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A General Model for Genetic Recombination

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ABSTRACT **A general model is proposed for genetic recombination. Its essential new feature is the hypothesis that recombination is initiated by a single-strand (or asymmetric) transfer, which may, after isomerization, become a two-strand (or symmetric) exchange. The likelihood of this transition from asymmetric to symmetric strand exchange determines certain characteristic features of recombination in any particular organism.**

In eukaryotes the segregation of an allele pair among the four products of meiosis occasionally departs from the usual 2:2 ratio while the segregation of well-separated markers on either side remains normal (i.e., 2:2). Frequently the flanking markers are recombined. This pattern, derived largely from genetic studies in fungi, probably means that the region of aberrant segregation is the actual site of chemical interaction between two recombining DNA molecules. Genetic analysis in fungi and prokaryotes, considerations of DNA structure, and the recognition of a number of enzymic mechanisms of DNA chemistry have made it possible to formulate rather specific hypotheses about molecular aspects of recombination, as we do in this paper (see refs. 1 and 2 for reviews).

The Holliday model

In 1964 Holliday proposed a model to explain recombination and aberrant segregation in fungi (3). Two homologous DNA duplexes, each corresponding to one of the four chromatids present at meiosis, undergo single-strand breakage and exchange single strands, forming heteroduplex DNA symmetrically for a limited distance as depicted in Fig. 1. Gene conversion, the unequal segregation of an allele pair among the four meiotic products, results from mismatch repair when a genetic marker happens to lie within the heteroduplex region. A 3:1 or 6:2 segregation requires repair on both chromatids. When repair occurs on only one, there is postmeiotic or 5:3 segregation; and if neither chromatid is repaired, there results a double postmeiotic segregation, called an aberrant 4:4. Termination of the interaction of DNA molecules by enzymic cleavage of the connecting pair of strands (*p,p* in Fig. 1) leaves the flanking arms in the parental configuration, whereas cleavage of the other pair of strands (*r,r*) yields products with the flanking arms in the recombinant configuration.

Recently, Sigal and Alberts (4) constructed a molecular model of the Holliday structure and found that it can be built with satisfactory bond lengths and angles, with no bases unpaired. The cross connection can move up or down by rotation of both duplexes in the same sense, a process that could be driven by rotary diffusion (5). Migration of the cross connection extends the region of heteroduplex DNA symmetrically on both chromatids, giving the postulated recombination intermediate (Fig. 1).

Heteroduplex DNA in one versus two chromatids

The equal formation of heteroduplex DNA on both chromatids, which is an intrinsic feature of the Holliday structure, should be reflected in the genetic data. The relevant evidence is mixed. The occurrence of aberrant 4:4 segregations in *Sordaria* indicates that heteroduplex DNA can form at the same site on both chromatids (6). At the *b2* locus in *Ascobolus*, Leblon and Rossignol (7) observed that the ratios of 6:2, 5:3, and aberrant 4:4 segregations were in good agreement with the hypothesis that heteroduplex DNA usually forms on both chromatids. In contrast, other studies are most simply interpreted to mean that heteroduplex DNA usually forms on only one chromatid. At the *arg4* locus in yeast the frequency of intragenic two-strand (i.e., two-chromatid) double exchanges is much less than that expected if heteroduplex DNA were formed initially on both chromatids followed by random repair of mismatches (8, 9). Similarly, at the W17 locus in *Ascobolus*, Stadler and Towe (10) found that among asci with uncorrected heteroduplex DNA on one chromatid, as evidenced by postmeiotic segregation, there was very seldom any genetic change on the homologous chromatid, thus suggesting that heteroduplex DNA had been formed on only one chromatid. The model presented here can accommodate both asymmetric and symmetric heteroduplex formation and provides a pathway for the generation of the Holliday structure.

The model

(*1*) A single-strand break in one DNA molecule becomes the site of strand displacement by a DNA polymerase. The displaced single-strand pairs with the complementary sequence in another molecule of DNA and induces a single-strand break in the latter.

(*2*) As shown in Fig. 2 A_p, further strand transfer results from the concerted operation of strand displacement and strand assimilation, respectively, by polymerase action at the arrow head and exonucleolytic action at the arrow tail. Because this reaction produces a tract of heteroduplex DNA on only one of the two interacting molecules, we call it *asymmetric strand transfer*. The necessary rotations of the duplexes are driven rapidly by the chemical energy available from the polymerization reaction.

(*3*) Dissociation of the enzyme allows structure A_p to rearrange, or isomerize (*i*), giving the structure A_r, as illustrated in Fig. 2. In structure A_p the arms that flank the site of exchange are in the parental configuration, whereas in structure A_r the arms are in the recombinant configuration.

(*4*) The cross connection in structure A_r is free to migrate in either direction as a consequence of rotary diffusion of the two DNA molecules. Whereas heteroduplex DNA was pre-

viously formed asymmetrically, strand transfer now is symmetric and heteroduplex DNA may form on both molecules (S_r, Fig. 2).

(5) Structure S_r, produced in the preceding step, may undergo a reversible isomerization (i') which is more complicated than that described above, but which also interchanges the parental and recombinant configurations of the flanking arms (ref. 4, see discussion). The structure S_p is physically but not genetically identical to the Holliday structure illustrated in Fig. 1.

(6) As suggested by Emerson (11) and Sigal and Alberts (4), isomerization of the Holliday structure interchanges the two pairs of like strands at the site of exchange, making them both susceptible to an endonucleolytic attack that terminates the exchange by cleaving the crossing strands. In the above, we have assumed that termination can occur only by cleavage of crossing strands, not noncrossing ones. If this restriction is relaxed (3), the isomerization i' no longer is required in order to obtain products with symmetrical regions of heteroduplex DNA and the parental configuration of flanking markers, since these could be produced directly by cleavage of structure S_r. Such products could also be obtained if rotary diffusion can be initiated without any prior isomerization, as indicated by the broken arrow in Fig. 2.

(7) Finally, this model, like most other current models of recombination, supposes that mismatched base pairs in heteroduplex regions are subject to enzymic repair.

Discussion

Interruptions in one or both strands of DNA have been correlated with increased recombination, but the process by which exchanges are initiated is unknown. The first step in our model, namely the invasion of a double-stranded molecule by a displaced single strand, is made plausible by (1) the existence of proteins that facilitate the melting and annealing of DNA (12, 1) and (2) the partially single-stranded character of superhelical DNA (13). Such superhelical DNA, which has been observed in the folded chromosome of *Escherichia coli* (14), might be present in recombining chromosomes.

Most of the subsequent steps in our model are based on reactions that have been demonstrated, although usually in circumstances that are simpler than the exchange of strands between molecules of DNA. These reactions or mechanisms include: (1) strand displacement (15–17); (2) strand assimilation (18, 19); (3) branch migration (20–22, 5); and (4) mismatch repair (23–25; J. Wildenberg and M. S. Meselson, manuscript in preparation). In addition, the polynucleotide chain interruption shown in Fig. 2 is presumed to be repaired by a ligase. As conceived here, strand displacement and strand assimilation comprise a concerted enzymic mechanism which, like migration of the cross connection, effects strand transfer without the exposure of single-stranded DNA. The two required enzymic activities, a polymerase and an exonuclease, might reside in a single molecule as in polymerase I of *E. coli* (26). The model suggests that the affinity of such an enzyme for the substrate A_p determines the extent of asymmetric strand transfer.

In addition to the reactions just listed, our model invokes two kinds of hypothetical molecular rearrangements or isomerizations. The first kind of isomerization, i, which might occur only after dissociation of the enzyme, can proceed by rotation of a pair of homologous arms 180° about an axis that

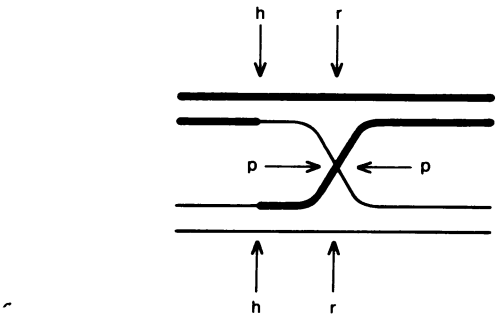

FIG. 1. The Holliday model for genetic recombination (3). Single-strand scissions are made at chemically identical sites on the homologous chromatids. Heteroduplex DNA is then formed symmetrically between *h* and *r* on both chromatids. The two pairs of like strands at the site of the crossover are considered by Holliday to be equivalent with respect to recognition by a DNase which terminates the exchange. Cleavages at the points marked *p* produce two molecules with the flanking arms in the parental configuration, whereas cleavages at the points marked *r* produce molecules with flanking arms in the recombinant configuration.

is between and parallel to them. This requires the interruption that is present in one of the four strands as represented in structure A_p and A_r (Fig. 2). Such isomerization and the onset of heteroduplex formation by rotary diffusion may be expected to occur rapidly following dissociation of the polymerase-nuclease. In contrast, the second kind of isomerization, i', requires more extensive motions of the arms and may be expected to occur less rapidly. Recently, while making an important correction of the original description of this kind of isomerization, Sobell questioned whether the required rearrangements could occur rapidly enough to play a role in recombination (27). In the following, we attempt to assess, on the basis of a simplified hydrodynamic model, the likelihood that this reaction might occur within the time available for genetic recombination. Starting from the Holliday struc-

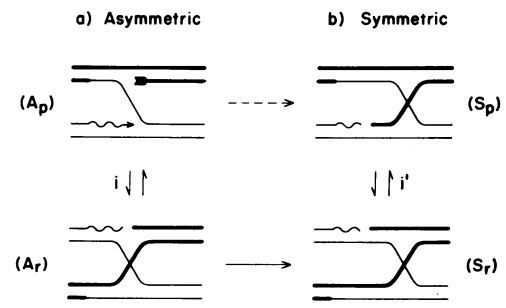

FIG. 2. The model described here: in (a) the *asymmetric phase*, heteroduplex DNA is formed on only one chromatid. In the first specified intermediate, A_p, the flanking arms are in parental configuration. By isomerization, i, the structure A_r is produced in which heteroduplex DNA is still restricted to one chromatid, but the flanking arms have acquired the recombinant configuration. Branch migration driven by rotary diffusion converts structure A_r to S_r and heteroduplex DNA is subsequently formed symmetrically on both chromatids. The configuration of the flanking arms in (b), the *symmetric phase*, can be rearranged by the isomerization i'. The structure S_p might also be produced directly from A_p by rotary diffusion (dashed arrow). The interruption shown in one strand in A_r is closed by a polynucleotide ligase at some unspecified time.

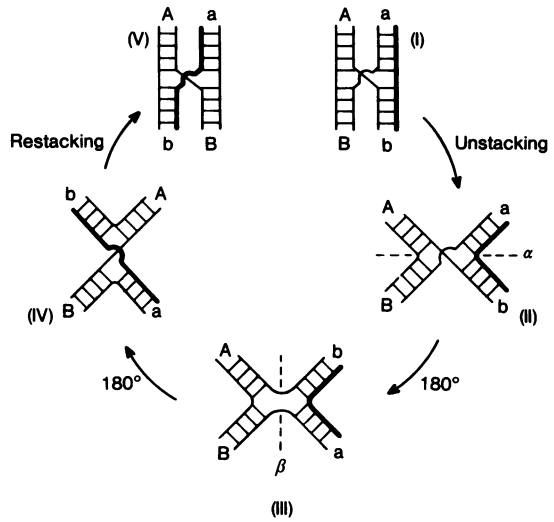

FIG. 3. The isomerization (i') proposed by Sigal and Alberts (4) and recently discussed by Sobell (27). The details of this figure are described under *Discussion*. A line representing one strand has been heavily shaded as an aid to following the rearrangements. The half-loop of one line in diagrams I, II, IV, and V labels the crossing strand that would be closer to the viewer in the corresponding molecular structure.

ture, as shown in Fig. 3 (I–V), this isomerization may be conceived as follows: (*1*) Unstacking of the four base pairs at the cross connection produces the symmetrical structure shown in Fig. 3 (II). (*2*) Rotation of the molecules ab and AB with respect to each other by 180° about an axis α which passes through the cross connection produces structure III. Examination of a molecular model reveals that this rotation can be completed in only one sense for steric reasons. (*3*) Rotation of the molecules aB and Ab with respect to each other by 180° about a second axis β which is perpendicular to the first gives structure IV. This rotation also can be completed in only one sense. (*4*) Restacking of base pairs on either side of the new cross connection gives the recombined structure V.

These rotations need not involve entire molecules but only the displacement of segments, the local motion of which is essentially independent of the rest of the molecule because of the flexibility of DNA. Since the time constants for unstacking and restacking a single base pair are of the order of a microsecond (28, 29), we shall ignore the time required for these steps. As a rough approach to estimating the rate of isomerization i′ we assume that the necessary rotations may be likened to the free rotary diffusion of a rod about a perpendicular axis at its center, where the length L of the rod is of the order of the diffusional segment length of DNA and d is the molecular diameter. With $L \gg d$, the time required for a root mean square average rotation of 360° is approximately (ref. 30, Eqs. 9, 10, and 37):

$$t = \frac{4\pi^3\eta L^3}{3kT[2\ln(2L/d) - 1]}$$

where k = Boltzmann's constant, 1.38×10^{-16} ergs/°K,
T = absolute temperature, 310°K,
d = 2 nm,
η = viscosity of the medium, dyne sec/cm².

Taking η as 0.01, the value for water, t = 1.2 msec if L = 1000 Å, and 0.75 sec if L = 10,000 Å. Since the statistical segment length of DNA in dilute salt solution is approximately 1000 Å (31), this result suggests that the mean time for the segmental rotations involved in the isomerization i′ may be considerably less than a second. The most questionable assumption in the calculation is the choice of the viscosity of free solvent as the appropriate value. If this is not too far wrong, the isomerization i′ could be rapid enough to occur during the time of at least several seconds presumed to be available for genetic recombination. Our estimate of the rate of isomerization i′ and estimates of the possible rate of migration of the cross-connection published elsewhere (5) are crude. However, they do suggest that our model cannot be dismissed *a priori* on kinetic grounds.

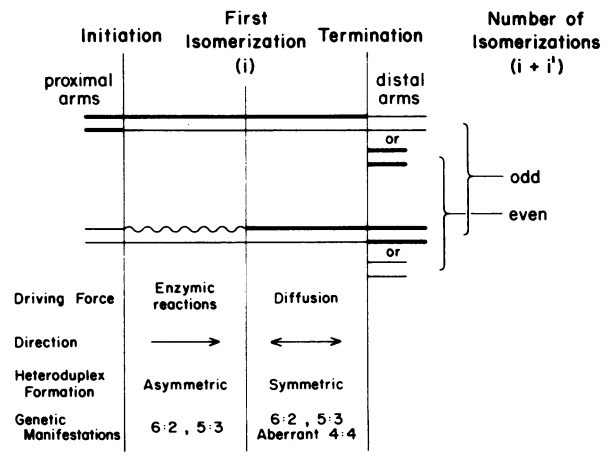

FIG. 4. A summary of some of the principal features and genetic consequences of the model.

Some genetic implications of the model are illustrated in Fig. 4. The first phase of recombination is asymmetric and enzymically driven. Depending on whether mismatched bases are corrected or not, the asymmetric formation of heteroduplex DNA would give rise respectively to 6:2 or 5:3 segregations adjacent to the point of initiation. The second phase which follows dissociation of the enzyme and the first isomerization (i) is symmetric and driven by rotary diffusion. The symmetic formation of heteroduplex regions may generate 6:2, 5:3, or aberrant 4:4 segregations, again depending on the particular pattern of repair of mismatched bases. It is during this second phase that the isomerization i' may occur one or more times. Depending on whether the total number of isomerizations, i plus i', is even or odd, the flanking markers will be in the parental or recombinant configuration, respectively. In addition to aberrant 4:4 segregations, the symmetric phase should generate another class of segregants that should not be generated during the asymmetric phase, namely intragenic or close two-strand double exchanges. We can account for the paucity of intragenic two-strand double exchanges in yeast (8) by supposing that asymmetric strand transfer makes a larger contribution to intragenic recombination than symmetric transfer, due to a particularly high affinity of the yeast polymerase-nuclease for its substrate. It may also be noted that when hybrid DNA is generated mainly in the asymmetric phase, reciprocal recombinants for close markers will be found mainly with the recombined configuration of outside markers observed by Fogel and Hurst (32).

As in other models (2), the preferential initiation of recom-

bination at fixed locations on the chromosome would manifest itself as genetic polarity. In our model, initiation at fixed locations would produce certain characteristic patterns of polarized segregation, in part because of the unidirectional nature of the asymmetric phase.

According to our model, we would expect that long deletions or substitutions should not exhibit aberrant 4:4 segregation or intragenic two-strand double exchange, since branch migration driven only by rotary diffusion should be blocked by long regions of nonhomology. By contrast, this barrier might be overcome by the enzymically catalyzed and energetically driven strand transfer that occurs during the asymmetric phase, giving 5:3 and 6:2 segregations of such mutations.

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