Testing the role of Maternal Haploid in the jewel wasp

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SCRIPPS COLLEGE

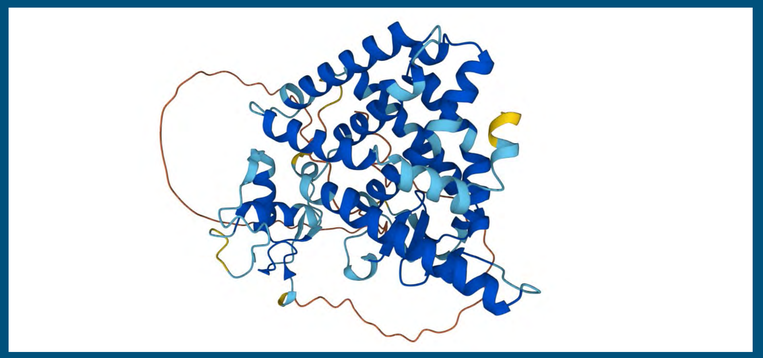
Introduction

In early reproduction, the sperm's DNA is packaged with highly basic proteins called protamines, an event that is needed to protect the genome from oxidative damage. After fertilization, the protamines are removed and repackaged with conventional histone proteins. A number of genes, including one called *maternal haploid* (MH), have been discovered in the fruit fly, *Drosophila melanogaster*, that play a role in this transition. In the fruit fly, MH helps to reverse DNA-protein crosslinks, and mutations in this gene cause abnormalities in the chromosomes donated by the sperm. In this study, I am using RNA interference (RNAi) to test if a homolog of MH in the jewel wasp, *Nasonia vitripennis*, functions in the same manner.

Methods

- Bioinformatic identification of the gene of interest
- Design primers specific for the gene, order them
- Amplify the coding sequence of the target gene using PCR
- Clean the PCR product with a kit
- Produce double stranded RNA by in vitro transcription
- Purify the dsRNA with a kit
- Inject the dsRNA into male wasp pupae
  (The dsRNA enters the developing germ cells and targets the MH gene in this case)
- The injected males become adults, which are crossed with normal females
- F1 progeny are scored for any effects

1. Predicted structure of the MH protein

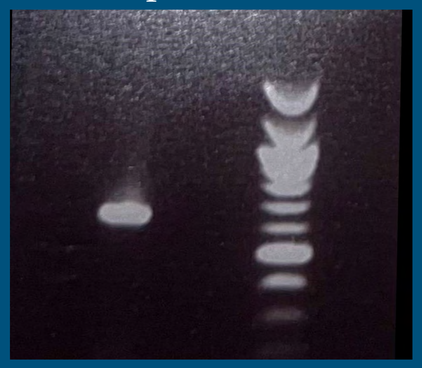


2. Maternal Haploid gene sequence

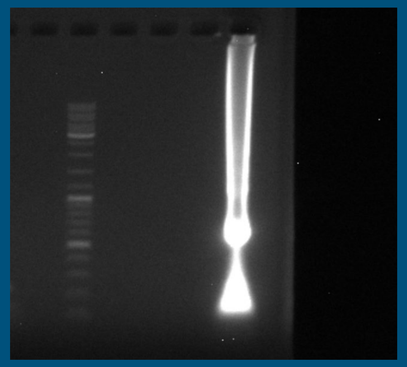
TCACTGAATCGAACTAATAAAACTCAAAAAACTAATTCAATGAATCTATCAAACTC
CATTAGTAAAATCTACAACCAGCACAAACAGCAGTTTCAGTGTTGAAACTGCCTCA
ATGCCCCATTTGTAACAAATCTGTTGATGAATTAAAAATTAATGAACATCTGGACA
CCGCAAGAGCCAGAAGTTATTTCAGATAGTTCACATAAAAAGAGGAAAAGTGAAGA
CAAATAAGAAACTAAAAGATGAAAATCAGCAGACATCTTCAGAAACAATAGATTGT
AAAATTACTGCCTGTCAATTTTAATGATCATTTGCAAAAATGTTTATCAAGTGACA
GATGATGTAGTGTCACTTCATGATTCTAGCTCTAGTTCTATAAATTTACATGACTC
TGATTTGCGATAAAAAATTAGAACCTGGCATGAGTTTGAATGAACATTTAGAAGAA
ATTTAATGAAAGTAATGACGATGATTTTGAAGAGAAAGACAGCAGTACTGGTGACA
GAGAAGGAAGTTTTGGAACGGTATCCCTGCCCTATTTGCATGTCATTAATTGAAGA
ACCACGTTGACATGTGCTTAGCCAAATCTAAATAAGTACAATTGTTATGCAGAGTT
TAGTGGTTACTTTATTCCAGCAAATTTGCTTTAGCTTTCAGTTTTTTACAGTACAT
ATCAAGCATATATTCACTTAACATTTTCGTTTTACATTTCATAGATTAGTTCATGTA
TTCAGGAGTTATTCAAAGTTCCTATATTGTCAATGTAAATATAAAAAAAAAACTTA
AATAGGATCCTGGTGATACTTTTAATTTAAACATAATTTATAATGTTATAATATAA

The shaded regions are forward and reverse primer annealing sites

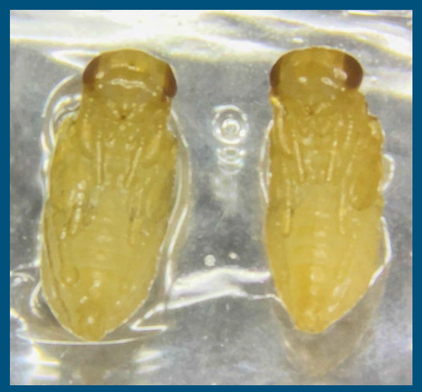
3. PCR product for MH



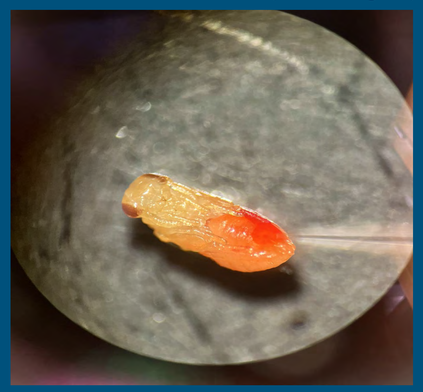
4. dsRNA for MH



5. Wasp pupae for injection



6. Injecting a wasp pupa with dsRNA (red dye)



4. *Nasonia* wasp adult stage

Results

- I successfully designed primers, amplified a PCR product, and synthesized dsRNA.
- I ran out of time before I could inject the dsRNA into wasp pupae and perform genetic crosses.
- My plan is to continue this work, conducting the injections and crosses and scoring F1 progeny.

Expectations

Strong mutations in the fruit fly MH result in a highly abnormal sperm nucleus that can be seen just after fertilization.
This effect results in embryos that have only one set of chromosomes.
This effect causes embryonic death because flies are diploids (they need two sets of chromosomes)
In the jewel wasp, females are diploid and males are haploid. Therefore, if I target MH with RNAi and this gene functions in the same way, loss of the sperm nucleus can be observed in two ways. First, I will be able to see all-male wasp broods (i.e., loss of the sperm nucleus will convert female-destined embryos into haploid males).
Second, I will be able to use microscopy to see sperm nuclear loss and perhaps also what happens to cause this effect.

Acknowledgments

I would like to thank for the assistance throughout this research aDr. P. Ferree nd to the Citrus College SRE program for the opportunity. Funding this project was provided by the US National Science Foundation (grant to P. Ferree).

Alternate Text

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Scripps College

'Testing the Role of Maternal Haploid in the Jewel Wasp'

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Figure 1. Predicted structure of MH protein.

Figure 2. Maternal haploid gene sequence.

Figure 3. PCR product for MH.

Figure 4. dsRNA for MH.

Figure 5. Wasp pupae for injection.

Figure 6. Injecting a wasp pupa with dsRNA (red dye).

Figure 7. Nasonia wasp adult stage.

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