Transient expression of *tryptophan oxygenase* gene from *Anopheles gambiae* in the green eye mutant of the housefly, *Musca domestica* (Diptera: Muscidae)

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Abstract

A plasmid construct containing the gene encoding *tryptophan oxygenase* (to) from *Anopheles gambiae* was introduced into green eye color mutants (ge) of the housefly, *Musca domestica*. Several variations in eye color were observed in 38.8% of the surviving G0 adults. The expression of *A. gambiae to* in the distantly related Diptera, housefly, implies conservation of the *tryptophan oxygenase* gene in the other Diptera and may be used for monitoring transformants as a marker in insects which have mutants homologous to *Drosophila melanogaster* vermilion.

Key words: *Musca domestica*, phenotypic marker, transient expression, *tryptophan oxygenase*, vermilion

INTRODUCTION

Since successful transformations by the P element in *Drosophila melanogaster* (Rubin and Spradling, 1982), many attempts to transform other insects have failed for several reasons. First, there is no established vector to carry DNA into the insect genome. The mobility of the P element, which is widely used for *Drosophila* transformation, is limited to only *drosophilids* (O’Brochta and Handler, 1988). Therefore, alternative transposable elements which have the potential to move in a broad range of organisms are required. Secondly, there is a lack of an appropriate selectable marker. In the genus *Drosophila*, the eye color genes, *white*, *cinnabar* and *rosy* are useful genetic markers, and combined with the P element, have been used as an effective genetic vector system. Such a system needs to be established for other insects. The third reason is the choice of a suitable promoter. The *Drosophila Hsp70* gene has been used in many insects, but it is known that this gene is not very effective in some species (Coates et al., 1996). The recent success of transformations in the mosquitoes, *Aedes aegypti* with mariner, *Hermes* and the medfly, *Ceratitis capitata* with piggyBac indicate the use of these transposable element vectors for a wide variety of insects (Coates et al., 1998; Handler et al., 1998; Jasinskiene et al., 1998). However, the second and the third problems still remain. In particular, it is important to identify selectable phenotypic markers. Resistance genes to toxic chemicals have previously been used (Miller et al., 1987). Nevertheless, germline transformation of *C. capitata* using a neomycin resistance marker was unsuccessful, and neomycin resistance as a marker has proved to be unsuitable for selection even in *Drosophila* (Ashburner, 1989). On the other hand, the first successful transformation other than *Drosophila*, has been reported in the medfly *C. capitata* with the *Minos* element (Loukeris et al., 1995). In this case, the white eye gene as a visible marker contributed to the efficient detection of transformants even though transformation frequency was low. Thus, the significance of eye color gene markers showed that phenotypic markers are required for establishing transformants and leads to the success of transgenesis.

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The *Drosophila melanogaster* tryptophan oxygenase gene, *vermilion* (v) serves as a selectable marker for *Drosophila* transformation (Fridell and Searles, 1991). Homologous mutants for *D. melanogaster vermillion* are green (ge) in the housefly, *Musca domestica*, and *vermillion* has been shown to complement the *tryptophan oxygenase* (to) mutant when injected into *ge M. domestica* embryos (White et al., 1996). Accordingly, *Autographa californica* baculovirus *ie-1* promoter drives expression in cell lines and may be useful as a promoter (Jarvis, 1993). *A. gambiae* to gene containing this baculovirus promoter was expressed in *D. melanogaster vermillion* (Besansky et al., 1997). In this paper, we investigated the function of *A. gambiae* to gene expressed from *ie-1* promoter in the diverged Diptera, housefly, to determine its potential as an effective marker.

**MATERIALS AND METHODS**

**Insect.** Green eye color mutant strain (ge), kindly provided by E. Bryant from The University of Houston, was used. The eye color of this mutant fly was yellowish green, while that of wild type fly was blackish red. Houseflies were kept as described by Zhang and Shono (1997) at 25°C with a relative humidity of 60% and a 16:8 light-dark cycle. Eggs were collected from adult *ge Musca domestica* 7 days after emergence.

**Microinjection and DNA construct.** pIE1-to plasmid which contains the *A. gambiae* to cDNA under baculovirus promoter (Besansky et al., 1997) was used. In short, the to cDNA was originally cloned from *A. gambiae* by Mukabayire et al. (1996) and a baculovirus promoter (ie-1) from *Autographa californica* (Cartier et al., 1994) was added upstream of the to. Then, this chimeric gene (IEI-to) was inserted into the NotI site of pBluescript to generate pIE1-to. Plasmid DNA was suspended in phosphate buffer (5 mM KCl, 0.1 mM NaPO₄, pH 6.8) and injected into the embryos as described by Yoshiyama et al. (2000).

**RESULTS AND DISCUSSION**

Microinjections were performed with two different concentrations of plasmid DNA. In the first experiment, using DNA construct at a concentration of 500 ng/µl, 17 surviving G₀ adults emerged from 51 injected embryos. No eye color changes were observed in this experiment. Next, 75 embryos were injected with DNA constructs at a concentration of 1,000 ng/µl. Of 49 adults that survived, 19 individuals showed phenotypic rescue in eye color. As a control, buffer alone was injected into 30 embryos. Twenty six G₀ adults survived and no eye color rescue was observed. The estimated volume of injection was almost the same, therefore, this suggests that the total quantity of to products is important and a concentration of 500 ng/µl was not sufficient to rescue eye color. Although we did not try other concentrations, it might be possible that the higher plasmid DNA concentrations drive a higher rate of transient expression. Further analysis is necessary to determine the best concentrations of plasmid DNA for transient expression of to gene.

In our results, 38.8% (19/49) of adults survived and showed several variations from yellow orange to red orange in the rescued eye color (Fig. 1). These eye colors were distinctively different from either ge or wild phenotypes. Similar color variations were also reported to result in eye color phenotype when *D. melanogaster vermillion* was injected into the housefly (White et al., 1996). The rescued color was evenly distributed throughout the entire area of each eye and no mosaic eyes were observed. In addition, the rescued eye color did not change as the housefly grew. None of the surviving G₀ flies were used for production of the next generation and the effect on fertility was not investigated.

The results of these experiments confirm that *A. gambiae vermillion* gene is expressed in the distantly related Diptera, the housefly, and the to gene appears to be functional among the Diptera.

Recently, the effectiveness of ie-1 baculovirus promoter has been shown in live *D. melanogaster* (Besansky et al., 1997). In this paper, we showed a comparable result using *M. domestica*, which predicts the utility of this promoter not only in the housefly but also in other insect species.

Homologous mutants for the *Drosophila melanogaster vermillion* gene can be easily de-
tected, because mutant eye color can be rescued by feeding the insects kynurenine. These mutants seem to exist in many insect species, such as *snow* in the honeybee, *Apis mellifera*, *salmon* in the Tsetsefly, *Glossina morsitans morsitans* and *yellowish* in the Australian sheep blowfly, *Lucilia cuprina* (Summers and Howells, 1978; Tucker, 1986; Gooding and Rolseth, 1987). Therefore, the *to* gene can be conserved among many insects and it would be an effective genetic marker in many insects which have homologous to *to* mutants. Finally, the construct combined with *ie-1* promoter and *to* gene would be a strong vector when used with a suitable transposable element.

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