



# *Perilitus coccinellae* Teratocyte Polypeptide: Evidence for Production of a Teratocyte-specific 540 kDa Protein

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The proteins synthesized by teratocytes of the braconid wasp *Perilitus coccinellae* were analyzed electrophoretically. Non-denaturing polyacrylamide gel electrophoresis (PAGE) revealed that the teratocytes contained a major polypeptide with a high molecular weight of about 540 kDa. SDS-PAGE indicated that the major subunit of this protein was approx. 94 kDa. The teratocytes were cultured *in vitro* in a medium containing <sup>35</sup>S-methionine. Both the proteins from the incubated teratocytes and the medium were subjected to PAGE followed by fluorography. The fluorogram revealed that the teratocytes synthesized the 540 kDa protein. RNAs extracted from the teratocytes, parasitoid larvae and host fat body were translated *in vitro* in a rabbit reticulocyte lysate. Only the translation products of teratocyte RNA reacted with the antibodies generated against the crude teratocyte extract. Changes in the total protein content of teratocytes and parasitoid larvae indicated that the teratocytes may have a role in providing nutrients for the developing parasitoid larvae during parasitism. This hypothesis was supported by the fact that the gut contents of the parasitoid larvae showed an immunoreaction to antibodies against crude teratocyte proteins.

Teratocyte Parasitoid–host interaction *Perilitus coccinellae* Braconidae *Coccinella septempunctata*  
Protein synthesis

## INTRODUCTION

When a braconid parasitoid larva hatches, spherical cells, most frequently called “teratocytes” are liberated into the host hemocoel. The teratocytes, which originate from an extraembryonic membrane, increase in size during growth of the parasitoid larva. The number circulating in the host either remains constant (Hashimoto and Kitano, 1971; Vinson and Lewis, 1973; Strand and Wong, 1991) or decreases (Sluss, 1968) during parasitism. Dahlman (1990) proposed three major functions of teratocytes including: (1) immunosuppression of host defenses; (2) secretion of enzymes and other factors influencing the host physiology; and (3) supplying nutrition to the parasitoid. So far there is little concrete evidence to prove any of these hypotheses, thus the function of teratocytes still remains unclear.

The braconid wasp *Perilitus coccinellae* Schrank parasitizes aphidophagous lady beetles and this species, like other braconids, has teratocytes (Hodek, 1973). The

teratocytes become hypertrophic to such an extent that their diameter increases by ten-fold from 50 to 500  $\mu\text{m}$  on average (Kadono-Okuda *et al.*, 1995). Sluss (1968) proposed that the main function of the *Perilitus* teratocytes is to absorb nutrients from the host because electronmicroscopic pictures show that the surface of teratocytes is densely packed with microvilli. These ultrastructural studies also suggested the teratocytes were actively engaged in protein synthesis due to the presence of extensive rough endoplasmic reticula (Sluss, 1968).

In this paper, we examine: (1) the pattern of teratocyte proteins of *P. coccinellae* electrophoretically; and (2) *de novo* protein synthesis by teratocytes by means of <sup>35</sup>S methionine incorporation *in vitro*. Finally, we discuss a possible function of the teratocytes in *P. coccinellae*.

## MATERIALS AND METHODS

### Insects

Adults of *Coccinella septempunctata brucki*, which is the main host of *P. coccinellae* (Maeta, 1969) were collected from the field in Tsukuba located at about 60 km northeast from Tokyo and kept in the laboratory at 25°C. After several days *Perilitus* larvae emerged from

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the hosts and made cocoons. The wasps were fed on 30% sucrose solution until used for parasitization. Unparasitized coccinellids were reared under short day (12L:12D) at 25°C, which are diapause averting conditions (Okuda and Hodek, 1983). Coccinellids were parasitized 7 days after adult eclosion by *P. coccinellae* in a Petri dish under careful observation to avoid super-parasitism. Under such conditions, *P. coccinellae* larvae complete pre-pupal development within about 19 days without undergoing diapause (Kadono-Okuda *et al.*, 1995). Teratocytes were sampled at either 8 or 11 days after parasitization.

Adults of *Harmonia axyridis*, which is another host of *P. coccinellae* (Maeta, 1969) were collected from the field in Tsukuba and kept under long day (16L:8D) at 25°C. After a week the coccinellids were parasitized and teratocytes were then collected from the parasitized host beetles at 11 days after parasitization.

#### Collection of teratocytes from the host

Parasitized host coccinellids were submerged in ice-cold Ringer's solution (110 mM NaCl, 1.8 mM KCl, 1.1 mM CaCl<sub>2</sub>, 2.4 mM NaHCO<sub>3</sub>) and dissected. The teratocytes were distributed in the host body cavity and dislodged from host tissues by pipetting with ice-cold Ringer's solution over the carcass several times. The teratocytes were then transferred to a watch dish and rinsed several times with the Ringer's solution to remove the host hemocytes. Teratocytes used in this experiment were more than 100 µm in diameter. Teratocytes of this size can be easily separated from the host hemocytes under microscope with a Pasteur pipet.

#### Polyacrylamide gel electrophoresis (PAGE) and fluorography

Non-denaturing and SDS-PAGE were performed as described by Davis (1964) and Laemmli (1970), respectively. Native and SDS-PAGE gels were stained with Coomassie blue. Fluorograms were processed according to Chamberlain (1979). Dried gels were exposed to Fuji X-ray film at -80°C for 3 days followed by development with a Fuji X-ray film developer.

#### In vitro incubation of teratocytes and the protein extraction

Protein synthetic activities of teratocytes were analyzed principally according to the methods previously described (Okuda and Chinzei, 1988). Teratocytes were dissected out in sterile Ringer's solution, and were cultured either in sterile Ringer's solution or Grace's medium without methionine containing 1 µCi of <sup>35</sup>S methionine (47.1 TBq/mmol, Amersham) at 25°C. After incubation for 1, 2 or 3 h, teratocytes and the culture medium were immediately cooled on ice and kept at -80°C. Frozen cells were homogenized in extraction buffer (×1) [0.05 M Tris-HCl, pH 8.5, 0.25 M NaCl, 1 mM phenylmethylsulfonyl fluoride (PMSF), 0.1% Triton X-100, 0.1% sodiumdeoxycholate (DOC)] with a Polytron homogenizer. The homogenate was centrifuged

at about 500 g for 15 min. The supernatant was stored at -80°C as crude teratocyte extract. Protein content of these extracts was determined by the Lowry method (Lowry *et al.*, 1951). Bovine serum albumin (BSA) was used as a standard protein for calibration. For counting the radioactivity incorporated into total teratocyte protein, 10 µl of crude teratocyte extract was placed on a 24 mm disc of Whatman 3MM filter paper. After drying completely, discs were put in cold 10% trichloroacetic acid (TCA) and washed three times with cold 5% TCA, once with ethanol-ether (3:1, v/v) and once with ether. Dried discs were put into scintillator (Amersham AGS II) and counted on a scintillation counter (Aloka 7000).

#### RNA extraction

Tissues were dissected into a ten-fold volume of saturated phenol and homogenized with a Polytron. The homogenates were vortexed for 5 min with the same volume of 100 mM Tris-HCl (pH 9.0) containing 0.5% SDS and the mixture was centrifuged at 750 g for 20 min at room temperature. The aqueous phase was removed and the phenol extraction was repeated three times. To the final aqueous phase, 1/10 vol. of 3 M sodium acetate and 2.5 vol. of cold ethanol were added and the mixture was kept at -20°C overnight to precipitate the RNA. To eliminate DNA and glycogen (Palmiter, 1974) the precipitate was washed with 70% ethanol and 3 M sodium acetate three times each. Finally the RNA was dissolved in sterile water for the *in vitro* translation experiment.

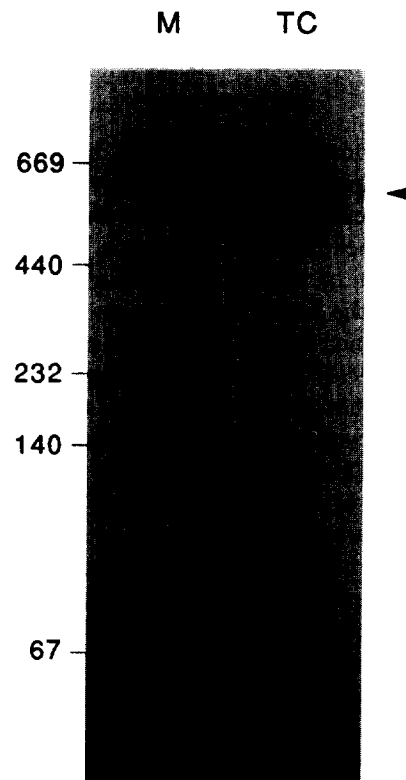


FIGURE 1. Profile of teratocyte proteins analyzed by native-PAGE on a 2.5–20% gradient gel. TC: crude teratocyte extract (24 µg) of day 11 after parasitization, M: standard marker proteins. Arrow indicates a 540 kDa teratocyte specific protein.

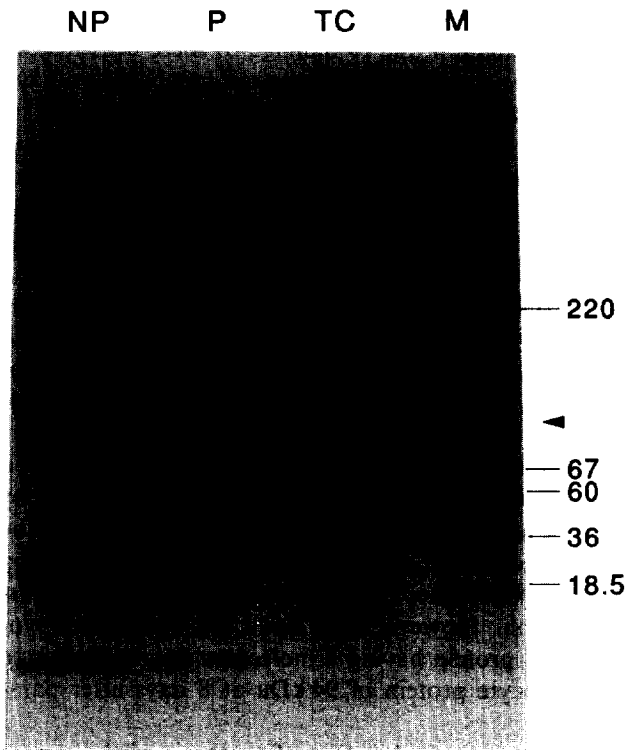


FIGURE 2. Profile of teratocyte protein and the host hemolymph proteins analyzed by SDS-PAGE on a 2.5–20% gradient gel. NP: hemolymph (1  $\mu$ l) of non-parasitized female host, P: hemolymph (1  $\mu$ l) of parasitized female host, TC: crude teratocyte extract (12  $\mu$ g) of day 11 parasitization, M: standard marker proteins. Arrow indicates a 94 kDa teratocyte specific polypeptides.

In a preliminary experiment, poly(A)<sup>+</sup> RNA was isolated from the total RNA by using an oligo(dT) cellulose (PL-Biochemicals, Milwaukee, WI) column chromatography as described (Aviv and Leader, 1972) and translated *in vitro* in a rabbit reticulocyte lysate in the presence of <sup>35</sup>S methionine. Analysis of translation products by fluorography showed that there was no significant difference between the patterns of polypeptides translated from poly(A)<sup>+</sup> RNA and those from total RNA. Thus, total RNA was routinely used as the template for the *in vitro* translation.

#### *In vitro* translation

*In vitro* translation was performed in a rabbit reticulocyte lysate pretreated with micrococcal nuclease (Wako Pure Chemicals, Osaka, Japan). Ten  $\mu$ l of the reaction mixture contained 5  $\mu$ g of RNA from each tissue, 8  $\mu$ l of reticulocyte lysate and 5  $\mu$ Ci of <sup>35</sup>S methionine. The translation reaction was performed at 30°C for 1 h. The reaction was stopped by the addition of 60  $\mu$ l of gel-loading buffer.

#### Immunoprecipitation procedures

Antisera to teratocyte proteins were raised in a rabbit using a crude teratocyte extract prepared from teratocytes isolated from hosts at 11 days after parasitization. Immunoprecipitation of translation products was performed as described by Watanabe and Price (1982) with

slight modifications. Twenty-five  $\mu$ l of translation products were mixed with 3  $\mu$ l of 10% SDS and 3  $\mu$ l of 20% Triton X-100. The mixture was then incubated at 30°C for 1 h with the diluted anti-teratocyte antibody (1/1500), followed by another 1 h incubation at 30°C with 100  $\mu$ l of 5% (w/v) staphylococcal protein A-Sepharose CL-4B (Pharmacia, Uppsalla, Sweden) suspended in Triton-saline (20 mM Tris-HCl/0.1% Triton X-100/0.14 M NaCl/1 mM EDTA). The immunoreaction mixture was transferred to a small plastic column and washed with 5 ml of Triton-saline and 3 ml of distilled water. The antigen-antibody complexes bound to the protein A beads were eluted with 50  $\mu$ l of 2 $\times$  gel-loading buffer, and the column was washed once with 50  $\mu$ l of distilled water. Pooled eluates were

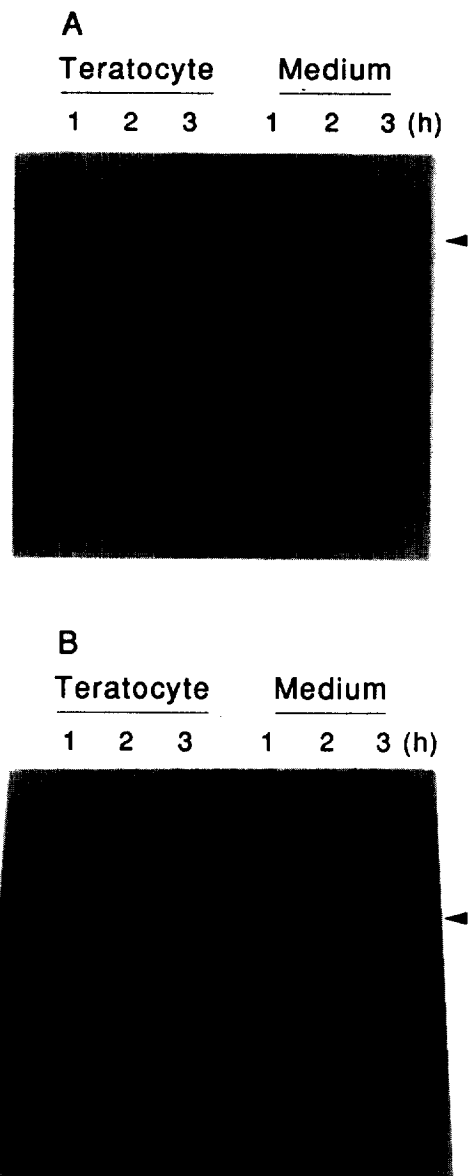


FIGURE 3. Fluorograms showing protein synthesis and release by teratocytes (day 11 after parasitization) after incubation for 1, 2 and 3 h in Ringer's solution containing <sup>35</sup>S-methionine: (A) native-PAGE on a 2.5–20% gradient gel, open arrow indicates position of the 540 kDa teratocyte specific protein; and (B) SDS-PAGE on a 2.5–20% gradient gel. Arrow indicates a 94 kDa teratocyte specific polypeptides.

analyzed by SDS-PAGE and fluorography as described above.

## RESULTS

### *Profile of teratocyte proteins*

Analysis of teratocyte proteins at 11 days after parasitization using non-denaturing PAGE on a 2.5–20% gradient gel with Coomassie blue staining (Fig. 1) revealed that teratocytes contained several proteins; one of the most prominent had a high molecular weight of about 540 kDa. The same sample was subjected to SDS-PAGE on a 2.5–20% gradient gel (Fig. 2) and the major subunit of the 540 kDa protein was approx. 94 kDa in molecular weight. The protein pattern of the host hemolymph of non-parasitized and parasitized females showed no qualitative difference but were quantitatively different.

### *Protein synthesis by teratocyte, host fat body and parasitoid larva*

Teratocytes recovered from hosts 11 days after parasitization were incubated in Ringer's solution containing <sup>35</sup>S methionine for 1, 2 and 3 h. After incubation, extracts of the cultured teratocytes and the medium were subjected to non-denaturing and SDS-PAGE on a 2.5–20% gradient gel followed by fluorography (Fig. 3). The fluorogram revealed that teratocytes incorporated <sup>35</sup>S methionine into a 540 kDa protein within 1 h of incubation. After 2 h of incubation, the release of the synthesized labeled protein could also be found in the medium. Since we observed that teratocytes became very fragile with maturation, it is possible that leakage of

teratocyte proteins into culture medium took place during incubation due to damage to these cells instead of by active secretion. We therefore incubated younger teratocytes sampled from hosts 8 days after parasitization. The fluorogram showed that day 8 teratocytes released very little synthesized teratocyte proteins into medium after 2 h incubation (Fig. 4). The same incubation was performed with Grace's medium without methionine which is richer in nutrition and better in osmotic balance than Ringer's solution. We obtained the same result as Ringer's solution (data not shown), which indicated that the protein release by maturing teratocytes was not due to the medium conditions but was stage specific. The profile of the proteins synthesized by the teratocytes was almost identical to that seen when total proteins in the teratocyte cells were analyzed by Coomassie blue staining (Fig. 1). Hence, most of the proteins contained in the teratocyte are synthesized by the cell itself, rather than being taken up from the host hemolymph. However, the host fat body also synthesized a protein having a molecular weight similar to the teratocyte protein of 94 kDa at 8 days after parasitization.

To determine the location of synthesis of the teratocyte protein we extracted RNA from maturing teratocytes, parasitoid larvae and host fat bodies, and translated the RNA samples *in vitro* in a rabbit reticulocyte lysate in the presence of <sup>35</sup>S methionine. The total translation products and the fraction of the translation products eluted by immunoaffinity chromatography with anti-teratocyte protein antibody were analyzed by SDS-PAGE (12% gel) followed by fluorography (Fig. 5). The fluorogram showed that neither the RNA extracted

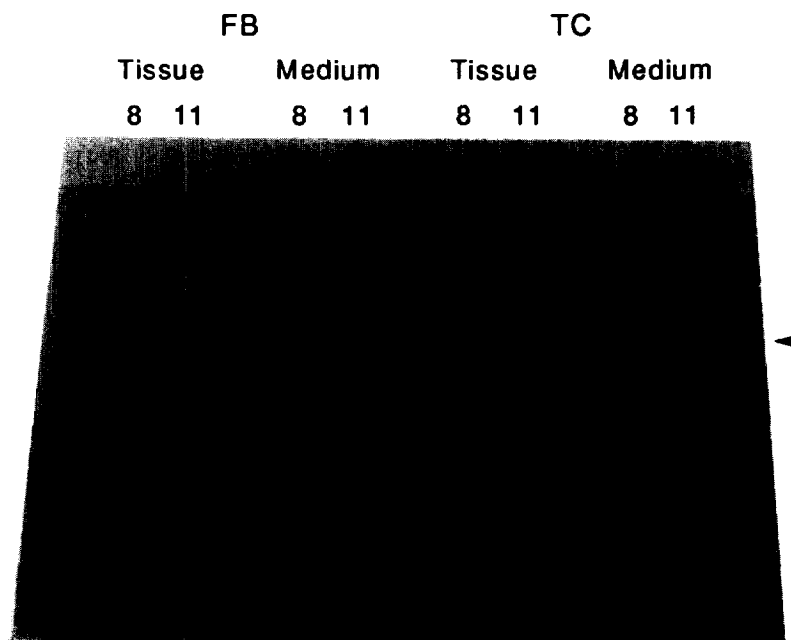


FIGURE 4. Fluorograms (SDS-PAGE on a 2.5–20% gradient gel) showing protein synthesis and release by teratocytes (TC) and the host fat body (FB) of days 8 and 11 post-parasitization after incubation for 2 h in Ringer's solution containing <sup>35</sup>S-methionine. The same volume of tissue extract and incubation medium were loaded per lane. Arrow indicates the major 94 kDa teratocyte specific polypeptides.

from the host fat bodies nor that from the parasitoid larva was capable of directing synthesis of proteins that were detected by the anti-teratocyte protein antibody. Only RNA extracted from teratocyte-translated protein reacted with the antisera. These results confirmed that the teratocyte proteins were exclusively of teratocyte origin.

*Teratocyte growth and the development of parasitoid larvae*

Changes in the relative protein content in the teratocytes and parasitoid larvae and total protein synthesis by teratocytes were measured during the course of parasitism (Fig. 6). The increase in protein content of the teratocytes mirrored the increase in protein synthesis rate up to 11 days after parasitization. Later, however, both declined abruptly due to a decrease of the number of teratocytes circulating in the host. In parasitoid larvae, by contrast, the protein content increased slowly

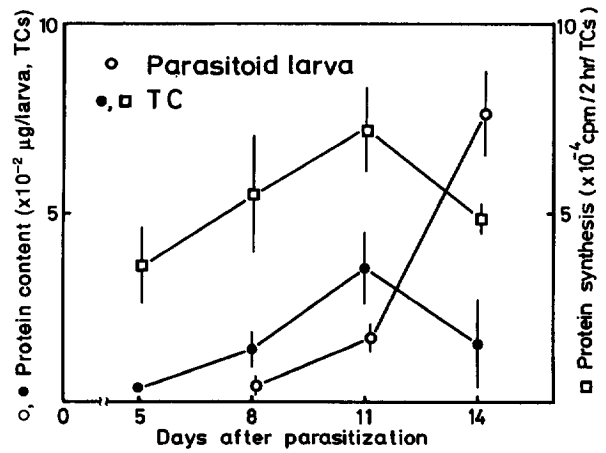


FIGURE 6. Changes in total protein content of teratocytes and parasitoid larvae and protein synthesis by teratocytes. Coccinellids were parasitized at 7 days after emergence and kept at 25°C (n = 5 at each sampling). Protein content of teratocytes declined abruptly by day 14 due to a decrease in the number of teratocytes circulating in the host. In contrast, the protein content of the parasitoid larvae increased dramatically after the larvae molted into the second instar. Vertical lines represent ±SD.

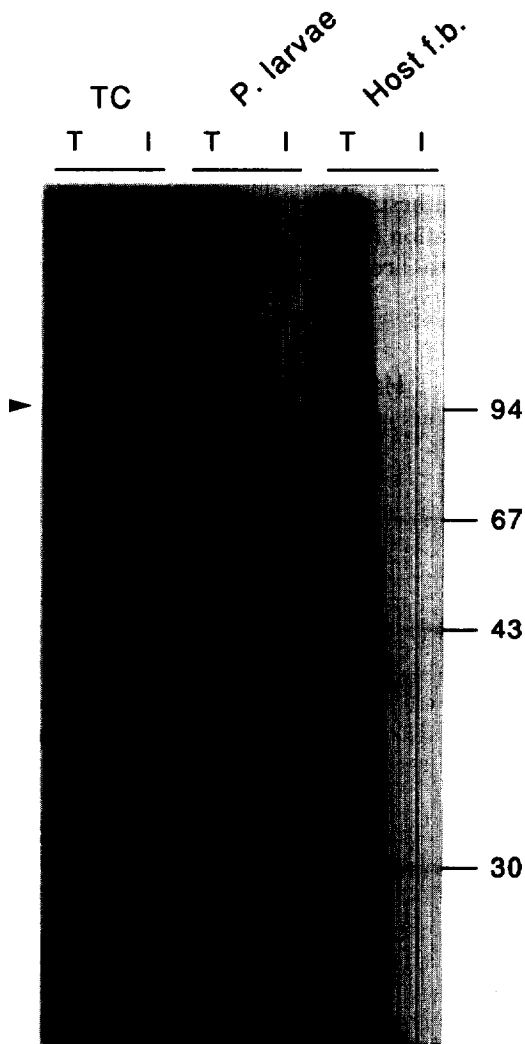


FIGURE 5. *In vitro* translation of RNA extracted from teratocytes, parasitoid larvae and host fat body in a rabbit reticulocyte lysate system containing <sup>35</sup>S-methionine. Translation products were subjected to SDS-PAGE on 12% gel followed by fluorography. (T): total translation products, (I): translation products immunoprecipitated by an antibody against crude teratocyte extract. Arrow indicates a 94 kDa teratocyte specific polypeptides.

during the first instar but then increased dramatically after the larvae molted into the second instar. Just prior to egression from the host the parasitoid larvae molted into the third (last) instar, and at this time few or no teratocytes were observed in the host body cavity. Thus, the drastic increase in the protein content of parasitoid larvae is associated with the loss of the teratocytes, which presumably are consumed by the parasitoid prior to exit from the host.

*Immunoreaction of the gut contents of parasitoid larvae and teratocyte proteins from different host species with anti-teratocyte antibody*

Changes in relative protein content in the teratocytes and parasitoid larvae (Fig. 6) suggested that teratocytes were consumed by parasitoid larvae during parasitization. To obtain direct evidence we examined whether the gut contents have an immunological reaction against the anti-teratocyte antibody by means of SDS-PAGE followed by Western blotting (Fig. 7). Although the major teratocyte protein of 94 kDa was not present in the parasitoid gut, probably due to degradation by the gut proteinases, several lower molecular weight immunoreactive bands were detected. Thus, we concluded that the teratocyte proteins were ingested by the parasitoid larvae.

*P. coccinellae* parasitizes about 30 species of aphidophagous lady beetles (Hodek, 1973). We obtained teratocytes from another host species, *H. axyridis*, on day 11 after parasitization and checked the protein profile and the immunoreactivity to the antibody using the method described above. We assumed that teratocyte protein profile and the immunoreaction should differ from host to host, if teratocytes absorbed proteins from each host's hemolymph. The teratocyte proteins of the two different host species were, however, almost

identical electrophoretically and immunologically (Fig. 7). This evidence further supports our hypothesis that teratocyte proteins are newly synthesized by the cell itself, rather than being taken up from the host hemolymph.

#### DISCUSSION

Ultrastructural studies demonstrated the presence of rough endoplasmic reticulum and other organelles in teratocytes (Sluss, 1968; Vinson and Scott, 1974; Lawrence, 1990; Tanaka and Wago, 1990), which provided circumstantial evidence for an active role in protein synthesis. In the fruit fly, *Biosteres longicaudatus*, a parasitism specific 24 kDa protein appears in the hemolymph several days after parasitization, and this protein appears identical to that synthesized by the teratocytes *in vitro* (Lawrence, 1990). In the present study we found that teratocytes in *P. coccinellae* contained a single major protein of 540 kDa (Fig. 1), with a subunit of approx. 94 kDa (Fig. 2). In *in vitro* studies, teratocytes rapidly incorporated <sup>35</sup>S-methionine into this 540 kDa protein (Fig. 3). This information, together with the result that only translation products from teratocyte RNA showed a positive immunoreaction with anti-teratocyte serum (Fig. 5), indicates that teratocyte proteins are exclusively synthesized by teratocytes. We also analyzed teratocyte proteins from a different host species, *H. axyridis* (Fig. 7). The protein pattern was electrophoretically and

immunologically quite similar to that of teratocytes from *C. septempunctata*. This evidence further supports the theory that teratocyte proteins mostly consist of newly synthesized teratocyte specific proteins, rather than being taken up from the host hemolymph; so far no evidence of pinocytosis has been observed (Vinson and Scott, 1974).

During parasitism, *P. coccinellae* teratocytes became hypertrophic and enlarged about ten-fold in diameter by the final stages of parasitism. Some teratocytes attained a maximum diameter of 820  $\mu$ m. The number of teratocytes, however, then decreased gradually and few or no teratocytes remained in the host body cavity after the parasitoid larva egressed (Sluss, 1968; Kadono-Okuda *et al.*, 1995). The drastic decrease of the total protein content of the teratocytes coincided with an increase of the total protein content of the parasitoid larva as the second instar progressed (Fig. 6). Since the gut contents of the parasitoid larvae showed a positive immunoreaction with the anti-teratocyte sera (Fig. 7), it is likely that parasitoid larvae ingest the teratocyte proteins. How is the material ingested? The parasitoid larvae may either feed directly on the teratocyte cells or they may take up those proteins that have been secreted into the host's hemolymph. Evidence for the latter possibility comes from the observation that mature teratocytes released the newly synthesized proteins into the culture medium during incubation (Fig. 3), while smaller teratocytes had a tendency to accumulate the synthesized teratocyte

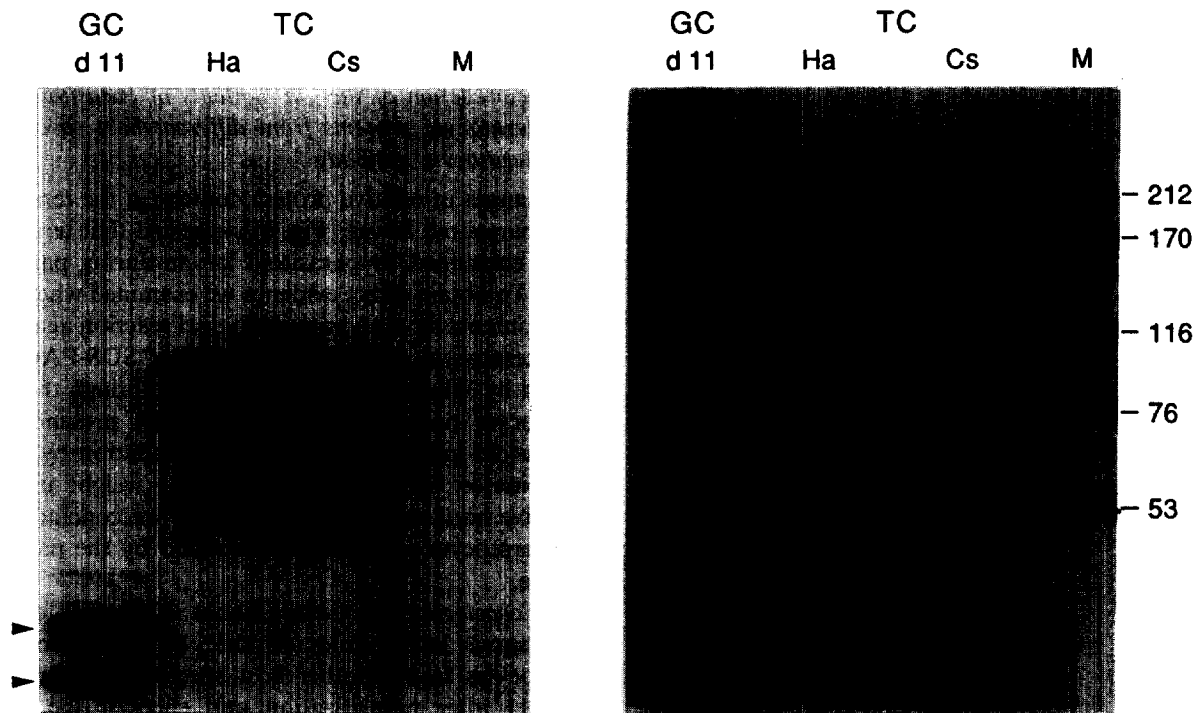


FIGURE 7. Immunoblot of crude extractions from teratocytes of two different host species and from the midgut contents of parasitoid larvae. The same samples were subjected to SDS-PAGE on two plates of 7.5% gels. The first gel was stained by Coomassie blue (right), the second gel was blotted onto a membrane and immunostained with antibodies against crude extract of teratocytes from the host, *C. septempunctata* (left). GC: midgut content of parasitoid larvae at the second instar in the host, *C. septempunctata*, TC: crude teratocyte extract of day 11 parasitization, Ha: crude extract of teratocytes from the host, *H. axyridis*, Cs: crude extract of teratocytes from the host, *C. septempunctata*, M: standard marker proteins. Arrows indicate proteins in the midgut of parasitoid larvae which are immunologically positive to antibodies against crude teratocyte protein.

proteins without releasing polypeptides (Fig. 4). Previous studies showed that teratocytes are secretory early in development (Strand *et al.*, 1986; Tanaka and Wago, 1990) and exhibit a storage function as they age (Sluss, 1968; Sluss and Leutenegger, 1968; Cohen and Debolt, 1984), a conclusion which is just opposite to our results. We observed that teratocytes became very fragile with maturation. It is possible that leakage of teratocyte proteins into culture medium took place during incubation due to damage to these cells instead of by active secretion. Further experiments are now under way to clarify the possibility of active secretion.

Dahlman (1990) proposed that teratocytes could serve three major functions: (1) immunosuppression of host defenses; (2) secretion of enzymes and other factors influencing the host physiology; and (3) supplying nutrition to the parasitoid. The evidence obtained in this study indicates that the teratocytes of *P. coccinellae* have a role in providing nutrition to the parasitoid larva. We would anticipate that the host viability might deteriorate rapidly during the dramatic growth phase of the parasitoid during its second instar (Fig. 4) if the parasitoid larvae took the required amount of nutrients directly from the host without utilizing teratocytes. This nutritive role of teratocytes in *P. coccinellae* may thereby have acted as a buffer against excessive damage to the host during the last stage of parasitization. To examine this hypothesis, we are currently characterizing the major teratocyte protein.

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