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Earlier evidence (1) indicated that the hyperactivity of the L chromosomes in the larval salivary glands of male Drosophila may be related to genetic dosage compensation. The three principal attributes of the male L chromosomes, namely, the increased width, high rate of RNA synthesis and faster replication (1,2,3) are probably causally interdependent. A direct demonstration of this relationship is not possible from studies on the normal glands. However, under several experimental conditions it is possible to reduce selectively the width of the normally inflated male X of the polytene nuclei of B. malayensis (2,5) and this provided an opportunity to investigate the above mentioned relationship. It has been demonstrated that consequent of X-irradiation of B. malayensis larvae, the width of the male X is reduced and comes to approximate that of the unirradiated X's of female nuclei (2).

In the present report, some aspects of the alterations induced in the replicative and transcription activities of the L chromosomes in male following X-irradiation of late third instar larvae of B. malayensis will be presented.

RNA SYNTHESIS

Oregon Re female and male larvae were irradiated with 1 ER as described earlier and sacrificed 3-4 h later. The excised salivary glands were labeled in vitro with [3H]uridine for 10 min and processed for radioautography (for details see (3)).

1. Work supported by University Grants Commission Fellowship no. F.1-85/62(EQ).
2. Present address: Cytogenetics Unit, Dept. of Zoology, University of Delhi, Delhi-7.
Earlier works (3, 5, 6) have demonstrated that the rate of DNA synthesis by the single X in males and the two X's in females is similar when compared with that by identical autosome segments in the two sexes. The data presented in Table 1 show that after X-irradiation the relative rate of DNA synthesis by the male X decreases compared to the autosome segment (3L) or the female X's. The different 3H/thymidine grain ratios in 1-3R male nuclei are consistently higher than those observed in 1-3R female nuclei (Table 1). These higher ratios in 1-3R male nuclei may be due either to an increased rate of DNA synthesis by the autosome segment, or to a depressed rate by the male X. The latter alternative is more likely since the 3H/thymidine grain ratios in 1-3R female nuclei remain the same as in normal female. This implies that the relative rate of 3H-thymidine uptake by the female 3L or the X's remains unaltered due to X-rays. This is to be expected since there is no nitrocellulose in the functional morphology either of the 3L or the X's in irradiated female.

The irradiation of third instar larvae (Oregon R) were X-irradiated with 130 kR as above and

3H-thymidine labeled salivary glands at different time intervals after X-irradiation, viz. 1, 2, 3, 6, and 8 hr later. The details of autoradiographic processing and the parameters used to evaluate the replicative organization have already been described in detail (4) for the normal male and female (5) and the same were used in the present experiments. For the sake of brevity, only one of the data will be presented here.

The detailed replication patterns of the 3-chromosome in D. melanogaster under normal conditions in male and female have been described (1) and the patterns observed in the present studies reveal very striking differences from the normal ones. The observed labeling patterns in 1-3R male at 1, 2, and 6-7 hr interval are presented in Tables 2 and 3, respectively. In ordering these patterns, it is assessed that replication begins simultaneously in all the 'replication sites' in the chromosomes, but notches at different times - so that, continuous labeling patterns after a pulse of 3H-thymidine.
patterns are seen in the initial 3-segment (left-hand side column in Tables 2 and 5) and discontinuous patterns in the later parts of the 5 (right-hand side column in Tables 2 and 5). Furthermore, it is assumed that these in an interval, the synthesis of 5A (in time vector) at any site, so that a site should not appear unlabeled at a period in the 5 when the 5A synthesis is not yet completed. Such patterns (c), however, sometimes observed to the normal except that they are termed 'exceptional' patterns on the basis of the above assumption (for further discussion see (1)).

It may be noted that in Tables 2 and 5 that at both the intervals after fertilization, the labeling patterns observed for the 2N segment can be arranged in occasional arrays beginning with continuous labeling and ending with discontinuous labeling with only few 'exceptional' patterns. The labeling patterns on 2N in 2K site normal are similar to those observed in the normal male. In the case of the late fertilization, however, the situation is different. Whereas, in the normal site, the 'exceptional' patterns were recorded for the X, in the 2K male normal the 'exceptional' patterns are much more frequent, especially in the 6-7th series. An analysis of these exceptional patterns in the 2K normal reveals that (a) in 1-2 K sera most common normal patterns are more frequent in the heavy discontinuous type of pattern (i.e., with 16 to 30 sites labeled on the 2K arm), while the late discontinuous patterns were similar to those observed in the normal male; (b) in 2K series (detailed data not included here) the exceptional patterns are seen in heavy discontinuous as well as in none of the late discontinuous patterns, and (c) in 6-7th series, on the other hand, the frequency of the exceptional patterns is very high and these are present in all the types of the patterns; in fact, very few of the labeled patterns (for 2N and 2K-segments) in 6-7th series male are identical to those observed in the normal male normal. It may also be noted that at 6-7th interval, several 'exceptional' sites (e.g., 15, 20, 25, 30, 40, 50, 60, 70, 90, 120, 130, 160, 180, 200), which in normal male were observed to be labeled only in males, where the 22-segment was continuously labeled (1), are now seen to be labeled in males in which the 22-segment shows discontinuous labeling.

Similarly, sites like 45, 60, 60, etc., were seen to be labeled after fertilization (2-3 hours) in males with very low doses.
normal male, nuclei with similar id labeling, these L-chromosomal sites appear unlabeled. Furthermore, whereas in normal male, the nuclei with 3 sites on the X (30,151,1200; 360) and 1 site on the 2 (36,101,1200; 30) and 3 sites on the 3 (30,151,100) labeled (5;3 pattern) are most frequent; this is not so in the irradiated male nuclei. In 1-2n series, 12 out of the 26, in 2-4n series, only 3 out of the 56, and in 4-7n series, only 2 out of the 58 labeled nuclei exhibited this pattern. It is worth noting here that in normal female glands too, this particular pattern (5;3) is very rare.

A comparative analysis of the present data and those obtained earlier for the normal male shows that in any of the observed nuclei in 1 ER male, the number of sites labeled on the X in relation to the number of sites labeled on the 28-segment is greater than that noted for the normal male. One such example is presented in Fig. 1. Here, the labeling pattern on the 28 in normal male (Fig. la) and 1 ER male (Fig. lb) is similar, yet the number of labeled sites on the X in 1 ER male is much higher than that seen on the normal male.

The significance of these data becomes apparent when considered in the light of the replicative organization of polytene chromosomes in normal conditions. It is now well known that the replication of chromosomes in a nucleus is co-ordinated and temporally controlled, and follows a specific sequence of labeling patterns (1,7,19), and that under normal conditions the replicative behavior of the polytene chromosomes in normal male shows very specific differences from that in female (1,9). The fact that the observed pattern on the 28-segment in 1 ER male and normal male are very similar to each other, suggests that the replicative organization of the 28-segment is not much altered due to irradiation. But the L-chromosomes in the same male nuclei show different labeling patterns in the 1 ER series. These alterations are indicative of the disturbed function organization of the male I. The deviations in the replicative behavior of the male I after 1 ER may be explained if we assume that (a) 2 irradiation delays or slows down the rate of DNA synthesis on the different sites on the male X; (b) the rate of DNA synthesis on the 28 may either be unaffected, or if the 28 is also affected, that on the X is slowed down to a greater degree; and (c) that the effect of X-rays on the 28
X in male has been induced by "dosage compensators" to do "extra" work, which, in Drosophila, may be represented by inhibitors of chromosomal activity; the observations also speak against a repressive action of the compensator gene in female since under this situation the metabolic activity of the male X would not be expected to be repressible under the action of inhibiting agents. It, however, remains to be seen whether alongwith the depressed RII and DNA synthesis by the male X, there is also a corresponding decrease in the activities of the enzymes determined by X-linked genes.

ACKNOWLEDGMENT

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REFERENCES

## Table I

Comparison of Relative Radiometric Incorporation of \( \text{L} \)-chromosomes in Male and Female Polytene Nuclei after \( \text{I} \)-Labeling

<table>
<thead>
<tr>
<th></th>
<th>( \overline{X} / X ) Mean</th>
<th>( S / X ) Mean</th>
<th>( S / X ) S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>0.90 ( \pm ) 0.06</td>
<td>6.60 ( \pm ) 0.02</td>
<td>0.94 ( \pm ) 0.03</td>
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<tr>
<td>(50)</td>
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<tr>
<td>Males</td>
<td>1.21 ( \pm ) 0.05</td>
<td>0.55 ( \pm ) 0.03</td>
<td>0.54 ( \pm ) 0.02</td>
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\( M \) = region of the left arm of chromosome \( 3 \) from band 51c to 52d;
\( S \) = region of the left arm of chromosome \( 3 \) from band 61b to 61d;
\( D \) = region of \( \text{L} \)-chromosome from band 20p to 31p;
\( D \) = region of \( \text{L} \)-chromosome from band 10p to 30d;
\( Y \) = region of \( \text{L} \)-chromosome from band 39 to 11.

Numbers in parentheses indicate the number of nuclei examined.
TABLE 2
Ordered Sequence of Labeling Patterns in Different 1/18 Hole Model (1.2 b Series)

<table>
<thead>
<tr>
<th>Labeling</th>
<th>Labeling patterns in different model (Each vertical column represents either one modified section on the X and Y.)</th>
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+ Indicates presence of labeling; -, absence of labeling; \#, presence of uncorrected labeling; 0, absence or corrected labeling.
<table>
<thead>
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<th>Table 3</th>
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<tr>
<td>Ordered Sequence of Labeling Patterns in Different Modules (6.2 mm Sections)</td>
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<table>
<thead>
<tr>
<th>Labeling Patterns in different module (Each vertical column represents one combined pattern on the 2 and 29.)</th>
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<tr>
<td>+ Indicates presence of labeling; - absence of labeling; $\phi$, presence of unexpected labeling; 0, absence of expected labeling.</td>
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... (continues)
Fig. 1: Representative $^3$H-TdR labeling patterns (HEAVY DISCONTINUOUS) in Normal (Fig. 1a) and 1 XR (Fig. 1b) male nuclei. The arrows point to the unlabeled sites on the 2R segment. In both nuclei the 2R segment has 16 sites labeled, but the X in 1 XR nucleus shows many more sites labeled.

Fig. 2: $^3$H-TdR labeling frequencies of selected sites on the X and 2R segments in Normal and 1 XR male nuclei.
V. C. Shah: 1. Do you really imply that size of k chromosomes involved have nothing to do as far as viability to radiation concerned but were the genetic activity irresponsible?

2. Have you studied RNA synthesis of the loci which are hit?

S. C. Lakhota: 1. Yes; this seems to be the case.

2. No; we are now trying to do this.