Elucidation of molecular mechanisms underlying sex determination and larval color pattern formation in the silkworm, *Bombyx mori*, using sophisticated genetic tools

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Abstract

Sericulture has entered a new era. Genetic engineering of the silkworm, *Bombyx mori*, developed new sericulture, i.e., production of functional silks and utilization of the silkworm as bioreactors. Furthermore, the Ministry of Agriculture, Forestry and Fisheries and the Minister of the Environment approved breeding of genetically modified silkworms at a farm. In this situation, we need more useful silkworms than before. To generate advanced silkworms, it is important to enhance both basic and applied sciences: understanding of molecular mechanisms and improvement of genetic engineering tools. Here, we identified sex- and collar pattern-determining genes to artificially control silkworm sex and larval pigmentation patterns in the near future.

Female silkworms carry W and Z sex chromosomes, whereas males carry a pair of Z chromosomes. The W chromosome has a dominant role in female determination, indicating that the existence of an unknown feminizing factor on this chromosome. Using comparative transcriptome analysis between molecularly sexed male and female embryos, we identified a W-chromosome-derived, female-enriched non-coding RNA. We also noticed that a single PIWI-interacting small RNA (piRNA) was produced from the W-chromosome-derived RNA. To understand the function of the female-specific piRNA, a piRNA inhibitor that is complementary to the piRNA was designed and injected into embryos. Inhibition of the piRNA-mediated signaling in female embryos led to the production of male-specific splice variants of *Bombyx mori doublesex* (*Bmdsx*), a gene which acts at the downstream end of the sex differentiation cascade. From these results, we named the piRNA precursor, *Feminizer* (*Fem*).

PIWI-piRNA complex usually targets transposable elements and cleavages them by the nuclease activity of PIWI protein. Interestingly, the cleavage target of *Fem*-derived piRNA was a protein-coding gene on the Z chromosome. siRNA-mediated silencing of this uncharacterized gene in male embryos caused expression of female-specific splice variants of *Bmdsx* and male-specific embryonic lethality. Upregulation of Z-linked genes was also observed in the siRNAs injected male embryos, indicating that this gene, which we named *Masculinizer* (*Masc*), controls not only masculinization but also dosage compensation. The masculinizing activity of *Masc* ortholog was also confirmed in another lepidopteran insect, *Trilocha varians* (Bombycidae) using embryonic RNAi. In addition, rescue experiments by injecting *in vitro* synthesized *Masc* cRNA into endosymbiotic bacterium *Wolbachia*-infected embryos demonstrated that the endosymbionts selectively kill male Asian corn borer, *Ostrinia furnacalis*, by targeting the host *Masc* gene and disrupting the dosage compensation.

Many color pattern mutants have been preserved in Japan. Responsible genes for these color mutants are available as phenotypic markers in order to establish transgenic silkworm strains and maintain them. Forward genetics approach and siRNA-mediated knockdown revealed that translucent larval skin of *ok* (*kinshiryu translucent*) and *otm* (*tanaka's mottled translucent*) mutants are caused by inability to incorporate and accumulate uric acid into the epidermal cells, respectively. Abnormal pigmentation patterns of *q* (*quail*) mutant were clearly reproduced by recently developed CRISPR/Cas9-mediated gene knockout.