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Dosage Compensation of X-Chromosome Activity in Interspecific Hybrids of *Drosophila melanogaster* and *D. simulans**

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Abstract. We have used the unstable ring X-chromosome of *D. melanogaster* to generate XX/X0 mosaics in the hybrid progeny from crosses between *D. melanogaster* females and *D. simulans* males. The functional properties of the polytene X-chromosome(s) in salivary glands of such X0/XX mosaic hybrid larvae have been analysed by autoradiography after ^{3}H-uridine or ^{3}H-thymidine labelling of the glands. The *simulans* X-chromosome in the hybrid X0 nuclei displays typical pale staining, enlarged diameter, higher rate of transcription (nearly two times higher than each of the Xs in the XX nuclei in the same gland) and a faster completion of replication as would be the case in the original parental X0 or XY nuclei. In the hybrid XX polytene nuclei, the *melanogaster* as well as the *simulans* X functions in the same manner as in female cells of the parents. The nucleolar transcription is also equal in the hybrid XX and X0 nuclei. Thus it seems that despite the evolutionary diversification between these two species, the regulatory system which brings about the dosage compensation of X-chromosome activity has been conserved.

Introduction

Since the pioneering study by Mukherjee and Beermann (1965), dosage compensation of X-chromosome activity in *Drosophila* has been extensively investigated. It is now well established that the equalisation of expression of X-linked genes in XY males and XX females is brought about by hyperactivity of the single X in male. This hyperactivity of the male X is manifested in polytene nuclei in its enlarged diameter and pale staining, in higher rate of transcription and in a faster completion of its replication (Mukherjee and Beermann 1965; Lakhotia and Mukherjee 1969, 1970; Lucchesi 1974). It has also been suggested that the level of X-chromosomal activity in a nucleus is determined by the relative dosages of the autosomal complement (Maroni and Plaut, 1973; Lucchesi, 1974).

* We dedicate this paper to Professor Dr. W. Beermann on the occasion of his 60th birthday

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As an evolutionary approach, we have examined the rates of RNA synthesis on polytene X-chromosome in the interspecific hybrids of *D. melanogaster* and *D. simulans*. Classical studies by Sturtevant (1920) had shown that in crosses between these two sibling species, the sex of the hybrid progeny is usually the same as that of the *melanogaster* parent. We have undertaken studies to examine if in such interspecific hybrids the dosage compensation mechanism is also undisturbed. In an earlier preliminary study in our laboratory (Lakkad et al., 1975) it was found that the unstable ring-X or the maternally acting *mit* mutant (Gelbart, 1974) of *D. melanogaster* can be utilized to generate gynandromorphs in the hybrid progeny of *D. melanogaster* ♀ × *D. simulans* ♂ and these gynanders range from complete bilateral mosaics to those having only a small patch of X0 or XX cells. It was also observed in this initial study that at the morphological level, the organization of polytene X-chromosome in XX and X0 nuclei is similar to that seen in the female and male cells of the paternal species. In the present work, we have extended these observations and utilizing the unstable ring-X of *D. melanogaster* to generate XX/X0 mosiac salivary glands in the interspecific hybrid larvae, the patterns of transcription and replication of the X-chromosome in single (X0) and double (XX) dose conditions have been examined. This genetic system permits a direct comparison of the activity of the same X-chromosome in male and female cells with identical physiological and genetic background (Lakhotia and Mukherjee, 1969). Our results suggest that despite the evolutionary divergence between these two species, the regulatory system for dosage compensation has not changed since the haploid autosomal complements of *D. melanogaster* and *D. simulans* together can cause the *simulans* X to be typically hyperactive in the X0 cells.

Materials and Methods

The unstable ring-X carrying stock R(1)w^{vc}/*y w spl*/B^{s}Y (for details of the genetic markers, see Lindsley and Grell, 1968) of *D. melanogaster* and a stock of *D. simulans* carrying as marker *y* (yellow body colour) on the X-chromosome, were maintained under standard conditions at 24° ± 1° C. Heterozygous ring-X virgin females, R(1)w^{vc}/*y w spl*, were collected, aged for 6–7 days and mass mated with *y* males of *D. simulans*. In successful crosses, the hybrid progeny larvae were grown to late third instar stages. In these crosses, usually only female larvae appear since the *melanogaster* parent is female (Sturtevant, 1920). Morphologically, female larvae of the two genotypes, i.e., (i) R(1)w^{vc}-*melanogaster* / *y-simulans* X, and (ii) *y w spl-melanogaster*/*y-stimulans* X, can be identified by the black mouth parts of the first and yellowish-brown mouth parts of the second types of larvae. Cytologically also, the polytene nuclei of the two types of female larvae can be distinguished by the presence of a ring- and a rod-X-chromosome in the first and two rod-X-chromosomes in the second type. For the present study, salivary glands from healthy late third instar larvae with black mouth parts (i.e., with ring-/rod-X chromosomes) were excised and pulse labelled with ^{3}H-uridine (250 µCi/ml, sp. act. 10.9 Ci/mM, BARC, Trombay) for 10 min at 24° C. The labelled glands from each larva were separately fixed and squashed. The squash preparations were processed for autoradiography with Ilford L4 nuclear emulsion in the usual manner. Salivary glands from a few of these larvae were pulse labelled with ^{3}H-thymidine (250 µCi/ml, Sp. act. 23.7 Ci/mM, BARC, Trombay) for 10 min and processed for autoradiography as above.

Observations

Out of 16 ^{3}H-uridine labelled autoradiographic preparations, 5 contained X0 polytene nuclei due to loss of the ring-X in some cells at early stages of develop-

ment. The X0 polytene nuclei could be easily distinguished from the XX nuclei in the preparations on the basis of the absence of the ring-X chromosome and the presence of only one rod-X chromosome derived from the *simulans* parent. In these preparations of salivary glands from 5 mosaic larvae, 3 had nearly equal proportions of XX and X0 nuclei, while in the glands from the other two larvae, all scorable nuclei were of the X0 type. Morphologically, the single X-chromosome in every X0 nucleus seen in these mosaic hybrids reveals the typical pale staining and enlarged width throughout its length while in the XX nuclei, the ring as well as the rod Xs show normal staining and size, characteristic of XY and XX nuclei, respectively, in the parent species (Fig. 1).

To ascertain the rates of RNA synthesis in these XX and X0 nuclei, silver grains were counted on segment 1A–3B of X-chromosome and segment 61A–63D of 3L in well spread XX and X0 nuclei. We had to restrict the grain count analysis to these relatively small chromosome segments since in interspecific hybrids many chromosome regions show asynapsis (Horton, 1939). Moreover, the inversion associated with the ring-X of *D. melanogaster* (see Lindsley and Grell, 1968) prevents an optimal spreading of all chromosomes in many nuclei and therefore, inclusion of long chromosome segments for grain count analysis would severely restrict the sample size of scoreable nuclei. Nevertheless, the general grain density along the entire X-chromosome is comparable to that scored on the 1A–3B segment of a nucleus. Moreover, earlier results (Mukherjee and Beermann, 1965; Lakhotia and Mukherjee, 1970) in *D. melanogaster* have shown that scoring the grain density on shorter or longer chromosome segments gives similar results with respect to the general rate of transcription of the X-chromosome. Thus we believe that the data on smaller segments are representative of the activity levels along the entire length of X-chromosome. The results of the grain count analysis in different XX and X0 nuclei in salivary glands of the 5 mosaic larvae are presented in Table 1. In it we have not included data on grain counts on the ring-X in XX nuclei because the 1A–3B segment of the ring-X remains close to the chromocenter and thus often can not be scored. It may, however, be noted that in 17 XX nuclei in which this segment of the ring-X could be clearly seen, the mean ratio of grain counts on 1A to 3B segments of ring- and rod-Xs respectively, has been found to be 1.07 ± 0.01. This shows that the *melanogaster* and the *simulans* Xs transcribe equally in the hybrid nuclei. Similarly, the *melanogaster* and *simulans* autosomes have been seen to transcribe equally in the hybrid nuclei since in all cases examined, the asynapsed autosomal segment showed equal grain density on the homologs.

The data in Table 1 and the examples in Figure 1a and b show that in each of the three XX/X0 mosaic glands, the rod-X derived from the *simulans* parent transcribes at a much higher rate in X0 nuclei than it does in the XX nuclei: the absolute rate of ^{3}H-uridine incorporation on the X-chromosome in X0 nuclei, on average, is twice that on the same X-chromosomes in XX nuclei (the pooled mean number of silver grains on the rod-X segment in XX nuclei is 64.96 ± 0.59 while that in X0 nuclei is 128.80 ± 1.54). The relative rate of ^{3}H-uridine incorporation on the X-chromosome (expressed as X/3L ratio) also shows a significant increase in the X0 nuclei. The pooled mean

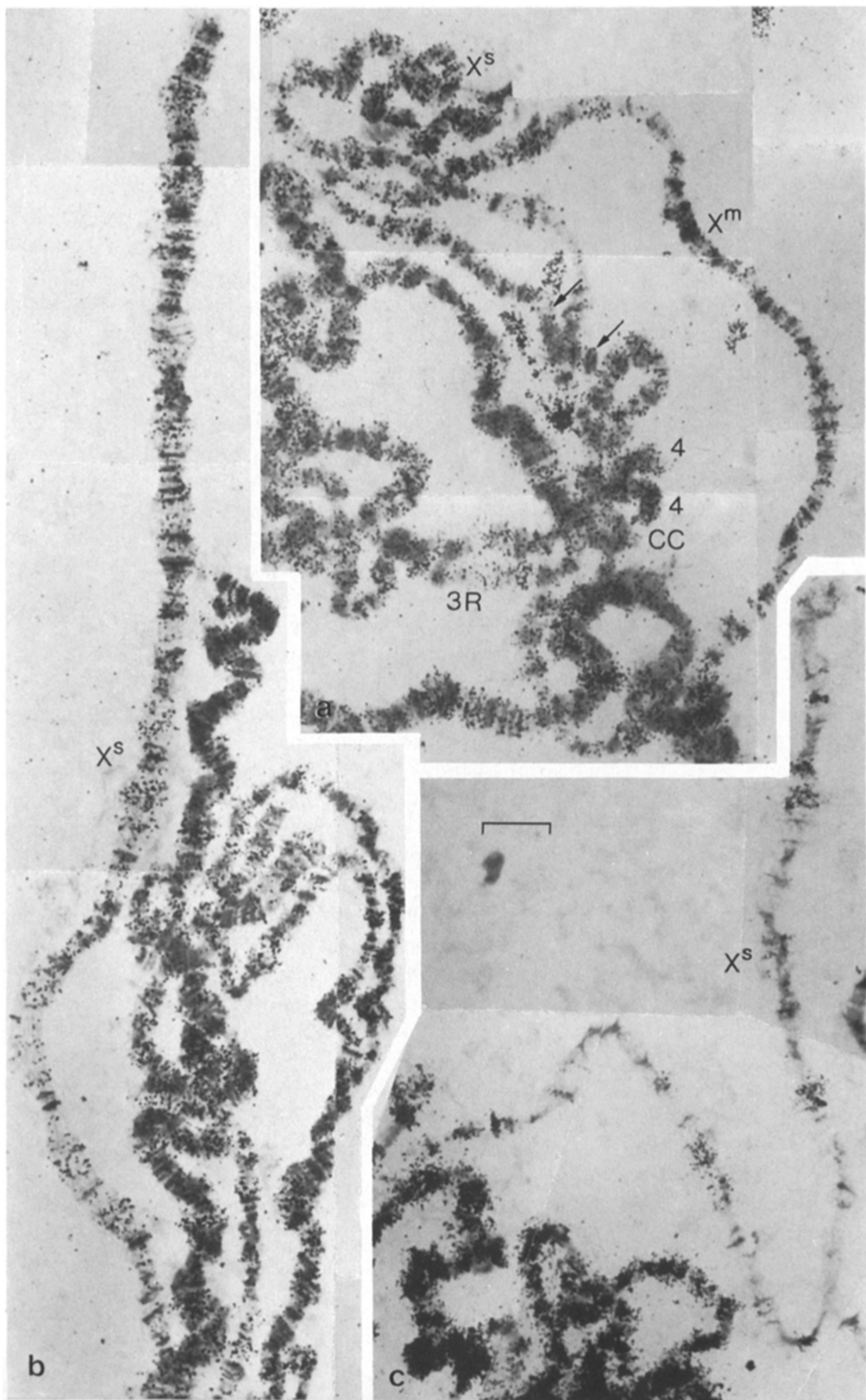
X^s
X^m
4
4
CC
3R
a
X^s
b
X^s
c

Table 1. ^{3}H-uridine labelling of segments of X- and the left arm of 3rd chromosome (3L) in XX and X0 nuclei in mosaic hybrid larvae (*D. melanogaster* ♀ × *D. simulans* ♂)

Mosaic larva No.	Nuclear type	No. of nuclei	Mean (±S.E.) No. of silver grains on[a]		Mean (±S.E.) X/3L ratio
			X-segment (1A–3B)	3L-segment (61A–63D)	
1	XX	7	51.10±1.03	114.70±3.41	0.47±0.02
	X0	6	103.80±6.24	123.30±7.87	0.86±0.02
2	XX	12	66.90±1.05	125.10±2.53	0.57±0.01
	X0	16	146.20±2.17	184.30±1.73	0.79±0.09
3	XX	6	77.20±2.22	158.50±4.19	0.50±0.02
	X0	3	142.70±17.50	168.00±14.33	0.90±0.16
Pooled means for larvae 1–3	XX	25	64.96±0.59	130.24±1.25	0.51±0.01
	X0	25	128.80±1.54	161.12±1.59	0.81±0.01
4 and 5[b]	X0	36	102.30±0.88	131.80±0.89	0.78±0.01

[a] For X-segment in XX as well as X0 nuclei, the silver grains present on the *simulans* derived rod-X only are presented here; for the 3L segment, the data represent labelling over the synapsed homologues from the two species

[b] Larvae 4 and 5 showed only X0 nuclei in salivary gland preparations; the data from the two are pooled here

X/3L grain ration in X0 nuclei (0.81) is 1.6 times more than that in XX nuclei (0.51, see Table 1). In the other larvae also which had only X0 nuclei in their salivary glands (individuals 4 and 5 in Table 1), the single X in all X0 nuclei shows the typical "male morphology" and incorporates ^{3}H-uridine at a higher level as in the X0 nuclei of XX/X0 mosaic salivary glands: the mean X/3L grain ratio in these X0 nuclei is not significantly different from that in the X0 nuclei of the mosaic glands (Table 1).

In salivary gland nuclei from hybrid larvae, the nucleolus is very prominent. Using an occular disc with square grid, the number of silver grains present in four separate 25 μm square regions of the nucleolus in 15 XX and 15 X0 nuclei in preparations from mosaic larvae nos. 2 and 3, were counted. The mean number of grains/25 μm^2 area of the nucleolus in these 15 XX nuclei is 26.09 while that in the 15 X0 nuclei is 26.15. Thus, the rate of nucleolar transcription in the XX and X0 nuclei is same.

Fig. 1. a and b. ^{3}H-uridine labelled autoradiograms of XX (**a**) and X0 (**b**) nuclei from mosaic salivary glands of a hybrid larva. In **a**, the rod-X from *simulans* parent is designated X^s, while the ring-X derived from the *melanogaster* parent is designated X^m; the 1A–3B segment of the X^m chromosome is near the chromocenter (*CC*) and is marked by two arrows; the asynapsed basal regions of 3R and 4 are also indicated. The single X from *simulans* parent (X^s) in **b** shows typical pale staining, enlarged width and a higher silver grain density than the X^s in XX nuclei (**a**). **c** Part of ^{3}H-thymidine labelled X0 polytene nucleus from mosaic salivary glands of a hybrid larva. The *simulans*-derived X^s shows labelling of very few band regions (1D type labelling pattern) while the autosomes show very few unlabelled regions (3D pattern) as is seen in parental species' male cells (see Berendes, 1966; Lakhotia and Mukherjee, 1970). Bar represents 10 μm

Among the autoradiographic preparations of ^{3}H-thymidine labelled salivary glands from ring-/rod-X heterozygous hybrid larvae also, some XX/X0 mosaics were seen. In these X0 nuclei, the X-chromosome appears to complete its replication faster than the autosomes as is seen in male polytene nuclei of *D. melanogaster* (Berendes, 1966; Lakhotia and Mukherjee, 1970) and *D. simulans* (unpublished observations): thus in all the labelled X0, but not XX nuclei, the X-chromosome shows an asychronously advanced stage of labelling pattern compared to the autosomes in the same nucleus. A representative example of such labelling pattern is shown in Figure 1c.

Discussion

The present observations show that the X-chromosome derived from the *simulans* parent can be as well dosage compensated in a cell autonomous manner (Lakhotia and Mukherjee, 1969) in the interspecific hybrid genetic milieu as in its own species' genetic background. This is evidenced in its typical pale staining, enlarged appearance and the significantly increased (nearly double) rate of ^{3}H-uridine incorporation in the X0 nuclei resulting from the somatic loss of the *melanogaster* derived ring X-chromosome. It may be noted that in the present data, the mean grain density on the 3L segment of X0 nuclei (particularly in larva no. 2, see Table 1) is slightly higher than in the XX nuclei and because of this the pooled mean X/3L grain ratio in X0 nuclei is only 1.6 times, and not twice that in the XX nuclei. This may give an impression that the single X in the hybrid X0 nuclei is incompletely dosage compensated. However, two considerations suggests that this apparently lower activity of the single X-chromosome in relation to 3L in the X0 nuclei is not to an incomplete dosage compensation but is probably related to sampling errors. Firstly, in two of the three mosaic salivary glands (nos. 1 and 3 in Table 1) observed, the mean X/3L ratios in X0 nuclei are nearly twice as high as those in the respective XX nuclei. Secondly, since we have scored only small chromosome segments which also include puff sites (2B on X and 62E on 3L segment), any small variation in ^{3}H-uridine labelling may cause X/3L grain ratios to vary appreciably in a non-specific manner. The typical faster replication of the X-chromosome in X0 nuclei further shows that the functional properties of the single X-chromosome in the interspecific hybrid genome are very similar to those seen in the male cells of the parental species.

It is known that in *D. melanogaster* the hyperactivity of the X-chromosome in males is regulated at the level of transcription in a "piecemeal manner" (Lakhotia, 1970; Lucchesi, 1974) and the relative dosages of the autosomal complement in the nucleus determine the level of transcriptional activity of the X-linked genes (Maroni and Plaut, 1973; Lucchesi 1973). As has been considered by Pierce and Lucchesi (1980), this regulatory mechanism may "involve special sequences associated with X-chromosome coding units" for their coordinated control. It is remarkable in this context that, as in the parental species, in the hybrid genome each of the two X-chromosomes in XX nuclei, derived respectively from *melanogaster* and *simulans* parents, and the *simulans* X in the X0 nuclei are regulated by the combination of the haploid autosomal

complements of each species to transcribe at a lower (in XX nuclei) or higher (in X0 nuclei) level depending upon the relative dosages (1:1 in XX and 1:2 in X0 nuclei) of the hybrid autosomal complemented. Apparently, the regulatory sequences involved in dosage compensation of X-linked genes have been specifically conserved during the evolutionary divergence of these two species.

Our observations show that the rate of ^{3}H-uridine incorporation in nucleolus in XX nuclei, with 2 nucleolar organiser regions (NORs) on the two Xs and in X0 nuclei with only one NOR on the single X is same. In the X0 polytene nuclei of *D. melanogaster* the number of rDNA sequences is the same as that in the XX polytene nuclei (Spear and Gall, 1973), Endow and Glover (1979) have suggested that this equal number of rDNA copies in polytene cells inspite of the initial dosage differences, is possibly due to polytenization of only one of the two NORs even in the normal XX and XY individuals. In view of that, it appears likely that in the present hybrid mosaic individuals also, the XX and X0 polytene nuclei have same number of rDNA copies and because of this the rate of nucleolar RNA synthesis is similar in them.

Classical observations by Sturtevant (1920) had shown that the hybrids of *D. melanogaster* and *D. simulans* do not survive unless they contain a *simulans* X. Apparently, the hybrid genome is somehow incompatible with the *melanogaster* X. Recently, it has been reported by Belote and Lucchesi (1980) that in *D. melanogaster* males which carry male specific autosomal lethals, the X-chromosome activity is not dosage compensated. In this context, it is to be noted that while our present results show that the *simulans* X is dosage compensated in the hybrid genome, they do not completely rule out the possibility that the *melanogaster* X is not properly dosage compensated in the hybrid genome if it lacks a *simulans* X and this could be one of the causes for the observed (Sturtevant, 1920) lethality of the hybrid progeny with only the *melanogaster* X or Xs.

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