Genet. Res., Camb. (1985), **46**, *pp.* 19–29
Printed in Great Britain

Cellular autonomy of hyperactivity in segmental *X* chromosomal aneuploids of *Drosophila* and dosage compensation

By JAYASHREE PRASAD-SINHA and A. S. MUKHERJEE

Genetics Research Unit, Department of Zoology, University of Calcutta, 35 Ballygunge Circular Road, Calcutta 700 019, India

(*Received 27 August 1984, and in revised form 5 March 1985*)

SUMMARY

Transcription in 9A-11A aneuploid mosaic female larvae of *Drosophila melanogaster* has been assessed autoradiographically. Eleven larvae were found to exhibit mosaicism out of sixty-six larvae scanned and the percentage of *XO* and *XX* nuclei varied from approximately 9 to 100. Irrespective of the number of *XX* nuclei present the *XO* nuclei (duplicated for 9A-11A) invariably showed hyperactivity for both the segments. The *XX* nucleus exhibited a dosage effect for all the three segments of 9A-11A. Results support the transcriptional constancy of the entire *X* chromosome, as proposed by Maroni and Lucchesi. Cellular autonomy of hyperactivity of the single *X* chromosome even at the level of segments of the *X* is thus evident from the present results.

1. INTRODUCTION

Mukherjee & Beermann (1965) showed that the *X* chromosome in *Drosophila* is twice as active in the male as the individual *X* chromosomes in the female and proposed that dosage compensation (that is equalization of *X*-coded gene products between the two sexes despite the difference in the number of *X* chromosomes and *X*-linked gene dosage) in *Drosophila* operates through the male, unlike mammals where it operates by inactivation of only one of the two *X*'s in the female (Lyon, 1961). Later, the proposition was corroborated by Kazazian, Young & Childs (1965) and Maroni & Plaut (1973) and Lucchesi and his co-workers (see Lucchesi, 1973) both at the level of transcription as well as of translation. Lakhotia & Mukherjee (1969) upon the use of *XX-XO* gynandric mosaic salivary glands which had otherwise normal diploid chromosome complement, showed very precisely that the *X* chromosomal hyperactivity in the male is cell-autonomous and that every individual *XO* cell regardless of its state of developmental timing expressed its hyperactivity.

Lucchesi (1973, 1977) in his review clearly pointed out that as the dosage of *X*-linked genes is increased (as duplication for one or more genes) in either sex, the effect is a 'dosage effect within each sex'. However, when the effect with a prescribed gene dosage is compared between the two sexes, one can clearly derive that the *X* chromosome in the male with the duplication shows hyperactivity when compared with the same gene dosage of *X*-linked genes in the female sex, and

therefore, shows 'dosage compensation between the sexes'. In our earlier publication (Prasad, Duttagupta & Mukherjee, 1981), we defined the difference between dosage effect and dosage compensation through the effect of additional dosage in segmental aneuploids.

Maroni & Lucchesi (1980) using various segmental aneuploids showed that while the segments of *X* chromosomes which were hyperploid did reveal a net increase in ^{3}H-UR silver grains, there was a decrease in the grain numbers on the haplo-*X* segments. Belote & Lucchesi (1980*a*) examined the G6PD level in the autosomal mutants *mle*^{ts} and *msl* which are male specific lethals in *Drosophila melanogaster*. They reported that the G6PD level in these mutant males (at sub lethal temperature) does not show the level that would be expected for dosage compensation. The polytene *X* chromosome in these mutants is also not typically puffy as in the normal male. They concluded that the regulation of dosage compensation is autosomal (since dosage compensation is operative through the males, and *mle*, *msl-1*, *msl-2* do not have any effect on transcription of the *X* chromosome in females).

In their paper on transcription of *X* chromosomal segmental aneuploids, Maroni & Lucchesi (1980) have shown that there is no change in overall transcription of the *X* chromosome in the segmental aneuploids, although the specific region involved in aneuploidy did show increased transcription. They explained this finding to be due to a kind of constancy in the level of transcription determined by the given signal from autosomal regulators. In our earlier work (Prasad *et al.* 1981) on transcription in segmental aneuploids, the transcription of the entire *X* chromosome did not show a significant difference between haplo-*X* and hyperploid *X* males, but we did not compare the result in the light of such constancy.

Since such discrepancy might result from the difference in the larval development of male, female and aneuploids, the present investigation was carried out to test the validity of the hypothesis of constancy in the level of transcription by using *XX-XO* mosaic salivary glands and comparing the results with those from non-mosaic complete *X* aneuploids. The results, while they support the constancy hypothesis of Maroni & Lucchesi (1980), leads us to contend that the hyperactivity of the *X* chromosome is strictly cell autonomous.

2. MATERIAL AND METHODS

(i) *Mosaic segmental aneuploid*

An unstable ring-*X* strain of *Drosophila melanogaster* R(1)w^{vC}/In(1)*dl-49*, *y w lz*^{s}/y^+*Y* was obtained from Bowling Green Stock Centre and used in these experiments to generate mosaics with segmental aneuploidy. Dp (9A-11A) was synthesized in the laboratory (Prasad *et al.* 1981).

Dp (9A-11A) segmental aneuploids were synthesized using two *X*; *Y* translocation stocks with displaced *X* and *Y* break points. In the F_1 progeny the resultant segmental aneuploidy is caused by overlapping of the same segments of the *X* chromosome borne by two separate free-moving *X* chromosomes with separate centromeres (Stewart & Merriam, 1975).

In most cases, except where they have been over-stretched due to pressure during

squash preparations, the segments involved in aneuploidy remain synapsed in both males and females, and the points of demarcation are very clear because of the major and prominent chromosomal bands involved at the break point as well as due to the presence of the heterochromatic piece of the translocated *Y* chromosome (plate 1, Prasad *et al.* 1981) attached thereto. The cytology is shown in Fig. 1*a*.

Five to six-day-old virgin females bearing unstable ring-*X* were crossed to Dp (9A-11A) males in culture bottles containing standard *Drosophila* medium supplemented with live yeast and maintained at 24 ± 1 °C. Well developed third instar female larvae (i.e. those crawling on the wall of the culture bottle) were selected and their salivary glands were dissected out in buffered Ringer (pH 6·8), taking sufficient care to keep every pair of salivary glands intact. Each pair of salivary glands were incubated in [^{3}H]uridine at a concentration of 500 μCi/ml (Sp. activity 13800 mCi/m-mole, B.A.R.C., India) for 10 min. Cytological squash preparations were prepared as described earlier (Lakhotia & Mukherjee, 1969). They were then covered with Kodak AR 10 stripping film, and stored in black bakelite boxes at 4 °C. After 14–15 days' exposure, slides were developed in Kodak D19b at 10 °C for 12 min. Developed slides were washed, fixed and air dried, stained in 1% toluidine blue in 30% alcohol and mounted on D.P.X.

Out of 66 pairs of salivary glands scanned, 11 pairs were found to exhibit mosaicism of various degrees. Grains could be counted from 9 pairs of glands only. From each pair the total number of *XO* and *XX* nuclei were scored and grains on the segments 1-3C, 1-8F, 9A-11A, 11B-20F, 15D-18A and on the entire *X* chromosome were counted from *XO* and *XX* nuclei for each pair of glands. Grains on the region 56E-60F of the corresponding nuclei were also counted as internal controls. Ratios were obtained by dividing the grain number on the *X* chromosome segments by the grain count of the corresponding 56E-60F segment of each nucleus. The means were calculated and compared with similarly obtained means from Oregon R$^+$ males and females, and Dp (9A-11A) males and females.

All markers are described by Lindsley & Grell (1968). Photographs were taken with a Zeiss Photomicroscope III.

3. RESULTS

Fig. 1*b*, *c* (Plate 1) show two [^{3}H]uridine autoradiograms, of normal male and female larval glands of *Drosophila melanogaster*, respectively. As stated in all earlier reports, the genes on the *X* chromosome in a male show hyperactivity while those on the *X* chromosomes in female show a dosage effect. In aneuploids for *X* chromosomal segments the same situation, that is hyperactivity of each homologous segment of the hyperploid section in the male and a dosage effect in the female, has been demonstrated (Prasad *et al.* 1981). In the present investigation, the pattern of transcription of segmental aneuploid has been examined simultaneously for male and female chromosomal complement in the same gland by examining the [^{3}H]uridine labelling in sex-mosaics. Figs. 2 and 3*a* (Plate II) showing two such autoradiograms under low magnification reveal the presence of *XX* and *XO* nuclei. Fig. 3*b* and *c* (Plate 2) show two of the nuclei in higher magnification. The nucleus in Fig. 3*b* has essentially female chromosomal complement with aneuploidy for

9A-11A, and that in 3*c* has male complement, with aneuploidy for 9A-11A. It is evident from the distribution of silver grains on the paired segments (9A-11A) in the autoradiograms that in presence of an additional 9A-11A segment in the female nucleus (Fig. 3*b*), each segment shows a dosage effect, while in the male nucleus, each of them shows hyperactivity. This pattern of expression is observed regardless of the number of *XX* and *XO* nuclei in the mosaic gland. A more quantitative evaluation of this interpretation is presented below.

Before presenting the data on hyperactivity in mosaic glands, it is necessary to establish the pattern of variation in normal males and females and in those with duplications.

Data in Table 1 are presented to compare the labelling density in different segments of the *X* chromosome in 1*X*2*A* and 2*X*2*A* (normal male and female), Dp (9A-11A) (aneuploid male and female). The *X*/2*R* grain ratios in the column 9A-11A show that each segment individually in both Oregon R^+ and Dp (9A-11A) male is hyperactive, whereas in the Oregon R^+ and Dp (9A-11A) females the segment reveals a dosage effect. Although the ratios on the flanking segments (1-8F and 11B-20F), in different genotypes are slightly lower in males than in females, the evidence is rather marginal.

Results on [^{3}H]uridine labelling in the nine mosaic salivary glands, obtained from the ring *X* heterozygote larvae, are presented in Table 2. The percentage of *XO* nuclei in these glands is based on the observation of all nuclei which could be resolved with clarity and varies from 9 to 100%.

Labelling density was computed from the ratio of grain number on the segments of the *X* chromosomes and that on the reference segment 56E-60F of the second chromosome. In order to avoid distributional and coincidental error, chromosomes which had no overlapping and least cytoplasmic materials were selected from the preparations, and grains were counted under a 100× oil immersion objective only from those labelled chromosomes which showed no superimposition of grains, such as shown in Fig. 1*b* and *c*. The chromosomes shown in Figs. 2 and 3 are presented to show the representative *XX* and *XO* nuclei in mosaic gland and the segments involved in aneuploidy.

Grains were counted under a 100× oil immersion objective from such magnified labelled chromosomes which showed no coincidence as in Fig. 4*a* and *b* (Plate 3).

Analysis of labelling density of the segment 9A-11A clearly reveals that regardless of the variation in the percentage of *XO* among all nuclei, the *XO* aneuploid nuclei having two doses of 9A-11A show hyperactivity for the paired aneuploid segment 9A-11A. In the two glands (2 and 9), however, the ratios of grains are not significantly different between females (with 3 doses) and males (with 2 doses).

A simple calculation reveals that except for glands 2 and 9, each segment of 9A–11A in aneuploid nuclei in *XO*2*A* is twice as active as each homologue in the aneuploid nuclei in *XX*2*A*. The calculation is as follows: Since in *XX*2*A* aneuploids, there are three 9A-11A segments, each ratio shown against *XX* under 9A-11A column may be divided by 3 to obtain the relative activity per segment. This activity per segment when multiplied by 4 (2 segments × 2 fold hyperactivity) should then give ratio similar to that shown against *XO* nuclei under the same

Table 1. *[^3H]UR mean grain count ratios* (X/2*R*) ± *S.E of different* X *chromosomal aneuploids and wild type* X *chromosomes of* Drosophila melanogaster

		Load of *X*	No. of 9A-11A segment	1-3C	2C1-3C7	1-8F	9A-11A	11B-20F	15D-18A	Entire *X*
Oregon R^+	♂	1·0	1	0·42 ± 0·02 (41)	—	1·46 ± 0·06 (17)	0·44 ± 0·02* (35)	1·31 ± 0·06 (20)	0·36 ± 0·03 (15)	3·42 ± 0·07 (41)
Oregon R^+	♀	2·0	2	0·52 ± 0·02 (31)	0·38 ± 0·03 (6)	1·75 ± 0·07 (12)	0·44 ± 0·02 (16)	1·61 ± 0·12 (12)	0·38 ± 0·02 (20)	3·76 ± 0·15 (12)
Dp (9A-11A)	♂	1·11	2	0·46 ± 0·10 (55)	—	1·39 ± 0·09 (13)	0·89 ± 0·04* (37)	1·21 ± 0·10 (13)	0·26 ± 0·01	3·48 ± 0·12 (41)
Dp (9A-11A)	♀	2·11	3	0·56 ± 0·04 (13)	—	1·54 ± 0·07 (13)	0·56 ± 0·06 (13)	1·25 ± 0·06 (13)	0·34 ± 0·02 (13)	3·32 ± 0·15 (13)

Numbers in parentheses represent number of segments scored.
* Probability < 0·05.

Table 2. [^3H]*UR mean grain count ratios* (X/2*R*) ± *S.E. from Dp 9A-11A segmental aneuploid mosaic of* Drosophila melanogaster

Gland number	Distribution of *XO*/*XX*	1-3C	1-8F	9A-11A	11B-20F	15D-18A	Entire *X*	No. of nuclei scored	Total no. of nuclei
1	10% *XO*	0·50 ± 0·05	1·37 ± 0·04	0·84 ± 0·05*	1·63 ± 0·13	0·29 ± 0·03	3·84 ± 0·23	9	18
	90% *XX*	0·59 ± 0·01	1·52 ± 0·02	0·68 ± 0·03*	1·56 ± 0·07	0·30 ± 0·02	3·72 ± 0·13	6	171
2	80% *XO*	0·48 ± 0·03	1·41 ± 0·10	0·64 ± 0·07**	1·28 ± 0·09	0·28 ± 0·03	3·37 ± 0·22	5	93
	20% *XX*	0·40 ± 0·04	2·46 ± 0·27	0·59 ± 0·06**	1·62 ± 0·11	0·34 ± 0·02	4·38 ± 0·44	4	23
3	64% *XO*	0·50 ± 0·02	1·51 ± 0·001	0·94 ± 0·07*	1·29 ± 0·14	0·24 ± 0·03	3·52 ± 0·13	2	26
	36% *XX*	0·54 ± 0·02	1·51 ± 0·05	0·66 ± 0·01*	1·37 ± 0·06	0·27 ± 0·02	3·55 ± 0·06	4	46
4	09% *XO*	0·56 ± 0·004	1·77 ± 0·04	0·85 ± 0·04	1·57 ± 0·01	0·35 ± 0·01	4·19 ± 0·10	2	12
	91% *XX***	—	—	—	—	—	—	—	123
5	19% *XO*	0·51 ± 0·03	1·39 ± 0·10	0·76 ± 0·06*	1·33 ± 0·13	0·33 ± 0·04	3·49 ± 0·22	5	39
	81% *XX*	0·53 ± 0·01	2·23 ± 0·35	0·60 ± 0·003*	1·60 ± 0·20	0·29 ± 0·02	4·44 ± 0·53	3	168
6	14% *XO*	0·49 ± 0·07	1·49 ± 0·37	0·85 ± 0·19***	1·12 ± 0·17	0·26 ± 0·03	3·46 ± 0·69	4	37
	86% *XX*	0·47 ± 0·04	1·42 ± 0·37	0·51 ± 0·06**	1·37 ± 0·03	0·39 ± 0·04	3·30 ± 0·44	3	234
7	60% *XO*	0·48 ± 0·02	1·32 ± 0·07	0·85 ± 0·12*	1·32 ± 0·16	0·26 ± 0·03	3·49 ± 0·17	3	101
	40% *XX*	0·54 ± 0·02	1·42 ± 0·07	0·60 ± 0·04*	1·56 ± 0·02	0·31 ± 0·02	3·59 ± 0·10	3	67
8	100% *XO*	0·39 ± 0·03	1·35 ± 0·05	0·86 ± 0·06	1·26 ± 0·10	0·18 ± 0·01	3·48 ± 0·21	6	121
	00% *XX*	—	—	—	—	—	—	—	—
9	50% *XO*	0·32 ± 0·06	1·12 ± 0·15	0·85 ± 0·02**	0·93 ± 0·12	0·17 ± 0·05	3·02 ± 0·45	3	36
	50% *XX*	0·49 ± 0·03	1·54 ± 0·02	0·80 ± 0·20**	1·25 ± 0·13	0·29 ± 0·01	3·59 ± 0·04	2	35

* Significant at 5% level; ** Not significant; *** Could not be scored due to lack of well spread and non-overlapping *XX* nuclei.

Table 3. *Mean* [^3H]*UR grain count ratios* (X/2*R*) ± S.E. *of different* X *chromosomal segments from mosaic Dp 9A-11A aneuploids and wild type* Drosophila melanogaster

			1-3C	1-8F	9A-11A	11B-20F	15D-18A	Whole *X*
Oregon R$^+$	♂		0·42 ± 0·02 (41)	1·46 ± 0·06 (17)	0·44 ± 0·02 (35)	1·31 ± 0·06 (20)	0·36 ± 0·03 (15)	3·42 ± 0·07 (41)
Oregon R$^+$	♀		0·52 ± 0·02 (31)	1·75 ± 0·07 (12)	0·44 ± 0·02 (16)	1·62 ± 0·12 (12)	0·38 ± 0·02 (20)	3·76 ± 0·15 (12)
Dp (9A-11A mosaics	♀	Nuclei *XO****	0·46 ± 0·03 (37)	1·37 ± 0·05 (37)	0·82 ± 0·03** (37)	1·32 ± 0·06* (37)	0·26 ± 0·02 (37)	3·52 ± 0·11 (37)
		Nuclei *XX*	0·52 ± 0·02 (25)	1·73 ± 0·10* (25)	0·63 ± 0·02** (25)	1·50 ± 0·04* (25)	0·31 ± 0·01 (25)	3·81 ± 0·13 (25)

* Significant difference at 5% level. ** Significant difference from the corresponding controls at 5% level. *** The gland from which *XX* nuclei data could not be taken has been omitted, and then means calculated.

Numbers in parentheses represent number of segments scored.

column. For example (in gland no. 1) 0·68/3 = 0·23, 0·23 × 4 = 0·92, (0·84). A summary of data in Tables 1 and 2 is given in Table 3.

The differences in ratios for the entire *X*, between *XO* and *XX*, or between *XY* and *XX*, are not significant (Tables 2 and 3). These data suggest firstly, if reduction in labelling density on external segments would take place for the maintenance of constancy in transcription, such reduction is also cell autonomous as the means of the ratios of grains on 1-8F and 11B-20F (Table 3) for the *XX* and *XO* nuclei in the mosaic gland, are highly comparable with those in normal female and male glands, respectively.

4. DISCUSSION

In an earlier communique (Prasad *et al.* 1981) we showed that in salivary glands of males aneuploid for the segment 9A-11A (i.e. with two doses), the total activity of that segment is twice that in *XO* and *XY2A* male. In this paper, the transcriptive activity, as measured by [^{3}H]uridine labelling, of the 9A-11A segment of the *X* chromosome in 1*X* (normal male), 2*X* (normal female),1·11*X* (aneuploid male) and 2·11*X* (aneuploid female), and in mosaics with 1·11*X* (aneuploid) and 2·11*X* (aneuploid) nuclei has been presented. The different quanta of the *X* chromosome (namely 1·11 or 2·11*X*) are based on the number of bands of the chromosome and for the sake of discussion this quantum is called the load of the *X* chromosome. Our results suggest that as in the case of diploid male (Lakhotia & Mukherjee, 1970; Chatterjee & Mukherjee, 1977) the hyperactivity of the *X* chromosome in aneuploid nuclei is maintained by a given load of the *X* chromosome (e.g. in *XY2A* normal male the load of *X* is 1, in an aneuploid male containing 10% additional bands it would be 1·1). In the *XX-XO* mosaics with aneuploidy the expression of hyperactivity is conserved atonomously.

On the basis of the results, we suggest that the uniformity might be a consequence of a stoichiometric utilization of the regulatory signals emanating from the regulatory gene or genes on the autosomes and those on the *X* chromosome (see also Mukherjee, 1982). The results on *X*-linked sex-specific lethals (e.g. Sxl^{f1}, Sxl^{M1}; Cline, 1978, 1979*a*, *b*, 1983; Lucchesi & Skripsky, 1981; Gadagkar *et al.* 1981, 1982) support such a possibility. Cline (1978) suggested a common genetic control for both sex differentiation and dosage compensation at the primary level of development. His later work with the *Sxl* showed that this locus is involved in sex determination (Cline, 1979*a*, *b*) as well as in dosage compensation (Cline, 1983). Lucchesi & Skripsky (1981) clearly demonstrated that the *Sxl* locus is involved in controlling the *X* chromosome transcription in female. The interaction between *da* and *Sxl* (Cline, 1978) leads one to predict that *da* would have similar effect on the *X* chromosome transcription as Sxl^{f1}. As the dominant Sxl^{M1} mutation is able to rescue females from the lethality of *da*, it can be expected to counteract the hyperactivity of the *X* chromosome (the cytological preparations of the Sxl^{M1} male polytene nuclei clearly reveal that the *X* chromosome in these males are much thinner than paired autosomes and, the mean [^{3}H]thymidine induced grain number was approximately four times less than that on the control *X* chromosome, that is the *X* chromosome of male Basc, Lucchesi & Skripsky, 1981).

The proper level of *X* chromosome function in males requires the wild type allele of at least three autosomal genes: *msl*-1 (male-specific lethal-1), *msl*-2 and *mle* (maleless) (Belote & Lucchesi, 1980*a*, *b*). These genes have no obvious major effect on the development of sexual characteristics. These male-specific lethal mutations interact with Sxl^{f1} allele to alter sexual differentiation. For example, $Sxl^{f1}/+$; *mle/mle* double mutant individuals often develop as females with sex-comb (Skripsky & Lucchesi, 1980).

Thus, experimental evidences show that autosomal genes (da^+, $msl\text{-}1^+$, $msl\text{-}2^+$ and mle^+, at least) and *X* chromosomal genes (Sxl^{F1}, Sxl^{M1}) are involved in an interaction to maintain the differential gene activity on the *X* chromosomes in the two sexes, which results in dosage compensation. Gadagkar *et al.* (1981, 1982) have proposed a model for regulation of dosage compensation as well as sex determination, taking into account the results of Cline, Lucchesi and co-workers and others involving the loci mentioned above.

Since the publication of works of Lucchesi & Maroni (1980), Stewart & Merriam (1975) and Prasad *et al.* (1981), efforts are being made to find out the mechanism by which the *X*-linked genes become hyperactive in the male. Belote & Lucchesi (1980*a*, *b*) have implicated the autosomal genes mle^+ (maleless), *msl*-1 (male specific lethal-1), msl^+-2 in the transcription of *X* chromosome in male. Mukherjee & his co-workers [Mukherjee, 1982; Ghosh & Mukherjee, 1983; Mukherjee & Ghosh (manuscript submitted)] on the other hand, suggested that certain inhibitory signals might originate from the *X* chromosome itself which might interact stoichiometrically with the enhancer signal emanating from the autosomal regulators (e.g. mle^+, msl^+ etc.). The evidence produced by them are as follows:

Firstly, in the mutant strain In(1)BM^2 (re-inverted mosaic) of *D. melanogaster*, the *X* chromosome in the male is nearly twice as hyperactive as the *X* chromosome in the normal male. When a duplication for the proximal region (15EF to 19D) of the *X* chromosome is combined by genetic cross with the In(1)BM^2, the aneuploid male so formed shows normal hyperactivity of the *X* chromosome rather than hyper-hyperactivity as found in the In(1)BM^2 (rv, mosaic) (Ghosh & Mukherjee, 1983; Mukherjee & Ghosh, submitted).

Secondly, in the hybrid male progeny of *D. miranda* × *D. persimilis*, the X_2 element of miranda partially synapses with the C element of *persimilis*. In this case, although other homologous autosomal elements are identical in the hybrid, the dosage compensation of the X_2 is perfect for all regions which are not homologous to those on the C element (Strobel *et al.* 1978; Das *et al.* 1982; Mutsuddi *et al.* 1984).

The work was supported by University Grants Commission – Special Assistance Programme to Department of Zoology, University of Calcutta and a Senior Research Fellowship to Jayashree Prasad-Sinha under the U.G.C. sponsored research project (Grant no. F.23-47/83-SR II of 11.2.83). to A.S.M.

REFERENCES

Belote, J. M. & Lucchesi, J. C. (1980*a*). Male specific lethal mutations of *Drosophila melanogaster*. *Genetics* **94**, 165–186.

Belote, J. M. & Lucchesi, J. C. (1980*b*). Control of *X* chromosome transcription by the maleless gene in *Drosophila*. *Nature* **285**, 573–575.

CHATTERJEE, R. N. & MUKHERJEE, A. S. (1977). Chromosomal basis of dosage compensation in *Drosophila*. IX. Cellular autonomy of the faster replication of the *X* chromosome in haplo *X* cells of *Drosophila melanogaster* and synchronous initiation. *Journal of Cell Biology* **74**, 168–180.

CLINE, T. W. (1978). Two closely linked mutations in *Drosophila melanogaster* that are lethal to opposite sexes and interact with daughterless. *Genetics* **90**, 683–698.

CLINE, T. W. (1979*a*). A product of the maternally-influenced sex lethal gene determines sex in *Drosophila melanogaster*. *Genetics* **91**, 522.

CLINE, T. W. (1979*b*). A male-specific lethal mutation in *Drosophila melanogaster* that transforms sex. *Developmental Biology* **72**, 266–275.

CLINE, T. W. (1983). The interaction between daughterless and sex-lethal in Triploids: a lethal sex-transforming maternal effect linking sex-determination and Dosage Compensation in *Drosophila melanogaster*. *Developmental Biology* **95**, 260–274.

DAS, M., MUTSUDDI, D., DUTTAGUPTA, A. K. & MUKHERJEE, A. S. (1982). Segmental heterogeneity in replication and transcription of X_2 chromosome of *Drosophila miranda* and conservativeness in the evolution of dosage compensation. *Chromosoma* **87**, 373–388.

GADAGKAR, R., NANJUNDIAH, V., JOSHI, N. V. & SHARAT CHANDRA, H. (1981). Measurement of the ratio of the number of *X* chromosomes to sets of autosomes in *Drosophila melanogaster*. *Current Science* **50**, 105–107.

GADAGKAR, R., NANJUNDIAH, V., JOSHI, N. V. & SHARAT CHANDRA, H. (1982). Dosage compensation and sex determination in *Drosophila*: Mechanism of measurement of the *X/A* ratio. *Journal of Bioscience* **4**, 377–390.

GHOSH, M. & MUKHERJEE, A. S. (1983). Regulation of dosage compensation in *Drosophila*. *XV*. *International Congress of Genetics*, Abstract no.**178**, p. 104.

KAZAZIAN, H. H., YOUNG, W. J. & CHILDS, B. (1965). *X*-linked 6-phosphogluconate dehydrogenase in *Drosophila* subunit association. *Science* **150**, 1601–1602.

LAKHOTIA, S. C. & MUKHERJEE, A. S. (1969). Chromosomal basis of dosage compensation in *Drosophila*. I. Cellular autonomy of hyperactivity of the male *X* chromosome in salivary glands and sex differentiation. *Genetical Research* **14**, 137–150.

LAKHOTIA, S. C. & MUKHERJEE, A. S. (1970). Chromosomal basis of dosage compensation in *Drosophila*. III. Early completion of replication by the polytene *X* chromosome in male: further evidence and its implications. *Journal of Cell Biology* **47**, 18–33.

LINDSLEY, D. L. & GRELL, E. H. (1968). Genetic variations of *Drosophila melanogaster*. *Carnegie Institute Washington*, publication no. 627.

LUCCHESI, J. C. (1973). Dosage compensation in *Drosophila*. *Annual Review of Genetics* **7**, 225–237.

LUCCHESI, J. C. (1977). Dosage compensation: Transcription level regulation of *X*-linked genes in *Drosophila*. *American Zoologist* **17**, 685–693.

LUCCHESI, J. C. & SKRIPSKY, T. (1981). The link between dosage compensation and sex differentiation in *Drosophila melanogaster*. *Chromosoma* **82**, 217–227.

LYON, M. F. (1961). Gene action in the *X* chromosome of the mouse (*Mus musculus* L.). *Nature* **190**, 372–373.

MARONI, G. & LUCCHESI, J. C. (1980). *X* chromosome transcription in *Drosophila*. *Chromosoma* (*Berl.*) **77**, 253–261.

MARONI, G. & PLAUT, W. (1973). Dosage compensation in *Drosophila melanogaster* triploids. II. Glucose-6-phosphate dehydrogenase activity. *Genetics* **74**, 331–334.

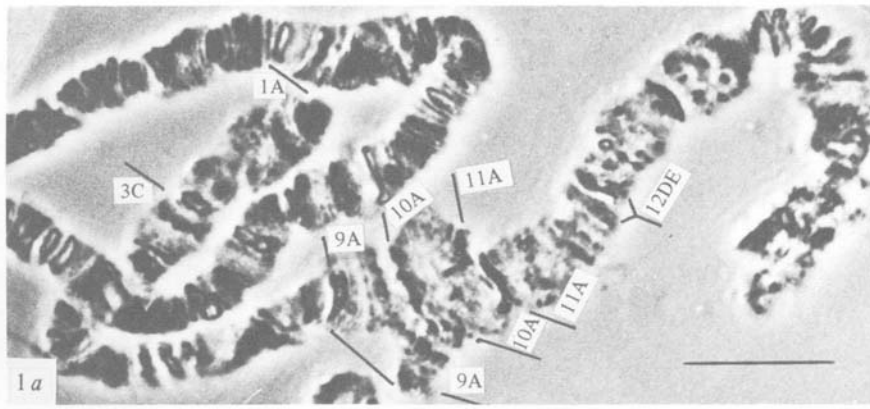
MUKHERJEE, A. S. (1982). Dosage compensation as a model system for genetic regulation in eukaryotes. *Current Science* **51**, 205–212.

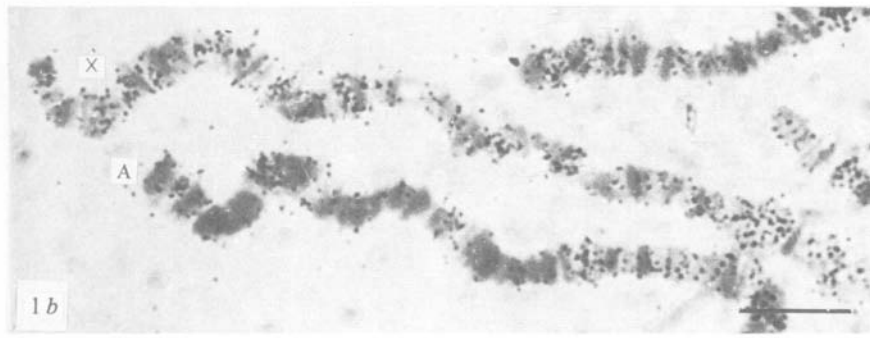
MUKHERJEE, A. S. & BEERMAN, W. (1965). Synthesis of ribonucleic acid by the *X* chromosomes of *Drosophila melanogaster* and the problem of dosage compensation. *Nature* **207**, 785–786.

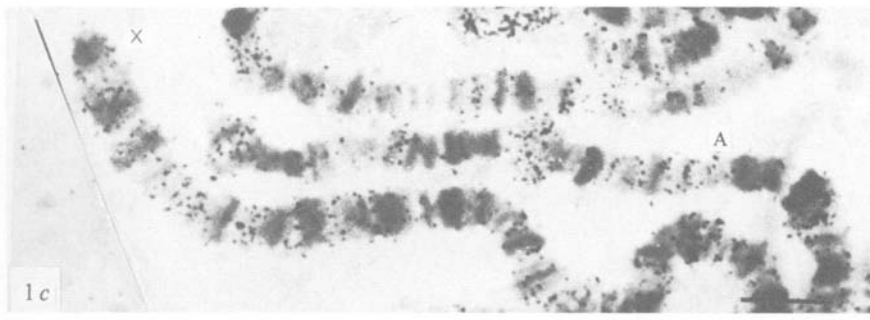
MUKHERJEE, A. S. & GHOSH, M. (1985). Enhanced hyperactivity of the *X* chromosome in In(1)BM^2 (RV) strain of *Drosophila melanogaster* and evidence for the existence of controlling elements in the *X* (Submitted.)

MUTSUDDI, M., MUTSUDDI, D., MUKHERJEE, A. S. & DUTTAGUPTA, A. K. (1984). Conserved autonomy of replication of the *X* chromosomes in hybrids of *Drosophila miranda* and *Drosophila persimilis*. *Chromosoma* **89**, 55–62.

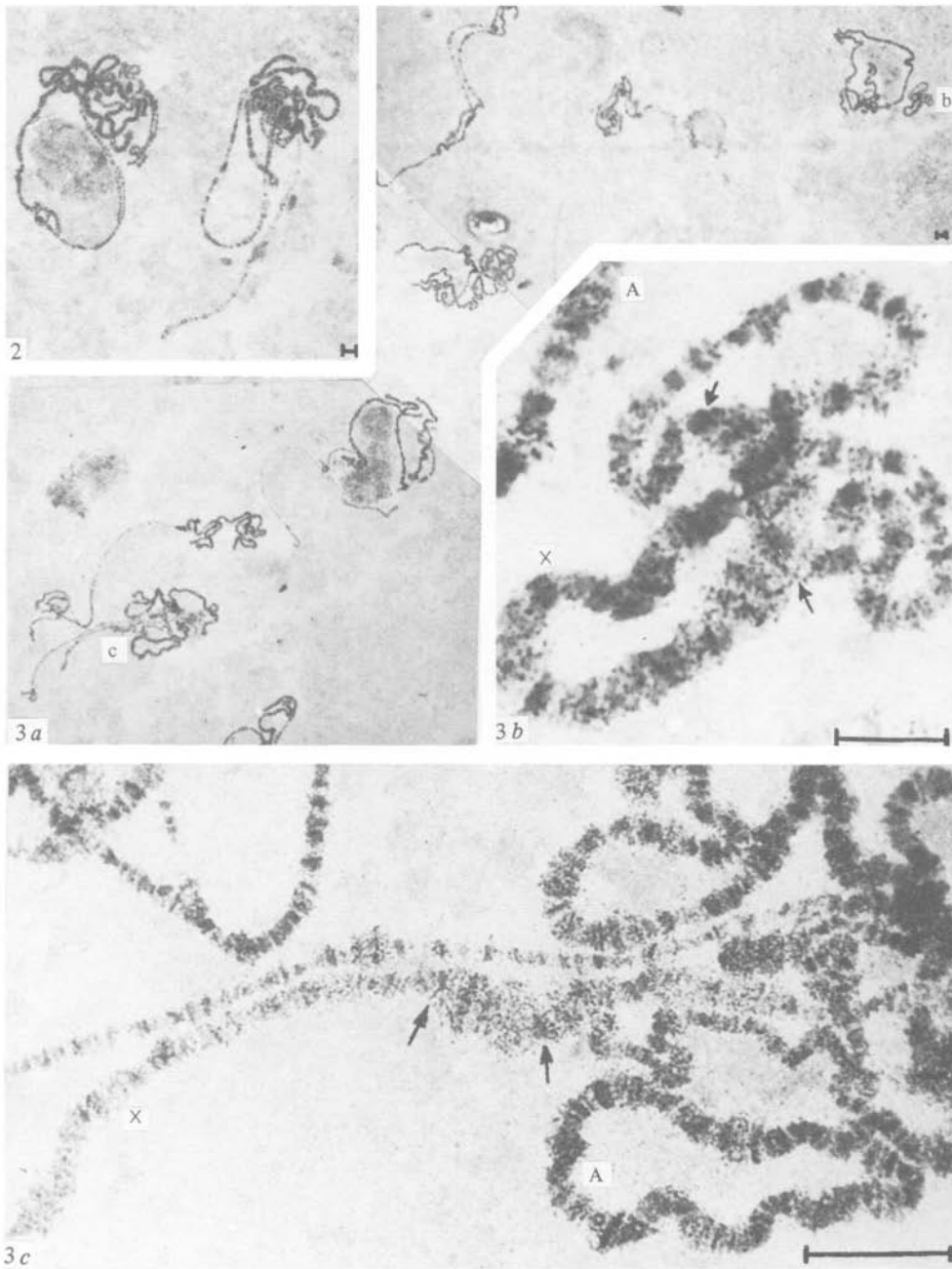
PRASAD, J., DUTTAGUPTA, A. K. & MUKHERJEE, A. S. (1981). Transcription in *X* chromosomal segmental aneuploids of *Drosophila melanogaster* and regulation of dosage compensation. *Genetical Research* **38**, 103–113.



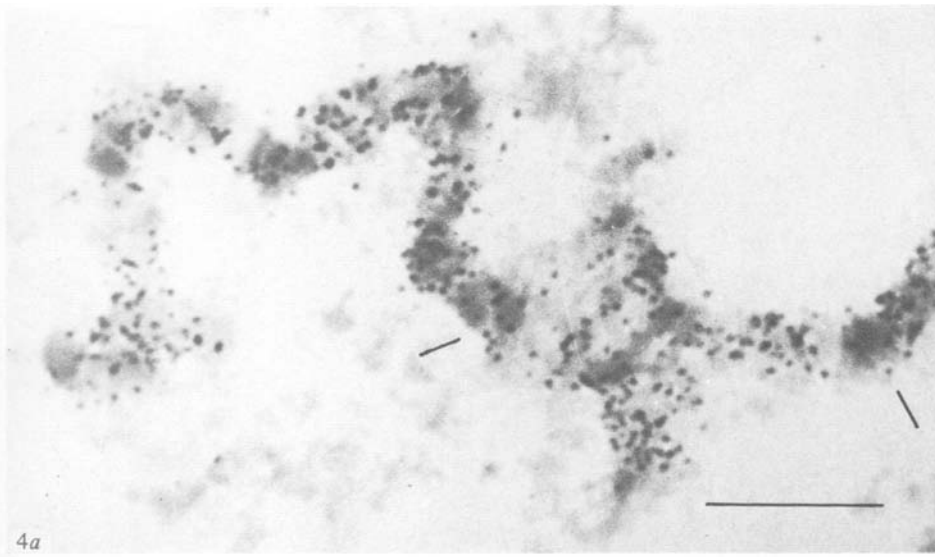




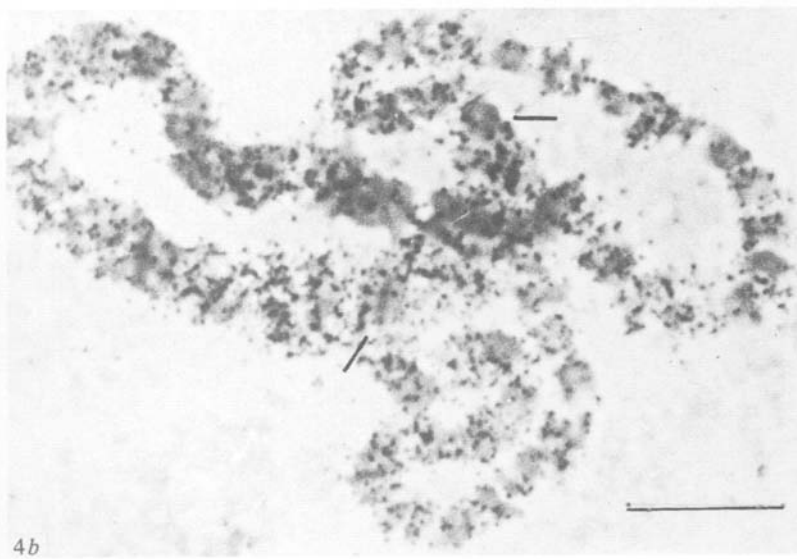
https://doi.org/10.1017/S0016672300022424 Published online by Cambridge University Press



https://doi.org/10.1017/S0016672300022424 Published online by Cambridge University Press



4*a*



4*b*

https://doi.org/10.1017/S0016672300022424 Published online by Cambridge University Press

Skripsky, T., Lucchesi, J. C. (1980). Females with sex-combs. *Genetics* **94**, 598.
Stewart, B. R. & Merriam, J. R. (1975). Regulation of gene activity by dosage compensation at the chromosomal level in *Drosophila*. *Genetics* **79**, 635–647.
Stewart, B. R. & Merriam, J. R. (1980). Dosage compensation. In *The genetics and biology of Drosophila*, vol. 2*d* (ed. M. Ashburner and T. R. F. Wright), pp. 107–140. Academic Press.
Strobel, E., Pelling, C. & Arnheim, N. (1978). Incomplete dosage compensation in an evolving sex chromosome. *Proceedings of National Academy of Science, U.S.A.* **75**, 931–935.

EXPLANATION OF PLATES

Plate 1

Fig. 1*a*. Photomicrograph showing morphology of the *X* chromosome of Dp 9A-11A male salivary gland.
Fig. 1*b*. A photomicrograph of a [^{3}H]uridine autoradiogram of the *X* chromosome of a polytene nucleus from the salivary gland of a male larva of *Drosophila melanogaster*. Note that the labelling density on the *X* (single) is very similar to that on autosome (paired).
Fig. 1*c*. The same from a female larva. Note the difference in stainability and grain distribution between the male and female *X* chromosome. The horizontal bars on this and all photographs represent 10 μm. *X*, *X* chromosome; *A*, autosome.

Plate 2

Fig. 2. A low magnification photomicrograph of a [^{3}H]UR autoradiogram of an aneuploid mosaic larva [R(1)w^{v^C}/Dp 9A-11A] showing *XX* (left) and *XO* (right) nuclei in the same field of the same preparation.
Fig. 3*a*. A low magnification photomicrograph of another [^{3}H]uridine autoradiogram from a mosaic salivary gland (Dp 9A-11A) showing the *XX* (3*b*) and *XO* (3*c*) nuclei.
Fig. 3*b*. The *XX* nucleus bearing one Ring-*X* magnified from the same nucleus. The three segments 9A-11A (two of the normal *X*'s and one of the duplication) are shown between arrows.
Fig. 3*c*. The *XO* nucleus magnified from the same nucleus. The two segments of 9A-11A (one of the normal *X* and one of the duplication) are shown between arrows.

Plate 3

Fig. 4. An autoradiogram of the *X* chromosomes in an *XO* aneuploid nucleus (*a*) and in an *XX* aneuploid nucleus (*b*) at higher magnification. The *XX* nucleus is a paired ring-*X* and Dp 9A-11A.