CHROMOSOMAL BASIS OF DOSAGE COMPENSATION IN *DROSOPHILA*, VI. TRANSCRIPTION AND REPLICATION IN MALE X-CHROMOSOME OF *D. MELANOGASTER* AFTER X-IRRADIATION

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The three principal attributes of *Drosophila* polytene X-chromosome in male, namely, increased width, high rate of RNA synthesis and faster DNA replication, are probably causally interdependent and related to genetic dosage compensation (see Lakhotia and Mukherjee 1970). A direct demonstration of this relationship is not possible from studies on the normal glands. However, an approach in this direction may be made since under certain circumstances like X-irradiation of larvae, the width of the male X is reduced to approximate that of the asynapsed X's of female nuclei (Lakhotia 1970*a*; Mukherjee *et al* 1968). In the present paper some aspects of the alterations induced in the transcriptive and replicative organization of the male X following X-irradiation of late third instar larvae of *D. melanogaster* will be presented, a preliminary account having been presented earlier (Lakhotia 1971).

Material and Methods

RNA Synthesis

Oregon R + male and female larvae, approximately 88-90h after hatching, were X-irradiated with 1 KR as described earlier (Mukherjee *et al* 1968) and sacrificed 3-4h later for *in vitro* labelling of a salivary gland with ^{3}H-uridine (^{3}H-UR) for 10 min (sp act 5·7 C/mM; activity 100 μC/ml). The labelled salivary glands were then processed for radioautography with Kodak AR 10 and exposed in dark for 20 days (see Lakhotia and Mukherjee 1969). The number of silver grains on specific areas of X and the 3L of a nucleus in male and female was scored and the 3L/X grain ratios were used as the parameter for evaluation of the transcriptive activity of the male X following X-irradiation. The entire X was considered in three parts: from section 11A to 20F (X^a), section 1A to 10F (X^b) and section 1A to 3B (X^c). The regions on the 3L were from section 62A to 63B (3L) and 61A to 61F (3L^t). The different grain ratios thus obtained were: 3L/X^a, 3L/X^b and 3L/X^c. The advantages and significance of comparing grain ratios for long and short chromosomal regions have already been pointed out by Mukherjee (1966). As the larval cultures were not fully synchronised, the preparations revealed some differences in their puffing patterns. As such, the labelled nuclei have been classified into two groups based on the puffing activity: group I nuclei correspond to the puffing stages 1-2 and the group II nuclei to the puffing stages 3-6 of Ashburner (1967).

DNA Synthesis

Oregon R + male larvae were X-irradiated with 1 KR as above and sacrificed for ^{3}H-thymidine (^{3}H-TdR) labelling at 1-2h, 2-4h and 6-7h after X-irradiation. The larval age at the time of X-irradiation was so adjusted that at the time of sacrifice, the larvae in each series were about 92h after hatching. The excised salivary glands were incubated in ^{3}H-TdR (100 μC/ml, sp act 6·7 C/mM) for 20 min and processed for autoradiography and evaluated as described earlier (Lakhotia and Mukherjee 1970). All the radioisotopes were procured from BARC, Trombay.

Observations

A. RNA Synthesis after X-Irradiation

The data on the 3L/X grain ratios for different chromosomal regions in the two groups of ^{3}H-UR labelled male and female nuclei show that the autosome-to-X grain ratios are significantly higher ($P < 0{\cdot}01$) in X-irradiated male nuclei when compared to the corresponding ratios in

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female nuclei (table 1). In irradiated female nuclei the 3L/X grain ratios are more or less the same as in normal female nuclei, the data for which have been published earlier (Lakhotia and Mukherjee 1969; Mukherjee 1966). This close similarity between control and X-irradiated female nuclei suggests that there is no alteration in the relative rate of RNA synthesis (^{3}H-UR incorporation) by the 3L or the X's in female polytene nuclei after X-irradiation. After X-irradiation, the width as well as the uptake of ^{3}H-UR by the male X(fig 1*a*) is relatively less as compared to the autosomes or the female X's (fig 1*b*).

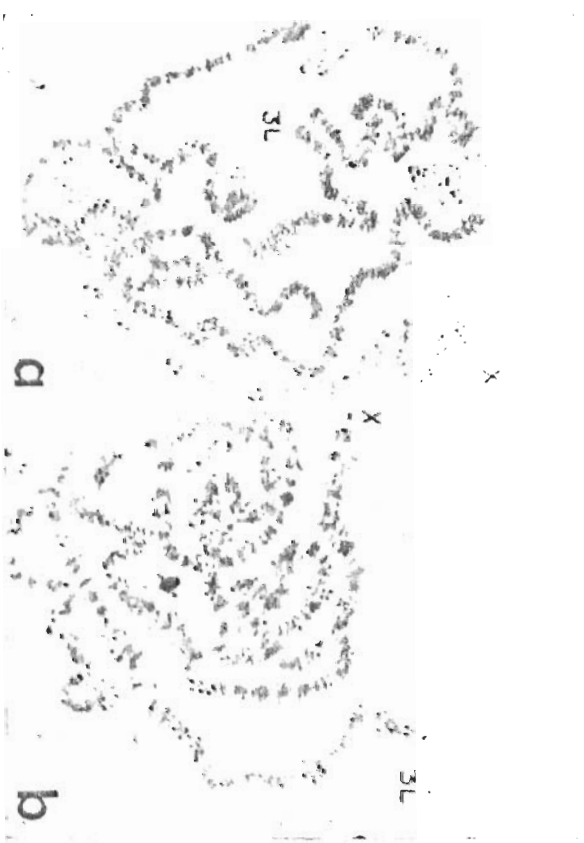


Fig. 1. Photomicrographs of ^{3}H-UR labelling in male (*a*) and female (*b*) nuclei after 1 KR.

Table 1. *Comparison of relative ^{3}H-uridine incorporation by X-chromosome in male and female polytene nuclei 3-4h after X-irradiation*

	Mean 3L/X grain ratio ± S.E.		
	3L/X[b]	3L/X[d]	3L/X[f]
Group I Nuclei			
Female	0·80 ± 0·01 (20)	0·63 ± 0·03 (21)	0·66 ± 0·01 (21)
Male	1·00 ± 0·05 (22)	0·72 ± 0·03 (16)	0·78 ± 0·06 (22)
Group II Nuclei			
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 (31)

For further details, see text; numbers in parentheses indicate the number of nuclei observed

B. DNA Synthesis by Male X After X-Irradiation

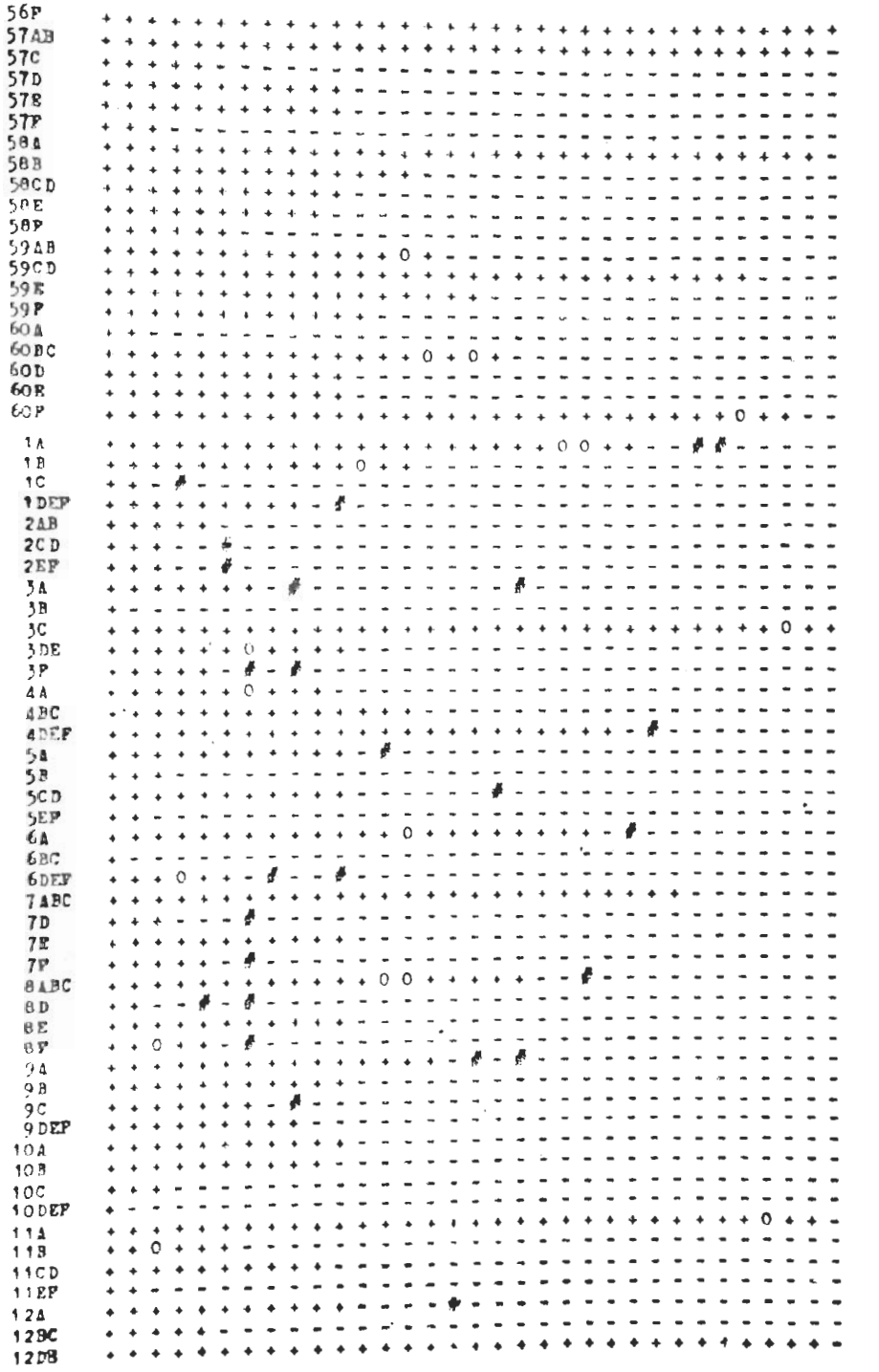
The detailed replication patterns of the X-chromosome in *D. melanogaster* under normal conditions in male and female have been described earlier (Lakhotia and Mukherjee 1970). The regions of the X-chromosome from 1A to 12E and of 2R from 56F to 60F were divided respectively into 45 and 20 independently replicating sites. The various labelling patterns were arranged in ordered arrays representing the time schedule of DNA synthesis in the individual 'replicon' sites, assuming that there is an uninterrupted DNA synthesis (in the time vector) at any site.

It has been proposed that replication begins simultaneously in all of them but finishes at different times so that continuous labelling patterns after a pulse of ^{3}H-TdR are seen in the initial S-period (tables 2, 3, 4, left side) and discontinuous patterns in the later parts of the S (right side in tables 2, 3, 4). Under normal conditions, the polytene X in male shows specific differences in its replicative organization with respect to that in female, being faster replicating (see Lakhotia and Mukherjee 1970).

Table 2. Ordered sequence of labelling patterns in 1 KR (1-2h) male nuclei

Observed patterns in different nuclei

Labelling sites	Each vertical column represents one pattern



+ = presence of labelling ; - = absence of labelling ;

≠ = unexpected presence of labelling, and O = unexpected absence of labelling at a site (see text)

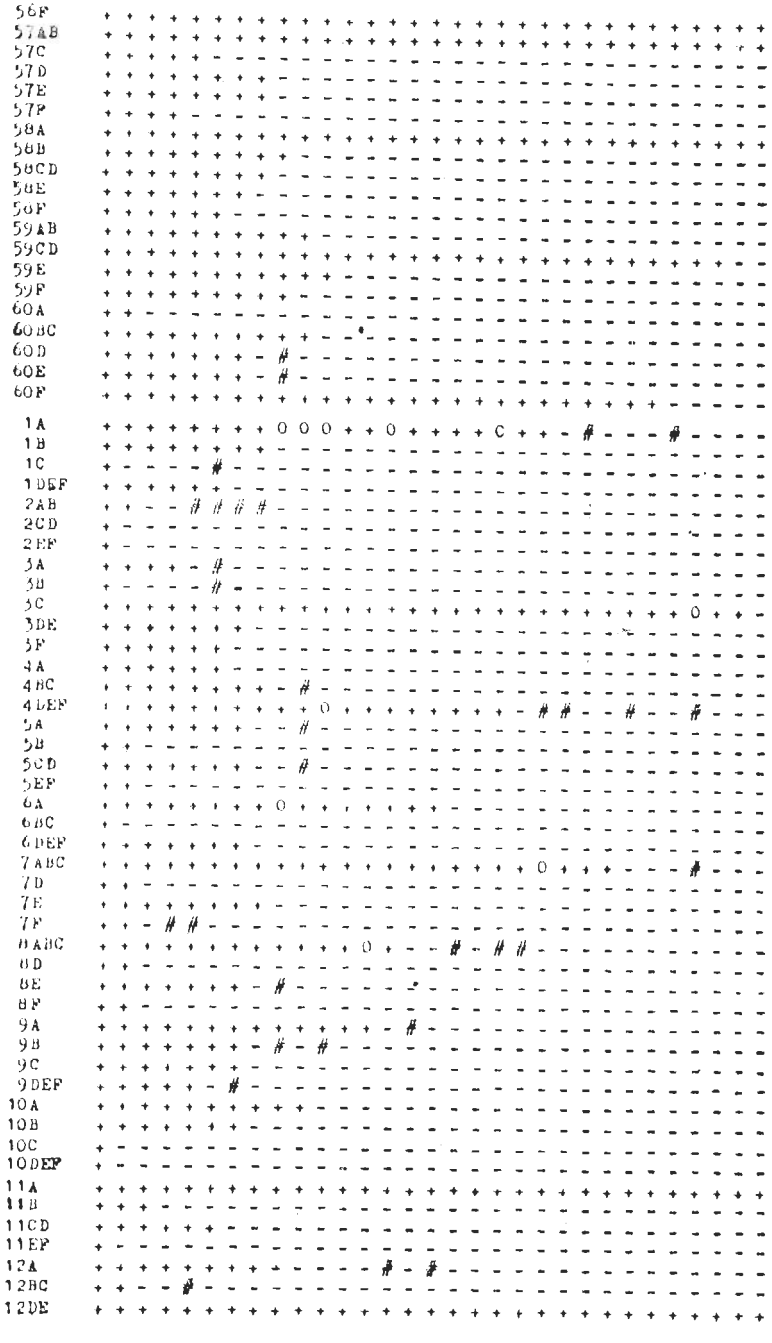


Table 3. Ordered sequence of labelling patterns in 1 KR (2-4h) male nuclei (symbols same as table 2)

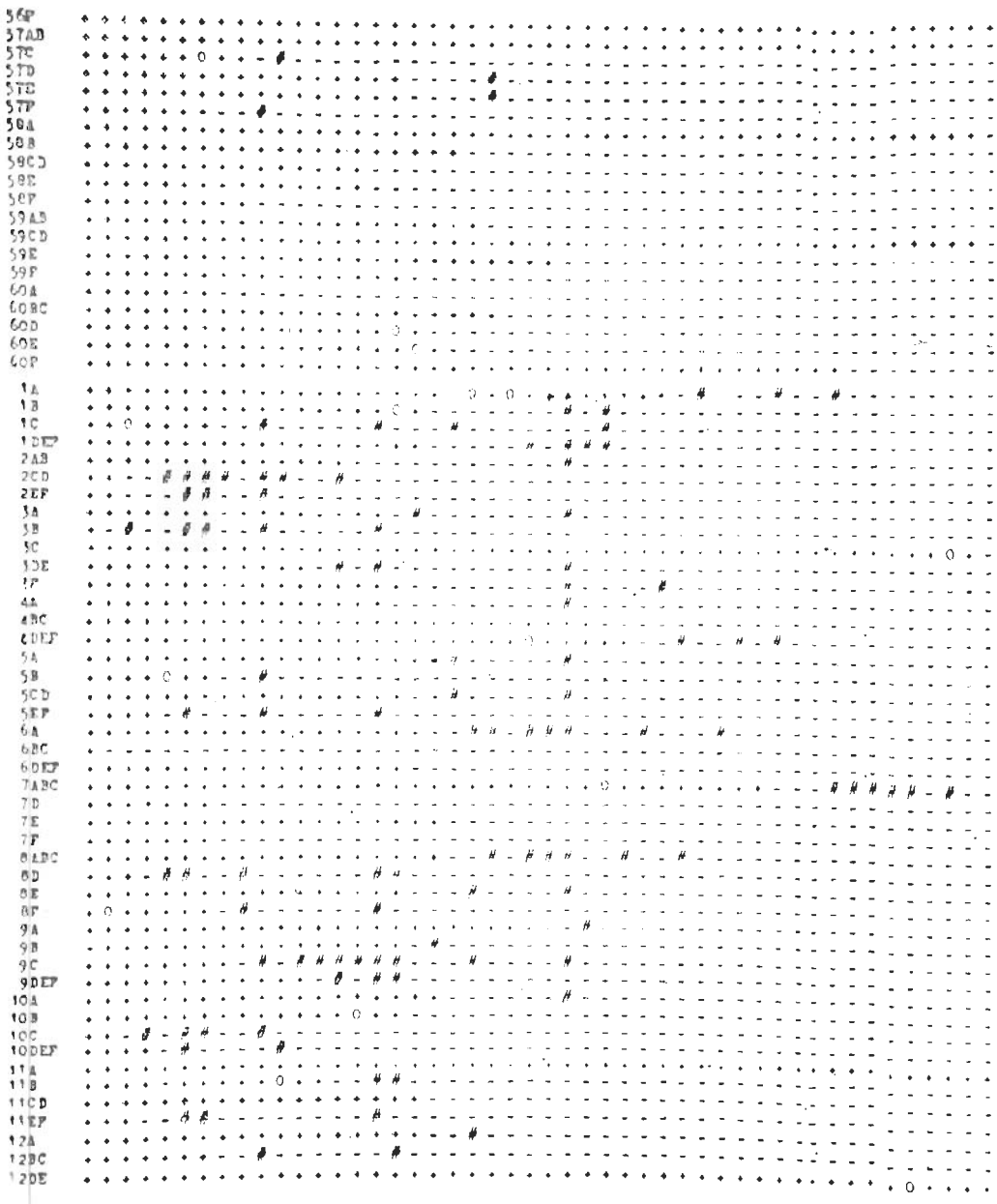


Table 4. Ordered sequence of labelling patterns in 1 KR (6-7h) male nuclei (symbols same as table 2)

The observed labelling patterns in 1 KR male nuclei at 1-2h, 2-4h and 6-7h (see tables 2, 3, 4), as in normal nuclei, are located in the ordered arrays on the basis of 2R-patterns; thus a pattern with more sites labelled on the 2R is placed to the left of a pattern with fewer sites labelled on the 2R. Several different labelling patterns on the X for a similar 2R labelling are serially arranged with the patterns having greater number of labelled sites on the X being placed to the left. With these parameters, very few 'exceptional' patterns are generated in the normal male and female (see Lakhotia and Mukherjee 1970). The patterns observed in 1 KR male nuclei reveal striking differences from the normal ones, particularly with reference to the X. At all the time intervals after X-irradiation, the labelling patterns for the 2R-segment can be arranged in sequential arrays with only few 'exceptional' patterns. The labelling patterns on the 2R in 1 KR male nuclei are similar to those observed in the normal male: the order of completion of replication by the different 'replicon' sites is identical to that in the normal. In the male X, however, in 1 KR nuclei, the 'exceptional' patterns (due to unexpected presence of labelling at a site) are much more frequent than in the normal, especially in 6-7h post-irradiation sample. An analysis of these 'exceptional' patterns on the X-chromosome of 1 KR male nuclei reveals that: (*i*) in 1-2h series, such exceptional patterns are more frequent in the heavy discontinuous type nuclei (i.e. with 16-20 sites labelled on the 2R-segment), while the late discontinuous patterns are more similar to those observed in the normal male; (*ii*) in 2-4h series, the 'exceptional' patterns are seen in heavy discontinuous as well as in some of the late discontinuous patterns, and (*iii*) in 6-7h series, the frequency of the 'exceptional' patterns is very high and these are present in all types of the patterns. At 6-7h interval, several X-chromosomal sites (viz., 1C, 2CD, 3B, 5B, 8D, 10DEF and 12BC), which in normal male are observed to be labelled only in nuclei where the 2R-segment is continuously labelled, are now seen to be labelled in nuclei in which the 2R-segment shows discontinuous labelling. Similarly, sites like 4EF, 7ABC, 8ABC etc., are seen to be labelled after X-irradiation (6-7h series) in nuclei with very late patterns on the 2R-segment; in normal male nuclei, with similar 2R-labelling, these X-chromosomal sites appear unlabelled. Whereas in normal male, the nuclei with 5 sites labelled on the 2R and three on the X are most frequent, this is not so in the irradiated male nuclei, especially in the later post-irradiation samples (table 5). The frequency of such 5:3 patterns gradually decreases in later post-irradiation samples. A comparison of the frequency of nuclei with 5 sites labelled on the 2R shows that this particular pattern remains nearly equally frequent in the normal and X-irradiated samples; however, the frequency of nuclei with only three sites labelled on the X shows a marked decrease in the 1 KR samples taken 2-4h and 6-7h after irradiation. Thus the decrease in the frequency of 5:3 patterns in 1 KR nuclei is not due to any change in the 2R labelling pattern, but is rather due to a progressive absence of X-chromosomes with only three sites labelled in 1 KR samples at different time intervals. Evidently the replicative organization of the 2R is not much altered by X-irradiation, unlike the male X.

In fig 2, the number of labelled sites on the region of X is plotted against that on the 2R segment of a nucleus for the normal and 1 KR (6-7h interval) male. In many of the X-irradiated nuclei the relative number of labelled sites on the X is more than the range encountered on the normal male X of corresponding 2R labelling pattern. At least in one nucleus in 6-7h series, a pattern was seen with the 5 sites labelled on the 2R and 21 on the X—a pattern typical for the normal female nuclei, but never observed in normal male. Figure 3 shows an example where there are relatively more sites labelled on the X in

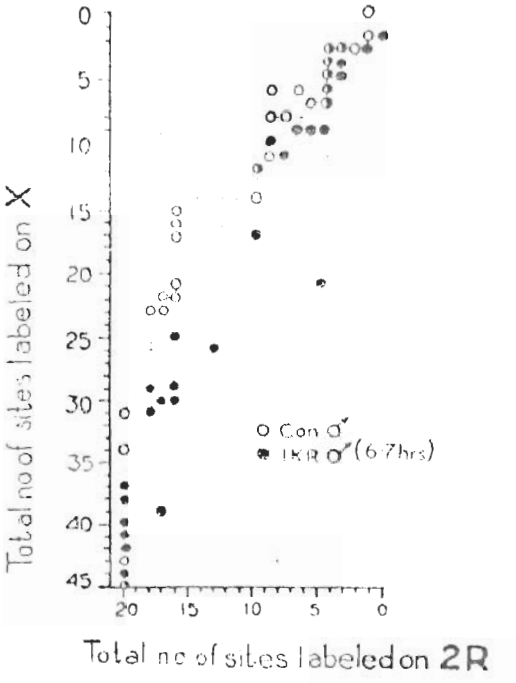


Fig. 2. Graphical representation of the number of ^{3}H-TdR labelled sites on the X- and 2R-segments in different nuclei in normal and 1 KR (6-7h) male.

irradiated male (fig. 3*a*) compared to the normal male (fig 3*b*).

A comparison of the labelling frequency of the different sites on the X and 2R in irradiated (1-7h: the data being pooled together) and normal male shows that many of the sites on the X are relatively more often labelled in the irradiated males, taking into consideration only those labelled nuclei with 1 to 16 sites on the 2R labelled. As pointed out by Arcos-Teran and Beermann (1968), this restriction to discontinuous patterns increases the sensitivity of the comparison. The labelling frequency of the 16 sites on the 2R and only some of the sites on the X have been compared (fig. 4). The labelling frequency of the 16 sites on the 2R is seen to be nearly the same in the normal and 1 KR male nuclei, while that of several sites on the X in 1 KR male is relatively high. The most striking increase in the labelling frequency after X-irradiation is for the sites 4DEF, 6A, 7ABC, 9A and 10A; 1A and 8ABC also show a slightly increased frequency after X-irradiation.

Discussion

A wealth of literature is available on the effect of X-irradiation on nucleic acid metabolism. Unfortunately, the conclusions reached through such studies have not been unequivocal. In general, *in vivo*

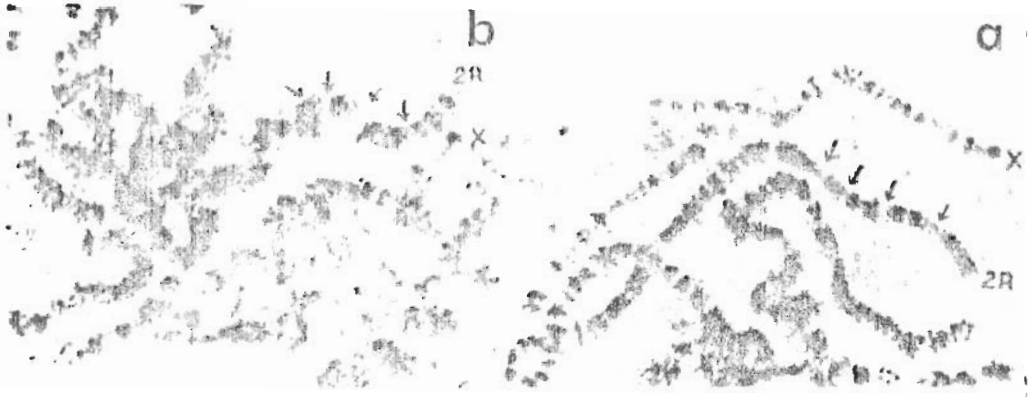


Fig. 3. Representative ^{3}H-TdR labelling patterns (heavy discontinuous) in 1 KR (a) and normal (b) male nuclei. The arrows point to the unlabelled sites on the 2R-segment. In both the nuclei the 2Rsegment has 16 sites labelled, but the X in 1 KR nucleus has many more sites labelled.

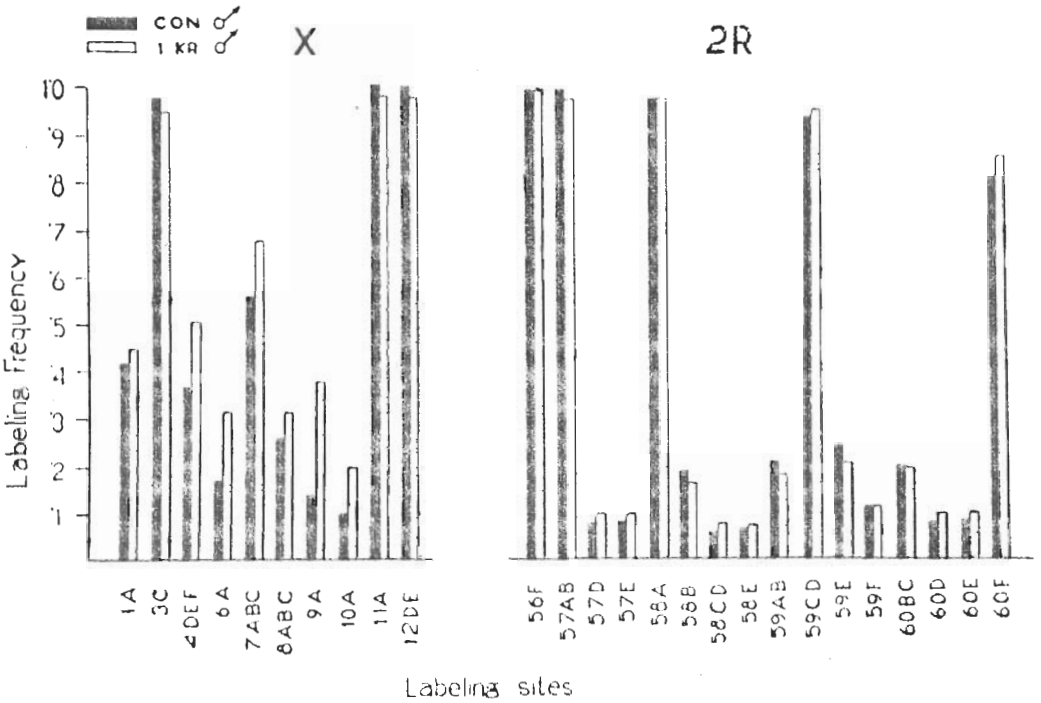


Fig. 4. ^{3}H-TdR labelling frequencies of selected sites on the X and 2R segments in normal and 1 KR male nuclei.

Table 5. *Comparison of the frequency of a late ^{3}H-thymidine labelling pattern in normal and irradiated male nuclei*

	% nuclei of the total number of labeled nuclei		
	(*a*) with 5:3 pattern	(*b*) with 5 sites on 2R labelled	(*c*) with 3 sites on X labelled
Normal male	20·0	51·0	31·3
1 KR 1-2h male	21·1	51·0	28·3
1 KR 2-4h male	6·7	55·0	13·3
1 KR 6-7h male	3·1	40·0	12·1

RNA synthesis is less sensitive to X-irradiation, while in *in vitro* systems, RNA synthesis is markedly inhibited by X-rays (Harrington 1964; Hennig 1967; Zimmermann *et al.* 1961). Bacchetti and Sinclair (1971) have provided evidence for a radiation-stimulated RNA synthesis in Chinese hamster cells in culture. Possibly, different test systems and different experimental conditions have an effect on the results. In the present study, a drastic reduction or inhibition of ^{3}H-UR uptake by salivary glands of *D. melanogaster* was not apparent 3-4h after X-irradiation. However, the data obtained suggest that there is a differential reduction in the rate of RNA synthesis by the male X as a result of the treatment. The work on the RNA synthesis by X-chromosome in gynandric salivary glands in *D. melanogaster* (Lakhotia and Mukherjee 1969) has demonstrated that as the relative rate of RNA synthesis in XY male and XX female (Chatterjee and Mukherjee 1971; Kaplan and Plaut 1968; Lakhotia and Mukherjee 1972; Mukherjee and Beermann 1965; Mukherjee 1966), the *absolute* rate of RNA synthesis by the X in male and female

nuclei is also the same. In the present experiments, after X-irradiation the 3L/X grain ratios in male are seen to be higher than those in female. The higher grain ratios in 1 KR male may be due either to an increased rate of RNA synthesis by the autosomal segment, or to depressed transcription by the male X. The latter alternative is more likely since the 3L/X grain ratios in 1 KR female nuclei remain nearly the same as in normal female, suggesting that the relative rate of ^{3}H-UR incorporation by the female 3L or the X's remains unaltered due to X-rays. This is to be expected since there is no change in the functional morphology of either the 3L or the X's in irradiated female nuclei.

Works of Hess and co-workers (Hennig 1967; Hess 1965; Hess and Meyer 1968) indicate that in *Drosophila* spermatocytes, X-irradiation may reversibly inhibit DNA-directed RNA synthesis. In the spermatocytes, the Y-chromosome loops are highly active in RNA synthesis and are damaged by X-rays and actinomycin D (Hess and Meyer 1968). Possibly while in general, *in vivo* RNA synthesis is less sensitive to X-rays, the loci which are very active in transcription may be more susceptible and this may apply to the present observations as well. The single X in polytene nuclei of male *Drosophila* is very active in RNA synthesis and the structure of the male X is also in some way 'damaged' (reduced width) by X-rays; this reduced width of the male X is also reversible (Mukherjee *et al* 1968). This parallel response of the Y-chromosome loops and the polytene X in male *D. melanogaster* to X-irradiation makes it very likely that after X-irradiation there is a reversible and selective repression of DNA-directed RNA synthesis by the male X.

X-irradiation is known to inhibit or slow down the rate of DNA synthesis in proliferating cells (Bacq and Alexander 1961; Dendy 1964; Goutier 1961; Kuzin 1964). Bacchetti and Sinclair (1971) have shown that in synchronized Chinese hamster cells in culture, the S is the most sensitive period in the cell cycle with respect to the effect of X-irradiation on DNA synthesis. Wong (1968) has reported that X-irradiation causes considerable inhibition of DNA synthesis in the salivary glands of *D. hydei*. A preliminary analysis of grain counts from the labelled nuclei made here indicates that there is some degree of reduction in the general uptake of ^{3}H-TdR. The significance of the present data on the effect of X-irradiation on replication becomes apparent when considered in the light of the replicative organization of polytene chromosomes in normal conditions. Replication of chromosomes in a nucleus is precisely co-ordinated and temporally ordered and follows a specific sequence of labelling patterns (Howard and Plaut 1968; Lakhotia and Mukherjee 1970; Mulder *et al* 1968); under normal conditions the replicative behaviour of the polytene X-chromosome in *Drosophila* male shows very specific differences from the female (Berendes 1966; Lakhotia and Mukherjee 1970). The similarity in the observed patterns on the 2R-segment in 1 KR and the normal male suggests that the replicative organization of the 2R (and presumably of other autosomes as well) is not much affected by the treatment: this treatment also fails to induce any change in the morphology (Mukherjee *et al* 1968) or transcriptive activity (present observations) of the autosomes. But the X-chromosome in the same male nuclei shows altered labelling patterns in the 1 KR series and these are significant in the context of the hyperactive organization of the male X (Lakhotia and Mukherjee 1970), indicating the disturbed functional organization of the male X. The deviations in the replicative behaviour of the male X following 1 KR may be explained by assuming that (*i*) X-irradiation delays or slows down the rate of DNA synthesis on the different sites on the male X; (*ii*) the rate of DNA synthesis on the 2R may either be unaffected, or if the 2R is also affected, that on the male X is slowed down to a greater extent; and (*iii*) that the effect

of X-rays on the different "replicon" sites of male X is not uniform, thus generating a high frequency of 'exceptional' patterns. This independent response of the differents ites on the male X is in agreement with the idea of 'piecemeal' regulation of the hyperactivity (Lakhotia 1970*b*).

Another possible factor responsible for the high frequency of the 'exceptional' patterns may be presumptive 'repair' synthesis following X-irradiation damage (Plaut 1969). However, the 'repair' synthesis may not explain all the observed 'exceptional' patterns on the male X, since such patterns would be expected with equal probability in different labelling patterns at various post-irradiation time intervals. They are random with respect to labelling site, but when considered with respect to labelling patterns (early or late), their distribution at the different time intervals is non-random, but in accordance with the expectation that continuous labelling is the beginning and that X-rays differentially slow down the rate of DNA synthesis by different sites on the male X. On this basis, in earlier post-irradiation samples, the continuous and/or heavy discontinuous patterns would show the 'disturbed' patterns, because the late patterns are expected to have completed most of their DNA synthesis prior to irradiation, while later samples (like 6-7h) would show more widely distributed 'exceptional' patterns. It seems, therefore, that the observed disturbances in the labelling with ^{3}H-TdR of the male X after I KR are mainly due to changes in its normal replicative organization itself.

Taken together, the present data indicate that after X-irradiation, there is a relative decrease in the transcriptive and replicative activities in the single X in polytene nuclei of male *D. melanogaster*: the autosomes are not affected to this extent. X-irradiation also selectively reduces the width of the normally wider polytene X in male *D. melanogaster* (Mukherjee *et al* 1968). This suggests that the enlarged width and the hyperactive organization are interdependent. Comparable effects on the male X in *D. melanogaster* are observed with certain other inhibitors of chromosomal activity (Lakhotia 1970*a*). At present it is not possible to dissociate the primary target of these treatments which leads to the observed effects. There are two possible factors that may be responsible for the enlargement and hyperactivity of the male X: either an accumulation of the transcription product, or a difference in the organization of the chromatin material in the male X. The second possibility seems likely. The role of chromosomal non-histone acid proteins remains to be examined in the present context. In view of the recent concepts regarding the relationship of genetic activity and the chromosomal acid proteins (Berendes and Beermann 1969), these chromosomal proteins are likely to be very important in the hyperactivity of the male X. In the puffs on Dipteran polytene chromosomes there is an accumulation of acid proteins prior to swelling and RNA synthesis (Berendes 1968) and it is possible that on the polytene X-chromosome of male also, a relatively greater accumulation of acid proteins has taken place to augment hyperactivity. Rudkin's (1964) data in this respect on the protein content of male and female X's are insufficient, since they are limited to a very small segment of the X in a highly inbred strain of *D. melanogaster*. More detailed observations are necessary about the protein content of the X-chromosome to establish their qualitative and/or quantitative differences between the X-chromosomes of male and female.

In *D. melanogaster* the greater sensitivity of metabolic activities of the male polytene X-chromosome suggests that the single X in male has been induced by the dosage compensators to do "extra" work which may be repressed by extraneous agents. All the available evidences thus indicate that in *Drosophila* dosage compensation is achieved by an hyperactivity of the single X in male and not by repression of the two

X's in female (Lakhotia and Mukherjee 1970) and that in *D. melanogaster* the increased activities are reversible by agents that repress the normal metabolism of chromosomes. 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 and/or content of the polypeptides determined by X-linked genes.

Summary

Replicative and transcriptive activities of the polytene X-chromosome of male *Drosophila melanogaster* have been examined autoradiographically after X-irradiation (1 KR) of late third instar larvae. There is a selective reduction in the relative rate of ^{3}H-uridine uptake by the male X 3-4h after the treatment. ^{3}H-thymidine labelling patterns were examined 1-2h, 2-4h and 6-7h after 1 KR, and the results have been interpreted to show relatively greater reduction in the rate of DNA synthesis by the male X compared to the 2R. Due to this the normally faster replicating male X exhibits various alterations in its replicative organization which are manifested in a high frequency of 'exceptional' patterns and relatively higher labelling frequency of several X-chromosomal sites in irradiated nuclei compared to normal male nuclei. The results suggest that the single X-chromosome in male is normally primed to do "extra" work to equalize its activity to that of the two X's of female and that this hyperactivation of the male X in *D. melanogaster* is reversible under circumstances which inhibit chromosomal activity.

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