GENE KNOCKDOWN IN LYGUS LINEOLARIS BY RNA INTERFERENCE

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Abstract

Three genes encoding polygalacturonase (PG) have been identified in *Lygus lineolaris* (Palisot de Beauvois) (Miridae: Hemiptera). Earlier studies showed that the three PG gene transcripts are exclusively expressed in the feeding stages of *L. lineolaris*. In an earlier report, it was shown that all three transcripts are specifically expressed in salivary glands indicating that PGs are salivary enzymes. Transcriptional profiles of the three PGs were evaluated with respect to diet, comparing live cotton plant material to artificial diet. PG2 transcript levels were consistently lower in cotton-fed insects than those reared on artificial diet. RNA interference was used to knock down expression of PG1 mRNA in adult salivary glands providing the first demonstration of the use of this method in the non-model insect, *L. lineolaris*. A second RNA interference project using a sequence similar to the inhibitor of apoptosis gene (IAP) confirmed the method and resulted in a lethal phenotype. RNAi is a powerful method to analyze gene function in *L. lineolaris*.

Introduction

The Biological Control of Pests Research Unit assembled a molecular genetics lab inside the Stoneville Research Quarantine facility. A primary goal for this facility during the last 5 years has been genetic manipulation of the tarnished plant bug, *Lygus lineolaris*. The focus on *L. lineolaris* is based on its substantial regional relevance; it has lately become a primary pest of Mississippi cotton. The goal of the research was to examine, on the molecular genetic level, how the insect functions, and use that insight to disrupt those functions that we humans find inconvenient.

For *Lygus,* feeding is inconvenient when they feed on our crops, including cotton. When *L. lineolaris* eats it injects enzymes that destroy flower buds and young fruit, and this results in decreased yield.

Materials and Methods

There are several methods to study genes at the functional level. Assuming some sequences are obtained from the organism, a scientist can:

- Compare the sequences to other known genes, and hypothesize that the gene will function in a similar fashion in your organism;
- Construct a probe from the putative gene sequence and use it to identify tissues or developmental stages in which the gene is plentiful;
- Extract transcripts from specific stages or tissues of the organism and measure the amounts relative to other genes;
- Deactivate the transcript and assess the effect of its loss:
    - this can be accomplished in a few model organisms by transgenic techniques, or, if the organism is not a model organism,
    - a more generalized technique like RNA interference may be employed (abbreviated RNAi here).

A minimal library of expressed sequence tags were prepared from *L. lineolaris* (Allen, 2007). Target genes were chosen based on comparisons to known insect genes. However, some unusual genes were noted; unusual because they did not appear to be insect genes. They were similar to fungus genes. Published biochemical analyses of *Lygus* saliva indicated presence of polygalacturonase (PG) enzymes, which degrade pectin. These enzymes are not found in Drosophila or mosquitoes or other genetic model insects because those insects do not require pectin degradation to feed. The function of extra-oral feeding on live plants with cell walls made of pectin dictates the presence of a gene product, or in this case more than one product, to break down cell walls.

Three PGs, very different from one another but conforming to the basic design of a polygalacturonase (Allen and Mertens, 2008). To summarize what we found out about PGs, we found them in all feeding stages (not eggs), that the expression in individual adults varied dramatically – speculatively based on readiness to feed, and transcripts were primarily present in salivary glands. To determine the functional relevance of the enzymes we performed RNAi experiments on the three sequences. We made double stranded RNA from each gene sequence, introduced it into the target organism, *L. lineolaris*, by microinjection (Walker and Allen, 2010). Hypothetically, this destroys the gene product during before it's translated and transcribed into the final enzyme. However, RNAi doesn't always work, and it doesn't usually eliminate the gene product entirely. We chose a second sequence to analyze and confirm our methods, a sequence that was similar to the "inhibitor of apoptosis" gene (IAP). This gene is involved in the controlled destructive recycling of cells during organismal and tissue development. Pridgeon et al. (2008) knocked down a similar sequence in mosquitoes and the effect was lethal. In other words, there was a measureable effect.

Results and Discussion

72 hours after injections, salivary glands were dissected, total RNA extracted and cDNA generated to feed into Quantitative Real Time PCR assays which serve to gauge expression levels (transcript quantities) of the experimental and control genes being analyzed. While expression levels of control genes did not differ in quantity under control and experimental conditions, PG1 gene expression differed in quantity. It was knocked down. We performed the experiments using each of the three PGs, and using five control genes. We also used different portions of the PG1 gene sequence. Knockdown could be measured for the PG1 treatments. However, we only identified knockdown using the PG1 double-stranded RNA, not PG2 nor PG3. Insects that were injected but not dissected for quantitative real-time PCR did not show any visible effects or loss of viability after injection with any PG double-stranded RNA (Walker and Allen, 2010). This was a bit disappointing, and we decided to look at some other gene targets. We chose a sequence that was similar to the "inhibitor of apoptosis" gene. Based on semiquantitative PCR, the IAP sequence appeared to be transcribed primarily in the adult stage. We followed similar procedures to the PG experiments. For the IAP sequence we used varying concentrations of dsRNA, but found no dose-response variation at the concentrations used. Both adult and nymph specimens were injected, and two different portions of the IAP sequence were evaluated; both demonstrated knockdown. More importantly, the IAP-injected insects exhibited a dramatically shortened lifespan after injection. All of those injected as nymphs died prior to adult eclosion (Walker and Allen, 2011).

Summary

While PG1 knockdown served as validation of the RNAi method, the lack of knockdown evidence for PG2 and PG3 was disappointing from a functional analysis perspective. Had knockdown been apparent for either of the other PGs a combination of knockdowns would have been assessed. The results from the IAP knockdown were more encouraging. Both qRT-PCR assays and survival assays supported the hypothesis that IAP serves a vital function in *L. lineolaris* as indicated in *Aedes aegypti*. Other potentially vital genes may be tested using the methods we have described. These vital functional genes may be exploited as agricultural biotechnology applications (Price and Gatehouse, 2008).

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