Ghosh, M., S. Banerjee and A.S. Mukherjee.
University of Calcutta, India. Activity of the X chromosome of the reinverted mosaic mutant larvae of **D.melanogaster** in in vitro culture.

Ashburner (1972, 1973) and Ashburner et al. (1974) have shown puffing activity in polytene chromosomes of Drosophila larval salivary gland grown in vitro synthetic culture medium with ecdysone. Majumdar & Mukherjee (1980) have reported induced puffing activity and replication of polytene chromosome in gland grown in modified Schneider's medium.

We have examined the puffing activity of the X chromosomes in male and female larval salivary gland of the mutant strain, $In(1)BM^2fB^{15}$, reinverted mosaic of **Drosophila melanogaster**, grown for 24-48 hr in Schneider's medium without yeast hydrolysate. In this mutant strain the X chromosome in in vivo, is extraordinarily hyperactive in male, and in extreme condition highly flabby and stumpy (Ghosh et al. 1982). In squash preparations of salivary glands of this strain, the X chromosome of the male appears in three morphological expressions, viz. (a) extremely wide, stumpy and puffy (flabby); (b) intermittently puffy

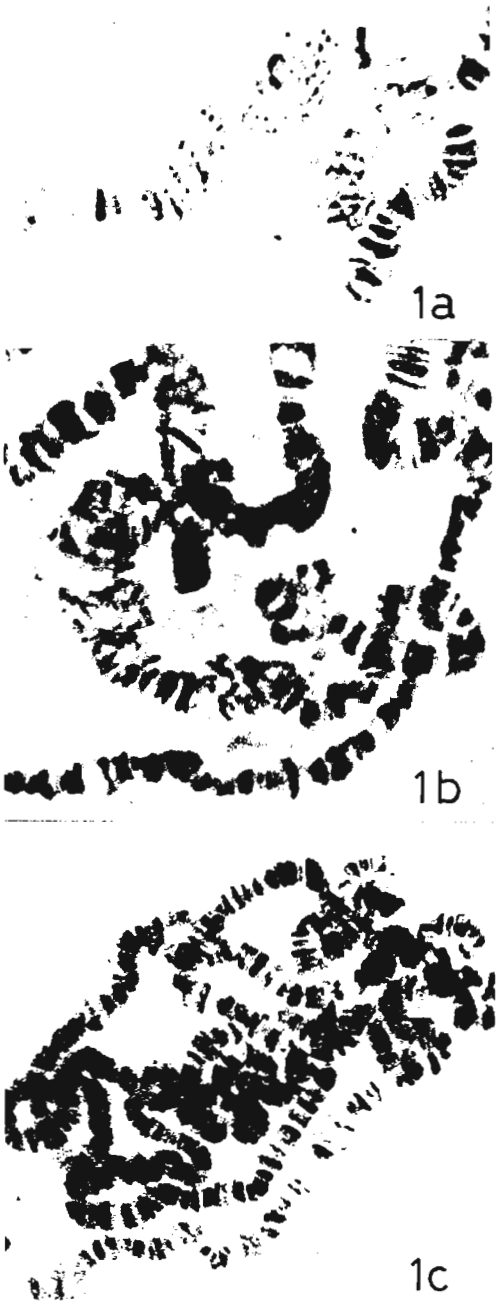

Figure 1. (a) Flabby X chromosome, (b) Intermediate X chromosome and (c) Near normal X chromosome of the mutant male $In(1)BM^2$ fB^{15} (rv, mosaic) grown in vivo.

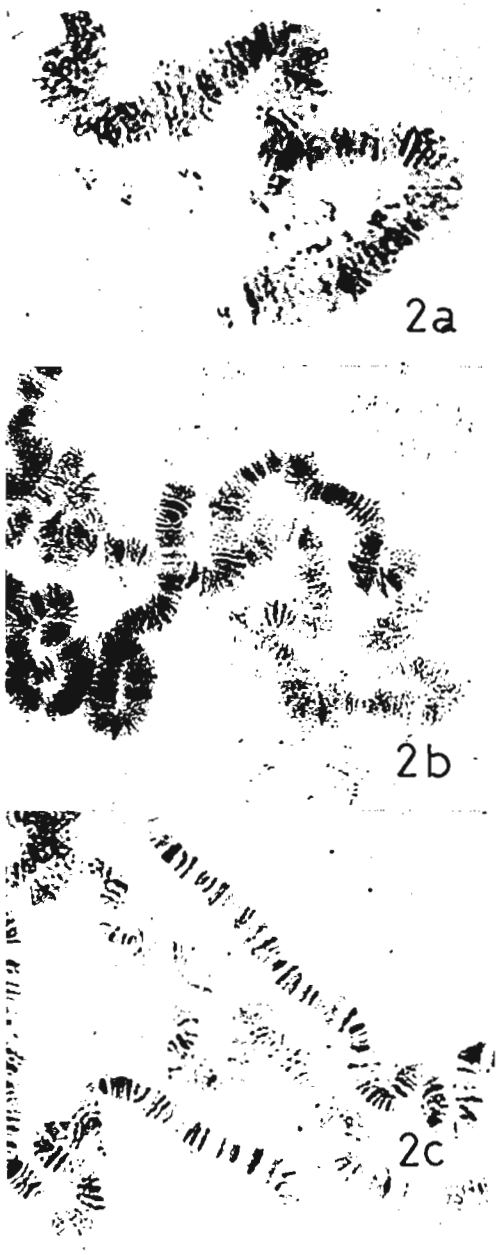

Figure 2. (a) Flabby X chromosome, (b) Intermediate X chromosome and (c) Near normal X chromosome of the male mutant larval glands grown in synthetic culture medium for 24 hr.

Table 1. Frequencies of different classes of X chromosomal activity in In(1)BM^{2}fB^{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

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.

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°±1°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°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 hyperactivty 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. Metzer & 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.

Ghosh, M. and A.S. Mukherjee. University of Calcutta, India. DNA replication in the X chromosome of In(1)BM^2 (rv,mosaic) of **Drosophila melanogaster.**

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}H-thymidine on puffs and interbands (Lakhotia & Mukherjee 1970; Chatterjee & Mukherjee 1975; Haegele & 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--reinverted 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}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