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Ultrastructure of Micropylar Cells in the Pre-ovulatory Follicles of Pacific Herring, *Clupea pallasi Valenciennes*

Hiromi OHTA* and Kazunori TAKANO*

Abstract

Micropylar cells in pre-ovulatory follicles of Pacific herring, *Clupea pallasi*, were observed ultrastructurally.

The micropyle of the herring was observed to be a shallow funnel-like depression of about 120 μm in diameter in the vitelline envelope at the animal pole of oocyte. Micropylar canal penetrated the entire vitelline envelope of about 30 μm in thickness at the bottom of the depression. A flattened micropylar cell, about 10 μm in height and 25 μm in width, was located in the depression, and extended an enlarged cytoplasmic process through the micropylar canal. The distal part of the process was wedged into the ooplasm and its surface was invaginated by several projections of the ooplasm. A bundle of microtubules was well developed and arrayed parallel to the long axis of the micropylar cell process. The micropylar cell was characterized by having dilated cisternae of rough endoplasmic reticulum containing amorphous material, mitochondria with partially tubular cristae, well-developed Golgi bodies and numerous smooth-surfaced vesicles in the cytoplasm. Possible functions of the cell were discussed in connection with these features of cytoplasmic organelles. Fine structural characteristics of granulosa cells overlying the micropyle area were also described.

Introduction

It is well established that, in teleost eggs, penetration of spermatozoa is successful only through a micropyle located at the animal pole of the vitelline envelope. In many cases, the narrow size of internal orifice of the micropyle permits the entrance of only one spermatozoon at a time[1)-5)]. It is conceivable, therefore, that the micropyle plays an important role as the first barrier in blocking the approach of supernumerary spermatozoa to the ooplasm.

In Pacific herring, *Clupea pallasi*, it was observed that spermatozoa were activated around the micropyle though they were almost motionless in sea water or in Ringer's solution[6)]. Moreover, it was suggested that such a sperm-activating factor, which was proteinaceous in nature, was present in the micropyle area of herring's egg[7)]. Although some light microscopic observations have been made in the herring on the micropyle of ripe eggs[8)] and on the micropylar cell of oocytes before ovulation[9)], there is no information concerning the ultrastructure of the micropylar cell in this species of teleost.

The present study was designed to examine fine structure of micropylar cell of pre-ovulatory follicles in the herring, *Clupea pallasi*, in order to add further information about its possible functions. Ultrastructural characteristics of

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granulosa cells in the micropyle area of the ovarian follicles were also observed in the present study.

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Material and Methods

The herring, *Clupea pallasi*, used in the present study were caught by a fixed net in Lake Notoro facing the Sea of Okhotsk, northern Hokkaido. They had migrated from the sea into the brackish lake for spawning in late April. Five mature females, ranging from 18.7 to 24.3 cm in body length, were used in the present observations.

After the sacrifice by decapitation, pieces of ovaries were fixed in Karnovsky's glutaraldehyde-paraformaldehyde mixture in 0.2 M cacodylate buffer (pH 7.4) for one or two days at room temperature, and postfixed in 1% osmium tetroxide in the same buffer for 2 hours at 4°C. After dehydration, the specimens were embedded in Epon. Ultrathin sections stained double with uranyl acetate and lead citrate were observed with a Hitachi HU-12 electron microscope. Parallel sections of about 1 μm thick were stained with methylene blue for light microscopic observations.

Results

The females used had ovaries containing two groups of oocytes; a group of oocytes of larger size was at the pre-maturation or migratory nucleus stages, and the other group of oocytes of smaller size was at the peri-nucleolus or less advanced stages.

The larger-sized oocytes, averaging 900 μm in diameter, were filled with a large bulk of minute yolk globules of less than 20 μm in diameter. In the peripheral ooplasm, yolk vesicles were arranged in one or two layers, except for the region near the inner orifice of micropylar canal where they were quite scarce in number (Fig. 1).

Micropyle area appeared as a shallow funnel-like depression of about 120 μm in diameter in the vitelline envelope at the animal pole of oocyte. Micropylar canal perforated the vitelline envelope averaging 30 μm in thickness at the region of the depression and measured in diameter about 3 μm at its outer end and about 1.5 μm at its inner end facing the ooplasm.

Vitelline envelope of oocytes was 45–50 μm in entire thickness and consisted of four distinct layers showing different stainability to methylene blue in semithin sections. The outermost layer, about 15 μm in thickness, which has been described as the adhesive layer[8),9)], showed a palisade structure in cross sections which decreased in height gradually toward the bottom of the depression and disappeared at the area around the outer orifice of micropylar canal. Underlying this layer, there were a thin, dark-stained layer measuring about 1.2 μm in thickness and,

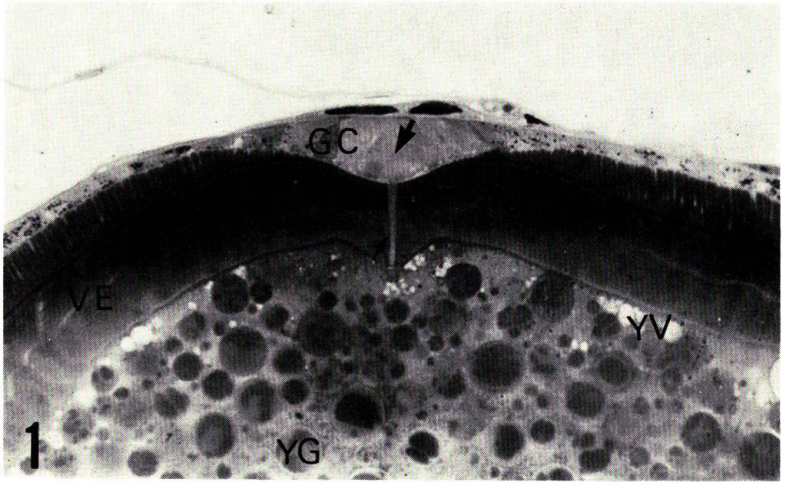


Fig. 1. Epon section (1 μm) of micropyle area of a pre-ovulatory oocyte of the herring. The vitelline envelope (VE) shows a four-layered organization and a shallow funnel-like dpression. A micropylar cell (indicated by a large arrow) located in the depression extends an enlarged cytoplasmic process (a small arrow) to the ooplasm. GC, granulosa cells; YG, yolk granule; YV, yolk vesicles. × 390.

arranged inward to this layer, two layers of about 30 μm in total thickness which displayed a pile of alternating light and dark laminae. Of the two layers, the outer one somewhat decreased in thickness toward micropylar canal, while the inner one showed a conical protrusion of about 10 μm in height and 30 μm in diameter toward ooplasm around the internal orifice of micropylar canal (Fig. 1).

The vitelline envelope was invested with a single layer of granulosa cells except for the micropyle area where the cells often formed two or more layers. A flattened micropylar cell about 10 μm in height and about 25 μm in width was located in a funnel-like depression of the micropyle. The cell had a round nucleus which was located eccentrically in the cytoplasm and stained deep with methylene blue (Fig. 1). Ultrastructurally, the micropylar cells were characterized by the presence of numerous dilated cisternae of the rough endoplasmic reticulum in the cytoplasm. These cisternae varying in size and shape contained amorphous material of low electron density. Well-developed Golgi bodies were present near the nucleus and were composed of several closely packed cisternae with many vesicles. Similar smooth surfaced vesicles containing electron-dense material were also found extensively throughout the cytoplasm. Round or rod-shaped mitochondria had poorly developed lamelliform cristae, though some of them possessed a few tubular cristae. Besides these organelles, free ribosomes were abundantly distributed in the cytoplasm (Figs. 2, 3).

The micropylar cell extended a thick cytoplasmic process through the micropylar canal to the ooplasm. The distal part of the process was wedged into the ooplasm to the depth of about 1 μm. The apical surface of the wedged process was often invaginated by several projections of ooplasm which appeared to be high in electron density (Fig. 5). In the cytoplasm of the micropylar process, a

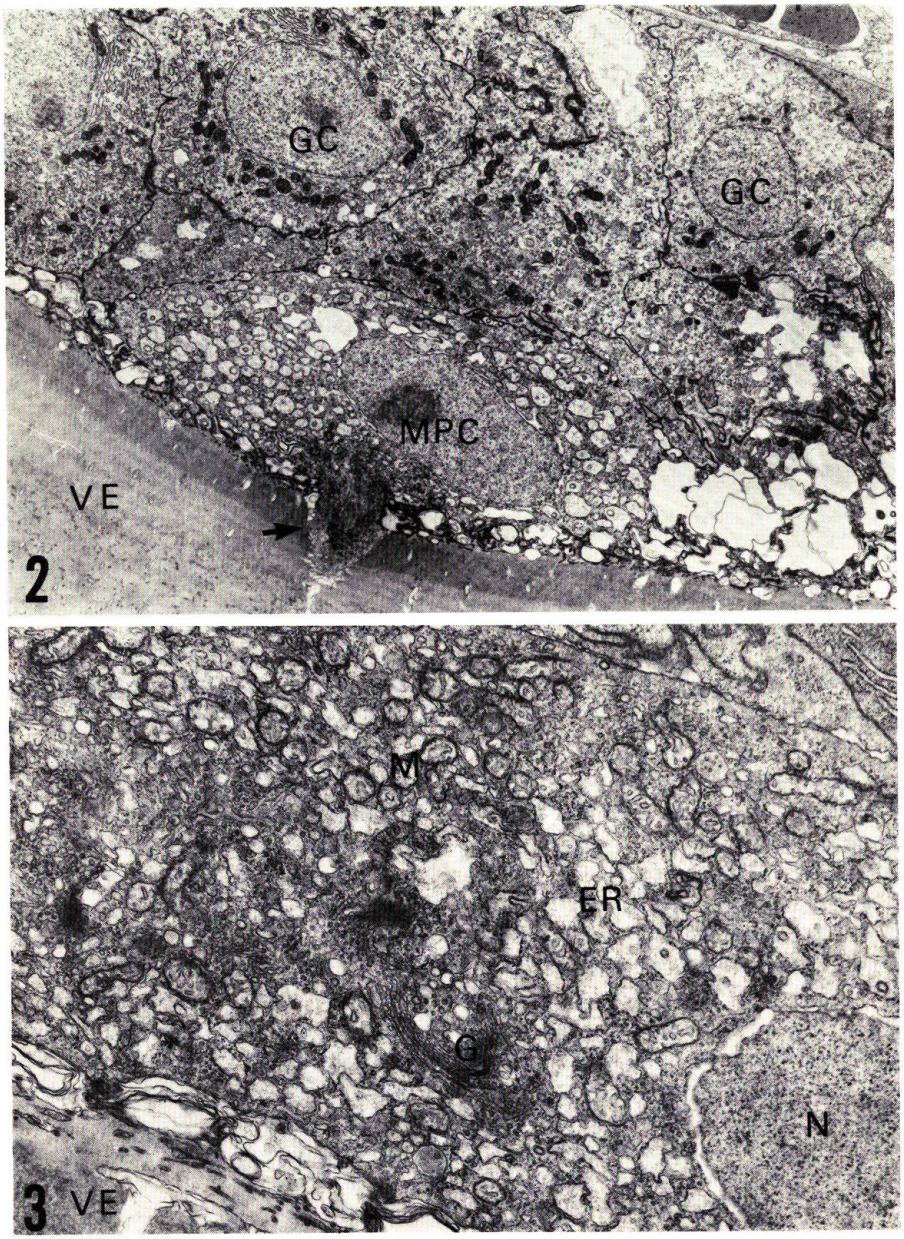
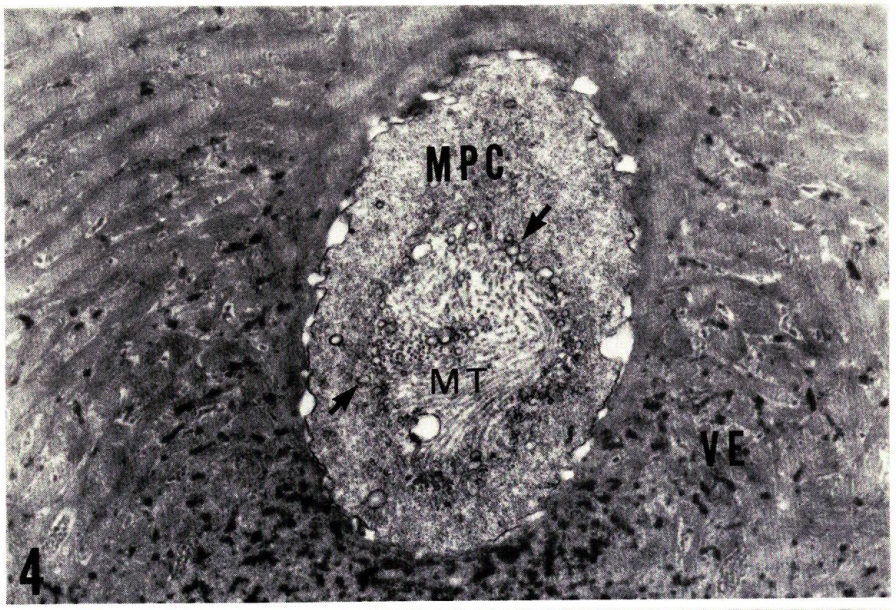


Fig. 2. Electron micrograph of micropyle area of the herring. Arrow indicates the basal region of the cytoplasmic process with a bundle of microtubules. GC, granulosa cell; MPC, micropylar cell; VE, vitelline envelope. × 3,500.

Fig. 3. Electron micrograph of a portion of a micropylar cell, showing well-developed rough endoplasmic reticulum (ER) containing amorphous material with low electron density and Golgi bodies (G). M, mitochondria; N, nucleus; VE, vitelline envelope. × 11,500.



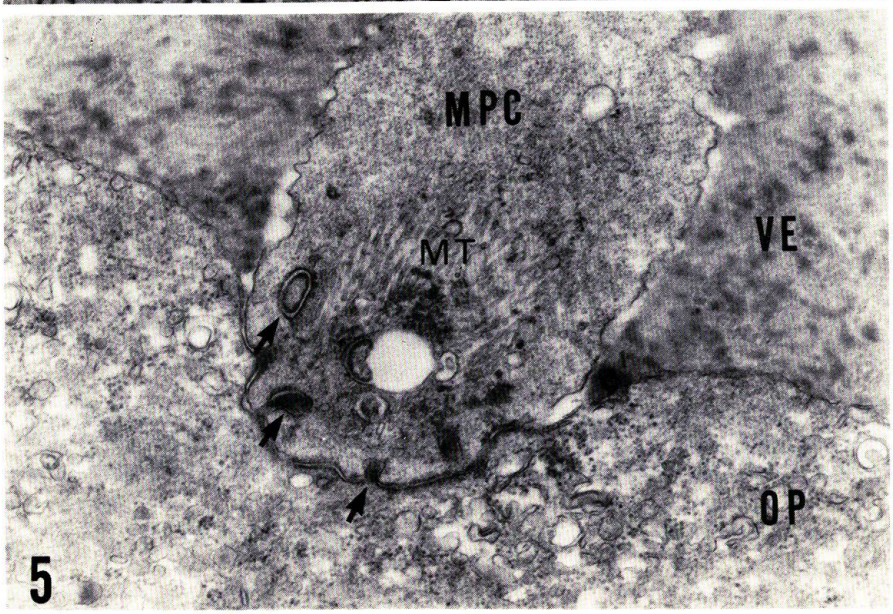


Fig. 4. Electron micrograph showing a cross section of the cytoplasmic process of a micropylar cell (MPC). A large number of microtubules (MT) are surrounded by many small vesicles (arrows). VE, vitelline envelope. × 13,700.

Fig. 5. Electron micrograph of the apex of the cytoplasmic process of a micropylar cell (MPC). Arrows indicate cytoplasmic invaginations on the surface of the process. MT, microtubules; OP, ooplasm; VE, vitelline envelope. × 24,500.

bundle of microtubules, about 25 nm in diameter, was arranged parallel to the long axis of the process, extending smoothly from the proximal region to the part near the apex of the process (Figs. 2, 5). In cross-sections of the process, it was observed that numerous smooth-surfaced vesicles of various sizes were arrayed around the bundle of microtubules occupying the center of the process (Fig. 4).

Granulosa cells overlying the micropyle area were fairly larger in size when compared with those surrounding, as a single layer, the whole surface of oocyte. The cells were clearly distinguished from the micropylar cell by their round or rod-shaped mitochondria with some parallel cristae and highly electron-dense matrix (Figs. 2, 6). In the cytoplasm of the granulosa cells, cisternae of rough endoplasmic reticulum usually assumed a tubular or a slightly dilated aspect, and contained electron-lucent material. Often, a few cisternae of the endoplasmic reticulum with scarce attaching ribosomes showed extensive dilation. Well-developed Golgi bodies consisting of several lamellae and a few vesicles were also commonly observed in the cytoplasm near a round or oval nucleus (Fig. 6).

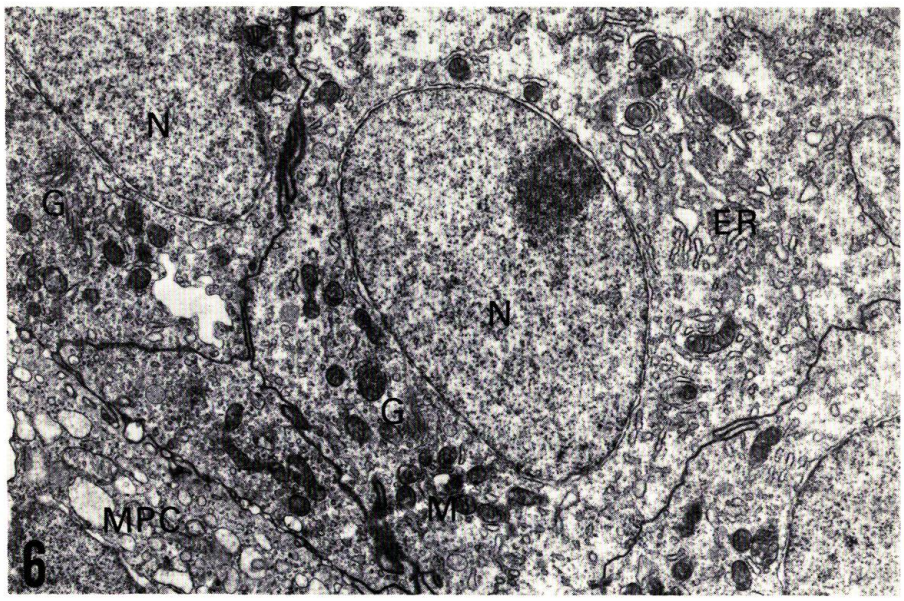


Fig. 6. Electron micrograph of granulosa cells overlying the micropylar cell (MPC). ER, rough endoplasmic reticulum; G, Golgi bodies; M, mitochondria; N, nucleus. × 7,100.

Discussion

Micropylar cells of the Pacific herring observed in the present study were characterized by having a markedly thick cytoplasmic process. The most conspicuous feature of the cells is that a well-developed bundle of microtubules extends linearly along the long axis of the process. Similar development of microtubules in the process of micropylar cells has been seen in several species of

teleosts such as the pond smelt, *Hypomesus transpacificus nipponensis*[10], the loach, *Misgurnus anguillicaudatus*[11], and the bitterling, *Rhodeus ocellatus ocellatus*[12]. The degree of the development of microtubules seems to differ in different species observed so far, probably in accordance with the length and shape of micropylar process in each species. The bundle of microtubules may be a common organelle universally found in the cytoplasm of micropylar cells of teleost fishes, and may play an important role as the principal cytoskeleton[13] in supporting the specialized architecture of micropylar cells. It is also interesting to note that the process of micropylar cell was invaginated by protrusions of ooplasm at several places on its apical surface. This peculiar structure may facilitate an intimate connection between micropylar cell and oocyte. No other junctional apparatus was detectable between the two cells.

Ultrastructurally, micropylar cells in the pre-ovulatory follicles of the herring were distinguished from neighboring granulosa cells by the presence of dilated cisternae of rough endoplasmic reticulum containing amorphous material in the cytoplasm of the former cells. In addition, well-developed Golgi bodies and numerous smooth-surfaced vesicles were observed in the micropylar cell cytoplasm. Such an ultrastructural profile is suggestive of the synthesis of proteinaceous material in the micropylar cells. Moreover, numerous smooth-surfaced vesicles of various sizes were arranged around the bundle of microtubules, as was also the case in the micropylar cell of the pond smelt[10]. In this regard, it is interesting to consider the possibility that microtubules may act as a system for intracellular transport of materials in various kinds of cells[13].

In mature oocytes at the pre-ovulatory stage, granulosa cells surrounding the oocytes were loaded by prominent electron-dense granules in the cytoplasm (unpublished observation). In contrast, granulosa cells at the micropyle area were mostly lacking in such granules and had some dilated vesicles of the rough endoplasmic reticulum in their cytoplasm.

These observations may suggest that, in the herring, micropylar cell and/or associated granulosa cells play some special roles in the mechanism of fertilization. However, which of two cell types actually contributes to produce a sperm-activating factor, the presence of which in the herring oocytes was suggested in the previous reports[6],[7], could not be determined in the present study.

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