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Recombination and Meiosis [and Discussion]

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Recombination and meiosis

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Although exchanges between sister chromatids are common in mitotic cells, those involving homologous chromosomes are rare. Since recombination between homologues is one of the functions of meiosis, it follows that one aspect of the differentiation of the meiocyte involves the synthesis of proteins or enzymes which facilitate synapsis and exchange. Mutants are known which seem to have constitutive levels of mitotic recombination between homologues, and these may be defective in the mechanism which normally represses mitotic recombination. It has been proposed that one component of the synaptonemal complex (s.c.) is a filamentous pairing protein with DNA binding sites which are base sequence specific. Synapsis occurs because the distribution of these sequences is the same in homologues. When only non-homologous chromsomes are present, as in haploid meiosis, only weak pairing can occur, since the base sequences are largely out of register. Although certain features of recombination at the molecular level are known, none of the models so far proposed suggest an explanation for interference between crossovers. It is suggested that interference may depend on the presence of a limited amount of another DNA binding protein which is specifically located within the s.c. A crossover between naked DNA molecules is initially a weak structure, which must be later converted into a visible and mechanically strong chiasma. It is assumed that this stabilization of a crossover is achieved by the DNA binding protein, which can diffuse freely within the s.c. and bind cooperatively to any recombinant DNA molecules within it. Depletion of the binding protein within the vicinity of a crossover makes it unlikely that the second crossover can be formed nearby.

Introduction

From a combination of genetical, biochemical and biophysical studies, substantial progress has been made in our understanding of the molecular mechanism of recombination and the associated process of repair. Nevertheless, three aspects of recombination in meiosis remain completely obscure: first, the specificity of pairing of homologues; second, the control of the initiation of genetic recombination, and third, the factors governing the interference between crossovers along the length of bivalents. In addition, little is known about the mechanisms which regulate the levels of recombination in meiosis and mitosis. In this speculative review, an attempt will be made to relate certain aspects of these problems to what is known about the mechanism and frequency of reciprocal and non-reciprocal recombination in meiosis and mitosis. The identity of crossovers and chiasmata will be assumed throughout.

The regulation of recombination in mitosis and meiosis

In eukaryotic organisms genetic recombination is largely or entirely restricted to one cell in the life cycle, the meiocyte. Mitotic crossing over between homologous chromosomes was first discovered in both sexes of *Drosophila* by Stern (1936). Although it was not possible to estimate its frequency with accuracy, it was clearly a very rare event in comparison to the frequency of crossing over in female meiocytes. Occasional mitotic crossing over has

also been recorded in higher plants (e.g. by Vig 1973); it may also occur in mammals, although the evidence assembled by Grüneberg (1966) is not compelling, since almost all the segregation events he cites could also be explained by mutation. More definitive experiments are needed to test for the occurrence of mitotic recombination in mammalian systems. The best quantitative data come from studies with fungi, since in several species it is possible to synthesize heterozygous or heteroallelic vegetative diploids (Pontecorvo 1958) and measure accurately the frequency of recombination between identical markers in mitosis and meiosis. These studies show that the frequency of recombination in meiosis is 10^2–10^4 times higher than in mitosis. Examples for allelic recombination are given in table 1. At the *nar*-1 locus of *Ustilago maydis* the meiotic frequency is about 600 times greater, and at the *inos*-1 locus about 6000 times greater than the mitotic frequencies.

TABLE 1. THE FREQUENCY OF ALLELIC RECOMBINATION AT THE *nar*-1 AND *inos*-1 LOCI DURING MITOSIS AND MEIOSIS IN WILD-TYPE (I.E. *rec*$^+$) AND *rec*-1 STRAINS OF *USTILAGO MAYDIS*

(Mitotic recombination frequencies are based on fluctuation tests, meiotic from the analysis of the random products of meiosis. The difference in meiotic recombination between normal and *rec*-1 crosses is not significant, although *rec*-1 does have other effects on meiosis, such as the disjunction of chromosomes (for full details of methods and results, see Holliday *et al.* (1976)).)

	wild-type		*rec*-1	
heteroalleles	mitosis	meiosis	mitosis	meiosis
nar 1–1/*nar* 1–6	1.7×10^{-6}	1.1×10^{-3}	9.5×10^{-6}	2.1×10^{-3}
inos 1–4/*inos* 1–5	2.9×10^{-7}	1.9×10^{-3}	4.9×10^{-6}	1.9×10^{-3}

These and many other observations must mean that the full machinery for recombination is not present in mitotic cells. We can therefore draw the obvious, although rarely stated conclusion, that at least one of the essential proteins or enzymes is repressed in mitotic cells and induced or derepressed during the differentiation of the meiocyte. Nevertheless, it has recently become apparent that one type of recombination does frequently occur in mitotic cells. This is reciprocal sister strand exchange. Giemsa or Hoescht 33258 staining of chromatids fully or half substituted with bromodeoxyuridine (BUdR) distinguishes the chromatids and exchanges are then easily scored (e.g. Perry & Wolff 1974; Latt 1974). Several exchanges per metaphase are seen in human lymphocytes or other cultured cells. It must be concluded that the enzymes necessary for crossing over between chromatids are indeed present in mitotic cells. Since the rare event is recombination between homologues, it seems likely that it is the mechanism for synapsis which is absent and which is therefore specifically induced in meiosis.

Indirect supporting evidence for the genetic regulation of recombination in mitosis and meiosis comes from two sources. First, it is known that treatment of mitotic cells with mutagens or recombinagens greatly increases recombination between homologues (e.g. Holliday 1964*a*; Zimmermann 1971), as well as between sister chromatids (Perry & Evans 1975). In the case of allelic recombination within a gene, the mitotic frequency can approach that seen in meiosis (Esposito 1968; Holliday, Halliwell, Evans & Rowell 1976). The supposition that this effect is due to the derepression of one or more proteins or enzymes essential for recombination mediated repair is supported by experimental evidence from *U. maydis* (Holliday 1971, 1975; Moore 1975). This induced recombination could, of course, facilitate exchanges between sister chromatids as well as between homologues. Second, mutant strains exist which have high levels of mitotic recombination. Such mutants may be defective in regulation, having a constitutive

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level of a protein essential for recombination between homologues. A striking example is known in *Aspergillus nidulans*. Parag & Parag (1975) isolated a mutant, *pop*, in a haploid strain containing one duplicated segment, with which it was possible to measure allelic recombination at the *ad*E locus. This mutant had a spontaneous frequency of mitotic recombination similar to the meiotic level. The mutant *rec*-1 of *U. maydis* may also be defective in regulation. In diploids homozygous for *rec*-1 the frequency of spontaneous mitotic recombination is increased about 6-fold at the *nar*-1 locus and about 17-fold at the *inos*-1 locus (table 1; for a full discussion see Holliday *et al.* 1976). Another possible example of a regulatory mutant is that causing Bloom's syndrome in man. Mitotic cells have a high frequency of chromosome abnormalities including many quadriradials, which almost certainly result from reciprocal exchange between homologues (German 1964). There is also a greatly increased frequency of sister strand crossing over (Chaganti, Schonberg & German 1974).

Unfortunately, it is not yet certain that any of these mutants are indeed defective in regulation, since it is also possible that in each case they are blocked in the repair pathway which does not require recombination. Spontaneous genetic damage which is normally removed by this pathway may therefore be diverted into one which is recombination mediated, hence the observed increase in mitotic cells. Nevertheless, it is clear that in special circumstances pairing and recombination between homologues can occur with very high frequency in mitotic cells, and the question therefore arises whether synaptonemal complexes (s.c.) or similar structures appear in such cells. The statement which is frequently made that the s.c. is specific for meiosis has not been based on studies of mitotic cells which are recombining at high frequency.

The problem of specific pairing

I suggested some years ago that a specific fibrillar DNA binding protein may be involved in the pairing of homologues (Holliday 1968). Subsequently the same proposal was made by Comings & Riggs (1971). Following the early work of Levan (1939), it has now been clearly demonstrated that colchicine treatment can prevent synapsis between homologues, which indicates that microtubules or similar structures are involved in this process (Driscoll & Darvey 1970; Dover & Riley 1973; Shepard, Boothroyd & Stern 1974). In its simplest form the hypothesis states that the specificity of pairing depends on a short sequence of bases and a single protein which binds specifically to this sequence. The sequence could be within repetitive DNA or unique DNA, or both. The essential point is that its distribution along the length of the chromosome would be identical in homologues and the sites would therefore be in register, whereas their distribution would be different in non-homologues. The interaction between homologues might be mediated by the protein over considerable distances, for instance in the colchicine sensitive pre-alignment phase of pairing in *Triticum* (Driscoll & Darvey 1970; Dover & Riley 1973). Alternatively, it might operate when the lateral elements of the homologues are within about 300 nm at zygotene. It is at this distance that the homologues are pulled together to complete the formation of the s.c. (Moens 1973). In *Lilium* the colchicine-sensitive stage is just prior to the formation of the s.c. (Shepard *et al.* 1974).

One possibility is that the filamentous or fibrillar protein has binding sites at each end and that when these are attached to DNA, condensation of the protein follows. Another, illustrated in figure 1, is that the protein has a single binding site and that molecules interdigitate or aggregate laterally, perhaps within the central element of the s.c. The longitudinal axes of the

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molecules might then be visible as transverse filaments. These two possibilities are applicable either to the pre-alignment phase of pairing or the later period of close synapsis.

The model predicts that in the absence of homologues, non-homologues will pair, since some binding sites would by chance be opposite each other, but this association would be weak. It accounts satisfactorily for the random distribution of s.c. material and regions of imperfect pairing seen at pachytene of meiosis in haploid barley (Gillies 1974). On the other hand, since it is most compatible with a zipper type mechanism for extending the regions of pairing, the model does not fully account for the multiple initiation sites which must occur in the formation of trivalents or quadrivalents and which have been seen, for instance, at zygotene during the pairing of bivalents in maize (Gillies 1975). Nor does it help solve the problem of the lack of interlocking of bivalents. Testing the hypothesis would require a search for a specific meiotic protein, which binds to a specific sequence of DNA and which may also bind colchicine.

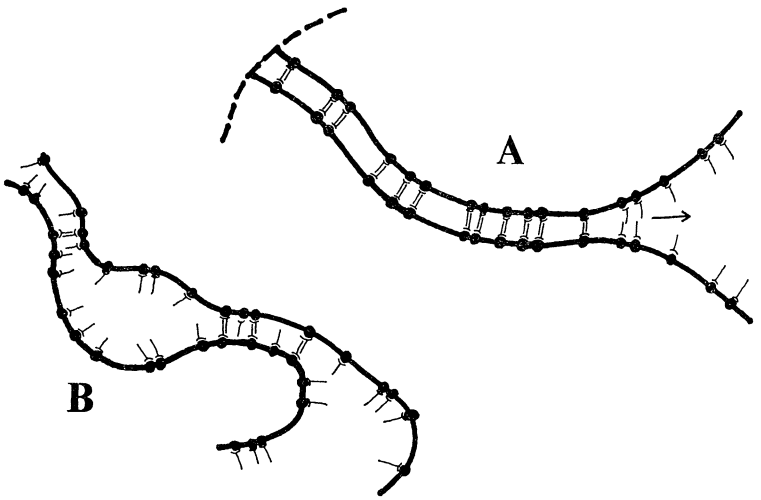

FIGURE 1. (A) Diagrammatic representation of the pairing of homologous chromosomes at zygotene, mediated by a DNA binding pairing protein. A filamentous or fibrillar protein attaches at one end to a specific base sequence which has identical distribution along the length of homologues. The protein molecules aggregate laterally, bringing the paired homologues to within a distance of about 100 nm, i.e. the width of the s.c. The particular distribution of protein–DNA binding sites produces two matching sets of cohesive protein filaments. From initial points of cohesion, pairing could spread by a zipper-like mechanism in either direction. (B) Non-homologues have as many protein–DNA binding sites, but they are not in register. Therefore the opportunities for cohesion between the filamentous proteins are much fewer. This results in weak pairing, with some extended regions of parallel alignment of chromosomes, and others where pairing fails and loops are formed, as has been seen in serial reconstruction of pachytene nuclei in haploid meiosis of barley (Gillies 1974).

THE INITIATION OF RECOMBINATION

The formation of the s.c. leaves the DNA of the duplicated homologous chromosomes separated by about 100 nm. Moreover, since the chromatin is relatively condensed, at any one point there may be several hundred strand equivalents of DNA (Westergaard & von Wettstein 1972). It is therefore extremely hard to visualize how recombination is initiated at homologous sites. It could be argued that recombination is a relatively infrequent event, with a range of 1–12 chiasmata in bivalents in different species, simply because it is hard for homologous sequences to find each other. But if this was so, then organisms with a low DNA content should have higher recombination frequencies than those with high DNA content, and this is certainly

not the case. It seems more likely that there is a specific mechanism governing the initiation of recombination.

It has been known for many years from studies of recombination at the fine structure level that there is marked polarity both in the frequency of gene conversion in different parts of the gene and in the pattern of outside marker exchange (see, for instance, Whitehouse & Hastings 1965). This evidence strongly suggests that there are defined initiation sites for recombination. Recently, a class of recombination mutants has been discovered in bacteriophage λ by Stahl and his associates which greatly increase the level of recombination in their vicinity (Stahl, Crasemann & Stahl 1975). These Chi mutants may provide base sequence specific signals for recombination enzymes. Similar mutants may exist in fungi (Gutz 1971; Catcheside 1974). The strongest evidence for a complex control system for meiotic recombination comes from extensive studies of Catcheside and his associates using naturally occurring *rec* mutants of *Neurospora* (for a recent review, see Catcheside 1974). These mutants do not affect recombination in their vicinity, but in particular regions of the genome elsewhere, each mutant having its own spectrum of effects. Since the recessive *rec* allele enhances recombination in each case, it is probable that the mutants are regulatory, and one possibility is that they control the synthesis of a series of specific endonucleases which, like restriction enzymes, may act on specific sequences, or 'recombinators', which are the initiation points for recombination (Holliday 1968). Indirect evidence for this comes from the identification of mutants such as *cog* on chromosome I, which in this case interacts with the *rec*-2 locus on chromosome IV. This interaction has striking effects on both recombination frequency and the polarity of recombination in the *cog* region of the chromosome (Catcheside 1974). The desynaptic mutant of *Hypochoeris*, which affects only one of the four bivalents (Parker 1975), also provides evidence for specific controls of recombination.

Sobell (1972) and Wagner & Radman (1975) have suggested that palindrome sequences (inverted duplications) are required for the initiation of recombination. Such palindromes can form base paired side arm or hair-pin loops projecting from the main axis of the DNA. For two such loops to meet in the centre of the s.c., the palindromic sequence would have to be at least 300 base pairs long (giving side arms of 150 base pairs or 50 nm). Recombinator sequences in these regions could provide a substrate for a specific endonuclease and provide the basis for the initiation of recombination. Alternatively, recombination may be initiated by a non-reciprocal event, as envisaged by Meselson & Radding (1975). A single strand detached from one chromatid would extend across the s.c. and become integrated into a recipient duplex. Recently, evidence has been obtained that single stranded pieces of DNA will form a hydrogen bonded complex with a supercoiled duplex, provided base homology is present (Holloman, Wiegand, Hoessli & Radding 1975).

Reciprocal and non-reciprocal recombination

It is well known that two types of genetic recombination can occur at the 4-strand stage of meiosis. The first is reciprocal crossing over, which is easily detected between widely spaced markers. The second is a form of non-reciprocal recombination in which a small fragment of genetic material is transferred from one homologous chromatid to the other, without the exchange of outside markers. This process can only be detected by fine structure analysis and/or analysis of tetrads or half tetrads. These two types of event occur with similar frequency in

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Drosophila (Carlson 1971), yeast (Hurst, Fogel & Mortimer 1972), *Neurospora* (see Fincham 1974), *Sordaria* (see Whitehouse 1974*b*) and *Aspergillus* (Pritchard 1955), but in different cases there may be a significant excess of non-reciprocal over reciprocal, or vice versa. The non-reciprocal event is detected as gene conversion (i.e. a 1:3 or 3:1 segregation for a particular site); the reciprocal one is associated with gene conversion in the close vicinity of the point of exchange. It is generally believed that an essential intermediate in both reciprocal and non-reciprocal recombination is hybrid or heteroduplex DNA, and that the correction of mismatched bases in this region gives rise to gene conversion (Holliday 1974; Whitehouse 1974*a*; Meselson & Radding 1975; Kushev 1974). Although the possibility of two or more molecular mechanisms for recombination cannot be ruled out, almost all the genetic data can be explained in terms of a unitary mechanism in which there is a given probability of generating exchange for outside markers.

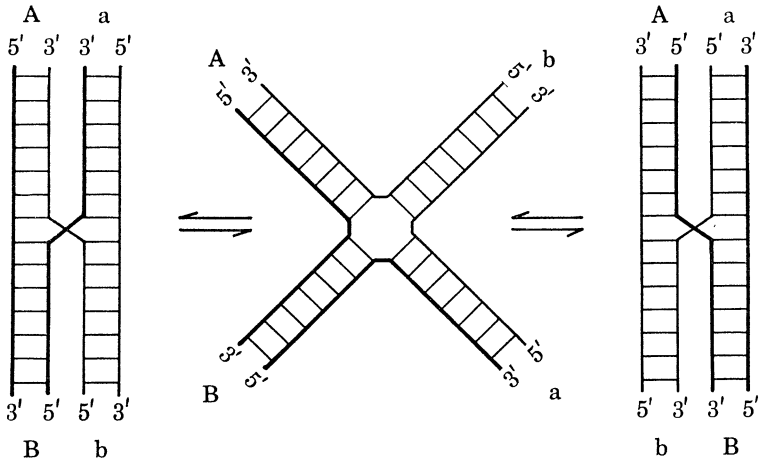

FIGURE 2. The reciprocal exchange of single DNA polynucleotide chains to form a half crossover. Heavy lines indicate strands from one parental chromatid, and light strands from the other; hybrid or heteroduplex DNA extends from the point of exchange on both chromatids. This exchange point may move by branch migration or rotary diffusion (see Meselson 1972). A/a and B/b represent outside markers where parental combinations are AB and ab. On the left, the strands which are on the inside of the structure are on the outside at the right; the change from one structure to the other is known as strand isomerization. For this to occur, two duplex arms must move through 180° to form the central configuration, where all strands are structurally equivalent, and then a further 180° rotation gives the alternative linear configuration. Breakage of inner strands of the structure on the left gives non-reciprocal exchange, whereas breakage of the inner strands of the structure on the right gives a crossover. Similarly, horizontal or vertical cuts of the opposite strands in the central structure will generate reciprocal or non-reciprocal exchanges. (For a full discussion, see Holliday 1964*b*, Sigal & Alberts 1972, Sobell 1974, Meselson & Radding 1975).

Whatever the mechanism of initiation of recombination, a likely intermediate is the 'half chiasma', in which polynucleotide chains of like polarity switch pairing partners at homologous points to give two reciprocal regions of hybrid DNA (figure 2). Such a structure has recently been visualized in recombining DNA molecules of bacteriophage λ (Valenzuela & Inman 1975). It has been shown by accurate molecular model building that the switching of strands does not disturb the hydrogen bonding between complementary bases and base stacking is preserved (Sigal & Alberts 1972). Two important properties are associated with this structure. First, the exchange point can diffuse in one or other direction by branch migration (Meselson 1972). Second, the strands which 'crossover' in the half chiasma, i.e. those on the inside, can

change partner with those that are on the outside through the process known as strand isomerization. Sobell (1974) pointed out that this isomerization requires a 360° strand rotation, but it is not yet clear whether this would be expected to occur rarely or frequently, or whether an intermediate molecular configuration where all four strands are structurally equivalent would be the most stable one (see figure 2).

Since these events are presumably occurring within, or possibly at the side of the s.c., the ends of the DNA molecules which are recombining are not free to change their anchorage points. We thus have three possible configurations, which are shown in figure 3. On the left, breakage of the inner strands would yield a non-reciprocal event and the two loops of DNA are free to move back to the sides of the s.c. In the centre, where strands have rotated through 180°, a horizontal cut of two strands gives the same result, but a vertical cut gives a crossover. On the right, breakage of the inner strands yields a crossover. In this configuration there must be some slack in the DNA to allow the necessary 360° rotation. The problem of the distribution of these reciprocal and non-reciprocal events along the length of the bivalents will be considered in the next section.

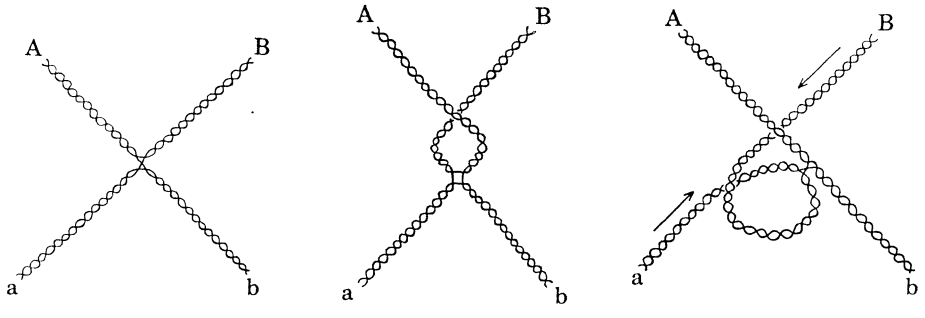

FIGURE 3. Representation of the structures in figure 2 showing the twisting of DNA duplices which must occur during strand isomerization. The ends of the molecules are anchored in the chromatin outside the s.c., and A/a and B/b represent outside markers. Cutting the inner strands of the structure on the left gives a non-reciprocal exchange, as does a horizontal cut of the strands in the central structure. Cutting the inner strands of the structure on the right gives a crossover, as does a vertical cut of the strands in the central structure.

INTERFERENCE BETWEEN CROSSOVERS

None of the molecular models for genetic recombination have provided an explanation of the widespread phenomenon of positive chiasma or chromosome interference. Early studies with three or more linked markers in *Drosophila* showed that the probability of double crossovers in adjacent intervals was much less than expected from the product of the frequencies of single exchanges. The coincidence of double exchanges, i.e. the ratio of the observed to the expected doubles, was almost zero over short intervals, increasing to 1.0 over longer ones (see, for instance, the results of Stevens 1936). With the possible exception of *Aspergillus nidulans* (Strickland 1958), interference has been seen in all organisms where appropriate genetic studies have been done. At the same time it is well known that the distribution of chiasmata in bivalents is not random. From the average chiasma frequency per bivalent, it can be easily shown that they do not follow a Poisson distribution; there is a much lower frequency of non-exchange bivalents (or univalents) or multiple exchanges than expected (Haldane 1931). The same is true when crossovers are scored genetically (e.g. Perkins 1962). In other words, some mechanism ensures that there is a high probability of one or a small number of chiasmata per bivalent. This is important

for the normal disjunction of homologues at metaphase I, although special mechanisms may exist for ensuring disjunction in the absence of crossing over (e.g. in males of *Drosophila* or females of *Bombyx*, see Rasmussen, this symposium). Crossover events in one bivalent are independent of those in another, i.e. there is no positive or negative interchromosomal interference, although this may not always be true for chiasmata (Mather 1936).

Much more recently it has become apparent that non-reciprocal recombination does not follow the same rules. Stadler (1959) showed that there was no interference between non-reciprocal gene conversion and crossing over in *Neurospora*, and the same effect has been well documented in *Drosophila* (Carlson 1971) and yeast (Mortimer & Fogel 1974). The data for yeast, which is based on extensive tetrad analysis, makes it clear that double non-reciprocal events occur on a random basis, and these do not interfere with crossovers or vice versa. On the other hand, double crossovers show classical interference. It is also apparent that interference is unrelated to the DNA content of chromosomes. For instance, yeast and maize have widely different genome sizes, yet similar interference. This makes it extremely unlikely that the phenomenon could be related to the simple mechanical stresses which might be generated for the crossover configuration shown in figure 3.

Some other features of interference deserve mention, although they are perhaps less fundamental. (1) When double exchanges do occur, they are distributed among the four chromatids almost at random. The frequency of two, three and four strand doubles is close to the expected 1:2:1 ratio, with a very slight excess of two strand doubles (Perkins 1962; Mortimer & Fogel 1974). (2) Interference does not extend across the centromere (Stevens 1936; Bole-Gowda, Perkins & Strickland 1962; and see Owen 1950). (3) There is no interference between sister strand exchanges at mitosis (Wolff & Perry 1974). (4) 'Negative interference' over short intervals (Pritchard 1955) is not due to multiple crossover events. It can be satisfactorily explained on the basis of single crossovers with associated conversion due to the correction of mismatched base pairs in hybrid DNA (Holliday 1968).

The idea that the phenomenon of interference might be related to a limiting amount of a particular substance or enzyme necessary for recombination is not a new one (see White 1973). Here I extend this hypothesis and make it more specific. A crossover between naked DNA molecules is initially a mechanically weak structure, which must in some way be converted into a visible chiasma, a mechanically strong one, since it holds together the diplotene chromosomes and resists the forces of repulsion which exist at this stage. I propose that this stabilization is achieved by a DNA binding protein which is not specific for base sequence, which binds co-operatively to DNA crossovers within the s.c., converting them into chiasmata, perhaps by a process involving condensation. (DNA binding proteins which are not sequence specific and which bind cooperatively to DNA are well known in microorganisms (see Alberts & Frey 1970; Banks & Spanos 1975).) The aggregation of protein to DNA could form the 'recombination nodules' reported by Carpenter (1975). The protein would be synthesised at or before zygotene in limited amount, and becomes part of the s.c. at pachytene. The completed s.c. is presumed to be a closed organelle, so once the protein is within it it cannot diffuse out; nor can it interact with the chromatin outside the s.c. It can, however, freely diffuse along the length of the s.c., possibly, but not necessarily, in the central element. However, the centromere, or a region of centromeric heterochromatin, may provide a barrier to free diffusion.

The consequence of this situation will be as follows. The initial events in recombination lead to an intermediate which can either become a non-reciprocal or a reciprocal exchange. The

DNA binding protein stabilizes crossovers, and it could even have a role in the process of strand isomerization. Since it binds cooperatively to the arms of the DNA extending from the exchange point, the pool of protein is depleted in their vicinity, making it unlikely that there can be stabilization of any other crossover nearby. On the other hand, non-reciprocal events, which do not require isomerization, either do not interact with the protein at all or are so transient that the DNA is rapidly withdrawn into the chromatids on the outside of the s.c. and no longer provides binding sites. This general hypothesis seems to be capable of accounting for the main features of interference. The synthesis of a defined amount of the protein provides a general mechanism for controlling the number of chiasmata, as there will be a high probability of at least one exchange per arm, or per bivalent, and a low probability of multiple exchanges. Organisms such as *Aspergillus*, which have no interference, would have an unusually high level of the protein. There would be no interference between crossovers and non-reciprocal events or between non-reciprocal events. It may also throw light on a paradox which is implicit in the data of Mortimer & Fogel (1974), but which has not been explicitly stated. When all recombination events within the *arg* 4 locus are examined, half are recombinant for outside markers (268 out of 549, see Hurst *et al.* 1972). This has been used as an argument in favour of the strand equivalence in the half chiasma structure shown in figure 2, since there is an equal probability of generating reciprocal and non-reciprocal events (Holliday 1974; Meselson & Radding 1975). However, since only crossovers interfere with each other, a second event close to the *arg* 4 locus is much more likely to be a non-reciprocal exchange than a crossover. Thus, in this region the ratio of reciprocal to non-reciprocal events must be less than 0.5. In general, therefore, if the interference data are correct, there cannot be exact equality of the two types of recombination, as indeed other data from yeast indicate (see Table 4 in Hurst *et al.* 1972).

Finally, this model for interference may help provide an explanation for one puzzling feature of the distribution of crossovers in the genome as a whole. It has been known for many years that structural heterozygosity in one bivalent leads to a significant increase in the number of crossovers elsewhere in the genome. In a recent review by Lucchesi & Suzuki (1968), which is largely concerned with extensive data from *Drosophila*, the authors conclude that any region of non-homology in one bivalent (or two, in a translocation heterozygote) will have this effect. Non-homology will result in the formation of an imperfect s.c. which is not a closed organelle; thus, the normal allocation of DNA binding protein will not be trapped within it. Rather, the molecules will be free to diffuse elsewhere at zygotene, and the upshot will be an increase in the concentration of the protein in other normal bivalents at pachytene and a corresponding increase in the probability of crossing over.

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Discussion

K. R. Lewis (*Department of Botany, University of Oxford*). Dr Holliday claimed that interference does not operate between bivalents in structural homozygotes. If I remember correctly, Mather found that where chiasma frequency was low, as in *Drosophila*, and, therefore, competition undesirable, it did not obtain : where supplies were limited, rationing was preferred. But where the chiasma frequencies were high and competition less likely to reduce a potential pair to a state of univalence, there was evidence of compensatory relationships. Is this the rule that proves the exception ?

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M. Hultén (*Regional Cytogenetics Laboratory, East Birmingham Hospital, Birmingham*). There are some aspects of your model I find very attractive. Thus, you try to explain crossover/chiasma interference, which so many people seem to have totally ignored, and further your model is appealing on account of its beautiful simplicity. However, when considering it in relation to the various genotypical and environmentally induced variations in crossing over and chiasma frequencies as well as distributions, I wonder if the story must not be more complex. Let me just ask you about the one example you mentioned, the interchromosomal effect on crossing over in a structural heterozygote. You say that an increased amount of a pairing enzyme would be available for other chromosomes since less is trapped in the heterozygote pairing configuration due to pairing difficulties. This would explain the increased crossover frequency often found on chromosomes not engaged in the interchange. But, how would you explain other types of interchromosomal effects, like a reduction in crossover frequency? I am not claiming the Schultz–Redfield hypothesis (1951) is wrong or you are wrong, I just react against your over-simplification.

R. Holliday. Nevertheless, the general rule in *Drosophila* and other organisms seems to be that the presence of structural heterozygosity *increases* recombination frequency in normal bivalents. However, there may well be some exceptions.

M. Hultén. Maybe Professor Lindsley would like to comment on this. However, I am positive all kinds of changes on different chromosomes have been reported, at least in *Drosophila* where this particular matter has been most thoroughly investigated (Steinberg & Fraser 1944; Ramel 1962, 1965; Suzuki 1962, 1963; Williamson 1966; Baldwin & Chovnick 1967; Valentin 1972). Most authors seem to find it impossible to explain all interchromosomal effects by any one mechanism, like pairing difficulties.

Lindsley. I agree.

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