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THE ENDING PATTERN OF DNA REPLICATION IN LARVAL BRAIN CELLS OF HOUSE FLY

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ABSTRACT

K. Y. Jan (1971) *The Ending Pattern of DNA Replication in Larval Brain Cells of House Fly*. Bull. Inst. Zool. Academia Sinica **10**:(2) 77-82. The ending pattern of DNA replication of the house fly larval brain cells was revealed by tritiated thymidine autoradiography. Often, auto- and allosomes replicated together, or the allosomes replicated alone. Whereas, the autosomes replicated alone was rare. In the XY cells, the X and Y replicated together or the X but not the Y replicated alone. In the XX cells, both X's replicated together or one X replicated alone. The genetic activities of the X and Y chromosomes were discussed.

The late DNA replication has been unfailably correlated with genetic inactivation (5). For instance, in the mammalian system, the X inactivation has been proposed as the mechanism for dosage compensation (12). In cells from both males and females one X remains euchromatic, non-condensed and early replicating, whereas the other X in the female complement becomes condensed, heterochromatic and late replicating (5, 7, 13, 16, 19). In the larval brain cells of the house fly, the allosomes seem to be late replicating (9). The present experiment was then undertaken for analysing the exact late replicating pattern, and this result was discussed in relation to the genetic activities.

MATERIALS AND METHODS

The techniques for fly (*Musca domestica* L. *ocra* strain) rearing, thymidine-methyl-H^3 (H^3TdR) autoradiography and chromosome identification were described previously (9). About 15 μC of H^3TdR (New England Nuclear, specific activities 18.9 C/mM) per larva was injected into the head region of the third instar larvae. The larval brains were sampled at 1.5 hours after H^3TdR injection for the preparation of microscopic slides. The slides were scanned for metaphase figures of which the allosomes were recognizable. These metaphase figures were then sketched, photographed and their locations on slides were recorded, These slides were then coated with Kodak NTB-3 liquid emulsion and exposed for 10 days. Metaphase figures with less than 4 silver grains or with high background grain counts within their immediate surroundings were not included in the present analysis.

RESULTS

In both XY and XX cells, the labelling occurred primarily on the auto- and allosomes

together or on the allosomes only (Table 1). There were two XY cells and three XX cells with autosomes labelled only. Their grain counts were 73 and 5 for the two XY cells and 10, 9, 8 for the three XX cells respectively. These grain counts make it difficult to account them due to background grains, although the cases with autosomes labelled only were rare.

TABLE 1.
Labelling frequencies for the autosomes and allosomes

	XY cells	XX cells
No. labelled metaphases	25	24
Autosomes labelled only	2	3
Auto- and allosomes both labelled	14	8
Allosomes labelled only	9	13
No. metaphases with allosomes labelled	23	21
Both allosomes labelled	15	13
One X labelled only	8	8
Y labelled only	0	—

The result as shown in Table 1 indicates that in the XY cells, both X and Y could be labelled or the X only was labelled, whereas cases with Y only labelled were not observed. Similarly, in the XX cells, two X's could be both labelled or one of the two X's was labelled. Some of the mitotic metaphase figures showing the exact labelling pattern are presented in Figs. 1a and b to 4a and b. The grain counts on each allosome and on all autosomes for each cell, are presented in Table 2. In cases where both allosomes were labelled the grain count for the less-grain allosome was not always one or two. Similarly, in cases where only one of the two allosomes was labelled, the grain count for this allosome was not always one or two. This checking gives certain confidence to conclude that at 1.5 hours after H^3TdR injection, the X and Y could be both labelled or the X only was labelled; the two X's could be both labelled or one X only was labelled.

DISCUSSION

The results of the present experiment may have at least four explanations: (A) Some of the cells may have both X's or both X and Y completing their DNA replication late, other cells may have only one X of the XX or X of the XY late. This explanation does not contradict to the finding that the allosomes of the larval brain cells are often (not always) heterochromatic (9). Barigozzi *et al.* (2) exposing the *Drosophila* embryonic cells *in vitro* to tritiated thymidine for 3.5 hours, also noted that in some female cells the centromeric regions of both X's are late in completing their DNA replication, in other female cells only one X in the centromeric region are late; whereas in the male cells, the entire Y and the centromeric region of the X are late (see also 20). (B) The fact that labelled metaphases were obtained at 1.5 hours but not at 1 hour after H^3TdR administration, led Jan and Boyes (8) to suggest that the minimum duration of G_2 plus prophase is 1.5 hours. Nevertheless, the

TABLE 2.
Grain counts for the labelled metaphases

XY cells				XX cells			
X	Y	Autosomes	Total	One X	Other X	Autosomes	Total
26	1	0	27	27	24	16	67
19	1	0	20	22	18	0	40
16	9	0	25	19	7	0	26
9	4	15	28	15	2	0	17
9	4	0	13	11	6	0	17
7	1	44	52	7	4	0	11
6	6	4	16	6	2	0	8
6	4	0	10	5	5	10	20
6	1	0	7	5	4	3	12
5	1	8	14	5	3	0	8
4	13	105	122	4	3	7	14
4	2	0	6	3	1	0	4
4	1	10	15	2	2	0	4
1	1	3	5	12	0	0	12
1	1	38	40	11	0	0	11
10	0	0	10	4	0	0	4
7	0	0	7	3	0	0	3
4	0	18	22	2	0	51	53
3	0	13	16	2	0	10	12
3	0	12	15	1	0	5	6
3	0	8	11	1	0	4	5
1	0	12	13	0	0	10	10
1	0	12	13	0	0	9	9
0	0	73	73	0	0	8	8
0	0	5	5				

possibility that the minimal duration of G_2 plus prophase is less than 1.5 hours but more than 1 hour, still exists. If this is the case, then the labelling of 1.5-hour sample was not the true ending pattern of DNA replication. Hence, labelling patterns other than the end of S period inevitably appeared in the 1.5-hour sample. (C) The uniformity of cell cycle time in the larval brain cells has been questioned (8). There is a possibility that one cell population has a G_2 plus prophase of 1.5 hours, whereas this duration is not followed by other cell population. (D) Of course the inaccuracy of autoradiographic technique may also account in part for the inconsistency.

Rubini and Palenzona (17) increased the number of X chromosomes in *M. domestica* L. *ocra* strain to six, by selective breeding and found that the flies with one Y chromosome and one or more X chromosomes

in their cells are always males but without a Y chromosome they are always females. There are no morphological difference between the flies having extra X chromosomes and those with the normal complement of X chromosome (*i.e.* one in males and two in females). This led Rubini and Palenzona (17) to conclude that the X chromosome may be functionless whereas the Y chromsome carries the male determining factor or factors. No genetic marker has yet been located on the X chromosome of *M. domestica* L. (21). These data together with late replication prefer the genetic inertness of the X chromsome. However, during the present experiment, the karyotypes of about 3,000 larvae have been examined. A karyotype without X chromosome has never been found, although 5 larvae have been found with XO allosomal complement. This would suggest that the X chromosome is not completely functionless. In the same sense the heterochromatic nature and late replication prefer the genetic inertness of the Y chromosome, but this again would have to compromise with the view that the Y chromosome palys a role in the sex determination. All these data point to the possibility that some euchromatic segments may exist in the X and Y chromsomes. Recently, the quinacrine fluorescent microscopy (3), Giemsa stain (1, 4, 15, 23) and the cytological localization of repetitive DNA (6) have been developed into powerful techniques for revealing the nature of heterochromatin. It is possible that these techniques may shed some light on this problem.

If genes are located on the X chromosome of the house fly and there is dosage compensation, then the finding that two X's in some XX cells and the one X in most XY cells are late in their DNA replication, seems to suggest that the one X inactivation is not the mechanism of dosage compensation. On the other hand the finding that one of the two X's in some XX cells is late in its DNA replication would prefer that the X inactivation is the mechanism of dosage compensation. The closest example available in this connection is in the salivary gland of *Drosophila*, where the dosage compensation is not accomplished by X inactivation (18, 22) but by the hyperactivity of the male X at the level of RNA synthesis (10, 11, 14).

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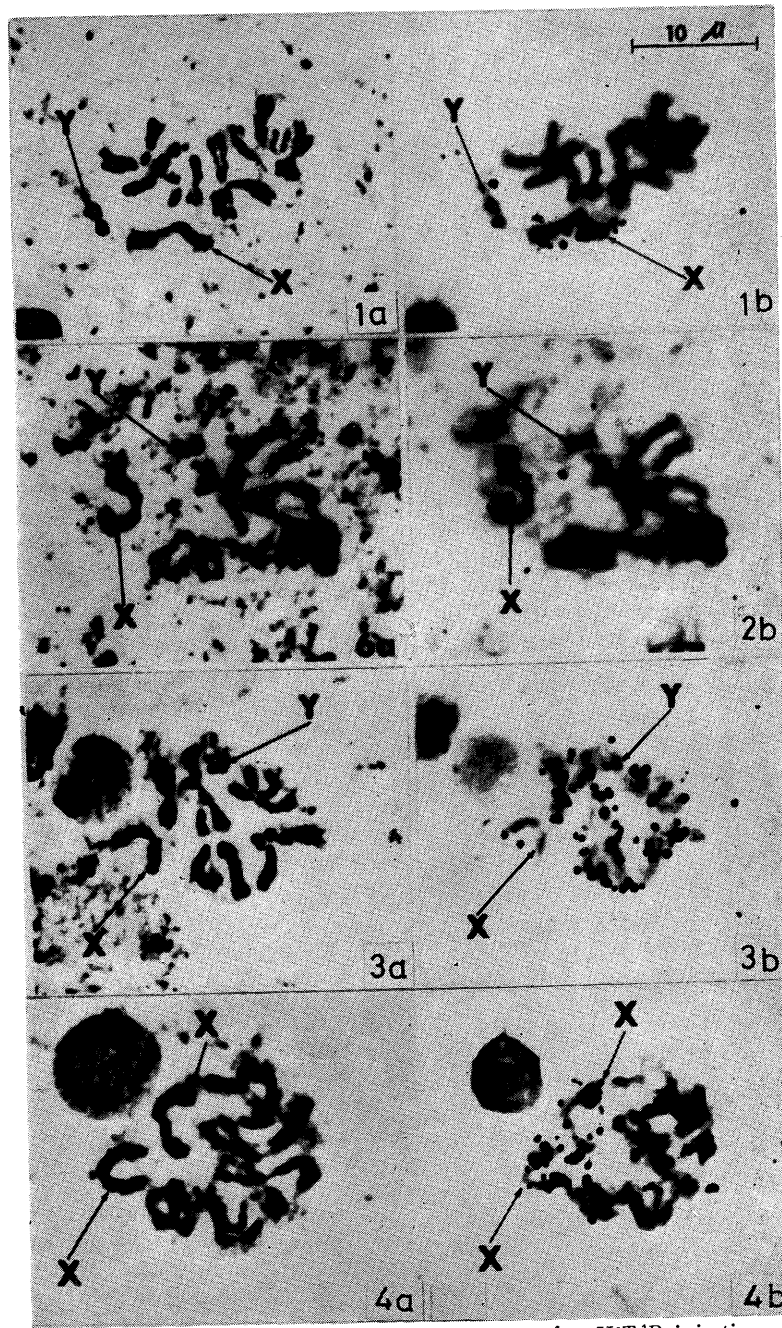

Mitotic metaphase figures sampled at 1.5 hours after H^3TdR injection

1a & b. the X and Y chromosomes only labelled.
2a & b. the X chromosome only labelled.
3a & b. the autosomes only labelled.
4a & b. two X chromosomes labelled.