Formation of the egg membranes of an idolothripine thrips, *Bactrothrips brevitubus* (Insecta: Thysanoptera)

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Abstract

Formation of the egg membranes of an idolothripine thrips, *Bactrothrips brevitubus* Takahashi was examined and described using an electron microscope. The vitelline membrane was a product of the follicular cells, as in many other insects. The formation of the vitelline membrane was completed by the time the yolk deposition commenced. The chorion was also derived from follicular cells and was composed of three-layered exochorion and unilayered endochorion.

Introduction

The insect egg or mature oocyte has two coverings that can be clearly distinguished, the vitelline membrane and the chorion or egg shell. Among them, the formation of vitelline membrane has long been attributed to the oocyte (Wigglesworth, 1972), but recent findings, on synthetic activities of the follicular cells and on the ultrastructure of vitelline membrane formation show that the vitelline membrane in most cases is a product of the follicular cells (cf. Schwalm, 1988).

In the Thysanoptera, the formation of egg membranes, especially on the origin of the vitelline membrane has not been reported in detail [Haga (1985) for chorion; Ananthakrishnan (1988) for vitelline membrane].

Herein, I describe the formation of the egg membranes of an idolothripine thrips, *Bactrothrips brevitubus,* with special reference to the origin of the vitelline membrane.

Materials and Methods

Adult females of *Bactrothrips brevitubus* were collected at Mt. Tsukuba, Ibaraki Prefecture, Japan. They were anaesthetized by submerging in 70% ethyl alcohol for a short time (10−20 sec), and the ovaries (oocytes and eggs) were dissected out of their bodies in Karnovsky's fixative. The ovaries were then post-fixed with 1% OsO_4. Fixed eggs and pieces of the fixed ovarioles were embedded in a water-miscible epoxy resin Quetol 651 (Nisshin EM), and made into ultrathin sections, which were stained with uranyl acetate and lead citrate, and then observed under a transmission electron microscope JEOL JEM 100CX−II at 80kV.

Results

Vitelline membrane formation

In the intercellular space between the early vitellogenic stage oocyte and follicular cells, well-developed interdigitation of oocyte and follicular microvilli was found (Fig. 1A). First, in the endoplasmic reticulum (ER) at the cortical cytoplasm of follicular cells, electron dense material was accumulated (Fig. 1B). Then, the material with the similar electron density to that accumulated in the ER of follicular cells came to be deposited in the intercellular space between the oocyte and follicular cells (Fig. 1C), and the vitelline membrane formed there.

On the deposition of electron dense material, the follicular microvilli disappeared, and the number of microvilli of the oocyte was reduced. The remaining microvilli of the oocyte may serve as molds of the vitelline membrane pores (0.05−0.08 μm in diameter) (Fig. 1D). These microvilli remained in the vitelline membrane pores until the chorion formed.

After its completion, the vitelline membrane became higher in electron density. The time of completion of

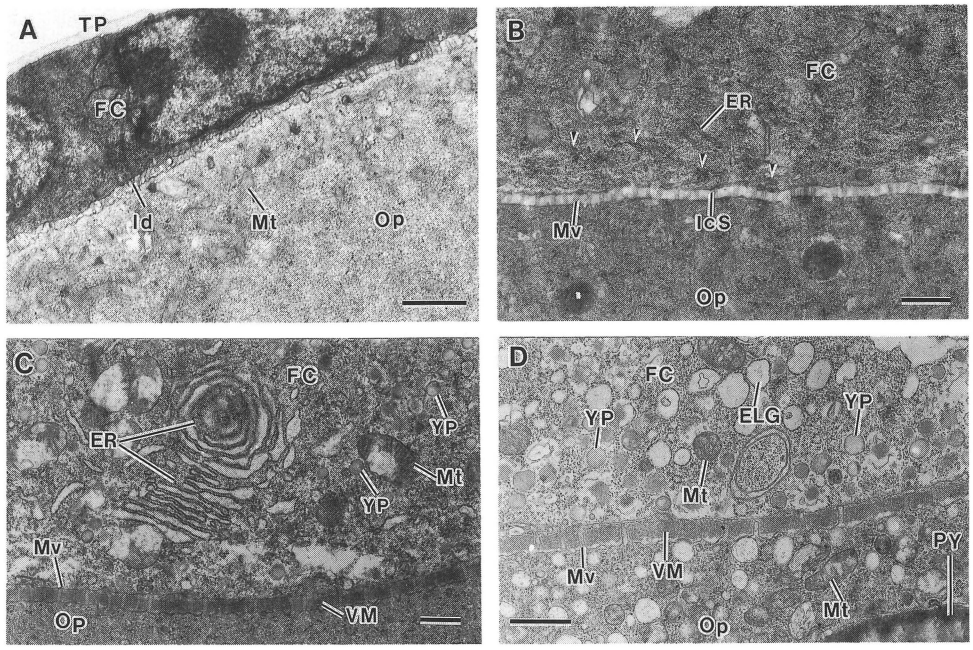

Fig. 1 Successive stages of vitelline membrane formation in *Bactrothrips brevitubus* (A–D). Arrowheads show the deposition of electron dense material in the endoplasmic reticulum (ER) at the cortical cytoplasm of follicular cells (FC). ELG: electron lucent granule, IcS: intercellular space, Id: interdigitation of microvilli of the oocyte and follicular cells, Mt: mitochondria, Mv: microvilli, Op: ooplasm, PY: proteid yolk, TP: tunica propria, VM: vitelline membrane, YP: yolk precursor. Scales = 1 μm.

vitelline membrane varied with the individual egg chamber, but it was approximately coincident with the commencement of yolk formation. Completed vitelline membrane was *ca.* 0.2 μm in thickness.

Chorion formation

The follicular cells, surrounding the oocyte in the late vitellogenic stage, started to discharge some electron dense granules (Fig. 2A), to deposit the chorion on the vitelline membrane. The chorion was composed of a unilayered electron lucent endochorion and three-layered exochorion with a little lower electron density than the vitelline membrane. The outermost, intermediate and innermost layers of exochorion measured *ca.* 0.5, 0.1−1.5 and 0.2 μm in thickness, respectively (Fig. 2B). The intermediate layer at first assumed a complex reticulated structure (Fig. 2C), and later the structure was reformed into one with microcanals of various sizes transversely running (Fig. 2D). The layers of exochorion exhibited similar electron density each other. The endochorion was between the exochorion and vitelline membrane, and it was amorphous in structure (Fig. 2D).

In the completed chorion, the exo- and endochorion were clearly defined with an electron dense boundary (Fig. 2D). A substance with a similar electron density to the boundary was observed to be deposited inside the exochorion as a few lines apart from the boundary by *ca.* 0.1 μm (Fig. 2D). After the completion of chorion, the follicular layer degenerated.

Discussion

Vitelline membrane

Although the vitelline membrane has been generally designated as a primary egg membrane

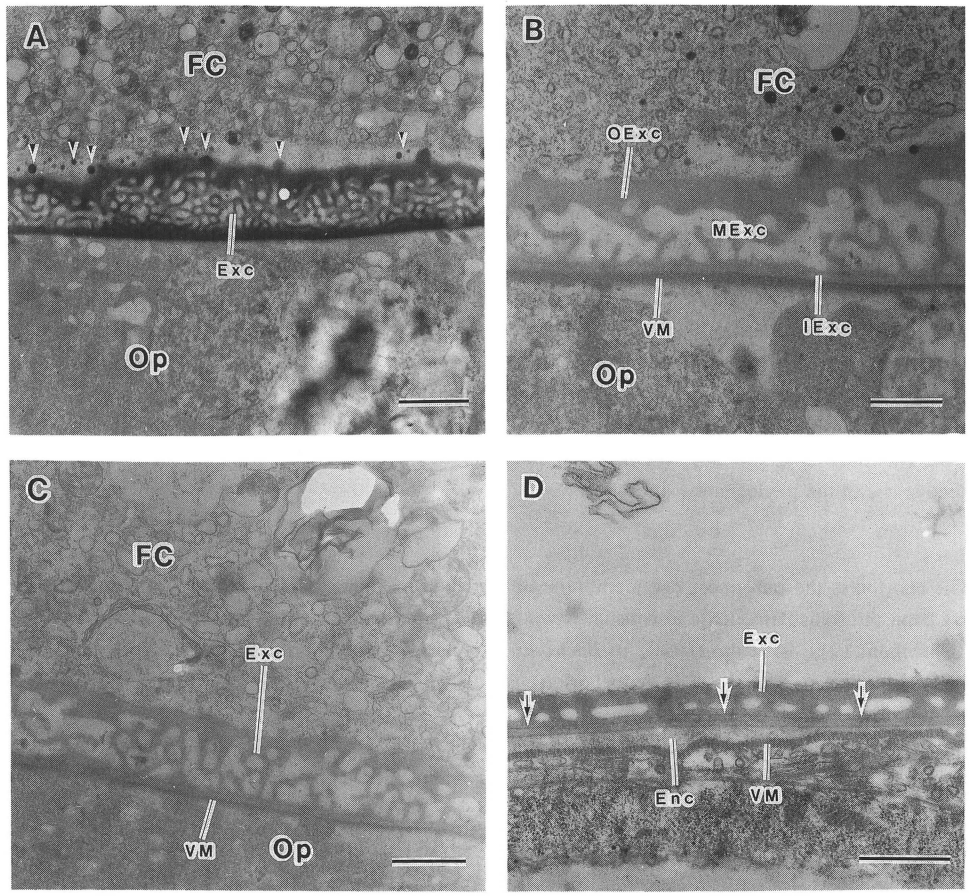

Fig. 2 Successive stages of chorion formation in *Bactrothrips brevitubus* (A–D). Arrows show the horizontal electron dense layers found in exochorion. Arrowheads show the deposition of chorion precursor. Enc: endochorion, Exc: exochorion, FC: follicular cell, IExc: innermost layer of exochorion, MExc: intermediate layer of exochorion, Op: ooplasm, OExc: outermost layer of exochorion, VM: vitelline membrane. Scales = A, 2 μm; B–D, 1 μm.

(Wigglesworth, 1972), now it is well known that the egg membrane called vitelline membrane in insects is exclusively derived from follicular cells, or it is a secondary egg membrane (cf. Matsuzaki, 1971; Schwalm, 1988), the only one exception being the silkworm *Bombyx* in which the vitelline membrane exclusively originates from the oocyte (Matsuzaki, 1968).

In *Bactrothrips brevitubus,* the material with electron density similar to that of the vitelline membrane later deposited is accumulated in the endoplasmic reticulum (ER) condensed at the peripheral area of follicular cells, prior to the formation of the vitelline membrane (cf. Fig. 1B). This strongly suggests that follicular cells play a principal role in the vitelline membrane formation. Similar precursor accumulation of the vitelline membrane in the ER of follicular cells has been reported for dragonflies *Aeschna* (Beam and Kessel, 1969) and *Sympetrum* (Matsuzaki, 1971). The follicular cell origin of the vitelline membrane is predominantly found in the insects, *e.g.,* other than the above-mentioned species, the silverfish *Lepisma* (Cone and Scalzi, 1967), cricket *Gryllus* (Matsuzaki, 1966, 1971), leafhopper *Ulopa* (Hamon, 1972), caddisfly *Parastenopsyche* (Matsuzaki, 1972), cecropia moth *Hyalophora* (King and Aggarwal, 1965), wasp *Nasonia* (Richards, 1968),

mosquito *Anopheles* (King, 1964) and the fruitfly *Drosophila* (Quattropani and Anderson, 1959; King and Koch, 1963). In a honeybee *Apis*, the vitelline membrane is deposited cooperatively by the follicular cells and oocyte (Hopkins and King, 1966).

In *B. brevitubus* the vitelline membrane is completed approximately the same time of or slightly before the commencement of yolk deposition, whereas in *Arrhenothrips ramakrishnae* its completion is at the last phase of yolk deposition (Ananthakrishnan, 1988). The vitelline membrane deposition in earlier stages as in *B. brevitubus* is known for *Aeschna* (Beam and Kessel, 1969), *Sympetrum* (Matsuzaki, 1971), *Parastenopsyche* (Matsuzaki, 1972), a lacewing *Chrysopa* (Matsuzaki, 1978) and *Drosophila* (King and Koch, 1963), and that in later stages as in *A. ramakrishnae* is known for *Gryllus* (Matsuzaki, 1971), a web spinner *Oligotoma* (Niwa and Matsuzaki, 1994), *Hyalophora* (King and Aggarwal, 1965) and *Nasonia* (Richards, 1968).

As for the Paraneoptera other than the Thysanoptera, the follicular cell origin of vitelline membrane is known for hemipteran *Ulopa* (Hamon, 1972) and *Bothrogonia* (Matsuzaki, 1975). Biliński and Jankowska (1987) reported that the chorion deposited in direct contact to the oocyte in another paraneopteran member, the Mallophaga (*Eomenacanthus*). This suggests that the vitelline membrane is not formed in this species. Thus, the vitelline membrane may vary in origin and formation even among the related taxa, and it may be not an effective cue in the phylogenetic discussion.

Chorion

The chorion is the outermost egg membrane or egg shell in animals. The chorion in insects is exclusively derived from the follicular cells, and is usually two-layered, composed of outer exochorion and inner endochorion (Chapman, 1969; Schwalm, 1988). In *Bactrothrips brevitubus*, the chorion is likewise derived from follicular cells and is composed of exo- and endochorion.

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