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主　論　文　の　要　旨

論文題目　Studies on the transcriptional regulation of genes involved in adult development in insects
（昆虫の成虫化に関わる遺伝子の発現制御機構に関する研究）

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論　文　内　容　の　要　旨

Insect molting and metamorphosis are controlled by molting hormone (ecdysone) and juvenile hormone (JH). The former induces each molt during post-embryonic development while the latter determines the nature of the molt. In the presence of JH, insects repeat the status quo (i.e. larva-larva) molts, preventing metamorphic changes, while they undergo metamorphic (i.e. larva-pupa and pupa-adult) molt once the concentration of JH in the hemolymph decreases. To date, a number of genes encoding for the transcription factors in hormonal signaling as well as the receptor protein have been identified.

The insect exoskeleton is composed of cuticle and wax layers, and the primary components of cuticle are chitin and cuticular proteins (CPs). Cuticle pigmentation and sclerotization occur in insect molting process. These are especially prominent in the adult development in beetles which have a hard exoskeleton and the elytra. Synthesis of adult cuticle followed by its tanning process (i.e. pigmentation and sclerotization) occurs during adult development. Genes encoding for enzymes involved in pigmentation and sclerotization, as well as structural CPs, have been identified and characterized in the red flour beetle *Tribolium castaneum*.

In previous studies in our laboratory using *T. castaneum*, it was reported that a topical application of a JH mimic (JHM) pyriproxyfen at pupation resulted in precocious dark coloration in legs. In the meantime, RNA interference (RNAi)-mediated knockdown of *ecdysone-induced protein 75B* (*E75*) gene, a transcription factor that works as one of the early ecdysone-response genes, resulted in precocious dark coloration in legs in *T. castaneum* pupae. Since the phenotype caused by JHM treatment resembled to that by *E75* knockdown, it raised a possibility that the genes involved in adult development are transcriptionally regulated by JH, and *E75* is involved in the signaling.

In this study, I first examined the hormonal regulation of adult cuticular pigmentation and sclerotization in *T. castaneum*. The effect of exogenous JHM treatment and *E75* knockdown on adult cuticular formation were examined in detail, in terms of morphological changes as well as altered expression of genes in hormonal signaling and in cuticular formation. In addition, expression analysis of *E75* and genes in cuticular formation was conducted in *Z. nevadensis* to obtain insights into its role in their development.

Study 1: Analyzing regulatory mechanism of genes directing adult development in the red flour beetle *Tribolium castaneum*

The red flour beetle *T. castaneum* is an important pest of stored grains. Apart from its importance as an insect pest, this insect has been used to unveil functions of various genes using RNAi techniques in each developmental stage. In this study, the molecular mechanism of adult cuticular formation was examined in detail.

An exogenous JHM treatment of Day 0 pupae did not inhibit pigmentation or sclerotization, but instead, induced precocious pigmentation of adult cuticle two days in advance. Quantitative RT-PCR analyses revealed that *E75* is downregulated in JHM-treated pupae. Meanwhile, *tyrosine hydroxylase* (*Th*), an enzyme involved in cuticular pigmentation and sclerotization, was precociously induced, whereas a structural cuticular protein *CPR27* was downregulated, by exogenous JHM treatment. RNA interference-mediated knockdown of *E75* resulted in precocious adult cuticular pigmentation, which resembled the phenotype caused by JHM treatment. Notably, upregulation of *Th* as well as suppression of *CPR27* were observed with *E75* knockdown. Meanwhile, JHM treatment suppressed the expression of genes involved in melanin synthesis, such as *Yellow-y* and *Laccase 2*, but *E75* knockdown did not result in marked reduction in their expression. Taken together, these results provided insights into the regulatory mechanisms of adult cuticular formation; the transcription of genes involved in adult cuticular formation proceeds in a proper timing with undetectable JH, and exogenous JHM treatment disturbs their transcription. For some of these genes such as *Th* and *CPR27*, *E75* is involved in transcriptional regulation. This study shed light on the molecular mode of action of JHM as insecticides; exogenous JHM treatment disturbed the expression of genes involved in the adult cuticular formation, which resulted in lethality as pharate adults.

Study 2: Analyzing the expression of *E75* and downstream genes during the caste development of dampwood termite *Zootermopsis nevadensis*

The dampwood termite *Z. nevadensis* is an invasive species introduced into Japan before 1999, and has been considered as a potential pest in Japan. To obtain insights into the role of *E75* in their development, expression analysis of *E75* was conducted.

Preliminary results revealed that the *E75* isoforms and *Th* were detected in the

different developmental stages of *Z. nevadensis*. The *E75C* had the highest expression level at third larval instar (L3) while the *E75D* had the highest expression level from L4 to 2nd nymphal stage (N2). The *E75E* had the lowest expression level all the studied developmental stage and almost undetectable at N2E stage. RNAi-mediated knockdown of each *E75* isoform is ongoing to reveal the roles of each isoform.

In summary, this study revealed the regulatory mechanism of genes that direct adult development of *T. castaneum*, and highlighted the significance of *E75*. In addition, preliminary results suggested that *E75* has an important role in post-embryonic development of *Z. nevadensis*, too. Besides these insights, this study shed light on the molecular mode of action of JHM as insecticides; exogenous JHM treatment at pupation disturbed the expression of genes involved in the adult cuticular formation, which resulted in lethality as pharate adults. Thus, this study has made a contribution to the field of plant protection.