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On the Mechanism of Ooplasmic Segregation upon Fertilization in *Oryzias latipes*

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Abstract Pulsating contraction of peripheral ooplasm of the eggs of a cyprinodontoid fish, *Oryzias latipes*, was observed after alveolar breakdown upon fertilization. The contraction is initiated at the animal pole region in the vicinity of the fertilization cone and propagates toward the vegetal pole. The movement of ooplasmic inclusions and of carbon particles injected into the peripheral ooplasm was examined after insemination, and it was suggested that ooplasmic segregation is induced by contractions of the peripheral ooplasm.

In fish eggs, ooplasmic segregation takes place following the cortical changes by fertilization and results in the formation of blastodisc (cf. Yamamoto, 1961). Studies in this regard have been made by some investigators (e.g., Sakai, 1965), but the mechanism by which the segregation occurs has not been clarified. According to Thomopoulos (1953), a contractive movement of peripheral ooplasm takes place upon fertilization in the stickleback egg, and Sakai (1961) observed a movement of ooplasmic inclusions and a change of egg surface tension following fertilization of the medaka (*Oryzias latipes*) egg. It seems possible that the ooplasmic segregation is the result of the contractive movement of peripheral ooplasm.

To test the above possibility, the following studies were made. First, the contractive movement of peripheral ooplasm which ensures immediately after insemination was carefully observed. Secondly, movement of ooplasmic inclusions or of carbon particles injected into the cytoplasm was followed during the ooplasmic segregation.

Materials and methods

Unfertilized ripe eggs of *Oryzias latipes* were obtained by the method devised by Yamamoto (1944). Females which had spawned in the morning were isolated from the males and were used the following day. The fish were laparotomized and the removed ovaries were placed in isotonic salt solution (Yamamoto, 1939, 1944). The ripe unfertilized eggs in the ovarian lumen were isolated with glass needles and kept in salt solution. Sperm suspension was prepared by smashing the testis of adult male with the blunt end of a glass rod in about 0.5 ml of salt solution.

An unfertilized egg was put on a slide glass with a small amount of salt solution. Two pieces of thread were set on both sides of the egg to keep its form spherical, and a cover slip was carefully applied. Under an ordinary microscope, the sperm suspension was introduced with a small pipette into the medium surrounding the egg.

Contractive movement of the egg upon insemination was observed in a hole slide glass under a binocular microscope (magnification, ×80).

Ooplasmic current in the peripheral region was observed by pursuing the movement of ooplasmic inclusions during ooplasmic segregation both in intact and fixed eggs. In living eggs, the position of marked ooplasmic inclusions was photographed from the amimal or equatorial sides in different eggs at intervals of 1/2 or 1 min from insemination until 40 min after insemination. The distance (μ) of movement was calculated from tracing the photographs which were enlarged 200 times; as the method described in Iwamatsu (1967). In paraffin-sectioned eggs which were fixed with cold Bouin's fluid (0～5°C) at intervals of 5 min after insemination, the position of the ooplasmic inclusions was observed after staining with Heidenhain's iron haematoxylin.

Some observations were made on fertilized eggs in which some cortical alveoli were embedded deeply in the ooplasm by means of centrifugation (ca. 1,500 g, 5 min) before insemination. Other observations were also made on the peripheral ooplasm into which a

small drop of the isotonic salt solution (ca. 20μ in diameter) which contained suspended carbon particles was injected immediately after insemination by the micromanipulation method of Hiramoto (1962) with some modification. All observations were made at water temp. 25～27°C, except for a case noted in the observation on the fertilization cone at 21～22°C. Stratiform regions which often appeared in the peripheral ooplasm were represented as layers.

Observations

1. Fertilization cone

Breakdown of the cortical alveoli started near the animal pole about 30 sec after a spermatozoon entered the ooplasm through a micropyle, as reported by Uwa (1967), and immediately followed thickening of the transparent ooplasm in the animal pole region (ca. 40 sec after insemination). About 5 sec after thickening, a blister-like fertilization cone appeared in the cortical region just beneath the end of the micropyle canal (Fig. 1), immediately after decrease in volume of ooplasm by exhaustion of alveoli. It began to withdraw about 1.5 min after the spermatozoon stuck the egg surface and disappeared 1 min later. This withdrawal was approximately synchronous with the completion of alveolar breakdown.

2. Contractive movement of the egg

When the fertilization cone appeared at the point of spermatozoon impact, the ooplasm began to contract partially around the animal pole region, and this partial constriction propagated slowly toward the vegetal pole, following the wave of the alveolar breakdown (Fig. 2). The whole course of this contractive movement took about 3 min.

3. Behavior of ooplasmic inclusions

Small granules (ca. 0.1μ) and oil droplets (ca. 35μ), which preexist in unfertilized eggs, moved temporarily and concurrently toward the animal pole about 2 min after insemination. Until about 10 min after insemination, they oscillated to and from their original position within a distance of about 10μ along the animal-vegetal axis of the egg. It was also observed that the gathering point of the ooplasmic inclusions corresponded to the animal pole (gv (*) in Fig. 3) where the polar bodies were protruded, but not to the point of spermatozoon-penetration. Migration (ca. 5μ/min) of small granules toward the animal pole began about 15 min after insemination, and then that of oil droplets toward the vegetal pole began about 15 min later (Fig. 4).

Also, the position of oil droplets in the peripheral ooplasm changed after insemination. In the unfertilized egg, the droplets were situated in the middle region in depth of the ooplasm. After the contents of the cortical alveoli were released into the perivitelline space by fertilization, the oil droplets protruded on the surface of the ooplasm for about 5 min. This change proceeded from the animal to the vegetal pole. About 10 min after insemination, the oil droplets had descended to the middle depth of the ooplasm and finally sunk into the

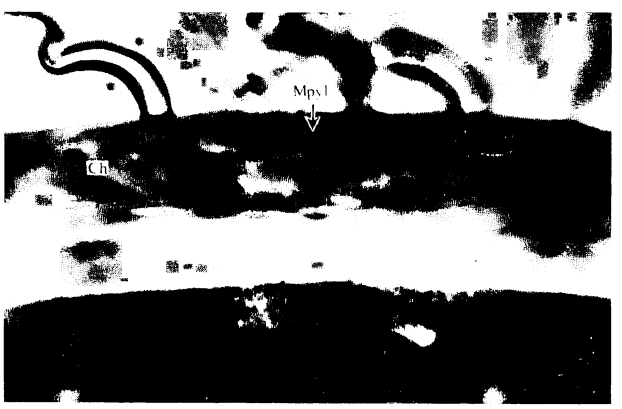

Fig. 1. A fertilization cone of a living egg about 60 sec after spermatozoon penetration (×1,500). Ch; chorion, Mpyl; micropyle.

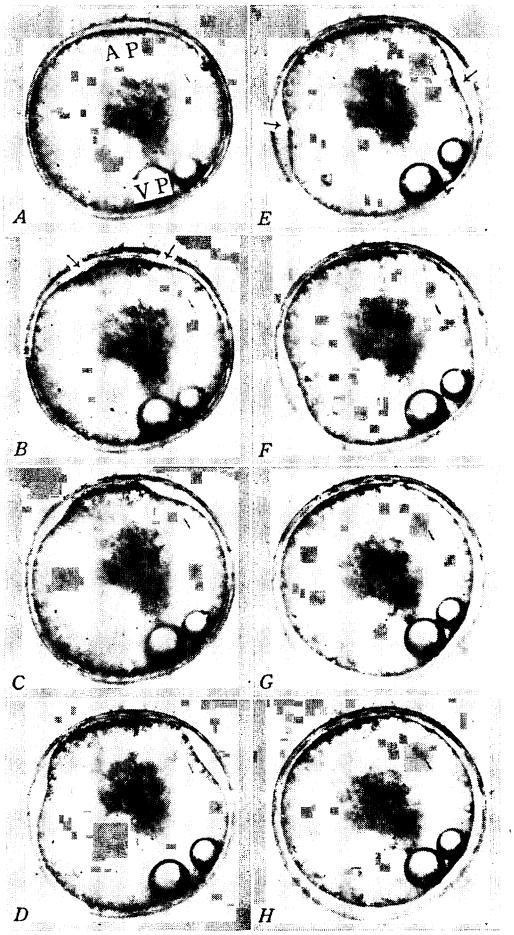

Fig. 2. Contractile movement of an egg, from about 45 sec (A) to about 150 sec (H) after spermatozoon penetration. (×33) AP; animal pole, VP; vegetal pole, Arrow; contracted region.

yolk mass.

4. Observations of ooplasmic segregation with some experimental aids

a) Behavior of injected carbon particles:

When carbon particles were injected into the peripheral ooplasm of unfertilized eggs, they dispersed among the ooplasmic inclusions. After fertilization, those particles situated in the inner region of the ooplasm moved toward the vegetal pole, whereas those in the outer region moved toward the animal pole (Fig. 5a). In some carbon injected eggs a kind of stratification was temporarily observed. As seen in Fig. 5b, four stratiform regions could be distinguished in the ooplasm; two homogeneous and gel-like

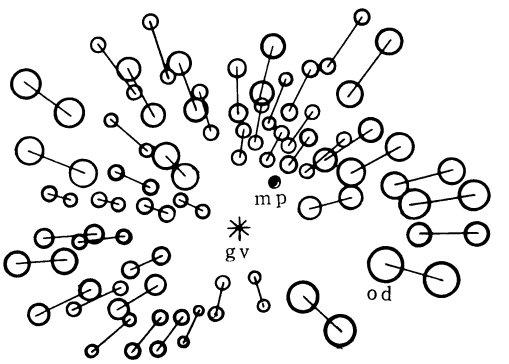

Fig. 3. Tracing of movement of oil droplets around the animal pole upon spermatozoon entry. Each solid line indicates direction of the shift of oil droplets. gv; position (*) where the germinal visicle disappeared. mp; micropyle, od; oil droplet.

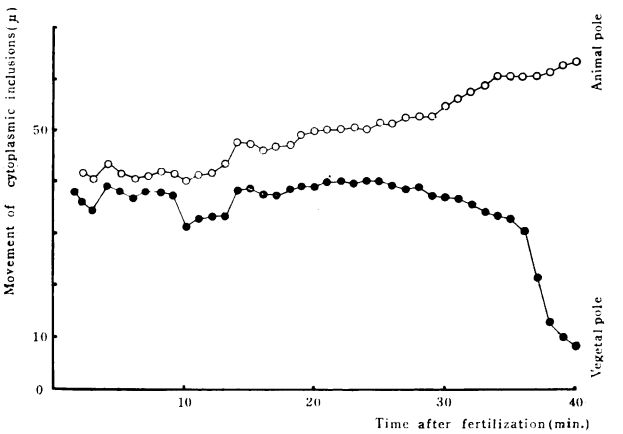

Fig. 4. Movement of cytoplasmic inclusions (small granules, 0.5-1μ; oil droplets, 20-40μ) of egg after insemination. Open circles, small granules; closed circles, oil droplets.

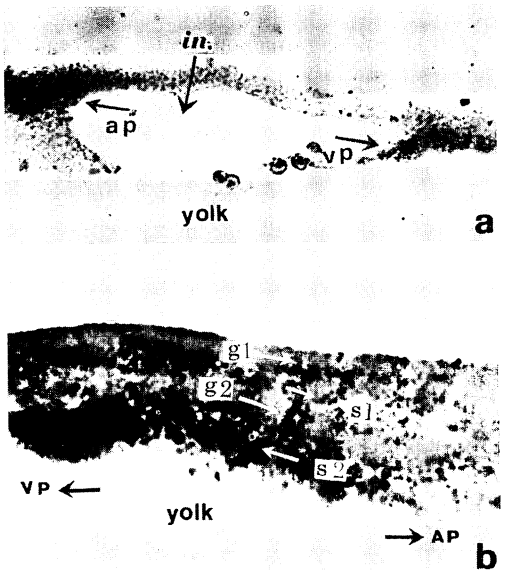

Fig. 5. Photographs of sectioned peripheral ooplasm of activated eggs which were injected with carbon particles. (×1,000) a: An egg 30 min after activation, showing the distribution of injected (*in*) carbon particles in the outer region to animal pole side(ap) and in the inner region to vegetal pole side(vp). b: An egg about 60 min after activation, showing homogeneous (g1, g2) and heterogeneous (s1, s2) regions in the ooplasm.

layers appeared in the outer and the middle regions within the ooplasm, and two heterogeneous sol-like layer, which consisted of some inclusions and carbon particles, appeared between the outer and middle layers and in the innermost region.

b) Behavior of ooplasmic inclusions in centrifuged eggs:

It has already been reported that cortical alveolus breakdown upon insemination is inhibited by acetone-treatment, and that these move toward the animal pole during ooplasmic segregation, and accumulate in the blastodisc (Sakai, 1964b, 1965; Iwamatsu, 1965). Such a inhibition of the alveolar break-down was also effected by centrifugation before insemination. The ooplasm containing cortical alveoli gathered at the centrifugal side, but oil droplets at the centripetal side. The majority of these alveoli failed to breakdown upon fertilization and moved gradually toward the animal pole, and the oil droplets moved toward the vegetal pole. Sectioning and staining of the eggs showed that these alveoli were situated in the outer or middle region of the ooplasm, and most small granules in the innermost region. Accumulation to the vegetal pole of small amount of ooplasm with numerous small granules was also observed upon fertilization in the eggs in which ooplasm had accumulated in the equatorial region by centrifugation. The oil droplets covered with a thin cytoplasm situated in the equatorial and centripetal sides, and then moved slightly toward the vegetal pole upon fertilization.

These observations suggest that two slow movements within the peripheral ooplasm arise during ooplasmic segregation: one toward the animal pole in the outer ooplasm region and the other toward the vegetal pole in the innermost ooplasm.

c) Ooplasmic segregation in constricted eggs:

When a small part of the vegetal hemisphere of the chorion of an unfertilized egg was cut off and the egg was inseminated, the egg gradually protruded out of the chorion and constricted into two parts, the animal and the vegetal spheres, connected to each other through a small pore of the chorion (Fig. 6A). The oil droplets in the animal sphere migrated toward the vegetal sphere and there fused, increasing in volume while decreasing in numbers; but these oil droplets did not move further into the vegeral sphere even after completion of the first cleavage (Fig. 6B). Oil droplets in the vegetal sphere, on the contrary, did not migrate their position to the vegetal pole side and after a long time, gathered together along the water surface side of egg. This seems to show that the constriction of the egg itself by a small pore of the chorion blocked the propagation of contractive movement from the animal to the vegetal pole and prevented the usual migration of the oil droplets toward the vegetal pole.

Discussion

When a medaka egg is fertilized, breakdown of the cortical alveoli is induced after the propagation of the invisible "fertilization wave" (Yamamoto, 1944). Decrease in ooplasmic volume takes place as a result of the extrusion of cortical alveoli into the perivitelline space (Yamamoto, 1940; Iwamatsu, 1965). As de-

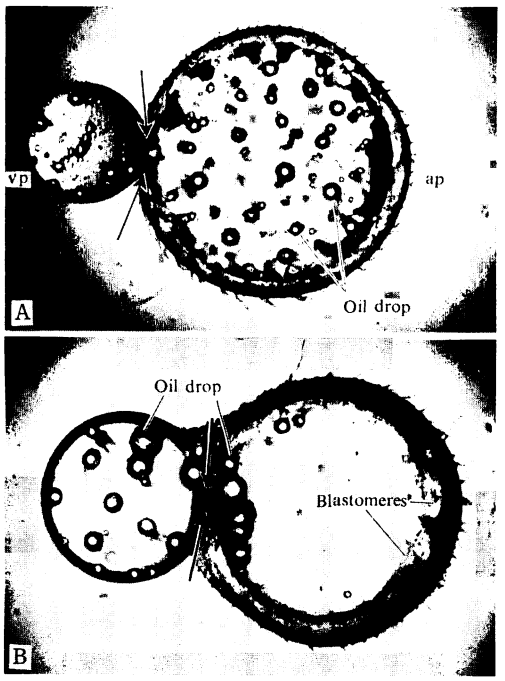

Fig. 6. Ooplasmic segregation in an egg constricted into the animal and the vegetal spheres upon fertilization. (×45)
A: Fertilized egg showing a similar distribution of oil droplets in both of the animal and the vegetal spheres about 5 min after insemination.
B: The egg in A showing the movement of oil droplets to the opposite side against the blastomeres in only the animal sphere. (Arrows indicate the constricted region)

scribed in the present observation, release of alveolar substance is followed by the contraction of the outermost part of the peripheral ooplasm which moves temporarily toward the animal pole. Concurrently, the oil droplets protrude on the surface of the peripheral ooplasm (see Sakai, 1961, pl. 5); this may be due to decreases both in the volume of peripheral ooplasm (Yamamoto, 1940) and in the tension of the egg surface (Sakai, 1961). The oil droplets oscillate around their original position alternatively along the animal-vegetal axis for about 20 min after insemination. This phenomenon has been described as the stationary stage by Sakai (1961) and also by Iwamatsu (1967).

It was also detected by the present observation that the contractive movement begins at the animal pole and spreads toward the vegetal pole side. The contractile layer in the ooplasm in the animal pole region would seem to play a primary role in this movement. After a conspicuous contractive movement, the protruding oil droplets on the egg surface are gradually withdrawn into the yolk mass and the egg surface becomes smooth.

A large amount of contractile component preexists around the animal pole region and may be segregated from the other components in the ooplasm upon fertilization. This contractile component around the animal pole connects with that which appears in the other areas of the peripheral ooplasm upon fertilization. The component appeared in the other areas change their position by its contraction toward the animal pole whereat the contraction is initiated. Consequently, some small inclusions situated between the outer and the middle layers, both of which are gel-like and contractile, may shift to the animal pole region by contraction of such layers. This contractile layer itself and small granules, or often large inclusions like cortical alveoli between the layers, are accumulated in a blastodisc, as described previously in Sakai (1964a, b) and Iwamatsu (1965). On the other hand, oil droplets in the innermost region of the ooplasm may be passively squeezed toward the vegetal pole by the pulsatile contraction of outer and middle gel-like layers in the direction of the animal pole, and also by the propagation of this contraction from the animal to the vegetal pole. The present observations on the pulsative movement of ooplasm and on the behavior of ooplasmic inclusions during bipolar differentiation suggest an important role of the contractile property of the peripheral ooplasm in ooplasmic segregation.

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魚卵の受精に伴う原形質内の分離の機構について

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メダカ *Oryzias latipes* 卵において，受精に伴っておこる表層胞の崩壊につづいて囲周原形質の律動的収縮がおこるのが観察された．この収縮は，受精丘のそばの動物極部域でおこり，植物極に向って進展してゆく．受精時にみられる卵原形質内の分離過程中，その原形質内の顆粒や外から注入された炭素粒の運動を調べた結果，その分離現象が卵の囲周原形質の収縮性によることが示唆された．

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