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Origin of repeats in *Drosophila* chromosomes

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1. INTRODUCTION

From the point of view of evolution, repeats (Rp) belong to the most important category of structural changes. They are assumed to provide a mechanism for the evolution of genes with new functions. Quite recently Smithies *et al.* (1962) have suggested that one of the human haptoglobin genes arose from previously existing ones by partial gene duplication. An estimation of the frequency with which Rp's occur and an understanding of the mechanism by which they originate is therefore of great interest.

Rp represents a type of duplication where a segment of one chromatid is inserted next to its homologous segment in the sister chromatid (see Fig. 1). This type of change was first described by Kaufmann & Bate (1938).

X-rays induce Rp's with a very low frequency. A detailed study of Rp's was made possible by the discovery that some chemical mutagens are capable of inducing Rp's with a high frequency. A complementary duplication-deficiency type of change has been found to be frequent in *Vicia faba* after treatment with nitrogen mustard (Ford, 1949) and in *Drosophila melanogaster* after treatment with formaldehyde food (Slizynska, 1957).

The mechanism responsible for the origin of Rp's may be deduced from a cytological study of different types of Rp and their relative frequencies. Such an analysis, based on formaldehyde-induced Rp's, is attempted in the present short note.

2. THE MECHANISM OF Rp FORMATION

The diagram of the proposed mechanism leading to the formation of Rp's and their complementary deficiencies (Df) is given in Fig. 1. Four types of Rp have been found during cytological analysis of the effects of formaldehyde food. While some of them *might* arise from a process other than chromosome breakage, e.g. from non-homologous crossing over, others require prior breakage. All of them *can* be explained by the mechanism shown in Fig. 1. This requires two breaks in a still undivided chromosome; after splitting of the chromosome, these breaks result in two pairs of isochromatid breaks. Depending on the type and the number of new rejoinings, different types of Rp are formed. All of them are accompanied by the same complementary Df. The expected ratio of Rp's to Df's is 1 : 1, i.e. in each case one of two daughter cells will contain a Rp, the other a complementary Df. When rejoining occurs during cleavage divisions, a mosaic is formed, in which a part of the body contains a Rp, and the other part, a Df. Such mosaics have been found after treatment with formaldehyde. Rejoining at meiosis does not give rise to observable mosaics but to two different spermatozoa, one carrying the Rp, the other the Df.

Closer quantitative analysis indicates that, in fact, most or all Rp's do arise from chromosome breaks by this mechanism. It will be seen from Fig. 1 that the unreversed Rp (type 1) is the only type that, theoretically, might arise instead from non-homologous

crossing over. Reversed Rp's (types 2, 3, 4) require actual breaks, and cannot arise from non-homologous crossing over.

If crossing over were, in fact, a major source of unreversed Rp's, one would expect this class to be much more frequent than the other three. The data show that this was indeed so for a sample of analysed Rp's. The observed frequencies were: twenty-nine for type 1, nine for type 2, four for type 3, one for type 4. Such a distribution is, however, expected from the numbers of new rejoinings required for the formation of these four types; for it is well known that new rejoining always occurs much less frequently than restitution in the original order. The numbers of new rejoinings required are one for type 1, two for types 2 and 3, and three for type 4. The actually observed numbers are approximately what would be expected on this basis; certainly the excess of unreversed Rp's over reversed ones is such that it does not call for the assumption that they may also arise from crossing over.

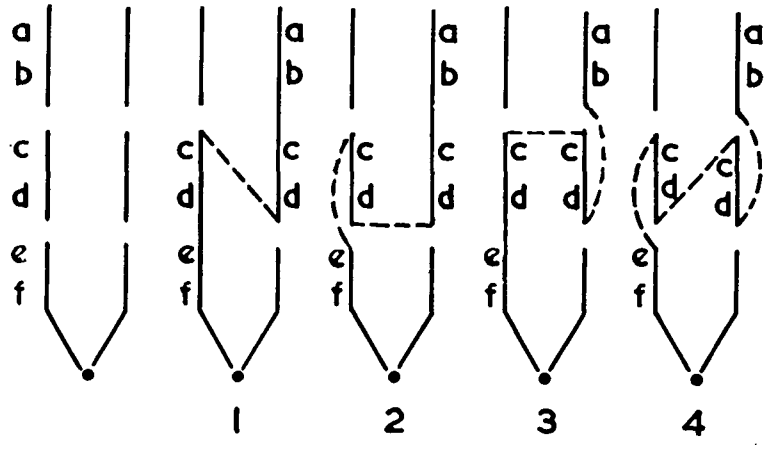

Fig. 1. The mechanism underlying the formation of repeats. All types of Rp have the same complementary Df: ab/ef; dotted lines mark new rejoinings.

It thus appears that, in general, Rp's arise from chromosome breaks: this is certainly true for reversed Rp's, and probably for most or all unreversed ones.

3. THE NATURE OF REPEAT-FORMING BREAKS

In order to result in a Rp with complementary Df, the breaks have to fulfil special conditions. They have to be available for reunion at a time when sister chromatids are closely apposed to each other, and they must affect both chromatids at the same level. For the latter condition to arise from 'hits' on the already separated chromatids would require a highly improbable coincidence, especially for a chemical mutagen. On the other hand, it would follow automatically from a previous hit on the still unsplit chromosome, if the broken ends remained available until chromatid separation. Observations on irradiated chromosomes show that such a delay in reunion is rare, at least for X-ray induced breaks. Although breaks induced in spermatozoa of *Drosophila* remain available until after fertilization, they then rejoin quickly and usually before chromosome splitting, as shown by the rarity of chromatid changes, in particular of Rp's (Bauer, 1939; Slizynska, unpub.). Results to be published presently show that this applies equally well to X-ray induced breaks in premeiotic chromosomes, which hardly ever persist long enough to give Rp's in meiotic prophase.

Although the situation may conceivably be different for chemically induced breaks, it

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seems more plausible to attribute the frequent induction of Rp's by certain chemicals to an already well-established peculiarity of chemical mutagens: their tendency to produce in the genetical material primary lesions that only subsequently—often after several replications—may give rise to mutations or chromosome breakage. Formaldehyde food, in particular, produces the majority of its structural effects on chromosomes by means of such 'potential breaks' (Auerbach & Moser, 1953*a*, 1953*b*; Slizynska, 1957). Similarly (personal communication from Dr J. Evans) a substantial amount of potential (delayed) breakage has been obtained with maleic hydrazide in *Vicia faba* roots. Potential chromosome breaks which become effective breaks immediately after the separation of sister chromatids fulfil ideally the conditions for the formation of Rp's. It seems likely that they are, indeed, the major source of Rp's.

Smithies *et al.* (1962) suggest that the intragenic duplication assumed to account for one of their haptoglobins arose through non-homologous crossing over. The mechanism proposed for formaldehyde induced Rp's provides an alternative explanation. It is probable that naturally occurring chemicals account for a proportion of spontaneous mutations and rearrangements; it is possible that they also produce Rp's by the mechanism discussed here.

4. THE FREQUENCIES OF SPONTANEOUS AND INDUCED REPEATS

The frequency with which Rp's arise *de novo* without treatment is not known. In *Drosophila*, it is very low, but it should be remembered that Rp's are detected only when accompanied by a dominant visible effect, e.g. duplication Bar in *Drosophila melanogaster*.

X-rays are capable of producing Rp's but with a very low frequency (2–6·5% of all changes) compared with other structural changes induced simultaneously by the same treatment (Slizynska, unpub.). Chemical mutagens, on the other hand, seem to produce Rp's with a much higher frequency than other kinds of structural change. It is probable that this is a characteristic feature of many chemical mutagens, but actual evidence is available only for nitrogen mustard in *Vicia faba* (Ford, 1949) and for formaldehyde food in *Drosophila melanogaster* (Slizynska, 1957). The relative frequencies of formaldehyde-food induced types of change are as follows: Rp's—35·3%, Df's—25·0%, translocations—17·7%, inversions–17·7%, other changes—4·3% of all changes.

For a study of Rp's, the salivary gland chromosomes analysis is the most suitable method. It allows a distinction to be made between reversed and unreversed Rp's and makes possible a very precise and exact location of breaks and their distribution along the chromosomes.

5. CONCLUSIONS

The following conclusions can be drawn: most or all repeats arise from actual chromosome breaks, which subsequently give rise to sister chromatid breakage followed by new rejoinings.

A model for the origin of repeats suggests that these may arise most readily from chromosome breaks which remain latent (potential) until separation into sister chromatids. This is in excellent agreement with the fact that formaldehyde, which produces mainly potential breaks, yields a high frequency of Rp's, whilst X-rays yield very few.

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