FINE STRUCTURE OF PIGMENT INCLUSIONS IN THE TEST CELLS OF THE OVARY OF *STYELA*

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In the tunicate ovary, both germ cells and follicle cells are generally believed to arise from the germinal epithelium (*cf.*, Seeliger (3) and Tucker (5)). The young germ cell moves internally from the periphery of the ovary and, together with several epithelial cells, constitutes the primary follicle. Initially, the germ cell is surrounded only by a flattened layer of epithelium. The cells of the epithelial layer, however, soon divide so as to form two layers of follicle cells, an inner and outer layer, and another follicle cell which becomes compressed in the periphery of the developing oocyte. The latter is referred to as the test cell and has no counterpart in any vertebrate ovary thus far examined. The test cell becomes separated from the follicle epithelium after formation of the vitelline membrane (chorion). A diagram illustrating these relationships is shown in Fig. 1. Alternative views concerning the origin of the oocyte-follicle cell complex in ascideans can be obtained by consulting Van Beneden and Julin (6), Spek (4), and Knaben (2). Recently, studies were made on the fine structure of the ovary of *Molgula*, with special reference to the changes occurring in the oocyte, follicle cells, and test cells during oogenesis (1).

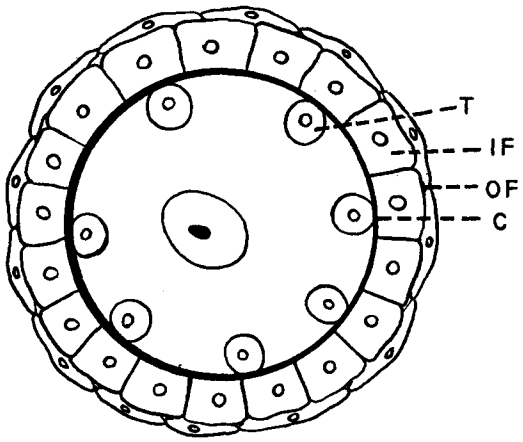


FIGURE 1

Schematic diagram showing relationship of test cell (*T*), inner follicle cell (*IF*), outer follicle cell (*OF*), and vitelline membrane (*C*) surrounding the developing oocyte of *Styela*.

In subsequent observations on the ovary of *Styela*, certain differences were noted especially with regard to the presence of pigmented inclusions with a unique fine structure in the test cell and are reported in this note.

MATERIALS AND METHODS

The tunicate, *Styela plicata*, was obtained from the Duke Marine Laboratory, Beaufort, North Carolina, during the month of May. The ovaries were excised and fixed in either a 1 per cent or 2 per cent solution of osmium tetroxide prepared with filtered sea water (pH 7.8) for 1 to 2 hours at 4°C. The ovaries were rapidly dehydrated in a graded series of alcohols and embedded in a mixture of butyl and methyl methacrylates. Sections were cut on a Porter-Blum microtome and supported on grids coated with 0.1 per cent Formvar in distilled ethylene dichloride and lightly stabilized with carbon. The microscopes used included the RCA EMU-3D and 3F.

OBSERVATIONS

Observations on the living oocytes of *Styela* reveal an accumulation of yellowish-orange inclusion material which appears localized in the test cells. The pigmented material was especially abundant in large oocytes which were almost ready for ovulation and appeared to fill completely the test cells. Fig. 4 is an electron micrograph of a portion of the test cell at a late stage in oogenesis when the oocyte is filled with yolk. In the micrograph, the test cell (*T*) is filled with oval masses of extremely electron-opaque material (*P*). The units comprising this material are both loosely and compactly arranged. In some instances, a thin membrane is observed surrounding the electron-opaque masses (Fig. 5, arrows). At low magnification, the inclusions appear to consist of rods which vary considerably in length. In suitable sections, the closely packed rods are preferentially oriented parallel with the longitudinal axis of the oval inclusion (Fig. 3, arrows). At somewhat higher magnification, more detail in structure is visible within the electron-opaque masses. The inclusions are now seen to consist of chains of small spheres (Fig. 5). The spherical units, in turn, contain an electron-opaque periphery with a central region which is lightly osmicated (Fig. 5). The spheres are approximately 38 to 40 mμ in diameter. Another inclusion is present in the test cell at this time and appears to be partially lipid in nature (Figs. 4 and 5, *L*).

It was not known at this stage whether the pigmented inclusions represented an endogenous material synthesized by the test cell prior to ovulation or whether it might represent a foreign material which had migrated into the cells. In order to obtain more information regarding the origin of the pigment, earlier stages of oocytes were examined with the electron microscope.

The young test cell contains small mitochondria, elements of the endoplasmic reticulum and Golgi material (Figs. 2, 3). Vacuoles are also present in the young test cell and contain various amounts of beaded filaments (Fig. 2, *F*). At later stages, the beaded filaments thicken and develop a structure which is characteristic of the fully formed pigmented material except that they are smaller in size (Fig. 3, arrows). Later, when the pigment is amassed within the test cell, the nucleus becomes compressed and the cytoplasm is noticeably depleted with respect to organelles (Fig. 4).

CONCLUSION

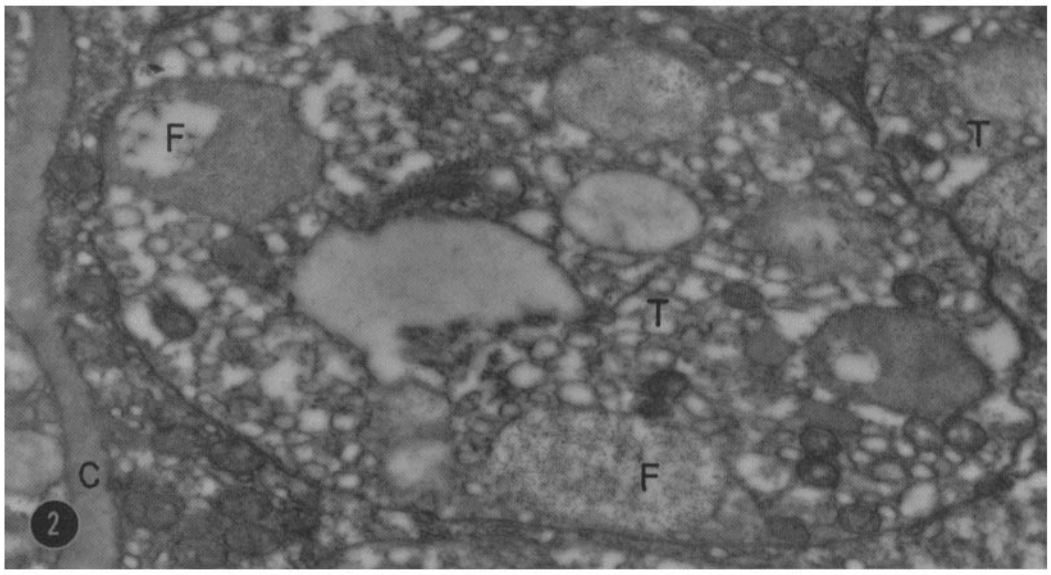
It thus appears that the inclusions described have their origin in the test cell in the form of fine, beaded filaments which are enclosed in a membrane-limited vacuole. The filaments increase in size, both in thickness and in length, and assume a rather specific orientation. Finally, closely packed chains of spherical units consisting of a dense periphery and a less dense interior are formed,

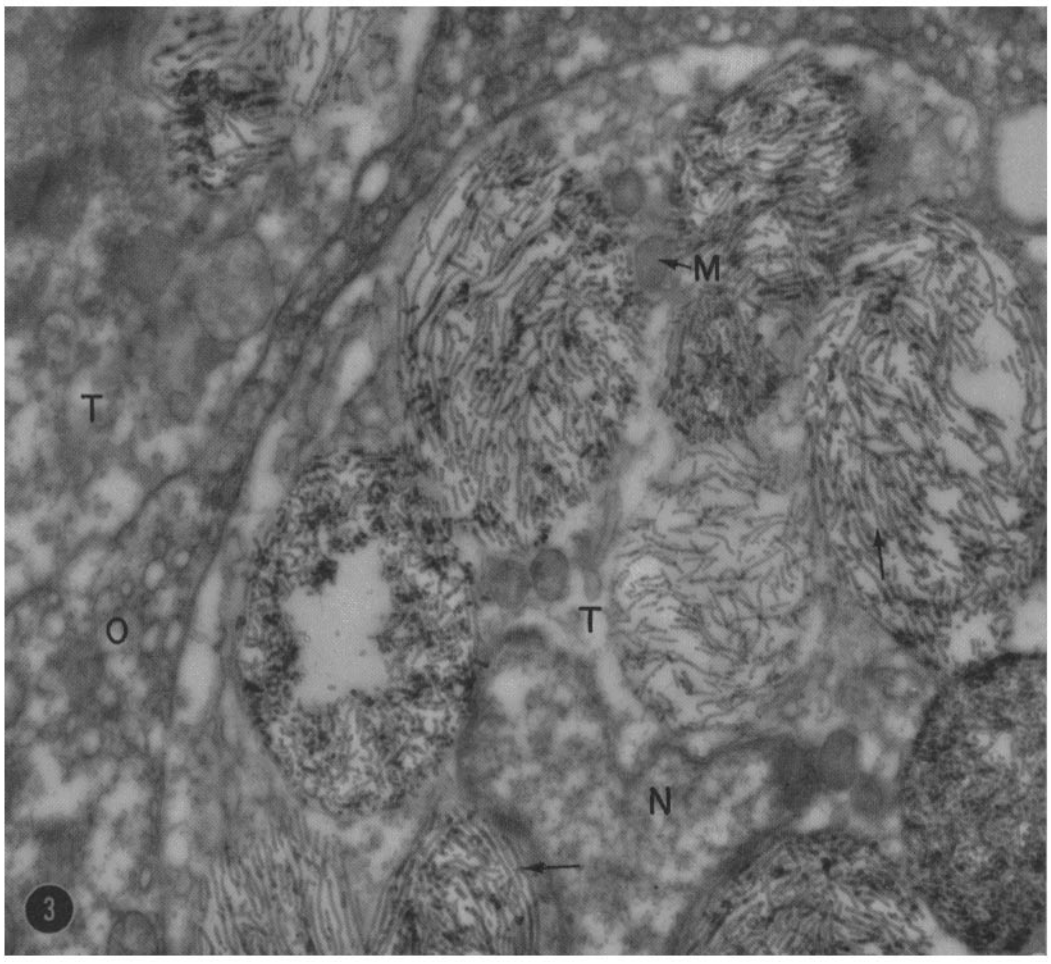
FIGURE 2

Portion of two test cells (*T*) early in oogenesis compressed in periphery of oocyte. Vacuoles containing various amounts of thin, beaded filaments are abundant (*F*). Vitelline membrane (*C*). × 14,000.

FIGURE 3

Portions of two test cells (*T*) showing intermediate stage in development of pigment inclusions. Mitochondria (*M*), nucleus of test cell (*N*), oocyte (*O*). Regions at arrows show structure of pigment at this stage. × 20,000.


F
T
T
C
F
2


M
T
T
O
N
3

as is characteristic of late stages and presumably represents the more mature condition. There is little doubt that the pigment which is observed in living test cells has the ultrastructure here described. However, it appears that a special type of pigment is represented in this case since it is structurally unlike any described in a variety of other cells. The functional significance of this material is not presently understood.

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