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# TEST CELL DIFFERENTIATION DURING OOGENESIS AND EARLY EMBRYOGENESIS OF *BOTRYLLUS SCHLOSSERI* (Ascidiacea)

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ABSTRACT. — Appearance and differentiation of test cells were followed in the ascidian *Botryllus schlosseri*, using colonies grown in the laboratory. Presumptive test cells were at first recognizable during late pre-vitellogenesis as isolated cells sited under the primary follicle layer. Then they embedded themselves in depressions of the oocyte being partially covered by its microvillar extrusions. Junctions definitely identified as such were never seen to be established between test cells and the oocyte.

The main features of differentiation consisted in the development of endoplasmic reticulum and Golgi apparatus composed of numerous long cisternae, and in the formation of dense round granules. The latter underwent morphological changes at the end of oogenesis and during early embryogenesis, when the test cells were freed into the perivitelline space. These observations suggest that the role of test cells in *B. schlosseri* is mainly played during these stages.

Key words: *Oogenesis. Embryogenesis. Cell differentiation. Colonial ascidia.*

## INTRODUCTION

Commonly in ascidians the growing oocyte is enveloped by outer (OFC) and inner follicle cells (IFC), acellular vitelline coat or chorion and test cells (TC). However, in solitary ascidians the OFC generally form a thin and only slightly differentiated layer (KESSEL, 1983), while in colonial ascidians such as *Botryllus schlosseri* and *Diplosoma listerianum* the OFC are well developed and are discharged at ovulation to form a sort of *corpus luteum* (ZANIOLO *et al.*, 1987; MARTINUCCI *et al.*, 1988).

The origin and differentiation of ascidian egg envelopes is still an open question. Some authors believe that they derive from the germinal epithelium, others from the blood cells, and others from both. In *Ciona intestinalis* for instance, according to MANCUSO (1965), TC originate from coelomic cells, while according to PÉRÈS (1954) and DE VINCENTIIS (1962) they derive from the germinal epithelium.

The role of TC has also long been discussed: during oogenesis they were considered to be involved in oocyte nutrition (KESSEL and KEMP, 1962), in providing yolk precursors (MANCUSO, 1965; GIANGUZZA and DOLCEMASCOLO, 1979), in forming pigment granules (KESSEL, 1962; KESSEL and BEAMS, 1965), and in accumulating vanadium or iron compounds to be transmitted to the embryo (KALK, 1963; HORI and MICHIBATA, 1981). During embryogenesis, TC are considered to play a role in larval tunic secretion (CAVEY, 1976; CLONEY and CAVEY, 1982; CAVEY and CLONEY, 1984) or in transmitting microorganism-like cells (REVERBERI, 1978; MANSUETO *et al.*, 1984).

The early appearance and differentiation of the TC in *B. schlosseri* during oogenesis and cleavage is here studied at the electron microscope.

## MATERIAL AND METHODS

*Botryllus schlosseri* Pallas, colonial ascidian of the order Stolidobranchiata, is an ermaphroditic species whose gonads are located in the body wall on either side of the zooid (Fig. 1).

The main oogenesis stages in colonies cultured in laboratory were observed with a stereomicroscope. Pieces of colonies were fixed in 1.5% glutaraldehyde buffered with 0.2 M cacodylate, pH 7.2 plus 1.6% NaCl.

After washing in buffer and postfixation in 1% osmium tetroxide in 0.2 M cacodylate buffer, the specimens were dehydrated and embedded in Epon. Thick sections (1 μm) were stained with toluidine blue. Thin sections, stained with uranyl acetate and lead citrate, were examined with a Hitachi H-600 electron microscope.

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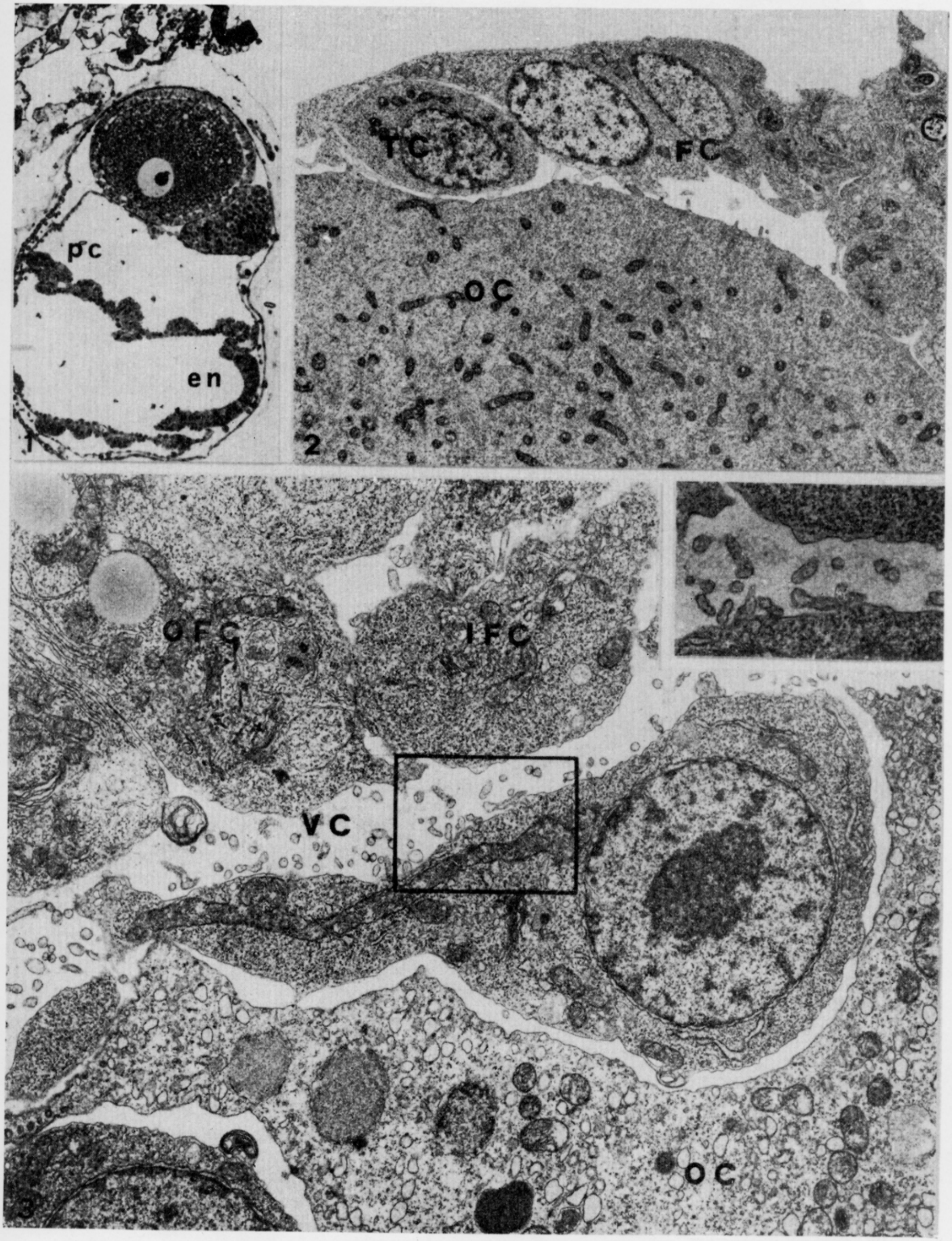
*Fig. 1.* — Transverse section of a *B. schlosseri* bud showing a vitellogenic oocyte sited in the mantle close to an undifferentiated testis (**t**). **p.c.**: peribranchial chamber; **en**: endostyle. Thick section. Toluidine blue. x 150.

*Fig. 2.* — Electronmicrograph showing detail of an oocyte at pre-vitellogenic stage. Round presumptive test cells (**TC**) are recognizable under the primary follicle layer (**FC**) surrounding oocyte (**OC**). x 4,000.

*Fig. 3.* — Early vitellogenesis. Test cells, encased in depressions of the oocyte (**OC**), are rich in ribosomes and do not show evidence of secretory granules. Outer (**OFC**) and inner (**IFC**) follicle cells and vitelline coat (**VC**) are recognizable. x 8,000. Inset: the loose vitellin coat and microvilli are shown in detail. x 12,000.


pc
en
1
TC
FC
OC
2
OFC
IFC
VC
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3

## OBSERVATIONS

The gonads of *B. schlosseri* appear in the body wall of the buds and the first germinal cells are grouped to form an undifferentiated gonadal blastema.

At the beginning of differentiation, the young oocytes are surrounded by a thin layer of undifferentiated follicle cells (primary follicle cells).

The oocyte presents a smooth oolemma with scanty short microvillar protrusions, and this pattern is also maintained throughout the first previtellogenic period when scattered cells appear underneath the primary follicle cells. They are roundish, only slightly differentiated, with small scarce mitochondria and distinguishable from the outer cells only by their position. They seem to be the presumptive test cells, preferentially located in shallow depressions on the oocyte surface (Fig. 2).

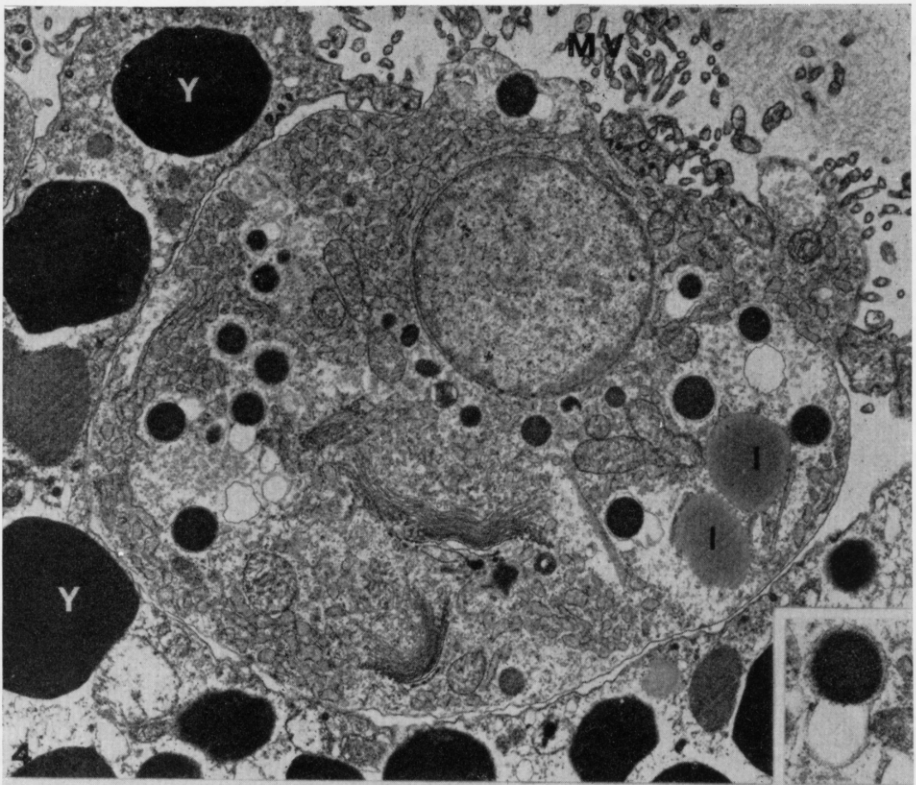
At the start of vitellogenesis, when the first yolk granules appear in the oocyte, short digitate microvilli rise over the oolemma surface. From this stage onwards and throughout vitellogenesis, the TC are progressively encased in the oocyte until they appear almost completely embedded and partially covered by the microvilli (Figs. 3, 4). At this stage the OFC and the IFC, separated from the TC by the vitelline coat, can be recognized around the oocyte (inset in Fig. 3). The vitelline coat appears during early vitellogenesis as a loose fibrillar weft.

The TC are encased in the oocyte, divided from each other by cytoplasmic portions rich in yolk granules. These cells, with smooth, regular cell membranes, unlike the follicle cells, never appear to establish unequivocal junctions with the oocyte. Electrondense granules form in the cytoplasm, increasing in number as ovulation approaches. They contain a large well-developed Golgi apparatus, composed of a large number of elongated cisternae (10-20), forming a fenestrate structure of smooth endoplasmic reticulum extending to the secreting surface. The Golgi apparatus grows in size at the end of vitellogenesis (Fig. 4); in concomitance the TC granules, probably derived from the Golgi cisternae, increase in number and size; their membrane often extends to form an apparently empty sac. (Fig. 4, inset).

In the cytoplasm, the rough endoplasmic reticulum is well developed

*Fig. 4.* — Late vitellogenic stage. A test cell is deeply encased in an oocyte and partially covered by ovular microvilli (**MV**). The test cell is characterized by numerous dense round granules and well-developed Golgi complex. x 6,300. Inset: a granule with its membrane extended to form an apparently empty sac. **l**: lipid droplet; **Y**: yolk granules. x 14,000.

*Fig. 5.* — Free test cell in perivitelline space after fertilization. Numbers 1 to 4 indicate possible different stages of maturation of granules. **G**: Golgi complex. x 21, 000.



MV
Y
Y
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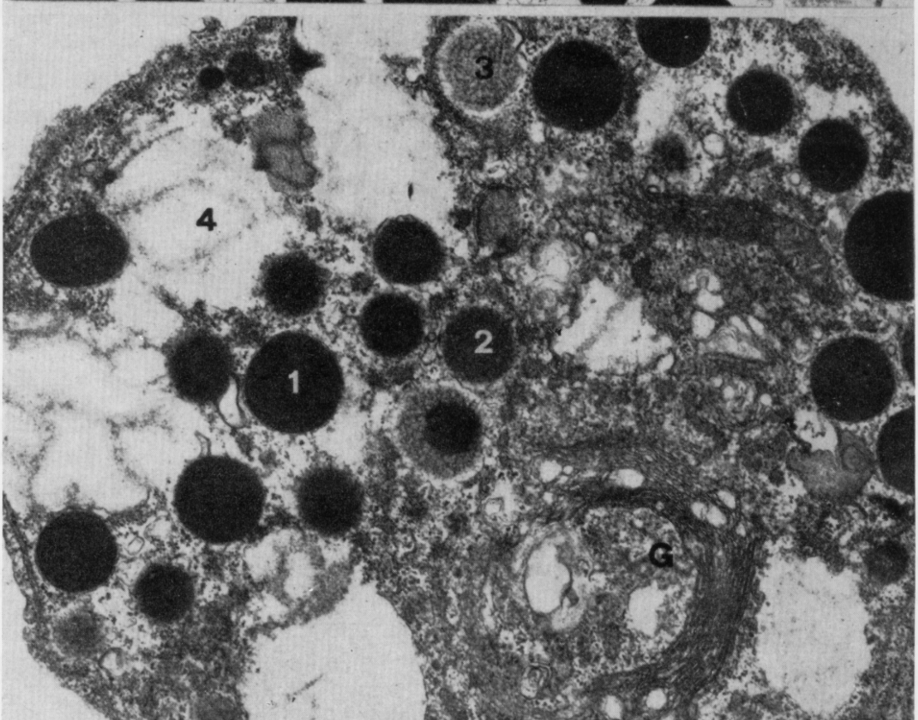

3
4
1
2
G
5

and the cisternae, with very few ribosomes are often enlarged to form vesicles filled with moderately electrondense material.

The TC remain encased in the oocyte even after ovulation, when the egg reaches the peribranchial chamber, where it is suspended through the placenta. Immediately after fertilization, the TC are released into the very limited perivitelline space. These cells are differentiated with respect to the previous stages: the Golgi apparatus is reduced in size, and the electron-dense granules progressively lose both their homogeneity and density, their matrix decreasing in compactness. Eventually this matrix becomes scattered in a loose weave inside the large vesicles originating from the swollen granule membranes (Fig. 5).

## DISCUSSION

In *B. schlosseri* during the pre-vitellogenic stage the presumptive TC appear at first interposed between the primary follicle cells and the oocyte. Before differentiation becomes evident, the TC embed themselves in depressions of the oocyte. They remain isolated from each other and are roundish in shape — unlike *Ascidiella aspersa*, where TC form a continuous layer and possess numerous pseudopodia-like extensions (MANSUETO and VILLA, 1983).

In the late pre-vitellogenesis of *Ciona intestinalis* (MANCUSO, 1965) and *Ciona savignyi* (SUGINO *et al.*, 1987), the TC possess dense granules, larger than those of the previous stage, and many RER profiles. This is not the case in *B. schlosseri* whose TC show granules only at the vitellogenic stage, when the follicular envelopes are differentiated. The delayed beginning of the secretory activity with respect to yolk formation may indicate that in *B. schlosseri* the TC are not involved in furnishing yolk precursors, as suggested for other ascidians (HARVEY, 1927; MANCUSO, 1965; GIANGUZZA and DOLCEMASCOLO, 1979).

In *Styela plicata* (JEFFERY, 1980) the TC show substantial levels of protein synthesis during oogenesis and are believed to be inactive during embryogenesis. In this and in other species of ascidians, they are freed into the perivitelline space at ovulation (REVERBERI, 1978; MARTINUCCI *et al.*, 1988). However in *B. schlosseri* most of them remain encased in the oocyte until late cleavage, when their granules show marked signis of maturation.

Our observations indicate that in this species the role of the TC is played mainly during embryogenesis, rather than oogenesis. This hypothesis is also suggested for other ascidians by several authors, since the TC are believed to participate in the formation of the larval tunic by furnishing constitutive elements (Kupffer, 1870) and controlling morphogenesis (Cavey, 1976; Cloney and Cavey, 1982; Cavey and Cloney, 1984), and/or to play an enzymatic role during larval hatching (Knaben, 1936; Berrill, 1950).

It cannot be excluded, as claimed by Reverberi (1978), that TC in different species have different roles, probably in relation to the modality of fertilization and the duration of embryogenesis.

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