The hyperactive X chromosome is not early replicating in mitotically active somatic cells of Drosophila nasuta males

S.C. Lakhota and J.K. Roy

Abstract: The temporal order of replication of the X chromosome(s) in mitotically dividing male and female cells in early embryos and in brain ganglia of Drosophila nasuta larvae was examined using [3H]thymidine pulse labelling and autoradiography. Both the X chromosomes in female cells and the single X chromosome in male cells replicated in complete synchrony with the autosomes in the nucleus. Thus, unlike the well-known early completion of replication by the hemizygous X chromosome in polytene nuclei in the salivary glands of male Drosophila larvae, the single X chromosome in mitotically dividing cells does not replicate earlier than the autosomes. We conclude that transcriptional hyperactivity of the single X chromosome required for dosage compensation in juvenile cells of male Drosophila is not dependent upon its early replication.

Key words: dosage compensation, hyperactive X chromosome, early replication.

Résumé: Le moment auquel s'effectue la réplication des chromosomes X de la mère dans les cellules de jeunes embryons et de ganglions cervicaux chez les larves de Drosophila nasuta a été examiné par marquage court ('pulse') et 4 la [3H]thymidine suivis d'autoradiographie. Les deux chromosomes X chez les femelles ainsi que l'unique chromosome X chez les mâles étaient repliqués en même temps que les gènes autosomiques dans le noyau. Ainsi, un soutien de la chronologie de la réplication dans les noyaux polycystiques des glandes salivaires de larves mâles de Drosophila, l'unique chromosome X présent dans les cellules en mère n'est pas répliqué avant les autosomes. Les auteurs en concluent que l'hyperactivité transcriptionnelle de l' X chromosome, requise pour compenser sa présence en plus seul égalant dans les cellules somatiques de Drosophila mâle, ne dépend pas d'une réplication précoc.

Mots-clés : compensation de dosage génique, chromosome X hyperactif, réplication précoc.

[Traduit par la rédaction]

Introduction
Dosage compensation for X-linked genes in males and females of Drosophila spp. is known to operate by hyperactivation of the single X chromosome in somatic cells of males (Mukherjee and Hirsch, 1979). The hyperactivation of the hemizygous X chromosome is directly manifested in polytenic nuclei of the salivary glands of male larvae by the increased width, Faster staining, and greater [3H]thymidine uptake of the hemizygous X chromosome when compared with autosomes of the paired X chromosomes in the female (Mukherjee and Lichtenberg, 1965; Lakhota and Mukherjee, 1969) and by the specific association of certain proteins (Kuroda et al., 1971; Patther et al., 1993). A greater transcription of X-linked genes in other somatic cells of male Drosophila has been directly documented through many studies dealing with specific gene transcripts or protein products (Lichti and Manning, 1983). It is also well established that the hemizygous X chromosome in polytenic nuclei of the salivary glands of male larvae completes a given replication cycle faster and earlier than autosomes in the males (Lakhota and Mukherjee, 1970; Chatterjee and Mukherjee, 1977). In view of the general correlation drawn between active genes and their replication in early S phase and vice versa (Goldsmith et al., 1984; Hollingsworth, 1989), it has generally been presumed that the early completion of replication by the male X chromosome in polytenic cells is causally related to its hyperactivity in relation to dosage compensation (Lichti and Manning, 1983; Mukherjee, 1980). In a recent report, Kar and Mukherjee (1990), using cell fusion to induce premature chromosome condensation (PCC), claimed that the X chromosome in mitotically dividing nuclei of Drosophila melanogaster males was also early replicating. On the other hand, Lakhota and Singh (1983) had suggested that the faster completion of replication by the hyperactive
Table 1. Synchroton in replication of autosome(s) and X chromosome(s) in male and female mite cells of *Lythrausta major*.

<table>
<thead>
<tr>
<th>Sex</th>
<th>X chromosome (I)</th>
<th>Y chromosome (I)</th>
</tr>
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<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
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<tr>
<td>Embryo</td>
<td>0</td>
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<td>Female</td>
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<th>Sex</th>
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<td>Female</td>
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**Note:** U, unlabelled; L, only euchromatin regions labelled; H, both heterochromatin regions labelled; O, only heterochromatin regions labelled; E, Y chromosome labelled.

X chromosome in male polytene nuclei could be a consequence of certain specific features of euchromatin chromosomes rather than being a causative factor for hyperactivity. In the present study, we have directly examined the limited order of replication of the X chromosome in early embryonic and mitotic larval brain cells of male and female *O. marmorata* using *H*-thymidine labelling. Our results show that in mitotic male cells, the single X chromosome is not early replicating, rather it replicates in complete synchrony with autosomes. In female cells also, the two X chromosomes coplate in synchrony with each other as well as with autosomes.

**Materials and methods**

Five and larvae of a wild-type strain (*Varunia*) of *O. marmorata* were reared on a standard *Drosophila* food medium at 24 ± 1°C. For *H*-thymidine labelling of embryonic cells, embryos 4-5 h old were removed from the food surface by bracing, washed in *Paeae* salt solution (Lakhotia and Mukerjee 1980), electrophoresed with 4% bleaching powder solution, and again washed several times with water followed by a wash with *Paeae* salt solution. Finally, the embryos were transferred to sterilized *Paeae* culture medium (Lakhotia and Roy 1980) in sterile vials and kept in fine steel needles into 2 to 3 pieces. In order to label the embryonic cells, *H*-thymidine (740 kBq/mL; specific activity 584.6 Gbq/mg, Indus Atomic Research Centre (IARC), Trombay, India) was added to the medium for 10 min, after which the embryo pieces were repeatedly washed with radioisotope-free medium supplemented with yeast extract (2 mg/mL) and "cold" thymidine (10 μg/mL). The washed embryo pieces were transferred to a fresh cavity slide and chated in the supplemented medium for 15 min to 4 h. Colchicine (1 μg/mL) was added to this medium for the last 15 to 30 min. Finally, air-dried preparations were made as described earlier (Lakhotia and Roy 1981). To label brain ganglia with *H*-thymidine, the ganglia were dissected from last 3rd instar larvae in modified *Paeae* medium and immediately pulse labelled with *H*-thymidine (740 kBq/mL; specific activity 584.6 Gbq/mg, IARC, Trombay, India) for 10 min, followed by washes with radioisotope-free medium supplemented with yeast extract and "cold" thymidine, as for embryos. The pulse-labelled ganglia were chated in the supplemented medium for 15 min to 24 h, when chased for longer than 2 h, colchicine (1 μg/mL) was added to the chase medium for the last 1 h of culture. Air-dried chromosome preparations were made at the end of the chase period, as described earlier (Lakhotia and Kumar 1978). Slides with labelled cells (embryonic or larval brain ganglia) were processed for autoradiography with Ilford L4 nuclear emulsion. Autoradiograms were stained with Giemsa and noted for the pattern of labelling in individual chromosome of every metaphase plate examined.

**Results and discussion**

The karyotype of *O. marmorata* reveals very easy identification of each individual chromosome owing to the distinctive shape, size, and heterochromatin content of the chromosomes (Lakhotia and Kumar 1978). The metaphase plates in different autoradiographic preparations were identified as male or female on the basis of their sex-chromosome constitution. Since the embryos or larvae were not pre-selected on the basis of their sex, this Table (1) turned out to be rather biased for female metaphases. It is known from earlier studies (Banigirdi 1968; Steinemanun
Fig. 1. Autoradiograms of 3H-thymidine pulse labelled metaphase plates from brain ganglia of male larvae of D. annulatus. (a) Metaphase with euchromatic regions of all chromosomes labelled (the heterochromatin Y chromosome (Y) is almost unlabelled). (b) Metaphase with both hetero- and euchromatin regions of all chromosomes labelled. (c) A normative metaphase chromosome after removal of silver grains from the autoradiogram are shown in the lower half of the figure to help identify each chromosome. (d) A metaphase with labelling on the euchromatin of all chromosomes. Note that in all cases labelling of the X chromosome (X) is similar to that on the autosome. Scale bar = 5 μm.

1984; Sprauling and Orr-Weaver 1987), that when pulse labelled with 3H-thymidine, chromosomes in metaphase cells of Drosophila show three distinct patterns of labelling: (1) E-labelled, in which only the euchromatin regions are uniformly labelled; (2) B-labelled, in which both euchromatin and heterochromatin regions are uniformly labelled; and (3) H-labelled, in which the label is restricted to heterochromatic regions only (see Fig. 1). As in other species, in Drosophila cells, the E-labelled metaphases represent early S phase, while the H-labelled metaphases represent the later S phase, and the B-labelled metaphases represent the interphase period (Burgus et al. 1985; Neirnann 1986; Sprauling and Orr-Weaver 1987). In agreement with this temporal order, in the present study, the H-labelled metaphases appeared first, in the early (15 min) chase samples, followed by the B- and E-labelled types, in that order, in later chase samples. However, in brain ganglia, the H-labelled metaphases persisted in later samples also. In nearly all the labelled metaphases examined from early embryos or brain ganglia, the different autosome showed remarkable synchrony in the labelling of their euchromatin, both euchromatin and heterochromatin, or heterochromatic regions, and accordingly, the labelled metaphases were classified as E, B, or H types respectively (Fig. 1; Table I). The labelling of the X and Y chromosomes (in the case of male cells) or of the two X chromosomes (in the case of female cells) in each of these different types of labelled metaphases was examined to see if any of these chromosomes replicated out of synchrony with reference to the autosome in the nucleus. The pooled data for different chase samples of the two tissues are presented in Table I. As for the autosome, the labelling of the X chromosome (X) was also classified as unlabelled (U), euchromatin labelled (E), both euchromatin and heterochromatin labelled (B), or heterochromatin labelled (H) type, while the Y chromosome was classified as unlabelled (U) or labelled (L) type (Table 1).

The data in Table 1 show that in all the embryonic male metaphases, the labelling of the autosome and the X chromosome was highly concordant. The mostly heterochromatin and late-replicating subcentric Y chromosome displayed low labelling of its tips in several metaphases with E- or H-type automorals and X-chromosomal labelling; the tips of the Y chromosome are known to be readily fluorescent with Hoechst 33342 (see Lakhotia and Kimura 1974). The X-chromosomal and automosomal labelling patterns were concordant in all the female embryonic metaphases examined except for two H-labelled types, one of which X chromosome was unlabelled, while the other showed weak labelling (Table 1).

In the 34 E-labelled metaphases from brain ganglia of male larvae, the C chromosome and autosome were similarly labelled. In 1 of the 36 H-labelled male brain metaphases, the C chromosome was a B-labelled type, while the labelling of the other 35 was concordant. In only 3 of the 84 H-labelled metaphases examined from brain ganglia of male larvae, was the X chromosome unlabelled; in all others the X chromosome, as well as the autosome, was H-labelled. In most of the metaphases from brain ganglia of female larvae, the X chromosomes were labelled in complete synchrony with the autosome; only in rare cases did out of the two X chromosomes appear unlabelled when the other X chromosome was labelled in synchrony with the autosome. In rare instances one or the other autosome also appeared
unlabelled or differently labelled from the others (details not presented but the overall incidence of such metaphases was comparable to that for the two X chromosomes in female nuclei).

In the more than 700 metaphases examined in preparations of centronuclear and larval brain ganglia cells in which the autosomes were unlabelled, the sex chromosomes (both the X chromosomes in female and the X and Y chromosomes in male cells) were also unlabelled.

If the single X chromosome in male cells was early replicating, as in larval salivary gland cells, one would expect the single X chromosome to show B- or H-type labelling in many nuclei with H-labelled autosomes and H-type labelling in nuclei with H-labelled autosomes and finally to see the male X chromosome unlabelled in many of the H-labelled metaphases. However, except for the two rare instances in which the X chromosome in male cells was out of sync with the autosomes (in one of the male X chromosomes (1) was "ahead" of the autosomes (H), while in the other, the male X chromosome (2) was "delayed" with reference to the autosomes (B) (see Table 1), in the nearly 150 others labelled metaphases from index, the X chromosome was always labelled in complete synchrony with the autosomes. Since a certain degree of inerchromosomal asynchrony was seen between the two X chromosomes in female cells or, also, within the autosome set, the two instances of asynchrony between the X chromosome and autosomes seen in male nuclei do not appear to have any significance.

In contrast with our present results with D. melanogaster (1973), we concluded that the X chromosome in male Drosophila melanogaster males early replicates, as does the Y chromosome. It is unlikely that these distinct results are the result of species differences as evidenced by the following two considerations: (i) the hemizygous X chromosome in the polytene nuclei of male D. melanogaster females also replicates very early (Roy and Laskiha, 1981) and (ii) in a parallel study in our laboratory (Bhattacharyya unpublished data) the X chromosome in mitotic cells of D. melanogaster males was also found to replicate in synchrony with the autosomes. As pointed out earlier (Laskiha et al., 1993), the conclusion of Kar and Mukherjee (1993) about early completion of the X chromosome in mitotic cells of male Drosophila may not be justified, since their experimental design to obtain PCC in Drosophila cells had several flaws, and also, the results presented by them were not unequivocal. Compared with the indirect approach of Kar and Mukherjee (1993), our present study directly examined the temporal order of replication of the X and Y chromosomes in mitotic cells and the results show unambiguously that neither the single X chromosome in male cells nor either of the two X chromosomes in female cells displays any kind of asynchrony we expect in replication of autosomes. Thus, we conclude that the single X chromosome in mitotically dividing cells of Drosophila melanogaster is not replicating earlier than the autosomes. This may appear to be at odds with the well-established, early completion of an endoreplication cycle by the hemizygous X chromosome in salivary gland polytene cells of male larvae (Laskiha and Mukherjee 1970; Chatterjee and Mukherjee 1977; Laskiha and Bhattacharyya 1987). However, Laskiha and Sinha (1983) suggested that the early completion of replication by the hemizygous X chromosome in polytene nuclei of male larvae was a special consequence of the unique requirements of the polytene structure, and that the early completion of replication was not a prerequisite for an increased rate of transcription of the somatically active X-linked genes in males to achieve dosage compensation. Another aspect of the organization of active replicons also needs to be considered in this context. In mammalian and other cells, adjacent replicons are organized as "replicon clusters" (Holland 1978) that are all activated synchronously at a given pair of the S phase and this clustering of replicons generates "replication bands" at the metaphase chromosome level (Holliday, 1980; Craig and Bickmore, 1993). Unlike the case in mammalian cells, replication clusters are not seen in Drosophila (Laskiha and Sinha, 1983; Steinmann, 1980). Laskiha and Tiwari (1985), further, all the active replicon origins along the entire euchromatin or heterochromatin regions appear to be "fired" together, so that the metaphase chromosomes of Drosophila, pulse labelled with [3H]thymidine during the S phase, do not display "replication bands" (Holmquist 1989; Ramam and Laskiha, 1989). In the absence of asynchronously replicating "replicon-clusters" in Drosophila nuclei, all chromosomes would be expected to replicate synchronously except in polyploid nuclei, where the special constraints of polyploidy result in asynchronous early replication of the hemizygous X chromosome in male nuclei (Laskiha and Sinha, 1983). Thus a synchronously replicating X chromosome in nonpolypotent cells of male Drosophila is not in conflict with its transcriptional hyperversatility.

References


