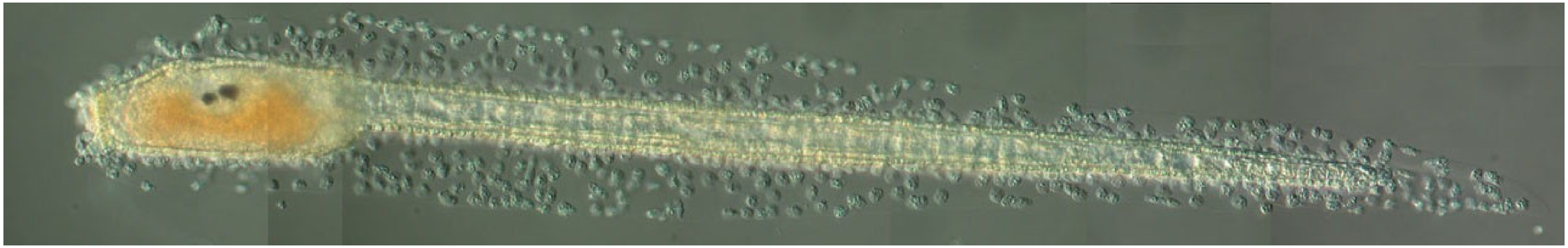
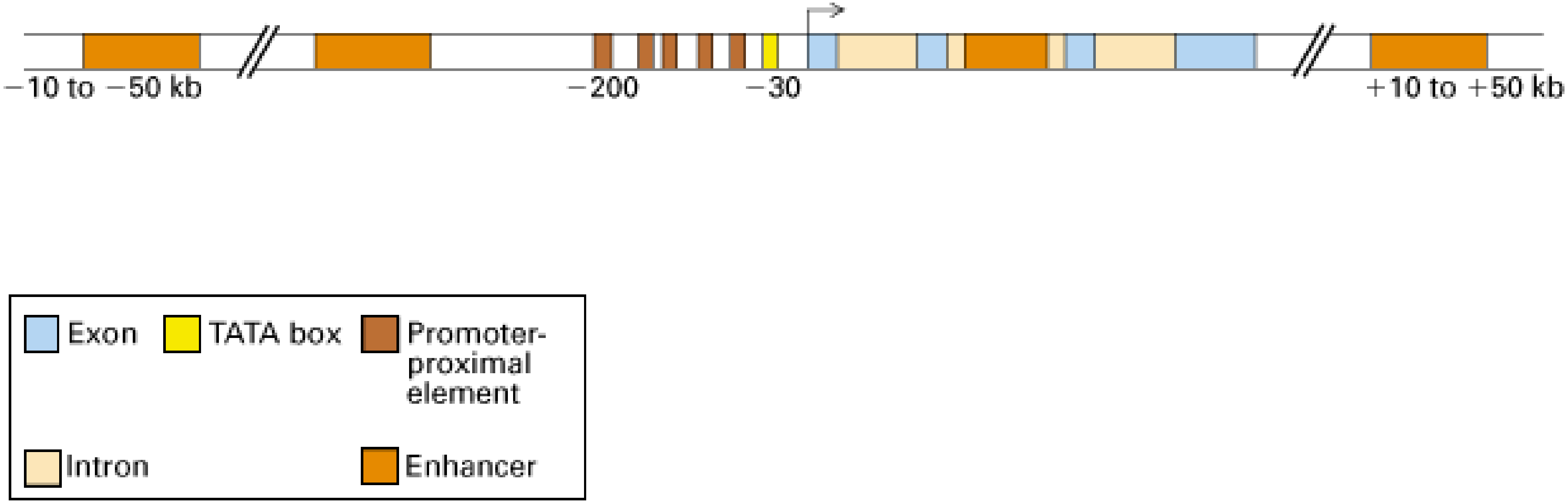
microRNAS: A new wrinkle on gene regulation



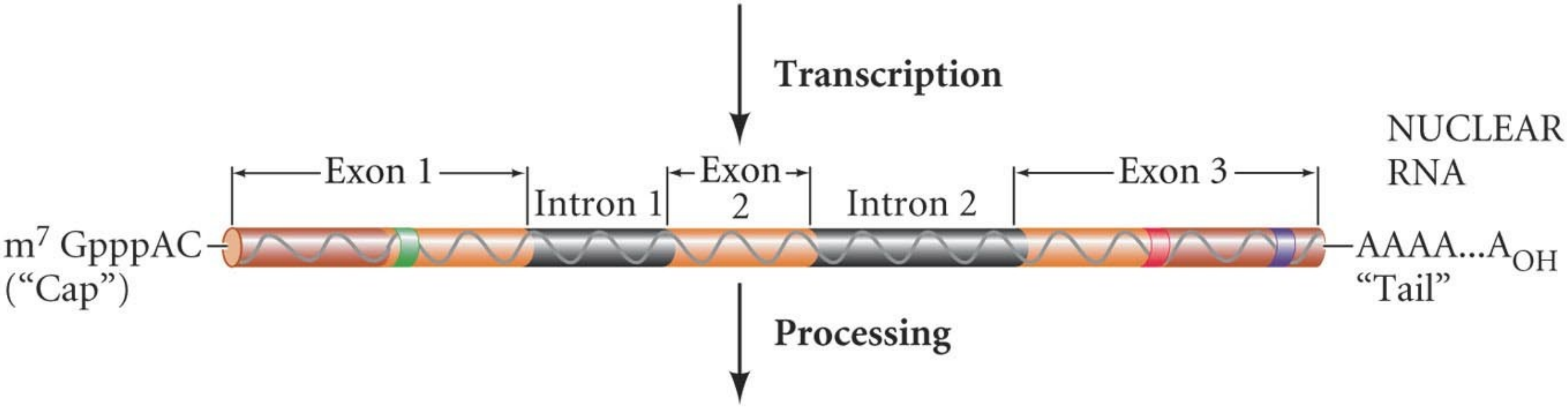
Robert W. Zeller
Center for Applied and Experimental Genomics
Department of Biology
San Diego State University
San Diego, CA

SDSU CSRC 2009

The structure of a typical eukaryotic gene

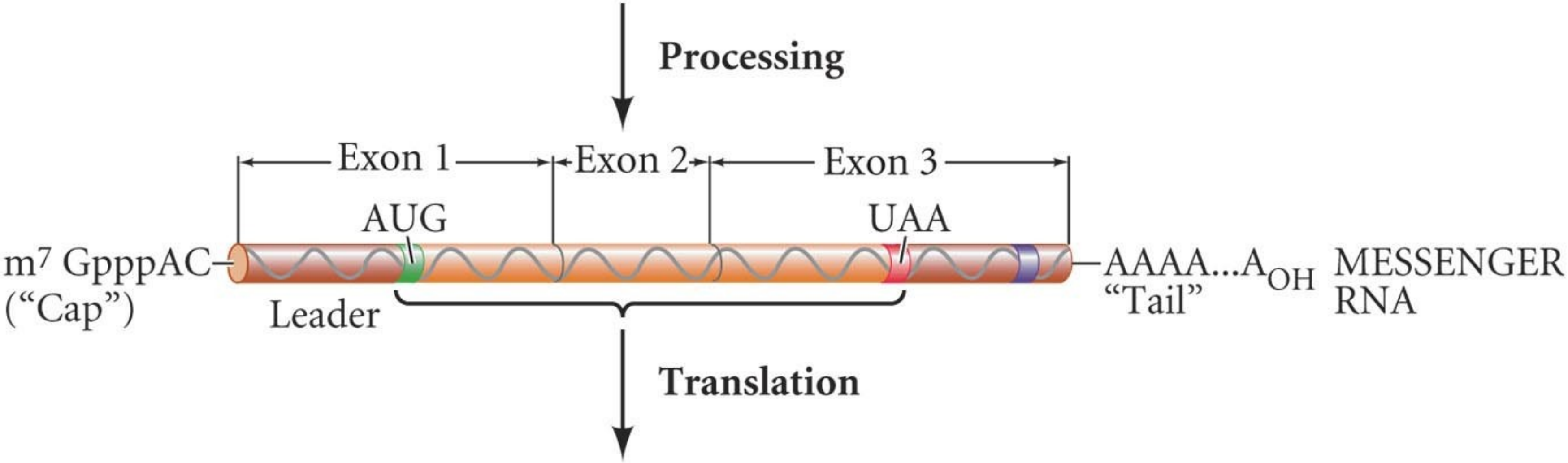


Transcribed regions of genes contain exons and introns



DEVELOPMENTAL BIOLOGY, Seventh Edition, **Figure 5.3 (Part 2)** Sinauer Associates, Inc.

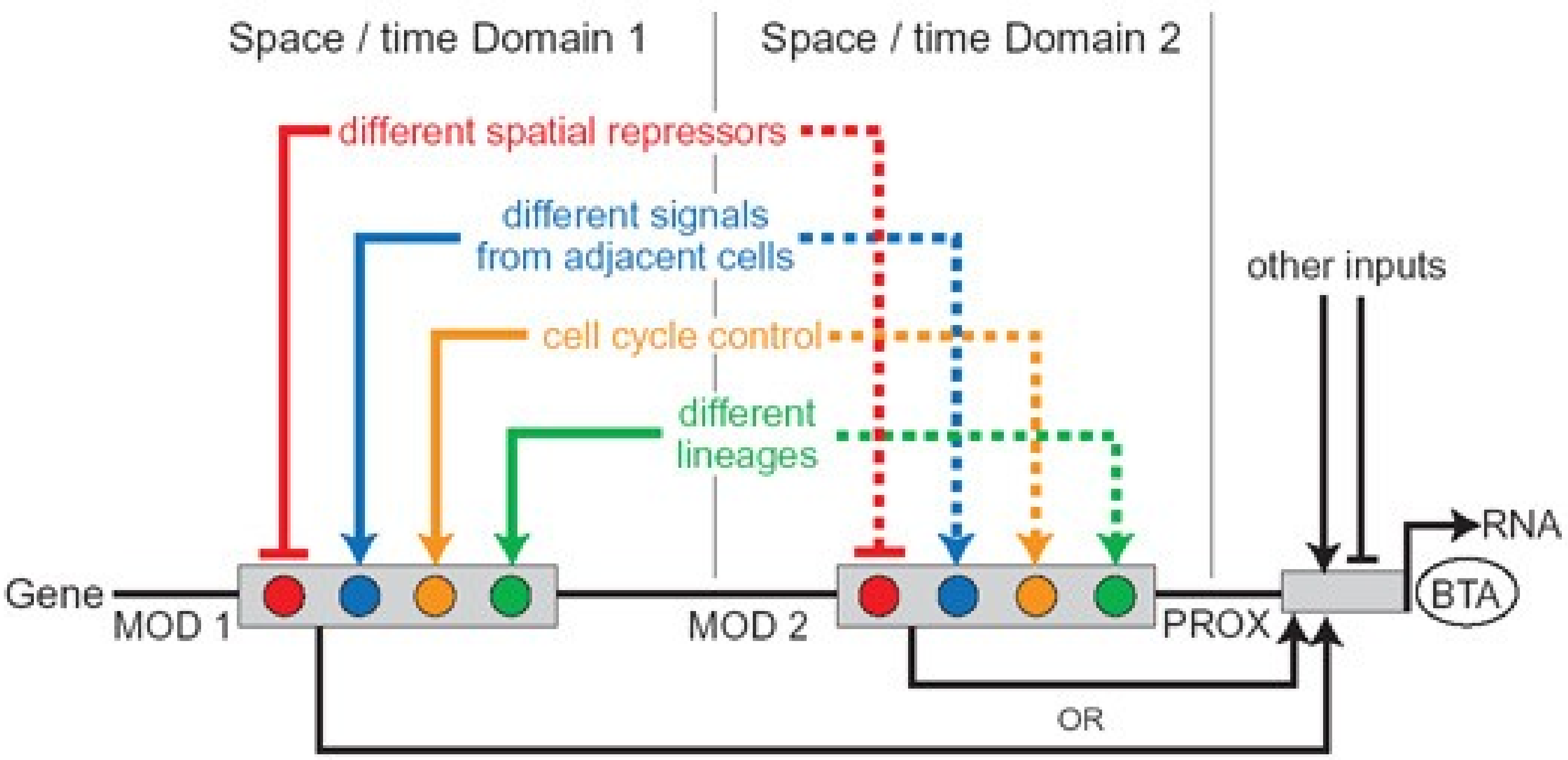
After splicing, mRNAs are translated into proteins



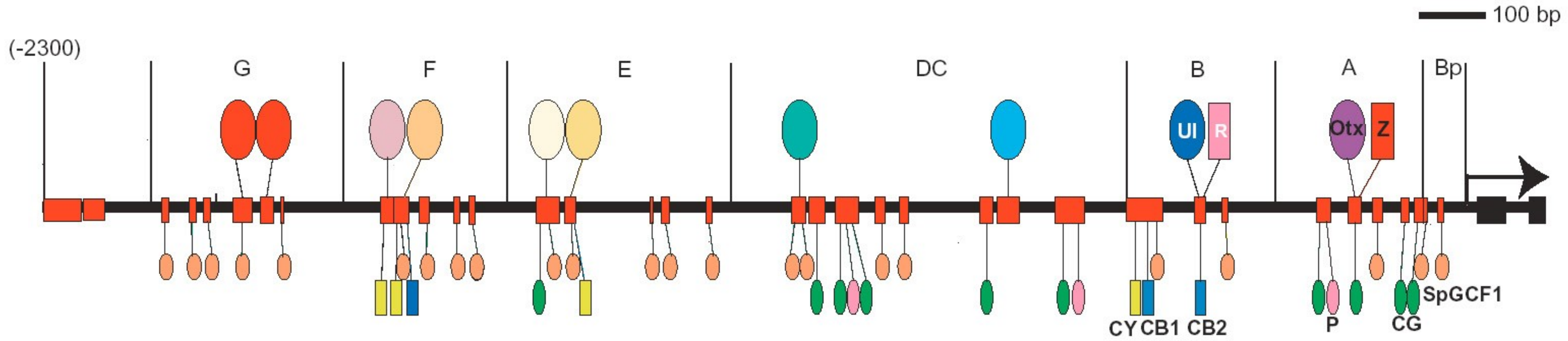
DEVELOPMENTAL BIOLOGY, Seventh Edition, **Figure 5.3 (Part 3)** Sinauer Associates, Inc.

cis-Regulatory regions are bound by transcription factors to regulate gene expression

Integration of information necessary for proper temporal and spatial expression of genes



Gene regulatory regions are typically complex

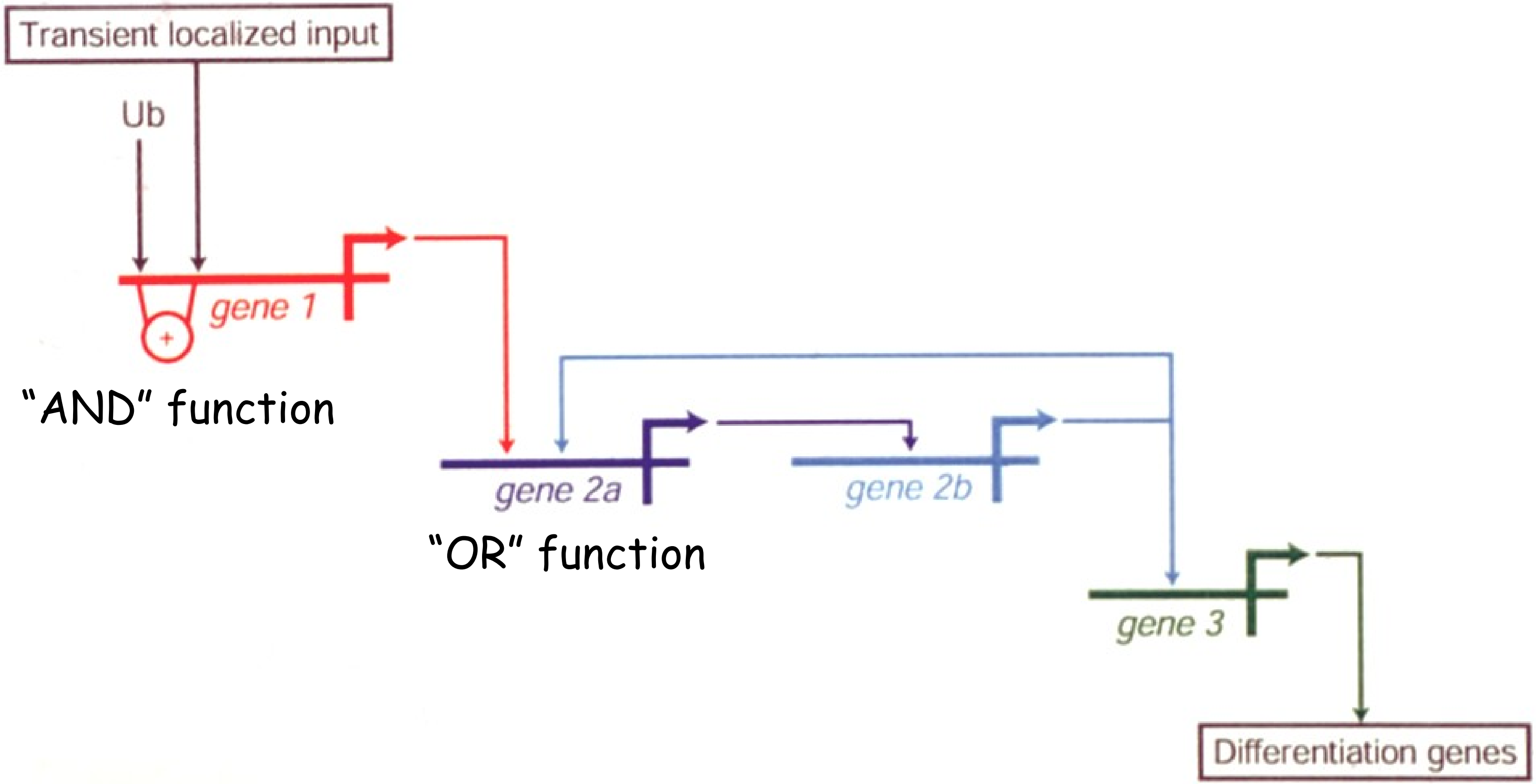


~20 proteins (balloons) regulating ~50 target sites (boxes)

Gene regulatory networks (GRNs):

- Genome - encoded elements (=parts) and instructions for using the parts
- transcription factors and signal transduction molecules (receptors and ligands)
- information is “digital” and “analog“
- Inputs and Outputs

A simple, hypothetical GRN



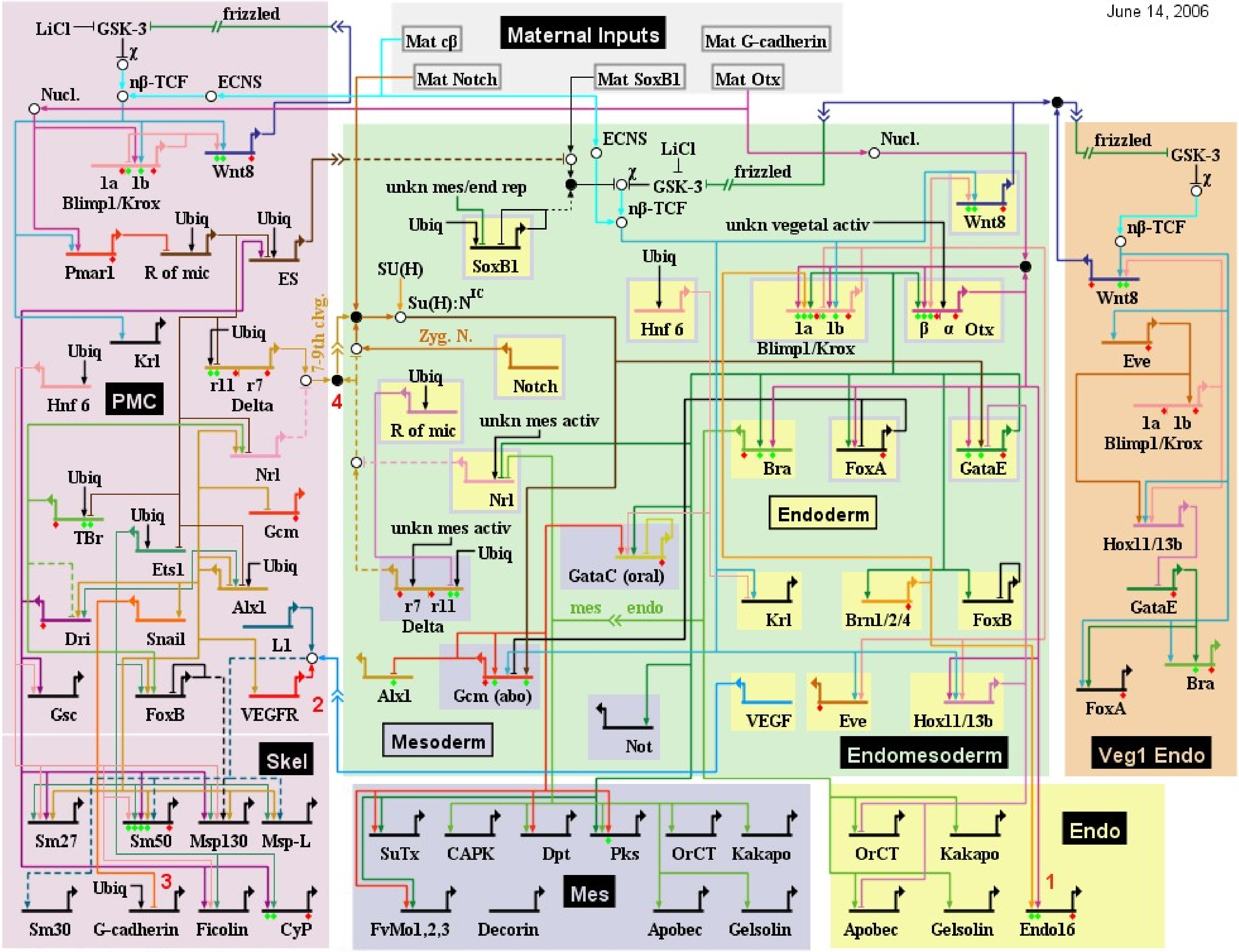
“AND” function

“OR” function

Davidson

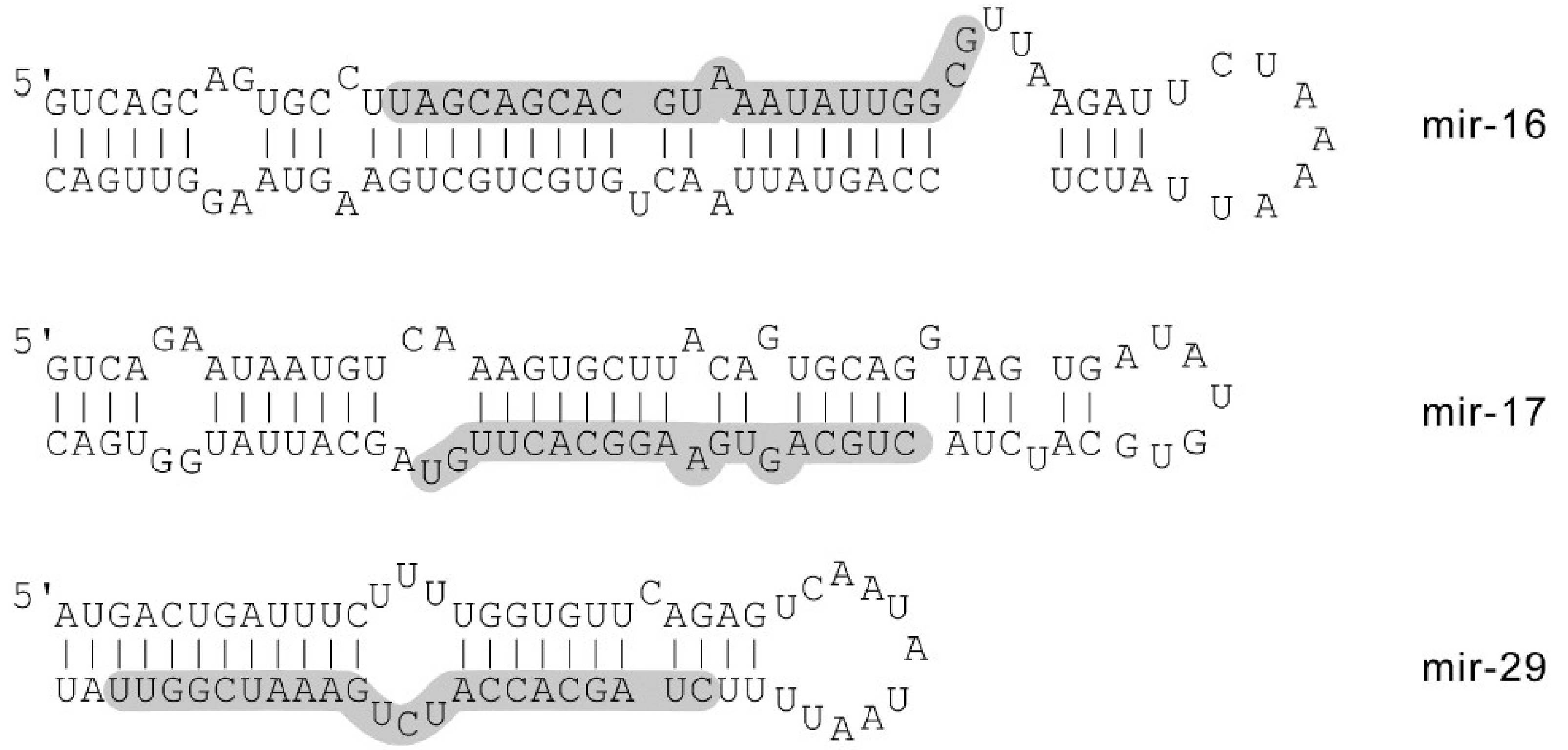
Endomesoderm Specification to 30 Hours

June 14, 2006



Ubiq=ubiquitous; Mat = maternal; activ = activator; rep = repressor;
unkn = unknown; Nucl. = nuclearization; χ = β-catenin source;
nβ-TCF = nuclearized b-β-catenin-Tcf1; ES = early signal;
ECNS = early cytoplasmic nuclearization system; Zyg. N. = zygotic Notch

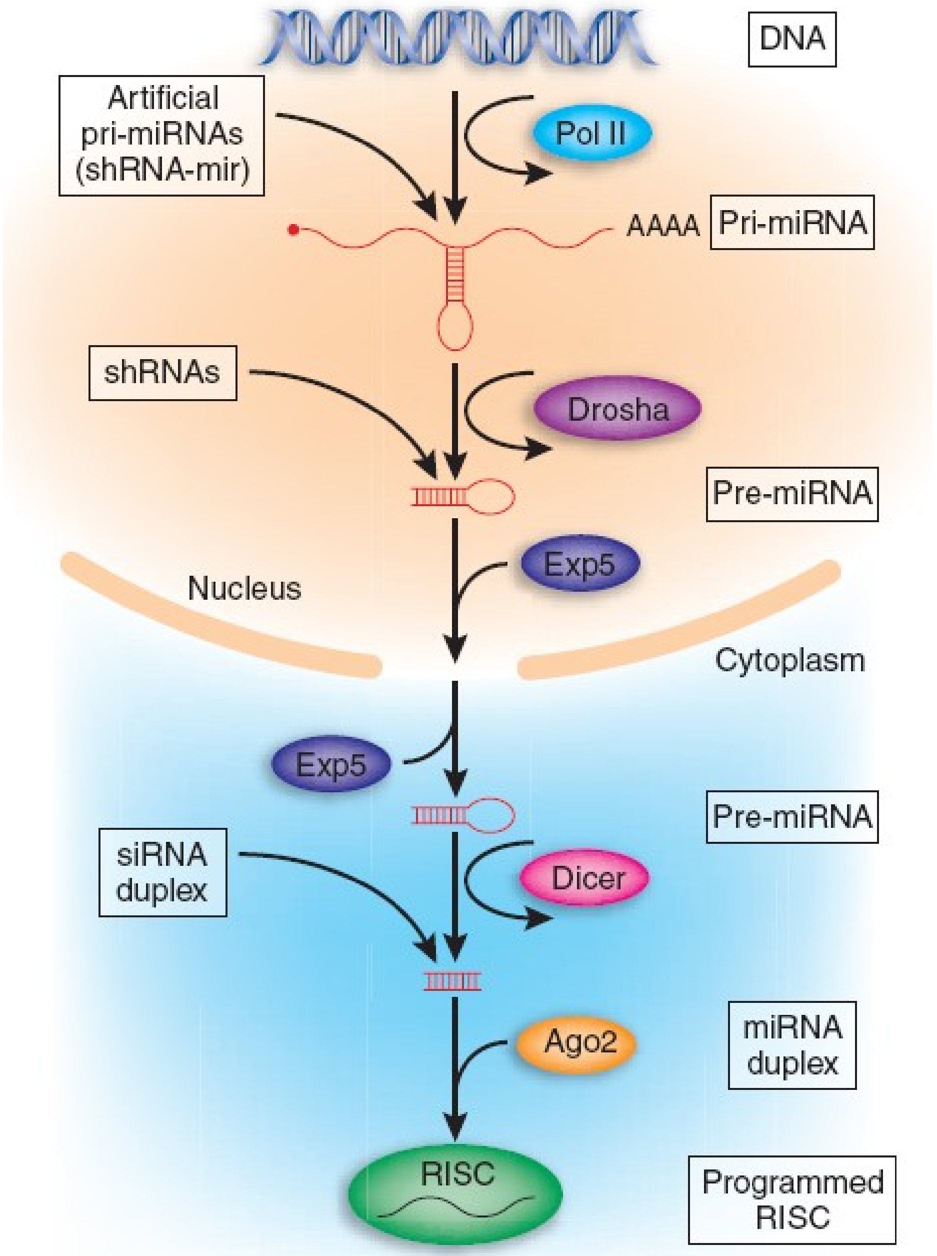
miRNAs :



miRs are typically found in introns, but can also be found in intergenic regions

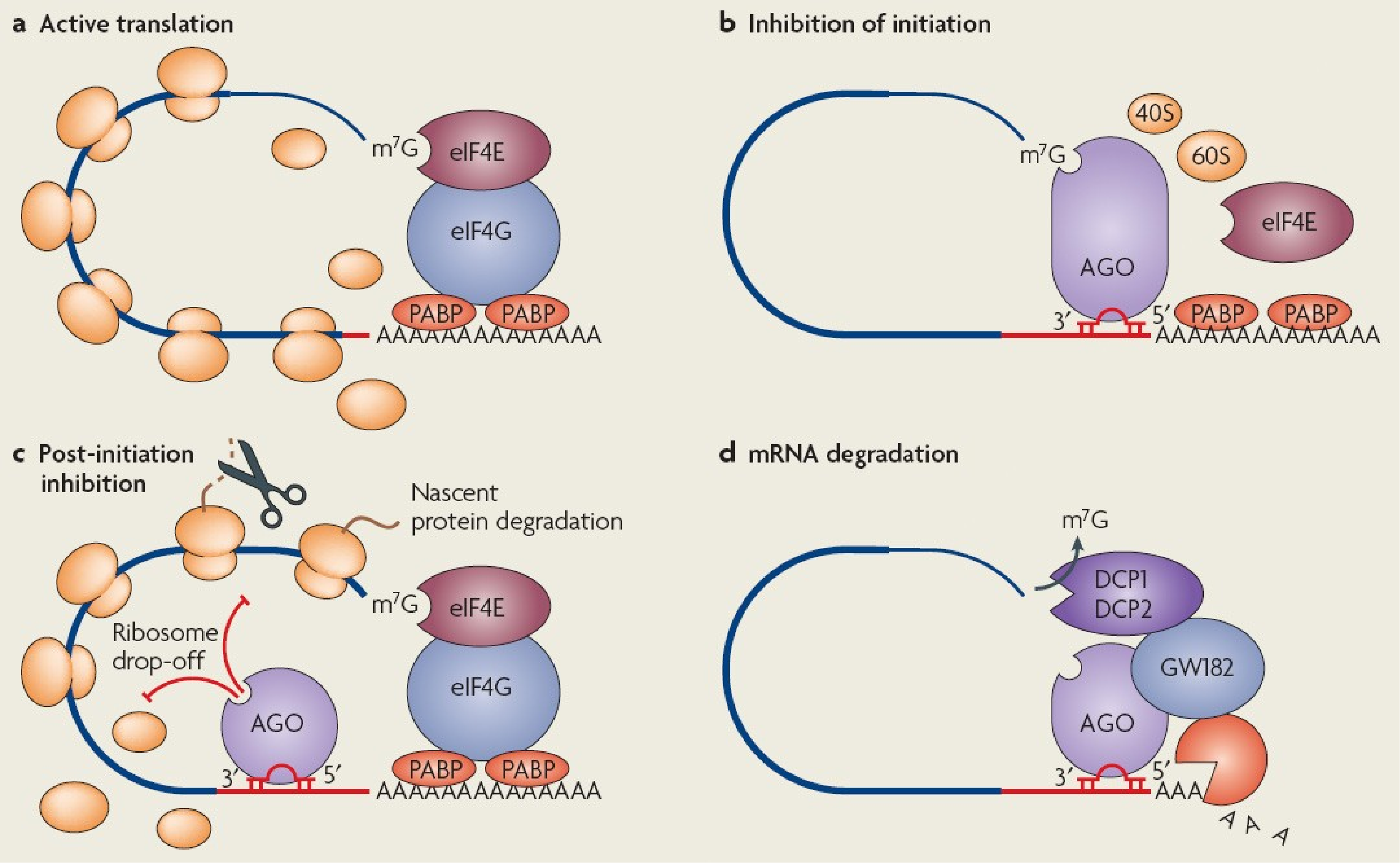
Bengert and Dandekar 2005

Micro RNAs



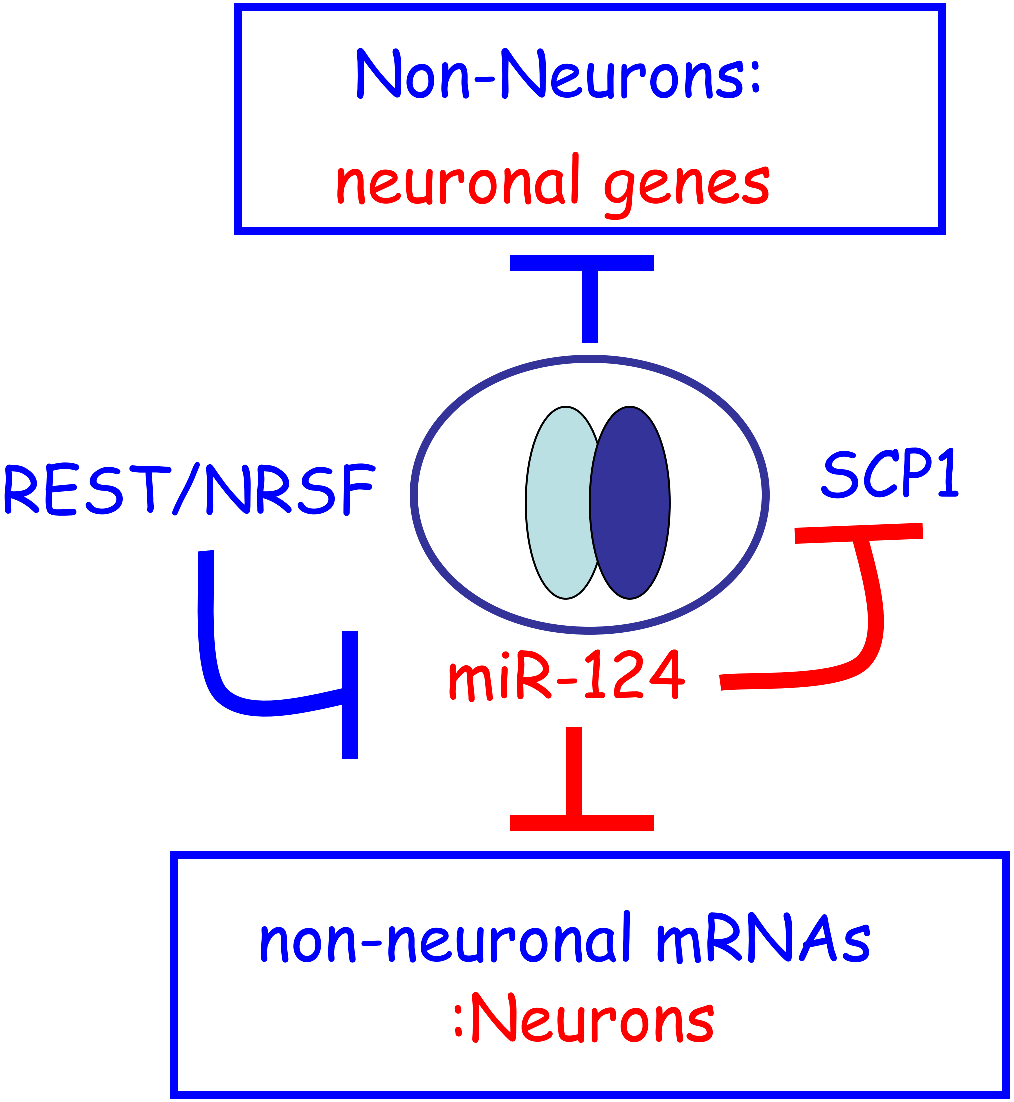
1) Dicer processes dsRNA into ~22 nt fragments (RNAse III)
2) siRNAs incorporated into RISC complex
3) RISC uses siRNA as template guide to target mRNA for cleavage

miRs regulate protein translation



Stefani and Slack, 2008

miR-124 is expressed in neurons and represses non-neuronal gene expression



Non-Neurons:

neuronal genes

REST/NRSF

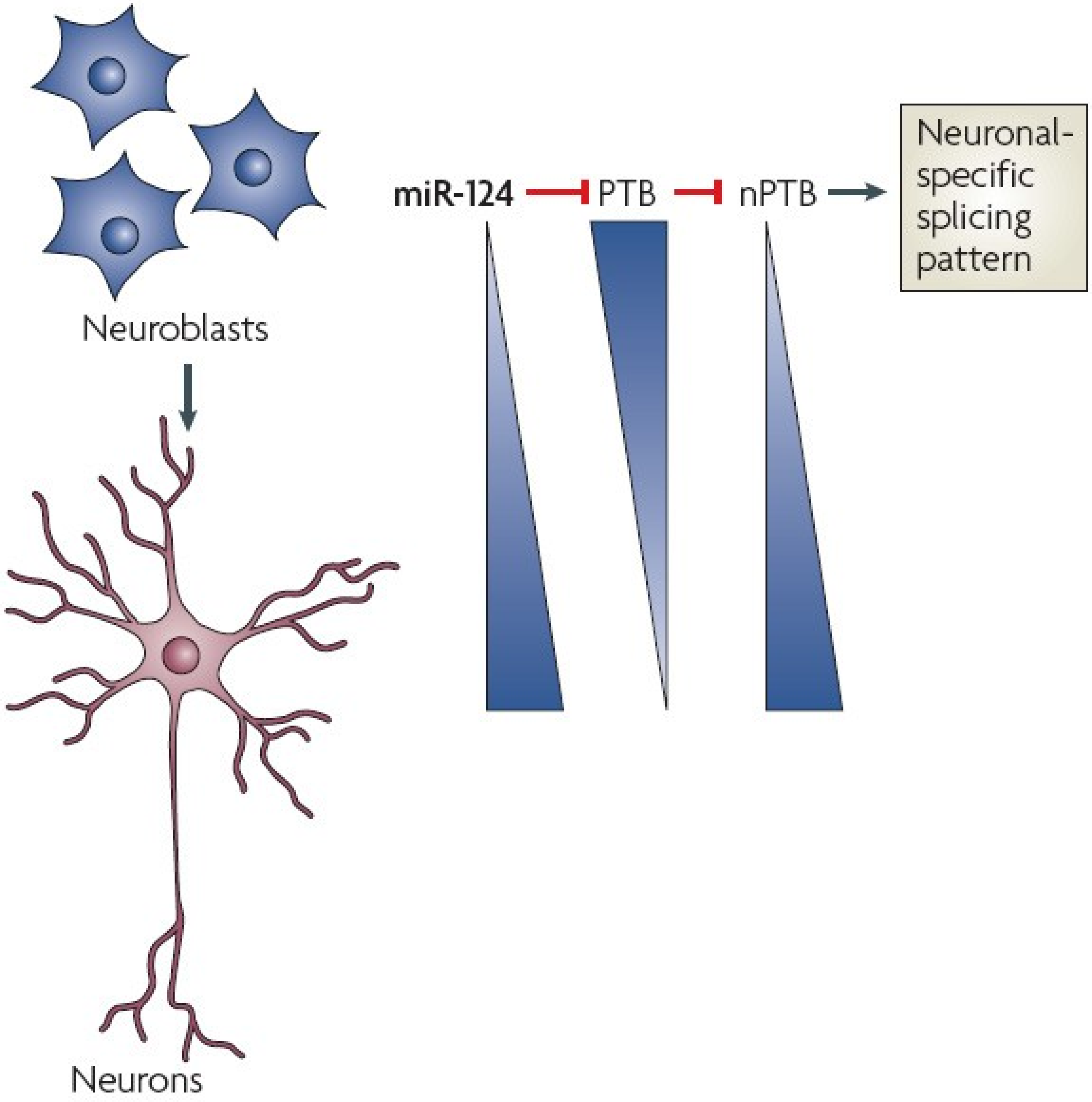
SCP1

miR-124

non-neuronal mRNAs

:Neurons

A specific miR-124 target regulates mRNA splicing



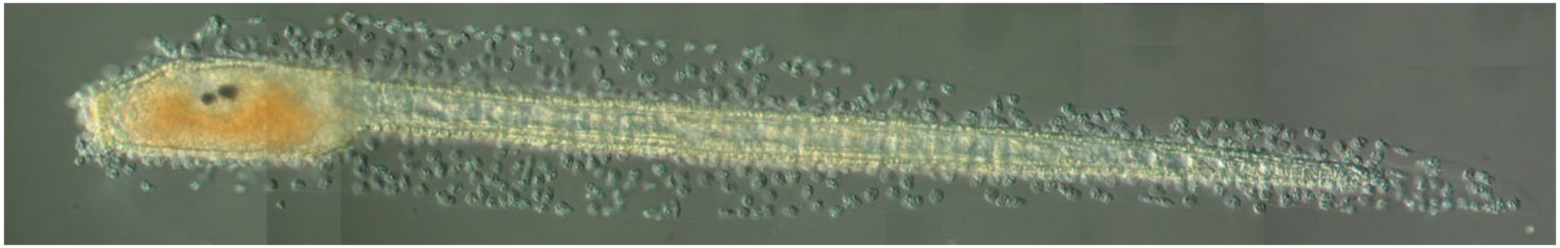
Neuroblasts

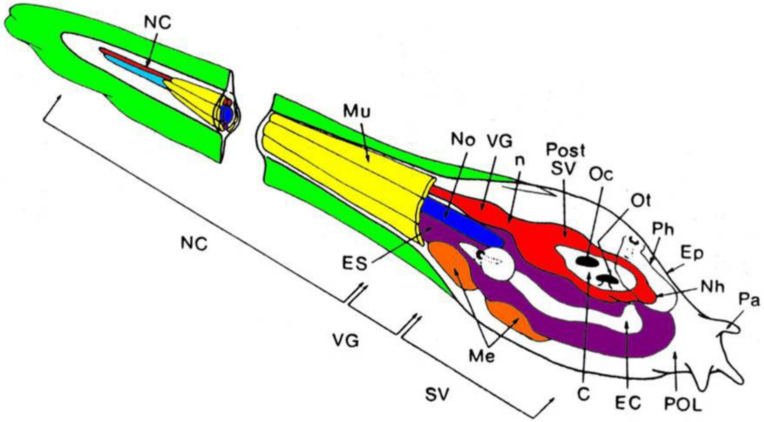
Neurons

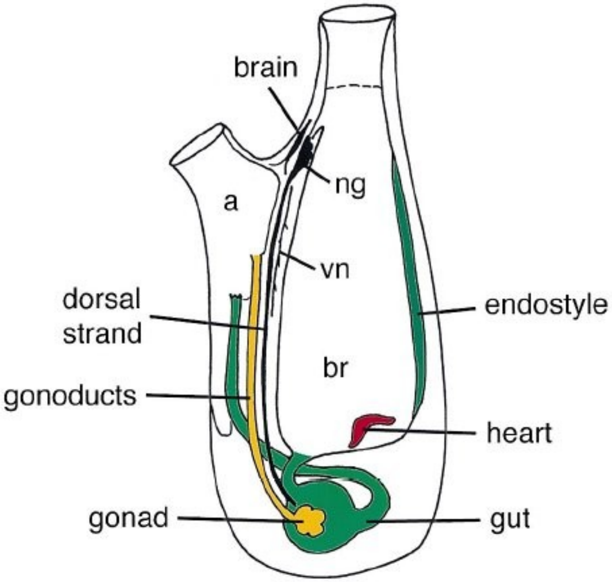
miR-124 ⊣ PTB ⊣ nPTB → Neuronal-specific splicing pattern

Stefani and Slack, 2008

Ascidians are invertebrate chordates





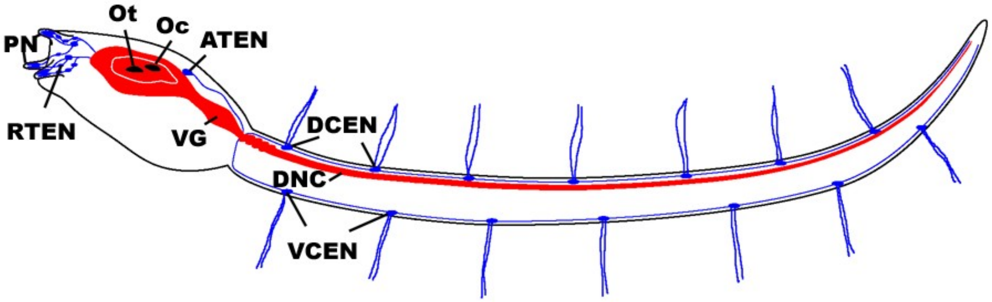


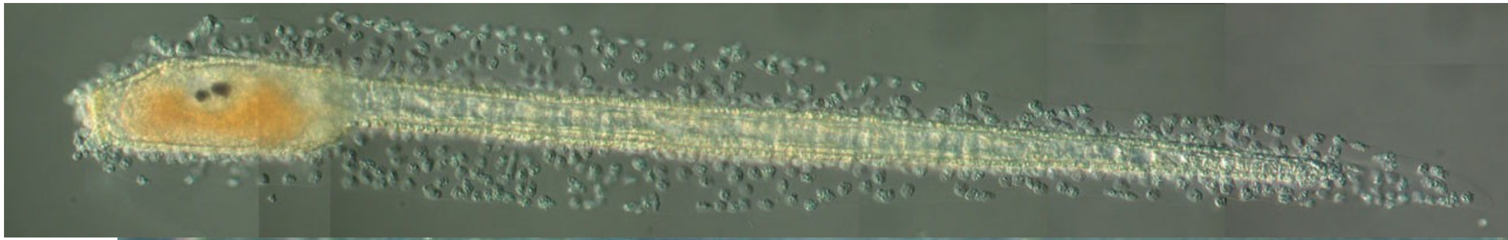
Baker and Bronner-Fraser, 1997

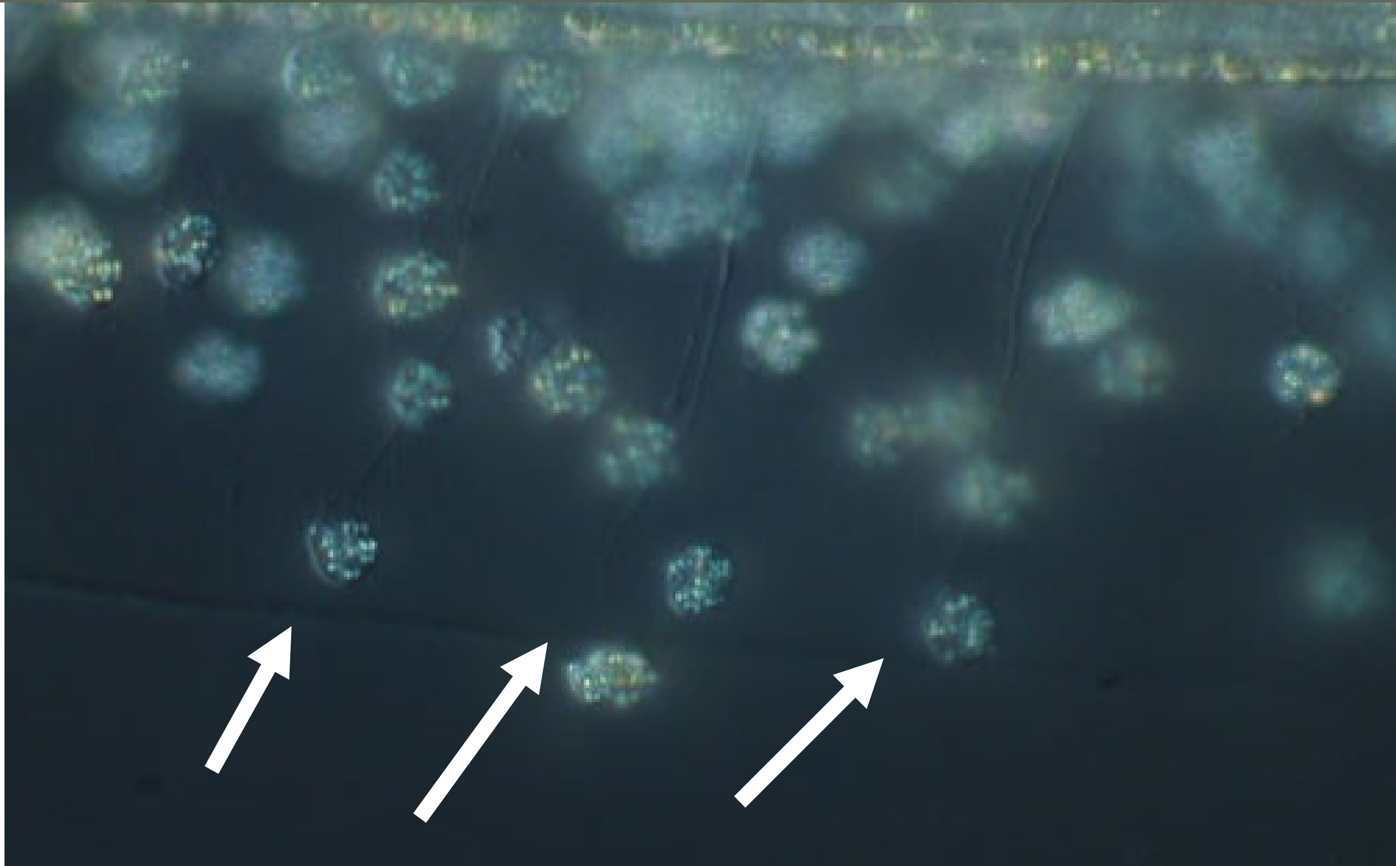
Epidermis : 800 cells
Mesenchyme: 900 cells
CNS: 330 cells
PNS: 50 cells

Muscle: 36 cells
Notochord: 40 cells
Endoderm: 500 cells

~2500 cells total

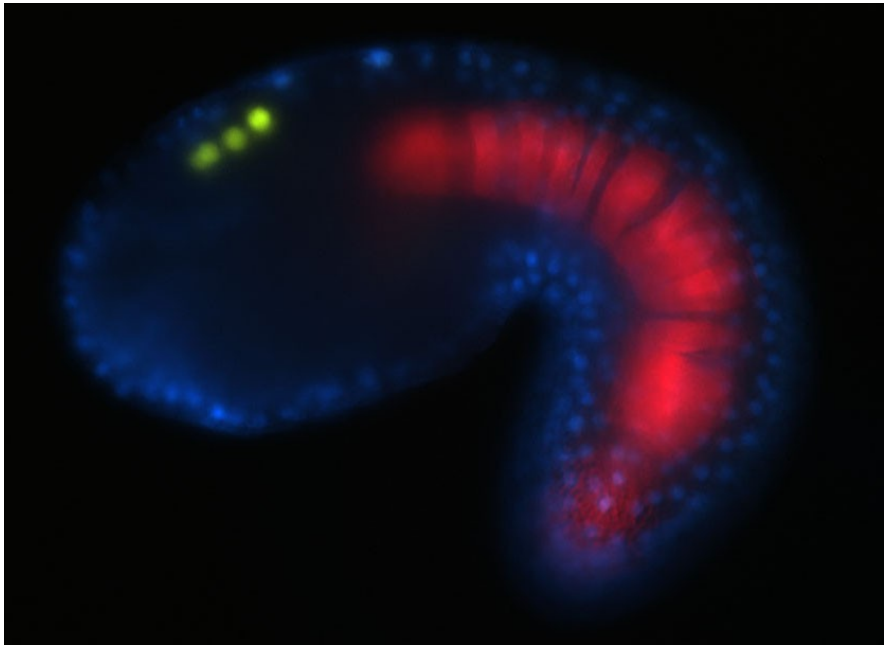






epidermal
sensory
neurons
ESNs

Ascidians are an excellent animal model for studying gene regulation



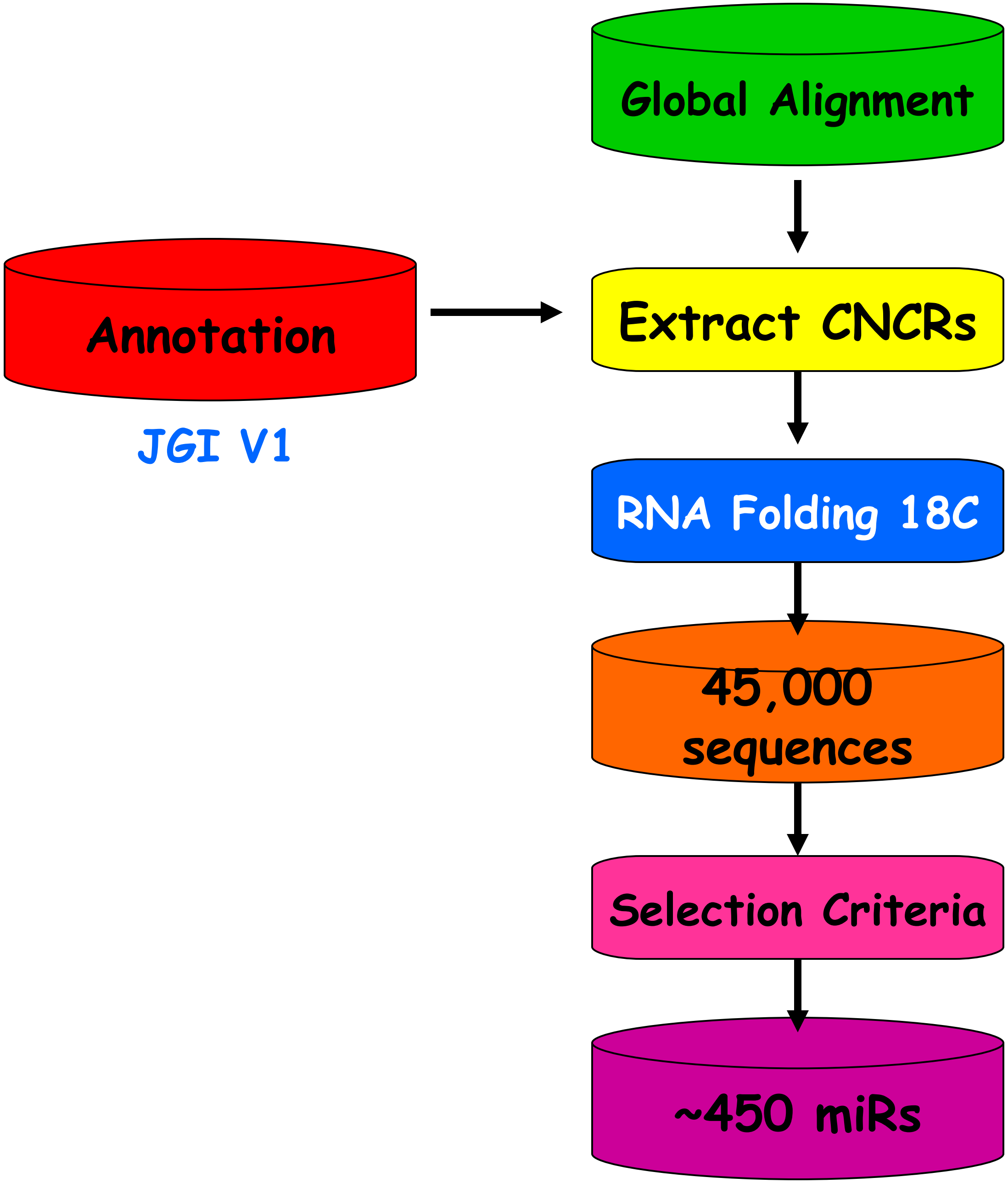
Small genome
Non-redundant genes
Genetics
Mutational screens
Comparative genomics

Simple to make transgenic
Rapid development
Single-cell resolution
Two genomes
Functional Genomics

Zeller, 2004, Zeller, et al 2006a, b

Satou et al., 2008


ASTM
JM90
8-14
8-14



Global Alignment

C. intestinalis
C. savignyi

Annotation

JGI V1

Extract CNCRs

~12M 100nt sequences

RNA Folding 18C

45,000 sequences

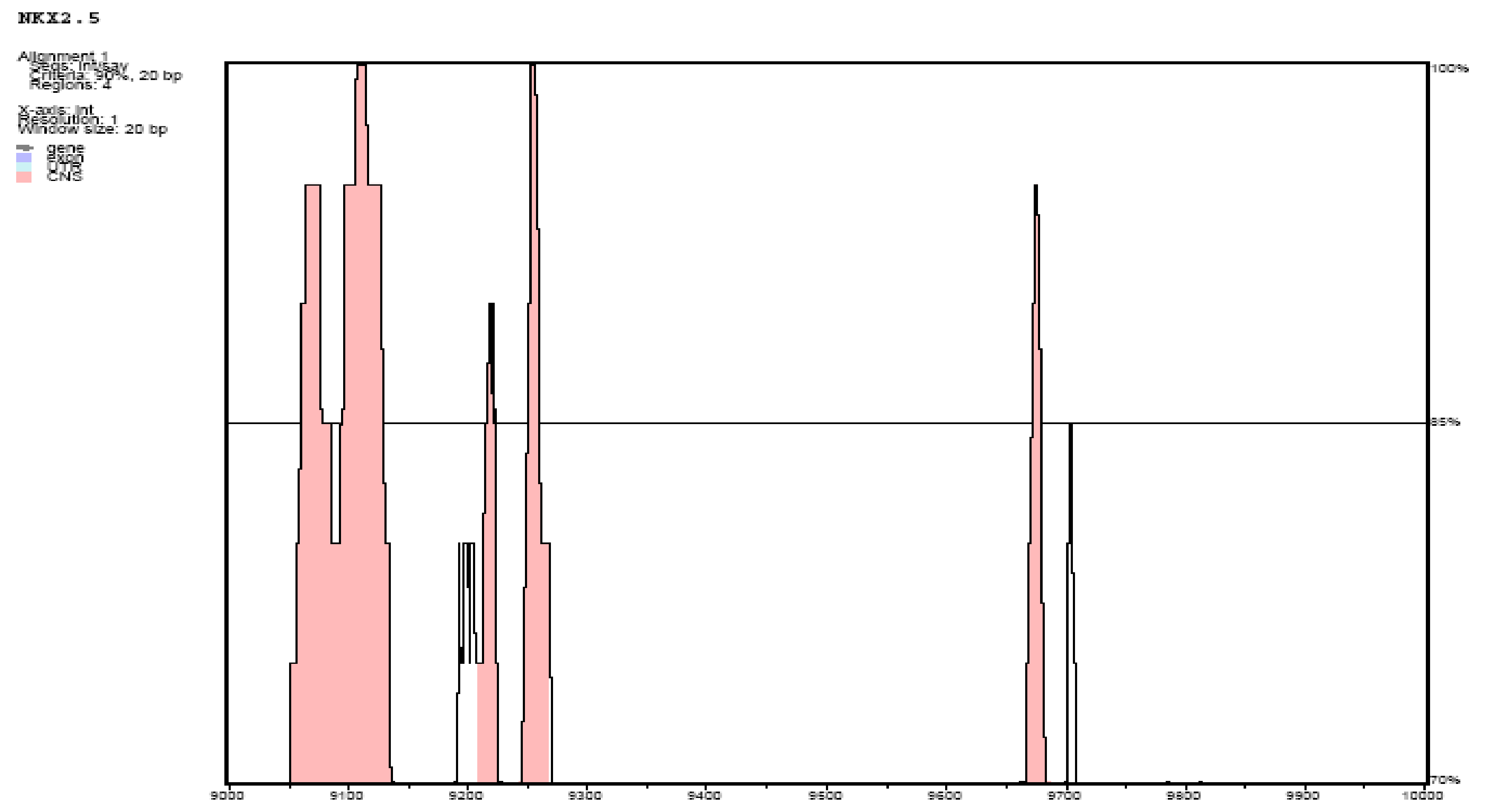
Fold like a hairpin at 18C

Selection Criteria

~450 miRs

Raja Keshavan

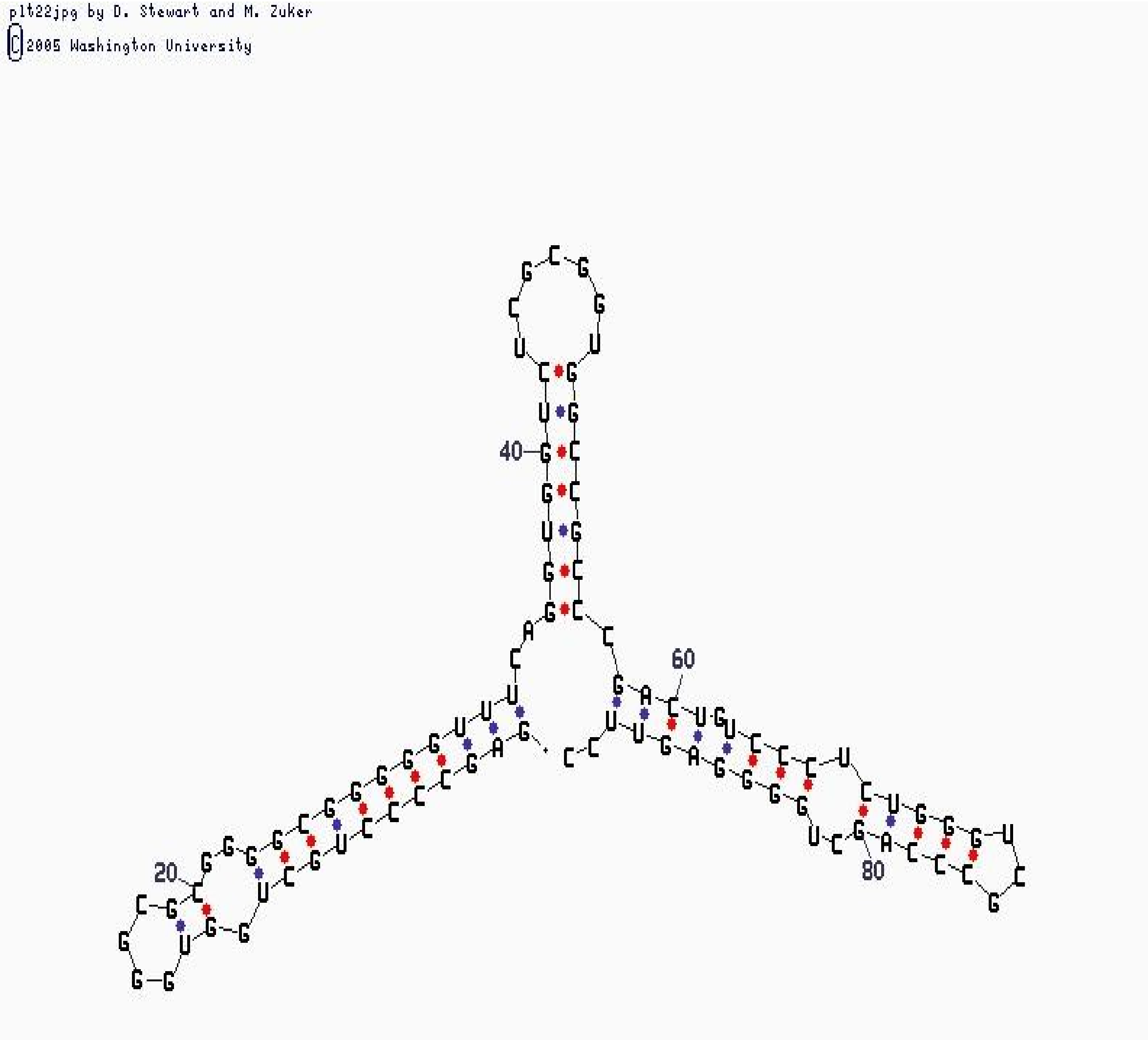
Phylogenetic footprinting identifies miR genes



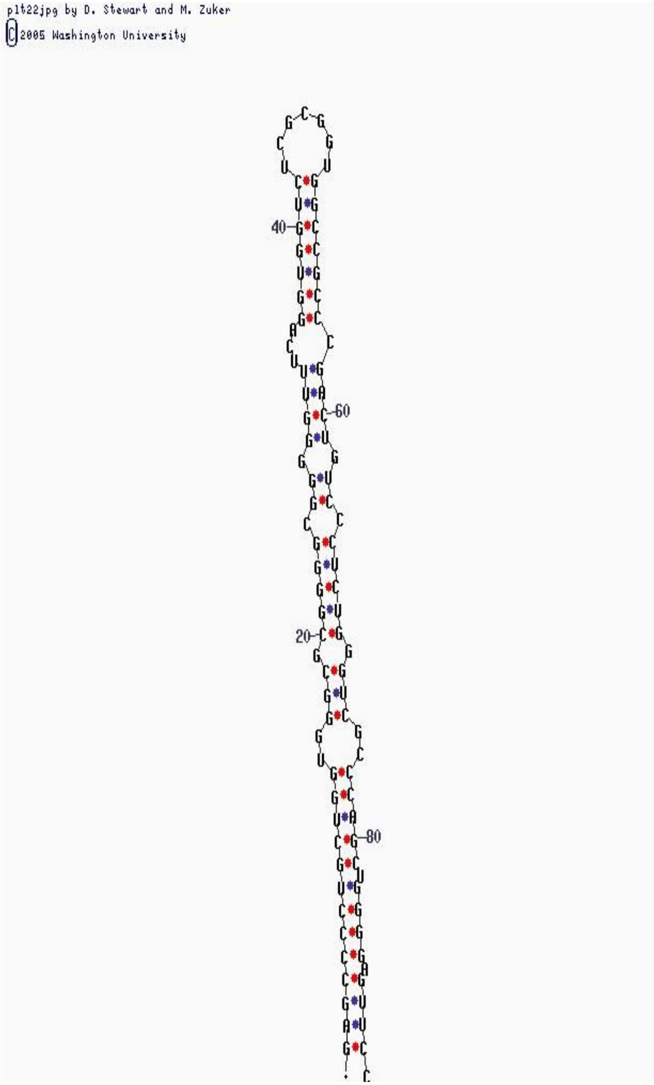
miR-125, Let-7 and miR-100, left to right

Raja Keshavan

Folding temperature matters for modeling!



miR-347 at 18C



miR-347 at 37C

Raja Keshavan

Predicted miRs fold the same in both species

Let-7

Scaffold_95:49190-49290 -

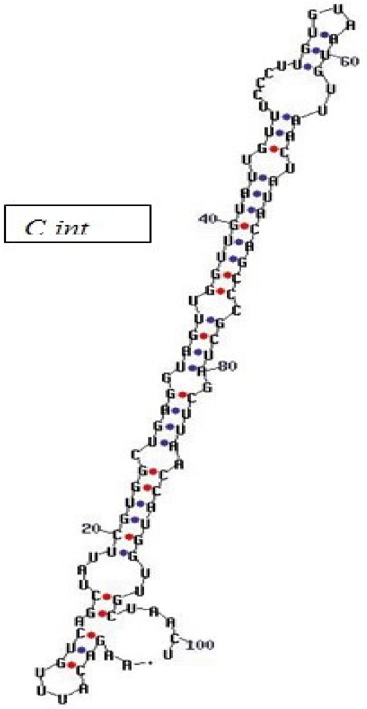
```
                               Let-7
                        |--------------------|
Ciona sav:  gctattcacagatgaggtagttggttgtattgttt
            |||||||   | |||||||||||||||||||||||
Ciona int:  gctattcgtggctgaggtagttggttgtattgttt
                        |||||||||||||||||| |||
fru-let-7d              UGAGGUAGUUGGUUGUAUGGUU
```

miR-100/99b

Scaffold_95:49640-49740 +

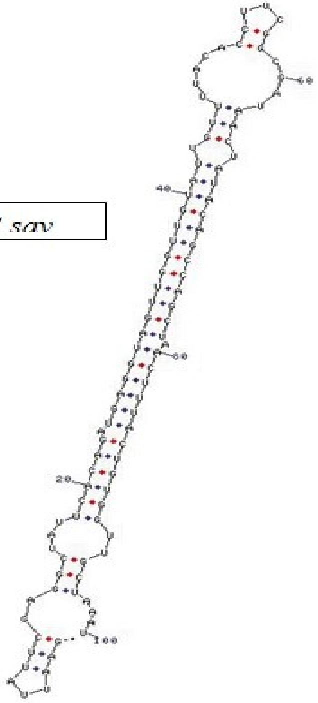
```
                           miR-100/99b
                        |--------------------|
Ciona sav:  cgttggatgcggcacccataaaaccgagcttgaga
            || | | | |  |||||||||| ||||||||||||
Ciona int:  cgcttggtccaacacccataaacccgagcttgaga
                        ||||| || |||||| |||| |
has-miR-99b             CACCCGUAGAACCGACCUUGCG
```

C int



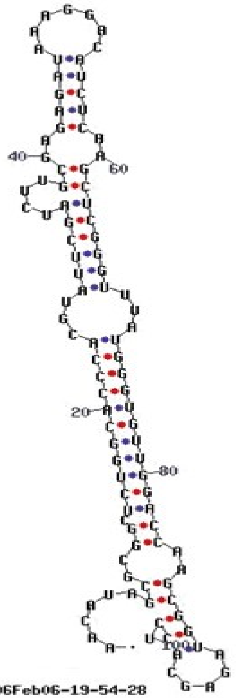
dG = -45.3 06Jan23-20-07-18

C sav



dG = -49.4 06Jan23-20-03-35

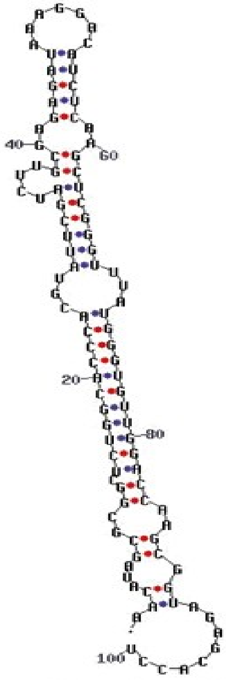
C int



dG = -46.3 06Feb06-19-54-28

dG = -46.3 06Feb06-19-54-28

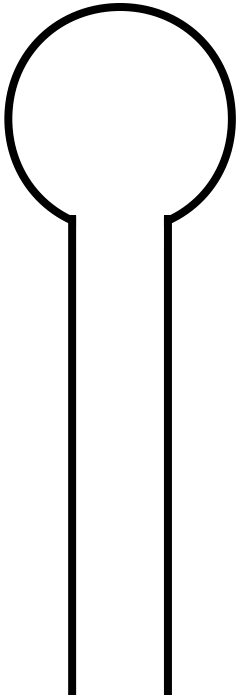
C sav



dG = -31.1 06Feb06-20-

Raja Keshavan

A tiled microarray validates miR expression in *Ciona*



450 predictions on array

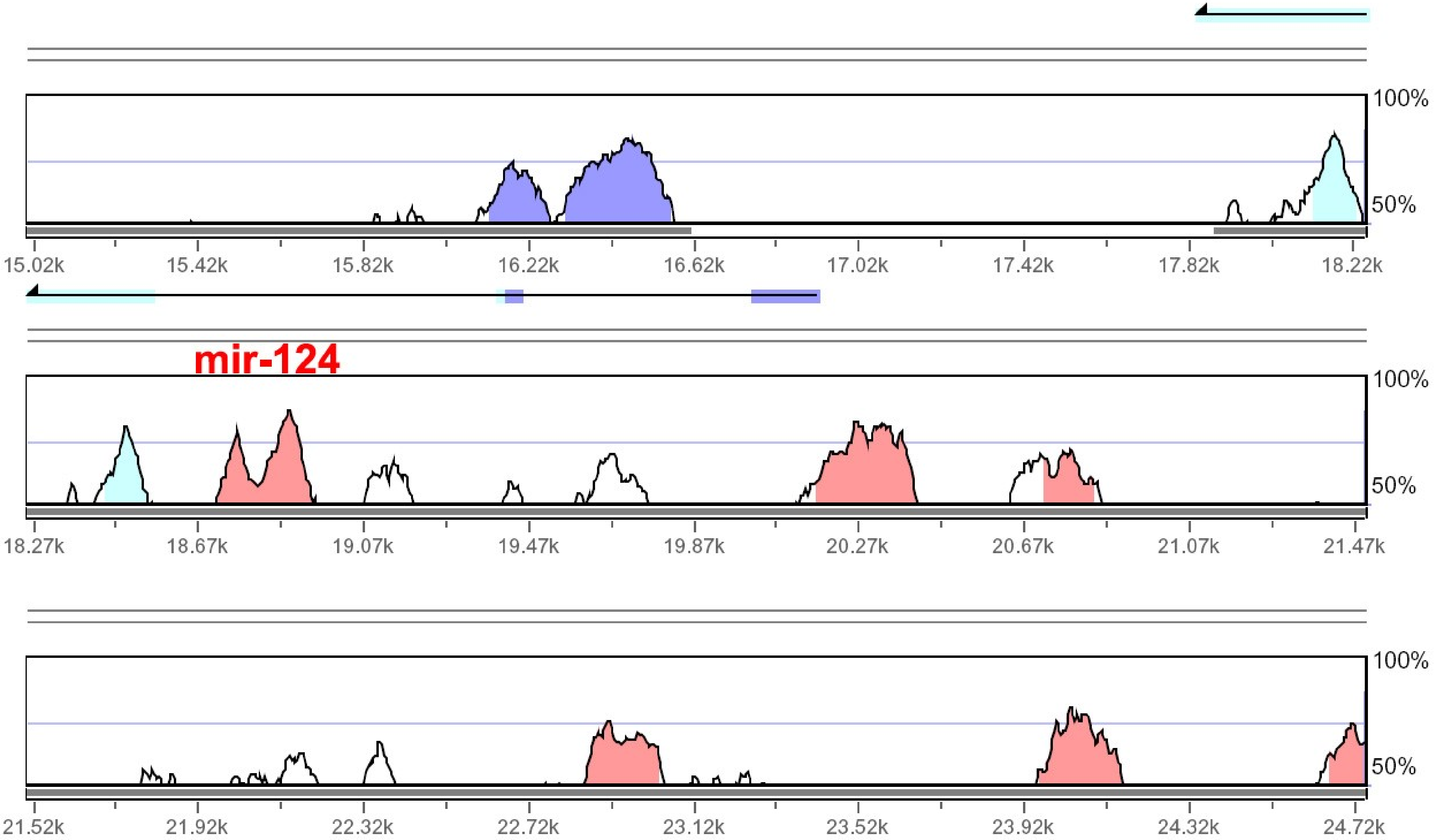
80 published miRs
- 12 Array (+)
- 29 Array (-) but predicted
- 39 not predicted

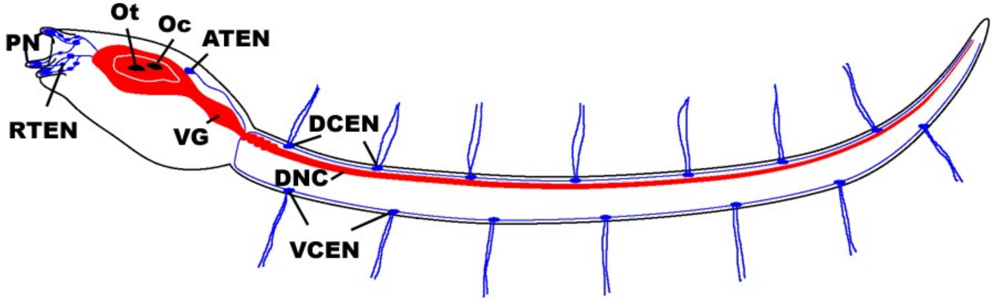
88 unique Array (+)
345 predicted but Array (-)

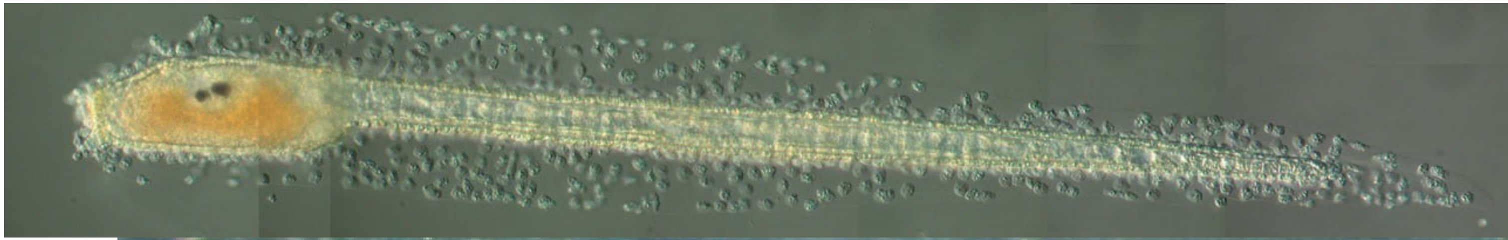
An evolutionarily conserved miRNA is expressed in the CNS and in the ESNs

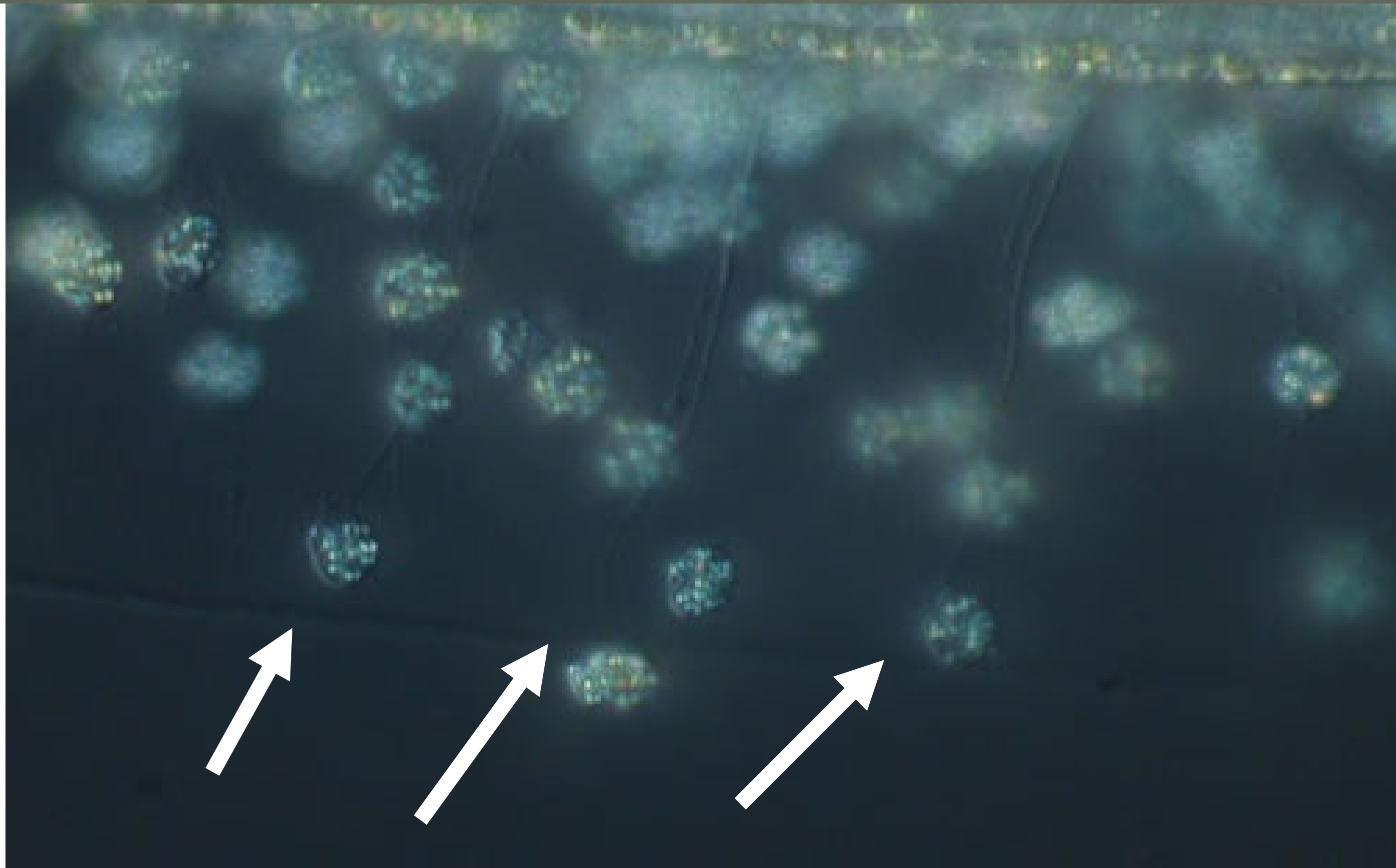
Jerry Chen

miR-124 is found within an intron of a non-protein coding gene



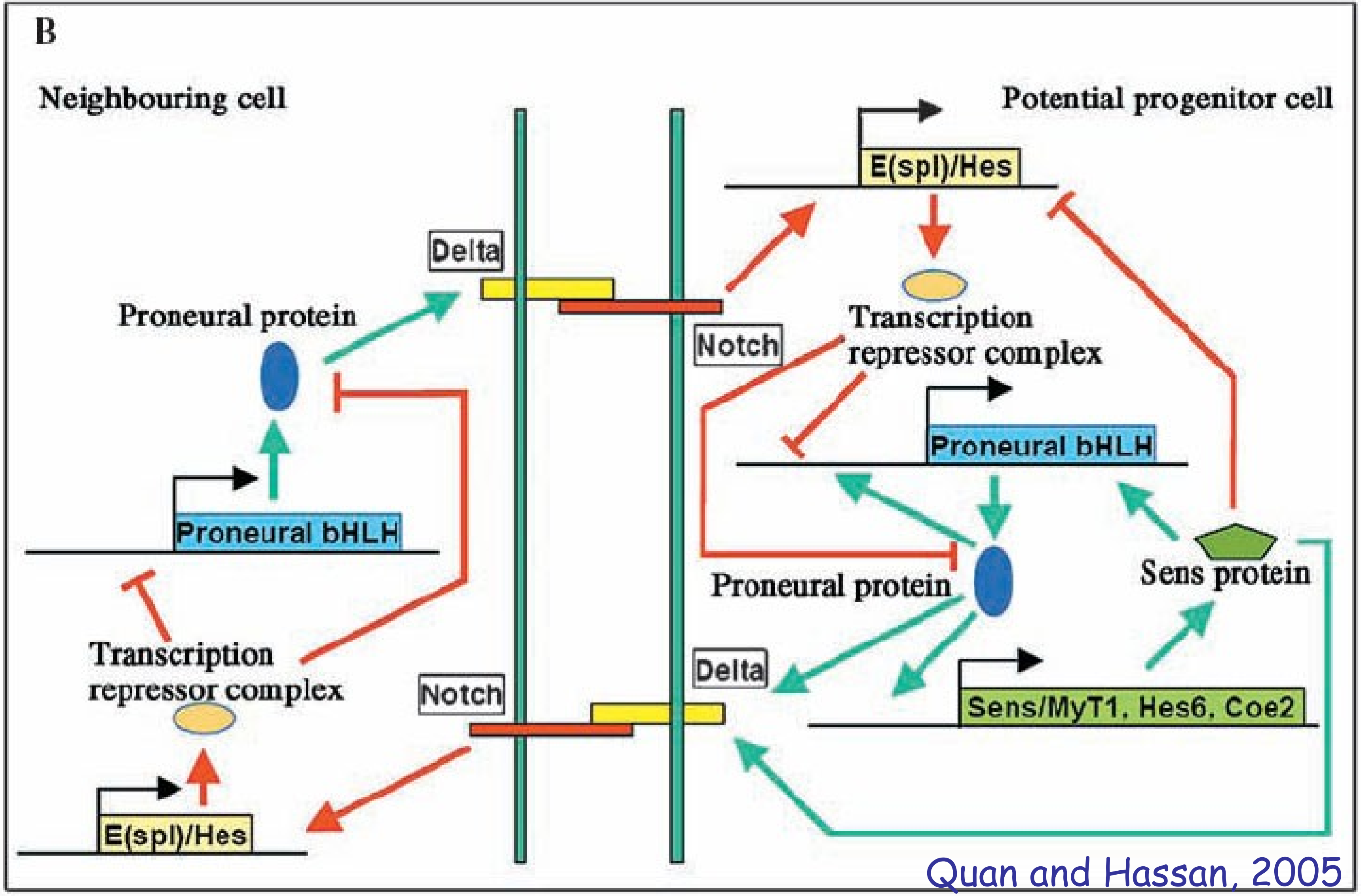




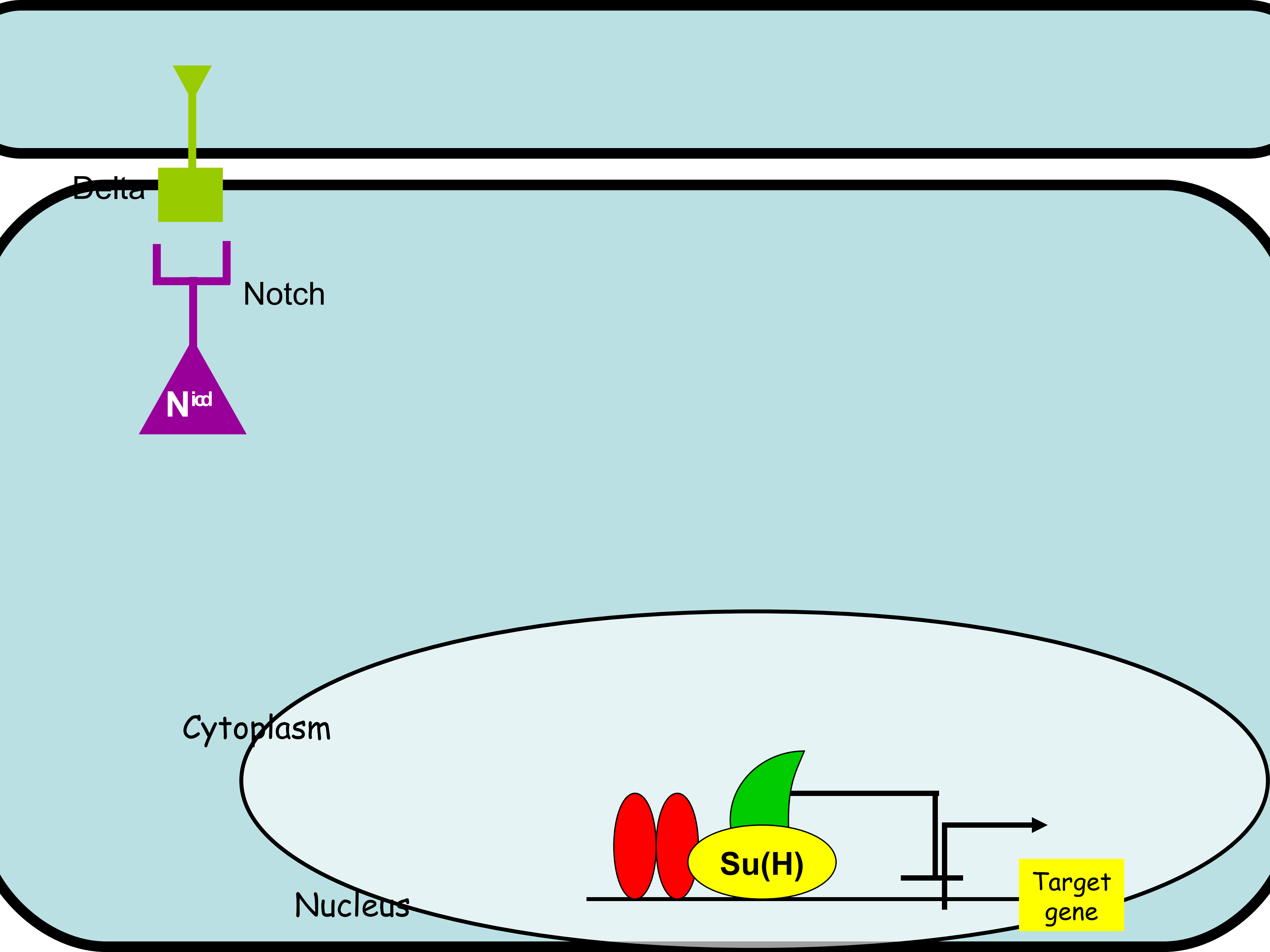


epidermal
sensory
neurons
ESNs

Notch signaling regulates ESN specification



Quan and Hassan, 2005



Delta

Notch

N^{icd}

Cytoplasm

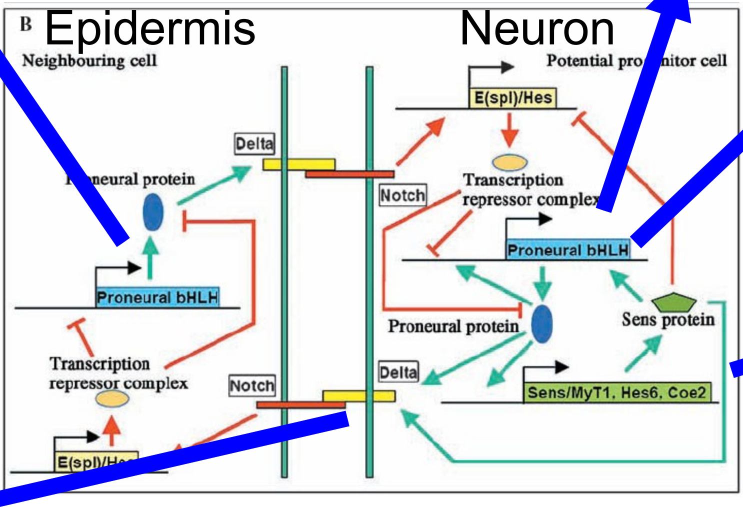
Nucleus

Su(H)

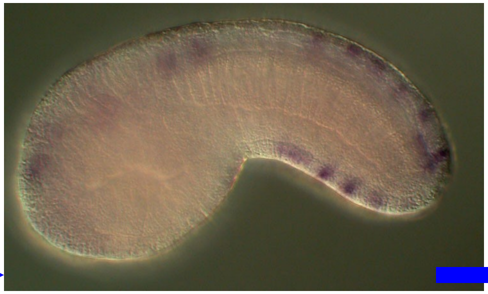
Target gene

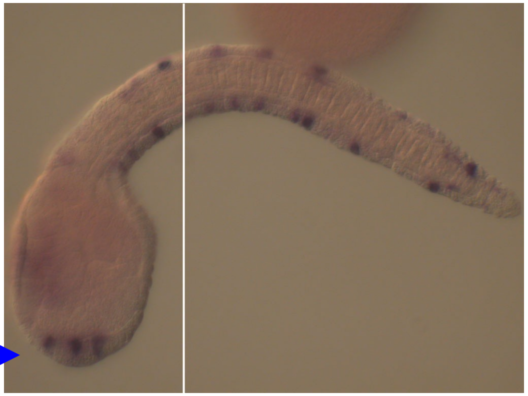
CiEpiB::dnSuH-CFP; Anti-acetylated tubulin; DAPI

The PNS “Network”



Quan and Hassan, 2005

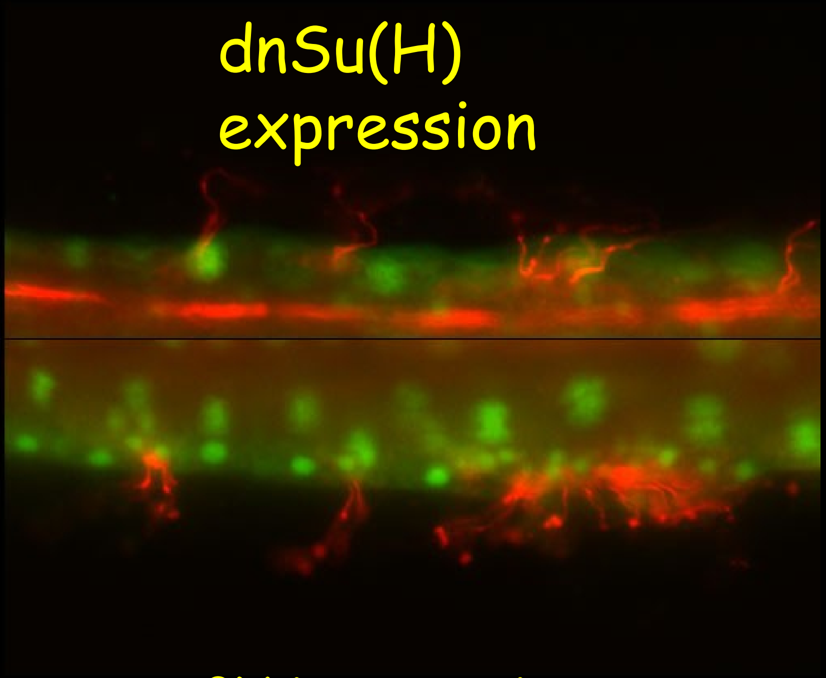




CiPou4 is expressed in the ascidian PNS

Blocking Notch signaling makes extra ESNs

Wild type

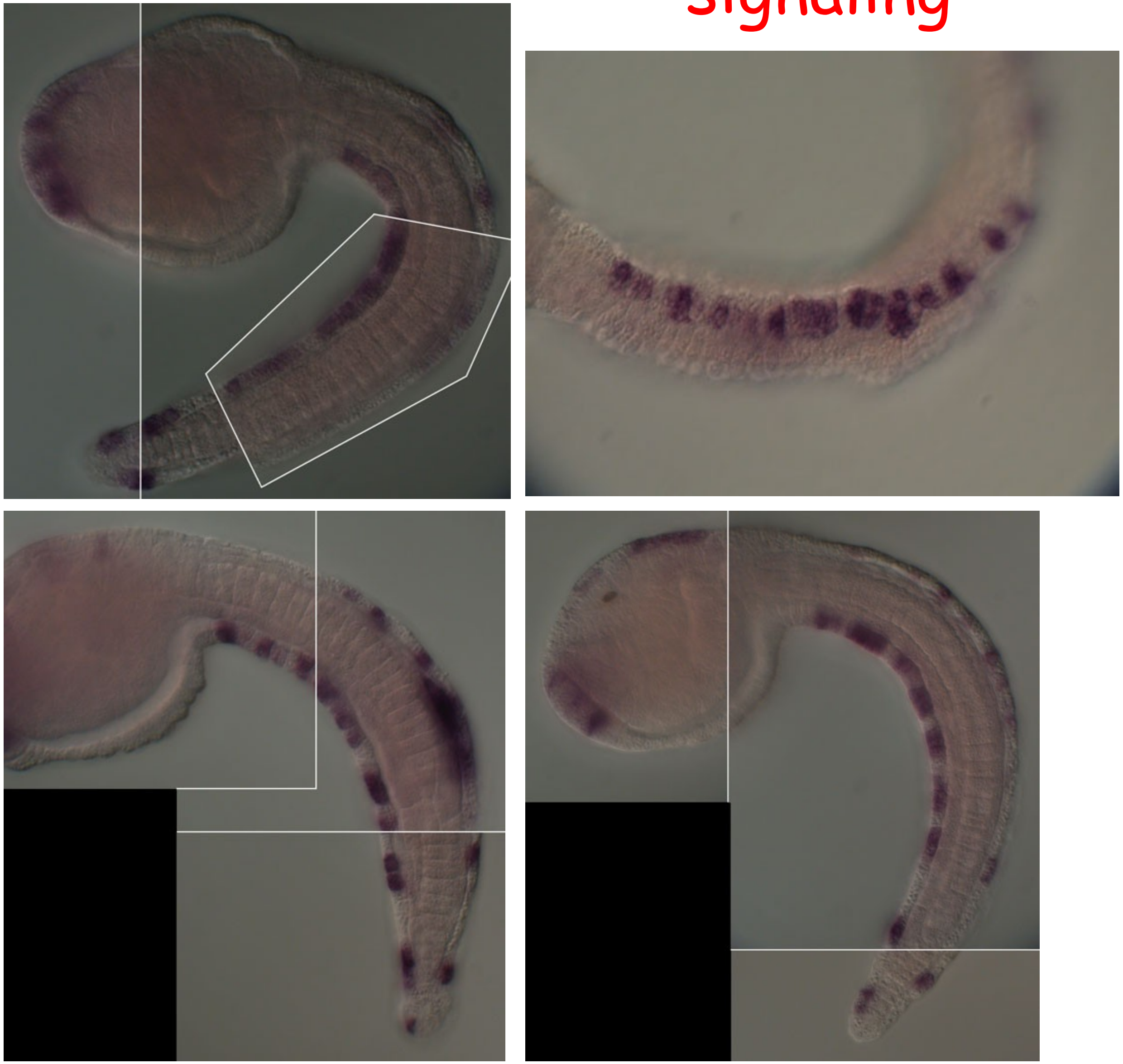


dnSu(H) expression

~2X increase in location of ESNs

Great expansion of ESNs at those locations

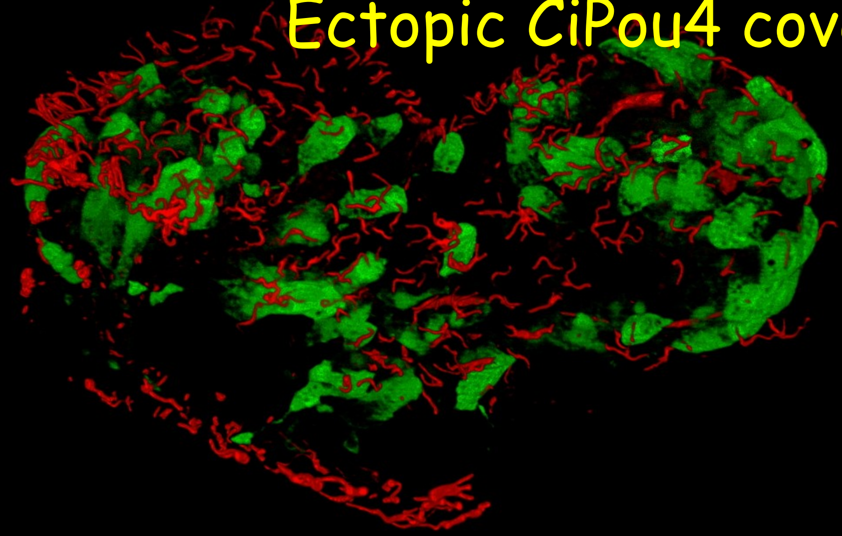
CiPou4 is activated downstream of Notch-Delta signaling



- CiPouIV is ectopically expressed along midlines
- Ectopic cilia are produced

Embyos express dnSuH throughout the epidermis

Ectopic CiPou4 coverts epidermis to ESNs

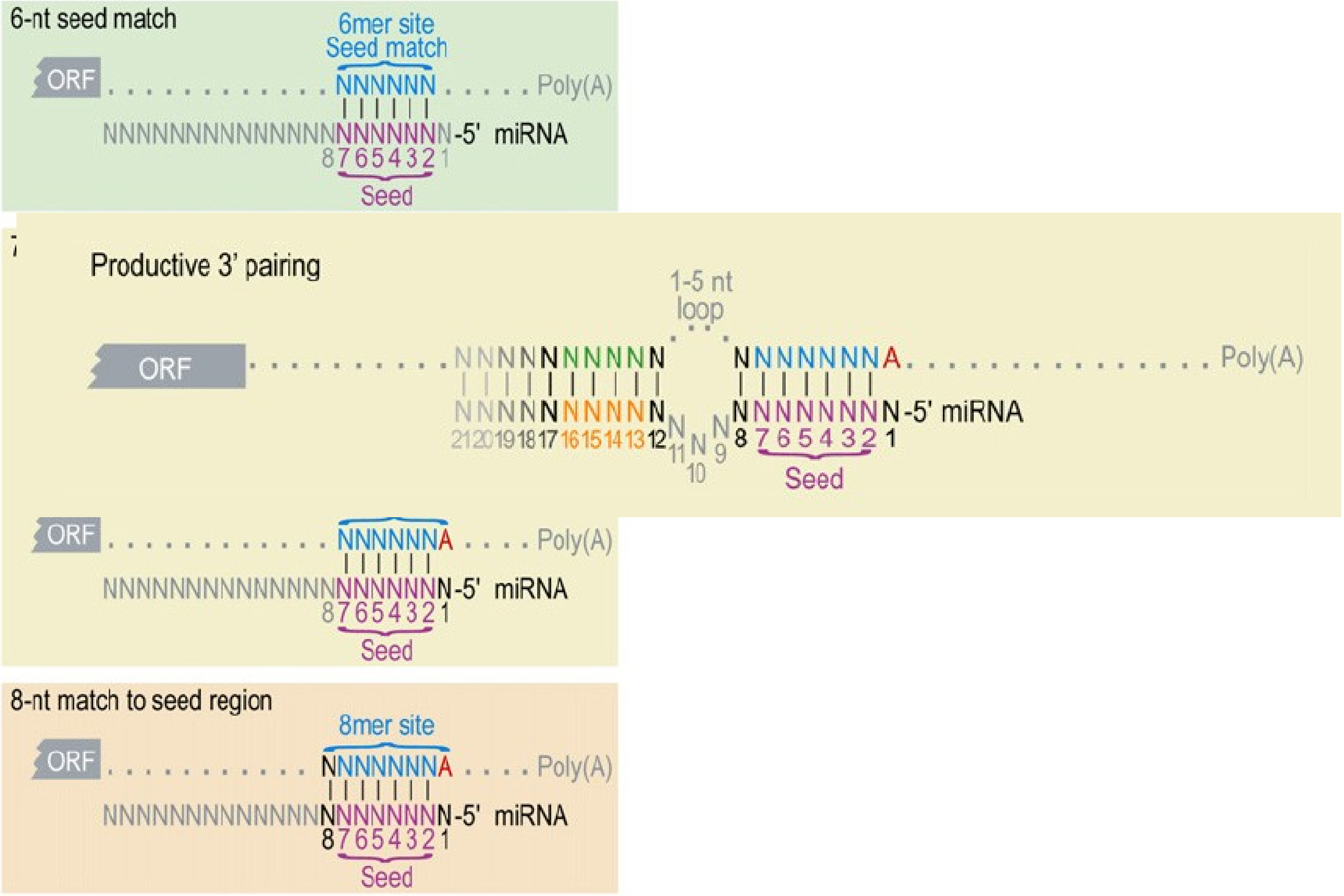


Advillin::GFP

Acetylated tubulin (cilia)

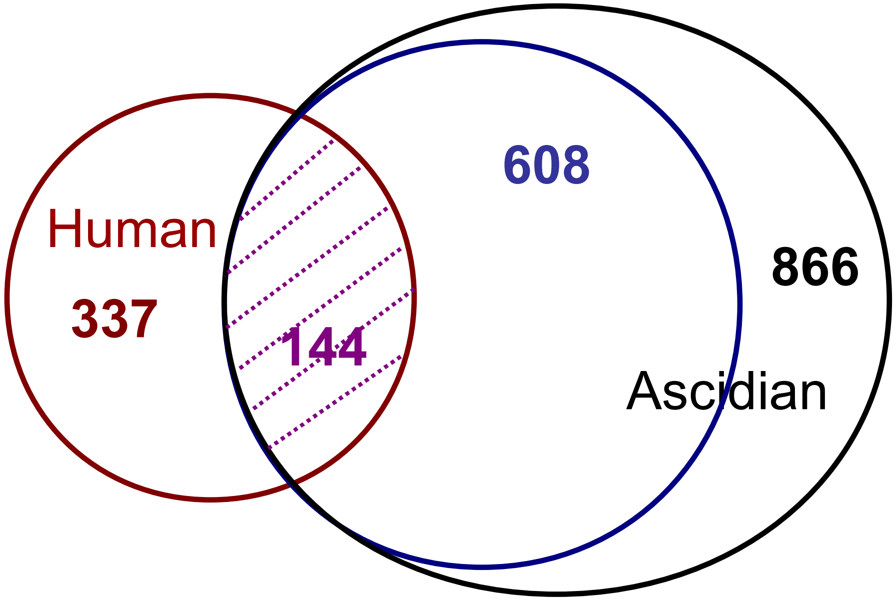
miR-124 is downstream of Notch signaling

miRs regulate their targets by matching a "seed" sequence on the mRNA 3' UTR



Grimson et al., 2007

Ciona miR-124 predicted targets



miR Seed sequence matches:

1-8 bp = best
2-8 bp = next best
1-7 bp = worst

⬛ Ascidian predicted mir124 targets (n = 866)

🟦 Ascidian predicted mir124 targets with human homologues (n = 608)

🟥 Human verified mir124 targets (n = 337)

🟪 Ascidian and human mir124 targets (n = 144)

Jerry Chen

Ciona miR-124 targets

866 unique targets

```
# FORMAT: >NAME  [#bp1-8, #bp2-8, #bp1-7]  score  #transcripts
>KH.L4.12.v1.A.ND1-1  [2, 0, 0] 17.9505832524 1
>KH.C2.863.v1.A.ND1-1  [2, 0, 0] 17.9412966657 1
```

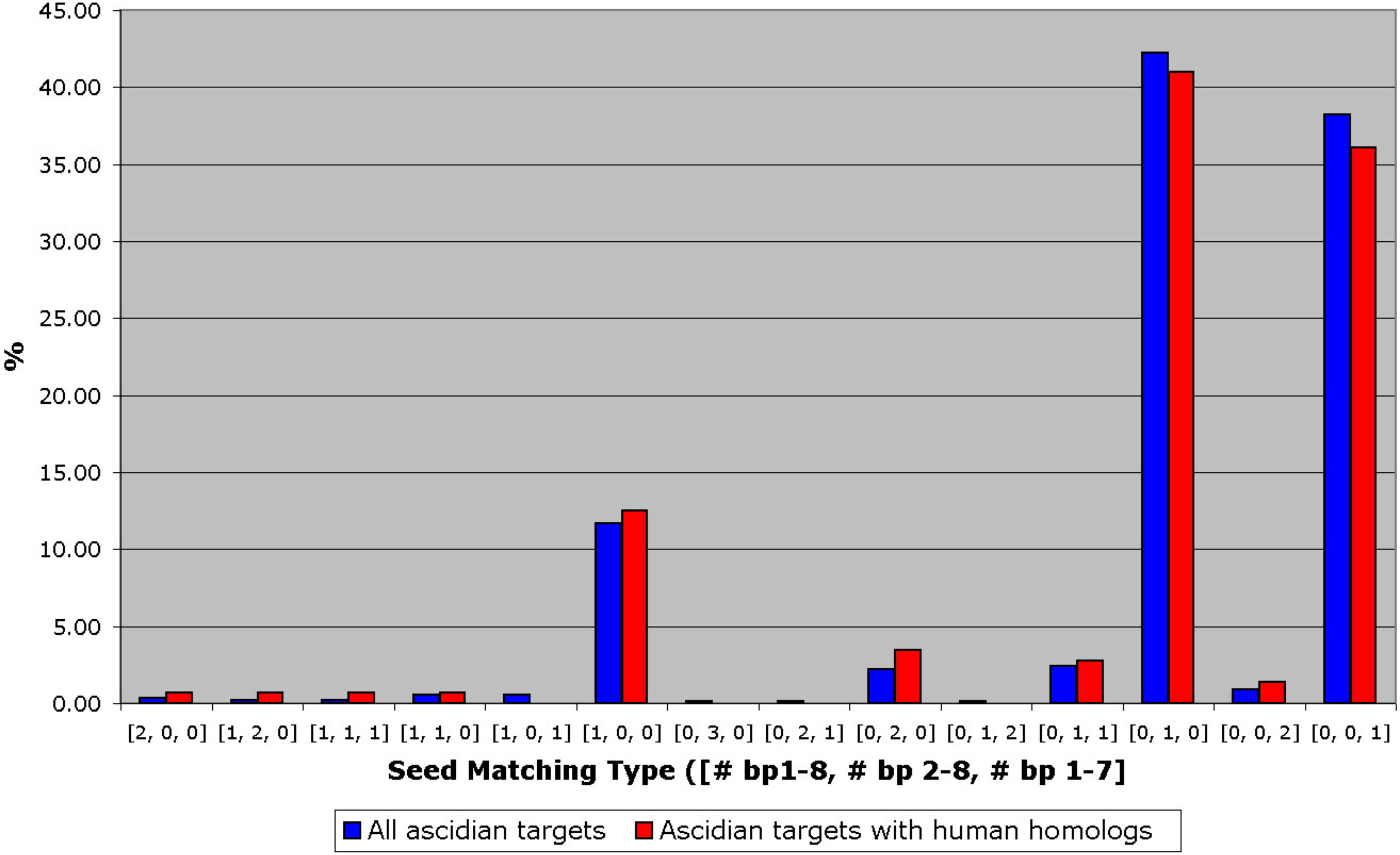
BEST

[#bp1-8, #bp2-8, #bp1-7]	# targets
[2, 0, 0]	3
[1, 2, 0]	2
[1, 1, 1]	2
[1, 1, 0]	5
[1, 0, 1]	5
[1, 0, 0]	101
[0, 3, 0]	1
[0, 2, 1]	1
[0, 2, 0]	19
[0, 1, 2]	1
[0, 1, 1]	21
[0, 1, 0]	366
[0, 0, 2]	8
[0, 0, 1]	331

Worst

Jerry Chen

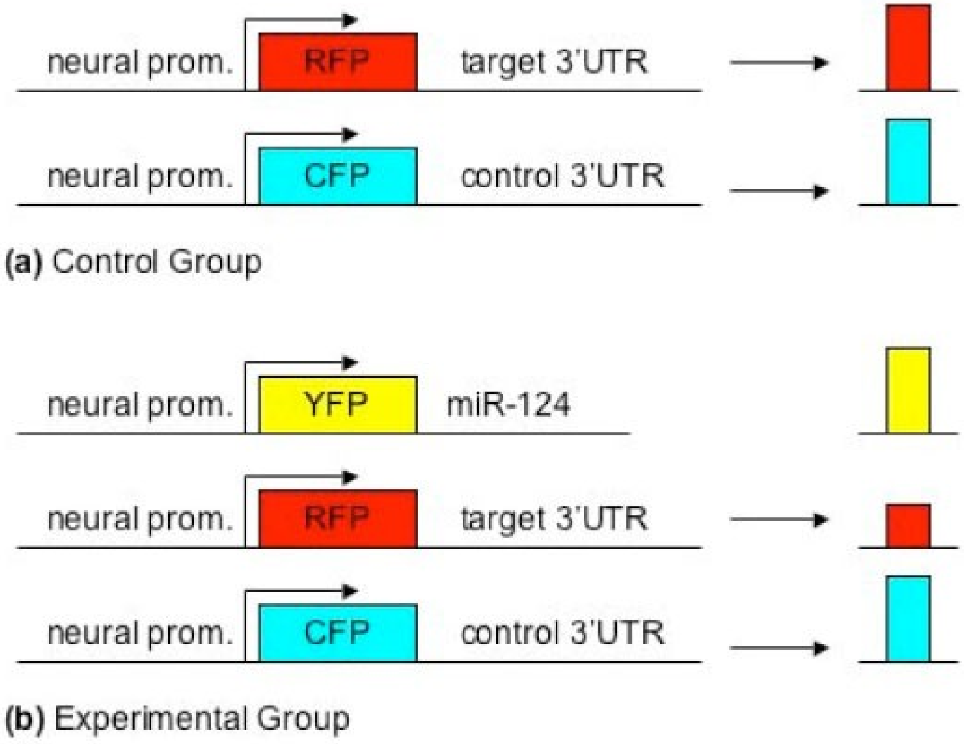
Ciona target composition is similar to human targets



“BEST” → “WORST”

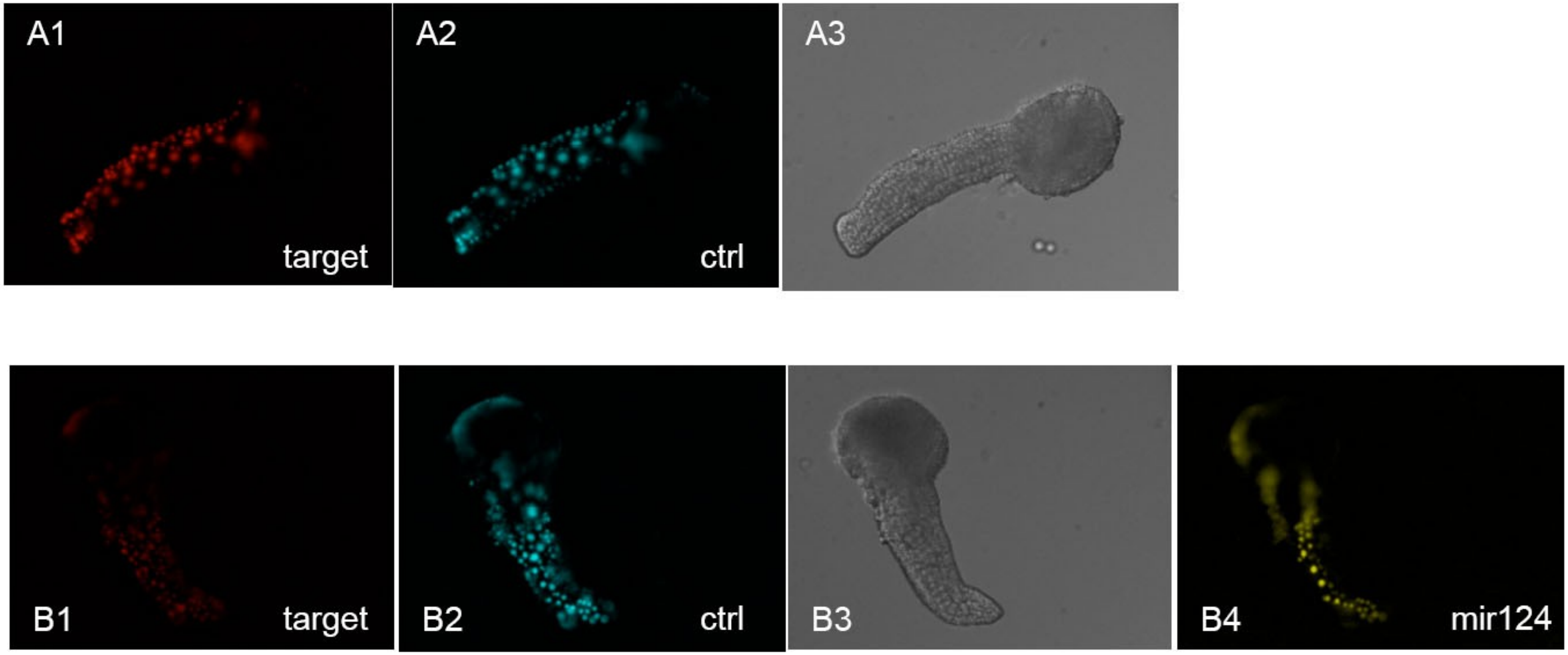
Jerry Chen

Biological assay of computationally predicted miR targets



Jerry Chen

Biological verification of miR targets



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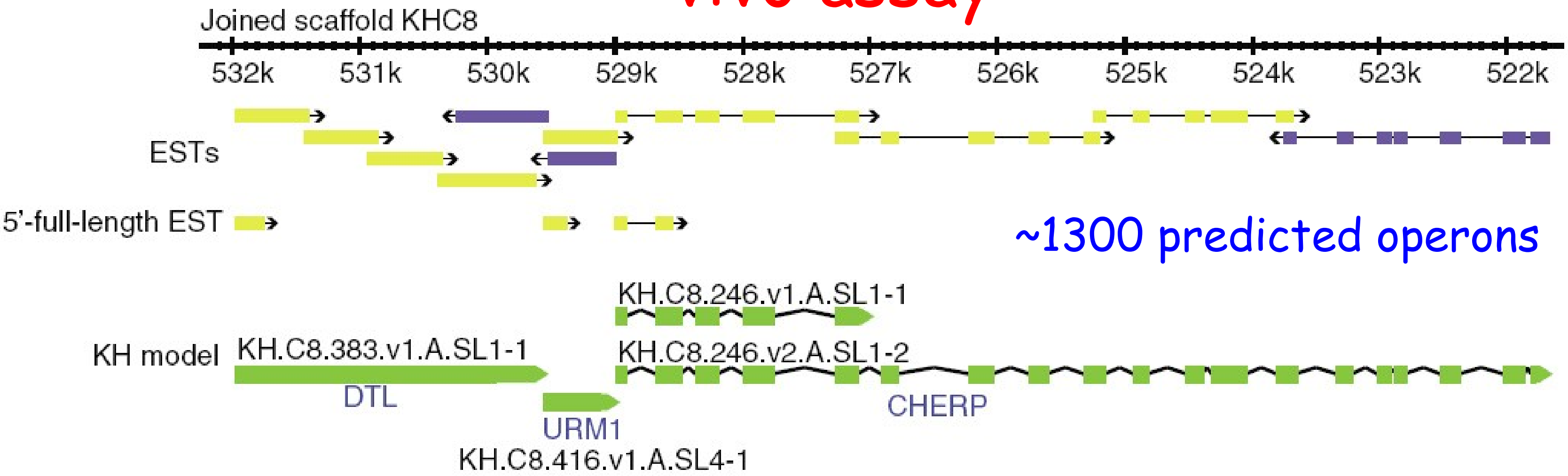
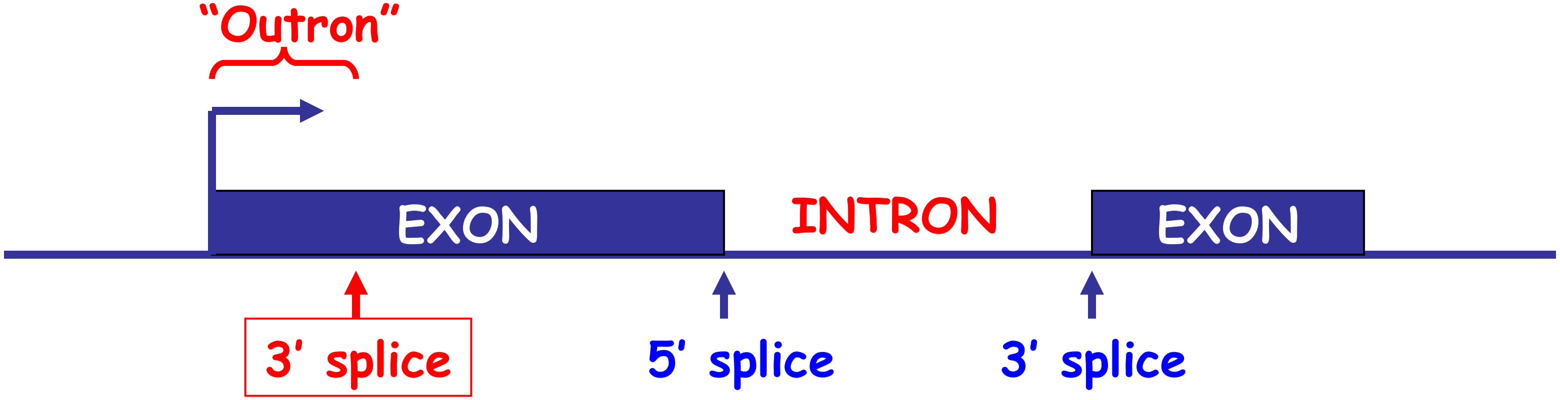
Future plans 1: exploit ascidian operons for in vivo assay



~1300 predicted operons

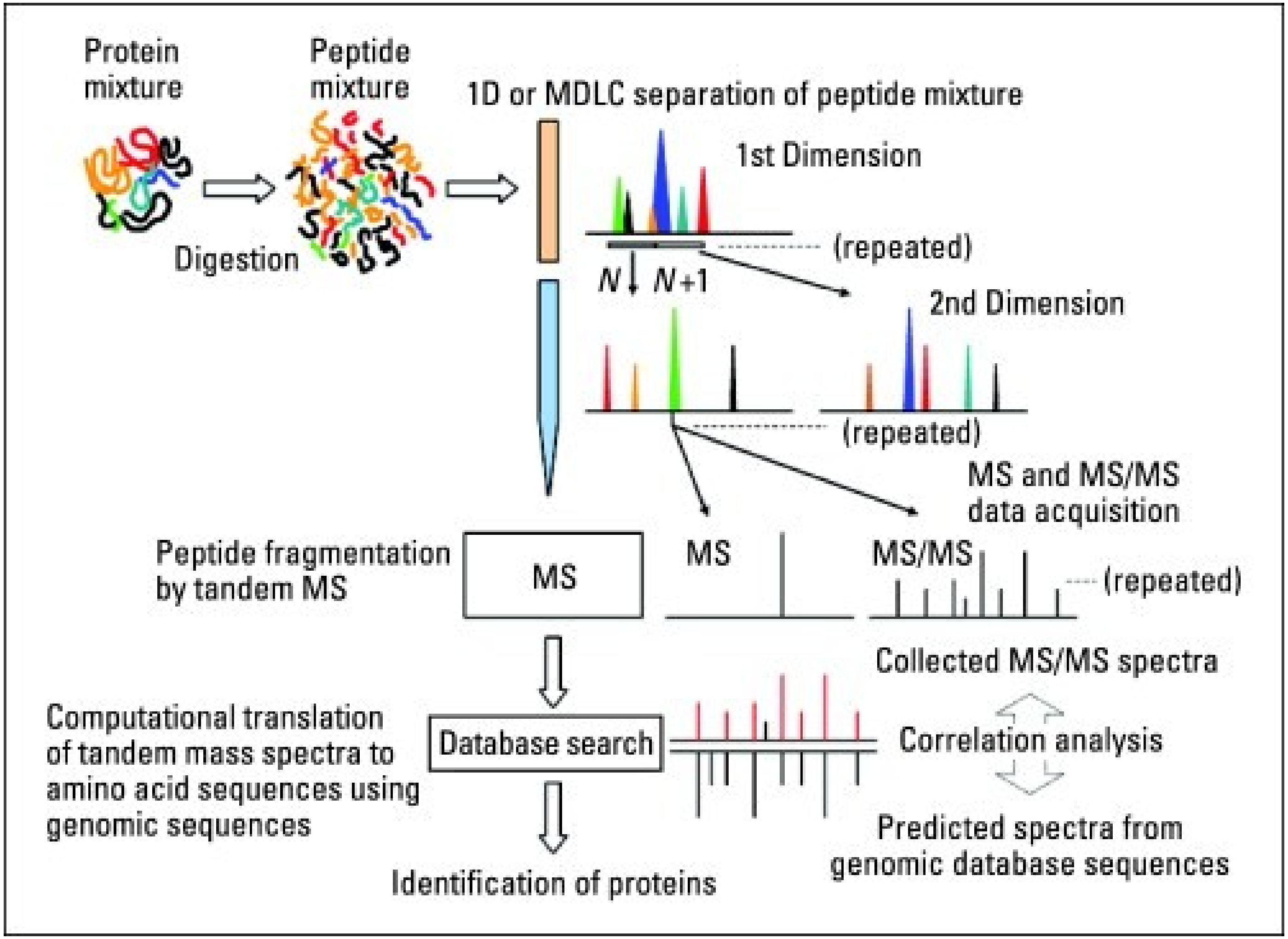


Satou et al., 2008

Artificial operon:

Future plans 2: proteomics analysis of miR targets - MudPIT

Multidimensional Protein Identification Technology



Motoyama and Yates, 2008

First MudPIT run:

- ~400 unique proteins identified
- 25 miR targets in sample

- ~50% sample is a 4200 amino acid protein that is probably a component of the yolk

- should be able to detect ~3000 proteins

Summary

- miRNAs add a new regulatory level to gene expression control
- Ascidians express a conserved neuronal miR - many target genes are shared with humans
- In vivo assays can be used to measure miR function and activity
- Proteomics for target ID

Jerry Chen
Raja Keshavan

National Science Foundation
WHERE DISCOVERIES BEGIN

San Diego State University
Center for Applied and Experimental
GENOMICS