The role of microRNA miR-124 in the regulatory network governing nervous system development using *Ciona intestinalis*

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The life and times of a sea squirt (ascidian)

'Bridge organism': closest invertebrate relative to vertebrates **Conserved**: Shares 80% of its genes with humans **Neural development**: shares many fundamental mechanisms **Bioinformatics**: Sequenced genome, simpler gene networks **Experiments**: electroporation of transgenic reporters

Tadpole larva



Juvenile



Adult



Impact of our research with these little guys



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Science News

Sea Squirt Offers Hope for Alzheimer's Sufferers

ScienceDaily (Mar. 3, 2010) — Alzheimer's disease affects an estimated 27 million people worldwide. It is the most common form of age-related dementia, possibly the most feared disease of old age. There is no cure, and the available drugs only help to relieve symptoms without slowing progression of the disease. One of the characteristic changes in the brains of Alzheimer's patients is the accumulation of plaques and tangles; currently, the best hope for curing or at least slowing the disease lies in developing drugs that target this buildup. Some drugs are already in clinical trials, but there is still a pressing need for more research, and for more and better drugs directed against both known and novel targets.

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Health & Medicine

 Alzheimer's Research

Healthy Aging

Mind & Brain

 One of the big problems in rapidly screening potentially useful drugs has been the lack of a good model system in which Alzheimer's plaques and tangles appear quickly.
 However, Mike Virata and Bob Zeller, scientists working at San Diego State University, California,





Ciona nervous system





CNS: "brain": ~100 neuron aggregate in the head; light, gravity sensing, motor response; "spinal cord": dorsal nerve cord along tail

PNS: ~10 pairs of ciliated epidermal sensory neurons (ESNs), evenly-spaced punctate pattern along dorsal and ventral midlines of the tail





The microRNA miR-124

- Sequence is well-conserved between invertebrates and vertebrates
- Nervous system specific



Neural-like cells

[Lim, 2005]



Non-specific HeLa cells

miR-124 knockdown



miR-124 overexpression

[Visvanathan, 2007]







Gene Expression and microRNA Function





- Watson-Crick base pairing
- 1st 8 nucleotides, "seed"
- inhibits translation (RNAi)

miRNA and Seed sequence

 Binding of miRNA seed region (first 8 bp) to 3'UTR most critical for functional efficacy

		87654321	(Seed)
Ci-miR-124	3′	AACCGUAAGUGGCGCACGGAAU	5′
Ci-generic 3'UTR	5′	CGCCCAGUAUCCAGGUGCCUUA	3′

Ci-miR-124	3′	NNNNNNNNNNNNN CACGGAAU	5′
bp 1-8	5′	.NNNNNNNNNNNNNCUGCCUUA	3′
bp 2-8	5′	.NNNNNNNNNNNNN CUGCCUUN	3′
bp 1-7	5′	.NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN	3′

Refs: Lewis (2003), Lewis (2005), Brennecke (2005), Krek (2005), Grimson (2007)

Target prediction algorithm

- 1. Extracted 3'UTRs from 24,000 genes, verified a/ ESTs
- 2. Scanned 3'UTRs for miR-124 seed-matching sites
- 3. Positive targets were scored for other binding criteria
- 4. Targets ranked and sorted

866 unique targets

Annotation of miR-124 gene targets

- 1. Computational BLAST analysis found top 10 homologous genes across all organisms
- 2. Used this information to give the gene an annotation using GO (gene ontology)

GO ID	Description	P-value
GO:0004708	MAP kinase kinase activity	0.002
GO:0030917	midbrain-hindbrain boundary development	0.006
GO:0042551	neuron maturation	0.006
GO:0007212	dopamine receptor signaling pathway	0.016
GO:0030902	hindbrain development	0.021
GO:0030425	dendrite	0.028
GO:000082	G1/S transition of mitotic cell cycle	0.028
GO:0021532	neural tube patterning	0.031
GO:0048749	compound eye development	0.034

Comparison with human targets

- Extracted gene IDs from two studies on human cell lines which identified human miR-124 targets using microarrays [Lim (2005), Karginov (2007)]
- 2. Used custom scripts along with NCBI web-based tools to extract official IDs and sequences for list of human miR-124 targets
- 3. Put these into a BLAST-formatted database
- 4. BLAST Ciona miR-124 targets against human miR-124 target database
- 5. Results:
 608 / 866 ci-mir124 targets have a human homolog (BLAST against human proteome, e-value < 1e-10)
 144 / 608 targets with human homologs are also human mir124 targets (BLAST against database of human targets, e-value < 1e-10)
 17% of our targets are also found as human targets with microarrays

Verification of targets - insight into miRNA function



Near-future projects

- **1. High throughput proteomics** Global identification of miR-124 targets
- 2. miR-tar-BLAST: web-based GUI for miRNA target prediciton algorithm
- 3. Improving scoring function of miRNA target prediction

High-throughput proteomics

• High-throughput identification of miR-124 targets by comparing protein levels with and without miR-124



Difference in peak areas shows relative abundance of sample vs. wild type (control)⁵

FIG: Cagney, et.al. Nature (2002) 20: 163

Thank you!

- Bob Zeller
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- Paul Paolini
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- NIW program
- NSF
- Dr. Castillo