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TRANSCRIPTIVE AND REPLICATIVE ACTIVITY OF THE X CHROMOSOME IN AN AUTOSOMAL SEGMENTAL HYPERPLOID IN *DROSOPHILA* AND ITS SIGNIFICANCE

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SUMMARY

In the present investigation the transcription and replication patterns have been examined in different segments of the X chromosome and in certain specific segments (88B–92A) of an autosomal segmental hyperploid in which an extra segment 88B–92A (3R) is translocated to the X chromosome in addition to the normal two doses. Transcriptive activity monitored by [^{3}H]uridine-labelling of these autosomal hyperploids reveals an enhanced hyperactivity of the male X chromosome while the female X chromosomes show no change in their activity. [^{3}H]thymidine autoradiograms reveal that while the labelling frequencies of most replicating sites are distinctly lowered in the autosomal hyperploid males, no change within sexes is resolvable with regard to labelling-intensity profile. Furthermore, the X-autosome labelling frequency relation shows a distinct deviation from linearity, suggesting multiple events that lead to a higher template form of the X chromosome. These findings lead us to suggest that the signals emanating from autosome(s) do not interfere with the primary modulation inherent in the X chromosome, but act on a modulated organization of the same at a second step evoking higher activity in the male X chromosome. The results further reveal that the gene activity of the X chromosome remains unaffected by the pattern of pairing of the autosomal segments.

INTRODUCTION

It has been demonstrated, with substantial supporting evidence, that dosage compensation in *Drosophila* operates by hyperactivity of the X chromosome in the male (Mukherjee & Beermann, 1965; Mukherjee, 1966; Lakhotia & Mukherjee, 1969; Chatterjee & Mukherjee, 1973; Korge, 1970*a,b*; Lucchesi, Rawls & Maroni, 1974; Maroni & Plaut, 1973*a,b*). The hyperactivity of the X chromosome remains unaltered when either an X segment is translocated to an autosome (Tobler, Bowman & Simmons, 1971), or an autosomal segment interrupts the contiguity of the X as an insertion (as evidenced by early completion of DNA replication of the X in such cases; Lakhotia, 1970).

Studies on transcription and enzyme activity in normal females and males, and in metafemales and metamales (Lucchesi *et al.* 1974; Lucchesi, Belote & Maroni, 1977), have provided evidence in favour of a role for some autosomal factors in the determination of hyperactivity of the X chromosome in male *Drosophila*. The

Key words: autosomal hyperploid, *Drosophila*, X chromosome.

discovery of the action of autosomal sex-specific lethal mutants has provided strong evidence in support of autosomal control (Belote & Lucchesi, 1980*a,b*; Cline, 1983).

Yet, evidence against exclusive autosomal control has come from results on the X-chromosome activity in different Sxl alleles (Lucchesi & Skripsky, 1981; Cline, 1983) and also from those on transcription in segmental hyperploids (Prasad, Duttagupta & Mukherjee, 1981), and from studies on sex-mosaics in hyperploids (Prasad-Sinha & Mukherjee, 1985). More direct evidence in favour of X–autosomal dual control has been claimed by the transcription pattern in the $In(1)BM^2$ (re-inverted) strain where the X chromosome is nearly four times as hyperactive and it is suppressible by a duplication of 16A–20D (Ghosh & Mukherjee, 1983; Mukherjee & Ghosh, unpublished data).

We have examined the transcription and replication patterns in different segments of the X chromosome and that of an autosomal segmental hyperploid in which a piece of the autosomal segment is translocated to the X chromosome in addition to the normal two doses. Results of this investigation should help us to determine whether or not a change of position and dosage in a given chromosome region leads to a concomitant change in the transcriptive and replicative behaviour of the X-chromosomal hyperactivity in male *Drosophila*, and whether chromosomal pairing of the autosomal hyperploid has any bearing on the activity of the X chromosome.

MATERIALS AND METHODS

Genetic procedures

The translocation stock used in these experiments was T(1,3)05 D/+; yf := of *Drosophila melanogaster*. The duplication-bearing males Dp(3;1)05 obtained from this stock carry a duplicated segment of autosome (88B–92A, 3R), one extra dose being inserted between 4F and 5A of Bridges' map of the X chromosome while the other two doses (88B–92A) are normally present on the 3R segment (Fig. 1A,B). These males are maintained with attached X females (Fig. 2A). To obtain segmental autosomal hyperploid female, Oregon R^+ virgins were crossed to Dp (3;1)05 D/+ males and the cross yielded daughters having three doses of the 88B–92A fragment (Fig. 2B).

Cytological procedure

Flies were reared on standard *Drosophila* food at 25°C. Larvae for cytological observations were grown on food supplemented with yeast. Salivary glands were dissected out from third instar larvae (either male or female) of the duplication strain in Ringer (pH 7·2). To measure the degree of RNA synthesis on the X chromosome and on the autosome, glands were labelled with [^{3}H]uridine (500 μCi ml^{-1}, sp. act. 9700 mCi mmol^{-1}; obtained from Bhabha Atomic Research Centre, Bombay) for 10 min, and, for replication, glands were incubated in [^{3}H]thymidine (400 μCi ml^{-1}, sp. act. 18800 mCi mmol^{-1}, obtained from BARC, Bombay) for 20 min. After labelling (with either [^{3}H]uridine or [^{3}H]thymidine) glands were fixed in 1:3 (v/v) acetic acid/methanol and squashed on 50% (v/v) acetic acid; coverslips were removed and autoradiography was performed by the usual technique with Kodak AR 10 stripping film (Lakhotia & Mukherjee, 1969). The autoradiograms were exposed for 15–16 days and developed in Kodak D19b. Preparations were stained with 1% Toluidene Blue. Oregon R^+ males and females and T(1,3)05 males were used as controls for the duplication males and females, respectively, labelled with either [^{3}H]uridine or [^{3}H]thymidine at the same concentrations, and processed for autoradiography using the same procedure. A minimum of three silver grains over a given chromosomal segment was deemed necessary to indicate significant labelling. Photographs were taken under oil-immersion objective (×100) in a Zeiss Photomicroscope III with bright-field transmitted or phase-contrast illumination.

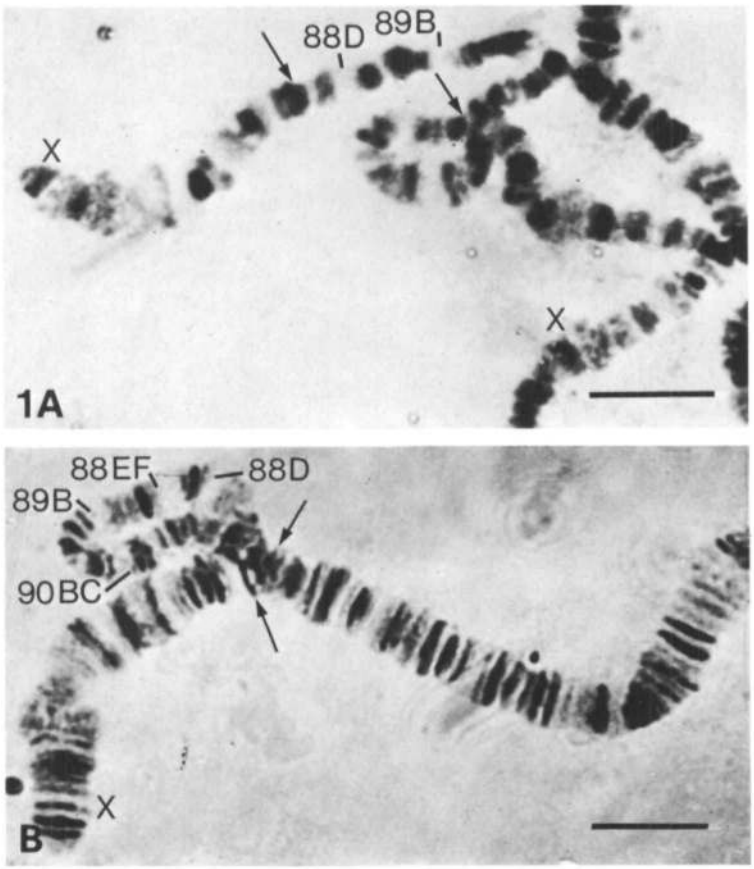


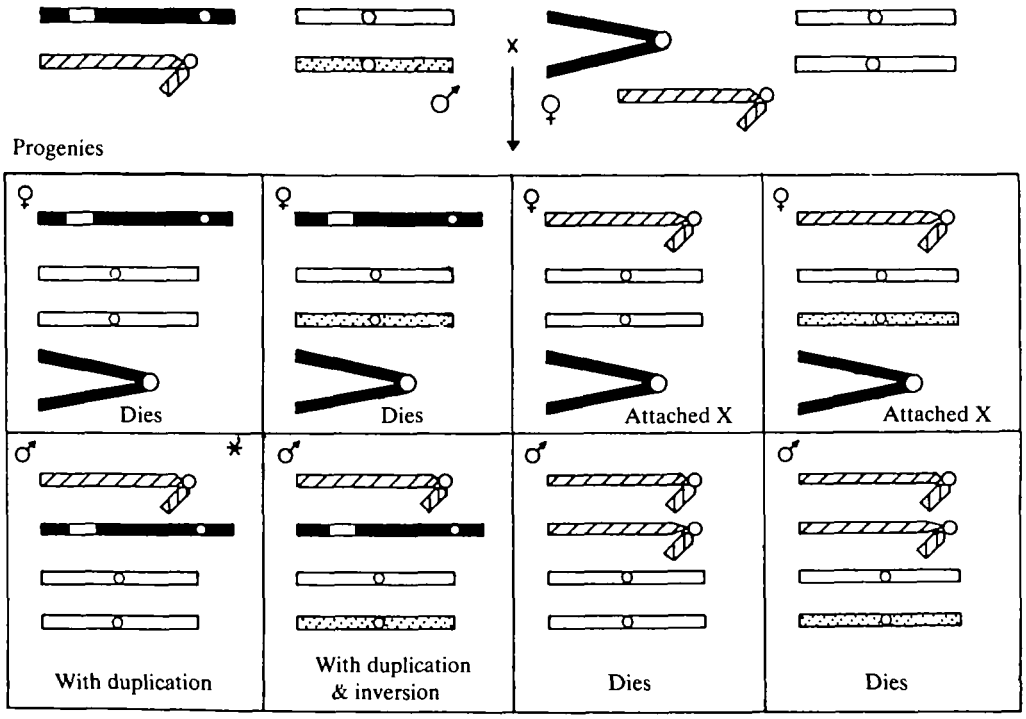
Fig. 1. Morphology of the distal part of the X chromosome, bearing the translocated segment of the autosome 3R, showing: A, the male X; and B, the female X.

RESULTS

Transcription in the autosomal hyperploid

In the present investigation RNA synthesis on larval salivary gland chromosomes has been examined autoradiographically in an autosomal segmental hyperploid (88B–92A) present as a duplication inserted in the X chromosome of *D. melanogaster*, and compared with two controls, Oregon R^+ and X–3 translocation T(1,3)05 with two doses of the segment 88B–92A. The intensity of silver grains due to [^{3}H]uridine was computed from actual grain counts on different parts of the X chromosome and the autosomal segment. The data (Table 1) reveal that the relative intensity of grains on 1A–4F of the X chromosome, i.e. the means of ratios of the number of silver grains on the X-chromosomal part and that on the part of the 2R (49E–56EF) of the corresponding nuclei is $0{\cdot}60 \pm 0{\cdot}04$ on Oregon R^+ male (Fig. 3A), $0{\cdot}66 \pm 0{\cdot}02$ in the T(1,3)05 X–3rd translocation male with two doses of 88B–92A (Fig. 3B) and $0{\cdot}80 \pm 0{\cdot}04$, in the hyperploid male nuclei in which the two normal segments were not paired with the inserted 3R segment (Fig. 3C); $0{\cdot}95 \pm 0{\cdot}07$, in

those hyperploid male nuclei in which one inserted and one normal 88B–92A segment were paired while the third dose was free (Fig. 3D); and $0{\cdot}92 \pm 0{\cdot}08$, in those in which all three doses of 88B–92A were paired together (Fig. 3E). The differences between the means of ratios of grains (1A–4F/49E–56EF) in the



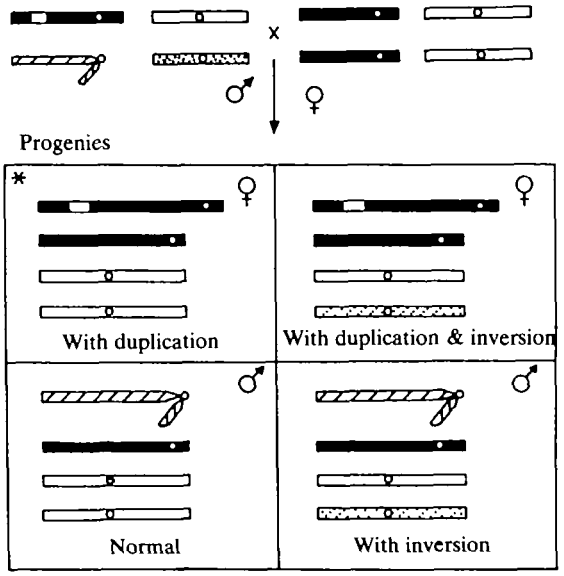


Fig. 2

hyperploid males under all three conditions of pairing and that in the Oregon R^+ male are significant ($P < 0{\cdot}01$, by F test), while the ratios in the X–3 translocation-bearing T(1,3)05 male with two doses of the segment and the Oregon R^+ male are not significantly different. Comparison of the X/2R ratios for the segment 5A–20F also reveals the same picture (Table 1). In the female, the corresponding ratios of grain numbers for 1A–4F and 5A–20F among hyperploids and Oregon R^+ are not significantly different. The [^{3}H]uridine labelling of the Oregon R^+ female X chromosome and the X chromosome with autosomal insertion under different conditions of pairing is shown in Fig. 4. On the other hand, the mean 88B–92A/49E–56EF grain ratios in Oregon R^+ male ($0{\cdot}86 \pm 0{\cdot}04$) and female ($0{\cdot}75 \pm 0{\cdot}03$) are significantly less than in the duplicated strain. The increased ratios in the hyperploid male and female are nearly 1·5–1·7 and 1·5–1·9 times (depending on the condition of synapsis) that in the Oregon R^+ male and female, respectively. Thus, these data clearly support a dosage effect for the autosomal segment (expected increase for dosage effect being 1·5 times) regardless of whether the segment is present in the X chromosome or the autosome. The relative incorporation of [^{3}H]uridine on the non-duplicated segment 96EF–100DE of the 3R has been analysed as the 3R/2R grain ratio and this serves as an internal control. The results show that there is no significant difference in the labelling intensity in the 96EF–100DE region in Oregon R^+ and hyperploid males and females (Table 1).

These findings imply that the addition of an autosomal piece to a diploid male (1X2A) tends to enhance the hyperactivity of the X chromosome in the male, but has no effect in the female. Furthermore, it indicates that the pairing condition of the autosomal segment has apparently no effect on the gene activity of the X chromosome.

Replication in an autosomal hyperploid

The DNA synthesis of Dp(3;1)05 male and female larvae has been compared with that of Oregon R^+ male and female larvae. The representative autoradiograms of [^{3}H]thymidine labelling in the Dp(3;1) female bearing the duplication is shown in Fig. 5A,B. For a comparison of the replicative behaviour, the labelling frequencies of the segments 1A–8ABC on X and 56EF–60D on 2R were scored. Results presented in Table 2 clearly establish that the labelling frequencies of most of the sites on the X chromosome of the Dp(3;1) males are less than those on that of the Oregon R^+ males and even much less than those on that of the Oregon R^+ females. In contrast, the labelling frequencies on 2R are highly comparable in both Oregon R^+ and Dp(3;1) male and female. A comparison of the intranuclear variation in [^{3}H]thymidine-labelling frequencies is presented in Fig. 6. Clearly both Oregon R^+

Fig. 2. Protocol of genetic crosses for generating duplication-bearing males and females. A. yf :=/Y (attached X/Y) females were crossed to Dp(3;1)05, D/+ of Oliver males. Filled bars represent the X chromosome, the dotted and open bars are the third chromosomes with and without inversion, respectively, and the hatched J-shaped figures are the Y chromosome. *, the progeny selected for study. B. Oregon R^+ virgin females were crossed to Dp(3;1)05, D/+ of Oliver males. All symbols are as in A.

Table 1. *Relative intensity of grains* ± S.E. *on different parts of the X chromosome and the autosome labelled with [^{3}H]uridine*

Stock	1A–4F/49E–56F		5A–20F/49E–56F		88B–92A/49E–56F				96EF–100DE/49E–56F	
	♂	♀	♂	♀	♂		♀		♂	♀
Oregon R^+	0·60 ± 0·04 (20)	0·50 ± 0·03 (20)	1·69 ± 0·06 (20)	1·60 ± 0·13 (20)	0·86 ± 0·04 (20)		0·75 ± 0·03 (20)		0·45 ± 0·02 (20)	0·46 ± 0·02 (20)
T(1,3)05 D	0·66 ± 0·02 (21)	—	1·58 ± 0·05 (21)	—	0·88 ± 0·05 (11)	[0·75 ± 0·03] (10)	—		0·57 ± 0·02 (21)	—
Dp(3;1)05 (1 dose 88B–92A in X and 2 doses 88B–92A in 3R)	0·80 ± 0·04* (20)	0·64 ± 0·02 (20)	2·10 ± 0·05* (20)	1·70 ± 0·07 (20)	1·50 ± 0·06* (20)		1·40 ± 0·11* (20)		0·45 ± 0·002 (20)	0·44 ± 0·01 (20)
					2 doses	1 dose	2 doses	1 dose		
					1·00 ± 0·04	0·57 ± 0·03	0·80 ± 0·02	0·46 ± 0·01		
Dp(3;1)05 (1 inserted and 1 normal segment 88B–92A paired, other dose in 3R free)	0·95 ± 0·07* (8)	0·60 ± 0·04 (5)	2·10 ± 0·13* (8)	1·70 ± 0·02 (5)	1·30 ± 0·07* (8)		1·30 ± 0·02* (5)		0·53 ± 0·15 (8)	0·60 ± 0·07 (5)
					2 doses	1 dose	2 doses	1 dose		
					0·97 ± 0·06	0·43 ± 0·02	0·89 ± 0·03	0·45 ± 0·008		
Dp(3;1)05 (3 doses of 88B–92A paired)	0·92 ± 0·08* (12)	0·60 ± 0·043 (12)	2·10 ± 0·15* (12)	1·50 ± 0·07 (12)	1·40 ± 0·09* (12)		1·15 ± 0·04* (12)		0·54 ± 0·04* (12)	0·44 ± 0·01 (12)

Number of observation is within parenthesis.
* Significant ($P < 0·01$).

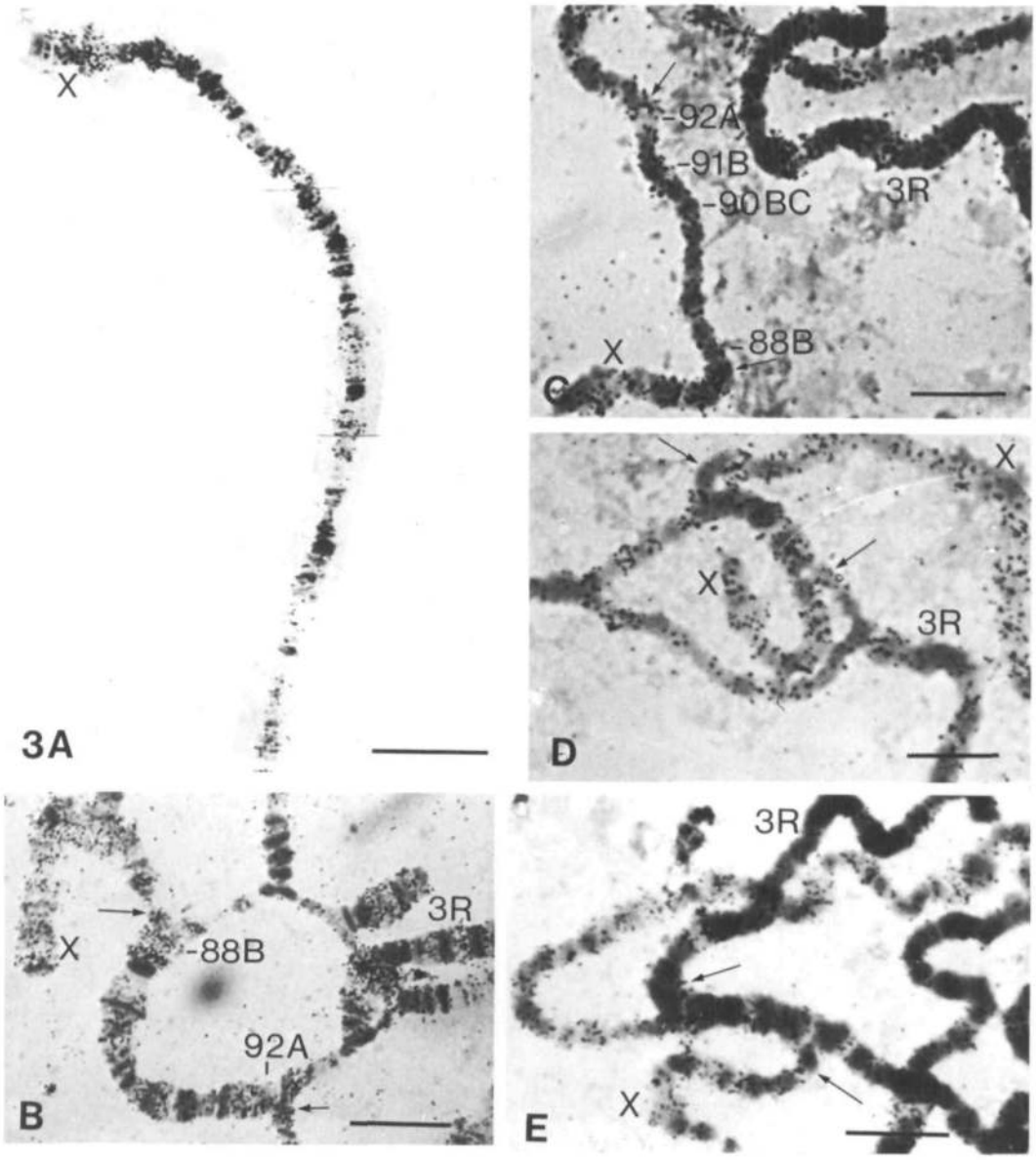


Fig. 3. A–E. Photomicrographs showing the [^{3}H]uridine labelling of the X chromosome of Oregon R^+ (A), T(1,3)05, D/+ (B) and Dp(3;1)05, D/+ (C–E) males. The translocated and, or, duplicated autosomal segments are indicated by arrows. The nucleus in B shows the X–3 translocation, in which the translocated segment (88B–92A) in X is paired with its homologue in 3R. In C the nucleus from Dp(3;1)05, D/+ male shows the inserted segment in X not paired with the two normal segments. In D the situation in another Dp(3;1)05, D/+ nucleus showing the inserted and one normal segment of 88B–92A paired, and the third dose free. In E a hyperploid male nucleus is shown where all three doses of the 88B–92A segment are paired.

male X and Dp(3;1) male X show a polynomial regression fitted to the equation: $Y = a + bX + cX^2$, in contrast to linear regression for the Oregon R^+ female X and the duplication-bearing female X chromosome. The deviation from linearity for the two

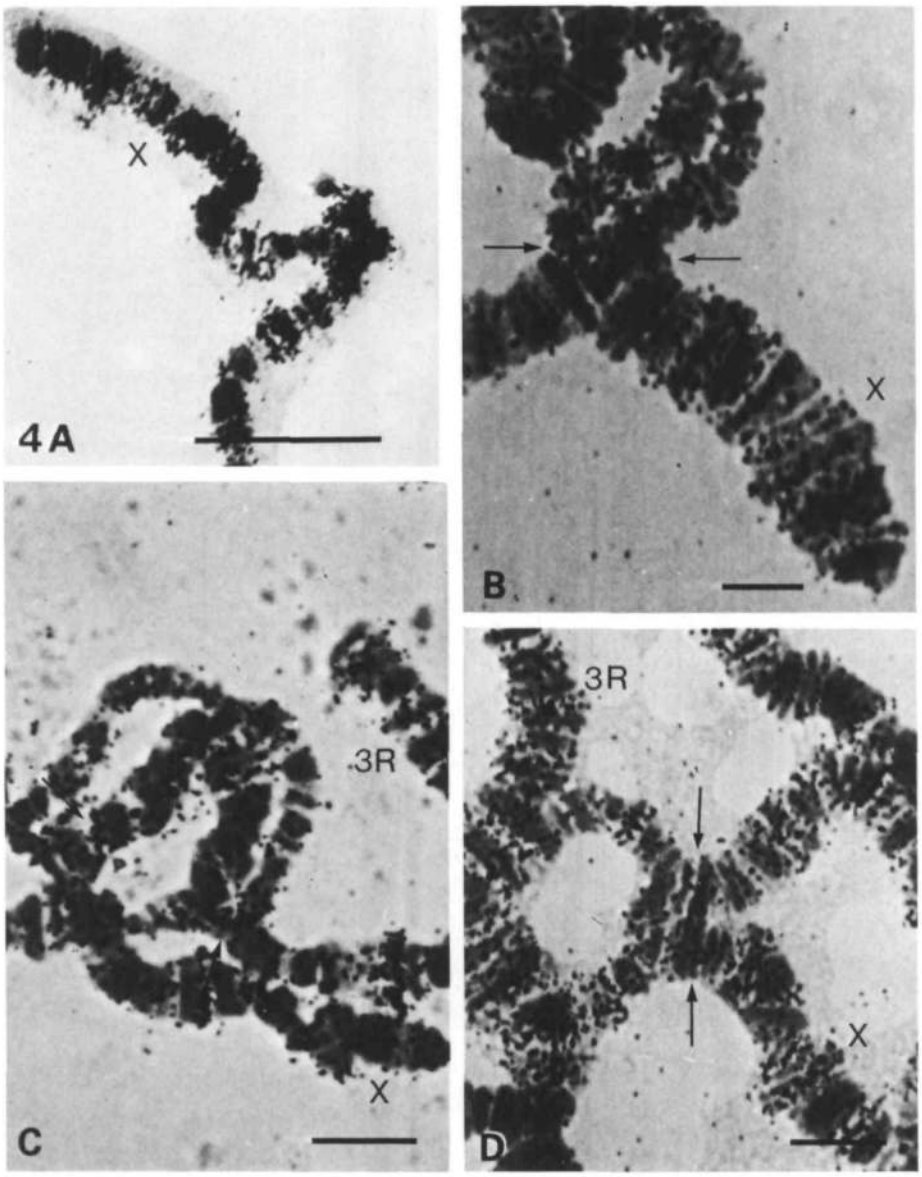


Fig. 4. A–D. Autoradiographs showing [^{3}H]uridine labelling of the X chromosomes of Oregon R^+ (A) and the Dp(3;1)05, D/+ (B–D) females. The duplicated autosomal segment is indicated by arrows. B. The nucleus of a hyperploid female in which the inserted autosomal segment in X is not paired with its normal homologues. C. The situation of pairing in a nucleus in which the inserted and one normal 88B–92A segment are paired and the third dose is free. D. All three 88B–92A segments are paired.

types of males is significant and, as resolved by the F values, the difference between the two males is also significant. Such a comparison strongly attests to the fact that the increased transcriptional competence of the X chromosome in the autosomal segmental hyperploid is decided at the template level and that the X chromosome of such a duplication-bearing complement replicates even earlier. Forty five different sites (see Lakhotia & Mukherjee, 1970) on the X chromosome (1A–12DE), 20 sites (delineated according to Nash & Bell, 1968) on the autosome 2R (56F–60F) and 18 sites on the duplication-bearing segment (88B–92A) were chosen for grain counting. To minimize the well-known difficulties of autoradiography and cytology (overlapping grains, dense packing of bands within the duplication loop of Dp(3;1)05, D/+ chromosomes etc.), only the nuclei at the terminal phase of replication (2D–1D pattern) were taken into account for a replication study of both the control (Oregon R^+) and the duplication strain. The labelling-intensity profile based on the ratio of silver grains on the specific replication units (1A, 1B, 1C etc...12DE) of the X chromosome to that on 56F of the autosome 2R (of the same nuclei) reveals that while more than 50% of the 45 sites of the X chromosome have a ratio greater than 0·4 in the female, over 80% of the sites have a ratio of less than 0·4 in the male in both Oregon R^+ and Dp(3;1) (Fig. 7A,B). No such differences could be detected for the autosomes.

However, no difference in the intensity profile is detectable for the X chromosome within the sexes. Thus, these data on the frequency of labelling and intensity profile

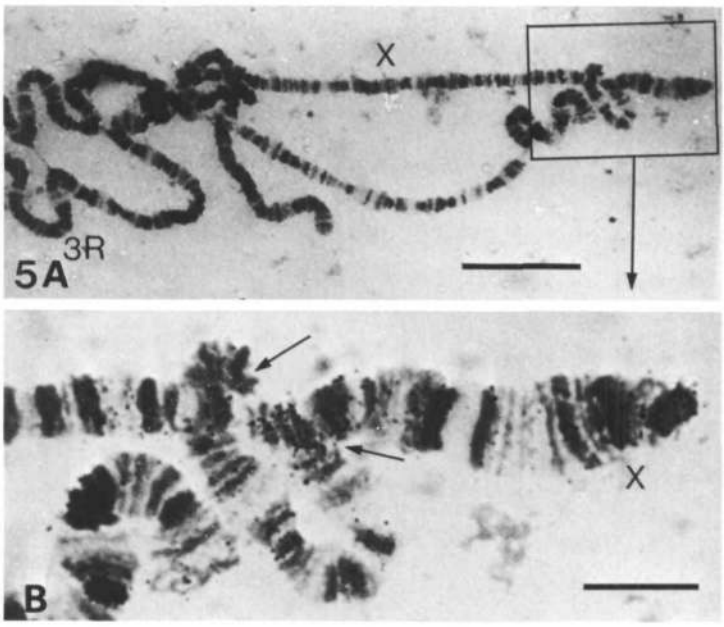


Fig. 5. Photomicrographs showing the [^{3}H]thymidine labelling (initial pattern) in a nuleus of the duplication-bearing female. Arrow indicates the enlarged view of the distal part of the X chromosome bearing the duplicated segment of 3R (88B–92A).

Table 2. *Frequency of [^{3}H]thymidine labelling (%) of different sites on the X chromosome and 2R of* D. melanogaster

	Sites on X chromosome												Sites on 2R				
Stocks	1A	1B	1C	3A	3C	4A	4BC	4DEF	5B	6A	7ABC	8ABC	56EF	58B	58E	60A	60D
Oregon R$^+$ ♂♂	96·6	46·6	6·6	16·6	100·0	30·0	26·6	76·6	16·6	43·3	96·6	76·6	100·0	90·0	46·6	36·6	73·3
Oregon R$^+$ ♀♀	96·6	76·6	36·6	50·0	100·0	80·0	80·0	96·6	43·3	90·0	96·6	83·3	100·0	93·3	43·3	43·3	76·6
Dp(3;1)05 ♂♂	63·3	23·3	0·0	1·0	96·6	26·6	3·3	43·3	0·0	13·3	33·3	3·3	100·0	93·3	43·3	33·3	70·0
Dp(3;1)05 ♀♀	100·0	70·0	30·0	36·6	100·0	73.3	76·6	100·0	43·3	83·3	93·3	83·3	100·0	90·0	40·0	33·3	73·3

taken together suggest that as the intensity is the same for the duplication-bearing male and the control male, and the frequency is considerably lower in the duplication male, the autosomal segmental hyperploidy does influence the level of activity of the X chromosome in the male. And, therefore, although the template form of X in the presence of autosomal duplication is indeed augmented, the replicative competence (as resolved by intensity profile) remains unaltered.

DISCUSSION

The results on the transcription and replication of the autosomal hyperploid strain presented here reveal that the addition of a piece of the third chromosome to an 1X2A genome enhances the hyperactivity and the template state of the male X chromosome.

On the other hand, the duplicated segment of the autosome (3R) shows a dosage effect as expected, while a non-duplicated segment of the autosome (96EF–100DE)

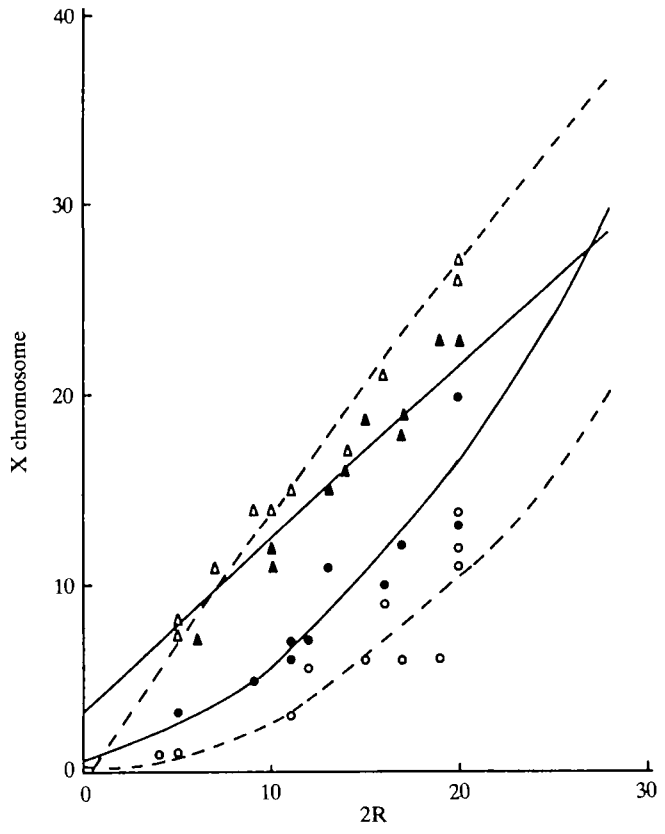


Fig. 6. Graphic representation of the number of [^{3}H]thymidine-labelling sites of the X chromosome relative to that of 2R in the Oregon R^+ and Dp(3;1)05 of Oliver males and females. The lines are curves calculated from the equation, $Y = a+bX+cX^2$, where Y axis represents the X chromosome, and x axis the autosome). (●—●) a^2 ♂; (○---○) Dp(3;1) ♂; (▲—▲) a^2 ♀; (△---△) Dp(3;1) ♀.

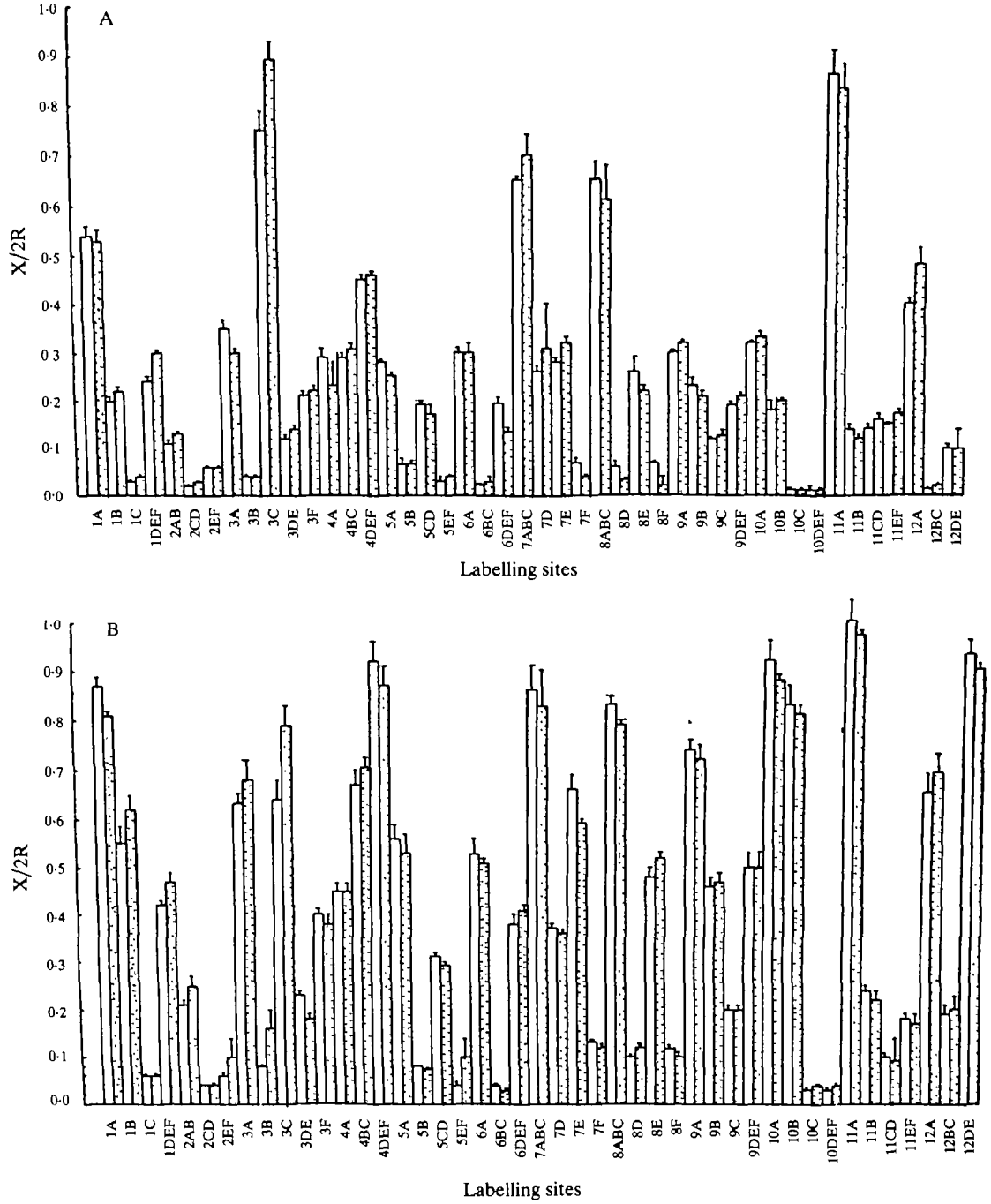


Fig. 7. A,B. Histograms showing the X/2R ratios of the intensity of [^{3}H]thymidine labelling of 1A–12DE of the X chromosome in male (A) and female (B) of Oregon R$^+$ and Dp(3;1)05, D/+ strains. Note that the relative intensity on the ordinate in the histogram for the male (A) includes most of the sites below 0·4, whereas that for the female (B) includes less than 50% of the sites below that level. The open and dotted bars represent the Oregon R$^+$ and Dp(3;1)05, D/+ strains, respectively.

of the duplication strain reveals no change from the normal. The transcriptive activity of the X chromosome and of specific segments of an autosome (88B–92A) has been analysed under various conditions of pairing between the duplicated segment of the autosome and its homologue in the genome. The result indicates that the transcriptional activity is independent of pairing.

Our results regarding transcription in a general way are in agreement with the earlier findings by Maroni & Plaut (1973*a*,*b*) and Lucchesi *et al.* (1974, 1977), which suggest the presence of regulatory factors of autosomal origin that might control the hyperactivity of the male X chromosome in *Drosophila*. However, since there is considerable evidence now that suggests that transcription of the X chromosome may be under dual regulatory control (Cline, 1983), it is conceivable that dislocation of the contiguity of the regulatory signals that may be interspersed on the X chromosome (Prasad-Sinha & Mukherjee, 1985; M. Ghosh & A. S. Mukherjee, unpublished data) may interfere with the realization of hyperactivity of the male X chromosome. In contrast, the present results reveal that the hyperactivity is not lowered due to the transposed segment of the autosome, but rather the autosomal hyperploid condition enhances it, as expected. In the case of diplo-autosomal translocation (T(X–3)05D) male the hyperactivity is maintained as in Oregon R^+. These facts rule out the possible position effect at the site of insertion on the X chromosome. Furthermore, as shown earlier by Lakhotia (1970) and as evident from the results, the X-chromosomal sections and the autosomal inserted segment manifest their own levels of activity, respectively, right from the site of insertion and there is no spreading effect. It may also be pointed out that there is no sex-related or sex-specific gene known to be located at the site of insertion, namely 4F–5A. The well-known dominant sex-specific lethal (Sxl^+) is at 6E1–7B7 (Belote & Lucchesi, 1980*a*). It may therefore be surmised that the X-chromosomal organization is predisposed by certain interacting regulatory genes.

Analysis of replication as done by the sitewise examination of the [^{3}H]thymidine labelling of the X chromosome and the duplicated part of the autosome reveals, as reported earlier by Lakhotia (1970), that while no change in the characteristic replication of the autosome is evident, the autosomal hyperploidy tends to enhance the template state of the X chromosome by an even earlier replication than euploidy.

In all earlier papers from this laboratory (Chatterjee & Mukherjee, 1973), it was reported that in whatever genetic situation, when the hyperactivity was altered, replication of DNA on the X chromosome was altered accordingly. For example, in different species of *Drosophila*, hyperactivity of the X chromosome in the male was always concurrent with the early completion of DNA replication of the X chromosome (Mukherjee & Chatterjee, 1975, 1976; Lakhotia & Mukherjee, 1970; Das, Mutsuddi, Duttagupta & Mukherjee, 1982).

No such observation has been made in metamales, but studies on replicative behaviour in metafemales have also confirmed this finding. In segmental hyperploids this parallelism has always been reinstated (Prasad-Sinha & Mukherjee, 1985). In hyperploids in which long segments from proximal ends of the X chromosome have been added, early completion and hyperactivity appear to be maintained up to the

addition of 0·62 length of the X (Mutsuddi, Mutsuddi, Duttagupta & Mukherjee, 1983), but beyond that hyperactivity is abolished and the X-chromosomal replication becomes synchronous. Recently, Goldman *et al.* (1984) convincingly established that genes that can be transcribed must replicate early, and the inactive genes must replicate and be transformed into a transcriptionally competent template form in order to be in the active state. The data presented here corroborate this relation between higher transcriptional competence and early replication.

It is conceivable on the basis of these observations that there is a parallel between enhanced transcriptional competence of the X chromosome in the male and its early completion of replication. However, the distinctly different regression (Fig. 6) for the Oregon R^+ and Dp(3;1) males, in contrast to the females, strongly suggests that the template forms resulting from the regulatory influence might be set differently in the two sexes.

What we therefore propose is that the autosomal signals do not interfere with the inherent primary modulation in the X chromosome. The autosomal signals act on the modulated organization of the X chromosome, guided by a modulator gene complex located on the X chromosome (Ghosh & Mukherjee, 1983; and unpublished) at a second step, evoking the higher activity of the male X chromosome. So an autosomal hyperploidy makes the male X more hyperactive. The findings of the present study lead us to conclude that a certain autonomously acting control system is inherent in the X chromosome, which may be influenced by the dosage of the autosome only at a second step preceding the hyperactivity. The results further indicate that the gene activity of the X chromosome is not influenced by the pairing of the autosome.

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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, Lond.* **285**, 573–575.

CHATTERJEE, S. N. & MUKHERJEE, A. S. (1973). Chromosomal basis of dosage compensation in *Drosophila*. VII. DNA replication patterns of puffs in male and female larval polytene chromosomes. *Cell Differ.* **2**, 1–19.

CLINE, T. W. (1983). The intersection between daughterless and sex lethals in triploids: A lethal sex-transforming maternal effect linking sex-determination and dosage compensation in *Drosophila melanogaster*. *Devl Biol.* **95**, 260–274.

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

GHOSH, M. & MUKHERJEE, A. S. (1983). Regulation of dosage compensation in *Drosophila*. *The 15th Int. Cong. Genet.*, New Delhi, abstr. no. 178, 104.

GOLDMAN, M. A., HOLMQUIST, G. P., GRAY, M. C., CASTON, L. A. & NAG, A. (1984). Replication timing of genes and middle repetitive sequences. *Science* **224**, 686–692.

KORGE, G. (1970*a*). Dosage compensation and effect for RNA synthesis in chromosome puffs of *Drosophila melanogaster*. *Nature, Lond.* **225**, 386–388.

KORGE, G. (1970*b*). Dosiskompensation und Dosiseffekt für RNA-synthese in Chromosomen Puffs von *Drosophila melanogaster*. *Chromosoma* **30**, 430–464.

LAKHOTIA, S. C. (1970). Chromosomal basis of dosage compensation in *Drosophila*. II. The DNA replication patterns of the male X-chromosome in an autosome X insertion in *Drosophila melanogaster*. *Genet. Res.* **15**, 301–307.

LAKHOTIA, S. C. & MUKHERJEE, A. S. (1969). Chromosomal basis of dosage compensation in *Drosophila*. I. Cellular autonomy of hyperactivity of male X-chromosome in salivary glands and sex differentiation. *Genet. Res.* **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. *J. Cell Biol.* **47**, 18–33.

LUCCHESI, J. C., BELOTE, J. M. & MARONI, G. (1977). X-linked gene activity in metamales (XY; 3A). *Chromosoma* **65**, 1–7.

LUCCHESI, J. C., RAWLS, J. M. JR & MARONI, G. (1974). Gene dosage compensation in metafemales (3X;2A) of *Drosophila*. *Nature, Lond.* **248**, 564–567.

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

MARONI, G. & PLAUT, W. (1973*a*). Dosage compensation in *Drosophila melanogaster* triploids. I. Autoradiographic study. *Chromosoma* **40**, 361–377.

MARONI, G. & PLAUT, W. (1973*b*). Dosage compensation in *Drosophila melanogaster* triploids. II. Glucose-6-phosphate dehydrogenase activity. *Genetics* **74**, 331–342.

MUKHERJEE, A. S. (1966). Dosage compensation in *Drosophila*: an autoradiographic study. *The Nucleus* **9**, 83–96.

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

MUKHERJEE, A. S. & CHATTERJEE, S. N. (1975). Chromosomal basis of dosage compensation in *Drosophila*. VIII. Faster replication and hyperactivity of both arms of the X-chromosome in males of *Drosophila pseudoobscura* and their possible significance. *Chromosoma* **53**, 91–105.

MUKHERJEE, A. S. & CHATTERJEE, S. N. (1976). Hyperactivity and faster replicating property of the two arms of the male X of *Drosophila pseudoobscura*. *J. Microsc.* **106**, 199–208.

MUTSUDDI, D., MUTSUDDI, M., DUTTAGUPTA, A. K. & MUKHERJEE, A. S. (1983). Replication and transcription in X-chromosomal hyperploids and segmental aneuploids of *Drosophila*. *The 15th Int. Cong. Genet.*, New Delhi abstr. no. 207, 118.

NASH, D. & BELL, J. (1968). Larval age and the pattern of DNA synthesis in polytene chromosomes. *Can. J. Genet. Cytol.* **10**, 82–90.

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

PRASAD-SINHA, J. & MUKHERJEE, A. S. (1985). Cellular autonomy of hyperactivity in segmental X-chromosomal aneuploids of *Drosophila* and possibility of existence of X-chromosomal regulatory signals for dosage compensation. *Genet. Res.* **46**, 19–29.

TOBLER, J., BOWMAN, J. T. & SIMMONS, J. R. (1971). Gene modulation in *Drosophila*: dosage compensation and relocated v^+ genes. *Biochem. Genet.* **5**, 111–117.

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