Roles of the *vasa* gene in developing *Thamnocephalus*

Jack Burgess[1*], AJ Stangl[1], Lisa Nagy[1]
[1]Molecular and Cellular Biology, University of Arizona, Tucson, AZ 85721

Abstract

This project focuses on determining the role of the *vasa* gene in development of the Beavertail Fairy Shrimp *Thamnocephalus platyurus*. The *vasa* gene is of particular interest for this objective because it is expressed in the germ line of related organisms, but may also play a role in stem cells. The Nagy lab studies the mechanisms that regulate segment addition in different arthropods. The best-studied arthropod, the fruit fly *Drosophila*, develops radically differently from most arthropods. *Drosophila* develops its segments all at once, while most arthropods, including *Thamnocephalus*, add segments sequentially from a posterior growth zone. Thus, genes expressed in stem cells may be important for sequential segment addition. In this project, I cloned a *Thamnocephalus vasa* gene and worked on determining its expression pattern.

Introduction

The purpose of my research is to identify candidate genes involved in posterior growth of *Thamnocephalus*. In related organisms, genes involved in germ cells have also been found to be expressed in stem cells (1 - 4), which may be involved in posterior growth. Three of these genes are *nanos*, *vasa*, and *piwi*. This research will create a clearer understanding of arthropod development.

This research is important because most developmental research has been done on model organisms such as the fruit fly, *Drosophila melanogaster*, which has a very different mode of development from other arthropods. *Drosophila* develops its segments almost simultaneously. Most other arthropods add segments sequentially, still a little-known process. During sequential segmentation, segments are added from the posterior. We hypothesize that this growth relies on stem cells and that these three genes, *vasa*, *nanos*, and *piwi* are essential to posterior growth.

In addition, as a more basal (ancestral) organism, research on *Thamnocephalus* will contribute to a greater pool of knowledge on arthropod development. By comparing *Thamnocephalus* to other arthropods, we can use our research on diverse arthropod groups to determine the evolution of varying methods of arthropod segmentation. A far downstream application of this research could be its application to the field of human biology.

My goal is to find orthologs of and design primers for these genes, amplify them from *Thamnocephalus* cDNA, clone them into plasmids and transform them into bacteria, synthesize riboprobes and conduct an *in situ* hybridization, and perform gene knockouts of these genes in *Thamnocephalus* using dsRNA with RNAi. With the information gained from these experiments, I can build a better picture of how vasa is involved in early Beavertail Fairy Shrimp development.

Results and Methods

Identifying gene orthologs

Sequences from *Drosophila melanogaster* were found for *vasa*, *nanos*, and *piwi* on the NCBI website. These sequences were then blasted to the *Thamnocephalus* Expressed Sequence Tag (EST) database to find putative gene orthologs. These putative orthologs were then blasted back to NCBI to determine if they returned respective gene sequences from related organisms, and could therefore be safely assumed to be orthologs. Orthologs for the *nanos* gene could not be found in this way.

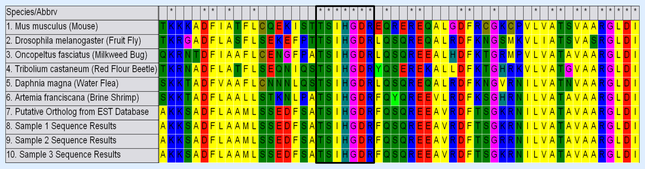

Figure 1: An alignment of *vasa* in various organisms. The selected portion represents a conserved region. Alignment indicates that the putative and later collected sequences (Sample 1, 2 and 3) all have highly conserved amino acid motifs common to other vasa othrologs.

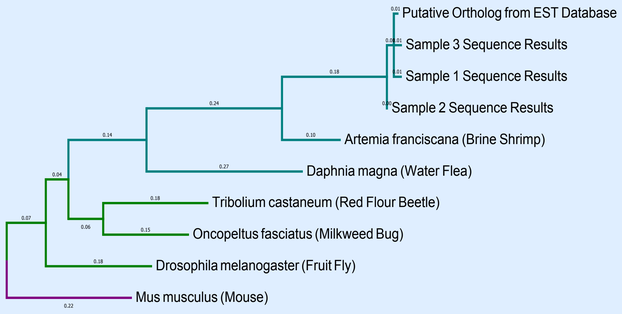

Figure 2. A phylogenetic tree depicting the sequences from the alignment. The close genetic distance and relationships between the samples and putative *Thamnocephalus vasa* gene adds support to the validity of the ortholog. Tree follows expected relationships, with the mouse as an outgroup and crustaceans branching away from insects in the arthropod branch.

PCR of *vasa* ortholog

A portion of the identified *vasa* ortholog was amplified out of *Thamnocephalus* cDNA with primers created using NCBI's primer designing tool. Only one primer pair, for *vasa*, produced a strong enough product.

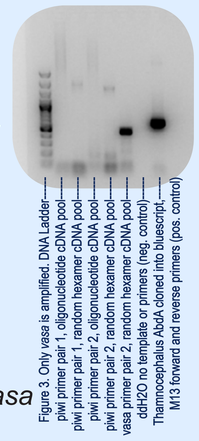

Figure 3. Only vasa is amplified.

Cloning of amplified sequence

The PCR product was cleaned up and cloned into plasmids, then transformed into bacteria. Colonies expected to contain plasmids with *vasa* inserts were selected, re-plated, and sent out for sequencing (results shown in alignment above). A colony whose sequence harbored the least SNPs was selected for riboprobe template PCR and grown out in a liquid culture for plasmid miniprep.

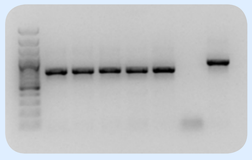

Fig. 4. Riboprobe template PCR gel electrophoresis. Right to left: DNA ladder, 4 samples of plasmid from colony with least SNPs, ddH_2O (neg. control), and *evx* plasmids (pos. control) All PCR samples ran with M13 forward and reverse primers.

In situ Hybridization

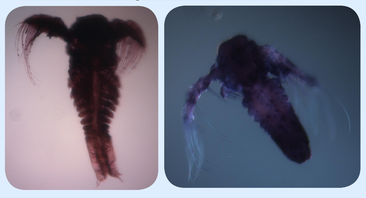

Figure 5. T7 on left, T3 on right. Both developed nonspecific binding.

Riboprobe synthesis was successful, but *Thamnocephalus* developed non-specific binding for T7 and T3 probes during *in situ* hybridization.

Discussion

Additional research is required for this project. A successful *in situ* hybridization is imperative to determine where *vasa* is expressed and develop a hypothesis on its function. The next step would be to perform a knockout of the gene with RNAi. If involved with stem cells or germ cells, absence of the posterior or germ line respectively is expected.

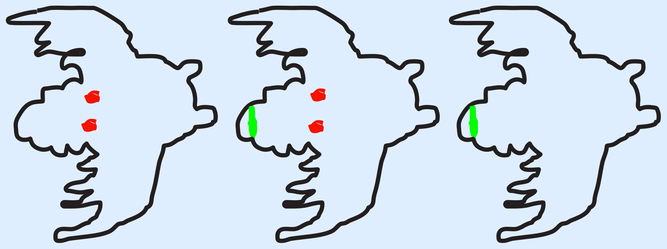

Figure 6. Three possible expected *in situ* hybridization results. Left: expected if *vasa* is expressed in only the germ line. Right: expected if *vasa* is only expressed in stem cells in the posterior growth zone. Center: expected if *vasa* is expressed in both germ line and stem cells in the posterior growth zone.

Materials

Thamnocephalus cysts from THAMNOTOXKIT F™, Nucleotides from Invitrogen, oneTaq from New England Biolabs, Cloning kit: STRATAGENE; StrataClonePCR Cloning Kit Catalog # 240205, PCR Cleanup kit: GE Healthcare illustra™ GFX DNA and Gel Band purification Kit, Primers:

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CCGTTCGACCAATCCTGTGA	Plus	20	875	894	60.04	55.00	4.00	1.00
Reverse primer	TCAAGGGAGAGCGAAACACC	Minus	20	1354	1335	59.97	55.00	2.00	0.00
Product length	480								

Thamnocephalus fixed according to protocol in The Beetle Book (Gregor Bucher 2009)

Acknowledgments

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