Letter to the Editor

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 synchrony and implications for dosage compensation" in this journal (Vol. 31, March 1993, pp. 210-214), A. Kari and A. S. Mulhern have reported that the single X-chromosome in males is allelic.

This was interpreted to suggest that an already known for polytene cells: the single X-chromosome in non-polytene cells of Drosophila males was also very easily 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 mg/ml) for 4.5 hr in Kinger's solution. No data are provided for the frequency of metaphase-arrested cells in this population of colchicine treated embryonic cells. The reference to the method for preparation of embryonic cells cited in the paper (their ref. no. 9, Sundberg 1971; D. S., 60 (1965) 219), is not at all related to preparation of embryonic cells. To date, PCC, one requires the population of mitotic cells to have a very high frequency (>= 90%, preferably much higher) of cells in metaphase as an absolute. 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 induc a higher incidence of polytene with extremely condensed chromosomes. Thus the example shown in Fig. 3 of the paper could as well be a polytene cell with greatly condensed chromosomes. Since the metaphase (interphase) cells were also derived from same species of Drosophila, how could the authors distinguish between treated 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 Kinger'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 cell type 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 labeled with [H]-thymidine. Labeling of embryonic cells couldn't be used to identify the cell cycle stages of fusion interphase cells. On the other hand, if the haemocytes were labeled with [H]-thymidine, one would have liked to see if these cells really got labeled? 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 [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 haemocytomem.

3. The illustrations in Fig. 3 of the paper are not at all convincing: as per the methods 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 a haemocyte haemocyte not labelled? [H]-thymidine as pointed out above? Identification of the alloplasm in S-G2 PCC in their figures 1c and 1d is very dubious - it appears almost impossible to ascertain what of the darker images is actually a chromosomes. 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 unambiguously identify specific chromosomes, one would not only need better PCC 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. 3 showed a PCC at all.
4. If the hermaphroditic 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 longer replicating in these cells.

5. In their enthusiasm to find a parallel with the known early replication of X-chromosome in polytenic nuclei in salivary glands of Drosophila larvae, the authors have ignored earlier similar observations (the expectation of premature chromosome segregation) labeling 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 polytenic cells is a special effect of the polynucle chromosome structure.1,2


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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 segregation in Drosophila: symptoms 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. All small samples from larvae and adult were used without colchicine as the source of larval phase 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, it 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 (Weyes et al., 1973). Furthermore, the proportion of mitotic to interphase cells used in fusion, was 3:1, implying that these were enough mitotic cells given.

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

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

4. The doubt raised by Prof. Lakhotia on "whether any of the examples showed a PCC at all", I think is unaware of the morphology of PCC's of insects cells and I refer him to the following articles: Raa and Johnson, 1972; PCC in mosquito cells and Haller and Vallenzenko, 1987; for PCC's induced in Drosophila, rat hepatocytes.

5. However, I submit that the photographic reproduction of the "H-Thymidine labelling" phase. This is always the case when the print is made as photo-offset or xerography.

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

6. The identification of X or Y in plate is clear enough to support the allochlocic 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, in a review, not gone through any reviewing by a referee.

I, therefore, stand by my conclusion, and if Prof. Lakhotia wishes to dispute 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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