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Chatterjee, R.N. University of Calcutta, India. Changes of DNA replication pattern of the polytene chromosomes of **Drosophila melanogaster** resulting from chromosomal rearrangements.

Bender et al. (1971) showed that replication of a duplicated region of polytene chromosome is changed as a result of a rearrangement. A similar situation has also been noted by Kalisch & Haegele (1973) who also observed that, at comparable replication phase, the labelling intensity of duplicated subdivision is different from those of homologous subdivisions in +/+ chromosomes even when the different DNA amount of both genotype was taken into consideration. Ananiev & Gvozdev (1974), on the basis of their work on DNA replication in a Eu-heterochromatin rearrangement of Drosophila, also indicated that the replicative behaviour of a replicating unit of the transposed region is altered from the parental type. The following sets of experiments were therefore carried out to characterize the factor or factors presumably responsible for determining the replication pattern in greater detail by genetically dissecting the units. For this pur-
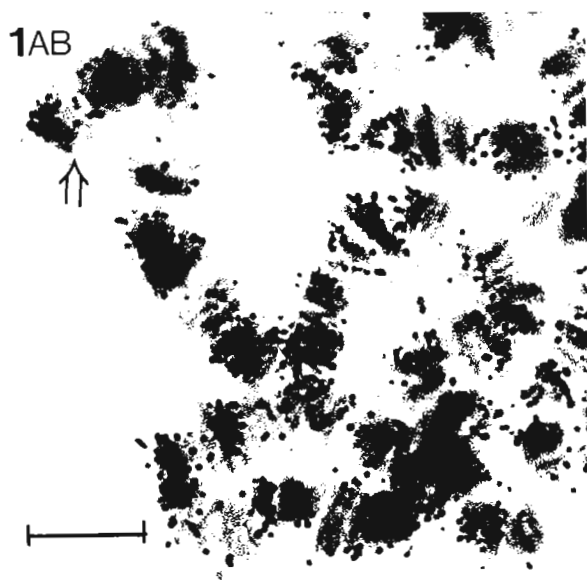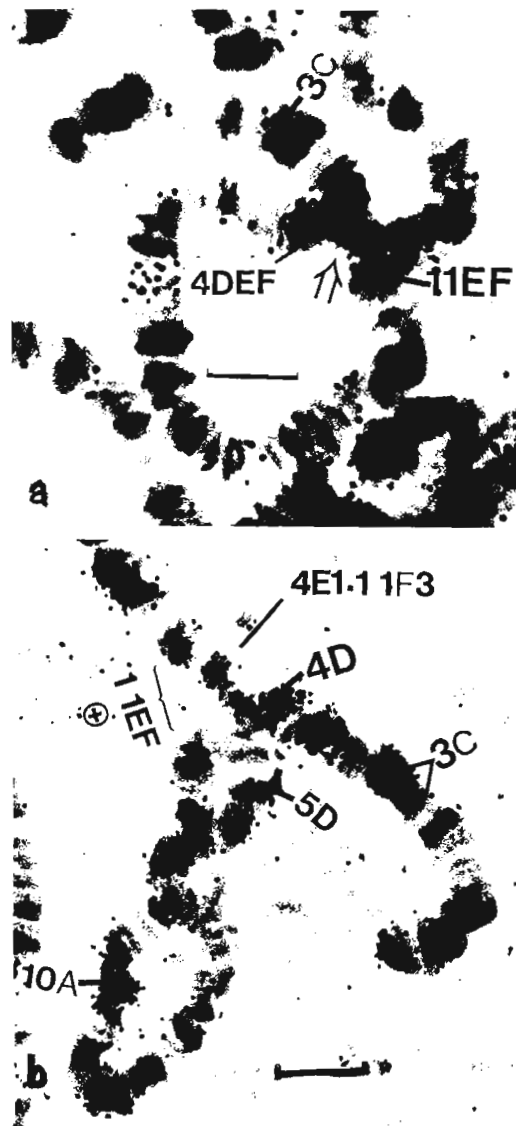

Figure 1. 3H-TdR labelled autoradiogram of inversion heterozygote In(1)d149, 1(1)J1/+, showing the 3D pattern in 1(1)J1/+. Arrow indicates asymmetric distribution of label at 1AB site. Scale indicates 10 μm.

Figure 2. 3H-TdR labelled autoradiograms of the X chromosomes of the inversion heterozygote In(1)d149, 1(1)J1/+. (a) An early pattern in In(1)d149/+. Arrow indicates absence of label at the point of break. (b) A terminal pattern in In(1)d149/+. Arrow points to the asynapsed homologues segments, one of which is unlabelled and the other labelled. Scale indicates 10 μm.
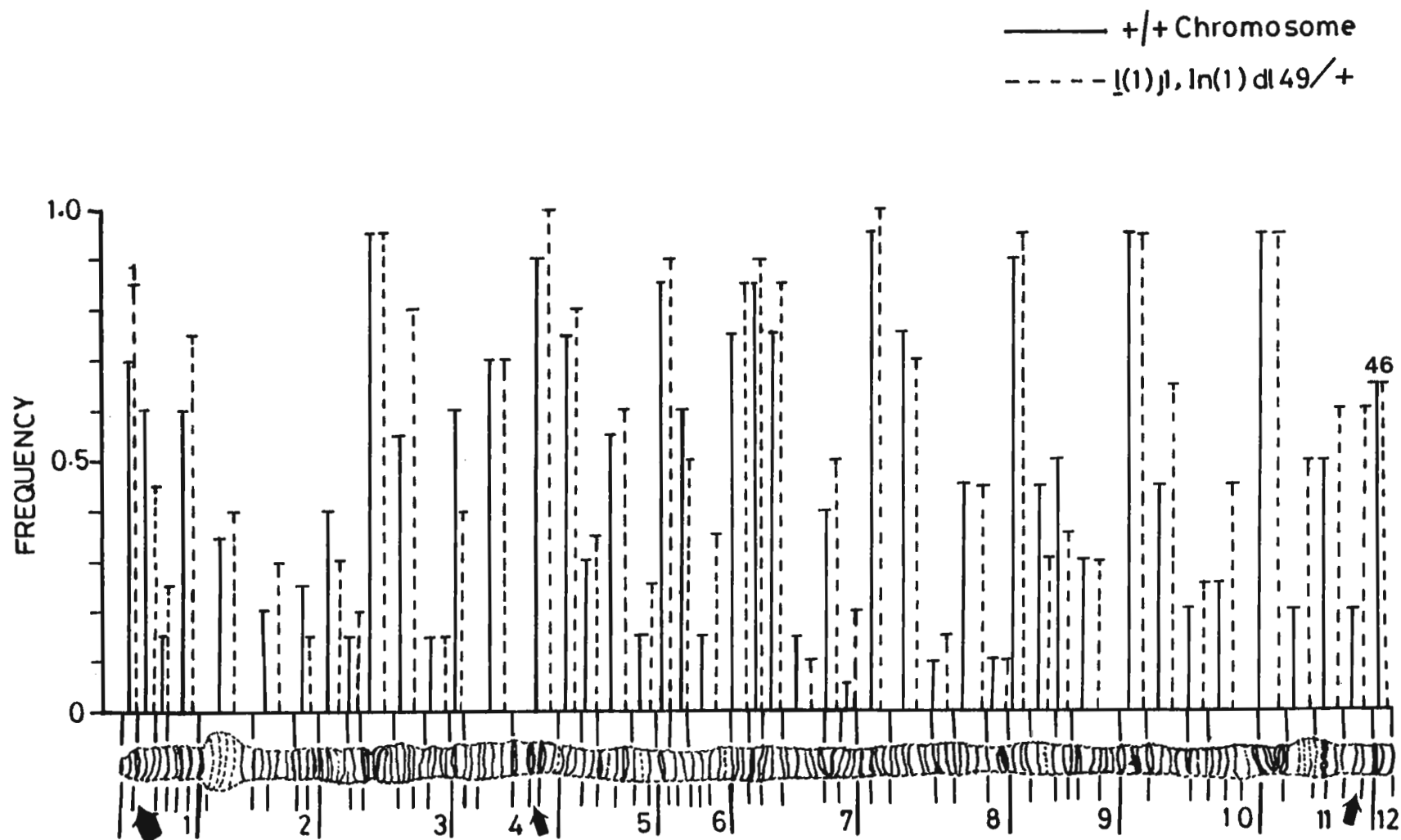
Figure 3. Histograms showing the frequencies of labelling of different replicating units in the normal and 1(1)J1, In(1)d149 bearing X chromosomes. Arrows at the bottom indicate the break points. Number 1 and 46 represent first and 46th replicating units.
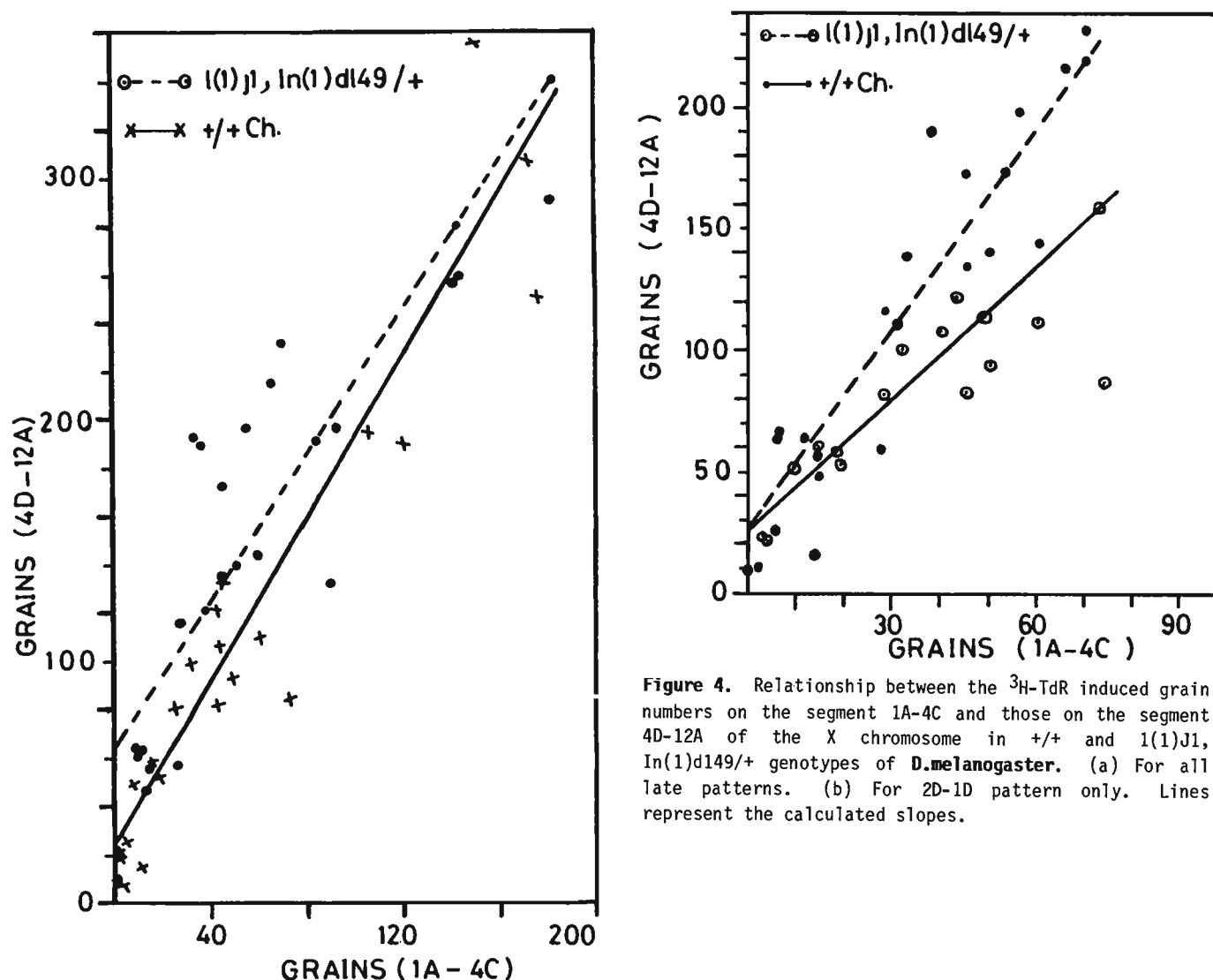
Figure 4. Relationship between the ^{3}H-TdR induced grain numbers on the segment 1A-4C and those on the segment 4D-12A of the X chromosome in +/+ and l(1)J1, In(1)d149/+ genotypes of **D.melanogaster**. (a) For all late patterns. (b) For 2D-1D pattern only. Lines represent the calculated slopes.

pose, DNA replication in genetically altered sequences such as those involving breaks within a replicating unit, has been examined.

For this series of investigations, the wild type strain (Oregon R$^+$) and the inversion heterozygote In(1)J1, y w 1z^S/+ stock of **D.melanogaster** were used. Excised salivary glands from late third instar larvae were dissected out in buffered Drosophila Ringer (pH 7.2) and incubated in 10 μl of Ringer's containing 5 μCi of ^{3}H-thymidine (Conc. 500 μCi/ml, Sp. activity 6,600 mCi/ml obtained from BARC, Bombay, India). Cytological preparations of chromosomes were then made and processed for autoradiography.

In this investigation, segment 1A-12A of the X chromosome have been analyzed for their order of replication in two altered sequences from the stock 1(1)J1, In(1)d149. In inversions, 1(1)J1, In(1)d149, the regions 1A5-1B5 and 4E1-11F2 have been inverted as two independent inversions, the resulting sequences in the two regions being 1A1-4.1B5-1A5. 1B6-4D7-11F2-4E1.11F3............20. Thus, here an altered sequence has formed within the two replicating units (Lakhotia & Mukherjee 1970) 1A and 1B and this alteration involves about 8 bands. On the other hand, the unit 4DEF has been split at 4D7-4E1, the new order of replicating units being 4D7-11F2 and 4E1.11F3. These inversions have been examined only in heterozygous condition due to the presence of the lethal mutant.

The autoradiogram in Figure 1 reveal the 3D pattern at the terminal phase of replication. The distribution of the grain on the tip of the X chromosome reveals that there is clear asymmetry in the distribution of lebel on the two homologues, one of which appears to finish the replication a little earlier than the other. Since it is known that the telomeric end of the X is always late replicating, it is reasonable to assume that half of the tip part of the X chromosome which shows relatively lower labelling (Fig. 1) or absence of labelling, is the altered sequence of the chromosome, bearing 1(1)J1. One reservation to be

made, however, is that the analysis does not rule out the possibility of existence of a deletion as the cause of its slight early finishing.

Figures 2a and b present an initial and a terminal labelling pattern, respectively, of the paired X chromosomes in In(1)d149/+ heterozygote. No study on the homozygous female bearing the inversion has been possible as the inversion bearing X also carries a lethal on the tip of it. In figure 2a, which shows an early pattern, the unit 4DEF, being late replicating is unlabelled in both homologues, whereas 11EF, being early replicating is labelled completely across the bands. In contrast, in Figure 2b (in late phase of replication) the 4D is completely labelled on both homologues, while 4F-4E1.11F3 segment is labelled on the inverted segment but in the normal homologue the 11EF part is unlabelled. The 4EF section is also labelled in the normal homologue (hidden under 4D in the band of the loop in Fig. 2b).

The histogram of the frequencies of labelling [obtained from 22 labelled nuclei of middle to late pattern of replication, each in control and 1(1)J1, In(1)d149/+ genome] presented in Figure 3, corroborates the observations on the autoradiogram. Here in the inversion bearing chromosome, the frequency of 4D1-7 (to the left of the arrow on the segment 4) remains unaltered, whereas that of 4EF.11F3 drastically increases by a factor of about 3. Thus, it appears that in this case the late replicating property is inherent in the 4EF section. It may be noted that some other sites (namely 6DEF, 7F, 9C, 10B, 10DEF and 11B) also show some difference in frequency from normal.

No data on intensity of site-wise labelling has been presented for this inversion heterozygote, as such intensity data may be incorrect due to introduction of change in geometrical configuration of the segments involved which might arise out of pairing in the inversion heterozygote. However, a total grain count data over 1A-4BC, which is outside the inversion, and that over 4D-12A which includes the inversion, has been presented in Figures 4a and b. It is interesting to note there that when analysis is made considering all middle to terminal pattern labelled chromosomes (3D-1D type), the regression slopes do not show significant deviation. However, when the analysis is delimited by considering only the grain range of 0-90, a limit up to which no super-imposition or "coincidence" is detectable, the regression slope of grain number in +/+ and the inversion heterozygotes significantly deviated from each other (Fig. 4b). This finding is a strong evidence to support that the alteration in the sequence of the constituent subunits of the replicating unit affects the terminal patterns more significantly than the initial patterns. The results of the present investigation have therefore revealed that the rearrangement of polytene chromosomes might affect the kinetics of DNA synthesis by establishing new patterns of control.

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Effect of α-amanitin on the DNA synthesis in the polytene chromosome of **Drosophila melanogaster**.

In the giant cells of dipteran larvae, α-amanitin, a potent inhibitor of eukaryotic RNA polymerase II, inhibits chromosomal RNA synthesis but not the nucleolar RNA synthesis at a concentration range of 20 to 30 μg/ml (Beermann 1971; Chatterjee & Mukherjee 1982, 1984). α-amanitin is also known to inhibit DNA synthesis in eukaryotes (Montecuccoli et al. 1973; Chatterjee & Mukherjee 1977). It has been suggested that the toxin, α-amanitin inhibits the initial and middle part of S phase probably through the inhibition of the primer RNA. An investigation was therefore undertaken to find out whether the inhibition of DNA synthesis by α-amanitin is causally related to the inhibition of RNA polymerase II by the inhibitor. For this reason, parallel pulse labelling with ^{3}H-UR and ^{3}H-TdR of the two contralateral glands of a pair was performed.

For this series of investigations, salivary glands from late third instar larvae of **Drosophila melanogaster** were dissected out in buffered Drosophila Ringer (pH 7.2) and incubated for 20 minutes in α-amanitin (Conc. 20 μg/ml). One gland from the pair was transferred to ^{3}H-UR (300 μCi/ml, Sp. activity 7500 mCi/mM obtained from BARC, Bombay, India) containing Ringer and the remaining one was transferred to ^{3}H-Tdr Ringer (Conc. 500 μCi/ml, Sp. activity 6500 mCi/m mole, obtained from BARC, Bombay, India). Cytological preparations of chromosomes were then made and processed for autoradiography.

Results of these experiments are presented in Table 1. Since it was noted that the amatoxin fails to produce a selective inhibition of DNA synthesis only in male or only in female nuclei, the data from both sexes have been pooled together. In Table 2, the glands receiving ^{3}H-TdR were designated as 'A' (contralateral gland) and those receiving ^{3}H-UR, in the contralateral counterparts were coded as 'B'. Data presented in Table 1, show that as it was observed earlier (Chatterjee & Mukherjee 1977), α-amanitin