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Preparation Of Mitotic Chromosome In Insect Brain Cells (Larval Brain): Drosophila

Pyush Thapak, Shalu Jain and Ramkumar Lodhi

School of Studies in Zoology, Jiwaji University, Gwalior-474011

Abstract: Mitotic chromosome cytology plays an essential role in many areas of *Drosophila* research. It is routinely needed for characterization of the mitotic phenotypes elicited by mutations affecting chromosome structure and/or behavior. Transfer actively crawling third instar larvae into drops of saline (0.7% NaCl in distilled water) placed on a siliconized slide. Make drops of about 50 and place 1-3 larvae in each of them. A major objective of the present study was to analysis cell cycle in the sub-population of cells that divides mitotically in larval brain ganglia by labeling the S-phase cells and chasing the label in individual chromosomes at metaphase stage. Our data on the labeling of metaphases in brain ganglia of Drosophila larvae present unusual and intriguing features which defy simple. Compared to our failure to define the mitotic cell cycle parameters in brain ganglia of D. melanogaster, Steinmann presented a computer-simulated typical labeled mitoses curve for brain ganglia of D. virilis larvae.

Keywords- Mitotic chromosome, metaphases, Drosophila, Gwalior.

I. INTRODUCTION

Mitotic chromosome condensation occurs as prelude to sister chromatid segregation that initiates at the onset of anaphase. The shortening of the chromosome length allows separated sister chroatids to be transported effectively by the mitotic spindle to opposite poles. Chromosomes must be significantly shorter than this distance, or they would extend into the middle of the dividing cell and be cut during cytokinesis (Swedlow and Hirano, 2003)

Two kinds of replication cycles, viz., endoreplication and mitotic, occur in different cell types of Drosophila larvae. Those at least four different cell types present in larval brain ganglia, only the neuroblasts and ganglion mother cells divide mitotically. Many cell in larval brain ganglia endoreplication with independent unequal replication of the heterochromatic (H) and euchromatic (E) regions. Although the dividing cells in larval brain are often used for a variety of studies involving metaphase chromosomes, comprehensive information on cell cycle in mitotically active brain cells (Lakhotia et al., 1995). GMCs divide symmetrically to produce pre-gangliaon and ganglion cells which differentiate into neurons. The

spatial distribution of dividing neuroblasts and GMCs follows a specific pattern during larval period Turman and Bate, 1988)

The well-characterized cell lineages, in combination with our tractable system to induce mis-segregation of chromosomes, offer a unique opportunity to trace the fate of aneuploid cells in real time and analyze their effect on the nervous system development. Through larval development, approximately 100 large neural stem cells called Neuroblasts (Mirkovi et al., 2019). Additionally, recent studies also indicate that the cellular response to aneuploidy is not uniform among different tissues (Knouse et al., 2017).

Some DNA- feulgency to photometric studies suggested the larval brain mitotic cells to be multistranded or polynomial while others questioned this claim. Although the debate concerning polynomial vsanaemic model of metaphase chromosomes in general is resolved in favor of a uninemic organization, some conflicting evidence regarding unnamed or polygene structure of the mitotic cells in brain ganglia of Drosophila larvae persist, especially because of the occurrence of endoreplication cycles in brain ganglia (Abad et al., 1992).

Jan and Boyes (1970) reported the presence of very large sized metaphase chromosomes in some mitotic cells in brain ganglia of Musca domestica larvae. The brain ganglia of late third instar l(2)gl 4 mutant larvae show high mitoic index and highly extended metaphase chromosomes (Radhakrishnan and Sinha 1987). A recent study observed the generation of embryonic optic neuroblasts (EONs) from the neuroepithelium overlying the developing embryonic brain (Hakes 2018). Surface glia in the developing Drosophila brain play essential roles in regulating the proliferation of neural stem cells, neuroblasts (NBs) In view of these we undertook a detailed and systematic analysis of cell cycle and DNA content in mitotically active cells in larval brain ganglia of D. melanogaster.

II. MATERIALS AND METHODS

Wild type stocks of D. melanogaster l (2) gl4or/SM5 stock of D. melanogaster were reared at 24± 1°C under standard laboratory conditions. The brain ganglia of late third instar l (2) gl4 larvae show tumorous growth, high mitotic index and highly extended metaphase chromosomes (Roy et al., 1995). Eggs from flies of each stock were collected at hourly intervals, and the larvae were grown in uncrowded dishes on yeast supplemented food at 24 ± 1°C. The l (2) gl4 homozygous larvae were differentiated from the l(2)gl4 or/SM5 heterozygotes on the basis of a prolonged larval life, sluggish movements and bloated appearance of the former. Brain ganglia from late third instar larvae of *D. melanogaster* (wild type) 10 days old homozygous larvae were aseptically excised in different cavity blocks containing. Mitotic chromosome preparations can be obtained from embryonic cells and gonial cells of both sexes, the tissue that provides the best mitotic figures is the larval brain. This tissue contains two major types of dividing cells: the neuroblasts and the ganglion mother cells (Hofbauer and Campos-Ortega, 1990). The neuroblasts divide either symmetrically, producing two neuroblast stem cells, or asymmetrically, producing another neuroblast and a smaller cell called the ganglion mother cell. The ganglion mother cell divides only once producing two daughter cells that differentiate into neurons. Several squashing techniques have been developed for preparation of larval brain mitotic chromosomes. These procedures are minor modifications of a basic technique developed 25 years ago (Gatti et al., 1974) and can be successfully used for preparing mitotic

chromosomes of various *Drosophila* and mosquito species. To characterize various aspects of mitotic chromosome morphology and behaviour, larval brains can be squashed either in aceto-orcein to obtain orcein-stained chromosomes, or in 45% acetic acid to obtain unstained preparations. Unstained material can be then stained with Giemsa to obtain permanent preparations, processed with a variety of banding techniques, or used for *in situ* hybridization (Bonaccorsi et al., 2000).

III. RESULT

In neural ganglia preparation, metaphase plates showed eight chromosomes including a pair of sex chromosomes (xx in female and xy in male), a pair of small 'dot' chromosomes and acrocentric with the proximal half being heterochromatic. The Y-chromosome, of similar size as the X, was sub-metacentric and in normal giemsa stained preparations, the tip of both arms of the Y-chromosome appeared lightly stained compared to the middle region which was typically heterochromatic. The diploid karyotype (2N = 8) of D. melanogaster (wild type and the l(2)gl4 mutant) consists of a pair of sex chromosome (XX/XY, X being large acrocentric and Y being large submetacentric), two pairs of large metacentrics (chromosome 2 and 3) and a pair of dot like 4th chromosomes. The karyotype of D. nasuta (2N = 8) consists of a pair of sex chromosomes (XX/XY), a pair of large metacentrics (chromosome 2), a pair of large acrocentrics (chromosome 3) and a pair of dot like 4th chromosome; the X-chromosome in this stock is either a large acrocentric or submetacentric type. In females, the two types of Xs can be present in homo- or in heterozygous condition.

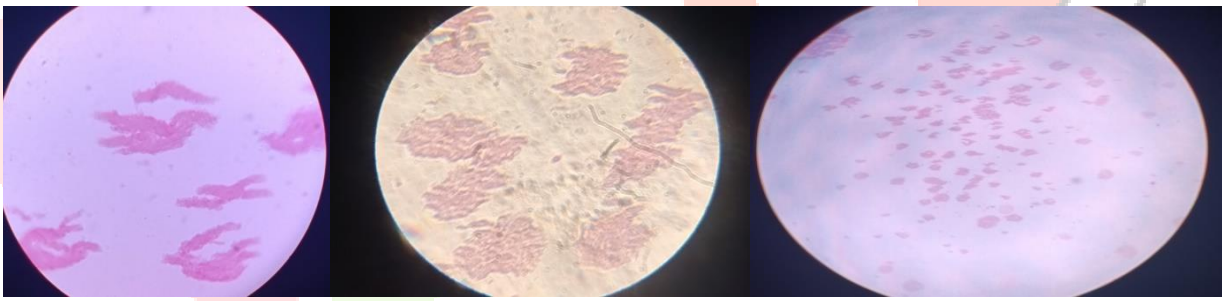

Fig. 1: Chromosomes in simple microscopes

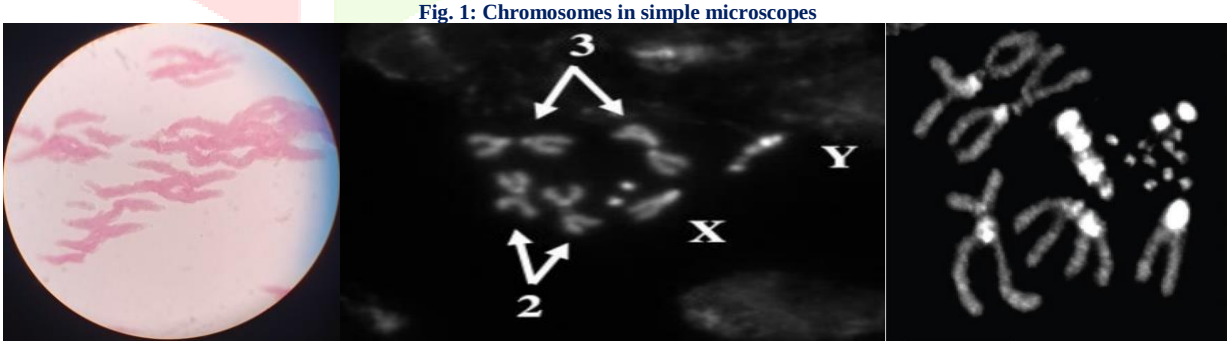

Fig. 2: Four pair of chromosomes in Drosophila

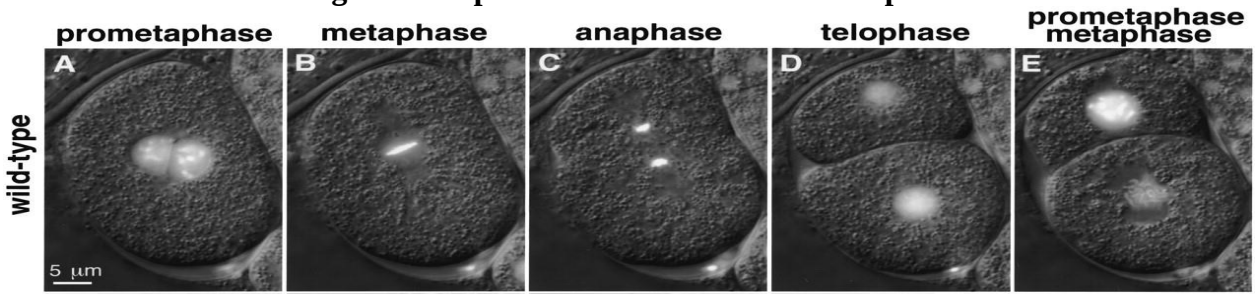

Fig. 3: Mitotic chromosome spindle formation divisional phase

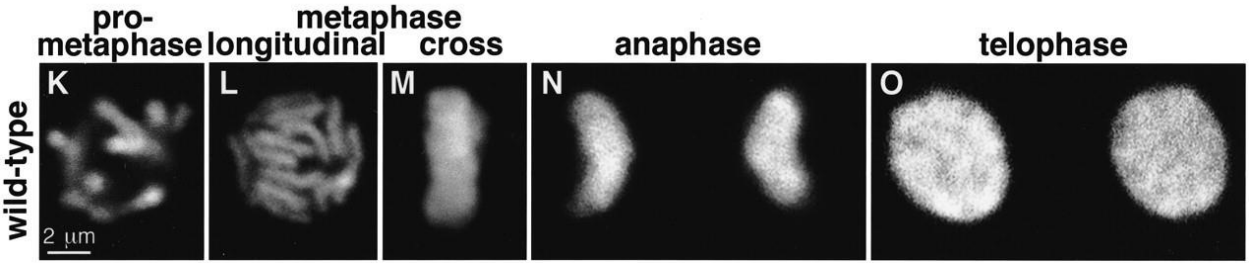

Fig. 4: Enlargement mitotic chromosome spindle formation

IV. Conclusion

The brain ganglia in Drosophila larvae comprise of cells with varying replication programmes. Besides the cells that do not engage in DNA synthesis, there are those that follow endoreplication cycles and those that traverse through mitosis. A major objective of the present study was to analyse cell cycle in the sub-population of cells that divides mitotically in larval brain ganglia by labeling the S-phase cells and chasing the label in individual chromosomes at metaphase stage.

V. References

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