Autonomous Fluorescence Localized at the Polar Lobe of Eggs of Japanese Oyster, *Crassostrea gigas*

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Under ultraviolet illumination, eggs of Japanese oyster *Crassostrea gigas* were found to emit specific yellow autonomous fluorescence in the vegetal cortical region of egg cytoplasm. The yellow fluorescing cytoplasm was composed of many granules and they were already concentrated at the vegetal pole region of fully matured oocytes stored in the ovary and remained there after fertilization. During first polar lobe formation, such granules localized at the vegetal cortical region of the lobe that appeared at the vegetal pole of the egg and then incorporated into the CD blastomere at the 2-cell stage. The localization of the granules was still recognizable but gradually became ambiguous during the 2-cell stage and the second polar lobe formation but there was no localization on and after the 4-cell stage.

Key Words: developmental biology, *Crassostrea gigas*, polar lobe, cytoplasmic determinants, autonomous fluorescence

Introduction

Eggs of some species of molluscs and annelids have been known to produce cytoplasmic protrusion, known as a polar lobe, at their vegetal pole during maturation divisions and/or early several cleavages. Cytoplasmic factors thought to be responsible for cell determination and morphogenesis are postulated to be shunt to specific cell lineage by protrusion and absorption of polar lobes and determinative cleavage (reviewed by [1-4]). Until now, considerable efforts have been made to identify the cytoplasmic factors and several structures localized specifically at the vegetal pole and the polar lobes of these eggs have been observed. Double membrane vesicles with a granular content seem to concentrate in the polar lobe as compared with elsewhere in *Ilyanassa* [5, 6]. Same structures are also found in *Nassarius* [7]. In *Bithynia* eggs, an RNA-containing 'vegetal body', which is composed primarily of small vesicles with a considerable number of multivesicular bodies, localizes at the vegetal pole cytoplasm and enters the polar lobe [8]. In *Crepidula*, the polar lobe contains a few small aggregates of vesicles [9]. In *Buccinum*, a large number of electron-dense vesicles fill the polar lobe. Polar lobes of *Crepidula* and *Buccinum* eggs contain germ plasm-like
structures consisted of a complex of small vesicles and dense granules [10]. The plasma membrane around the polar lobe and the vegetal pole of some eggs is also known to have specific properties; the local accumulation of apparently symbiotic bacteria in Dentalium [11], local folding of the surface in Nassarius, Buccinum, and Crepidula [12] and in the polychaeta Sabellaria [13], locally increased excitability of the membrane in Dentalium [14], and increased numbers of intramembraneous particles in Nassarius [15].

In this study, granules emitting autonomous yellow fluorescence under ultraviolet (UV) illumination are found to be localized at the vegetal cortical plasm of oocytes of Japanese oyster Crassostrea gigas. The behavior of the granules during early cleavage stages will be described and discussed.

**Materials and Methods**

**Animals.** Japanese oyster, Crassostrea gigas, was examined in this study. Adults of C. gigas were collected around Kada Bay, Wakayama, Japan and maintained in temperature-controlled aquaria (22°C). Oocytes were washed out from dissected ovaries in Millipore-filtered artificial seawater (MFASW), Jamarin U (Jamarin Lab., Osaka), and rinsed two or three times with MFASW. They were fertilized with a diluted suspension of sperm collected from dissected testes and fertilized eggs were reared in petri dishes filled with MFASW.

**Fluorescence microscopy.** Living specimens were mounted with MFASW on microscope slide on which pieces of Scotch tape were put in parallel to hold about 100 μm space between the slides and the cover slips. To observe autonomous fluorescence of the specimens, a Nikon Optiphot equipped with an epifluorescence optic unit EFD2 was used. The EFD2 unit for UV illumination was composed of a mercury lamp house (Osram HBO-100W/2), Nikon CF Fluor objective lenses for epifluorescence microscopy, and an UV excitation filter cassette. The cassette was an assemblage of a dichroic mirror DM400, an excitation filter EX330–380, and a barrier filter BA420, and of which the main wavelength was 365nm. Photomicrographs were taken with Kodak Ektachrome 200 or T–MAX 100 film.

**Centrifugation of oocytes.** Oocytes were placed with MFASW on the bed of Percoll (Sigma, St. Louis) prepared at the bottom of 1.5-ml microcentrifuge tube. The tube was centrifuged at 2000×g for 10min at room temperature. Centrifuged oocytes were withdrawn from the surface of Percoll bed and the centrifugal direction of them was examined under a dissecting microscope. Localization of autonomous fluorescence in the centrifuged oocytes was examined as described above.

**SEM observation.** Ovarian tissues were cut out from individuals with scissors and fixed with 2.5% glutaraldehyde in MFASW for 2 hr at 4°C. The fixed tissues were rinsed
several times in distilled water, immersed in the mixture of 2% guanidine hydrochloride and 2% tannic acid for 8 hr at room temperature, rinsed again several times in distilled water, and then immersed in 2% Osmic acid for 8 hr at room temperature for conductive block staining [16]. The stained specimens were dehydrated through a graded series of ethanol solutions, cracked with fine forceps in ethanol, and dried by the critical-point technique using CO2 with a JEOL JCDP-5 apparatus. The dried specimens were mounted on brass stubs with the cracked face upward, coated with a thin layer of gold by means of a JEOL JFC-1100E ion spatter-coater, and then examined with JEOL JSM-840A SEM. Photographs were taken with Fuji NEOPAN SS film.

Results

**Autonomous fluorescence in oocytes and cleaving eggs**

Fluorescence microscopical observations carried out on *C. gigas* oocytes demonstrated that there were some cytoplasmic structures with autonomous fluorescence (Figs. 1a and b). Under UV illumination, it was found that the granules with intense yellow autonomous fluorescence were localized at the cortical cytoplasmic region of one pole of the oocytes and that the remainder of cytoplasm of the oocytes emitted weak pale blue autonomous fluorescence (Figs. 1b, c, and d). On the other hand, the germinal vesicles emitted no autonomous fluorescence. The same fluorescent pattern was also observed in oocytes of related species, *Saccostrea echinata* but not in another bivalve, *Mytilus edulis* (preliminary observation). The cortex where the fluorescing granules were localizing and the cytoplasmic region adjoining the cortex were more transparent than the remaining cytoplasmic region of oocytes under a normal transmission light microscope (Fig. 1a). It was also observed that the yellow fluorescing granules were scattered over the remaining cortical region of the oocytes when focused on the surface of them (Fig. 1e). It was unknown that these were the same granules as ones localizing at one pole of oocytes.

These kinds of autonomous fluorescence faded under continuous UV illumination within a few minutes. The autonomous yellow fluorescence recognizable under UV illumination was not detected with violet excitation (main wavelength, 405nm), blue excitation (main wavelength, 495nm), nor green excitation (main wavelength, 546nm) (data not shown). When eggs were fixed with ethanol, the yellow autonomous fluorescence disappeared and the pale blue autonomous fluorescence became more intense. On the other hand, the localized yellow autonomous fluorescence remained intact and the pale blue background fluorescence of cytoplasm was weakened after 10% formalin fixation (data not shown).

Oocytes dissected from the same ovary of *C. gigas* had diverse morphologies (Fig. 1a and b). Some oocytes were spherical (Fig. 1a, b and c) but others were pear-shape with a cytoplasmic protrusion (Fig. 1a, b and d). The oocytes immediately after dissection from the ovary were polygonal but became round gradually as the time passed. In an
Fig. 1. Autonomous fluorescence of oocytes, fertilized eggs, and early cleavage stage embryos of Japanese oyster, C. gigas. (a) and (b) are the same magnification and from (c) to (k) are the same magnification. Scale bars indicate 50μm. (a) Morphology of oocytes dissected from matured ovary. Some oocytes (arrow) are pear-shape with a cytoplasmic protrusion of which cytoplasm is more transparent than the remaining cytoplasmic region except for germinal vesicle region. (b) Fluorescent microphotograph of the same frame as that of (a) under UV illumination. Granules with intense yellow autonomous fluorescence are localized at the cortical cytoplasmic region of one pole of the oocytes (arrowheads). Some oocytes show no localization of yellow autonomous fluorescing granules (asterisk). (c) Spherical type of oocytes under UV illumination. Intense yellow fluorescing granules localize at the cortical cytoplasmic region of one pole of oocytes. (d) Pear-shape type of oocyte under UV illumination. Intense yellow fluorescing granules accumulate at the apical cortical region of the protrusion of the oocyte. (e) Fluorescent microphotograph of spherical type of oocyte where the surface is in focus. Yellow fluorescing granules are scattered over the cortical region of the oocyte. (f) Polar body forming fertilized egg under UV illumination. This photograph clearly shows that polar body (arrow) extrudes at the opposite side of the localization of yellow fluorescing granules. (g) Trefoil stage embryo under UV illumination. In this egg, intense yellow fluorescing granules are scattered over the cortical region of polar lobe. (h) In this trefoil stage embryo, the localization of fluorescing granules shifts to the cleavage plane. (i) Two-cell stage embryo showing localization of yellow fluorescing granules at the vegetal cortical region of CD blastomere that has absorbed polar lobe. (j) Four-cell stage embryo still shows localization of yellow fluorescing granules at the vegetal cortical region of D blastomere that has absorbed second polar lobe. (k) Eight-cell stage embryo shows no localization of yellow fluorescing granules.
extreme case, the morphology of pear–shape oocytes was looked like baseball bats or maracas. The ratio of spherical oocytes and pear–shape ones was different between each batch. Among spherical oocytes examined, more than half of them exhibited the localization of the yellow fluorescing granules at the periphery of them. The cortical cytoplasm exhibiting the localization of the yellow fluorescing granules was more transparent than the remaining cytoplasmic region. The remains, however, showed no localization of fluorescence and their peripheral cytoplasm was opaque uniformly (Fig. 1b; Table 1). On the other hand, almost all the pear–shape oocytes exhibited the localization of yellow fluorescing granules at the apical cortex of their protrusions (Figs. 1b and d; Table 1) and the cytoplasm of the protrusion was also more transparent than other cytoplasmic region of the oocyte (Fig. 1a).

In the case of spherical oocytes, they were thought to orient toward random direction in the space between the slide and the cover slip. When the aggregation of the yellow fluorescing granules was located on the upper or lower face of oocyte, it became difficult to identify it. On the other hand, almost all the pear–shape oocytes located their protrusions at the periphery because this orientation seemed to be most stable under these conditions. Therefore the localization of fluorescing granules was recognized in almost all the pear–shape oocytes examined. This seemed to be the reasons why the ratio of spherical oocytes exhibiting the localization was lower than that of the pear–shape ones.

The protrusion of the pear–shape oocyte was the vegetal pole because the polar bodies would always appear at the opposite side of the oocyte after fertilization and because the protrusion remained only at the vegetal region of the first and second polar lobes in fertilized eggs (data not shown). Almost all the spherical eggs exhibiting the localization of yellow fluorescing granules also protruded polar bodies at the side of the oocytes opposite to the region of the fluorescence localization (Fig. 1f). Therefore, the yellow fluorescing granules always localized at the cortical cytoplasm of vegetal pole of oocytes.

After fertilization, oocytes underwent germinal vesicle breakdown and clear cytoplasmic region appeared at the animal pole where the first and second polar bodies would be pinched off (Fig. 1f). During germinal vesicle breakdown and polar body formation, yellow fluorescing granules and clear cytoplasm remained localizing at the cortical cytoplasm of the vegetal pole of fertilized eggs (Fig. 1f). On the other hand, newly appeared clear cytoplasmic region of animal pole did not have pale blue autonomous fluorescence or yellow autonomous fluorescing granules (Fig. 1f).

Just prior to the first cleavage, C. gigas eggs made a large cytoplasmic protrusion, the first polar lobe, at the vegetal pole, and before completion of the first cleavage the polar lobe was absorbed to one of the sister blastomeres to which the polar lobe connect with cytoplasmic bridge. The polar lobe was composed of the transparent cytoplasm that had been localized at the vegetal pole region of oocytes. During formation and absorption of the first polar lobe, the yellow fluorescing granules still remained localizing at the vegetal cortical region of the polar lobe of more than 80%
Table 1 Percentages of oocytes, eggs and early embryos with localization of yellow fluorescing granules in *Crassostrea gigas*

<table>
<thead>
<tr>
<th>Egg shape</th>
<th>No. of oocytes, eggs and embryos with the localization of yellow fluorescing granules*</th>
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<tbody>
<tr>
<td></td>
<td>Oocytes</td>
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<tr>
<td>Spherical</td>
<td>739/1248</td>
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<tr>
<td>(59.2%)</td>
<td>(96.2%)</td>
</tr>
<tr>
<td>Pear-shaped</td>
<td>175/180</td>
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<tr>
<td>(97.2%)</td>
<td>(100%)</td>
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* Total number of batches examined was 7. Except for oocytes, only eggs and embryos of which animal pole was clearly recognizable in view of their polar bodies were counted. The spherical oocytes were examined at random because it was difficult to identify their animal pole. Only pear–shape oocytes and eggs of which the protrusion was clearly seen at their periphery were counted.

** Not counted.

of eggs (Table 1; Figs. 1g, h, and i). In many eggs, these granules localized at the most vegetal region of the polar lobe, but sometimes the localization somewhat shifted to the region near the cleavage furrow (Fig. 1h). Some eggs still had cytoplasmic protrusion at the most vegetal region of the polar lobe as described previously and these granules localized at the protrusion and the adjacent cortical cytoplasm (data not shown).

At the 2-cell stage, one blastomere, named CD, which had absorbed the polar lobe, was twice or more as large as another one, named AB (Fig. 1i). The yellow fluorescing granules were localized at the vegetal cortical region of the CD blastomere (Fig. 1j). The vegetal cytoplasm of the CD blastomere where the polar lobe was absorbed was more transparent than remaining cytoplasm or the cytoplasm of the AB blastomere. The AB blastomere did not exhibit the localization of the yellow fluorescing granules (Fig. 1i).

At the end of the 2-cell stage, the vegetal hemisphere of the CD blastomere protruded again and formed the second polar lobe but it was not constricted so clearly as the first one in *C. gigas*. After protrusion of the second polar lobe the AB blastomere and the remaining animal part of the CD blastomere divided almost equally. The second polar lobe was absorbed into one of the daughter blastomeres of the CD, which was named D blastomere, and then the embryos entered the 4-cell stage. During these period, the yellow fluorescing granules still localized at the vegetal cortical region of the CD blastomere, the second polar lobe, and the D blastomere (Fig. 1i and j), but the percentage of embryos which exhibited the apparent localization of the yellow fluorescing granules became less than 50% (Table 1). The degree of localization of the yellow fluorescing granules in the embryos still showing recognizable localization also decreased and there were little embryos that showed the localization as clearly as oocytes did (Fig. 1j). On the other hand, yellow fluorescing granules scattered over the
cortical cytoplasm of A, B, and C blastomeres and of the animal half of the D blastomere became conspicuous (data not shown).

On and after the 4-cell stage, it became difficult to distinguish the localization of yellow fluorescing granules in cleaving embryos. At the 8-cell stage, there was no localization of the granules not only at the vegetal cortical region of the largest blastomere, 1D, but also at the other region of the embryo (Fig. 1k).

Change of distribution of autonomous fluorescence in centrifuged oocytes.

In order to examine whether the yellow fluorescing granules adhered firmly to the cortex of the vegetal pole, oocytes were centrifuged on the bed of Percoll until the cytoplasm stratified. The oocytes centrifuged at 2000 × g for 10 min became four-layer construction (Fig. 2a). In the stratified oocytes, the most centripetal layer, named layer I, was dark brown and composed of opaque cytoplasmic components. The boundary between the layer I and the next intermediate layer, named layer II, was distinct (Fig. 2a). In some stratified oocytes the germinal vesicle was situated in this boundary embedding about 2/3 part of itself in the layer II, but in others the germinal vesicle was disappeared probably because of mechanical breakdown of it by centrifugal force. The germinal vesicle centrifuged was transparent, showed no stratification and emitted no autonomous fluorescence under UV illumination. The intermediate layer II was composed of transparent cytoplasmic components with scattered granules (Fig. 2a). The boundary between this layer II and the next intermediate layer III was indistinct. The layer III was the thinnest one and composed of translucent granular cytoplasm (Fig. 2a). The boundary between the layer III and the most centrifugal layer IV was also somewhat indistinct. The layer IV was composed of opaque light brown cytoplasmic components (Fig. 2a). Some pear-shape oocytes kept their cytoplasmic protrusions after centrifugation. The centrifugal pole did not always agree with the protrusion, which indicate that the direction of stratification was independent of any axis of oocytes (Fig. 2c).

During observation of the stratified oocytes under UV illumination, the yellow fluorescing granules was also found to be stratified at the most centripetal region of the layer IV (Fig. 2b). The pale blue autonomous fluorescence emitted by the whole cytoplasm of uncentrifuged oocytes occupied the whole region of the layer I, III, and IV (Fig. 2b). The yellow fluorescing granules sometimes moved as a mass under centrifugal force to the periphery of the most centripetal region of the layer IV and did not form a layer (Fig. 2c). In some case, a mass of yellow fluorescing granules was situated at the centrifugal pole of oocytes and remaining yellow fluorescing granules was stratified at the most centripetal region of the layer IV. In these oocytes, the vegetal pole may have coincided with the centrifugal pole, and the centrifugal force may not be strong enough to tear off the aggregate of yellow fluorescing granules from the cortex.

About 20 min after oocytes were stratified by centrifugation, the stratification composed of four layers of cytoplasm gradually became ambiguous and about 1 hr
Fig. 2. Autonomous fluorescence in centrifuged oocytes of *C. gigas*. Upside of photographs coincides with centripetal direction. Scale bar indicates 50 μm. (a) Oocyte centrifuged at 2000 × g for 10 min. Four layers were identified and named layer I, II, III, and IV respectively from centripetal pole. (b) Localization of autonomous fluorescence in centrifuged oocyte. The same specimen that is in (a) was observed under UV illumination. Intense yellow autonomous fluorescing granules stratified at the most centripetal region of layer IV. Cytoplasm of layers I, III, and IV emits pale blue autonomous fluorescence, but there is no autonomous fluorescence in layer II. (c) Centrifuged and stratified oocyte with a mass of intense yellow fluorescing granules attaching to the cortical region of the most centripetal of layer IV. This region does not seem to coincide with vegetal pole because the protrusion of vegetal pole remaining in this oocyte situates at the equator region of layer II.

After centrifugation, the stratification nearly disappeared. The yellow fluorescing granules moved from the layer where they sedimented and concentrated again in the cortical region of cytoplasm. The localization of the yellow fluorescing granules was restricted mainly to the cortical region where the layer IV had situated, but the distinct localization of the granules like that of uncentrifuged oocytes was not observed in the recovered oocytes. Although distinct localization of the yellow fluorescing granules was observed in some recovered oocytes, it was not known whether the re-localization occurred. A mass of the yellow fluorescing granules described above may be merely remaining at the periphery of oocyte.

**SEM observation of the ovary**

In order to examine the correlation between the localization of the yellow fluorescing granules at the vegetal pole region of oocytes and the situation of oocytes in the ovary, the fractured ovary of *C. gigas* was observed with scanning electron microscope. In the ovary, almost all the matured oocytes observed in the fractured section assumed the shape of a polygonal teardrop and attached to the acinar wall of ovary with the apex of their long narrow protrusions (Figs. 3a and b). Under light microscope, some oocytes were teardrop-shape just after dissection from ovary and resembled ones observed in the ovary under scanning electron microscope. In those oocytes, the apex of the protrusion coincided with the vegetal pole because polar bodies always
Fig. 3. Scanning electron microscopic observation of ovarian oocytes of *C. gigas*. (a) Fracture face of ovary. Many matured oocytes with polygonal teardrop shape are attaching to the acinar wall of ovary with the apex of their long narrow protrusions. Scale bar indicates 50 μm. (b) High magnification of the region where oocyte attaches to the acinar wall. The surface of oocyte is covered with extracellular structures composed of many granules connecting each other with thin fibers. On the other hand, there is no such extracellular structure at the apical surface of the oocyte embedding in the acinar wall. Scale bar indicates 5 μm.

eXtruded just at the opposite side of the oocyte. These observations may suggest that oocytes attach to the acinar wall with their vegetal pole.

There were little follicular-like cells over the surface of the ovarian matured oocytes in view of the SEM photograph (Fig. 3a). The apical part of the protrusion of oocyte was embedded in the cell layer of acinar wall and the remainder was exposed directly to the lumen of the acinus (Fig. 3b). Although the surface of the embedded region of the oocyte was in contact with acinar wall cells directly, the rest of the surface was covered with extracellular structures composed of many granules connecting with each other by thin fibers (Fig. 3b).

Discussion

The polar lobe of molluscan eggs is thought to carry cytoplasmic determinants responsible for the differentiation of apical tuft and posttrochal region of trochophora and some mesodermal structures of veliger (for reviews, see [1-4]). The lobe protrudes at the vegetal pole of egg and D lineage blastomere at meiotic divisions and/or
at early several cleavages and is thought to be composed of vegetal part of cytoplasm, cortex, and plasma membrane of eggs. As clearly shown in this study, cytoplasmic granules emitting yellow autonomous fluorescence under UV illumination localize at the vegetal pole cortex of oocytes of Japanese oyster *Crassostrea gigas*. These granules remain at the vegetal pole cortex of eggs after fertilization. During first and second polar lobe formation, these granules are incorporated into the lobe and localize at the vegetal cortex of the lobe. The localization of the granules becomes gradually ambiguous during 2-cell stage and seems to disappear after 4-cell stage. In view of the prospective role of the polar lobe in the segregation of the cytoplasmic determinants, the behaviour of the yellow fluorescing granules during first two cleavages is very interesting. From the D lineage blastomere deletion experiments in *Ilyanassa* [17] and in *Dentalium* [18], it is inferred that the morphogenetic determinants localized in the polar lobe are progressively shunted during cleavage to the specific micromeres where they will function. The yellow fluorescing granules may be segregated into specific blastomere during following cleavages.

Until now, a special category of cytoplasmic constituents, such as RNA-containing ‘vegetal body’ in *Bithynia* [8], double membrane vesicle with a granular content in *Ilyanassa* [5, 6], a few small aggregates of vesicles in *Crepidula* [9], and a large number of electron-dense vesicles in *Buccinum* [10], has been observed localizing specifically at the vegetal plasm and the polar lobe. Several specific properties of plasma membrane, such as the local accumulation of symbiotic bacteria [11] and locally increased excitability of the membrane in *Dentalium* [14], local folding of the surface in *Nassarius, Buccinum*, and *Crepidula* [12] and in the polychaeta *Sabellaria* [13], and increased numbers of intramembraneous particles in *Nassarius* [15], are also investigated around the polar lobe and vegetal pole of eggs and specific blastomeres. The localization and behavior of the granules with yellow autonomous fluorescence that observed in this study seem to resemble those of the structures described above.

Although the polar lobe of *Bithynia* egg is small, removal of the ‘vegetal body’ containing polar lobe causes lack of adult structures such as shell, foot, eyes, and tentacles [19]. The ‘vegetal body’ is present in the uncleaved egg and in the first polar lobe but cannot be detected in histological sections of the CD blastomere [20]. Furthermore, it is known that the abilities to differentiate tissues and organs segregate gradually from D line macromere into respective micromere in each cleavage in *Ilyanassa* [21]. The localization of the fluorescing granules described in this study is also apparent in oocyte, uncleaved egg, and the first polar lobe, but become gradually ambiguous during 2-cell stage and seems to disappear after second cleavage.

When centrifuged at 2000×g for 10min, the oocyte of *C. gigas* was stratified into four layers. In most of these oocytes, the yellow fluorescing granules stratified at the most centripetal region of the most centrifugal layer, layer IV, but in some of them the granules moved as a mass to the most centripetal region of layer IV. Occasionally, they stuck to the centrifugal region of the oocytes. The ‘vegetal body’ in *Bithynia* is also reported to be removed as a whole by centrifugal force at about 1400×g [10].
thirty of seventy centrifuged eggs, ‘vegetal body’ was no longer found in the polar lobe, but in the cytoplasm of one of the blastomeres. After removal of the polar lobe in centrifuged eggs not only was abnormal embryos of characteristic lobeless type obtained but also completely normal embryos [10]. This result indicates that in Bithynia eggs, the morphogenetic determinants are localized in the ‘vegetal body’ and move with the ‘vegetal body’ by such degree of centrifugal force. On the other hand, Clement [22] centrifuged the matured eggs of Ilyanassa obsoleta at 2000×g and produced two types of light fragments, one type is animal hemisphere fragments with light cytoplasmic components and the other vegetal hemisphere fragments with light cytoplasmic components. The former fragments cleaved equally without formation of polar lobe and differentiated into partial larvae that lacked lobe-dependent structures. However, the latter underwent unequal first cleavage with a polar lobe and differentiated lobe-dependent structures. He concluded that the morphogenetic influence of the vegetal pole area is able to withstand centrifugal force sufficient to displace lipid droplets, yolk particles, and nuclei [22]. Therefore, it is uncertain that there is any relation between the fluorescing granules and the morphogenetic abilities. The development of the stratified oocyte of C. gigas should be examined in further investigation.

SEM photographs show that the ovary is filled with pear-shape oocytes that attach to the ovarian wall with their vegetal protrusions. This connection of oocyte at the vegetal pole with the ovarian wall and the localization of the granules with yellow autonomous fluorescence at the vegetal protrusions of pear-shape oocytes suggest that these granules may be transported from the ovarian wall cells into the oocyte through the connection and fixed there. The junctional structures between the plasma membrane of oocyte and that of ovarian wall cell should be examined with transmission electron microscope. Furthermore, permeability of molecules between them should be also examined with intracellular injection of low molecular-weight maker, such as lucifer yellow, in further investigation.

Visible markers of specific cytoplasm in eggs and embryos such as fluorescing granules described in this investigation are thought to be useful to examine the behavior and the function of such cytoplasm in development. We have already reported the specific autonomous fluorescence of ‘myoplasm’ in living eggs and embryos of the ascidian, Ciona intestinalis [23] and used it as a marker for isolation of the myoplasm from living fertilized eggs to make monoclonal antibodies which recognize the myoplasm specifically [24] The autonomous fluorescence of cortical cytoplasmic granules of polar lobe might be used as a convenient marker for isolation of polar lobe specific cytoplasm in the oyster eggs and improve our knowledge on the cytoplasmic determinants that are thought to be localized at the polar lobe.

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References

Autonomous Fluorescence of the Polar Lobe of C. gigas


マガキ卵の極葉に局在する自家蛻光

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マガキ Crassostrea gigas の卵を UV 赤起の蛻光顕微鏡で観察したところ、植物極表層の細胞質が特異的な黄色自家蛻光を持っていることがわかった。この自家蛻光を発しているのは、その部域に局在する多数の顆粒状構造である。この局在は卵巣内の成熟した卵母細胞で既に認められ、受精後も保持されている。第一極葉形成時にこの顆粒は極葉の植物極表層に局在し、2細胞期には CD 割球に取り込まれる。この顆粒の局在は2細胞期及び第二極葉形成時にも認められるが徐々に不明瞭になり、4細胞期以降にはこのような局在は認められなかった。