

Transcriptome analysis of soldier differentiation in the incipient colony of the damp-wood termite *Zootermopsis nevadensis*

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Termite soldiers are a unique caste among social insects in terms of their morphology and role. Soldier differentiation involves many developmental changes, but proximate mechanisms remain unclear. It is because we cannot identify the individual which will differentiate into a soldier in the natural condition. In incipient colonies of the damp-wood termite *Zootermopsis nevadensis*, however, the oldest 3rd instar larva (No. 1 larva) always differentiate into a presoldier, and the next 3rd instar (No. 2 larva) into the 4th instar (Maekawa et al. 2012). This is the first case showing that a particular individual differentiated into a presoldier before the molt. Thus, to elucidate molecular mechanisms of soldier differentiation, we focused on the presoldier differentiation from No. 1 larva, and compared the data with the molt into 4th instar from No. 2 larva. We performed transcriptome analyses using next-generation sequencing (RNA-seq), and expression and function analyses of the candidate gene during presoldier differentiation.

First, RNA-seq analyses showed that there was a significantly highly expressed gene *ZnNLaz*, the *Drosophila NLaz* homolog, in No. 1 larva. The ZnNLaz protein structure was inferred using the Phyre2 software, and shown to be completely similarity with the lipocalin family. We searched the particular protein regions in the sequence using pfam database, and identified that ZnNLaz had a conserved lipocalin 2 domein with a specific beta barrel structure. Next, we performed the RNA interference (RNAi) analysis to know if *ZnNLaz* functions in soldier differentiation. Double-stranded siRNAs or DDW were injected into No. 1 larva, and we observed the resulting molted individual phenotypes. Results showed that ZnNLaz RNAi strongly decreased presoldier differentiation rates, and most individuals molted into 4th instar. In the latter case, proctodeal trophallaxis from reproductives to No. 1 larva was significantly lower than the case for those undergoing presoldier differentiation.

Our results suggest that *ZnNLaz* is the candidate genetic determinant for soldier differentiation in *Z. nevadensis*. Soldier differentiation is known to be regulated by insect hormone (e.g. juvenile hormone) titers. Based on the gene expression analyses of hormone signaling, we will discuss about role of *ZnNLaz* and the proximate mechanisms during soldier differentiation in termites.