Transformation and Gene Silencing Technologies to Control *Helicoverpa armigera*

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The results in this thesis are, except where otherwise acknowledged, my own original work.

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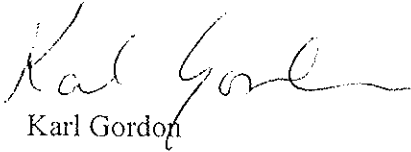
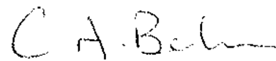


Karl Gordon



Carolyn Behm

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Abstract:

Stable transformation is an essential tool for molecular biologists working on non-model organisms. The ability to introduce and express genes of choice in an organism provides a means to investigate important molecular questions such as gene function, biochemical pathway analysis, reporter gene studies and developmental processes. My PhD studies have focused on the transformation of the pest *Helicoverpa armigera* with the reporter gene EGFP (enhanced green fluorescent protein). There are essentially two parts to transformation, 1) DNA delivery and 2) target gene integration. Biolistics is a technique for DNA delivery that involves coating microscopic gold particles with the DNA of choice and accelerating them at high velocity into cells. Biolistics has been widely used to transform many kinds of plant tissue, and has had mixed success transforming *Drosophila* embryos. Extensive attempts to adapt biolistics to transform *H. armigera* embryos proved fruitless, with too many technical hurdles to overcome. These difficulties led me to use microinjection delivery of DNA into embryos. Compared to biolistics, microinjection is a lower-throughput technique delivering DNA to individual embryos, however, this method is well established, with none of the technological hurdles raised by biolistics. Results for microinjection were encouraging, with a high frequency of transient EGFP expression and the generation of two putative EGFP stably transformed *H. armigera* lines. Following DNA delivery, integration of target genes into insect genomes is commonly mediated by transposon-based gene movement. I used the class II transposon *piggyBac* to facilitate the movement of the EGFP reporter gene into the genome of *H. armigera* embryos as a visual proof of integration.

The development of an effective microinjection technique also allowed exploration of the role of RNA interference (RNAi) in *H. armigera*. This highly specific silencing technique was used with a view to knocking down the expression of genes essential for the growth and development of this insect. This in turn will form the basis for the development of a targeted genetic control mechanism. By co-injecting an EGFP construct and either siRNA or dsRNA against EGFP into embryos, I observed a significant reduction in the frequency and level of EGFP fluorescence in embryos. Quantitative real time PCR validated these observations, showing a reduction in EGFP transcript upon co-injection with dsRNA or siRNA. These results suggest that the RNAi pathway is conserved in *H. armigera* and provide a basis for testing phenotypic effects of silencing specific genes in this insect.

For RNAi to be developed as an effective pest control mechanism, the parameters of RNAi in specific pests must be thoroughly understood. In particular, is systemic RNAi functional in *H. armigera*? For RNAi to be most effective, the silencing signal must be able to spread throughout all cells in the organism. One gene identified in *C. elegans*, known as SID-1, plays a role in mediating systemic spread of the RNAi signal, which may involve the cell-cell movement of siRNAs. Not all organisms contain a SID-1 gene. For example, no SID-1 homologue has been identified in *Drosophila*, and as a result systemic silencing is absent. I identified two different SID-1-like genes in *H. armigera*, strongly suggesting the possibility of systemic RNAi in this organism and supporting further studies into the use of RNAi as a pest control mechanism.

Abbreviations:

1156 R	- The cuticular chitin synthase *Helicoverpa armigera* homologue.
1156 R3	- The midgut chitin synthase *Helicoverpa armigera* homologue.
CHS	- Chitin synthase
Ct	- Cycle threshold
DPCR	- Days post chorion removal
DPF	- Days post feeding
DPI	- Days post injection
DsiRNA	- DICER generated siRNA
EcR	- Ecdysone receptor
GAPDH	- Glyceraldehyde-3-Phosphate Dehydrogenase
GFP	- Green fluorescent protein
HaSNPV	- *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus
IRM	- Insecticide resistance management
JHE	- Juvenile hormone esterase
LB	- Luria broth
miRNA	- Micro RNA
RISC	- RNA induced silencing complex
RNAi	- RNA interference
RsiRNA	- RNaseIII generated siRNA
SH	- Sodium hypochlorite
SID-1	- Systemic RNAi deficient 1
siRNA	- Small interfering RNA
USP	- Ultraspiracle