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Molecular Mechanisms of the Origin of Chromosome Aberrations and the Structural Organisation of Eukaryotic DNA

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Summary. A new hypothesis on the appearance of exchange chromosomal aberrations has been suggested. According to this hypothesis, temporal duplex polynucleotide structure should arise during G_1 and G_2 phases during the correction of DNA. The size of the duplex, as a rule, should be restricted to the size of complementary nucleotide sequences in the regions of repetitions. Any polynucleotide break in a duplex zone would result in chromosome breakage and if complementary broken ends interact with each other, then exchange chromosome aberrations may be formed. This hypothesis would explain such previously obscure phenomena as extremely high frequencies of exchanges after mutagen treatment, the nature of mitotic crossing-over, negative interference, change of aberration types before replication, the low frequency of damaged structural genes during aberration formation, etc.

Key words: Chromosome Aberrations - Eukaryotes - DNA - Polynucleotide Structure - Repetitive and Unique DNA Sequences - Mitotic Crossing-over - Mutagen Treatment

Introduction

Two basic processes are involved in the formation of new genotypes - mutagenesis and recombination. Mutations are divided into three basic types: genic or point mutations; structural chromosomal changes or aberrations of chromosomes; and numerical changes or genome mutations.

Structural chromosomal changes play an important part in natural selection. Of late, it has become evident that chromosomal rearrangements serve, even to a greater extent than point mutations, as a source of large evolutionary changes in higher organisms (Wilson et al. 1974). Numerous data (see for review Vachtin 1973) also indicate that chromosomal aberrations play an important role in carcinogenesis. Studying the mechanisms of the appearance of chromosomal aberrations is also very important for a new branch of modern biology - genetic engineering. Recently, certain advances have been achieved in genetic engineering of prokaryotes and initial studies have been carried out on eukaryotes, particularly on insects (Fox and Yoon 1966; Nawa and Yamada 1968) and plants (Ledoux et al. 1974; Turbin et al. 1975; Soyfer and Turbin 1974). Until now, in the studies on eukaryotes, attention has been centred on the possibility of changing heredity by means of DNA injection, and on the functions of exogenous nucleic acids and their fragments in host cells, while the possibilities of chromosomal engineering have been left out of consideration. But in all probability, for genetic engineering in eukaryotes, transplantation of chromosomes or, more important, parts of chromosomes may prove to be no less important a method than manipulation of individual structural genes, or fragments of DNA. This will certainly require a more precise knowledge of the molecular mechanisms of chromosomal rearrangements, particularly the steps leading to formation of new chromosomal structures and new sets of chromosomes. It is obvious that control of heredity and morphogenesis in eukaryotes cannot be realised without resolving the mechanisms of chromosomal mutagenesis at the molecular level.

Some progress is being made in understanding the nature of the origin of chromosome breakages. One of the first attempts to describe the molecular mechanisms was made by Soyfer (1969) and Dubinin and Soyfer (1969). The authors proposed that chromosomal breaks appeared as a result of errors by

the nucleases (in particular, the enzymes of excision repair) and pointed out the possible role of erroneous cutting of DNA in the regions with an altered secondary structure. After the discovery in eukaryotes of the endonuclease giving single-strand breaks (Slor and Lev 1973), Bender and co-workers (1974) further developed the idea and used it to interprete many cytogenetic facts. Further development of the molecular genetic theory of chromosomal mutagenesis became possible after the realisation of the role in mutagenesis not only of repair but also of some other enzymes operating on the DNA (see, for example, Soyfer 1970, 1975). It was shown that other enzymes which trigger genetic processes such as replication and recombination may also be involved in chromosomal mutagenesis. The possibility of the enzymes being involved in the production of point mutations had been considered by a number of authors (Setlow 1964; von Borstel 1969; Witkin 1969; Holliday 1970; etc.) and an analysis of the role of these enzymes in chromosomal mutagenesis was undertaken by Soyfer (1976). To form exchange aberrations two interacting chromosome fragments should connect with each other. Such connection was bound to happen due to the recombination-like process.

As was established in experiments with prokaryotes, two polynucleotide chains in the process of recombination have to recognise each other; in other words, the single-stranded regions of polynucleotide chains should be complementarily paired (Szybalsky 1964; Howard-Flanders and Boyce, 1966). A similar complementary interaction is possible in eukaryotes during meiosis, when the longitudinal fusion of DNA chains of two homologous chromosomes takes place. At this time the exchange of chromosome regions, i.e. recombination, can - and actually does - occur, but before recombination the single-stranded breaks and the following degradation of single DNA strands in each of the two synapting chromosomes take place.

The process of chromosome aberration in mitotic cells differs significantly from meiotic recombination. The exchanges may arise both within one chromosome and between two chromosomes; however, as a rule these chromosomes are non-homologous and it seems that complementary interactions of the single-stranded regions of the DNA of these two chromosomes are impossible.

The classical Sax-Lea hypothesis of chromosomal aberration (Sax 1938; Lea 1955) and Revell's exchange hypothesis (Revell 1954; see also Evans 1962), as well as some other concepts of the origin of chromosomal rearrangements, were not in fact concerned with molecular change of the chromosomal structures or the participation of any enzymes in the process of chromosomal mutagenesis, but treated these phenomena as purely topological. The chromosomes were considered as an ideal continuum, i.e. the chromonema without any discontinuities. However, the study of the molecular organisation of chromosomes reveals some structural peculiarities changing during cell cycles. These peculiarities should be taken into consideration when explaining the molecular nature of chromosomal rearrangements. Besides, the data on the intracellular activity of enzymes interacting with DNA (exo- and endonucleases, polymerases, polynucleotidlygases, etc.) should be used in this analysis.

If it is possible to outline the universal molecular theory of chromosomal rearrangements, then such theory must fit the kinetic regularities observed in the spontaneous and induced mutagenesis of higher organisms; and this theory must satisfactorly interpret in molecular terms differing temporary and topological peculiarities of chromosomal mutagenesis.

In the present work the possibility of using some data on molecular-genetic chromosome organisation and its functioning are discussed in relation to the mechanisms of origin of chromosome rearrangements.

Molecular Organisation of Chromosomal DNA of Eukaryotes and Formation of Exchange Aberrations

With the development of biochemical techniques for preparing and analysing nucleic acids, it became possible to elaborate the procedure for isolating unbroken molecules of DNA from chromosomes.

The amount of DNA per chromosome in various organisms was accurately measured by highly sensitive methods. For instances the results of the viscosimetric determination of the molecular weight of DNA in cell lysates of *Drosophila melanogaster* (Kavenoff et al. 1974) demonstrated that the largest DNA molecules had a size equal to the total amount of DNA in the second chromosome, the largest in the chromo-

some complement. Since the degradation of chromosomal DNA takes place only after DNase (and not pronase) treatment, it was concluded that, in agreement with many other findings (see for review Prescott 1970; Akifiev and Makarov 1974), only one DNA molecule extended along the eukaryotic chromosome. If so, then each chromosome exchange must be attended not only by breakage of the DNA molecules before their connection into a new chromosomal structure, but also by the local synthesis of DNA in the gaps remaining after the connection of the fragments, and by joining the polynucleotide chains with lygases to form a single DNA structure. From this point of view it is very important that Kavenoff et al. (1974) also demonstrated subsequent joining of the fragments into a single structure and showed that the molecular weight of DNA in the recombinant chromosomes changes in accordance with the hypothesis of discontinuity. For example, in symmetric translocations the molecular weight of chromosomal DNA was equal to the summary molecular weight of the fragments involved in the exchange. This means that the process of forming exchange aberrations should be completed by the joining of the recombinant parts of DNA into a single DNA molecule. It needs to be underlined that the preservation of the polarity of the DNA chains is an indispensable condition of chromosomal fragments joining (Soyfer 1969, 1970, pp. 200-201; Brewen and Peacock 1969). In the case of inversions, enzymatic digestion of terminal nucleotides must take place to permit joining of the sugar-phosphate bonds in the new site (Soyfer 1970, 1971).

The most important requirement of aberration formation is complementary association of the ends of DNA molecules of interacting chromosome fragments. Earlier geneticists believed that chromosome fragments were connected according to an "end to end" type. However, no example of the joining of two double-stranded DNA molecules without cohesive ends has been reported; and there is only one possibility of association of broken ends: the complementary interaction of single-stranded ends of two duplex molecules, as was suggested in the first hypothesis on the molecular basis of recombination (Szybalsky 1964; Howard-Flanders and Boyce 1966). In this case aberration formation may be promoted by the introduction of a point lesion of DNA which would initiate repair reaction (Soyfer 1969). At any rate the necessary condition of exchange interactions is the appearance of single-stranded regions at the DNA ends of interacting fragments containing complementary nucleotide sequences. Complementarity may be full, then apparently the paired region may be short (a few dozen nucleotide pairs), or else a more or less extended region of partial pairing (up to several thousands of nucleotides) may occur in the recognition region.

Until recently it was unclear whether complementary nucleotide sequences exist capable of pairing their single-stranded chains in the same chromosome, or in some regions of different chromosomes. The possible existence of these complementary regions was revealed by Britten and Kohne in 1968. They established that the genome of eukaryotes consists of unique (i.e. non-repetitive) nucleotide sequences and some parts of repetitions. About 50 % of the genome of eukaryotes is regular alternation (so-called interspersion) of unique sequences of 800-1100 nucleotide pairs and relatively short repetitions of 200-400 nucleotide pairs; 20 % of the genome normally has longer intervals between repetitions; and, finally, in the remaining 20 %, repetitive sequences seem to be altogether absent (Chamberlin et al. 1975).
Bonner et al. (1974) postulated that each unique sequence, in the part of the genome in which interspersion occurs, has a pair of short tandem repetitions at both sides. Denaturation maps of eukaryote DNA described by Taylor and co-workers (see Evenson et al. 1972) also bear out the interspersial principle of genome organisation in the regions of partial denaturation; and Degtyarev and Akifjev (1975) demonstrated that repetitions are capable of complementary interaction with both sequences in the adjacent denaturation region, and the identical regions of at least some other chromosomes.

These data on thin DNA structures suggest an important idea: if primary breaks in chromosomal DNA arise in unique (non-repetitive) sequences of the nucleotides of two molecules of DNA, then the possibility of the formation of exchange aberrations is much less than in the case arising from breaks in repetitive groups of nucleotides.

If this statement is valid, then for the sake of convenience of calculation we shall disregard the cases

of unique sequences and consider only the interactions of the fragments having the regions of repetition at the ends. Since the average length of the repetitive regions is one third of that of the unique one (Davidson et al. 1974), the probability of primary breakage occurring in the region of repetitions is $P = 0.3$. Even if two chance breaks coincide in time and in space, the probability of exchange interaction will not be higher than $P = 0.3 \times 0.3 = 0.09$. In other words the maximal frequency of chromosome aberrations of the exchange type, which may be calculated on the basis of the data on the molecular structure of the genome in eukaryotes, may not be more than 9%. However, the experimentally observed quantity of exchange aberrations is generally at least 4-5 times higher than expected. In some cases [e.g. after irradiation of dry seeds of *Crepis capillaris* (Nemtseva 1965) and human leucocytes in vitro (Wolff 1973)] over 90% of rearrangements were attributed to the exchange type. At the same time, without taking into consideration a special mechanism, it can be supposed that both spontaneous and induced chromosome breaks must arise with the same probability in unique and in repetitive regions. Only the breaks located in repetitive zones can be used as a basis for the appearance of exchange aberrations, whereas the breaks of unique regions do not lead to the appearance of exchange rearrangements, if we consider the special mechanism.

This conclusion, taking into account the molecular organisation of eukaryotic DNA, seems to be very important, but three circumstances, as before, need to be explained. First, for a single exchange aberration to appear, two breaks in the repetitive regions located in different points of a chromosome should simultaneously take place in the DNA. Secondly, for the subsequent joining of chromosome fragments into a single structure it is necessary that in each fragment, at the ends, one of the DNA chains should be cut by exonucleases to make possible the complementary joining of these chromosome fragments. However, for this phenomenon to take place each fragment should have at the ends not any single stranded region but only the ends complenting each other. And third, for the exchange aberrations to appear, it is necessary that the fragments should be able to meet each other in the cell. But the realisation of this requirement would decrease the probability of the appearance of chromosome aberrations.

Thus although the account of the interspersial structure of DNA and the repetitions in DNA open the possibility of explaining in principle, the appearance of exchange aberrations, the total number of aberrations and the extremely high frequency of exchange aberrations compared with the breakages of chromosomes are still to be interpreted. At the same time, many of these difficulties could be eliminated if we suppose that a certain process (such as the correction of DNA) develops in the cells before DNA replication. In the course of this process the breaks arising in DNA induce simple chromosome aberrations as well as exchange aberrations.

Explanation of the Mechanism of the Appearance of Chromosome Aberrations on the Basis of the Hypothesis of Temporary Duplexes

As mentioned before, chromosomal DNA has numerous repeated nucleotide sequences, frequently limiting on both sides the regions of unique sequences. It must be underlined that the correction (or "rectification") of genetic material may constitute an important mechanism providing for the identity of the chromosomal organisation in successive cell generations. According to the initial hypothesis of Callan (1967) and Whitehouse (1967), two complementary regions of chains of chromosomal DNAs may approach each other and be matched (corrected, or rectified) with the help of hypothetical correcting enzymes (of the repair enzyme type). Although these hypotheses do not have definitive experimental support so far, we may attempt to further develop the basic principle of correction to explain chromosome aberration formation, paying special attention to the following.

According to Callan (1967) and Whitehouse (1967), DNA regions in the course of correction are compared to one so-called master replica of the locus. We think the correction is an absolutely random process; it may spread over any two complentary regions of DNA, adjacent or scattered along one or several homologous or even non-homologous chromosomes. Let us consider this model in more detail.

If chromosomal DNA has repetitive sequences, it stands to reason that the process of chromosomes' coming together and subsequent correction of their

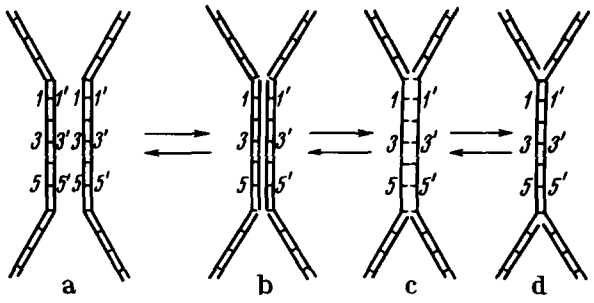

Fig.1. Formation of duplex structures in the regions of different chromosomes having identical repeated nucleotide sequences a) connection of chromosomes in homologous regions; b) single-strand cutting of DNA in opposite sites; c) partial degradation of single-strands of DNA in the complementarily regions of repeated nucleotide sequences; d) formation of synapse

DNA may be initiated just in these regions. As a result of their coming together, identical regions may "recognise" each other and form a synaptic structure with the aid of special proteins (of the type of the gene 32 product of phage T4 facilitating the unwinding of a double-stranded DNA).

The possibility of such a process in eukaryotes seems realistic enough at least because the DNA unwinding proteins have been found both in meiotic (Stern and Hotta 1974) and mitotic (Alberts 1974) cells. Naturally, chromosome convergence and formation of synaptic complexes (Fig.1) are most effective between the complementary regions of chromosomal DNA. Finally, in the synaptic region the two DNA molecules of different chromosomes will join together to form a single structure with hydrogen bonds between the chains (Fig.1d). The necessary provision is the presence of single strand breaks in both double-stranded DNAs (Fig.1b) and subsequent degradation of one strand of each (necessarily antiparallel, Fig.1c). Distant regions of a chromosome containing repeated sequences may be involved in the process as well. After the formation of a duplex and matching of the DNA chain of one chromosome with the other, the duplex will disintegrate, while the markedly large single-strand gap will be resynthesized. Akifiev and Aingorn (1972) found that the synthesis of DNA in g_1 phase of human leucocytes in vitro differed from replicative and reparative synthesis. Yet for a while the gaps will undoubtedly remain the target for endonuclease attack (especially if separate modified bases or their groups are present in the region), resulting in hydrolysis of the sugar-phosphate chain of DNA and breakage of the chromosomes.

Endonucleases may certainly produce single-strand breaks in the duplex also, which would promote recombination of chromosomes due to erroneous joining of the DNA strands before the disintegration of the the duplex.

As well as occurring spontaneously, breaks in the duplex regions may be induced by various chemical mutagens and by ionising radiation.

Thus, the duplex formed for the correction of chromosomal DNA may represent a real molecular structure giving the most favourable conditions for chromosomal rearrangement. Furthermore, since all earlier discussions of the mechanisms of the formation of chromosomal aberrations were based on the assumption that single-strand gaps and different endonucleases played a certain part in the formation of breaks, none of these hypotheses took into account the specificity of the molecular organisation of chromosomes or the possibility of regular formation of extended gaps in the course of normal correction of DNA.

The type of exchange, i.e. symmetrical or asymmetrical, is determined by the type of repeated sequence forming a duplex. In the case of generally simple repetitions, symmetrical exchanges occur; when the duplex consists of interacting inverted repetitions, asymmetrical exchanges take place. It should be mentioned that the only possible mechanism of joining of the two DNA molecules, which preserves the polarity of the strands in the course of asymmetrical exchanges, is complementary interaction of inverted repetitions (Fig.2).

Thus, the process of correction, apparently occurring in all eukaryotic cells prior to DNA replication or following it, may contribute not only to eliminating premutational lesions, but also to production of chromosomal aberrations.

When discussing the nature of exchange rearrangements, we noted that DNA conjugation is necessary for initiation of chromosomal recombination. Since conjugation is attended by complementary interaction of two DNA strands of different chromosomes, which is facilitated (in non-homologous chromosomes) in the regions of repeated DNA sequences, it was reasonable to restrict the calculation of the maximum exchange frequency to recombination involving only re-

gions of repetition. Thus, a maximum exchange frequency of 9% was estimated. The value, calculated on the assumption of independent and fully random interaction of chromosomal regions, is a result of the multiplication of probabilities of the frequency of repetitions in the DNA of one chromosome.

However, consideration of the mechanism of duplex formation invalidates the suggestion of independent interaction of nucleotide sequences in the repetition regions. If the assumption that DNA regions are corrected with the aid of temporarily formed duplexes in true, then interaction of DNA regions will take place during each correction event, and the probabilities of occurrence of such repetitive regions must be added up. But since correction may be multiply repeated during one cell cycle, the frequency of formation of temporary duplexes may increase, so that the possibility of exchanges may be higher and approach unity.

In mutagenic treatment, the frequency of exchanges also depends upon the nature of the mutagen applied. For example, administration of the agents inducing breaks of DNA at a high frequency increases the frequency of exchanges.

Thus, the hypothesis of the formation of temporary duplexes during DNA correction may be useful in explaining one of the intriguing questions of chromosomal mutagenesis, namely, the paradox of "exchange aberrations". It will be shown further that the duplex hypothesis may be helpful in the explanation of some other features of genetic processes.

Finally, we shall outline the real enzymic systems capable of correcting genetic material. The discovery of a number of endonucleases, part of which interacts only with double-stranded and the rest only with single-stranded DNA molecules, the finding of several different types of endonuclease, DNA polymerases and of the enzymes promoting effective unwinding of double-stranded DNAs, indicate the diversity of enzymic reactions with DNA, and the broad scope of operations of DNA in the cell. Among the most important evidence of the existence of DNA correction in eukaryotes was, undoubtedly, the finding of a special process of maintenance of the molecular structure of DNA, i.e. repair. Repair, recently reported to take place both in animals (Regan et al. 1968; Horikawa et al. 1968; Soyfer and Yakovleva 1972) and in plants (Soyfer and Cieminis 1974; Howland 1975), suggests that this system of enzymes may be involved in the process of correction. So correction, presumably, represents specialised repair which came into being at a certain stage in the evolution of living matter to provide for the prolonged function of the genetic material with such a complex organisation as the eukaryotic chromosome.

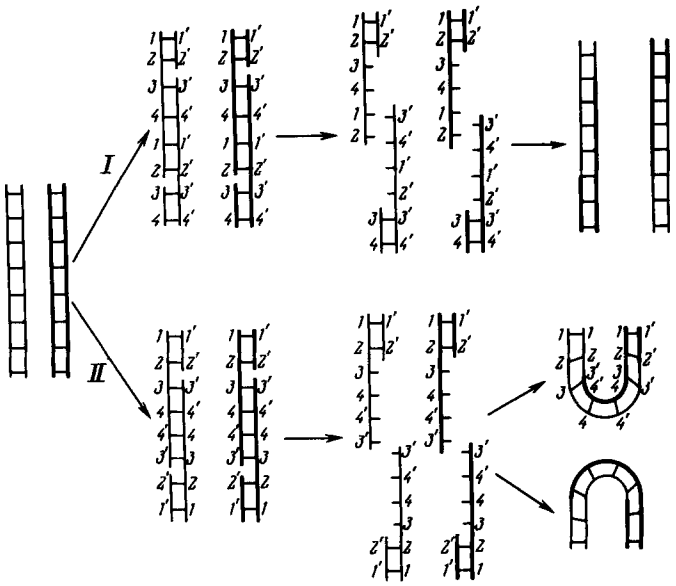

Fig. 2. Interactions of nucleotide sequences at the time of formation of symmetric and asymmetric changes. I) symmetric changes arising in the regions of simple repeats; II) asymmetric changes arising in the regions of inverted repeats

In the whole of the discussion we were concerned with DNA correction in the region of repeated sequences. It is becoming more and more evident that this region, in contrast to the regions of unique (non-repetitive) segments, forms the regulatory zone of the genome. Efficient correction in this zone must be of great importance for such processes as cell differentiation in higher organisms, aging, removal of primary carcinogenic changes, so it is quite obvious that the rigorous correction of the regulatory zone of the genome may not be less important, on the evolutionary level, than repair of structural genes. It should be stressed that this hypothesis might clarify the differences in frequency and spectrum of the aberrations arising at different stages of the cellular cycle.

It is quite obvious that such an approach to the nature of chromosomal aberrations must take into

consideration the phases of the cell cycle. Since at different phases of the cell cycle intrachromosomal processes are maintained by the phase-specific enzymes, different enzymes will be involved in aberration formation at different phases. For instance, if repair enzymes are involved in the formation of temporal duplexes, their (repair enzymes) inhibition before S-phase will influence the mutagenesis frequency, not only through direct action on repair activity but also via normal formation of duplexes. That different enzymes are involved in chromosomal mutagenesis at different phases has been proved in several experimental models. For instance, after studying the duration of the phases of the cell cycle in germinating barley seeds by direct biochemical methods (Soyfer et al. 1973), and the determination of the hydroxyurea concentrations most effectively inhibiting replicative synthesis of DNA in plants, the authors showed that the most pronounced cytogenetic effect of repair inhibition by caffeine occurred in G_1, whereas the greatest increase of the aberration frequency as a result of replication inhibition by hydroxyurea was in the S-phase (Soyfer et al. 1977).

Using a specific method of inhibiting the beginning of DNA replicative synthesis in meristem cells of *Crepis capillaris*, Shavelzon et al. (1976) succeeded in synchronising a majority of cells over a rather short period of the cycle, i.e. at the boundary of G_1 and S phases. Since recombination of single-stranded DNAs is hardly feasible, it could be expected that ionizing radiation, which normally induces exchanges of the chromosomes in this case. The expectation was fully confirmed by experiment. Upon irradiation of normal seeds with all cells in early G_1, all aberrations in the first mitosis were chromosomal exchanges. After synchronization of cells at the boundary G_1 - S, the spectrum of radiation-induced mutations changed drastically: of the total of 107 rearrangements induced by radiation, only 6 were chromosomal exchanges; among the other 101 chromatid aberrations there were no exchanges, but only simple and isolocus breaks. Thus it was shown that there was a period of the cell cycle over which the contribution of exchanges was negligible. It is possible that the temporal duplexes of chromosomes leading to the exchange aberrations were formed in early and middle G_1 phase but not at the end of G_1.

Interpretation of Some Unclear Phenomena of Aberration Production and Crossingover in Terms of the Hypothesis of Temporary Duplexes

The hypothesis of temporary duplexes may be used in explaining some vague questions of chromosome aberration theory.

Let us consider briefly some phenomena of chromosomal mutagenesis so far unexplained.

1) Mitotic Crossingover

During meiosis homologous chromosomes join together (conjugate) to form a synaptonemal complex (see for review Lyapunova and Bogdanov (1975)], thereby producing a molecular structure to mediate meiotic crossingover. As mentioned previously, during meiosis a single structure is formed (conjugation occurs between homolcgous chromosomes over all their length).

It seemed that in mitosis there was no such stage at which chromosome conjugation could be possible, so it was reasonably suggested that mitotic crossingover in somatic cells was not feasible. But then partial chromosome conjugation was demonstrated by C. Stern (1936) in *Drosophila*, and later by workers using other subjects, although its molecular mechanism has so far not been understood.

We may attempt to explain mitotic crossingover on the basis of the temporary duplex model proposed here; the breaks in the duplex, followed by erroneous joining of the broken ends of the strands, may give rise to recombinant chromosomes.

2) Negative Interference

It was a hard and fast rule of classical genetics that the probability of crossingover of the two markers of homologous chromosomes was higher the farther apart the loci of the markers were spaced. Yet it was found by a study of crossingover of tightly linked genes that, with a decreasing distance between them and upon passing through a certain limit, the frequency of exchanges between several tightly linked genes tended to increase. This phenomenon was called negative interference. Its nature remains unexplored. We

suppose that regardless of where a duplex is formed - in somatic or generative cells (including prophase of the first meiosis) - the probability of exchange with the parts of tightly linked genes will increase, hence the process, referred to as negative interference, will take place. In passing, it also follows that a similar phenomenon must take place in somatic crossingover.

3) Quantitative Proportion of Exchange and Breakage Rearrangements of Chromosomes

A spontaneous or induced single strand break of chromosomal DNA formed outside the duplex region and not subjected to the action of repair enzymes, may give rise only to chromatid breakage mutations. Under the action of repair enzymes, broken strands will be resynthesised, or in the case of their erroneous operation, chromosomal breakage will ensue.

The situation will be quite different in the case of breakage in the duplex region. If the breakage persists to the beginning of the duplex divergence, chromosomal aberration will ensue. If breaks occur in both strands, an exchange aberration may arise. Finally, upon the divergence of the duplex, even with several breaks in both strands, exchange aberrations will hardly take place.

The ratio of breakage and exchange aberrations will, therefore, be determined not only by the number of breaks, but also by the stage at which the duplex is formed.

It seems that the hypothesis of temporary duplexes permits an explanation of several observations involving aberration types, and also helps to explain the reason for the sharp shift in the proportion of the types of chromosome mutation caused by the use of inhibitors of nucleic acid metabolism (Soyfer et al. 1977; Shavelson et al. 1976).

The temporary duplex hypothesis also allows an explanation of the observation that an increase in the frequency of chromosomal deletions and exchanges will not necessarily submit to a standard and quaternary dependence, as required by the calculations of Leenhouts and Chadwick (1974), because the probabilities of chromosome deletions and exchanges, upon the appearance of single-strand breaks in the newly formed duplex, are quite close.

4) Changes in the Aberration Types under Mutagenic Treatment Long before and Immediately before Replication

It was demonstrated by Evans and Savage (1963) that the proportions of chromosome and chromatid aberrations after the mutagenic treatment at different periods of G_1 varied. For example, if the cells entering the synthetic phase were exposed to X-rays, only chromatid and isochromatid aberrations were induced. This fact, which seems strange at first, may be easily explained from the standpoint of the temporary duplexes hypothesis. Since all duplexes disintegrate before the beginning of DNA synthesis and the gaps occurring in them are resynthesised until a double-stranded DNA structure is recovered, quite obviously, at this stage only chromatid breaks, and no other types, may appear.

5) Low Frequency of Lesions of the Structural Genes in the Course of Chromosomal Exchanges

It has been a long time since geneticists paid attention to the fact that the frequency of lesions in the structural genes is negligible (e.g. Thomas 1970; Lefevre 1974). It seems that there are no structural genes at the point of convergence of two chromosomes. This becomes comprehensible if it is considered that chromosomal rearrangements [not only exchanges, but also deletions, as was stressed by Thomas (1970)] may predominantly occur in the duplex regions consisting of complementary sequences, making up a middle or highly repetitive fraction (a regulatory zone) of the genome. So, one of the functions of the repetitions is prevention of the unique segments, containing structural genes, from participating in chromosomal breaks. Conceivably, the low frequency of changes in the structural genes in contrast to the high frequency of aberrations in the repetitive sequences, causing changes in the regulation and coordination of individual genes in eukaryotes, was an important acquisition to the genetic apparatus of higher organisms in the course of the evolution of living matter.

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