Electron Microscopic Observation of the Breakdown of Cortical Vesicles in the Chum Salmon Egg

By

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(*With 23 Text-figures*)

Following the fertilization or parthenogenetic stimulation of the eggs of various fish species, conspicuous membranous structures in the cortical ooplasmic layer —— the cortical vesicles —— undergo a drastic change (Yamamoto, 1961; Iwamatsu and Ohta, 1976; Iwamatsu and Keino, 1978; Hart and Yu, 1980; Brummett and Dumont, 1981). The contents of these vesicles are discharged from the egg and play important roles in the formation of perivitelline space (cf. Laale, 1980) and in the prevention of polyspermy (Kanoh, 1957; Ginsburg, 1961; Brummett and Dumont, 1979, 1981). It is known that the salmonid egg does not initiate the embryonic development in an isotonic salt solution after sperm penetration into the ooplasm; immersion in water is a *sine qua non* for the induction of this development (Kusa, 1950, 1964; Ginsburg, 1963; Yamamoto, 1976). The property of salmonid eggs is therefore different from that of the eggs of such fish species as *Oryzias* and *Pungitius* whose eggs react to the sperm entry in the salt solution and initiate the embryonic development (Yamamoto, 1961; Kusa, 1953). Furthermore, the salmonid egg shows breakdown of cortical vesicles parthenogenetically after incubation in water (Kanoh, 1950; Yamamoto, 1951). No change of the ooplasm can be detected, however, in the unfertilized *Oryzias* egg following incubation in water, although the fertilizability of the eggs is reduced considerably within a short time (Yamamoto, 1961). In order to obtain a clue to the understanding of the physiological property of the salmonid eggs, a fine structural study was performed on the breakdown of cortical vesicles in chum salmon eggs. Furthermore, the spatiotemporal course of breakdown was also followed.

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Materials and Methods

The materials used in this study were samples of chum salmon, *Oncorhynchus keta*, obtained from the Hokkaido Salmon Hatchery at Chitose, Hokkaido. Batches of the gametes from the fish were stripped into separate glass vessels without contamination from water and stored at 1°C.

Immediately before use, the ripe eggs were washed in several changes of Salmon-Ringer solution (SRS) (Yamamoto, 1976). An aliquot of each batch was directly fixed in 5%glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) containing 2.5%sucrose for 16 hours at room temperature (intact eggs). The remaining eggs were divided into two groups; one of them was fertilized by "dry" sperm, while the other remained unfertilized. Both groups were then immersed in tap water (13°C) to induce breakdown of the cortical vesicles. Since the breakdown is independent of the presence of sperm in the egg, both developing (fertilized) eggs and parthenogenetically activated eggs were obtained by this method. After incubation in tap water for the allotted time, the eggs were fixed in the same fluid that had been used for the fixation of intact eggs.

For the purpose of scanning electron microscopy (SEM), various portions of the cortical ooplasm were excised from the rest of the egg after the careful removal of the overlying envelope (*zona pellucida*). The specimens were rinsed in the cacodylate buffer followed by postfixation in 1%OsO_4 in the same buffer at 4°C. These were then dehydrated in acetone, critical-point dried in CO_2, sputter- coated with gold and examined in a JEOL JSM-T20 scanning electron microscope.

For transmission electron microscopy (TEM), small pieces of the cortical ooplasm with or without the overlying envelope were excised from various portions of the glutaraldehyde-fixed eggs and rinsed in the cacodylate buffer. The specimens were postfixed in cacodylate-buffered 1%OsO_4 (4°C), dehydrated in acetone and embedded in Epon 812. Thin sections stained with uranyl acetate and lead citrate were examined in a JEOL JEM-100S electron microscope.

Results

The structure of the cortical ooplasmic layer in the intact egg

The ooplasm of the chum salmon egg surrounds the central mass of yolk and forms a layer, the cortical layer. The layer is thickest at the animal pole and gradually decreases toward the opposite end of the egg. The outer surface of the cortical layer is closely associated with the overlying egg envelope; there is no clear space between the envelope (*zona pellucida interna*) and the ooplasm. The association of both structures is so intimate that it is difficult to separate the envelope from the ooplasm without causing disruption of the surface structure of the layer. The structure of the cortical layer in the intact eggs was, therefore,

examined in thin sections only.

The cortical layer is divided into two regions, a peripheral region and an inner one. The peripheral region, which is 0.2-0.5 μm thick, shows a compact structure and does not contain such membranous organelles as mitochondria or smooth endoplasmic reticulum (SER). There are, however, nonordered filaments and free ribosomes in this region of the cortical layer (Figs. 1,2). Numerous microvilli that start from the surface are found either invading the lumen of the radial canals or sandwiched in between the envelope and the ooplasm (Fig. 1). Although the length of the villi is shorter in the latter case, it seems changeable within the same egg; the villi in the radial canals apparently decrease in length after long-term incubation in SRS. The fine structure of the microvilli is, however, identical in both cases and resembles that observed in the peripheral region of the cortical layer.

In contrast to the peripheral ooplasm, the inner region of the cortical layer possesses a number of mitochondria, tubular and vesicular profiles of SER and a few number of small yolk spherules (Fig. 1). A conspicuous constituent of this region is the cortical vesicle (CV) measuring 2-25 μm in diameter. The vesicles are arranged in a single layer which runs parallel to the ooplasmic surface. Serial sections disclose that the CVs are usually prevented from making direct contact with the egg plasma membrane by the peripheral ooplasm (Figs. 1, 7a). A few number of CVs, however, closely appose their upper edge to the egg plasma membrane (Fig. 2). Although the inner region of the cortical layer does not contain any CVs beneath the micropyle of the egg envelope, it possesses a number of ellipsoidal vesicles (Kobayashi and Yamamoto, 1981, 1985).

Each CV is membrane-bounded and contains crisscrossed fibrils and fine particles. The fibrils generally show a uniform distribution throughout the lumen of the small vesicle but frequently form an aggregate in the large vesicle. In the latter case, the aggregate looks like a core of the vesicle (Fig. 1).

Breakdown of cortical vesicles

I was able to observe drastic changes of the ooplasmic surface during the first few minutes of the incubation in tap water of both fertilized and unfertilized (parthenogenetically activated) eggs; these changes were associated with breakdown of the CVs which had been observed in the cortical layer of the intact egg. I was able to observe various stages of the breakdown of individual CVs in the animal pole area of a single egg fixed at 60-120 seconds postincubation in tap water. It is therefore possible to reconstruct the process of the breakdown by

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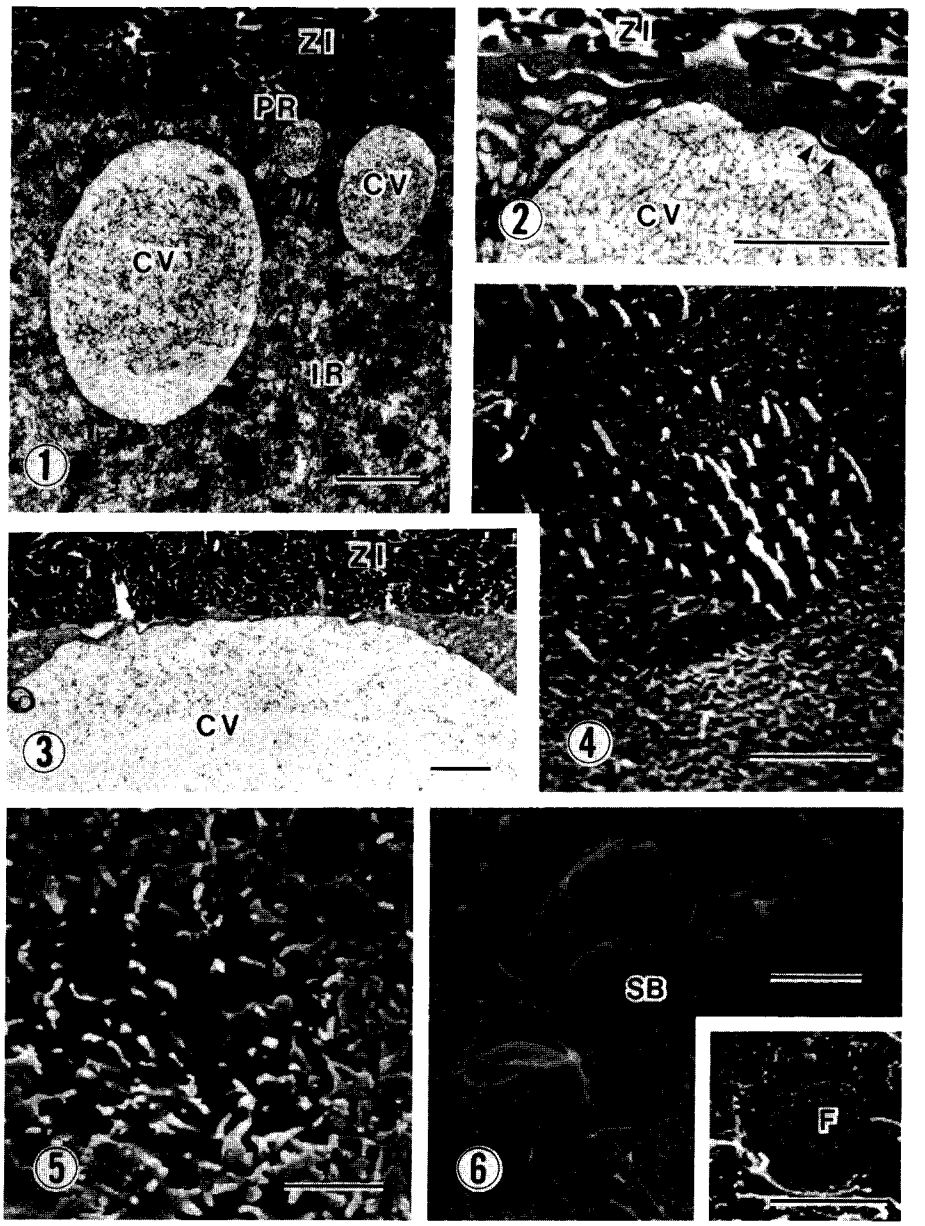
Abbreviations

CI, indentation of ooplasmic surface (previous cortical vesicle lumen); CO, compact ooplasm; CV, cortical vesicle; F. fibrillar material or aggregate; IR, inner region; MF, microfilaments; PR, peripheral region; PS, perivitelline space; SB, spherical body; ZI, *zona pellucida interna*.



Figs. 1 and 2. Sections through the animal pole area of the intact egg showing the peripheral, compact and inner, loose ooplasmic regions in the cortical layer. Note the presence of a closely-packed mass of fibrils in the central region of large-sized CVs in Fig. 1. Close contact of the upper edge of CV with the egg plasma membrane is observed, as

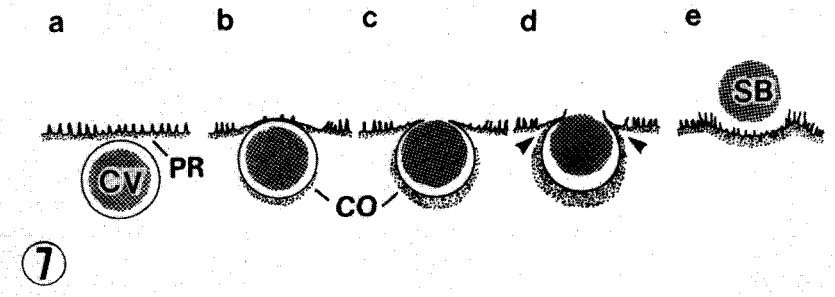


Fig. 7. Schematic illustration of the distribution of compact ooplasm in the cortical layer of the egg during the CV breakdown. a: intact egg. When the CV approaches the plasma membrane, a small area of the CV membrane is decorated with the compact ooplasm (b). During the discharge of the contents, the CV membrane with this ooplasm increases in area (c). The compact ooplasm finally joins with the peripheral region of the cortical layer (d arrowheads, e).

means of SEM and TEM micrographs of the eggs fixed after they had been incubated for 1-2 minutes.

Initially, the CVs located underneath the peripheral region of the cortical ooplasmic layer approach the egg plasma membrane (Figs. 3, 7b). This step may be virtually absent in the CVs whose upper edge had closely apposed the plasma membrane of the intact egg. The approach is identified by SEM by the appearance of circular areas on the ooplasmic surface where the number of microvilli is smaller than over the rest of the surface (Fig. 4); these villi are relatively short and burly. Careful observation of these eggs by TEM reveals the existence of a small area of the CV membrane decorated with compact ooplasm. It is observed at the site opposite to the contact of CVs with the plasma membrane (Fig. 7b).

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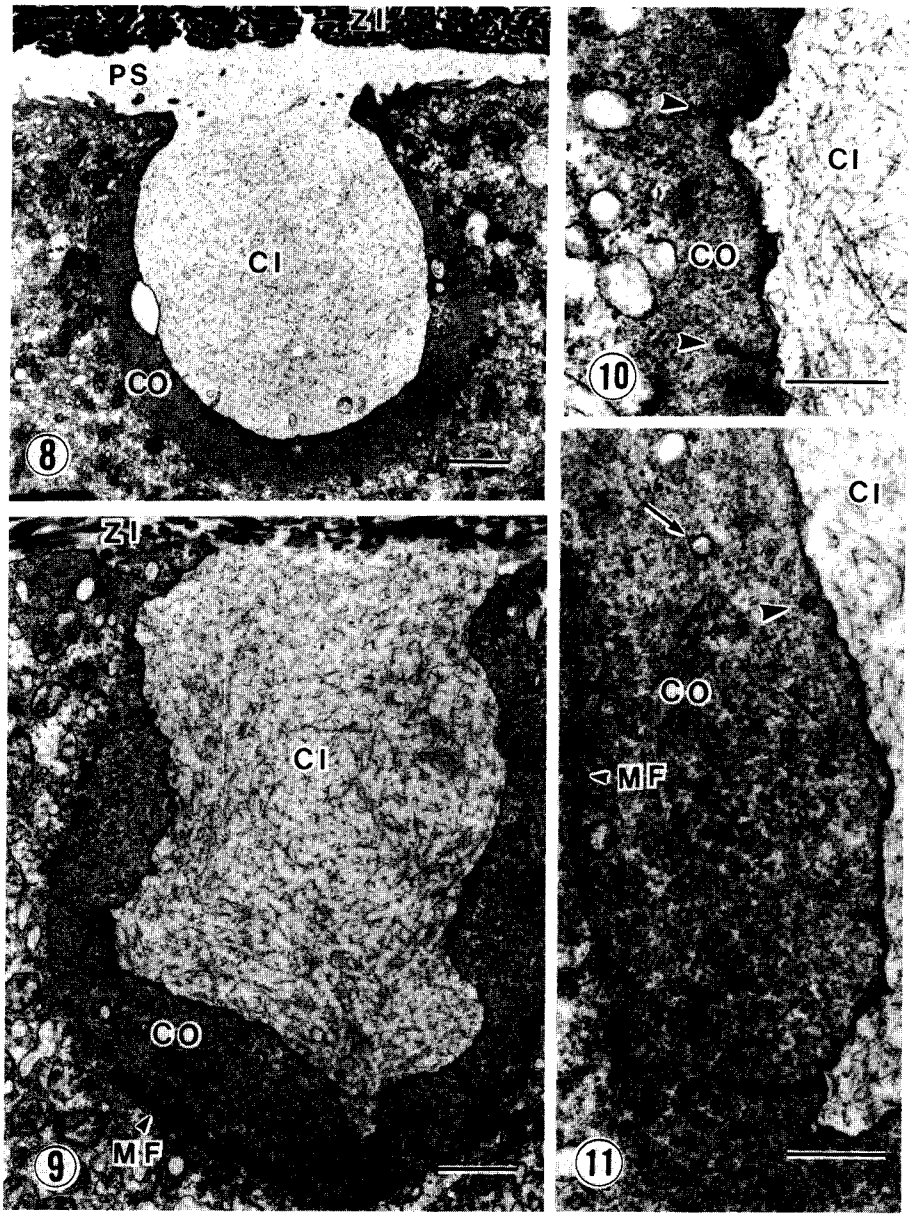
indicated by arrowheads in Fig. 2. Bar=2 μm.

Fig. 3. Section through the cortical layer of the animal pole area fixed at 60 seconds postincubation in tap water showing the initial dislocation of the cortical vesicles. Bar= 2 μm.

Fig. 4. SEM of the ooplasmic surface of the animal pole area fixed at 60 seconds postincubation. Note the circular areas of the ooplasmic surface with short and burly microvilli. Bar=5 μm.

Fig. 5. SEM of the animal pole area of the egg fixed at 60 seconds postincubation indicating small apertures of the CVs on the ooplasmic surface. Bar=2 μm.

Fig. 6. SEM of the animal pole area of the egg fixed at 90 seconds postincubation showing apertures on the ooplasmic surface. Note two or three openings over a single CV. The CV contents are seen as smooth-surfaced spherical bodies. Bar=10 μm. Inset: A small indentation of the ooplasmic surface in the animal pole area. It is filled with a mass of loosely-packed fibrils. Bar=5 μm.



Figs. 8-11. Sections through the cortical layer of the animal pole area fixed at 90 seconds postincubation. 8). The deep indentation of the ooplasmic surface. Note a thick layer of the compact ooplasm decorating the lining membrane of previous CV. Bar=2 μm. 9). Bundles of microfilaments in the periphery of the compact ooplasmic layer. Bar = 1 μm. 10&11). High magnification of the compact ooplasmic layer showing

The fine structure of the compact ooplasm resembles that observed in the peripheral region of the cortical layer. During the next stage, the upper edge of the CV membrane coalesces with the egg plasma membrane to externalize the inner surface of the CV membrane (Fig. 7c); the externalization can be recognized by SEM by the formation of small apertures on the ooplasmic surface (Fig. 5). At an early stage of the aperture formation, several small openings can frequently be observed on the surface overlying a single CV (Fig. 6); the increase in diameter of these openings, however, results in the formation of an aperture in each CV. A part of the contents of the CVs is visible by SEM through this aperture on the ooplasmic surface. A small-sized CV contains a loosely-packed mass of fibrils, whereas a large-sized one possesses a relativaly smooth-surfaced spherical body (Fig. 6). Careful observations, however, indicate that the spherical body also consists of densely-packed fibrils. In sections, the CV which have coalesced with the egg plasma membrane is recognized as a deep indentation of the ooplasmic layer (Figs. 7d, 8). During the increase in diameter of the aperture, the compact ooplasm previously observed in the inner region of the cortical layer increases in size along the periphery of the indentations; the lining membrane of the indentation (the previous CV membrane) decorated with this ooplasm increases in area during the course of the CV breakdown (Fig. 7c). Finally the compact ooplasm joins with the peripheral region of the cortical layer at the margin of the aperture (Fig. 7d). It contains, as does the peripheral ooplasm in the cortical layer, nonordered filaments and free ribosomes but possesses no such membranous organelles as mitochondria or SER (Fig. 8). Bundles of microfilaments are formed in the vicinity of the indentations during the increase in diameter of the aperture (Fig. 9). These are usually located peripherally in the compact ooplasm that decorates the lining membrane of indentations and show an arrangement parallel to the membrane (Figs. 9, 11).

Careful observation of the wall of the indentations at this stage reveals the presence of coated invaginations of the membrane lining the wall (Figs. 10, 11). Furthermore, the surface of the indentation frequently possesses uncoated tubules (15-20 nm in diameter) with coated blind ends of hemispherical shape in a deep region of the compact ooplasm (Fig. 10). This region occasionally also contains both coated vesicles (about 75 nm in diameter) (Fig. 11) and relatively large, smooth vesicles (Figs. 8, 11). Although similar structures are detected in the peripheral region of the cortical layer at this stage, their number is considerably larger in the vicinity of the indentations.

With the shallowing of the indentations, their contents are expelled from the previous CV lumen (Fig. 7e). When the contents are removed from the surface, a mosaic constitution of the plasma membrane can be clearly recognized by SEM of the cortical layer (Figs. 12,13); the plasma membrane now consists of the lining

endocytotic activity of the wall of the previous CV. Note coated membranous structures (arrowheads) and a smooth-surfaced vesicle (arrow). Bar=0.5 μm.

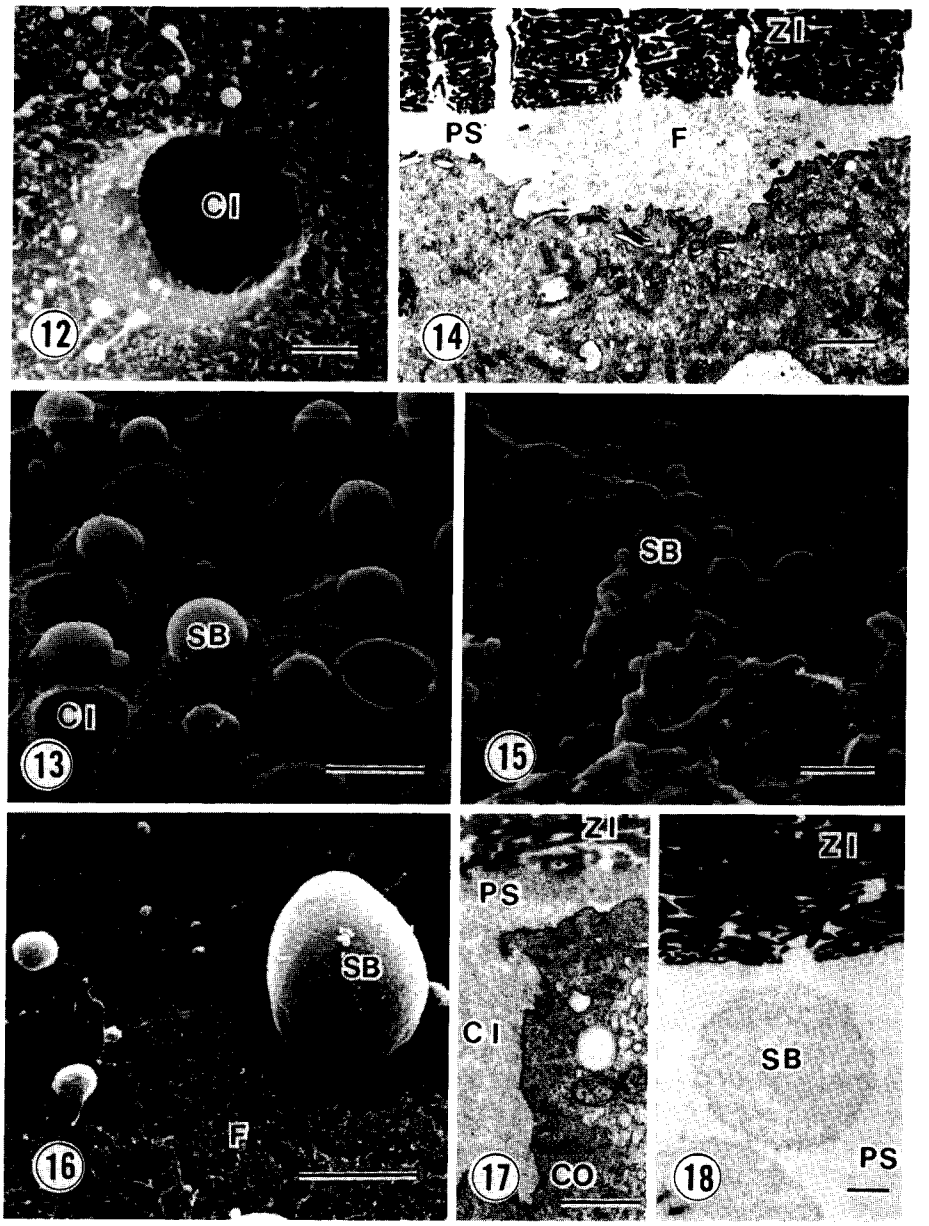


Fig. 12. SEM of the animal pole area fixed at 120 seconds postincubation showing the indentation of the ooplasmic surface. Note elongate microvilli at the margin of the indentation. Bar=5 μm.

membrane of the shallow indentations and the original egg plasma membrane. The former is dark and smooth-surfaced, whereas the latter is light and bears numerous microvilli. The boundary of these areas is characterized by the presence of elongate microvilli (Fig. 12). As the bottom of the indentations attains to nearly the level of the original plasma membrane, the surface of the previous indentation begins to show undulations. The microvilli marking the boundary of the two areas of the plasma membrane begin to decrease in length. With the formation of numerous microvilli on the membrane lining the previous indentations, the distinction of two areas on the egg surface becomes hard to observe (Fig. 13).

Concurrently with the discharge of the contents from the previous CV lumen, the compact layer of ooplasm which decorates the membrane lining the indentation gradually decreases in thickness (Fig. 7e). At the same time, the peripheral region of the cortical layer increases in width (Fig. 14). After the completion of the CV breakdown, therefore, the peripheral region of the cortical layer which contains neither mitochondria nor SER shows the same width throughout the animal pole area. Although it still contains coated, membranous structures, their number is apparently smaller than that in the compact ooplasm decorating the membrane of indentations during the preceding stage.

In the perivitelline space of the activated eggs, the CV contents remain unchanged or coalesce with each other to form amorphous materials; by SEM of the fixed egg, these can be observed either on the ooplasmic surface (Fig. 15) or on the inner surface of the egg envelope (Fig. 16). Transverse sections of the envelope from these eggs disclose thick accumulations of fibrillar and particulate material on its inner surface (Fig. 17). I was not able to detect these accumulations in any of the eggs incubated in tap water for 2 hours (Fig. 18). The perivitelline space of these eggs, however, still contains a small number of the spherical bodies.

Fig. 13. SEM of the animal pole area fixed at 120 seconds postincubation showing indentations of the ooplasmic surface. The shallow indentations are microvillous and show the same structure of the surface as the original plasma membrane of the egg. Bar = 25 μm.

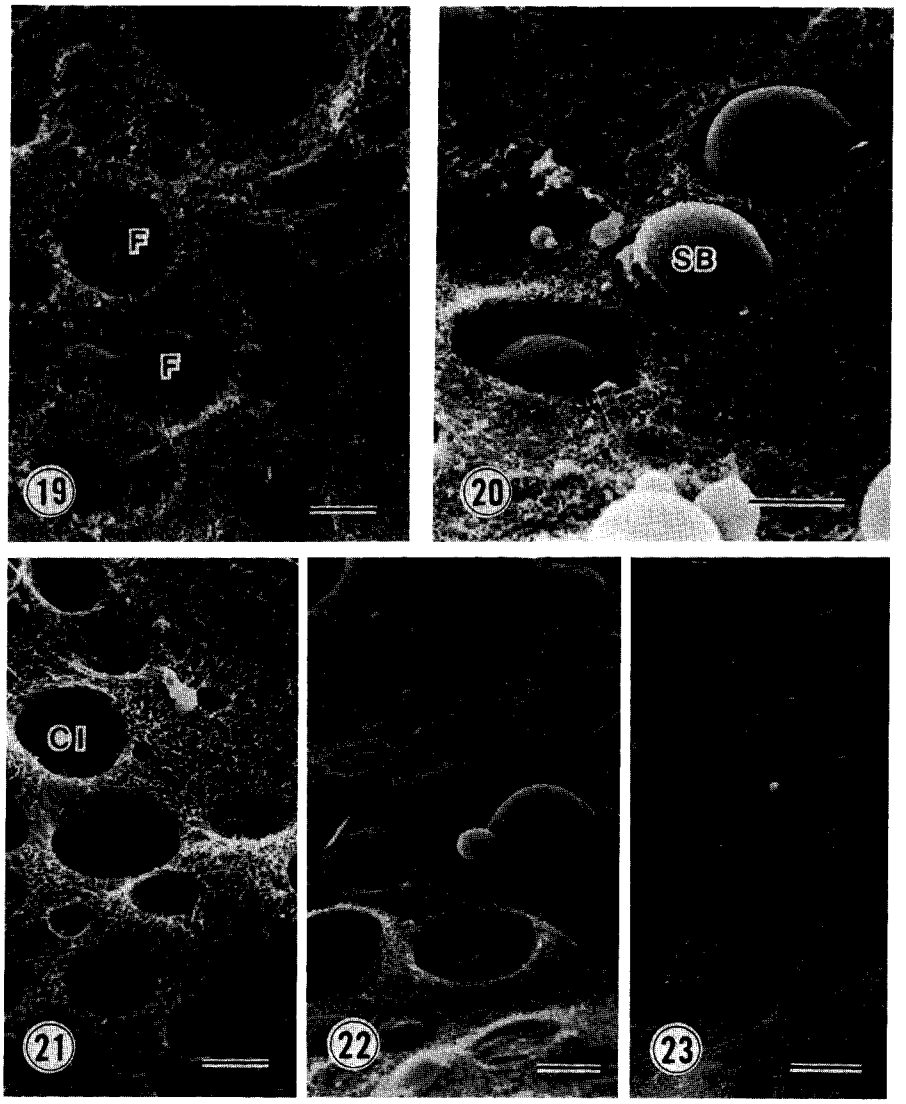
Fig. 14. Section through the animal pole area fixed at 120 seconds postincubation showing the microvillous surface of indentation. Bar = 2 μm.

Fig. 15. SEM of the animal pole area fixed at 120 seconds postincubation showing the coalescence of spherical bodies on the ooplasmic surface. Bar = 25 μm.

Fig. 16. SEM of the inner surface of the egg envelope from the egg fixed at 120 seconds postincubation. Bar = 5 μm.

Fig. 17. Section through the animal pole area of the egg fixed at 120 seconds postincubation showing accumulation of fibrillar and particulate materials in the perivitelline space. Bar = 1 μm.

Fig. 18. Transverse section through the perivitelline space of the egg fixed at 2 hours postincubation showing spherical bodies. Bar = 1 μm.



Figs. 19-23. The temporal course of the CV breakdown in the animal pole area of parthenogenetically activated eggs. 19). 30 seconds postincubation showing the initiation of the breakdown of small-sized CVs. Note indentations filled with fibrillar material. Fibrillar aggregates are seen on the ooplasmic surface. Bar=5 μm. 20). 60 seconds postincubation showing the initiation of the breakdown of large-sized CVs. Bar=10 μm. 21). 120 seconds postincubation showing large indentations of the ooplasmic surface. Bar=10 μm. 22). 180 seconds postincubation showing the shallowing of indentations. The surface of some indentations is still smooth but that of others is microvillous. Bar=10 μm. 23). 360 seconds postincubation showing a mosaic constitution of the ooplasmic surface. Dark patches represent the surface of the shallow indentations. Bar=25 μm.

The spatiotemporal course of the surface changes in the eggs incubated in tap water

In order to examine the spatiotemporal course of the CV breakdown on the ooplasmic surface, SEM was performed on the eggs fixed at selected times of the incubation in tap water. The separation of the envelope from the glutaraldehyde-fixed eggs however caused mechanical disruption of the structure in some areas of the egg. Transmission electron microscopy revealed that the CV breakdown has still not begun in these areas of the egg. Observation of the restricted areas of the ooplasmic surface was possible, however, by the time some of the changes accompanying the breakdown had occurred in the cortical layer.

Parthenogenetically activated eggs: The CV breakdown can first be recognized at the animal pole area of the eggs fixed at 30 seconds postincubation in tap water; a number of apertures are observed on the ooplasmic surface of this area (Fig. 19). Since a loosely-packed mass of fibrils is seen through the aperture, there can be no doubt in these eggs that small-sized CVs in the area are broken down. Fibrillar material is also observed on the ooplasmic surface (Fig. 19); this may be discharged from the lumen of small-sized CVs with apertures. In contrast to the state of the animal pole area, no aperture can be observed on the equatorial or vegetal surfaces of the egg. The breakdown of large-sized CVs in the animal pole area which contain spherical bodies can be recognized in the eggs fixed at 60 seconds postincubation in tap water. Each spherical body fits its own lower hemisphere into an indentation (the lumen of previous CV) of the ooplasmic surface (Fig. 20). Some of the CVs distributed in the equatorial region also initiate the breakdown of these eggs. In the eggs fixed at 90-120 seconds postincubation in tap water, the animal pole area possesses a number of indentations on the ooplasmic surface (Fig. 21); these contain neither masses of fibrils nor spherical bodies. This observation indicates that the CV contents had been discharged from the indentations of this area. In the equatorial region of the egg, however, various-sized indentations with spherical bodies can be observed on the ooplasmic surface. The indentations on the animal surface decrease in depth in the eggs fixed at 180-240 seconds postincubation in tap water (Fig. 22); these can now be recognized as shallow depressions of the surface. The surface structure of the equatorial region in these eggs is nearly the same as that of the animal pole area of the egg fixed at 90-120 seconds postincubation. The breakdown of CVs on the vegetal surface is first detected in the eggs fixed at 180-240 seconds and that on the animal surface is nearly completed in those at 300-360 seconds postincubation (Fig. 23). After completion of the CV breakdown (7 minutes postincubation), the whole surface of the cortical layer is covered with a plasma membrane which bears numerous microvilli. The distribution of the villi is uniform in every region of the egg. These observations indicate that the CV breakdown in the chum salmon egg begins at the animal pole area and ends at the vegetal pole area.

Fertilized eggs: The spatiotemporal course of surface changes is essentially the same as that described for the parthenogenetically activated eggs, although the

formation of several apertures on the animal pole area can be recognized in the eggs fixed at 15 seconds postincubation in tap water. It is thus clear that the CV breakdown is initiated earlier than in the parthenogenetically activated eggs.

Discussion

As in the unfertilized *Cyprinus* and *Carassius* eggs (Kudo, 1976), the cortical layer of the chum salmon egg shows an apparent structural difference between its peripheral and inner regions. Since the CVs in the intact egg were mostly aligned beneath the narrow, peripheral region in the cortical layer, they may be dislodged after low-speed centrifugation of the eggs (cf. Yamamoto, 1961). In sea urchins and amphibians, however, the same treatment is not able to displace a similar component of the cortical layer, the cortical granule (CG), which is closely apposed to the egg plasma membrane (Hylander and Summers, 1981; Katagiri, 1960). It is apparent that the structure of the cortical layer in the fish egg differs from that of the sea urchin and amphibian eggs.

The process of CV breakdown in the chum salmon egg is nearly the same as that reported for other fish species (Iwamatsu and Ohta, 1976; Iwamatsu and Keino, 1978; Hart and Yu, 1980; Brummett and Dumont, 1981). At the initial phase of the fusion of the CV membrane with the egg plasma membrane, I frequently observed several small openings on the ooplasmic surface overlying a single CV. A similar observation has been reported by Brummett and Dumont (1981) who studied the CV breakdown in the glutaraldehyde-fixed eggs of *Fundulus*. These openings might indicate the occurrence of multiple fusions of the CV membrane with the egg plasma membrane. This is not the case, however, because a quick-freezing and freeze-fracture study of the cortical change in the sea urchin egg by Chandler and Heuser (1979) demonstrated that, during the dehydration process for the preparation, the plasma membrane in the glutaraldehyde-fixed egg shows "vesiculation"; perhaps the CV or CG membrane and the egg plasma membrane undergoing the membrane fusion tend to be disrupted in the fixative.

When the CVs located in the inner region of the cortical layer approached the egg plasma membrane and established close contact with the latter immediately before the formation of apertures on the ooplasmic surface, a small area of the CV membrane was decorated with the compact ooplasm having filamentous material (Fig. 7b). During the discharge of the CV contents, the CV membrane with this ooplasm increased in area (Fig. 7c, d). The appearance of a similar ooplasm during the CV breakdown has also been reported by Iwamatsu and Ohta (1976) for the *Oryzias* egg, although they did not describe its origin. These observations suggest that the compact ooplasm plays a role in the process of the breakdown. The fine structure of the compact ooplasm resembles that observed in the peripheral region of the cortical layer in the intact egg. Since the ooplasm in animal eggs contains actin, it is probable that the compact ooplasm as well as the peripheral region of the cortical layer in the chum salmon egg possesses similar

proteins. Perhaps contractile components, which may be distributed sparsely in the inner region of the cortical layer, are concentrated and organized into microfilamentous bundles in the vicinity of CVs and exclude membranous organelles from the site immediately before and during the CV breakdown. The presence of microfilaments in the compact ooplasm would account well for the initial dislocation of CVs and the subsequent discharge of their contents in the chum salmon egg.

The breakdown of a few number of small-sized CVs in the animal pole area was recognized in the fertilized egg fixed at 15 seconds postincubation in tap water. This is apparently earlier than the breakdown in the parthenogenetically activated egg. The early breakdown in the fertilized eggs may be related to the presence of sperm nucleus in the ooplasm. A preliminary observation shows that the sperm penetration into the ooplasm induces the breakdown of a small number of the CVs in the animal pole area of the egg kept in SRS (unpublished). Yamamoto (1966) demonstrated that the local application of tap water to the ooplasmic surface of the chum salmon egg causes the CV breakdown only in the area to which it is applied; the stimulation of whole surface by water is necessary for the complete breakdown of CVs. These observations may explain the initiation of the CV breakdown at the area of the egg's animal pole. Since the animal pole of the egg possesses a channel, the micropyle, contact with water at this site of the ooplasmic surface may occur immediately after incubation in water (Kobayashi and Yamamoto, 1981). It is therefore clear that the early initiation of CV breakdown at the animal pole area of the fertilized egg results from a twofold stimulation (by sperm penetration and tap water) of the ooplasmic surface of this area.

Recent studies of fertilization of the eggs of several animals have indicated that the CV breakdown is preceded by an abrupt rise of the intracellular free calcium level (Gilkey et al., 1978; Gilkey, 1983; Eisen et al., 1984). This may also be the case for the chum salmon eggs; Kanoh (1956) and Yamamoto (1979) observed an efflux of calcium ions from these eggs following incubation in deionized water; they speculated that the efflux results from an increased level of free calcium ions in the ooplasm. The rise in the intracellular free calcium level may trigger the CV breakdown in the chum salmon eggs. This assumption is supported by the fact that the incubation of intact chum salmon egg in the SRS containing calcium ionophore A23187 is capable of inducing the CV breakdown in this fish (unpublished).

Following the breakdown of CVs, the limiting membrane of these structures was inserted into the original surface of the cortical layer. Furthermore, the volume of egg cell proper decreased after the discharge of the CV contents. It is therefore reasonable to suppose that the chum salmon egg possesses a mechanism that is able to retrieve any excess plasma membrane. A similar mechanism has been also postulated in the developing sea urchin eggs (Eddy and Shapiro, 1976; Chandlar and Heuser, 1979; Fisher and Rebhun, 1983); this mechanism seems to

be concerned with the elongation and formation of microvilli and the appearance of membranous structures in the compact, perivesicular ooplasm (Iwamatsu and Ohta, 1976; Iwamatsu and Keino, 1978; Nuccitelli, 1980; Hart and Yu, 1980; Donovan and Hart, 1982). In the chum salmon egg, the removal of excess membrane by means of the formation of the membranous structures (endocytosis) is initiated as early as the occurrence of the elongation of microvilli and is active during the shallowing of indentations on the ooplasmic surface. The simultaneous progress of microvillar elongation and the removal of the excess membrane may be related to the large size of CVs in the chum salmon egg.

Summary

The fine structure of the cortical ooplasmic layer and its change following the incubation of the eggs in water were observed in the chum salmon, *Oncorhynchus keta*. A single layer of cortical vesicles (CVs) ran parallel to the ooplasmic surface and was usually prevented from making direct contact with the egg plasma membrane by the compact ooplasmic region where no membranous organelles are to be found. Each CV was membrane-bounded and contained fine particles and crisscrossed fibrils.

After incubation of the eggs in water, the CVs approached the egg surface. As a result of the fusion of the CV membrane with the overlying egg plasma membrane, numerous apertures were formed on the ooplasmic surface. The CV contents were then discharged from the egg through these apertures. A thick layer of the compact ooplasm with filamentous components invariably appeared in the vicinity of the CV membrane. I suggest that the initial dislocation of CVs and the subsequent discharge of the CV contents is caused by the contractile activity of the compact ooplasm.

Although the ooplasmic surface increased in area owing to the insertion of the previous CV membrane, the elongation of the preexisting microvilli at the edge of the CV aperture and the formation of new microvilli on the previous CV membrane during the CV breakdown may serve to accommodate the excess plasma membrane. Furthermore, the excess plasma membrane was removed by the endocytotic activity of the ooplasmic surface.

The CV breakdown began at the animal pole area of the egg and ended at the vegetal pole area. Although the spatiotemporal course of the breakdown in the fertilized eggs was nearly the same as that in the parthenogenetically activated ones, the early appearance of a few number of small apertures was recognized on the animal pole area of the former eggs.

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