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Function of the *decapentaplegic* and *twisted gastrulation* Genes in Wing Vein Formation of the Sawfly, *Athalia rosae ruficornis* Jakovlev (Hymenoptera)*

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Decapentaplegic (Dpp) protein acts as a morphogen that is secreted into the extracellular region and specifies cell fate in a concentration-dependent manner. In the early embryonic stage of *Drosophila melanogaster*, Dpp protein plays an important role in establishment of the dorso-ventral axis. Dpp protein forms a complex with Twisted gastrulation (Tsg) and Short gastrulation (Sog) proteins and is transported over a relatively long distance (O'Connor *et al*., 2006). Such molecular mechanism is also involved during wing vein formation at the pupal stage in *Drosophila*. Dpp protein is expressed in longitudinal vein cells and transported by forming a complex with Crossveinless (Cv; a homologous protein of Tsg) and Sog proteins to particular cells of the intervein region, and finally differentiates these cells into crossvein cells (Shimmi *et al*., 2005; O'Connor *et al*., 2006).

Function of the *dpp* gene in the early embryonic stage has also been investigated in certain other insect species (Sanchez-Salazar *et al*., 1996; Yamamoto *et al*., 2004; Angelini and Kaufman, 2005; Zee *et al*., 2006). The findings suggest that a similar regulatory mechanism involving the Dpp protein in establishing the embryonic dorso-ventral axis is probably conserved in insects. However, conservation of this mechanism in wing vein formation remains largely unknown in insects other than *Drosophila*. The sawfly, *Athalia rosae ruficornis* Jakovlev is a suitable species to address questions about the relationship between morphological diversity of wing veins and molecular mechanisms of wing vein development, because gene functional analyses in larval-pupal and pupal-adult transitions are feasible in this species by gene knockdown using RNA interference (RNAi) by introducing double-stranded RNA (dsRNA). In addition, the complicated vein patterns of *A. rosae ruficornis* compared to those of *Drosophila* and differences in venation between the forewing and hindwing are favorable features for investigating interspecific and intraspecific modification of the mechanisms. As the first step, we analyzed the function of *dpp* and *tsg* genes using RNAi method.

First, we injected a 252-bp-long dsRNA targeting the *dpp* gene and a 440-bp-long dsRNA targeting the *tsg* gene to the last instar larvae in which wing veins are not yet formed, and observed their phenotypes in the wing primordia at the late prepupal stage, when wing vein patterns are established. Injection of each dsRNA to the last instar larvae resulted in failure of the proper development of wing veins. After injecting 3 μg of *dpp* dsRNA, wing veins were completely lost (n = 10, 9 survived and examined). Three micro-grams of *tsg* dsRNA injection resulted in a relatively milder phenotype in which parts of wing veins residually developed (n = 9, 6 survived and examined). When doses ranging from 30 ng to 6 μg of *tsg* dsRNA were injected, the phenotypes appeared similar to those after 3 μg-injection. These findings indicate that *dpp* and *tsg* genes are involved in wing vein formation of *A. rosae ruficornis*. In *Drosophila*, Dpp protein synthesized in the longitudinal veins is transferred by Cv and Sog proteins to intervein cells nearby and contributes later to the formation of crossveins (Shimmi *et al*., 2005; O'Connor *et al*., 2006). To see if the similar molecular regulation works in *A. rosae ruficornis*, we injected these dsRNAs at later stages: early prepupal stage when wing primordia just start forming some veins, or at the mid prepupal stage when their wing veins are formed to some degrees. Injection of *dpp* dsRNA to early prepupae resulted in defective wing vein formation in both longitudinal and

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crossveins, while *tsg* dsRNA injection to early prepupae only slightly affected the phenotype of wing veins those were formed nearly normally. After mid-prepupal injection of *dpp* dsRNA and *tsg* dsRNA, normal wing veins were formed and there were no apparent knockdown effects on their phenotypes. These findings suggest that the *tsg* gene acts at earlier stages to form longitudinal veins in *A. rosae ruficornis*. Involvement of the *dpp* and *tsg* genes in wing vein formation is conserved, although the detailed molecular interactions seem to differ slightly between *A. rosae ruficornis* and *Drosophila*.

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