Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 762–766, February 1982
Biochemistry

Polar branch migration promoted by recA protein: Effect of mismatched base pairs*

(genetic recombination/strand exchange/heteroduplex joints/gene conversion)

CHANCHAL DASGUPTA AND CHARLES M. RADDING

Departments of Human Genetics and Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut 06510

Communicated by Francis H. Ruddle, October 13, 1981

ABSTRACT *Escherichia coli* recA protein makes joint molecules from single-stranded circular phage DNA (viral or plus strand) and homologous linear duplex DNA by a polar reaction that displaces the 5′ end of the plus strand from the duplex molecule [Kahn, R., Cunningham, R. P., DasGupta, C. & Radding, C. M. (1981) *Proc. Natl. Acad. Sci. USA* 78, 4786–4790]. Growth of the heteroduplex joint, which results from strand exchange or branch migration, stopped at the borders of regions of nonhomologous DNA that were variously located 145, 630, or 1202 nucleotides from the end. Accumulation of migrating branches at heterologous borders demonstrates that their migration is not the result of random diffusion but is actively driven by recA protein. Growth of the heteroduplex joint was blocked even when a heterologous insertion was located in the single-stranded DNA, a case in which the flexible single-stranded region might conceivably fold out of the way under some condition. The recA protein did not make joint molecules from phage φX174 and G4 DNAs, which are 70% homologous, but did join phage fd and M13 DNAs, which are 97% homologous. In the latter case, heteroduplex joints extended through regions containing isolated mismatched base pairs but stopped in a region where the fd and M13 sequences differ by an average of 1 base pair in 10. These results suggest that in genetic recombination the discrimination of perfect or near-perfect homology from a high degree of relatedness may be attributable in part to the mechanism by which recA protein promotes strand transfer.

The heteroduplex joint, a splice containing paired strands from each of two DNA molecules, is a key feature of homologous genetic recombination (1, 2). A body of genetic evidence supports the view that the formation and processing of these heteroduplex joints is largely responsible for the classical phenomena of meiotic gene conversion and aberrant meiotic segregation, phenomena that are associated with reciprocal crossing-over (3, 4). According to this theory of gene conversion and recombination, there are mechanisms of strand transfer that permit the inclusion of mismatched bases, even extensive insertions or deletions (5), and there are mechanisms that can correct mismatches prior to replication. Conversion of extensive insertions and deletions has been observed (3, 6, 7), and the correction of mismatched bases has been demonstrated by transfection of bacteria with hybrid or heteroduplex DNA containing various mismatches (1, 2, 8).

Recent observations on the recA protein of *Escherichia coli* have made it possible to study the formation of heteroduplex joints in a precise way *in vitro*. The recA protein is a DNA-dependent ATPase (9, 10) that promotes homologous pairing of DNA molecules *in vitro* (11). Experimentally, we have distinguished two aspects of this pairing activity: *synapsis*, which pairs a single-stranded region of DNA with homologous duplex DNA,

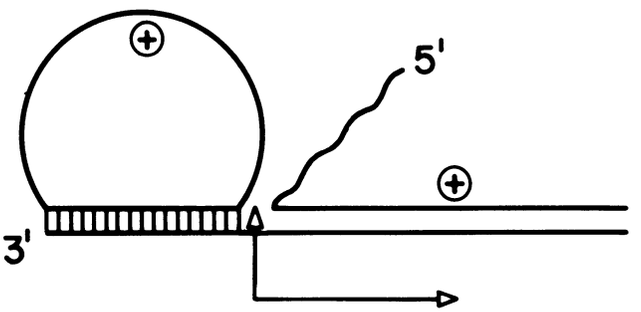

FIG. 1. Polar strand exchange or branch migration promoted by *E. coli* recA protein. The recA protein pairs a circular single strand of phage DNA with its complement in duplex DNA, displacing the 5′ end of the linear viral or plus strand and making a heteroduplex joint that begins only from the 3′ end of the complementary strand (12, 14). For emphasis, the heteroduplex region or heteroduplex joint is marked by short vertical lines. The vertical arrow signals the branch point or point of strand exchange, and the horizontal arrow indicates the direction of net migration of the branch.

even in the absence of a free end in any strand of DNA, and *strand transfer* or strand exchange, which causes the formation of long heteroduplex joints in some circumstances (12, 13); in particular, recA protein acting on circular single-stranded DNA and linear duplex DNA promotes the polar formation of heteroduplex joints. The circular plus strand replaces its homolog in the linear duplex DNA starting only from the 5′ end of the linear plus strand (see Fig. 1 and ref. 14). Thus, this combination of substrates provides a unique opportunity to study heteroduplex formation that starts from a defined site and proceeds in one direction. To determine some of the characteristics of heteroduplex formation, we have examined the effects of small and large mismatches on the growth of heteroduplex joints.

METHODS

Formation of Joint Molecules by recA Protein. The standard 20-μl reaction mixture contained 31 mM Tris·HCl (pH 7.5), 1.2 mM ATP, 20 mM $MgCl_2$, 4–6 μM each single-stranded and duplex DNA, and 2–3 μM recA protein (M_r 37,800). Concentrations of DNA are expressed as the molar concentration of nucleotides. Incubation was at 37°C for 10–30 min in different experiments.

Assays. The method of assaying joint molecules, called assay D or the D-loop assay, was as described (12), except that we omitted the detergent or heat prior to filtration.

Electron Microscopy. Samples were prepared for microscopy and molecules were measured as described by Cunningham *et al.* (13, 15).

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Abbreviation: SSB, single-stranded-DNA binding protein.

* This is paper no. 13 in a series on the recombination-like activities of recA protein. Paper no. 12 is ref. 14.

Preparation of DNA. Single-stranded and replicative form DNA from phages fd, M13, M13G*ori*1 and M13mp2 were prepared as described by Cunningham *et al.* (15). Phage G4 single-stranded DNA was prepared as described by Beattie *et al.* (16).

Enzymes. The recA protein was purified as described (17, 18). Purified *E. coli* single-stranded-DNA binding protein (SSB) was a gift from John Chase and phage T4 gene *32* protein was a gift from Navin Sinha. Restriction endonucleases *Hpa* I and *Bam*HI were purchased from Bethesda Research Laboratories. Restriction endonucleases *Bgl* I, *Hgi*AI, and *Sau*96I were purchased from New England BioLabs.

RESULTS

Accumulation of Branch Points at the Border of Heterologous DNA. For these experiments, we used DNA from the chimeric phage M13G*ori*1 (19), which has an insert of 2216 base pairs of phage G4 DNA in the genome of phage M13. Using restriction endonucleases, we cut the duplex circular replicative form of M13G*ori*1 to yield the chimeric products illustrated in Fig. 2, in which the 3′ end of the complementary strand was located in G4 DNA. We allowed these molecules to react with single-stranded circular phage G4 DNA and recA protein for 10 or 15 min at 37°C. The electron microscopic appearance of joint molecules corresponded to the interpretation in Fig. 1 (12) and readily permitted identification and measurement of the length of the heteroduplex regions. For both of the molecules of M13G*ori*1 DNA diagrammed in Fig. 2, the length of most heteroduplex regions was equal to the length of G4 DNA at the 3′ end of the complementary strand, within the limits of error (*ca.* ±100 base pairs). None of the heteroduplex regions extended beyond the heterologous block. The observed accumulation of branch points at the boundary of heterologous DNA supports our previous conclusion about the polarity of the reaction as diagrammed in Fig. 1. The lengths of the heteroduplex regions illustrated in Fig. 2*B* corresponded to the length of G4 DNA between the 3′ end of the G4 complementary strand and the beginning of M13 sequences. There were no heteroduplex regions that exceeded the length of the 3′ end of G4 complementary strand and that corresponded instead to the length of the G4 DNA at the other end of the molecule.

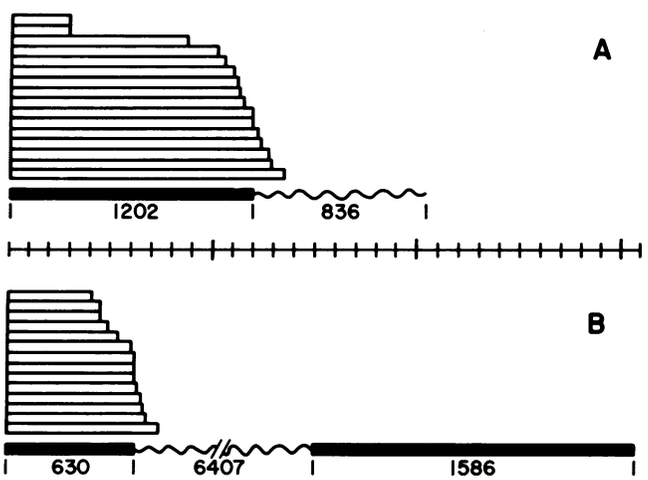

FIG. 2. Accumulation of branch points at the border of heterologous DNA. At the bottom of *A* and *B* are maps of the linear duplex DNA involved in each experiment. The molecule diagrammed in *A* was obtained by cutting M13G*ori*1 superhelical DNA with restriction endonuclease *Hpa* I, which produces two fragments. Because of the polarity of the reaction, only the smaller fragment reacts with circular single-stranded G4 DNA (14). The molecule diagrammed in *B* was obtained by cutting M13G*ori*1 DNA once with endonuclease *Bgl* I. The maps are oriented with the 3′ end of the complementary strand at the left. Solid bars represent phage G4 DNA, and wavy lines represent M13 DNA. The circular single-stranded DNA was from phage G4. The joint molecules were made as described in *Methods*, except that incubations were at 37°C for 15 min for *A*, and 10 min for *B*. Each open horizontal bar represents the length of the heteroduplex region in a single joint molecule. According to previous findings (14), these heteroduplex joints start to form from the 3′ end of the complementary strand (see Fig. 1). Each division of the horizontal scale corresponds to 100 base pairs.

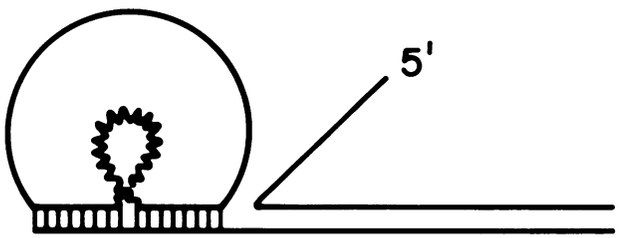

FIG. 3. Hypothetical disposition of a heterologous insert in single-stranded DNA involved in a strand exchange. The saw-toothed line represents heterologous DNA.

Arrest of Strand Exchange at an Insertion in the Donor Single-Stranded DNA. An insertion in a single-stranded donor molecule might present less of an impediment to continued growth of a heteroduplex joint than does an insertion in the duplex recipient molecule. In the former case, the heterologous insert need only loop back on itself to allow homologous sequences to present themselves at the growing junction (Fig. 3). We explored this idea by using the chimeric phage M13mp2, which contains an insertion of 789 bases of *lac* DNA in M13 (20). Single-stranded M13mp2 DNA was allowed to react with duplex M13 DNA that had been cleaved only by endonuclease *Sau*96I. This cleavage put the insert in the single-stranded DNA at a site corresponding to 145 bases from the 3′ end of the complementary strand. Within the limits of error, none of the heteroduplex joints extended beyond the site of the insert (Table 1). In a control, consisting of circular single-stranded M13 DNA reacting with the same duplex M13 DNA, 13 heteroduplex joints varied in length between 350 and 3000 base pairs (data not shown).

Because helix-destabilizing proteins, both *E. coli* SSB protein and T4 gene *32* protein, augment the formation of D loops when the amount of recA protein is limiting (21, 22), we tested the possible effect of these proteins on the arrest of strand ex-

Table 1. Arrest of heteroduplex growth at an insertion in the single-stranded DNA

Length of heteroduplex DNA, base pairs	Number of molecules			
	recA protein	recA protein + SSB	recA protein + T4 gene *32* protein	Totals
0– 50	0	0	0	0
50–100	3	6	0	9
100–150	13	12	14	39
150–200	6	1	10	17
>200	0	0	0	0
				65

In the standard reaction mixture for 30 min at 37°C, recA protein formed joint molecules from circular single-stranded M13mp2 DNA and linear duplex M13 DNA produced by cleaving the replicative form with endonuclease *Sau*96I. The M13mp2 DNA contains a heterologous insertion of 789 nucleotide residues at a site corresponding to 145 nucleotides from the 3′ end of the complementary strand in the duplex molecule.

change. Under controlled conditions in which SSB and gene 32 protein stimulated the formation of joint molecules by circular single-stranded DNA and linear duplex DNA (control data not shown, previous data in ref. 21), the two helix-destabilizing proteins did not relieve the block imposed by a heterologous insert in the single-stranded DNA (Table 1). In addition to the molecules that we measured (Table 1), we examined qualitatively about 250 other joint molecules, but we did not detect any with long heteroduplex joints. The failure of recA protein to push a migrating branch past an insertion in the single-stranded donor molecule emphasizes the concerted nature of the strand exchange (14).

The data in Table 1 and Fig. 2 show that the branch points in joint molecules (see Fig. 1) accumulated at boundaries of nonhomologous DNA located respectively 145, 630, or 1202 nucleotides from the 3′ end of the complementary strand. By contrast, in the absence of a heterologous block, branch points are evenly distributed (see Fig. 5 *B* and *D* below and ref. 12). In the case of those molecules in which the boundary of nonhomologous DNA was 1202 nucleotides from one end, we could readily have detected shorter heteroduplex joints (as in Fig. 2*B* and Table 1), but most branches migrated to the nonhomologous boundary and stopped (Fig. 2*A*). Thus, in the formation of joint molecules from circular single-stranded DNA and linear duplex DNA, recA protein promotes a unidirectional strand exchange or branch migration.

Effects of Mismatched Base Pairs. The DNAs of phages φX174 and G4 are 70% homologous (23, 24), whereas those of fd and M13 are 97% homologous (25, 26). Elsewhere we will report in detail the observation that several combinations of φX174 and G4 DNA did not form stable joint molecules when circular single-stranded DNA was allowed to react with linear duplex DNA. By contrast, mixed pairs of fd and M13 DNA formed joint molecules about as well as the homologous combinations (Fig. 4). In the nucleotide sequences of fd and M13 DNA there are 193 differences, consisting of 179 changes of a single base, 5 changes of two consecutive bases, 1 change of three consecutive bases, and one extra base in fd (25, 26). The formation of joint molecules by fd and M13, the known sequences of these two phages, and the known polarity of the recA reaction made it possible to look for an effect of relatively sparse base changes on elongation of heteroduplex joints by recA protein. As in the experiments described above, by cleaving duplex fd or M13 DNA with different restriction enzymes, we varied the site at which heteroduplex formation began. The production of the expected cleavage products also served as a control on the authenticity of our stocks of fd and M13. In the following description we will use the term *mismatched combination* to distinguish any pair of fd and M13 molecules from completely homologous pairs that serve as controls.

Endonuclease *Bam*HI cleaves M13 DNA at a single site (26). We allowed this linear molecule to react with circular single-stranded M13 DNA and with circular single-stranded fd DNA. In the case of the mismatched combination, the heteroduplex joints did not extend beyond 1400 base pairs from the end in 21 out of 22 joint molecules (Fig. 5*C*). By contrast, in the homologous control the heteroduplex joints extended beyond 1400 base pairs in 10 out of 12 molecules (Fig. 5*D*).

In another experiment, we cleaved fd circular duplex DNA with endonuclease *Hgi*AI, which cuts fd DNA at a single site.

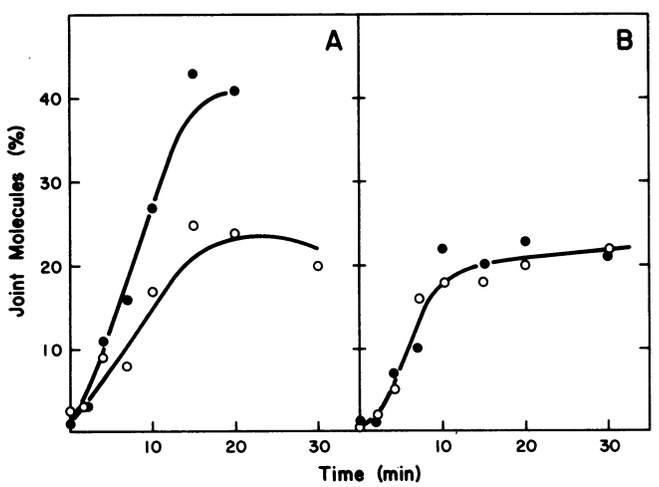

FIG. 4. Time course of formation of joint molecules by mismatched pairs of fd and M13 DNA (○) and completely homologous pairs of fd or M13 DNA (●). (*A*) Circular single-stranded M13 DNA and linear duplex fd DNA made by cleavage with endonuclease *Hgi*AI (○). The control is circular single-stranded fd DNA allowed to react with the same duplex fd DNA (●). These are the same substrates described in Fig. 5 *A* and *B*. (*B*) Circular single-stranded fd DNA and linear duplex M13 DNA made by cleavage with endonuclease *Bam*HI (○). The control is circular single-stranded M13 DNA allowed to react with the same linear duplex molecule (●). These are the substrates described in Fig. 5 *C* and *D*.

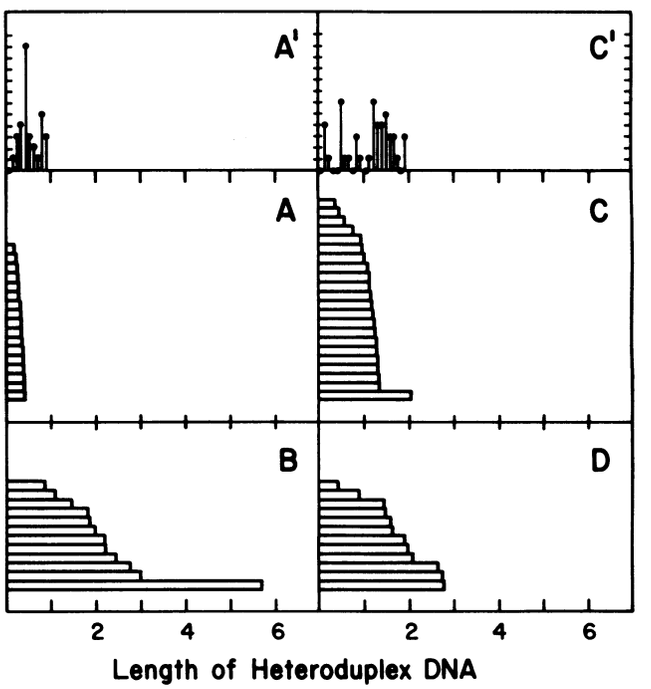

FIG. 5. Effects of mismatched base pairs on the elongation of heteroduplex joints by recA protein. The DNA in these experiments were pairs of fd and M13 molecules. Joint molecules were made as described in *Methods*. Incubation was in the standard reaction mixture for 30 min at 37°C. Each horizontal bar represents the length (in kilobase pairs) of heteroduplex DNA in one joint molecule. (*A*) Circular single-stranded M13 DNA and linear duplex fd DNA made by cleavage with restriction endonuclease *Hgi*AI. (*A'*) The distribution of mismatched base pairs in the first 900 base pairs from the 3′ end of the complementary strand in duplex DNA. Each vertical line represents the number of mismatches per 90 nucleotide residues; each division of the vertical scale is a single mismatched base pair. The horizontal scale is the same as for the rest of the figure. (*B*) The homologous control for *A*: circular single-stranded fd DNA paired with the same duplex fd DNA as in *A*. (*C*) Circular single-stranded fd DNA and linear duplex M13 DNA made by cleavage of M13 replicative form by endonuclease *Bam*HI. (*C'*) Like *A'*, the distribution of mismatched base pairs in the first 1980 base pairs from the 3′ end of the complementary strand. (*D*) The homologous control for *C*: circular single-stranded M13 DNA that had reacted with the same linear duplex DNA as in *C*.

Table 2. Arrest of polar strand exchange by mismatched base pairs

Length of heteroduplex DNA, base pairs	Number of molecules			
	recA protein	recA protein + SSB	recA protein + T4 gene *32* protein	Totals
0–100	0	0	0	0
100–200	1	1	3	5
200–300	8	6	15	29
300–400	8	8	7	23
400–500	1	3	0	4
>500	0	0	0	0
				61

The same combination of DNA molecules described in Fig. 5*A*. A mismatched trinucleotide is present 354 bases from the 3′ end of the complementary strand in the linear duplex molecule, followed by 13 mismatches in the next 100 base pairs. Incubation of the standard reaction mixture was for 30 min at 37°C.

When this linear duplex fd DNA reacted with circular single-stranded M13 DNA, the heteroduplex joint did not extend beyond 400 base pairs in 19 molecules that were measured (Fig. 5*A*). In the homologous control, all of the heteroduplex joints were longer, ranging between 850 and 5700 base pairs (Fig. 5*B*).

As in the case of the insertion of 789 bases in M13mp2 described above (Table 1), neither the *E. coli* SSB protein nor T4 gene *32* protein had an effect on the arrest of heteroduplex joints in the mismatched pair consisting of duplex fd DNA cleaved by *Hgi*AI and circular single-stranded M13 DNA (Table 2). In addition to the molecules that we measured (Fig. 5*A*), we examined qualitatively about 200 joint molecules each made by recA protein, plus SSB protein and by recA protein plus T4 gene *32* protein. We found only one long heteroduplex joint.

DISCUSSION

Previous experiments have shown that when recA protein pairs circular single strands with linear duplex DNA, it forms heteroduplex joints in a polar fashion, in the sense that recA protein forms heteroduplex DNA only from one specific end of the molecule (14). This observation could mean either that recA protein recognizes a specific end, following which the heteroduplex joint grows by random diffusion, or that recA protein drives a strand exchange in a specific direction, presumably by some sort of polar and cooperative binding. The present observations favor the latter explanation by showing that branch points accumulate at the border of heterologous regions, an observation that is not consistent with a random one-dimensional walk unless one postulates in addition that the nonhomologous DNA is an absorbing boundary. Recently Cox and Lehman (27) observed that in the presence of *E. coli* SSB the growth of heteroduplex joints requires the continuous activity of recA protein. The two sets of observations, with and without SSB, support the same conclusion, namely that recA protein actively drives a strand exchange involving a single strand and a duplex molecule. On the basis of the substrate specificity of recA protein and the structure of the products of the reaction, we inferred that recA protein has a strand transferase activity as well as a pairing activity (12, 28). The present findings confirm that view.

The polar formation of heteroduplex joints in such a well-defined system provides an opportunity to study a complicated process that lies at the heart of homologous genetic recombination. The experiments described here represent an attempt to define the effect of mismatched bases on the formation of heteroduplex DNA. We examined two combinations of fd and M13 DNA in which heteroduplex formation started from different restriction sites. According to the published nucleotide sequences of fd and M13, beginning from the *Bam*HI site in M13 there are 31 differences in the first 1431 bases, 29 of which involve nonadjacent bases; 14 of these mismatches are in the last 19% of the region (See Fig. 5*C′*). Among the 22 migrating joints, 17 stopped between 948 and 1366 base pairs from the end (Fig. 5*C*). In this region of 418 base pairs, there are only 12 single mismatched base pairs. Differences in the nucleotide sequences are most concentrated in the 90 base pairs between 1187 and 1277, where there are 9 mismatches. This run of mismatches coincides approximately with the mean length of the 17 joints that stopped between 948 and 1366 base pairs. With regard to the second mismatched combination, the published nucleotide sequences show that 3 sequential bases differ at a position 354 nucleotides from the *Hgi*AI site in fd. Between the end and the mismatched trinucleotide there are only 4 single mismatches. In the 100 base pairs beyond the triplet, however, there are 13 differences, including two mismatched dinucleotides (Fig. 5*A′*). In our experiments, the growth of heteroduplex DNA starting from the *Hgi* AI site was strikingly arrested (Fig. 5*A* and Table 2). The mean length of 61 heteroduplex joints was 290 ± 150 (2SD), which correlates with the dense concentration of mismatches beyond nucleotide residue 354 (Fig. 5*A*). From these two experiments, we conclude on the one hand that recA protein, using the energy of ATP, is able to drive a strand exchange through some single pairs of mismatched bases, and on the other hand that the extension of heteroduplex joints by recA protein can be stopped by relatively minor degrees of mismatching, possibly by a mismatched trinucleotide (Fig. 5*A*), or by a density of single mismatches as low as an average of 1 base pair in 10 (Fig. 5*B*). Helix-destabilizing proteins did not enable recA protein to overcome this barrier under the conditions of our experiments.

The substrate containing a heterologous insertion in the single-stranded DNA (Fig. 3) is a particularly interesting one with regard to genetic recombination and the possible inclusion of deletions or insertions in heteroduplex regions (see Introduction). Although in the present experiments recA protein did not push the heteroduplex joint past such a discontinuity, other factors, such as superhelicity, affect the growth of heteroduplex joints and may eventually prove to overcome various discontinuities.

Note Added in Proof. Polarity in the formation of joint molecules by recA protein has recently been described also by Cox and Lehman (29) and West *et al.* (30).

We gratefully acknowledge the expert technical assistance of Lynn Osber and the helpful comments on our manuscript by Jonathan Blum, John Flory, Roger Kahn, and Anna Wu. This research was sponsored by Grant NP 90G from the American Cancer Society and Grant CA 16038-07 from the National Cancer Institute.

1. Radding, C. M. (1978) *Annu. Rev. Biochem.* **47**, 847–880.
2. Fox, M. S. (1978) *Annu. Rev. Genet.* **12**, 47–68.
3. Fogel, S., Mortimer, R., Lusnak, K. & Tavares, F. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 1325–1341.
4. Rossignol, J.-L., Paquette, N. & Nicolas, A. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 1343–1352.
5. Radding, C. M. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 1315–1316.
6. Fink, G. R. & Styles, C. A. (1974) *Genetics* **77**, 231–244.
7. Lawrence, C. W., Sherman, F., Jackson, M. & Gilmore, R. A. (1975) *Genetics* **81**, 615–629.
8. Wagner, R., Jr., & Meselson, M. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 4135–4139.
9. Ogawa, T., Wabiko, H., Tsurimoto, T., Horii, T., Masukata, H. & Ogawa, H. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 909–915.

10. Roberts, J. W., Roberts, C. W., Craig, N. L. & Phizicky, E. M. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 917–920.
11. Radding, C. M. (1981) *Cell* **25**, 3–4.
12. DasGupta, C., Shibata, T., Cunningham, R. P. & Radding, C. M. (1980) *Cell* **22**, 437–446.
13. Cunningham, R. P., Wu, A. M., Shibata, T., DasGupta, C. & Radding, C. M. (1981) *Cell* **24**, 213–223.
14. Kahn, R., Cunningham, R. P., DasGupta, C. & Radding, C. M. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 4786–4790.
15. Cunningham, R. P., DasGupta, C., Shibata, T. & Radding, C. M. (1980) *Cell* **20**, 223–235.
16. Beattie, K. L., Wiegand, R. C. & Radding, C. M. (1977) *J. Mol. Biol.* **116**, 783–803.
17. Shibata, T., DasGupta, C., Cunningham, R. P. & Radding, C. M. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1638–1642.
18. Shibata, T., Cunningham, R. P. & Radding, C. M. (1981) *J. Biol. Chem.* **256**, 7557–7564.
19. Kaguni, J. & Ray, D. S. (1979) *J. Mol. Biol.* **135**, 863–878.
20. Gronenborn, B. & Messing, J. (1978) *Nature (London)* **272**, 375–377.
21. Shibata, T., DasGupta, C., Cunningham, R. P. & Radding, C. M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2606–2610.
22. McEntee, K., Weinstock, G. M. & Lehman, I. R. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 857–861.
23. Godson, G. N., Fiddes, J. C., Barrell, B. G. & Sanger, F. (1978) in *The Single-Stranded DNA Phages*, eds. Denhardt, D. T., Dressler, D. & Ray, D. S. (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York), pp. 51–86.
24. Godson, G. N. (1978) in *The Single-Stranded DNA Phages*, eds. Denhardt, D. T., Dressler, D. & Ray, D. S. (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York) pp. 671–695.
25. Beck, E., Sommer, R., Auerswald, E. A., Kurz, C., Zink, B., Osterburg, G., Schaller, H., Sugimoto, K., Sugisaki, H., Okamoto, T. & Takanami, M. (1978) *Nucleic Acids Res.* **5**, 4495–4503.
26. van Wezenbeek, P. M. G. F., Hulsebos, T. J. M. & Schoenmakers, J. G. G. (1980) *Gene* **11**, 129–148.
27. Cox, M. M. & Lehman, I. R. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3433–3437.
28. Radding, C. M., Cunningham, R. P., DasGupta, C., Shibata, T. & Osber, L. (1981) *Cold Spring Harbor Symp. Quant. Biol.* **45**, 385–390.
29. Cox, M. M. & Lehman, I. R. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 6018–6022.
30. West, S. C., Cassuto, E. & Howard-Flanders, P. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 6149–6153.