Evaluation of Several Genes as Targets for RNAi in German Cockroach

Rachel Wilkins[1,2], Kun Yan Zhu[1], and Kristopher Silver[1]

[1]Department of Entomology, College of Agriculture, Kansas State University
[2]Department of Biology, College of Arts and Sciences, Kansas State University

Abstract

RNA interference (RNAi) was first discovered in nematodes when exogenous double-stranded RNA (dsRNA) complementary to a specific gene suppressed expression of that gene [1]. Subsequently, much effort has been devoted to developing RNAi as a highly specific tool for therapeutic interventions and control of insect pests [2]. However, there are still many challenges associated with using RNAi to control insects, including efficient delivery and selection of appropriate targets. In this study, we evaluated three genes as potential targets for causing mortality via RNAi in German cockroach, *Blatella germanica*. German cockroaches are ubiquitous structural pests that can serve as reservoirs for species of pathogenic bacteria, viruses, or fungi in humans [3,4] and is an excellent organism for exploring insect control using RNAi. Injection of dsRNA complementary to either tubulin (Tub, a cytoskeletal structural protein), VATPase subunit 1 (Vha, an integral membrane protein), or Snf7 (an ESCRT III protein) caused decreased survival with Snf7 causing the greatest and fastest mortality (LT50 = 8.2 days). Our results demonstrate that when suppressed with RNAi, these genes could be effective targets for cockroach control. Furthermore, knowing these genes can be effectively used for RNAi, we can now attempt to understand why methods other than injection for RNAi delivery are less efficient in an effort to improve the utility of RNAi in insect control.

Purpose

The purpose of this research is to evaluate specific genes as potential targets for RNAi-based control of German cockroach.

Questions, Hypotheses, and Predictions

Question: Can RNAi of tubulin, Vha, or Snf7 be used to control German cockroach?

Hypothesis: If German cockroaches are injected with dsRNA specific to tubulin, Vha, or Snf7, then we will observe significant levels of cockroach mortality

Prediction: We expect that German cockroaches injected with dsRNA complementary to tubulin, Vha, or Snf7 will die at much higher rates than cockroaches injected with dsRNA complementary to green fluorescent protein (GFP)

Study System

German cockroaches are easily maintained in the laboratory, have a sequenced genome that will soon be available publicly, and are highly susceptible to injected dsRNA, but less so to dsRNA delivered by other methods [5] making them an excellent model organism for our experiments

Figure 1. Study design

Methods and Experimental Design

Cockroaches were kept in plastic containers at 27 C with a 16:8 h light/dark cycle and provided food (dog food) and water (in the form of moist cotton balls in a dish) *ad libitum*. Prior to experimentation, cockroaches were placed at -20 C for 5-10 min to ease collection for injection. Cockroaches were kept on ice until they were injected, using a microinjector (PB600 Dispenser, Hamilton, Reno, NV), just posterior to the last leg pair with water or a 1 µg/µL solution of dsRNA (prepared previously, Z. Xu). Initial efforts evaluated the ability of adult cockroaches to survive injection with 1 or 2 µL of water (15 cockroaches per group) over a 48 h period to determine the best volume to use in subsequent experimentation with dsRNA.

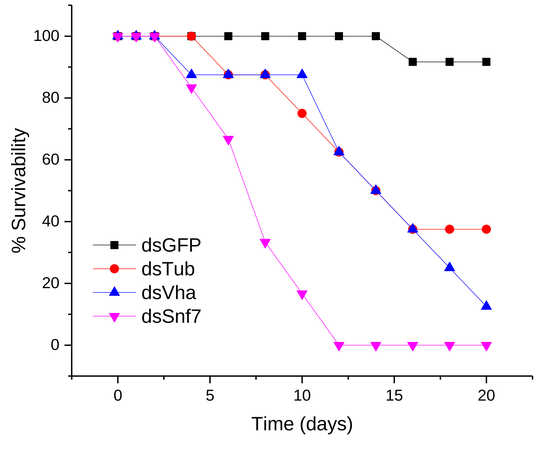
For dsRNA experiments, each cockroach was injected with 2 µL of dsRNA complementary to GFP (control, is not complementary to any gene in German cockroaches, 16 injected), Tub (10 injected), Vha (10 injected), or Snf7 (8 injected). Cockroaches were then kept in plastic containers with food and water provided (see Fig. 1) and live cockroaches were counted daily for 72 h and then every other day up to 20 days.

Results

Table 1. Number of cockroaches surviving injection with different volumes of water. Survival of cockroaches injected with 1 or 2 µL of water was not different than survival of uninjected cockroaches over the 48 h period following injection.

		Days Post Injection	
Volume	**# Injected**	**1**	**2**
0 µL	15	15	15
1 µL	15	14	14
2 µL	15	15	15

Figure 2. Percent survival of cockroaches injected with dsRNAs. The number of surviving adult cockroaches injected with dsTub, dsVha, or dsSnf7 began to decrease between days 3 and 5 and continued to decline (37.5, 12.5, or 0 % for dsTub, dsVha, or dsSnf7, respectively) over the course of the observation period. The median lethal times (LT_{50}) calculated for dsTub, dsVha, or dsSnf7 treatment groups were 15, 15, or 8.2 days, respectively.



Conclusions

1. Two µL dsRNA is an appropriate injection volume for adult cockroaches.
2. dsTub, dsVha, dsSnf7 caused decreased survival in adult cockroaches and are viable dsRNAs for future experiments exploring cockroach control with RNAi.

Future Directions

In continuing this research, the next step would be to repeat this experiment. The methods and design of the experiment would be similar; however, a larger number of German cockroaches would be used, possibly 30-40 roaches per treatment group. Testing on more roaches would create clearer results with stronger distinctions of effects between each treatment group. Increasing numbers and repeating also will permit statistical comparisons between groups to determine if the observed differences are real or simply statistical anomalies.

Further experimentation should also be directed at understanding the mechanisms that are responsible for the reduced sensitivity of cockroaches to RNAi via the oral route as opposed to by injection. Injection, while useful in experimental settings, is not practical in applied settings or for control of cockroaches in a structure, and subsequent experiments designed to understand the obstacles that currently limit the utility of RNAi in insect control is critical.

References

1. Fire, A., *et al*. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature,* **1998**, *391*, (6669), 806-811.
2. Kim, Y.H., *et al*. RNA interference: Applications and advances in insect toxicology and insect pest management. *Pestic Biochem Physiol,* **2015**, *120*, 109-117.
3. Elgderi, R.M., *et al*. Carriage by the German cockroach (Blattella germanica) of multiple-antibiotic-resistant bacteria that are potentially pathogenic to humans, in hospitals and households in Tripoli, Libya. *Ann Trop Med Parasitol,* **2006**, *100*, (1), 55-62.
4. Rosenstreich, D.L., *et al*. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. *N Engl J Med,* **1997**, *336*, (19), 1356-1363.
5. Lin, Y.H., *et al*. Oral delivery of dsRNA lipoplexes to German cockroach protects dsRNA from degradation and induces RNAi response. *Pest Manag Sci,* **2016**.

Acknowledgements

We thank Dr. Jeremy Marshall for organizing and initiating the Undergraduate Research Experience program as well as the Department of Entomology for supporting this initiative.