Development of RNAi methodology in *Megachile rotundata*

Cameron Kolthoff[1] and **Alex S. Torson**[2]

Department of Biology, Coe College[1] and USDA-ARS[2]

Looking for a graduate student?

Introduction

- RNA interference (RNAi) is the immune response which allows an organism to defend itself from transposons and foreign RNA.
- Our work focuses on the exogenous siRNA pathway which defends against viral dsRNA. We're taking advantage of this pathway by introducing *in vitro* dsRNA rather than the normal viral dsRNA.
- The process of introducing the *in vitro* dsRNA varies widely across species.
- Methodology must be developed for *Megachile rotundata* to investigate gene function through RNAi.
- **The purpose of this project was to investigate whether injection of dsMucin RNA into post-diapause quiescent prepupae, which were stored at 6°C, would result in knockdown of the Mucin1 gene.**

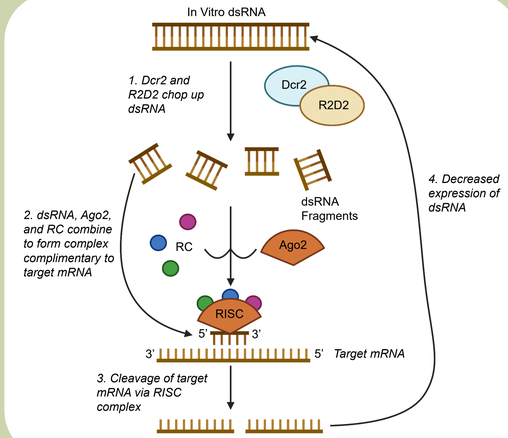
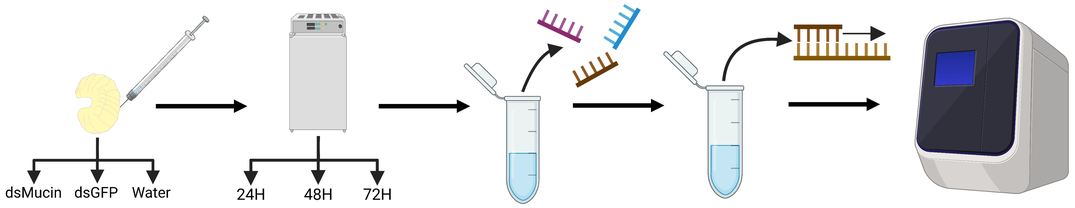


Figure 1. General overview of exogenous snRNAi pathway

Methods

1. **Larvae were injected** with 1.5uL of dsMucin, dsGFP, or nuclease free water (n = 30 for each treatment) at 10ug.
2. **Individuals were chilled** at 6°C for three time periods (24H, 48H, and 72H) at n = 10 per treatment and time group.
3. **Total RNA was extracted** via RNAzol. Quantity and quality was assessed using a Nanodrop One and Agilent TapeStation 4150.
4. **Synthesis of cDNA** via SuperScript IV Reverse Transcriptase was conducted following DNase treatment.
5. **qPCR** was used to determine effectiveness of knockdown using a Roche 480.



A260/280 Average: 2.04
A260/230 Average: 1.74

A260/280 Stdv: 0.026
A260/230 Stdv: 0.15

**Standard*
A260/280: 1.8 – 2.1
A260/230: ~2.0

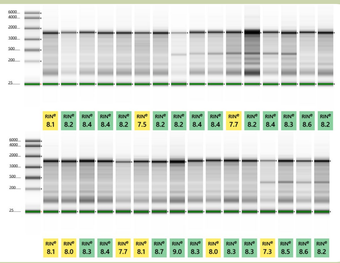


Figure 2. Average RNA purity ratios and example RIN values

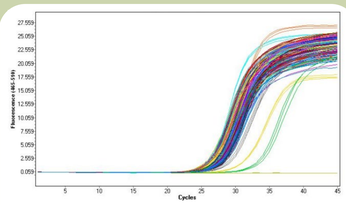


Figure 3. Amplification curve from Roche 480 showcasing consistency of technical replications (Average stdv: 0.09 cycles)

Results and Conclusions

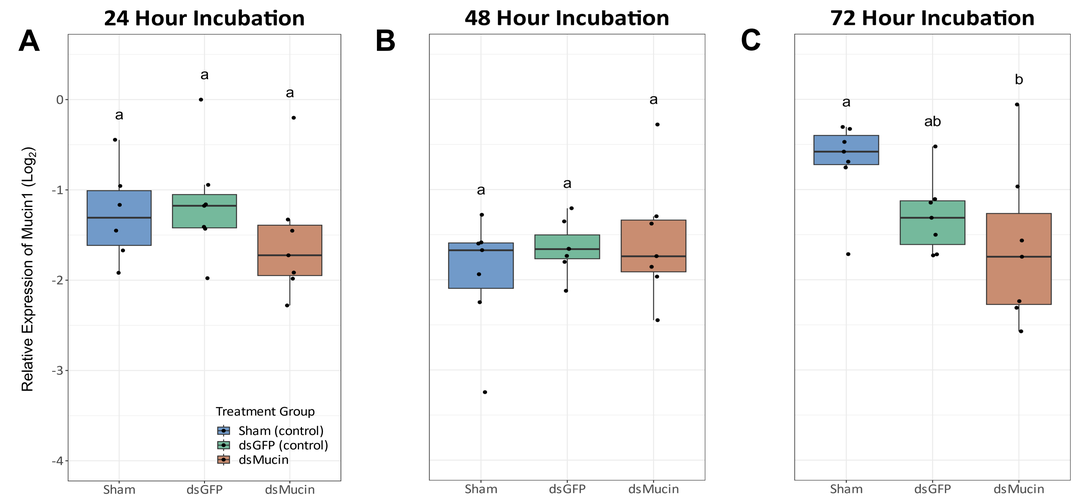


Figure 4. Relative expression of Mucin1 in post-diapause quiescent Megachile rotundata prepupae over 3 time periods. An ANOVA and post-hoc Tukey's Test were performed to determine significant difference ($p < 0.05$) between treatment groups of time periods (denoted by "a" and "b").

- No apparent knockdown was observed between treatment groups over the course of 24 and 48 hours.
- The significantly different expression of Mucin1 between dsMucin and Sham at 72H indicates a possible knockdown. However, 72H dsGFP did not show significant difference when compared to the other treatments.
- The observed decrease in the average expression of Mucin1 for both dsGFP and dsMucin compared to Sham at 72 hours could be the result of an off-target response of the RNAi machinery.

Future Directions

- When taking into consideration the preliminary 1-week trial (Figure 5.) compared to 72 hours, the 3-day to 1-week period should be investigated.
- A formal reference gene selection should be performed on the individuals from this trial.
- Trials should be run again with individuals stored at warmer temperatures as metabolisms may have been too slow at 6°C for RNAi to occur.
- A trial where the sex is controlled should be performed to see if sexual differences are a factor in the observed variation in expression within targets
- Investigate other gene targets with consistent moderate to high expression and that will not result in death of the individual if suppressed.

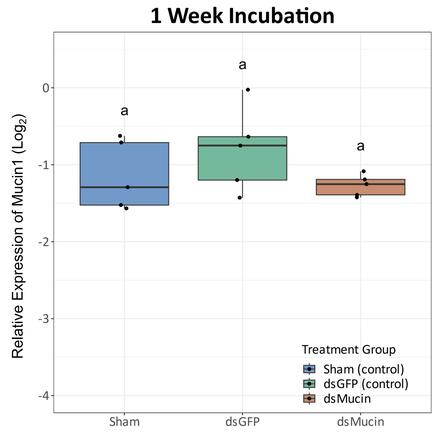


Figure 5. Preliminary 1-week trial results

We would like to thank Marnie Larson and Melanie Tischleder for their assistance with molecular techniques, Gagandeep Brar for synthesizing the dsRNA constructs, Pollination Nation for funding the project through NSF-EPSCoR – 1826834 and USDA-ARS project 3060-21220-03200D, and BioRender for their online graphics software.