Role of the clock gene *period* in the circadian rhythm and photoperiodism of the silkmoth *Bombyx mori*

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Introduction

Many organisms have ability of adaptation to daily and annual environmental changes. The circadian clock and the photoperiodism allow organisms to anticipate the daily environmental changes and to prepare accordingly. A molecular model of the circadian clock was first proposed in the fruit fly *Drosophila melanogaster*. This clock model producing the approximately 24 h period is described as an ensemble of interlocked negative transcription/translation feedback loops. Bünning (1936) first proposed a hypothesis that a circadian clock is involved in photoperiodism, and this concept is now wildly accepted.

period (*per*) is a clock gene that plays a role as an important negative regulator of the circadian clock in the *Drosophila* model. In Lepidoptera, although the presence of *period* has been shown, the PERIOD temporal localization pattern is different between several species and from *Drosophila melanogaster*. Thus, it remains necessary to examine the circadian clock in Lepidoptera, with special reference to the role of *per*. Involvement of clock genes in photoperiodism has been shown in several insects. Results that suppression or dysfunction of clock genes disrupts photoperiodism have been obtained exclusively in insects with photoperiodism controlling larval (nymphal) diapause or adult diapause. The juvenile hormone (JH) plays important roles in larval and adult diapause, and it is reported that a clock gene has a role in peripheral tissues at the downstream of the JH signaling pathway. Therefore, it is still unclear whether pleiotropic effects of clock genes or a circadian clock consisting of these genes causes the loss of photoperiodism by knockdown or knockout of the genes.

To solve these problems, I focus on the silkmoth, *Bombyx mori*, because both behavioral circadian rhythms and photoperiodism have been reported and highly efficient genome editing techniques have been established in *B. mori*.

Materials and Methods

per knockout strains were established from a nondiapause strain, *pnd w-1*, and a bivoltine strain, *Kosetsu*, by using TALEN.

To examine whether *per* plays a role in circadian rhythms, eclosion and hatching rhythms were compared between wild type strains (*pnd w-1* and *Kosets*) and *per* knockout strains produced from them. Quantitative real-time PCR was performed to analyze temporal expression changes of *per* and another clock gene, *timeless* (*tim*) mRNA.

To examine whether *per* is involved in photoperiodism, larvae of *Kosetsu* and the *per*-knockout strain derived from *Kosetsu* was reared under long-day or short-day conditions at 25°C, or short-day conditions at 20°C, and the diapause status of their progeny eggs was assessed.

Results and Discussion

The generated *per* knockout alleles were considered null, because in knockout from *pnd w-1* the allele encoded an extensively truncated form of PERIOD and in knockout from *Kosetsu* a new stop codon came up in the insertion allele. I used these *per* knockout strains for further experiments.

The wild type strains showed clear circadian rhythms in eclosion and hatching, whereas the *per* knockout strains showed arhythmic eclosion and hatching under constant darkness. In these strains, moreover, temporal expression changes of clock genes, *per* and *tim*, were disrupted. These results indicate that *per* is indispensable for circadian rhythms.

The wild type *Kosetsu* showed a clear long-day response: When larvae were reared under long-day and short-day conditions at 25°C, adults produced nondiapause and diapause egg, respectively. However, the *per* knockout strain originating from *Kosetsu* had lost the sensitivity to photoperiod and laid nondiapause egg under both conditions at 25°C. Moreover, when larvae of the *per* knockout strain were reared under short-day conditions at 20°C, some adults laid diapause eggs. Thus, *per* plays an important role in photoperiodism but not in the induction of diapause in *B. mori.*

Conclusion

The clock gene, *per*, is indispensable for behavioral circadian rhythms and photoperiodism in *B. mori*. The negative feedback loop of the circadian clock involving *per* functions for the production of circadian rhythms and the circadian clock consisting of *per* is involved in photoperiodism.