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Embryonic and Early Post-embryonic Development of the Parasitic Wasp, *Trichogramma chilonis* (Hymenoptera, Trichogrammatidae)

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Synopsis

The embryonic development, from the germ layer formation to the organogenesis, and the early post-embryonic development of *Trichogramma chilonis* were described. The central mass which occupies the core of the embryo at the blastoderm stage was seen to stream out from the embryonic dorsum, and then became flattened between the surface of the embryo and vitelline membrane. It leaves from the embryo at hatching and eventually degenerated in the yolk of the host egg. A longitudinal groove appeared on the ventral surface of embryo, and cells at the surface of embryo sank and increased in number. They differentiated into the mesodermal and endodermal cells, but most of them degenerated the next stage of the development. The stomodaeum was formed an invagination of the ectoderm, and the mid-gut epithelium originated from the endodermal cells which lie near the center of embryo and the proctodaeum was formed by the growing of the depression which appeared at the posterior end of the embryo. The primordial germ cells just appeared were located outside the blastoderm, and then they moved ventrally and sank inward in the posterior pole of the embryo. The nervous system originated from the ectodermal cells derived from the ventral part of the embryo, but neither ventral ganglion nor ventral nerve cord was formed.

Introduction

In the previous paper, the author described the state of the host eggs, the number of parasites within a single host egg, and the duration of embryonic and post-embryonic development, also the egg structure about a half hour after oviposition and the early embryonic stage; from fertilization to the completion of blastoderm, of the parasitic wasp, *Trichogramma chilonis* Ishii.

So far as the author is aware, no paper concerning the organogenesis of *Trichogramma* has been published except those by Silvestri (1908) and Gatenby (1917).

In the present paper, the author intends to describe the embryonic development, especially the germ layer formation and the organogenesis, and the early post-embryonic stage of *T. chilonis.*

Materials and Methods

The materials and methods used in the present study are the same as described in the previous paper (Tanaka, M., 1985).

Results

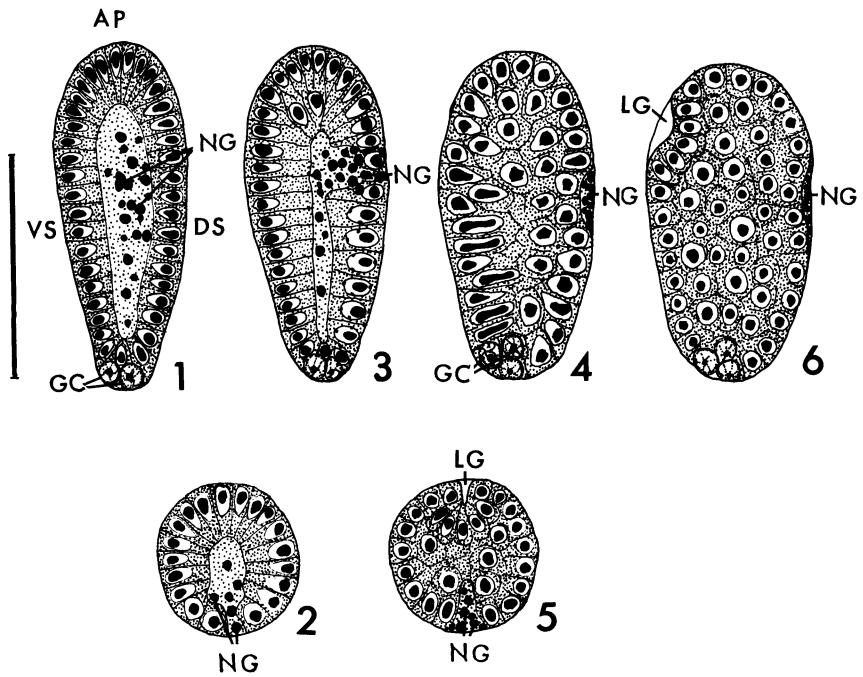
1. Germ layer formation

At about 10 hr after oviposition the eggs of *T. chilonis* are elongated oval in shape, about 80 μm in length and 35 μm in width. At this time the eggs are at the blastoderm stage (Fig. 1). Size of the blastoderm cells is different from place to place, that is, the blastoderm cells having a spherical large nucleus distributing in the dorsal and posterior regions of the egg are larger than those in other regions. Each of these nuclei has a single large chromatin mass which lies closer to the egg surface. With the lapse of time the height of blastoderm cells increases, and at the same time, the eggs become slightly shorter but remarkably broader. At this time the number of nuclei is about 20 in the longitudinal section through the dorsal part of the blastoderm (dorsal blastoderm), about 22 through the ventral part (ventral blastoderm) and about 23 in a cross section through the major axis of blastoderm. Fourth to fifty nucleolar granules are seen scattered in the core of the egg surrounded by the blastoderm, being termed as central mass hereafter, and some of them lie closer to the depresssion occurred in the dorsal blastoderm (Fig. 3).

The primordial germ cells, spherical in shape and about 7 μm in diameter, are located outside of the blastoderm in the posterior end of the egg (Figs. 1, 3) and clearly differ from another cell in their position, size and affinity for stains.

As the axial part becomes narrow by ingrowing of the blastoderm cells, the central mass containing the nucleolar granules gradually streams out to the dorsal periphery with some blastoderm cells. The mass expands and breaks through the dorsal blastoderm at the position between one-third and two-thirds from the anterior end of the egg (Fig. 3). This out-streaming of central mass the arrangement of larger blastoderm cells found in the dorsal part of the blastoderm is disturbed.

At this stage the cells in the posterior half of the dorsal blastoderm become larger and larger. The primordial germ cells change their position toward the ventral edge in the embryonic posterior pole and they stay there for a while. When the central mass has streamed out and lies in the space between the blastoderm cells, the core of the egg is occupied by the ingrowing and multiplying cells. After the expulsion of the central mass the enlargement of the chromatin mass in each nucleus takes place in parallel with that of nuclear volume, and the rapid multiplication of cells occurs. The elongated nuclei with long chromatin mass are found in the peripheral cell layer (Fig. 4). No mitotic division is observed during this process. Through the growth and multiplication of cells the embryo



Figs. 1 - 6. Longitudinal and cross sections through the major axis of egg, showing successive changes from the blastoderm stage to the germ layer formation.

1. Longitudinal section showing blastoderm stage.
2. Cross section showing blastoderm stage.
3. Longitudinal section showing out-streaming of central mass.
4. Longitudinal section showing germ layer formation.
5. Cross section showing longitudinal groove.
6. Longitudinal section at slightly later stage than that shown in Fig. 5.

AP anterior pole DS dorsal side, GC primordial germ cell, LG longitudinal groove, NG nucleolar granule, VS ventral side. Scale : 50 μm.

broadens to form an elongated oval body.

At the stage when the egg measures about 75 μm by 40 μm in major axes, the cells at the egg surface retains regularity of their arrangement, whereas the inner ones are disposed irregularly. The primordial germ cells located in the postero-ventral region of embryo are enclosed by the somatic cells. Soon after, on the ventral surface which is approximately one-third from the anterior end of embryo, a logitudinal depression or groove apperars (Figs. 5, 6). This groove is about 7 μm in length, 3 μm in width and 5 μm in depth. Each of oval cells arranged regularly around this groove has a large chromatin mass and is distinguishable from other cells in their form and distribution (Figs. 5, 6). This state has a striking resembrance to the inner layer formation in other insect embryos. These cells, soon after, gradually sink inward and increase in their number.

When the longitudinal groove on the ventral surface is formed, the extruded mass containing nucleolar granules lies between the dorsal peripheral cells (Fig. 4). Such a stagnation of the out-streaming does not last long, owing to the growth of embryo and the increase in somatic cell number. The extruded mass is pressed out on the surface of the peripheral cell layer and flattens between the embryo and the vitelline membrane. The space formed by the out-streming is soon closed up, and the gap once appeared in the peripheral cell layer is repaired.

At about 15 hr after oviposition the embryo is ovoid in shape, about 75 μm by 45 μm in major axes, composed of polygonal cells with in which single, large chromatin mass situates at their center, and several tiny granules on their inner surface. Most of the somatic cells arranged irregularly have almost the same shape and size. It is difficult to distinguish the cells destined to form the mid-gut epithelium *i. e.*, endodermal cells, from the mesodermal ones by their shape and size. Now the depression on the dorsal surface of embryo has disappeared, and neither any metameric segmentation nor a cavity is found in the embryo. The flattened extruded mass containing the nucleolar granules and some somatic cells covers the surface of embryo. By this time the primordial germ cells stained faintly and embraced by the somatic cells sink inward and are situated in the ventral edge of posterior pole of the embryo (Figs. 4, 6, 7).

The amnion and serosa are not found in any stages of the development.

2. Organogenesis

Alimentary canal

At about 15 hr after oviposition the stomodaeum appears as a depression of the ectoderm on the antero-ventral surface of embryo. Shortly after its appearance the stomodaeal invagination rapidly extends dorso-posteriorly, forming a long and narrow lumen (Fig. 8). And then the extremity of the stomodaeal invagination impinges on the anterior end of the mid-gut epithelium and connects with the mid-gut lumen at the region about one-third of embryo (Fig. 9). No stomodaeal valve is formed. In the first instar larva which is about 115 μm by 60 μm in major axes, the stomodaeum or the fore-gut is about 45 μm in length and its dorsal wall is thicker than the ventral one. Soon after the invagination of stomodaeum occurred a narrow lumen of the mid-gut appears in the center of the embryo, and it is situated slightly dorsally. This lumen is surrounded by about 9 cells in cross section and

about 11 cells in longitudinal one. With the development of mid-gut lumen the cells consisting of the mid-gut epithelium and the neighboring cells become larger than the others. On the other hand, a lumen of the proctodaeum begins to be formed in the posterior end of embryo and it develops rapidly (Fig. 9). At this time single-layered blind end of proctodaeum is closely in contact with that of the mid-gut epithelium. By the longitudinal section of this stage the proctodaeum is U-shaped, and it measures about 11 μm in width and 15 μm in depth. The author failed to determine whether the proctodaeum is formed with the same way as that observed in the case of stomodaeum formation or not.

Even when the body cavity has been formed, the demarcation between proctodaeum and mid-gut epithelium still remains intact (Fig. 13). The wall of the anterior half as well as the blind end of proctodaeum is thick and consists of the large cells having a striking resembrance to the mid-gut epithelial cells, while that of its posterior half or opening is extremely thin. This state persists until the further development of the first instar larva occurs.

After the formation of stomodaeum, mid-gut and proctodaeum the endodermal cells which do not take part in the formation of mid-gut epithelium and most of the mesodermal cells gradually degenerate, which results in appearance of the body cavity.

Extruded nucleolar granules

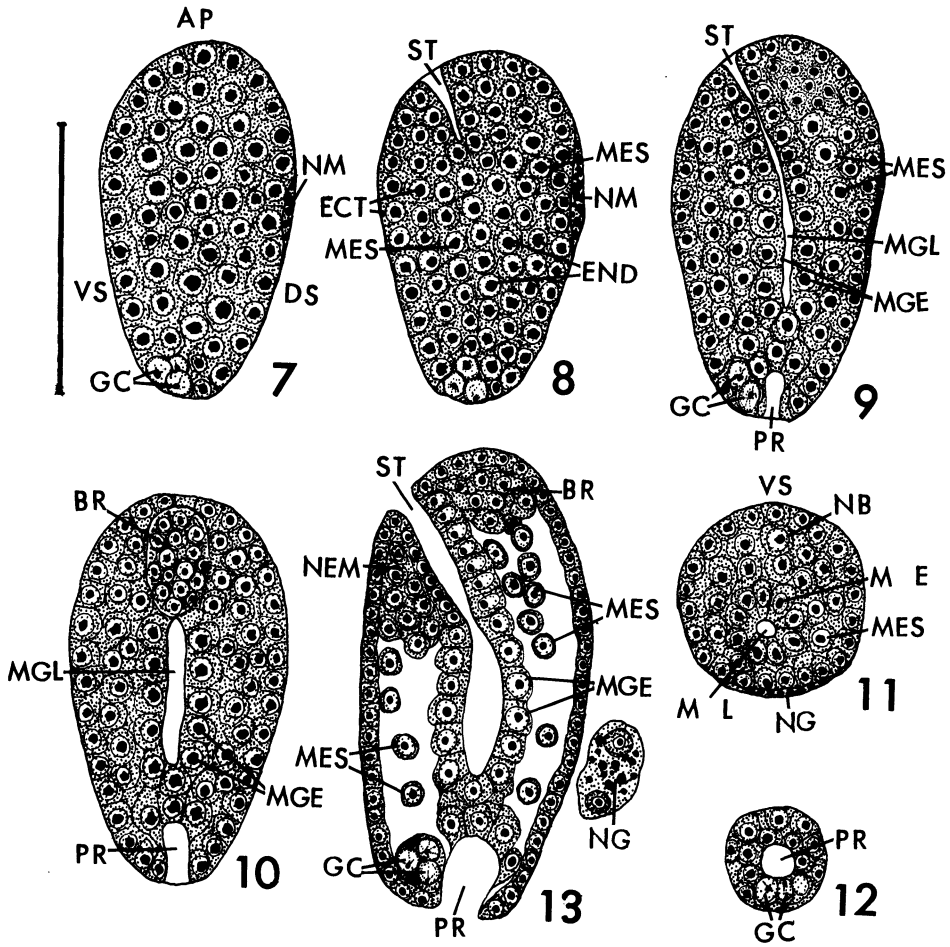
In the embryo which has finished the germ layer formation, the extruded mass flattens between the periphery of embryo and the vitelline membrane, and covers the dorsal surface of the embryo as if it is an embryonic envelope. When the alimentary canal has been completed and the body cavity begins to be formed, the beginning of hatching occurs. As the chorion is torn and cast off posteriorly, the flattened extruded mass leaves from the embryo. Then it becomes a spherical body containing nucleolar granules and several somatic nuclei or cells, and is found by the dorsal body wall of the embryo (Fig. 13). This body ramains intact for a while, but eventually it degenerates in the yolk of the host egg.

Primordial germ cells

Just before the proctodaeum invagination, about seven primordial germ cells lost an affinity for staining and surrounded by the somatic cells are observed in the ventral edge of posterior end of the embryo. After the proctodaeum formation they are embraced by the flattened cells between the ventral proctodaeal wall and body wall of embryo (Figs. 9, 12, 13).

Mesoderm

After the alimentary canal formation a large number of cells sink inward from the periphery of the embryo and increase in number during the germ layer formation. They, however, degenerate except the cells which have taken part in the formation of alimentary canal and nervous system. Consequently a small number of loose spherical cells with a chromatin body and protoplasmic network is found in the newly formed body cavity (Fig.



Figs. 7 - 9. Longitudinal sections through the major axis of egg, showing successive formation of alimentary canal.

Fig. 10. Horizontal section through the major axis of egg at the stage little later than that shown in Fig. 9.

Fig. 11. Cross section through the major axis of egg at the same stage shown in Fig. 10.

Fig. 12. Cross section through the proctodaeum at the same stage shown in Fig. 11.

Fig. 13. Longitudinal section of a first instar larva and a extruded mass.

Ap anterior pole, BR brain, ECT ectodermal cell, END endodermal cell, GC primordial germ cell, MES mesodermal cell, MGE mid-gut epithelium, MGL mid-gut lumen, NB neuroblast, NEM nerve cell mass, NG nucleolar granule, PR proctodaeum, ST stomodaeum, VS ventral side. Scale : 50 μm.

13). These cells are probably mesodermal cells. Neither the aggregation of these cells nor the formation of mesoblastic somite was observed at any stages of the development.

Nervous system

When the formation of mid-gut lumen begins, the ventral two-cell layer and dorsal mono-cell layer are recognizable between the rudimental mid-gut epithelium and the peripheral layer of embryo in cross section (Fig. 11). The cells consisting of the ventral outer layer probably correspond with the neuroblasts in other insect embryos, and the inner and the dorsal layer are the mesoderm, but most of which are destined to degenerate during the following development. As the development proceeds, nearly all outer cells also degenerate. In the oldest embryo or the newly hatched first instar larva large masses of the nerve cells, namely the brain and suboesophageal ganglion, may be obserbed.

The brain develops over the antero-dorsal side of the fore-gut, and the suboesophageal ganglion is formed beneath the fore-gut wall (Fig. 13). The former is elliptic, about 25 μm in length and 15 μm in width, and the latter is smaller than the former. Though there a few number of cells are found on the inner surface of the ventral body wall, neither the ganglion nor the nerve cord differentiates.

Discussion

1. Primordial germ cells

According to Silvestri's (1908) illustrations, the primordial germ cells in the eggs of *Oophthora semblidis* are situated in the posterior part of the blastoderm, and when the stomodaeum begins to invaginate, the germ cells are located in the postero-central region of embryo, being sandwitched by the peripheral layer and inner somatic cells.

In *T. chilonis,* the primordial germ cells differentiated outside the blastoderm at the posterior pole of the egg, and they remained intact for a while. After the expulsion of nucleolar granules they changed their position to the ventral edge in the embryonic posterior pole and sank inward during the formation of germ layer. These observations agree with Gatenby's (1917) descriptions on *T. evanescens.*

2. Alimentary canal

In *T. chilonis,* before the formation of proctodaeum and mid-gut the stomodaeum began to invaginate as a depression of the ectoderm, and then it extruded dorso-posteriorly. Shortly afterward the end of the stomodaeum impinged on the anterior end of the developing mid-gut, and the stomodaeal lumen became continuous with the mid-gut lumen.

Silvestri (1908) illustrated the similar feature of the stomodaeal invagination of *O. semblidis.* Gatenby (1917), however, stated that, although the stomodaeum was normally formed, the cell mass situated between the stomodaeal and mesenteron cells remained unbroken till the formation of body cavity occurs.

As to the origin of the mid-gut epithelium Gatenby (1917) described that a longitudinal groove regarded as a early blastopore appeared on the dorsal surface of the embryo and it became deeper to form the mid-gut.

In *T. chilonis* this groove invaginated at the ventral surface, and the cells arranged around it sank inward and increased in number. Although the fate of these cells is not yet definitely known, it is possible to say that these cells take part in the formation of the fore- or mid-gut epithelium.

Regarding the proctodaeum formation Gatenby (1917) noted that, in *T. evenescens* it was difficult to ascertain the occurrence of invagination as observed by the time of stomodaeum formation, and the demarcation between the mid-gut and proctodaeum was quite indistinct. Though Silvestri (1908) has given an illustration of the sagittal section of *O. semblidis* embryo in which the alimentary canal has been formed, he did not describe the earlier stages of proctodaeum formation.

In *T. chilonis,* in which the mid-gut lumen was formed, the proctodaeum appeared at the posterior end of the embryo, and it grew rapidly. The invagination of proctodaeum was not confirmed by the present author. When the proctodaeum became clear after the occurrence of large proctodaeal cavity the single cell layer which forms proctodaeal blind end was closely in contact with the posterior mid-gut epithelium. These epithelial cells in the extremity of the newly formed proctodaeum had a striking resemblance to those in the mid-gut. In this species the contacted area of proctodaeal end and the end of the mid-gut is kept intact and opening which may join two lumens may not appear at this stage, and the demarcation between the proctodaeal and mid-gut epithelium was indistinct for a while, but the former became distinguishable from the latter in the stage of the body cavity formation.

From the above-mentioned facts it is conceivable that the proctodaeum may be formed by the growing of the lumen which appears at the posterior end of the embryo, and not formed by the general invagination of the ectoderm. The epithelial cells in the extremity of proctodaeum probably originates from the endodermal cells.

3. Embryonic Envelope

In *T. chilonis* neither amnion nor serosa is formed at any stages of the development.

Silvestri (1908) described that a 'pseudoserosa' was formed by the delamination of the embryonic cells in the eggs of *O. semblidis* and indicated that it covered the embryo. Gatenby (1917) denied Silvestri's explanation on the pseudoserosa and stated that the embryonic membrane did not exist in *Trichogramma.* However, it could be observed in *T. chilonis* that when the chorion was torn and cast off, the embryo floated free in the yolk and changed its form into a round body as observed in *T. evanescens* (Gatenby, 1917). This fact shows that that the flattened, extruded cells observed over these embryos are not true embryonic envelope.

Therefore the author agrees with Gatenby's conclusion above-mentioned.

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