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Humoral mediator-dependent activation of the *Sarcophaga* lectin gene

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Sarcophaga lectin is a defence protein synthesized by the fat body and secreted into the hemolymph in response to injury of the body of third instar larvae of *Sarcophaga peregrina* (flesh-fly). In this paper, we demonstrate that the stimulus of body injury is first transmitted to a certain tissue present in the anterior part of the body, and from there a mediator molecule that interacts directly with fat body cells is secreted into the hemolymph. On interaction with this mediator molecule, the fat body begins to synthesize mRNA for *Sarcophaga* lectin.

Lectin gene; Humoral mediator; RNA blot hybridization; (*Sarcophaga peregrina*, Fat body)

1. INTRODUCTION

In previous papers we demonstrated that a galactose-binding lectin and at least three groups of antibacterial proteins are induced in the hemolymph of *Sarcophaga peregrina* larvae when their body wall is pricked with a hypodermic needle. These proteins are acute-phase inflammatory proteins and are likely to participate in the defence mechanism of this insect, since damage to the body wall increases the chance of invasion by parasites and other foreign substances. Of these proteins, we have purified a lectin called *Sarcophaga* lectin and three antibacterial proteins termed sarcotoxin I, II and III to homogeneity [1–4]. Moreover, in experiments with antibody against *Sarcophaga* lectin, we found that this lectin participates in the exclusion of externally introduced sheep red blood cells [5].

Recently, we isolated a cDNA clone for *Sarcophaga* lectin (pLE10) and determined the complete amino acid sequence of the lectin [6]. RNA blot hybridization with this cDNA as a probe showed that the *Sarcophaga* lectin gene is expressed transiently twice during the life of *Sarcophaga*; namely in the early embryonic, and pupal stages [7]. Expression of the gene at these stages is indispensible for development of this insect, whereas that occurring in response to body injury is to cope with emergency and is rather unusual. Thus, the *Sarcophaga* lectin seems to have functions in both normal development and emergency. As the *Sarcophaga* lectin gene is a single-copy gene [6], there must be some mechanism that regulates its expression under different physiological conditions.

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2. MATERIALS AND METHODS

2.1. *Animals*

The flesh-fly *S. peregrina* was kept at 27°C with dry milk, sugar cubes and fresh water. Larvae were reared on pork liver, and when they crawled upward at the third instar, they were collected, washed well and kept in a plastic container with a small amount of water.

2.2. *RNA blot hybridization*

RNA was subjected to electrophoresis (10 μg RNA/lane) in a horizontal slab gel of 1.2% (w/v) agarose containing 2.2 M formaldehyde in 20 mM morpholinopropanesulphonic acid (Mops), 5 mM sodium acetate and 1 mM EDTA, pH 7.0. Before application to the gel, the RNA was heated to 60°C for 7 min in the above Mops-sodium acetate buffer supplemented

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with 50% (v/v) formamide and 2.2 M formaldehyde. After electrophoresis, RNA was transferred to a nitrocellulose filter as described by Thomas [8], and hybridized with nick-translated pLE10 (a cDNA clone for *Sarcophaga* lectin [6]) under the conditions in [7], at 42°C for 12–16 h. The filter was washed successively with 2 × SSC containing 0.1% SDS for 15 min at room temperature, and 0.5 × SSC containing 0.1% SDS for 15 min at 42°C, followed by autoradiography at −80°C.

3. RESULTS AND DISCUSSION

We are interested in the mechanism of transmission of the stimulus of body injury to a target organ in which the *Sarcophaga* lectin gene is selectively activated. The fat body is the largest and most well-developed organ in the larvae and synthesizes most hemolymph proteins. To determine whether *Sarcophaga* lectin mRNA is synthesized in the fat body or in other tissues in response to body injury, we carried out RNA blot hybridization with RNA extracted from the fat body of injured larvae and from the rest of the carcass free from the fat body using pLE10 as a probe. As evident from fig.1, the *Sarcophaga* lectin gene was found to be expressed exclusively in the fat body, and not in other tissues, indicating that the fat body is the only organ that synthesizes *Sarcophaga* lectin in response to body injury.

Why is the *Sarcophaga* lectin gene activated in fat body cells when the body wall is injured? There are at least two possible explanations. Insects have an open vascular system and their tissues are surrounded by hemolymph, so one possibility is that some mediator that interacts directly with fat body cells is secreted from the damaged body wall. Another is that some substance secreted from the damaged body wall stimulates another tissue to secrete a mediator molecule that interacts with fat body cells. We examined these possibilities by ligation experiments.

The middle of the body of third instar larvae was ligated with cotton thread, the posterior part then being pricked with a hypodermic needle. After 3 h, the anterior and posterior parts were separated at the position of ligation, RNA was extracted from both parts, and the contents of *Sarcophaga* lectin mRNA were examined by RNA blot hybridization. As summarized in fig.2, no appreciable *Sarcophaga* lectin mRNA was detected in either the posterior or anterior part of the fat body of unligated and undamaged larvae, but, after injury

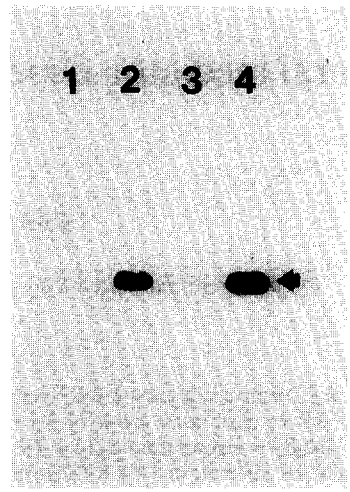


Fig.1. Fat body specific expression of the *Sarcophaga* lectin gene. The fat bodies of 10 third instar larvae were excised under a binocular microscope and combined. The remainders of the larvae without the fat body were also combined. RNA was extracted from the pooled tissues and analyzed by RNA blot hybridization. RNA extracted from: (lane 1) whole body of normal larvae; (lane 2) whole body of injured larvae (RNA was prepared 3 h after pricking the body wall with a hypodermic needle); (lane 3) carcass of injured larvae; (lane 4) fat body of injured larvae. Arrow indicates the position of *Sarcophaga* lectin mRNA.

of the posterior part of the body wall, both the posterior and anterior fat body were found to synthesize almost the same amount of *Sarcophaga* lectin mRNA. A significant amount of *Sarcophaga* lectin mRNA was also detected in ligated larvae without injuring the body wall, possibly due to the body injury caused by ligation. In these larvae, much more *Sarcophaga* lectin mRNA was synthesized in the anterior part of the fat body than in the posterior part, suggesting the participation of some tissue in the anterior part of the body in activation of the *Sarcophaga* lectin gene in the fat body. Probably, a substance secreted from the body wall when it is damaged by ligation is transmitted to this tissue in the anterior part and promotes the secretion of a mediator molecule that stimulates fat body cells to synthesize *Sarcophaga* lectin mRNA. This notion was supported by the finding that pricking the body wall of the posterior part after ligation did not result in any obvious increase in lectin mRNA in the posterior part.

These results suggested that the hemolymph

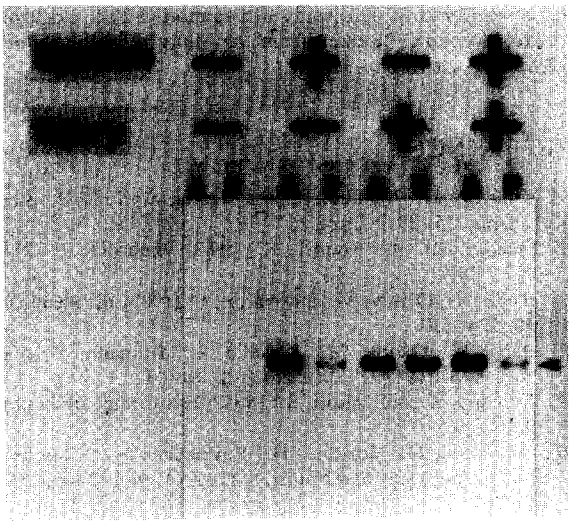


Fig.2. Effect of ligation on expression of the *Sarcophaga* lectin gene. The middle of the body of third instar larvae was ligated with cotton thread, and the posterior body wall was pricked with a hypodermic needle when necessary. The larvae were kept for 3 h at 27°C, and then posterior and anterior parts were separated with fine scissors and pooled separately. RNA was extracted from the two parts, which contained the posterior and anterior parts of the fat body, respectively, and analyzed by RNA blot hybridization. Since the fat body is the only organ that synthesizes *Sarcophaga* lectin mRNA, the RNA detected by blot hybridization reflects the amount of *Sarcophaga* lectin mRNA in the fat body. Four larvae were used in each experiment. (+) Ligation or injury; (−) no ligation or injury; (A) anterior and (P) posterior parts of the fat body. Arrowhead indicates the position of *Sarcophaga* lectin mRNA.

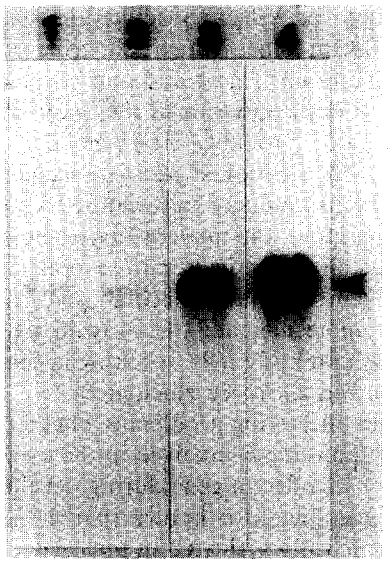


Fig.3. Expression of the *Sarcophaga* lectin gene in the posterior part of the fat body on injection of hemolymph. Larvae were ligated in the middle of the body, and the anterior half was cut off with fine scissors. Then 2-μl samples of hemolymph were injected through the ligated wound into the posterior body sac. After incubation of this sac for 2 h at 27°C, RNA was extracted and analyzed by RNA blot hybridization. Four larvae were used for each experiment. The hemolymph injected was collected from normal third instar larvae, or larvae 3 h after pricking the body wall. Posterior body sac: (lane 1) of normal larvae (control); (lane 2) posterior body sac into which a hypodermic needle had been inserted via the wound (mock injection); (lane 3) after injection of hemolymph from normal larvae; (lane 4) after injection of hemolymph from injured larvae. Arrowhead indicates the position of *Sarcophaga* lectin mRNA.

from injured larvae contains a mediator molecule that activates fat body cells to synthesize *Sarcophaga* lectin mRNA. To test this possibility, we investigated the effect of hemolymph prepared from injured larvae in activating the *Sarcophaga* lectin gene in fat body cells. For this a larva was ligated in the middle of the body, and then the anterior half was cut off. The body in the posterior half of the larvae did not synthesize *Sarcophaga* lectin mRNA, as described above. The effect of injecting hemolymph through the ligated wound into this body sac containing the posterior part of the fat body on the expression of the *Sarcophaga* lectin gene was examined by RNA blot hybridization. As shown in fig.3 (lane 2) no appreciable mRNA synthesis was detected in the fat body after simply inserting a hypodermic needle into the body cavity, but after injection of hemolymph from injured larvae, the *Sarcophaga* lectin gene was clearly activated (lane 4).

Injection of hemolymph from uninjured larvae also activated the *Sarcophaga* lectin gene, although to a lesser extent, as shown in lane 3 of fig.3. This activation may have been due to contamination of the hemolymph with mediator molecules produced during the preparation of hemolymph, since it is impossible to collect hemolymph without injuring the body wall and the specific tissue in the anterior part of the body that secretes the mediator molecule was probably stimulated during collection of the hemolymph which was usually done by centrifuging the larvae after cutting off their anterior tip.

These results strongly suggest that injuring the body wall induces some tissue in the anterior part of the larvae to produce a mediator molecule that

is essential for selective activation of the *Sarcophaga* lectin gene. Many endocrine organs, including the brain, ring gland and corpus allatum, are located in the anterior of the body, and one of these organs probably releases the mediator molecule. As mentioned above, sarcotoxins I, II and III are also induced in response to body injury [2–4]. Therefore, the same mediator molecule may be responsible for activation of several acute-phase protein genes. The molecular nature of this mediator molecule, and the way in which it interacts with fat body cells, resulting in specific gene activation remain to be elucidated.

Another interesting problem is whether the same mediator molecule is involved in the expression of the *Sarcophaga* lectin gene during development of *Sarcophaga*, since it is known that the same gene is also transiently activated in the early embryonic and pupal stages [7].

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