

Notes on the ultrastructure of female germ cells in the adult ovary in *Argulus japonicus* (Crustacea: Branchiura)

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We have studied the adult and larval ovaries of a branchiuran crustacean, *Argulus japonicus*, and ascertained that the growing oocytes are located not in the ovarian lumen but on the outside of the ovarian wall, protruding into the hemocoel. We have also found some anatomical and histological similarities in the ovarian structure and oogenetic mode of *A. japonicus* to those in chelicerates and pentastomids rather than to those in other crustaceans (Ikuta and Makioka, 1993, 1994). However, we have no comparative ultrastructural studies between these animals, because of lack of ultrastructural knowledge on *A. japonicus* ovaries. The present study provides ultrastructural descriptions on the female germ cells of adult *A. japonicus* ovaries.

The adult females of *A. japonicus* were collected from the body surfaces of mirror carps reared in the Ibaraki Prefectural Freshwater Experimental Station. They were dissected with razor blades and their ovaries were fixed with Karnovsky's solution followed by 1% OsO₄, with 2.5% glutaraldehyde followed by 1% OsO₄, or only with OsO₄. Small pieces of the fixed ovaries were embedded in low viscosity epoxy resins, and made into ultrathin sections, which were stained with uranyl acetate and lead citrate, and then observed under a transmission electron microscope.

The youngest germ cells in the germarium have few organelles in the cytoplasm, and their large nuclei have a prominent nucleolus and dispersed chromatin particles (Fig. 1). We have not yet observed mitotic figures of these germ cells, but found a cytoplasmic bridge between these cells, which may possibly be a telophasic figure of the oogonial cell division (Fig. 1, inset).

Growing oocytes of various sizes swarm around the outer surface of the ovarian wall, covered by an extended basement membrane of the ovarian epithelium. Many microvilli develop around the surfaces of the previtellogenic oocytes. Some aggregations of mitochondria are found in the cytoplasm near the germinal vesicle, and many small vesicular structures in the periphery (Fig. 2).

In the perinuclear region of the early vitellogenic oocytes, some large lipid droplets are formed before occurrence of the proteinaceous yolk granules. The proteinaceous yolk granule consists of the peripheral thin layer and central electron-dense core (Fig. 3). The larger the yolk granules are, the thinner the peripheral layer is (Figs. 4, 5). The late vitellogenic oocytes are filled with the large yolk granules and with numerous fragments of the lipid droplets (Figs. 4, 5). The yolk granules and lipid droplets are more concentrated in the central region of the oocytes (Fig. 5) than in the peripheral region (Fig. 4). Mitochondria and rough endoplasmic reticulum are predominantly found in the periplasm (Fig. 4).

The mitotic activity was not demonstrated in the youngest germ cells with nucleoli in the nuclei, but the presence of a cytoplasmic bridge between these cells is thought to represent their oogonial nature.

The growing oocytes have been confirmed to be sandwiched by the ovarian epithelium and its basement membrane at the electron microscopic level. Such a condition of growing oocytes as found in *A. japonicus* may be unique among crustaceans but popular in pentastomids (Nørrevang, 1972; Walldorf and Riehl, 1985) and chelicerates (Makioka, 1988).

Characteristic aggregations of mitochondria in the previtellogenic oocytes of *A. japonicus* closely resemble those in some pentastomids, which are called Balbiani bodies or yolk nuclei (Nørrevang, 1972; Walldorf and Riehl, 1985). The presence of developing microvilli in the periplasm of late previtellogenic and vitellogenic

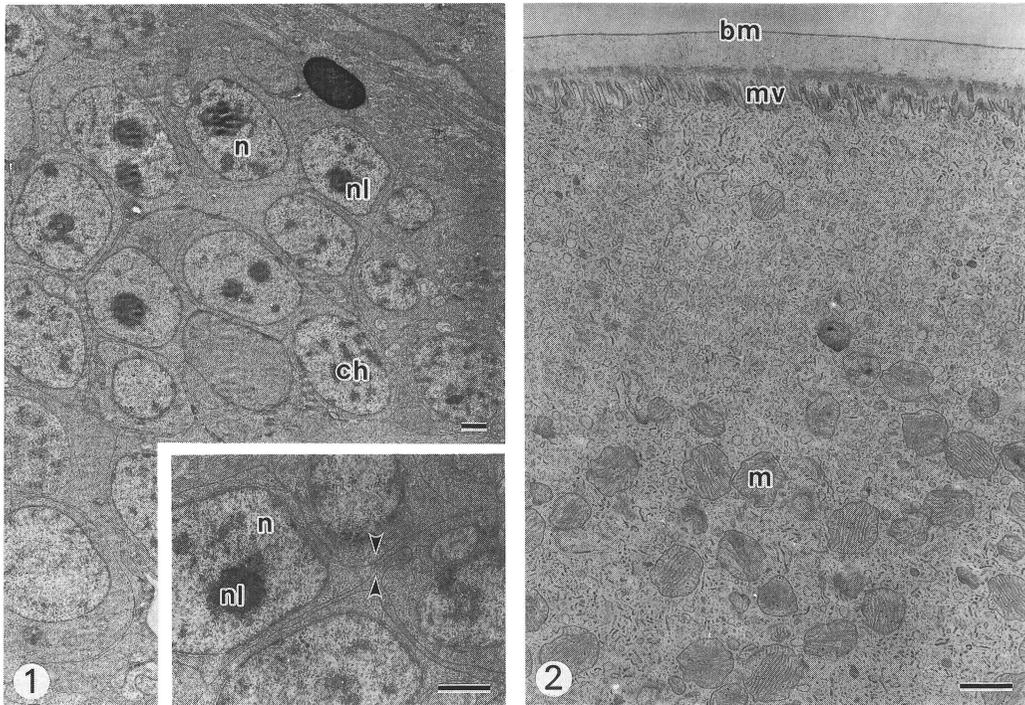


Fig. 1 Electron micrograph of germarium in adult *Argulus japonicus* ovary. Glutaraldehyde (GA)-OsO₄ fixation. Inset: cytoplasmic bridge of oogonia (arrowheads).

Fig. 2 Previtellogenic oocyte. OsO₄ fixation.

bm: basement membrane, ch: chromatin, m: mitochondrion, mv: microvilli, n: nucleus, nl: nucleolus. Bars = 1 μ m.

oocytes suggests that the oocytes take yolk precursors from the hemolymph by themselves. The absence of auxiliary cells, nurse cells or follicle cells (Ikuta and Makioka, 1993), may support this idea.

Thus, we found the close resemblance in some aspects of oogenesis between *A. japonicus* and pentastomids, and this may suggest the closer phylogenetic affinity between the branchiurans and pentastomids.

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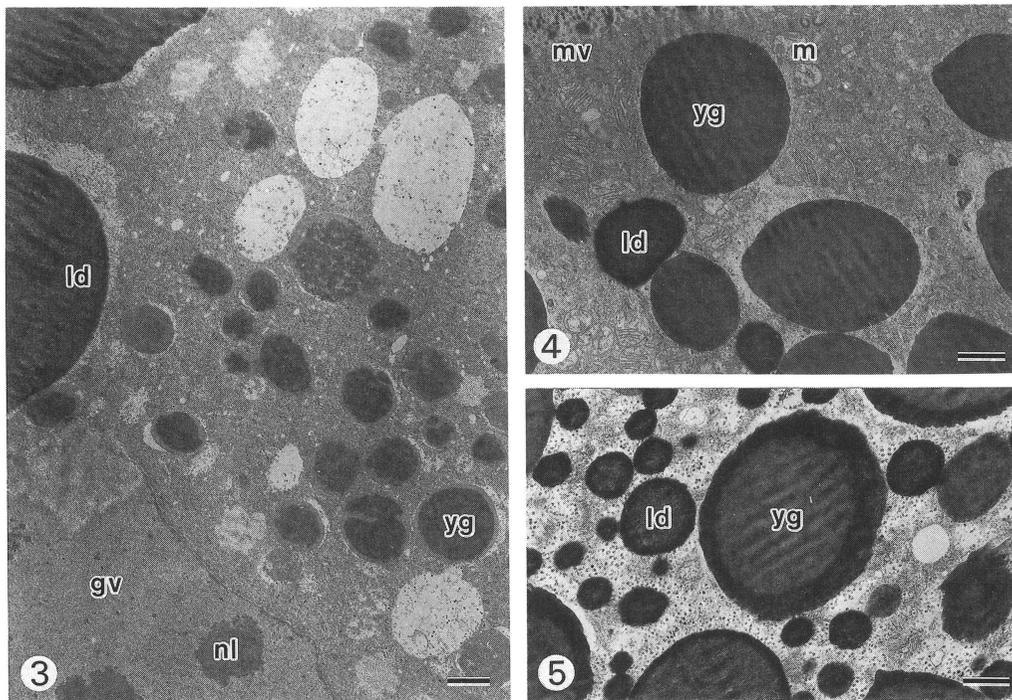


Fig. 3 Perinuclear region of early vitellogenic oocyte in adult *Argulus japonicus*. Karnovsky's solution-OsO₄ fixation.

Fig. 4 Peripheral region of late vitellogenic oocyte. GA-OsO₄ fixation.

Fig. 5 Central region of late vitellogenic oocyte. GA-OsO₄ fixation.

gv: germinal vesicle, ld: lipid droplet, m: mitochondrion, mv: microvilli, nl: nucleolus, yg: yolk granule. Bars = 1 μ m.