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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

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ABSTRACT

[^{3}H]Thymidine labeling patterns have been examined in gynandric mosaic salivary glands of *Drosophila melanogaster*. The Ring-X stock, R(1) w^{vc}/In(1)dl 49, *l* (1) J1 *y w* lz^s, was used for this purpose. 365 labeled XX2A and 40 labeled XO2A nuclei were obtained from a total of 624 nuclei in nine pairs of mosaic salivary glands. It was observed that in all but those nuclei which had DD, 1C, and 2C patterns, the X chromosome of the XO2A nuclei always had fewer sites labeled than the X chromosomes of the XX2A nuclei, for a given pattern of the autosomes in either sex. Such asynchronous labeling of the X chromosome in the XO2A (male) nuclei was observed regardless of the proportion of the XO2A cells (2.0–73.7%), in the mosaic glands. Moreover, while the frequency of [^{3}H]thymidine labeling for all of the 39 replicating units except the two late replicating sites (3C and 11A) in the X chromosome of the XO2A nuclei, was consistently lower than in the X chromosome of the XX2A nuclei, the mean number of grains on the X chromosome was relatively (to autosomes) similar in both XX2A and XO2A cells. The results, therefore, suggest that, as in XY2A larval glands, the X chromosome in the XO2A cells also completes the replication earlier than autosomes and that the XO2A nuclei show cellular autonomy with respect to the early replication of the X chromosome, like its counterpart, RNA transcription. Absence of the asynchrony during the initial phase (DD-2C) further supports the contention (*a*) that the single X chromosome of the male not only completes the replication earlier but that the rate of replication of its DNA is possibly faster, and (*b*) that there might be a common regulation with respect to the initiation of replication of different chromosomes in a genome.

Mukherjee and Beermann (14) demonstrated that the X chromosome of male *Drosophila* produces twice as much RNA as the individual X chromosomes of female *Drosophila*. This hyperactivity of the X chromosome in the male is, according to them, the cytological counterpart of dosage compensation in *Drosophila* at the primary level of gene action. Their proposal opened up a series of

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questions with respect to the molecular and chromosomal basis of dosage compensation. The most important question, whether dosage compensation is a phenomenon related to the physiology and development of the sex itself, has been answered by Lakhotia and Mukherjee (7). They showed by use of XX-XO mosaic salivary glands of *Drosophila* larvae that the X chromosome in XO nuclei is always hyperactive regardless of the proportion of male tissue in the gland, indicating a cellular autonomy of hyperactivity in the haplo-X condition. Since then, the hyperactivity of the single dose of the X-chromosomal genes in the male has been demonstrated at both the transcriptional and the translational levels (2, 11, 12). Two very interesting features have been found to be invariably associated with the hyperactivity vis-à-vis dosage compensation in *Drosophila*: (*a*) the X chromosome in the larval salivary gland of the male is almost as wide as the two paired X's of the female (19), and this feature is also autonomous (7). The actual significance of this enlarged width of the X chromosome in male *Drosophila* is still conjectural, although evidence indicates that it would be due to an extra amount of nonhistone protein (21, and our unpublished data). (*b*) The X chromosome in all species of *Drosophila* studied so far (3, 4, 8, 9, 15, 16) completes replication earlier in the male than in the female with respect to the autosomes. Recent evidence (15, 16), which shows that the X chromosome(s) of both male and female start replication simultaneously with the autosomes of the respective complement, suggests that the X chromosome not only completes replication earlier in the male, but does so at a faster rate. In this report we wish to present the results of an investigation of the DNA synthesis in gynandric mosaics (XX-XO), in order to substantiate that (*a*) DNA replication in the X chromosome is completed earlier and perhaps also with a faster rate in haplo-X cells, and that (*b*) the haplo-X cells show cellular autonomy with respect to the early replicating property, as they show for enlarged width and hyperactive transcription (7).

MATERIALS AND METHODS

For obtaining gynandric mosaics, the unstable Ring-X stock of *Drosophila melanogaster* consisting of R(1) w^{vc}/ In(1) dl 49, *l*(1) J1 *y w* lz^s females and In(1) dl 49, *l*(1) J1 *y w* lz^s males was used. A detailed description of the stock is given in Lindsley and Grell (10). 5- to 6-day-old virgin females from the Ring-X stock were mated to *y w* sn^3 males. The Ring-X-containing female larvae produced in the F_1 of the cross could be distinguished from the homozygous *y w* larvae by the color of the mouth parts and malpighian tubules. Thus, the ring-bearing female larvae were identified and their salivary glands were dissected out in *Drosophila* Ringer's at pH 7.2 (1). The wild type strain, Oregon R+, of *D. melanogaster* was used to obtain complete male and female larvae. For obtaining larvae of reasonably similar age, eggs were collected in plastic petri dishes (7.5 cm in diameter), and late third instar larvae of the female sex were collected. Adults as well as all developmental stages were reared at 22° ± 1°C in a thermostatically controlled incubator and raised on *Drosophila* culture medium.

Excised salivary glands from fully grown late third instar larvae were dissected out in *Drosophila* Ringer's and incubated in 10 μl of Ringer's containing 5 μCi of [^{3}H]thymidine ([^{3}H]TdR, 500 μCi/ml, sp act, 6,600 mCi/mmol, obtained from Bhabha Atomic Research Centre, Bombay, India) for 20 min. The glands were fixed in aceto-ethanol (1:3) mixture, stained in aceto-carmine aceto-orcein (2:1), and squashed in 50% acetic acid. Cover slips were removed in a 1:1 mixture of 50% acetic acid and absolute ethanol, and preparations were processed for autoradiography using Kodak AR 10 stripping film according to the procedure described earlier (8). The autoradiographs were developed in Kodak D 19 b, fixed in X-ray fixer, and stained again in toluidine blue. The exposure time was 15 days.

The labeling patterns were identified on the basis of the distribution and intensity of silver grains on the chromosomes and the chromocenter, and classified as DD, 1C, 2C, 3C, 3D, 2D, and 1D as described earlier (4, 15, 16). A brief description of these patterns and their sequential relation is given below.

Autoradiographs were examined and photographs were taken under a Zeiss photomicroscope III with ordinary transmitted light or phase contrast as appeared suitable.

RESULTS

About 100 pairs of salivary glands from suspected mosaic larvae were processed for autoradiography. Only nine pairs of them yielded recognizable mosaicism in the salivary gland. Among 624 nuclei examined from the nine pairs of mosaic glands, 71 were XO2A, of which 40 were labeled with [^{3}H]TdR and 31 were unlabeled, and 550 were XX2A, of which 365 were labeled and 185 unlabeled. The remaining three of the 624 cells appeared to have a higher than diploid polytenic value. The percentage of XO nuclei (labeled and unlabeled) ranged from ~2–73.7 (Table I) in the different glands.

The average labeling index of the nuclei in these mosaic glands is 66.4% for XX2A and 56.3% for XO2A cells, as compared to 80.4% in completely

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TABLE I

Distribution of Mosaicism in the Gynandric Mosaic Salivary Glands of Drosophila melanogaster Larvae

Gland pair no.	Total no. of nuclei observed	XX2A nuclei	XO2A nuclei	Male nuclei (XO2A)
				%
1	100	89	11	11.0
2	57	54	3	5.3
3	113	94	19	16.8
4	77*	72	2	2.6
5	32	31	1	3.2
6	19	5	14	73.7
7	32	22	10	31.3
8	152	149	3	2.0
9	42	34	8	19.0
Total	624*	550	71	12.9

* Three of these nuclei had greater than 2X2A chromosome complement.

female and 64.13% in completely male larval glands examined earlier. This difference in the average labeling indices may be attributed to the developmental stage of the larvae and is perhaps not due to the mosaicism of the salivary glands, as the labeling indices in XX and XO cells are proportionately similar to those in completely female and male larval glands, respectively. Observations show that, as reported by Lakhotia and Mukherjee (7), the width of the X chromosome in the XO cells is autonomously enlarged.

The cells of larval salivary glands of *Drosophila* are an asynchronous population of cells which are in different parts of the S phase. When salivary glands are labeled with [^{3}H]TdR, about 20-30% of the cells do not take the label. The remaining labeled cells show different labeling patterns in different cells (Fig. 1). In a broad sense, these patterns can be classified as: (*a*) disperse labels on interbands, some puff sites, and some thin bands and nucleolus (DD pattern, Fig. 1*a*, nucleolus not shown); (*b*) labels on the nucleolus, interbands, puff sites, thin and dark bands, and very little on the chromocenter, with a uniformly single layer of grains (1C pattern, Fig. 1*b*); (*c*) labels on the whole chromosome and the chromocenter with moderately intense labeling (2C, Fig. 1*c*); (*d*) intense labeling on the whole chromosome and the chromocenter (3C and 3C-3D, Fig. 1*d* and *e*); (*e*) heavy labeling on all dark bands and chromocenter, but no labeling on puffs, thin bands, or interbands (3D, Fig. 1*f*); (*f*) more unlabeled gaps on the chromosomes, chromocenter labeled, and poor or no labeling on the nucleolus (2D, Fig. 1*g*); (*g*) only a few very dark bands labeled, and the chromocenter labeled (1D, Fig. 1*h*); and (*h*) labels only on the chromocenter (not shown). The details of these patterns and the reasoning behind arranging them in sequential order have been given earlier (3, 4, 6, 8, 15, 20). Suffice it to mention here that, on the basis of the late replicating property (late start and late finish) of the chromocentric heterochromatin, it has been possible to place the DD to 1C patterns at the beginning and 1D to CL patterns at the terminal end of replication (4, 8). The middle patterns 2C-3C-3D-2D can then be arranged in that order on the basis of the increasing intensity of chromocentric and chromosomal labeling at first (up to 3C) and then gradual completion of replication of individual bands or groups of bands, without interruption (see reference 4).

The autoradiographs of the mosaic glands (Figs. 2 and 3) reveal that in those XO cells which have been classified as intermediate or late patterns, i.e., 3C to 1D, on the basis of their autosomal labeling, the labeling pattern of the X chromosome is invariably different from that of the autosomes of the respective nucleus. Such lack of synchrony in the [^{3}H]TdR labeling pattern between the X chromosome and autosome(s) is not detectable in the XO cells with early patterns, i.e., those showing DD to 2C labeling (Fig. 4). In none (but one exceptional cell described later) of the XX type was such asynchrony observed. This implies that in the XO cells with 3C to 1D type labeling the replication pattern of the X chromosome is out-of-synchrony with the replication patterns in the autosomes. The asynchrony of the X chromosome in the XO cells (with 3C to 1D pattern) is observed regardless of the proportion of the XX and XO cells in the mosaic glands.

The data on the frequency distribution of the X-chromosomal and autosomal labeling patterns in XX2A nuclei indicate that while in XX2A nuclei there is complete parity in the absolute frequency of each pattern between X and 2R (Fig. 5*a*), in XO2A nuclei such parity is observed only for the patterns DD, 1C, and 2C. From 3C onward up to 2D, the frequencies of X-chromosomal patterns are drastically reduced, and for 1D the frequency is greater by nearly four-fold in the X than in the 2R (Fig. 5*b*). The frequency distribution for the pooled data, per se, does not give a clear picture

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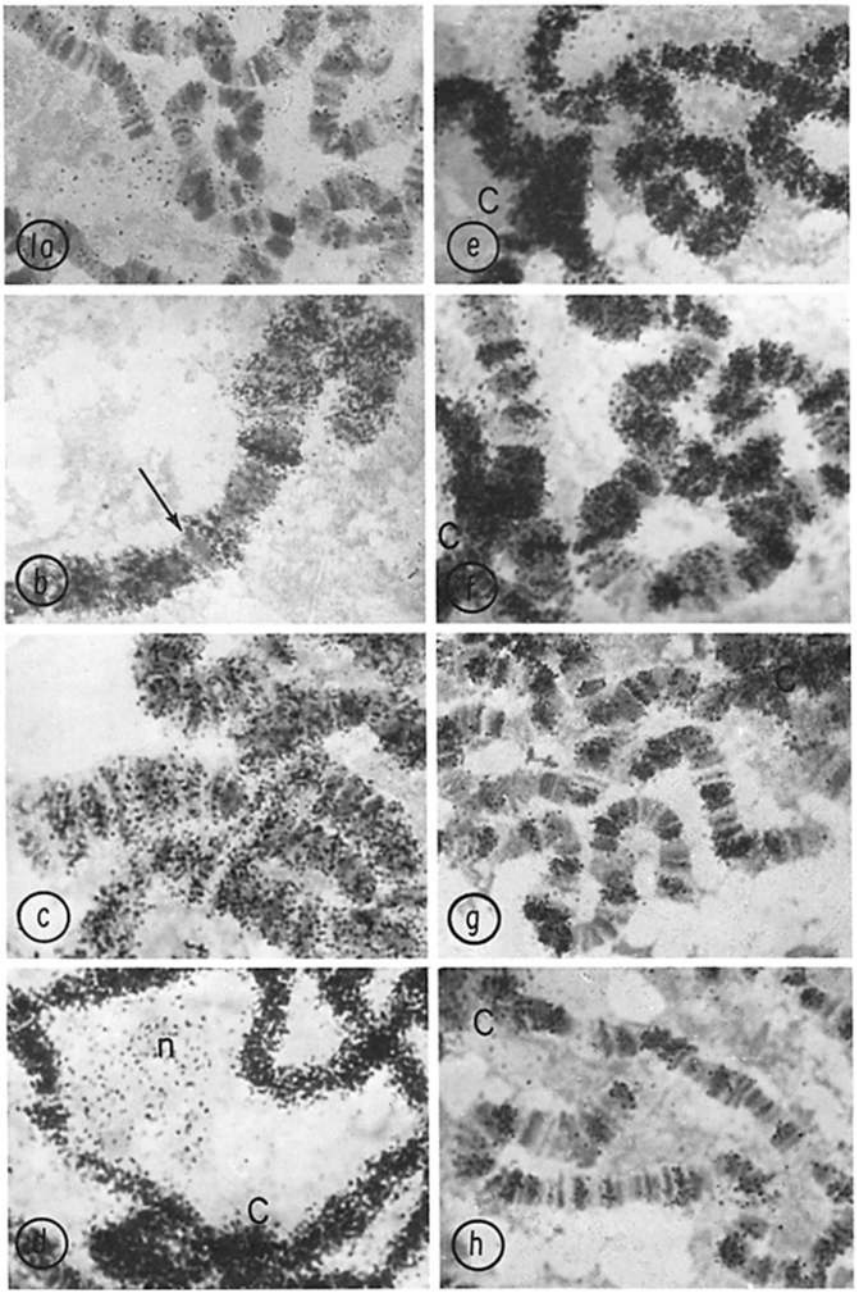

FIGURE 1 [^{3}H]TdR autoradiographs of completely female larval salivary glands of *Drosophila melanogaster*, showing different labeling patterns. (*a*) Early DD pattern, with only puffs, interbands and some thin bands labeled; (*b*) almost a 1C pattern, with labels all over the chromosome, except for only one dark band (arrow); (*c*) a 2C pattern showing grains on all bands and interbands; (*d*) a 3C pattern with intense labeling on the whole chromosome and chromocenter, nucleolus (*n*) moderately labeled; (*e*) a 3C–3D pattern with dense labeling on the chromosomes and on the chromocenter, with some narrow gaps; (*f*) an early 3D pattern; (*g*) a 2D pattern; (*h*) a 1D pattern. C = chromocenter. The chromocenter is labeled in (*d–h*). (*a*) × 600; (*b–h*) × 1,000.

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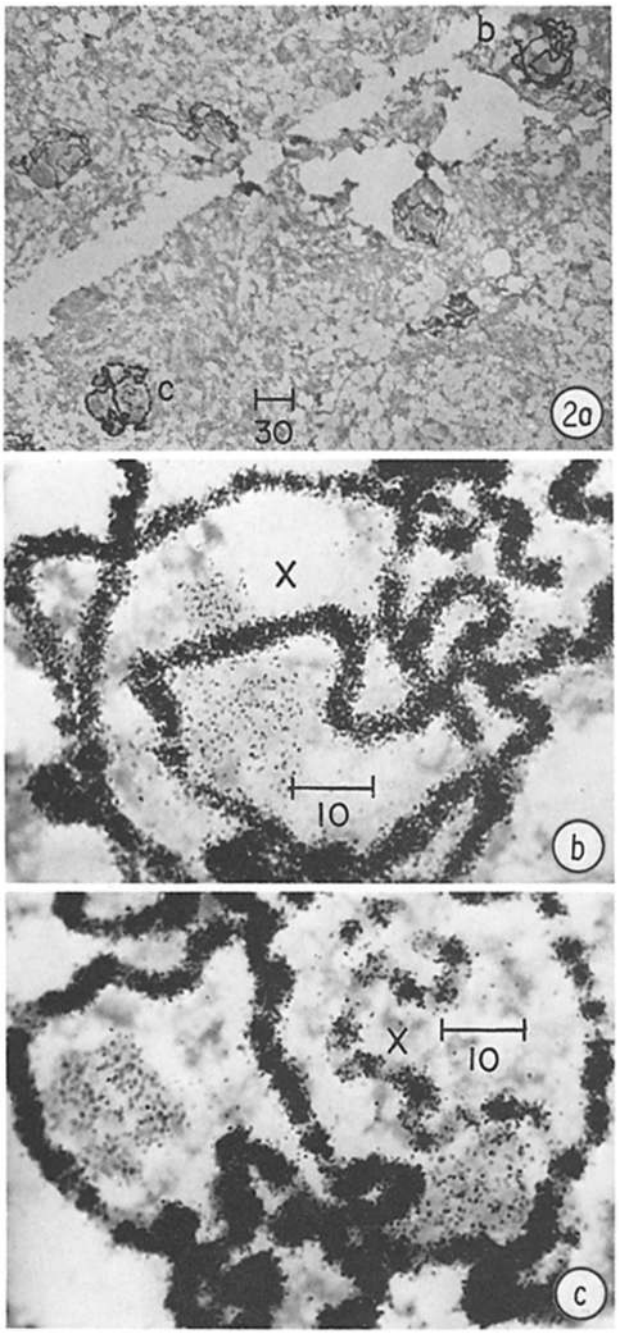

FIGURE 2 [^{3}H]TdR autoradiograph of a mosaic salivary gland (no. 1) of *Drosophila melanogaster* with only 11% XO2A nuclei. (*a*) The autoradiograms of the mosaic gland in low magnification showing different nuclei; (*b*) an XX2A nucleus in higher magnification from (*a*), showing the autosomes and paired X chromosomes all in 3C stage; and (*c*) an XO2A nucleus from (*a*), showing the autosomes with 3C-3D type labeling and the X chromosome with 3D type labeling. The scale is given in μm. (*a*) × 120; (*b* and *c*) × 1,000.

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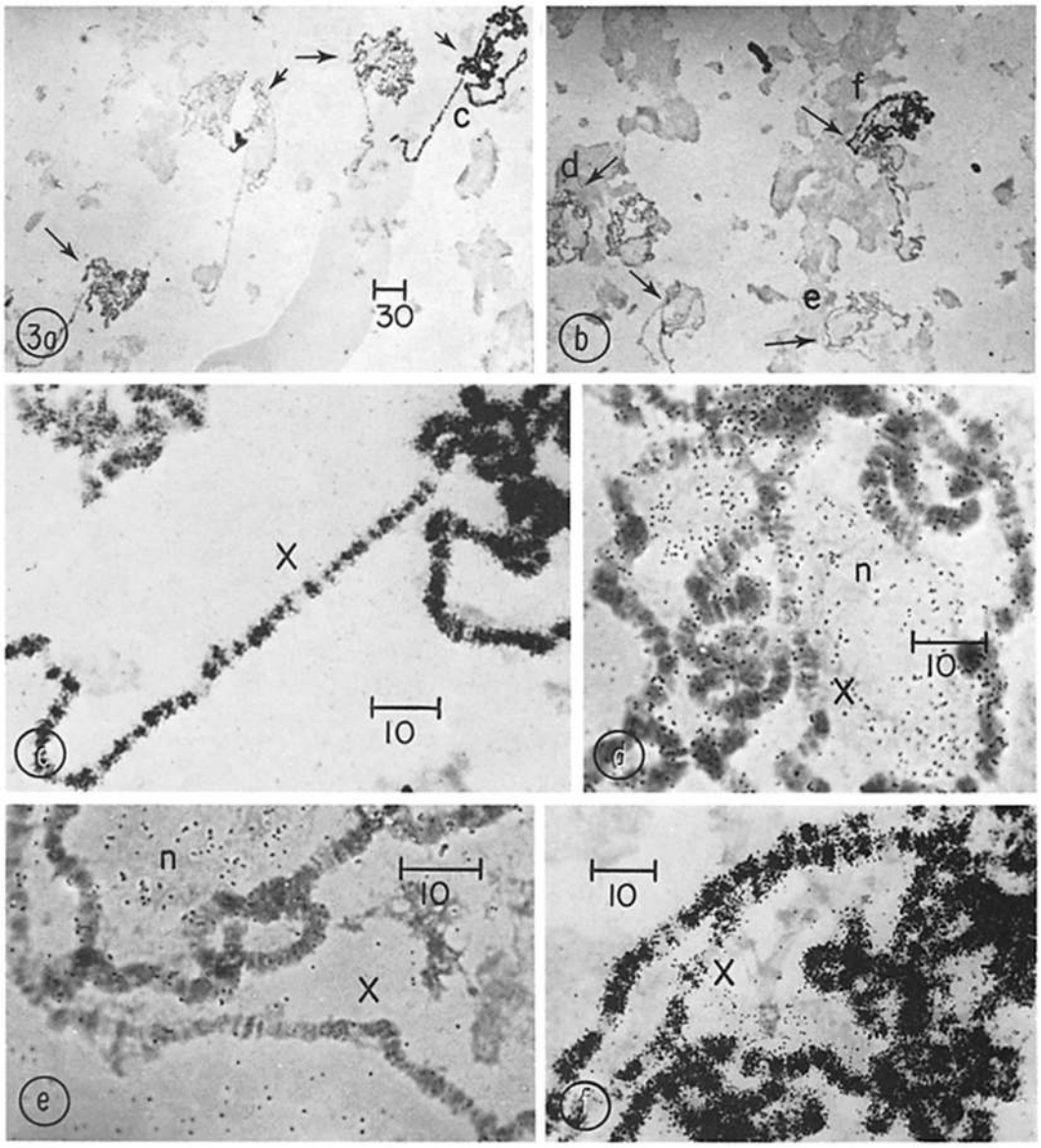

FIGURE 3 [^{3}H]TdR autoradiograph of a mosaic salivary gland (no. 6) of *D. melanogaster* with about 75% XO2A nuclei. (*a*) The autoradiograph of the mosaic salivary gland in low magnification showing four male (XO2A) nuclei in the field (arrows); (*b*) the same mosaic gland under low magnification showing four XO2A nuclei in the field (arrows); (*c*) a male nucleus (marked *c* in Fig. 3*a*) under higher magnification, showing distinct asynchrony of the male X; (*d*) a male nucleus (marked *d* in Fig. 3*b*) at DD stage showing both X and autosomes similarly labeled; (*e*) a male nucleus (marked *e* in Fig. 3*b*) showing autosomes with 2D labeling and the X with 1D labeling; (*f*) a male nucleus (marked *f* in Fig. 3*b*) showing autosomes with 2D labeling and the X with 1D labeling; (*f*) a male nucleus (marked *f* in Fig. 3*b*) showing autosomes at 3C-3D and the male X at 3D stage. n, nucleolus. The scale is given in μm. (*a* and *b*) × 120; (*c–f*) × 700.

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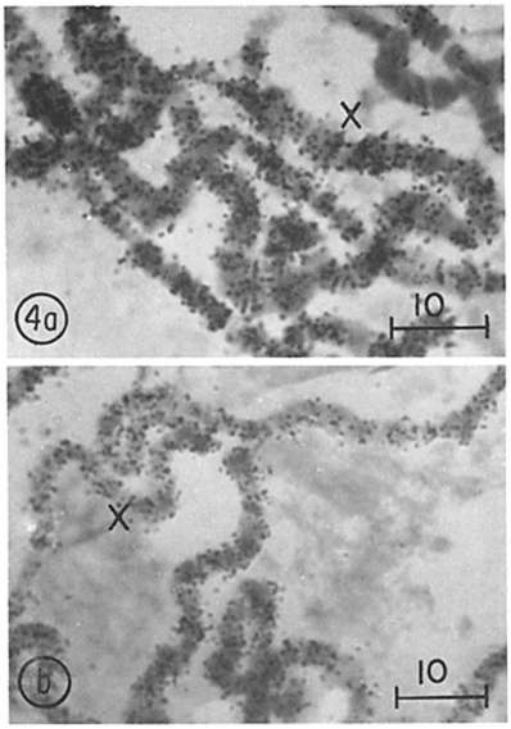

FIGURE 4 [^{3}H]TdR autoradiographs of a mosaic salivary gland (gland no. 3, with about 17% XO2A cells) showing 1C pattern in an XX2A (*a*) and an XO2A nucleus (*b*). No asynchrony between the X and autosomes is apparent in either of these two nuclei. Note the paired 2X's in (*a*) and the single X in (*b*). The scale is given in μm. (*a*) × 1,000; (*b*) × 800.

on the intranuclear asynchrony, but shows that at least for most of the haplo-X nuclei the labeling frequency for the X chromosome is distinctly lower than the frequency for the autosomes, and suggests that the X chromosome in the XO2A cell population might have a tendency to complete the replication earlier. The increase in the frequency of 1D pattern for the X chromosome in XO2A could be attributed mostly to the late replicating sites mentioned later (Fig. 7, e.g., nos. 10 and 39).

The detailed labeling patterns of the X chromosome and autosome (2R) in the XX2A and XO2A nuclei for individual gland pairs are shown in Table II. The results show that the labeling patterns of the X chromosomes in the XX2A conform with those of the autosome, in almost all gland pairs. There is only one exception, i.e., in gland no. 1 there were 16 nuclei with 3C type autosome(s), but in one of the 16 nuclei the X chromosome had one or two very short unlabeled or poorly labeled gaps, like 3C–3D (e.g., Fig. 1*e*). Such unlabeled gaps, however, might arise due to several reasons, for example, local stretching or unconventional absence of synthesis or unscheduled synthesis (3). The data therefore reveals that in none of the nuclei is there any evidence for a differential labeling in the X chromosome and autosome(s) in XX2A nuclei. On the contrary, in XO2A nuclei, while the labeling patterns of the X

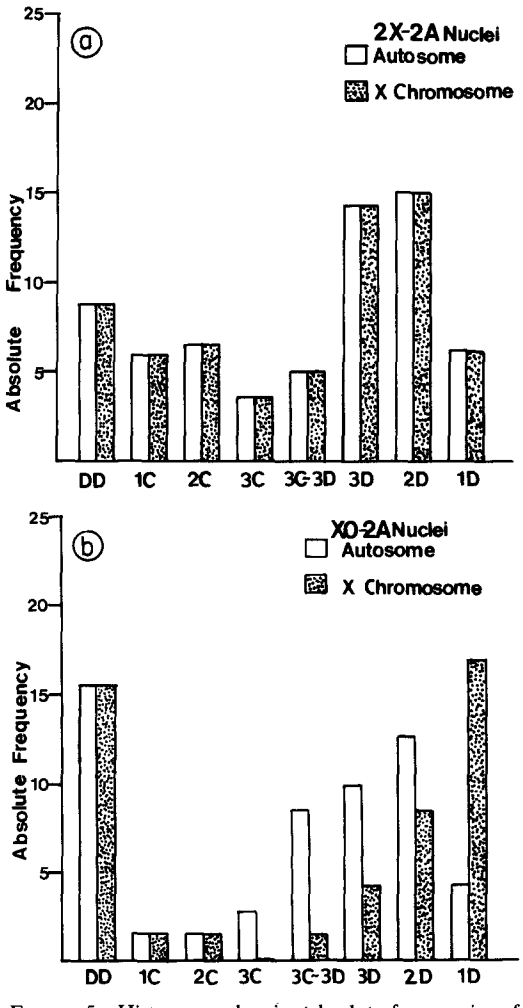

FIGURE 5 Histograms showing absolute frequencies of different [^{3}H]TdR labeling patterns of the X chromosome in relation to that of the corresponding autosomes in the XX2A (*a*) and XO2A (*b*) nuclei in the mosaic salivary glands of *D. melanogaster*, among 365 XX2A and 40 XO2A nuclei observed. Five unlabeled X chromosomes have not been included among 1D of (*b*). Abscissa = [^{3}H]TdR labeling patterns (DD-1D).

TABLE II

Distribution of [^{3}H]TdR Labeling Patterns in the Mosaic Salivary Glands Examined

		XX2A nuclei												XO2A nuclei											
Gland no.	Chromosome	N	NL	DD	1C	2C	3C	3C-3D	3D	2D	1D	CL	UL	N	NL	DD	1C	2C	3C	3C-3D	3D	2D	1D	CL	UL
1	A	89	–	–	–	2	16	13	40	15	3	–	–	11	–	–	–	–	2	–	5	2	–	–	–
	X		–	–	–	2	15 (+1)	13	40	15	3	–	–		–	–	–	–	–	–	1	4	5	–	–
2	A	54	–	–	–	–	1	3	7	23	8	4	8	3	–	–	–	–	–	–	1	1	1	–	–
	X		–	–	–	–	1	3	7	23	8	4	8		–	–	–	–	–	–	–	–	1	–	2
3	A	94	–	6	12	30	1	4	19	16	1	–	5	19	–	3	–	1	–	–	1	2	1	–	8
	X		–	6	12	30	1	4	19	16	1	–	5		–	3	–	1	–	–	1	2	2	–	10
4	A	72*	2	30	20	3	1	4	6	2	1	–	3	2	–	1	–	–	–	–	–	–	–	–	1
	X		2	30	20	3	1	4	6	2	1	–	3		–	1	–	–	–	–	–	–	–	–	1
5	A	31	–	–	–	–	–	–	1	–	–	–	30	1	–	–	–	–	–	–	–	–	–	–	1
	X		–	–	–	–	–	–	1	–	–	–	30		–	–	–	–	–	–	–	–	–	–	1
6	A	5	–	1	–	–	–	–	–	–	–	–	4	14	–	7	1	–	–	–	–	2	1	–	3
	X		–	1	–	–	–	–	–	–	–	–	4		–	7	1	–	–	–	–	–	2	–	4
7	A	22	–	–	–	–	–	1	1	–	–	–	20	10	–	–	–	–	–	–	–	–	–	–	10
	X		–	–	–	–	–	1	1	–	–	–	20		–	–	–	–	–	–	–	–	–	–	10
8	A	149	1	10	–	–	–	3	4	27	20	4	80	3	–	–	–	–	–	1	–	2	–	–	–
	X		1	10	–	–	–	3	4	27	20	4	80		–	–	–	–	–	–	1	–	2	–	–
9	A	34	–	1	–	–	–	–	–	1	–	–	32	8	–	–	–	–	–	–	–	–	–	–	8
	X		–	1	–	–	–	–	–	1	–	–	32		–	–	–	–	–	–	–	–	–	–	8

DD, 1C-3C, and 3D-1D are described in the text. CL = chromocenter labeled, UL = unlabeled, and NL = nucleus only labeled. The symbol A represents the autosome and X the X chromosome.

* Three others in this gland had higher polytenic value.

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chromosome and the autosomes were similar from DD to 2C, they were distinctly dissimilar from 3C to 1D. For example, in gland no. 3 (Table II) there were three XO2A nuclei with DD patterns, and in those nuclei both the autosome 2R and the X chromosome had DD type labeling. On the other hand, in the same gland, three other XO2A nuclei had the 3C-3D type pattern in the autosome, but in one of them the X chromosome was the 3D type and in the other two it was the 2D type.

A more direct intranuclear relationship between the X and the autosomal labeling pattern is shown in Table III. In column 2, the numbers of autosomes with the particular labeling patterns are shown for XX2A (left) and XO2A (right). In the XX2A nuclei the numbers of X chromosomes showing the specific pattern match exactly those of the autosomes, with the single exception (row 3C) mentioned earlier. In contrast, for the XO2A nuclei, the numbers of X chromosomes with a specific pattern match the numbers of autosomes with the same pattern (numbers on the right of column 2), from DD to 2C; thereafter, there is a differential labeling of the X and 2R in every nucleus. For example, among the two XO2A nuclei, under 3C type (row 4), the autosomes were 3C type but the X chromosome was 3C-3D in one and 3D in the other. Similarly, among nine 2D type XO2A nuclei, the autosomes were 2D in all the nine, but seven of them had the 1D pattern in the X chromosome, and in the remaining two, the X was completely unlabeled. These data, therefore, clearly reveal that an intranuclear asynchrony between the X and the autosome(s) exists in the XO2A nuclei of the mosaic glands, regardless of the proportion of XO2A cells among the total number of cells.

The segments 1A to 11A of the X chromosomes and 56F to 60F of the autosome 2R (10) have been examined with respect to the labeling frequency of the individual sites. Since each segment may have one or more independently labeling subsegments, these segments of the X and 2R have been divided into 39 and 20 replicating units, respectively (Fig. 6), on the basis of the arguments discussed elsewhere (4, 8, 15). The replicating units are in no way comparable to replicons, but may simply represent a series of synchronously replicating bands and interbands. It is evident from the data in Fig. 7 that the frequencies of labeling for the 20 replicating units of 2R are concordant in both XX2A and XO2A nuclei. On the other hand, of the 39 X-chromosomal units all but two (nos. 10 and 39, which are the well-known late replicating sites) show clearly lower frequency in XO2A than in XX2A nuclei.

The grain count data of the two specific segments, 1A-11A of the X chromosome and 56F-60F of the autosomal arm, 2R, are presented in Table IV. Only the data of the chromosome segments with the 3D to 1D patterns are shown here, since the numbers of silver grains on the chromosomes showing the DD to 3C type are highly variable or unaccountably large (Fig. 1*a–e*). Both mean grain number per segment (columns a and c) and total intensity of labeling of each segment with a specific pattern (i.e., mean grain number X

TABLE III

[^{3}H]TdR Labeling Pattern(s) of the X Chromosome of Female (XX2A) and Male (XO2A) Nuclei of the Mosaic Glands for a Specific Pattern of Labeling on the Autosome (2R) Showing Intranuclear Association

Labeling pattern of autosome (2R)	Number (♀ + ♂)	Labeling patterns of X	
		XX2A	XO2A
DD	(48 + 11)	DD (48)	DD (11)
1C	(32 + 1)	1C (32)	1C (1)
2C	(35 + 1)	2C (35)	2C (1)
3C	(19 + 2)	3C (18 + 1)	3C-3D (1), 3D (1)
3C-3D	(28 + 6)	3C-3D (28)	3D (2), 2D (4)
3D	(78 + 7)	3D (78)	2D (2), 2D-1D (1)*, 1D (4)
2D	(84 + 9)	2D (84)	1D (7), UL (2)
1D	(33 + 3)	1D (33)	UL (3)
CL	(8 + 0)	CL (8)	–

Numbers in parentheses indicate number of nuclei with specific patterns.
* Included under 1D in Table II and Fig. 5*b*.

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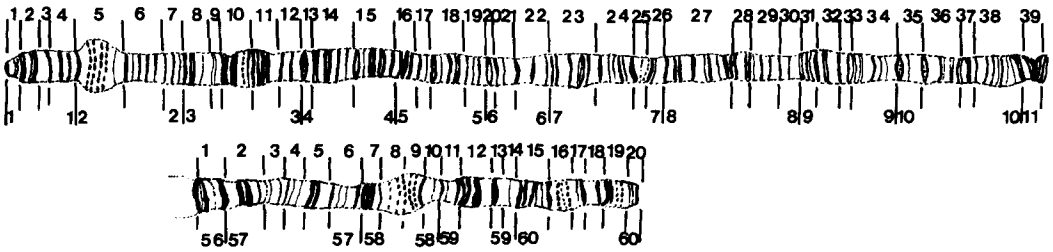

FIGURE 6 The segments 1A-11A of the X chromosome (top) and 56F-60F of the 2R autosomal arm (bottom) of *D. melanogaster*, observed in the study. The different replicating units (1-39 of the X and 1-20 of the 2R) (8) corresponding to their cytological extent on Bridges' map of salivary gland chromosomes of *D. melanogaster* (see reference 10) are shown in the upper divisions of each section.

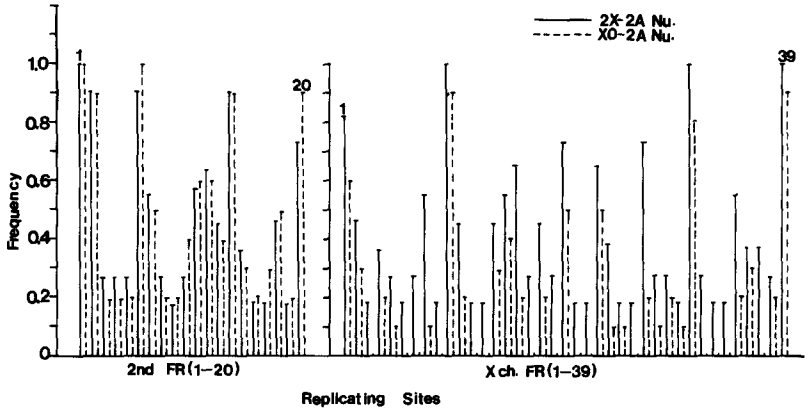

FIGURE 7 Histograms showing the frequencies of [^{3}H]TdR labeling of the 20 replicating units of the 2R segment and the 39 replicating units of the X chromosome segment of the XX2A (female) and XO2A (male) nuclei in the mosaic salivary glands of *D. melanogaster*.

TABLE IV

Analysis of [^{3}H]TdR Labeling Intensity on the X and 2R Segments of XX2A and XO2A Nuclei of Mosaic Glands

Labeling pattern of the nuclei*	X chromosome (1A-11A)			Autosome (56F-60F)			X/A ratio of total intensity (ab/cd)
	Mean grain no./ segment (a)	Frequency of pattern (b)	Total intensity (a × b)	Mean grain no./ segment (c)	Frequency of pattern (d)	Total intensity (c × d)	
		%			%		
XX2A nuclei							
3D (5)	280.4	14.2	3981.7	221.4	14.2	3143.9	1.26
2D (4)	102.6	15.3	1569.8	86.8	15.3	1328.0	1.18
1D (2)	46.5	6.0	279.0	25.5	6.0	153.0	1.82
XO2A nuclei							
3D (4)	156.0‡	4.2	665.2	168.0	9.9	1663.2	0.40
2D (2)	86.0	8.5	731.0	79.5	12.7	1009.7	0.72
1D (2)	10.0	16.9	169.0	27.0	4.2	113.4	1.49

Numbers in parentheses denote the number of nuclei scored for grain count.

* Classification of labeling pattern is based on the labeling pattern of autosomes.

‡ For XO2A nuclei the mean grain number on the X has been multiplied by 2 to allow adjustment for haploidy of the X.

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frequency, in percent, of each pattern among all labeled cells) in the X and 2R have been compared. Data show that the relative mean numbers of grains on the X chromosome (adjusted for the haploidy of the X in the XO2A cells) with respect to that on the 2R segment of XX2A and XO2A nuclei are similar. When the total intensity of silver grains on each X chromosome segment is compared in the XX2A and XO2A (column 4), it is evident that the total intensity of labeling on the X chromosome in XO2A nuclei is distinctly less than that in XX2A nuclei, even after adjustment for the haploidy of the X in XO2A nuclei. This is also evident from the comparison of the X/A ratios of the total intensity (last column) in XX2A and XO2A nuclei. The high X/A ratio (1.49) for the 1D pattern in the XO2A nuclei is obviously due to a higher frequency of the X chromosome with the 1D pattern, and this higher frequency is attributable mostly to the two late replicating sites (nos. 10 and 39) mentioned earlier.

The two sets of data, the consistent lower labeling frequency and total labeling intensity in the X chromosome than in the autosome in the XO2A nuclei during 3C to 2D (and even up to 1D, if we ignore the two late replicating sites), on the one hand, and the consistent intranuclear asynchrony in the labeling pattern between the X chromosome and the autosome(s) during the same period on the other, regardless of the proportion of XO2A nuclei in the mosaic glands, strongly suggest that the single X chromosome in XO2A nuclei of the mosaic glands completes the replication earlier than autosomes, and, like the hyperactivity of the X chromosome, the early completion of replication by the X chromosome in haplo-X cells is also cell autonomous. Moreover, a comparable mean grain number on the X chromosomes (adjusted for the haploidy) in XO2A and XX2A nuclei, relative to that on the autosome segment of 2R, supports the contention that the rate of replication of the X chromosome of the male may be relatively faster than that of the autosomes of the complement, a contention derived from the observation that the initial labeling patterns of the X chromosome and autosomes are synchronous in the XO2A nuclei.

DISCUSSION

Lakhotia and Mukherjee (7) demonstrated that both the enlarged width and hyperactivity of the X chromosomes of male *Drosophila melanogaster* are cell autonomous. Their data were derived from a wide range of mosaicism for XX2A and XO2A nuclei. In the present investigation the range of mosaicism varied from about 2–74% XO2A nuclei. It must be remembered that even in completely female or male larvae, the [^{3}H]TdR labeling index is never 100%. In the mosaic glands examined, it is even less. The different nuclei are in different sequential parts of the S period. Nevertheless, the asynchrony of labeling between the X chromosome and autosomes was observed in those XO2A nuclei which had the 3C to 1D type patterns and the asynchrony was not influenced by the number or proportion of the XX2A cells around the XO2A cells.

The data presented here reveal that as in the complete males (8), in the XO2A nuclei of gynandric mosaic salivary glands of *Drosophila* larva also, (*a*) the number of [^{3}H]TdR labeled sites on the X chromosome was less than that on the autosome(s) beyond the 2C stage, (*b*) the labeling frequency of the different patterns of the X chromosome was distinctly lower than those of the autosomes in XO2A nuclei which were classified as 3C to 2D on the basis of their autosomal labeling, (*c*) the asynchrony could not be detected earlier than 3C, either by the labeling pattern or by the frequency of the early patterns (DD to 2C), and (*d*) the adjusted mean grain number on the X chromosome relative to the number on the autosome was proportionately similar in both XX2A and XO2A nuclei, for the 3D to 2D patterns. We conclude from these data that the X chromosome in XO2A nuclei completes the replication earlier than the autosomes and, conversely, also earlier than the X chromosomes in the XX2A nuclei. This early completion of replication by the X chromosome of haplo-X cells is also cell autonomous.

There may be three alternative possibilities regarding the operational means of achieving the early completion of replication of the X chromosome in haplo-X cells: (*a*) it may be brought about by a faster rate of replication of the single X chromosome in XO2A or XY2A cells, as compared to the autosome, and such faster replication of DNA probably would correspond to a rapid polynucleotide chain elongation; (*b*) it may be achieved by an early initiation of replication in the X chromosome of the haplo-X cells; or (*c*) it may be due to a larger number of replicating units in the X chromosome of haplo-X cells than in the XX2A cells. Our data presented earlier (3, 8, 15, 16) and here strongly support the first possibility. Firstly, neither in the XO2A nuclei of the mosaic

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glands nor in the completely male larval glands (15, 16) was the asynchrony between the X chromosome and autosome observed earlier than the late DD stage. Since it was possible to demonstrate the asynchrony of labeling between the X chromosome and the autosome in male larval gland cells of *D. pseudoobscura* during the late DD and 2C (see Fig. 2 of reference 15), it is reasonable to assume that had they existed at an early stage, they could indeed have been cytologically recognized. Secondly, no nucleus has been recorded so far by us or previous workers as having only the X chromosome labeled in the haplo-X cells. Recently, Meer (13) has pointed out a case of only the X chromosome being labeled in the hybrid of *D. athabasca* and *D. azteca,* but a similar case has not been found individually in either of the two species. Thirdly, the grain count data mentioned above provide additional support along the same line. Finally, autoradiographs of actinomycin D-pretreated glands have revealed a number of DD patterns, possibly because the drug caused a block and subsequent release of the DNA replication (17). In these autoradiographs, the different chromosomal elements (X and autosomes) have a reasonably similar DD pattern in both sexes. These findings stand strongly against the second alternative.

On the other hand, if one assumes that each replicon must receive a signal for the initiation of the S, then there should be a finite time lag between the initiation of the first and the last set of homologous replicons. This would then imply that all of the replicons in a segment of an X chromosome in a haplo-X nucleus would, on the average, have been initiated before all of the (twice as many) replicons had been initiated in a segment of the diplo-autosomal remainder of the chromosomes in the complement. Then, for identical rates of replication (measured in lengths of chromatid per time unit), one would expect that the haplo-X would finish replication relatively (to autosomes) sooner than the diplo-X. However, our earlier observations (3, 4, 8) and the results of Mulder et al. (18) suggest that neither the initiation nor the termination is guided by the number or size of the replicon or replicon series (cf. reference 22). The initiation and termination of the two late replicating units (10 and 39) of the X chromosome are not different in XX2A and XO2A (or XY2A), despite there being presumably twice as many replicons in the former as in the latter. These points, we believe, limit the theoretical expectation of early initiation and early completion of replication of the X chromosome in haplo-X cells.

The third possibility that there may be a larger number of replicating units in the X chromosome of haplo-X cells than in the X chromosome of diplo-X cells can also be ruled out by the observed data from the autoradiographs of mosaic glands presented here as well as those of complete male and female larval glands reported earlier (3, 8, 15, 16). No X chromosome in the XO2A nuclei was observed in which a particular replicating unit (such as no. 10 or 15) had revealed two or more labeled sequences, as against only one in XO2A. Whether this altered rate of replication of DNA in the X chromosome in haplo-X cells is due to a larger endogenous pool of TdR remains to be examined. However, this is an issue relating to the mechanism, and if we assume the randomness of incorporation of the precursors, this should not interfere with our interpretation.

Since the early completion (or a faster rate) of replication by the X chromosome in the XO2A nuclei is observed here under otherwise similar physiological and genetic conditions, we believe that this is a more direct support to the contention that both hyperactivity and early completion of replication are two cytological counterparts of the genetic phenomenon of dosage compensation in *Drosophila*. The correlation between hypertranscriptive activity of the X chromosome of the male and hypertranslational activity of the X-linked genes of male *Drosophila* has been demonstrated by Lucchesi et al. (11). The present finding on the cellular autonomy of faster replication (or at least early completion of replication) implies further that dosage compensation in *Drosophila* is not dependent on the development or physiology of the sex.

The more interesting outcome of the present investigation is perhaps the observation that the single X chromosome of male *Drosophila* very likely starts replication synchronously with the autosomes at the very beginning of replication but that the asynchrony is detected only during the 2C-3C stage. In *D. pseudoobscura,* Mukherjee and Chatterjee (15, 16) provided evidence for an early asynchrony, and they could detect the differential labeling in XY2A cells at late DD and 1C-2C. These data suggest that, like the asynchrony reported for the autosomes (5), the replication of the X chromosome starts initially synchronously with the autosomes in both sexes and becomes

asynchronous in haplo-X cells only when all or most sites become active for the synthesis of DNA. It may be possible that the point of asynchrony might be set differently for different species. If this were actually so, this would imply that a common control of initiation of the replication for a particular genome holds true for both autosome and X chromosome and for both sexes.

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