FOLLICLE CELL BRIDGES IN THE MOSQUITO OVARY: SYNCYTIA FORMATION AND BRIDGE MORPHOLOGY

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SUMMARY
In the mosquito, Culex pipiens quinquefasciatus, the follicle cells enveloping the oocyte and the nurse cells are connected by intercellular bridges. The bridges are formed by incomplete cytokinesis, and they persist for more than 30 h after their formation. Reconstructions from serial sections showed that one syncytial group contained at least 32 cells; several cells continued outside the series. The cells in a syncytium divide asynchronously; this results in an irregular, branched organization. The bridges may be either embedded in the cytoplasm of the cells, or they may form an extracellular connexion.

INTRODUCTION
Syncytial arrangement of oogonia and oocytes or spermatogonia and spermatocytes have been described in both vertebrates and invertebrates (e.g. Koch & King, 1966; Ruby, Dyer, Gasser & Skalko, 1970; Dym & Fawcett, 1971; King & Akai, 1971). In insects with meroistic ovaries (ovaries with nurse cells) the oocyte is connected to one or more nurse cells. The nurse cells feed RNA and possibly organelles into the oocyte and thereby facilitate the rapid development of the oocyte (Telfer, 1975). Insects with meroistic, polytrophic oogenesis have each oocyte with its attendant nurse cell(s) enveloped by a layer of follicle cells, which are thought to be of somatic origin (mesodermal). The oocyte and the nurse cells are interconnected by cytoplasmic bridges, which arise by incomplete cytokinesis (Telfer, 1975). In the polytrophic ovaries of mosquitoes the follicle cells of the ovariole divide from the time of emergence of the adult female until vitellogenesis is well under way (Laurence & Simpson, 1974). The enlarging nurse chamber is thus covered by an increasing number of follicle cells.

Intercellular bridges have been found between the follicle cells in the mosquito Aedes aegypti and the stable fly Stomoxys calcitrans (Meola, Mollenhauer & Thompson, 1977), in the honeybee Apis mellifica (Ramamurty & Engels, 1977), and possibly in Drosophila immigrans (Mahowald, 1972). The present paper traces the formation of follicle cell syncytia in the mosquito, Culex pipiens quinquefasciatus, and shows some morphological aspects of bridge formation.
MATERIALS AND METHODS

The mosquitoes were reared at 20–22 °C. Larvae were fed dog pellets, and the adults
maintained on a 5 % glucose/sucrose solution. After emergence the oocytes develop to a
resting stage, and completion of oogenesis occurs only after a blood meal has been taken. The
ovaries were dissected out in the fixative, 2 % glutaraldehyde in 0-05 or 0-08 M phosphate buffer.
They were fixed for 2 h, washed, and postfixed in 1 % osmium tetroxide. After dehydration they
were embedded in Epon. Fixation and embedding were done at room temperature. The
reconstructions of follicle cell bridges were based on series of about 150 sections (30 h after
emergence), and 300 sections (12 and 24 h after the blood meal).

Fig. 1. Diagram of follicle cell syncytia. At 24 h after the blood meal there are no more
cell divisions. ———, extracellular bridge (as in Fig. 8); →, bridge rims lie in the
cytoplasm of one cell (arrows have the same polarity as in Fig. 2); □, cell continues
outside the series.

RESULTS

Follicle cell clusters

At emergence of the adult female the oocytes are at pachytene. The flattened follicle
cells barely cover the egg chambers, which each contain one oocyte and 7 nurse cells.
Mitotic divisions occur among the follicle cells until about 20 h after the blood meal;
at this time the oocytes are at diplotene and vitellogenesis is progressing rapidly.
The frequency of mitotic divisions among the follicle cells was very low from
emergence till the resting stage 3–4 days later (oocytes at late pachytene–early
diplotene). Intercellular bridges in 3 follicle cell groups were traced at 30 h after
emergence. Two groups consisted of 2 cells each, and one of 3 cells (Fig. 1). Many
Fig. 2. 12 h after the blood meal. Intercellular bridges (arrows) connect 5 follicle cells. 

$d$, dividing cell. $\times 7000$.

Fig. 3. 12 h after the blood meal. Cell which has recently divided; the midbody (arrow) has started to form. $c$, chromatin; $n$, nucleolus. $\times 21,000$. 
cells at 10 h and 30 h after emergence had 2 bridges, indicating the presence of at least 3 interconnected cells.

After the blood meal there is an increase in follicle cell divisions. Among the about 50 cells observed 12 h after the blood meal 2 were dividing, and several others had recently divided. These cells can be identified by their dense cytoplasm (Figs. 2, 7). Two complete cell clusters were traced. The clusters contained 6 and 8 cells respectively, which were arranged in linear order (Figs. 1, 2).

In ovaries fixed 22 and 24 h after the blood meal the mitotic divisions among the follicle cells had ceased; several bridges still contained midbodies, which indicates that the bridges had recently formed (Fig. 5). One cell cluster traced at 24 h after the blood meal consisted of 32 cells; 7 of these continued outside the series, and it is likely that at least some of them were connected to new cells, making the cluster larger than the 32 cells observed. Among the 32 cells (7 of which may have additional bridges) there was one cell with 5 bridges, one with 4 bridges, 3 cells with 3 bridges, and the remaining had 1 or 2 bridges (Fig. 1). The 7 cells which continue outside the series comprise 3 groups containing 1, 2 and 4 cells (the cells marked with squares in Fig. 1). The groups are widely separated on the surface of the egg-chamber.

The clusters observed at 30 h after emergence and at 12 h after the blood meal were located partly above the oocyte and partly above the nurse cells. The cells of the cluster observed 24 h after the blood meal were all located above the oocyte, which at this stage is larger than the 7 nurse cells combined.

Bridge morphology

A dividing follicle cell at telophase is shown in Fig. 3. The midbody (arrow) has just started to form; microtubules are embedded in the 2 chromosome masses through openings in the nuclear membranes. After the bridge is formed the midbody persists for some time (Figs. 4, 5), and then it disappears. For comparison a newly formed cystocyte bridge with remnants of the midbody is shown in Fig. 6. The diameter of the follicle cell bridges is 0.2–0.5 μm, whereas the cystocyte bridges are 2–4 μm (compare Figs. 5 and 6, which are at the same magnification).

As indicated on Fig. 1 and shown in Figs. 2 and 4, many of the bridges have their rims entirely in the cytoplasm of one of the 2 interconnected cells. This type of bridge is seen most frequently (Fig. 1); when the bridge is not perpendicular to the plasma...
membranes the rim lies in the cytoplasm of both cells (Fig. 7). Finally the bridge may form an extracellular cytoplasmic connexion (Fig. 8).

The mitotic divisions stop around 20 h after the blood meal and presumably no new bridges are formed after this time. The existing bridges contain ribosomes, a few microtubules, and sometimes rough or smooth endoplasmic reticulum. They remain unaltered for at least 30 h. During this time tritiated thymidine is incorporated by all the follicle cells and they are thus becoming polyploid.

DISCUSSION

Woodruff & Telfer (1973) showed that in the *Cecropia* moth an electrical gradient is present between the nurse cells and the oocyte, and that this gradient may contribute to the polarized transfer of material from the nurse cells into the oocyte. Functional differentiation also exists among the follicle cells: pinocytosis occurs at the oocyte surface towards the follicle cells (Roth & Porter, 1964; Anderson & Spielman, 1971), but not at the nurse cell surfaces, and the vitelline membrane and chorion are formed only above the oocyte, not above the nurse cells.

Most of the material taken up pinocytotically by the oocyte is synthesized in the fat body (Hagedorn, 1974), but the follicle cells may contribute some proteins, as has been found in *Cecropia* (Anderson & Telfer, 1969). During early vitellogenesis all the follicle cells incorporate radioactive histidine and leucine (unpublished results), and the synthesized proteins may be transported through the bridges to the cells covering the oocyte, and subsequently transferred to the oocyte. Later, when the synthesis of the vitelline membrane begins, most of the 500 or more follicle cells (Laurence & Simpson, 1974) are associated with the oocyte, and it is unlikely that the few cells which are not could play an effective ‘nurse’ role.

Thus the bridges may during some stages of oogenesis have the same function as the cystocyte bridges, and allow polarized transfer of material.

The ovarioles are not rigid structures; they increase continuously in volume, and sections of them show that they are squeezed and deformed by neighbouring ovarioles. During the movements the follicle cells are variously stretched and squeezed, and when 2 interconnected cells pull apart the bridge may change from a cytoplasmic position (Fig. 7) to an extracellular one (Fig. 8). Similar variations in bridge morphology has been observed in the ovary of the mouse (Ruby, Dyer & Skalko, 1969).

The bridges do not synchronize the mitotic divisions, as is obvious from the asymmetry of the cell cluster observed 24 h after the blood meal. Whether a cell goes through one or several divisions seems to be determined at the level of the particular cell. A similar asynchrony in cell divisions among interconnected cells was reported in the spermatogonia and spermatocytes of the rat (Moens & Hugenholtz, 1975). The bridges may facilitate the synchronization of other events, such as the switch from mitotic divisions to polyploidization and the almost simultaneous start of vitelline membrane synthesis. It seems, though, that these functions might be regulated through communicating junctions (Mahowald, 1972).
REFERENCES


(Received 10 October 1977)