Morphology and Regional Distribution of the Primordial Germ Cells in the Giant Freshwater Prawn, *Macrobrachium rosenbergii*

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ABSTRACT Primordial germ cells (PGCs) of early developing embryos of *Macrobrachium rosenbergii* were detected at 1.5-2.5 days of development. They were large round cells containing a large nucleus with prominent nucleoli and granulated cytoplasm. They were not reactive to periodic acid-Schiff reaction and showed negative alkaline phosphatase activity. In 6.5-day-old embryo, a cluster of PGCs occupied the dorso-medial region behind the yolky portion. In 14.5-day-old embryo, they were observed at the posterior of the heart. The PGCs were not observed either in the testis or ovary. Only type I spermatogonia showed certain resemblance to the embryo PGCs.

KEYWORDS: Primordial germ cell, embryo, ovary, testis, *Macrobrachium rosenbergii*.

INTRODUCTION

Primordial germ cells (PGCs) are progenitor cells for gametes. The morphology and histochemical properties of the PGCs from embryos of vertebrates such as chicks,[1-2] quails,[3-4] frogs,[5] carp,[6] mice,[7] marsupials[8] and humans[9] have been reported. The PGC is characterized by its large size and round shape, and by the large round nucleus with conspicuous nucleoli. The studies on the ultrastructure of PGCs of vertebrates showed that they contained heterochromatic nuclei.[3, 6, 9] The cytoplasm contained abundant ribosomes and mitochondria, poorly developed RER and Golgi complex.[3, 6, 9] It is of great interest that the PGCs which show similar cytological characteristics in all vertebrate species, nevertheless show certain differences in immunohistochemical properties.[10, 11] Human and chick PGC contain a large amount of glycogen granules and lipid droplets in the cytoplasm.[1, 2, 9] In quail PGCs, there is no glycogen granule in the cytoplasm, but considerable amount of lipid vesides were found.[3-4]

Little information is available on the PGCs of invertebrates. The studies on PGCs of fruit flies, *Drosophila melanogaster,*[12-13] and nematodes, *Caenorhabditis elegans*[14] showed similarities in morphology to that of vertebrate species. Because of their potentials for genetic manipulation, the *in vitro* culture of PGCs[15-17] and production of germline chimeras by transferring of PGCs to the recipient embryos[18-23] have been extensively studied.

Macrobrachium rosenbergii, the giant freshwater prawns, are one of the most important economic animals in Thailand. The methods of cultivation had already been established for a number of years. However, the studies on genetic manipulation and *in vitro* culture are still limited. Very little is known on the crustacean PGCs which have a great potential for genetic manipulation. Hence, the present study reported on the morphology and regional distribution of the PGCs in developing embryos of *M. rosenbergii*. In addition, the ovaries and testes were also examined for the presence of PGCs.

MATERIALS AND METHODS

Mature giant freshwater prawns, *M. rosenbergii*, were obtained from a prawn farm in Supanburi Province, Thailand. They were acclimatized in large fiberglass tanks under a controlled temperature at 26°C for 2 days before transferring to individual chambers and laboratory-maintained as previously described.[24] Mating and oviposition were allowed under laboratory conditions to ensure the accurate timing of developing embryos. Oviposition usually occurred at night (8.00-12.00 p.m). Embryos on the day after oviposition were timed as 0.5-day-old.

Clusters of embryos at various days after oviposition were collected from the abdomens of the brooding females. The embryos were placed on a glass slide and covered with a coverslip. The morphology of the PGCs was examined with a phase-contrast

microscope. Cytochemical staining for periodic acid-Schiff (PAS) reaction was prepared. The embryos were smeared and air-dried before fixing with formalin-ethanol fixative for 5 min. The slides were rinsed in slowly running tap water for 1 min and were then incubated in 1% periodic acid for 15 min. After several changes of deionized water, the slides were stained in Schiff reagent for 10 min. They were then washed in 3 successive changes of tap water and counter-stained briefly with hematoxylin. For the assessment of alkaline phosphatase activity, the air-dried slides were fixed with citrate-acetone-formaldehyde fixative and stained for the activity using the alkaline phosphatase reagent kit (Sigma) and the methods according to the manufacturer's protocol.

Embryos of early stage of development at 6.5 days, and late stage at 14.5 days after oviposition, were collected. Testes from mature males at pre-molting stage, which were spermatogenically active[25] were removed. Ovaries at stage 1 of the ovarian development which had pale orange color and contained all developmental stages of oocytes,[24] were dissected from the mature females. Developing embryos, testes and ovaries were processed for histological study. Specimens were fixed in Bouin's fluid for 24 h at room temperature. Then, they were dehydrated in a graded series of ethanol, infiltrated and embedded in paraffin. Sections of 7 μm thick were cut on a rotary microtome and stained with hematoxylin and eosin. Staining for PAS reaction was also repeated histochemically.

Results

PGCs of early developing embryos are easily distinguished from other somatic cells under the phase-contrast microscope. They can be detected in embryos as early as 1.5-2.5 days of development. They are large, relatively spherical cells with the size of 15-18 μm in diameter (Fig 1). The nucleus of the PGC is large and comprises 2-4 prominent nucleoli and the cytoplasm is granulated (Fig 1). Neither PAS reaction nor alkaline phsophatase activity was detected in the PGCs of developing embryos. A cluster of PGCs occupy the dorso-medial region behind the yolky portion of the 6.5-day-old embryos. Two strands of PGCs leading from the yolky portion toward the posterior region can be detected (Fig 2). At 14.5 days of development, a mass of PGCs, which will eventually coalesce with the primordium of the gonad, is observed at the posterior of the heart (Figs 3, 4). The morphology of the PGCs at 14.5 days of development slightly differs from that of the early stages. They are relatively smaller and are not as strikingly different from the somatic cells as those of the early stages (Fig 4).

Spermatogonia resembling PGCs observed in the embryos are also detected in the testicular tubes from the mature males. A zone comprised of two different types of spermatogonia (type I and type II) occupies the border on one side of the testicular tube, while the spermatocytes of different meiotic stages fill the inner part of the tube (Fig 5). Type I spermatogonia which resemble PGCs in the embryos seem to be the precursor cells for type II spermatogonia. Type II spermatogonia can be distinguished from type I spermatogonia by exhibiting the absence of prominent nucleoli and increased clumping of chromatins which are closely associated with the nuclear membrane (Fig 6). Active mitotic activity of the spermatogonia is frequently observed (Fig 7).

Ovaries of *M. rosenbergii* are divided into lobules. Oogonia and all stages of the oocytes can be detected in each lobule (Fig 8). The oogonia and the pre-vitellogenic oocytes containing sparse cytoplasm are located at the internal region of the ovary (Figs 8-9), while the vitellogenic oocytes containing a large amount of cytoplasm are arranged at the external region. The mature oocytes are positioned at the most external region of the ovary. The morphology of the oogonia resembles that of the type II spermatogonia. They are characterized by their short blocks of heterochromatin which are closely associated with the nuclear membrane (Fig 9). The oogonia containing prominent nucleoli are occasionally found.

Discussion

PGCs of the giant freshwater prawn, *M. rosenbergii*, are separated from the somatic cell lines early in the embryonic stage. Predetermination of the PGCs during early stages of development is common in invertebrates. They are generated within the first hour after the onset of cleavage in nematode, *C. elegans*[14] and are separated cells prior to the formation of somatic cells in *D. melanogaster*.[12] Formation of the PGCs in vertebrates commences later. Chick PGCs are formed at blastodermal stage,[2,4] while mammalian PGCs first appear at the primitive streak stage.[7, 9]

During early stages of embryonic development, PGCs of the giant freshwater prawn, *M.rosenbergii,* are easily discernable. Their morphology is similar to those of other vertebrates and invertebrates described so far.[1-2, 7, 9, 12] They are large, round cells containing a large nucleus with conspicuous nucleoli. However, in PGC of *M. rosenbergii*, the cyto-

plasm is granulated, resembling those observed in the insect, *D. melanogaster*,[12, 26] and quails.[3] The presence of RNA in the granules has been described in *D. melanogaster*,[12] The PGCs of chicks and mammals such as mice, do not show such characteristic, but, rather, contain large amount of lipid vesicles.[9, 27-28]

Cytochemical studies were performed on the PGCs of chicks, mice and human.[1-2, 7, 9, 29] Chick PGCs could be cytochemically identified by their significant amount of glycogen content which was positive with PAS reaction.[1-2, 29] Strong PAS activity had been reported in the PGCs of human embryos[9] but only a slight reaction was found in those of mice[7]. In addition, positive alkaline phosphatase activity, the characteristic of mammalian PGCs [9, 30] was not detected in the PGCs of the giant freshwater prawn.

In quails, the PGCs contain granulated cytoplasm and show a negative reaction with PAS indicating a lack of glycogen content.[3, 4] The PGCs of the giant freshwater prawn also show granulated cytoplasm similar to that of the quail PGCs. This may implicate the similarity in the mechanism of the PGC migration. Low level of glycogen, reflecting a low level of energy source, in mouse PGCs has been suggested to be the cause of their passive migration along with the morphogenetic movement during gastrulation.[31] PGCs migrate across the gut primordium and assemble into the gonad primordium where active increase in the number by mitotic division[32] occurs.

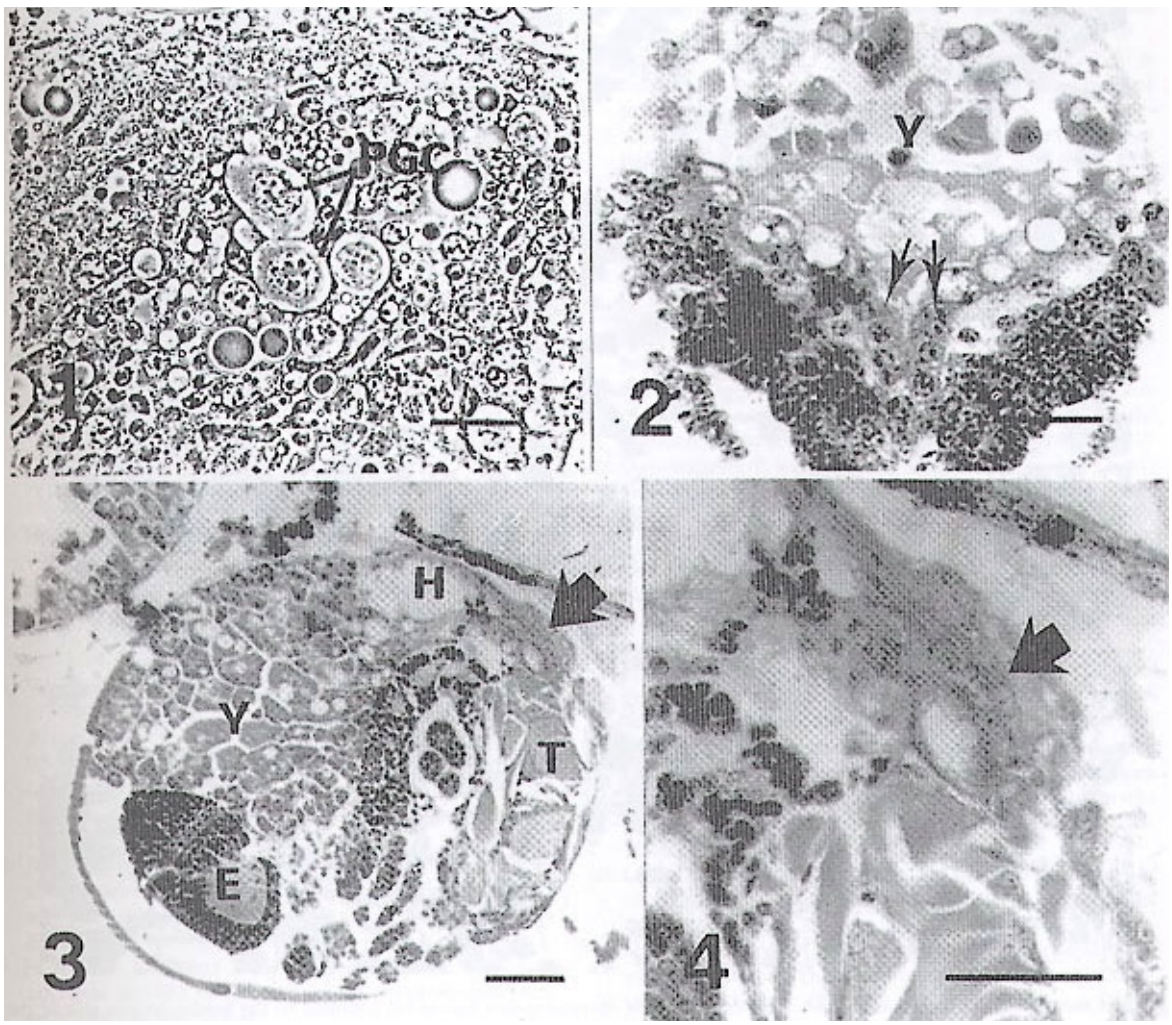

Fig 1. Phase-contrast micrograph of PGCs from 6.5-day-old embryo. The cells (PGC) are large and contain granulated cytoplasm. The nucleus contains 2-3 dense nucleoli. The surrounding cells are strikingly smaller than the PGCs. Bar = 20 μm.

Fig 2. Horizontal section of 6.5-day-old embryo. A cluster of PGCs are found at the dorso-medial region behind the yolk (Y). Two strands of PGCs leading posteriorly can be detected (arrows). The yolky portion (Y) is located anteriorly. Bar = 50 μm.

Fig 3. Longitudinal section of 14.5–day-old embryo. A mass of PGCs (arrow) are located behind the heart (H). The yolky portion (Y) is found dorsally behind the eye (E) and the tail muscle (T) curves postero-ventrally. Bar= 50 μm.

Fig 4. High magnification of Fig 3 showing the mass of PGCs (arrow) behind the heart. Bar = 20 μm.

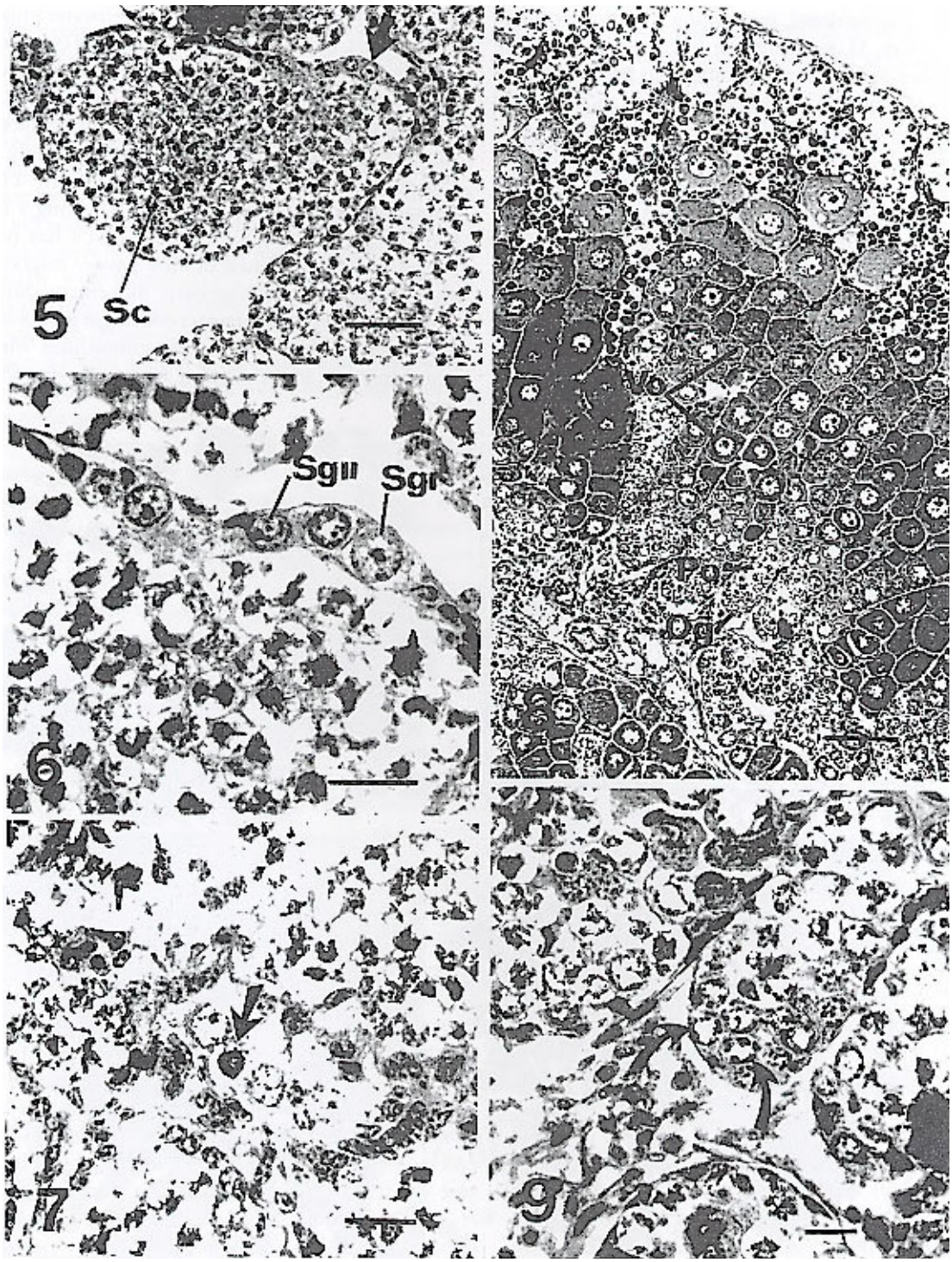

Fig 5. Cross section of a testicular tube. A zone containing spermatogonia (arrow) is confined to the border on one side of the testicular tube. The rest of the tube, the lumen, is filled with spermatocytes (Sc). Bar = 50 μm.

Fig 6. High magnification of the peripheral zone containing two types of spermatogonia. Type I spermatogonia (SgI) contain prominent nucleoli while type II spermatogonia (SgII) lack this structure. Bar = 20 μm.

Fig 7. Cross section of a testicular tube showing the region of mitotically active spermatogonia (arrow). Bar = 20 μm.

Fig 8. Cross section of an ovary comprising all stages of oocytes and oogonia. The oogonia (Og) and the previtellogenic oocytes (Po) are located in the middle portion. The vitellogenic oocytes (Vo) are found in the external region of the ovary. Bar = 100 μm.

Fig 9. High magnification of the middle region of ovary (from boxed area in Fig 8) showing oogonia (arrows) which are characterized by dense chromatins associated with nuclear membrane. Bar = 20 μm.

The present study also reported on the morphological characteristics of spermatogonia in the testis and oogonia in the ovary. And comparison of the PGCs to spermatogonia and oogonia may indicate their presence in either the testis or the ovary. In the testis, type I spermatogonia were found to resemble PGCs in the embryos. In the ovary, the oogonia, which contained prominent nucleoli, were rarely found. It is apparent that the ovary did not contain PGCs. Even though certain oogonia showed some resemblance, but they were present in such a small number.

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