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Chromosomal basis of dosage compensation in *Drosophila*

II. The DNA replication patterns of the male *X*-chromosome in an autosome—X insertion in *D. melanogaster**

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SUMMARY

The functional morphology and the replication pattern of the male *X*-chromosome in an autosome-*X* insertion stock (*T*(1;3) 05) of *Drosophila melanogaster* have been examined. In larval salivary glands carrying this insertion neither the enlargement and pale staining of the single male *X*, nor the characteristic early completion of replication cycle, as revealed by ^{3}H-*TdR* autoradiography is in any way changed. The normal properties of the inserted autosomal segment are also unaltered. The results appear to support a 'piecemeal' type of dosage compensation mechanism in *Drosophila* operating through the male.

1. INTRODUCTION

Evidence has already been presented to suggest that enlargement and hyperactivity of the polytene *X*-chromosome in male larval salivary glands of *Drosophila melanogaster* is the chromosomal basis of dosage compensation, and that this hyperactivity is independent of sex differentiation (Lakhotia & Mukherjee, 1969). The present communication provides support for the idea that dosage compensators are not limited to any particular region of the *X*-chromosome in *Drosophila*—a suggestion derived originally from genetic studies using various *X*-autosome translocations and duplications or deficiencies of small segments of the *X* (see Muller, 1950). Previous cytological studies on *X*-autosome translocations (Schultz, 1965; Muller & Kaplan, 1966) have shown that the functional morphology of the male *X*, i.e. the pale staining and enlargement, remains unaltered following translocation.

Dosage compensation in mammals is achieved by the inactivation of one of the two *X*'s of the female (Lyon, 1961; Russell, 1961) and this process is thought to be controlled by an 'inactivation centre'. Autosomal material transferred to the neighbourhood of this centre becomes inactivated (Ohno & Cattanach, 1962; Russell, 1963; Russell & Montgomery, 1965). The inactive *X*-chromosome is also late replicating and in translocations or insertions the autosome may also acquire this late replicating property (Evans *et al.* 1965). If the hyperactivity of the *X* in male *Drosophila* were also controlled by one or several closely grouped controlling centres, the *X*-autosome translocations would be expected to disturb the con-

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tinuity of action of the controlling centre(s). In such a situation the two outstanding attributes of the male X in larval salivary glands—namely hyperactivity (Lakhotia & Mukherjee, 1969) and early replication (Berendes, 1966; Rodman, 1968; Arcos-Terán & Beermann, 1968; and present observations)—should also be disturbed. Conversely, if a piece of autosome is inserted in the male X, the inserted segment should become hyperactive and early replicating under the influence of the hyperactive male X.

2. MATERIAL AND METHODS

The translocation stock used was $T(1;3)$ 05, $D/+$ & $yf :=$. For abbreviations and a cytological description of the translocation see Lindsley & Grell (1968). This stock has a piece of the right arm of the third chromosome ($3R$) inserted into the X. A pericentric inversion is also associated with the third chromosome in the stock used but this in no way influences our study.

Mature male third instar larvae were used for salivary gland chromosome studies. The width of the regions proximal and distal to the autosomal insertion (X^p and X^d) was measured by the method of Mukherjee, Lakhotia & Chatterjee (1968); so too was the width of the inserted autosomal section when in an asynapsed condition. The replicative behaviour of both the X and the inserted autosome was studied by the use of tritiated thymidine (^3H-TdR) labelling. For this purpose excised salivary glands from male larvae were incubated for 20 min in *Drosophila* Ringer containing 3 μci of ^3H-TdR (Sp. Act. 5·7 ci/mM) and autoradiographed by the usual technique with Kodak AR 10 Stripping Film. The exposure time was 20 days. For a comparison with the normal X, excised salivary glands from male larvae of Oregon $R+$ wild-type stock of *D. melanogaster* were also autoradiographed by the same technique.

3. OBSERVATIONS AND DISCUSSION

The enlargement and pale staining of the male X—both distal and proximal to the insertion—are very similar to the standard male X. The inserted segment of $3R$ is also similar to its homolog in the standard Oregon $R+$ stock. This holds true whether the inserted segment pairs, or fails to pair, with its homolog (see Plate 1, figs. *a*, *b*). The mean ratio of the width of the two regions of the $X(X^p/X^d)$ is 1·01 (S.E. = $\pm$ 0·026; N = 30) and the mean ratio of the width of the inserted autosome segment, when in an asynapsed condition, to that of whole X-chromosome is 0·70 (S.E. = $\pm$ 0·012; N = 30). The two ratios (1·01 and 0·70) are significantly different from each other ($P < 0{\cdot}001$). Note that a very similar value (0·69 $\pm$ 0·023) has already been obtained in normal *D. melanogaster* for the width of an asynapsed autosome and the single male X (Mukherjee, A. S. *et al.* 1968).

The labelling pattern of the X-chromosome in the insertion stock was studied in detail in about 50 nuclei selected on the basis of clear recognizability of the different chromosomal segments. The labelling pattern in the X-chromosome of the insertion stock observed in the present study is precisely the same as that

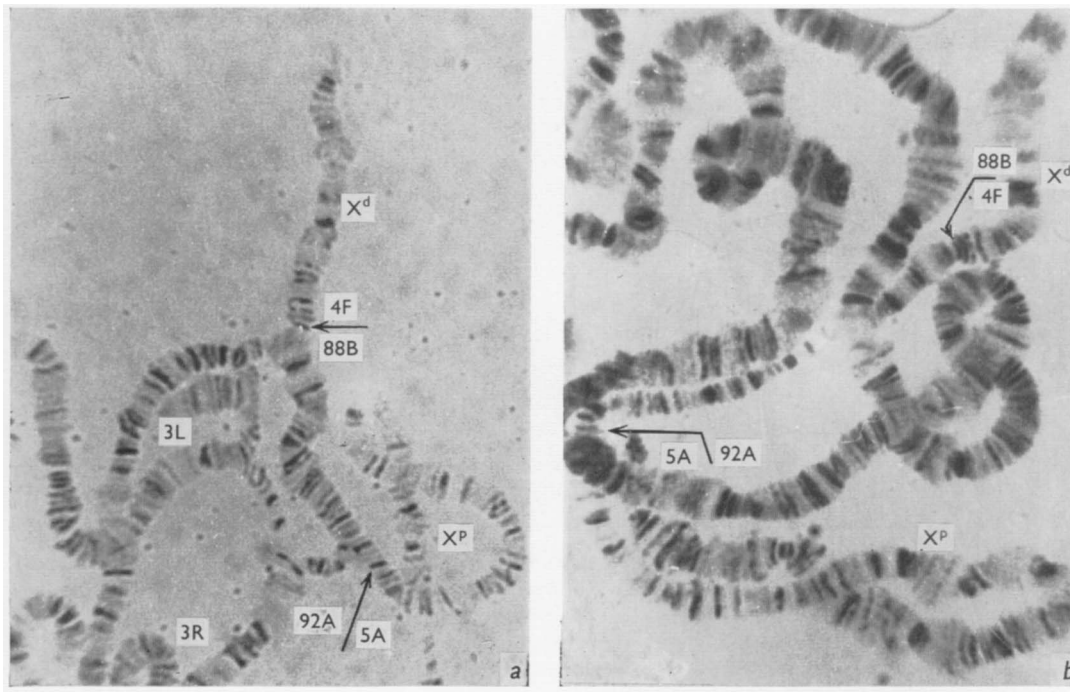

Plate 1. The salivary gland chromosomes of $T(1;3)05$ male larvae. (*a*) The inserted autosomal segment, indicated by band numbers and arrows, is paired with its homolog; the pericentric inversion in the third chromosome is also shown. (*b*) The inserted autosomal segment is unpaired and is distinctly less wide, and more deep staining, than the single male X.

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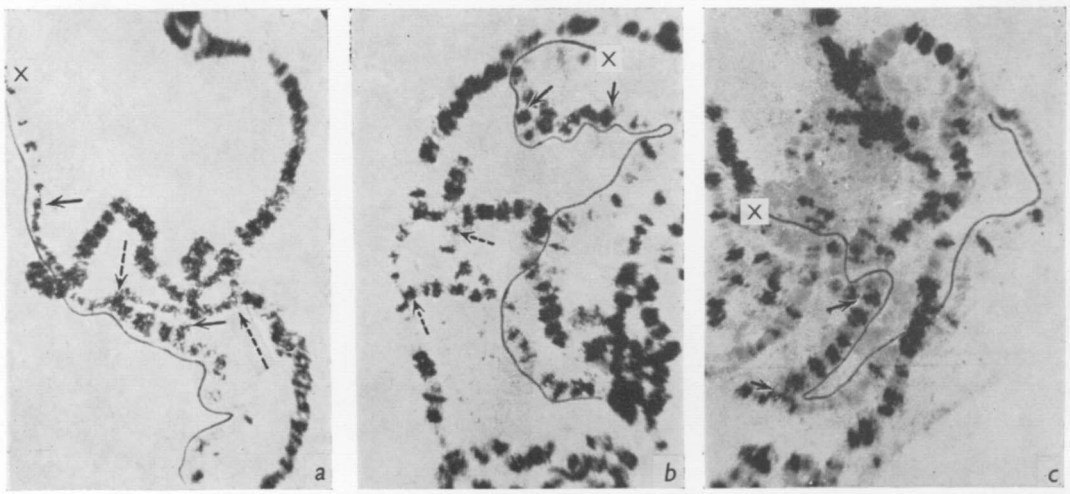

Plate 2. ^{3}H-*TdR* labelling patterns in salivary gland chromosomes of $T(1;3)05$ male larvae. Solid line indicates the orientation of the X; solid arrows the inserted autosomal segment and broken arrows the non-translocated homolog of the insertion in unpaired condition. (*a*) Autosomes and the insertion are continuously labelled, the X shows discontinuous label. The insertion is partly paired with its homolog. (*b*) and (*c*) Autosomes and the insertion showing discontinuous label, while the X shows label at still fewer sites. In (*b*) the inserted segment is completely unpaired, in (*c*) completely paired. Note the similarity of labelling patterns in the inserted segment and its homolog.

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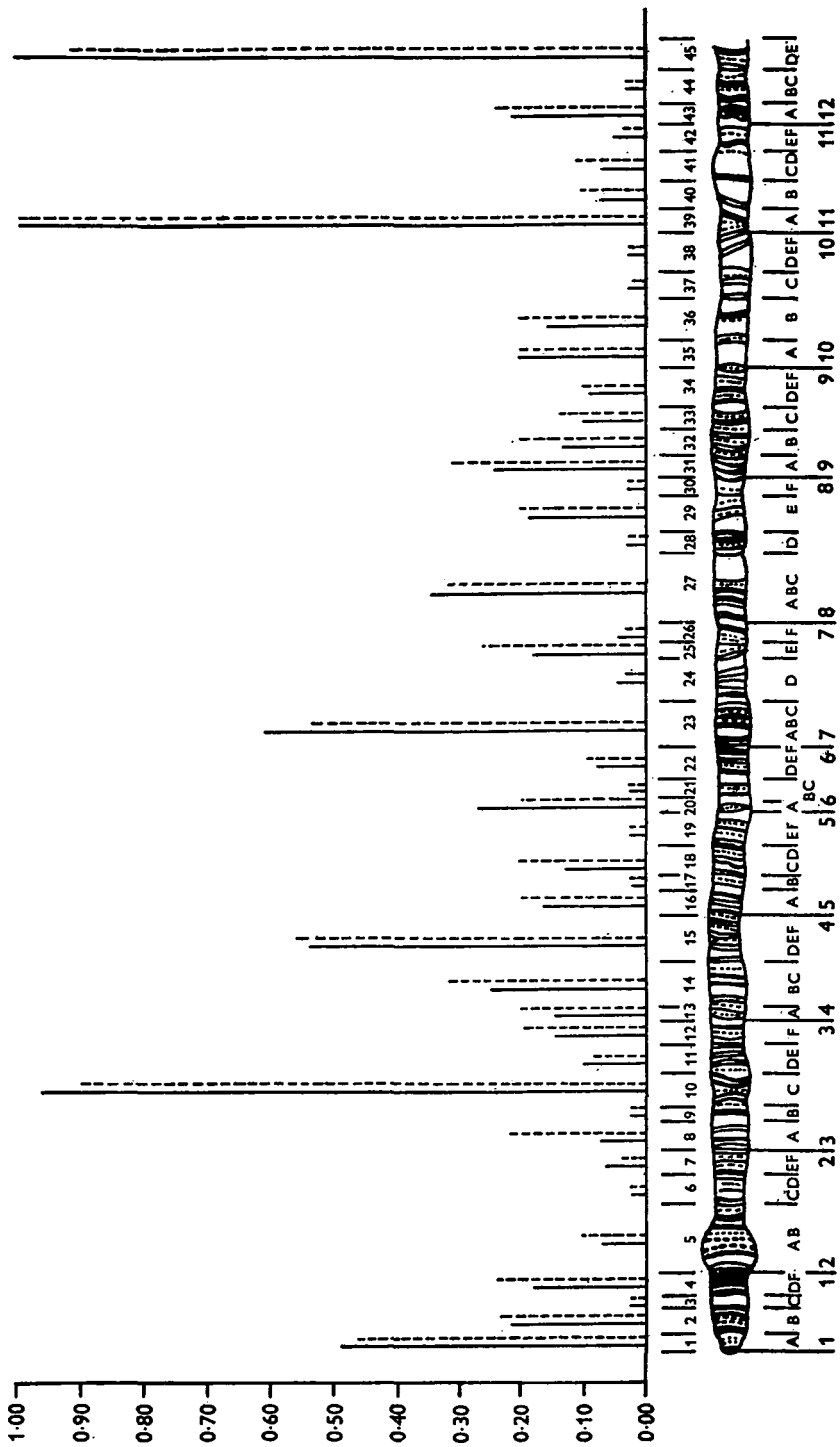
reported by Arcos-Terán & Beermann (1968) in the normal, non-translocated *X*-chromosome of male *D. melanogaster*.

Earlier observations by Berendes (1966), confirmed here, show that the male *X* in larval salivary glands exhibits a distinct asynchrony in its replication. Similarly, in the insertion stock it has been observed that in nuclei where the autosomes, including the inserted segment, are continuously labelled, the *X*-chromosome very often shows distinctly discontinuous labelling both proximal and distal to the autosomal insertion (Plate 2*a*). On the other hand, in all cases where the inserted autosomal segment and the other autosomes in the nucleus are discontinuously labelled, the male *X* invariably shows labelling at still fewer sites (Plate 2*b*, *c*). Significantly, the pattern of labelling of the inserted autosomal segment is always identical to that of the non-translocated homolog, whether the two are paired (Plate 2*a*, *c*) or unpaired (Plate 2*b*). An asynchrony in the replication of the proximal and distal regions of the *X* was never observed. The replication cycles of both male *X* and the autosomes thus persist unmodified following insertion.

For a precise comparison of the replicative behaviour of the *X*-chromosome in the present insertion stock with that in the standard male *X*, labelling frequency of the various sites and the total number of grains on the *X*-chromosome (from section 1A to 12E of Bridges's map (Bridges, 1935)) in different types of labelling patterns were scored for both the *T*(1;3) 05 and Oregon *R*+ male *X*'s. From the same nuclei, labelling frequency of the different sites and the total number of grains on the region 56F to 60F of the right arm of the second chromosome (2*R*) were also scored. On the *X*-chromosome 45 different sites were made out between 1A to 12E which could be seen to have independent labelling (see Text-fig. 1*a*); on the 2*R* (56F–60F) 20 sites (Text-fig 1*b*) were delineated after Nash & Bell (1968). Text-fig. 3*a* presents the labelling frequency of the 45 sites on the *X* in Oregon *R*+ (71 labelled nuclei) and the insertion stock (44 labelled nuclei). Text-fig. 1*b* shows the labelling frequency of the 20 sites on 2*R* in the two stocks. It should be noted here that to consider any site as labelled a minimum of three grains was taken as the lower limit. It is clear that in general the frequency of labelling of the different sites in the *X* as well as the 2*R* is similar in both the cases. The minor variations that are there may be attributable to the difference in the number of nuclei analysed in the two stocks and possibly also to some slight difference in the larval age. That the differences are not due to the insertion as such is borne out by the fact that some variations are also noticeable in the labelling frequency of different sites of the 2*R* in the two stocks.

A comparison of the ratios of total number of ^3H-*TdR* grains on the 2*R*(56F–60F) and the *X*(1A–12E) in normal and insertion stock also demonstrates the similarity of the replication pattern. Text-fig. 2 shows the mean 2*R*/*X* grain ratio plotted against different labelling patterns of the *X*. In both the normal and the insertion *X*-chromosome the 2*R*/*X* grain ratio shows a strikingly similar increase as the number of labelled sites on the *X* decreases. In this respect both of these differ from the female *X*'s, where the 2*R*/*X* grain ratio remains more or less constant throughout the replication cycle (S. C. Lakhotia, unpublished). This inverse



Text-fig. 1*a*

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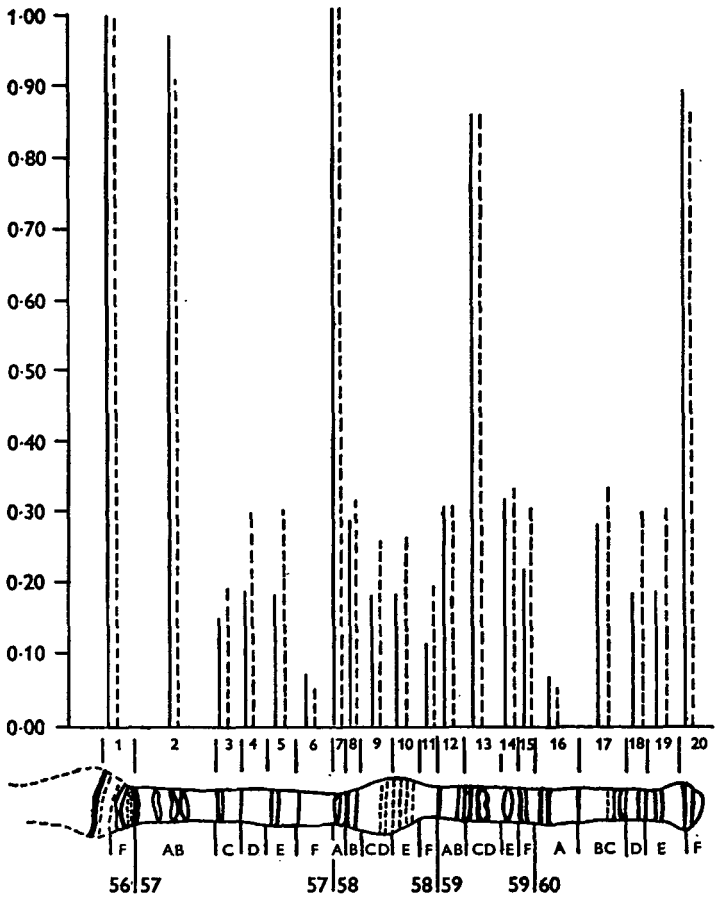


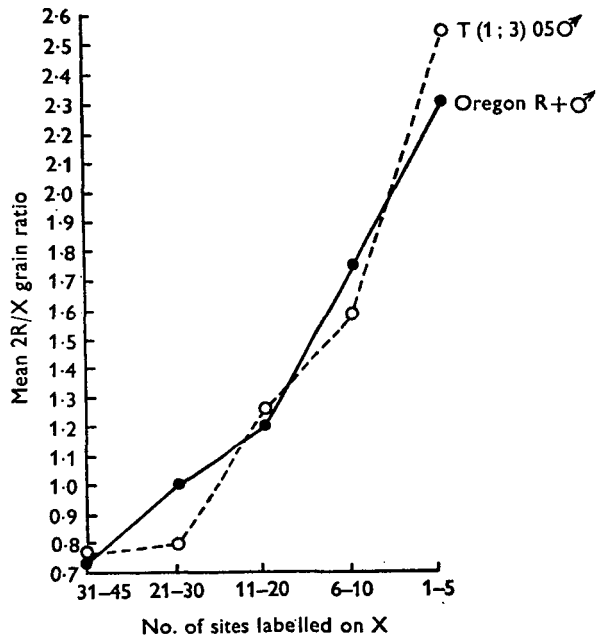
Text-fig. 1*b*

Text-fig 1.(*a*) ^{3}H-*TdR* labelling frequency of the 45 different sites on the *X*-chromosome from section 1A to 12E of Bridges's map. Solid line = Oregon *R*+ male *X*; broken line = *T*(1; 3) 05 male *X*. (*b*) ^{3}H-*TdR* labelling frequency of the 20 different sites on the terminal region of 2*R* (section 56F to 60F of Bridges's map). Solid line = Oregon *R*+ male and broken line = *T*(1; 3) 05 male.

relationship between the number of labelled sites and the $2R/X$ grain ratio in the male suggests the interpretation that, after simultaneous initiation of replication in all the chromosomes, the *X*-chromosome in the male replicates faster, so that in the male at any *subsequent moment* in the S-phase more units have completed their replication, as compared to the female *X*'s. This results in *comparatively* reduced incorporation of ^{3}H-*TdR* by the male *X* as the replication cycle advances.

The early completion of the replication cycle by the male *X* has an important bearing on the mechanism of dosage compensation in *Drosophila*. There is an inverse relation between genetic activity and the condensation of the chromosome. In general, the condensed and late replicating material is considered to be genetically inactive (Schultz, 1965; Lima-de-Faria & Jaworska, 1968; Barigozzi, 1968; Mukherjee, B. B. *et al.* 1968). The *X*-chromosome in the larval salivary glands of

male *Drosophila* has been shown to be more diffuse and more active in RNA synthesis than the single X's of the female (see Lakhotia & Mukherjee, 1969). The early completion of replication by the male X also attests to its hyperactivity. This implies that the mechanisms underlying enlargement and hyperactivity are operative at the level of DNA organization in the polytene chromosomes (see Berendes, 1966). This is further suggested by observations that agents which selectively reduce the width of the male X-chromosome (e.g. X-rays, see Mukherjee, A. S. *et al.* 1968) also cause specific alteration in its replication pattern (S. C. Lakhotia, unpublished).



Text-fig. 2. Mean $2R/X$ ^3H-TdR grain ratio in different labelling patterns of the male X in normal and insertion stock.

Our findings lead one to presume that the hyperactivity and the property of early replication of the male X in *Drosophila* are not achieved by a process similar to 'wholesale' inactivation of one of the X's in female mammals. In *Drosophila* the same end result, namely the equality of expression of sex-linked genes in the two sexes, is arrived at 'piecemeal' (see Muller & Kaplan, 1966). However, contrary to Muller's hypothesis of repression in the female, the 'piecemeal' mechanism is thought to operate through the male. Each X-linked gene is influenced by specific compensator loci distributed throughout the X-chromosome and possibly also the autosomes (Cock, 1964), in such a way that they are activated to an identical level in the two sexes (Lakhotia & Mukherjee, 1969).

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