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Structural Changes in the Endoplasmic Reticulum of Starfish Oocytes during Meiotic Maturation and Fertilization

LAURINDA A. JAFFE*†‡ AND MARK TERASAKI†‡§

*Department of Physiology, University of Connecticut Health Center, Farmington, Connecticut 06032; †Marine Biological Laboratory, Woods Hole, Massachusetts 02543; ‡Bodega Marine Laboratory, Bodega Bay, California 94923; and §Laboratory of Neurobiology, NINDS, National Institutes of Health, Bethesda, Maryland 20892

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The endoplasmic reticulum (ER) of live starfish oocytes was observed during meiotic maturation and fertilization. The ER was visualized by injection into the cytoplasm of an oil drop saturated with the fluorescent lipophilic dye DiI; DiI spread throughout the oocyte endoplasmic reticulum and the pattern was imaged by confocal microscopy. The ER in the immature (germinal vesicle stage) oocyte was composed of interconnected membrane sheets. In response to 1-methyladenine, the sheets of ER appeared to become associated with the yolk platelets, forming spherical shells. A few of these spherical shells could sometimes be seen in immature oocytes, but their number was much greater in the egg at the first meiotic spindle stage. At about the time that the first polar body formed, the spherical shells disappeared, and the ER returned to a form like that of the immature oocyte. The spherical shells did not reappear during the second meiotic cycle. During maturation, the ER also began to move; the movement was apparent by the time of germinal vesicle breakdown and continued throughout both meiotic cycles and in eggs with second polar bodies. When eggs at the first meiotic spindle stage were fertilized, the form of the ER changed. Within 1 min after sperm addition to the observation chamber, the circular cross sections of the spherical shells of the unfertilized egg ER were no longer distinct. At this point, the form of the ER could not be discerned with the resolution of the light microscope; however, the rate of spreading of DiI from an injected oil drop decreased, providing strong evidence that the ER had become fragmented. The ER remained in this form for several minutes and then gradually, the appearance of the ER and the rate of DiI spreading returned to be like those of the unfertilized egg. Injection of inositol trisphosphate caused a similar change in the ER structure. These results indicate that the ER is a dynamic structure, the form of which changes during oocyte maturation and fertilization.

INTRODUCTION

The endoplasmic reticulum (ER)[1] has recently been visualized in live sea urchin eggs by confocal microscopy after injection of an oil drop saturated with the fluorescent lipophilic dye, DiI (Terasaki and Jaffe, 1991, 1993; Jaffe and Terasaki, 1993a). DiI diffuses from the oil drop into the continuous membrane of the ER, but does not label other cytoplasmic organelles. Evidence that this technique specifically stains the ER is that other membrane bound structures that can be identified by light microscopy (cortical granules, yolk platelets, microvilli) are not labeled. Also, the pattern of staining observed with DiI closely resembles the pattern of ER staining seen by immunofluorescence using an antibody to a calsequestrin-like protein (Henson *et al.*, 1989). With this DiI labeling method, dramatic structural changes in the ER have been seen during fertilization (Terasaki and Jaffe, 1991; Jaffe and Terasaki, 1993a).

In the present study, we have used the DiI labeling technique to examine the structure of the ER during starfish oocyte maturation and fertilization. Starfish oocytes, which are arrested at first prophase, resume meiosis in response to the hormone 1-methyladenine (Kanatani *et al.*, 1969). In the species used in the present study (*Asterina miniata*), the nuclear envelope of the germinal vesicle (GV) breaks down about 20–30 min after 1-methyladenine is applied, at 20°C. After germinal vesicle breakdown (GVBD), starfish oocytes then proceed through the two meiotic divisions, forming two polar bodies. In addition to causing the resumption of meiosis, 1-methyladenine causes the oocytes to acquire the ability to undergo normal monospermic fertilization; normal fertilization can occur from about the time of GVBD to about the time of first polar body formation (see Fujimori and Hirai, 1979).

The structure of the ER during oocyte maturation and fertilization is of particular interest because the ER releases Ca at fertilization, and because the ability to re-

[1] Abbreviations used: ER, endoplasmic reticulum; GV, germinal vesicle; GVBD, germinal vesicle breakdown; DiI (1,1'-dihexadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate or 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate); IP_3 (inositol trisphosphate); MPF, maturation promoting factor (or mitosis and meiosis promoting factor).

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lease Ca develops during oocyte maturation (Chiba *et al.*, 1990; see Jaffe, 1995). In addition, components involved in the meiotic cell cycle may be associated with the ER (Duesbery and Masui, 1993).

MATERIALS AND METHODS

Starfish (*A. miniata*) were collected near the Bodega Marine Laboratory (Bodega Bay, CA). Pieces of ovary or testis were removed through a small hole in the dorsal body wall, made with a 3.0-mm sample corer (Fine Science Tools, Foster City, CA). Follicle-free oocytes were obtained by incubating the pieces of ovary in calcium-free seawater on ice, followed by several washes in natural seawater (see Jaffe *et al.*, 1993). Oocytes obtained by this procedure are at first meiotic prophase (germinal vesicle stage) and are referred to as "immature." Oocyte maturation was stimulated by addition of 1 μM 1-methyladenine (Sigma Chemical Co., St. Louis, MO). We refer to an oocyte that has proceeded to formation of the first meiotic spindle or beyond as an "egg," since at about this stage, the oocyte attains the ability to undergo normal fertilization. Sperm were obtained from the excised testis and were diluted ~1:1000 in natural seawater before addition to the front of an observation chamber containing eggs.

Most experiments were carried out at 18–20°C, although for some experiments, the temperature was 22–24°C. At 18–20°C, *A. miniata* embryos developed normally, while at 22–24°C, maturation and fertilization appeared to occur normally, but cleavage did not. However, oocytes matured and fertilized at 22–24°C developed normally when they were subsequently returned to 18–20°C. Results concerning the events of maturation and fertilization were the same at the two temperatures, except that events occurred more rapidly at 22–24°C.

Microinjection and observation were carried out with oocytes held between two coverslips separated by two pieces of double-stick tape; the coverslips were held on a plastic support slide with a U-shaped cutout (Kiehart, 1982; Terasaki and Jaffe, 1993). Microinjection was done using a constriction pipet (Hiramoto, 1974; Kishimoto, 1986; Terasaki and Jaffe, 1993) filled with a saturated solution of DiI in Wesson oil (Pelican Plaza Grocery and Deli, Bodega Bay, CA; A & P Market, Falmouth, MA; Stop & Shop, W. Hartford, CT) (see Terasaki and Jaffe, 1993). For injection of fluorescein dextran or inositol trisphosphate (IP_3), we used either a constriction pipet filled with silicon oil (dimethylpolysiloxane, 100 centistokes, Sigma) or a pipet backfilled with a drop of mercury (Hiramoto, 1962; Kiehart, 1982). Injection volumes were calibrated by measuring the diameter of the DiI oil drop or, for the aqueous solutions, by measuring the diameter of an equivalent volume of oil. $DiIC_{16}(3)$ [full name: 1,1′-dihexadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate] and $DiIC_{18}(3)$ [full name: 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate] were obtained from Molecular Probes (Eugene, OR). Similar results were obtained with each; we use the term DiI to refer to either. The 70-kDa fluorescein dextran was obtained from Molecular Probes. IP_3 was obtained from Calbiochem (San Diego, CA).

Confocal microscopy was carried out using a Bio-Rad MRC600 system (Bio-Rad Laboratories, Hercules, CA) coupled to either a Zeiss Axioskop with a 40X, 1.3 N.A. PlanApo objective (Carl Zeiss Inc., Thornwood, NY), or to an Olympus IMT-2 inverted microscope with a 20X, 0.7 N.A. DPlanApo objective, or a 60X, 1.4 N.A. SPlanApo objective (Olympus Corp., Columbia, MD), or to a Nikon Diaphot inverted microscope with a 60X, 1.4 N.A. PlanApo objective (Nikon, Inc., Melville, NY). Procedures for collecting and storing data were as described by Terasaki and Jaffe (1993). The following correction in Table 1 of Terasaki and Jaffe (1993) should be noted: Column 2, line 4 should read, "live filter a 1," and column 2, line 10 should read, "wait % 1."

RESULTS

DiI Labeling of the Starfish Oocyte ER

The ER of immature starfish oocytes was labeled with DiI by injecting an oil drop saturated with DiI. By about 45 min, the dye had spread uniformly throughout the ER, labeling a network of cisternae (Fig. 1A) as well as the nuclear envelope of the germinal vesicle. In the cytoplasm within approximately 1 μm of the oocyte surface (the "cortex"), the ER appeared to be an irregular network of tubules. Images of the ER were of the best quality in the outer 10–20 μm of the cytoplasm, but interpretable images of ER cisternae could be obtained to a depth of about 30–50 μm. Except for the cortex and nuclear envelope, the ER structure appeared to be uniform throughout the cytoplasm, with no obvious differences related to depth from the surface or to animal–vegetal polarity.

Structural Changes in the Oocyte ER during Meiotic Maturation

In the mature egg at the first meiotic spindle stage, the ER included a large number of structures that appeared in optical sections as circular profiles, about 1–2 μm in diameter (Figs. 1B, 2A, 3A, and 6A). These profiles were observed throughout the cytoplasm, except in the cortex, where the ER was composed of tubular membranes. Focusing through adjacent planes showed that these structures had the shape of spherical shells. The spherical shells appeared to be interconnected, since they were labeled when a DiI-saturated oil drop was injected into eggs at 20 min after GVBD.

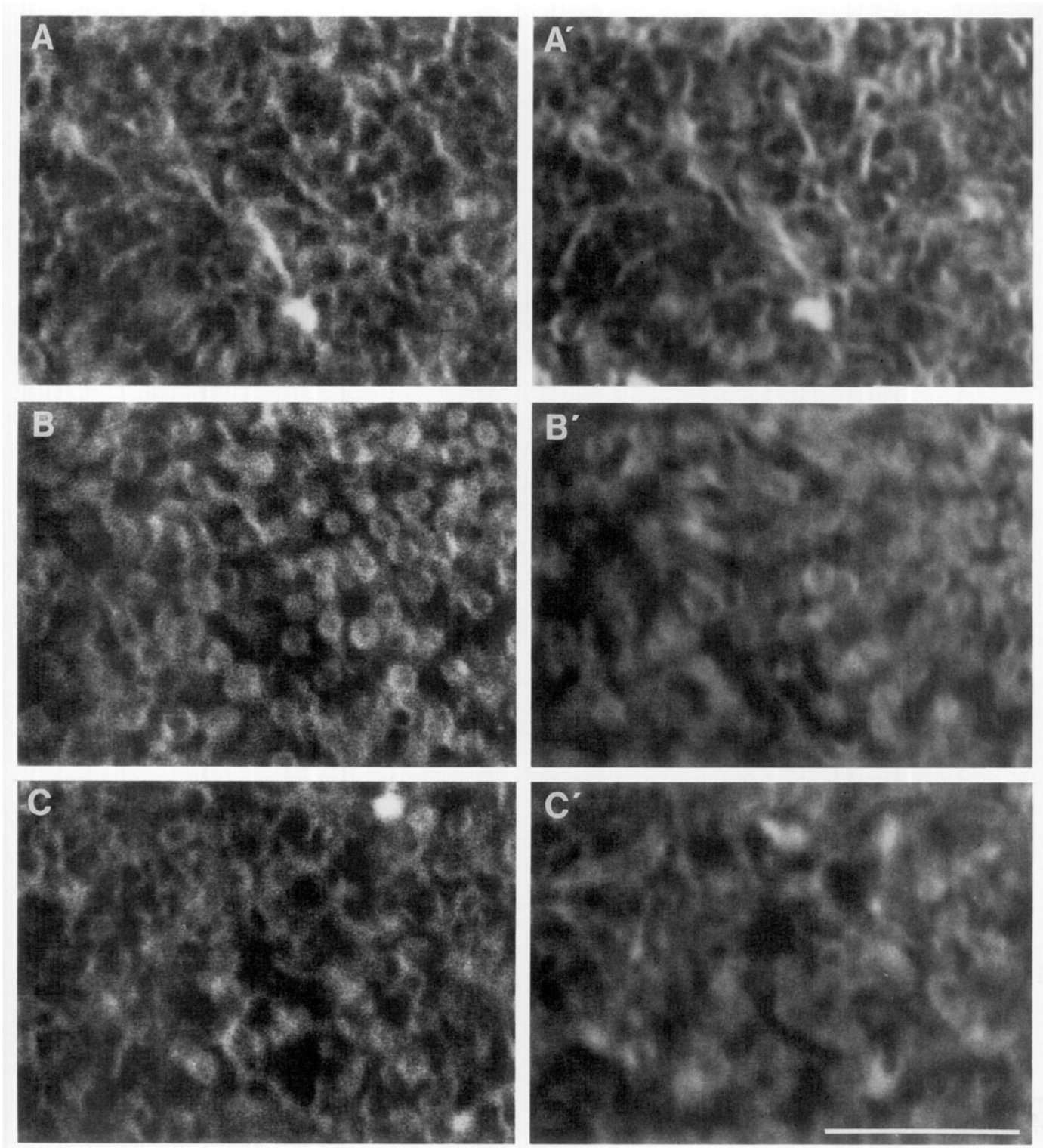

FIG. 1. Change in ER structure and motility during oocyte maturation. All images are from a single oocyte, at 8 μm below the cell surface. Each pair of images shows on the left data collected by accumulating 2 sequential 3.1-sec scans and on the right data collected by accumulating 10 sequential 3.1-sec scans. (A) ER in the immature oocyte. The absence of significant blurring of the 10-scan image (A′) compared to the 2-scan image (A) indicates that the ER was undergoing relatively little movement. (B) ER in an egg at the first meiotic spindle stage, 30 min after GVBD. Relative to the image of the immature oocyte in (A), there were many more 1- to 2-μm spherical structures. The blurring of the 10-scan image (B′) compared to the 2-scan image (B) indicates that the ER was moving. (C) ER in an egg at the first polar body stage (97 min after GVBD, 33 min after first polar body formation). Relative to the image of the egg at the first meiotic spindle stage in (B), there were many fewer 1- to 2-μm spherical structures. The blurring of the 10-scan image (C′) compared to the 2-scan image (C) indicates that the ER was moving. Temperature, 18°C. Bar, 10 μm.

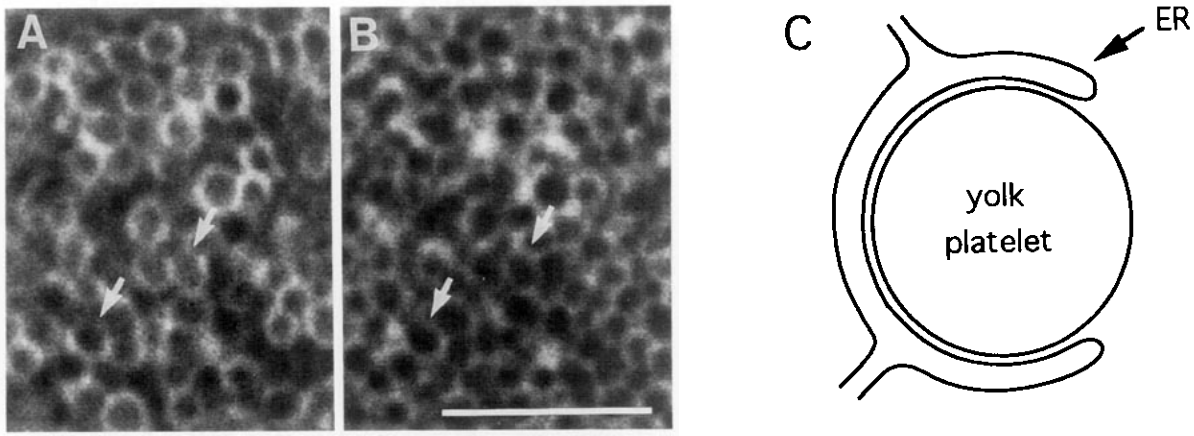

FIG. 2. Evidence that the spherical shells of ER are associated with yolk platelets. An oocyte was co-injected with 70-kDa fluorescein dextran (3.5 μM) and DiI dissolved in oil and then exposed to 1-methyladenine and incubated until it had reached the first meiotic spindle stage. (A) DiI image. (B) Fluorescein dextran image. The dextran diffuses throughout the cytosol; most if not all of the negative images are likely to represent yolk platelets, since these are the only organelles in the oocyte interior that have a comparable size and distribution (see Longo *et al.*, 1982). The DiI-labeled spherical shells correspond to regions that exclude the dextran, providing evidence that the ER is associated with yolk platelets. Several of the DiI-labeled spherical shells do not appear to be complete (arrows). Images in (A) and (B) were taken at 5 μm below the cell surface. Temperature, 20°C. Bar, 10 μm. (C) A drawing of the proposed ER–yolk platelet association. The ER cisternae are now arranged so that they partially envelop yolk platelets and consequently appear in optical cross sections as circles or partial circles. Note that the two bilayer membranes separated by the ER lumen are seen with the light microscope as a single line.

The spherical shells had the approximate size and distribution of yolk platelets (see Longo *et al.*, 1982). To examine the localization of the ER with respect to the yolk platelets, we would ideally have used a fluorescent dye specific for yolk platelets. In the absence of such a dye, we visualized the yolk platelets by injecting oocytes with 70-kDa dextran; this inert polysaccharide diffused through the cytoplasm and showed the yolk platelets as negative images (Fig. 2B). We concluded that the regions where dye was excluded were occupied by yolk platelets by comparison with electron microscopic images (see Longo *et al.*, 1982), which showed no other organelles of comparable size in the starfish oocyte interior. We excluded the possibility that the negative images were simply images of the spherical ER shells, because the fluorescent dextran images were similar in immature oocytes without spherical ER shells and in mature eggs in which spherical ER shells were present. The fluorescein dextran images were also similar at later stages when the spherical DiI-labeled structures were no longer present (see below). When the eggs were doubly labeled with DiI and dextran, the nonfluorescent interiors of the DiI-stained spherical shells were seen to correspond with negative images of the yolk (Figs. 2A and 2B). This indicated that the spherical shells of ER were associated with yolk platelets. We cannot, however, exclude the possibility that the spherical shells of ER could be surrounding both yolk platelets and smaller organelles in the space between the ER and the yolk platelet membranes.

Many of the DiI images of the spherical shells of ER were not uniformly bright around the circumference (arrows in Fig. 2A), and by focusing, it was seen that some of the spherical shells appeared to be uniformly bright in one optical section but not in another. These results suggested that many of the spherical shells of ER that surround yolk platelets are incomplete (see Fig. 2C).

A small and variable number of spherical shells were sometimes present in immature oocytes from different animals, but there were always many fewer than in eggs at the first meiotic spindle stage. The formation of the spherical shell-shaped structures was observed in 36/36 oocytes from 11 animals. The time at which the spherical shells became most abundant corresponded to about the time that the first meiotic spindle first became easily visible in DiI-stained eggs. The spindle could be observed as a barrel-shaped structure, around which ER was accumulated, but which contained little ER in the interior (Terasaki, 1994). This was also approximately the time at which the oocyte first acquired the ability to undergo normal monospermic fertilization (see Chiba *et al.*, 1990). We found that the capacity to undergo normal fertilization did not develop immediately after GVBD. In two experiments, oocytes were inseminated at either 0–5 min after GVBD or at 15–20 min (at 20°C). In both experiments, the 0–5 min set was polyspermic, as determined by the occurrence of irregular first cleavage, while the 15–20 min set was monospermic.

At about the time that the first polar body formed (about 60–70 min after nuclear envelope breakdown at 18–20°C), the spherical ER structures disappeared, and

the ER returned to a form similar to that of the immature oocyte (Fig. 1C). Disappearance of the spherical shells at first polar body formation was observed in 12 eggs from four animals and occurred whether or not the egg had been fertilized while it was at the first meiotic spindle stage. We did not observe a reappearance of the spherical shells between the formation of the first and second polar bodies, in either unfertilized or fertilized eggs (3 eggs from one animal were observed at 3-min intervals; 2 eggs from another animal were observed at 10-min intervals).

Increase in Movement of the ER during Meiotic Maturation

Movement of the ER was visualized by making time-lapse video sequences. The ER of immature oocytes was relatively stationary and began to move during maturation. The movement consisted of apparently random shifting and was very similar to movements seen previously in sea urchin eggs (Terasaki and Jaffe, 1991). In order to document the movements, we averaged 10 consecutively scanned images (3.1 sec per scan) and compared this with the average of 2 consecutively scanned images; movement was detected as blurring in the 10-scan image compared with the 2-scan image. Relatively little movement was seen in the ER of most immature oocytes from most animals (Fig. 1A'). Usually, an increase in ER movement occurred during maturation (Fig. 1B'). Comparing immature oocytes with eggs at the first meiotic spindle stage (at 20–40 min after GVBD at 18–20°C), 19/23 pairs from six animals showed a clear increase in ER movement. In the other cases, there was not a detectable increase, because the ER of the immature oocyte was already moving significantly. In 9/9 eggs from three animals, the movement of the ER persisted after first polar body formation and after the spherical structures disappeared (Fig. 1C'). The ER also continued to move during and after the second meiotic division.

Transient Fragmentation of the ER after Fertilization or Injection of IP_3

DiI-labeled starfish eggs (at the first meiotic spindle stage) were inseminated, and images of the ER were recorded at subsequent times. As in sea urchin eggs (Terasaki and Jaffe, 1991; Jaffe and Terasaki, 1993a), fertilization caused a change in the form of the ER. Within 1 min after sperm addition to the observation chamber, the circular cross sections of the spherical shells of the unfertilized egg ER (Fig. 3A) were no longer distinct. By 1 min after the ER change was first detected, the ER attained the form shown in Fig. 3B. The ER remained in this form for several minutes and then gradually, returned to a form like that of the unfertilized egg (Fig. 3C). Fertilization was seen to cause these changes in ER structure in 8/8 eggs from three animals.

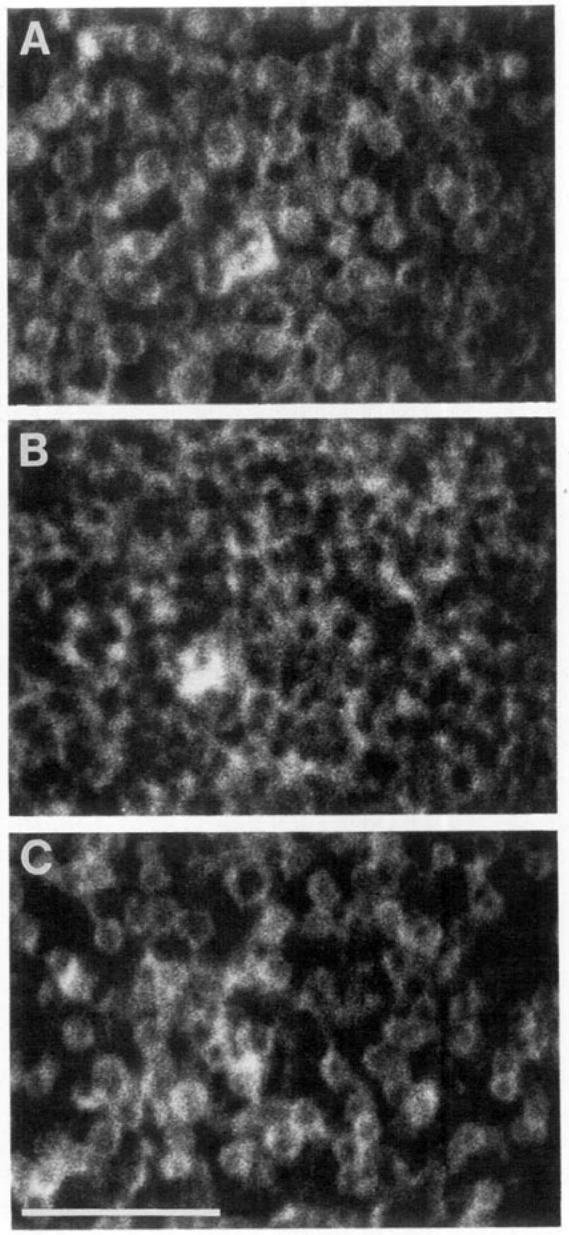

FIG. 3. Changes in ER structure during fertilization. (A) An unfertilized egg (16 min after germinal vesicle breakdown). (B) The same egg at 1.0 min after the change in ER structure was first detected. (The ER change was first seen at 0.7 min after sperm were added to the observation chamber; sperm were added at 17 min after germinal vesicle breakdown). (C) The same egg at 15 min after fertilization; the ER has returned to its original form. All images were taken at 8 μm below the cell surface. Temperature, 24°C. Bar, 10 μm.

To examine whether the change in the appearance of the ER represented the development of discontinuities in the ER network, the spreading of DiI from an injected oil drop was compared in unfertilized eggs and in eggs injected at 1 min after the elevation of the fertilization

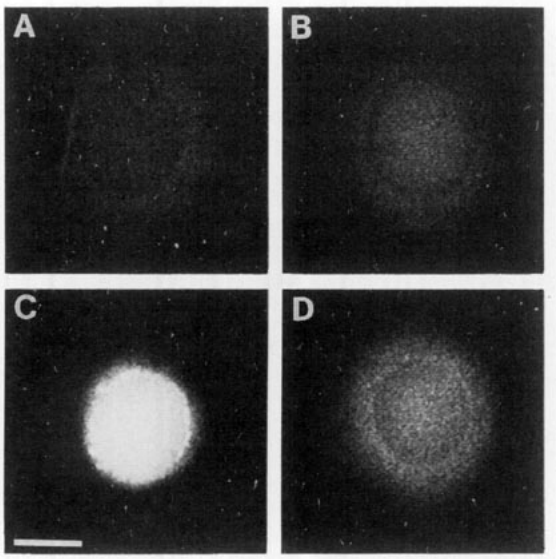

FIG. 4. Spreading of DiI from oil drops injected into oocytes and eggs at different stages of maturation and fertilization. All images were taken 3.0 min after injection of an oil drop of ~250 pl volume. (A) An immature oocyte. The central gray circle is the oil drop, surrounded by a halo of spreading DiI. The black region on the left is the space occupied by the germinal vesicle. DiI does not spread into this space because there is no ER within the GV. (B) An unfertilized egg, injected 14 min after GVBD. (C) A fertilized egg, injected 1.1 min after the first appearance of the fertilization envelope (this was at 16 min after germinal vesicle breakdown). The oil drop is bright because very little DiI has spread out of the oil. (D) A fertilized egg, injected 20 min after fertilization. Temperature, 22°C. Bar, 50 μm.

envelope was first observed. Images taken at 3 min after injection showed that in comparison to the unfertilized egg, much less spreading of DiI from the oil drop occurred in the just-fertilized egg (Figs. 4B and 4C). When the fluorescence intensity of the oil drop was plotted as a function of time after injection, the just-fertilized egg showed a plateau in the curve, indicating a transient period after fertilization when DiI spreading occurred much more slowly (Fig. 5). The spreading of DiI was also examined in eggs injected at 18–36 min after fertilization. Images taken at 3 min after injection showed that DiI spread from the oil drop at a rate approximately the same as that in the unfertilized egg (Figs. 4B and 4D). This could also be seen in the graph of fluorescence intensity of the oil drop as a function of time after injection (Fig. 5). Similar results were obtained from 4/4 sets of eggs (unfertilized, 1 min, 18–36 min) from two animals. These findings indicate that at fertilization, the continuity of the ER is transiently decreased.

We also compared the rates of DiI spreading from the oil drop in immature oocytes and in eggs at the first meiotic spindle stage (Figs. 4A, 4B, and 5). In each of the four pairs of cells examined, the dye left the oil drop somewhat more rapidly in the immature oocytes, suggesting that some decrease in ER continuity may occur during maturation, but this change, if significant, was much less than that at fertilization.

Since starfish oocytes undergo a transient rise in free calcium at fertilization (Eisen and Reynolds, 1984; Chiba *et al.*, 1990) or in response to injection of IP_3 (Picard *et al.*, 1985a; Chiba *et al.*, 1990), we examined whether the change in form of the ER after fertilization was also seen after a calcium rise caused by injection of IP_3. DiI-labeled eggs at the first meiotic spindle stage were injected with 36 pl (1% of the egg volume) of 100 μM IP_3. Images of the ER at successive 1-min intervals showed that the ER structure changed with a pattern and time course similar to that observed at fertilization (Fig. 6). Similar results were obtained from 6/6 eggs from two animals.

DISCUSSION

Using confocal microscopy of live DiI-labeled starfish oocytes, we have visualized a sequence of changes in the form of the endoplasmic reticulum during maturation and fertilization. The ER of the immature oocyte interior is composed of interconnected membrane sheets. In response to 1-methyladenine, the sheets of ER appear to become associated with the yolk platelets, forming spherical shells. A few of these spherical shells can sometimes be seen in immature oocytes, but there are

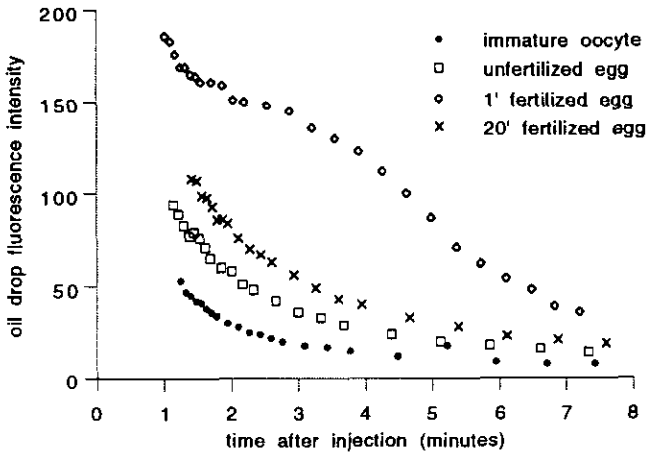

FIG. 5. Time course of DiI leaving an oil drop injected into oocytes and eggs at different stages of maturation and fertilization. Data were collected from the same cells shown in Fig. 4. The average fluorescence intensity in the drop was determined for a square area that was about half the diameter of the oil drop. (For these 8-bit images, the maximum intensity is 256). DiI left the oil drop rapidly in the immature oocyte, unfertilized egg, and 20' fertilized egg, but slowly in the 1' fertilized egg, indicating that ER continuity is greatly decreased in the period just after fertilization. The slight difference between the unfertilized and the 20' fertilized egg was not seen consistently in different experiments. The difference between the immature oocyte and the unfertilized egg (at the first meiotic spindle stage) was seen consistently, but was always much smaller than the difference between the 1' fertilized egg and the other stages.

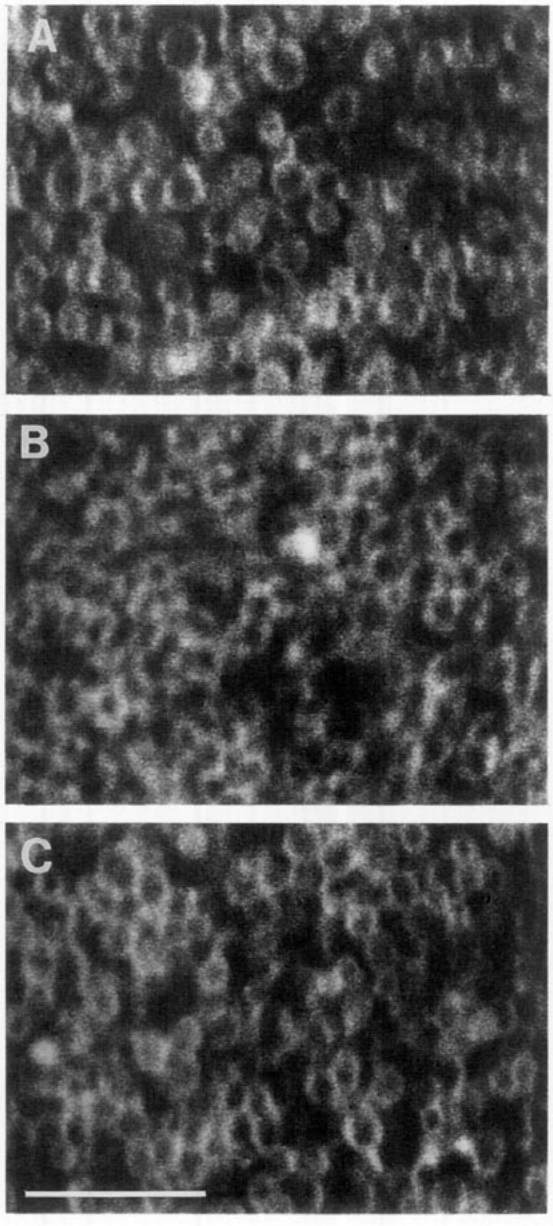

FIG. 6. Changes in ER structure after injection of IP_3. (A) An egg at 20 min after germinal vesicle breakdown. (B) The same egg at 1.2 min after injection of 36 pl of 100 μM IP_3 (1% of cell volume) (23 min after germinal vesicle breakdown). The ER has undergone a change in structure similar to that at fertilization. (C) The same egg 16 min after IP_3 injection; the ER has returned to its original form. All images were taken at 8 μm below the cell surface. Temperature, 18°C. Bar, 10 μm.

many more in the egg at the first meiotic spindle stage. Like the ER of the immature oocyte, the spherical shells of the mature egg appear to be part of an interconnected membrane system. The spherical shells of ER remain until the formation of the first polar body, when they revert back to the sheet form.

The timing of the appearance of the spherical shells correlates approximately with the development of the ability of the ER to release Ca in response to fertilization or IP_3 injection (Chiba *et al.*, 1990) and the resulting ability of the egg to undergo normal monospermic fertilization. However, it seems unlikely that the change in ER form fully accounts for this change in its ability to release Ca, since the spherical shells disappear at the time of first polar body formation, yet in first polar body eggs, cortical granule exocytosis occurs apparently normally, and the ability of the egg to prevent polyspermy is reduced only slightly (Fujimori and Hirai, 1979). In addition, in sea urchin eggs, which have completed meiosis at the time of fertilization and the associated release of Ca, the ER does not have the form of spherical shells. Direct measurements of the starfish egg's ability to release Ca before and after disappearance of the spherical ER structures have not been made.

The appearance of the spherical ER shells during oocyte maturation is accompanied by an increase in movement of the ER, which persists even after the spherical shells have disappeared. Recent experiments suggest that these movements are actin dependent and that one function of the movements is to move the ER into the former germinal vesicle region following GVBD (Stricker and Schatten, 1991; Terasaki, 1994).

Fertilization causes another quite different sequence of changes in ER form. The DiI-spreading experiments indicate that this involves a transient fragmentation of the ER membranes. The fragmentation appears to be caused by Ca release from the ER, since the period when the ER is fragmented correlates temporally with the period when cytoplasmic Ca is high (Chiba *et al.*, 1990; Shilling *et al.*, 1994), and since IP_3 injection causes a similar change in ER structure. The ER changes seen at fertilization in starfish eggs are like those previously described in sea urchin eggs (Terasaki and Jaffe, 1991; Jaffe and Terasaki, 1993a), although the period of ER fragmentation and the period of elevated calcium (compare Shilling *et al.*, 1994, and Shen and Buck, 1993) are somewhat longer in starfish. Thus, the transient ER fragmentation may be a fundamental event of Ca release at fertilization.

Whether the fragmentation plays a role in the Ca release process is unknown, but it could be speculated, for example, that ER fragmentation stimulates further Ca release by increasing the curvature of the membranes in which Ca transport proteins are located. The increase in membrane curvature could mechanically stretch an ion channel in the ER membrane, causing it to open, in a way analogous to stretch-induced opening of plasma membrane ion channels (Morris, 1990). Many such stretch-activated channels have been identified, including some that can also be activated by chemical messengers (Vandorpe and Morris, 1992). It is possible that Ca channels in the ER (IP_3-gated, ryanodine-sensitive, or other) could be opened by stretch, thus allowing Ca release. Possible functions of the ER fragmentation in re-

lation to other events of fertilization have been previously speculated upon (Terasaki and Jaffe, 1991; Jaffe and Terasaki, 1993a,b), but remain unknown.

ER Structure and the Cell Cycle

As mentioned above, the appearance and disappearance of spherical shells of ER corresponds with the period of the cell cycle when the first meiotic spindle is present. Changes in ER form associated with the cell cycle have been previously observed by electron microscopy in rat thyroid epithelial cells undergoing mitosis (Zeligs and Wollman, 1979). In interphase, the ER of these cells is in the form of flat cisternae. However, from prometaphase to anaphase, the ER appears to be composed of rounded vesicles. Then during telophase, the flat cisternae reform. A similar change in ER morphology has been reported to occur during mitosis in 3T3 fibroblasts (Koch *et al.*, 1988), although whether it occurs in all mitotic cells is unknown. The ER changes differ in these mammalian mitotic cells and in the starfish oocyte during first meiosis (a formation of vesicles vs a wrapping of ER cisternae around another structure), but both involve a distinctive ER morphology associated with the metaphase period of the cell cycle. Cell-free preparations of ER also show different morphologies associated with metaphase or interphase conditions (Allan and Vale, 1991). Whether the form of the ER in other species of oocytes undergoes changes during meiosis analogous to those we see in starfish is unknown, but in both frog and mouse, the amount of ER in the cortical region of the oocyte increases between the GV stage and second metaphase (Campanella *et al.*, 1984; Charbonneau and Grey, 1984; Larabell and Chandler, 1988; Ducibella *et al.*, 1988). Interestingly, the ER in the frog egg at second metaphase is wrapped around the cortical granules (Campanella *et al.*, 1984; Charbonneau and Grey, 1984; Larabell and Chandler, 1988), a morphology that resembles the ER-yolk platelet association in the starfish egg.

These observations raise many questions about how cytoplasmic factors, such as MPF ("maturation" promoting factor or "mitosis and meiosis" promoting factor, see Murray and Hunt, 1993), cytoskeletal elements, or calcium, may cause changes in ER structure. A role for MPF in stimulating the formation of the spherical shells of ER is suggested by the approximate similarity in the timing of the appearance and disappearance of spherical shells and MPF during the first meiotic cycle (Kishimoto and Kanatani, 1976; Doreé *et al.*, 1983; Picard *et al.*, 1985b). However, MPF reappears during the second meiotic cycle, while spherical shells have not been detected during this phase. Some evidence indicates that the amount of MPF is less during the second compared to the first meiotic cycle (see Kishimoto and Kanatani, 1976).

In various cells, the ER has been found to have structural interactions with microtubules and/or actin filaments (reviewed in Terasaki, 1990). Microtubules in starfish oocytes undergo changes related to the meiotic cell cycle during maturation (Schroeder and Otto, 1984; Shirai *et al.*, 1990). Also, cortical actin undergoes changes during maturation (Schroeder and Stricker, 1983), and cytochalasin inhibits the completion of germinal vesicle breakdown (Stricker and Schatten, 1991). However, formation of the spherical shells does not appear to require actin filaments or microtubules, since neither cytochalasin (1 μg/ml) (Terasaki, 1994) nor nocodazole (0.1 mg/ml) (Terasaki and Jaffe, unpublished) prevents the formation of the shells.

The relationship between Ca and ER structure during fertilization suggests that a similar relationship between Ca and ER structure might exist during oocyte maturation. However, there is no evidence that cytosolic Ca differs in immature oocytes and in eggs after GVBD (Witchel and Steinhardt, 1990). A small and transient increase in free cytosolic Ca is sometimes seen when 1-methyladenine is applied to starfish oocytes (Witchel and Steinhardt, 1990; Kikuyama and Hiramoto, 1991), but this transient is not seen under conditions like those used in our experiments, in which the 1-methyladenine is applied by allowing it to diffuse to the oocyte (Witchel and Steinhardt, 1990). In starfish, the available evidence also indicates that Ca in the ER lumen does not change during the transition from the immature oocyte to the first metaphase egg (Chiba *et al.*, 1990, but for mouse, see Tombes *et al.*, 1992). Whether there are changes in cytosolic or lumenal Ca that would be detected by more sensitive methods, or that accompany later stages of oocyte maturation, is unknown.

The possible functional significance of the changes in ER structure to the cell cycle is also unknown. However, it could be speculated that biochemical reactions involving ER-associated proteins, or within the aqueous cytoplasm near the ER, are influenced by the form of the ER. For example, reactions within the restricted space between the membranes of an ER spherical shell and a yolk platelet could differ from reactions in less restricted cytosol.

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