

Table 1. Frequencies of different classes of X chromosomal activity in  $In(1)BM^2fB^{15}$  reinverted mosaic male larval salivary glands grown in Schneider's medium and in vivo.

	Total no. of cells examined	(% freq. of X chromosomal types		
		Flabby	Intermediate	Near normal
A] In vivo				
10°C	1430	62.2	31.5	6.3
18°C	2534	50.0	24.9	25.1
23°C	1770	24.8	50.1	25.1
B] In vitro (23°C)				
24 hr	1700	25.0	37.7	37.3
48 hr	1242	18.7	31.4	49.9

Salivary glands were dissected out from male and female late third instar larvae, and transferred to a T-flask containing Schneider's medium (minus yeast hydrolysate) and kept at  $23^{\circ}\pm 1^{\circ}\text{C}$  in an incubator, for 24 hr and 48 hr. Thereafter, the glands were transferred to *Drosophila* Ringer (pH 7.2) after giving an interval of 5 min for acclimatization. Squash preparations of chromosomes were made as described (Lakhotia & Mukherjee 1969).

The mosaic expression of the X chromosome of the mutant male larva in vivo is presented in Figure 1a-c and that of the X chromosome of male larval gland grown in vitro culture is shown in Figure 2a-c. Evidently, all three classes of nuclei, containing flabby, intermediate and near-normal condition of the X chromosome in 1X2A nuclei, are manifested as observed in vivo.

However, as evident from Table 1, the frequencies of these classes of nuclei are slightly different in in vitro culture, as compared to in vivo. The results in Table 1 reveal that the extreme flabby condition of the X chromosome of the mutant is predominant in in vivo specially when grown at  $10^{\circ}\text{C}$ . In in vitro culture, more near-normal class appear, especially after 48 hr of culture.

Apart from the difference in the frequency of the three classes, it is quite evident that the mosaic condition of the extra hyperactive X chromosome is maintained in in vitro culture, i.e., even outside the larval body. It may be suggested that continuous presence and supply of larval hormone such as ecdysone may not be needed to maintain the extraordinary hyperactivity of the X chromosome in the male. The mosaic expression appears also to be cell autonomous.

**References:** Ashburner, M. 1972, *Chromosoma* 38:255-282; \_\_\_\_\_ 1973, *Devel. Biol.* 35:47-61; Ashburner, M., C. Chihara, P. Metzger & G. Richards 1974, *Cold Spr. Harb. Symp. Quant. Biol.* 38:655-662; Ghosh, M. & A.S. Mukherjee 1983, XVth Int. Congr. Genet. Abstr. 178:104; Ghosh, M., D. Bose & A.S. Mukherjee 1982, Vth All Ind. Cell Biol. Conf. Abstr. 28:17; Majumdar, D. & A.S. Mukherjee 1980, *DIS* 55:159-160.

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& Mukherjee 1970; Chatterjee & Mukherjee 1975; Haegeler & Kalisch 1974) and such labelling pattern is called DD pattern (which is identified by disperse label on puffs and interbands and lack of label on chromocentric heterochromatin). Earlier works from this laboratory have revealed that the X chromosome in *Drosophila* male is early replicating (Lakhotia & Mukherjee 1970) and also faster in rate of chain growth (Chatterjee & Mukherjee 1978). The X chromosome of the male in all *Drosophila* species examined is hyperactive, puffy and faster replicating (see Mukherjee 1982). While searching for the mechanism of the hyperactivity of the X chromosome, we came across with a mutant strain,  $In(1)BM^2$ --reinvert mosaic. The X chromosome in the male larval salivary gland of this strain is extremely puffy in 30 to 50% of the cells. It has been shown that the X is indeed superhyperactive as compared to that of the wild type, and synthesizes 3 to 4 times as much RNA as the individual X chromosomes of its female counterpart.

In this report we are presenting the replicative behaviour of the X chromosome in this strain. DNA replication has been monitored by autoradiography using  $^3\text{H}$ -thymidine. Results shown in Table 1 reveal that while in the reinverted mosaic female the labelling frequencies of all sites except two (viz., 6DEF and

in sections of the chromosome; and (c) nearly normal puffy structure as in wild type male. Thus, the different nuclei manifest as mosaic expression for the X chromosome. All other autosomes appear normal. In squash preparations from female larval glands, all chromosomes are normal. Such expression has been explained as an expression of some modulator for dosage compensation (Ghosh & Mukherjee 1983, and in prep.).

The intention of the present investigation was to find out whether in the simplified culture medium such extraordinary hyperactivity of the male X chromosome of the mutant strain is maintained and expressed as mosaic.

It has been well documented that as in all eukaryotes, in *Drosophila* DNA replication is also initiated at multiple initiation sites (Blumenthal et al. 1974; Lakhotia & Mukherjee 1970). Cytologically, such multiple initiation is manifested as disperse labelling of  $^3\text{H}$ -thymidine on puffs and interbands (Lakhotia

Table 1. Labelling frequencies of moderately late and very late replicating X chromosomal sites of Oregon R<sup>+</sup> male, female and reinverted mosaic male and female (data taken from 2D-1D labelling patterns).

Chromo-somal sites	Oregon R <sup>+</sup> male	Oregon R <sup>+</sup> female	Reinverted mosaic male	Reinverted mosaic fem.
A] Late replicating sites				
1A	85	100	100	100
3C	90	100	100	100
11A	100	100	100	100
12EF	100	100	100	100
14A	70	95	55	95
15DEF	40	80	40	90
19EF	35	90	90	100
20AB	45	95	75	100
B] Moderate replicating sites				
3DE	15	50	35	45
4DEF	30	100	60	100
6DEF	50	85	75	65
7ABC	60	95	10	50
8ABC	85	75	80	100
9A	30	95	15	85
9C	10	90	30	85
12A	85	100	45	85
13A	40	95	90	95
13DEF	60	70	35	70
18A	45	60	35	60

Table 2. Labelling frequencies of relatively early replicating X chromosomal sites of Oregon R<sup>+</sup> male, female, reinverted mosaic male and female (data taken from 2D-1D type labelled patterns).

Chromo-somal sites	Oregon R <sup>+</sup> male	Oregon R <sup>+</sup> female	Reinverted mosaic male	Reinverted mosaic fem.
1DEF	30	60	0	30
2AB	35	35	0	10
3A	15	50	0	45
4A	50	70	0	60
4BC	45	55	0	20
5CD	40	50	0	45
6A	35	70	5	70
7E	0	65	0	50
8E	20	30	10	30
8F	0	20	0	20
9EF	60	80	5	80
12D	20	40	5	40
14DE	25	35	0	45
16A	35	55	0	55
16DE	20	40	0	40
17A	10	20	10	40
17DEF	15	40	10	40
18DE	20	80	0	80
18F	5	60	0	60
19A	35	95	5	95
19D	10	75	0	75

7ABC) are similar to those in the wildtype (Oregon R<sup>+</sup>) female, in the reinverted mosaic male, frequencies of all but the late replicating sites (1A, 3C, 11A and 12EF) are lower than those in the reinverted mosaic female. However, the frequencies for most of the sites are greater than those of the corresponding sites in Oregon R<sup>+</sup> male.

A critical evaluation of the percent frequencies of the labelling presented in Tables 1 and 2, reveal that the different replicating sites or clusters can be classified into three groups. In Table 1 are shown the two classes: (A) those which are late replicating sites and (B) those which include early to moderately late replicating. The sites included in (A) do not show considerable change in labelling frequency in the mutant male and female. The sites included in (B) contain regions which do not show a change in the mutant or show an increased labelling frequency in the male. In contrast, the sites presented in Table 2, which include mostly early replicating regions in normal male, are highly early replicating in the mutant male such that no labelling is virtually observed on the X chromosome of the mutant male, while they do not reveal a considerable change in the X of the mutant female.

Thus, nearly 50% of the replicating sites of the X chromosome of the mutant male shows a relatively more early replicating character as compared to the early replicating property of the Oregon R<sup>+</sup> male.

**References:** Blumenthal, A.B., H.J. Kriegstein & D.S. Hogness 1974, Cold Spr. Harb. Symp. Quant. Biol. 38:205-223; Chatterjee, S.N. & A.S. Mukherjee 1975, Ind. J. Exp. Biol. 13:452-459; \_\_\_\_\_ 1978, Ind. J. Exp. Biol. 16:1027-1031; Haegeler, K. & W.E. Kalisch 1974, Chromosoma 47:403-413; Lakhotia, S.C. & A.S. Mukherjee 1970, J. Cell Biol. 47:18-33; Mukherjee, A.S. 1982, Curr. Sci. 51(5):205-212.

