CONDITIONAL LETHAL GENES TO CONTROL COTTON PESTS

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Abstract

A mutation of the common *Notch* gene acts as a temperature dependent lethal during development when exposed to temperatures below 20° C during embryogenesis. The mutant gene is dominant and a single copy expresses the lethal phenotype in the presence of two copies of the normal *Notch* gene. Transformation strategies are being used to construct pink bollworm strains that contain copies of the mutant *Notch* gene. These strategies employ the newly discovered transposable elements, *Hobo*, *Hermes* and *PiggyBac*.

Introduction

Ever since the P elements were demonstrated to selectively transform the vinegar fly, *Drosophila melanogaster*, in 1982, a means has been sought for routine transformation of insects to construct strains for a variety of purposes. These include developing insecticide resistant strains of beneficial insects to improve integrated pest management, improving honey production in honey bees, improving pollination by Hymenoptera, improving the sterile insect technique by using sexing techniques with genetic markers, and developing other genetic control techniques.

Since the P elements were inactive in other insects, other transposable elements (TEs) were sought as substitutes. TEs have no phenotype and are therefore very difficult to identify in insects. Nevertheless, a growing number of TEs have been discovered in insects that have served as vehicles to drive insect transformation (Robertson and Lampe, 1995). A partial list of TEs is given in Table 1. along with the year of successful transformation. Accompanying references provide the published citation. A question mark indicates a transformation that was reported but not published. We include only reports from reliable workers, but must be considered preliminary until actually published.

The first non-Drosophiloid insect transformation is considered to be that of Mediterranean fruit fly reported by Loukeris, et al. (1995). This work reported the use of the transposable element, Minos, originally isolated from *Drosophila hydei*, to transform the Medfly. For a marker system to help analyze the putative transformants, the white eye gene was used along with a mutant white eye strain of Medfly. Successful or partially successful transformation was indicated by the amount of wild type eye color the inserted plasmid was able to rescue.

We have been studying the Notch gene of animals with a view toward developing a conditional lethal gene to insert into insects. Once inserted, the strain obtained could serve as a genetic control tool suitable for use in the field. The *Notch* gene is highly conserved in all animals examined. A specific mutation, numbered 60gll was obtained by surveying survivors of gamma irradiation from a *Drosophila* colony.

Methods

Female pink bollworms oviposit on glass slides that are placed on top of oviposition containers. A barrier of wax is placed around the eggs and filled with oil. Glass microcapillaries are pulled on a Sachs-Flaming PC-84 microelectrode puller, filled with DNA solution, attached to a World Precision Instruments PV830 pneumatic picopump and mounted on an Eppendorf 5171 manipulator (Peloquin, et al., 1997). Eggs are penetrated, injected near the pole cell end, then harvested and placed on rearing medium. Dechorionation of the eggs is unnecessary for pink bollworm eggs. About 6% of the sham injected eggs develop to the point of eclosion, but then die probably from side effects cause by oil contamination.

Stabs of cDNA for *Notch* were obtained as gifts from Spyros Artavanis-Tsakonis' lab which included the *Notch* promoter region. Hobo derived plasmids were obtained from Al Handler in Florida. The *Notch* gene was truncated near the same region as the original 60gll mutant *Notch* gene, inserted into the hobo element and injected along with a helper plasmid that contained the transposase coding region of the hobo element. A genomic DNA Pink bollworm library was surveyed using probes made from hobo itself and none were found. Thus once inserted, the mutant Notch gene should be unable to move due to inherent transposable element activity.

Results and Discussion

Electro-mechanical injection of pink bollworm eggs was compared and found greatly superior to mechanically operated injection (Table 2.). From these results, it was obvious that mechanical manipulation of the injection needle cause severe damage to the eggs. The Electro-mechanical device provided a smooth motor driven entry, injection and withdrawl along the same axis as penetration of the egg. The electro-mechanical device is expensive, but advantages in its use outweight the cost. Notice in particular the number of adults obtained by both methods compare to the numbers of eggs injected.

The first strains obtained from modified Notch injections included an adult male that had a rough eye phenotype. This is exactly the type of phenotype obtained from the

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original mutant Notch *Drosophila* strain that the Notch gene came from. We were skeptical that the rough eye phenotype signified transformation because rough eye traits are know to occur spontaneously in culture.

Other groups involved in insect transformation are using selectable marker genes to help with strain isolation. Presence of the opd resistance trait can be used to protect transformed insects against organophosphorus insecticide treatment, for example. However, we are leery of introducing a resistant gene into a major pest insect like pink bollworm, and hope to use normal Notch phenotypic expression to guide selection.

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Table 1. Transposable elements used in insect transformation and sources.

Name	Source	Transformation
P	*D. melanogaster*	*D. melanogaster* (1982)
hobo	*D. melanogaster*	*D. melanogaster* (1989)
		D. virilis (1996)
		Pectinophora gossypiella?
Mariner	*D. mauritiana*	*D. melanogaster* (1993)
		D. virilis (1996)
Minos	*D. Hydei*	*D. melanogaster* (1995)
		Ceratitis capitata (1995)
Hermes	*Musca domestica*	*D. melanogaster* (1996)
		M. domestica?
piggyBac	GmMNPV culture	*Trichoplusia ni*?
		Plodia interpunctella?
		Cydia pomonella?
		Ceratitis capitata?

The question mark indicates an unpublished, but reliable reference. The *D.* is *Drosophila*.

Table 2. Percent survivors of DNA injections into eggs by mechanical (Narashigi) manipulator and by electro-mechanical (Eppendorf) manipulator.

	Number		Percent survivors	
	Narashige	Eppendorf	Narashige	Eppendorf
Injected	10,101	880	100%	100%
Hatched	2,292	642	24	73
Adults	852	401	8.4	45