SOMATIC CELLS SUPPORTING GAMETOGENESIS IN DEEP-SEA CLAM *CALYPTOGENA PACIFICA*

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Introduction

In bivalve molluscs, as well as in other Metazoa, the developing germ cells are closely associated with specialized somatic cells (Guraya 1995, 1998). These are intraacinar accessory (in males) or follicular (in females) cells, which create a specialized microenvironment around developing germ cells and participate in regulation of their development (Pipe 1987; Eckelbarger, Davis 1996a, 1996b; Yurchenko, Vashchenko 2010). In addition to accessory and follicular cells, there are interacinar cells, mainly, adipogranular (ADG) cells and cells of vesicular-connective tissue (VCT-cells), that are crucial for nutrient accumulation and storage (Lowe et al. 1982; Peek, Gabbott 1990; Mathieu, Lubet 1993). Some bivalve molluscs accumulate nutrients in the muscles and use these nutrients to supply germ cells development (Mathieu, Lubet 1993, Galap et al., 1997, Barber and Blake, 2006; Vite-García and Saucedo, 2008).

Species of the genus Calyptogena are often dominant in deep-sea communities, reaching a density of up to several hundreds of individuals per square meter (Barry et al. 1996, 2007; Fujikura et al. 2002). These molluscs have reduced digestive system and maintain chemosynthetic bacterial endosyrnbionts in their gills. It significantly differs the Calyptogena genus from other bivalves. At the same time, their fecundity is rather high (Berg 1985 in Fiala-Medioni and Le Pennec, 1989) that allow suggesting well-developed “infrastructure” to support gametogenic processes.

This study aims to describe somatic components in the gonadal area of a deep-sea bivalve mollusk *Calyptogena pacifica* empathizing interacinar connective tissue cells, female and male intracinar cells.

Material and Methods

Mollusks were collected using a Comanche 18 remotely operated vehicle from the southern summit of the Piip submarine volcano (55.3821° N, 167.2613° E), located within the Vulkanolog Rise, Bering Sea,at a depth of 463 m during the expedition of the A.V. Zhirmunsky National Scientific Center of Marine Biology, Far Eastern Branch, Russian Academy of Sciences (NSCMB FEB RAS), aboard the R/V Akademik M.A. Lavrentyev (cruise LV-82) in June 2018. For light and electron microscopy the material was prepared in order to standard technique. Semi-thin sections were stained with methylene blue–azure 2 basic fuchsine

Results and Discussion

The gonad consists of numerous acini united by an excurrent duct. On a section, we found acini at various stages of reproductive cycle (active gametogenesis or pre-spawning) that was not described in shallow-water species with a clear reproductive cycle.

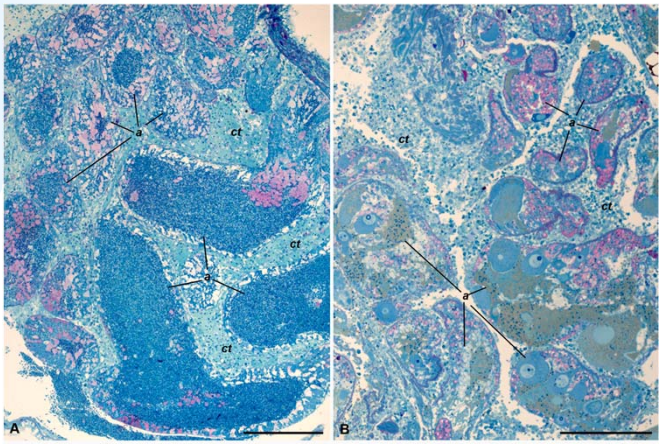

Fig. 1. General morphology of gonad tissue. Light microscopy. (A) Overview of male gonad area. Numerous acini (*a*) are surrounded by connective tissue (*ct*). Note the acini with high or low content of spermatozoa. (B) Overview of female gonad area with numerous acini with sex cell at the various stage of their development. Scale bars: A,B = 200 μm

The morphology of accessory cells in males is very variable. Sometime these cells have enlarged basal part spanning up to several tens of micrometers. On the other hand, bodies of accessory cells often extended towards the acinus lumen. The developing germ cells located in small pockets along the accessory cells. Accessory cells were described in molluscs, but they were significantly smaller (Eckelbarger and Davis, 1996, Yurchenko and Vashchenko, 2010).

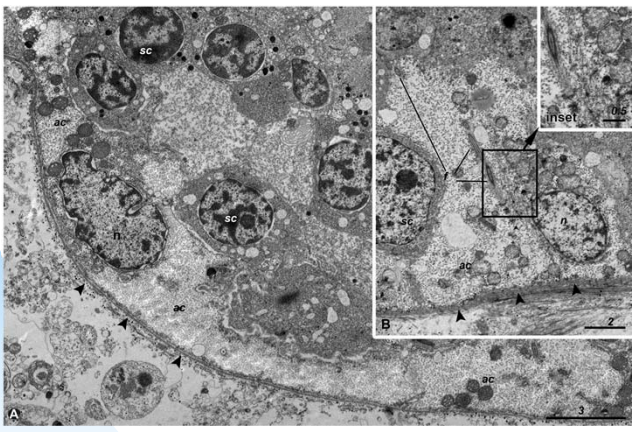

Fig. 3. Male intraacinar accessory cells. TEM. (A) An accessory cell (ac) located along an acinus wall (arrowheads), beneath spermatogenic cell (sc). (B) Accessory cell with flagellum (f); body of the cell directed toward an acinus lumen. Arrowheads point to acinus wall. (inset) high magnification of the basal part of the flagellum. Scale bars: A = 3 μm; B, C = 2 μm

There are several types of haemocytes, namely, erythrocytes and granulocytes, in the interacinar space in both males and females. There were no any other cell types similar to ADG-cells or VCT-cells.

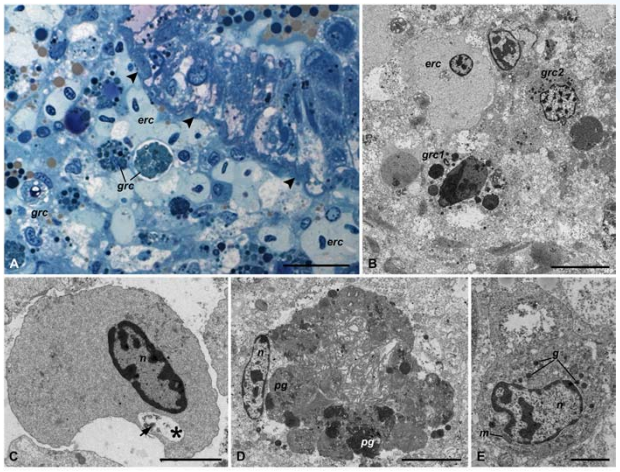

Fig. 2. Morphology of haemocytes in gonad area. (A) Light microscopy, (B-E) TEM. (A) Erythrocytes (erc) and granulocytes (grc) near a female acinus (arrowheads). (B) Haemocytes with various morphology: erythrocytes and two types of granulocytes (grc1, grc2). (C) Erythrocyte with discoid nucleus (n), homogenous flacked cytoplasm and a vacuole (asterisk) contained electron-dense material (arrow). (D) Granulocyte I with peripherally located elongated nucleus and cytoplasm filled with numerous phagosomes (pg). (E) Granulocyte II contained mitochondria (m) and small electron-dense granules (g). Scale bars: A = 20 μm, B = 5 μm, C, D = 3 μm, E = 2 μm

In female, the somatic accessory cells are located along the acinar wall and do not form follicle s around developing oocytes. Their shape varies from cuboidal to slightly elongate. In general, somatic cells arranged into two layers in the proximity of privitellogenic oocytes and form a single layer beneath vitellogenic oocytes. Endoplasmic reticulum is well-developed and could occupy till half of cytoplasm volume..

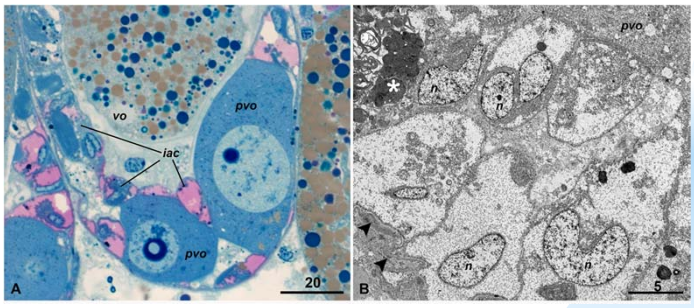

Fig. 4. Female intraacinar accessory cells. (A) Light microscopy, (B) TEM. (A) Overview of female acinus with previtellogenic (pvo), vitellogenic oocytes (vo) and somatic cells (iac). (B) Two-layer somatic cells with variously shaped nuclei (n) and moderately developed endoplasmic reticulum (er); the cells are located between acinus wall (arrowheads) and previtellogenic oocyte. Asterisk indicates a phagosome . Scale bars: A = 20 μm; B = 5 μm

Thus, *C. pacifica* demonstrates a well-developed system of somatic cells involved in gametogenesis. All cell types presented in *C. pacifica* have been described in shallow-water molluscs (Franco et al., 2010; Agnese et al., 2013, Rosati, 2018, etc) however, the studied species showed the absence of interacinar stationary (ADG-, VCT) cells and typical intraacinar female follicular cells. As it has been shown in pectinides, haemocytes during gametogenesis, deliver nutrients from an adductor muscle or intestinal loop (the main storage places in the scallops) to the acini with germ cells (Le Pennec et al., 1991). Because no interacinar stationary cells and other places of nutrient deposition were observed in C. pacifica, the transport of nutrients is probably carried out from the gills by haemocytes circulating in a highly developed haemoceole. The presence of specific somatic cells in the female acini of *C. pacifica*, different from the typical follicular cells, does not exclude their involvement in vitellogenesis. This is evidenced by intercellular contacts between oocytes and somatic cells and a highly developed endoplasmic reticulum.

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