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Research paper

Ultrastructural study of oogenesis in the acotylean *Echinoplana celerrima*, (Platyhelminthes, Polycladida)

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ABSTRACT

Here, the ultrastructure of the female gonad of the marine polyclad *Echinoplana celerrima* was investigated using both light and electron microscopy. The female gonads are dispersed in the dorsal parenchyma between the digestive ramifications. Each ovary is separated from adjacent tissues by a homogeneous basal lamina and is characterized by the presence of a germinative zone, growing oocytes at the previtellogenic stage, and at the vitellogenic stage.

The germinative zone is located at the dorsal part of the gonad and is characterized by the presence of undifferentiated young germ cells or oogonia having a large nucleus with a well-shaped nucleolus. Growing oocytes at the previtellogenic stage are characterized by the production and accumulation of eggshell granules. Indeed, the cytoplasm is gradually packed with cytoplasmic organelles and electron-dense inclusions that coalesce to form eggshell granules. When maturing, the latter become formed in their center by an electron-dense core surrounded by very electron-dense areas of variable shape. During the vitellogenesis stage, the oocytes undergo a notable increase in the cytoplasmic volume due to the accumulation of specific organelles such as free ribosomes and endoplasmic reticulum. These specific cytoplasmic organelles are probably involved in the production of vesicles containing a medium-dense and granular material. Repeated coalescence of these vesicles gives rise to the yolk globules. At this stage, the cytoplasm also displays some multivesicular bodies (MVBs). The origin of these MVBs and their function is discussed.

1. Introduction

Polyclads are simultaneous hermaphrodites with a brief protandric phase as free-living Platyhelminthes. Contrary to the neophoran worms that possess female gonads consisting of germaria (ovaries) producing oocytes and vitellaria producing vitellocytes, polyclads belong to Archoophora, in which the structure of the homocellular female gonads remains primitive without separation between germaria and vitellaria; the eggs are entolecithal (Karling, 1940). The female gonad is homocellular having a double function, that is, to elaborate germ cells and to produce yolk globules. Each ovarian follicle appears to develop from a single oogonial cell (Rieger et al., 1991).

The evolutionary history of the phylum from the Archoophora to the Neoophora level of organization could be reconstructed by investigating different modifications of the female gonad (Gremigni, 1997). It is interesting to note that the interrelationships between the Archoopora and Neoophora have seen little attention from molecular phylogenetic research point of view. Indeed, to our knowledge, only one recent molecular phylogenetic study has examined this interrelationship between the two groups (Laumer and Giribet, 2014). According to this study, polyclads are one of 3 platyhelminth higher taxa (the 2 others are Catenulida and Macrostomorpha) that retain a plesiomorphic (endolecithal) condition, producing a single major cell type during oogenesis. However, in the ectolecithality mechanism, vitellocytes are the responsible for the synthesis and storage of yolk and shell (Gremigni, 1988; Swiderski and Xylander, 2000) and thus should be considered as a deeply consequential developmental innovation, unique to Platyhelminthes (Laumer and Giribet, 2014)

Despite the taxonomic and phylogenetic importance of the ultrastructural data of the female gonads (Karling, 1967, 1974;

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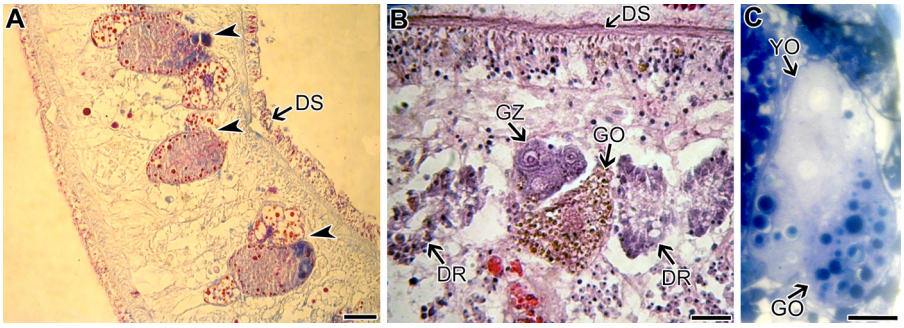

Fig. 1. Light micrographs showing sagittal sections of *Echinoplana celerrima*. (A) Distribution of ovaries (arrows) throughout the body at the dorsal side (DS); scale bar = 20 μm. (B) Details of an ovary comprising a germinative zone (GZ) located in the dorsal part of the ovary that contains young germ cells and growing oocytes (GO), which are pushed towards the ventral side; DO: digestive ramification, DS: dorsal side; scale bar = 16 μm. (C) Semithin section of an ovary showing young oocytes (YO) at the dorsal germinative zone and the growing oocytes (GO) situated more ventrally; scale bar = 10 μm.

Gremigni, 1988; Gremigni and Falleni, 1998), the oogenesis characteristics of many archoophoran species are still unknown and most investigations concern oocytes or vitellocytes of neoophoran flatworms (Gremigni and Nigro, 1983; Tekaya et al., 1998, 1999; Falleni et al., 2006, 2009; Charni et al., 2010; Harrath et al., 2011, 2013, 2014). In the Archoophora, detailed ultrastructural investigations are scarce and were carried out on the ovary of some species of the order Polycladida (Boyer, 1972; Domenici et al., 1975; Gremigni, 1983; Thomas, 1986; Ishida and Teshirogi, 1986; Liana and Litvaitis, 2009) and Macrostomida (Gremigni et al., 1987; Smith et al., 1988; Kuales et al., 2011).

Using light and transmission electron microscopy, the main objective of this study is to document the ultrastructural characteristics of the oogenesis in the polyclad *Echinoplana celerrima* to elucidate the cellular features of reproduction in "Turbellaria", mainly in Polyclads. These characteristics of oogenesis could help to improve our understanding of the phylogenetic relationships with the members of "Turbellaria", in particular, the species with plesiomorphic endolecithality. Moreover, it has been demonstrated that mature ovarian oocytes have large amounts of protein yolk globules that are autosynthetically produced within the ooplasm of the polyclad species *Prostheceraeus floridanus* (see Boyer, 1972) and *Notoplana alcinoi* (see Domenici et al., 1975). The autosynthetic mechanism of yolk production, which is the primitive mechanism that first appeared in Metazoa (Gremigni and Nigro, 1983), is discussed in the present study.

2. Material and methods

2.1. Sampling

Sexually mature specimens of *E. celerrima* were collected under rocks at the Lake of Tunis, a natural lake situated between the city of Tunis and the gulf of Tunis (36°48′24.40″N; 010°14′54.53″E). The lake of Tunis with an area of 4500 ha is a highly eutrophic, saline lake, with water depths that seldom exceed 2 m.

2.2. Preparation for microscopy

2.2.1. Light microscopy

Mature animals were fixed in Bouin's fixative and embedded in paraffin. The histological sections were prepared at intervals of 6–8 μm and stained in eosin and toluidine blue or Mallory–Cason stain.

2.2.2. Transmission electron microscopy (TEM)

Worms were fixed overnight at 4 °C in 3% glutaraldehyde in 0.1-M phosphate buffer (pH 7.2). After fixation, the material was post-fixed in 1% osmium tetroxide in the same buffer for 2 h at room temperature, dehydrated using ascending grades of ethanol series, impregnated in propylene oxide and resin mixture, and embedded in pure resin. The semi-thin sections were cut and stained with 1% toluidine blue. The ultrathin sections of silver shades (60–70 nm) were cut using an ultra-microtome (Leica, UCT) equipped with a diamond knife; sections were then placed on copper grids and stained with uranyl acetate (20 min) and lead citrate (5 min). Stained sections were observed with a TEM (JEOL JEM-1011) operating at 80 kV. Both the micrographs and electron micrographs were digitized using Adobe Photoshop by adjusting the contrast and the brightness balance.

3. Results

3.1. Light microscopy

As in all polyclad flatworms, ovaries of *E. celerrima* are numerous. They occur laterally throughout the dorsal parenchyma (Fig. 1A) and are scattered between the digestive ramifications (Fig. 1B). They form individual rounded follicles of approximately 40–70 μm in diameter (Fig. 1B). Each ovarian follicle is surrounded by a proper basal lamina and is filled with germ cells at various stages of differentiation. We can distinguish two parts inside every follicle. The germinative zone is located at the dorsal part of the ovary and contains few young germ cells, whereas the remaining ventral part of the gonad is filled with developing oocytes (Fig. 1B and C).

3.2. Electron microscopy

Each ovary is delimited from adjacent tissues by a homogeneous electron-dense basal lamina (Fig. 2A). The accessory cells are found at the periphery of the ovary in contact with the basal lamina and extended between oocytes (Fig. 2B). The cytoplasm of these cells and its extensions are rich in ribosomes and some electron-dense masses. The intercellular junctions present between accessory cells and oocytes are very tight and cannot be distinguished easily.

The oogonia and immature oocytes have an oval and a relatively large nucleus (approximately 13 μm in diameter) with diffuse chromatin and a well-structured nucleolus of approximately 5 μm in diameter (Fig. 2A). Their poorly differentiated cytoplasm contains numerous ribosomes and some mitochondria with a translucent matrix surrounding dense masses in the perinuclear area of the cytoplasm (Fig. 2C). Some translucent vesicles are also observed.

The ventral part of each gonad contains growing previtellogenic oocytes at different stages of maturation. These cells undergo an increase in their cytoplasmic volume, which is gradually packed