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AN ULTRASTRUCTURAL STUDY ON THE OVARIOLE, DEVELOPMENT IN THE ORIENTAL FRUIT FLY[1]

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Wen-Yung Lee, C. K. Tseung and T. H. Chang (1985) An ultrastructural study on the ovariole development in the oriental fruit fly. *Bull. Inst. Zool., Academia Sinica* **24**(1): 1-10. The ovarioles of the oriental fruit fly, *Dacus dorsalis* belong to the polytrophic type of the ovariole. There are 30 to 48 ovarioles in each ovary. The ovariole consists of two major parts: the germarium and the vitellarium with 2-5 egg chambers. Each chamber contains 16 cystocytes, of which one will be the oocyte and other 15 are the nurse cells, surrounded by the cuboidal follicular cells. The ovariole is circled by the tunica propria and the cellular external sheath.

The germarium consists of two kinds of cells: The dark cell, or the reproductive cells and the light cell, or the mesodermal cells. In the anterior portion of the germarium, a dark cell is the oogonium which divides by mitosis into one oogonium and one cystoblast. The cystoblast can migrate to the posterior part of the germarium and the further imcomplete divisions occur, so that the complex interconnecting cells are produced, named syncytium. The mesodermal cells can also migrate to the areas between the syncytia as the follicular cells consequently. The further incomplete divisions continue until to obtain 16 cells in the chamber. Then, the egg chamber leaves to the vitellarium from the germarium.

In the egg chamber, the oocyte can be recognized from the 15 nurse cells by the appearence of the granules in the bottom cell of the egg chamber in the fourth or the fifth day old flies. While the oocyte well develops to become an egg, the nurse cells degenerate into the remnants and the follicles also degenerate as a membrane-like ribbon. A vitelline membrane is formed around the egg by the condensation of the secretions from the follicular cells and the oocyte. A chorion is also formed outside the vitelline membrane.

By whether the nurse cells are existent or not, the ovariole of an insect can be divided into Panoistic type and Meroistic type. From the difference in the arrangement of nurse cells and oocyte, the meroistic ovariole is divided into Polytrophic type and Telotrophic type (Snodgrass, 1937). The fruit fly possesses polytrophic meroistic type of ovariole.

In the polytrophic ovariole, the nurse cells and the oocyte arise from the division of an oogonium. The oogonium divides into an oogonium and a cystoblast. The cystoblast, through consecutive divisions, becomes a cluster of connected cystocytes, and later form an egg chamber. One cell of the egg chamber develops into an oocyte and the other cells develop into nurse cells (Brown and King, 1964). The number and developmental process on an ovariole varies from species to species (Chapman, 1971). The present paper describes the oogenesis and morphology of an ovariole in

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the oriental fruit fly *Dacus dorsalis* (Diptera: Tephritidae). Observations have been made using the transmission electron microscope.

MATERIALS AND METHODS

The flies reared at Kuan-Hsi Citrics Experimental Station were used in this study. The pupae were brought to Academia Sinica and kept in the incubator under 25°±2°C, RH 70–75%. The flies were collected as soon as they emerged. The photoperiod in the incubator was the same with that of the natural circumstance. The flies were fed with water and a diet (Sugar: Peptone=5:1). Random selection of 10–20 females were dissected each day.

0–10 days old ovaries were prefixed in a mixture of 2.5% Glutaraldehyde (buffered with 0.1 M Cacodylate to pH 7.2) at 4°C for one hour, washed with the same buffer (to which 8% sucrose was added) for half an hour, and postfixed with 1% osmium tetroxide (buffered with 0.1 M Cacodylate to pH 7.2) for one hour. The ovaries were stained with 4% Uranyl acetate for 2 hours. At this moment the solution with specimens was restored to room temperature. Then the specimens were dehydrated in a gradient of alcohol from 50% to 100%, shifted into propylene oxide and infiltrated and embedded in low viscosity Spurr medium (Spurr, 1969). Serial sections 1 μ thick were cut on a LKB ultramicrotome using glass knives. The sections were stained with Methylene blue and Basic Fuchsin and were observed under light microscope (Ann Preece 1978). The target sites were determined and sectioned into slices of 400 Å–800 Å in thickness. The sections were put on single-hole grids with Formvar supporting films (0.4% in 1, 2-Dichloroethane), stained with Lead citrate for 5 minutes (Glauert 1979) and then observed with the transmission electron microscope of Hitachi 11A and Zeiss 109.

RESULTS

1. Morphology of an ovariole and the formation of an egg chamber.

There are two ovaries in an oriental fruit fly. Each ovary is composed of 30–48 ovarioles. An ovariole can be divided into an anterior part of germarium and a posterior part of vitellarium (Fig. 1, Grm and Vtl). The germarium (Grm) attaches to the terminal filament (Tf). The terminal filament is full of spindle-like cells. There are dark cells and light cells in the anterior portion of the germarium. The dark cells, which are oogonia (Oog) and cystoblasts (Cy), possess tight cytoplasm, the light ones called mesodermal cells (Mdc), few of which are in the middle portion of the germarium, possess loose cystoplasm. A stem-line oogonium gives rise to a stem-line oogonium and a cystoblast. The cystoblast gives rise to cystocytes (Cc). The cystocytes develop into a cluster called Syncytium (Sy). In the posterior portion of the germarium (Fig. 1), the syncytium (Sy), the cells of which connected closely, is encircled by the mesodermal cells, forming a pro-egg chamber (PEgCb). These mesodermal cells increase in the number and the size and become follicular cells.

From the ultrastructural observation, the follicular cells do not cease growing until the egg chamber completely develop. Meanwhile the cystocytes in the pro-egg chamber undergo their cell divisions. In the vitellarium (Vtl) of the ovariole, there are the outer well-arrayed follicular cells (Fc) and the inner 16 cystocytes (Cc). In each egg chamber (Egcb), of the 16 cystocytes, one will become an oocyte and the others will be nurse cells.

The ovariole is surrounded by two layers, an inner tunica propria (Tp) and an outer external sheath (ExS). The tunica propria is a membrane, but the external sheath is a cellular layer. Observed from over the magnification of 3000× under transmission electron microscope, many cells are found in the external sheath.

2. The formation of an egg

As soon as a fly emerged, its ovaries are sac-like cone-shaped. The ovarioles are full of dark cells and light cells. On the second day after emerged, the dark cells and the

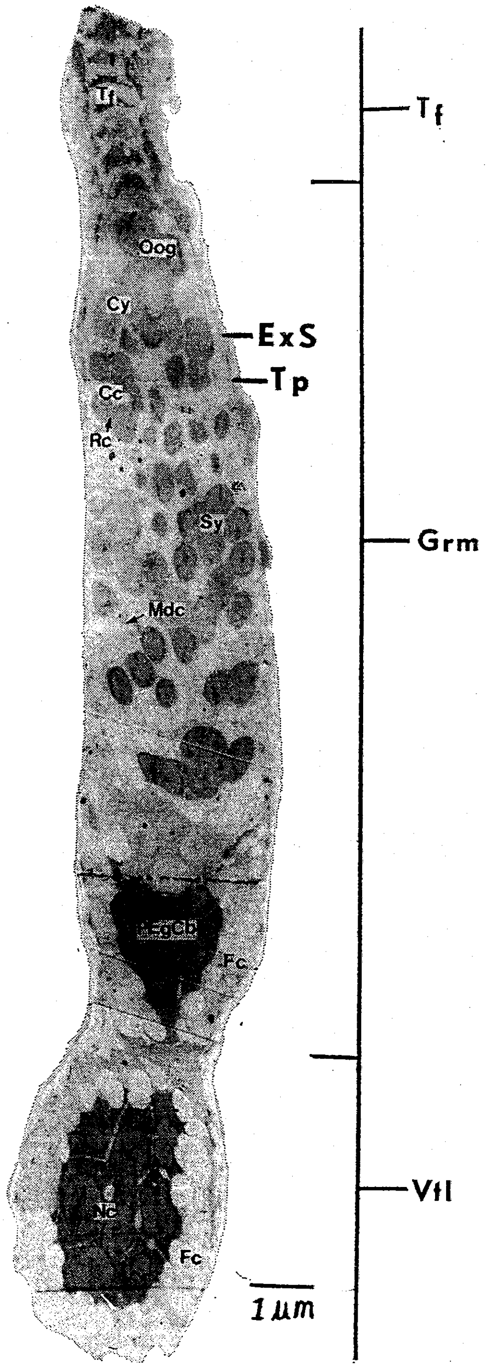


Fig. 1. Transmission electron microscopic observation of the ovariole in *Dacus dorsalis.* The upper part is terminal filament (Tf), the middle is germarium (Grm) and the lower is vitellarium (VtL). In germarium the dark cells are oogonia (Oog), cystoblasts (C) and cystocytes (Cc). Cystocytes inter-connect by ring canal (Rc) to form a syncytium (Sy). The light cells are measodermal cells. Later, they become cuboidal follicular cell (Fc) surrounding the pro-egg chamber (PEgCb). The ovariole is encircled by tunica propria (Tp) and a cellular external sheath (ExS).

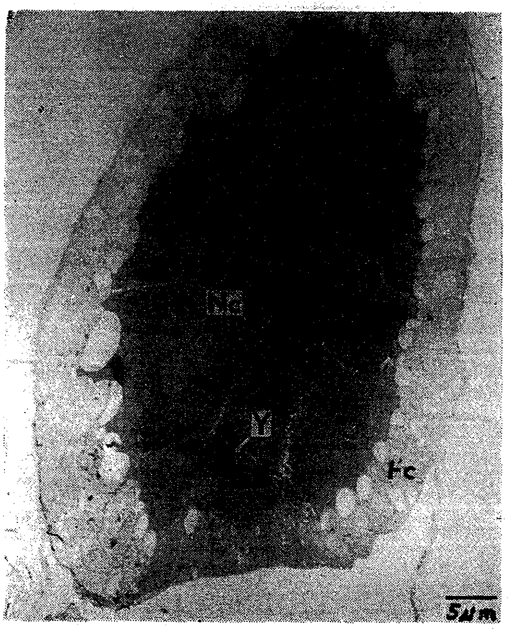


Fig. 2. An egg chamber of a four-days old adult, oocyte with yolk particles (Y) is located at the posterior egg chamber. Nc:nurse cells, Fc:Follicular cells.

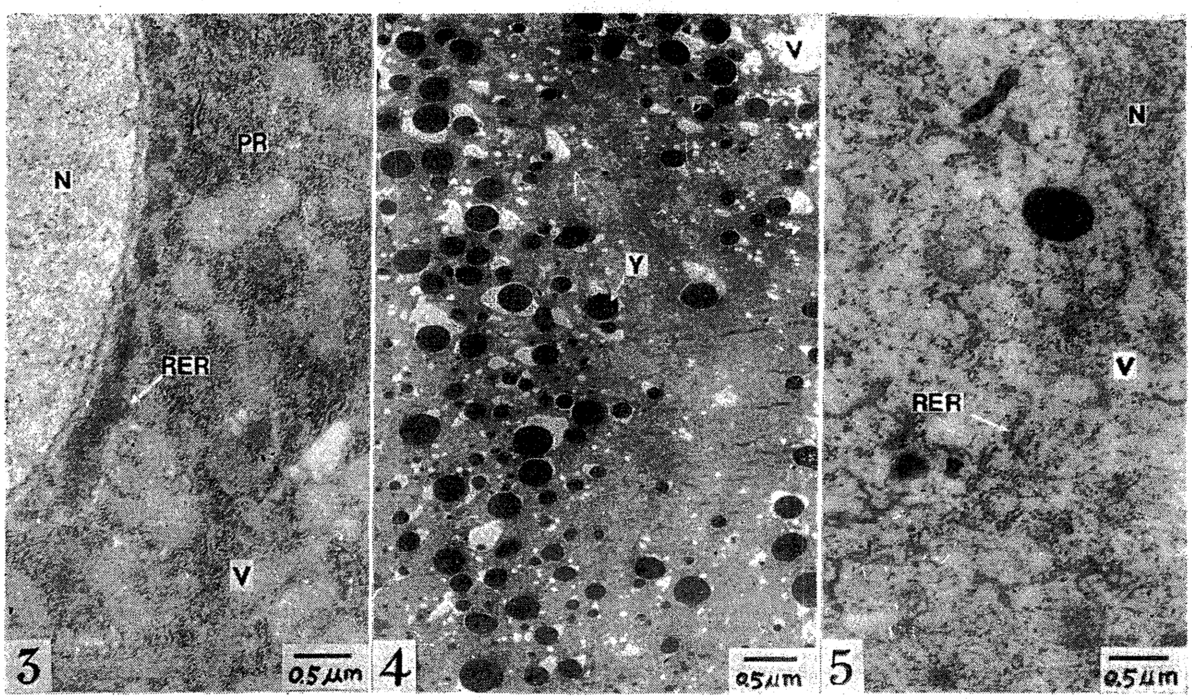
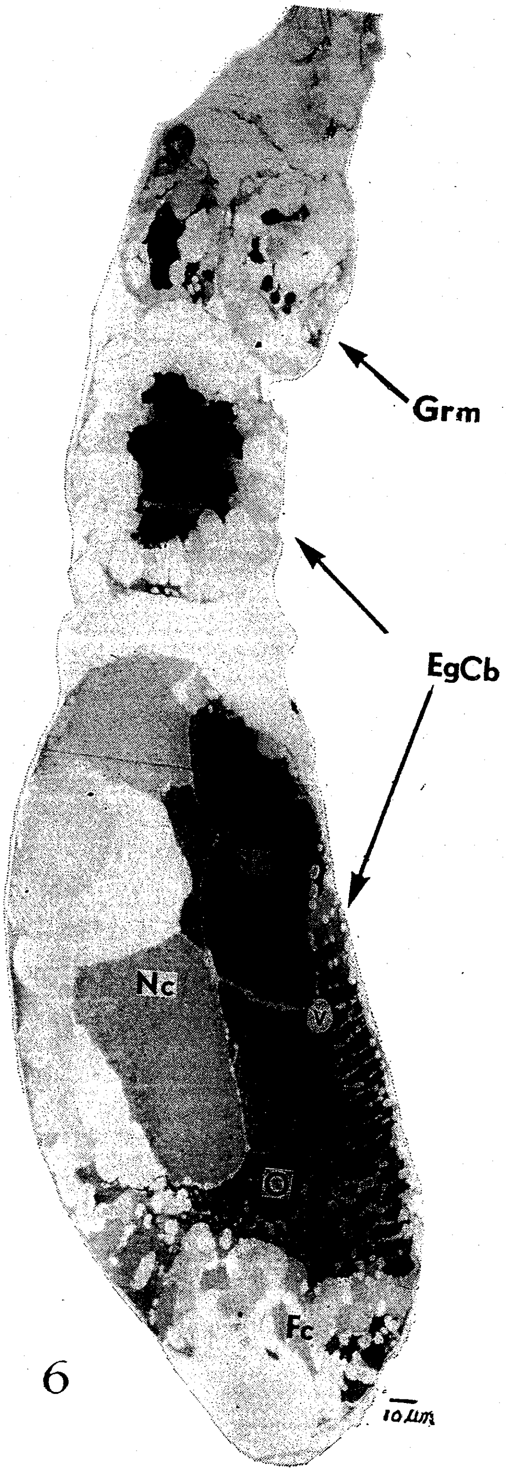


Fig. 3. Magnified micrograph of a part of nurse cell, cytoplasm is full of rough endoplasmic reticula (RER), vacuoles (V) and polyribosomes (PR). N:nucleus.

Fig. 4. Magnified micrograph of a part of oocyte, cytoplasm is full of yolk particles (Y) vacuoles (V) and inconspicuous endoplasmic reticula.

Fig. 5. Magnified follicular cells, cytoplasm is full of rough endoplasmic reticula (RER). N:nucleus, Ed:electric dense.



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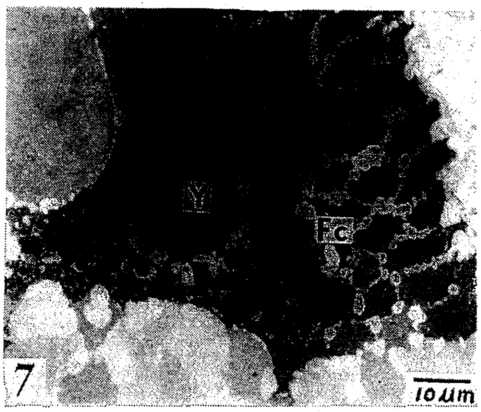

Fig. 6. An ovariole of a six-days old adult, oocyte (O) of the first egg chamber develops, and the surrounding follicular cells (Fc) become wide and flat. Nc: nurse cell, V: vacuole, Grm: germarium, EgCb: egg chamber.

Fig. 7. A magnified part of the figure 6 shows yolk particles (Y), follicular cells (FC) and vacuoles (V).

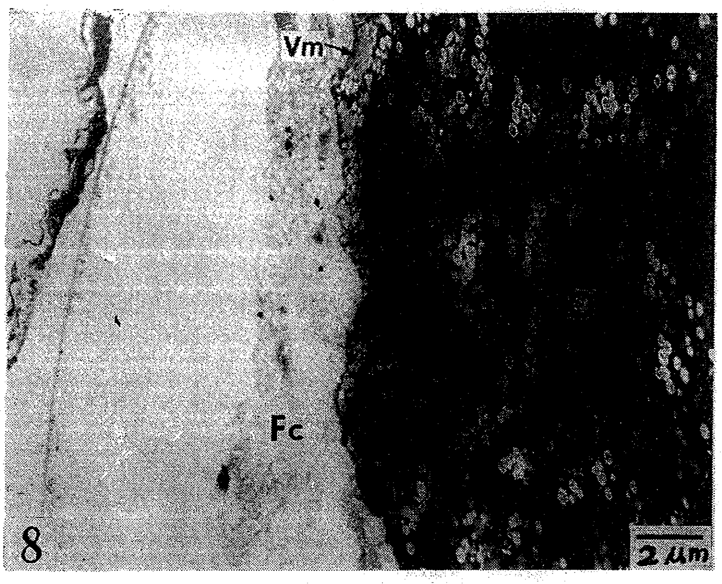

Fig. 8. Eight days after emergence, oocyte (O) fully develops. Cytoplasm is occupied with yolk particle and vacuoles. The secretions of oocyte and follicular cells (Fc) condense as a vitelline membrane (Vm).

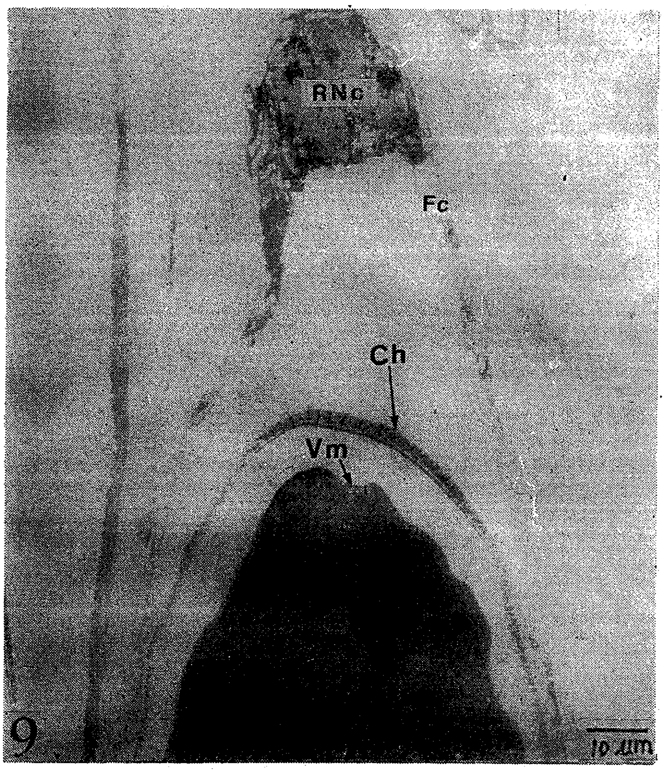

Fig. 9. At the end of egg formation, nurse cells have diminished into remnants (RNC). Follicular cells (Fc) reduce into a belt-like membrane. Chorion (Ch) is formed. Vitelline membrane (Vm) is on the edge of the cytoplasm of oocyte.

light cells increase by cell divisions and migrate posteriorly. The pro-egg chambers can be found in some ovarioles. In the 3 days-old flies, the spherical egg chamber has completely developed and left the germarium for the vitellarium. Yet hereto the oocyte can not be distinguished from the nurse cells. There may have been two egg chambers in the growing state. In the 4 days-old flies, yolk particles (Y) can be found in the basal part of the second egg chamber (Fig. 2). Hence the oocyte can be identified. With the magnification of 32,500× in micrographs, there can be seen a lot of rough endoplasmic reticula (RER) and vesicles (V) both in the nurse cells (Fig. 3) and in the follicular cells (Fig. 5). However the cytoplasm in the nurse cell is denser with abundant ribosomal granules than that in the follicular cells. There are a lot of small and large yolk particles (v) in the oocyte (Fig. 4), where the cytoplasm is dense but the rough endoplasmic reticula are few. Six days after the emergence, the egg chamber becomes elongate (Fig. 6), the oocyte (o) occupies more space of the egg chamber, and the follicular cells become wide and flat (Fig. 7, Fc). The yolk particles increase in numbers and size (Fig. 7, Y). The oocyte has been growing and the nurse cells diminish gradually. In the 7 days-old flies (Fig. 8), the oocytes (o) are almost mature and full of dense yolks and vesicles. The follicular cells (Fc) almost lose their shape. A vitelline membrane (Vm) is formed in the intercellular space between the follicular cells (Fc) and the oocyte (O). From the micrograph of the Figure 8, the membrane (Vm) is condensed from the materials contributed by the follicular cells and the oocyte. 9–10 days after the emergence (Fig. 9), all nurse cells reduce into remnants (RNC). The follicular cells diminish into a belt-like structure (Fc). The oocyte develops completely to become an egg, surrounded by a separate membrane of chorion (Ch) about 2–5 μ in thickness. The mature egg departs from diminished nurse cells, shifts into the oviduct and is laid.

DISCUSSION

Ovarioles of all insects in Dermaptera and the lice (Anoplura) throughout the holometabolous orders except the insects of Siphonaptera (Mead-Briggs 1964) belong to polytrophic type (Chapman 1971). The germarium of the *Drosophila* is full of oogonia in the distal end where there are about 50 oogonia (Chapman 1971). In *Drosophila melanogaster*, Koch and King (1966) stated that there is a stem-line oogonium. The stem-line oogonium divides mitotically into two cells. One is an oogonium and the other is a cystoblast. The cystoblast gives rise to cystocytes. The stem-line oogonium in the anterior portion is stationary, yet the cystoblast migrates downwards in the consecutive divisions. The cystoblast cannot be distinguished from the oogonium by morphological features, but the migration occurs only in cystoblast. The cystoblast undergoes four imcompleted division and becomes 16 connected cells. This cluster of the associated cells is called syncytium. The time of division in cystoblast varies from species to species, three in mosquito (Roth & Porter, 1964) four in muscoid and four or more in Hymenoptera (Reviewed from Brown and King, 1964). A mutant of *Drosophila* divides once more than the normal. Therefore, there are 32 cystocytes in the egg chamber of the mutant (Gill, 1963).

The complete formation of the egg chamber in *Drosophila* takes place in 48 hours after the pupation (King *et al*, 1968); the egg formation occurs in 3 days after the emergence in the oriental fruit flies. Each egg chamber contains one oocyte and several nurse cells. The numbet of nurse cells varies from different species. The nurse cells in *Drosophila* (Brown and King, 1964) and *Dytiscus* (Reviewed from Chapman, 1971) are 15, *Aedes* and *Melophagus* (Diptera) (Saunders, 1964) and the *Hyalophora* moth (King &Aggarwal 1965) are 7, *Apis* and *Bombus* (Hymenoptera) 48, and *Carabus* (Coleoptera) (Review from Banhag, 1958) 127. In the Earwig *Anisolabis maritima* (Dermaptera) the

8 cystocytes generate of 4 oocyte-nurse cell complex, which consists of an oocyte and a nurse cell (Yamauchi & Yoshitake, 1982). As *Drosophila* does, the cystoblast of the oriental fruit fly undergoes 4 incomplete divisions and becomes 16 cells: one oocyte and 15 nurse cells.

Brown and King (1964), and Koch and King (1966) report the formation of an egg chamber in *D. melanogaster* includes three steps: (1) 4 divisions of cystoblast in the anterior portion of the germarium giving rise to clusters of syncytia with 16 cells. (2) The mesodermal cells extend and grow between syncytia. (3) the mesodermal cells encircle the syncytium and hence complete the formation of an egg chamber, the mesodermal cells themselves become spindle-like (Profollicular cells). So the egg chamber leaves the germarium, the profollicular cells become well-arrayed follicular cells.

The process of egg formation in the oriental fruit fly is not quite the same with that in the *Drosophila*. The cystoblasts migrate posteriorly and undergo divisions, the mesodermal cells enlarge and encircle the syncytium to form a pro-egg chamber. After this, the cystocytes in the syncytium keep on dividing until the four divisions are completed. The cystocytes grow up and leave the germarium and become a complete egg chamber, entering the vitellarium. The number of egg chambers in the ovariole is determined by the nutrients, temperature, humidity, ventilation and population density in the larvae stage while the insects are reared (Koch & King, 1966). The average number of the egg chambers of an ovariole, in *Hyalophora cecropia* is 68 (King & Aggarwal, 1965), in *Drosophila* is 5–6 (Koch and King, 1966).

So the egg chamber departs from germarium, the Oocyte usually occupies the posterior portion (Chapman; 1971). But Sang and King reported in 1961 that the oocyte of *D. melanogaster* fed with a medium without pynodoxine, would be located in the middle part of an egg chamber. Gill reported in 1963 that a gene, called chromatic oocyte determinant determined which cell would be the oocyte and others would be the nurse cells, King and Aggarwal (1965) studied the oogenesis of the *Hyalophora* moth and found that the oocyte could be recognized in each cyst by the extensive glycogen in the cytoplasm. In the early stage, the oocyte and the nurse cell connected directly side by side and in the late stage the oocyte was in the most posterior part of the cystocyte's group. Koch and King (1966) believed that the first cystoblast in the *Drosophila* flies with undergoing 4 divisions was associated with other cells by 4 ring canals. This cystoblast would be the oocyte. Koch *et al.* (1967) and Kinderman and King (1973), Judged that the oocyte before the formation of an egg chamber by the thickening of the ring canals of the oocyte. However, this study gives no hint at the distinguishing of the oocyte from the nurse cells in the pro-egg chamber. The mechanism of the oocyte formation of the oriental fruit flies could be the same with that of *D. melanogaster*. Four days after the emergence the oocyte can be identified by the appearance of yolk particles. Observed under 80 KV, a magnification of 7000× shows that the cytoplasm of the oocyte is full of a variable yolk particles and a few polyribosomes. The nurse cells are full of polyribosomes and rough endoplasmic reticula. Cytoplasm of the oocyte in Smith and King's (1967) micrograph shows polyribosomes and fibrils, which are considered DNA by Koch and King (1966).

There are three types of yolk particles in the oocyte-protein-yolk, protein-starch complex and lipid-yolk. Protein-yolk is synthesized in nurse cells, sometimes in the oocyte. Lipid-yolk can be synthesized in Golgi complex and stored in the vacuoles while the oocyte is in growth. Lipid-yolks are provided by nurse cells (Chapman 1971). Telfer (1960, 1961) stated that the majority of the proteinaceous yolk sphere of the *Cecropia* moth, oocytes in later stages than 5 contain molecules manufactured outside the ovary and transported to it by the hemolymphs. These proteins have passed through the follicular cells and both the epithelial sheath and the tunica propria.

With the growth of the oocyte, the nurse cells start to diminish and are cut out from the oocyte by the follicular cells. The oocyte and the follicular cells secrete materials, which condensed to form a vitelline membrane (King and Aggarwal, 1966). This layer prevents the inclusion of cystoplasm out of the oocyte from penetration. But the follicular cells keep on secreting and form the chorion (Telfer & Smith, 1980). According to this study, the secretion of the occyte and the follicular cells forms the vitelline membrane (Vm). Outside this membrane there is also a chorion. The oocyte leaves from the remnants of the nurse cells. It hereto becomes a fully grown egg and is ready to be ovulated.

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利用穿透性電子顯微鏡研究東方果實蠅微卵管發育過程

李文蓉　曾宗國　張桃興

東方果實蠅微卵管係屬於營養細胞與卵細胞交互間列型 (polytrophic ovarioles)。一個卵巢約有 30～48 條微卵管，每一條微卵管的構造有生殖原質部 (germarium) 和卵黃部 (vitellarium)。卵黃部含有 2～5 個卵室 (egg chambers) 卵室內有 16 個囊細胞 (cystocytes)，其中一個演變爲卵細胞 (oocyte) 和 15 個營養細胞 (nurse cells)。卵室外被排列整齊的包囊細胞 (follicular cells) 包圍著。微卵管從頂端之端線帶 (terminal filament)，被兩條膜狀體包圍內膜稱披膜 (tunica propria)，外膜稱外鞘 (external sheath)。

生殖原質部內有深淺色的兩種細胞，深色細胞在生殖原質部頂端爲卵原細胞 (oogonium)，卵原細胞分裂產生卵原細胞和原胚囊 (cystoblast)，原胚囊向生殖原質部底部移動並產生囊細胞 (cystocytes)，囊細胞再行不完全分裂形成囊胞堆 (syncytium)，淺色細胞爲中胚層細胞 (mesodermal cells) 經分裂向生殖原質部內部及底部移動，最後把細胞堆包圍而成前卵室 (pro-egg chamber)，囊細胞在前卵室繼續分裂成爲16個細胞。前卵室離開生殖原質部，卵室內16個細胞，其中一個將發育爲卵細胞 (oocyte)，15個爲營養細胞 (nurse cells)，成蟲羽化後第四天或第五天卵巢微卵管之卵室底部細胞呈現卵黃顆粒，由此確定該細胞爲卵細胞，卵細胞漸漸長大，至羽化後第九天成爲卵子 (egg)，營養細胞退化變爲殘渣 (remnants of nurse cells)，包囊細胞亦退化爲膜帶狀物，卵子的細胞質周邊有包囊細胞及卵細胞分泌物形成之卵膜 (vitelline membrane)，卵膜與退化之包囊細胞間有由包囊細胞分泌形成之卵殼 (chorion)。