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Dynamic Unwinding of DNA Helices: A Mechanism for Genetic Recombination

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A model of recombination is discussed in which the process is initiated by the unwinding of the DNA helix rather than by breakage of one or both of the component strands of the helix. It is argued that initial breakage is unattractive as the causal event in recombination from the evolutionary point of view and that it creates difficulties in interpretation even when considered against the background of the present day cellular environment. In contrast it is shown that initial unwinding is capable of producing a configuration which specifies precisely the positions at which breaks must occur and which is thereby able to generate recombination events in any situation in any organism. Considerable support, both theoretical and experimental, exists for the concept of helix unwinding, and particularly for the idea that DNA helices exist in solution in a dynamic state in which short regions are continually being unwound and rewound. The model of recombination developed from these arguments accounts for the generally observed features of the process at both intragenic and intergenic levels, and in addition provides explanations for more subtle phenomena like polarization, allele-specific marker effect and positive interference. The basic assumptions of the model are shown to be capable of forming the basis of explanations of inter-chromosomal effects like synapsis and the production of chromosomal aberrations. The arguments so developed, again having a firm foundation in the known biophysical characteristics of DNA, suggest an unusual reason for chromosomal organization and structure. Possible experimental tests of the concepts on which the various suggestions are based are indicated.

1. Introduction

In the past decade, a number of models which seek to explain the process of genetic recombination have been proposed. For the most part, such models have originated with a desire to formalize an explanation for a relatively small number of particular effects. Thus the copy-choice model (Lederberg, 1955) was proposed essentially to provide an explanation for non-reciprocal genetic exchange in bacteriophage; the hybrid-DNA models (Whitehouse,

1963, 1966; Whitehouse & Hastings, 1965; Holliday, 1964, 1968) to explain meiotic gene conversion in fungi and to suggest how non-reciprocality could be reconciled with a breakage-reunion process; the "sex-circle" model of Stahl (1969) to account for discrepancies in reciprocality and the inability to demonstrate repair of mismatching (a fundamental feature of hybrid-DNA models) in bacteriophage recombination; the "single strand copy-choice" model of Paszewski (1970) to avoid the formation of hybrid DNA and emphasize the asymmetry of gene conversion; the "molecular model" of Hotchkiss (1971) to stress the restrictions imposed by molecular and enzymic processes; and the "initial unwinding" model of Moore (1972) to avoid difficulties posed by initial strand breakage. Just as models of recombination put forward in the 1930's and 1940's were based solely on intergenic events, so the models of today are based mainly on intragenic events.

Despite their limited origins, though, these models attempt to explain a process which must be considered a fundamental genetic phenomenon; a phenomenon which has wide-reaching effects at both the intra- and intergenic levels. For example, any model, if it is to be considered adequate, must be capable of explaining positive interference as effectively as negative interference. Yet most of the current models, while successfully explaining the latter (it being one of the "design criteria") find difficulty in accounting for the former. There are other similar examples and this paper is an attempt to reconsider the case for just one of the current models of recombination—the initial unwinding model—and to see how well it fits into this wider theoretical framework.

2. The Universality of Recombination

Recombination of genetic material must be a general process since it is known to occur in almost as many organisms as are known to possess genetic material. There are very few exceptions to this rule; *Drosophila* males and the RNA bacteriophage $f2$ may be cited as well known examples of organisms in which general recombination seems to be absent. However, such exceptions as these do not seriously affect the above generalization for on the one hand it is clear that in *Drosophila* the absence of recombination is an imposed restriction on an otherwise normal genetic system whereas in $f2$ phage the entire genetic system is most unusual. Moreover, in the case of $f2$, only very low recombination frequencies can be expected in a genome consisting of just three genes, and bearing in mind the high mutation rate of the RNA genome it is entirely possible that there is a technical restriction on the recognition of recombination rather than a fundamental restriction on the occurrence of the process. Thus, recombination does seem to be a general process and it

appears equally reasonable to suggest that in addition it is also a fundamental process.

In its strongest form this suggestion implies that the possession of organized genetic information carries with it a requirement for the reorganization (recombination) of that information. In the evolutionary sense this would mean that as soon as individual segments of genetic information became associated together into single structures (primitive chromonemes) there would have been selective advantage in a process which facilitated the rearrangement of the genetic segments within those primitive structural associations. This interpretation leads to the view that recombination as a process is almost as primitive as the polynucleotide-based genetic information itself, and this has a corollary which is important to considerations of the mechanism of recombination. For, if the process did indeed originate at such an early stage in the evolution of living systems, then like the genetic code and its relationship to the metabolic machinery, its basic features are likely to be universal. This is not to say, of course, that modifications across the spectrum of living organisms are unlikely, indeed a reasonable supposition would be that the process would become sophisticated in parallel with increasing complexity of cellular structure; but rather that differences between organisms of different grades of organization *are* likely to be sophistications and not fundamental alterations to the process. This view necessarily requires that mechanisms which are considered as basic parts of any model of recombination must be reasonable components of a very primitive cellular (or even pre-cellular) system. It means essentially that recombination must be provided for in a cellular environment which initially completely lacks any organization which is specifically designed to facilitate this process. Such organization could only have arisen after the selective advantage of the process had been demonstrated. Any model of recombination must, in consequence, be founded upon mechanisms which are feasible in such a primitive environment and this seems to require that, in essence, such foundation mechanisms be the result of errors in the normal functioning of the primitive cell.

Recombination is a process which consists of a train of molecular events whose end-product is a rearrangement of the genotype. Regardless of the "substrate" on which the process works this must be the case now and must always have been the case since however the stages are arranged and whatever their finer points there is a basic need for some such sequence as: break genetic material—rearrange genetic material—rejoin genetic material. For recombination, as for any train of events like this, it is the initial event which is the most important. Subsequent events may well be responsible for the actual production of recombinant genotypes but they, by definition, cannot proceed unless the initial event has taken place. It is at the initial stage, therefore, that

the causality of the process lies and it is this stage which must be most carefully scrutinized.

3. Initial Strand Breakage?

There is general agreement that recombination of DNA molecules involves separation of the two strands of a double helix, events subsequent to this separation and the mechanism which promotes it provide the major differences between different models. Strand breakage is usually suggested as providing the origin of this strand separation, but the break itself cannot be the initial stage for it must be caused by some previous event. Provision must therefore be made for the creation of strand breaks if these are considered a major component of recombination. Various mechanisms have been suggested in recent models, including fixed opening points at linkers between genes (Whitehouse, 1963, 1966; Holliday, 1964), symmetrically arranged polynucleotide base sequences which interact with specific structural proteins (Sobell, 1972), specific recombinase enzymes and their corresponding "substrate" sequences scattered along the DNA molecule (Holliday, 1968), and gaps resulting from regionalized delay in replication during early meiosis (Hastings, 1972). The difficulty with these suggestions is that they all imply an advanced grade of organization either of structure or of metabolism or both. They cannot, therefore, be envisaged as a reasonable component of a primitive system. Even as candidates for the present day stage of organization suggestions of this type are argumentative. It is difficult to reconcile the molecular precision and regularity envisaged for these processes with the apparently contradictory situation that, in eukaryotes at least, recombination events are extremely rare in relation to the large amount of substrate (in the form of DNA helix) which exists in the cell. It has been known for many years that chiasma frequencies can be fairly directly related to genetic recombination frequencies at least in a general sense (e.g. Darlington, 1934; Rhoades, 1950) and that a physical exchange of chromatid segments is demonstrable (Taylor, 1958, 1965). But even if chiasmata are visible representations of only a small proportion of successful recombinations it is still a significant fact that despite the large amount of DNA contained in chromosomes even the largest of them rarely contain more than four or five chiasmata. The observed infrequency of chiasmata relative to the total amount of DNA available for recombination suggests that if recombination has as its causal event any form of enzymic process then that process must have a far lower efficiency than is usually expected of enzyme-mediated reactions. It is possible to envisage such a system existing today, but it is not easy to see how it could have originated at a primitive grade of organization without conflicting with other structural

and functional features of the evolving system. For this reason one is inclined to discard the specific enzymic initiation of recombination and consider instead some sort of situation in which the necessary causal configuration for recombination is regularly established along the length of the DNA molecule but only infrequently made use of.

If DNA is synthesized discontinuously then a considerable number of strand breaks must be produced as a by-product of DNA replication. Current evidence, though, indicates that these breaks are closed very quickly in prokaryotes as well as in eukaryotes (Okazaki *et al.*, 1968; Schandl & Taylor, 1969), and that the rapid closure of such breaks is an integral part of the replicative machinery. One might suppose, however, that the closure of breaks produced during replication might not have been so certain nor so rapid at a primitive level of organization, so that breaks formed in this way could have persisted for a sufficient time for them to be utilized to initiate recombination events. There are two points which argue against this possibility. First, if recombination is to depend on replication for the provision of its initiating event then it might be expected that the two processes would evolve in concert, becoming more intimately linked as the whole system increased in complexity. Yet the reverse appears to have taken place. Only in viral infections does recombination occur contemporaneously with replication; in other prokaryotes it probably takes place independently of replication and in eukaryotes recombination is certainly separated from replication in time and in some cases (like the Ascomycete fungi) in space as well. More fundamentally, though, breaks in genetic material threaten the integrity of that material and conflict with the requirements of its basic function—the reliable archival storage of genetic information. One would presume that at such an early stage of development the protection of the integrity of the genetic message would be a matter of prime importance. There would consequently be a greater selective advantage in the improvement of mechanisms for the repair of strand breaks than in the development of secondary mechanisms which make use of them. Moreover, once an effective "supervisory and repair" system has evolved it is difficult to see how spontaneous breaks could occur. Particularly since they must always somehow occur at least in related pairs, one in each of two completely independent molecules.

The preoccupation with initial strand breakage evident in current theorizing about recombination is understandable on two counts. First, enzymic processes concerned with polynucleotide synthesis and management feature prominently in research at this time. Second, it is self-evident that polynucleotide strands must be broken at some stage during recombination. However, the arguments outlined above indicate that strand breakage, as the causal event in recombination, is just as incompatible with the highly evolved cell as it is with a

primitive-cellular system. Leaving aside completely discussion of the plausibility of the mechanisms which are proposed to account for initial strand breakage, current arguments encounter logical difficulties which argue against them. This aspect can be illustrated by reference to just two, diametrically different, types of approach. At one extreme there are models of recombination which commence with a gap or break in a DNA molecule but which neither offer, nor accept the need to offer, an explanation of the way in which that break arose. Such models present only half the story and dismiss from consideration the most important step in the sequence—the initiating step. Faith in accounts of this nature is not encouraged by such assurances as "The number of arbitrary strand breakages we have assumed is fewer than in almost any other model for recombination" (Hotchkiss, 1971). It is illogical to accept an arbitrary foundation for a construction which attempts to portray a fundamental cellular phenomenon. At the other extreme there are models in which the initial severance of the DNA strand is achieved with great ingenuity. Probably the most elegant of this class is the model described by Sobell (1972) in which specific structural proteins recognize specifically differentiated segments of DNA and organize a structure that is susceptible to nuclease attack. The breakage is thereby accounted for but the very high level of organization envisaged creates its own difficulties. It is argued in the present discussion that recombination is a rare event. This basic feature requires the imposition of restrictions to the operation of models which, like Sobell's, postulate sophisticated enzymic and structural organization. One may suggest recombinase enzymes *but* they cannot be very efficient; or structural proteins *but* there cannot be too many of them; or differentiated nucleotide sequences *but* only a few can be open to recombine at any one time. What, then, determines the efficiency of the enzymes, the number of structural proteins, or the availability of recombinator sequences? These models fail strictly in terms of logic. They explain recombination too well and have to be made more complex in order to make them less efficient.

It is necessary to consider a mechanism which, by making use of the native properties of the molecules concerned, will provide a configuration which could cause strand breakage, spontaneously or enzymically, in precisely related positions in two separate molecules. Initial unwinding of duplexed polynucleotide chains is just such a mechanism.

4. Initial Unwinding of DNA Helices

The thermodynamic feasibility of large scale unwinding has been demonstrated on a number of occasions (e.g. Levinthal & Crane, 1956; Fong, 1964). For recombination it is not necessary to consider unwinding of extensive

regions. Since in its native form the DNA double helix usually has 10 base pairs/turn, the unwinding of just 10 turns could free a length equivalent to around 10% of a gene specifying a polypeptide of over 300 amino acid residues. It is necessary to emphasize this point to make it clear that throughout this discussion the unwinding events envisaged involve segments which are extremely short in relation to the average length of the molecules involved, and short even in relation to average gene length. The basis of the mechanisms must be essentially similar to the earliest steps of DNA denaturation. In particular it is considered that the recombination process makes use of that dynamic equilibrium which consists of alternate unwinding and rewinding of short regions of the double helix which has been called "breathing" (Printz & von Hippel, 1965). As this is an equilibrium which is actively dynamic under physiological conditions and temperatures (von Hippel & Printz, 1965) the DNA contained in a cell must be seen as a molecule which is in a continuous state of flux rather than as a completely stable molecule which exists perpetually in the form of a rigidly maintained double helix. Under these normal conditions, of course, the equilibrium favours the double helix but at any one point in time there must be a large number of short unwound segments and the distribution of these segments through the length of the DNA molecule must change from one point in time to the next.

This dynamic unwinding equilibrium is a feature which depends very much on the nature of the molecular structure of the polynucleotide. This being so it must be a feature not only of all DNA molecules in solution at the present time, but also of all DNA molecules which have been in solution throughout evolutionary time. As is explained below, dynamic unwinding is capable of producing error configurations which have recombination potential; and since it is an inherent property of the DNA helix it represents a plausible evolutionary base for the whole process of recombination. Moreover it is a feature which can provide the basis for explanations of a number of different phenomena and the arguments which can be developed are also outlined below. It is important to note, however, that since these arguments have as their foundation an effect which results solely from the nature of the molecules concerned they are the simplest possible arguments. Because of this essential simplicity these arguments must be discounted before any more complex mechanism can be sensibly considered.

5. Initial Unwinding as a Prelude to Recombination

That initial unwinding of the DNA helix could provide a configuration capable of being resolved as a recombination event was originally proposed by Cross & Lieb (1967) to explain prophage detachment in lysogenic bacteria.

More recently it has been shown that the mechanism can provide a configuration which can also explain many features of intra- and inter-genic recombination in eukaryotes (Moore, 1972). The essential basis for the model is the assumption that continuous rounds of unwinding and rewinding of short (short in proportion to average gene length) segments of DNA helices take place spontaneously. It is considered that the unwinding events occur at random with respect to both time and spatial position, although as will be pointed out below, it is probable that the randomness is of the qualified form indicated by Hotchkiss (1971). An important point, however, is that the unwinding takes place in a completely intact molecule. No covalent bonds need be severed to promote unwinding and it is equally likely to occur interstitially at any point along the length of the helix as at the ends of the helix. For any segment to unwind the parts of the helix immediately adjacent on either side must rotate. Such short segments are being unwound that the rotation will be limited in extent and may be accommodated by a small degree of overwinding or other molecular deformation. This must be the case if the helix is not free to rotate (as it would not be in a circularized molecule, and as it might not be if it is organized into a chromosomal structure) or if the amounts of energy involved in unwinding are not sufficient to rotate the entire structure. In any of these events the adjacent regions will be under tension (essentially they are storing all or part of the potential energy for rewinding). Since the atomic packing of the natural helix is already close it is likely that the tendency for deformation will have to be dissipated over some considerable distance so that the regions under tension will be much larger in extent than the unwound segment itself.

If these events occur in an isolated helix then there is no hindrance to their immediate reversal. However, if two homologous helices are lying side by side in close molecular proximity interactions between the two become possible. The unwinding event produces two portions of single stranded DNA which by definition are not involved in hydrogen bonding. If two such sections from different molecules come into contact complementary base pairing can take place to establish illegitimate, hybrid, double-stranded regions. This interaction (illustrated in Fig. 1) would prevent the immediate reversal of the unwinding sequences and establish an initial configuration capable of providing for recombination.

The recombination itself results from the resolution of this illegitimate pairing between different molecules. Leaving aside the re-establishment of the original "natural" configuration (which must be at least equally probable but which is effected simply by retracing the steps already indicated) the error situation which now exists can only be resolved by breaking the polynucleotide strands. The breaks will occur at the ends of the illegitimately paired

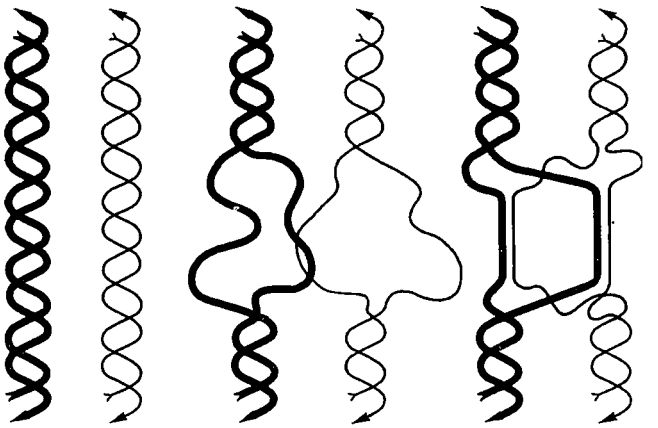

FIG. 1. Formation of regions of illegitimate pairing. Complementary segments of two adjacent DNA helices (non-sister chromatids) unwind and the single strands so produced cross-anneal by hydrogen bonding to form associations in which a strand from one helix is paired with a strand from the second helix. In all of these figures only two of the four chromatids of the bivalent are drawn, and the polarity of the strands in each helix is indicated by small arrow heads.

hybrid regions for it is in these places that the strands exchange their partners and where, in consequence, the error is most extremely expressed. The tangled strands at each end of each hybrid segment thus specify precisely the positions where breaks must occur without the need for structural or functional differentiation in the DNA. As shown in Figs 2 and 3, the breaks release both molecules from their entanglement and the disposition of the four breaks required among the four strands involved produces either a chiasma with hybrid DNA fragments or two recombinant molecules containing hybrid fragments but not involved in a reciprocal chiasmatic interchange.

It is unlikely that the breaks are produced without enzymic intervention. However, the entangled strands at each end of the illegitimately paired segments provide an error signal which could well be recognized and acted upon by DNA repair systems. The particular system involved cannot be reliably speculated upon at present. But it is noteworthy that distortions in the DNA helix are feasible substrates for some of the enzymes which are currently thought to be components of a repair system (Friedberg & Goldthwait, 1968; Setlow, 1968). Neither is it likely that the breaks referred to above will remain as simple single-bond breaks. A simple "cut" will allow resolution of the illegitimate pairing but would then probably be repaired by a process which excised and then replaced portions of the broken strand on either side of the cut. As the envisaged hybrid region is very short the

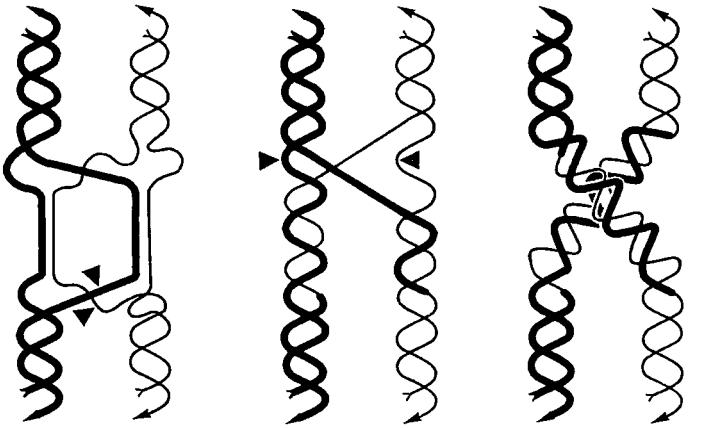

FIG. 2. Resolution of illegitimately paired segments to form a chiasma containing regions of hybrid DNA. Breaks in two strands (arrow heads) in the region where they are exchanging partners release both helices and allow partial rewinding. Breaks in the two strands not so far involved complete the resolution of the illegitimate pairing and result in the formation of a chiasma-like interchange which has segments of hybrid DNA.

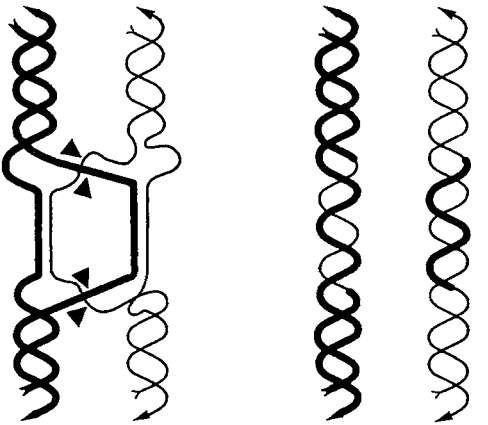

FIG. 3. Resolution of illegitimately paired segments to form regions of hybrid DNA but without a chiasma. If the four breaks which are necessary to resolve the illegitimate pairing occur in the same two strands (at the arrow heads) then the two original helices effectively exchange short segments of one of their component strands.

excised and replaced portion may well, in some cases at least, amount to the entire hybridized segment.

The events described above are figured as though occurring between two homologous DNA helices which constitute two non-sister chromatids, but no restriction to this situation is implied. An exactly similar series of events

could equally well occur between DNA helices which radically differ in size (as is the case in bacterial exconjugants and transductants, or in prophage attachment), between different parts of the same helix (prophage detachment, and perhaps in the formation of chromosomal aberrations), and even between a helix and a single strand, as in bacterial transformation, when a configuration similar to that postulated by Fox (1966) would be produced.

6. Intragenic Recombination Phenomena

The most important aspect of the model as far as intragenic recombination is concerned is that it establishes segments of hybrid DNA in the parent molecules. The evidence for the involvement of hybridity has been extensively reviewed (see references in Davern, 1971) and it is here assumed to be a necessary component of recombination. In as much as the model does provide for the formation of hybrid DNA its predictions do not materially differ from those of other models which also rely on DNA hybridity. There are differences, however, in that the length of the hybrid region is here considered to be very much less than that proposed by either Whitehouse & Hastings (1965) or Holliday (1968). In common with these two original hybrid–DNA models intragenic recombination is here envisaged to result from conversion events caused by repair of base mismatching when the hybrid region contains a mutant site. But whereas other models suggest that the repair is promoted by the base mismatch itself (Fincham & Holliday, 1970) the repair here is seen as a by-product of the break–and–excision process responsible for resolving the original tangling of the illegitimately paired molecules. It has been pointed out (Moore, 1972) that this interpretation does not preclude the possibility that mutant sites may affect the repair process. There is a good deal of evidence (Norkin, 1970; Gutz, 1971; Leblon, 1972) that recombination frequencies and conversion spectra are influenced by the nature of the mutant site and by the surrounding coding pattern. But the data so far available do not define the way in which these effects arise; this is still a matter of interpretation rather than fact. Indeed recent work shows that this so-called marker effect is quite complex. Marker effect sites may either enhance or reduce recombination frequencies, and there appear to be at least two different types of marker effect enhancement site (Moore, 1972, 1973). The interpretation presented here is that the *primary* error recognized by the excision-repair system involved in recombination is the disorganization and tangling of the strands at either end of the illegitimately paired segments of DNA and not any base mismatch that may be present. Once the "attention" of the repair system is directed to the region by this massive error it will be in a position to recognize and repair the relatively minor disturbance caused by the base mismatch.

The randomness of the unwinding events which initiate the whole process and their small size in relation to the size of the gene would both contribute to an overall correspondence between observed intragenic recombination frequencies and the actual physical distances between mutant sites. The very fact that reliable allele maps can generally be constructed on the basis of recombination frequencies between pair-wise combinations of mutant alleles suggests just such a correspondence. In a few particularly favourable cases a correspondence has been demonstrated (Yanofsky, Drapeau, 1967; Sherman *et al.*, 1970; and see references in Thomas, 1966). There seems good reason to believe, therefore, that allele maps constructed from recombination frequencies do represent, fairly directly, the physical structure of the gene. This can only be the case if recombination events are more-or-less randomized and involve segments of DNA that are short in relation to the overall length of the gene, for only if these conditions are met will the observed recombination frequency be a reliable measure of the size of the interval between mutant sites. That the size of the hybrid region is small is further supported by such evidence as that of Fogel & Mortimer (1969) who showed that the frequency of co-conversion increased linearly as the distance between two mutant sites was reduced; thus indicating that two mutant sites are only jointly affected by the hybrid repair mechanism if they are closely adjacent.

The interstitial unwinding model of recombination therefore seems to have, in common with any hybrid-DNA model, the ability to explain the basic features of gene conversion; but in addition it provides better than most others for a correspondence between recombination frequency and physical distance. In this context the temporal sequence of unwinding and strand breakage is irrelevant, it is the manner and extent of hybrid DNA formation that is important. However, for the explanation of some other features which have been revealed by studies of intragenic recombination, particularly polarization of recombination frequencies, it may appear that initial breakage of DNA strands (rather than initial unwinding) provides a more realistic basis. This is not the case. The explanations of polarization using initial strand breakage which have so far been advanced all rely on further speculations. On the other hand, an explanation using initial unwinding as its basis can be presented which is founded upon the known biophysical characteristics of DNA.

Comparison of the characteristics of different loci makes it clear that the position with regard to polarization is now quite complex. There seems to be no generalization which is adequate to describe all genes. In most cases polarization is barely evident, in others it has not been observed at all. Where it is observed it may appear as a simple gradient extending from one end of the gene to the other, but it may also be bidirectional (see references and

discussion in Paszewski, 1970). A previous comment on this point (Moore, 1972) suggested simply that polarization could be accommodated by postulating fixed or preferred unwinding points in the DNA molecule. No further explanation was offered; this is presented in the following paragraph.

Since the unwinding of DNA helices requires that hydrogen bonds be severed and stacking forces between the bases dissipated the unwinding events which constitute the dynamic equilibrium and provide a prelude to recombination cannot occur with strict randomness. The number of hydrogen bonds possible between pairs of bases depends on their identity, so the number of hydrogen bonds which exist per unit length of DNA helix will vary according to the coding pattern. In consequence the likelihood of unwinding will also vary according to the coding pattern. It is known, for example, that the melting temperature of natural DNA depends strongly on its guanine–cytosine content, that the melting temperatures of synthetic polymers depend on their base compositions and that treatments with chemicals which affect the bases also affect the melting temperature profiles; broadly speaking guanine–cytosine base pairs are more stable in the natural helix than are adenine–thymine pairs (see Szybalski, 1967). If these effects can be recognized at the gross level of overall denaturation of large molecules it is reasonable to suppose that they will have considerable influence on the more subtle events leading to unwinding of short lengths of the helix. The relative likelihood that any one segment will tend to unwind more-or-less frequently than average will depend on its base composition and this in turn depends on the overall coding of the gene and on the position of the segment within the gene. Just as the coding patterns differ between different genes and between different parts of the same gene so the tendency to unwind and promote recombination will also differ from gene to gene as well as within genes. In general this would not be expected to grossly affect the postulated correspondence between recombination frequency and physical distance since this is an overall relationship resulting from the relative placing of populations of unwinding events. It is possible, though, to foresee situations where the coding pattern is such as to determine either the direction or the frequency of unwinding to such an unusual extent that it becomes detectable as an altered pattern of recombination. If, for example, a region rich in guanine–cytosine pairs is juxtaposed to one rich in adenine–thymine pairs then unwinding might be more frequent in the latter than in the former; and if this coding discontinuity occurs in the intervals between different pairwise combinations of allelic mutants, then a gradient in recombination frequencies may result. The severity, extent and direction of the gradient will depend on the coding sequence of the gene. But this will not be because the coding is specifically differentiated to promote or direct recombination; rather it is due to the

coding sequence developed to specify a particular polypeptide having the ability to secondarily affect the otherwise random recombination events. Obviously this basic phenomenon is open to further development. Specific coding sequences could be developed in order to direct recombination in particular situations. For example regulatory mechanisms could be introduced since the interaction of effector molecules (whether they be protein or nucleic acid) with specific coding sequences would be a means of directing helix unwinding in a positive sense. The important point is that such processes are not absolute necessities to explain the phenomenon; it can be accounted for quite adequately on the basis of the natural activities of DNA molecules so that any differentiation in the coding pattern and any involvement with a regulatory molecule can be seen as an evolutionary adaptation which is a specific addition to the recombination process rather than a fundamental feature of it.

There is also an explanation for marker effect in this interpretation. Whereas the recognition of a gradient requires some similarity in the effect over a considerable length of the gene, individual mutational changes may well produce local alterations in the unwinding pattern which are identifiable as allele-specific effects. As a trivial example consider the possibility that the sequence —AAGTT— is one which is quite unexceptional in that it neither promotes nor hinders the occurrence of unwinding events. If a mutation occurs which substitutes A—T for the G—C base pair a consecutive sequence of five A—T pairs will be produced and this sequence may well promote unwinding events at a frequency that is higher than is usual for that gene. The mutant allele will thus be involved in single site conversion more often than normal and will be identified as a site which shows marker-effect enhancement of recombination frequencies.

7. Intergenic Recombination

The model provides for the random occurrence of reciprocal recombination (because it assumes randomized unwinding events) and thereby accounts for the relationship between recombination frequency and distance which is observed in chromosomal mapping. On the other hand, the ability of the DNA coding pattern to influence unwinding potential in such a way as to skew the randomized process in one direction or another explains why this relationship is only approximate and why the degree of relatedness differs from one chromosomal region to another.

The sequence of events which are postulated above to result in recombination is numerically unlikely and this may seem to argue against the plausibility of the model. Unwinding must occur in two independent molecules in the same place at the same time; the molecules must be sufficiently close for

illegitimate pairing to be established; this state must be maintained, and not simply reversed; repair enzymes (perhaps components of a generalized DNA repair system in lower organisms, though more likely components of an adaptive "recombination repair system" in chromosomal organisms) must recognize the errors in this pairing and correct them; and finally, the errors must be corrected in such a way as to produce a reciprocal chiasma–like configuration. The improbability that this sequence of unlikely events will be successfully completed is a virtue of the model rather than an embarrassment. For, as indicated earlier, if the chromosomal frequency of chiasmata is a reliable index then recombination is indeed an exceedingly rare event. Recombination frequencies are usually expressed in terms which are related to the numbers of progeny, nuclear divisions or rounds of replication. These frequencies give an artificially high impression of the true recombination frequency. This is because the equations used to establish them ignore, for technical convenience, a large and important constant—the total amount of DNA. Since this is a constant from generation to generation the fact that it is ignored does not matter for practical purposes. But it does matter to a discussion of the absolute probability of recombination. The quintessential step in recombination is the breakage of the phosphodiester bond between adjacent sugar moieties of the DNA backbone. The true frequency of recombination, therefore, would relate the number of successful breaks (recombinants, chiasmata, etc.) to the total number of such bonds which are available for breakage. A chromosomal organism possessing an amount of DNA equivalent to 10^9 nucleotide pairs would have to show 10^7 chiasmata per nucleus per meiosis for recombination *not* to be considered a rare event. Observed chiasma frequencies indicate, in contrast, that true recombination frequencies fall in the range 10^{-8} to 10^{-10} and that, it is maintained, suggests that successful recombination is rather rare. This conclusion is valid despite the fact that much of eukaryote DNA may be in some reiterated form which is not available for recombination [as suggested in various ways by the models of Callan (1967), Whitehouse (1967), Edstrom (1968) and Britten & Davidson (1969)]. For even if only 1% of the total amount of DNA is available for recombination, and this is probably a gross underestimate, then the above frequencies would only be changed by two orders of magnitude.

A feature of intergenic recombination which is frequently encountered when relatively closely-linked genes are considered is that recombination in one interval reduces the observed frequency of coincident recombination in the immediately adjacent intervals. This positive interference was indicated in the introduction to be a potential discriminator between different models of recombination since it imposes a restriction on their operation. Models which envisage initial strand breakage (whether or not this is associated with

localized DNA replication) cannot easily explain positive interference unless further conjectural mechanisms are added to those of the basic model. In contradistinction to this situation the original basic proposals of the unwinding model themselves predict positive interference. Since it is not only proposed that unwinding initiates recombination but that the unwinding is facilitated by rotational deformation of the adjacent regions it follows that the occurrence of one unwinding event which becomes fixed by illegitimate pairing will preclude the possibility of a second unwinding event in the immediate vicinity. It is argued above that the tension developed in the regions adjacent to an unwound segment will have to be dissipated over a considerable length of the helix, this would result in the effect being recognizable over intergenic distances. The effect would be exercised in both the dynamic and temporal senses. At some distance from the primary unwinding event further unwinding would be less likely than usual but not completely prevented, while in the immediately adjacent area recombination would be impossible because unwinding could not occur until the repair mechanism had resolved the primary illegitimately-paired and tangled strands. The position would not be completely restored by this resolution however, since the time dimension must also be important. The length of time now available for unwinding in these neighbouring regions has been reduced and they can never compensate for the reduction. Inevitably, then, there will be a gradient in the effect, with the greatest reduction in unwinding potential being seen close to the initial event. This would be detected as a progressive increase in the intensity of positive interference as successively smaller intervals were examined. Eventually, of course, the interval would be reduced to the level where negative interference, contributed by the coincidence of chiasma–like reciprocal recombination and conversion in the associated hybrid DNA, is recognized.

8. Interchromosomal Phenomena

A necessary requirement for any form of recombination is that the participating parental structures be brought into close proximity. Furthermore the structures must pair in an homologous fashion in exact linear register. It has been suggested that this process of synapsis is a feature of chromosomes, but not of DNA molecules (Thomas, 1966). This suggestion was made on the basis that the fidelity of DNA crystals indicated the absence of any exterior differentiation between DNA helices which could provide for sequence specific interaction. This, while true for crystalline DNA, is not true for DNA in solution. The dynamic unwinding equilibrium to which DNA helices are subject ensures an almost infinite variety in external differentiation of the helix. Moreover since unwinding generates single stranded regions it also

provides for the precise association of the polynucleotide chains through the annealing reaction. Of course the unwinding is transitory and involves very short segments; but it is a continuous and repetitive process so that there could be an overall contribution towards a very strong attractive force. The small size of the segments does not appear to be restrictive either since it has been demonstrated that the length of polynucleotide strand necessary for the formation of reasonably stable duplexes (the "stable minimum length") can be as small as 10 to 20 nucleotide pairs (Thomas, 1966).

This thus seems to represent a reasonable mechanism for arranging synapsis between both DNA molecules and chromosomes. The interaction between chromosomes may well be altered as a result of their complications in structure. Indeed, if the affinity between single-stranded polynucleotides is as great as annealing studies suggest then some restriction on the process may be necessary in most organisms and particularly in those which possess a very large amount of DNA that must be regularly ordered in cell division. So the development of chromosomal structure, and in particular the intimate association of proteins with DNA, could perhaps have resulted from a need to limit the degree of association possible between native DNA molecules. It is difficult, however, to see the need for the development of a secondary mechanism for establishing precisely complementary synapsis; this must be the role of the DNA itself. Once molecular synapsis is attained though, there must be some provision for its eventual nullification. In viruses this could be a partial function of the aggregation of head membrane proteins on the organized DNA which takes place in the earliest stages of maturation; in bacteria the reason for the apparent involvement of membrane growth in the separation of DNA molecules; while in eukaryotes the investment of the DNA with protein may well, after the initial alignment of the DNA molecules, allow organizational control of the structures to pass unhindered to the centromeres and spindle apparatus.

Since this interpretation of synapsis merely provides a basis for the type of annealing interaction discussed by Thomas (1966) it has the same corollaries with regard to the general structure of the genetic message as are considered by this author and his associates. Essentially if synaptic annealing involves recognition events between short lengths of transiently single stranded DNA then efficient and error-free complementary pairing can only obtain if the DNA molecules involved are constructed from non-repetitive nucleotide sequences. This does not necessarily prejudice the interpretation of repetitive satellite DNA since the specific function of this fraction of the genome may be of such a nature as to outweigh its effects on synapsis; indeed if satellite DNA is generally associated with centromeric regions (Jones, 1970) it may even be involved in a specific aspect of the phenomenon. But the need for a general

absence of repetition in the genetically active DNA does lead to further logical speculations concerning the origin of insertions, deletions and inversions as well as to the prediction that compartmentalization (into chromosomes) must be achieved if a cell is to contain more DNA than is allowed by the repetition limit (Thomas, 1966). Furthermore, if non-homologous chromosomes do share identical sequences then explanations for synaptic associations between non-homologous chromosomes and for the rare and erroneous recombination events which lead to exchange of segments between non-homologous chromosomes can also emerge naturally from the simple original assumption that native DNA helices exist in a dynamic ferment of unwinding events.

9. Conclusion

The speculations contained in this discussion are based on what is thought to be a more realistic appreciation of the form in which DNA molecules are to be found in the cellular environment. Initially the potential of postulating initial strand breakage is contrasted with initial unwinding in a model of recombination and the latter is argued to be the more plausible. It is suggested that spontaneous unwinding is a common and natural feature of DNA helices and shown that it is a feature which can be used as the starting point for explanations of a wide range of genetic and cellular phenomena. Breakage of DNA strands must sometimes occur and this model does not preclude the possibility that if a break occurs and is not repaired immediately then it might generate unwinding which could result in recombination. This sort of effect could account for the influence of ionizing radiations which are known both to produce strand breaks and to increase recombination. It is important, though, to distinguish the usual from the unusual and it is implicit in this discussion that the usual route to recombination starts with initial unwinding and not with initial breakage. Recombination promoted by initial strand breakage is seen as resulting from a completely abnormal process.

The specific suggestions made here should not be taken as inviolable models, though the author naturally hopes that they are correct. Rather, they are primarily intended to illustrate the diversity of argument which can logically be developed once the idea that DNA forms a rigidly fixed helix is disposed of. The explanations offered depend on the biophysical characteristics of DNA, but they may not be as difficult to test as this could imply. Many types of treatment are known which have influence on either DNA denaturation or annealing *in vitro*. Some are too extreme to be applied *in vivo* but there are others which might be applicable to the *in vivo* situation. One is thinking here, for example, of the effects of temperature, pH, various inorganic ions and of such organic compounds as alcohols, amides and urea.

Such treatments could be systematically applied to a wide range of different organisms and their effects studied in a range of different ways (inter- and intragenic recombination analyses, chiasma frequencies, synaptic associations, transformation, prophage integration and detachment, etc.). There would inevitably be numerous idiosyncratic variations, but if these arguments are even partially correct then general trends interpretable as due to the effect being exercised on the capacity of the DNA to unwind should be readily apparent.

REFERENCES

BRITTEN, R. J. & DAVIDSON, E. H. (1969). *Science, N.Y.* **165,** 349.
CALLAN, H. G. (1967). *J. Cell Sci.* **2,** 1.
CROSS, R. A. & LIEB, M. (1967). *Genetics, Princeton* **57,** 549.
DARLINGTON, C. D. (1934). *Z. indukt. Abstamm.-u. VererbLehre* **67,** 96.
DAVERN, C. I. (1971). *Prog. Nucleic Acid Res. Mol. Biol.* **11,** 229.
EDSTRÖM, J. E. (1968). *Nature, Lond.* **220,** 1196.
FINCHAM, J. R. S. & HOLLIDAY, R. (1970). *Molec. gen. Genet.* **109,** 309.
FOGEL, S. & MORTIMER, R. K. (1969). *Molec. gen. Genet.* **105,** 164.
FONG, P. (1964). *Proc. natn. Acad. Sci., U.S.A.* **52,** 239.
FOX, M. S. (1966). *J. gen. Physiol.* **49,** 183.
FRIEDBERG, E. C. & GOLDTHWAIT, D. A. (1968). *Cold Spring Harb. Symp. quant. Biol.* **33,** 271.
GUTZ, H. (1971). *Genetics, Princeton* **69,** 317.
HASTINGS, P. J. (1972). *Genet. Res.* **20,** 253.
VON HIPPEL, P. H. & PRINTZ, M. P. (1965). *Fedn Proc. Fedn Am. Socs. exp. Biol.* **24,** 1458.
HOLLIDAY, R. (1964). *Genet. Res.* **5,** 282.
HOLLIDAY, R. (1968). In *Replication and Recombination of Genetic Material,* pp. 157–174 (W. J. Peacock & R. D. Brock Eds). Canberra: Australian Academy of Sciences.
HOTCHKISS, R. D. (1971). *Adv. Genet.* **16,** 325.
JONES, K. W. (1970). *Nature Lond.* **225,** 912.
LEBLON, G. (1972). *Molec. gen. Genet.* **115,** 36.
LEDERBERG, J. (1955). *J. cell comp. Physiol.* **45,** Suppl. 2, 75.
LEVINTHAL, C. & CRANE H. R. (1956). *Proc. natn. Acad. Sci., U.S.A.* **42,** 436.
MOORE, D. (1972). *Genet. Res.* **19,** 281.
MOORE, D. (1973). *Genet. Res.* **22,** (in press).
NORKIN, L. C. (1970). *J. molec. Biol.* **51,** 633.
OKAZAKI, R., OKAZAKI, T., SAKABE, K., SUGIMOTO, K., KAINUMA, R., SUGINO, R. & ZWATSUKI, N. (1968). *Cold Spring Harb. Symp. quant. Biol.* **33,** 129.
PASZEWSKI, A. (1970). *Genet. Res.* **15,** 55.
PRINTZ, M. P. & VON HIPPEL, P. H. (1965). *Proc. natn. Acad. Sci., U.S.A.* **53,** 363.
RHOADES, M. M. (1950). *J. Hered.* **41,** 59.
SCHANDL, E. K. & TAYLOR, J. H. (1969). *Biochem. biophys. Res. Commun.* **34,** 291.
SETLOW, R. B. (1968). *Progr. Nucleic Acid Res. Mol. Biol.* **8,** 257.
SHERMAN, F., STEWART, J. W., PARKER, J. H., PUTTERMAN, G. J., AGRAWAL, B. B. L. & MARGOLIASH, E. (1970). *Symp. Soc. exp. Biol.* **24,** 85.
SOBELL, H. M. (1972). *Proc. natn. Acad. Sci., U.S.A.* **69,** 2483.
STAHL, F. W. (1969). *Genetics, Princeton* **61,** Suppl., 1.
SZYBALSKI, W. (1967). In *Thermobiology* (A. H. Rose, Ed.). London: Academic Press.
TAYLOR, J. H. (1958). *Proc. 10th Int. Congr. Genet., Montreal* **1,** 63.
TAYLOR, J. H. (1965). *J. Cell Biol.* **25,** 57.
THOMAS, C. A. (1966). *Prog. Nucleic Acid Res. Mol. Biol.* **5,** 315.

WHITEHOUSE, H. L. K. (1963). *Nature, Lond.* **199,** 1034.
WHITEHOUSE, H. L. K. (1966). *Nature, Lond.* **211,** 708.
WHITEHOUSE, H. L. K. (1967). *J. cell. Sci.* **2,** 9.
WHITEHOUSE, H. L. K. & HASTINGS, P. J. (1965). *Genet. Res.* **6,** 27.
YANOFSKY, C., DRAPEAU, G. R., GUEST, J. R. & CARLTON, B. C. (1967). *Proc. natn. Acad. Sci., U.S.A.* **57,** 296.