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Blastoderm Formation in the Silkworm, *Bombyx mori* (Lepidoptera, Bombycidae)

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Synopsis

The mechanism of blastoderm formation in the silkworm, *Bombyx mori*, has been investigated not only by light and electron microscopy, but also by immunofluorescence microscopy.

In *B. mori* eggs, blastoderm cells are formed in a mechanism different from that usually seen in many other insect species; that is, the formation of a syncytial blastoderm and the typical cleavage furrows cannot be observed. In the egg of *B. mori*, when a cleavage nucleus arrives near the periphery of the egg, the egg surface is raised into a hillock over the nucleus. Cleavage nuclei continue to migrate further toward the surface and protrude beyond the initial level of the egg surface. The periplasm fused with their associated cytoplasm is partitioned. Each nucleus is separated by a laterally-invading limiting membrane from the yolk granule-occupied layer to yield a blastoderm cell.

It has been believed that the mode of blastoderm formation is essentially similar among many insect species (Anderson, 1962). A typical and well-studied example is found in *Drosophila*, where, after the syncytial blastoderm is formed, cleavage furrows grow inward between from the egg surface to finally yield blastderm cells (Huettner, 1923; Ede & Counce, 1956; Mahowald, 1963; Fullilove & Jacobson, 1971; Sanders, 1975; Turner & Mahowald, 1976). Iwasaki (1931) reported that in *Bombyx* also blastoderm cells are formed in the same manner as in *Drosophila*. Takesue *et al.* (1980) have found, however, that blastoderm cells are formed in a different way in *Bombyx* in this species neither typical syncytial blastoderm nor cleavage furrows are formed. Recently, a similar mode of blastoderm formation has been reported in *Callosobruchus* (Miyamoto & Van der Meer, 1982).

In this paper I will summarize our studies on the morphological aspect of blastoderm formation in *Bombyx* egg and discuss the relevant structural elements. The results on *Bombyx* eggs have been published (Keino & Takesue, 1982; Takesue *et al.*, 1976, 1980, 1982, 1983, 1984). The morphological features observed during blastoderm formation in

Bombyx are illustrated in Fig. 1.

Eggs up to 9 hr after oviposition In the egg just after oviposition are seen three kinds of yolk granules: yg_1, located in the inner region of the egg and intensely stained with toluidine blue; yg_2, located in the narrow peripheral region beneath the periplasm; and *ygd*, small intensely-stained dot, located in the very thin periplasm. A similar dot (yg_2-*d*) is also seen in yg_2. Fluorescence microscopy using anti-vitellin showed that yg_1, *ygd* and yg_2-*d* are very rich in vitellin but yg_2 has a negligible, if any, amount of the protein. The whole surface of the egg is covered with fine finger-like microprojections (microvilli).

When the egg is incubated at 29°C, there are seen no significant morphological changes in the interior of the egg, except for migration of cleavage nuclei, until 6 hr. On the other hand, the egg surface changes considerably. In scanning electron microscopy the surface of the 4-hr egg appears covered with numerous microvilli of 3 μm long and 1.3 μm in diameter. In the 6-hr egg the microvilli have almost completely been replaced by thick and abundant ruffle-like microprojections, implying intensive synthesis of the plasma membrane during this period.

In the 8.5-hr egg many cleavage nuclei, surrounded by a little of the cytoplasm, have already migrated near the peripheral yg_2-occupied region but still remained in the yolk-rich yg_1-occupied region. In *Drosophila* the initially thin periplasm becomes gradually thicker and thicker during the period of migration of cleavage nuclei, but no such thickening of the periplasm or the yg_2-occupied region occurs in *Bombyx*.

Up to 9 hr, cleavage nuclei with the associated cytoplasm have migrated into the yg_2-occupied region at the anterior part. This nuclear invasion of the egg periphery is later at the posterior part. Since blastoderm formation proceeds in the same manner in the two regions irrespective of this difference in timing, I will focus on the processes occurring at the anterior part of the egg.

Blastoderm cell formation Blastoderm cells are formed 9-10 hr after oviposition at the anterior part. When a cleavage nucleus arrives near the periplasm, the egg surface is raised into a hillock over the nucleus and the periplasm begins to fuse with its associated cytoplasm. Cleavage nuclei continue to further migrate toward the surface. When they protrude beyond the initial level of the egg surface, the periplasm that has already fused with the nuclear associated cytoplasm is partitioned among and gathered around the nuclei. Then each nucleus is separated by a membrane, which invades laterally under the nucleus, from the yg_2-occupied region to give a blastoderm cell. Neither syncytial blastoderm nor typical cleavage furrow formation as seen in many insect species occurs in the *Bombyx* egg. The apical portion of the surface of a newly-formed blastoderm cell is covered with ruffles, but many interdigitating microvilli are seen in the groove region between neighboring blastoderm cells. The apical cytoplasm is abundunt in mitochondria but poor in vacuoles, while in the cytoplasm under the nucleus there are *ygd*s, many vacuoles of different sizes and, sometimes, some yg_2s.

At this stage the number of blastoderm cells is not enough to completely cover the whole surface of the egg, so that the yg_2-occupied region becomes situated outermost at the surface between blastoderm cells, though there is sporadically residual periplasm there. Newly formed blastoderm cells divide tangentially on the egg surface, which becomes completely covered with blastoderm cells by 12 hr after oviposition. Ruffles on the apical portion of

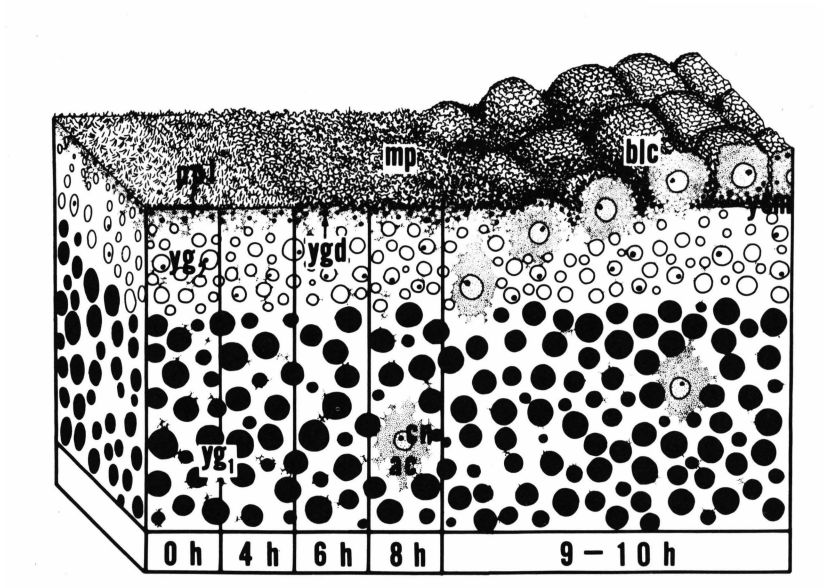

Fig. 1. Summary of morphological features observed during blastoderm formation in the *Bombyx* egg.
ac associated cytoplasm, blc blastoderm cell, cn cleavage nucleus, mp microprojection, ppl periplasm, yg_1 and yg_2 inner yolk granules, ygd small yolk granules, ysm yolk-sac membrane.

blastoderm cells become flattened, possibly owing to their proximity to the vitellin membrane.

Interestingly, the yg_2-occupied region remains almost the same in width at least up to this stage. Vitellin has been thought to serve as a main source of nutrients for the embryonic development. As described above, however, blastoderm cells develop on the periphery that is separated from the extremely vitellin-rich yg_1-occupied interior region by the very vitellin-poor yg_2-occupied region, suggesting an important role of yg_2s in early embryogenesis (Takesue *et al.*, 1983).

Possible involvement of cytoskeletons in blastoderm formation Blastoderm formation involves migration of cleavage nuclei and remarkable changes of the egg surface. These processes are possibly controlled by cytoskeletal systems, because cellular movement is generally believed to be regulated by these systems. The results as described below support this possibility.

In the associated cytoplasm of a cleavage nucleus which has migrated near the periplasm are found many long microtubules extending to the periphery of the associated cytoplasm from the perinuclear region, where the microtubules appear associated with the nuclear membrane. In a cleavage nucleus which is about to protrude from the egg surface the microtubules radially extend from each of the centrioles located above and under the nucleus. In a newly-formed blastoderm cell the microtubules are found only under the nucleus. A similar distribution of microtubules has been reported in the gall midge, *Wachtliella persicariae* (Wolf, 1980). No microtubules are seen outside the nuclear associated cytoplasm on electron microscopic sections. In indirect immunofluorescence microscopy, however, antibodies against tubulin stain the perivitellin space between the egg surface and chorion in addition to nuclear associated cytoplasm.

No microfilaments are also detected even in the microprojections covering the egg surface on electron microscopic sections. In indirect immunofluorescence microscopy antibodies against actin stain the perivitellin space, the periplasm, yg_2s and the gap regions among yg_1s, but not cleavage nuclei in the 8.5-hr egg, indicating that actin exists in the internal and peripheral regions of the egg.

When the 8.5-hr egg is exposed to colchicine for 3 hr, nuclear division arrests and cleavage nuclei remain in the yg_1-occupied region as in the 8.5-hr egg, but the microprojections on the egg surface become wider and larger as in the normally developing egg. After 7-hr exposure, many blastoderm-cell-like but nucleus-lacking structures are found on the egg surface. These results suggest that microtubules are involved in migration of cleavage nuclei but not in changes of the microprojections during blastoderm formation and, furthermore, that the changes of the egg surface is independent of the nuclear migration.

Cytochalasin B inhibits the morphological change of the microprojections and decreases the migration rate of cleavage nuclei. When the 8.5-hr egg is exposed to the drug for 5 hr, cleavage nuclei have arrived at the egg surface but the microprojections have disappeared. The same results have been obtained with cytochalasin D. These results suggest that the morphological changes of the egg surface during blastoderm formation are controlled by microfilaments. The microfilaments are also probably involved in nuclear migration at least near the egg surface.

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