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Structural changes of oviduct of freshwater shrimp, *Macrobrachium nipponense* (Decapoda, Palaemonidae), during spawning*

LU Jian-ping (卢建平)†, ZHANG Xiao-hui (张晓辉), YU Xiao-yun (于晓云)

(*School of Life Sciences, Zhejiang University, Hangzhou 310029, China*)

†E-mail: jplu@zju.edu.cn

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Abstract: The structural change of the oviduct of freshwater shrimp (*Macrobrachium nipponense*) during spawning was examined by electron microscopy. The oviduct wall structural characteristics seem to be influenced significantly by the spawning process. Before the parturition and ovulation, two types of epithelial cells (types I and II) are found in the epithelium. The free surfaces of type I and type II cells have very dense long microvilli. Under the type I and type II cells, are a relatively thick layer of secreting material and a layer of mostly dead cells. After ovulation, two other types of epithelial cells (types III and IV) are found in the oviduct wall epithelium. The free surface of type III cells only has short microvilli scattered on the surface. The thick layer with secreting material and the dead cell layer disappeared at this stage. In some type III cells, the leaking out of cytoplasm from broken cell membrane led to the death of these type III cells. The transformation of all four types of epithelial cells was in the order: IV→I→II→III.

Key words: *Macrobrachium nipponense*, Crustacean, Oviduct, Cell, Structure
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INTRODUCTION

Although there are many studies on the female reproductive system in Decapoda (Hartnoll, 1968; Zhao *et al*., 1994; 1998; Jensen *et al*., 1996), the structure and function of the oviduct are poorly understood. The structure of the oviduct of Decapoda was considered simple, with the oviduct being merely a duct through which eggs pass on their way to the gonopore or the spermatheca (Hartnoll, 1968; Zhao *et al*., 1998). However, the material secreted by the Phyllopoda oviduct may have some physiological roles: lubrication of the oviduct, influence on the first meiotic division, and formation of the eggshell (Trentini and Scanabissi, 1982; Munuswamy and Subramoniam, 1985). So it is important to understand the structure and function of the oviduct of Decapoda. To the authors' knowledge, there were no publication concerning oviduct structural changes and cell type constitution in *Macrobrachium nipponense*. In this paper, we report the structural changes of the oviduct of the shrimp *M*. *nipponense* during spawning process. Our hypothesis is that the change of cell type constitution in the oviduct wall is an adaptation to physiological functions of oviduct during the spawning process.

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MATERIALS AND METHODS

Animals

Female *Macrobrachium nipponense* were purchased from the local farmer market and maintained in tanks with circulating water at 21~25 °C. The oviducts were obtained from adult females before parturition and exuviation (in which the ovary developed up to the 1st~2nd teeth of the rostrum) and 1 d after the spawning.

Methods

For light microscopic study, oviducts were fixed in Bouin's solution for 2 d, and embedment in paraffin. Seven-μm thick sections were cut on a regular microtome. Sections were stained with hematoxylin-eosin (HE). For electron microscopic study, samples were fixed in 2.5% (*V*/*V*) glutaraldehyde-2% (*w*/*V*) paraformaldehyde (0.1 mol/L phosphate buffer, pH 7.2) for 1~2 h at 4 °C, then post-fixed in 1% (*w*/*V*) OsO_4 (4 °C), dehydrated in acetone, infiltrated and embedded in Epon 812. Thin sections were cut by a LKB-III ultramicrotome, double stained with uranium acetate and lead citrate, then observed and photographed using a JEM-1200EX transmission electron microscope.

RESULTS

Structure of the oviduct wall before parturition and exuviation

In *M. nipponense*, a pair of oviducts originate from the anterolateral part of the ovary, and end at the female genital pores at the basipodite of the 6th thoracic appendage. Muscles linked to the surface of both ends of the oviducts.

The oviduct wall that is folded in some areas consists of a thin layer of connective tissue (CT), a basal lamina (BL), a layer of columnar epithelial cells, and a layer of secreting substance (SS) (Fig.1a and Fig.1b).

In the oviduct wall are two types of epithelial cells, one with clear and another with dark cytoplasm. The cells with clear cytoplasm are called epithelial cell type II, which comprised the majority of epithelial cells in the oviduct wall (Fig.1c). The cells with dark cytoplasm are called epithelial cell type I, only comprise a small portion of the cells in the oviduct wall (Fig.1c).

The elliptical nucleus at the center of type II cells, occupy most of the cell volume (Fig.1d). There are two types of nuclear envelope pores, one being several times larger than the other (Fig.1d). Under light microscope and electron microscope, a vacuolated zone was found between the nucleus (N) and basal lamina (Fig.1a and Fig.1e). This vacuolated zone consists of finger-like protuberances (P) of the epithelial cells and the basal lamina (BL). Many microvesicles and small vesicles from the endoplasmic reticulum (ER) appeared in the cytoplasm of the protuberances (Fig.1f).

The cytoplasm of type I epithelial cell is much darker than that of type II. In the centre and at the basal part of the epithelium, type-I cells extended out many thin and long protuberances between the type II epithelial cells (Fig.1c).

The free surface of the epithelium (cell types I and II) had long and dense microvilli (MV). This microvilli layer was as thick as the epithelial cell layer itself (Fig.1a and Fig.2a). There was a much thicker secreing substance layer under this microvilli layer (Fig.1a and Fig.2a). In the secreting substance layer, we found many large, blob-shaped vacuoles (Fig.1a). Under light microscope, the secretion from this vacuoles seems to contain fibrous and granular material (Fig.1a and Fig.1b), but under electron microscope it looked like a homogeneous, amorphous substance (Fig.2a). Another granular substance was seen lying on the inner surface of the secreting substance layer (Fig.1b). This consisted of dead cells. Many large vesicles were observed, surrounding the nucleus (N) of these dead cells (Fig.2b).

Structure of the oviduct wall after spawning

After spawning, the significant changes in the structure of the oviduct wall of *M. nipponense* were the disappearance of the secreting substance layer and the dead epithelial cells layer (Fig.2c). Only residues of the secreting substance layer could be seen between the folds in the oviduct walls (OW). Under light microscope some residues looked like broken blobs (Fig.2c), and under electron microscope, it looked as if the residues were cytoplasm leaking out from the dead cells (Fig.2d).

Two other types of epithelial cells, different from those seen prior to the parturition and moulting, were now found in the oviduct wall epithelium. The clear cells were named type III epithelial cell (Fig.2d), and the other with dark cytoplasm was called type IV epithelial cell (Fig.2e).

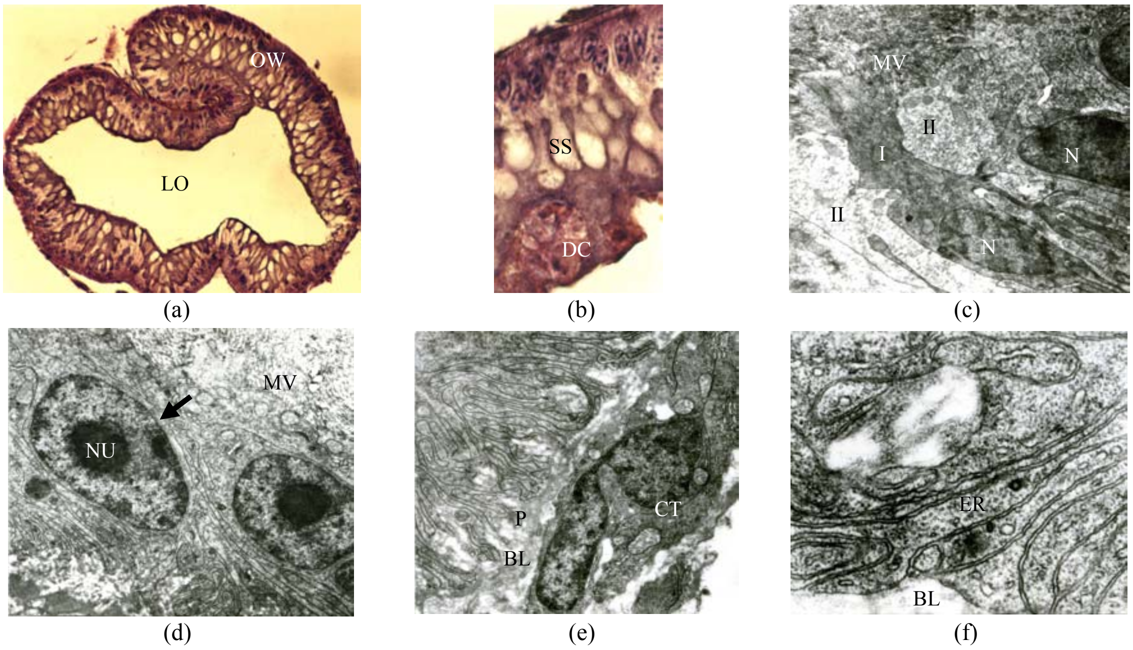

Fig.1 Structure of the oviduct of freshwater shrimp, *M. nipponense* before the parturition and ovulation (a) Transverse section of oviduct, ×40, HE stain; (b) Part of transverse section of oviduct wall, ×160, HE stain; (c) Oviduct wall epithelial cells, ×5000; (d) Epithelial cells type I, ×5000; (e) Outer part of epithelial cell type I, ×8000; (f) Outer part of epithelial cell type I, ×30000

BL: Basal lamina; CT: Connective tissue; DC: Dead cell layer; ER: Endoplasmic reticulum; I: Epithelial cell type I; II: Epithelial cell type II; LO: Lumen of the oviduct; MV: Microvilli; N: Nucleus; NU: Nucleolus; OW: Oviduct wall; P: Protuberance; SS: Secreted substance; Arrow: Nuclear envelope pore

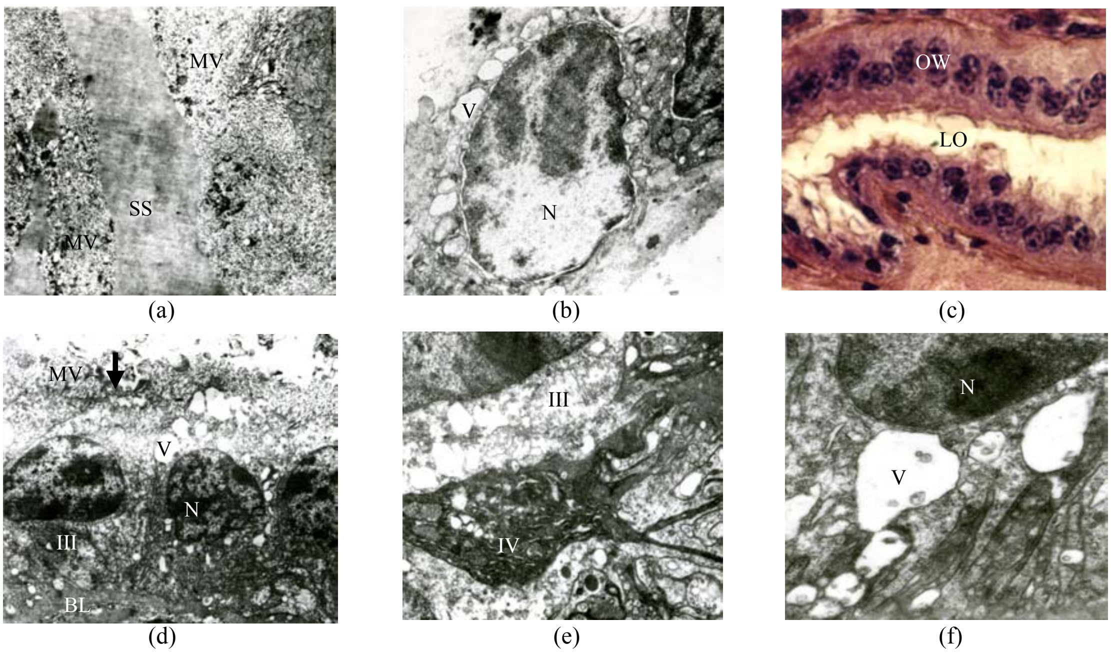

Fig.2 Structure of the oviduct of freshwater shrimp *M. nipponense* before the parturition and ovulation (a, b) or after laying eggs (c~f) (a) Inner part of oviduct wall before parturition exuviating, ×4000; (b) Dead cells, ×8000; (c) Part of transverse section of oviduct, ×160, HE stain; (d) Part of oviduct wall, ×3500; (e) Part of oviduct wall, ×8000; (f) Outer part of an epithelial cell type III, ×12000

III: Epithelial cell type III; IV: Epithelial cell type IV; BL: Basal lamina; LO: Lumen of the oviduct; MV: Microvilli; N: Nucleus; OW: Oviduct wall; SS: Secreted substance; V: Vesicle; Arrow: The cytoplasm leaked from the epithelial type III

The free surface of the epithelium only had short and scattered microvilli (MV) (Fig.2e). The type IV cells and some of the type III cells even lacked microvilli.

The due to spawning structural variations of the organelles in the epithelial cells could be found easily. The nucleus zone of type III epithelial cell was surrounded by many big vesicles (V), especially in the area between the nucleus (N) and the free surface of the cell (Fig.2e). This was similar to the situation in the dead cell (Fig.2e). The endoplasmic reticulum (ER) appeared as small vesicles in type III cell, but the vesicles were larger than those before the parturition moulting (Fig.2d). The vacuolated zone between nucleus and basal lamina disappeared (Fig.2c and Fig.2d). Many different sized vesicles appeared on the surface of the cells and located near the basal lamina (Fig.2f). Compared with those cells before the parturition and exuviation, the cytoplasm of type III epithelial cells was transparent. It was found that the cell membrane of some epithelial cells broke and cytoplasm leaked to cause the death of the cells (Fig.2d).

The cytoplasm of type IV epithelial cell, which was rich in mitochondria and endoplasmic reticulum (ER), was darker than that of the type I epithelial cell (Fig.1c and Fig.2e). The type IV cell was smaller than the type I epithelial cell, and had fewer protuberances.

DISCUSSION

Oviduct wall

The structure of oviduct wall of *M. nipponense* is similar to that found in other shrimp (*Macrobrachium carcinus*, *Macrobrachium rosebergii*) (Deng, 2000; Yu and Lu, 2005), but is different from that of crabs (Decapoda, Braclyrora) and Isopoda (Hartnoll, 1968; Hryniewiecka-Szyfter and Tyczewska, 1992; Jensen *et al.*, 1996).

The oviduct of shrimp, *M. carcinus* or *M. rosebergii*, is also composed of a thin layer of connective tissue and a layer of columnar epithelial cells (Deng, 2000; Yu and Lu, 2005). While the oviduct of the Dungeness crab, *Cancer magister*, is composed of a layer of columnar epithelium cell with a thin chitin lining, and the gonoduct wall is composed of three layers: the columnar epithelium, chitin, and connective tissue (Jensen *et al.*, 1996). In the isopod, *Saduria entomon*, the oviduct wall is composed of a layer of epithelium cells and a layer of muscle cells, and the apical surface of the epithelium is a cuticula layer (Hryniewiecka-Szyfter and Tyczewska, 1992). However, the oviduct of *M. nipponense* does not have a layer of cuticle on the apical surface of the epithelium, and the epithelial cells do have a secretory function. The role of the oviduct of *M. nipponense* may be more complex than the oviduct in crabs or isopods, in which oviducts are apparently only a passage for the eggs. The morphology and structure of the epithelial cells is also different from that of the wall of the spermatheca of the snow crab, *Chionoecetes opilio*, which has multiple layers of epithelial cells (Sainte-Marie and Sainte-Marie, 1998).

The lateral pouch epithelial cells of the oviduct of the fairy shrimp *Streptocephalus dichotomus* (Munuswamy and Subramoniam, 1985), of the follicle duct wall of *Triops cancriformis* (Trentini and Scanabissi, 1982), and of the oviduct wall of *M. carcinus* (Deng, 2000), *M. rosebergii* (Yu and Lu, 2005) and *M. nipponense* consists of secretory cells. While the lateral pouch epithelial cells of the oviduct of fairy shrimp *S. dichotomus* form a layer of flattened to cylindrical cells surrounded by a single layer of longitudinal muscle cells and some circular muscles lie at regular intervals (Munuswamy and Subramoniam, 1985). And the follicle duct wall of *T. cancriformis* is a single layer of closely packed epithelial cells lying on the basal lamina (Trentini and Scanabissi, 1982). However the oviduct wall of *M. nipponense* contains two layers of epithelium and connective tissue.

The epithelial cell ultrastructure of the oviduct wall of *M. nipponense* is also similar to that of *M. rosebergii* (Yu and Lu, 2005) but different from that of the follicle duct wall of *T. cancriformis* (Trentini and Scanabissi, 1982). The epithelial cells of the oviduct wall of *M. nipponense* before laying eggs contain Golgi complex and rough endoplasmic reticulum appearing in microvesicle form. Whereas no Golgi complex and developed rough endoplasmic reticulum are found in *T. cancriformis* (Trentini and Scanabissi, 1982). But they all have a vacuolated zone in the cytoplasm between the nucleus and the basal lamina. In *T. cancriformis*, the vacuoles of the vacuolated zone arise from cytoplasmic membranes in the intercellular spaces (Trentini and Scanabissi, 1982). It is possible that the vacuolated zone is an area in which the organic matter in blood is taken into the oviduct. So in *M. nipponense*, the oviduct epithelial cells could probably have another role. Besides the secretion function, the epithelial cells also function in absorbing some material from blood, similar to that of the anchoring stratum of spermathecal wall of snow crab (Sainte-Marie and Sainte-Marie, 1998).

Transformation among the oviduct wall epithelial cells

The renewal of the oviduct wall epithelial cells in *M. nipponense* occurs during the spawning process. According to the changes of the cytoplasmic density and organelles, the transformation among the four types of epithelial cells may from IV→I→II→III. The type IV epithelial cell has dense cytoplasm, with the basal part of the cell projecting several finger-like protuberances between or among type III epithelial cells. The type I epithelial cell has size similar to that of the type II or III epithelial cell, and denser cytoplasm and more organelles. Many microvilli appear on the free surface of the type I cell. The type II epithelial cell has clear cytoplasm and many organelles, such as microvesicle-like endoplasmic reticulum. Many finger-like protuberances appear at the base of the type II cell and with many microvilli also appearing on the cell's free surface. The type II epithelial cells account for most cells of the oviduct wall before spawning. The type III epithelial cell cytoplasm is clearer and contains many vacuoles. The type III epithelial cell tends to break and die during spawning. It was found that most oviduct wall epithelial cells die during spawning and but are renewed after spawning. This process of renewal of epithelium related to spawning is probably economically effective in energy and can reduce the harm of spawning to the oviduct or body. In *M. rosebergii*, the renewal process of epithelial cells is also found in the oviduct wall (Yu and Lu, 2005). Although it needs to be proved, the epithelial cells of the oviducts of other crustaceans with many times egg-laying in the life circle may probably have a similar renewal process.

Function of shear stress

From the disappearance of the secretion substance layer and scattered microvilli, the oviduct wall and eggs of *M. nipponense* were probably exposed to high shear stress during spawning. Although subjected to such physical stresses as they passed through the oviduct, the eggs have no visible damages and their fertilization rate and hatchability are still high. In echinoid, the viscosity of the eggs and the presence of extracellular layer on eggs have a potential function either to minimize the shear stress in the oviduct or to reduce the damage to the eggs exposed to high shear stress (Thomas and Bolton, 1999). The eggs are highly deformable spheres surrounded by jelly coats that, as a whole, behave like a fluid. Besides the contribution of eggs, some properties of the freshwater shrimp oviduct wall may have capacity to reduce damage (to eggs and oviduct wall) caused by shear stress. The secretion substance can lubricate the oviduct, and so, reduce the viscosity of the eggs and prevent direct contact between the eggs and the oviduct wall epithelial cells and prevent damages to each other partially. Moreover, the thick micovilli layer possibly has a similar role as the secretion substance too.

Physical stresses are probably important selective factors in the evolution of gamete properties of echinoid and other free-spawning invertebrates (Thomas and Bolton, 1999). Although *M. nipponense*, living in freshwater or salt water, suffer less stress from its surroundings than echinoid, it is also possible that physical stress is an important selective factor in the evolution of gamete properties of *M. nipponense*.

Function of secretion substance

Secretion substances in crustacean may have three potential roles: (1) lubrication of the oviduct; (2) formation of the eggshell; (3) influence on the development of egg. The secretion substances in Anostraca seemed to lubricate the passage of the pouched oviduct lumen to the ovisac (Anderson *et al*., 1970; Criel, 1980; Munuswamy and Subramoniam, 1985) and were thought of as having influence on the first meiotic division of oocytes (Criel, 1980). However, in *T. cancriformis*, the epithelium produces the eggshell material, with the egg covering beginning to form as the egg passes through the follicular duct accumulated with this material (Trentini and Scanabissi, 1982). In *M. nipponense*, the disappeared oviduct wall secretion substances will adhere to the surface of eggs as they pass through the oviduct. It is possible that the main role of *M. nipponense* secretion substances is the lubrication of the oviduct during spawning, and it is also possible the secretion substances probably have influence on the interactions between sperm and egg.

The present study provides new knowledge of the female *M. nipponense* oviduct. Our findings on the structural changes of the *M. nipponense* oviduct in relation to spawning have never been noted in other Decapoda. Further investigation, however, would be necessary to provide a comprehensive understanding of the structure and function of the oviduct in Decapoda.

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