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Interchromosomal Asynchrony of DNA Replication in Polytene Chromosomes of *Drosophila pseudoobscura**

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Abstract. Analysis of ^{3}H-thymidine autoradiograms of late third instar larval salivary glands of *Drosophila pseudoobscura* revealed a unique example of asynchrony of replication in the autosome complement. The two autosomal arms, 2 and 3, show similar labeling pattern during the initial phases, DD to 3C, and thereafter, the chromosome 3 has fewer labeled sites than chromosome 2 until the most terminal pattern, 1D. Detailed sitewise analysis of ^{3}H-thymidine labeling shows that while nearly 54% of the sites examined in chromosome 2 have a labeling frequency greater than 50%, only 13% of all sites in chromosome 3 have labeling frequency at that range. The number of labeled sites on chromosome 3 plotted against that on chromosome 2 shows a hyperbolic profile rather than a linear relationship. The silver grain ratio of the 2nd to 3rd increases from 1.5 to 3.1 through different stages of the cycle. These results suggest that both chromosomes start replication simultaneously but the third chromosome appears to complete the replication earlier than the second. These data open up the possibility of separate control mechanisms for the initiation and termination of DNA replication in polytene chromosomes.

Introduction

DNA replication in polytene chromosomes of Diptera has been examined autoradiographically in many species by various groups of workers. In *Drosophila* polytene chromosomes a general pattern of synchronous replication among different sites of the same chromosome and between different chromosomes has been reported (Plaut *et al.*, 1966), with the exception of the X-chromosome of the male. Although different sites or replicons may complete replication at different time, implying an independent organization of replicating units, groups of replicons appear to follow a synchronized sequential order. However, asynchronous replication is known to exist in *Drosophila* polytene nuclei, namely under-replication of the nucleolus organizer (Spear and Gall, 1973; Laird, 1973), lack of replication of α-heterochromatin (Lakhotia, 1974) and faster replication of the male X (Lakhotia and Mukherjee, 1970; Mukherjee and Chatterjee, 1975). Nothing precisely is known about the regulation of these cases of asynchronous replication although much work has been done on the significance of such asynchrony (Lakhotia and Mukherjee, 1970; Mukherjee, 1973; Laird, 1973; Gall and Rochaix, 1974; Tartof, 1974). In *D. melanogaster* polytene chromosomes in which DNA replication has been studied in detail (Lakhotia and Mukherjee, 1970; Hägele, 1973; Hägele and Kalisch, 1974), no asynchrony between nonhomologous chromosomes has been reported. In *D. hydei*, Chatterjee and Mukherjee (1973) did not find any evidence of interchromosomal asynchrony. In highly polymorphic

* This paper is dedicated to the memory of the late Prof. H. D. Berendes.

species like *D. willistoni*, *D. pseudoobscura*, *D. ananassae* and others, such analysis has not been made. Recently, we have examined DNA replication in one such species, *D. pseudoobscura* (Chatterjee and Mukherjee, 1975) and have provided evidence of X chromosomal asynchrony in males (Mukherjee and Chatterjee, 1975). This paper presents some information on interchromosomal asynchrony in DNA replication between two autosomes of *D. pseudoobscura*.

Material and Methods

The wild type ST-322 strain of *Drosophila pseudoobscura*, obtained from the University of Texas, Austin, Texas, has been used for the present investigation. Cultures were maintained in standard *Drosophila* medium and reared at $22 \pm 0.5°C$ in a B.O.D. incubator. Mature well-fed third instar larvae were used for chromosome preparation.

Cytological and Autoradiographic Procedure. Conventional cytological procedure described earlier (Chatterjee and Mukherjee, 1975; Mukherjee and Chatterjee, 1975) has been used. Salivary glands obtained from partially synchronised late third instar larvae were excised in *Drosophila* Ringer at pH 7.2 and incubated in ^{3}H-thymidine (^{3}H-TdR, Specific activity–5,700 mCi/mM, BARC, Trombay, India) for 20 minutes. Two pairs of glands were incubated in 10 µl of Ringer containing 4 µCi of ^{3}H-TdR (400 µCi/ml), fixed in ethanol-acetic acid (3:1), rinsed in 50% acetic acid, stained lightly for a short time in a 2:1 mixture of aceto-carmine-acetic orcein (for detection of chromosomes) and squashed on a drop of 50% acetic acid. Coverslips were removed in aceto-alcohol vapour and in acetic acid, and the slides were covered with Kodak AR 10 stripping films. Preparations were exposed for 16–17 days and developed in Kodak D19b developer and fixed in Kodak acid fixer. The usual procedure for scanning the autoradiograms has been used (Mukherjee and Chatterjee, 1975).

Results and Discussion

The ^{3}H-thymidine (^{3}H-TdR) labeling patterns on the segments 81 to the last two dark bands of 74 (74D) of the autosome 3 and 62 to the last two dark bands of 56 (56D) of the autosome 2 of *D. pseudoobscura* have been examined from the autoradiograms. These two segments have been divided into 24 and 28 sub-divisions respectively, on the basis of the previous record on their length and pattern of termination as reckoned by Plaut *et al.* (1966), a method that has been discussed in earlier publications (Lakhotia and Mukherjee, 1970; Chatterjee and Mukherjee, 1973, 1975; Mukherjee and Chatterjee, 1975). These units are presented schematically in Fig. 1. It must be emphasized, that in the light of the observations of Pelling (1966) and Sorsa (1974), these units are not strictly comparable to replicating units, but may simply represent one or more replicating units which are synchronously ordered with respect to their initiation, length and termination pattern.

Figs. 2–5 represent the ^{3}H-TdR labeling patterns of the initial (disperse discontinuous, DD or interband), middle (continuous, C) and terminal (discontinuous 3D–2D) patterns on the arms of chromosomes 2 and 3. On the basis of the arguments presented elsewhere (Chatterjee and Mukherjee, 1975) and those presented by Hägele and Kalisch (1974), the DD or interband-puff pattern is the initial phase. 1C–3C are the middle parts and 3D–1D are the terminal phases of the replication cycle.

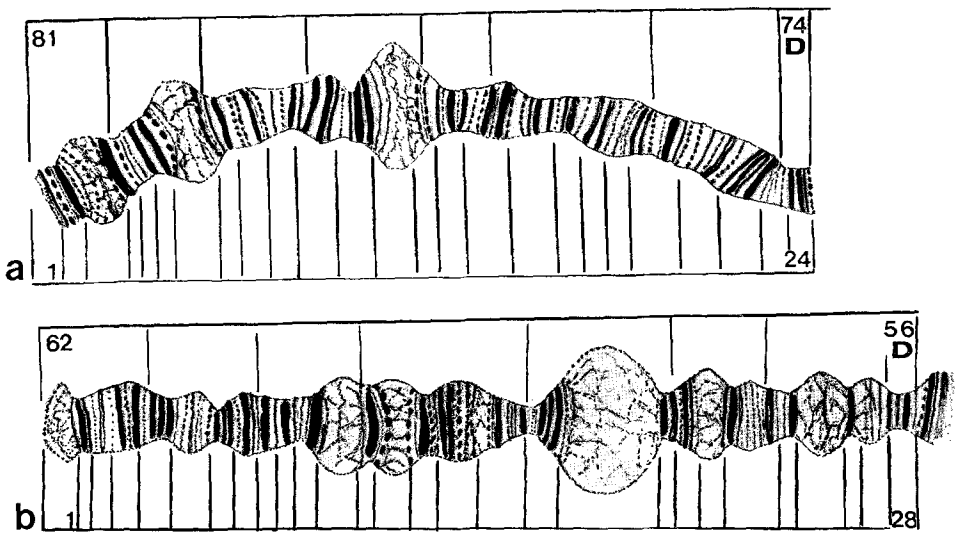

Fig. 1 a and b. Camera lucida drawing of the segments 56D to 62 (tip) of the second chromosome (b) and 74D to 81 (tip) of the third chromosome (a) of the late third instar larval salivary gland of *Drosophila pseudoobscura*. Sections on the upper part of each chromosome represent the main segments of Stocker and Kastritsis (1972). Fine and newer bands shown have been mapped by Duttagupta and co-workers in this laboratory. The sections shown in the lower part of each chromosome, namely 1 to 28 for chromosome 2 and 1 to 24 for chromosome 3, are the "replicating units" described in the text

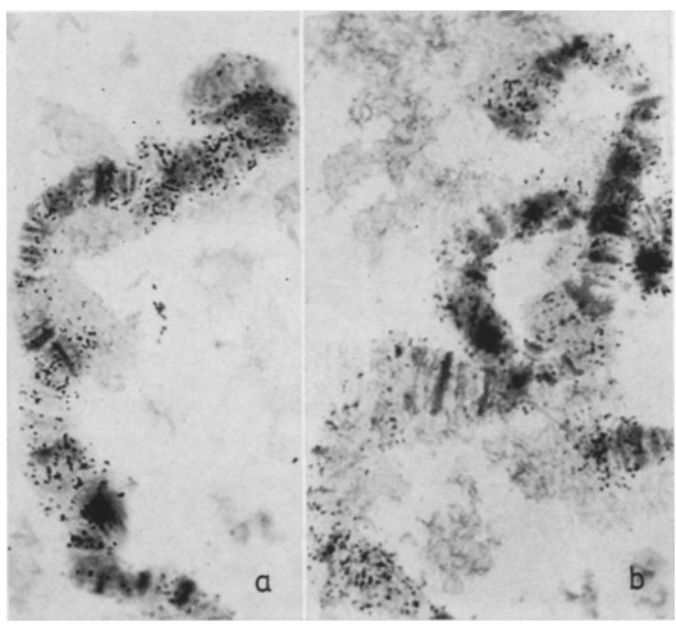

Fig. 2 a and b. ^{3}H-TdR autoradiogram of the chromosomes 3 (a) and 2 (b) showing the initial interband or DD pattern. Both chromosomes showing similar labeling patterns and grain intensity. Dark bands in both chromosomes are unlabeled; puffs, thinbands and interbands are labeled

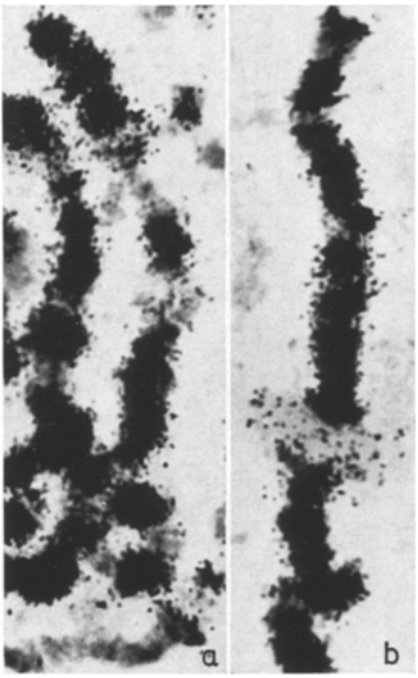

Fig. 3a and b. ^{3}H-TdR autoradiogram showing 3C–3D transition pattern on chromosome 2 (b) and 3D pattern on chromosome 3 (a). The third chromosome has distinctly fewer labeled sites than chromosome 2

Our observations on the autoradiograms showed that no distinct difference between chromosomes 2 and 3 could be recognised during the initial part (Fig. 2). Both arms showed similar labeling pattern with puffs and interbands labeled and dark bands unlabeled.

For most of the 3C labeling patterns also no difference was apparent. In some cases, however, 20 to 23 sites in chromosome 3 were labeled when all 28 sites on chromosome 2 were continuously labeled (see Fig. 7 and p. 122). After this stage a clear difference in the labeling pattern could be resolved between the two arms in 100% of the nuclei (Figs. 3–5). The number of unlabeled gaps on the arms of chromosome 3 is always progressively more and more in the later phases of the cycle. It appears, therefore, that both autosomes are initiated to replicate at the same time but their various sites tend to complete the replication asynchronously. Such asynchrony of chromosome 3 during the phases from 3D onward has been observed in preparations from both sexes.

The relative frequencies of ^{3}H-TdR labeling patterns of the 24 sites of chromosome 3 and 28 sites of chromosome 2, respectively, among the total number of labeled nuclei examined ($N = 68$) are shown in Fig. 6. These nuclei included all kinds of labeled nuclei (*viz.*, DD, C and D). As no difference between the male and female was observed either from the labeling patterns or frequency estimation, the data from both sexes have been pooled together. Data in Fig. 6 reveal

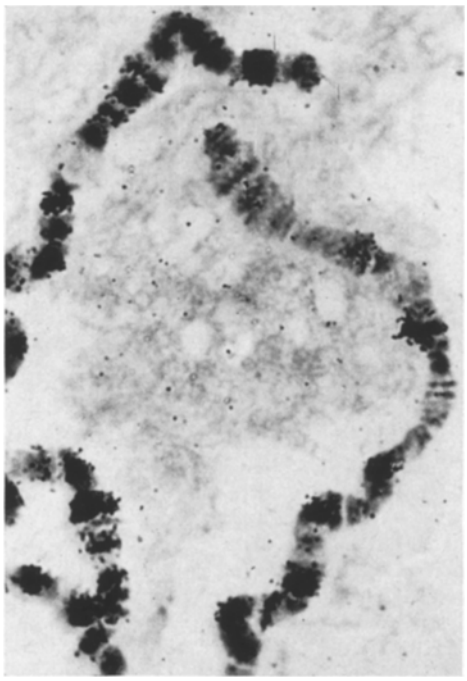

Fig. 4. ^{3}H-TdR autoradiogram of chromosomes 2 (left) and 3 (right) during the 2D stage, with more unlabeled gaps on the third than on the second

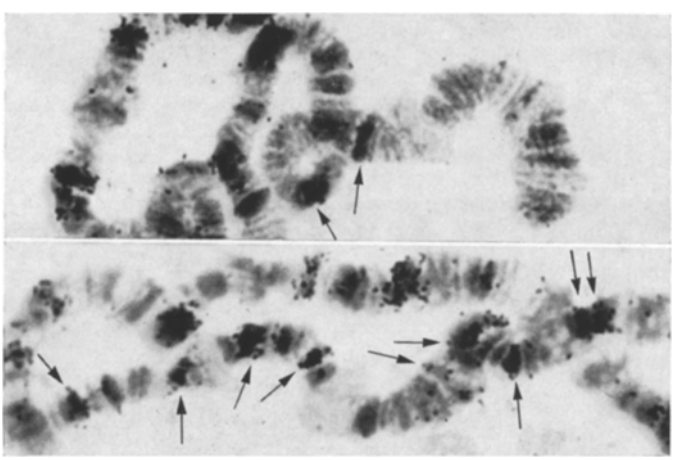

Fig. 5. ^{3}H-TdR autoradiogram showing a 2D–1D pattern with labeled sites (arrows), 9 on chromosome 2 (below) and only 2 on chromosome 3 (above)

that only 3 of the 24 sites (12.5%) in the third chromosome segment have labeling frequency greater than 50%, while 15 out of the 28 sites (53.6%) in the second autosome segment have labeling frequency greater than 50%. Similarly, there

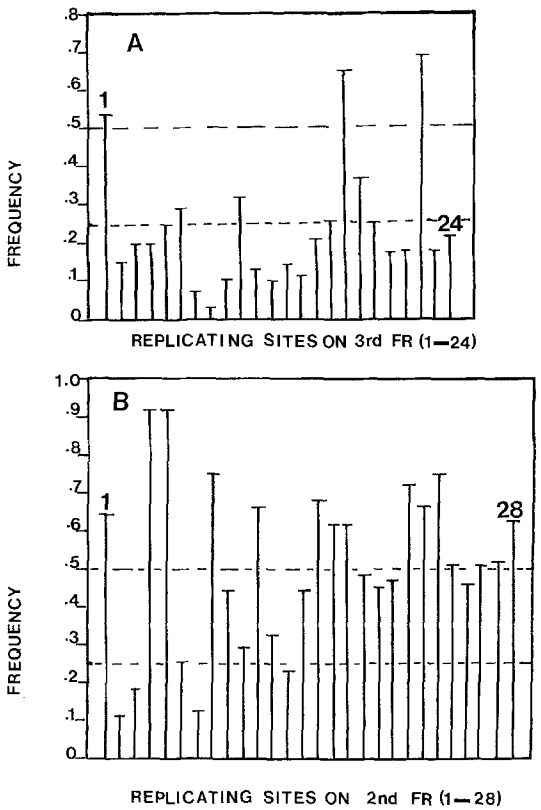

Fig. 6A and B. Histograms showing the frequency of ^{3}H-TdR labeling of the 24 sites on chromosome 3 (A) and 28 sites on the chromosome 2 (B). The broken lines represent the 50% and 25% frequency levels

are considerably fewer sites labeled between 25 to 50% in the third than in the second chromosome. The majority of the sites in the third chromosome have labeling frequency less than 25% as compared to only 4 out of the 28 sites in the second chromosome.

Results on the analysis of the number of labeled sites on the third chromosome plotted against that on the second, for each nucleus, reveal further that the relationship is far deviating from linearity and the curve could be best fitted to a hyperbola (Fig. 7). The curve fits closely with the calculated hyperbolic curve for both male and female. Results presented in Fig. 7 further show that for some but not all C type patterns on chromosome 2 (28 sites labeled), the labeling pattern of chromosome 3 is also continuous. This finding implies that the divergence between the timing of replication between the two chromosomes starts sometime during the C pattern.

When the intensity of silver grains due to ^{3}H-TdR incorporation is examined in the two autosomal segments, it becomes evident that for all labeling classes (3D to 1D) in which grain counts could be done the mean number of grains is

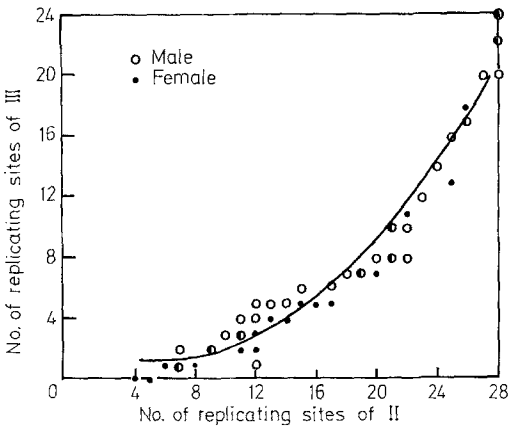

Fig. 7. Chart showing the number of ^{3}H-TdR labeled sites on chromosome 3 plotted against that on chromosome 2 of each nucleus of *D. pseudoobscura*. The observations were taken separately for male and female, and plotted accordingly. Note that for some continuous patterns (28 sites labeled on the chromosome 2) only, the chromosome 3 also shows continuous labeling (24 sites labeled)

Table 1. Analysis of the intensity of incorporation over the second and third chromosomal segments of *D. pseudoobscura* with reference to the number of labeled sites on the third chromosome

No. of labeled sites on chromosome 3	No. of silver grains over chromosome		Grain ratio 2/3
	2	3	
24–21 (—) (1C–3D)	a	a	—
20–17 (2) (3D)	1,337	886	1.5
16–13 (2) (3D)	689.5	440	1.6
12– 9 (5) (3D–2D)	384.6	138.8	2.8
8– 5 (12) (2D)	291.6	94.5	3.1
4– 1 (12) (1D)	126.2	53.2	2.4

[a] Impossible to count.

consistently lower in the segment of chromosome 3 than in that of chromosome 2 (Table 1). This lower number of grains in chromosome 3 is not in proportion to the relatively slightly smaller size of the segment of the 3rd chromosome examined. This is evident from the ratio of the grain numbers on the segment of chromosome 2 to those on 3. The ratio increases from 1.5 to 3.1 at the 5–8 sites labeled pattern and then slightly decreases but never becomes anywhere close to one. These data might imply that fewer replicative forms of DNA are progressively available in the third chromosomal segment than in that of the second. However, since a higher labeling intensity of chromosome 3 than 2 has not been observed either during the continuous phase or earlier, among the autoradiograms, the actual significance of this lower intensity during the discontinuous phases remains

obscure. Furthermore, there is no evidence with respect to a higher DNA content in the second chromosome than in the third.

Spectrophotometric measurements of the DNA content of the labeled and unlabeled bands during different phases of ^{3}H-TdR labeling as have been done by Mulder *et al.* (1968), may be useful to resolve this paradox. This may be specially important as it has been observed that there are considerably more puffs on the third than on the second chromosome (Stocker and Kastritsis, 1972).

Nevertheless, at the face of the observations on the autoradiograms and the data on the labeling frequency as well as pairwise analysis of the number of labeled sites of the two chromosome segments it seems reasonable to interpret that the third chromosome of *D. pseudoobscura* begins its replication synchronously with other autosomes but tends to complete the replication cycle earlier than the latter. The unequal distribution of the long and late replicating sites (Fig. 6) of the two chromosomes strongly supports this conclusion. It may be noted here that in the male, the X-chromosome is even faster or rather more early replicating than the third chromosome of this species (Mukherjee and Chatterjee, 1975).

If the findings presented above are real, this would indicate that in addition to the asynchrony existing for the rDNA (Spear and Gall, 1971), the α-heterochromatin (Lakhotia, 1974) and the male X (Lakhotia and Mukherjee, 1970; Mukherjee and Chatterjee, 1975), an interchromosomal asynchrony might also be present. This finding challenges the existence of a single central control mechanism proposed earlier for both initiation and termination of the DNA synthesis by the different chromosomes (Plaut *et al.*, 1966). This would rather imply that individual control mechanisms might exist for each chromosome and separate sets of control mechanism might be possible for initiation and termination.

The mechanism of such asynchrony between chromosomes regardless of the sex is not quite clear. What it means is, however, important. In reality it would imply that such control mechanism might exist in *Drosophila* system that would render the intrasynthetic timing shorter or longer. The significance of this fact is stressed by the well established high degree of polymorphism of chromosome 3 in this species (Dobzhansky, 1951) and a greater number of active puff sites in the same chromosome (Stocker and Kastritsis, 1972). Analysis of this asynchrony between the autosomes in different polymorphic varieties should be of considerable significance. A knowledge of this aspect may reveal the structural and functional organization of the genome of this species, and its relation to other species of *Drosophila* (Laird, 1973).

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