Journal of Invertebrate Pathology **76,** 79–80 (2000)
doi:10.1006/jipa.2000.4950, available online at http://www.idealibrary.com on IDEAL®

NOTE

Two Collagenases Are Secreted by Teratocytes from *Microplitis mediator* (Hymenoptera: Braconidae) Cultured *in Vitro*

Teratocytes, derived from serosal cells of some parasitoid wasp embryos after the parasitoid egg hatches, have many putative functions that mediate the relationship between host and parasitoid (D. L. Dahlman and S. B. Vinson, *In* N. E. Beckage, S. N. Thompson, and B. A. Federici, Eds., "Parasites and Pathogens of Insects," Vol. 1, pp. 145–165, New York, Academic Press, 1993). Their ability to secrete has been investigated extensively, especially the secretion of some polypeptides or proteins (E. J. Schepers, D. Dahlman, and D. Zhang, *J. Insect Physiol.* **44,** 767–777, 1998; S. B. Vinson, A. K. Mourad, and D. K. Sebesta, *Arch. Insect Biochem. Physiol.* **26,** 197–209, 1994). However, D. L. Dahlman and S. B. Vinson (In N. E. Beckage, S. N. Thompson, and B. A. Federici, Eds., "Parasites and Pathogens of Insects," Vol. 1, pp. 145–165, New York, Academic Press, 1993), in their comprehensive review describing teratocytes, suggested that the cells probably released collagenase that attacked the collagen sheath surrounding the fat body to permit selective release of fat body cells for the nutrition of the parasitoid larvae, which would not kill the host. Based on this hypothesis, we detected collagenase activity by zymograph analysis in the medium of teratocytes from *Microplitis mediator* cultured *in vitro.* The enzymes' activity also existed in the hemocoel of the host *Pseudaletia separata* during the later stages of parasitism.

M. mediator is a solitary endoparasitoid wasp parasitizing some Noctuidae and Geometridae insect larvae, including important pest insects, such as *Helicoverpa amigera, Pseudaletia separata,* and *Brathra brassicae* (A. P. Arthur and P. G. Mason, *Can. Entomol.* **118,** 487–491, 1986). In this study, we examined the *M. mediator–P. separata* system to investigate the collagenase secretion function of teratocytes. Parasitoid *M. mediator* larvae were maintained in *P. separata* larvae, which were reared on an artificial diet at a 16:8 h photoperiod, with a 26 ± 0.5°C temperature regimen. The young teratocytes were obtained from *in vitro* hatching of the embryos, which were dissected from the host 32 h after the female wasp's oviposition (F. Pennacchio, S. B. Vinson, and E. Tremblay, *Int. J. Insect Morphol. Embryol.* **23,** 93–104, 1994). Mature cells (4 days old) were collected from the hemocoel of the host by a modification of the method of T. Tanaka and H. Wago (*Arch. Insect Biochem. Physiol.* **13,** 187–197, 1990). The cells were cultured in Sf 900 insect cell culture medium (Gibco BRL Life Technologies, Inc. Grand Island, NY) under sterile conditions for 12 h (4-day cells) or 24 h (young cells) at 27 ± 0.5°C. The supernatant of the cultured medium was analyzed by gelatin zymography (S. J. Fisher and Z. Werb, *In* M. A. Haralson and J. R. Hassell, Eds., "Extracellular Matrix: A Practical Approach, pp. 260–287, IRL Press, Oxford, 1995). Secretion by human trophoblasts served as a positive control.

Zymography of the supernatant (Fig. 1) showed that young teratocytes secreted a 72-kDa gelatinase whose molecular weight was equal to that of gelatinase A (*EC 3.4.24.24*), while a 92-kDa gelatinase, whose molecular weight was equal to that of gelatinase B (*EC 3.4.24.35*), was secreted by 4-day teratocytes. Gelatinase A and gelatinase B are two important collagenases belonging to the matrix metalloproteinase family. They play crucial roles in the degradation of the extracellular matrix and the remodeling of the tissues of mammals (P. Reponen, C. Sahlberg, P. Huhtala, T. Hurskainen, I., Thesleff, and K. Tryggvason, *J. Biol. Chem.* **267,** 7856–7862; 1992; P. Reponen, C. Sahlberg, C. Munaut, I. Thesleff, and K. Tryggvason, *J. Cell Biol.* **124,** 1091–1102, 1994). We believe the two collagenases secreted by the teratocytes are gelatinase A and gelatinase B, respectively, though more evidence should be presented. Furthermore, the presence of parasitoid larvae enhances collagenase secretion (Fig. 1, lane 1). This observation is consistent with the findings of M. R. Strand, S. B. Vinson, W. C. Nettles, Jr., and Z. N. Xie

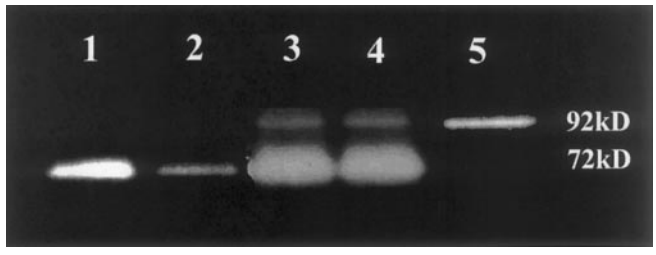

FIG. 1. Zymography of teratocytes cultured *in vitro* (lane 1, neonate parasitoid larvae and young teratocytes cultured for 24 h; lane 2, young teratocytes cultured for 24 h; lanes 3 and 4, human trophoblasts cultured for 48 h; lane 5, 4-day teratocytes cultured for 12 h).

0022-2011/00 $35.00

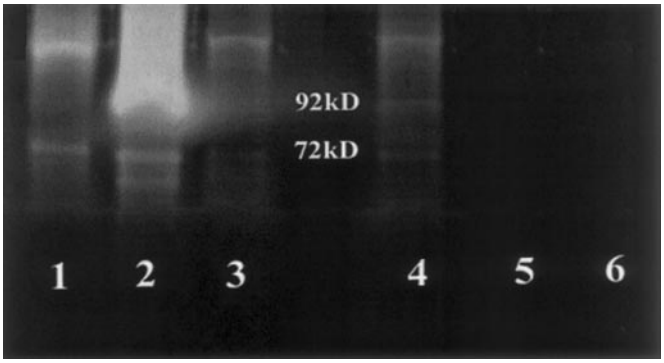

FIG. 2. Zymography of hemolymph plasma of *P. separata* (lanes 1–3, hemolymph plasma of *P. separata* 5, 6, and 7 days after being parasitized, respectively; lanes 4–6, hemolymph plasma of an unparasitized host reared in parallel with the parasitized hosts 5, 6, and 7 days after parasitization occurs. The enzyme's activity in lane 4 is relatively lower than that of lanes 1–3. Several experiments show that low collagenase activity presents in hemolymph plasma of unparasitized *P. separata* sometime in its lifetime.

(*Ent. Exp. Appl.* **46,** 71–78, 1988) and P. D. Greany, S. M. Ferkovich, and W. R. Clark (*Southwest Ent.* **12,** 89–94, 1989), who found that the presence of parasitoid larvae was beneficial to the growth of the teratocytes cultured *in vitro.*

To compare *in vitro* and *in vivo* secretion, collagenase activity was also found in the hemolymph plasma of the parasitized host (Fig. 2), especially in plasma samples taken at 6 days after parasitization. At this time, the parasitoid larvae were in their maximum growth phase (data not shown). This finding suggests that collagenases may play an important role in the nourishment of parasitoid larvae.

Key Words: collagenase; *Microplitis mediator; Pseudaletia separata;* teratocytes; parasitoid wasp; zymography; cell culture; secretion function.

We thank Dr. Yunge Zhao and Dr. Ping Xu, State Key Lab of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, for their kind help in preparing zymograph analysis. This work is supported by National Natural Sciences Fundation Grant 39870099 and a program of the State Key Lab of Integrated Management of Insects and Rodents (No. 9912).

QiLian Qin
He Gong
Tsuey Ding

State Key Lab of Integrated Management of Insects and Rodents
Institute of Zoology, Chinese Academy of Sciences
Beijing, China 100080
E-mail: qinql@panda.ioz.ac.cn

Received January 6, 2000; accepted May 5, 2000