

Endocytotic Activity during the Cortical Granules Genesis in Marine Planarian Oocytes (Platyhelminthe, Tricladida)

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Introduction

Cortical granules are membrane-bound, electron dense cell inclusions known in oocytes of many animals, invertebrates [1-5] and vertebrates [6]. They are distributed in the peripheral ooplasm forming a monolayer beneath the plasma membrane [1,3,5]. These organelles are interpreted as Golgi complex productions [1,3,4,7,8]. In some metazoan (as crustacean), it is suggested that mitochondria take part in the origin of cortical granules [3]. Cortical granules contents differ among species (glycoproteins, mucopolysaccharide etc.) but their functions of preventing polyspermy remain similar [3,5,9,10] in the fertilization process. In planarians (Platyhelminthes, Tricladida), these inclusions are signalized for several species [4]. We present here an ultrastructural analysis of their genesis process in the marine gonochoristic triclad *Sabussowia dioica* where we demonstrate a novelty represented by the contribution of accessory cells in the formation of their peripheral content using an endocytotic process.

Material and Methods

The ovary of the gonochoristic planarian *S. dioica* is studied by light and transmission electron microscope as detailed in [1].

For morphological studies, mature females specimens were fixed with 4% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 2 h at 4°C. They were then postfixed in 2% OsO₄ in the same buffer for 1 h at room temperature, dehydrated in a graded series of ethanols and embedded in Spurr or Epon -Araldite resins. Semi thin sections (1-2 µm thick) were stained with 1% toluidine blue and 1% methylene blue in 1% sodium tetraborate solution. Ultrathin sections obtained with a diamond knife, were stained with uranyl acetate and lead citrate and examined with a Jeol 100 SX transmission electron microscope.

The cytochemical investigations for glycoproteins and polysaccharides are made using the Thiéry method: Ultrathin sections obtained from blocs used for morphological studies were firstly incubated in 2% thiocarbohydrazide (TSH) in 2% acetic acid for 1-72 h and then in 1% silver proteinase for 30 mn in the dark and finally observed without further contrast staining.



Figure 1: *Sabussowia dioica* Ovary structure on semithin section showing Growing germ cells (Gc) and accessory cells (Heads of arrows). Scale bar =50 µm.

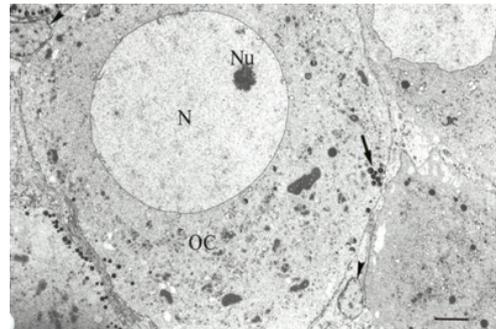


Figure 2: Growing oocyte (OC) and accessory cells (heads of arrows). A cluster of cortical granules is seen (Arrow). N: nucleus; Nu: nucleolus. Scale bar =5 µm.

Results

The ovary structure

It is made by growing germ cells surrounded with elongated accessory cells (Figures 1 and 2).

Oocytes show several stages of development

- The young oocytes exhibit a smooth surface and a developed Golgi apparatus (Figure 3).
- In intermediate stages, small granules are scattered through the ooplasm (Figures 2 and 4).
- The ooplasm of submature and mature oocytes contains two types of inclusions: fibrogranular material surrounded by a double smooth membrane scattered in the whole ooplasm (Figure 5) and small granules (0.5- 0.7 µm in diameter).
- In Mature oocytes, the small granules were located in a peripheral monolayer. Irregular wall with invaginations (Figures 4 and 6) and pinocytotic vesicles (Figures 4, 5 and 7) were noticed.

Cortical Granules Genesis

In *S. dioica*, cortical granules are characterized by a central fenestrated area of medium electron density embedded in a homogeneous component surrounded by a narrow cortex (Figure 8).

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In Young and intermediate stages growing oocytes, the developed Golgi apparatus (Figure 3) is involved in the production of small vesicles (Figure 9) that the repeated coalescence gives rise to roundish membrane bounded granules of medium electron density scattered through the ooplasm of intermediate stages (Figures 2 and 9).

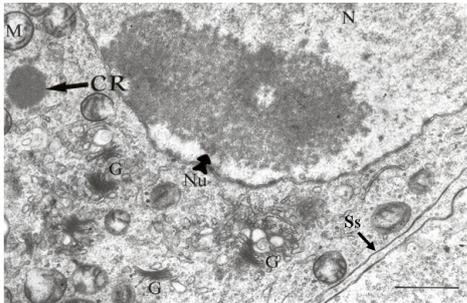


Figure 3: Part of a young oocyte showing a developed Golgi apparatus (G), a smooth surface (Ss). CR: Chromtoid body, N: nucleus, Nu: Nucleolus. Scale bar = 1 μ m.

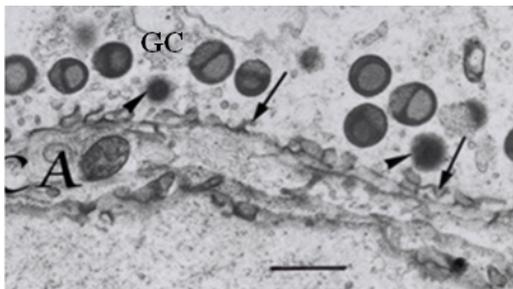


Figure 4: Cortical granules (GC), pinocytotic vesicles (Head of arrows) and invaginations (arrows) in the periphery of a mature oocyte. CA: accessory cell. Scale bar = 1 μ m.

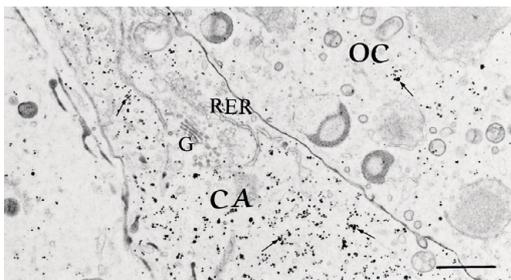


Figure 5: Golgi complex (G), RER and glycogen particles (thin arrows) in accessory cell (CA) close to a submature oocyte (OC).

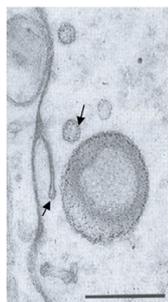


Figure 6: endocytotic vesicles and long invagination (arrows) of the plasma membrane near a forming cortical granule. Scale bar = 0,5 μ m.

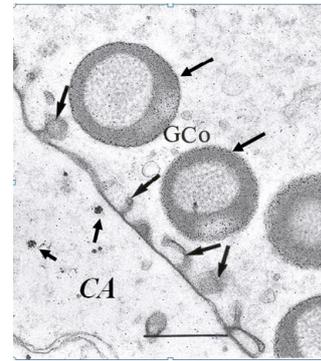


Figure 7: glycogen particles in the cytoplasm of accessory cells (CA) and in the cortical Granules (GCo), particularly in the peripheral dense component and in the invagined Vesicles (arrows). Scale bar = 0.5 μ m.

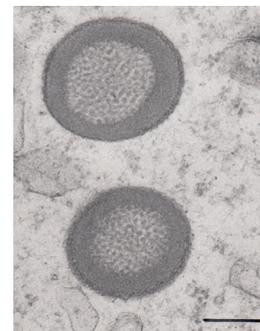


Figure 8: Cortical granules structure. Scale bar = 0.25 μ m.

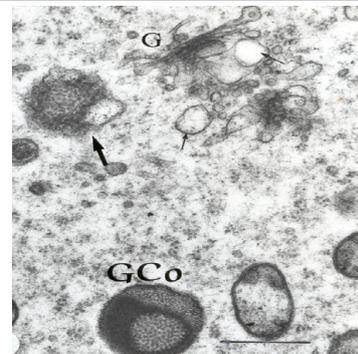


Figure 9: Part of intermediate stage Oocyte showing Golgi productions (thin arrows), a nascent granule (thick arrow) and cortical granules scattered in the ooplasm (GCo.). Scale bar = 1 μ m.

Submature and mature oocytes (OC) surface shows many irregularities and invaginations corresponding to endocytotic vesicles coming from the accessory cells (CA, Figures 4 and 5). The former, exhibit developed RER, Golgi complex and glycogen particles. Endocytotic vesicles fuse with cortical granules and participate to their peripheral component (Figures 5, 6 and 10).

The Thiéry cytochemical test shows glycogen particles more numerous in the cytoplasm of accessory cells that in the oocytes (Figure 5). In oocytes, glycogen particles are located in cortical granules, particularly in the peripheral dense component and in the invagined vesicles (Figure 7).

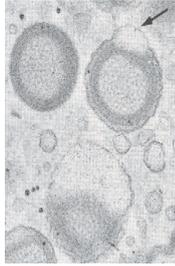


Figure 10: Fusion of Endocytotic vesicles (arrows) with cortical granules. Scale bar = 0.5 μ m.

Discussion

Cortical granules are usually interpreted as endogenous Golgi complex productions [1,3,4,7,8]. In some metazoan (as crustacean), it is suggested that mitochondria take part in the origin of cortical granules [3]. The novelty discovered here is the contribution that seems to come from endocytotic activity at the oocyte surface from the accessory cells, particularly the glycogenic peripheral component. Among Platyhelminthes, an endocytotic activity related to yolk formation has been described [11] and another during embryonic development [12]. Details are given in ref. [1,13].

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