Studies on Regulatory Mechanisms of Pupal Metamorphosis in the Silkworm Using Genomics and Genome Editing.

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Insect molting and metamorphosis are intricately governed by two hormones, ecdysteroids and juvenile hormones (JHs). JHs prevent precocious metamorphosis and allow the larva to undergo multiple rounds of molting until it reaches the appropriate size for metamorphosis. Although it has been widely accepted that this "*status quo*" action of JHs is required to maintain the larval status throughout the larval stage, several attempts to deplete JHs using classic and modern techniques have failed to induce precocious metamorphosis during the very early larval instars.

I have been working on the hormonal control of insect molting and metamorphosis using the silkworm, *Bombyx mori*. Using the whole genome sequence of the silkworm, I identified the gene responsible for the *mod* (*dimolting*) mutant, in which pupal metamorphosis is induced precociously [1]. Silkworms typically have five larval instars, but *mod* mutant larvae undergo pupal metamorphosis after the third or fourth larval instar. I showed that the *mod* mutant carries a null mutation in the cytochrome P450 gene *CYP15C1*. Biochemical experiments demonstrated that CYP15C1 is essential for the biosynthesis of JHs, and *mod* larvae lack JHs in the hemolymph. This finding raised a fundamental question why JH-deficient larvae are not able to metamorphose after the first or second instar. To address this question, I applied genome editing techniques to the silkworm and established knockout lines having null mutations in JH biosynthetic enzymes or JH receptors. Using these lines, I provided definitive evidence that embryogenesis and maintenance of juvenile status during the early larval stages are largely independent of JHs or the JH signaling pathway [2]. Furthermore, the results also suggest that an unidentified factor or a signal is required to acquire the competence for metamorphosis.

I am hopeful that my research will lead to a better understanding of molecular bases of insect metamorphosis and to the design of target-specific, biorational chemicals for insect pest control.

References

[1] Daimon T, et al. (2012) Precocious metamorphosis in the juvenile hormone-deficient mutant of the silkworm, *Bombyx mori*. PLoS Genet 8(3):e1002486.

[2] Daimon T, Uchibori M, Nakao H, Sezutsu H, & Shinoda T (2015) Knockout silkworms reveal a dispensable role for juvenile hormones in holometabolous life cycle. Proc Natl Acad Sci U S A 112(31):E4226-4235.