

Electron Microscope Observations on the Formation and Development of Cortical Granules in *Xenopus laevis* Oocytes

Norio YOSHIZAKI

Department of Biology, Faculty of General Education,
Gifu University, Gifu, 501-11 Japan.

(Received October 4, 1993)

ABSTRACT

The origin and development of cortical granules were observed by treating sections of *Xenopus laevis* oocytes with a rabbit antiserum against cortical granule lectins and with a gold-conjugated goat antiserum against rabbit IgG. In stage I oocytes, gold particles were present on small numbers of cortical granules of 200–600 nm size. In stage II and III oocytes, they appeared on granules in the Golgi complexes as well as on large numbers of cortical granules, ranging in size from 200 nm to 1.4 μ m, in the cortical cytoplasm. Some of these cortical granules showed an irregular shape, indicating fusion of small granules into a large one. Gold-labeled granules disappeared from the Golgi complexes at stage IV. Cortical granules in stage IV and later oocytes consisted exclusively of large granules (1.4–1.5 μ m), and those in stage VI oocytes were aligned in a single row beneath the oolemma. These results suggest cortical granules form in the Golgi complexes of stage I to III oocytes and coalesce in the cortical cytoplasm of stage II to IV oocytes.

INTRODUCTION

The presence of cortical granules (CG) has been reported in the oocytes of many animals though not in all. Their origin was ascribed to Golgi complexes because immature granules in the Golgi complexes bore morphological features resembling those of mature CGs ⁽⁴⁾. This notion about origin might be acceptable in the case of CGs like those of sea urchins ⁽¹⁾, which possess characteristic internal structures that can be compared. However, the CGs of animals like *Xenopus* possess rather homogeneous contents and thus lack appropriate markers. Consequently, specific marker molecules of CG must be identified and their localization in the granules of Golgi complexes proved before the CG origin can be determined.

Upon fertilization in *Xenopus*, a CG exudate interacts with a prefertilization layer and forms a fertilization layer which prevents polyspermy ^(8,12). Among the constituents

of the exudate are CG lectins ^(5,8,9) which have been shown to appear in the CGs of unfertilized eggs and in the fertilization layer of fertilized eggs ⁽¹⁰⁾. To clarify in the present paper the origin and development of CGs, sections of *Xenopus* oocytes were treated with a rabbit antiserum against the lectins but also with a gold-labeled goat antiserum against rabbit IgG. To my knowledge, the formation of CGs in the Golgi complexes has never before been demonstrated by immunoelectron microscopy. Parallel observations were made on specimens which were doubly fixed with glutaraldehyde and OsO₄, in order to control for the structure of individual organelles, since the quality of fixation for immunoelectron microscopy is usually low. Observations made on the doubly fixed specimens might also lead to revision of the descriptions made in earlier times by electron microscopy ⁽²⁾ when its methodology was still undeveloped.

MATERIALS AND METHODS

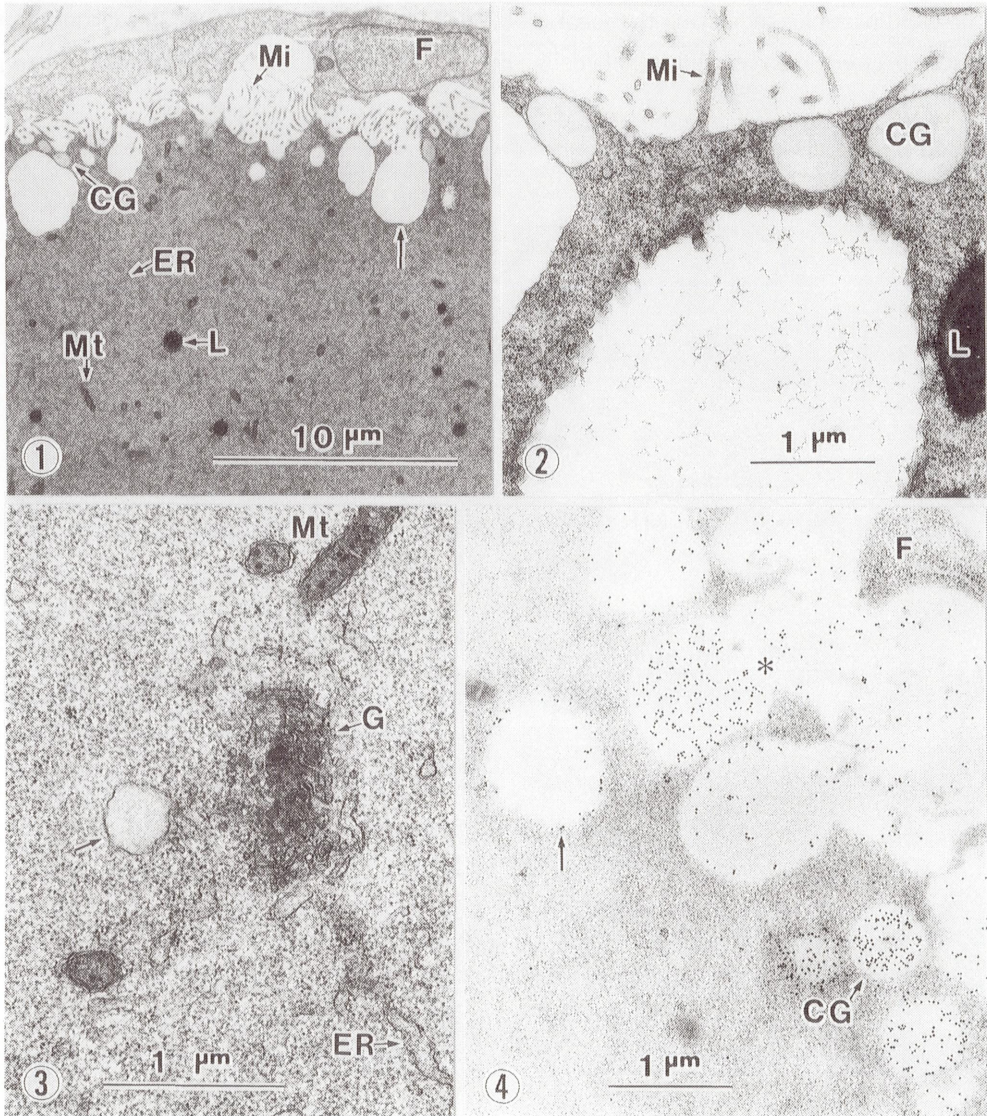
Electron microscopy: *Xenopus laevis* were purchased from a dealer in Hamamatsu. Ovaries were dissected from a female and fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.4) for 3 hr at 4° C. Individual oocytes were separated with forceps during the fixation. They were rinsed with the same buffer, postfixed in 1% OsO₄ in cacodylate buffer for 2 hr, dehydrated in acetone, and embedded in Epok 812. Sections were stained with uranyl acetate and lead citrate. Developmental stages of oocytes were determined by the morphological criteria of Dumont ⁽³⁾.

Immunoelectron microscopy: Glutaraldehyde-fixed oocytes were dehydrated in ethanol and embedded in Lowicryl K4M (Sigma) at -20° C according to the manufacturer's instructions. Thin sections were mounted on collodion-coated nickel grids. The sections were blocked for 10 min with 0.5% BSA in isotonic phosphate-buffered saline (PBS; pH 7.2) and washed with PBS. They were then incubated for 1 hr at room temperature with a 1/2000 solution of antiserum against cortical granule lectin. The preparation of the lectin and production of its antiserum in a rabbit were described previously ⁽⁹⁾. The sections were subsequently washed with PBS and treated for 1 hr with a gold-conjugated goat antiserum against rabbit IgG (E-Y Lab.). Then they were washed with PBS and with distilled water and were stained with uranyl acetate and lead citrate. After staining, the sections were dried and coated with carbon vapor. Control sections were treated with rabbit non-immune serum and then gold-conjugated goat antiserum. There was no significant labeling on the control sections.

RESULTS

Stage I oocytes

Oocytes extrude long microvilli toward surrounding follicle cells. The oolemma of stage I oocytes also showed huge indentations at the base of the microvilli (Fig. 1).

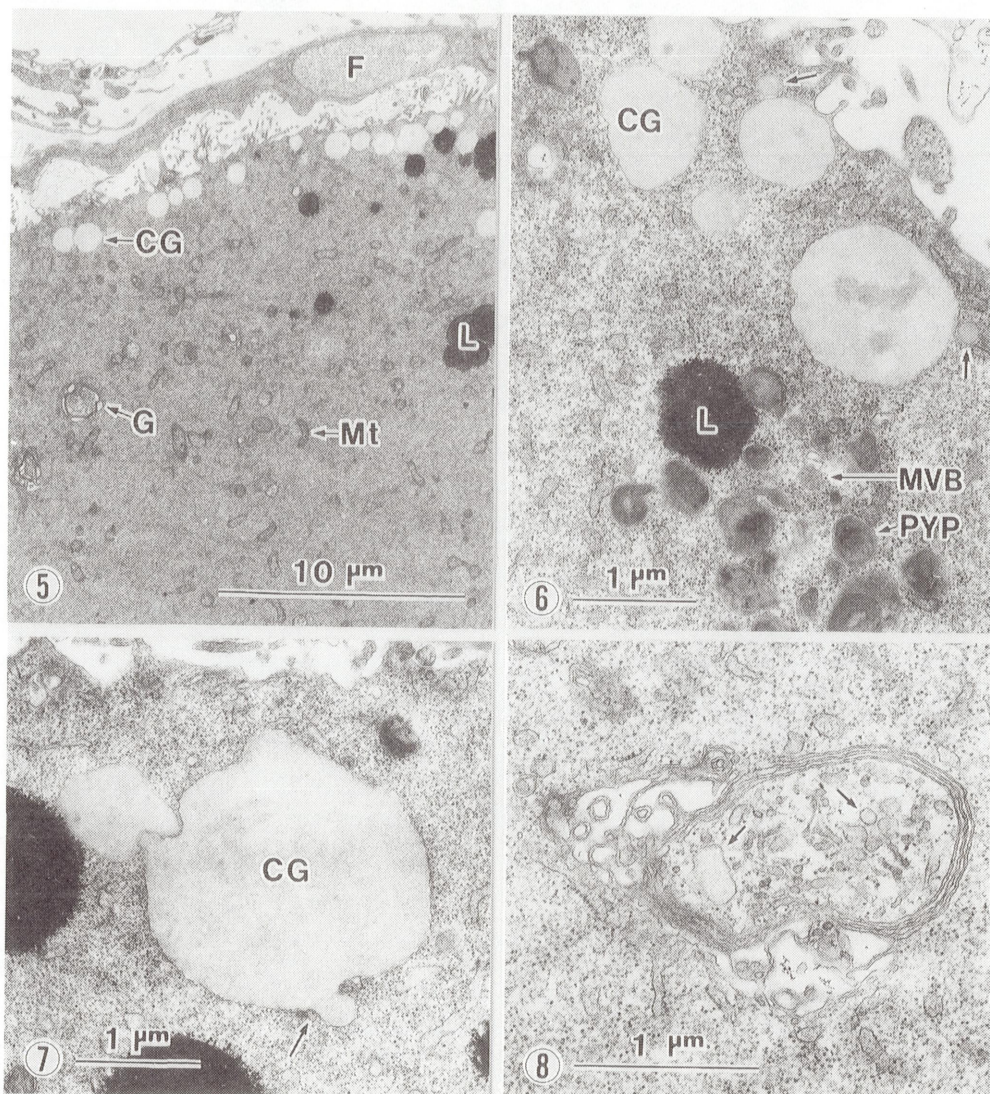


Figs. 1-3. Ultrastructures of stage I oocytes. The oolemma shows huge indentations (arrow) and outward protrusions of microvilli (Mi; Fig. 1). There are small cortical granules (CG) 600 nm in size beneath the oolemma (Fig. 2) and a small granule (arrow) near the Golgi-like membrane cluster (G) in subcortical cytoplasm (Fig. 3). ER, endoplasmic reticulum; F, follicle cell; L, lipid droplet; Mt, mitochondrion.

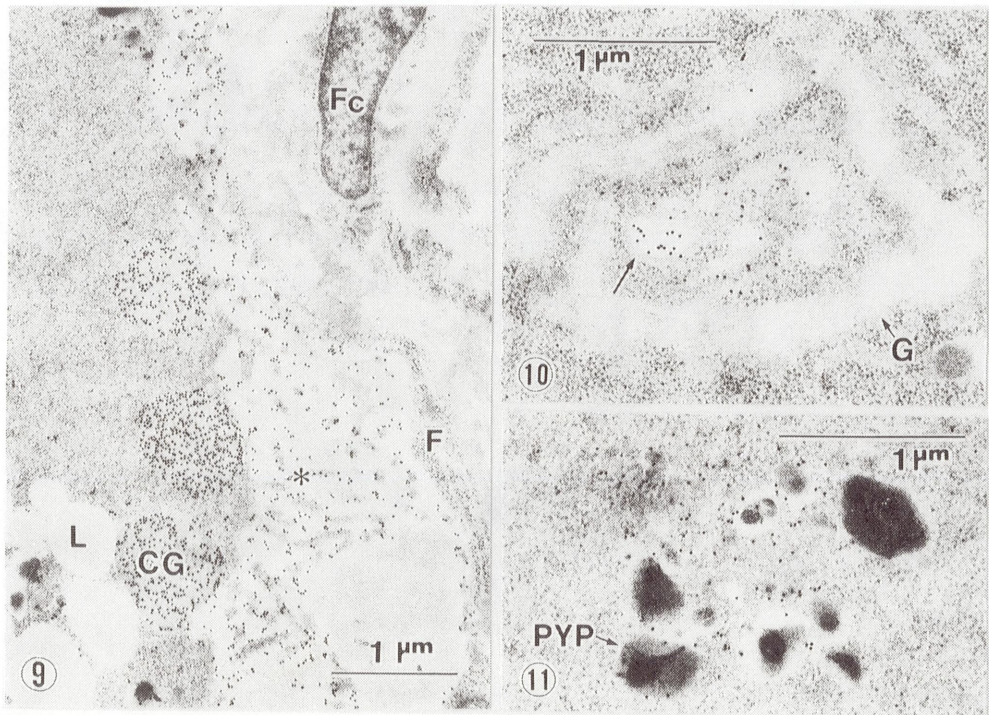
Fig. 4. Immunoelectron microscopy of stage I oocyte. Gold particles are present on cortical granules (CG) and in some of the space (*) between oocyte and follicle cells (F). An arrow indicates a cross section of indented oolemma which is also labeled with gold particles.

There were a few cortical granules (CG) 200–600 nm in size just beneath the oolemma (Fig. 2). Stage I oocytes were characterized by highly electron-dense ground cytoplasm with mitochondria, endoplasmic reticula and lipid droplets scattered within it (Fig. 1). Golgi-like membrane clusters were occasionally found in subcortical cytoplasm with small granules nearby. Fig. 3 shows such a granule of 350 nm in size.

Gold particles indicating the location of CG lectin can be seen in small CGs and in spaces between oocytes and follicle cells (Fig. 4). Preferential labeling was noticed on



Figs. 5–8. Ultrastructures of stage II oocytes. Cortical granules (CG) of various sizes line the underside of the oolemma (Figs. 5 and 6). Free arrows in Fig. 6 point out the smallest CGs of 200 nm. Fig. 7 shows an irregularly shaped CG, presumably the result of fusion between a small CG and a large one (arrow). There are clusters of multivesicular bodies (MVB) and primordial yolk platelets (PYP) (Fig. 6). Golgi complexes (G) located in the subcortical cytoplasm (Fig. 5) contain small granules (arrows in Fig. 8). F, follicle cell; L, lipid droplet; Mt, mitochondrion.



Figs. 9-11. Immunoelectron microscopy of stage II oocytes. Gold particles are present on cortical granules (CG) and in the space between follicle cell (F) and oocyte (*; Fig. 9). Small granules (free arrow in Fig. 10) in the Golgi complex (G) and clusters of primordial yolk platelets (PYP; Fig. 11) are also labeled with gold particles. Fc, fibrocyte; L, lipid droplet.

the oolemma. A Golgi complex could not be found by immunoelectron microscopy at this stage.

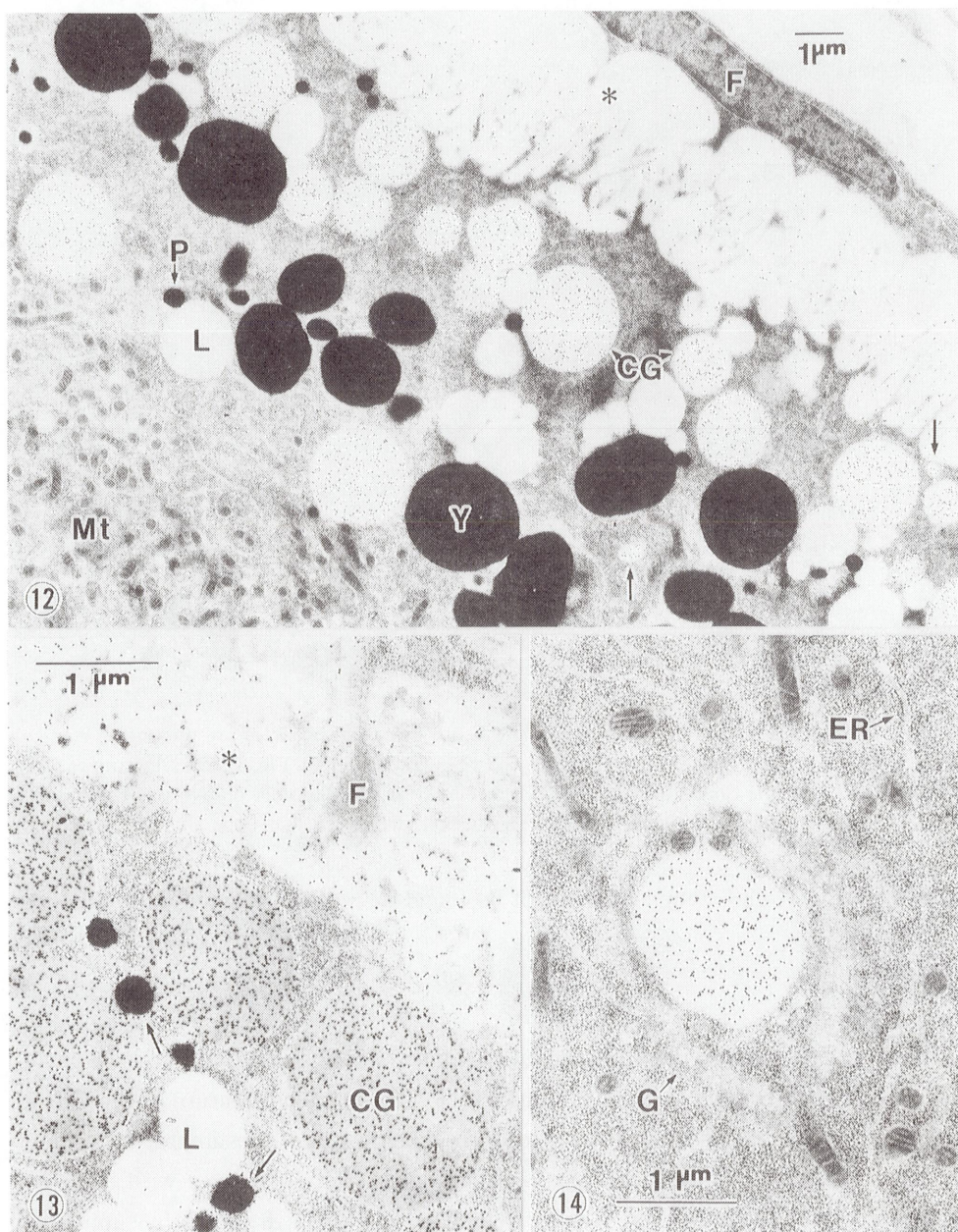
Stage II oocytes

A layer of CGs was present beneath the oolemma of stage II oocytes (Fig. 5). Their size varied from 200 nm to 1.2 µm (Fig. 6). A prominent feature of the CGs in these oocytes was that some of them were irregular in shape, presumably because of the fusion of small CGs with a larger one (Fig. 7). Golgi complexes with a typical lamellar structure were located in subcortical cytoplasm (Fig. 5) and a few contained small granules inside (Fig. 8). Clusters of multivesicular bodies (MVB) and primordial yolk platelets (PYP) appeared in these stage II oocytes (Fig. 6).

Immunoelectron microscopy disclosed significant labeling by gold particles on granules in the Golgi complex (Fig. 10) and the PYP clusters (Fig. 11) as well as the CGs and in the spaces between oocytes and follicle cells (Fig. 9).

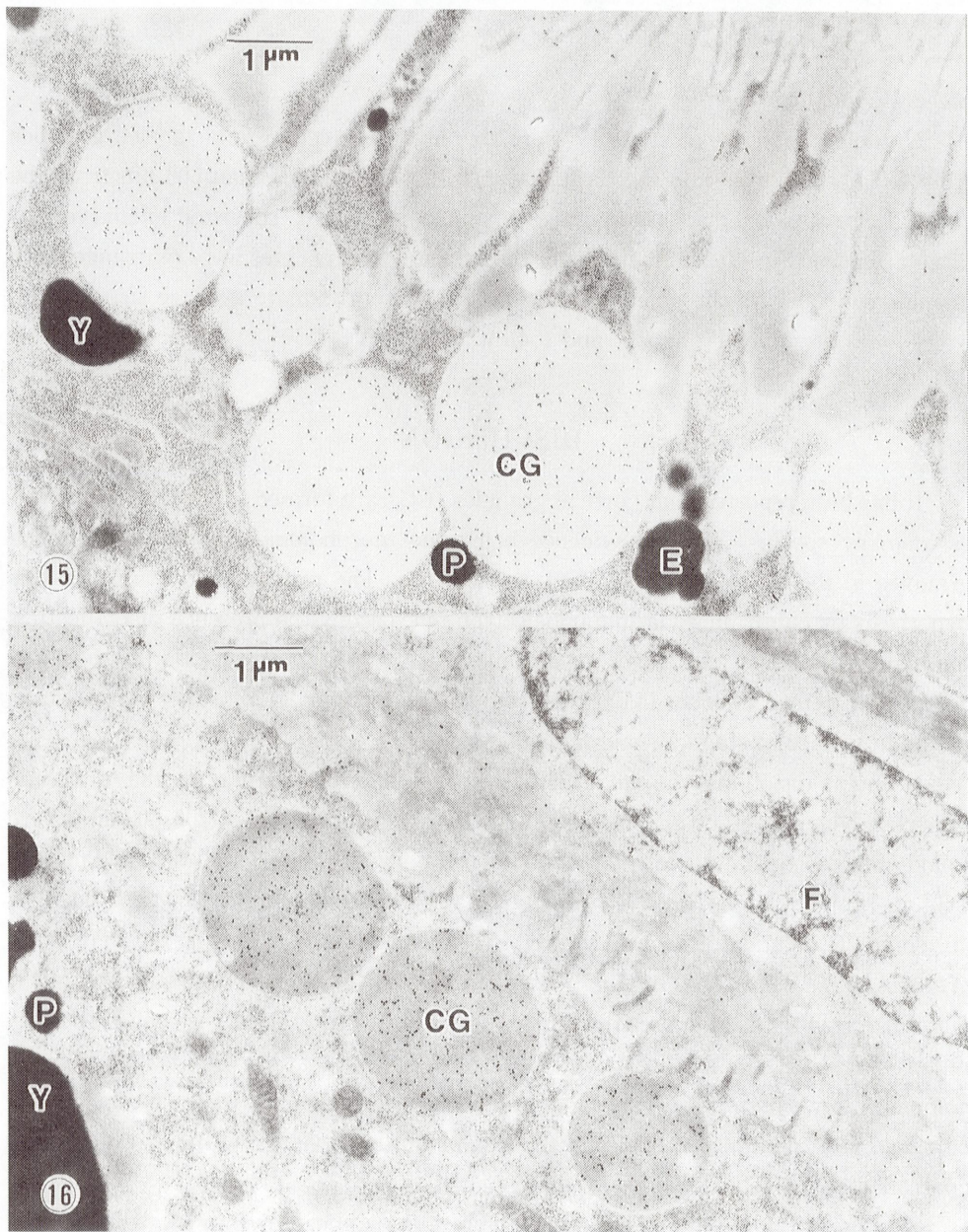
Stage III

The number of gold-labeled CGs increased in the cortical cytoplasm of stage III



Figs. 12–14. Immunoelectron microscopy of stage III oocytes. Cortical granules (CG) accumulate in the cortical cytoplasm (Fig. 12). CGs and intrafollicular spaces (*) are labeled with gold particles (Figs. 12 and 13). Arrows in Fig. 12 show the smallest CGs and those in Fig. 13 show pigment granules (P), both of which are labeled. Fig. 14 shows a Golgi complex (G) containing a gold-labeled granule approximately the same size as a mature CG. ER, endoplasmic reticulum; F, follicle cell; L, lipid droplet; Y, yolk platelet.

oocytes (Figs. 12 and 13), where they were distributed into several rows. The largest of them reached $1.4\mu\text{m}$ (calculated from doubly fixed specimens). Small CGs of 200–600 nm were present as well. CGs were also found in the subcortical cytoplasm.



Figs. 15-16. Immunoelectron microscopy of stage IV (Fig. 15) and VI oocytes (Fig. 16). Cortical granules (CG) smaller than 600 nm cannot be seen in the cortical cytoplasm of a stage IV oocyte (Fig. 15). CGs in the stage IV oocyte consist exclusively of large granules (Fig. 15), and those in a stage VI oocyte are aligned into a single row beneath the oolemma (Fig. 16). The CGs were labeled with gold particles. E, endosome; F, follicle cell; P, pigment granule; Y, yolk platelet.

The development of the Golgi complexes was the highest at this stage during the oogenesis. Granules of various sizes were observed inside them, the largest one being 1.4 µm in size (Fig. 14). Pigment granules, which appear from stage III, were also

labeled by gold particles (Fig. 13).

Stage IV and later oocytes

Notable in oocytes at stage IV were the disappearance of gold-labeled granules from Golgi complexes and the disappearance of CGs smaller than 600 nm from the cortical cytoplasm. Thus CGs in stage IV and later oocytes consisted exclusively of large granules (Fig. 15), and moreover those in stage VI oocytes were rearranged into a single row beneath the oolemma (Fig. 16). The approximate average size of them was 1.5 μm at stages IV and V, and 1.4 μm at stage VI.

DISCUSSION

It has been reported that cortical granules (CG) are formed in the Golgi complexes in many species (4), the evidence being the morphological similarity between granules in the Golgi complexes and mature CGs. The present study supports this hypothesis by confirming via immunoelectron microscopy the presence of CG lectins, the major constituent of CGs, in the granules. In *Xenopus*, CG production in the Golgi complexes seemed to occur in oocytes from stage I to stage III, most actively at stage III, and to cease in stage IV and later oocytes.

Granular fusion may be the structural phenomenon accounting for the formation of such a huge secretory granule as CG, since (a) only small CGs (200–600 nm) were found in the cortical cytoplasm of stage I and II oocytes, mixed small-size and mature CGs (1.4 μm) in stage III oocytes, and no small ones at all in stage IV and V oocytes; (b) some CGs in stage II oocytes showed irregular contours, suggesting fusion of small granules into a large one. In Golgi complexes, the granules found at stages I and II were also small, but at stage III granules of various sizes, the largest being approximately the same as a mature CG. It would thus appear that the CGs leave the Golgi complex after enlarging to varying degrees and then coalesce with each other in the cortical cytoplasm until they reach the size of mature CGs. How the size of CGs is controlled is not yet known.

The OsO_4 fixation and methacrylate embedding adopted by Balinsky and Devis⁽²⁾ for electron microscopy gave rise to fragmentation and vesicularization of membranous structures such as the endoplasmic reticulum and Golgi lamella. Thus in their figures many small vesicles appear around mature CGs in the cortical cytoplasm. In the *Xenopus* figures, it was impossible without appropriate labeling of specific molecules either to distinguish small CGs from vesicles originating from other organelles or to observe fusing granules. Balinsky and Devis may have hesitated for these reasons to propose that the coalescence of small CGs is a mechanism of CG growth.

Gold particles observed in the present study in the spaces between follicle cells and oocytes and on clusters of multivesicular bodies and primordial yolk platelets indicate the presence of materials which have the same epitopes as those of the CG lectins there. It is known that CG lectins possess the same sugar specificity as serum lectin⁽⁶⁾ and yolk lectin⁽¹¹⁾ in *Xenopus*. It has been further shown that the yolk lectins administered to stage II oocytes *in vitro* were taken into the clusters of multivesicular bodies and primordial yolk platelets but not into the CGs⁽¹¹⁾. Thus those gold particles might show the existence of yolk lectin or its precursor which will be carried into yolk platelets in company with the vitellogenin. The gold particles on pigment granules may be explained in the same way, because pigment granules are also formed in the clusters of multivesicular bodies⁽²⁾.

The appearance in stage I oocytes of membrane clusters I described as Golgi-like structures suggests that typical Golgi complexes are not maintained in pre-vitellogenic, young oocytes but are newly formed concomitant with CG formation. Membrane clusters without CGs were frequently observed in cortical cytoplasm of stage I and II oocytes (unpublished). It is well-known that Golgi complexes play a key role in the production and transportation of materials such as secretions, cellular membranes and lysosomal enzymes. The production of cellular membranes and lysosomal enzymes might be a principal activity of vitellogenic oocytes since the oocytes show very rapid growth and a large amount of vitellogenin is processed by lysosomal enzymes⁽⁷⁾. However, it is still obscure how the Golgi complexes play roles in their production. Study is needed to clarify the origin and development of Golgi complexes in young oocytes.

Acknowledgement : I wish to thank Dr. H. Kubota of Kyoto University for instruction in immunoelectron microscopy, and Ms. M. Lynne Roecklein for reading through the manuscript.

REFERENCES

1. Anderson, E., 1968. Oocyte differentiation in the sea urchin, *Arbacia punctulata*, with particular reference to the origin of cortical granules and their participation in the cortical reaction. *J. Cell Biol.*, 37, 514-539.
2. Balinsky, B. I. and R. J. Devis, 1963. Origin and differentiation of cytoplasmic structures in the oocytes of *Xenopus laevis*. *Acta Embryol. Morphol. Exper.*, 6, 55-108.
3. Dumont, J. N., 1972. Oogenesis in *Xenopus laevis* (Daudin). I. Stages of oocyte development in laboratory maintained animals. *J. Morphol.*, 136, 153-180.
4. Guraya, S. S., 1982. Recent progress in the structure, origin, composition, and function of cortical granules in animal egg. *Intern. Rev. Cytol.*, 78, 257-360.
5. Nishihara, T., R. E. Wyrick, P. K. Working, Y. H. Chen and J. L. Hedrick, 1986. Isolation and characterization of a lectin from the cortical granules of *Xenopus laevis* eggs. *Biochemistry*, 25,

6013-6020.

6. Roberson, M. M., A. P. Wolffe, J. R. Tata and S. H. Barondes, 1985. Galactoside-binding serum lectin of *Xenopus laevis*. J. Biol. Chem., 260, 11027-11032.
7. Wall, D. A. and I. Meleka, 1985. An unusual lysosome compartment involved in vitellogenin endocytosis by *Xenopus* oocytes. J. Cell Biol., 101, 1651-1664.
8. Yoshizaki, N., 1986. Properties of the cortical granule lectin isolated from *Xenopus* eggs. Develop. Growth Differ., 28, 275-283.
9. Yoshizaki, N., 1989. Comparison of two lectins isolated from *Xenopus* cortical granules. Zool. Sci., 6, 507-514.
10. Yoshizaki, N., 1989. Immunoelectron microscopic demonstration of cortical granule lectins in coelomic, unfertilized and fertilized eggs of *Xenopus laevis*. Develop. Growth Differ., 31, 325-330.
11. Yoshizaki, N., 1990. Localization and characterization of lectins in yolk platelets of *Xenopus* oocytes. Develop. Growth Differ., 32, 343-352.
12. Yoshizaki, N. and Ch. Katagiri, 1984. Necessity of oviducal pars recta secretions for the formation of the fertilization layer in *Xenopus laevis*. Zool. Sci., 1, 255-264.