Blastoderm formation in the silkworm egg
(Bombyx mori L.)

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SUMMARY

Embryogenesis in the egg of the silkworm, Bombyx mori, up to 24 h after oviposition was studied by light microscopy with special reference to nuclear migration and blastoderm formation. In Bombyx eggs blastoderm cells seem to form in a mechanism different from that usually seen in many other insect species; that is, in Bombyx eggs no typical cleavage furrows were seen. Cleavage nuclei which had migrated, accompanied by the associated cytoplasm, to the egg surface pushed up the plasma membrane and protruded beyond the initial level of the periplasm. The periplasm fused with their associated cytoplasm was partitioned among and pulled around the nuclei. Then each nucleus was separated by a laterally-invading limiting membrane from the yolk-granules-occupied region to yield a blastoderm cell.

INTRODUCTION

There have been few morphological studies on early developmental stages up to 24 h after oviposition, i.e. until the stage of germ-band formation, in the egg of the silkworm, Bombyx mori. In a previous paper (Takesue, Keino & Endo, 1976), we have reported that in Bombyx eggs blastoderm and yolk cells begin to form about 6 and 24 h, respectively, after oviposition at 27 °C.

Anderson (1962) has stated that the mechanism of blastoderm formation is essentially similar among eggs of different insect species. A typical and well-studied example can be found in Drosophila eggs, where the blastoderm is formed by cleavage furrows growing inward from the egg surface between nuclei located in a row in the periplasm (Huettner, 1923; Mahowald, 1963; Fullilove & Jacobson, 1971; Sanders, 1975). Iwasaki (1931) reported that the blastoderm is formed in Bombyx in the same manner as in Drosophila. In studying the early developmental stages of Bombyx eggs, however, we have...
made observations which suggest that blastoderm cells in Bombyx eggs might be formed in a mechanism different from that proposed by Iwasaki (1931).

In the present work we have paid special attention to the movement of cleavage nuclei leading to blastoderm formation. A preliminary note of these results has been published previously (Takesue, Onitake & Keino, 1977).

MATERIALS AND METHODS

Non-diapause eggs from the cross between Daizo and Japanese 106 bivoltine races of the silkworm, Bombyx mori, were used as the material. The eggs were obtained from female moths which had hatched from eggs incubated at 15 °C in the dark. Moths were allowed to lay eggs for 30 min at 25 °C and the eggs were incubated at 25 °C, not at 27 °C used in the previous work (Takesue et al. 1976), in order to slow down the rate of development of the eggs.

As described previously (Takesue et al. 1976), the eggs were deprived of the chorion and vitelline membrane with fine forceps in 2-5 % glutaraldehyde–0-1 M potassium phosphate buffer (pH 7-5) and fixed in the same buffer for 2 h, followed by post-fixation with 2 % osmium tetroxide for 1-5 h. After block staining with uranyl acetate and dehydration in an ascending ethanol series and propylene oxide, they were embedded in Epon 812. Sections about 0.5 μm thick were cut with a glass knife on an ultramicrotome and stained with 1 % toluidine blue in 1 % borax for examination in the light microscope.

In preliminary experiments, the eggs were punctured with a sharp needle at one site on each of the dorsal and ventral sides and at the anterior and posterior poles (four sites in all) and fixed by immersion in 2-5 % glutaraldehyde–0-1 M potassium phosphate buffer (pH 7-5) for 20 h, followed by post-fixation with 0-5 % osmium tetroxide for 18 h. After fixation, the chorion was removed and the eggs were block-stained with uranyl acetate, followed by the procedures as described above. Essentially the same morphological features were observed in the eggs fixed by the two procedures. Since the latter consumed much more time than the former, we have adopted the former fixation procedure in this work.

RESULTS

In the egg less than 30 min after oviposition (Fig. 1A), the whole surface of the egg was covered with microprojections and two kinds of yolk granules were seen: some (yg1) were intensely stained and located in the inner region of the egg, and the others (yg2), located in the peripheral region beneath the periplasm, were weakly stained, but had small densely-stained dots inside them (Takesue et al. 1976). In the periplasm there were a lot of small dots similar in density and size to those in yg2. In the egg 8 h after oviposition (8 h egg) (Fig. 1B), the microprojections became abundant and thick, implying intensive
Blastoderm formation in the silkworm egg

Fig. 1. Light micrographs of the eggs 0 h (A) and 8 h (B) after oviposition. The whole surface of the eggs was covered with microprojections increasing in number and height with time. Cleavage nuclei accompanied by the associated cytoplasm had already appeared in the 8 h egg (B). yg₁, yolk granule in the inner region of the egg; yg₂, yolk granule in the peripheral region; lp, lipid particle; ppl, periplasm; mp, microparticle; cn, cleavage nucleus; ac, associated cytoplasm.

synthesis of the plasma membrane during this period. Many cleavage nuclei, surrounded by the associated cytoplasm, were seen among yg₁, not yg₂, near the anterior pole. Up to 9 h after oviposition, these cleavage nuclei with the associated cytoplasm had migrated into the peripheral region of the egg, though this nuclear invasion of the egg surface was not simultaneous over the entire periphery (Toyama, 1909).

The blastoderm formed 9–10 h after oviposition at 25 °C, though development of cleavage nuclei into blastoderm cells was not simultaneous over the entire periphery, consistent with the non-simultaneous nuclear invasion of the egg periphery described above. Figure 2 shows the process of blastoderm formation occurring in the eggs 9–10 h after oviposition. When a cleavage nucleus arrived near the periphery of the egg, the egg surface was raised into a hillock, still covered with abundant microprojections, over the nucleus and the periplasm fused with its associated cytoplasm (Fig. 2A and B). As cleavage nuclei continued to further migrate toward the surface and protruded beyond the initial level of the egg surface, the periplasm which had been previously
Fig. 2. Light micrographs showing the process of blastoderm formation in the eggs 9–10 h after oviposition. See the text for the details. \( yg_{1} \) and \( yg_{2} \), yolk granules; \( yg_{d} \) and arrows, small densely-stained dot; \( cn \), cleavage nucleus; \( ac \), associated cytoplasm; \( ppl \), periplasm; \( mp \), microprojection; \( bcle \), blastoderm cell; \( ysm \), yolk-sac membrane.
located above the yg2-occupied area was partitioned among and gathered around the cleavage nuclei, as indicated by the presence of small densely-stained dots scattered around the nuclei. Then each nucleus was separated by a laterally-invading membrane from the yg2-occupied area to give a blastoderm cell (Fig. 2C and D). The processes of syncytial blastoderm and typical cleavage furrows as seen in many insect species could not be observed in Bombyx eggs.

Newly-formed blastoderm cells divided tangentially on the egg surface, their microprojections becoming broader and shorter (Fig. 2D). In fact, these structures were not finger-like but ruffle-like (Keino & Takesue, in preparation). This change of microprojections gave an impression of their merging with each other and becoming incorporated into the plasma membrane of newly-formed cells. At this stage the number of blastoderm cells was not enough to completely cover the whole surface of the egg, so that the yg2-occupied area became situated outermost at the surface between the blastoderm cells, though there was sporadically residual periplasm there (Fig. 2D).

The morphology of the just-completed blastoderm cell was very different above and under its nucleus (Fig. 2D). Above the nucleus the cytoplasm was dense, full of a great number of mitochondria and poor in vacuoles, and microprojections on the surface were few and small. On the other hand, under
the nucleus there were small densely-stained dots, a lot of vacuoles of different sizes and, sometimes, some yg₂.

Figure 3 shows light micrographs of a 12 h egg. The whole surface of the egg became covered with completed blastoderm cells and, interestingly, the weakly-stained peripheral layer which yg₂ occupied remained almost the same in width at least up to this stage (Fig. 3A). Microprojections became very few and small on the apical surface of the cells, but they developed well on the free surface opposing neighbouring cells. Under the nucleus more complicated networks were seen connected with the yolk-sac membrane.

DISCUSSION

In the present work the chorion and vitelline membrane were removed from eggs immersed in the fixation solution to have the eggs fixed as fast as possible. After post-fixation with osmium tetroxide, the eggs were embedded in Epon 812. These procedures made it possible to obtain much clearer light micrographs than those obtained by previous investigators (Toyama, 1909; Iwasaki, 1931; Miura, 1932) using paraffin embedding and yielded some interesting observations on the migration of cleavage nuclei and blastoderm formation in Bombyx eggs.
Anderson (1962) has stated that the mode of blastoderm formation is essentially similar among different insect species, in contrast to the high variety of the number of synchronous nuclear divisions taking place during cleavage and of the timing of nuclear invasion of the egg surface. In fact, Iwasaki (1931) reported that blastoderm formation in *Bombyx mori* is similar to that in *Drosophila*; that is, the development of cleavage nuclei into blastoderm cells is performed by cleavage furrows growing inward from the egg surface between the nuclei located in a row in the periplasm. However, the results obtained in the present work are clearly different from those reported for *Drosophila* eggs, as follows.

First, in *Drosophila* the periplasm is initially very thin but it becomes gradually thicker and thicker, with concomitant segregation of typical cytoplasmic organelles to the periphery of the egg and of the yolky constituents to the central region, during the time cleavage nuclei migrated toward the egg surface (Turner & Mahowald, 1976). No such thickening of the periplasm or yg2-occupied area was observed during the period of migration of cleavage nuclei in *Bombyx mori*. Second, no typical syncytial blastoderm was formed. Third, no typical cleavage furrows could be observed when cleavage nuclei were transformed into blastoderm cells. In *Bombyx* eggs the plasma membrane outside the periplasm does not seem to invaginate in the periplasm or the yg2-occupied area. Instead, the cleavage nuclei with the associated cytoplasm push up the plasma membrane and migrated beyond the initial level of the thin periplasm. The periplasm fused with their associated cytoplasm is partitioned among and pulled around the nuclei. The plasma membrane limiting the periplasm enters laterally between the nuclei and the yg2-occupied area, so that each nucleus becomes isolated in its own cytoplasm to give a blastoderm cell. This presumed process of blastoderm cell formation in *Bombyx* eggs is schematically depicted in Fig. 4. There is a possibility that the protrusion of blastoderm cells might be an artefact due to the mechanical removal of the chorion and vitelline membrane during fixation of eggs. This can be ruled out, however, by the finding that the same phenomena are seen in eggs which have been fixed before removal of the chorion and vitelline membrane as mentioned earlier. Furthermore, no apparent changes in width of the weakly-stainable peripheral layer up to the stage of blastoderm formation may be consistent with the thought that there occur no typical longitudinally-invading furrows in *Bombyx* eggs. Thus the mechanism of blastoderm formation we have proposed above for *Bombyx* eggs is inconsistent with the conclusion of Iwasaki (1931). The reasons for this discrepancy are unknown at present. His (printed) light micrograph of a cleavage nucleus developing into a blastoderm cell is not clear enough to support his conclusion and he reinforced his conclusion by an analogy between blastoderm and yolk-cell formation.
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REFERENCES


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