Expression and function of *Krüppel homolog 1* gene in *Riptortus pedestris*
ホソヘリカメムシにおける *Krüppel homolog 1* 遺伝子の発現と機能に関する研究

Li Dong

Introduction

Juvenile hormone (JH) has two major functions in insect development, i.e., suppression of metamorphosis in the larval stage and promotion of reproduction in the adult stage. *Krüppel homolog 1* (*Kr-h1*) encoding a zinc finger transcription factor acts downstream of the JH receptor complex, and therefore is known as an early juvenile hormone-response gene. The expression pattern and function of *Kr-h1* gene have been clarified in several insect species. The bean bug, *Riptortus pedestris,* shows a clear photoperiodic response in ovarian development: Female adults develop their ovaries under long-day conditions, but enter diapause with suppression of ovarian development under short-day conditions. This suppression of ovarian development is due to the cessation of JH secretion. In the present study in *R. pedestris*, I examined the expression pattern of *Kr-h1* and the effect of knockdown of clock genes on the expression of *Kr-h1*. Moreover, I examined the function of *Kr-h1* in adults and nymphs of *R. pedestris* by knockdown of the gene.

Materials and methods

R. pedestris were reared under short-day (SD, 12-h light and 12-h darkness) or long-day conditions (LD, 16-h light and 8-h darkness). The temporal expression pattern throughout a day was examined and the daily expression of *Kr-h1* was compared between SD and LD. After application of a JH analogue (JHA) or injection with dsRNA of *Kr-h1*, *Clock* (*Clk*) or *cryptochrome-m* (*cry-m*) to adults, they were dissected for

examining the reproductive status and relative transcript levels of *Kr-h1*, *Clk or cry-m*. Relative transcript levels of these genes were examined by qRT-PCR. After injection with dsRNA of *Kr-h1* to fourth (penultimate) instar nymphs, they were reared under LD until the molting to fifth (final) instar nymphs. The morphological characters of these nymphs were compared with normal fifth instar nymphs.

Results

Kr-h1 mRNA showed a slight fluctuation with a peak at Zeitgeber time (ZT) 8 and a trough at ZT 20 under LD, but the difference was not statistically significant. The expression levels of *Kr-h1* transcripts increased after day 5 and peaked at day 7 after transfer to LD. Under SD, JHA application induced ovarian development and increased the expression of *Kr-h1* transcripts to a level similar to that of intact insects under LD. *Clk* RNAi is effective for the suppression of *Clk* expression. *Clk* RNAi resulted in a significantly lower proportion of insects with ovarian development and significantly decreased the expression level of *Kr-h1* after transfer of the insects to LD. *cry-m* RNAi decreased the expression level of *cry-m*. *cry-m* RNAi resulted in a significantly higher proportion of ovarian development. The expression level of *Kr-h1* was significantly increased by *cry-m* RNAi in all experimental regimes.

The expression level of *Kr-h1* was significantly decreased by *Kr-h1* RNAi in the abdomen but not in the head. *Kr-h1* RNAi resulted in a lower proportion of ovarian development, but the difference was not statistically significant. *Kr-h1* RNAi is effective for suppression of *Kr-h1* expression in nymphs. Several adult features were found in the fifth instar nymphs with injection of *Kr-h1* dsRNA. The nymphs with adult features did not emerge even though they were reared under LD over 1 month.

Discussion

The daily *Kr-h1* expression patterns indicate that an increase in *Kr-h1* expression

precedes ovarian development. Application of JHA caused ovarian development and an increase of the expression level of *Kr-h1*, suggesting that both ovarian development and *Kr-h1* expression are induced by JH. The change of the expression level of *Kr-h1* induced by *Clk* or *cry-m* RNAi indicates that *Kr-h1* expression is regulated by a photoperiodic response in which a circadian clock with these clock genes is involved.

The necessity of *Kr-h1* for ovarian development is elusive based on the above results. The results that adult morphological features were induced by suppression of *Kr-h1* expression show that *Kr-h1* expression prevents metamorphosis in nymphs.

Conclusion

The above results demonstrated that expression of *Kr-h1* is JH dependent and strongly associated with a photoperiodic response in ovarian development. Although the function of *Kr-h1* in adults is still unclear, *Kr-h1* plays an important role in suppression of metamorphosis in nymphs.