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## Mechanisms determining the outline shape of the adult lepidopteran wings

Ryuji Kodama

Wings of the lepidopteran insects (butterflies and moths) develop from the wing imaginal disc, which is a hollow sac made of simple epithelium. When the pupariation is completed, the wing, which was hidden inside the body wall of the larvae, is exposed on the surface of the pupa, which gradually turns into the adult wing. The outline shape of the adult wing is often different from that of the pupal wing. This difference is brought about by the programmed cell death of the marginal area of the pupal wing, while the internal area develops as adult wing blade. The marginal dying area is called the degeneration region and the internal area is called the differentiation region, hereafter.

The cell deaths in the degeneration region proceeds very rapidly and completes in a half to one day period in *Pieris rapae* or several other species examined. It was shown that the dying cells in the regeneration region have two characteristics common with the apoptotic cell death in mammalian cells. These are i) the presence of apoptotic bodies, which are heavily condensed cells or their fragments engulfed by other cells or macrophages, shown by transmission electron microscopy and ii) the presence of conspicuous accumulation of fragmented DNA evidenced by the TUNEL histological staining (Kodama, R. et al., Roux's Arch. Dev. Biol. 204, 418-426, 1995).

The cells in the degeneration region are actively engulfed by the macrophages in the cavity beneath the wing epithelium. Moreover, the macrophages seem to be concentrated beneath the degeneration region by the strong adhesion between basal surfaces of the dorsal and ventral epithelium in the differentiation region. By injecting the india ink or ferritin solution to the body cavity of the pupa, we have confirmed that this adhesion is tight enough to exclude the macrophages from the differentiation region, because the injected probes was found mostly concentrated in the degeneration region when observed several minutes later (Yoshida, A. (Biohistory Research Hall) and Kodama, R., unpublished).

Studies using another lepidopteran species, *Orgyia recens approximans*, provided by Drs. Y. Arita and K. Yamada (Meijo University) is underway. In this species, the wing is normally formed until the beginning of the pupal period, but becomes conspicuously degenerated only in the female adult. In our preliminary study, it was shown that the pupal wing is normally formed both in male and female pupa, but after about two days, female pupal wing starts degeneration on its margin, as if the degeneration region is continuously formed deep into the center of the wing (Kodama, R. et al., unpublished). It is thus suggested that the control mechanism which demarcates the region to be degenerated is defective in the female in this species. Further investigation using this species might give important insight into such mechanisms.

Fig.1. The tracheoles (fine threads) and the primary trachea (thick tube in the center) at the late stage of the pre-pupa

Another collaborative work with the laboratory of Dr. K. Watanabe (Hiroshima University) concerns mostly on the development of trachea and tracheole pattern in the swallow tail butterflies. Trachea and trcheoles are both important in delivering air into the wing and their pattern coincide with that of the boundary of degeneration and differentiation zones at the distal end of the wing. According to the observations, the pattern formation of wing epithelium is often dependent on tracheal and tracheole patterns. Basic research on the development of tracheal pattern formation is being done through the scanning electron microscopy and the bright field light microscopy of the fixed or fresh specimens to describe the exact pathway and the time course of the formation of elaborate pattern of trachea and tracheoles and to establish the cytological and developmental relationship between the formation of tracheal pattern and epithelial cell pattern, such as scale cell pattern.

The figure depicts how the tracheoles protrude from the primary trachea at the pre-pupa stage. These fine threads are arranged with even spaces and may closely related with the scale cell pattern formation (Fig. 1).

## Protein palmitoylation and developmental mechanism at embryogenesis

Kohji Ueno

We have studied the molecular mechanisms of the development of cells and organs in the silkworm *Bombyx mori* and have found that a high molecular weight protein (p260/270) was expressed in abdominal leg cells during early embryonic stages. p260/270 was identified to be a protein palmitoylase which transfers palmitate to cysteine residues of proteins. Almost of small GTP-binding, heterotrimeric G, and G-protein-linked receptor proteins are known to be modified with palmitate through thioester linkages. These dynamic modifications are thought to be important in regulation of signal transduction.

To understand the molecular mechanism how the modification of protein palmitoylation regulates the development of cells and organs, a search for a homolog of p260/270 in vertebrate was undertaken. Homology search of an ESTdb (Expressed Sequence Tags data base) with the amino acid sequences of p260 and p270 identified mouse embryonic cDNA clones which were highly homologous to the amino acid sequences of p260 and p270. Since analysis of these clones revealed that the cDNA contained a long open reading frame encoding 2504 amino acids which showed 94% homology to rat fatty acid synthase (FAS), it was concluded that a homologue of *Bombyx* p260/270, i.e. FAS, is expressed during mouse embryogenesis.

I*n situ* hybridization of mouse embryos revealed that the transcripts are detected mainly in the central and peripheral nervous system in mouse embryos from embryonic day 11.5. Immunocytochemical analyses of cultured mouse primary embryonic brain cells were performed to identify which cells express mouse FAS. This analysis revealed that mouse FAS was expressed specifically in neural cells in which growth-associated protein (GAP)-43 was expressed. GAP-43, by protein palmitoylation, regulates $G_o$ signal transduction and neural axonal growth. In a cell-free system, purified FAS from mouse embryos transferred palmitate to GAP-43 through cysteine residues. Furthermore, cerulenin, an inhibitor of FAS, reduced axonal growth and *in vivo* palmitoylation of GAP-43 in cultured neurons. Figure 2 shows the effect of cerulenin on neuron morphology.

From these results, mouse FAS was speculated to be responsible for the palmitoylation of GAP-43 and subsequent regulation of axonal growth in mouse embryos. Since we also found that p260/270 was expressed in brain cells in insect embryos, a protein palmitoylase was speculated to regulate the axonal development during embryogenesis in invertebrate and vertebrate.

### Publication List:

Kubo-Irie, M., Irie, M., Nakazawa, T. and Mohri, H. (2000) Spermiogenesis in the stag beetle, *Aegus lavicollis* Waterhouse (Coleoptera, Lucanidae), with special reference to the centriole adjunct. *Invert. Reprod. Develop*..**37**, 223-231.

Ueno, K. (2000) Involvement of fatty acid synthase in axonal development in mouse embryos. *Genes. Cells* **5**, 859-869

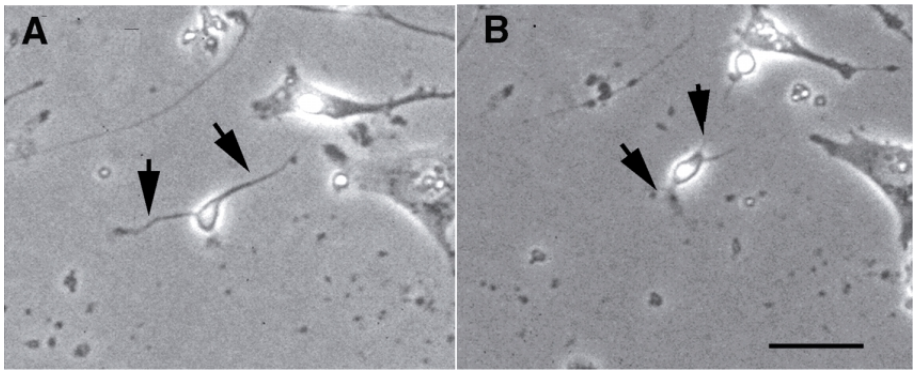


Fig.2. Effect of cerulenin on cultured primary neurons. Morphology was observed by phase-contrast microscopy after the addition of 400 µM cerulenin. (A); 0 min., (B) 120 min. Axonal outgrowths started to disintegrate and disappeared by 120 min. Arrows indicate axonal outgrowths. Bar represents 25 µm.