Proc. Natl. Acad. Sci. USA
Vol. 75, No. 8, pp. 3698–3702, August 1978
Biochemistry

In vitro system from *Escherichia coli* that catalyzes generalized genetic recombination

(reciprocal recombination/plasmid DNA/figure 8 structures/chi forms/initiation of recombination)

HUNTINGTON POTTER AND DAVID DRESSLER

Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts 02138.

Communicated by Arthur Kornberg, May 30, 1978

ABSTRACT **This paper reports an *in vitro* system for studying generalized genetic recombination. The system uses extracts from *Escherichia coli* as a source of enzymes and plasmid DNA molecules as substrates. Unit-size plasmid DNA rings are converted into genomes fused at a region of DNA homology at a frequency of about 5–10% over a period of hours. That the fused structures are the result of recombination is supported by two lines of evidence. When two partially homologous plasmids of different sizes are used as substrates for the *in vitro* system, intermediates containing one plasmid of each size are obtained. Furthermore, fused structures are not formed with high efficiency in extracts from recombination-deficient (Rec A$^-$) cells.**

DNA synthesis does not appear to be required for the formation of the recombination intermediates; it is possible to omit DNA precursors from the reaction mixture and, furthermore, to develop the fused structures even in the presence of chain-termininating dideoxynucleoside triphosphates.

The structures formed *in vitro* have the basic properties of recombination intermediates previously recovered from intact cells. That is, two genomes are demonstrably fused at a region of homology. However, in one way the molecules formed *in vitro* have a property less frequently observed *in vivo*—the fused genomes often appear to be connected over an extended region of homology ranging up to several hundred base pairs in length. This extended region of pairing may indicate the presence of two crossover connections very close together and, as will be discussed, may provide an insight into the mechanism by which the recombination intermediate is formed.

One touchstone for analyzing the mechanism of genetic recombination is based on the isolation of structures actively engaged in the recombination process. Fig. 1 shows a set of plasmid DNA molecules that have been recovered from intact cells and identified as intermediates in recombination. These forms were obtained when recombination-proficient cells were lysed, their plasmids were isolated, and the DNA was examined in the electron microscope (8–10). With this procedure, which allows the study of individual DNA molecules, it was possible to observe structures shaped like a figure 8 and (after linearization with the restriction enzyme *Eco*RI) structures shaped like the Greek letter chi (χ). These forms were judged to be recombination intermediates because (*i*) they contained two genome-length elements; (*ii*) these elements were connected at a region of homologous DNA; and (*iii*) these forms were absent in recombination-deficient (Rec A$^-$) bacteria.

About 1500 *in vivo* plasmid χ forms were photographed and analyzed. In 100 instances, it was possible to observe the nature of the polynucleotide strand connections in the region of the crossover. An example is shown in Fig. 1*g* where the individual strands can be seen, crossing over from one genome to the other. These molecules appear to have a single crossover connection, and the arrangement of the polynucleotide strands provides physical evidence in support of a particular recombination intermediate first proposed on genetic grounds in 1964 by Holliday (1–3).

In addition to the plasmid DNA molecules shown in Fig. 1, physical evidence in support of the Holliday recombination intermediate comes from the work of Warner and Tessman and their colleagues with S13 and G4 (5, 11), Benbow *et al.* with ϕX174 (12, 13), and Valenzuela and Inman with λ (14).

Having confidence in the structure of an intermediate stage through which two DNA molecules pass during recombination, one would like to make the transition from the level of molecular biology to the mechanistically more specific level of enzymology. In the end, one would like to understand recombination as a series of steps through which known enzymes process two DNA molecules, leading to an exchange of genetic information. This paper reports our initial results in developing a system that carries out generalized recombination *in vitro*.

Formation of fused structures *in vitro*

The figure 8 and derived χ form shown in the upper electron micrographs in Fig. 1 were in fact formed *in vitro*. These molecules consist of fused genomes and resulted when monomer-size plasmid DNA rings were incubated over a period of hours in an extract prepared from recombination-proficient *E. coli* cells.

The method for the preparation of the cell extract is given in detail in the legend to Fig. 2. In brief, cells were grown in nutrient broth and harvested during logarithmic growth. The cells were then broken open by lysozyme digestion and freeze-thaw treatment. After the lysate was cleared of cell debris and host DNA by a high-speed centrifugation, the resulting supernatant was filtered to remove unlysed cells and passed over a Sephadex column to remove small molecules (Fig. 2). To the freshly prepared cell extract purified monomer-size DNA rings of the plasmid pMB-9 were added to a final concentration of 100 μg/ml. Based on the molecular mass of the plasmid (3.4×10^6 daltons), the concentration of the DNA molecules in the reaction mixture can be calculated as 1.5×10^{13}/ml. This corresponds closely to the concentration of plasmid molecules within the intact cell where 20 plasmids exist in a volume of 5×10^{-13} ml. Whereas the plasmid DNA concentration in the reaction mixture approximated that found *in vivo*, the cellular enzymes were present at least a 1:10 dilution. The cell extract and the added monomer-size plasmid DNA rings were incubated at 37°, and aliquots were withdrawn over a period of hours for analysis by electron microscopy.

The plasmid DNA was stable during the incubation period: 98% of the DNA molecules continued to appear as circles for periods up to 16 hr. Only at later times (25–30 hr) was there evidence for substantial degradation of the DNA.

During the incubation an increasing number of monomer rings were converted to multimeric circles. The percentage of multimers increased from less than 0.2% at 0 hr to about 10% at 16 hr (Table 1). The appearance of these fused structures, in

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

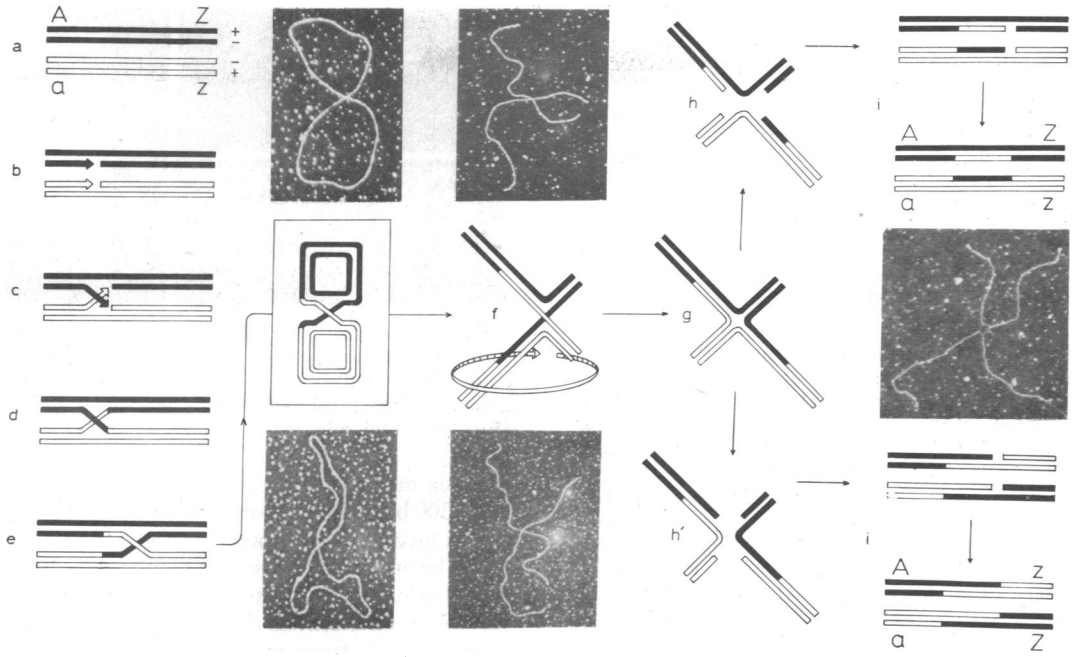

FIG. 1. When plasmids are isolated from recombination-proficient *E. coli*, one finds, at a level of about 1%, fused genomes that appear to be intermediates in genetic recombination. These molecules have the shape of a figure 8. When cut with the restriction enzyme *Eco*RI, the figure 8s assume the shape of the Greek letter chi (χ), thus showing that they consist of two genomes held together at a point of DNA homology. Such molecules can be fit into a prototype scheme for genetic recombination. In its simplest form the two genomes to be recombined may be pictured as undergoing a set of nicking and reciprocal strand exchange events leading to the production of a single crossover ($a \rightarrow d$). Such an intermediate was proposed by Holliday on genetic grounds because of the ability of the crossover connection to move laterally to create regions of heterozygous DNA ($d \rightarrow e$) (1–5). Although there is physical evidence for the existence of the Holliday recombination intermediate, the specific strand-nicking and exchange mechanism shown here for forming the intermediate remains hypothetical. As will be discussed, the data of the current paper suggest that a different initiation mechanism may in fact be responsible for the formation of the recombination intermediate.

Panels g–i show the proposed maturation of the recombination intermediate. Because of its symmetry, it is expected that the intermediate can be processed in either of two related ways to yield different pairs of recombinant chromosomes (3, 6, 7). This dual maturation potential is most easily visualized if one considers the intermediate in the planar representation shown in g. One maturation pathway cuts diagonally across the intermediate along a north-south axis and leads to the formation of a pair of reciprocally recombinant chromosomes in which the genes on either side of the crossover emerge in a new linkage (h'). In the alternative maturation pathway, nicking along an east-west axis leads to the separation of the two recombining chromosomes—but this time the genes on either side of the crossover are left in their original linkage (h).

The three electron micrographs in the lower part of the diagram came from intact cells; those in the upper part were formed *in vitro*.

more than 20 independent cell extracts, provided an initial indication of a "recombinase" activity *in vitro*.

Examples of multimeric circles formed *in vitro* are shown in Fig. 3. At early times, the multimers had the shape of a simple figure 8 (Fig. 3 *A* and *B*). Later, more complex multimers arose (Fig. 3*C*); these involved several circular plasmid genomes and were interpreted as resulting from the interaction of monomers with previously formed multimers.

Multimers formed *in vitro* consist of pairs of unit-length genomes fused at a region of DNA homology

The figure 8, although a highly suggestive structure, is inherently ambiguous. Although such a geometry could represent two genomes covalently held together at a region of DNA homology (as in Fig. 1*d*), two alternative interpretations are also possible: the figure 8 could result from a double-length circle accidentally crossing itself in the middle or from two monomer circles interlocked like links in a chain. When dealing with this problem *in vivo*, we removed the ambiguity associated with the figure 8 by cleaving the plasmid DNA forms with the restriction enzyme *Eco*RI prior to electron microscopy. Whereas double-length circles and interlocked monomer rings are expected to separate into two unit-size rods upon enzyme digestion, the persistence of χ-shaped structures with two pairs of equal-length arms indicates two genomes held together at a point of DNA homology. We have used the same method to determine the nature of the plasmid figure 8s formed *in vitro*. As shown in Fig. 1 and Table 1, when the plasmid DNA forms were cut with *Eco*RI, the preparations were found to contain 5–10% χ forms amid a simple background of unit-length plasmid rods. By this criterion, the fused structures made *in vitro* have one of the basic properties of the recombination intermediates recovered from intact cells.

A cell-free system derived from frog oocytes has recently been developed by Benbow and Krauss (15; see also 16) and, like the bacterial system described here, appears to be active in fusing circular DNA molecules.

Recombination or replication?

Although the fused structures formed *in vitro* involve two genomes held together at a point of DNA homology, one must be concerned that processes other than recombination could produce them. In fact, it was evident that DNA replication was occurring in the reaction mixture, giving rise to Cairns type replicating intermediates (Fig. 4 *left*). Because, in the terminal stage of replication, the two daughter genomes of an almost-duplicated Cairns form are still held together at a point of DNA homology, the structure will have a figure 8 appearance and is expected to give rise to a χ form when cut by *Eco*RI (Fig. 4 *right*).

Three lines of evidence, however, indicate that the fused structures are not the product of replication, but rather represent recombination.

(*i*) An initial indication that the χ forms were not derived from near-terminal replicating intermediates came from the fact that the point of contact between the two genomes varied

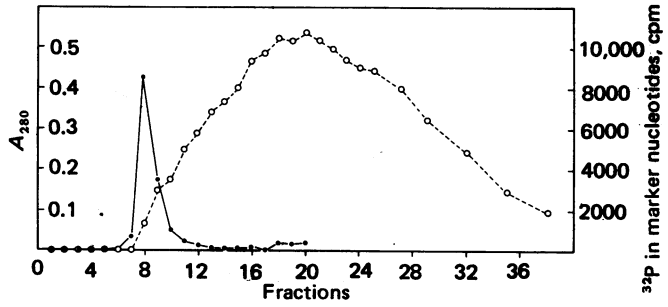

FIG. 2. Preparation of the reaction mixture. Recombination-proficient *E. coli* (MM 294 from Matthew Meselson) were grown in nutrient broth (10 g of Bactotryptone, 1 g of yeast extract, and 8 g of NaCl per liter) to about 4×10^8 cells per ml at 37° with aeration. The cells were harvested by centrifugation ($4000 \times g$ for 10 min at room temperature). The drained pellets from 300 ml of cells were then resuspended at room temperature in 0.5 ml of 50 mM potassium phosphate buffer (pH 7.4) containing 10% sucrose, quick-frozen in liquid nitrogen, and thawed on ice. Lysozyme and KCl were added to 500 μg/ml and 80 mM, respectively, and the mixture was incubated for 30 min on ice. After a second freezing in liquid nitrogen and thawing on ice, the cell lysate was cleared of host DNA and cell debris by a high-speed centrifugation (57,000 × *g*, 2°, 30 min). The resulting supernatant was filtered through a Uni-pore polycarbonate filter (0.2-μm pores) to remove unlysed cells. The filtrate was then loaded on a Sephadex column (G-25 coarse) equilibrated with 50 mM potassium phosphate buffer (pH 7.4) containing 10% sucrose and 1 mM 2-mercaptoethanol and eluted with the same buffer. As judged by the addition of radioactive ATP (O - - - O), the column was effective in removing about 98% of the cellular metabolic precursors originally present in the extract. Fractions were collected, and those containing the A_{280} peak material (●—●) were pooled and used directly in the reaction mixture.

To make the final reaction mixture, the cell extract was diluted 1:1 with potentially useful metabolites to give final concentrations of 100 μM deoxyribonucleoside triphosphates, 4 mM ATP, 200 μM for the other ribonucleoside triphosphates, 1 mM each for NAD^+, $NADP^+$, and NADPH, 2 mM phospho*enol*pyruvate, 5 mM spermidine, 6 mM mercaptoethanol, 20 mM $MgCl_2$, and 150 mM potassium phosphate buffer (pH 7.8).

(compare the χ forms in Fig. 1 and Fig. 6). The point of contact was not confined to the terminus of DNA replication which, in this colicin E1-related plasmid, is 20% from the *Eco*RI cleavage site. Moreover, the addition of rifampicin to 15 μg/ml (which inhibits the RNA polymerase priming required for a round of plasmid DNA replication) did not prevent the formation of multimers (Table 1).

(*ii*) Next, to provide direct evidence that the fused structures represented a recombination event, we supplemented the reaction mixture with two different but partially homologous plasmids (pMB-9 and pBR-322, both related to the plasmid colicin E1). These plasmids were readily distinguishable in the electron microscope because of their difference in size (5500 and 4300 base pairs). As shown in Fig. 5 and Table 1, these reaction mixtures developed composite figure 8 molecules that could be cut by *Eco*RI to give composite χ forms.

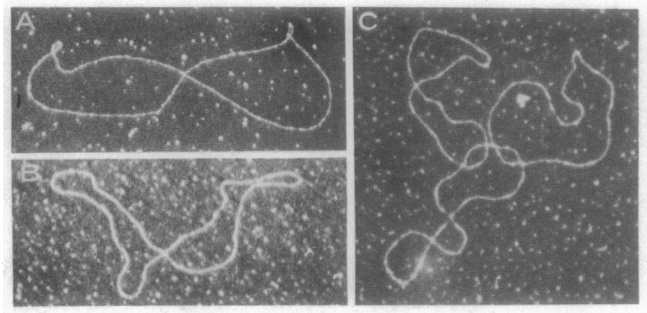

FIG. 3. (*A* and *B*) Figure 8s formed *in vitro*. (*C*) A multimer consisting of three fused monomer rings. The fused structures in this study are not likely to be the result of accidental overlaps between monomer rings, because the DNA was spread for the electron microscope at high dilution (see top line, Table 1).

(*iii*) Finally, as expected on the basis of our previous *in vivo* results, fused plasmids did not arise with high efficiency in extracts made from recombination-deficient (Rec A^-) cells (strain MM 152, see Table 1).

On the basis of these data, the fused structures formed *in vitro* appear to be products of recombination, not replication.

A difference between the structures observed *in vivo* and *in vitro*

In carrying out these experiments we were interested in whether the *in vitro* system would only support the pattern of events deduced from *in vivo* studies or whether a new observation might provide a further clue to the recombination mechanism. In fact, the molecules formed *in vitro* have a property less frequently observed *in vivo* which may be related to the mechanism by which the recombination intermediates are formed.

When studying χ forms recovered from intact cells, it was possible to observe about 100 molecules in which the polynucleotide strand connections in the region of the crossover could be seen. These molecules had the structure shown in Fig. 1*g* and were interpreted as containing a single crossover of the type suggested by Holliday. However, such open molecules were very rare among the forms produced *in vitro*. Among 1095 χ forms, we found only 2 that contained an open crossover.

In vitro, instead of open molecules, we commonly observed (about 60%) that the two genomes in the fused structure were connected over a rather long region of homology ranging up to about 500 base pairs in length. χ forms with extended regions of pairing at the area of the crossover are shown in Fig. 6.

We have considered two possible explanations for the ex-

Table 1. Representative data on formation of recombination intermediates *in vitro*

Exp.	Extract	DNA	Additions*	Incubation, hr	Multimers, %	χ forms, %
1	Rec^+	pMB-9	Complete	0	<0.2 (0/500)	—
				12	4 (30/750)	4 (29/800)
				16	13 (134/1034)	11 (112/1012)
2	Rec^+	pMB-9	+Rif	16	13 (107/800)	9 (110/1200)
3	Rec A^-	pMB-9	+Rif	16	0.4 (2/500)	—
4	Rec^+	pMB-9 +pBR-322	+Rif	9.5	6.5 (108/1650)	5 (41/841)
5	Rec^+	pMB-9	+Rif, −dXTPs +ddXTPs	12	11 (110/1010)	10 (55/555)

Cell extracts were prepared and incubated as described in the legend to Fig. 2. In exp. 4, in which two partially homologous plasmids were incubated together, the percentage of composite figure 8s and χ forms was about 30%. The data in this table were obtained from incubations using the metabolites given in the legend to Fig. 2. However, these metabolites are not essential.

* Rif, rifampicin.

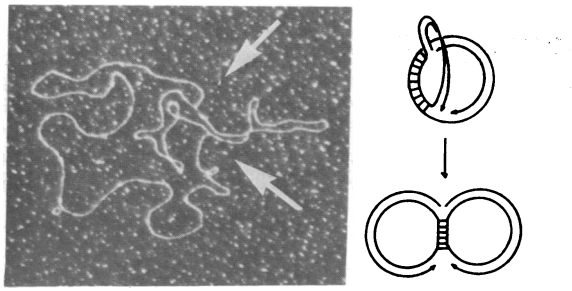

FIG. 4. (*Left*) A replicating Cairns form made *in vitro*. (*Right*) Diagram of a near-terminal Cairns form, showing its figure 8-like appearance.

tended region of pairing in the area of the crossover. One explanation is that the fused structures are not recombination intermediates of the Holliday type but rather are of the type proposed by Broker and Lehman (17). They suggested that a recombination intermediate is formed when two molecules, one gapped in the positive strand and one in the negative strand (at homologous regions), become hydrogen bonded to each other (as in Fig. 7). We consider this explanation for the fused structures unlikely. The strongest piece of evidence we have on this point is that a preparation containing 10% figure 8s can be completely denatured (pH 13) and renatured with the recovery of 2–5% intact structures. These figure 8s may then be converted into χ forms by digestion with *Eco*RI. Such stability through the denaturation is not expected for a Broker–Lehman recombination intermediate because only two covalent strand connections exist in the region of the crossover (Fig. 7).

Their ability to withstand alkali denaturation indicates that the fused structures must be connected at the crossover region in a way that maintains the continuity of all four polynucleotide chains. Therefore we favor a second explanation for the extended region of pairing—the presence of two covalent crossed strand connections close together. The two crossovers could be formed by the nick and reciprocal strand invasion mechanism shown in Fig. 1, applied twice in succession. However, another alternative is also attractive: both crossovers might be formed in a single concerted event. In this case, two DNA molecules would become locally denatured at homologous places and the pairs of exposed positive and negative strands would become intertwined (Fig. 8; see also refs. 18 and 19). This initiation mechanism is supported by the data just given and is also consistent with the recent finding by Champoux (ref. 20; also K. Kirkegaard and J. C. Wang, personal communication) that two single-stranded circles (the positive and negative strands of simian virus 40) can wind into each other to form a covalently closed duplex ring in a reaction catalyzed by a DNA "untwisting" enzyme. Champoux has considered this enzymatic reaction in terms of its possible role in the initiation of recombination.

The molecules formed *in vitro* (as in Fig. 6) may have resulted from monomer DNA rings that became interwrapped after localized denaturation at homologous places. After the initial contact, strand interwrapping would be extended for several hundred base pairs, with transient nicks being introduced by the untwisting enzyme to allow the necessary interwrapping. The structure shown in Fig. 8 *B* and *D* results and is characterized by two reciprocal regions of heteroduplex DNA. Two crossover connections of the type shown in Fig. 1 exist, one at either end of the wrapped segment (compare Fig. 8*B* with Fig. 1*d*). Such a wrapped structure, because of its two crossover connections, could not be readily stressed open in the region of pairing during spreading for the electron microscope—hence, our inability to find single open crossover molecules of the type shown in Fig. 1*g*.

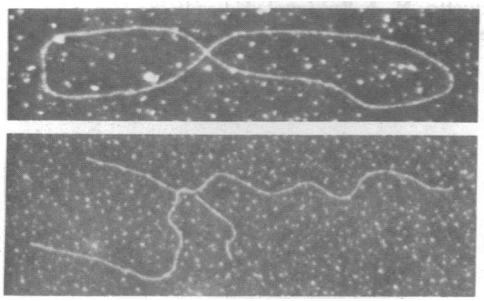

FIG. 5. A figure 8 and derived χ form made *in vitro* from two plasmids of different sizes that are partially homologous.

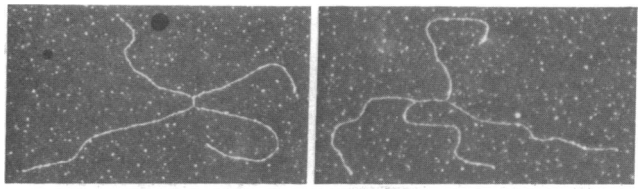

FIG. 6. χ forms that appear to have increasing extents of pairing in the region of the crossover.

Although the wrapped structure is 2-fold more complex, it is not fundamentally different from the single crossover structure shown in Fig. 1. We imagine that such an intermediate may be matured by the same type of strand-nicking events that have been proposed for the Holliday-type recombination intermediate (refs. 1, 6, 7; see also legend to Fig. 1). A pair of maturation nicks would be introduced independently at each of the crossover positions. After one crossover is matured, the structure shown in Fig. 1*f* would result and could rotate into a planar configuration that allows the individual DNA strands in the crossover to be seen (as in Fig. 1 $f \rightarrow g$). Maturation at the second crossover would finally separate the two recombinant genomes. Each of these molecules will have a region of heterozygous DNA in the area of the crossover, which is known to be a property of recombinant chromosomes from genetic studies (21–25). And, depending on the type of cutting that occurs at the two crossovers (see Fig. 1), there would be either a retention of the flanking genes in their parental linkage or, with equal probability, the production of a recombinant linkage for the flanking genes [again in accord with genetic findings about recombinant chromosomes (21, 22)].

The interwrapping initiation mechanism makes an important prediction—that no DNA synthesis should be required for the formation of the recombination intermediate. This is in contrast to several ingenious initiation mechanisms—for instance, the one proposed by Meselson and Radding (26)—that do require DNA synthesis. We have tested this prediction in two ways. First, we found that the multimeric structures could be developed with equal efficiency in reaction mixtures from which DNA precursors had been omitted (Table 1). And further, guarding against the possibility that DNA precursors might be generated in the reaction mixture, we included dideoxynucleoside triphosphates at a concentration of 100 μM for each.

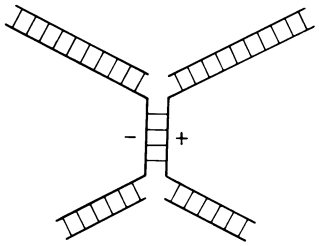

FIG. 7. The structure of the recombination intermediate proposed by Broker and Lehman (17).

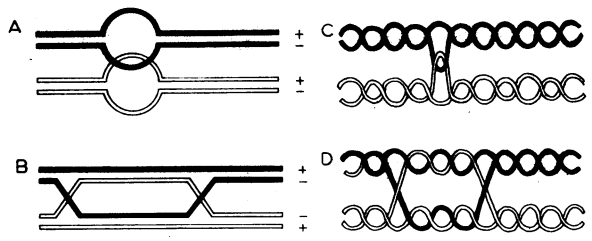

FIG. 8. A possible initiation mechanism for the formation of a recombination intermediate containing two crossover connections. Each individual crossover is of the type shown in Fig. 1. The structures shown may be compared with the electron micrographs in Fig. 6.

Control experiments showed that the dideoxynucleotides were effective in prematurely terminating DNA strands—even in reaction mixtures with added DNA precursors, there was a blockage in the conversion of G4 single-stranded circles to the duplex ring state, a reaction that readily occurred in the absence of the dideoxynucleoside triphosphates. In such reaction mixtures, multimeric plasmid forms always arose (Table 1). Thus, we conclude that very little, and perhaps no DNA synthesis at all, is required for the formation of the structures we observed.

Asymmetric heteroduplex regions

If the interwrapping initiation mechanism is correct, then the recombination intermediate formed will contain two reciprocally symmetric regions of heterozygous DNA. However, several genetic studies in fungi indicate that the regions of heterozygous DNA in the finished pair of recombinant chromosomes are not perfectly reciprocal (22, 27). These studies were, in fact, the impetus for the proposal of Meselson and Radding (26) who sought to explain the asymmetric heterozygous regions by suggesting that strand nicking, followed by DNA synthesis and the displacement of a single-stranded tail, initiates the formation of the recombination intermediate: the displaced tail invades the other double helix and provokes a reciprocal nicking and strand invasion at a slightly different place. After the filling-in of any resultant gaps and the trimming of any remaining tails, this would yield asymmetric heteroduplex regions at the outset of recombination. How can the genetic data be accommodated without relying on this DNA synthesis? We suggest that the formation of asymmetric heterozygous regions arises not at the stage of the formation of the recombination intermediate but rather at the stage of maturation, when the wrapped intermediate is broken apart (see also 19). Nicks are then introduced, which necessarily occur at the borders of the heteroduplex DNA. If nick translation occurs at this time (prior to sealing of the recombinant chromosomes by ligase), this will generate dissimilar regions of heterozygous DNA in the pair of recombinant chromosomes, and thus account for the genetic data.*

* Even if a recombination intermediate is formed with a single crossover as shown in Fig. 1 ($c \rightarrow d$), nick translation at the time of maturation is still able to give asymmetric heteroduplex regions and remains an alternative to the model of Meselson and Radding (26).

We thank John Wolfson for reading the manuscript and for spirited discussions over a period of years dealing with the possibility that recombination intermediates might be formed by the interwrapping of two double helices in a reaction catalyzed by a DNA untwisting enzyme. The work of our laboratory is made possible by Research Grants GM-17088 from the National Institutes of Health and NP-57 from the American Cancer Society and by the Dreyfus Teacher Scholar Fund.

1. Holliday, R. (1964) *Genet. Res.* **5**, 282–304.
2. Holliday, R. (1968) in *Replication and Recombination of Genetic Material*, eds. Peacock, W. & Brock, R. (Australian Academy Science, Canberra, Australia), pp. 157–174.
3. Holliday, R. (1974) *Genetics* **78**, 273–287.
4. Meselson, M. (1972) *J. Mol. Biol.* **71**, 795–798.
5. Thompson, B., Camien, M. & Warner, R. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2299–2303.
6. Emerson, S. (1969) in *Genetic Organization* (Academic, New York), Vol. 1, pp. 267–360.
7. Sigal, N. & Alberts, B. (1972) *J. Mol. Biol.* **71**, 789–793.
8. Potter, H. & Dressler, D. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3000–3004.
9. Potter, H. & Dressler, D. (1977) in *DNA Insertion Elements, Plasmids, and Episomes*, eds. Bukhari, A., Adhya, S. & Shapiro, J. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
10. Potter, H. & Dressler, D. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4168–4172.
11. Doniger, J., Warner, R. & Tessman, I. (1973) *Nature (London) New Biol.* **242**, 9–12.
12. Benbow, R., Zuccarelli, A., Shafer, A & Sinsheimer, R. (1974) in *Mechanisms in Recombination*, ed. Grell, R. (Plenum, New York), pp. 3–18.
13. Benbow, R., Zuccarelli, A. & Sinsheimer, R. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 235–239.
14. Valenzuela, M. & Inman, R. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3024–3028.
15. Benbow, R. & Krauss, M. (1977) *Cell* **12**, 191–204.
16. Atlardi, D., Mattoccia, E. & Tocchini-Valentini, G. (1977) *Nature* **270**, 754–756.
17. Broker, T. & Lehman, R. (1971) *J. Mol. Biol.* **60**, 131–149.
18. Cross, R. & Lieb, M. (1967) *Genetics* **57**, 549–560.
19. Sobell, H. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 2483–2487.
20. Champoux, J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5328–5332.
21. Kitani, Y., Olive, L. & El-ani, A. (1962) *Am. J. Bot.* **49**, 697–706.
22. Hurst, D., Fogel, S. & Mortimer, R. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 101–105.
23. White, R. & Fox, M. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 1544–1548.
24. Enea, V. & Zinder, N. (1976) *J. Mol. Biol.* **101**, 25–38.
25. Lam, S., Stahl, M., McMilin, K. & Stahl, F. (1974) *Genetics* **77**, 425–433.
26. Meselson, M. & Radding, C. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 358–361.
27. Paquette, N. & Rossignol, J.-L. (1978) *Mol. Gen. Genet.*, in press.