Lighting Up Water: Characterizing the origin and diversification of the light organ in the bioluminescent ostracod Vargula hilgendorfii

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This study characterizes the origin and diversification of transcription factors that differentiate secretory cell types in Metazoan organisms.





• It is well known from developmental studies that transcription factors control cell differentiation.

- Transcription factors interact with one another and other regulatory elements such as cis-regulatory elements to regulate gene expresssion.
- The Gene Regulatory Network

(GRN) determines the identity of

the cell. The GRN is thought to

• Understanding the evolution of

a novel complex trait such as

the light organ requires first to

identify the components of the

be composed of transcription

factors

Developed a computational pipeline to use the **BLAST** software on the command line.

Step1: Blast our transcription factor sequence against our custom database. blastn -query Sex_comb_TF.fa -db /home/Imesrop/secretory_cell_tfs/050520_mist1_genomedb_fixed/databaseBL AST -evalue 1e-10 -num_threads 4 -outfmt '6 sacc' | uniq > sex_comb_TF_blast_e10.txt

#Step 2: Retrieving fasta sequences from our custom database. blastdbcmd -db

/home/Imesrop/secretory_cell_tfs/050520_mist1_genomedb_fixed/databaseBL AST -dbtype prot -entry_batch sex_comb_TF_blast_e10.txt -outfmt %f -out sex comb TF blast e10 fasta.fasta

Results & Conclusions

Putative T-box protein 30/42 [Caenorhabditis elegans] utative Brachyury protein-like protein (Hypsibius dujardir Tbox transcription ex transcription factor T homolog 1-like [Anneissia japor factor TBX19 [mus musculus] - master regulator - differentiates othetical protein LOTGIDRAFT 154800 [Lottia gigant pituitary cells. One ortholog in Zebrafish ex-containing protein TBXT-like (Eurytemora affinis (T-box transcription othetical protein DAPPUDRAFT 44582 (Daphnia pul REDICTED: brachyury isoform X1 [Tribolium castaneu _____ factor TBXT); A few rachyury [Tribolium castaneum trachyenteror isoforms exist for this brachventeror gene. Tbox originated in REDICTED: brachyury protein homolog 1-like (Hydra vulgaris hypothetical protein TRIADORAFT 25521 (Trichoplax adhaerens Chordates. The paralog ranscription factor T-box T-box transcription factor T-A [Danio rerio brachyury TF has a box transcription factor T [Mus muscul achyury protein isoform X3 (Danio rerio different function, rachyury protein isoform X1 (Danio rerio involved in notochord

Figure 1:

- Ec, Euphilomedes carcharodonta; SI, Skosbergia lerneri; Vh, Vargula hilgendorfii; Pe, Photeros EGO; Ft, Vargula *Tsujii;* Kh, Kornickeria hastingai; Hs, H-group SVU. Study species 🛠
- Evolution of the upper lip in ostracods within the Cypridinidae family offers an opportunity to investigate cell-type origination.
- The upper lip is used for bioluminescence and luminous courtship signaling.
- light producing Luminescent ostracods emit compounds from a light organ within the upper lip.
- Light reaction involves a substrate, luciferin, and a luminesce enzyme, luciferase, each of which is synthesized in separate gland cells and discharged in a mucus-like substance from the upper lip¹.
- The mucus also contains digestive enzymes which may be secreted from gland cells in the upper lip or exported from the gut.





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Research Question

- (1)What are the identities of transcription factors that are responsible for the differentiation of exocrine secretory cells?
- (2)What is the history of the transcription factors involved in secretory cell

- **#Step 3:** Replace the spaces in the fasta headers with an underscore; this will be easier to annotate the tree later. sed 's/ /_/g' sex_comb_TF_blast_e10_fasta.fasta > sex_comb_TF_blast_e10_fasta_underscore.fasta

#Step 4: Align the sequences using the mafft software. mafft sex_comb_TF_blast_e10_fasta_underscore.fasta > sex comb TF blast e10 fasta underscore mafft.fasta

#Step 5: Run the IQtree. Iqtree -s sex_comb_TF_blast_e10_fasta_underscore_mafft.fasta

#Step 6: Submit iqtree job. sbatch iqtree murad.sh

#Step 7: View our gene tree in iTOL.

Phylogenies of transcription factors known to differentiate secretory cell types.

scaling factor that universally Originated in chordates. Closely related gene such as 'dimmed similar function in where it also

MIST1 BHLH [mus musculus] establishes secretory morphology protein' in Drosophila performs a functions as a scaling factor and differentiates neuroendocrine cells

| | T-box transcription factor TBX19 [Mus musculus] | interred intr |
|---|--|----------------|
| | T-box transcription factor TBX19 isoform X1 [Mus musculus] | differentiatio |
| | T-box-containing protein TBXT-like [Danio rerio] | unerentiatio |
| | T-box-containing protein TBXT isoform X1 [Danio rerio] | |
| | T-box transcription factor TBX19 isoform X2 [Danio rerio] | |
| | hypothetical protein TRIADDRAFT_53394 [Trichoplax adhaerens] | |
| | hypothetical protein TRIADDRAFT_17171 | |
| | T-box transcription factor TBX15 [Nematostella vectensis] | |
| | PREDICTED: T-box protein 2-like [Hydra vulgaris] | |
| L | T-box transcription factor T (Nematostella vectensis) | |
| | T-box transcription factor TBX19-like [Nematostella vectensis] | |
| | T-box protein 2-like [Dendronephthya gigantea] | |
| | uncharacterized protein LOC114530264 [Dendronephthya gigantea] | |
| | PREDICTED: T-box-containing protein TBXT-like (Amphimedon gueenslandica) | |
| | uncharacterized protein LOC100632202 [Amphimedon gueensiandica] | |
| | | |

Figure 4: Phylogeny of the TBX19 transcription factor. TBX19 highlighted in purple. Red bracket indicates the clade that contains TBX19. TBX19 originated in Chordates.

- The MIST1, SREBP1 and TBX19 transcription factors are known to differentiate and establish the secretory phenotype of secretory cells and glands in well studied model systems such as mouse and zebrafish.
- Understanding whether the orthologs of these transcription factors are also present in the upper lip of ostracods will reveal important information about the diversification of secretory cell types in the light organ of bioluminescent Ostracods.
- The MIST1, SREBP1 and TBX19 transcription factors originated in vertebrates. We did not find the orthologs of these genes in other animals suggesting

differentiation? (3)How can this help us understand the origin of the light organ in the bioluminescent ostracod Vargula hilgendorfii?

Methods

- Performed a literature survey to identify transcription factors known to differentiate secretory cells or glands in Metazoan organisms.
- Downloaded the FASTA protein sequences of our transcription factors of interest, MIST1, SREBP1 and TBX19, from NCBI.
- Built a computational pipeline to run the BLAST software on the command line.
- Searched the protein sequence against our custom genome database that includes annotated genomes from animals and ostracod transcriptomes to determine if any orthologs if our transcription factor of interest is also present in the upper lip of ostracods. Species of ostracod transcriptomes in our study are



Figure 2: Phylogeny of the MIST1 transcription factor. MIST1 highlighted in purple. Red bracket indicates the clade that contains MIST1. MIST1 originated in Chordates.

sterol regulatory element-binding protein 1 isoform X1 [Nematostella vectens SREBP1 class D bHLHL i regulatory element-binding protein 1-like [Dendronephthya gigan [zebrafish] - regulates the EDICTED: sterol regulatory element-binding protein 1-like (Amphimedon gueens EDICTED: sterol regulatory element-binding protein 1-like [Amphimedon queenslands expression of key metabolic DICTED: starol regulatory element-binding protein 1-like isoform X3 [Priapulus cauda enzymes necessary for lipid REDICTED: sterol regulatory element-binding protein 1-like isoform X2 [Priapulus cauda ____ thetical protein CAPTEDRAFT 165299 (Capitella teleta production. One ortholog in tical protein LOTGIDRAFT_224211 [Lottia gigante mouse (sterol regulatory element-binding protein); A few erol regulatory element-binding protein 1-like isoform X2 (Anneissia japonic isoforms exist for this gene. rol regulatory element-binding protein 1-like isoform X1 [Anneissia japon SREBP1 originated in the ol regulatory element-binding protein 2 (Mus muscul Chordates. ding protein 1 isoform b Mus muscu rol regulatory element-binding protein 1-like isoform X3 (Eur PREDICTED: sterol regulatory element-bit arol regulatory element binding protei sterol regulatory element binding protei sterol regulatory element binding protei erol regulatory element binding prote sterol regulatory element-binding protein 2-lik up c-1316 length 3061 cov 139.463 g756 i1.p ula hilgendorfi-TRINITY DN26067 c0 g1 i4 ia higendorfi-TRINITY DN26087 c0 g1 i5

Figure 3: Phylogeny of the SREBP1 transcription factor. SREBP1 highlighted in purple. Red bracket indicates the clade that contains SREBP1. SREBP1 originated in Chordates.

that the identity of transcription factors that differentiate and establish secretory cells in the light organ of ostracods might be different.

Future Wor



Using the homologs generated from the BLAST output, we will build a phylogenetic tree using the IQTree program.

The tree will then be viewed and analysed in iTOL viewer.

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- 3. Huvard, A. L. (1993). Ultrastructure of the light organ and immunocytochemical localization of luciferase in luminescent marine ostracods (Crustacea: Ostracoda: Cypridinidae). Journal of Morphology, 218(2), 181-193.

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