Structure of Egg Cortex Relating to Presumptive Embryonic and Extraembryonic Regions in Silkworm, *Bombyx mori* (Bombycidae: Lepidoptera)

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and

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Synopsis

In the egg cortex of the mature *Bombyx* egg, large pyronin-positive granules exist attached to the periplasm. These granules are not distributed evenly, but are localized in the definite region. With the beginning of development, these granules break down and the regional difference of the egg cortex disappears. However, this distribution pattern in the mature egg coincides with the extent of the embryonic and extraembryonic regions at the early germ band stage, and this fact suggests that the granules might play an important role for differentiation of both regions.

Introduction

Ooplasm of the insect egg usually consists of a cortical thin layer, the periplasm, including no yolk spheres, and the reticuloplasm, a network containing yolk spheres in its mesh. Of these two cytoplasmic structures, the periplasm seemed to contribute chiefly to organization of the basic body pattern of the embryo and have been studied from various viewpoints, resulting in establishment of fate map in some species (see Sander, 1976). However, from the morphological point of view, researches of structures essential for embryonic pattern formation hardly succeeded, except for some structures, one of which was the posterior polar plasm relating to the pole cell segregation. Especially in *Drosophila*, structure and function of the posterior polar plasm have been studied vigorously and many interesting results are reported (see Okada, 1986).

In *Bombyx*, Takami (1942, 1946) analyzed the embryonic development with cauter-

ization method and made a rough fate map. Subsequently, Miya (1958) pursued the problems on germ cell differentiation with the same method and suggested the region and the time of germ cell segregation, but any characteristic structure similar to the posterior polar plasm could not been confirmed. Afterward, Suzuki (1969) found that pyronin-positive granules distributed within the egg cortex in the newly-laid egg and observed their change with the progress of development, and Miya (1978) studied their ultrastructure and obtained the conclusion that these granules were a kind of rough-surfaced endoplasmic reticulum, rER, constructing concentric whorl. Furthermore, Yamauchi is studying their origin by electron microscopy (personal communication). The granules are not distributed evenly within the egg cortex, but are localized at the specific region. The present paper deals with the distribution pattern of these granules.

Material and Method

For light microscopic observation, non-diapause eggs of the strain "Daizo" were used as material. The eggs were fixed with Carnoy's fluid and cut $6-15$ μ m thick with the usual paraffin method. The sections were stained with Mayer's acid hemalum or Kurnick's methylgreen — pyronin. As the counterstaining, the sections were treated in RNase solution diluted with buffer of five thousand times at 55℃ for 1 hr. In order to calculate the extent of the region where the pyronin-positive granules exist in the mature egg and the germ anlage appears at the early germ band stage, the sections were transcribed by a projector and measured by a curvimeter.

For electron microscopy, the same method as in the previous report was used (Miya, 1978).

Results

1. Distribution of the pyronin-positive granules in mature egg

The mature egg is covered by the pyronin- and hematoxylin-negative vitellin membrane lying just under the chorion. The egg cortex is composed of the homogeneous layer stained lightly with pyronin and hematoxylin, and the region containing large pyronin- and hematoxylin-positive granules. These granules sometimes coagulate together with the surrounding cytoplasmic inclusions to form very large structures.

The granules are distributed unevenly within the egg cortex. In the cross section the granules are distributed numerously in both lateral sides and only few and/or smaller granules are observed in the ventral side, but hardly any exist in the dorsal side of the egg (Figs. 1, 2, 6 B). In the frontal section the granules are observed in both lateral sides, within the range of about $10-90\%$ of the egg length from the posterior pole, but in the anterior and posterior regions granules hardly exist (Fig. 6 A).

In the anterior region a pyronin-positive discoidal structure is recognized and this structure is correspondent to the specialized arrangement of rER (Miya, 1978, 1984, 1985). Figures 3 and 4 represent the ultrastructure of the pyronin-positive granules.
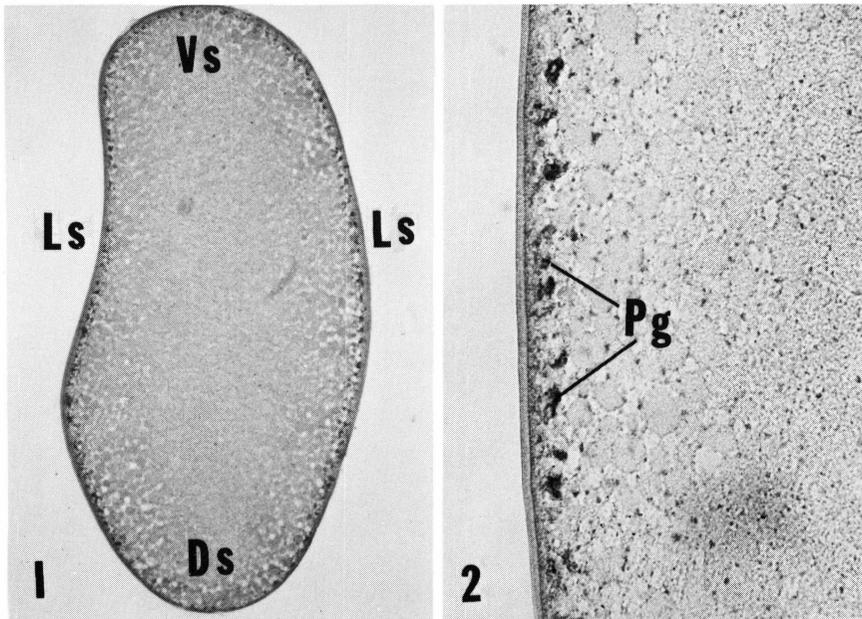
Fig. 1. Cross section of the mature egg. Ds: dorsal side, Ls: lateral side, Vs: ventral side of the egg. × 60.

Fig. 2. Magnified figure of the lateral side of the egg shown in Fig. 1. Pg, pyronin-positive granule. × 240.

These granules are constructed with large concentric whorls of rER, in which no mitochondria exist, but sometimes dense organelles connecting with rER are observed (Fig. 4, Ea). Figure 5 is an electron micrograph showing the enlarged structure of the rER.

As shown in Fig. 6, when the extent of the egg cortex without pyronin-positive granules is indicated as the ratio for the entire circuit, the following values are obtained: 20.3 ± 1.7 % for the anterior polar region, 16.8 ± 0.8 % for the posterior polar region, and 15.6 ± 1.6 % for the dorsal side of the egg.

2. *Structural change of the pyronin-positive granules with beginning of development*

Electron-microscopically, the first structural change by entry of sperms into the egg appears in the vitellin membrane and a distinct fertilization membrane is formed (Miya, 1978, 1984). However, from light microscopical observation, resumption of the maturation division, which rests at the metaphase of the first division, is characteristic and the direction of the spindle becomes from parallel to vertical to the egg surface. Fifteen to 30 minutes after oviposition, the division comes at anaphase and then about 1 hour after the second division begins. At this stage the structure of egg cortex changes remarkably; the vitellin membrane changes to become the fertilization membrane and the homogeneous layer stained lightly disappears, resulting in approach of
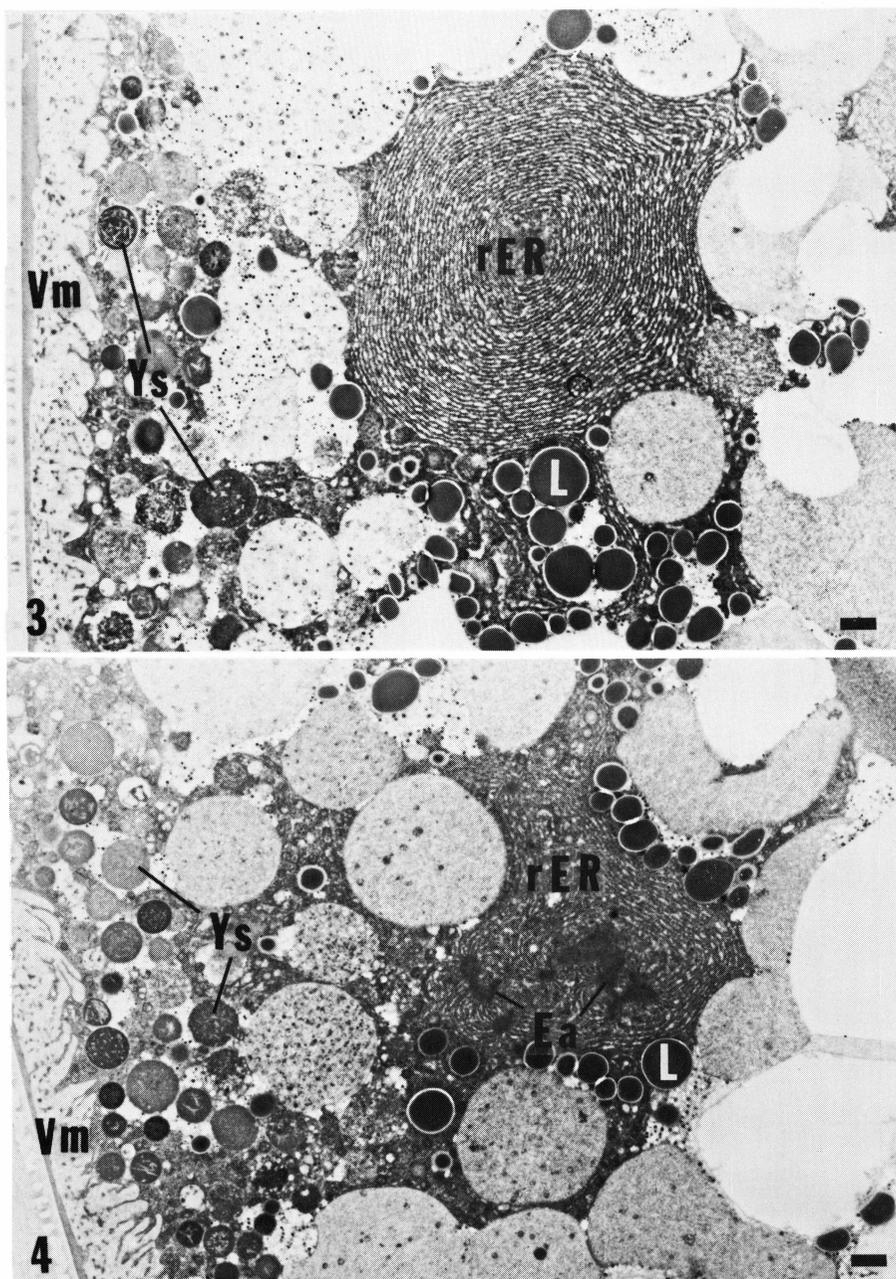
Figs. 3, 4.   Electron micrographs representing egg cortex of the mature egg. L, lipid dro-
plet; rER, rough-surfaced endoplasmic reticulum; Vm, vitellin membrane; Ys,
small yolk sphere. Scale: 1 μ m.
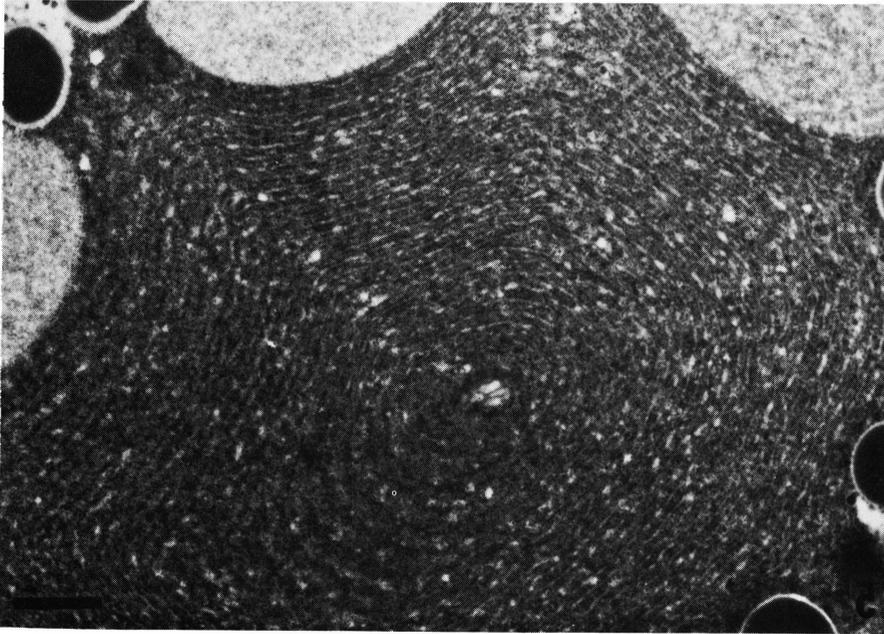
Fig. 5.   Electron micrograph representing the magnified concentric whorl of rER. Scale: 1 μ m.

the pyronin-positive granules just under the fertilization membrane. With the progress of development, the pyronin-positive granules disappear and a relatively homogeneous layer stained with pyronin becomes recognizable under the fertilization membrane (Figs. 7, 8). Figures 9 and 10 are electron micrographs representing this process; the large whorl of rER attaching to the periplasm begins to break down to fuse together with the compoments of the periplasm and at the same time the cisternae of rER change from the lamellar to the tubular and/or vesicular structure.

3. *Penetration of the cleavage nuclei into the periplasm and formation of the blastoderm and the germ anlage*

About 12 hours after oviposition, the cleavage nuclei enclosed with cytoplasmic coat reach the egg cortex and penetrate into the periplasm, but this process does not occur at the same time in all region of cortex as reported by Miya (1958). In the present observation the cleavage nuclei penetrate into the periplasm at the lateral sides near the anterior pole at first, and then the penetration extends posteriorly and dorsally, and to the posterior pole at last. After penetration of the cleavage nuclei into the periplasm, nuclear divisions parallel to the egg surface occur and the cell membrane is formed around each nucleus in the same order as of penetration into the periplasm. Figures 11 − 14 represent this situation in the cross section; formation of the cell membrane begins at the lateral side (Fig. 12), on the other hand, the penetrating nuclei are

$$\frac{mc}{Nc} \times 100 = 20.3 \pm 1.7 \, (\%)$$

$$\frac{lc}{Lc} \times 100 = 15.6 \pm 1.6 \, (\%)$$

$$\frac{nc}{Nc} \times 100 = 16.8 \pm 0.8 \, (\%)$$
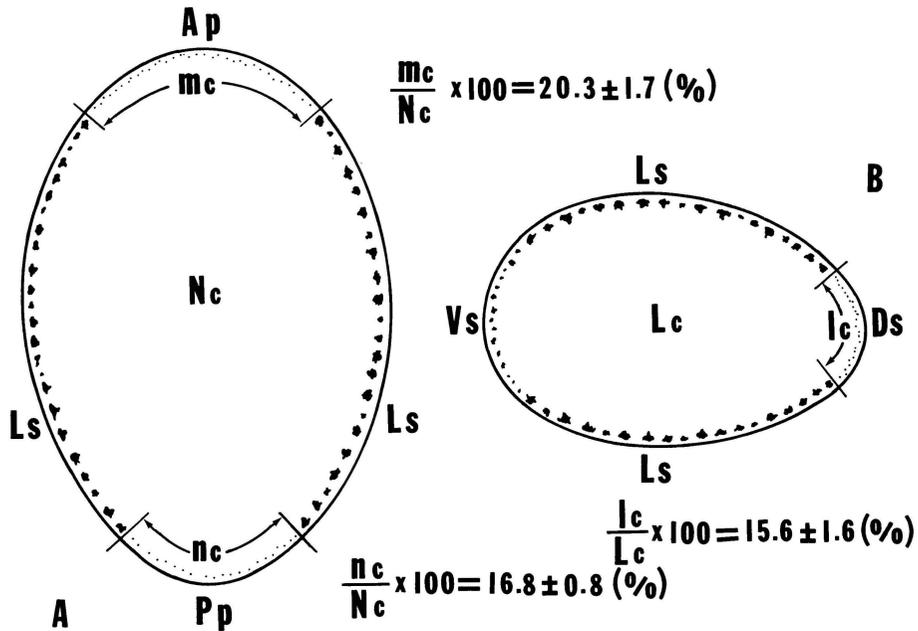
Fig. 6. Diagrammatic figures of the frontal (A) and cross (B) sections representing distribution pattern of the pyronin-positive granules. Ap: anterior pole, Pp: posterior pole, Ds: dorsal side, Ls: lateral side, Vs: ventral side of the egg, Nc, Lc: length of the entire circuit, mc, nc, lc: extent of the regions lacking pyronin-positive granules at the anterior, posterior and lateral sides of the egg, respectively.
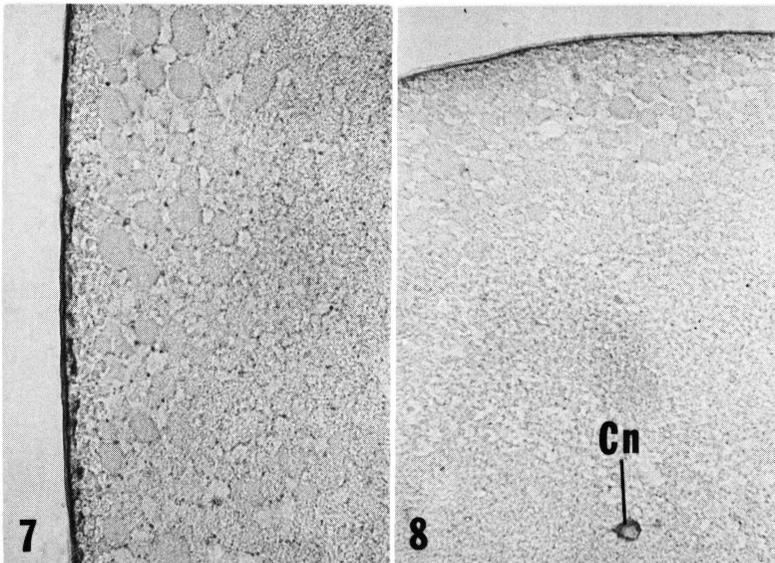
Fig. 7. Cross section of the 3-hours old egg. × 240.
Fig. 8. Cross section of the 7-hours old egg. Cn, cleavage nucleus. × 240.
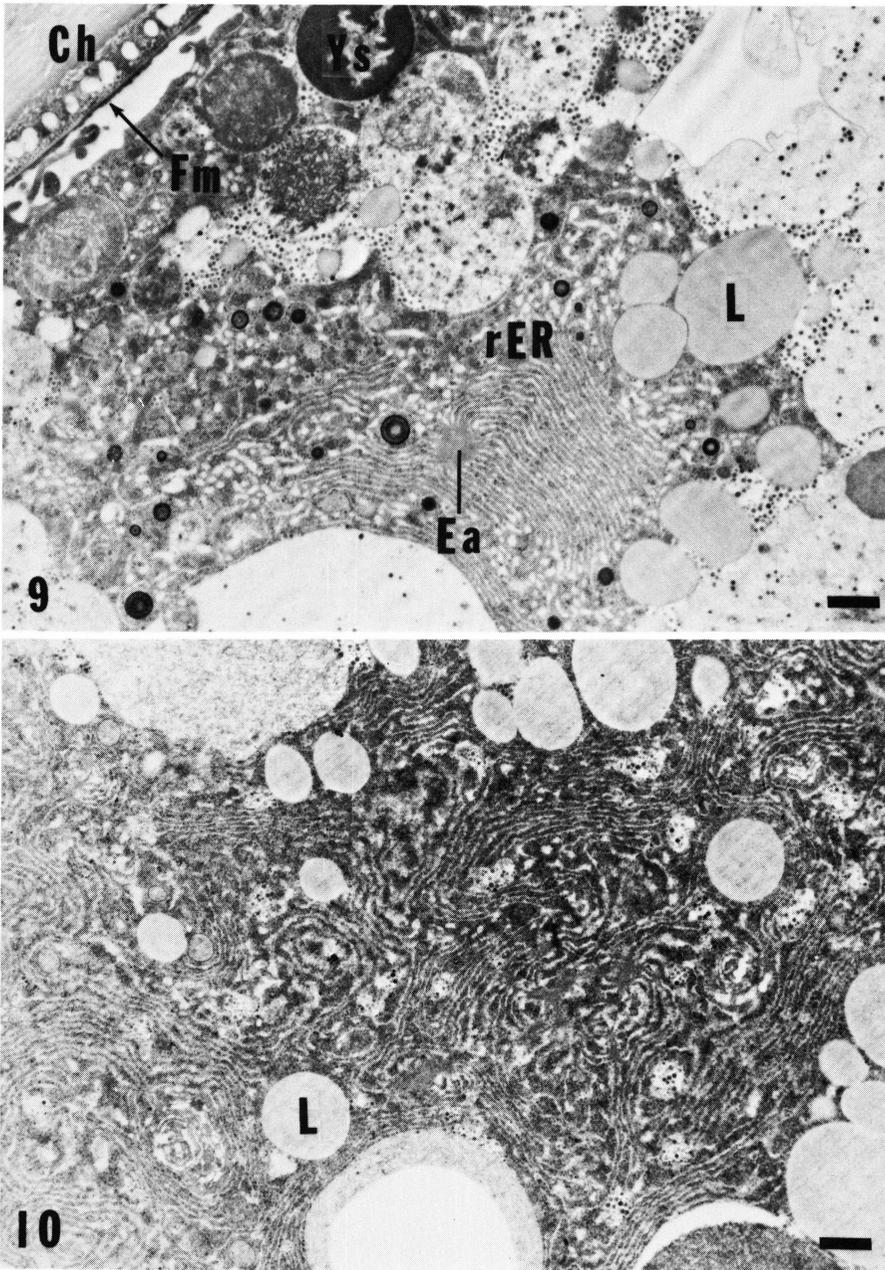
Figs. 9, 10. Electron micrographs representing structural change of rER. Ch, chorion; Ea, dense organelle connecting with rER; Fm, fertilization membrane; L, lipid droplet; rER, rough-surfaced endoplasmic reticulum; Ys, small yolk sphere. Scale: 1 μm.
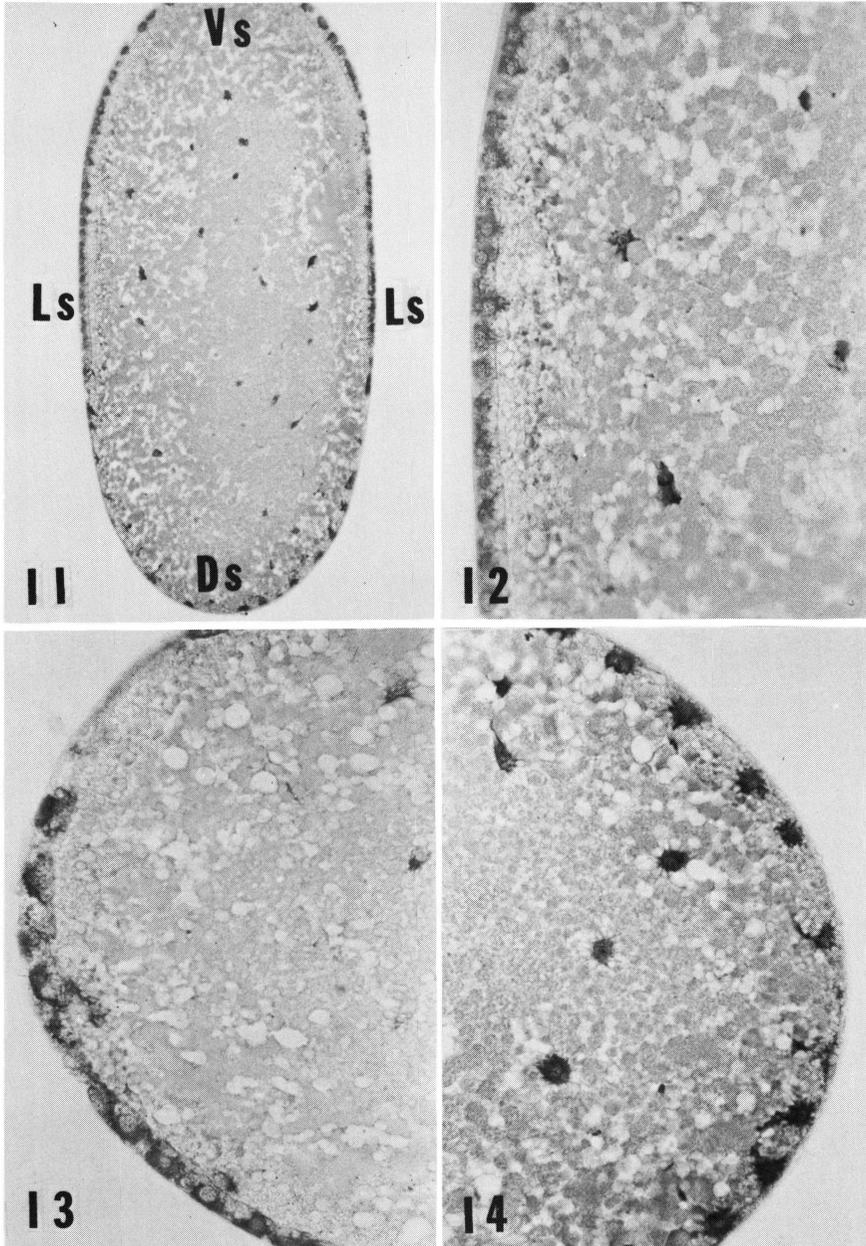
Figs. 11—14. Cross section (11) and its magnified figures at the lateral (12), ventral (13) and dorsal (14) sides of the 13-hours old egg. Ds: dorsal side, Ls: lateral side, Vs: ventral side of the egg. Fig. 11, × 60; Figs. 12—14, × 120.
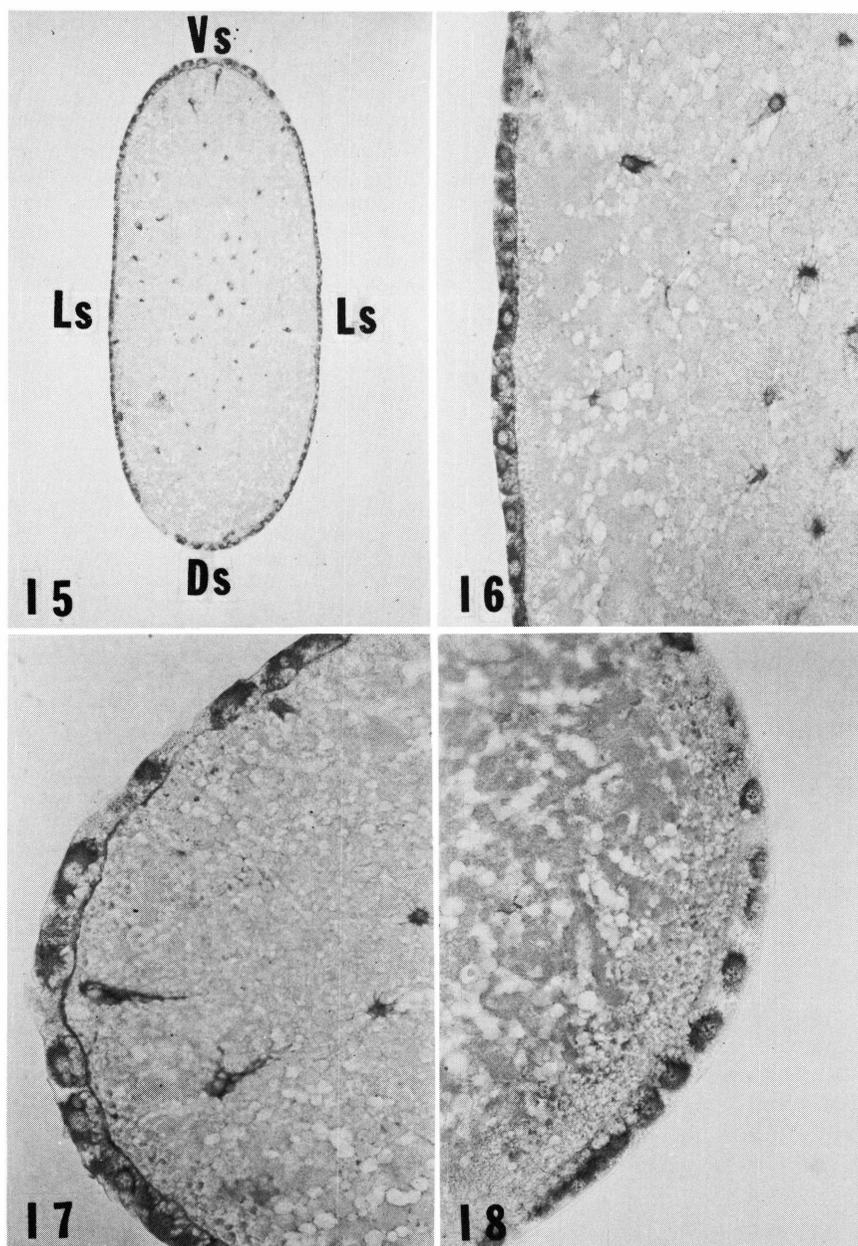
Figs. 15 — 18. Cross section (15) and its magnified figures at the lateral (16), ventral (17) and dorsal (18) sides of the 15-hours old egg. Ds: dorsal side, Ls: lateral side, Vs: ventral side of the egg. Fig. 15, × 50; Figs. 16 — 18, × 120.
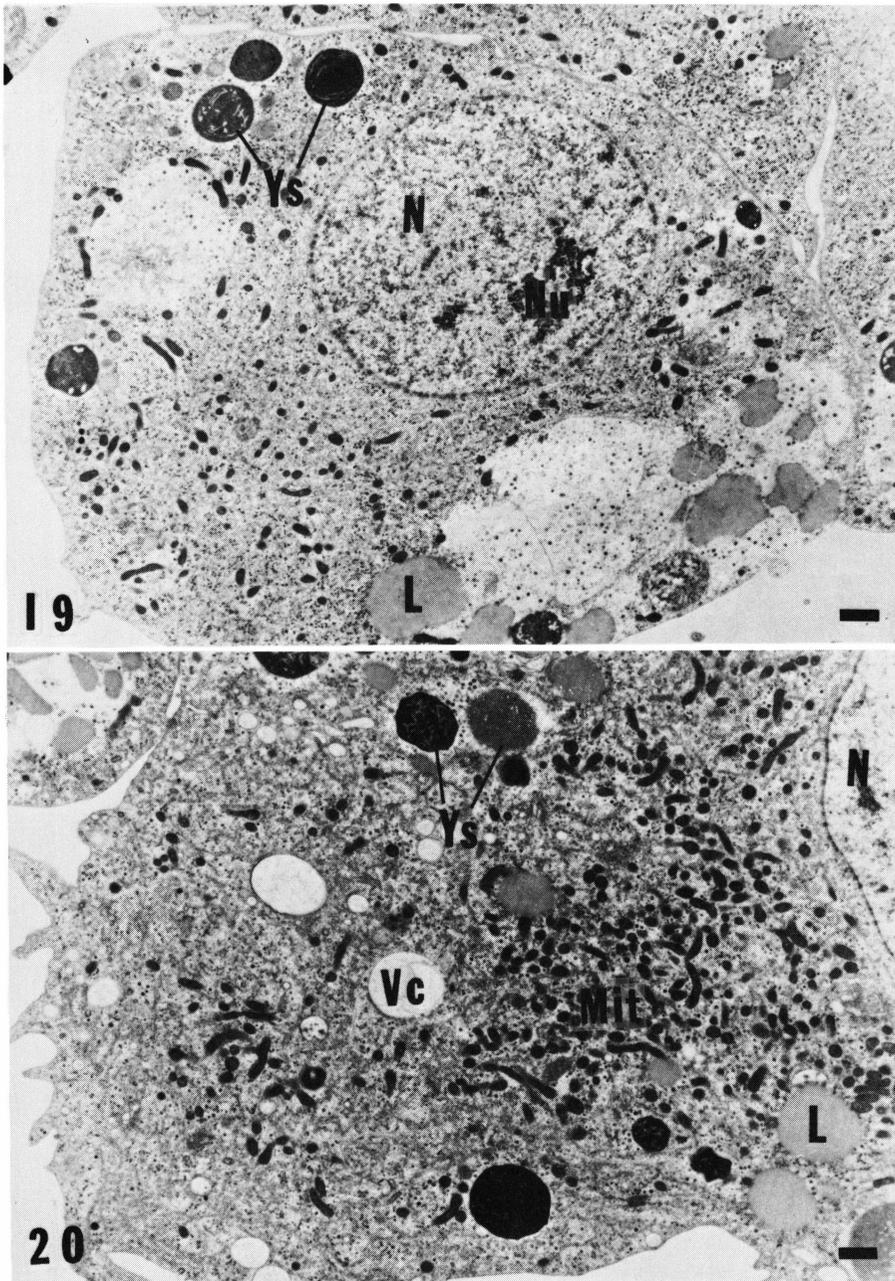
Figs. 19, 20.   Electron micrographs representing the cell of the germ anlage (19) and the cell of the extraembryonic region (20). L, lipid droplet; Mit, mitochondria; N, nucleus; Nu, nucleolus; Vc, vacuole; Ys, small yolk sphere. Scale: 1 μm.

few and formation of cell membrane does not occur yet at the dorsal side (Fig. 14). During this process one or two nucleoli appear in the nucleus, suggesting beginning of activation of genes originated from the zygote. In *Bombyx* the above process is not carried out by formation of typical cleavage furrow, but by protrusion of the cleavage nuclei beyond the initial level of the egg surface (Takesue *et al.*, 1980; Takesue, 1985).

Through the above-mentioned processes the cellular blastoderm is completed, but the structure of the blastoderm cells is different corresponding to the egg regions; the cells lying at the ventral and both lateral sides of the egg are smaller in size and are distributed densely, while the cells at the dorsal side are larger and exist separately. The former cells are the cells of the embryonic region, which form the germ anlage later, and the latter cells are the cells of the extraembryonic region, which develop the embryonic envelope (Figs. 15—18).

Figures 19 and 20 are electron micrographs representing the cell of the germ anlage and that of the extraembryonic region, respectively. The latter is larger and contains more numerous mitochondria, vacuoles, and other cytoplasmic inclusions than the former one.

As shown in Fig. 21, when the extent of the extraembryonic region in the cross and frontal sections is indicated as the ratio for the entire circuit, the following values are obtained: 21.8 ± 5.7 % for the anterior region, 14.4 ± 1.6 % for the posterior region, and 13.7 ± 5.2 % for the dorsal side of the egg. When these values are compared with those of corresponding values shown in Fig. 6 and examined by t-test, in all cases any significant differences are not recognized. Consequently, the egg cortex in which pyronin-positive granules are distributed seems to pre-indicate structurally the embryonic region in the mature egg.

Discussion

The periplasm of the insect egg has been considered to play an important role for formation of the basic body pattern of the embryo and the fate map is described in several insect species (*e. g. Drosophila*, Poulson, 1950; *Tenebrio*, Anderson, 1972; Sander, 1976). However, relation between the egg architecture and the basic body pattern has not been clarified, except for the posterior polar plasm and the anterior polar plasm. Especially, as to the function of the posterior polar plasm for formation of the pole cells, many investigations have been carried out from the various points of view, and Okada (1986) reviewed recently many results on cytoplasmic function of the posterior polar plasm for segregation of germ line in *Drosophila*. On the other hand, as to the anterior polar plasm, relation between its architecture and function is not analyzed enough. Zissler and Sander (1973, 1982) reported in *Smittia* egg that the periplasm protrudes inward as a cone-shaped plug at the anterior pole, and Ripley and Kalthoff (1983) obtained from the temporal change of the ratio of the double cephalons, induced by UV-irradiation, the following results; the cytoplasmic ribonucleoprotein particles existing in a yolk-free cytoplasmic cone observed behind the anterior pole of the newly laid egg, diffused posteriorly with the progress of development, and then migrated into the periplasm of the anterior part of the egg.

In *Bombyx* there is no specialized structure within the periplasm except the anter-

ior polar plasm, but it has been considered that the periplasm seemed to play an important role during the early embryogenesis, the same as in other insect. Takami (1942, 1946) analyzed the role of the periplasm by cauterization and suggested the existence of the regional difference within the periplasm, Miya (1958) obtained the similar possibility as to the germ cell differentiation by the same procedure, and Kobayashi (1980, 1981) suggested the significance of the periplasm for the early embryogenesis by UV-irradiation.

In the anterior polar plasm of *Bombyx* egg, arrangement of the various organelles, especially rER, is different from that in the other region of the periplasm (Miya, 1978, 1984, 1985), but this specialized structure seems to relate to reception of sperms, and the evidence concerning to determination of the longitudinal body pattern as in *Smittia* was not obtained yet.

The pyronin-positive large granules, a characteristic structure seen only in the mature egg of *Bombyx*, are distributed widely, attached to the periplasm and dispersed with activation of the egg by sperm entry (Suzuki, 1969). These granules constitute large concentric whorls or rER electron-microscopically, and the stacks of cisternae change to the tubular and/or vesicular structure with entry of sperms. From the present results, these structures do not distribute evenly with the egg cortex, but seem to be absent in the presumptive extraembryonic region lying at the anterior, posterior and
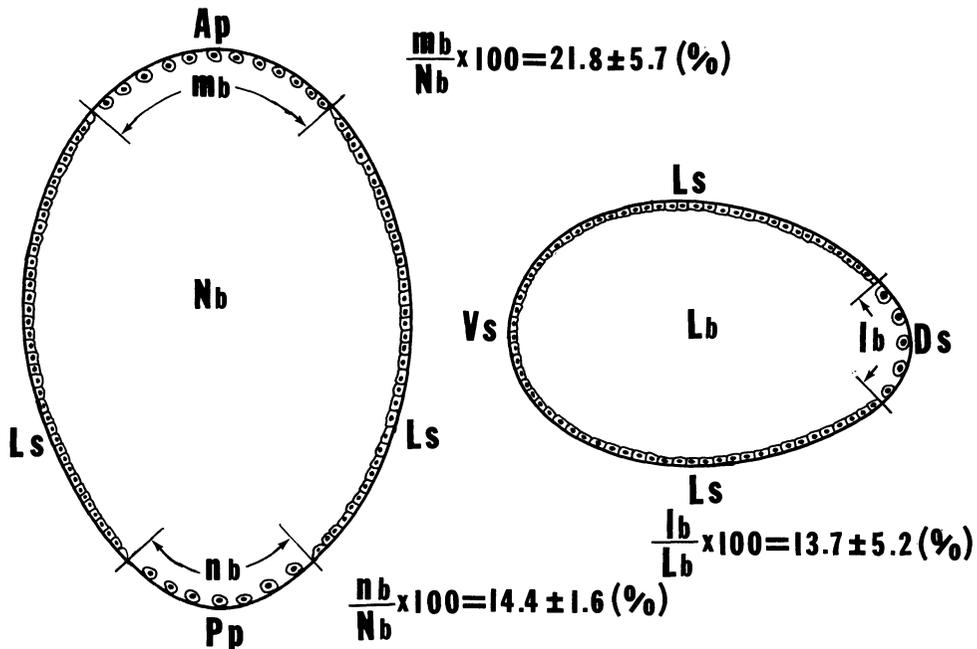
Fig. 21. Diagrammatic figures of the frontal (A) and cross (B) sections representing the embryonic and extraembryonic regions. Ap: anterior pole, Pp: posterior pole, Ds: dorsal side, Ls: lateral side, Vs: ventral side of the egg, Nb, Lb: length of the entire circuit, mb, nb, lb: extent of the extraembryonic region at the anterior, posterior and lateral sides of the egg, respectively.

dorsal side of the egg. Furthermore, the distribution pattern of these granules coincides with that of the germ anlage and the presumptive embryonic envelope at the early germ band stage. Though future investigation will be necessary, the unequal distribution of rER is one of the regional differences of the periplasm and this architecture might play an important role for formation of the basic body pattern of the embryo, through qualitative and/or quantitative differences of protein synthesis.

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