Changes in the Distribution of Endoplasmic Reticulum in Porcine Oocytes during Meiotic Maturation

Teruo Maeda* and Tetsuki Yagyu

Faculty of Applied Biological Science, Hiroshima University, Higashi-Hiroshima 739, Japan

Abstract: *Changes in the distribution of endoplasmic reticulum (ER) in porcine oocytes during meiotic maturation were examined. In the Germinal Vesicle (GV) stage oocyte, there were many bright clusters of ER present in the peripheral cytoplasm, and some of them formed a mass. This mass was absent in the Prometaphase I (Pro-MI) and Metaphase I (MI) stage oocytes, and the clusters became fewer than in the GV stage oocyte. ER accumulations that appear to be generated by scattering of the cluster or the mass were uniformly distributed in the peripheral cytoplasm and formed a thin layer beneath the plasma membrane. In the Metaphase II (MII) stage oocyte, the layer became more evident and wider, and a space with a relatively sparser distribution of ER was observed beneath the layer. From these results, it is clear that the distribution in the ER of porcine oocytes is changing during miotic maturation.*

Key words: *Endoplasmic reticulum, Porcine oocyte, Meiotic maturation.*

It is well known that the endoplasmic reticulum (ER) is not only the site of protein synthesis and assembly [1] and lipid synthesis [2, 3] but also the site of a Ca^{2+} store [4–6]. It has been demonstrated that the ER contains inositol 1,4,5-trisphosphate (IP_3) receptors (IP_3Rs) and ryanodine receptors, both of which mediate Ca^{2+} release from the ER [7–9]. It has also been shown that a dramatic increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) at fertilization initiates activation of the eggs, and then a series of repetitive $[Ca^{2+}]_i$ rises (Ca^{2+} oscillations) continues during fertilization [10]. Both the $[Ca^{2+}]_i$ and the Ca^{2+} oscillations are caused by IP_3-induced Ca^{2+} release (IICR) from intracellular stores, as demonstrated with a function-blocking monoclonal antibody to the IP_3R [11]. Moreover, it has been reported that the IICR mechanism develops during maturation of hamster [12] and mouse [13] oocytes as a factor in the acquisition of the ability of an oocyte to undergo normal fertilization. These findings make it clear that the ER developing during maturation plays a very important role in fertilization.

Fluorescent lipophilic dye (DiI) diffuses from an oil drop into the continuous membrane of the ER, but does not label other cytoplasmic organelles, so that dramatic structural changes in the ER of living oocytes can be visualized after injection of an oil drop saturated with DiI [14]. Recently, structural changes in the ER of the oocytes in some species (starfish [15], mouse [6] and hamster [16]) during meiotic maturation were investigated with the DiI labeling method, but there are few reports on porcine oocytes.

In the present study, the ER of porcine oocytes matured *in vitro* and then labelled with DiI was observed by confocal microscopy to clarify the distributional changes in the ER during meiotic maturation.

Materials and Methods

Oocyte collection and culture: Ovaries were collected from prepubertal gilts at a local slaughterhouse and were transported to the laboratory in 0.85% NaCl with 0.1 mg/ml kanamycin (Meiji Seika, Tokyo, Japan) at about 30°C within 2 hr. Oocytes were collected from follicles measuring from 3 to 8 mm in diameter by dissection with a razor blade. Oocytes possessing a complete, compact cumulus mass were selected and washed twice in Dulbecco's phosphate buffered saline (PBS) supplemented with 1% bovine serum (Nacalai Tesque, Kyoto, Japan) and 0.1 mg/ml kanamycin. They were then further washed two times in the culture medium. Fifteen to 20 oocytes were transferred to a 100 μl droplet of culture medium covered with mineral oil

Received: August 15, 1997
Accepted: September 4, 1997
*To whom correspondence should be addressed.

(Sigma Chemical Co., St. Louis, MO) in a 35-mm polystyrene culture dish and cultured at 39°C in a humidified atmosphere of 5% CO_2 in air. The culture medium was TCM-199 with Earle's salts (Gibco BRL, Grand Island, NY), supplemented with 10 IU/ml hCG (Teikokuzouki, Tokyo, Japan), 10 IU/ml PMSG (Teikokuzouki), 1 μg/ml 17β-estradiol (Sigma), 50 μg/ ml gentamicin (Sigma) and 10% fetal calf serum (Gibco). The medium was sterilized by filtration through 0.45-μm Millipore filters (Millipore Ltd., Bedford, MA).

Treatment of oocytes and labelling of ER with DiI: Before and after cultivation (24 and 48 hr), oocytes with cumulus cells were denuded enzymatically with 0.1% hyaluronidase (Sigma) and mechanically with a finely drawn pasteur pipette. Labelling the ER with DiI was basically done according to the method described by Shiraishi *et al.* [16]. To inject fluorescent dye, the denuded oocytes were transferred one by one to a 20 μl drop of PBS in a 35-mm polystyrene culture dish. The dish was mounted on an inverted microscope (Leitz, Flovert FU, Germany) with two micromanipulators (Leitz, Micromanipulator M, Germany) and heated to 37°C. Injection of DiI was carried out on the dish. To stain the ER, a saturated solution of DiI (Molecular Proves Inc., Eugene, OR) was prepared by dissolving the dye in 100 μl of soybean oil. The DiI solution was injected into the denuded oocyte with holding and injection pipettes attached to the micromanipulators. According to the procedure described by Hogan *et al.* [17], these pipettes were made of microglass tubes (Microcaps, Drummond Scientific Co., U.S.A.) and their tips were prolonged by a puller (Leica, Micropipette Puller, Model PC-98, U.S.A.) and they were finely finished with a microforge (Leica, Microforge, Model MF-1, U.S.A.). The injection was done with a cell injector (Shimadzu, Model C1J-1, Japan), and approximately 9 pl of DiI solution was injected into an oocyte.

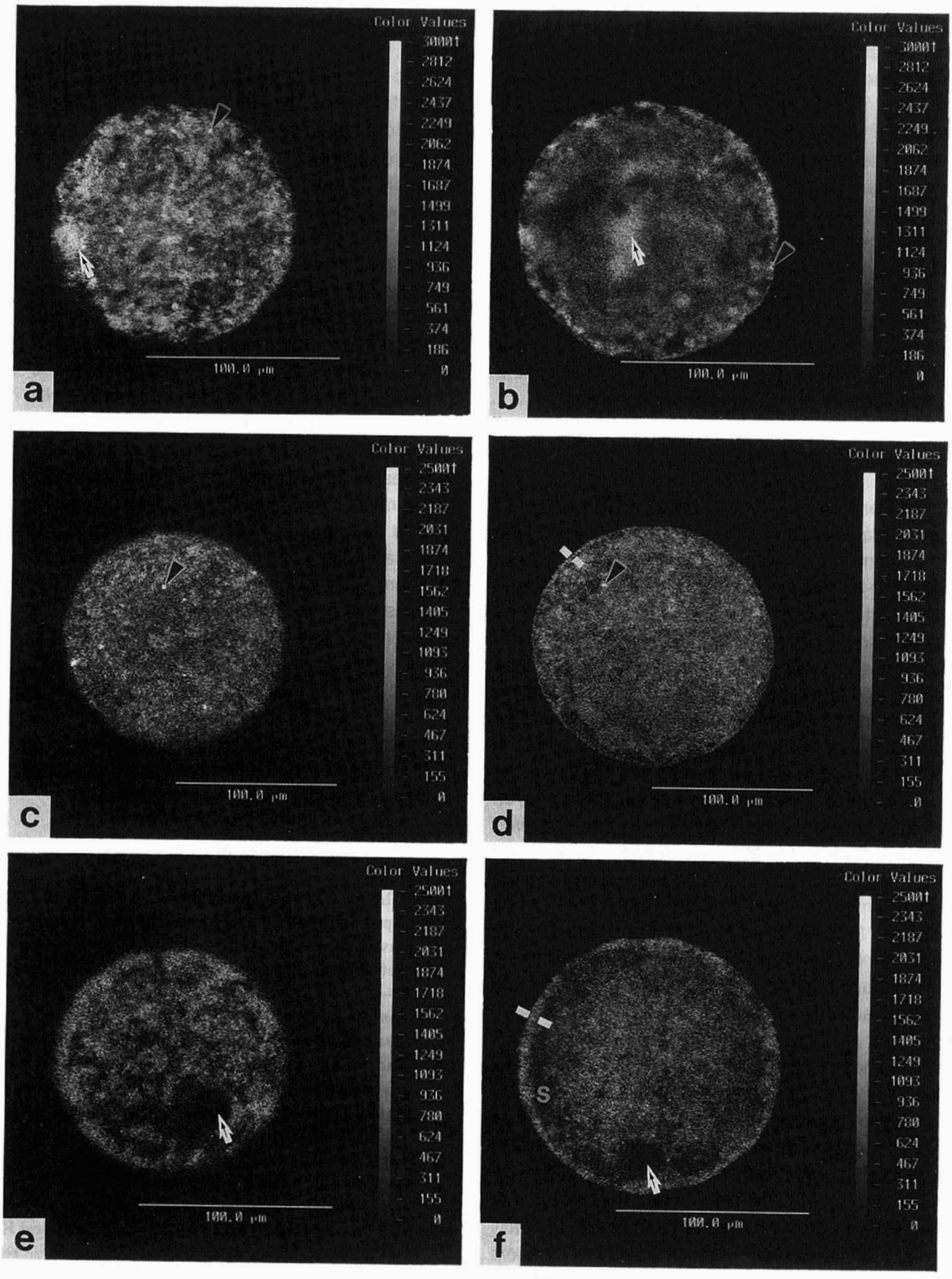
Observations of the ER and nuclear stage of oocytes: DiI-injected oocytes were cultured with the abovementioned culture medium for 1–2 hr to allow DiI to diffuse through the oocyte and then washed several times in PBS. They were observed under a confocal laser scanning microscope (CLSM; Meridian, ACAS-570, U.S.A.). After focusing on the top surface of the oocyte four to six optical sections were recorded in each oocyte. The depth of each section was 3.6 μm. The object lens of the laser microscope was used at a magnification of × 100. The excitation was blue with 488 nm emission. The wavelength of the detectors was 630 nm. Laser power was 200 mW. After observation of the ER with the CLSM, the oocytes were fixed for 48 hr in acetic acid and ethanol solution (at a ratio of 1:3, v/v) at 10°C, stained with 1% lacmoid in 45% acetic acid, and examined with a phase-contrast microscope to clarify their nuclear stage.

Results

ER in oocytes at the Germinal Vesicle (GV) stage: The GV stage oocytes were obtained from non-cultured oocytes. Examination of the immature, GV stage oocyte with CLSM after DiI staining revealed a reticular network. In addition, there were many bright clusters of ER present in the peripheral cytoplasm of the oocyte (Fig. 1a). Although most of the clusters were uniformly distributed in the peripheral cytoplasm of the oocyte, some of them formed a mass (Fig. 1a). Scanning deeper into the oocyte revealed the presence of a mass in the peripheral and the central cytoplasm of the oocyte (Fig. 1b). The mass exhibited no polarity. There was no ER in the germinal vesicle of the oocyte.

ER in oocytes at the Prometaphase I (Pro-MI) and Metaphase I (MI) stages: The Pro-MI and MI stage oocytes were obtained from the oocytes cultured for 24 hr. In the Pro-MI oocyte, the mass was absent and the clusters became fewer than in the GV stage oocyte (Fig. 1c). ER accumulations that appear to be generated by scattering of the cluster or the mass were observed in the peripheral cytoplasm of the Pro-MI stage oocyte (Fig. 1c). In addition, the accumulations were uniformly distributed in the peripheral cytoplasm of the oocyte and formed a thin layer (about 2 μm in width) beneath the plasma membrane (Fig. 1d). The ER in the central cytoplasm in the Pro-MI stage oocyte became more uniform than that in the GV stage oocytes (Fig. 1d). There was no ER near the chromosomes. Distribution of the ER in the MI stage oocyte was almost same as that in the Pro-MI stage oocyte (data not shown).

Fig. 1. Distribution of the DiI-stained ER in oocytes at the GV (a and b), Pro-MI (c and d), and MII (e and f) stages. Confocal sections of the oocytes in the peripheral cytoplasm (a, c and e). Confocal sections of the same oocytes at a location 14.4 μm from the peripheral cytoplasm (b, d and f). Each arrow head in a, b, c and d indicates a cluster of ER. Each arrow in a and b shows the cluster mass. The arrow in e indicates the polar body. The arrow in f indicates the injected oil drop. "an opening of a white line" in d and f shows the layer the ER accumulation. "S" in f shows the space in which the distribution of ER is relatively sparse.


Color Values
3000↑
2812
2624
2437
2249
2062
1874
1687
1499
1311
1124
936
749
561
374
186
0
100.0 μm
a
b
Color Values
2500↑
2343
2187
2031
1874
1718
1562
1405
1249
1093
936
780
624
467
311
155
0
100.0 μm
c
d
e
f
S

ER in oocytes at the Metaphase II (M II) stage: The mature MII stage oocytes were obtained from the oocytes cultured for 48 hr. The mass and/or the clusters observed in the GV and Pro-MI stage oocytes were absent from the MII stage oocyte. In the MII stage oocyte, the layer observed in the Pro-MI and MI stage oocytes became more evident and was reorganized as a fine, reticular net work beneath the plasma membrane (Fig. 1e). It was 6–8 μm in width (Fig. 1f), and a space 10–13 μm in width, in which the distribution of ER was relatively sparse was observed beneath the layer (Fig. 1f). The ER in the central cytoplasm in the MII stage oocyte was distributed uniformly same as in the Pro-MI and MI stage oocytes (Fig. 1f). There was no ER in the polar body or near the chromosomes (Fig. 1e).

Discussion

There were many bright clusters of ER present in the peripheral cytoplasm of the GV stage oocyte, and some of them formed a mass. The mass was absent from the Pro-MI stage oocyte, and the clusters became fewer than in the GV stage oocyte. ER accumulations that appear to be generated by scattering of the cluster or the mass were uniformly distributed at the peripheral cytoplasm and formed a thin layer beneath the plasma membrane in the Pro-MI and MI stage oocyte. The mass and/or the clusters observed in the GV, Pro-MI and MI stage oocytes were absent and the layer became more evident and wider in the MII stage oocyte. A space containing relatively few ER was also observed beneath the layer in the MII stage oocyte. From these results, it is considered that during maturation the clusters of ER migrated and scattered near the plasma membrane, forming a uniformed layer beneath the plasma membrane. The formation of the layer might also be attributed to the migration of ER present in the subcortical area of the oocyte to the peripheral cytoplasm. After all, it is clear that the distribution in the ER of porcine oocytes changes during meiotic maturation.

Concerning the changes in the distribution of the ER of mammalian oocytes during miotic maturation, it has been demonstrated in mouse oocytes that cortical ER accumulation comes into existence [6]. Moreover, Shiraishi *et al.* [16] have also shown that in hamster oocytes after the Pro-MI, surface ER masses gradually disperse to form a number of much smaller ER clusters near the surface and are incorporated into thicker ER networks. ER accumulations in the peripheral cytoplasm of oocytes during meiotic maturation therefore seem to be a common phenomenon in mammalian oocytes.

It has been shown in hamster [10], mouse [18] and human [19] oocytes that during the period of sperm-induced Ca^{2+} oscillation, each individual $[Ca^{2+}]_i$ invariably begins from a focus in the oocyte periphery and spreads throughout the entire peripheral region before propagating to the central ooplasm. The developmental changes in the distribution of the ER and IP_3Rs which are associated with $[Ca^{2+}]_i$ [11] and the spatial pattern of Ca^{2+} release during maturation of hamster oocytes were examined by Shiraishi *et al.* [16]. Their results demonstrate that when immature oocytes (GV stage) were inseminated, clear $[Ca^{2+}]_i$ did not occur and the distribution of IP_3Rs changed dramatically during maturation, in parallel with the distribution of the ER. They have also shown that the ER shifted to the peripheral surface during maturation in apposition to cortical granules. From these reports and the present results, it is considered that the changes in the distribution of ER in porcine oocytes during meiotic maturation, such as the formation of the layer at the peripheral cytoplasm, be closely related to immediate $[Ca^{2+}]_i$ and cortical granule exocytosis at fertilization. The formation of the layer in the peripheral cytoplasm may account for the acquisition of the ability of porcine oocytes to undergo normal fertilization.

Acknowledgment

The authors thank Dr. R.J. Etches of the Department of Animal and Poultry Science, University of Guelph, Canada, for kindly reviewing the manuscript.

References

1) Palade, G. (1975): Intracellular aspects of the process of protein synthesis. Science, 189, 347–358.
2) Davidowicz, E.A. (1987): Dynamics of membrane lipid metabolism and turnover. Annu. Rev. Biochem., 56, 43–61.
3) Van Meer, G. (1993): Transport and sorting of membrane lipids. Curr. Opin. Cell Biol., 5, 661–673.
4) Han, J.K. and Nuccitelli, R. (1990): Inositol 1,4,5-trisphosphate-induced calcium release in the organelle layers of the stratified, intact egg of *Xenopus laevis*. J. Cell Biol., 110, 1103–1110.
5) Terasaki, M. and Sardet, C. (1991): Demonstration of calcium uptake and release by sea urchin egg cortical endoplasmic reticulum. J. Cell Biol., 115, 1031–1037.
6) Mehlmann, L.M., Terasaki, M., Jaffe, L.A. and Kline, D. (1995): Reorganization of the endoplasmic reticulum during meiotic maturation of the mouse oocyte. Dev. Biol., 170, 607–615.
7) McPherson, S.M., McPherson, P.S., Mathews, L., Campbell, K.P. and Longo, F.J. (1992): Cortical local-

ization of a calcium release channel in sea urchin eggs. J. Cell Biol., 116, 1111–1121.

8) Kume, S., Muto, A., Aruga, J., Nakagawa, T., Michikawa, T., Furuichi, T., Nakade, S., Okano, H. and Mikoshiba, K. (1993): The *Xenopus* IP_3 receptor: Structure, function, and localization in oocytes and eggs. Cell, 73, 555–570.

9) Parys, J.B., McPherson, S.M., Mathews, L., Campbell, K.P. and Longo, F.J. (1994): Presence of inositol 1, 4, 5-trisphosphate receptor, calreticulin, and calsequestrin in eggs of sea urchins and *Xenopus laevis*. Dev. Biol., 161, 466–476.

10) Miyazaki, S., Shirakawa, H., Nakada, K. and Honda, Y. (1993): Essential role of the inositol 1,4,5-trisphosphate receptor/Ca^{2+} release channel in Ca^{2+} waves and Ca^{2+} oscillations at fertilization of mammalian eggs. Dev. Biol., 158, 62–78.

11) Miyazaki, S., Yuzaki, M., Nakada, K., Shirakawa, H., Nakanishi, S., Nakade, S. and Mikoshiba, K. (1992): Block of Ca^{2+} wave and Ca^{2+} oscillation by antibody to the inositol 1,4,5-trisphosphate receptor in fertilized hamster eggs. Science, 257, 251–255.

12) Fujiwara, T., Nakada, K., Shirakawa, H. and Miyazaki, S. (1993): Development of inositol trisphosphate-induced calcium release mechanism during maturation of hamster oocytes. Dev. Biol., 156, 69–79.

13) Ducibella, T., Kurasawa, S., Duffy, P., Kopf, G.S. and Schultz, R.M. (1993): Regulation of the polyspermy block in the mouse egg: Maturation-dependent differences in cortical granule exocytosis and zona pellucida modifications induced by inositol 1,4,5-trisphosphate and an activator of protein kinase C. Biol. Reprod., 48, 1251–1257.

14) Terasaki, M. and Jaffe, L.A. (1993): Imaging endoplasmic reticulum in living sea urchin eggs. Methods Cell Biol., 38, 211–220.

15) Jaffe, L.A. and Terasaki, M. (1994): Structural changes in the endoplasmic reticulum of starfish oocytes during meiotic maturation and fertilization. Dev. Biol., 164, 579–587.

16) Shiraishi, K., Okada, A., Shirakawa, H., Nakanishi, S., Mikoshiba, K. and Miyazaki, S. (1995): Developmental changes in the distribution of the endoplasmic reticulum and inositol 1,4,5-trisphosphate receptors and the spatial pattern of Ca^{2+} release during maturation of hamster oocytes. Dev. Biol., 170, 594–606.

17) Hogan, B., Costantini, F. and Lacy, E. (1986): Introduction of new genetic information into the developing mouse embryo. In: Manipulating the mouse embryo (Hogan, B., Costantini, F. and Lacy, E., eds.), pp. 151–204, Cold Spring Harbor Laboratory, U.S.A.

18) Carroll, J., Swann, K., Whittingham, D. and Whitaker, M. (1994): Spatiotemporal dynamics of intracellular $[Ca^{2+}]_i$ oscillations during the growth and meiotic maturation of mouse oocytes. Development, 120, 3507–3517.

19) Tesarik, J., Sousa, M. and Mendoza, C. (1995): Sperm-induced calcium oscillations of human oocytes show distinct features in oocyte center and periphery. Mol. Reprod. Dev., 41, 257–263.