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A System to Control Gene Expression in the Transgenic Sawfly, *Athalia rosae ruficornis* Jakovlev (Hymenoptera)*

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Stable germline transformation utilizing transposon-derived vectors has been achieved in more than 20 insect species across four orders, Diptera, Coleoptera, Lepidoptera and Hymenoptera (review: Kramer, 2004). Functional analysis of a gene by its conditionally induced misexpression has become much easier with transgenic techniques in insects other than experimental model species such as the fruit fly, *Drosophila melanogaster*. The sawfly, *Athalia rosae ruficornis* Jakovlev is one of the non-model species in which germline transformation is feasible (Sumitani *et al.*, 2003). We have been studying the underlying molecular mechanisms of egg maturation, egg activation, fertilization, early embryonic development and sex-determination in *A. rosae* (review: Oishi *et al.*, 1995, 1998). A technique for the targeted expression of transgenes is the key to unraveling gene functions, especially if the gene of interest affects viability. However, there is the issue of the scarcity of known promoters that drive spatiotemporal gene expression in *A. rosae* as well as in other non-model species. Here we demonstrate that a system for targeted gene expression based on a bacterial tetracycline-resistance operon is applicable to *A. rosae*.

The system examined is the one that is based on the tetracycline-controlled transactivator (tTA) and its response element (TRE). The tTA activates gene expression by binding to a TRE placed upstream of a target transgene. The advantage of this system is that the targeted gene expression is negatively controlled by tetracycline (Tet-Off system). Tetracycline bound to tTA forms a complex and interferes with the binding of tTA to TRE, resulting in silencing of the target gene. The effectiveness of this system was shown in some dipteran species (review: McGuire *et al.*, 2004). Tetracycline can be applied as a food supplement (Bello *et al.*, 1998).

Two types of transgenic strains of *A. rosae* were established: one bore the *Drosophila heat shock protein 70 (hsp70)* gene promoter-driven tTA marked with the enhanced cyan fluorescent protein (ECFP) gene with eye-specific expression (tTA strain), and the other bore the enhanced green fluorescent protein (EGFP) gene as a reporter gene placed downstream of the TRE and marked with the red fluorescent protein (DsRed) gene (TRE-EGFP strain). Both strains were viable and quite normal in fecundity. Individuals bearing both transgenes (tTA;TRE-EGFP) were produced by crossing the two strains and screening for the progeny with eye fluorescence. EGFP was detected in embryos bearing both transgenes (tTA;TRE-EGFP), but not in embryos bearing either one of the transgenes (tTA;+ and +;TRE-EGFP) regardless of heat-shock treatment. EGFP was expressed even when tTA;TRE-EGFP embryos were reared at low temperature (20°C; usual rearing temperature is 25°C). The results indicated that this system worked to induce targeted gene expression, while the *Drosophila hsp70* gene promoter was not able to regulate gene expression strictly in a temperature-dependent manner in *A. rosae*.

Suppression of the targeted gene was examined by supplementing tetracycline to the diet. Tetracycline was dissolved in diluted honey at a final concentration of 100 μg/ml and given to parental females. Oral application of tetracycline did not affect their viability and fertility. EGFP expression was completely suppressed in all tTA;TRE-EGFP embryos produced by crossing tetracycline-fed females of one strain and tetracycline-unfed males of the other strain. We concluded that the Tet-Off system was applicable to *A. rosae* as an

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effective tool to regulate the expression of transgenes independent of promoters.

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