論文の内容の要旨

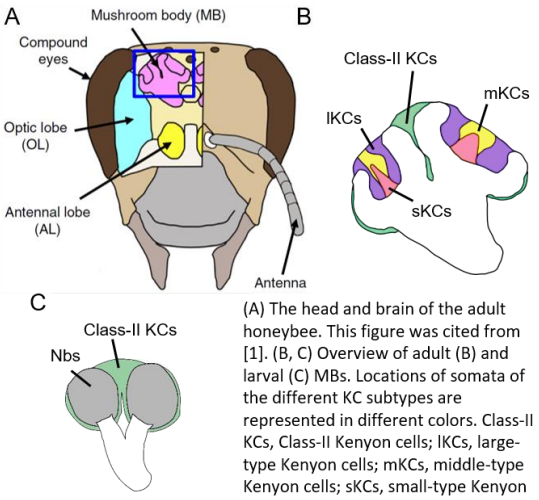
Expression analysis of genes expressed preferentially in the honeybee mushroom bodies during metamorphosis and functional analysis of PLC in learning and memory.

(ミツバチ脳キノコ体で選択的に発現する遺伝子の変態期での発現と PLC の記憶学習における機能の解析)

氏名 末次　翔太

The European honeybee (*Apis mellifera* L.) is a social insect, and its larvae cannot live alone, while the adult workers are engaged in various tasks to maintain colony activity. It is unknown how the brain structure and function underlying the honeybee social behaviors develop during its ontogeny. The mushroom bodies (MBs) are a higher order center of the insect brain (Fig. 1A) and the honeybee MBs compromise four Kenyon cell (KC)

Figure 1. Overview of the brain and MBs of the honeybee.

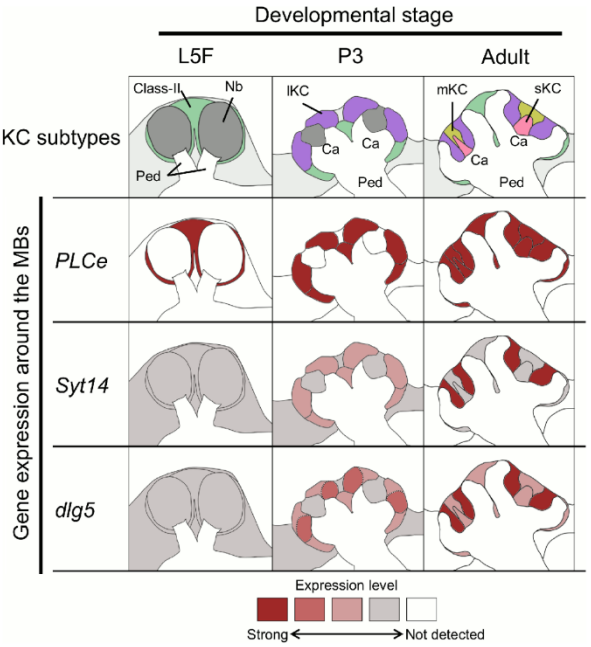


(A) The head and brain of the adult honeybee. This figure was cited from [1]. (B, C) Overview of adult (B) and larval (C) MBs. Locations of somata of the different KC subtypes are represented in different colors. Class-II KCs, Class-II Kenyon cells; lKCs, large-type Kenyon cells; mKCs, middle-type Kenyon cells; sKCs, small-type Kenyon cells; Nbs, neuroblasts.

subtypes (the class I large- [l], middle- [m], small- [s], and class-II KCs) that have distinct gene expression profiles (Fig. 1B) [1]. On the other hand, the larval honeybee MBs are consisted of only class-II KCs (Fig. 1C) [2]. However, it was unknown whether the larval and adult MBs are different in terms of gene expression. In my master course studies, I showed that *Phospholipase C epsilon* (*PLCe*), *Synaptotagmin 14* (*Syt14*), and *discs large 5* (*dlg5*) are expressed almost selectively in the MBs of the adult worker honeybee brains. Based on these results, in my doctoral course study, I first analyzed the expression of these genes in the larval and pupal brains.

In Chapter I, I used quantitative reverse-transcription polymerase chain reaction (qRT-PCR) to examine the expression levels of *PLCe*, *Syt14*, and *dlg5* in the brains of the larval and pupal honeybees. Expression of *PLCe*, *Syt14*, and *dlg5* increased from the larval to late pupal stage, suggesting that these genes play important roles in the later pupal and adult stages [3]. *In situ* hybridization analysis revealed that *PLCe* was preferentially expressed in the class-II KCs in the late larval brain while the preferential expression of *PLCe* was detected in both of the class-II and differentiating lKCs in the early pupal brain, indicating that *PLCe* is expressed in all KC subtypes irrespective of the developmental stages (Fig. 2) [3]. In contrast, while expression of *Syt14* and *dlg5* in the late larval brain was

Figure 2. Schematic illustration of expressions of *PLCe*, *Syt14*, and *dlg5* in the MBs in metamorphosis.

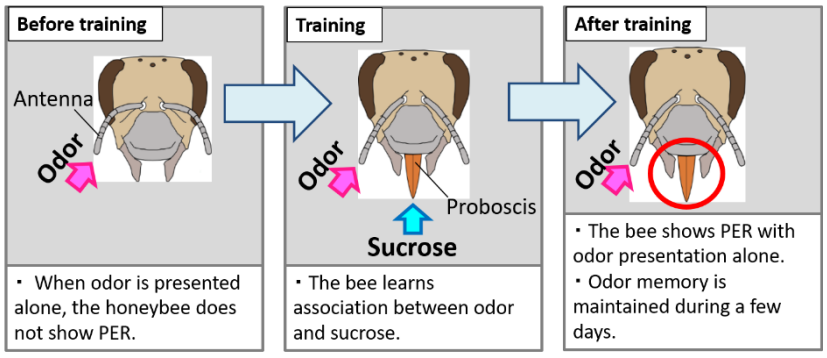


This figure was cited and modified from [3]. L5F, larval fifth-instar feeding stage; P3, pupal day 3 after pupation; Class-II, class-II Kenyon cells; Nb, neuroblasts; Ped, peduncle; lKCs, large-type Kenyon cells; Ca, calyx; mKCs, middle-type Kenyon cells; sKCs, small-type Kenyon cells.

very low, *Syt14* and *dlg5* started to be selectively expressed in the lKCs at the late pupal stage, indicating that the lKCs-selective expressions of *Syt14* and *dlg5* are acquired during midpupal to adult stages (Fig. 2) [3]. These results indicated that the larval and adult honeybee MBs have distinct gene expression profiles.

In Chapter II, I analyzed the role of PLC in learning and memory in the adult worker honeybee. For this, I planned to use pharmacologic inhibitors targeting PLC. Quantitative RT-PCR revealed that *PLCe* and the genes for other two PLC subtypes are expressed more strongly in the MBs than in other brain regions, proboscises and antennae, suggesting that PLC subtypes act mainly in the MBs in the worker brains. Furthermore, biochemical analysis revealed that two pharmacologic agents, edelfosine and neomycin, which are used as common PLC inhibitors in various animals, effectively inhibit PLC activity in the honeybee brain homogenate. Then I analyzed possible involvement of PLC in olfactory associative learning of the honeybee (Fig. 3) using these two inhibitors. When the honeybees were injected with edelfosine or neomycin into their heads, memory formation in training was declined in comparison to the mock-injected control groups. On the other hand, memory maintenance at 1 h and 24 h after training was not different from the control groups, suggesting that PLC is involved in the early stage of memory formation but not memory maintenance till 24 h in the adult worker honeybees.

Figure 3. Olfactory–proboscis extension reflex (PER) associative learning in the honeybee.



Although preceding studies showed that calcium/calmodulin-dependent protein

kinase II (CaMKII) is involved in the maintenance of odor memory in the honeybee [4, 5], my study first showed that PLC, an upstream factor in the calcium signaling, is involved in acquisition of memory in the honeybee. Considering that *PLCe* is also expressed in larval class-II KCs as well as adult MBs, it is possible that PLC also function in learning and memory even in the larval stages.

In summary, in my doctoral course study, I first indicated that larval and adult honeybee MBs have distinct gene expression profiles, and PLC functions in learning and memory in adult worker honeybees. I expect that these findings could contribute to give insights into the molecular and neural bases underlying honeybee social behaviors.

[References]

1. Kaneko, Suenami and Kubo. *Zoological Lett*. 2016; 2. Farris, Robinson, Davis, et al. *J Comp Neurol*. 1999; 3. Suenami, Paul, Takeuchi, et al. *PLoS ONE*. 2016; 4. Matsumoto, Sandoz, Devaud, et al. *Learn Mem*. 2014; 5. Scholl, Kübert, Muenz, et al. *J Exp Biol*. 2015