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MOLECULAR ASPECTS OF GENETIC EXCHANGE AND GENE CONVERSION

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SOME ten years ago molecular models were proposed which attempted to explain both recombination at the fine structure level and gene conversion in fungi (HOLLIDAY 1962, 1964; WHITEHOUSE 1963; WHITEHOUSE and HASTINGS 1965). These models shared the following features: (1) Recombination occurred by the breakage and reunion of homologous DNA molecules; (2) Heteroduplex or hybrid DNA structures were an essential intermediate in recombination, complementary base pairing providing the necessary specificity for homologous pairing at the molecular level; (3) Mismatched bases within hybrid DNA were corrected or repaired to give normal base pairing; (4) The models were unifying in attempting to explain both reciprocal crossing over and associated non-reciprocal gene conversion by one overall mechanism.

It has now been demonstrated, particularly by the experiments of TAYLOR (1965), that recombination at meiosis does indeed involve breakage and reunion of chromatids. In addition, many of the features of recombination on which the models were based are now known not to be unique to fungi. CARLSON, together with CHOVNICK and associates have now demonstrated that gene conversion with or without associated crossing over, polarized recombination and fine structure map expansion occur also in Drosophila (CARLSON 1971; CHOVNICK *et al.* 1970; BALLANTYNE and CHOVNICK 1971; SMITH, FINNERTY and CHOVNICK 1970). This makes it much more likely that all eukaryotes have the same general mechanism for recombination, although, as we shall see, there are important differences in detail between species. In this paper I will discuss certain molecular aspects of hybrid DNA models in the light of observations which have been made within the last decade, but no attempt will be made to review all the pertinent recombination experiments.

HETERODUPLEX DNA

It has now been generally accepted that heteroduplex or hybrid DNA (hDNA) is an intermediate in recombination. Using polynucleotide ligase and DNA polymerase deficient mutants of phage T4, BROKER and LEHMAN (1971) were able to observe recombination intermediates by electron microscopy. Many of these molecules are branched, and it is obvious that one of the arms of any Y-shaped structure must be a heteroduplex. Furthermore, they suggested that the branch point where one chain of a double stranded molecule switches to pair with another chain can migrate along the chromosome by the breakage and formation of

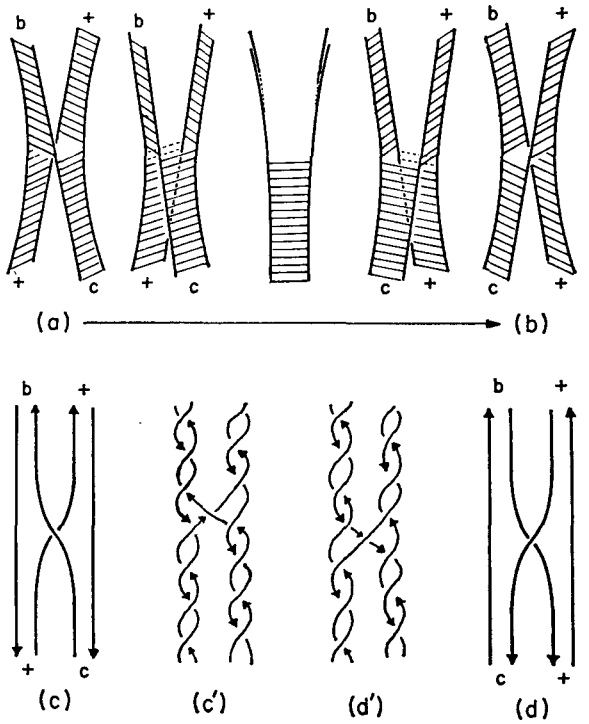

FIGURE 1.—Half chromatid chiasmata (EMERSON 1969; based on HOLLIDAY 1964). Above: a purely diagrammatic three-dimensional representation of the exchange of single strands between DNA duplexes, where the horizontal lines indicate hydrogen bonds between polynucleotide chains. Rotation of the figure (a → b) causes different chains to appear to cross one another. Below: two dimensional representations and coiled polynucleotide chains, where c and c′ show one isomeric form and d and d′ the other. Isomerization does not require the breakage and formation of hydrogen bonds, so the two forms are in equilibrium (SIGAL and ALBERTS 1972).

new hydrogen bonds. Intermediates in recombination between ϕX174 double stranded circular molecules (replicative form DNA) have also been seen by electron microscopy (BENBOW, ZUCCARELLI and SINSHEIMER (1974a). In these studies two parental molecules could be identified because one of them contained a small deletion. Since the recombining circles are held together by a region thicker than a single DNA molecule, it is possible that there are two parallel hDNA structures at the region of interaction. By using an appropriate deletion and wild-type phage, clear evidence for branch migration has been obtained *in vitro* (KIM, SHARP and DAVIDSON 1972). MESELSON (1972) has calculated the rate of formation of hDNA by random diffusion of the initial exchange point along the molecule. For molecules the size of λ genomes, heteroduplex DNA 1,000 nucleotides long could be formed in about 20 seconds. For larger molecules, the formation of long regions of hDNA may well be impeded by the absence of points of free rotation, but nevertheless the formation of hDNA long enough to explain genetic recombination data, say one or a few genes, seems to present no mechani-

cal problems. Larger stretches might well be formed in the presence of a protein of the type discovered by WANG (1971), which brings about the unwinding of supercoiled DNA.

The question of the actual structure of the exchange point has been answered by building accurate models. SIGAL and ALBERTS (1972) and also JEGGO (1973) demonstrated that the hydrogen bonding can be completely preserved on each side of the switch point. This, of course, makes it easy to see how the actual making and breaking of hydrogen bonds will lead to branch migration. But in addition, SIGAL and ALBERTS showed that the inner strands are not in a unique configuration, but are structurally equivalent to the outer "nonexchanged" strands. This is shown diagramatically in Figure 1, taken from EMERSON (1969). The reversible exchange of inner and outer strands is called isomerization. If the inner strands always break, then in the one configuration outside markers are recombined and in the other configuration outside markers remain parental. This strand equivalence was, of course, implicit in the original model (HOLLIDAY 1964). The extensive data on gene conversion and crossing over assembled by HURST, FOGEL and MORTIMER (1972) demonstrated that half of all conversion events are associated with crossing over. Amongst 549 conversions, 268 were recombinant for outside markers.

It should be noted that the breakage of two strands of like polarity does not have to be at precisely equal points. Breaks between non-homologous pairs of nucleotides will lead to a small single strand overlap and a small single strand gap. These defects can undoubtedly be repaired by exonuclease, repair polymerase and ligase action. The important point is that regions of hydrogen bonded overlap ensure that exchanges can occur without any deletion or addition of genetic material.

THE ORIGIN OF HETERODUPLEX DNA

The question of the origin of hDNA remains unanswered. Polarity in gene conversion and outside marker recombination has often been interpreted to mean that there are defined initiation points in recombination. The following suggestions have been made:

1) After the premeiotic S phase, single strand gaps are left at the ends of replicons and these provide defined initiation points (WHITEHOUSE 1969; HASTINGS 1972).

2) A nuclease binds to specific base sequences distributed at random along the chromosome. The enzyme has two binding sites and cuts at the same time strands of like polarity at homologous points (HOLLIDAY 1968).

3) An endonuclease cuts single strands at the ends of stabilized GIERER loops (see GIERER 1966). Such loops may occur at operator regions (SOBELL 1972).

4) Recombination is initiated by single stranded "aggression" on a recipient duplex. This assumes that some kind of transient homologous association between three strands occurs which is followed by a nick in one of the recipient strands (HOTCHKISS 1971; BENBOW, ZUCCARELLI and SINSHEIMER 1974b).

Experiments by CATCHESIDE and his associates with Neurospora may help to throw light on the problem of the initiation of recombination (ANGEL, AUSTIN and CATCHESIDE 1970; CATCHESIDE and AUSTIN 1971; SMYTH 1973; CATCHESIDE, personal communication). Three different *rec* loci have been identified which control recombination frequency in specific parts of the genome, each locus having its own spectrum of effects. In addition, a mutant *cog* strongly influences recombination in its own vicinity and interacts with the *rec-2* locus. These results can be understood if the activity of at least three endonucleases is under genetic control and each enzyme acts on specific base sequences. The mutant *cog* would then be introducing a new substrate or target for one of these enzymes. Mutants in other organisms which strongly stimulate recombination in the gene in which they lie, such as M26 in the *ade-6* locus in Schizosaccharomyces (GUTZ 1971), may also act in the same way.

Whatever the mechanism for initiating recombination, it is clear that DNA would neither unravel nor anneal under normal physiological conditions. Melting to form single strands occurs only at high temperatures, and single strands loop back on themselves to form irregular hydrogen bonded secondary structures that cannot pair with similar homologous structures. These difficulties are avoided in the presence of sufficient quantities of a DNA binding protein like those discovered by ALBERTS and his associates (ALBERTS and FREY 1970; DELIUS and MANTELL and ALBERTS 1972). Such "gene 32" proteins show nonspecific but cooperative binding to single stranded DNA. They have the property of lowering the melting temperature of double stranded DNA as well as facilitating the annealing of single stranded DNA. It is known that the gene 32 protein is essential for recombination in phage T4. DELIUS, MANTELL and ALBERTS (1972) showed that melting of lambda DNA in the presence of gene 32 protein begins at high AT rich regions. It is possible, therefore, that the preferred initiation points in fungi are simply rich in AT sequences. Recently a protein with properties similar to the gene 32 product has been purified from vegetative cells of the smut fungus *Ustilago maydis* (G. R. BANKS and A. SPANOS, in preparation). Previously, HOTTA and STERN (1971) had detected a similar protein in meiocytes of Lilium, but not in vegetative tissues.

THE CORRECTION OF MISMATCHED BASE PAIRS

Results obtained with spore colour mutants in *Ascobolus immersus* provide strong support for the correction hypothesis (LEBLON 1972a, 1972b; LEBLON and ROSSIGNOL 1973). It is now quite clear that the pattern of conversion and postmeiotic segregation is dependent on the type of mutation used in the cross. Base substitution mutants show high frequencies of postmeiotic segregation, whereas addition/deletion mutants show low frequencies. If one such mutation designated + gives an excess of conversions to the wild-type allele, then the mutation of opposite sign (−) gives the reverse conversion spectrum. These mutations are known to be an addition and a deletion, because they partially suppress each other's phenotype in *cis* configuration. The basic conversion frequency, i.e., total

frequency of aberrant octads, remains the same irrespective of the type of mutation. This suggests that there is a given probability of a genetic region being included in hDNA, but that subsequent events involving repair of mismatched bases depends on the nature of the mutation. In the case of heteroduplexes containing one unpaired base (for instance, an addition paired with normal DNA), repair in one direction is efficiently triggered; for mismatched bases the repair is inefficient, and this results in postmeiotic segregation. The situation in yeast is different, since a large number of mutants show equality in the frequency of conversion to the mutant or to the wild-type allele (FOGEL, HURST and MORTIMER 1971), and this implies that correction is random in this organism.

SPATZ and TRAUTNER (1970) and TRAUTNER *et al.* (1971) have shown that artificial heteroduplex molecules of bacteriophage SPP1 of *B. subtilis*, which contain single mismatches, frequently give rise after transfection to only mutant or wild progeny phage rather than mixed bursts. This is probably due to repair which preferentially occurs in one or another strand and does not depend on the type of base substitution. In some instances, however, the given site is always corrected to mutant, irrespective of the strand it is in. The overall results are most easily explained if the repair is actually triggered by the mismatch rather than being the consequence of some general DNA turnover of single strands. Recent additional evidence for correction of mismatched bases comes from transformation experiments with *B. subtilis* (BRESLER, KRENEVA and KUSHEV 1971), and results with bacteriophage ϕX174 are interpreted on the same basis (BAAS and JANSZ 1971, 1972; BENBOW, ZUCCARELLI and SINSHEIMER 1974a).

Although it now seems likely on genetic grounds that correction occurs, a DNase has not yet been detected that can specifically act on a DNA substrate containing one or more mismatched base pairs. It is known, however, that DNA polymerase I in synthesizing DNA is able to discriminate between normal pairing and incorrect pairing and cut out the offending base (BRUTLAG and KORNBERG 1971).

Ingenious experiments by FOGEL and MORTIMER (1970), using ochre and ambre suppressors of yeast, have demonstrated the fidelity of gene conversion. The converted chromatid always carries the base pair of one or other parent. This rules out the possibility that correction operates by the removal and random replacement of bases without scission of either polynucleotide gene.

THE EVOLUTION OF A MECHANISM FOR THE REPAIR OF MISMATCHED BASES

Mismatched bases can occur in DNA either because the reading mechanism of DNA polymerase fails, or because heteroduplex DNA is formed by recombination. In either case it is impossible to believe that any enzyme could distinguish the wild type from the mutant strand. If correction occurs at random, the wild type base pair is reestablished in half the cases. Yet the same result follows if no correction occurs, since then one daughter molecule will be wild type and the other mutant. It is therefore clear that there is no selective advantage in having an enzyme which recognizes mismatches, since this does not eliminate mutants.

Why then would such a mechanism exist? A likely answer is that the primary role for an excision enzyme is the repair of abnormal or damaged bases, and that this enzyme is also capable of detecting—sometimes with rather low efficiency—the minor distortions in the DNA duplex caused by the mispairing of normal bases. Recently evidence has been obtained that the spontaneous loss of purine residues from DNA leads to excision repair (VERLY, PAQUETTE and THIBODEAU 1973). It is also known that cytosine in DNA spontaneously deaminates to uracil under physiological conditions (L. E. ORGEL, personal communication). The consequence of this could be serious in higher organisms if a mechanism did not exist for removing uracil and replacing the normal base.

CO-CONVERSION

When two mutant alleles are crossed, the recombination is related to the distance between the mutant sites. This would not be so if the correction process merely involved removal and replacement of an offending base, since then mutants close together could give almost as many recombinants as those further apart (FINCHAM and HOLLIDAY 1971). Fine structure maps are explicable if the repair triggered by mismatched bases results in the degradation of one strand followed by repair replication. This would result in the co-conversion of closely linked sites. FOGEL and MORTIMER (1969) first clearly demonstrated that co-conversion does occur in yeast and that its frequency decreases as the distance between the two mutants increase. More recently LEBLON and ROSSIGNOL (1973) have demonstrated in Ascobolus that in crosses involving both a frameshift and a base substitution mutation within the b_2 spore color locus, 93% of conversion events covered both sites. Moreover, the conversion spectrum of the frameshift mutation (very low frequency of postmeiotic segregation) overrides that of the nearby base substitution mutation. This result is explicable if correction in hDNA is most usually triggered by the frameshift mutation and results in single stranded excision extending over the second site. Repair synthesis then generates one or other parental chromatid.

MAP EXPANSION AND MARKER EFFECTS

The non-activity of fine structure maps in fungi has been explained on the basis of correction of mismatched bases, with single stranded breakdown and resynthesis of a section of DNA of fairly constant length (HOLLIDAY 1968; FINCHAM and HOLLIDAY 1971). Mutants closer together than the length of the excised region give additive maps, whereas those further apart give a higher frequency of recombination than expected from the sum of all the smallest intervals. Apart from fungi, map expansion has now been demonstrated in Drosophila (CARLSON 1971), but it appears not to occur in prokaryotes. The absence of map expansion in fungi such as Coprinus (GANS and MASSON 1969; MOORE 1972) does not imply that this general model is incorrect; it may simply mean that the length of the repaired region is very variable.

It should be emphasized that map expansion is a marker effect, and it is incor-

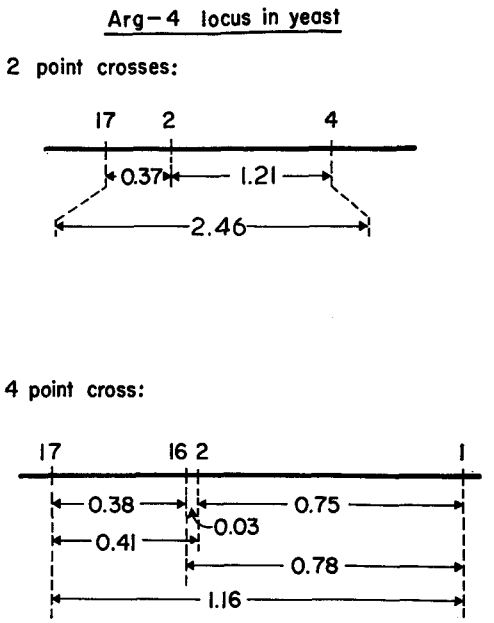

FIGURE 2.—Fine structure maps of the *arg4* locus in yeast derived from non-selected tetrads (data of HURST, FOGEL and MORTIMER 1972). Recombination values (%) are calculated by treating the individual members of the tetrads as random progeny. A cycle of three 2-point crosses yields a map which differs significantly from additivity, but this *map expansion* is absent in the fine structure map obtained from one cross in which all four alleles are segregating. (In multipoint crosses it is in fact mathematically impossible for the outermost sites to have a higher recombination frequency than the sum of intervening intervals. On the other hand *map contraction* would have been seen had conversion of inner sites occurred without exchange of outer sites. Amongst 1505 tetrads, none of this type was detected).

rect to suppose that it could be explained merely by the random conversion of segments of DNA (FOGEL and MORTIMER 1970). The marker effect could be due either to repair triggered by mismatched bases or alternatively to the inhibition of hDNA formation by heterozygous sites. In multipoint crosses, where all the products of meiosis are analysed, it is impossible to generate map expansion. This is clearly seen in the data of HURST *et al.* (1970) in their extensive analysis of recombination at the *arg4* locus in yeast using unselected tetrads. The 4-point cross gives the fine structure map shown in Figure 2. (In this map recombinants between sites are scored as follows: a crossover between sites yields two recombinants per tetrad; a conversion at one site and not the other yields one recombinant per tetrad; co-conversion yields no recombinants). It would be very desirable to have comparison between these maps and those generated by 2-point crosses using the same mutants, since the latter would probably give a map showing expansion, i.e., the outermost markers give a higher than expected frequency of recombination. Unfortunately the 2-point crosses carried out involved different mutant sites. Map expansion is evident from the cycle of three 2-point crosses involving *arg4* (HURST *et al.* 1970) as shown in Figure 2, and

it has also been demonstrated by 2-point crosses in the *his1*, *ade8* and *ade3* loci in yeast (FOGEL and HURST 1967; ESPOSITO 1968; JONES 1972). Discrepancy between the fine structure maps between two- and multi-point crosses is explicable on the basis of the correction theory. A mutant site flanked by two others will sometimes trigger co-conversion of two or all three sites, and this reduces the overall yield of recombinants.

The effect of markers is seen by comparing the recombination frequency between two adjacent sites, *arg4-1* and *arg4-2*, in the presence and absence of other *arg4* alleles proximal to this interval. In the 2-point cross, recombination frequency is 1.84%, but when mutants *arg4–16* and *17* are also present in the cross, the recombination between *arg* alleles *1* and *2* is reduced to 0.72% (see Table 1). This effect could be entirely due to co-conversion of these alleles, yielding no recombinants, triggered by the nearby *16* and/or *17* heterozygous sites. The proportion of co-conversions for *1* and *2* is clearly much greater in the 4-point cross than the 2-point one (Table 1). Comparable behavior of the alleles *16* and *17* is seen in the presence of either the allele *19*, which is some distance away, or the alleles *2* and *1*, which are much closer. In the latter case, recombination between *16* and *17* is reduced 2.3 fold and the conversion frequency increased relative to single site conversion (Table 1). These marker effects are expected if mismatches are removed by an excision repair process.

AHMAD and LEUPOLD (1973) have suggested that the marker effect of map expansion could be due to an effect of heterozygous sites on the initial stability of hDNA. Another possibility is that branch migration is significantly impeded

TABLE 1

Recombination and conversion at the arg 4 *locus in yeast: the effect of nearby sites on recombination and conversion of adjacent mutants* arg 4–1 *and* 4–2 *and* arg 4–16 *and* 4–17 *(data of* HURST, FOGEL and MORTIMER 1972; MORTIMER, *personal communication)*

Cross	No. tetrads	Conversion of *1* and/or *2* Single-site	Co-conversion	Ratio ss/co-con.	Crossovers between *1* and *2*	Total recombination between *1* and *2* (%)
$\frac{1\ +}{+\ 2}$	483	25	24	1.04	5	1.81
$\frac{1\ 2\ +\ +}{+\ +\ 16\ 17}$	1,505	39	76	0.51	3	0.75

Cross	No. tetrads	Conversion of *16* and/or *17* Single-site	Co-conversion	Ratio ss/co-con.	Crossovers between *16* and *17*	Total recombination between *16* and *17* (%)
$\frac{19\ +\ 17^*}{+\ 16\ +}$	2,566	69	124	0.56	10	0.87
$\frac{1\ 2\ +\ +^*}{+\ +\ 16\ 17}$	1,505	23	105	0.22	0	0.38

* In these crosses the recombination between adjacent alleles *16* and *19* is 1.61% and between *16* and *2* is 0.03%.

when a mismatched base pair is encountered. However, if the overall conversion frequency is a measure of the probability of mutant sites being included in hybrid DNA, then the total frequency of co-conversion and single site conversion should be reduced as more markers are introduced into the cross. HURST, FOGEL and MORTIMER (1972) have measured conversion frequencies at the *arg4* locus in crosses in which *1*, *2*, *3* or *4* alleles are segregating and there is no evidence for this effect.

HYBRID DNA ON ONE OR TWO CHROMATIDS?

One of the strongest reasons for proposing that hDNA is formed reciprocally was the detection of aberrant 4:4 octads in Sordaria (KITANI, OLIVE and EL ANI 1962). In these, postmeiotic segregation occurs in two pairs of spores. This result would be expected if mismatched bases within hDNA on both chromatids were not corrected and segregated out at the first postmeiotic division. If segregation occurs from only one chromatid, then 5:3 or 3:5 octads are observed. From the frequency of 3:5 and 5:3 octads one can calculate the expected frequency of aberrant 4:4's if hDNA is formed reciprocally. Early data from Sordaria (KITANI, OLIVE and EL ANI 1962) showed that the frequency of these octads is close to expectation (WHITEHOUSE and HASTINGS 1965). More recently it has been possible to detect aberrant 4:4's in the unordered octads in *Ascobolus immersus*. Again, the frequency is just what would be expected if hDNA is always formed on both chromatids (LEBLON and ROSSIGNOL 1973). However, in another strain of Ascobolus, STADLER and TOWE (1971) obtained evidence that postmeiotic segregation was derived primarily from a recombination intermediate with only one region of hDNA.

If two regions of hDNA are formed and repair of mismatched bases is random, then a number of classes of tetrads should be observed, including, for instance, that of the following type (where *1* and *2* are mutant alleles and A and B are outside markers):

Cross					Tetrad			
A	1	+	B	→	A	1	+	B
a	+	2	b		A	+	2	B
					a	1	+	b
					a	+	2	b

In formal terms, this is equivalent to a 2-strand double exchange tetrad. Such tetrads are observed in yeast at a much lower frequency than the reciprocal hDNA model would predict (FOGEL, HURST and MORTIMER 1971), although in Sordaria KITANI and OLIVE (1967) have shown that the equivalent octad, aberrant 4:4 with parental outside markers, is often detected. Similarly, reciprocal hDNA with random repair should often generate reciprocal recombinant genotypes within a gene without exchange of outside markers; but in yeast these are rarely seen (FOGEL, HURST and MORTIMER 1971; FOGEL and HURST 1967). Failure to find significant numbers of these tetrads in yeast is explained if hybrid DNA forms on only one chromatid. Nevertheless, it is now known that post-

meiotic segregation occurs in yeast (ESPOSITO 1971), and aberrant 4:4 tetrads have been detected (FOGEL, personal communication).

The problem can be solved if hDNA instead of remaining static at a particular region of the chromosome, in fact diffuses back and forth by branch migration. In this case, a mutant could be present transiently in hDNA. It would only remain in an hDNA region until the next round of DNA synthesis if it happened to be within that region at the time the final breakage and reunion of polynucleotide chains occurred. These events fix the final length and position of hDNA. Since postmeiotic segregation is known in several fungi, including yeast, it seems probable that the initial step in repair is rate limiting, all subsequent steps occurring rapidly (see also FINCHAM and HOLLIDAY 1970). Therefore the chance of correction occurring in reciprocal regions of migrating hDNA may be relatively small in yeast, and the overall genetic result is almost the same as if hDNA were formed on only one chromatid. This illustrated in Figure 3. In Figure 3A there is just one exchange point which diffuses back and forth, which is essentially the same variant of the original model which was proposed to explain the strict polaron in Ascobolus (Figure 5 in HOLLIDAY 1964). The other possibility is suggested by the model of SOBELL (1972). In this case there are two exchange points and the length of hDNA between them is fixed. SOBELL has pointed out that this structure can diffuse by branch migration along the paired chromatids.

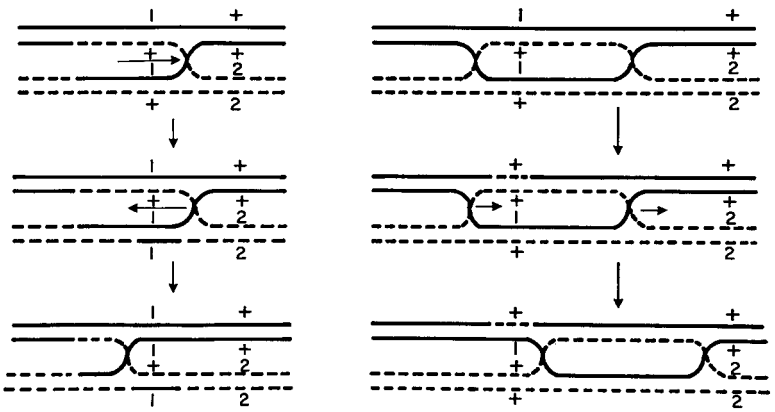

FIGURE 3.—The effect of branch migration on gene conversion and crossing over. A: the hDNA diffuses past mutant site 1, providing an opportunity for correction of mismatches, but frequently it reverses direction and diffuses back to the position indicated, or is lost altogether. If no correction occurs, then there is normal 2:2 segregation for site 1. Correction of one mismatch, as illustrated, yields on re-annealing one parental and one mismatched strand. A second repair event generates a conversion or a parental tetrad. The genetic result is the same as if only one region of hDNA had been formed (see text). Initial correction of both mismatches (not illustrated), which may be relatively rare, can yield tetrads of the "forbidden" type, e.g., reciprocal cross overs within the gene without outside marker exchange (see text). B: Two half chromatid exchange points yield a bubble which can migrate into the centre of the gene (SOBELL 1972). If there is no correction at site 1, then breakage and reunion of strands generates in half the cases a reciprocal exchange between sites 1 and 2. Nonreciprocal exchange events will not be detected. Correction of mismatches at site 1 during the diffusion of the bubble yields the same results as in Figure 3A.

This idea is appealing for several reasons. Although the initiation of recombination may occur at a defined point, hence giving rise to polarity in recombination, the hDNA bubble may move from this point and sometimes end up within a gene. With mutants on either side not included in hDNA, resolution of the structure by breakage and reunion will give in half the cases a classical reciprocal exchange without gene conversion. Such tetrads have often been observed in yeast (FOGEL and HURST 1967; FOGEL, HURST and MORTIMER 1971; AHMAD and LEUPOLD 1973). Another type of tetrad the migrating bubble will account for is that in which a conversion and a crossover are separated by a region of DNA with normal 2:2 segregation:

Cross					Tetrad			
A	1	+	B	→	A	1	+	B
a	+	2	b		A	+	+	b
I	II	III			a	+	2	B
					a	+	2	b

The second member of this tetrad arises—on a formal interpretation—from three exchanges. A static hDNA model can explain the tetrad only if both mutants are included in hDNA: mutant 2 is corrected by change to the parental configuration, whilst mutant 1 converts. But if hDNA is initiated in region I and migrates to region III, there is a given probability of conversion of either or both mutants en route. The structure is finally resolved in region III to give a reciprocal exchange.

The difference between Ascobolus or Sordaria and yeast could lie mainly in the amount of branch migration which occurs. The faster the exchange point is resolved by breakage and reunion enzymes, the greater the chance that reciprocal hDNA will be fixed in position and the smaller the chance that a region of hDNA will wander across heterozygous sites.

CONCLUSIONS

Hybrid DNA models for recombination were put forward to explain existing data and to make predictions which could be tested experimentally. They have on occasions been interpreted too literally, leading authors to conclude that their data were incompatible with a particular model. Yet even ten years ago it was evident that different species of fungi differed in their pattern of recombination at the fine structure level, and that no single rigidly defined model could explain all the experimental observations.

As we have seen, several of the essential features of hybrid DNA models are now supported by experimental evidence, although this evidence is usually indirect. There is still considerable ignorance about many of the processes involved. The initiating events in recombination are quite obscure and the many studies of the synaptonemal complex have not yet shown how this structure may facilitate the interaction between homologous chromosomes. Although it is generally believed that hybrid DNA must be an intermediate in recombination, it has not yet been demonstrated in any eukaryotic organism. The genetic evidence in cor-

rection of mismatched bases is very strong, but so far no mutant has been shown to be defective in correction, nor has an enzyme been identified which is capable of acting specifically on DNA substrates containing mismatches. However, biochemical studies of recombination have now been initiated in eukaryotic organisms, such as Lilium and Ustilago. An endonuclease appears in meiocytes of the former at the pachytene stage (see STERN and HOTTA 1973), and in Ustilago mutants deficient in an endonuclease are defective in recombination (BADMAN 1972; HOLLOMAN 1973; HOLLOMAN and HOLLIDAY 1973). A DNA binding protein with properties similar to the gene 32 protein of bacteriophage T4 has been identified in meiocytes of Lilium (HOTTA and STERN 1971) in vegetative cells of Ustilago (BANKS and SPANOS, in preparation). Many recombination defective mutants are now available in Drosophila and various fungi, and one obvious future approach is to correlate biochemical with genetic malfunction.

Recent studies on DNases required for genetic recombination reveal that they react with their substrates in a complex manner (CASSUTO and RADDING 1971; HOLLOMAN 1973; KARU *et al.* 1973). Models for recombination may therefore be useful in helping to decide which DNA substrates should be synthesized for further testing of the properties of these enzymes.

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