DIFFERENTIATION OF OVARIOLAR FOLLICULAR
CELLS AND FORMATION OF PREVITELLINE-MEMBRANE
SUBSTANCE IN SIMULIUM VITTATUM ZETTERSTEDT
(DIPTERA: SIMULIIDAE)

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Abstract—In the cytoplasm of ovariolar follicular cells of newly emerged black flies, free
ribosomes were predominant and few strands of endoplasmic reticulum were observed.
Golgi complexes were well developed, but no electron-dense secretions were seen within
the Golgi vesicles. At the end of day 2, while vitellogenesis was most active and endo-
plasmic reticulum prevailed in the cytoplasm of the follicle cells, Golgi vesicles containing
dense material were observed. Dense droplets were abundant in the cytoplasm adjacent
to the microvilli, as well as among the microvilli. At the end of day 3, during which
vitellogenesis was complete, the Golgi complexes, containing both dense and fibrous
materials, became very prominent in the follicular-cell cytoplasm. The cisternae of the
endoplasmic reticulum became dilated and some long strands of endoplasmic reticulum
were organized into concentric whorls. At this stage the deposited, previtelline-membrane
substance between the follicular cells and the oocyte began to coalesce and gradually
formed the vitelline membrane.

Index descriptors—(in addition to those in the title): oocyte oogenesis, vitellogenesis,
ribosomes, Golgi complexes and vesicles, endoplasmic reticulum, vitelline membrane.

INTRODUCTION

Oocyte differentiation and vitellogenesis have been studied extensively in a wide range of
animals (see reviews: Raven, 1961; Nørrevang, 1968). In contrast, the structure and function
of the ovariolar follicular cell during oogenesis have received much less attention. The
primary function of follicular cells is the formation of the egg envelope (Raven, 1961; King
Controversy exists concerning the origin of vitelline membrane in insects, some studies
indicating that it is a product of the oocyte (Okada and Waddington, 1959), of the follicular
cells (Goodman et al., 1968) or of both (Hopkins and King, 1966). These differences may
relate to the species under study. However, recent studies (Favard-Séreré, 1966; Beams and
Kessel, 1969) have obtained clear ultrastructural evidence of the formation of vitelline-
membrane substance in the follicular cells. The present paper reports new aspects of the
functionally related, ultrastructural alterations in the ovariolar, follicular cells before and
during formation and deposition of vitelline-membrane substance.

MATERIALS AND METHODS

Larvae and pupae of the black fly, Simulium vittatum, were collected and reared in the
laboratory (Liu and Davies, 1972). Ovaries were removed from flies of different ages, fixed
for 1 hr in 5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.4) at 4°C, and briefly washed

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FIG. 1. Thin sections of ovaries of newly emerged black flies. (1) Two follicular cells each with a large nucleus (Nu), free ribosomes (distributed throughout the cytoplasm), a few strands of rough endoplasmic reticulum (Er), and mitochondria (M) with an electron-lucid matrix. (×23,000).
Figs. 2-3. Thin sections of ovaries of newly emerged black flies. (2) A well-developed Golgi complex (G) in a follicular cell. (× 80,100) (3) A Golgi complex (G) in an oocyte and peripheral microvilli (MV) (× 80,100).
with the same buffer before being post-fixed in 1% osmium tetroxide for 1 hr. Fixed ovaries were dehydrated through a graded series of ethanol and embedded in Araldite. Sections were double stained with uranyl acetate and lead citrate, and examined in a Zeiss EM9 electron microscope.

**OBSERVATIONS**

Ovariolar follicular cells of newly emerged black flies possessed a large nucleus with a prominent nucleolus consisting of chromatin material centrally located, which was surrounded by some densely packed chromatin material. The chromatin consists of small particles, which resemble the ribosomes in the cytoplasm (Fig. 1). Free ribosomes fill the cytoplasm but some are beginning to attach to membranous elements (Fig. 2). A few strands of rough endoplasmic reticulum are occasionally seen in the cytoplasm (Fig. 1). Well-developed Golgi complexes are observed in both follicular cells and the oöcytes (Figs. 2, 3). However, no electron-dense material was ever observed within the Golgi vesicles of the follicular cells at this stage, nor was there any electron-dense material observed in the extracellular space between the follicular cells and oöcytes among the oöcyte microvilli (Fig. 3). Mitochondria in follicular cells have an electron-lucid matrix with well-developed cristae.

At the end of day 2 (48-56 hr after emergence), which was the most active period of yolk-protein deposition in the oöcyte, the follicular cells were much different than those of previous stages. The nucleus had the chromatin material dispersed throughout the nucleoplasm, but mostly associated with the nuclear membrane (Fig. 4). In the cytoplasm, rough endoplasmic reticulum was the predominate feature in contrast to the earlier stages. Golgi vesicles, containing dense material are seen in the cytoplasm (Figs. 4, 5), as well as dense droplets, especially towards the oöcyte surface and among the microvilli between the follicular cells and oöcyte (Fig. 6). Mitochondria also showed changes; mainly the mitochondrial matrix had become more electron dense compared to that of previous stages.

At the end of day 3 (between 66-72 hr after emergence) vitellogenesis was complete, but during this period further changes occurred within the follicular cells. The Golgi complex became most prominent, containing dense and fibrous materials (Fig. 7). Electron-dense material, bounded by a membrane, and membrane-bound multivesicular bodies were found along the periphery of the follicular cells towards the oöcyte. At the same time the deposited, previtelilline-membrane substance became an irregular, highly packed mass between the follicular cells and the oöcyte. The microvilli of the oöcyte had largely disappeared (Fig. 8). Changes in rough endoplasmic reticulum in the follicular cells were marked; in addition to the more dilated cisternae, long strands of endoplasmic reticulum were organized into concentric whorls (Fig. 10). Frequently lipid droplets were observed, enclosed within these concentric whorls of rough endoplasmic reticulum (Fig. 9).

**DISCUSSION**

Functionally related structural changes of mitochondria in simulid oöcytes during lipid-yolk deposition have been described previously (Liu and Davies, 1973). The present study further illustrates the functional significance of the structural transformations within the developing ovary. There seems to be little doubt that the structural alterations of the follicular cells during the period described above, are related mainly to vitelline-membrane formation. There is no structural evidence to show that the follicular cells contribute
Fig. 4. Thin sections of black fly ovaries during period of most active yolk-protein deposition. (4) Two follicular cells each having a nucleus (Nu) with chromatin material dispersed throughout nucleoplasm, a network of rough endoplasmic reticulum (Er), Golgi complexes (G) containing a dense secretion, and mitochondria (M) with matrix more electron-dense than at earlier stage (× 32,800).
Figs. 5-6. Thin sections of black fly ovaries during period of most active yolk-protein deposition. (5) Follicular cell showing greater detail of Golgi vesicles (G) which contain electron-dense material, and a mitochondrion (M) (× 55,100). (6) Note electron-dense droplets (arrows) in a follicular cell near the microvilli (MV) and among microvilli between the follicular cell and an oocyte (OOC) (× 23,000).
FIGS. 7–8. Thin sections of black fly ovaries at the end of yolk deposition. (7) A Golgi complex (G) in a follicular cell. Note endoplasmic reticulum (Er) with distended cisternae, and a mitochondrion (M) (× 65,200). (8) An irregular, highly packed mass (arrow) of vitelline-membrane substance between follicular cells and oöcyte. Note a dense secretion (D), a multivesicular body (arrowhead) and disappearance of the microvilli (× 65,200).
Figs. 9-10. Thin sections of black fly ovaries at the end of yolk deposition. (9) A follicular cell showing concentric whorls of endoplasmic reticulum (Er) within which lipid droplets (L) are enclosed (x 61,600). (10) A follicular cell showing concentric whorl of endoplasmic reticulum (x 61,600).
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materials for yolk deposition in these simulids nor that oocytes contribute materials for vitelline-membrane formation. Structurally the mechanism of synthesis and deposition of previtelline-membrane material is similar to that described by Beams and Kessel (1969) in the dragonfly. The brief account by Favard-Séréréno (1966), the first ultrastructural work which describes the follicular cell, showed two stages of secretion, the first during vitelline-membrane secretion when the cell had much rough endoplasmic reticulum and typical Golgi complexes, and the second during chorion synthesis when the Golgi saccules became highly vacuolated. His observations were supported by Beams and Kessel (1969).

In the black fly, the follicular cells showed 3 stages of change at the time of vitelline-membrane development. The first stage was before previtelline-membrane formation, when there were signs of development of rough endoplasmic reticulum, along with well-developed Golgi complexes. These changes led to the second stage where the cells possessed a network of rough endoplasmic reticulum in which protein was presumably synthesized and transported to the Golgi complexes, as suggested by Beams and Kessel (1969). This process has long been known to exist in pancreatic exocrine cells (Jamieson and Palade, 1967). In the Golgi apparatus, complex carbohydrate is formed in the pancreatic exocrine cells (Peterson and Leblond, 1964). Beams and Kessel (1969) suggested that the complex carbohydrate was then discharged by reverse pinocytosis into the extracellular spaces between the follicular cells and oocyte. The third stage in structural transformation within the follicular cells of the black fly was the appearance of concentric whorls of rough endoplasmic reticulum. This change may represent a functional transformation of these cells. Nickerson and Curtis (1969) suggested that in the adrenocortical cells of the Mongolian gerbil, the concentric whorls of endoplasmic reticulum served as a reserve which would be utilized for the synthesis of enzymes for steroid synthesis. In the follicular cells of black flies, the association of concentric whorls of rough endoplasmic reticulum with lipid may indicate a shift from synthesis of complex carbohydrate to synthesis of a lipoprotein complex, because it is known that the vitelline membrane is composed of protein, carbohydrate and lipid (King and Koch, 1963). Thus these ultrastructural observations with black flies may indicate that synthesis of the carbohydrate and lipid components of the vitelline membrane are divided into 2 stages.

The appearance of multivesicular bodies at the later stage may relate to their involvement in the final processes of vitelline-membrane formation. Perhaps these bodies contribute enzymes for the fusion of previtelline-membrane materials into a complete membrane and for the fusion of previtelline-membrane materials into a complete membrane and for the breakdown the microvilli on the oocyte surface. Multivesicular bodies are known to contain lysozymes (Hugon and Berger, 1967; Locke and Collins, 1968). The lysozymes are synthesized presumably in the Golgi complexes and transferred to the site of utilization (Northcote, 1971).

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REFERENCES


