Evolution and Expression of TSR-domain proteins in cnidarian-dinoflagellate symbiosis

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Abstract

The symbiotic relationship between cnidarians, such as corals and sea anemones, and dinoflagellates plays a crucial role in supporting the diversity and productivity of coral reefs. Critical to the symbiosis is the recognition process between host and symbiont at the onset of symbiosis, which involves the host innate immune system. One potential group of immune proteins in cnidarians are those that contain the thrombospondin type-1 repeat (TSR). In vertebrates, TSR domain-containing proteins are involved in diverse functions including immunity, however not much is currently known about these proteins in cnidarians. Previous work on the sea anemone *Aiptasia pallida* demonstrated that TSR domain-containing proteins potentially play a role at the onset of symbiosis, however the specific TSR domain-containing protein involved in this process is unknown. Thus, we have chosen two specific TSR domain-containing proteins, semaphorin-5 and trypsin-like, to investigate. We have characterized semaphorins from a diversity of invertebrates and generated a phylogenetic tree to develop an understanding of semaphorin evolution in metazoans. Lastly, we measured gene expression of two TSR-domain-containing proteins, a semaphorin-5 and trypsin-like sequence, in *A. pallida* at the onset of symbiosis using qPCR. Overall, the research presented here suggests that there is a distinct clade of cnidarian semaphorin-5 sequences that is separate from the other metazoan semaphorin-5 sequences. Finally, semaphorin-5 showed an initial upregulation at the onset of symbiosis, indicating that it potentially plays a role in the phagocytosis of symbionts.

Background

Symbiosis and Coral Reefs

- Dinoflagellates are housed inside the host cells and are surrounded by a membrane (symbiosome)
- The symbiosis is centered around nutrient exchange[1]
- Important to study from an ecological point of view (coral bleaching)[2]

Previous Research

- Treatment of aposymbiotic *A. pallida* with an antibody to the TSR domain of human thrombospondin-1 displayed decreased recolonization rates at the onset of symbiosis[3]
- Treatment of aposymbiotic *A. pallida* with a peptide designed to a TSR domain-containing-protein yielded a significant increase in recolonization rate at the onset of symbiosis[3]
- As there is great diversity of TSR domain-containing proteins found in *A. pallida*, there is uncertainty in the specific TSR domain-containing protein involved at the onset of symbiosis[3]

Objectives

1. Generate a phylogenetic tree of invertebrate semaphorin sequences to better understand their evolutionary history
2. Measure gene expression of TSR-domain-containing proteins at the onset of symbiosis in *A. pallida*
   - We hypothesize that the TSR-domain-containing proteins will be highly expressed during the onset of symbiosis

Objective 1: Phylogenetic Analysis of Invertebrate Semaphorin Sequences

Experimental Methods

BLASTp of invertebrate genomes through JGI genome portal and Ensembl using queries of known *Homo sapiens* and *Drosophila melanogaster* semaphorin sequences

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Pfam domain characterization

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Alignment with Muscle using MEGA

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Maximum Likelihood Phylogenetic Analysis

Results

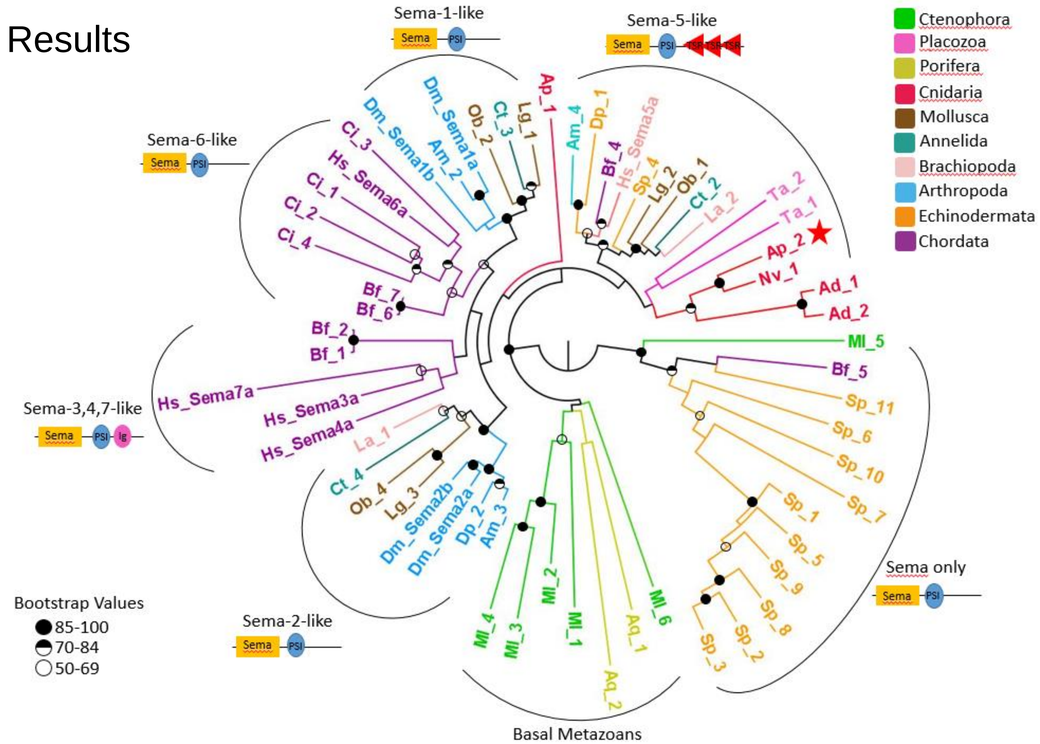
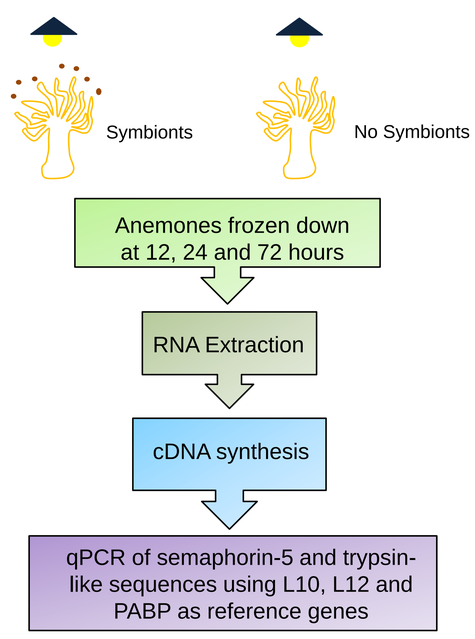


Figure 1: Phylogenetic tree of invertebrate semaphorin sequences. Abbreviations are as follows: Ml: *Mnemiopsis leidyi*, Ta: *Trichoplax adhaerens*, Aq: *Amphimedon queenslandica*, Ad: *Acropora digitifera*, Ap: *Aiptasia pallida*, Nv: *Nematostella vectensis*, Lg: *Lottia gigantea*, Ob: *Octopus bimaculoides*, Ct: *Capitella teleta*, La: *Lingula Anatina*, Am: *Apis mellifera*, Dp: *Daphnia pulex*, Dm: Drosophila melanogaster, Sp: *Strongylocentrotus purpuratus*, Bf: *Branchiostoma floridae*, Ci: *Ciona intestinalis*, Hs: *Homo sapiens*

Objective 2: TSR domain-containing-protein Gene Expression

Experimental Methods



Results

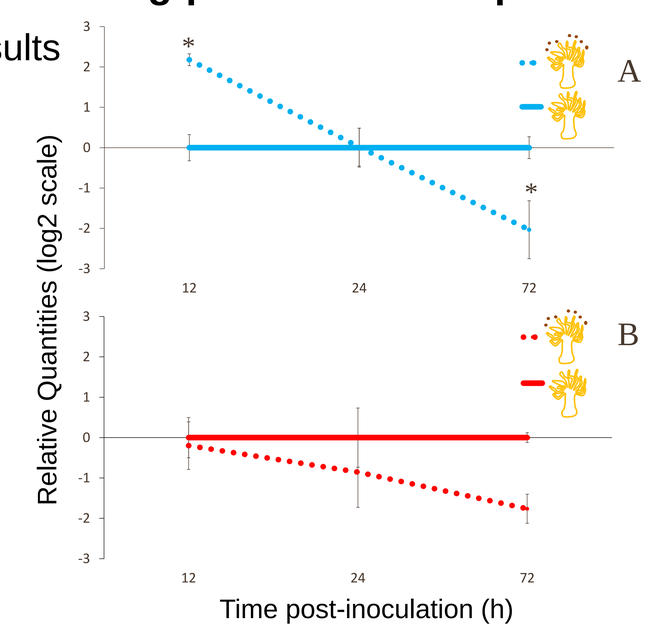


Figure 2: Gene expression of Semaphorin-5 (A) and Trypsin-like (B) at multiple time points. Stars indicate a significant difference in gene expression at each respective time point between symbiont and no symbiont treatments

Discussion

Objective 1 – Phylogenetic Tree Analysis of Semaphorin

- Semaphorin-5 is found in protostomes and deuterostomes but there is a specific, distinct clade of cnidarian sequences
- Most semaphorins group into protostome and deuterostome specific clades
- Sea urchins (*Sp*) displayed a lineage specific expansion of sema-only proteins

Objective 2 – TSR Gene Expression

- Semaphorin-5 plays a role at the onset of symbiosis, potentially through initiating phagocytosis of symbionts
- Trypsin-like displays a downward trend with no significant difference between treatment groups

Literature Cited

1.) **Davy, S. K., D. Allemand, and V. M. Weis**. 2012. Cell biology of cnidarian-dinoflagellate symbiosis. Microbiology and Molecular Biology Reviews76:229-261
2.) **Douglas AE**. The Productivity of Corals. Oceanography.
3.) **Neubauer EF, Poole, AZ, Davy SK, Weis VM**. (in prep)- The thrombospondin structural homology repeat (TSR) domain is important in successful dinoflagellate infection of the anemone Aiptasia sp.

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