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Letter to the Edtior

Is the X-chromosome early replicating in mitotic cells of *Drosophila* males?

In recently published paper "Induction and characterization of premature chromosome condensation in *Drosophila* synkaryons and implications to dosage compensation" in this journal (Vol. 31, March 1993, pp. 210-214), A. Kar and A.S. Mukherjee have reported that the single X-chromosome in males was allocyclic. This was interpreted to suggest that as already known for polytene cells, the single X-chromosome in non-polytene cells of *Drosophila* males was also early replicating (in relation to dosage compensation of X-chromosome activity in males and females). This is an interesting approach which, if successfully accomplished, is expected to provide very useful results.

In my view, however, the experimental plan used by the authors and the data presented in the paper have serious flaws which do not justify the above conclusions. The following points are to be considered in this context:

1. The authors used embryonic cells of *Drosophila* as the mitotic cells for fusion with adult haemocytes (interphase cells). The embryonic cells were treated with colchicine (5 µg/ml) for 4-5 hr in Ringer's solution. No data are provided for the frequency of metaphase-arrested cells in this population of colchicine treated embryonic cells [incidentally, the reference to the method for preparation of embryonic cells cited in the paper (their ref. no. 9, Kambysellis MP, *D.I.S.*, 60 (1985) 219), is not at all related to preparation of embryonic cells]. To induce PCC, one requires the population of mitotic cells to have a very high frequency (> 50%, preferably much higher) of cells in metaphase. One is not sure if the authors checked their samples of embryonic cells for incidence of metaphase-arrested cells. Moreover, the high concentration of colchicine given for 4-5 hr may induce a high incidence of polyploidy with extremely condensed chromosomes. Thus the example shown in Fig. 1b of the paper could as well be a polyploid cell with greatly condensed chromosomes. Since the other (interphase) cells were also derived from same species of *Drosophila*, how could the authors distinguish between fused and polyploid cells? Another question with this part of the methodology concerns the extent of cell proliferation when embryonic cells were incubated for 4-5 hr in Ringer's rather than in a complete culture medium — it is doubtful if the embryonic cells would divide actively when incubated in simple salt solutions. Furthermore, no data were provided on the numbers and concentration of the two cell types used for hybridization and the frequency of hybrid cells. These information are essential for the repetition of this methodology.

2. The paper did not clearly state which cell type (the embryonic mitotic or the adult haemocyte) was labelled with ^{3}H-thymidine. Labelling of embryonic cells could not be used to identify the cell cycle stages of fusing interphase cells. On the other hand, if the haemocytes were labelled with ^{3}H-thymidine, one would have liked to see if these cells really got labelled? This question arises in context of the well established notion that no cells outside gonads are replicating in adult flies—if this indeed was true for haemocytes from adult flies as well, there was no point in using ^{3}H-thymidine to label the haemocytes of adult flies! That the haemocytes do not proliferate actively in adult flies is also suggested by their very low numbers in adult haemolymph[1].

3. The illustrations in Fig. 1 of the paper are not at all convincing: as per the metods described, these illustrations were expected to be autoradiograms of Feulgen-stained cells showing PCC. However, no autoradiographic labelling was discernible in any of the figures. Was it because the haemocytes could not be labelled with ^{3}H-thymidine as pointed out above? Identification of the allocyclic X in S-G_2 PCC in their figures 1c and 1d is very dubious—it appears almost impossible to ascertain which of the darker images in the figures are chromosomes, leave aside the question of specifically identifying one of these as an X- or Y-chromosome! Even if one agrees that the identified element was an X or a Y-chromosome, how would one know that this did not belong to the embryonic mitotic cell? To be able to unambiguously identify specific chromosomes, one would not only need better PCs and photomicrographs but more importantly, fusion between cells with distinct karyotypes. The present illustrations leaves one wondering if any of the examples in their Fig. 1 showed a PCC at all!

4. If the haemocytes from adult flies were not cyclic mitotically, as appears to be the case, their fusion with mitotic cells cannot provide any information on the time of replication of a chromosome simply because the chromosomes were no more replicating in these cells.

5. In their enthusiasm to find a parallel with the known early replication of X-chromosome in polytene nuclei in salivary glands of *Drosophila* larvae, the authors have ignored certain earlier observations (especially using ^{3}H-thymidine or 5-bromodeoxyuridine labelling of mitotic cells in brain ganglia of *Drosophila* larvae) which actually indicated that the X-chromosome in mitotic cells of male *Drosophila* was not early replicating. It has been argued earlier that the early replication of the X-chromosome in male polytene cells is a special effect of the polytene chromosome structure[2,3].

1 Rizki T M, in *The genetics and biology of Drosophila*, Vol. 2b, edited by M Ashburner and T R M Wright (Academic Press, London, New York, San Francisco), 1978, 397.
2 Lakhotia S C and Sinha P, *Chromosoma*, 88 (1983) 265.
3 Raman R and Lakhotia S C, in *Trends in chromosome research*, edited by T Sharma (Narosa Publ. House, New Delhi, Springer-Verlag, Berlin, Heidelberg), 1990, 69.

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Author's Explanation

I have carefully read the comments made by Prof. S.C. Lakhotia on our paper entitled: Induction and characterization of premature chromosome condensation in *Drosophila* synkaryons and implications to dosage compensation .

The manuscript was reviewed by one of the well known *Drosophila* geneticist in India, and also before that by another reputed geneticist abroad. I therefore, do not accept the comments made by Prof. Lakhotia, which I feel are highly biased.

Prof. Lakhotia has failed to understand the technical details:

1 Total cell samples from larvae and adult were used (without colchicine) as the source of interphase cells, and for the mitotic cells, the samples were treated with colchicine to get the metaphase-arrested cells samples. Thus, there is no relevance to the induction of polyploidy, as even if the mitotic cells were polyploid, this would not affect the different types of PCC induction, rather that would be advantageous.

2. The mammalian data on the required concentration of mitotic cells is not applicable to *Drosophila* systems (Wyss et al., 1973). Furthermore, the proportion of mitotic to interphase cells used in fusion, was 3:1, implying that there were enough mitotic cells given.

It has been correctly pointed out by Prof. Lakhotia that prolonged incubation in colchicine results in condensed metaphase plates. Such condensed metaphase chromosomes are apparent in numerous figures of PCC's published by other authors (Rao and Johnson, 1972, 1974; Rao, 1976).

3 The replicative phases were indeed available from the haemocytes although at a low rate, but we had also used non-metaphase arrested embryonic cells.

4. The doubt raised by Prof. Lakhotia on "identification of allocyclic X in S-G2 PCC (Fig. 1c-1d) is very dubious"—raises the question about his predisposed negative attitude.

As regards his comment on "whether any of the examples showed a PCC at all", I think he is unaware of the morphology of PCC's of insects cells and I refer him to the following articles; Rao and Johnson, 1972: PCC in mosquito cells and Halfer and Vallenzasca, 1987: for PCC's induced in *Drosophila* rat heterokaryons.

5. However, I admit that the photographic reproduction of the ^{3}H-Thymidine labelling is poor. This is always the case when the prints are made as photo-offset or xeroxing.

Furthermore, we regret the wrong reference given for the method of preparations of embryonic cells, which was over looked during proof reading. The reference should read 7 (Kar and Mukherjee 1987) and not 9 as printed.

6. The identification of X or Y in plate is clear enough to support the allocyclic nature, whether it is labelled or not.

7. Lastly, Prof. Lakhotia's comments appear to me highly biased as he has not given any relevant reference except his own, that is a review, not gone through any reviewing by a referee.

I, therefore, stand to my conclusion, and if Prof. Lakhotia wishes to disprove it otherwise I suggest him to perform the experiment by himself or better in collaboration with some one who has the expertise in cell hybridization, and I wish him good luck.

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