A MECHANISM FOR GENETIC RECOMBINATION GENERATING ONE PARENT AND ONE RECOMBINANT*

By Thierry Boon and Norton D. Zinder

ROCKEFELLER UNIVERSITY, NEW YORK

Abstract.—A mechanism is proposed for generating one parental and one recombinant genome in a single recombination event between two DNA molecules. Three stages of the event are described: initiation, replication, and return. Initiation requires breakage and joining of strands. Replication proceeds through a biparental "replication fork" generated in initiation. Return also involves breakage and joining of strands. Some of the implications of such a mechanism for genetic recombination are discussed.

Introduction.—We have undertaken a study of the products of recombination of bacteriophage f_1[1] under conditions in which the parental genomes cannot replicate in the cell prior to recombination. The results obtained suggest that the products of one recombination event between two parental genomes are one parental genome and one recombinant genome.

Previous studies of genetic recombination in bacteriophage did not demonstrate a correlation between the yields of reciprocal recombinants produced in single cells.[2-5] Similarly, recombination between closely linked markers is often nonreciprocal in Neurospora and other fungi.[6,7] Although the results with phage f_1 are still preliminary, we wish to propose a mechanism of recombination having main features that are compatible with such nonreciprocal events. This mechanism involves breakage and joining of DNA strands and also requires DNA synthesis.

Description of the Mechanism.—The DNA of phage f_1 (similar to M_{13} and fd) is single stranded and circular.[8] It has a molecular weight of about 1.7×10^6.[9] After entrance into the host cell this DNA is transformed into a circular double-stranded form.[10,11]

As the mechanism to be proposed is primarily aimed at understanding the recombination events in phage f_1, we describe it, using two double-stranded circular DNA molecules as parents. We refer to these DNA molecules as "chromosomes"; each of which is marked by a single mutation. Figure 1 depicts successive stages of a recombination event leading to the formation of a wild-type recombinant. We divide the process into three stages: initiation, replication, and return.

(a) *Initiation* (*I, II, III of Fig. 1*): Initiation involves breakage, pairing, and joining of DNA strands. We will not give a detailed sequence for the steps involved in passing from the first stage to the third stage. What is required is a break in one strand of one chromosome and a break in both strands of the other chromosome. The three breaks must take place in approximately the same region. Pairing of complementary strands occurs in this region. Although the breaks could be independent of each other, it seems possible that some of the breaks could be promoted and specified by the other breaks and by the pairing.

The important point is that the chromosome with the double-stranded break will be "extended" to form a recombinant whereas the chromosome with a single-stranded break will be "repaired" to emerge as a parent. *A priori*, two classes of recombination events are possible: those that initiate "inside" the markers and those that initiate "outside" the markers (see Fig. 1). In each class, four combinations of strand breaks are possible.
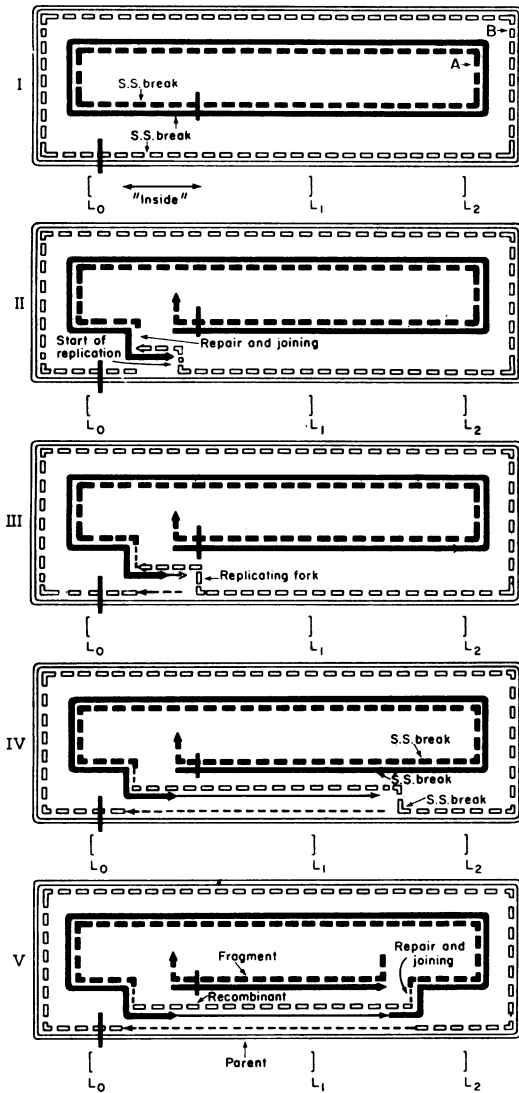
FIG. 1.—Successive stages in a recombination event. The figure illustrates only one of the possible combinations leading to a wild-type recombinant. The two parental DNA molecules are labeled A (*thick solid lines*) and B (*thick hollow lines*). They are drawn with their genetically homologous regions aligned.

The thick intact lines represent "$+$" strands while the thick broken lines represent "$-$" strands. New synthesis is represented by the corresponding thin lines. All $+$ strands have the same $5'$ to $3'$ direction (given here as counterclockwise), so do the $-$ strands (clockwise). Arrows indicate the $3'$ ends at free ends and at boundaries between strands of different origin.

The vertical bars position two mutations. "Inside" refers to the lesser of the two arcs determined by the mutations. The designations "SS (single-strand) break," "repair and joining," and "start of replication" are topological not chronological. The symbols L_0, L_1 represent the ends of two linear molecules recombining without a return; L_0 L_2 with a return.

A combination is defined by specifying which of the four strands remains intact. This determines which of the parental genomes will emerge in association with the wild-type recombinant. It is possible to introduce specific requirements that would reduce the number of allowed combinations.

As is apparent in II and III of Figure 1, an initiation results in the formation of a structure that is similar to the "replicating fork" proposed by Cairns[12] to be the structure involved in DNA replication.

(b) *Replication (III, IV of Fig. 1)*: DNA synthesis proceeds through the replicating fork. The chromosome with the double-stranded break is extended by acquiring a strand from the other chromosome and a newly synthesized copy of this strand. The intact strand of the other chromosome is copied to replace the strand transferred to the recombinant.

The rules of, and the problems raised by, this replication are the same as those relevant to all DNA synthesis.[13]

One could impose the restriction that DNA synthesis can proceed only in one direction. Recombination events yielding one of the parents would then have their initiation "inside" the markers, whereas events yielding the other parent would have their initiation "outside" the markers.

(c) *Return (IV, V of Fig. 1)*: The return event involves breakage and joining of three strands, as did initiation. A recombination event involving circular chromosomes can produce, among other products, DNA molecules that are physically identical to the parental molecules (double-stranded, circular, continuous, and the same length). To yield such products, the return event *must* involve the breakage of the same three strands that were broken during initiation ("correct" return) and *must* occur before the replicating fork reaches the initiation point. Absence of return, incorrect return, or return after more than one round of synthesis would produce a set of more or less abnormal molecules including, for instance, double-length circles as the least "monstrous."

The effectiveness of this mechanism and, we think, its plausibility thus depend on two probabilities: that of the return during the first round of synthesis, and that this return is correct.

The return event could take place ahead of the replicating fork; alternatively it could involve the replicating fork. The replicating fork has free ends and may also have single-stranded regions. These structures could have a greater tendency to interact with the other chromosome than would have a regular DNA duplex. This would promote the frequency of the return event. Moreover, the growing strands (free ends) in the replicating fork were specified by the pattern of strand breaks of the initiation. In the example given in the figure, strand B+ was left intact. Therefore, the strands extending A+ and B— have free ends in the replicating fork, while their complementary strands might have single-stranded regions (not yet copied). Thus the asymmetries in initiation permit the introduction of rules that could restrict the choice of strands broken in the return event. We, therefore, consider it plausible that the replicating structure could promote not only the frequency but also the "correctness" of the return event.

Linear double-stranded DNA molecules have no absolute requirement for a return event. Figure 1 indicates the result of a recombination event, without a return (L_0,L_1) and with a return (L_0,L_2).

Discussion.—We wish to state and discuss briefly the main attributes of the mechanism just described.

(a) The outcome of an individual recombination event is one parental genome and one recombinant genome.

(b) Recombinant molecules contain double-stranded DNA derived from one parent; between the initiation point and the return point they contain a single strand of DNA from the other parent along with a newly synthesized complementary strand. The integration of parental DNA into the recombinant is compatible with the results of numerous studies of viral and bacterial recombination systems.[14–17] Evidence will be presented that this applies to phage f_1.

(c) Whatever the precise mechanism for return is, the presence of a replicating structure could have an influence on the location of the return point relative to the position of the initiation point. Indeed, a plausible, though not obligatory, assumption is that a replicating structure traveling along a chromosome has a probability of returning in the unit-length segment just ahead of it that depends only on the types of DNA molecules involved (e.g., specific for f_1).

This assumption can be expressed as follows: Let us consider a population of recombining chromosomes that have their initiation points at x and their replicating forks proceeding in the direction indicated in Figure 2. We call R_ℓ the fraction of chromosomes that have not yet returned when their replicating forks reach

$\ell = \frac{1}{2}$

$\ell = \frac{3}{4} \rightarrow$

Direction of
replication

$\leftarrow \ell = \frac{1}{4}$
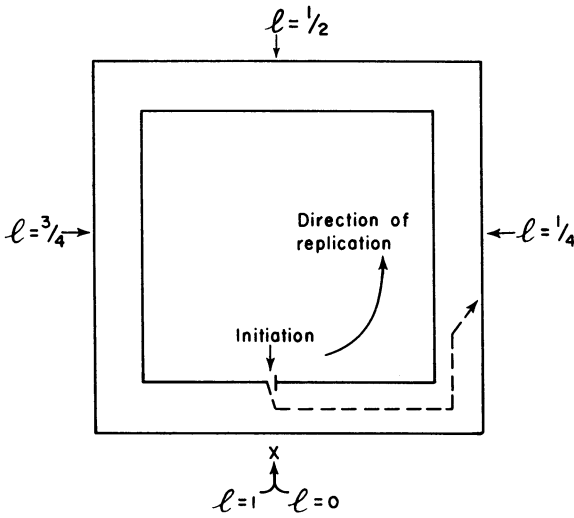
Initiation

$\ell = 1$　$\ell = 0$

X

FIG. 2.—Schematic diagram of the polar movement of a replicating fork after initiation of recombination at point x (details in III of Fig. 1). The length of the whole chromosomes is arbitrarily taken to be equal to 1.

a point situated at a distance ℓ (taken in the direction of replication) from x. The assumption stated above is equivalent to: $dR\ell/d\ell = -kR\ell$; then k being a constant for the type of DNA molecules involved. Thus, $R\ell = e^{-k\ell}$.

If this assumption is correct, a recombining structure that has initiated at point x has a probability of returning at a point situated at a distance ℓ from x, proportional to $e^{-k\ell}$. Also, the probability that a recombining structure returns before its replicating fork reaches the initiation point is equal to $1 - e^{-k}$ (the length of the whole chromosome being taken as equal to 1).

Thus, the higher the value of k, the higher the probability of return and also the higher the probability that the return occurs near the initiation point. We feel that these considerations may be relevant to the observations of negative interference.[18]

The mechanism described above can be extended to recombination between a double-stranded DNA molecule and a single-stranded DNA molecule. Such recombination might occur in bacterial transformation[19, 20] and in conjugation.[21-24]

Note added in proof: What has been called in this paper a correct return is one in which that branch of the replicating fork which links one DNA molecule to the other is the one which returns to the parent molecule (A in Fig. 1). If instead, the other branch of the fork joins this parent, there will be a reciprocal exchange of markers located outside the region of synthesis. In such a return, circular molecules generate double-length rings; however, linear molecules and circular molecules with specific sites of opening would give rise to recombinants of unit size.

[1] Zinder, N. D., R. C. Valentine, M. Roger, and W. Stoeckenius, *Virology*, **20**, 638 (1963).
[2] Hershey, A. D., and R. Rotman, *Genetics*, **34**, 44 (1949).
[3] Wollman, E. L., and F. Jacob, *Ann. Inst. Pasteur*, **87**, 674 (1954).
[4] Bresch, C., *Z. Naturforsch.*, **10b**, 545 (1955).
[5] Kaiser, A. D., *Virology*, **1**, 424 (1955).
[6] Mitchell, M. R., these PROCEEDINGS, **41**, 215 (1955).
[7] Lissouba, P., and G. Rizet, *C. R. Acad. Sci.*, **250**, 3408 (1960).
[8] Marvin, D. A., and H. Schaller, *J. Mol. Biol.*, **15**, 1 (1966).

[9] Marvin, D. A., and Hoffmann-Berling, *Z. Naturforsch.*, **18b**, 884 (1963).

[10] Ray, D. S., H. Bscheider, and P. H. Hofschneider, *J. Mol. Biol.*, **21**, 473 (1968).

[11] Pratt, D., and W. Erdahl, *J. Mol. Biol.*, **37**, 181 (1968).

[12] Cairns, J., *J. Mol. Biol.*, **23**, 191 (1963).

[13] *Cold Spring Harbor Symposium on Quantitative Biology*, **33** (1968).

[14] Kellenberger, G., M. L. Zichichi, and J. Weigle, these PROCEEDINGS, **47**, 869 (1961).

[15] Meselson, M., and J. Weigle, these PROCEEDINGS, **47**, 857 (1961).

[16] Fox, M., *Nature*, **187**, 1004 (1960).

[17] Siddiqi, O., these PROCEEDINGS, **49**, 589 (1963).

[18] Chase, M., and A. Doermann, *Genetics*, **43**, 332 (1958).

[19] Lacks, S., *J. Mol. Biol.*, **5**, 119 (1962).

[20] Fox, M., and M. Allen, these PROCEEDINGS, **52**, 412 (1964).

[21] Bonhoffer, F., and W. Vielmetter, *C.S.H. Symp. Quant. Biol.*, **33**, 623 (1968).

[22] Cohen, A., W. D. Fisher, R. Curtiss, III, and H. Adler, *C.S.H. Symp. Quant. Biol.*, **33**, 635 (1968).

[23] Rupp, W. D., and G. Ihler, *C.S.H. Symp. Quant. Biol.*, **33**, 647 (1968).

[24] Ohki, M., and J. Tomizawa, *C.S.H. Symp. Quant. Biol.*, **33**, 651 (1968).