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REPLICATIVE AND TRANSCRIPTIVE ACTIVITIES OF THE POLYTENE X-CHROMOSOME OF MALE DROSOPHILA MELANOGASTER AFTER X-IRRADIATION[1]

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Earlier evidence (1) indicated that the hyperactivity of the X-chromosome in the larval salivary glands of male Drosophila may be related to genetic dosage compensation. The three principal attributes of the male X-chromosome, 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 D.melanogaster(2,4) and this provided an opportunity to examine the above mentioned relationship. It has been demonstrated that consequent of X-irradiation of D.melanogaster larvae, the width of the male X is reduced and comes to approximate that of the asynapsed X's of female nuclei(2). In the present report, some aspects of the alterations induced in the replicative and transcriptive activities of the X-chromosome in male following X-irradiation of late third instar larvae of D.melanogaster will be presented.

RNA SYNTHESIS :

Oregon R+ female and male larvae were irradiated with 1 KR as described earlier(2) and sacrificed 3-4 h later. The excised salivary glands were labeled in vitro with ^{3}H-uridine for 10 min and processed for radioautography (for details see,(3)).

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Earlier works[3,5,6] have demonstrated that the rate of RNA synthesis by the single X in male and the two X's in female is similar when compared with that by identical autosomal segments in the two sexes. The data presented in Table 1 show that after X-irradiation the relative rate of RNA synthesis by the male X decreases compared to the autosomal segment (3L) or the female X's. The different 3L/X ^{3}H-uridine grain ratios in 1 KR male nuclei are consistently higher than those observed in 1 KR female nuclei (Table 1). These higher ratios in 1 KR male nuclei may be due either to an increased rate of RNA synthesis by the autosomal segment, or to a depressed rate by the male X. The latter alternative is more likely since the 3L/X grain ratios in 1 KR female nuclei remain the same as in normal female. This implies that the relative rate of ^{3}H-uridine uptake by the female 3L or the X's remain unaltered due to X-rays. This is to be expected since there is no noticeable in the functional morphology either of the 3L or the X's in irradiated fem

DNA SYNTHESIS :

Late third instar larvae (Oregon R+) were X-irradiated with 1 KR as above and sacrificed for *in vitro* ^{3}H-thymidine labeling of salivary glands at different time intervals after X-irradiation, viz., 1-2h, 2-4h, and 6-7h later. The details of radioautographic processing and the parameters used to evaluate the replicative organization have already been described in detail for the normal male and female[1] and the same were used in the present experiments. For the sake of brevity, only some of the data will be presented here.

The detailed replication patterns of the X-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 KR male at 1-2h and 6-7h interval are presented in Tables 2 and 3, respectively. In ordering these patterns, it is assumed that replication begins simultaneously at all the 'replicon' sites [illegible] chromosomes, but finishes at different times - so that, continuous labeling patterns after a pulse of ^{3}H-thy

midine are seen in the initial S-period (left-hand side columns in tables 2 and 3) and discontinuous patterns in the later parts of the S (right-hand side columns in tables 2 and 3). Furthermore, it is assumed that there is an uninterrupted synthesis of DNA (in time vector) at any site, so that a site should not appear unlabeled at a period in the S when its DNA synthesis is not yet completed. Such patterns are, however, sometimes observed in the normal nuclei and they were termed 'exceptional' patterns on the basis of the above assumptions (for further discussion see, (1)). It may be noted in Tables 2 and 3 that at both time intervals after X-irradiation, the labeling patterns observed for the 2R segement can be arranged in sequential arrays beginning with continuous labeling and ending with discontinuous labeling with only few 'exceptional' patterns. The labeling patterns on 2R in 1 XR male nuclei are similar to those observed in the normal male. In the case of the male X-chromosome, however, the situation is different. Whereas, in the normal male, few 'exceptional' patterns were recorded for the X, in the 1 XR male nuclei the 'exceptional' patterns are much more frequent, especially in the 6-7h series. An analysis of these exceptional patterns in the 1 XR nuclei reveals that : (a) in 1-2 h series such exceptional patterns are more frequent in the heavy discontinuous types of nuclei (i.e., with 16 to 20 sites labeled on the 2R-segment), while the late discontinuous patterns are more similar to those observed in the normal male; (b) in 2-4h series (detailed data not included here) the exceptional patterns are seen in heavy discontinuous as well as in some of the late discontinuous patterns, and (c) in 6-7h 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 combined patterns (for X and 2R-segments)in 6-7h series male are identical to those observed in the normal male nuclei. It may also be noted that at 6-7h interval, several X-chromosomal sites (viz., 1C,2CD,3B,5B,8D,10DEF,12BC), which in normal male were observed to be labeled only in nuclei where the 2R-segment was continuously labeled (1), are now seen to be labeled in nuclei in which the 2R-segment shows discontinuous labeling. Similarly, sites like 4EF, 7ABC, 8ABC etc., are seen to be labeled after X-irradiation (6-7hlater) in nuclei with very late disco[illegible]

normal male, nuclei with similar 2R labeling, these X-chromosomal sites appear unlabeled[1]. Furthermore, whereas in normal male, the nuclei with 5 sites on the 2R (56F,57AB,58A,59CD and 60F) and three on the X (3C,11A and 12DE) labeled (5;3 pattern) are most frequent, this is not so in the irradiated male nuclei. In 1-2h series, 12 out of the 56, in 2-4h series, only 3 out of the 45, and in 6-7h 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[1].

A comparative analysis of the present data and those obtained earlier for the normal male shows that in many of the observed nuclei in 1 KR male, the number of sites labeled on the X in relation to the number of sites labeled on the 2R-segment is greater than that noted for the normal male. One such example is presented in fig. 1. Here, the labeling patterns on the 2R in normal male (fig. 1a) and 1 KR male (fig. 1b) is similar , yet the number of labeled sites on the X in 1 KR male is much higher than that seen on the normal male X.

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 ordered, and follows a precise sequence of labeling patterns[1,7,8], and that under normal conditions the replicative behaviour of the polytene X-chromosome in Drosophila male shows very specific differences from that in female[1,9]. The fact that the observed patterns on the 2R-segment in 1 KR and normal male are very similar to each other, suggests that the replicative organization of the 2R-segment is not much altered due to X-irradiation. But the X-chromosome in the same male nuclei shows different labeling patterns in the 1 KR series. These alterations are indicative of the disturbed functional organization of the male X. The deviations in the replicative behaviour of the male X after 1 KR may be explained if we assume that : (a) X-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 2R may either be unaffected, or if the 2R is also affected, that on the X is slowed down to a greater degree; and (c) that the effect of X-rays on the ma

X in male has been induced by 'dosage compensators' to do 'extra' work, which, in D.melanogaster, may be repressed by inhibitors of chromosomal activity; the observations also speak against a repressive action of the compensator genes in female since under this situation the metabolic activities 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 RNA and DNA synthesis by the male X, there is also a corresponding decrease in the activities of the enzymes determined by X-linked genes.

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TABLE V

COMPARISON OF RELATIVE ^{3}H-URIDINE INCORPORATION BY X-CHROMOSOME IN MALE AND FEMALE POLYTENE NUCLEI AFTER X-IRRADIATION

	MEAN 3L/X GRAIN RATIO ± S.E.		
	$3L/X^p$	$3L/X^d$	$3L^f/X^f$
FEMALE	0.90±0.06 (20)	0.64±0.02 (20)	0.44±0.03 (20)
MALE	1.21±0.05 (28)	0.88±0.03 (34)	0.54±0.02 (34)

3L = region of the left arm of chromosome 3 from band 68B to 62A;

$3L^f$ = region of the left arm of chromosome 3 from band 61F to 61A;

X^p = region of X-chromosome from band 20F to 11A;

X^d = region of X-chromosome from band 10F to 3C;

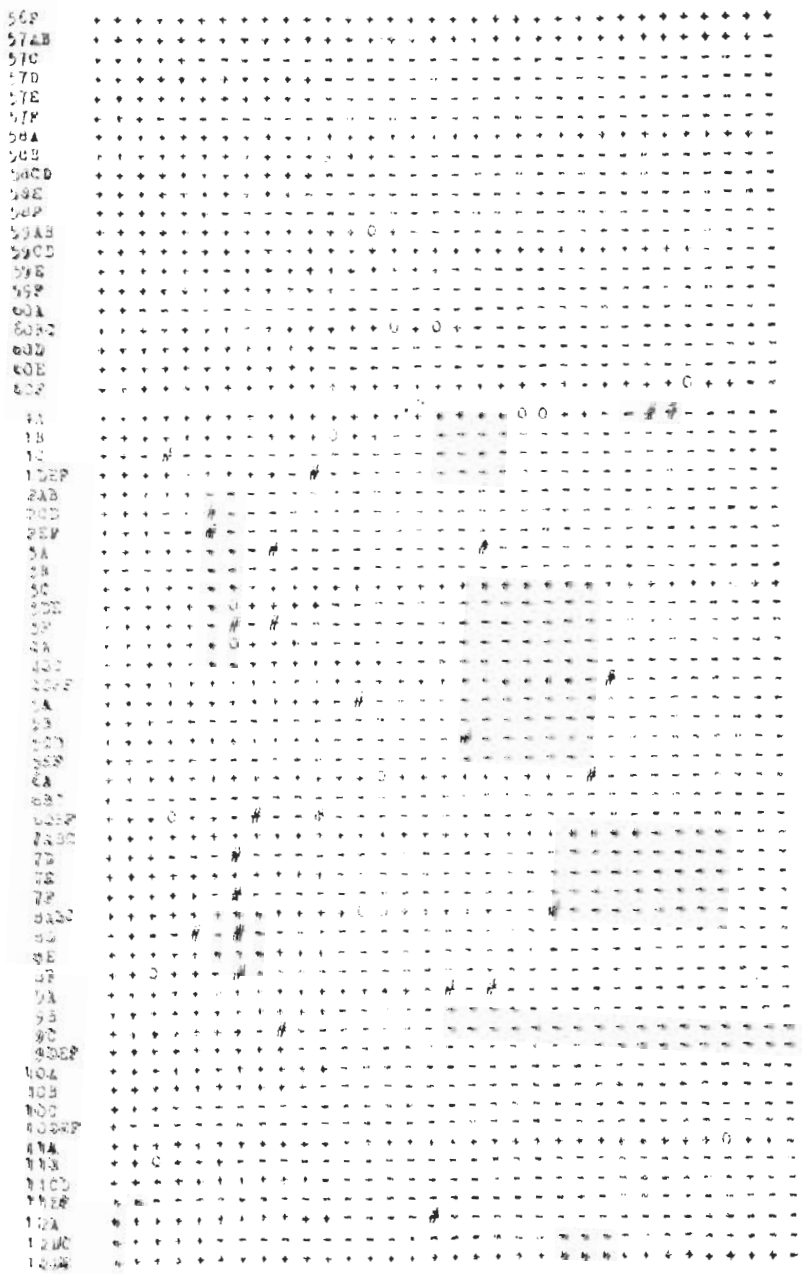
X^f = region of X-chromosome from band 3B to 1A.

Numbers in parentheses indicate the number of nuclei examined.

TABLE 2.

Ordered Sequence of Labeling Patterns in Different 1 KR Male Nuclei (1-2 h Series)

Labeling sites	Labeling patterns in different nuclei (Each vertical column represents one combined pattern on the X and 2R)

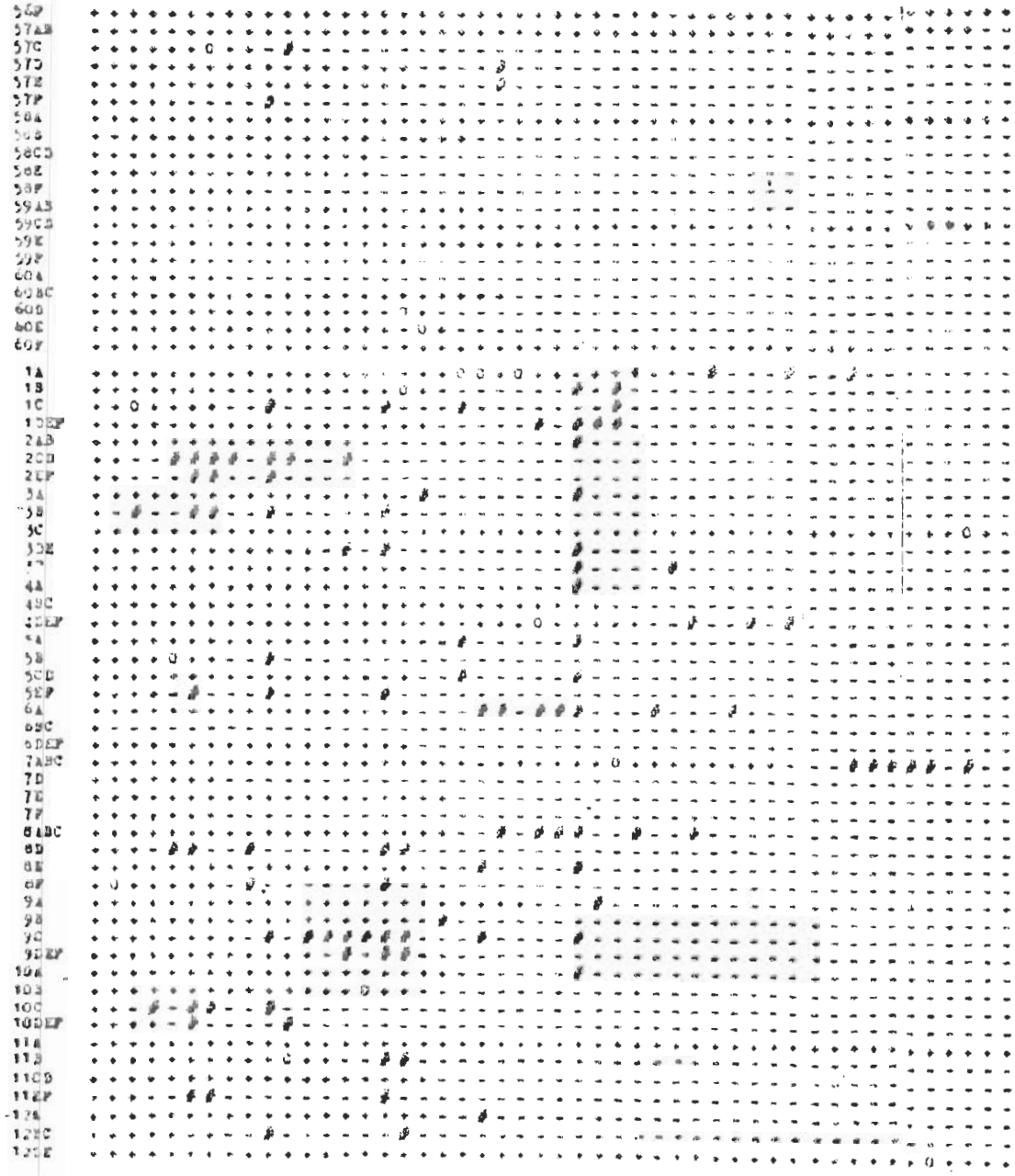


+ indicates presence of labeling; -, absence of labeling; #, presence of unexpected labeling; 0, absence of expected labeling.

TABLE 3

Ordered Sequence of Labeling Patterns in Different 1 KR Male Nuclei (6-7 h Series)

Labeling sites	Labeling patterns in different nuclei (Each vertical column represents one combined pattern on the X and ZH)



+ indicates presence of labeling; -, absence of labeling; #, presence of unexpected labeling; O, absence of expected labeling.

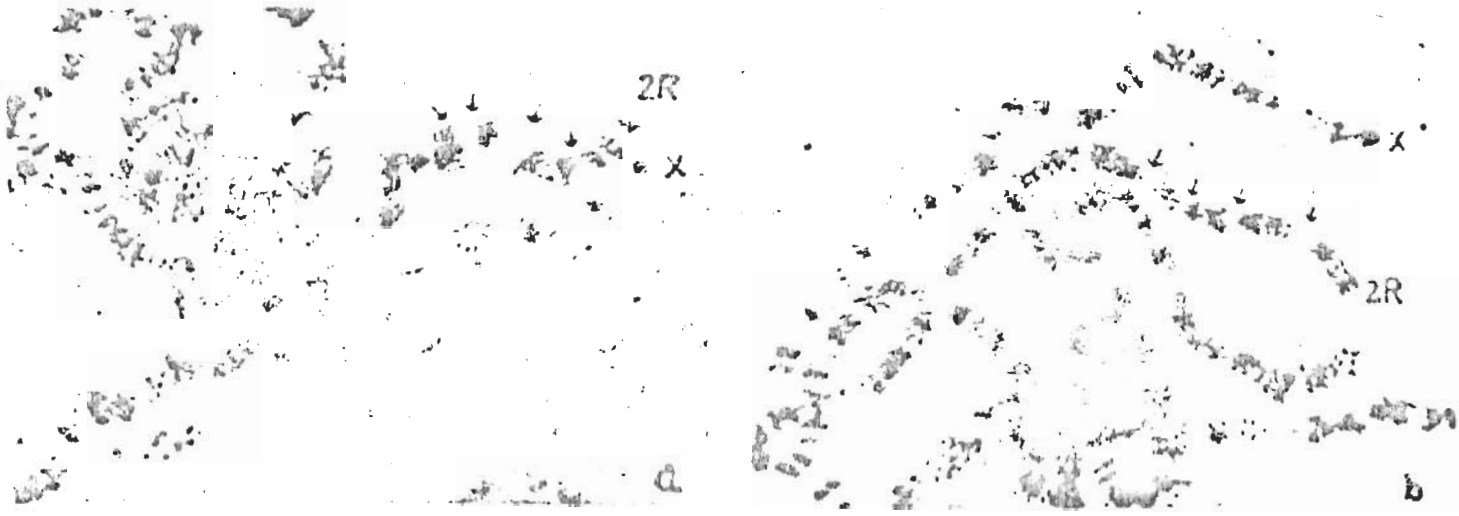


Fig. 1: Representative ^{3}H-TdR labeling patterns (HEAVY DISCONTINUOUS) in Normal (Fig. 1a) and 1 KR (Fig. 1b) male nuclei. The arrows point to the unlabeled sites on the 2R-segment. In both nuclei the 2R-segment has 16 sites labeled, but the X in 1 KR nucleus shown many more sites labeled.

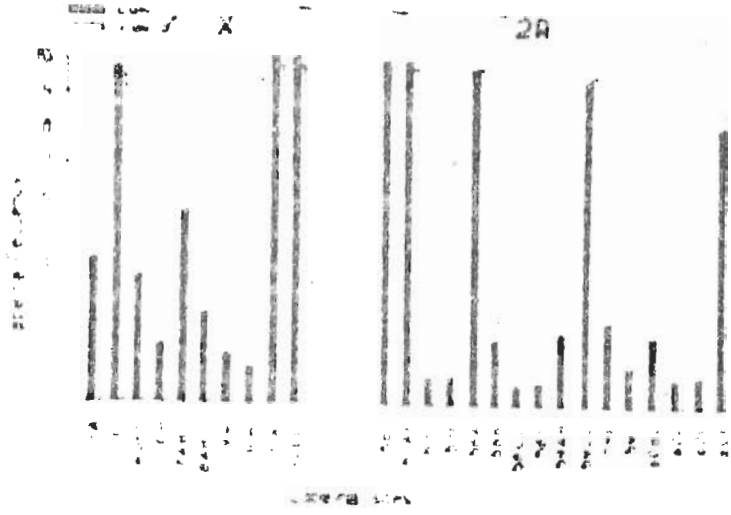


Fig. 2: ^{3}H-TdR labeling frequencies of selected sites on the X and 2R-segments in Normal and 1 KR male nuclei.

DISCUSSION

V. C. Shah : 1. Do you really imply that size of x-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. Lakhotia : 1. Yes; this seems to be the case.

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