Early Embryonic Development of Golden Apple Snail *Pomacea canaliculata* (Lamarck, 1822)

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Abstract

In histological study, the first cleavage division occurred after laying the egg mass and this cleavage type of zygote or fertilized egg was holoblastic type with unequal in both vertical and horizontal plane. Then, the first cleavage (2-celled embryo) underwent into second unequal division of cleavage until the embryo reached the 4-cell stage. After that, those cells still continued to undergo the cleavage and gave rise a mass of unequal cells which was called morula. After morula stage, the embryo developed into blastula stage that had no blastocoel and was stereoblastula. In blastula stage, there was an increase in the number of small blastomeres. They spread over and invested in the large blastomeres by the method of epiboly which resulted in the formation of gastrula stage.

Key Words: blastomere, zygote, morula, blastula, gastrula

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Introduction

The developing embryos of *Pomacea canaliculata* utilized some material within the shells as a source of nutrition because the eggs contained carotenoproteins (Heras et al., 2007), so the eggs hatched in the form of young snails (Kume and Dan, 1968). In addition, they were found that egg development was divided into many stages such as morula, blastula, gastrula, organogenesis, juvenile and adult stage. However, the early development of molluscan egg was described by Conklin (1897). He studied the development of *Crepidula* and reported that the egg was divided into four blastomeres according to the spiral mode of cleavage. The four blastomeres divided into eight blastomeres (four macromeres and four micromeres).

The four micromeres were on the animal pole side (upper part) of four macromeres. Each blastomere divided again. The macromere divided into macromere and micromere whereas micromere gave rise into micromeres. The group of blastomeres was called morula stage. From Kume and Dan (1968) they reported that blastocoel formation of blastula stage occurred in egg with little yolk was called coeloblastula. If the blastocoel was very small, the blastula was called stereoblastula. After blastula stage, the embryo developed into gastrula stage. In this stage, the embryo was composed of an inner and an outer cell layer. The outer cell layer became the ectoderm while the inner cell layer became the endoderm of adult snail (Kume and Dan, 1968).

At present, there is not any reports about embryonic development of *P. canaliculata*, especially the stage of early development. So the results of this study will be useful to students who study about invertebrate development. Moreover, the farmers will protect their rice seedlings from damaging by young snails, if they learn about the development of *Pomacea canaliculata*.

Materials and Methods

Preparation of Embryos

The snails, *Pomacea canaliculata* were collected from rice field in Khet Bang khen, Bangkok, Thailand. They were maintained in an aquarium at temperatures 29-30 °C. The pink reddish egg masses were laid on the walls of aquarium. The egg masses were removed and placed in petri dishes at room temperature (32-33°C).

Preparation of permanent slide

After the eggs were laid for one hour, the egg shells were cracked with small forceps and the embryos were removed and fixed in 10% formalin. Embryos were collected every hour for two days. After the embryos were fixed in 10% formalin for 24 hours, they were dehydrated with 50%, 70%, 80%, 95% and 100% ethyl alcohol, respectively. Specimens were embedded in paraffin
blocks and sectioned with microtome. The serial sections (6 micrometer in thickness) were stained by hematoxylin and eosin staining method and examined under light microscope.

Results and Discussion

The embryo developed within egg shell because there was carotenoproteins oворubin and lipoproteins which were the most abundant source of proteins and lipids in the egg (Garin et al., 1996). In addition, the carotenoproteins oворubin was the most important source for bright color of eggs (Heras et al., 1998). Moreover, carotenoproteins could protect egg from the damaging effects of light (Dreon et al., 2004).

Stage of early development

Zygote

The fertilized egg or zygote was examined under light microscope (Figure 1). It was surrounded by thick membrane or fertilization membrane (Kume and Dan, 1968). The zygote divided by mitosis. The first cleavage division gave rise into unequal cells: a larger blastomere and the smaller one (Figure 2). This result was the same as to Desrosiers et al. (1996). They found that the first division of giant scallop Placopecten magellanicus was unequal. The two-celled embryo underwent the second division of cleavage and the embryos reached the 4-cell stage producing four large blastomeres called macromeres (Figure 3). The four-celled embryo underwent the third cleavage and the third cleavage was horizontal (i.e., equatorial). The third cleavage was unequal, so that the cells of the upper tier (micromere) were smaller than those of the lower tier (macromeres) (Figure 4). This cleavage pattern was holoblastic and spiral. This result had been supported by Page (2009). He reported that molluscs exhibited a highly conserved pattern of spiral cleavage.

Morula

Morula stage showed a mass of cells resulting from cleavage of the zygote preceding the formation of the blastula. It could be noticed that the micromeres were on the animal pole side (upper part) of macromeres in form of inclination and this pattern was called spiral. It could be explained that macromere gave rise into macromere ( ma: →) and micromere (mi: ←) (Figure 4).

Blastula

Blastula stage was spherical embryo which was composed of a single layer (Kume and Dan, 1968). In Pomacea egg, it cleaved unequally (Figure 5-6), as in Crepidula (Conklin, 1897). There were large blastomeres (macromeres) at the vegetal side and small blastomeres (micromeres) at the animal
pole region (upper part), and no blastocoel. This type of blastula was called stereoblastula, as in *Crepidula* (Conklin, 1897).

**Gastrula**

There was an increase in the number of small blastomeres (micromeres) in the developing embryo (Figure 7-8). These micromeres spread over and invested the macromeres in the developing embryo by the method of epiboly (Kume and Dan, 1968), as in *Crepidula* (Conklin, 1897). This was the formation of gastrula stage (Kume and Dan, 1968). In addition, Kume and Dan (1968) also reported that the large cells or macromeres which were enclosed to be the endoderm and the micromeres or outer cell layer which surrounded the large cells to be the ectoderm.

In conclusion, this histological study of early development in *P. canaliculata* will be useful for snail biology and development.

**Figure 1.** A. Fertilized egg or zygote of *Pomacea canaliculata* after laying egg

**Figure 2.** B. First cleavage; two-cells stage

**Figure 3.** C. Four cells stage of developing embryo

**Figure 4.** D. Morula stage of developing embryo (top view) with inclination; structures in this figure are indicated as: *mi*, micromere; *ma*, macromere
Figure 5. A. Blastula stage of developing embryo (side view); structures in this figure are indicated as: mi, micromere; ma, macromere.

Figure 6. B. Blastula stage of developing embryo (side view); structures in this figure are indicated as: mi, micromere; ma, macromere.

Figure 7. C. Gastrula stage of developing embryo; structures in this figure are indicated as: ect, ectoderm; end, endoderm.

Figure 8. D. Gastrula stage of developing embryo structures in this figure are indicated as: ect, ectoderm; end, endoderm.

References


