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Triplications and the problem of non-homologous crossing-over

By H. SLIZYNSKA
Institute of Animal Genetics, Edinburgh, 9

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1. INTRODUCTION

The importance of duplications as the main source of the genic material for evolution was recognized very early (Bridges, 1919; Muller, 1935) and is now generally accepted by geneticists. In nature the most frequent type of duplication is a repeat (tandem duplication), where the duplicated segments lie adjacent to each other; they may or may not be inverted in the relation to each other and to the rest of the chromosome. Repeats have been found at different structural levels and may involve groups of genes, single genes, or even only parts of a gene. The examples of repeats found in micro-organisms, *Drosophila*, higher plants and mammals are numerous (Lewis, 1967). So far, their study has been limited mainly to an analysis of repeats already established in nature and interesting evolutionary models have been based on the consequences of crossing-over within a duplicated region. The origin of such regions is usually ascribed to non-homologous crossing-over but, as will be discussed below, this is a rather implausible assumption. It is therefore of obvious interest to study the mechanisms that can give rise to repeats. This can be done best by inducing new changes in normal chromosomes. Numerous new duplications of the repeat type were found in *Drosophila melanogaster* after treatment with formaldehyde (Slizynska 1957) and their mode of origin has been discussed. In the present communication, two triplications found after treatment with other chemical mutagens will be described and discussed.

2. DESCRIPTION OF TRIPLICATION

The following abbreviations will be used: Tr, a triplication; 2L, 2R, 3L and 3R, left and right arms of the 2nd and 3rd chromosomes of *Drosophila melanogaster*; T2–3, a translocation between 2nd and 3rd chromosome; *bw*, brown, the eye colour gene in 2R; TEM, triethylene melamine.

Tr-I occurred in an experiment designed to test whether storing of the TEM-treated germ cells increases the frequency of all structural changes and not only that of translocations (Snyder, 1963). Injected males were kept with females for 2 days to ensure that only mature spermatozoa were utilized; treated spermatozoa were stored in the untreated inseminated females for 14 days and then sampled. Among structural changes in fifty-seven cytologically analysed F_1 larvae, one triplication was found in a male larva, which also carried a small inversion in 3R.

Tr-I is located in 2L and extends from 33A to 40B. The linear arrangement is as follows: 21A to 33A/40B to 33A/40B to 33A/40B to 33A/40B to the centromere; all members of Tr-I are inverted in relation to the rest of the chromosome, i.e. not inverted in relation to each other. The total number of bands in the salivary chromosomes of Tr-I larva is 5660 in comparison with 5026 in the wild type; about 30% of the bands in 2L are represented three times.

Tr-II occurred after the treatment of males with mustard gas (Sonbati & Auerbach, 1960) and was found in the course of the cytological examination of genetically scored translocations. Tr-II accompanied a translocation which had been scored in the first brood; therefore, like Tr-I, this change must have been induced in the postmeiotic haploid germ cells, presumably in spermatozoa.

Tr-II is located in 2R and, cytologically, the sector from 41A to 44D is represented three times. The linear arrangement is as follows: centromere to 41A/41A to 44D/44D to 41A/41A to 44D/44D to 60. The middle member of the Tr-II is inverted. About 14% of the bands in 2R is triplicated, increasing the total number of bands in salivary chromosomes from 5026 to 5183.

Both triplications have some features in common. Both were induced by chemical mutagens in a haploid chromosome complement. Since Tr-I occurred in all salivary cells of the F_1 larva, and Tr-II in all gonadic cells of the F_1 fly, both must have been established not later than in the embryonic cells of the F_1 zygote. The most important fact is that both contain inverted segments. It is evident that actual chromosome breaks must be involved in the formation of inverted repeats; non-homologous crossing-over cannot account for the change in the original sequence of genes.

3. MODELS OF THE ORIGIN OF THE TRIPLICATIONS

The formation of a duplication requires the presence of two chromosome threads with identical breaks. The model for the origin of duplications (Slizynska, 1963*a*) postulates two latent breaks in the still-undivided chromosome; after splitting of the chromosome, these breaks open and two pairs of isochromatid breaks are obtained, from which a repeat can easily be formed (Fig. 1A–C). It is not easy to imagine a mechanism, by which a triplication could be produced in one step from a single affected chromosome, because this would require the simultaneous presence of three chromosome threads with identical breaks. There are, however, several mechanisms which may lead to the formation of a triplication by successive steps. One of them is exemplified by the origin of double-Bar from Bar. Double-Bar is a triplication of the 16A subdvision in the *X*-chromosome and is produced with a low frequency but regularly from the homozygous Bar duplication. This is usually connected with recombination of the outside markers. The occasional appearance of non-recombinant double-Bar and normal chromosome in the Bar cultures has been explained by crossing over between sister chromatids (Peterson & Laughnan, 1963). This involves oblique, but homologous pairing of duplicated segments.

Origin of Tr-I. Tr-I could have been formed in this way, i.e. by the following

steps (Fig. 1 A–E). (A) Induction by TEM of two latent breaks in the still undivided chromosome. (B) The opening of latent breaks in sister chromatids and the formation of the inverted duplication and the complementary deficiency. (C) The formation of two daughter cells, only one of which—that carrying the duplication—survives; as the maximum length for a viable heterozygous deficiency in the third instar larvae was found to be about eighty bands (Slizynska, 1957), the complementary deficiency for more than 200 bands would almost certainly be cell-lethal. (D) Oblique, but homologous crossing-over between duplicated segments in the sister chromatids. (E) Crossing-over resulting in mosaic tissue: Tr-I in the salivaries, and the complementary inversion—presumably located in the posterior parts of the larva.

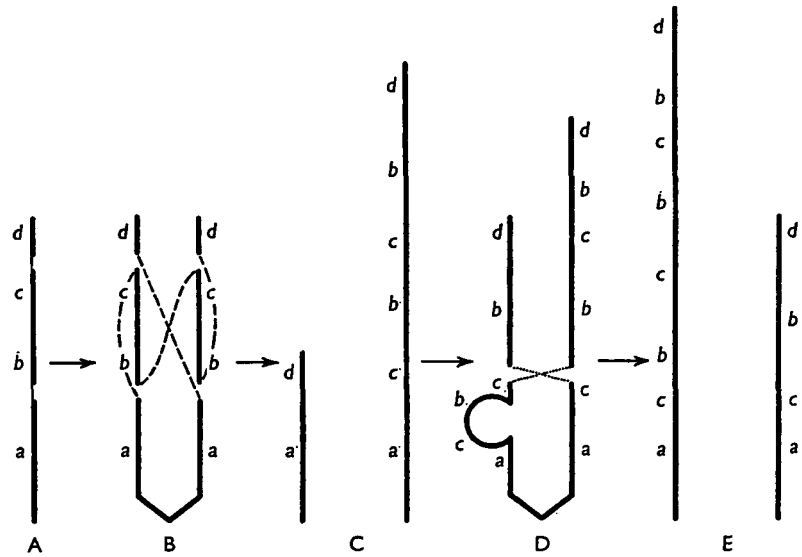

Fig. 1. The formation of Tr-I. Oblique, but homologous sister chromatid crossing-over following the formation of a duplication.

Origin of Tr-II. While all triplications with the sequence *a–bc–bc–bc–d* as in double-Bar, or *a–cb–cb–cb–d* as in Tr-I, can be explained by the model shown in Fig. 1, Tr-II, in which only the middle segment is inverted, cannot be accounted for in the same way. A duplication-repeat postulated as above would have the sequence *a–bc–cb–d*. Sister chromatid crossing-over in such duplication will produce dicentric and acentric chromatids, as in the case of a heterozygous inversion. Since Tr-II could not arise from the duplication formed as the intermediate step, the mechanism involving rejoining of breaks between three chromosome threads must be looked for.

The model which may explain the origin of Tr-II is presented in Fig. 2. In this model, the locations of the '*bw*' gene and its normal allelomorph are marked; the reason for this will be explained later. The successive steps (Fig. 2, A–E) are as follows. (A) Induction of two latent breaks by mustard gas. (B) Interchromosomal

somatic crossing-over, prior to the opening of breaks; two latent breaks are transferred to the normal, untreated chromatid. (C) Two daughter cells are formed; one of them carries the latent breaks on both chromosomes. (D) Splitting of the chromosomes, opening of breaks and the formation of Tr-II. (E) Two daughter cells are formed; the homozygous deficiency will be lethal, only the cell with the triplication will survive. The mosaic will be formed at stage 'C'; as all gonadic cells contained Tr-II and the normal chromosome, the anterior part of the F_1 fly presumably contained two normal chromosomes. There are, however, additional difficulties in applying this model in the case of Tr-II, because this particular triplication was found in the translocation (T2–3), which was first scored genetically. Therefore,

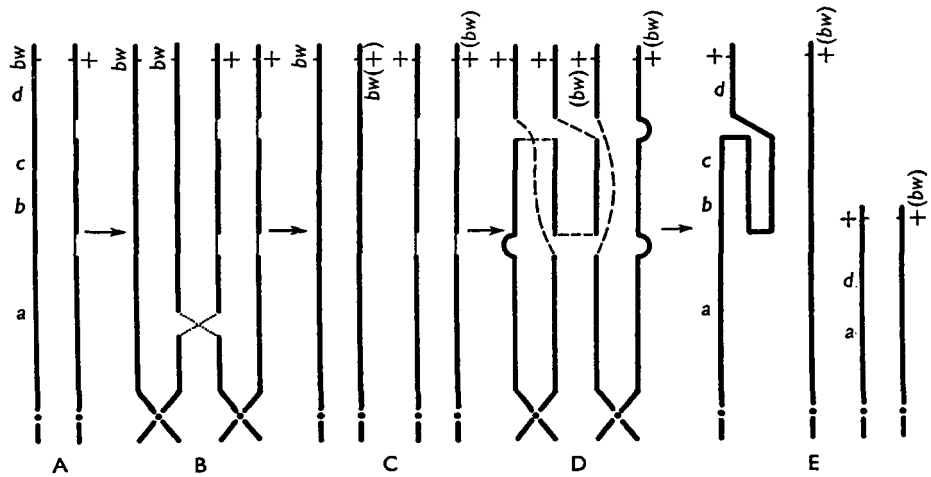

Fig. 2. A model by which the origin of Tr-II can be explained. Crossing-over takes place prior to the opening and rejoining of latent breaks. This model can account for the origin of any type of triplication, by assuming the appropriate kinds of rejoining between broken ends. A, untreated normal chromosome and the treated one with two latent breaks. B, Crossing-over transferring both latent breaks to the untreated chromatid; two pairs of sister chromatids are now identical: each has one normal chromatid and one with two latent breaks. C, After the segregation of chromatids, one of the daughter cells carries latent breaks on both chromosomes. D, Splitting of the chromosomes, opening and rejoining of breaks; as a result, one centromere carries Tr-II and a deficiency, the other a deficiency and a normal chromatid. E, Two daughter cells: one carrying a triplication and a normal chromosome, the other a homozygous deficiency.

the F_1 male must have had in his gonadic cells one 2nd chromosome carrying T2–3 and Tr-II, and its normal homologue carrying '*bw*', one of the marker genes used in the translocation tests. It is easy to see that, without the second crosssing-over between the '*bw*' locus and the distal break, the '*bw*' gene would not be present at all in the gonadic cells. This additional crossing-over is not marked in Fig. 2; instead the symbols in brackets, (*bw*) and (+), indicate the results of such exchange. As the segment between '*bw*' and the distal break represents more than half of the total length of 2R, the occurrence of an additional crossing-over in such

a long segment is quite probable. The model illustrated in Fig. 2 receives some support from a case found in one of the TEM experiments and shown in Fig. 3. The F_1 male larva, son of a TEM-treated father, carried two changes in the same chromosome: an inverted repeat involving about 90 bands and located in the proximal half of 2L, and a translocation (T2–Y) with the break in 2L near the free end (Fig. 3, I*a*). Three types of cell (Fig. 3, II*a*, *b* and *c*) were found in the salivary glands of this larva. All nuclei carried the repeat, but only some of them (II*a*) contained both translocated chromosome segments; the remaining two groups of cells were aneuploid for one or the other half of T2–Y (II*b* and *c*). This kind of mosaic can be obtained only if interchromosomal somatic crossing-over has transferred the translocation break of 2L into an untreated chromatid; the details can be followed easily on the diagram.

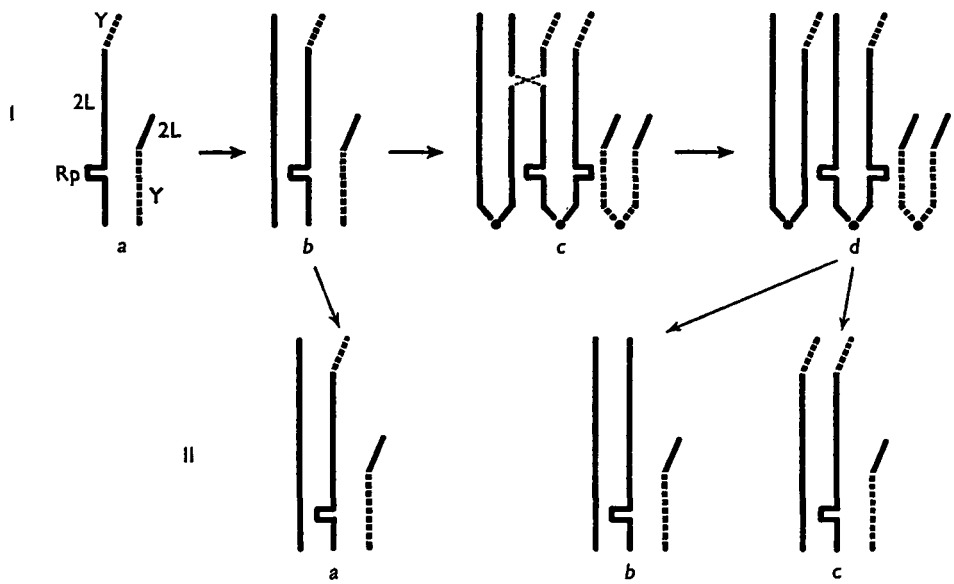

Fig. 3. I. (*a*) Treated spermatozoon carrying a repeat and T2L–Y. (*b*) F_1 zygote; untreated *X*-chromosome is omitted from the drawing; two lines of cells are formed: II*a*—without crossing-over, and Ic—in which somatic crossing-over occurred. (*c*) Crossing-over between untreated and treated 2L chromatids. (*d*) The result of crossing-over: part of the translocation is transferred to the untreated chromatid. II. (*a*) Line of cells without crossing-over. (*b*), (*c*) Two types of aneuploids resulting from crossing-over.

The assumptions made in formulating the model shown in Fig. 2 are not unreasonable. First, somatic crossing-over is not uncommon; in addition, the postulated crossing-over has to be located between the proximal break and the centromere; that is, in the region where somatic crossing-over is most frequent (Stern, 1936; Kaplan, 1953). Secondly, in contrast to X-rays, many chemical mutagens, including mustard gas, induce delayed mutations and chromosome breakage (Auerbach, 1949, 1951; Auerbach & Moser, 1953). At the same time, chemical

mutagens—but not X-rays—frequently induce repeats (Slizynska, 1963*b*, and unpublished data).

Only some triplications can, and probably do arise in the way shown in Fig. 1; but most or all triplications arising *de novo* may be explained by the model presented in Fig. 2. This model seems to be more plausible than the alternative, unsupported assumption that in the male pronucleus or in the embryonic cells the affected chromosome consists of four half-chromatids with identical breaks which rejoin independently.

4. DISCUSSION

In the preceding interpretations chromosome breakage had to be accepted as the first step in the formation of the triplications in question. Chromosome breaks, however, are rarely mentioned in the literature in connexion with the origin of repeats; the most frequent interpretation offered is that tandem duplications arise by occasional pairing and crossing-over between non-homologous segments. Several points may be raised in connexion with this hypothesis. It is known that strict homology is obligatory in meiotic pairing and crossing-over; it is observed in salivary chromosomes and used there as the means of detecting structural changes. Wherever the pairing takes place, homology is the general rule. In addition, if crossing-over is to follow, pairing must be 'effective' (exchange pairing); the superficial, distributive pairing (Grell, 1964), similar to that observed in the meiotic divisions in Drosophila males (Slizynski, 1964), would not be sufficient. In view of the fact that precise homology is a necessary condition for the pairing of chromosome segments, it is difficult to imagine a mechanism which would induce a chromosome segment not only to abandon its normal pairing affinity, but also to acquire the ability for intimate pairing with a structurally dissimilar segment.

Reported cases of non-homologous crossing-over lend themselves to alternative interpretations. There is always a possibility that when pairing affinity is shown by the apparently non-homologous segments of the chromosome, one is actually dealing with an old repeat. This is exemplified by the relations between *zeste* and *white* loci (Gans, 1953; Green, 1959, 1961). It has been shown that the manifestation of the zeste phenotype depends upon two doses of w^+, and that its expression is influenced by some mutant alleles of white. This functional relationship is accompanied by the infrequently but regularly occurring pairing of the zeste and white regions, followed by crossing-over. As a result, identical deficiencies and complementary duplications for the chromosome segment between *zeste* and *white* are produced. This parallelism between function and pairing affinities led Green to the conclusion that most probably these two loci represent a repeat. Although this does not preclude the possibility that also genuine cases of non-homologous crossing-over may be found, proof for them is difficult to obtain.

Even granting that recombination between non-homologous chromosome segments is possible, the question arises to what extent such mechanism can be responsible for the origin of repeats. There is not much doubt that all inverted repeats, including the two triplications described here, *must* have originated from

chromosome breaks. It has been found that 14 out of 43 different formaldehyde-induced repeats, i.e. about one-third, were of this type (Slizynska, 1963*a*). If inverted and uninverted repeats are formed with similar frequencies from breaks, the origin of at least two-thirds of cases can be accounted for by breaks. The remaining one-third could have arisen by non-homologous crossing-over; but they can be explained just as well by the chromosome breaks. It must be concluded therefore that chromosome breaks rather than non-homologous pairing and crossing-over constitute the major source of repeats. It should be stressed that the above conclusions refer to repeats longer than one or two bands in the salivary chromosomes, which probably involve several genes or gene complexes. It is not impossible that a different mechanism might be responsible for the formation of very small repeats duplicating only part of a gene, such as Smithies, Connel & Dixon (1962) have assumed to have occurred in the evolution of haptoglobins.

It was mentioned that many repeats, some of them inverted, are known to be established in nature. This raises the question of 'spontaneous' breaks. That such breaks are not always rare is indicated by the high rates of 'spontaneous' chromosomal aberrations occurring occasionally in nature. Levitan (1962), who described an aberration-inducing factor in one of the laboratory strains of *Drosophila robusta*, quotes several cases reported in the literature. Recently several instances of extreme chromosome damage induced by viruses have been reported. An interesting fact, which may have some bearing on the formation of repeats, emerged from the work on chromosome damage induced by the measles virus in human cells *in vitro*. Nichols, Levan, Aula & Norby (1964) observed that many cells exhibited extreme fragmentation of the chromosomes. In addition, however, some cells, otherwise undamaged, showed only single chromosome breaks, usually of the delayed isolocus type. This is just the type of break on which the formation of repeats depends; if such cells multiply, they may form a source of 'spontaneous' repeats.

SUMMARY

Two triplications induced in a normal halpoid chromosome complement by TEM (Tr-1) and by mustard gas (Tr-II) are described. Two models which may lead by successive steps to the formation of a triplication are suggested. The induction of two latent breaks is essential in both of them. The origin of repeats in general is discussed. It is concluded that chromosome breaks rather than non-homologous crossing-over constitute the major source of repeats.

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Fig. 1. Karyotype of the male *Bufo regularis regularis* Reuss.

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