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Technical Tips

Vector-Dependent Gene Expression Driven by Insulated P-Element Reporter Vectors

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Drosophila, transgenic, reporter gene assay, cis-regulatory element, Su(Hw), insulated vector, salivary gland

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ABSTRACT

The Pelican and Stinger series of P-element transformation vectors are a popular choice for reporter gene expression in transgenic flies. We report here as a cautionary note that these vectors on their own can drive reporter gene expression in the larval and pupal salivary gland.

INTRODUCTION

Transgene insertions in *Drosophila* are subject to chromosomal position effects, which can lead to different levels of transgene expression in independent transgenic lines. The use of transformation vectors carrying insulator sequences that buffer against position effects, such as the Su(Hw) insulator (reviewed by West et al.[1]), can alleviate this problem and insure similar transgene expression levels for different chromosomal insertions of the same construct. A widely used set of vectors for this purpose is the Pelican and Stinger series of vectors constructed by Barolo et al.[2,3] These are insulated P-element transformation vectors with extensive cloning sites that can be used to generate reporter gene expression by promoter fusion or by insertion of regulatory sequences in front of a minimal *hsp70* promoter. Versions with *lacZ*, cytoplasmic and nuclear *GFP*, and *DsRed* reporter genes are all available. We report here the previously undocumented observation that the pH-Stinger and pRed-H-Stinger vectors by themselves mediate reporter gene expression in the salivary glands of late third instar larvae and pupae.

RESULTS

We recently generated a series of transgenic flies to test the regulatory activity of putative cis-regulatory elements from five different loci. Three of these were constructed using pH-Stinger and two using pRed-H-Stinger. In all of these transgenic flies, including multiple independent insertions of the same construct, we observed similar reporter gene expression in salivary glands during late larval and early pupal stages (Fig. 1A and B). However, the endogenous genes were not expressed in salivary glands during the same stage as assessed both by RNA in situ hybridization and RT-PCR. Based on this observation, we speculated that the GFP/RFP expression in salivary glands was vector driven.

To evaluate the effects of the vectors, we generated transgenic flies bearing vector only. Because pRed-H-Stinger is similar to pH-Stinger except for the replacement of the *EGFP* gene with the *DsRed* gene, and same reporter gene expression pattern was observed in transgenic flies containing enhancer fused with either vector, only pH-Stinger was tested. We obtained three independent pH-Stinger transgenic lines, and GFP expression can be identified clearly in each line in salivary glands during late larval and pupal stages (Fig. 1C and D). GFP expression can also be observed in adult flies (Fig. 2). No GFP or RFP expression is detected in wild-type Oregon R flies (Fig. 1E and F and data not shown).

CONCLUSION

Our data suggest that the salivary gland expression we observed with our initial series of enhancer-containing reporter constructs was an artifact of the pH-Stinger and pRed-H-Stinger vectors only. Caution should therefore be taken when using these vectors to assay the activity of putative regulatory sequences, as the salivary gland expression driven by these two vectors can lead to the erroneous assignment of regulatory activity to non-regulatory sequences.

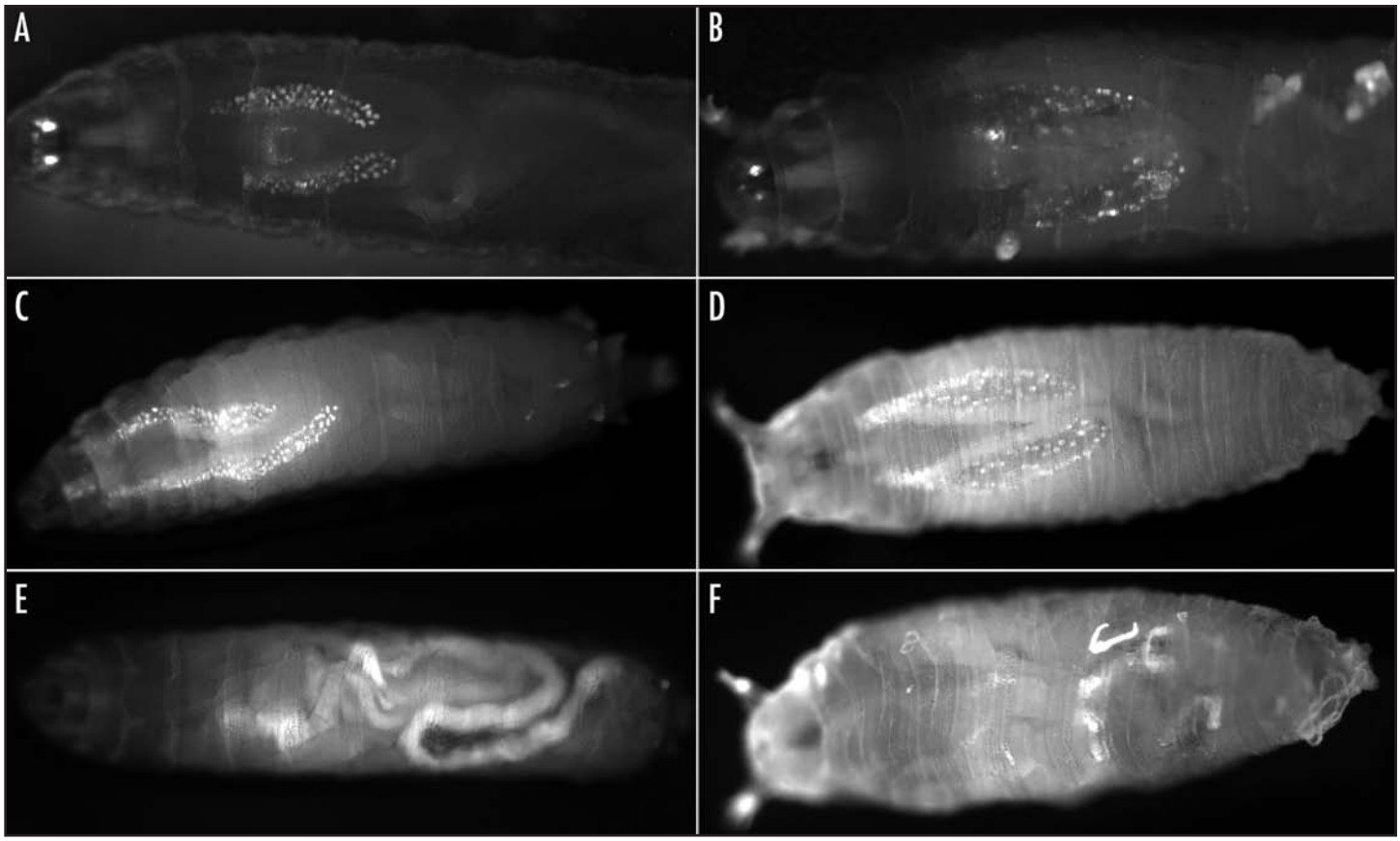

Figure 1. (A and B) DsRED expression was observed in larva (A) and pupa (B) of transgenic flies containing a reporter gene construct made using the pRed-H-Stinger vector. (C and D) GFP expression was observed in larva (C) and pupa (D) of transgenic flies containing the pH-Stinger vector only. (E and F) No GFP expression can be detected in larva (E) or pupa (F) of wild-type Oregon flies. All panels show ventral views. Pictures were taken using a Leica MZFLIII stereomicroscope, Q-imaging digital camera and OpenLab imaging software.

Figure 2. Lateral view of adult transgenic flies containing pH-Stinger vector only.

References

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