient production of molecules like φX replicative form II (RFII). Inasmuch as *recA* protein-promoted branch migration is relatively slow and kinetically distinguishable from D-loop formation, this system has served as a model with which to study these two phases of genetic recombination.

MATERIALS AND METHODS

Materials. *recA* protein was purified to homogeneity by a recently described procedure (21). Concentrations of *recA* protein were determined by using an $A_{280}^{1\%}$ of 5.16 based upon its amino acid composition (12, 13). SSB was the generous gift of J. Kaguni of this department. Hexokinase, pyruvate kinase, and nuclease S1 were purchased from Sigma. Restriction endonuclease *Ava* I was purchased from New England BioLabs.

φX DNA was isolated from φX*am3*-infected *E. coli* C. Preparation of circular (viral) single strands has been described (22). Supercoiled φX RFI DNA was prepared by a procedure described for isolation of plasmid DNA (ref. 23, p. 116). In all cases, the DNA was banded to equilibrium at least twice in CsCl gradients containing ethidium bromide. Labeling of DNA with [^{3}H]thymidine was carried out as described (24). Full-length linear duplex φX DNA was prepared by endonuclease *Ava* I cleavage of φX RFI followed by phenol extraction; φX DNA contains only a single *Ava* I site. Bacteriophage M13 single-stranded circular DNA and bacteriophage P22 duplex DNA were generous gifts from J. Kobori and K. McEntee, respectively, of this department.

ATP, ADP, phospho*enol*pyruvate, and glucose 6-phosphate were purchased from Sigma. ^{3}H-Labeled nucleotides were purchased from Amersham. Adenosine 5′-[γ-thio]triphosphate (ATP[γS]) was purchased from Boehringer Mannheim. Nitrocellulose filters (HAWP, 0.45 μm pore diameter) were from Millipore.

Methods. Four different assays were used to monitor the fate

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Abbreviations: φX, bacteriophage φX174; RFI (replicative form I), supercoiled double-stranded DNA; RFII (replicative form II), circular double-stranded DNA containing a nick in one strand; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; SSB, *E. coli* ssDNA-binding protein; ATP[γS], adenosine 5′-[γ-thio]triphosphate.

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of the various reaction components during strand exchange. The standard reaction mixture in each case contained 25 mM Tris·HCl, 80% cation (final pH = 7.2); 5% (vol/vol) glycerol; 10 mM $MgCl_2$; 1 mM dithiothreitol; 1 mM ATP; 5.6 μM linear duplex ϕX DNA; 3.3 μM ϕX circular ssDNA (viral strands); 2.0 μM *recA* protein; and 0.3 μM SSB. Where indicated, ATP[γS] was used at a concentration of 200 μM. All reactions took place at 37°C and were initiated by the addition of *recA* protein after preincubation of other components.

Nitrocellulose filter binding. This assay is based on the method of Beattie *et al.* (25) and was used to measure the fraction of ^{3}H-labeled linear duplex ϕX DNA molecules that acquired D loops. At the times indicated, aliquots (50 or 100 μl) were removed. $NaDodSO_4$ and EDTA were added immediately to final concentrations of 0.5% and 12 mM, respectively, followed after 20 sec by 1 ml of a solution containing 2 M NaCl and 0.15 M sodium citrate. Within 10 min the mixtures were filtered through nitrocellulose filters and rinsed with an additional 2 ml of 2 M NaCl/0.15 M sodium citrate. The filters were dried and assayed for radioactivity. In each experiment one reaction mixture was set up without *recA* protein or ssDNA. Aliquots of this mixture were treated with $NaDodSO_4$ and EDTA as described above. Several (two to four) of these were heated at 100°C for 4 min and cooled quickly in ice. After addition of 1 ml of 2 M NaCl/0.15 M sodium citrate they were filtered as before. Values obtained with these samples were used to define 100% strand assimilation. Those aliquots filtered without heating define the background retention of ^{3}H-labeled duplex DNA (less than 2% in all cases).

Resistance to S1 nuclease. This assay, which is based on a method used previously for measurement of *recA* protein-catalyzed DNA renaturation (6), measures heteroduplex base pair formation during branch migration. ^{3}H-Labeled ϕX circular ssDNA was used in the standard reaction mixture, and its incorporation into S1 nuclease-resistant material was measured.

Aliquots (50 μl) were removed from the reaction mixture at the times indicated. $NaDodSO_4$ was added to a final concentration of 0.8%, and the mixtures were rapidly cooled in ice. After at least 15 min the mixtures were diluted 1:13 with a solution containing 500 mM NaCl, 50 mM sodium acetate at pH 4.6, 1 mM zinc acetate, denatured calf thymus DNA at 30 μg/ml, and S1 nuclease at 2.0 Sigma units/ml. (One unit is defined as the amount of S1 nuclease needed to digest 1 μg of ssDNA in 1 min at 37°C.) After incubation at 37°C for 30 min, carrier DNA (20 μl of calf thymus DNA at 5 mg/ml) was added and the mixture was diluted 1:2 with cold 10% trichloroacetic acid. After 30 min on ice the mixtures were filtered through Whatman GF/C filters. The filters were rinsed with 3 ml of 10% trichloroacetic acid, followed by 1 ml of 95% (vol/vol) ethanol, then dried and assayed for radioactivity. Production of S1 nuclease-resistant, ^{3}H-labeled DNA was completely dependent upon the presence of complementary sequences within the duplex DNA. Control reaction mixtures lacking duplex DNA were included in each experiment and define the S1 nuclease-resistant background (<2% in all cases). To determine the maximal amount of acid-precipitable ^{3}H present, a reaction mixture was treated identically except that no S1 nuclease was added. ssDNA is in slight excess so that if all of the linear duplex molecules in the standard reaction mixture were converted to RFII, only 85% of the single-stranded circles would be utilized, and thus become S1-nuclease-resistant. The acid-precipitable maximum was therefore corrected by a factor of 0.85, and this value taken as 100% heteroduplex formation.

Agarose gel electrophoresis. Aliquots (35 μl) of the standard reaction mixture were adjusted to 10% (vol/vol) glycerol, 12 mM EDTA, 1% $NaDodSO_4$, and 0.01% (wt/vol) bromphenol blue. Electrophoresis was performed in 0.8% agarose gels using a Tris/acetate buffer system as described (ref. 23, p. 148). Linear duplex ϕX DNA is easily separated from ϕX RFII under these conditions.

DNA-dependent ATPase. This assay was performed as described (6) with [^{3}H]ATP (40 μCi/ml; 1 Ci = 3.7×10^{10} becquerels).

RESULTS

The complete exchange of strands between circular single-stranded and linear duplex ϕX DNA molecules promoted by *recA* protein yields a full-length linear single strand and a nicked circular duplex (ϕX RFII). The demonstration that the *recA* protein will promote strand exchange is provided in Fig. 1. A band that comigrates with ϕX RFII was evident after 20 min; another band corresponding to the displaced linear single strand appeared at the same time. This result suggests that unwinding of the linear duplex and formation of heteroduplex are tightly coupled. As shown in Fig. 1, the time at which RFII-like molecules appeared was independent of the concentration of *recA* protein. The reaction required *recA* protein, ATP, Mg^{2+} (not shown), and homologous single strands. Both the rate and ex-

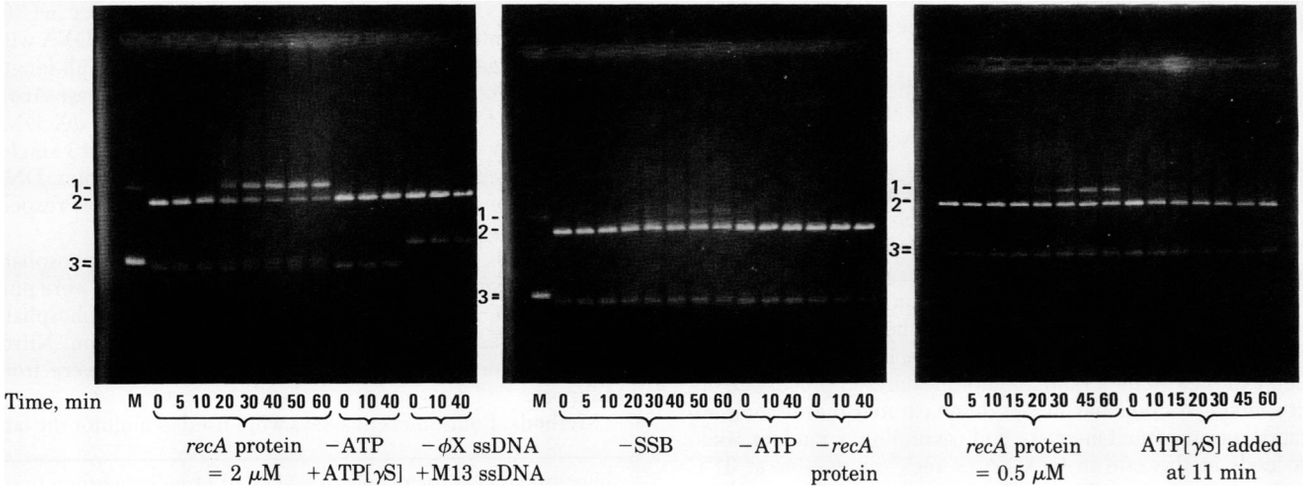


FIG. 1. *recA* protein-promoted strand exchange measured by agarose gel electrophoresis. Markers (M): 1, ϕX RFII; 2, ϕX linear duplex DNA; 3 (upper), ϕX linear single strands; 3 (lower), ϕX circular (viral) single strands. The lower band in the M channel is ϕX RFI DNA, which migrates close to ssDNA in the buffer system used.

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tent of reaction were greatly reduced in the absence of SSB. Electron microscopy verified that a high proportion of RFII-like molecules were produced in the reaction (not shown).

***recA* Protein-Promoted Strand Exchange Is a Two-Phase Reaction.** Although most of the duplex molecules in the reaction contained D loops within 5 min, as judged by their retention on nitrocellulose filters (Fig. 2), relatively few heteroduplex base pairs were formed as shown by the continued susceptibility of the ssDNA to S1 nuclease (Fig. 2). Heteroduplex formation proceeded slowly and was complete only after an additional 20 min. Completion of heteroduplex formation corresponded closely to the appearance of RFII as judged by agarose gel electrophoresis (Fig. 1). Thus, conversion of D loops to RFII appears to be a slow, largely synchronous process. This interpretation is supported by another experiment shown in Fig. 1. When ATP[γS] was added 11 min after the beginning of the reaction, 20% of the ssDNA had been incorporated into S1 nuclease-resistant material; however, RFII-like forms were undetectable by agarose gel electrophoresis (<5%). Thus, the net extension of D loops by branch migration is a kinetically significant phase of strand exchange and is measured directly by the S1 nuclease assay. Strand exchange may therefore be used as a model system for the analysis of two phases of homologous recombination—e.g., strand assimilation leading to D-loop formation, and branch migration.

D-Loop Formation. As shown in Fig. 3, D-loop formation measured by nitrocellulose filter binding was completely dependent upon ATP and *recA* protein. It was also stimulated by SSB (26, 27). D loops were not formed when nonhomologous ssDNA (M13) was used in place of ϕX single strands, confirming that the nitrocellulose filter assay measures formation of D loops with base-paired heteroduplex regions. D loops were also formed when the nonhydrolyzable ATP analog ATP[γS] replaced ATP, confirming an earlier result (26). Substitution of M13 ssDNA for ϕX ssDNA eliminated the reaction in the presence of ATP[γS] just as it did with ATP, so that base-paired heteroduplex regions are generated in both cases. Thus, the *recA* protein-dependent homologous pairing reaction resulting in the formation of D loops requires ATP but not its hydrolysis.

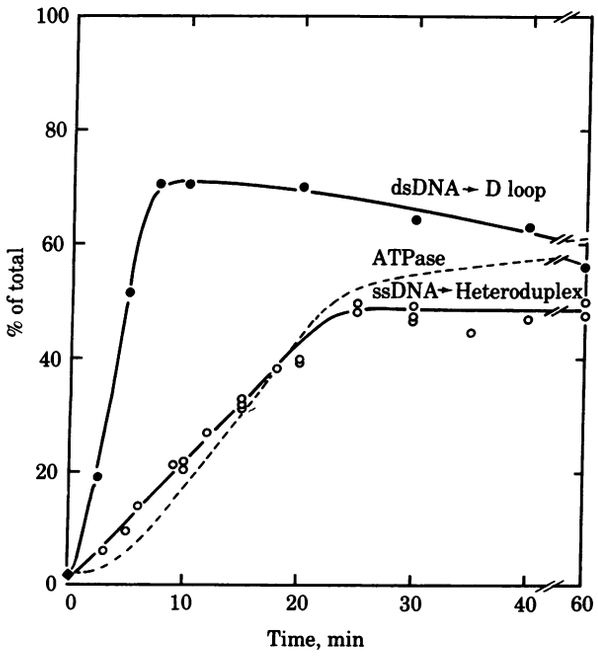


FIG. 2. D-loop formation and branch migration promoted by *recA* protein. dsDNA, double-stranded DNA.

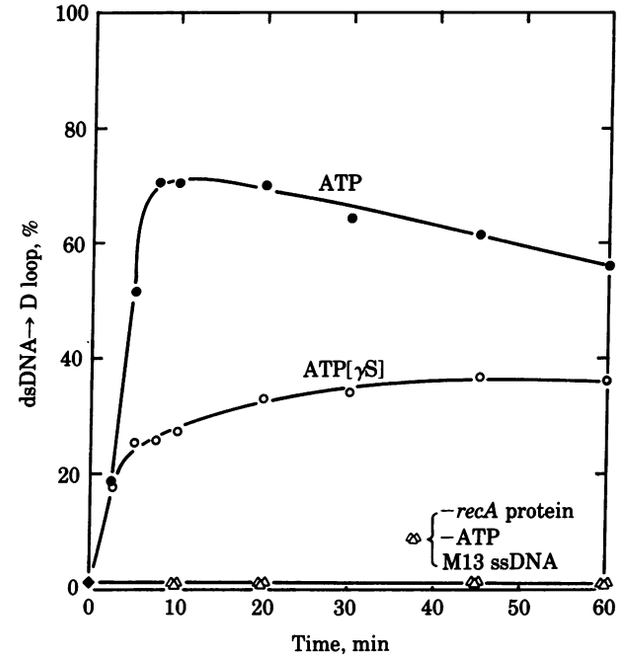


FIG. 3. D-loop formation promoted by *recA* protein in the presence of ATP or ATP[γS]. Control experiments in which M13 ssDNA was substituted for ϕX single strands yielded identical results for reactions with ATP or ATP[γS].

Branch Migration. Extension of D loops as measured by conversion of ^{3}H-labeled ϕX ssDNA to S1 nuclease resistance was also completely dependent on *recA* protein and ATP (Fig. 4). The heterologous duplex DNA from bacteriophage P22 could not substitute for ϕX dsDNA, demonstrating that S1 nuclease resistance is a valid measure of base pair formation and not a

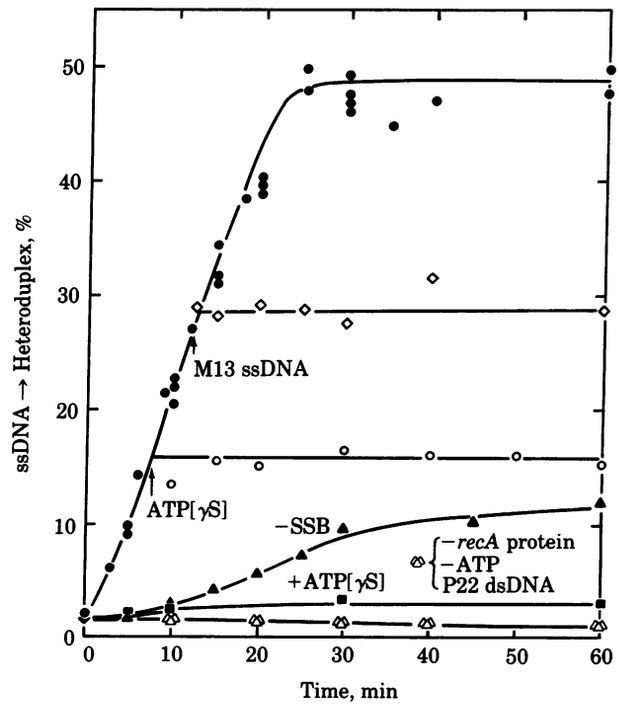


FIG. 4. Heteroduplex formation via branch migration promoted by *recA* protein. ●, Standard reaction; ◇, 20-fold excess of M13 ssDNA added at the time indicated; ○, 200 μM ATP[γS] added as indicated; ▲, no SSB; ■, ATP[γS] in place of ATP; △, controls as indicated (when P22 dsDNA was added, ϕX dsDNA was omitted).

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consequence of nonspecific protection of the ssDNA by SSB or *recA* protein. When ATP[γS] was substituted for ATP, about 3% of the ssDNA became S1 nuclease resistant, consistent with the formation of a large number of relatively small D loops that were not further converted to RFII-like molecules. Assuming that an average D loop contains 300–500 base pairs of heteroduplex, and that D-loop conversion to RFII is complete within 20 min, we estimate the rate of branch migration under these conditions to be about 4 base pairs per sec.

Addition of ATP[γS] to an ongoing reaction abruptly halted branch migration. Inasmuch as ATP[γS] is a potent inhibitor of the DNA-dependent ATPase activity associated with *recA* protein (6), this result implies that there is a continuous requirement for ATP hydrolysis during *recA* protein-promoted branch migration. This conclusion is strengthened by the finding that addition of sufficient glucose and hexokinase to convert all of the ATP to ADP with a half-time of $\approx$10 sec also promptly halted the net extension of heteroduplex regions (data not shown). Hexokinase, glucose, glucose 6-phosphate, or ADP separately had no effect on the reaction.

Net heteroduplex entension was also halted by the addition of a 10-fold excess of M13 ssDNA (Fig. 4). Because *recA* protein has a high affinity for ssDNA, the excess heterologous DNA would be expected to sequester any free *recA* protein in the reaction.

The *recA* protein in these experiments was present at a ratio of 1 *recA* protein monomer per 1.4 nucleotides of ssDNA (1 per 4.5 total nucleotides). As shown in Fig. 5, both the apparent rate and extent of branch migration were directly dependent on the *recA* protein concentration. This effect is not due to depletion of the ATP, because it occurred in the presence of an ATP-regenerating system (phospho*enol*pyruvate and pyruvate kinase). The *recA* protein therefore acts stoichiometrically rather than catalytically during branch migration, with approximately 1 *recA* protein monomer required for every 2 base pairs of heteroduplex formed.

As shown in Fig. 4, branch migration is strongly stimulated by SSB. In the absence of SSB, approximately 10% of the ssDNA was incorporated into heteroduplex, and fewer RFII-like molecules could be detected by agarose gel electrophoresis (Fig. 1). Decreasing the concentration of SSB to less than $\frac{1}{3}$ inhibited the reaction, whereas a 4-fold increase had no apparent effect (data not shown).

DISCUSSION

The system described here for the measurement of strand exchange has a number of useful features. (*i*) Both reactants and products are well defined and easily separable by gel electrophoresis. (*ii*) All of the single strands are viral strands and have no complement except within the duplex DNA. Thus, *recA* protein-promoted renaturation of single strands does not compete with strand exchange. (*iii*) The fate of each of the substrates in the reaction can be followed quantitatively. Two groups have recently reported related *recA* protein-dependent strand exchange reactions using somewhat different duplex substrates (20, 28).

With this system we have demonstrated that strand exchange can be separated into two kinetically distinct phases, consisting first of strand assimilation to generate D loops, followed by branch migration to extend the heteroduplex regions and complete the exchange (Fig. 6). Several lines of evidence point to a direct role for *recA* protein in branch migration. (*i*) Replacement of ATP by ATP[γS] results in the formation of D loops containing short heteroduplex regions (300–500 base pairs). However, these D loops are not extended to produce RFII-like structures, indicating that conversion does not occur via un-

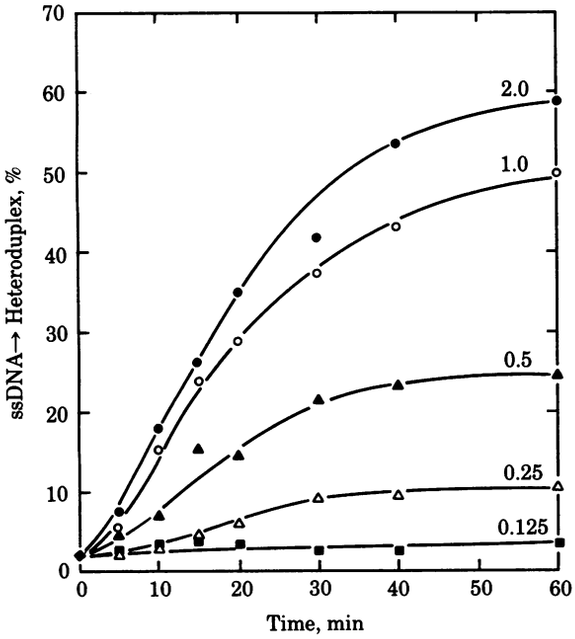


FIG. 5. Effect of *recA* protein concentration on the rate and extent of heteroduplex formation via branch migration. Standard reaction conditions were used except that SSB was 0.23 μM. An ATP-regenerating system consisting of 1.5 mM phospho*enol*pyruvate and an amount of pyruvate kinase (determined empirically) that converted 1 mM ADP to ATP with a half-time of $\approx$10 sec was included in all cases. Under these conditions no net ATP hydrolysis occurred for 50 min. The numbers on the curves refer to the concentration of *recA* protein, in μM.

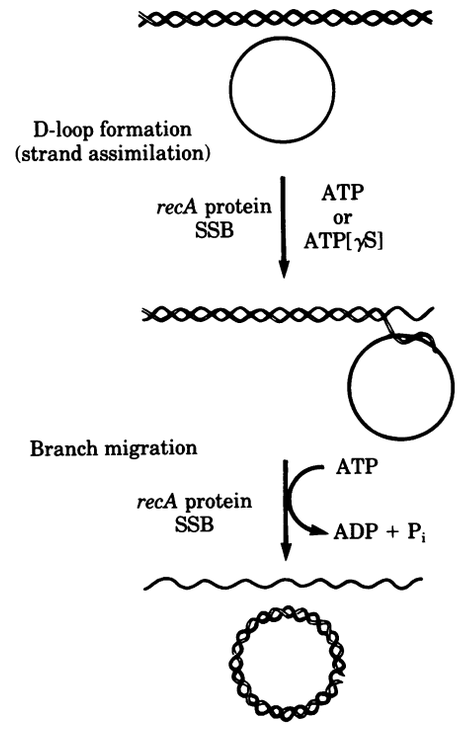


FIG. 6. DNA strand exchange promoted by *recA* protein. The linear duplex and circular ssDNAs are converted in the first phase to a D-loop structure shown here at one end of the duplex. Branch migration subsequently yields a full-length linear single strand and RFII.

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catalyzed or SSB-promoted branch migration. (*ii*) ATP hydrolysis is required throughout the reaction. (*iii*) The net extension of heteroduplexes is halted by the addition of an excess of heterologous ssDNA, a treatment that should not affect uncatalyzed branch migration.

D-loop formation does not require ATP hydrolysis under these conditions (i.e., high concentrations of *recA* protein and SSB). It has been shown previously that ATP[γS] will not support this reaction at lower concentrations of *recA* protein or in the absence of SSB (29, 30). Because ATP hydrolysis is not an absolute requirement for D-loop formation, we believe it is unlikely that processive movement of *recA* protein along the DNA is involved in this phase of strand exchange.

One plausible mechanism to account for *recA* protein-driven branch migration involves extension of a D loop by processive movement of the protein along the DNA. However, the finding that excess heterologous ssDNA rapidly inhibits extension of the heteroduplex argues strongly against a mechanism of this kind. A processive mechanism is also difficult to reconcile with the requirement for stoichiometric amounts of *recA* protein. Moreover, distributive mechanisms, in which *recA* protein acts transiently at a branch point of the D loop, are equally difficult to reconcile with the dependence of the extent of reaction on *recA* protein concentration.

A mechanism that is consistent with our findings is based on the observation that *recA* protein forms extensive and highly structured filaments under appropriate conditions (unpublished data). Thus, the *recA* protein involved in D-loop formation could serve as a core onto which additional *recA* protein could bind to form a growing filament. Branch migration would then result from extension of the filament with no requirement for movement of *recA* protein along the DNA. The reaction might be analogous to the ATP-dependent formation of actin filaments (31). Removal of free *recA* protein by the addition of excess ssDNA would halt filament growth and thereby block branch migration. We do not yet understand the role of ATP hydrolysis in this reaction.

The requirement that *recA* protein be added in stoichiometric amounts to promote strand exchange is not inconsistent with the levels of *recA* protein normally found in *E. coli*. Recent measurements indicate that several thousand *recA* protein molecules are present during normal cell growth (C. Paoletti, personal communication), a level that can be increased by almost two orders of magnitude after treatments that damage the DNA. Our findings provide one rationale for the constitutive presence of these large quantities of *recA* protein.

The rate of *recA* protein-promoted branch migration in this system, $\approx$4 base pairs per sec, was estimated by assuming unidirectional branch migration. On the basis of the recent results of DasGupta *et al.* (20), as well as a consideration of topological constraints, it is reasonable to assume that strand exchange begins with a D loop formed at one end of the linear duplex. In this situation, a random walk mechanism could generate only a few percent RFII, regardless of the rate of the reaction. The conversion of more than 50% of the D loops to RFII therefore implies that directionality is an important characteristic of *recA* protein-promoted branch migration. Directionality is also inherent in the filament mechanism described above.

The stimulation of branch migration by SSB is more difficult to explain. Previous results indicated that SSB stimulated D-loop formation by low concentrations of *recA* protein by removing excess ssDNA (26). In contrast, SSB stimulates branch migration many fold even though sufficient *recA* protein is present to saturate the ssDNA. This aspect of strand exchange requires further study.

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1. Stahl, F. W. (1979) *Genetic Recombination* (Freeman, San Francisco).
2. Hotchkiss, R. D. (1974) *Annu. Rev. Microbiol.* **28,** 445–468.
3. Radding, C. M. (1978) *Annu. Rev. Biochem.* **47,** 847–880.
4. Sodergren, E. J. & Fox, M. S. (1979) *J. Mol. Biol.* **130,** 357–377.
5. McEntee, K., Hesse, J. E. & Epstein, W. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 3979–3983.
6. Weinstock, G. M., McEntee, K. & Lehman, I. R. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 126–130.
7. Ogawa, T., Wabiko, H., Tsurimoto, T., Horii, T., Masukata, H. & Ogawa, H. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **43,** 909–915.
8. Roberts, J. W., Roberts, C. W., Craig, N. L. & Phizicky, E. M. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **43,** 917–920.
9. Shibata, T., DasGupta, C., Cunningham, R. P. & Radding, C. M. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1638–1642.
10. Clark, A. J. (1973) *Annu. Rev. Genet.* **7,** 67–86.
11. Holloman, W. K. & Radding, C. M. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 3910–3914.
12. Horii, T., Ogawa, T. & Ogawa, H. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 313–317.
13. Sancar, A., Stachelek, C., Konigsberg, W. & Rupp, W. D. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2611–2615.
14. McEntee, K. & Weinstock, G. M. (1981) in *The Enzymes*, ed. Boyer, P. (Academic, New York), 3rd. Ed., in press.
15. Broker, T. R. & Lehman, I. R. (1971) *J. Mol. Biol.* **60,** 131–149.
16. Lee, C. H., Davis, R. W. & Davidson, N. (1970) *J. Mol. Biol.* **48,** 1–22.
17. Radding, C. M., Beattie, K. L., Holloman, W. K. & Wiegand, R. C. (1977) *J. Mol. Biol.* **116,** 825–839.
18. Thompson, B. J., Camien, M. N. & Warner, R. C. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 2299–2303.
19. Warner, R. C. & Tessman, I. T. (1978) in *The Single-Stranded DNA Phages*, eds. Denhardt, D. T., Dressler, D. & Ray, D. S. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 417–432.
20. DasGupta, C., Shibata, T., Cunningham, R. P. & Radding, C. M. (1980) *Cell* **21,** 437–446.
21. Cox, M. M., McEntee, K. & Lehman, I. R. (1981) *J. Biol. Chem.* **256,** in press.
22. Cunningham, R. P., DasGupta, C., Shibata, T. & Radding, C. M. (1980) *Cell* **20,** 223–235.
23. Davis, R. W., Botstein, D. & Roth, J. R. (1980) *Advanced Bacterial Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
24. Pagano, J. S. & Hutchison, C. A., III (1971) in *Methods in Virology*, eds. Maramorosch, K. & Koprowski, H. (Academic, New York), Vol. 5, p. 91.
25. Beattie, K. L., Wiegand, R. C. & Radding, C. M. (1977) *J. Mol. Biol.* **116,** 783–803.
26. McEntee, K., Weinstock, G. M. & Lehman, I. R. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 857–861.
27. Shibata, T., DasGupta, C., Cunningham, R. P. & Radding, C. M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2606–2610.
28. West, S. C., Cassuto, E. & Howard-Flanders, P. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2100–2104.
29. McEntee, K., Weinstock, G. M. & Lehman, I. R. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 2615–2619.
30. Shibata, T., Cunningham, R. P., DasGupta, C. & Radding, C. M. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5100–5104.
31. Simpson, P. A. & Spudich, J. A. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 4610–4613.

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