The marvelous unfolding story of microRNAs

Greetings from Worcester Massachusetts!

UMass Chan MEDICAL SCHOOL | Morningside Graduate School of Biomedical Sciences

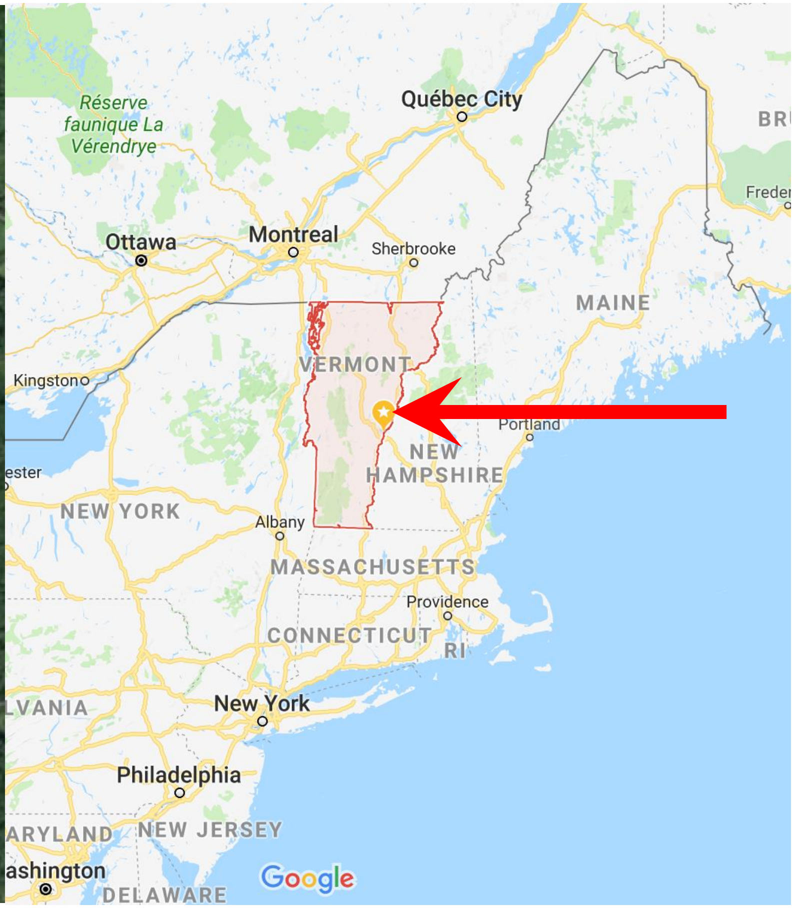
The marvelous unfolding story of microRNAs

More than 30 years ago

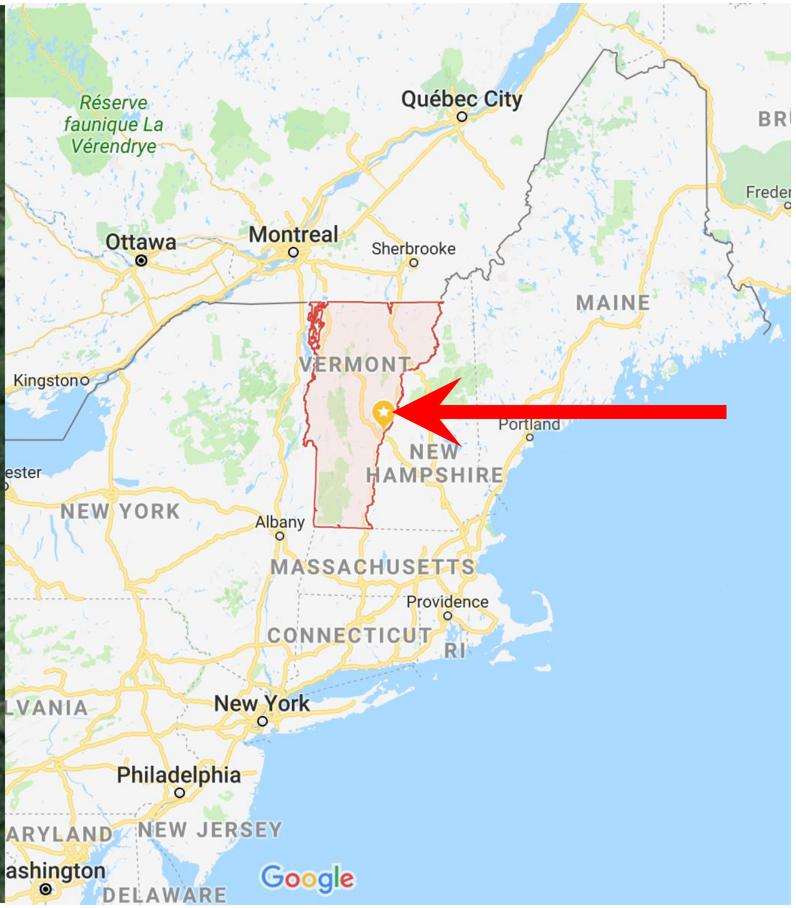
Rosalind Lee, Rhonda Feinbaum and V. Ambros (1993) The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14* Cell 75

Rosalind Lee and Rhonda Feinbaum

How did I get here?



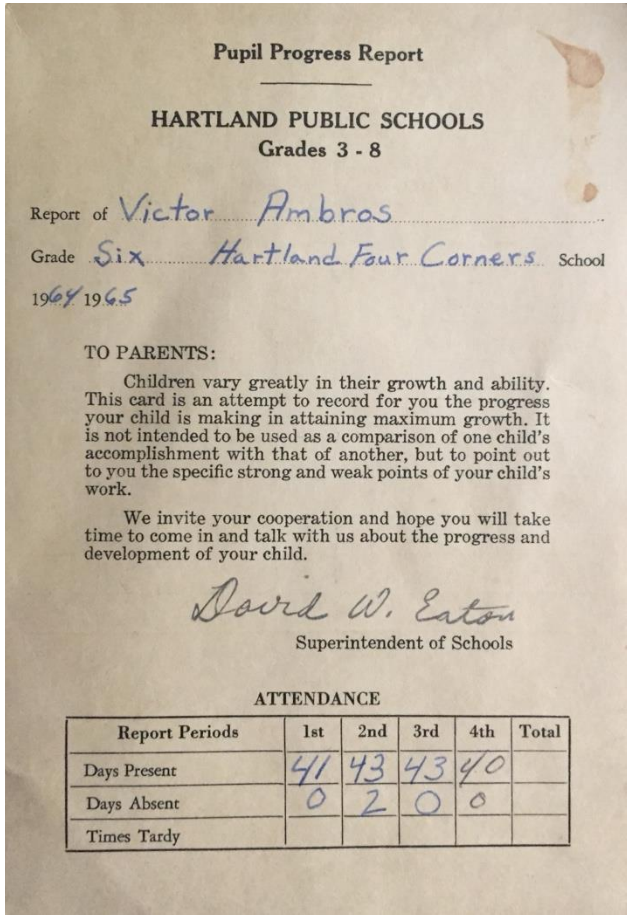
I grew up on a farm in Vermont (USA)



I started out as a scientist, learning to figure things out

Encouraged by my dad, Longin Ambros

The young scientist goes to school



Pupil Progress Report

HARTLAND PUBLIC SCHOOLS
Grades 3 - 8

Report of Victor Ambros

Grade Six Hartland Four Corners School

1964 1965

TO PARENTS:

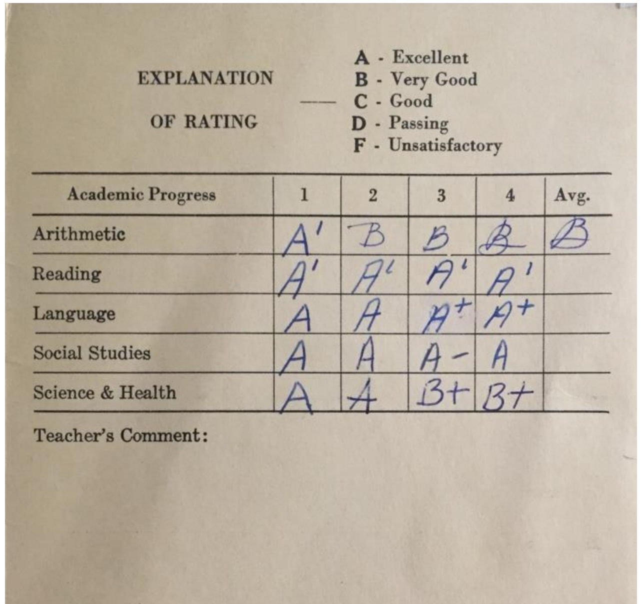
Children vary greatly in their growth and ability. This card is an attempt to record for you the progress your child is making in attaining maximum growth. It is not intended to be used as a comparison of one child's accomplishment with that of another, but to point out to you the specific strong and weak points of your child's work.

We invite your cooperation and hope you will take time to come in and talk with us about the progress and development of your child.

David W. Eaton
Superintendent of Schools

ATTENDANCE

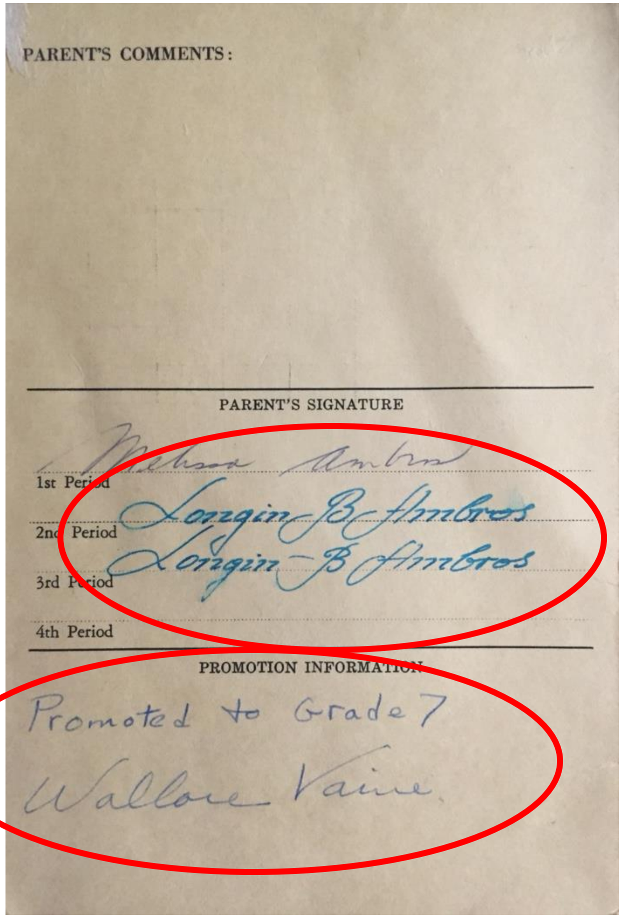
Report Periods	1st	2nd	3rd	4th	Total
Days Present	41	43	43	40	
Days Absent	0	2	0	0	
Times Tardy					



EXPLANATION OF RATING — A - Excellent, B - Very Good, C - Good, D - Passing, F - Unsatisfactory

Academic Progress	1	2	3	4	Avg.
Arithmetic	A'	B	B	B	B
Reading	A'	A'	A'	A'	
Language	A	A	A+	A+	
Social Studies	A	A	A-	A	
Science & Health	A	A	B+	B+	

Teacher's Comment:



PARENT'S COMMENTS:

PARENT'S SIGNATURE

1st Period Melissa Ambros

2nd Period Longin B. Ambros

3rd Period Longin B. Ambros

4th Period

PROMOTION INFORMATION

Promoted to Grade 7

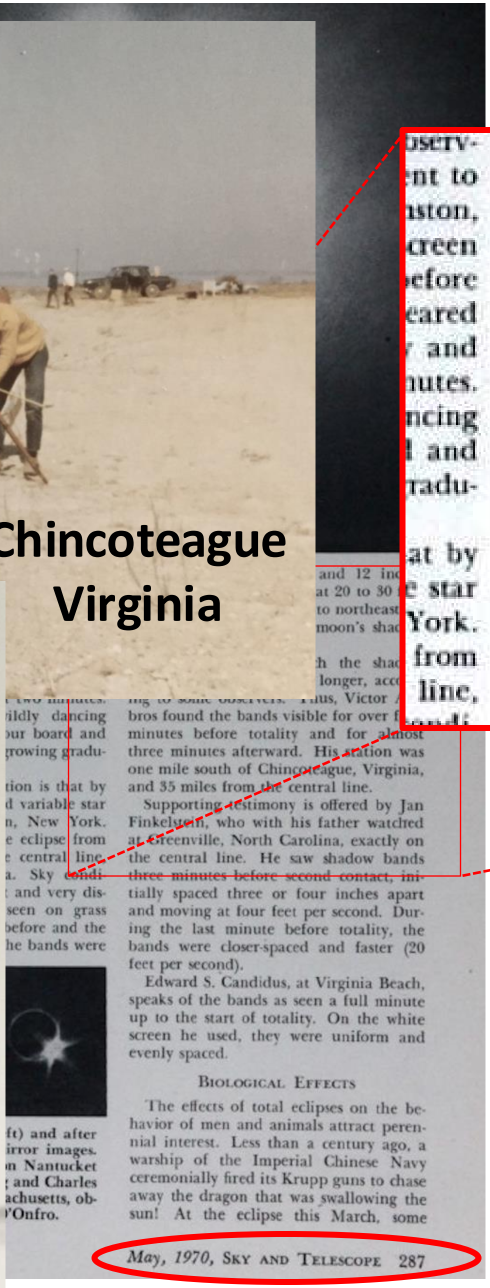
Wallace Vaine

I was an amateur astronomer

Astrophoto of
Comet Bennett
I took from my
backyard

May 15, 1970

My first published scientific result!


bros found the bands visible for over four minutes before totality and for almost three minutes afterward. His station was one mile south of Chincoteague, Virginia, and 35 miles from the central line.

Supporting testimony is offered by Jan Finkelstein, who with his father watched at Greenville, North Carolina, exactly on the central line. He saw shadow bands three minutes before second contact, initially spaced three or four inches apart and moving at four feet per second. During the last minute before totality, the bands were closer-spaced and faster (20 feet per second).

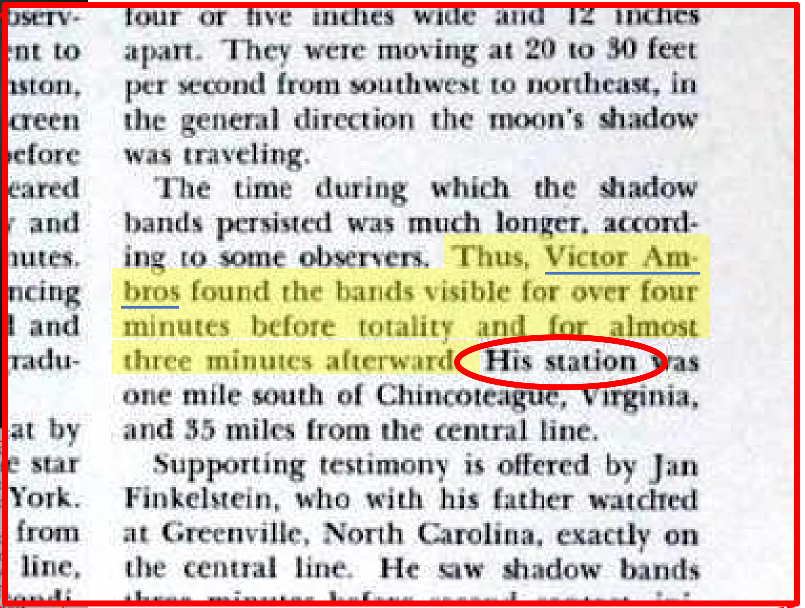
Edward S. Candidus, at Virginia Beach, speaks of the bands as seen a full minute up to the start of totality. On the white screen he used, they were uniform and evenly spaced.

BIOLOGICAL EFFECTS

The effects of total eclipses on the behavior of men and animals attract perennial interest. Less than a century ago, a warship of the Imperial Chinese Navy ceremonially fired its Krupp guns to chase away the dragon that was swallowing the sun! At the eclipse this March, some

May, 1970, SKY AND TELESCOPE 287

Sky and Telescope
May 1970


four or five inches wide and 12 inches apart. They were moving at 20 to 30 feet per second from southwest to northeast, in the general direction the moon's shadow was traveling.

The time during which the shadow bands persisted was much longer, according to some observers. Thus, Victor Ambros found the bands visible for over four minutes before totality and for almost three minutes afterward. His station was one mile south of Chincoteague, Virginia, and 35 miles from the central line.

Supporting testimony is offered by Jan Finkelstein, who with his father watched at Greenville, North Carolina, exactly on the central line. He saw shadow bands

March 7, 1970
Total Solar Eclipse

Observations of Shadow Bands

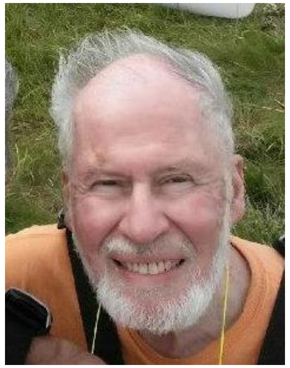
My scientific career path

1971 MIT

Inspiration from other students
Kevin Struhl, Gary Struhl

Research experience

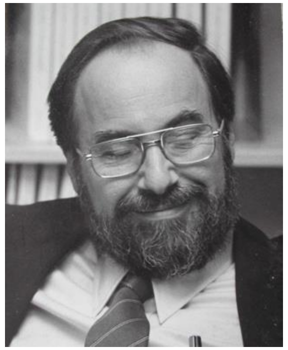
Encouragement from mentors



Ed Gruberg

BS 1975

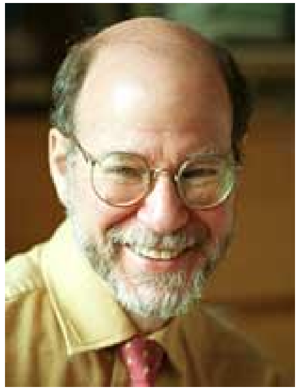
MIT grad school



David Baltimore

Ph.D. 1979

Postdoctoral research

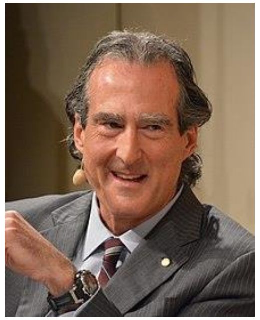


Bob Horvitz

Faculty @Harvard 1985

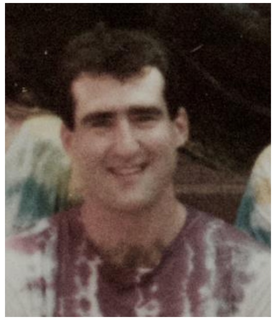
@Dartmouth 1992

@Umassmed 2008



Craig Mello

My PhD student Craig Mello



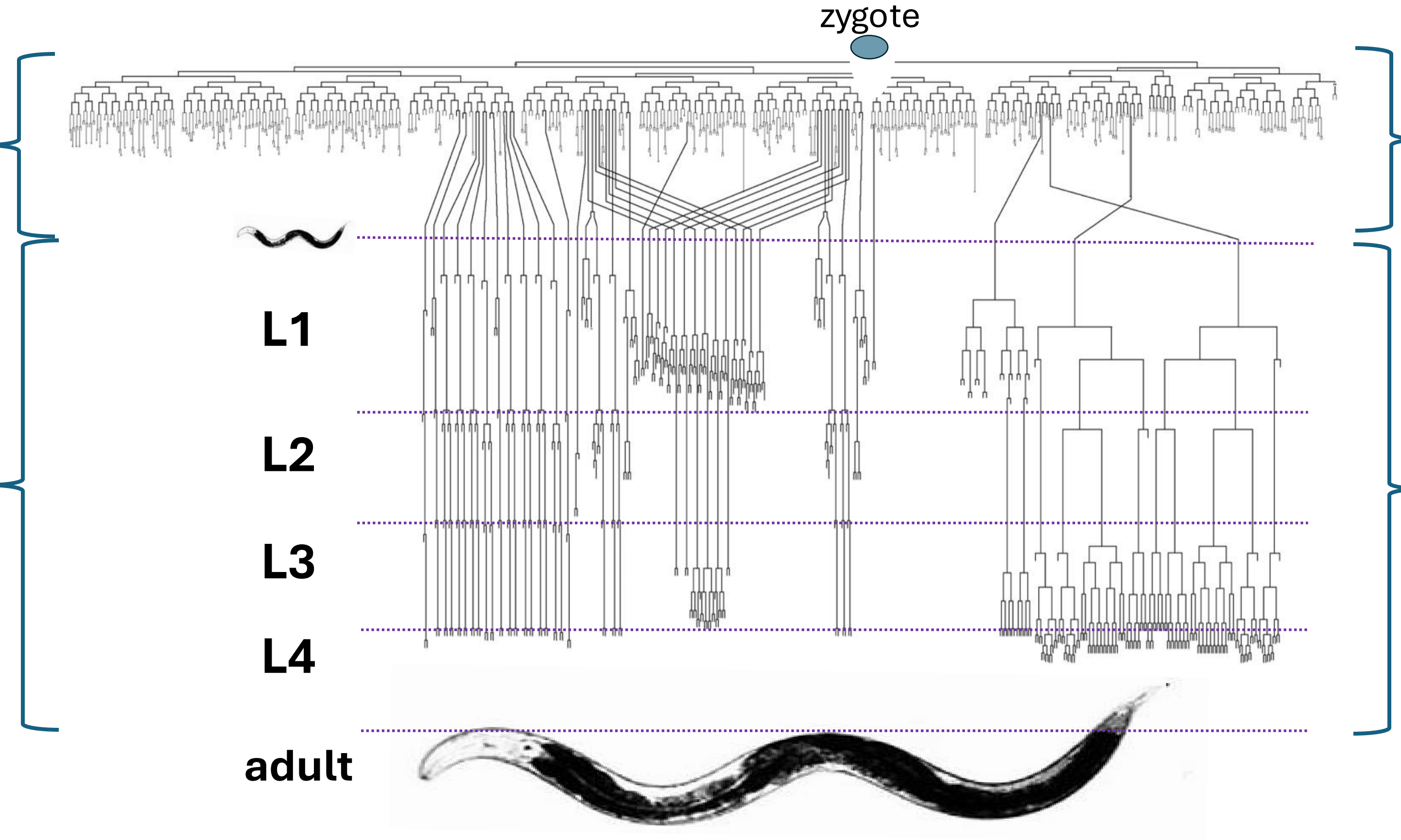
This Improbable Life!

In the beginning - circa 1980 - Bob Horvitz's lab @ MIT

C. elegans cell lineages

embryogenesis

invariant
Post-embryonic
stages

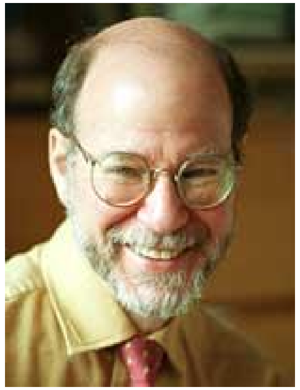


Sulston et al 1983

Sulston and Horvitz 1977

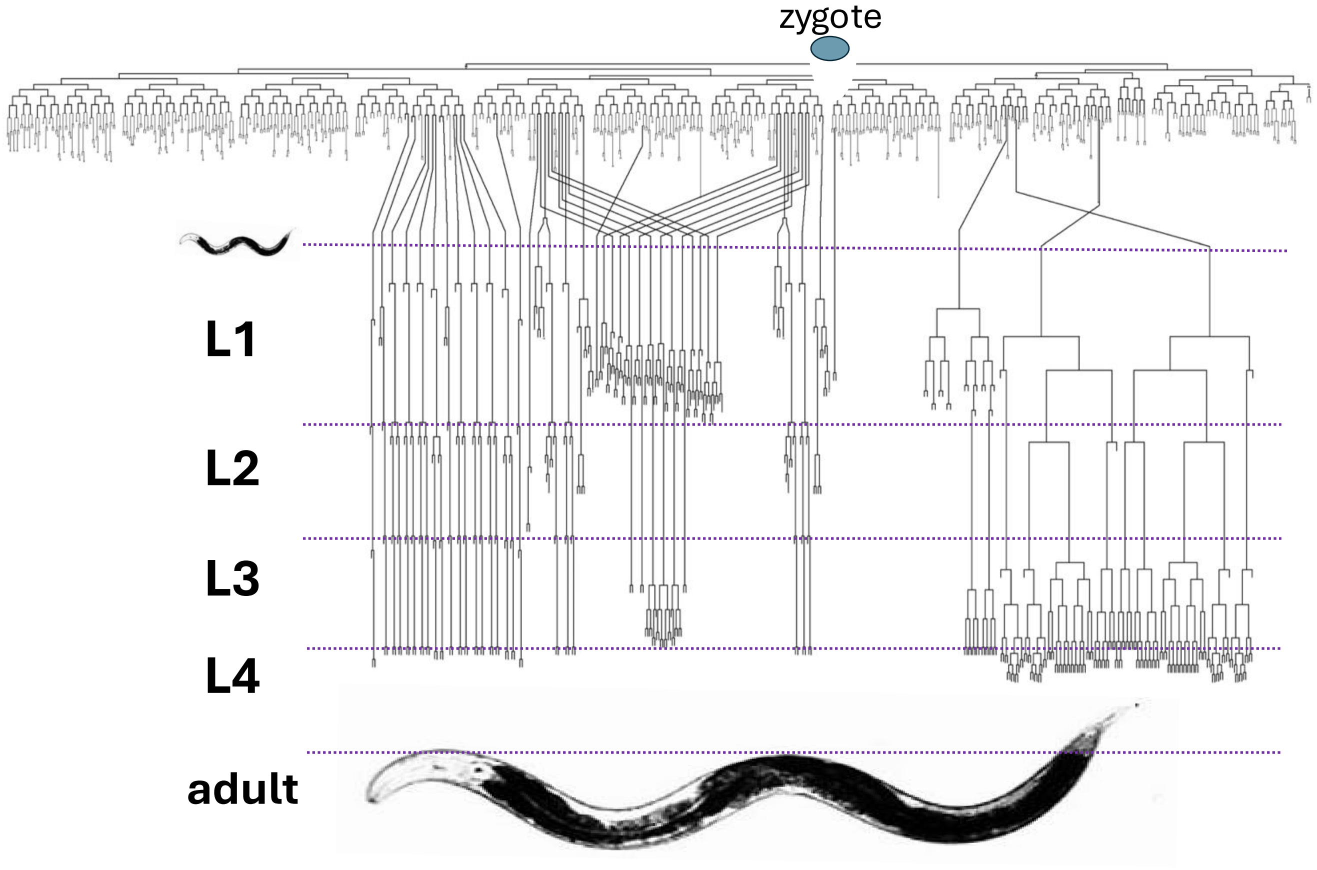
How is the development of an animal encoded in its genome?

Genetic Approach: Identify mutants with developmental defects. Find out what they can teach you.



Model Organism Developmental Genetics

C. elegans cell lineages



embryogenesis — Sulston et al 1983

invariant Post-embryonic stages — Sulston and Horvitz 1977

L1

L2

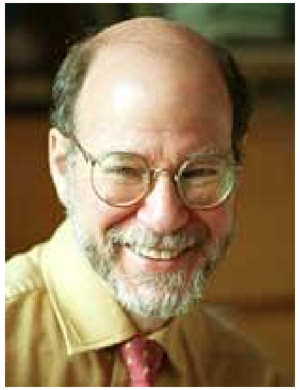
L3

L4

adult

How is the development of an animal encoded in its genome?

Genetic Approach: Identify mutants with developmental defects. Find out what they can teach you.



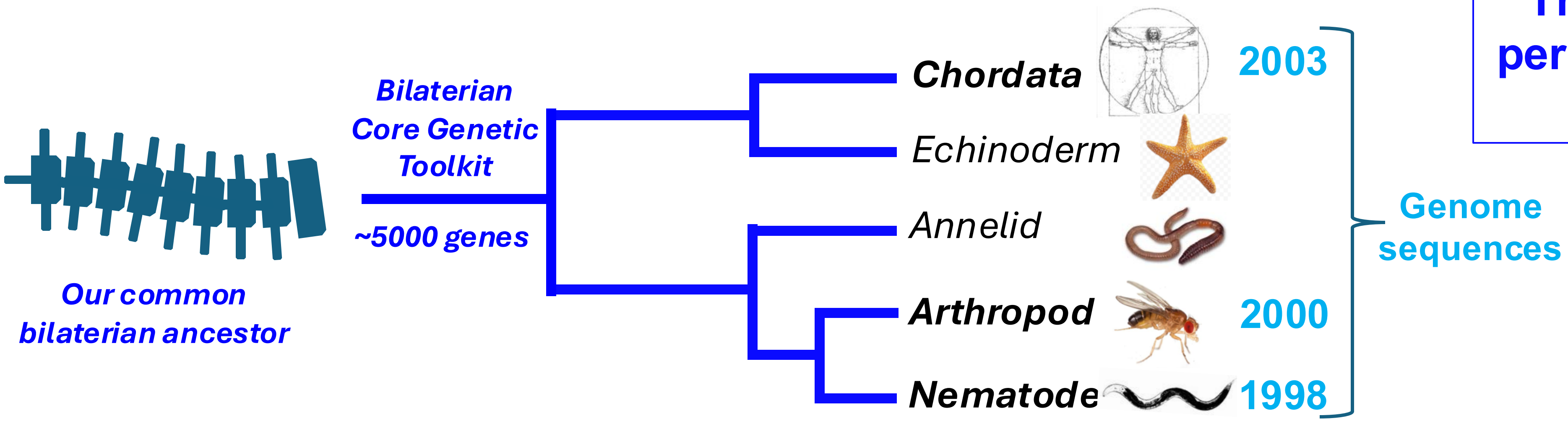
Model Organism Developmental Genetics

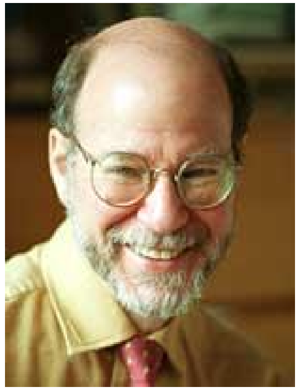
The Model Organism Premise

Developmental mechanisms in one animal → Can apply to distant animals

Evolutionary adaptation of the 'Core Genetic Toolkit' → animal complexity and diversity

"The worm is a tiny person in disguise."
--Bob Horvitz





How is the development of an animal encoded in its genome?

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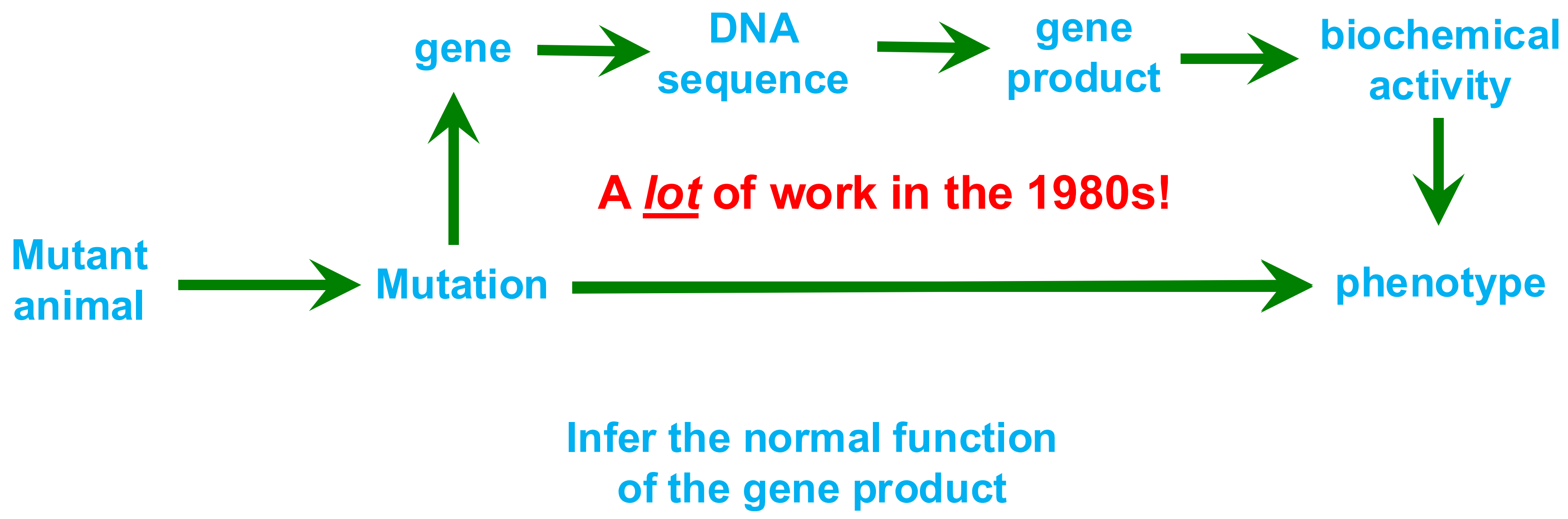
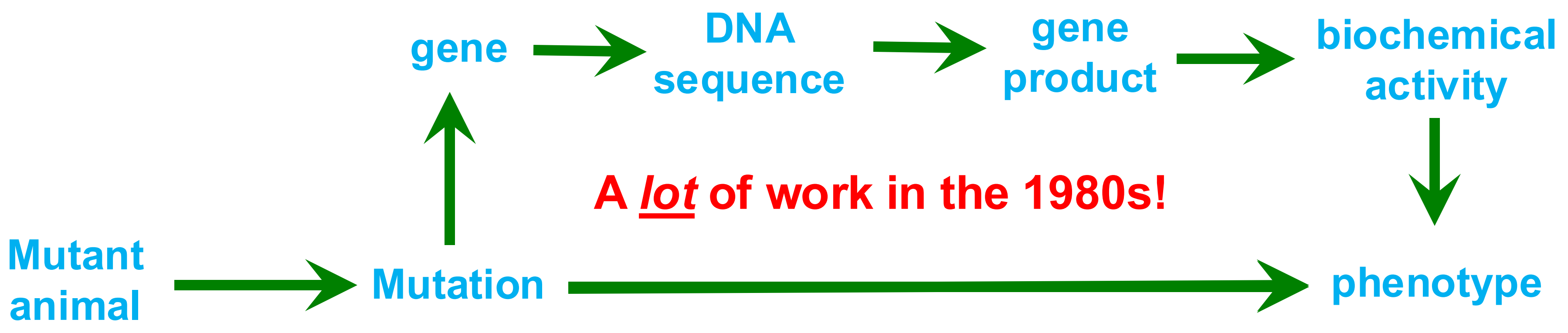
Model Organism Developmental Genetics



Genetic Approach: Identify mutants with developmental defects; find out what they can teach you.

Choosing what mutants to follow

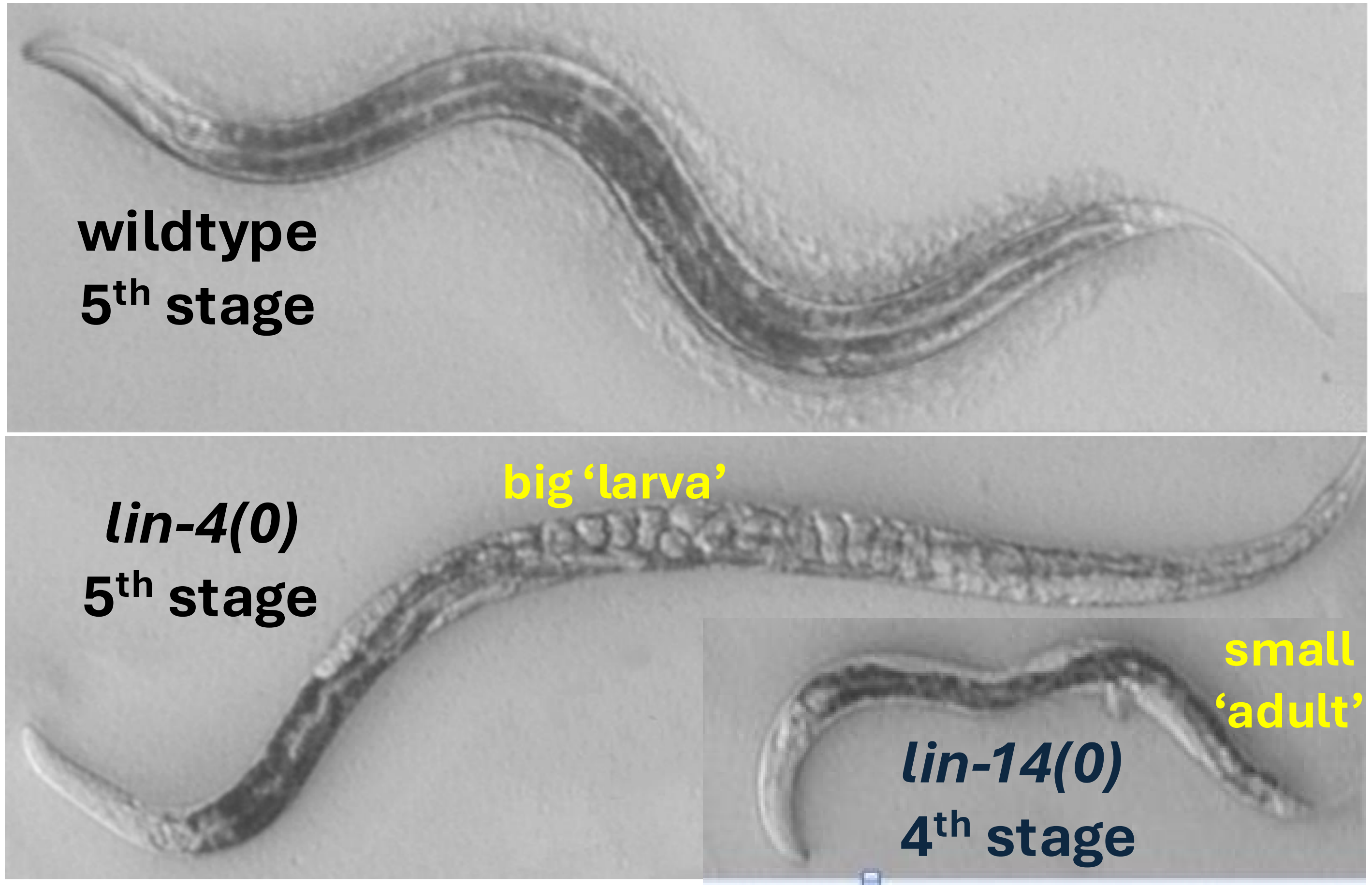


Infer the normal function
of the gene product

Genetic Approach: Identify mutants with developmental defects; find out what they can teach you.

Choosing what mutants to follow

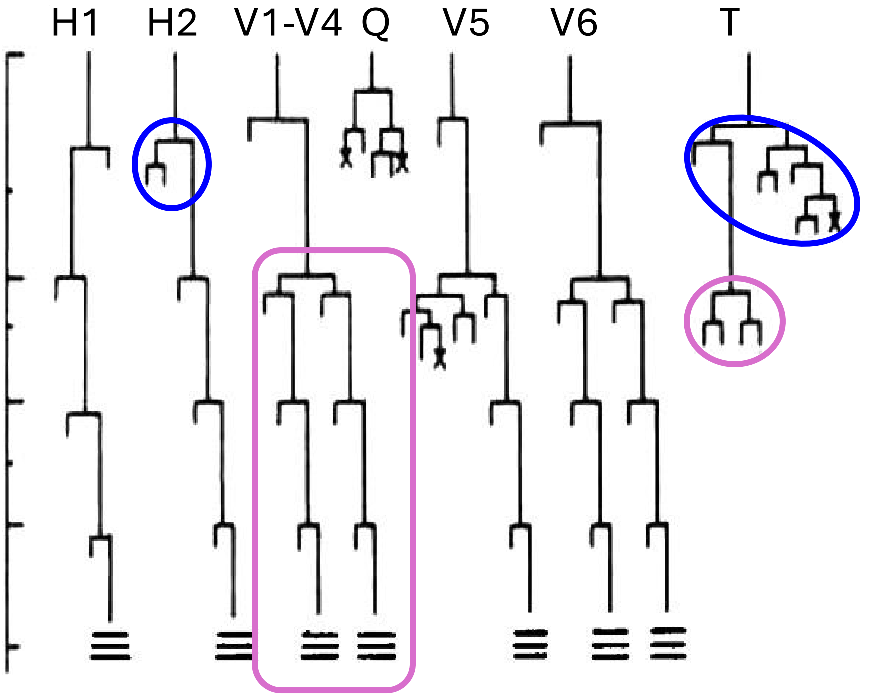
Bob's genetic screens → *C. elegans* Developmental Timing Mutants



Chalfie, Horvitz & Sulston (1981)

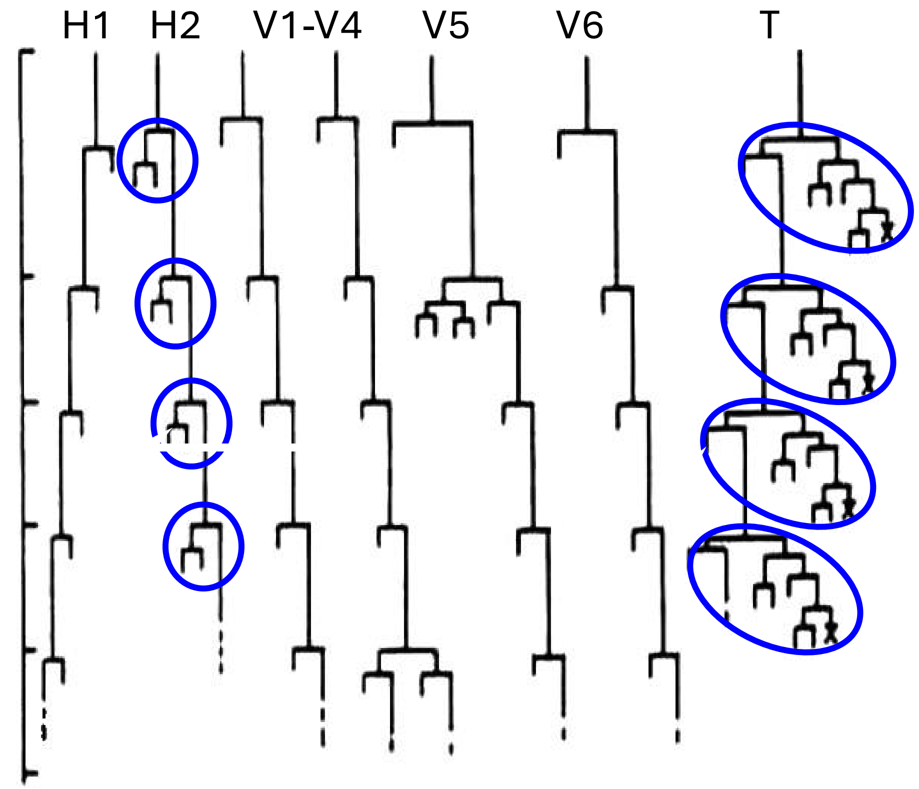
C. elegans Developmental Timing Mutants

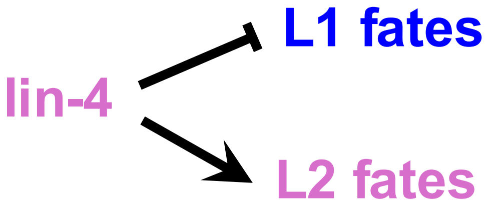
Wild type



“Retarded” development

lin-4(0) mutant

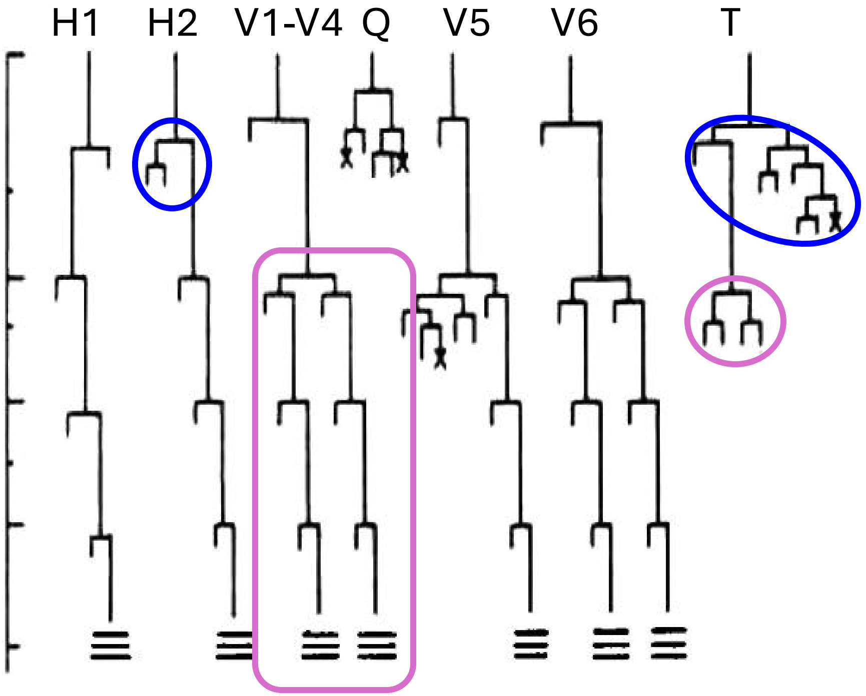




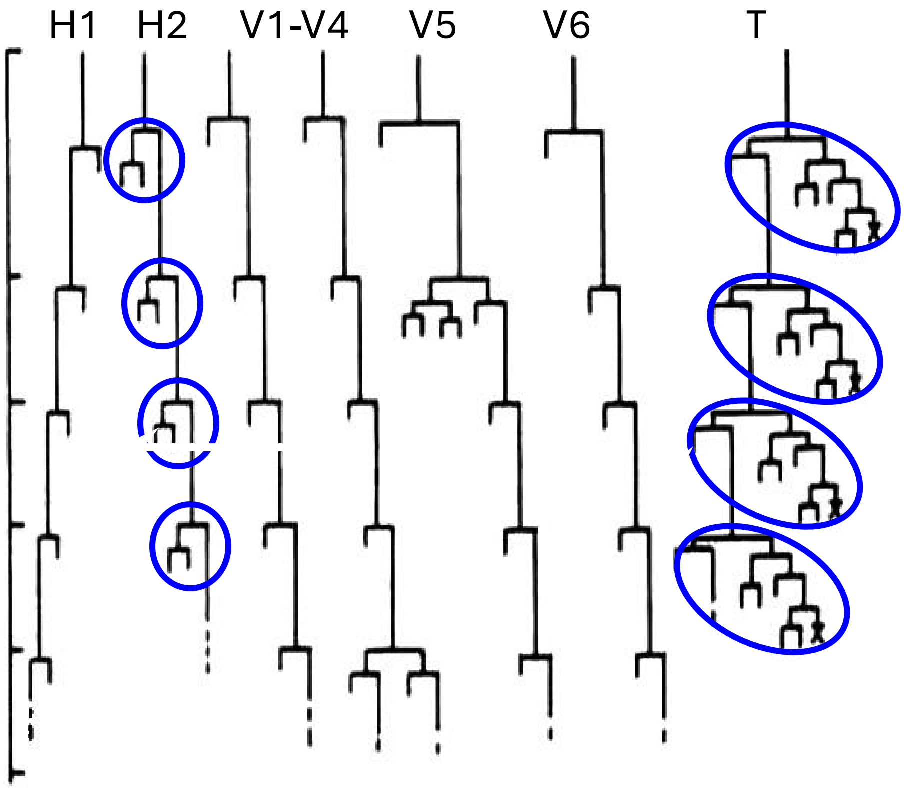
Chalfie, Horvitz & Sulston (1981)

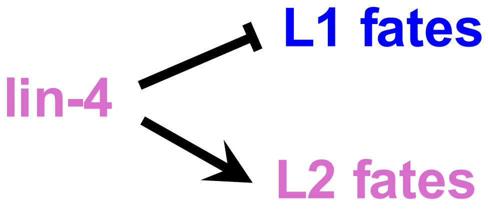
C. elegans ‘Heterochronic’ Mutants

Wild type



“Retarded” development
lin-4(0) mutant

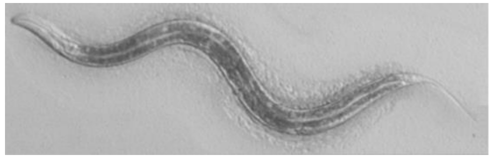




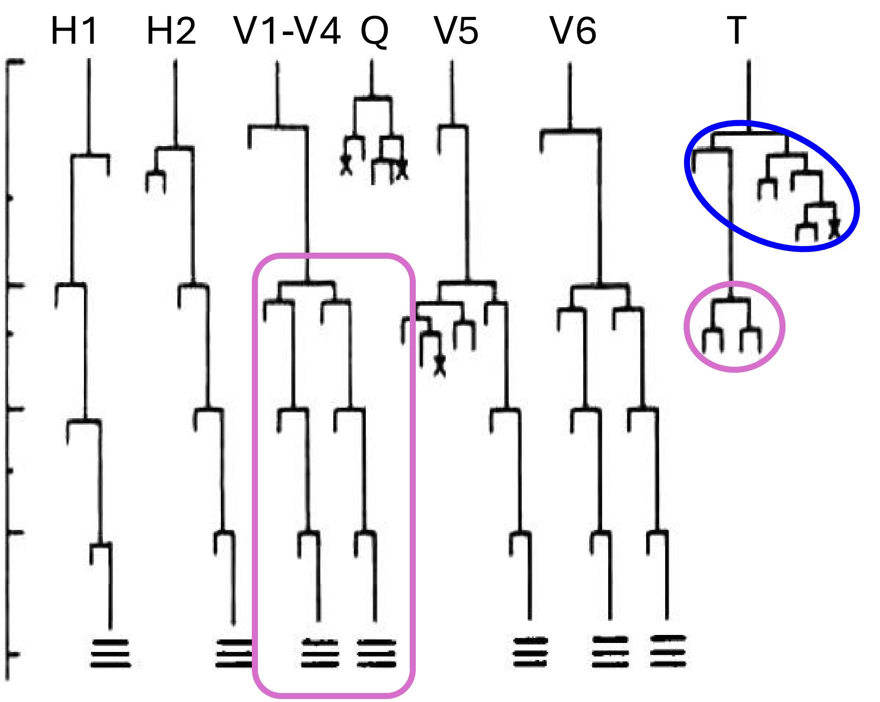
Retention of ancestral juvenile characteristics

Chalfie, Horvitz & Sulston (1981)

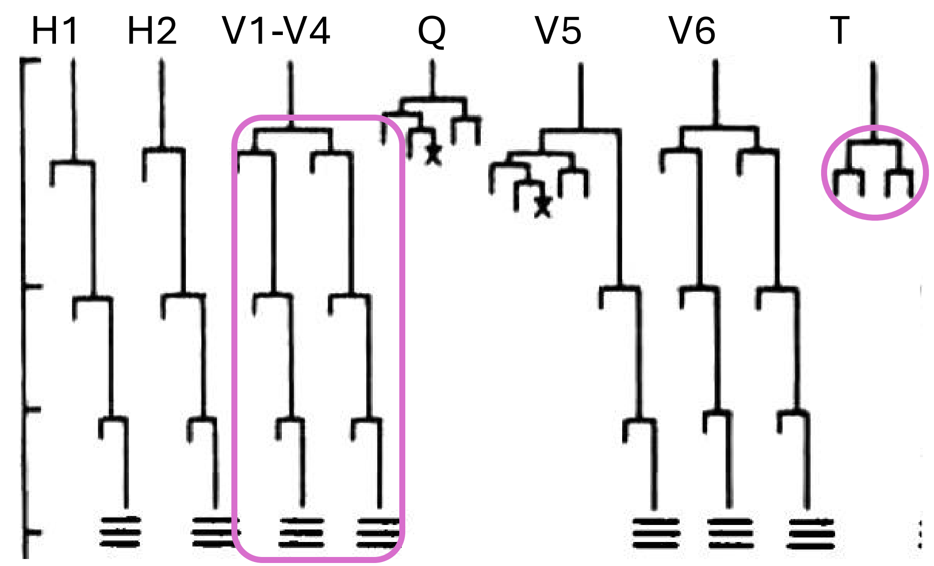
An opposite class of Heterochronic mutant phenotype



Wild type



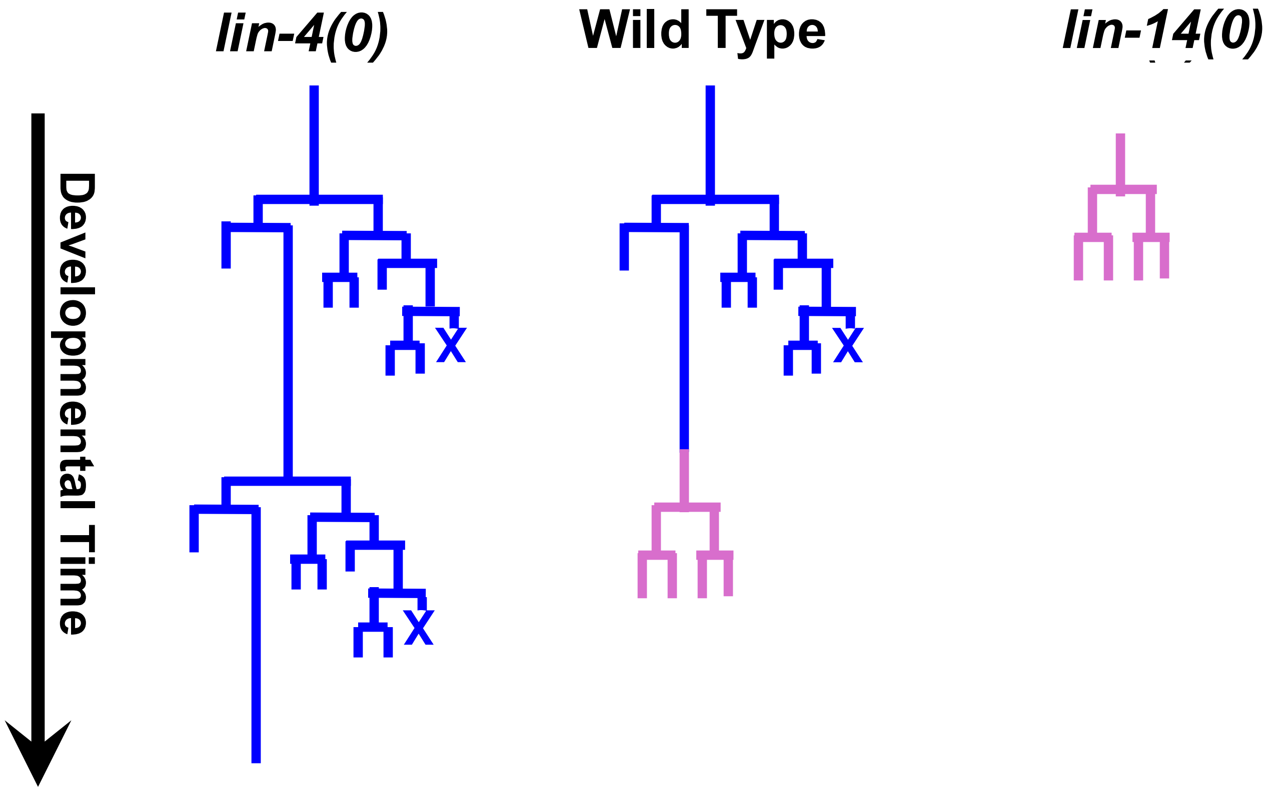
"Precocious" development
lin-14(0) mutant



LIN-14 → **L1 cell fates**

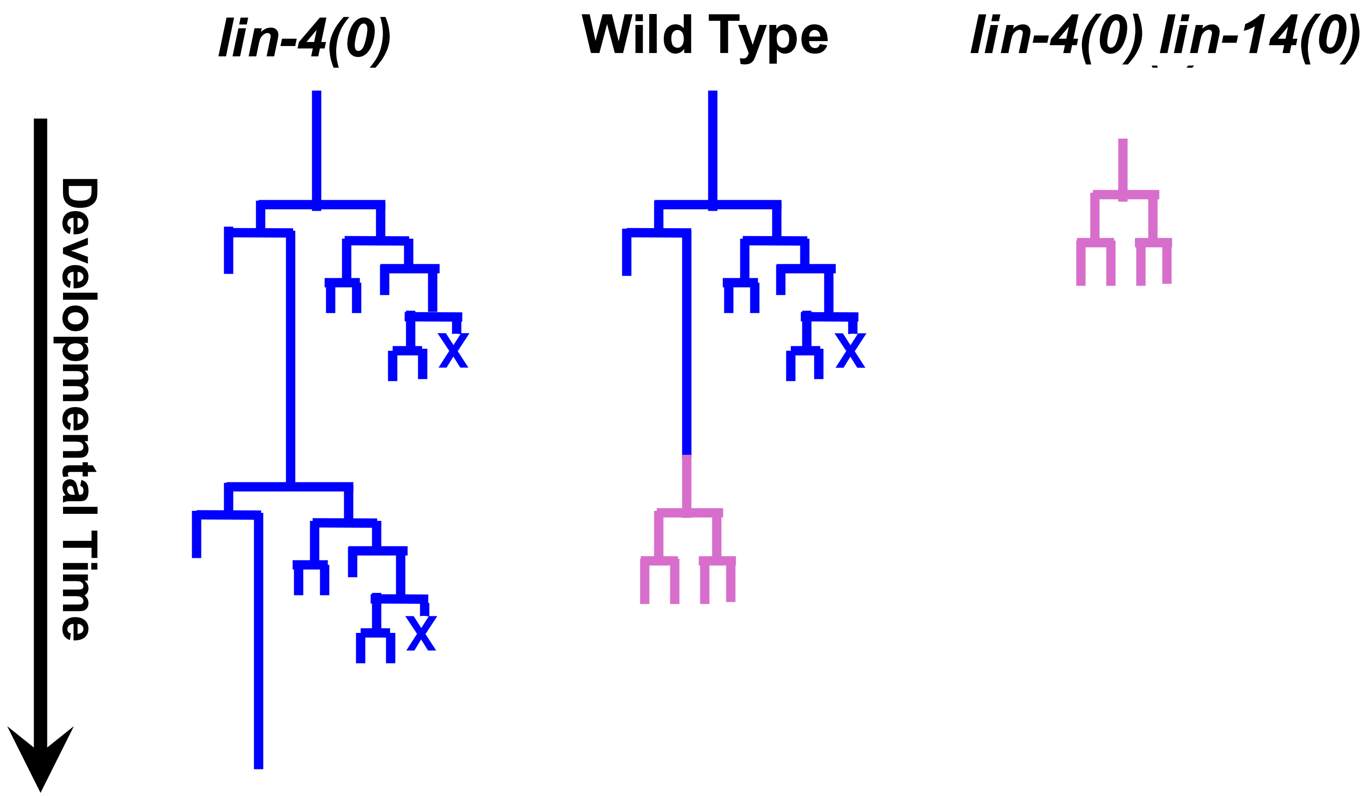
LIN-14 ⊣ **L2cell fates**

lin-4(0) and *lin-14(0)* mutants have opposite heterochronic phenotypes



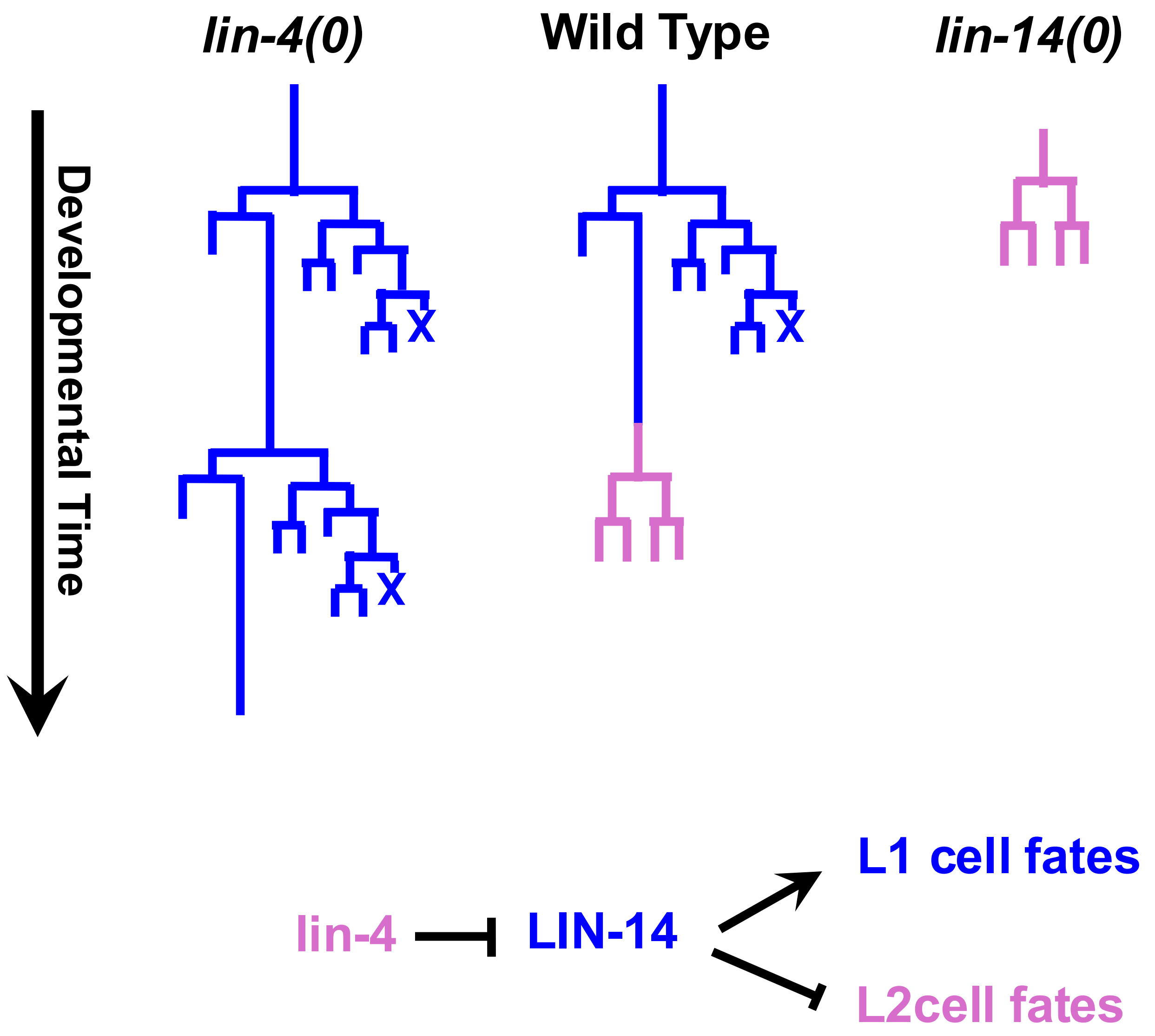
Which Model is Correct?

lin-4(0) and *lin-14(0)* mutants have opposite heterochronic phenotypes

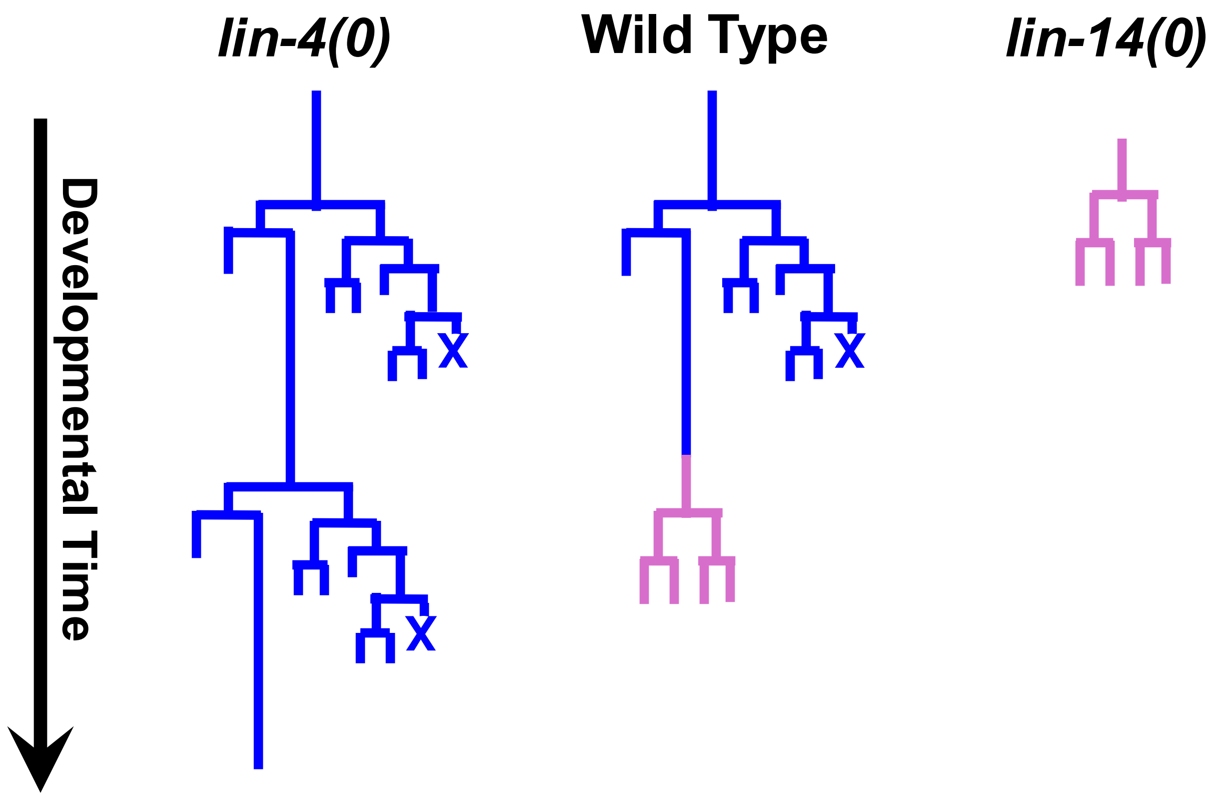


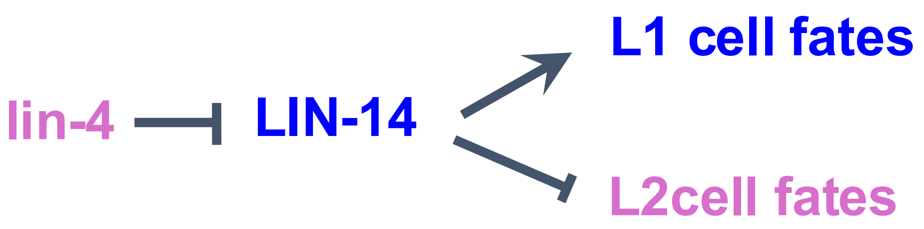
Which Model is Correct? **This one!**

Opposite temporal transformations in cell fate in *lin-4(0)* and *lin-14(0)* mutants

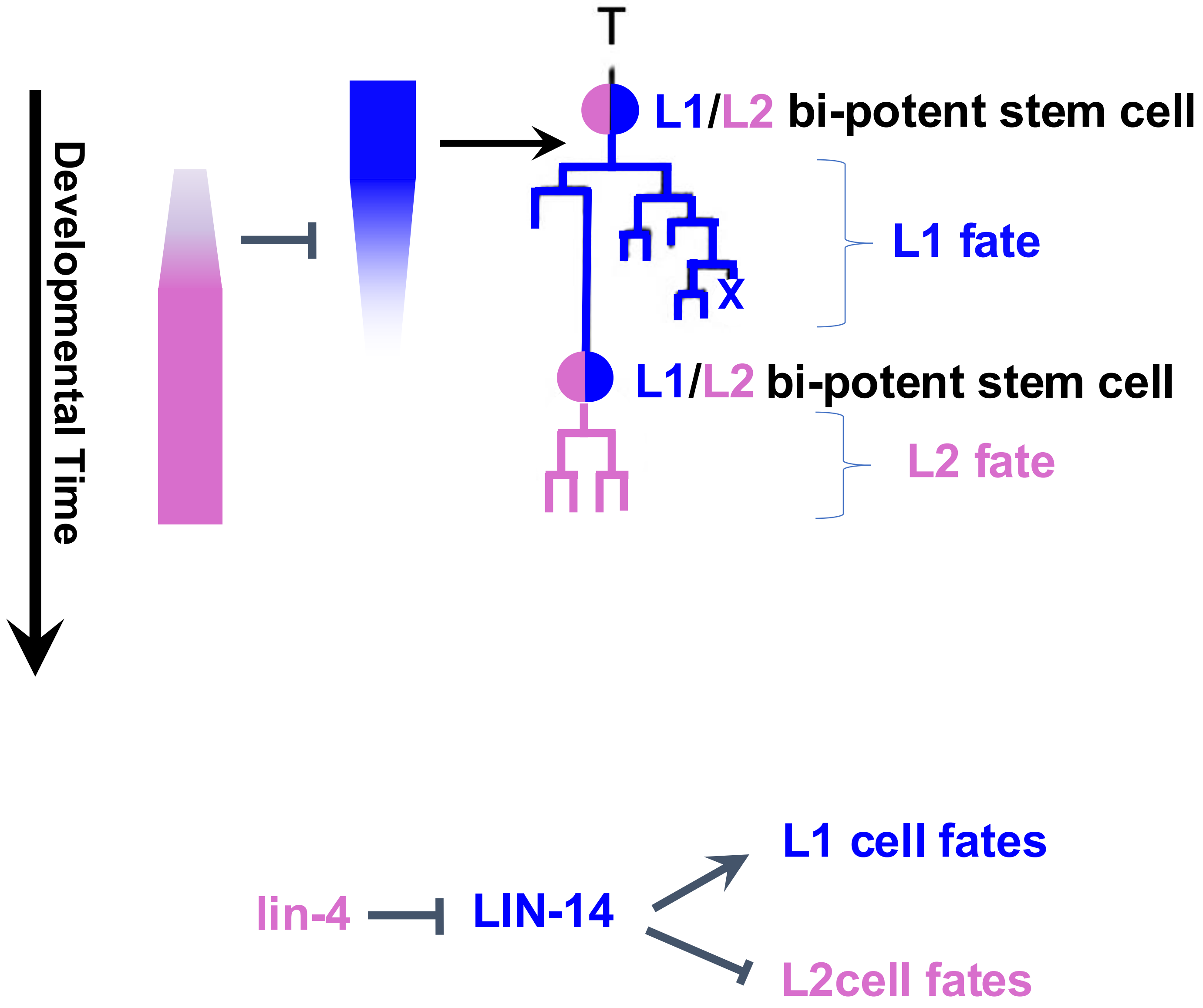


Lineally-related stem cells with multiple *potential* temporal cell fates

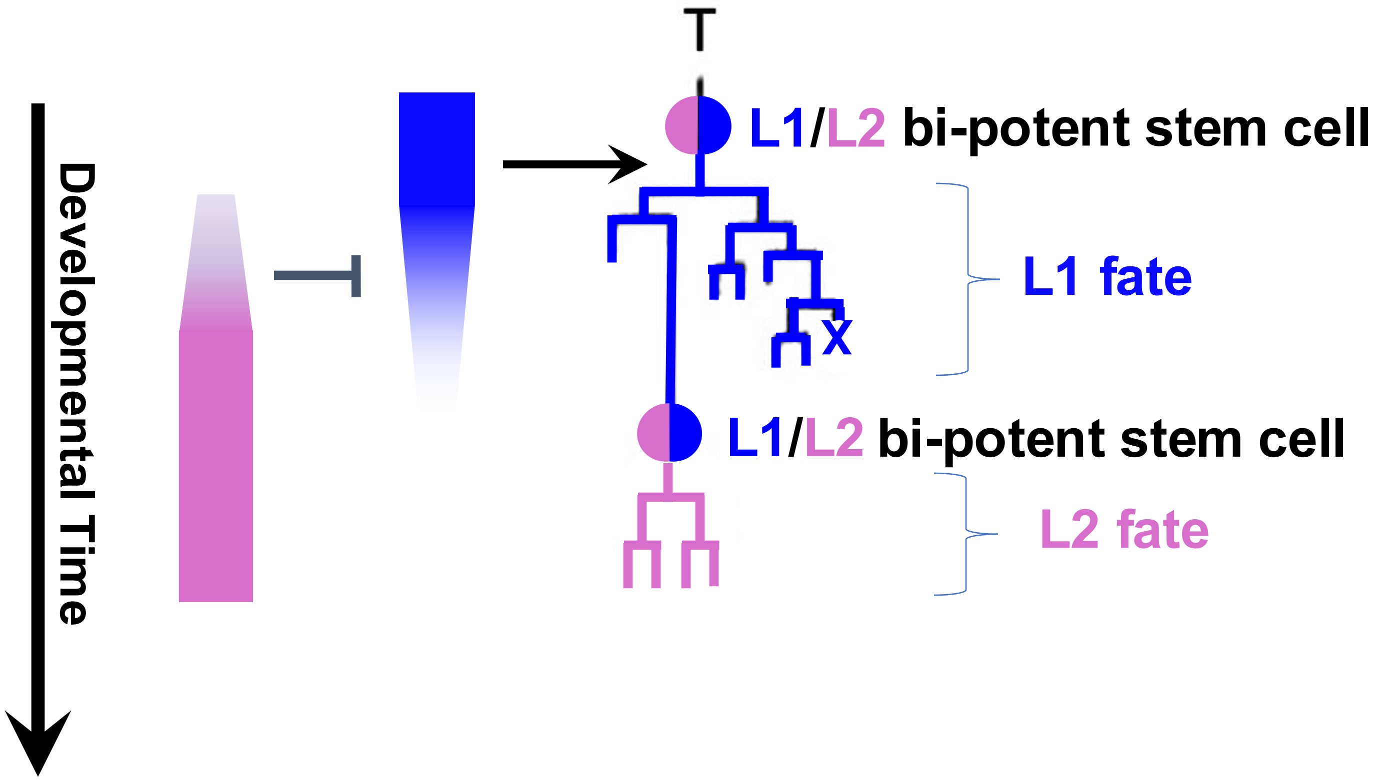


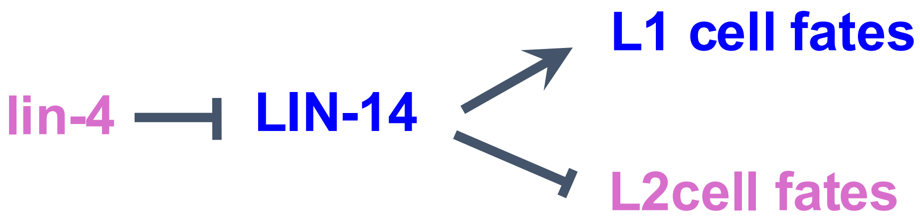


Lineally-related stem cells with multiple ***potential*** temporal cell fates



The *lin-4* and *lin-14* genes and their developmental context was super-interesting

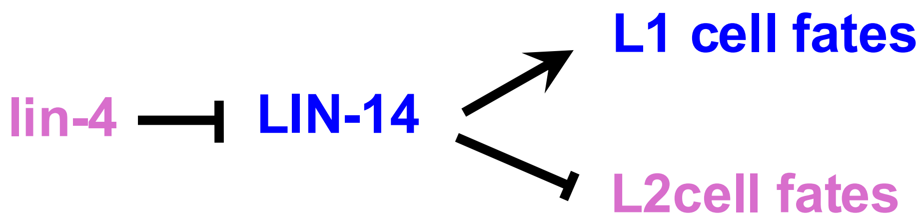




Setting out to clone the *lin-4* and *lin-14* genes

Ambros Lab
(Harvard Cambridge)
lin-4

Ruvkun Lab
(Harvard Boston)
lin-14



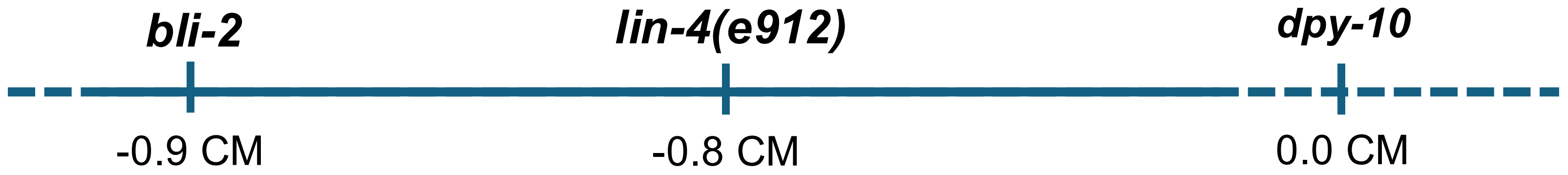
***lin-4(e912)* mutant**

***lin-14(0)* mutants**

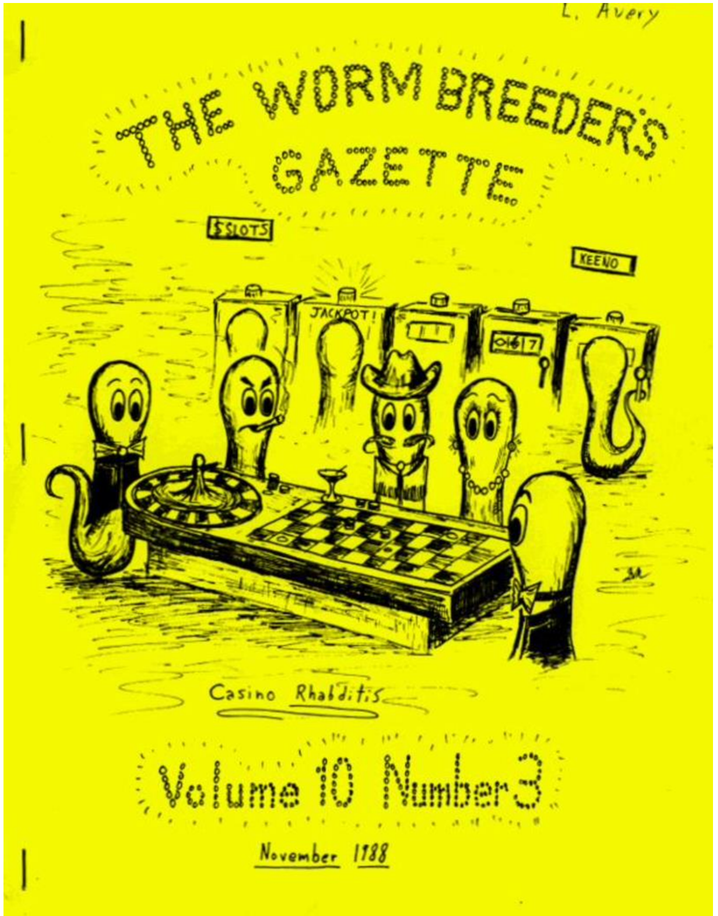
and

***lin-14(gain of function)* mutants**

Getting started ~ 1988: Collecting cloned DNA in the *lin-4* region of LG II



Getting started ~ 1988: Collecting cloned DNA in the *lin-4* region of LG II



The MRC *C. elegans* Physical Mapping Project ~ *1988*

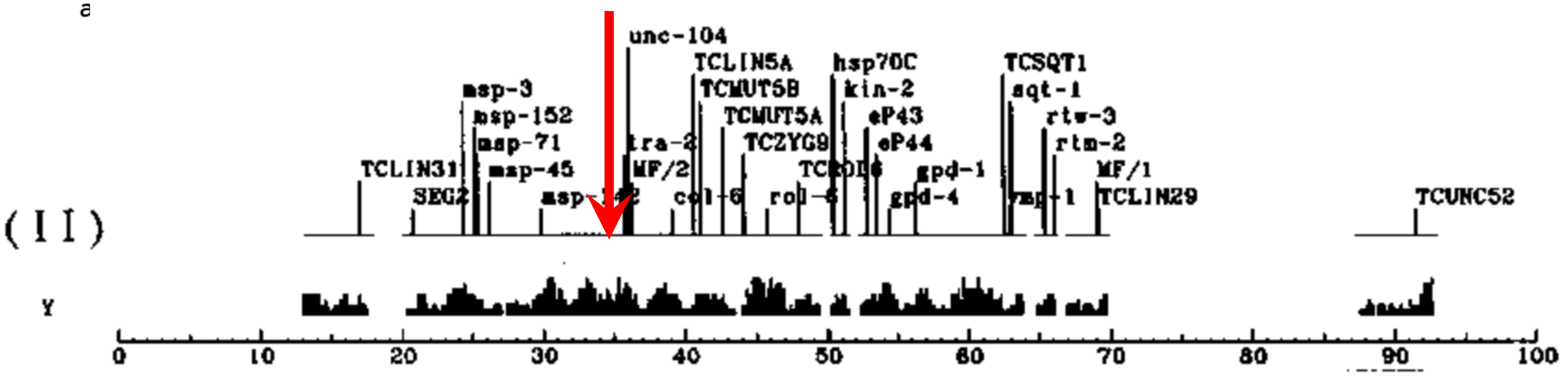
Worm Breeder's Gazette 10(3) November 1988

The Genome Map

Alan Coulson, John Sulston, Yiji Kohara, Donna Albertson, Rita Fishpool, Bob Waterston, Humaira Ameer

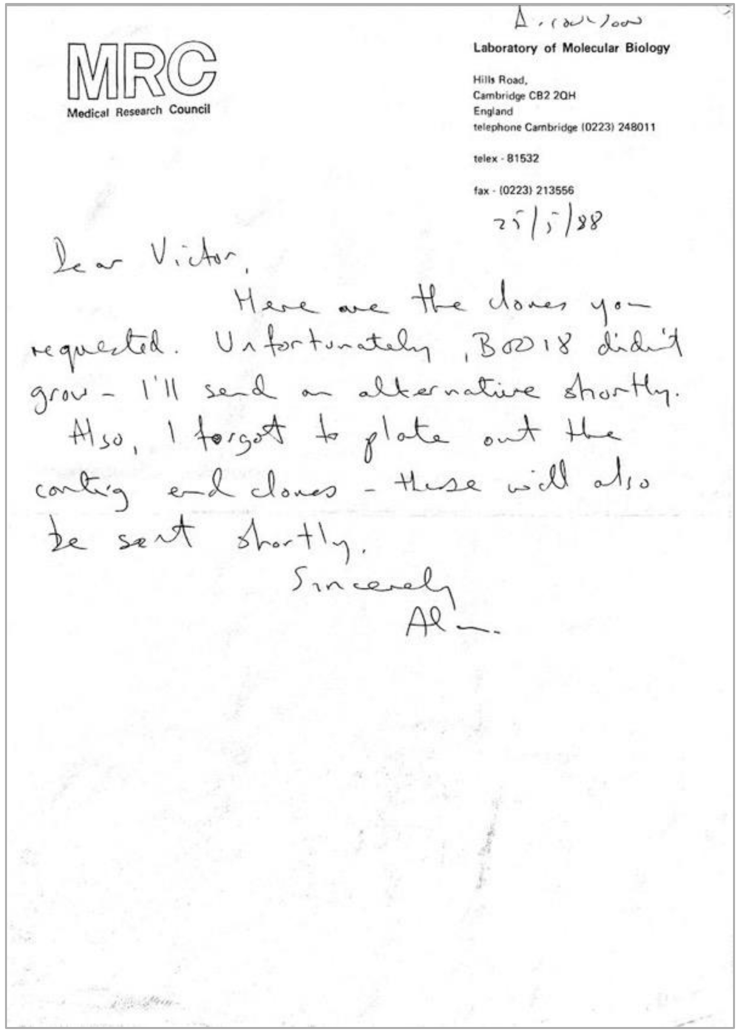
```
contigs=
251
mean contig size=
340kb
Since our previous report, 260 joins have been made.  Mostly, we
have been probing cosmid grids with YAC clones taken at random from a
new bank of median insert size 250kb.  The genetic cluster on each
chromosome is now represented fairly completely by seven contigs on
```

……



Getting started ~ 1988: Collecting cloned DNA in the *lin-4* region of LG II

Alan and John send clones and helpful hints



A. Coulson

MRC
Medical Research Council

Laboratory of Molecular Biology
Hills Road,
Cambridge CB2 2QH
England
telephone Cambridge (0223) 248011
telex - 81532
fax - (0223) 213556

25/5/88

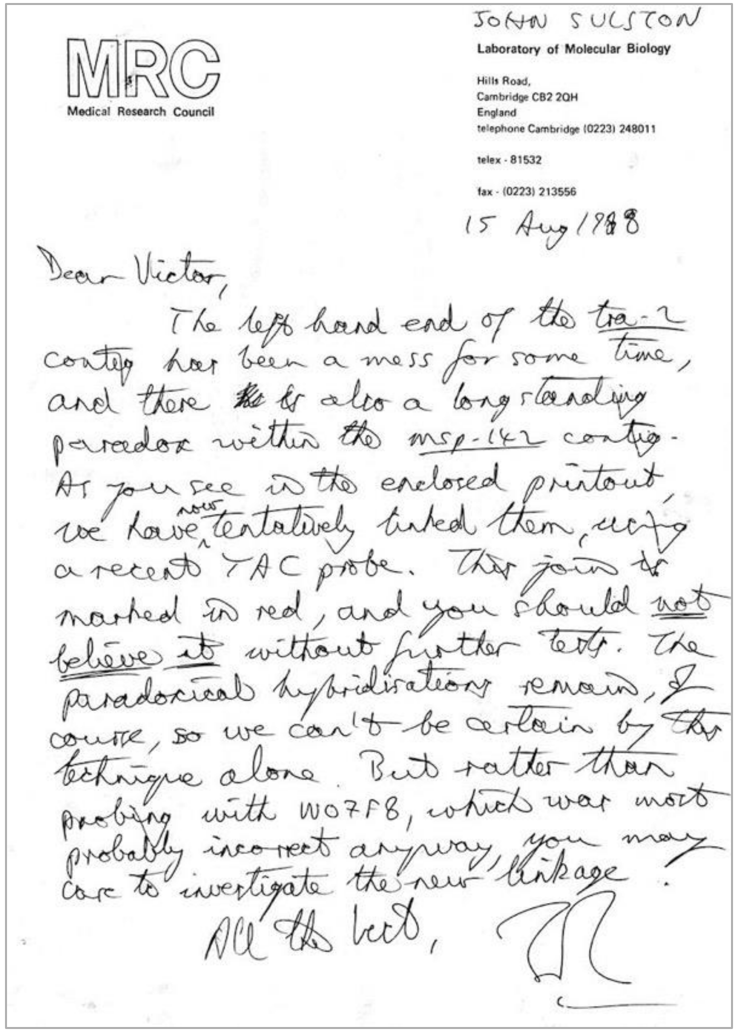
Dear Victor,

Here are the clones you requested. Unfortunately, B0218 didn't grow - I'll send an alternative shortly.

Also, I forgot to plate out the contig end clones - these will also be sent shortly.

Sincerely

Alan.



JOHN SULSTON

MRC
Medical Research Council

Laboratory of Molecular Biology
Hills Road,
Cambridge CB2 2QH
England
telephone Cambridge (0223) 248011
telex - 81532
fax - (0223) 213556

15 Aug 1988

Dear Victor,

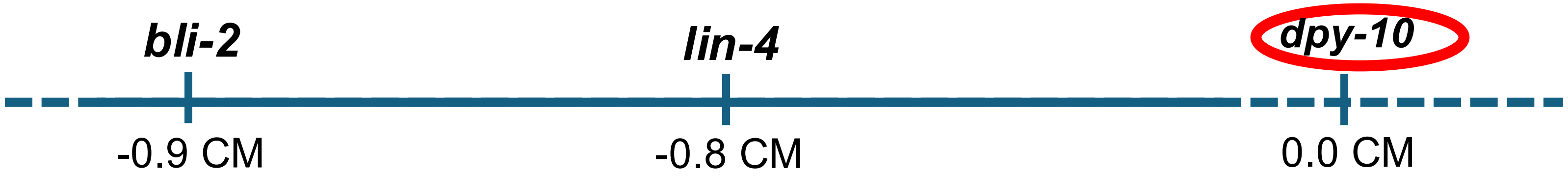
The left hand end of the tra-2 contig has been a mess for some time, and there is also a longstanding paradox within the msp-142 contig. As you see in the enclosed printout, we have now tentatively linked them, using a recent YAC probe. The join is marked in red, and you should not believe it without further tests. The paradoxical hybridisations remain, of course, so we can't be certain by this technique alone. But rather than probing with W07F8, which was most probably incorrect anyway, you may care to investigate the new linkage.

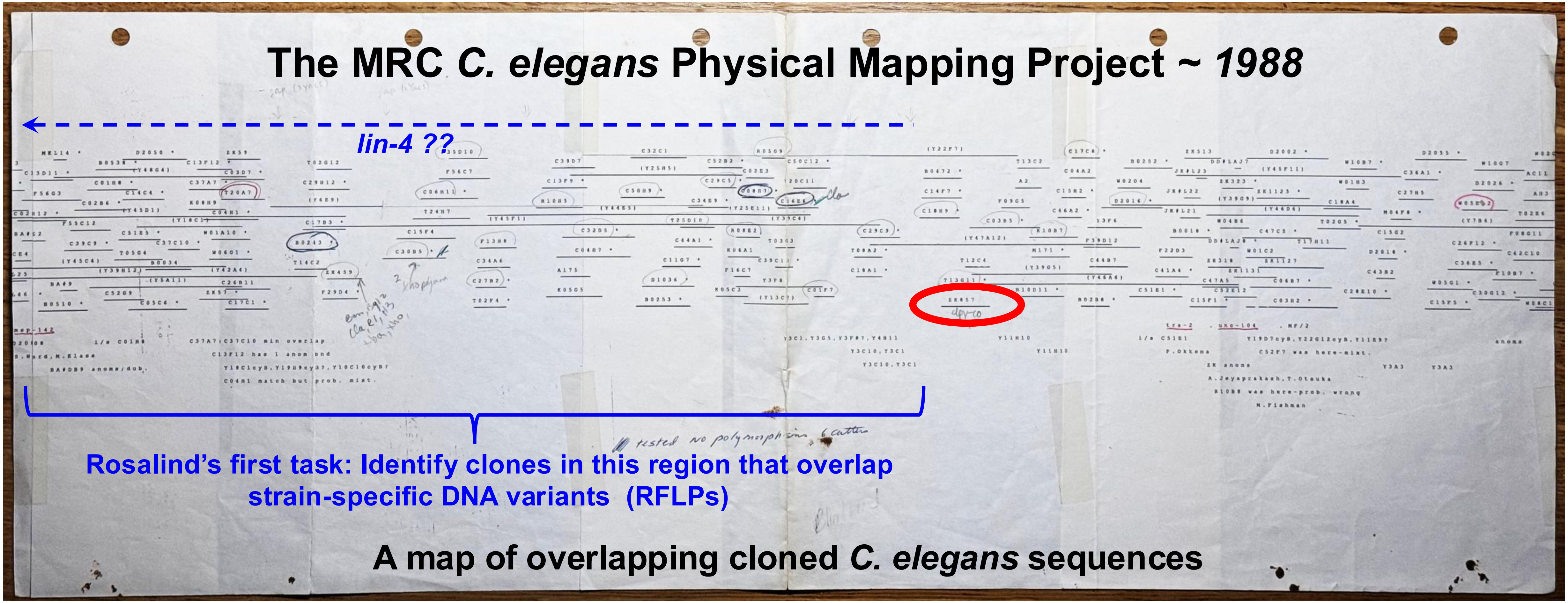
All the best,

John

Pro Bono

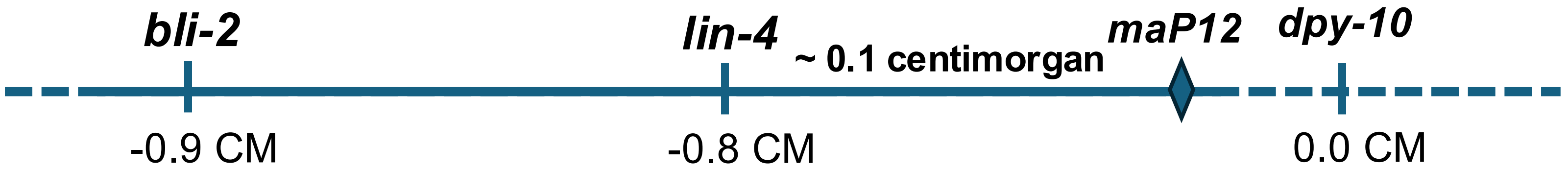
Getting started ~ 1988: Collecting cloned DNA in the *lin-4* region of LG II



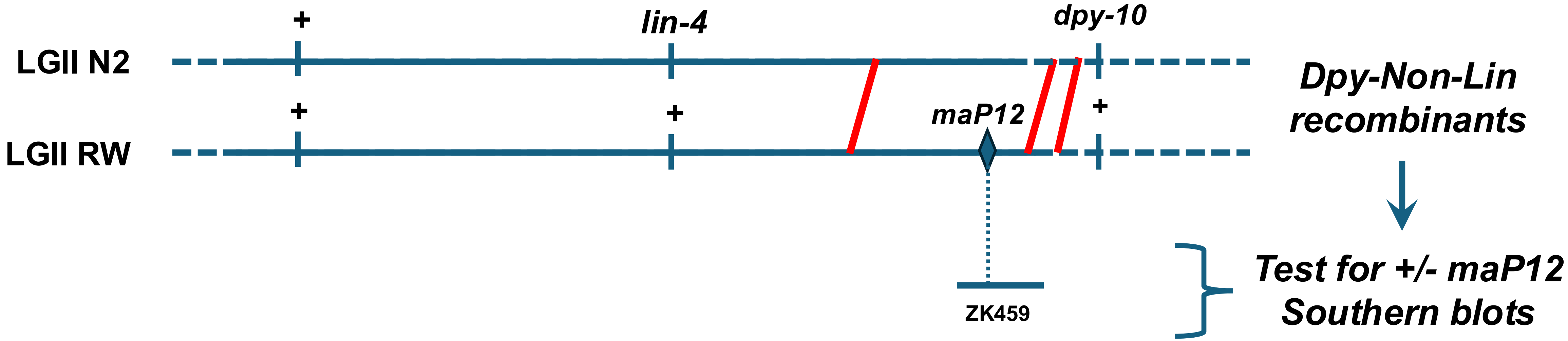


Rosalind Lee

Getting started ~ 1988: Genetic/molecular mapping in the *lin-4* region of LGII

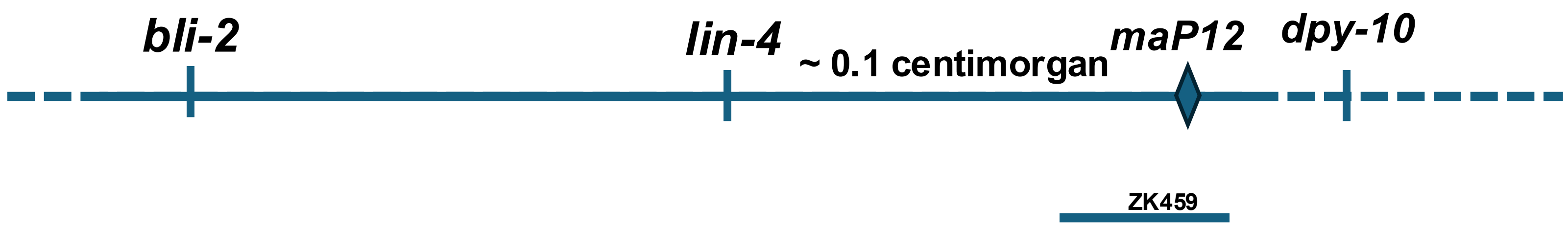


Mapping DNA polymorphisms (RFLPs) detected by Southern blots to genomic DNA



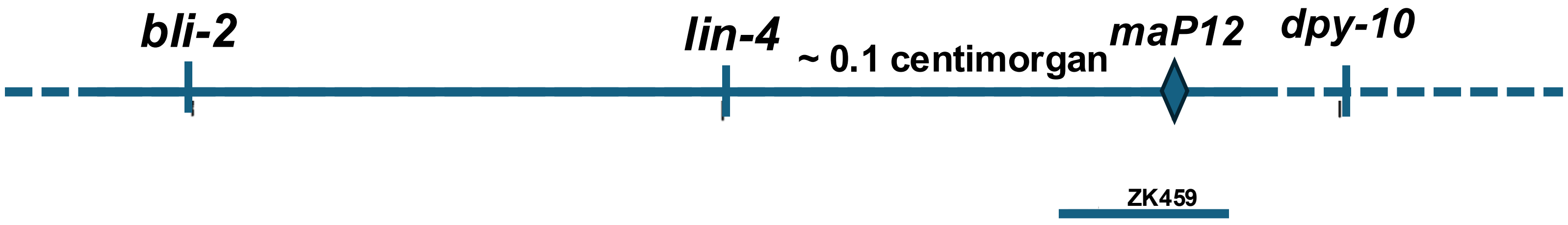
Rosalind Lee

maP12 → toehold close and to the right of *lin-4* at *maP12*



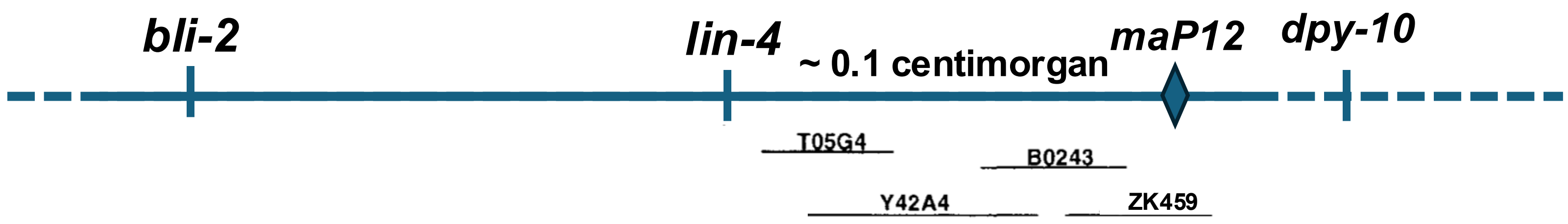
Rosalind Lee

Chromosome walking from *maP12* to *lin-4* facilitated by the MRC



Rosalind Lee

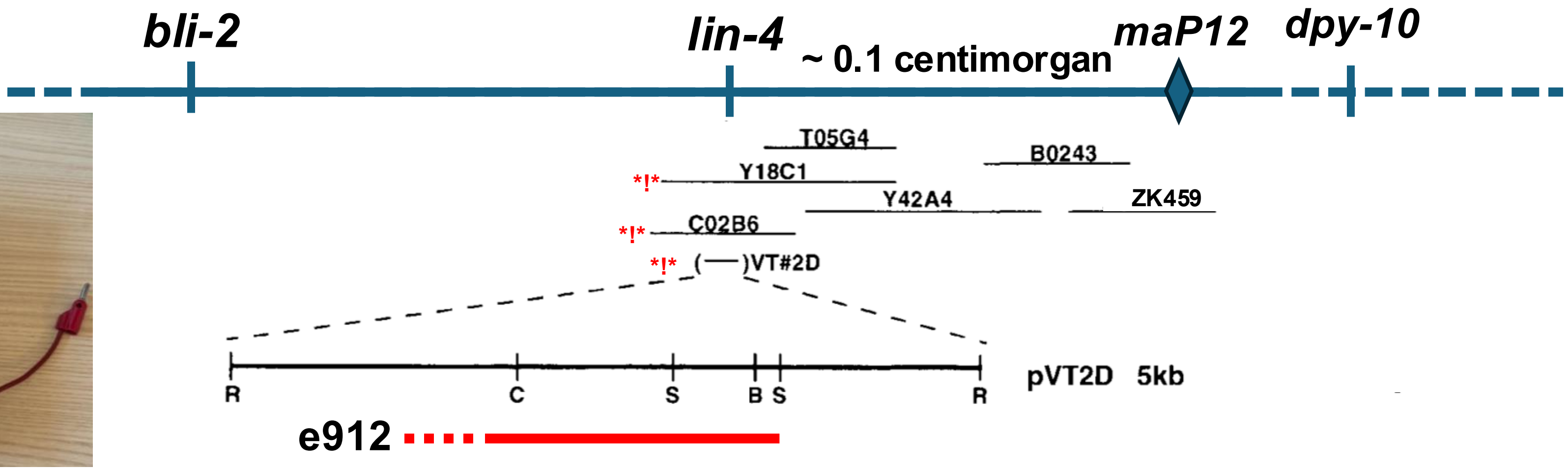
Chromosome walking from *maP12* to *lin-4* facilitated by the MRC



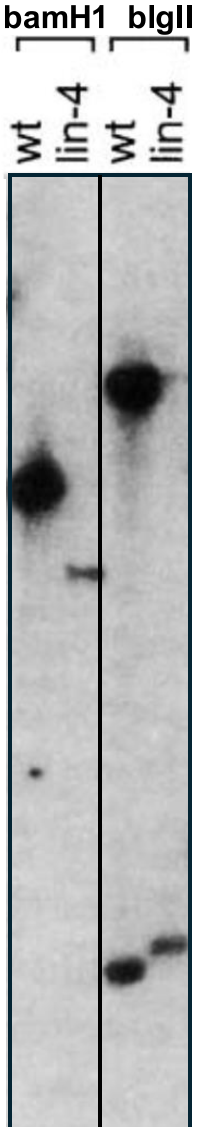
Rosalind Lee

Finding the *lin-4(e912)* DNA lesion

MJ Research
PPI 100
Pule-field gel
electrophoresis



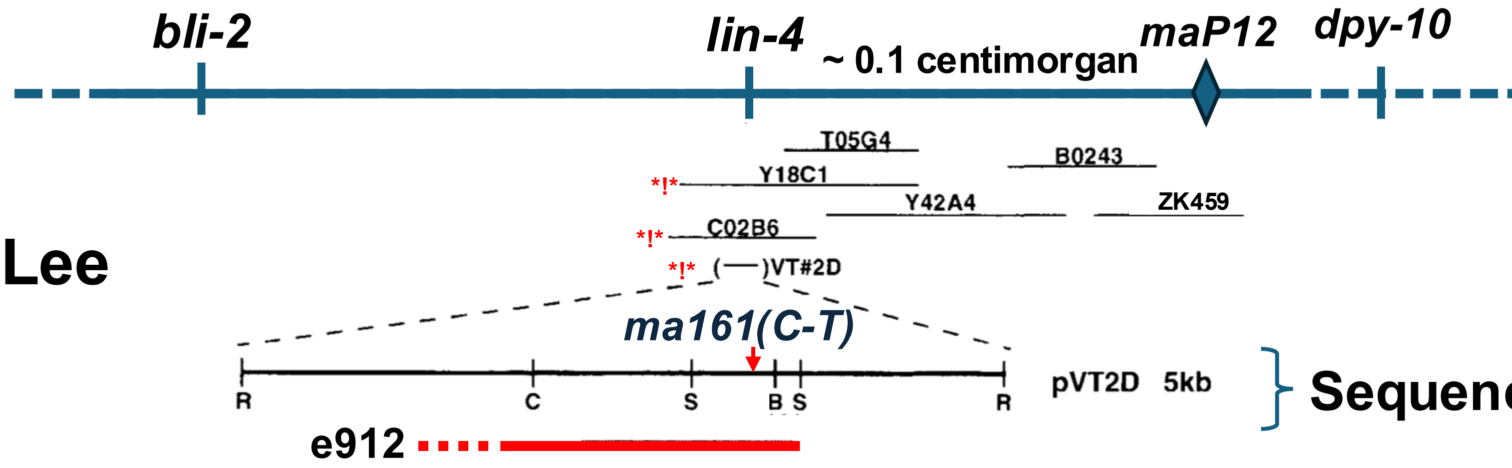
Southern Blots with genomic DNA lin-4(e912) vs WT



Rosalind Lee

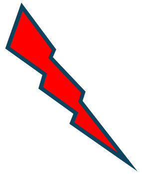
Finding the *lin-4(e912)* DNA lesion

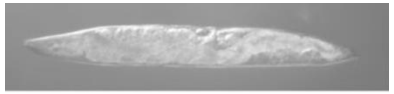
Rosalind Lee



Sequencing 5,000 bp clone

Screening for new *lin-4* (ideally) *point* mutations





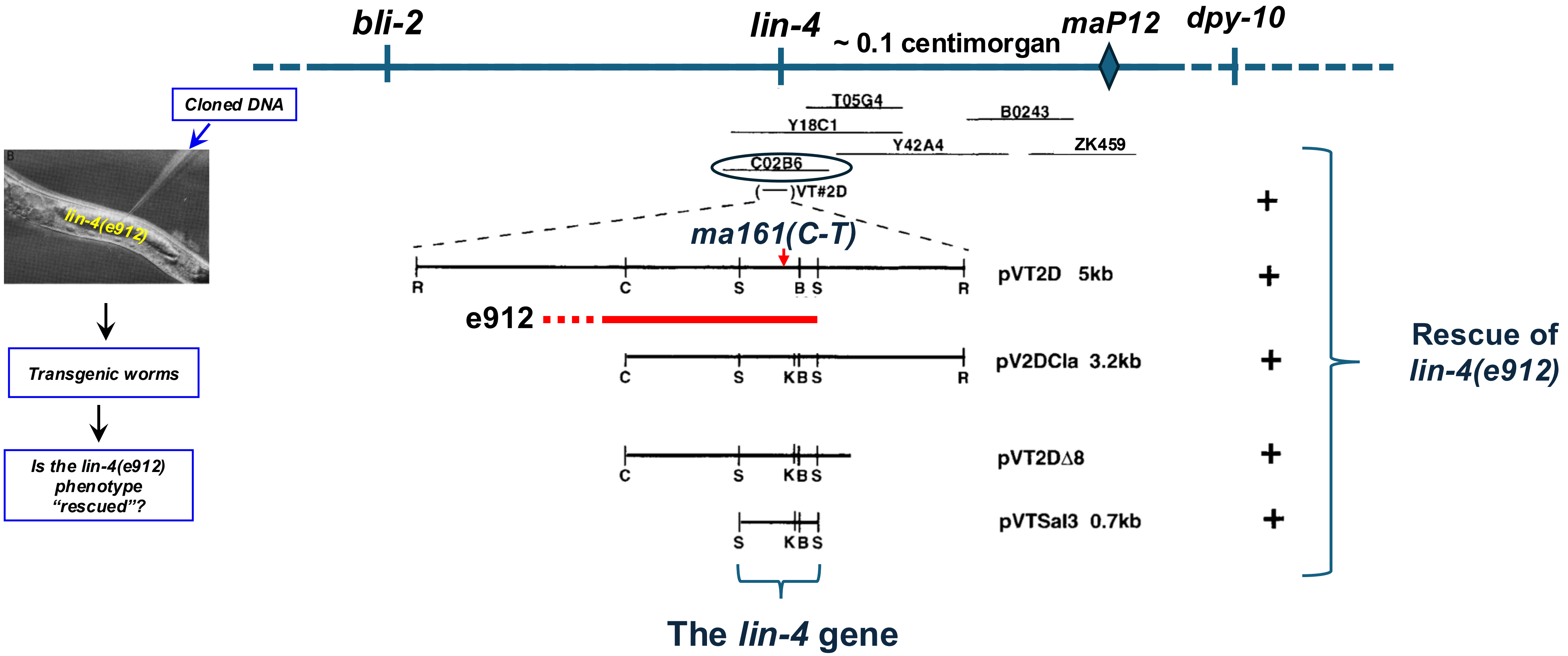
WT males X lin-4(e912) dpy-10

~20,000: *lin-4(e912) dpy-10* / + +

1: *lin-4(e912) dpy-10* / *lin-4(new)* +

Rhonda Feinbaum

Mapping the boundaries of *lin-4* by transgenic rescue of *lin-4(e912)*



Rhonda Feinbaum and Rosalind Lee

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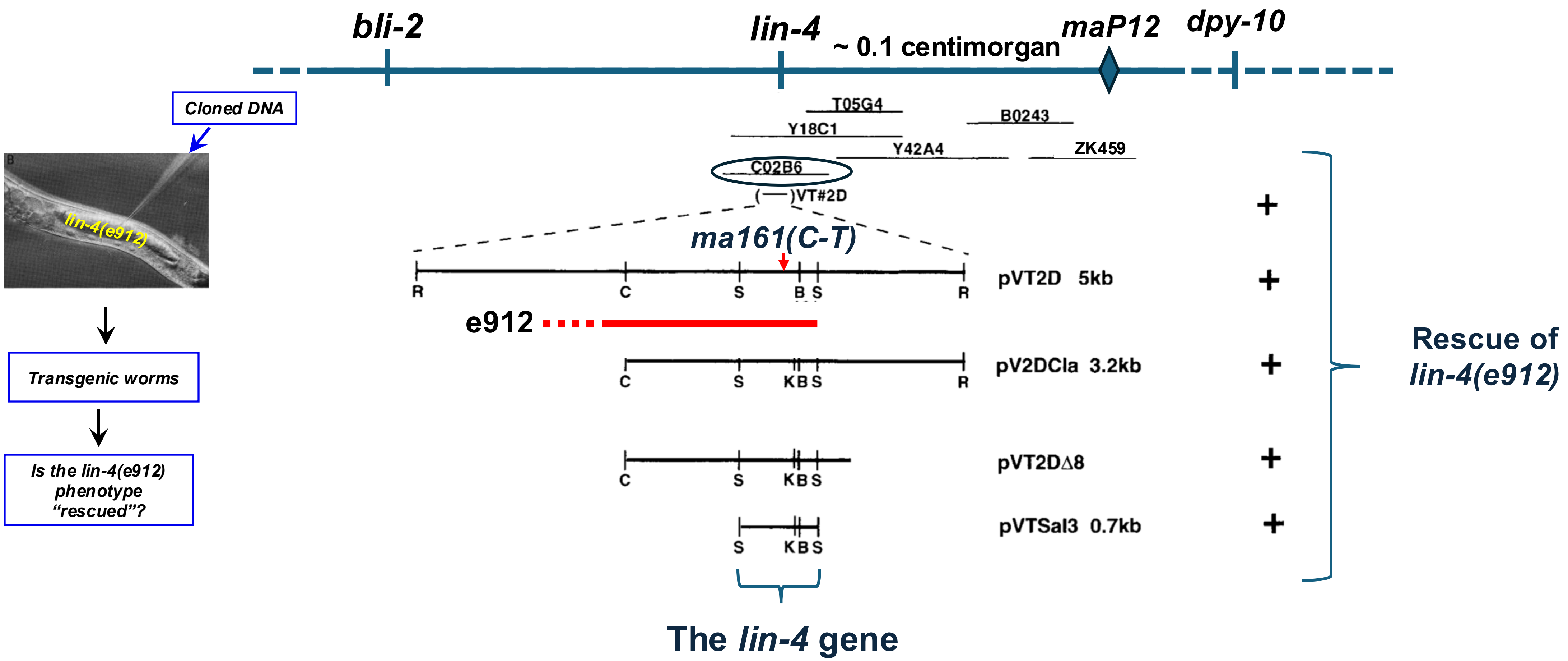
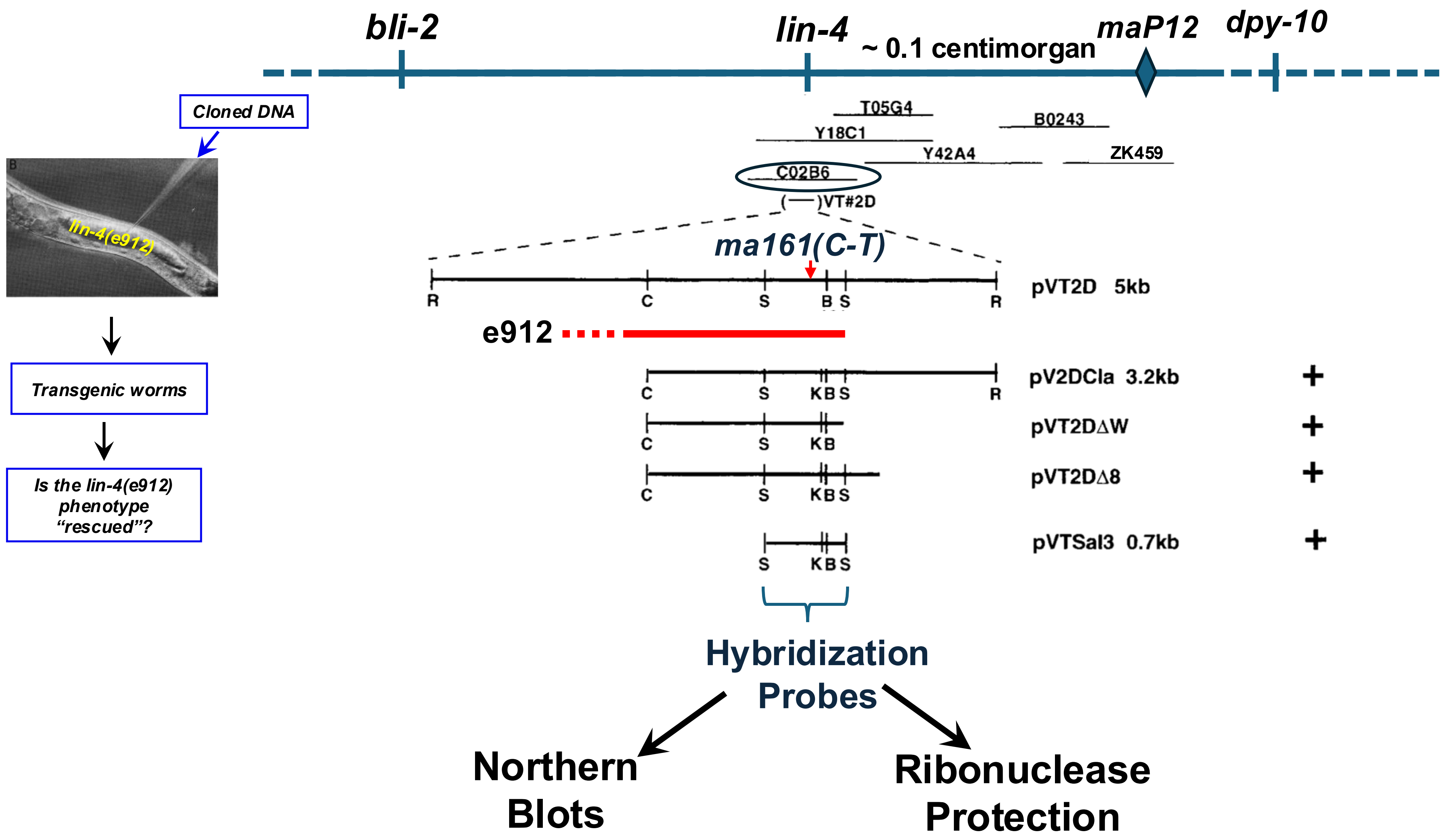


Rhonda Feinbaum and Rosalind Lee

Detection and mapping of *lin-4* transcripts



Rhonda Feinbaum and Rosalind Lee

Detection and mapping of *lin-4* transcripts

A

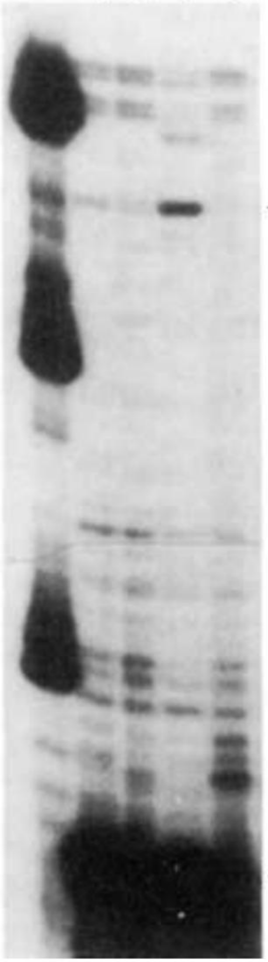
wild type lin-4 rescued

61nt ◄lin-4L

22 nt ◄lin-4S

Northern Blots

wild type lin-4 rescued tRNA



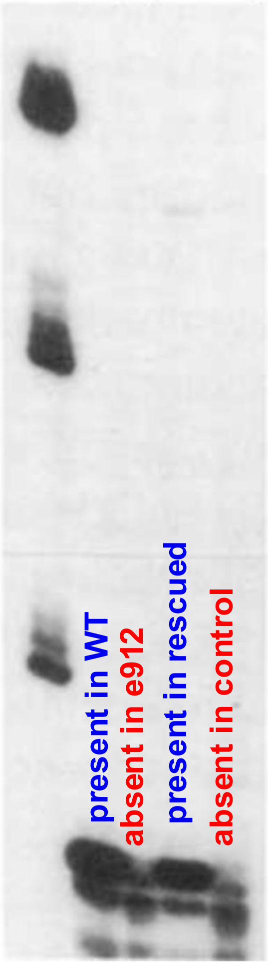
~55 nt!

←~ "What's that"?

Ribonuclease Protection

C

wild type lin-4 rescued tRNA



60–

46–

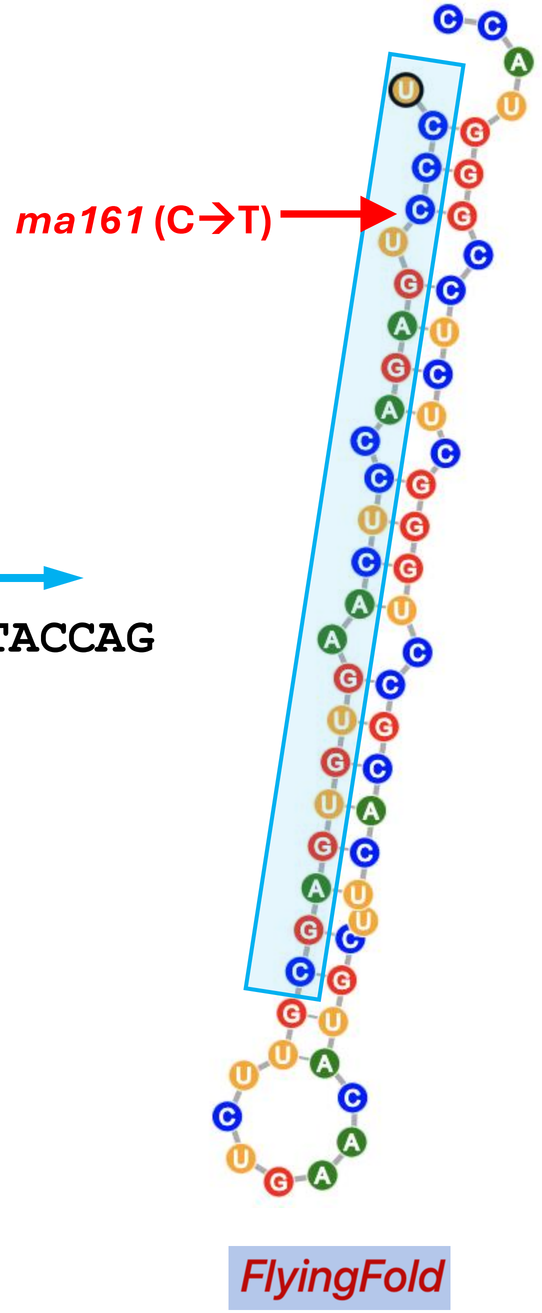
28–

◄lin-4L

◄lin-4S

Rhonda Feinbaum **Victor Ambros**

22 nucleotide and 61 nucleotide *lin-4* transcripts

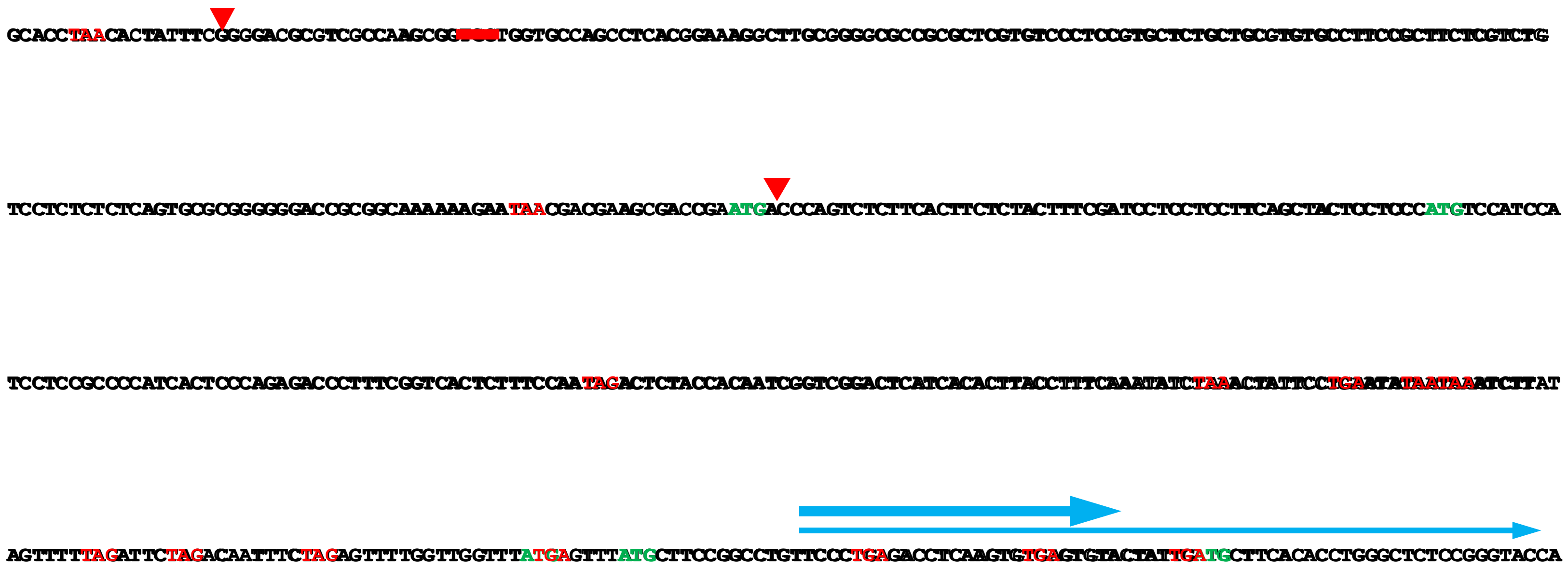


CCTGTTCCCTGAGACCTCAAGTGTGAGTGTACTATTGATGCTTCACACCTGGGCTCTCCGGGTACCAG

ma161 (C→T)

Rhonda Feinbaum and Rosalind Lee

Ruling out putative *lin-4* protein coding capacity



There are some start codons

And numerous stop codons

We introduced frameshifts, and a stop → Still <u>rescues *lin-4(e912)*</u>

"Evolutionary mutagenesis" → clones from *C. briggsae*, *C. remanei*, and *C. vulgaris* <u>rescue *lin-4(e912)*</u>

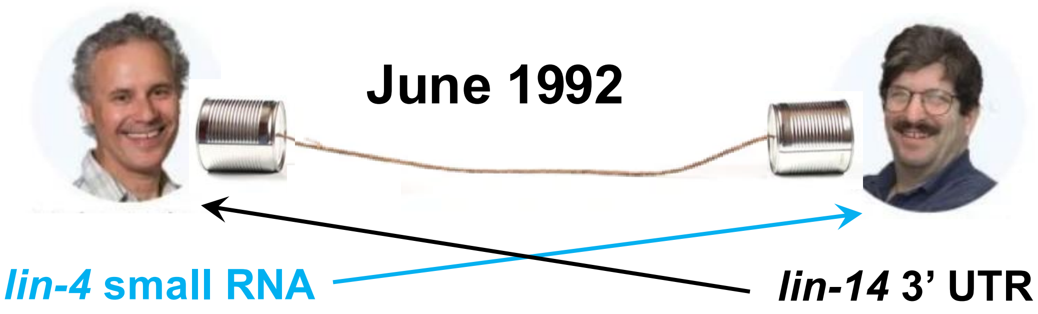
Rhonda Feinbaum and Rosalind Lee

Evolutionarily divergent *lin-4* sequences rescue *C. elegans lin-4(e912)*

```
elegans     GCACCTAACACTATTTCGGGGACGCGTCGCCAAGCGGTGGTGGTGCCAGCCTCACGGAAAGGCTTGCGGGGCGCCGCGCTCGTGTCCCTCCGTGCTCTGCTGCGTGTGCC----TTCCGCTTCTCGTCTGTCCTCTCTCTCA
briggsae    CGAGCGCGGCGCCCCGCAAGGACGCGTCGCCAAGCGGTGGTGGTGCCAGCCTCACGGAAAGGCTTGCGGGGCGCCGCGCTCGTGTCCCTTCGTGCTCC-CTGCGTGTGCCTGCTTTCCGCTTC---TCTGTCCTCTCTCCCA
remanei     GCAGAGCGCGCTTTCAGAGGGTCGCGTCGCCAAGCGGTGGTGGTGCCAGCCTCACGGAAAGGCTTGCGGGGCGCCGCGCTCGTGTCCCTCGGTGCTCC-CTGCGTGTGCCCGCTTTCCGCTTCTCGTCTGTCCTCTCTCTCA
vulgaris    GACCCATTATGTCTCGTAGGGACGCGTCGCCAAGCGGTGGTGGTGCCAGCCTCACGGAAAGGCTTGCGGGGCGCCGCGCTCGTGTCCCTTCGTGCTCC-CTGCGTGTGCCCGCTTTCCGCTTCTCGTCTGTCCTCTCTCTCA
                             ** ****************************************************************   ******* ***********    *********   ************* **

elegans     GTGCGCGGGG-----GGACCGCGGC-----AAAAAAGAATAACGACGAAGCGACCGAATGACCCAGTCTCTTCACTTCTCTACTTTCGATCCTCCTC----------------CTTCAGCTACTCCTCCCATGTCCATCCA
briggsae    GTGCGCGGTGAA---GGACCGCGGCAGCAAAAAAAAAGAATAACGACGAAGCGGCCGAATGACCCAGTCTCTTCACTTCTCTACTTTCGATCCTCCTCCCCTTCTTTTTTGCT-GCTTCTTCTACTCCTCCCATGTTCATCCA
remanei     GTGCGCGGTGAAGGGGGACTGCGGCAG--AAAAAAAGAATAACGACGAAGCGACCGAATGACCCAGTCTCTTCACTTCTCTACTTTCGATCCTCCCATCTTATTTTCTGCT--TCTTCTTCTACTCCTCCCATGTTCATCCA
vulgaris    GTGCGCGGTGAAGGGGGACTGCGGCAG--AAAAAAAGAATAACGACGAAGCGACCGAATGACCCAGTCTCTTCACTTCTCTACTTTCGATCCTCCCCAATCTTATTTCTGCTTCTTCTTCTACTCCTCCCATGTTCATCCA
            ******** *     **** *****      ********************** ***************** ************************                ****  *************** ******

elegans     TCCTCCGCCCC-ATCACTCCCAGAGACCCTT-TCGGTCACTCTTTCCAATAGACTCTACCACAATCGGTCGGACTCATCACACTTACTTTCAAATATCTAAACTA-TTCCTGAA-TATAATAAATCTTATAGT-------TT
briggsae    TC--CCGCCCCAATCGCTCCCAGAGACTCACACCGGTCACTCTAACTATTAGTCTTCACCGCAACCGGT-------ATCATTCCAACTC---CATCCATC--CTA-TTTCTAAACTACAGTAATCC---------------
remanei     TC--CCGCCCCAATCGCTCCC--AGACCCCGTCGGTCACTCT--CTATTAGACCCTTCCTCAATCTGTCG--------------ACTC---AATCTACCAACTATTTCCTGGGCTACAGTAACCCGAAGAGTATCATATTC
vulgaris    TC-CCGCCCCAATCGCTCCC---AGACCCCGTCGGTCACTCT--CTATTAGACTCTTCCTCAATCTGTCG-------------AACTC---AATCTACCAACTATTTCCTGGGCTACAGTAACCCGAAGAGT---------
            **   ******* *** *****   **** *    **********  * * *** *    ** ***                    ***      **        *** ** **     ** * ***  *

elegans     TTAGTTTATAGTTTTTAGATTCTAGACAATTT---CTAGAGTTTTGGTTGGTTTATGGTT----TATGCTTCCGGCCTGTTCCCTGAGACCTCAAGTGTGAGTGTACTATTGATGCTTCACACCTGGGCTCTCCGGGTACCA
briggsae    ----------TTTAAAAAATCCTAAAACT-------AGAATTTTTGGTTGGTTTATATGTATCAGATGCTTTCGGCCTGTTCCCTGAGACCTCAAGTGTGAGCGTTCTGAACATGCTTCACGCCTGGGCTCTCCGGGTACAG
remanei     ATCAATCATATTCATCACATTCTAGACTTTCTGAGAAAGACTTTTGGTTGGTTTATATGT-TCTGATGCTTTCGGCCTGCTCCCTGAGACCTCAAGTGTGAGCGCACTGTTGATGCTTCACGCCTGGGCTCTCTGGGTACAG
vulgaris    ---TATCATATTCATCACATTCTAGACTTTTCGAGAAAGACTTTTGGTTGGTTTATATGT-TCTCATGCTTTCGGCCTGCTCCCTGAGACCTCAAGTGTGAGCGCACTGTTGATGCTTCACGCCTGGGCTCTCTGGGTACAG
                      *    * ** *** *             * ***************   *     ****** ******* ********************* *  **    ********* *********** ********
```

"Evolutionary mutagenesis" → clones from *C. briggsae*, *C. remanei*, and *C. vulgaris* rescue *lin-4(e912)*

Complementarity between *lin-4 RNA* and *lin-14* 3' UTR sequences




```
5'GUUCCCUGAGACCUCAAGUG.UGAG          lin-4
3'CAAG.GACUC......UCGU-ACUC
  UAAG.GACUC.-.........ACUU
  CAAGGGACUC.-...UUUAC-GCUC          lin-14
  UAAG.GACUC.-.......U.ACUC          3'UTR
  CAAGGGACUC.......CAU..CUU
  CAAG.GACU..-.....UGU.-UUC
  CA.GGGACUC.-.........ACUC
```

Lee, Feinbaum & V. Ambros (1993) The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. Cell 75

Wightman, Ha, Ruvkun (1993) Posttranscriptional regulation of the heterochronic gene *lin-14* by *lin-4* mediates temporal pattern formation in *C. elegans*. *Cell* 75

Today's perspective:

```
U
 CCCUGAGACCUCAAGUGUGA
.GGGACUC..........ACU...

U
 CCCUGAGACCUCAAGUGUGA
.GGGACUC......UUACGCU...

U
 CCCUGAGACCUCAAGUGUGA
.GGGACUC.......CAU.CU...

UC
  CCUGAGACCUCAAGUGUGA
..GGACUC..........ACU...

UC
  CCUGAGACCUCAAGUGUGA
..GGACUC......UCGUACU...

UC
  CCUGAGACCUCAAGUGUGA
..GGACUU........GU.UU...

UC
  CCUGAGACCUCAAGUGUGA
..GGACUC..........ACU...
```

Ambros lab heterochronic activities 1993 ~ 2000

Rougvie and Ambros (1995) The heterochronic gene *lin-29* encodes a zinc finger protein that control a terminal differentiation event in *C. elegans*. *Development* 121

Moss, Lee, and Ambros (1997) The cold shock domain protein LIN-28 controls developmental timing in *C. elegans* and is regulated by the *lin-4* RNA. *Cell* 88

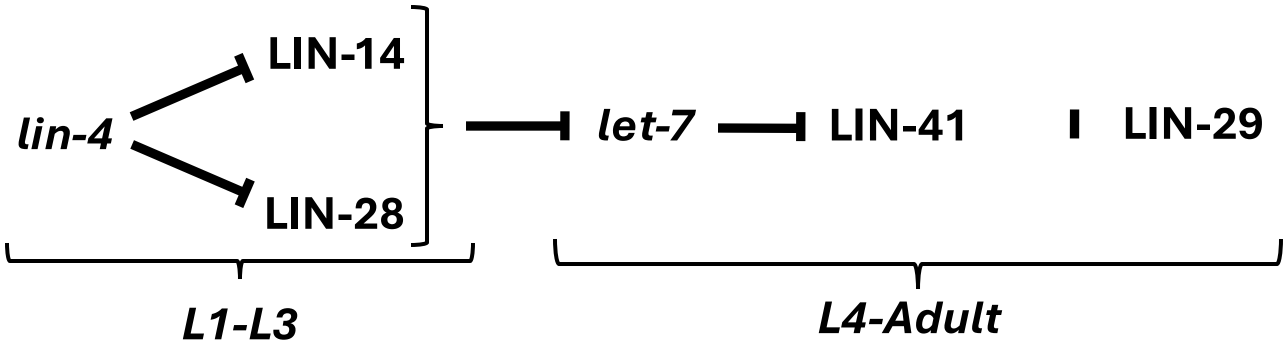
Feinbaum and Ambros (1999) The timing of *lin-4* RNA accumulation controls the timing of postembryonic developmental events in *C. elegans*. *Developmental Biology* 210

Olsen and Ambros (1999) The *lin-4* regulatory RNA controls developmental timing in C. elegans by blocking LIN-14 protein synthesis after the initiation of translation *Developmental Biology* 216

Slack, Basson, Liu, Ambros, Horvitz, Ruvkun (2000) The LIN-41 RBCC gene acts in the C. elegans heterochronic pathway between *let-7* regulatory RNA and the LIN-29 transcription factor. *Molecular Cell* 5

Ambros lab heterochronic activities 1993 ~ 2000

1999: *lin-4* and *let-7* are the only known microRNAs, and only *C. elegans.*



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1998 – 2001 Apprehending the scope and significance of microRNAs

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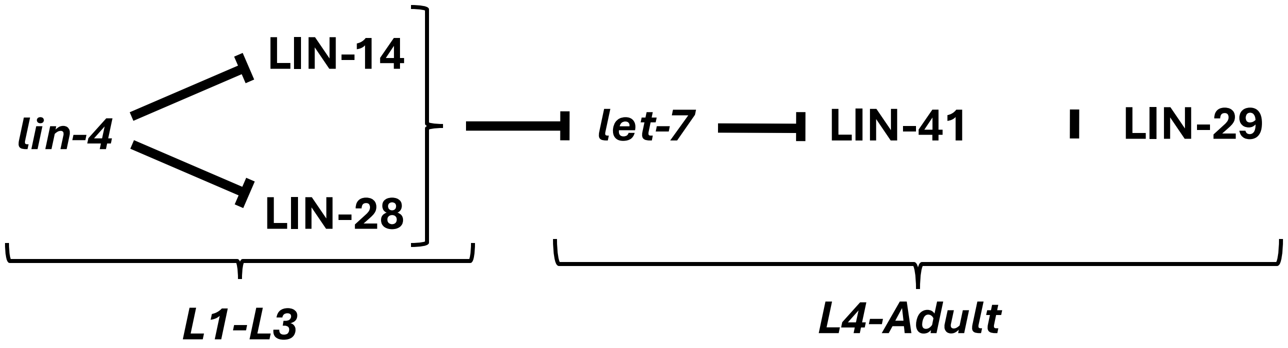

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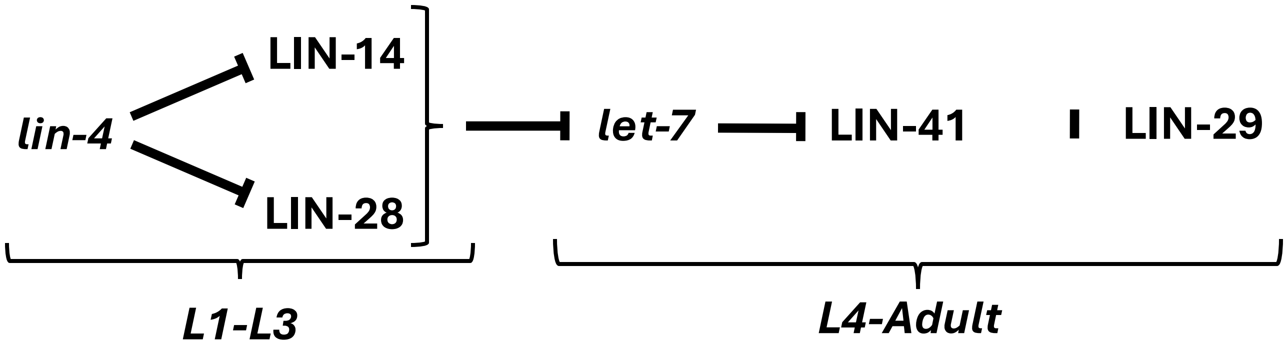
1998 – 2001 Apprehending the scope and significance of microRNAs

Consilience of microRNA and RNAi phenomena



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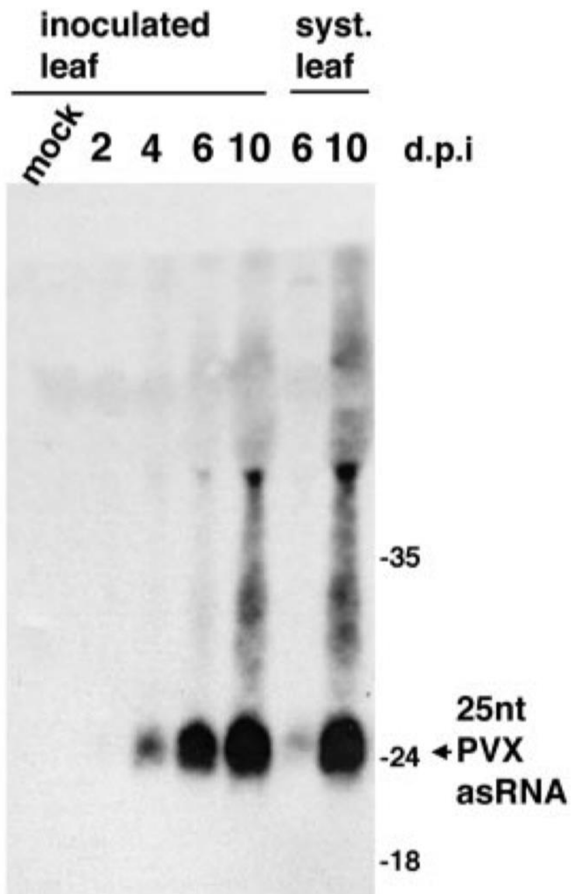
1998 – 2001 Apprehending the scope and significance of microRNAs

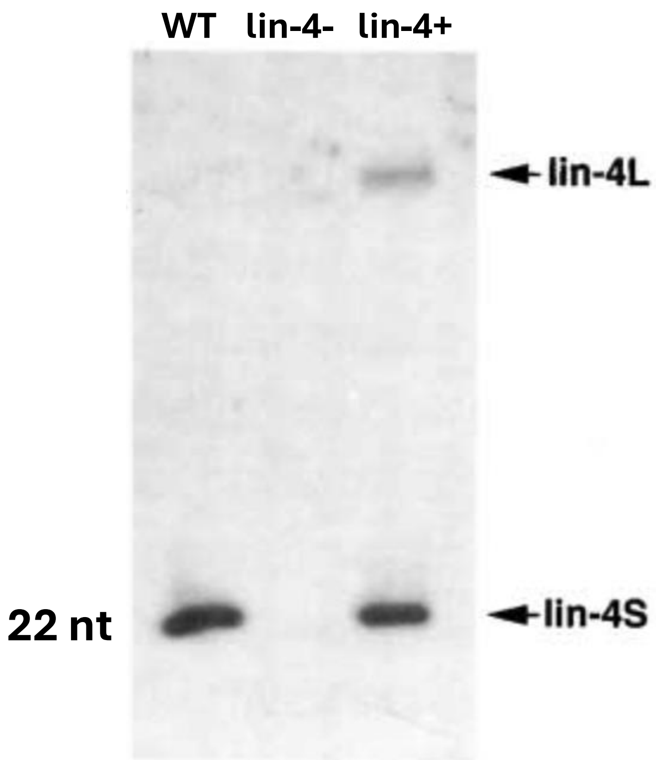
Consilience of microRNA and RNAi phenomena

Fire et al Mello (1999) Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans *Nature*

Hamilton & Baulcombe (1999) A species of small antisense RNA in posttranscriptional gene silencing in plants *Science*



Lee Feinbaum Ambros 1993



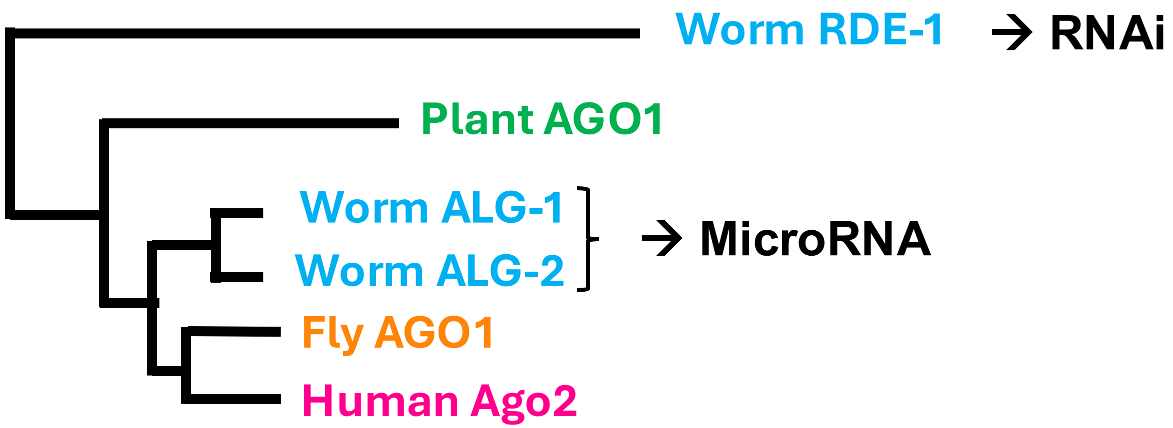
1998 – 2001 Apprehending the scope and significance of microRNAs

Consilience of microRNA and RNAi phenomena

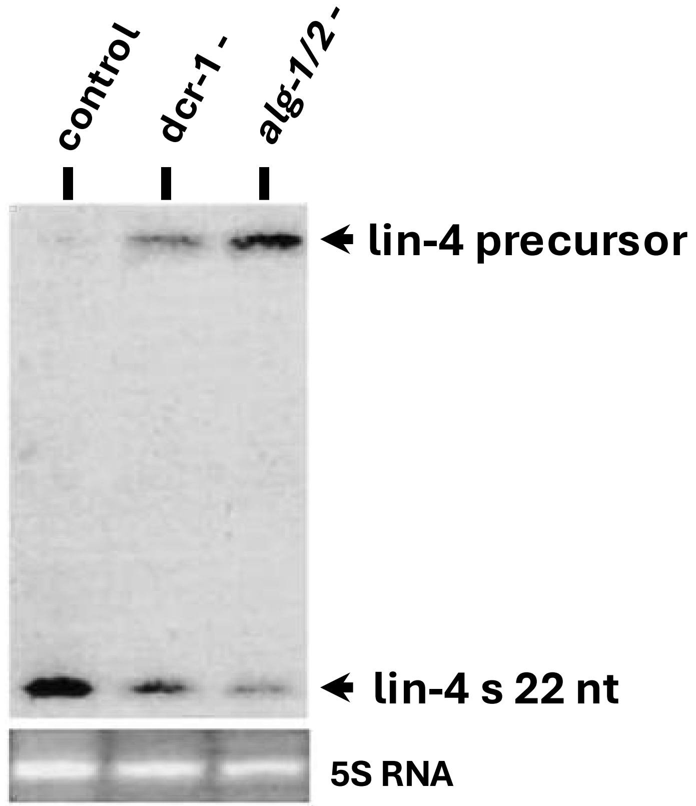
RNAi and microRNAs are mechanistically related?

RNAi ← Argonaute proteins → microRNA

Tabara etal Mello (1999) *C. elegans rde-1* is required for RNAi and encodes a conserved Argonaute family protein



Grishok et al Ruvkun Mello (2001) *C. elegans* Argonautes ALG-1 and ALG-2 are required for *lin-4* and *let-7* function



1998 – 2001 Apprehending the scope and significance of microRNAs

Consilience of microRNA and RNAi phenomena

? microRNAs: Evolutionary adaptation of RNAi for gene regulation ?

RNAi ← Argonaute proteins → microRNA

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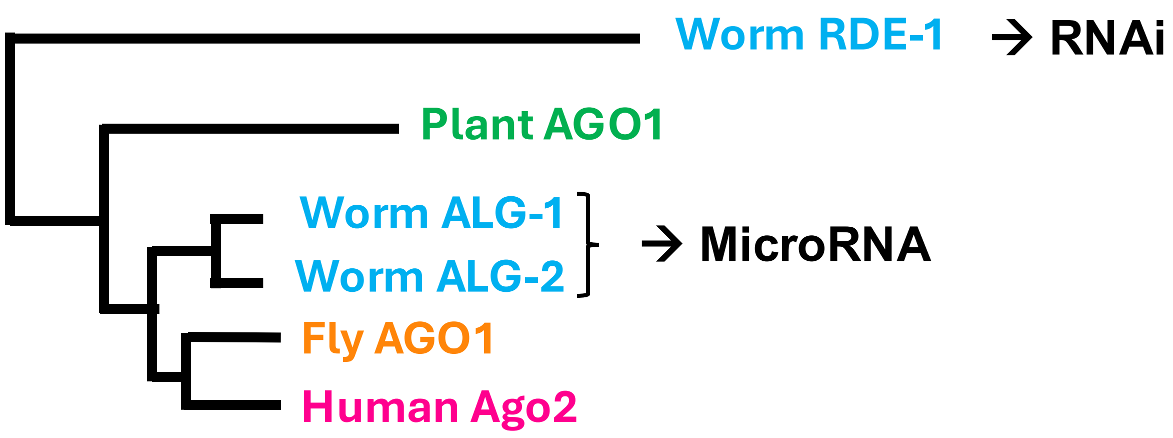

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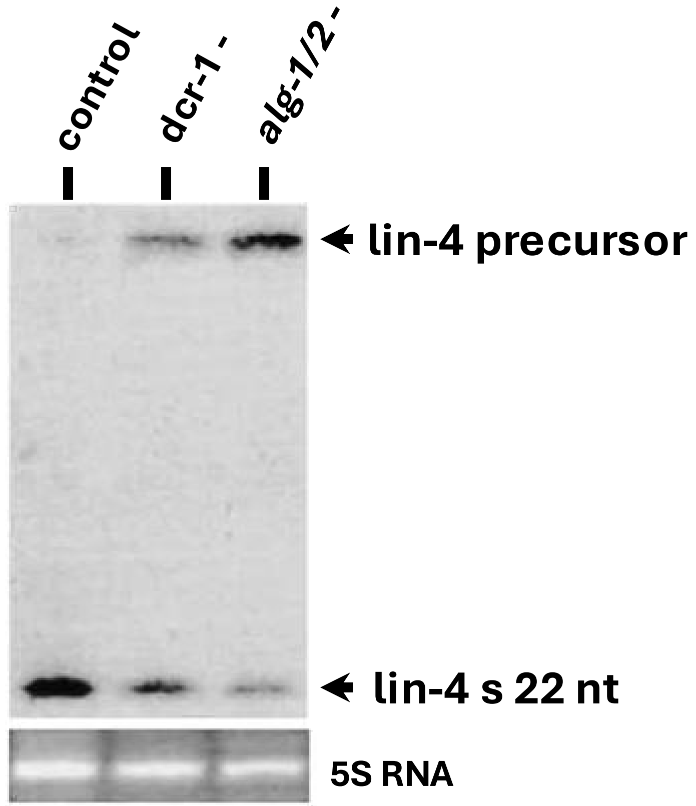
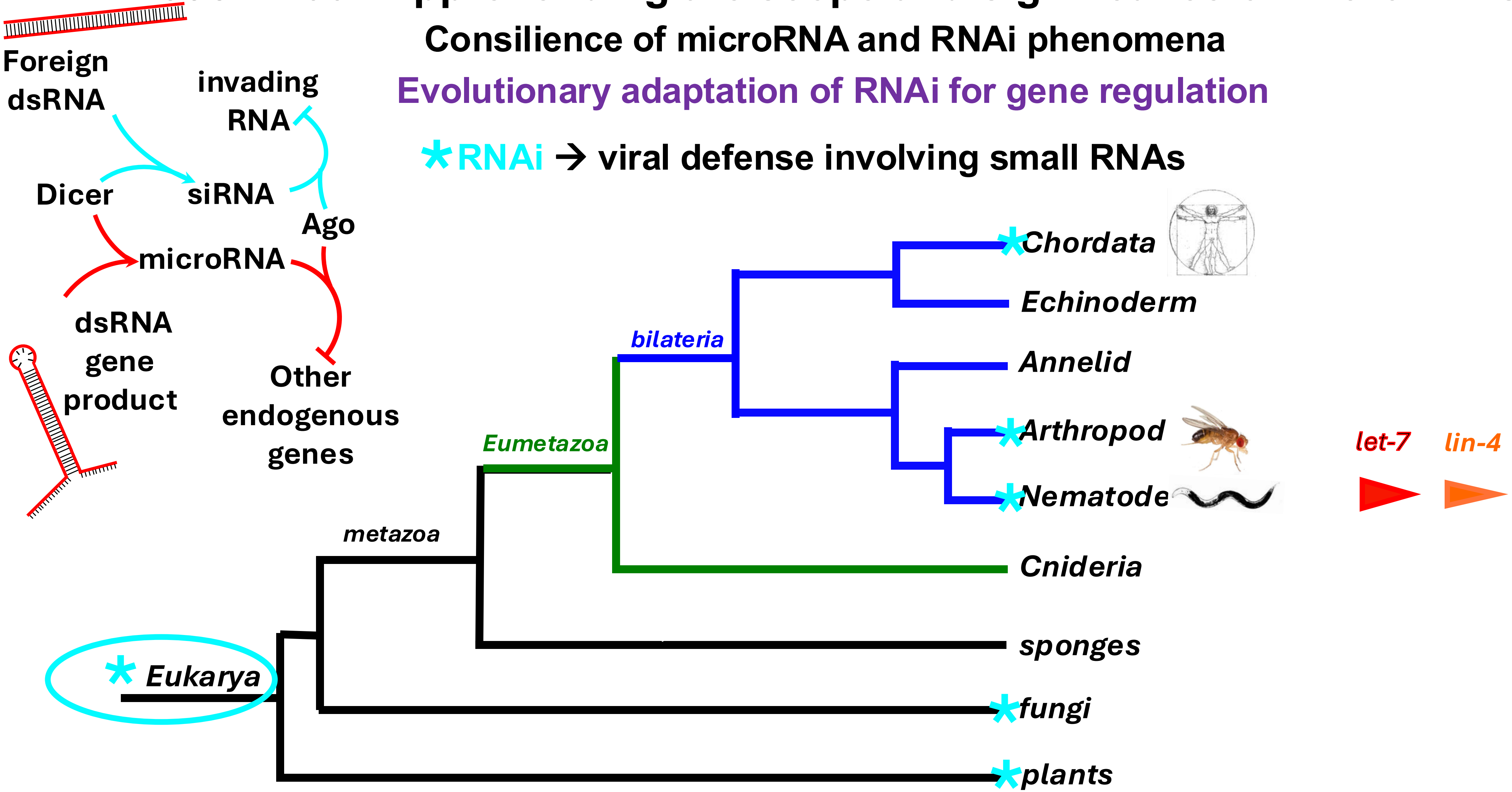


1998 – 2001 Apprehending the scope and significance of microRNAs

Consilience of microRNA and RNAi phenomena

Evolutionary adaptation of RNAi for gene regulation

*RNAi → viral defense involving small RNAs

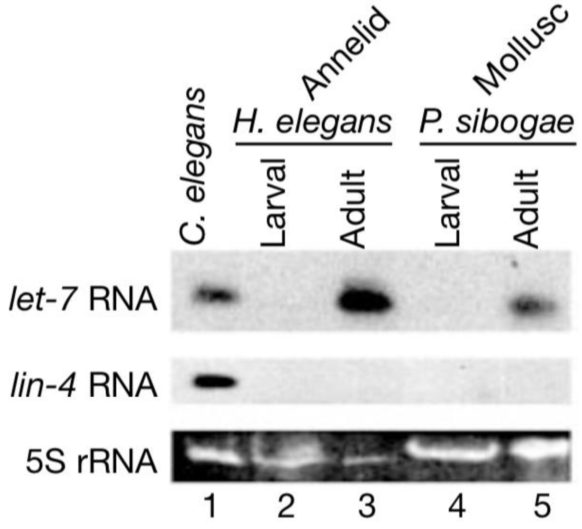


1998 – 2001 Apprehending the scope and significance of microRNAs

let-7 microRNA is ancient and derived from RNAi

let-7 microRNA is conserved across bilaterian animals!

Pasquinelli et al … Ruvkun (2000) Conservation of the sequence and temporal expression of _let-7_ heterochronic regulatory RNA. *Nature*

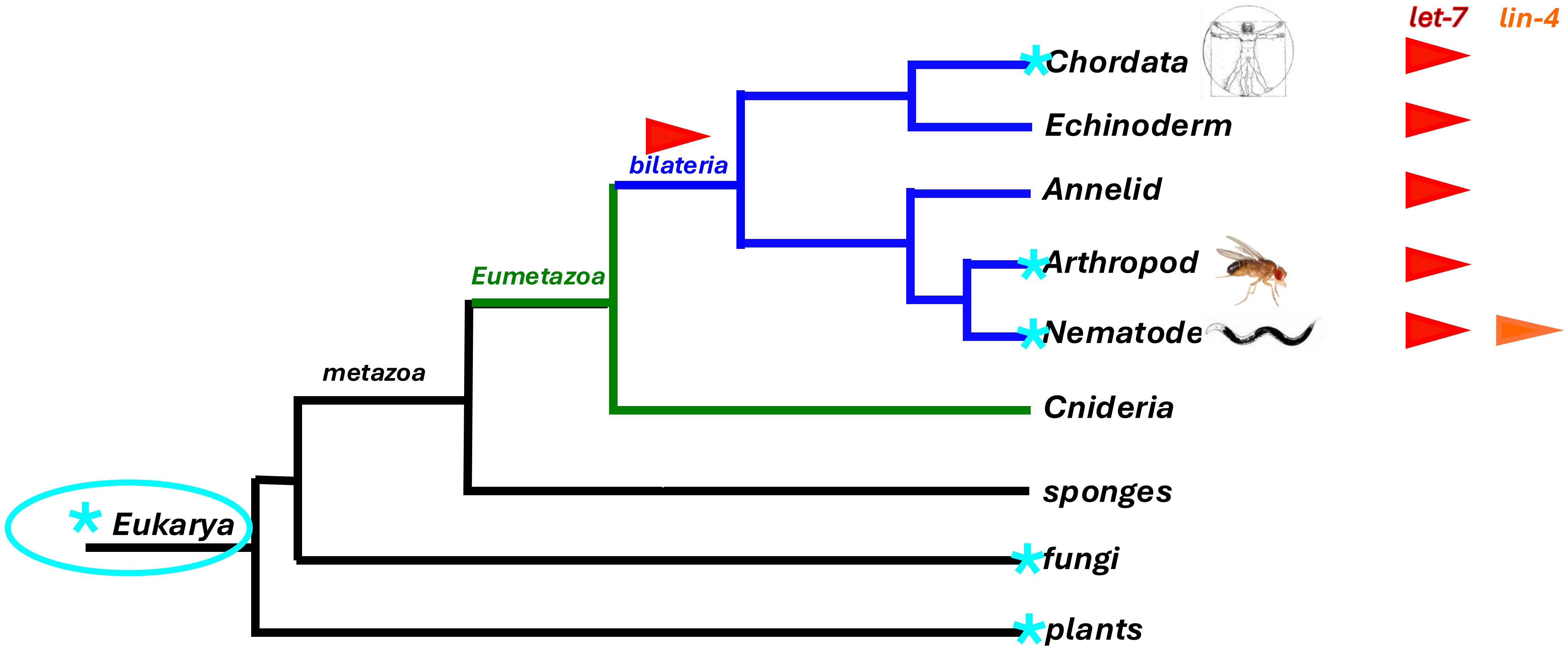



```
Fish let-7a UGAGGUAGUAGGUUGUAUAGUU
 Fly let-7a UGAGGUAGUAGGUUGUGUGGUU
Worm let-7a UGAGGUAGUAGGUUGUAUGGUU
```

There *must* be other microRNAs besides *let-7* and *lin-4*!

let-7 microRNA is ancient and derived from RNAi

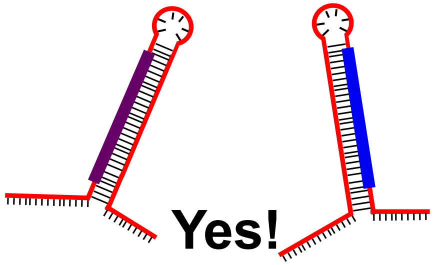
let-7 microRNA is conserved across bilaterian animals!



There ***must*** be other microRNAs besides *let-7* and *lin-4*!

circa 2000 …
Cloning and sequencing small RNAs
(Rosalind and Victor)

C. elegans **RNA** → **~ 22 nt RNA** → **~ 22 nt cDNA** → **Library of clones** → **~22 nt sequences** → **Match to genome**



Yes! We found them

We should have realized ….
There must be **other labs doing this!**

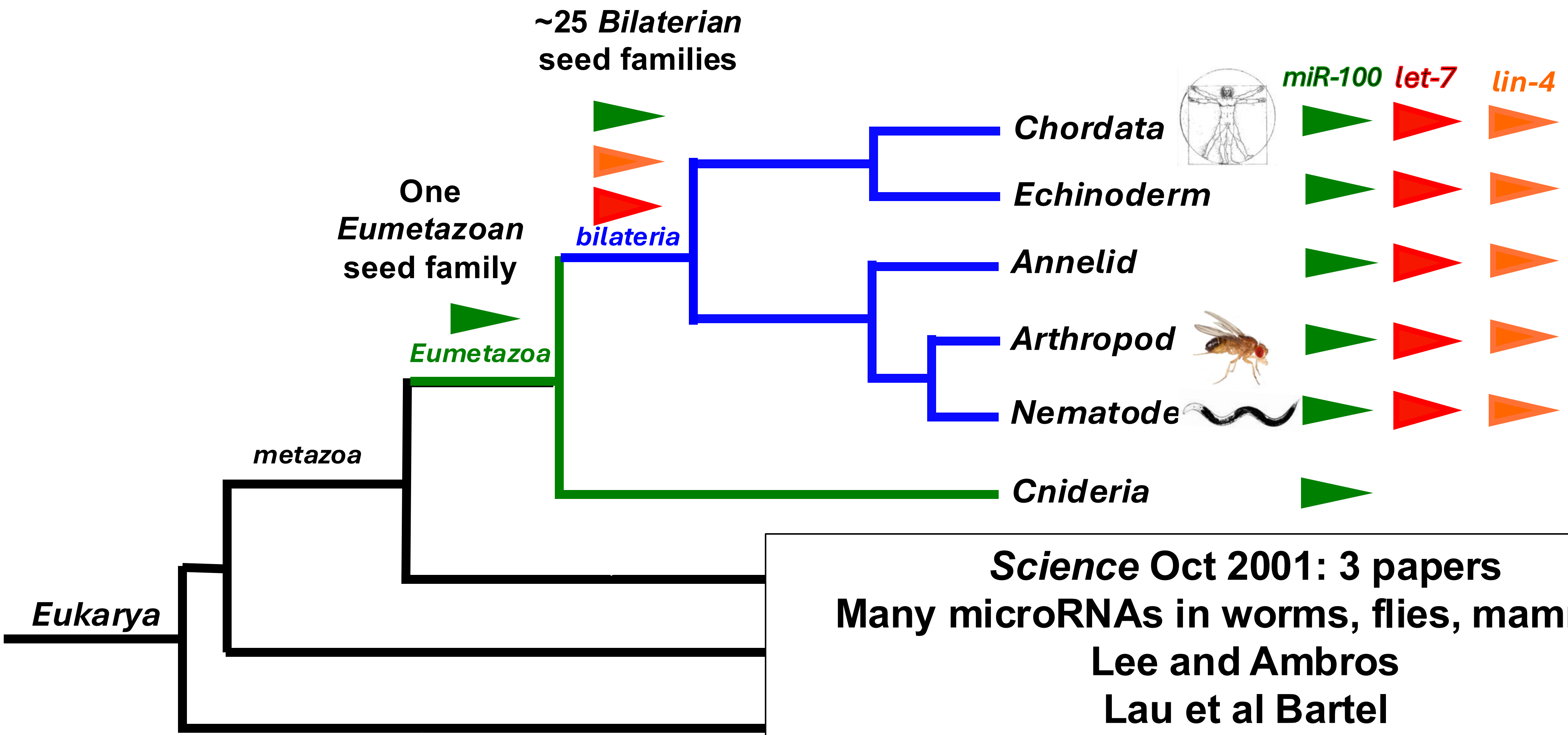
By the skin of our teeth →

Science **Oct 2001: 3 papers**
Many microRNAs in worms, flies, mammals
Lee and Ambros
Lau et al Bartel
Lagos-Quintana et al Tuschl

MicroRNAs across Eukaryotes

```
Hsa-mir-100 AACCCGUAGAUCCGAACUUGUG
Cel-mir-52  CACCCGUACAUAUGUUUCCGUGCU
```

'Seed' nt 2-8



Science **Oct 2001: 3 papers**

Many microRNAs in worms, flies, mammals

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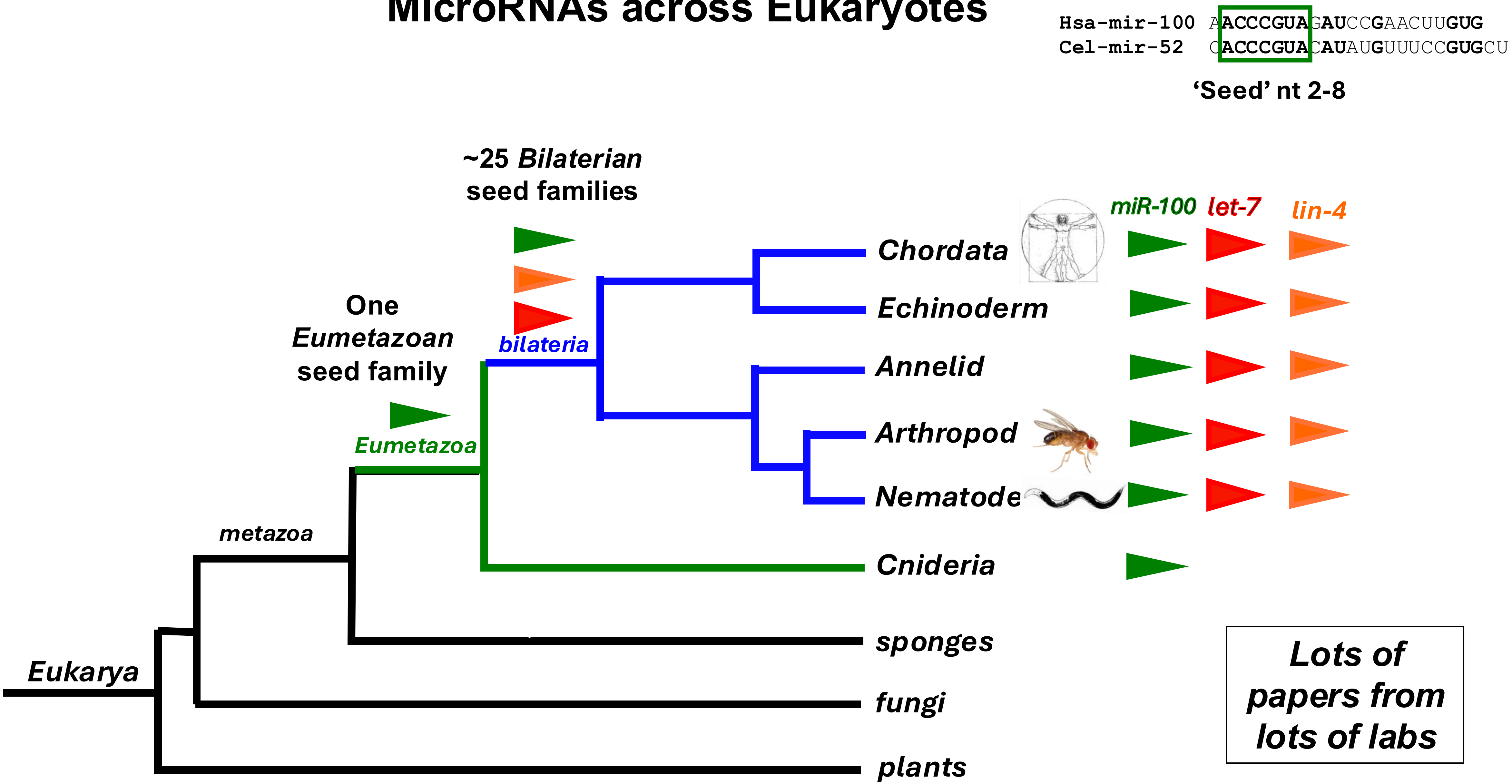
MicroRNAs across Eukaryotes

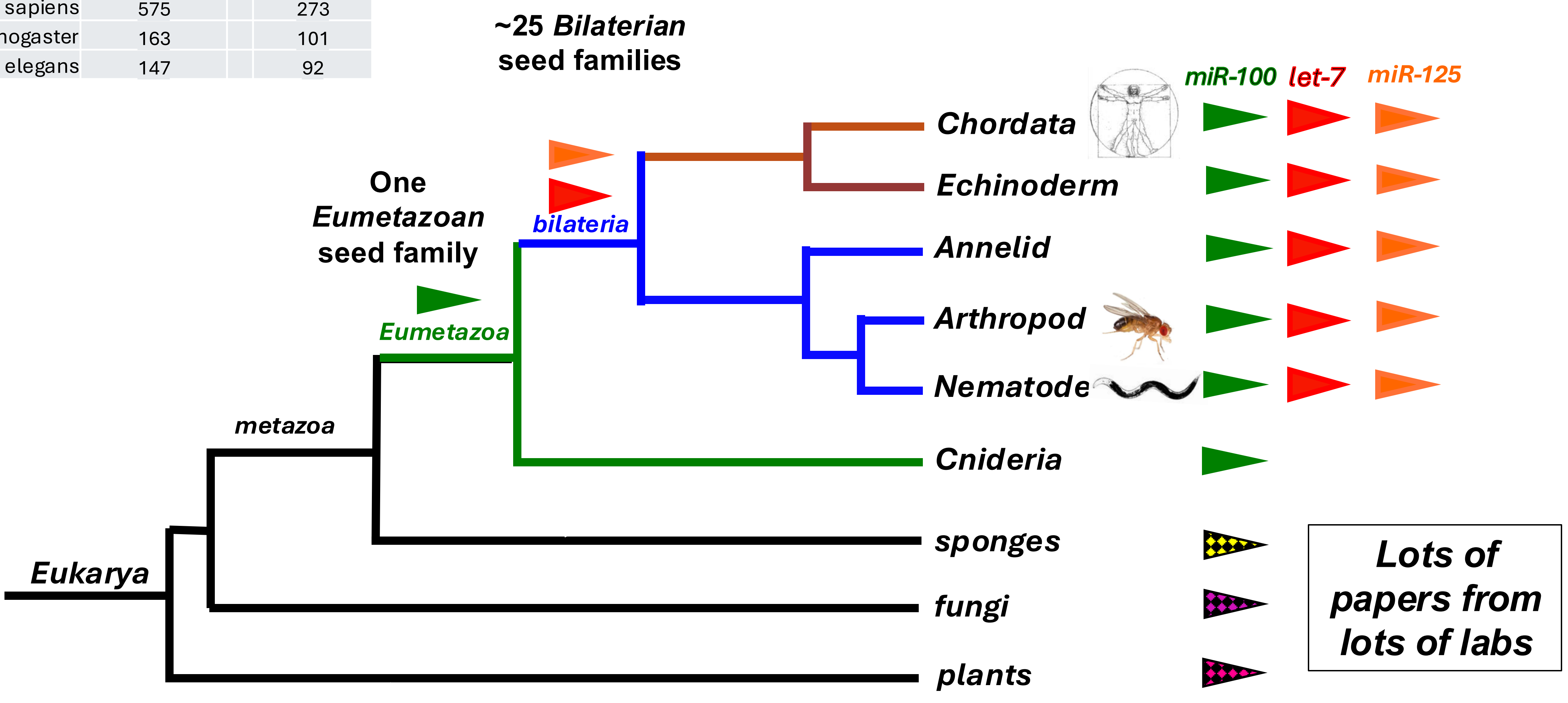


MicroRNAs across Eukaryotes

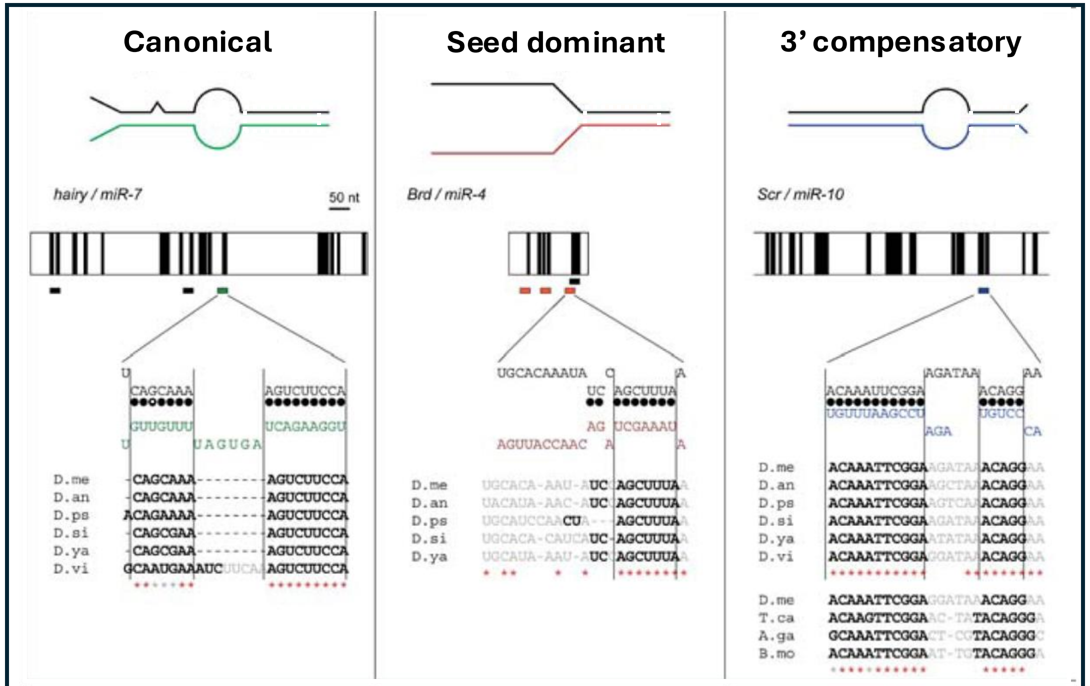
```
Hsa-mir-100  AACCCGUAGAUCCGAACUUGUG
Cel-mir-52   CACCCGUACAUAUGUUUCCGUGCU
```

'Seed' nt 2-8

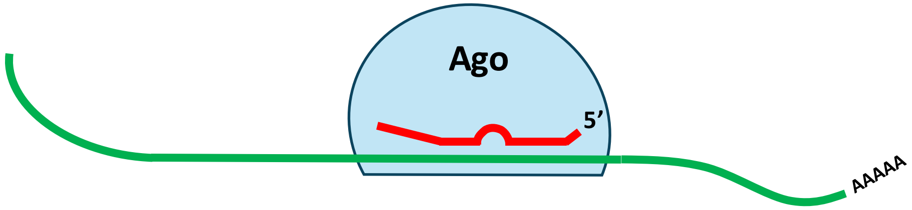
	microRNA loci		seed families
H. sapiens	575		273
D. melanogaster	163		101
C. elegans	147		92



How do microRNAs recognize their mRNA targets?



Brennecke et al Cohen (2005)

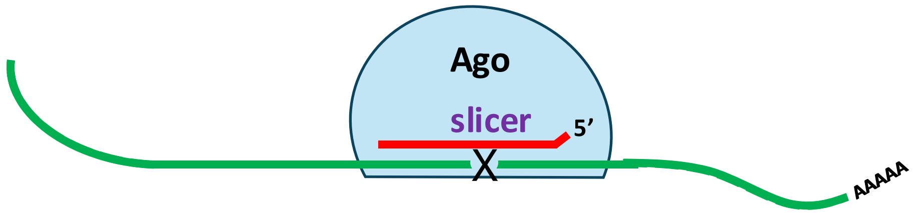


Partial complementarity – seed match (+)

How do microRNAs repress their mRNA targets?

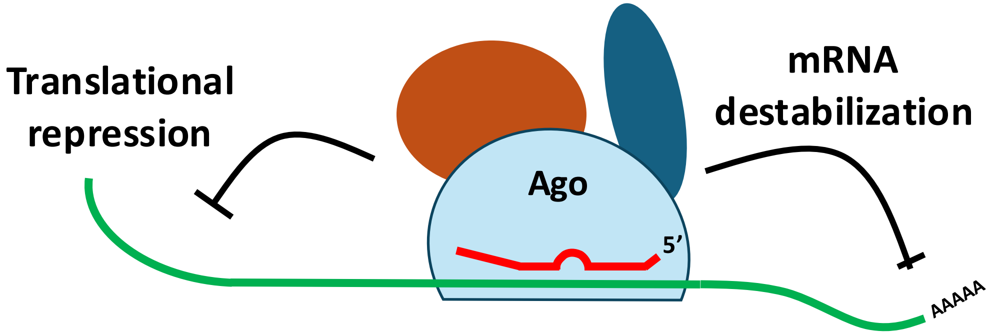
In animals, microRNAs generally do not ***slice*** their mRNA targets

RNAi:



Full complementarity → mRNA is destroyed

MicroRNAs:

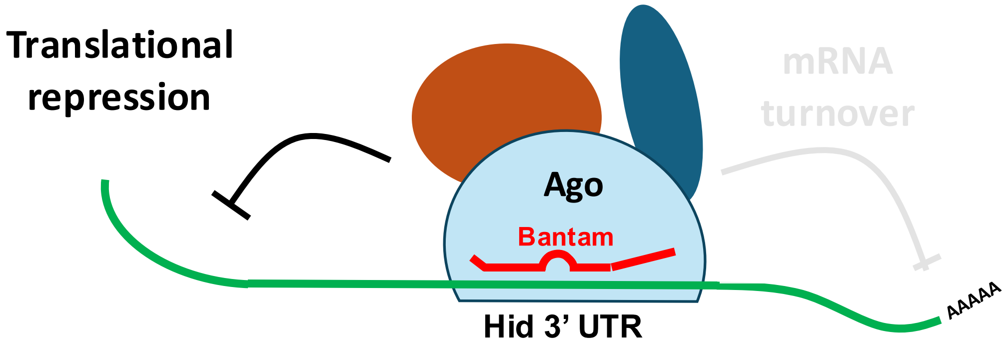


Partial complementarity – seed match (+) : No Slicing

Programmable repression by other means:

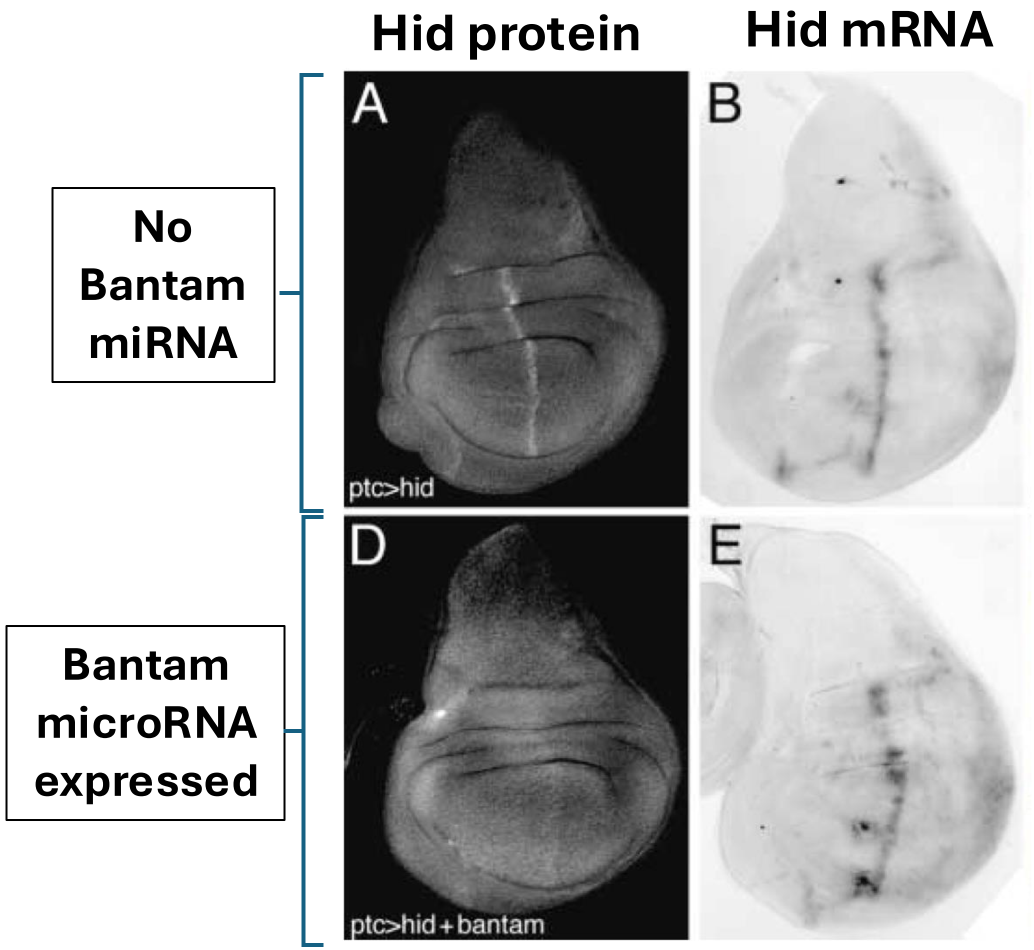
Translational repression without mRNA target destabilization

Bantam ---| Hid → apoptosis



Programmable repression by other means

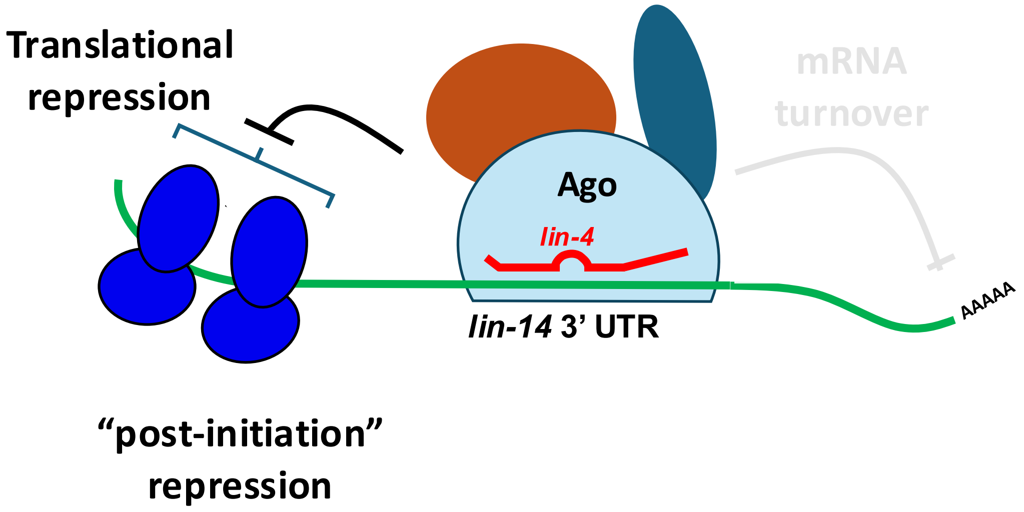
Brennecke et al Cohen (2003)

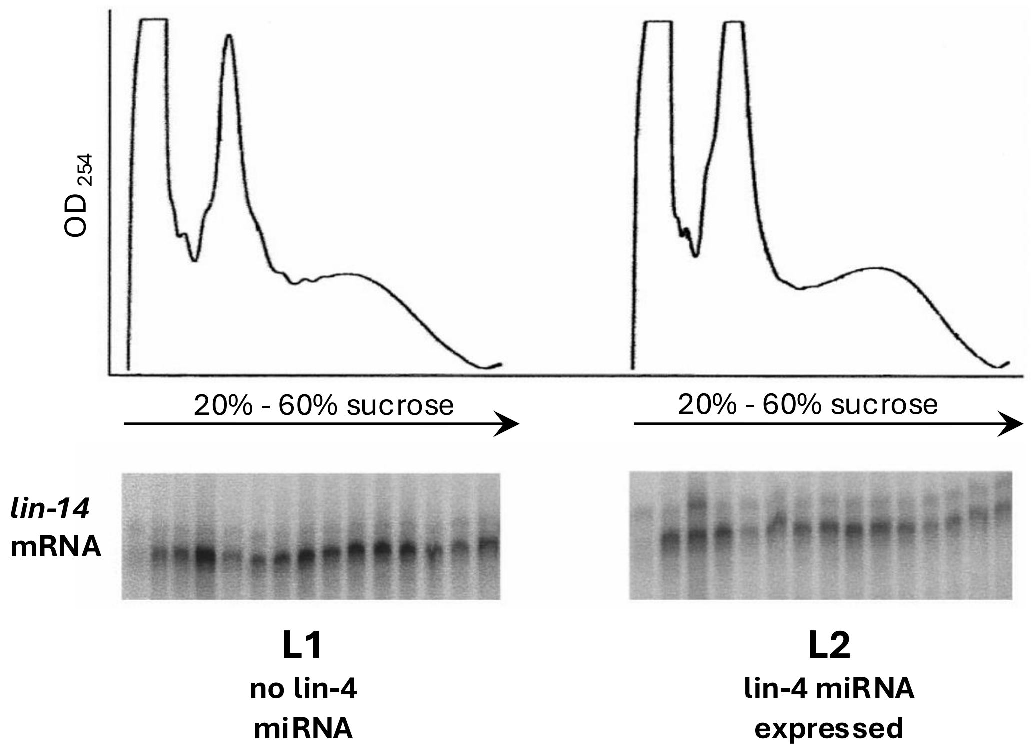


Translational repression without mRNA target destabilization

lin-4 ---| LIN-14 → early cell fates

Philip H. Olsen and Victor Ambros (1999)
Developmental Biology 216





Phil's conlusions supported and refined by:

Stadler et al Fire (2012) Contributions of mRNA abundance, ribosome loading, and post- or peri-translational effects to temporal repression of C. elegans heterochronic miRNA targets. *Genome Research* 12

Identification of microRNA -- target regulatory circuits

Computational searches for seed matches in annotated mRNAs

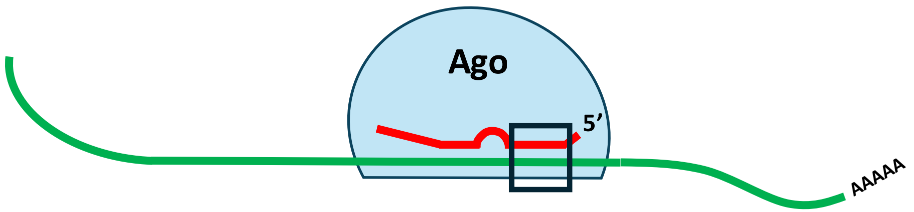
In many animals each microRNA seed can match <u>hundreds of genes</u>

C. elegans let-7	→	201 sites	in	134 genes
D. melanogaster let-7	→	113 sites	in	94 genes
H. sapiens let-7	→	5479 sites	in	4140 genes

Targetscan.org

↑

Search in 3' UTR sequences for complementarity to nt 2-8 of the microRNA



Partial complementarity -- seed match (+)

Evolutionary patterns of of miRNA--target interactions

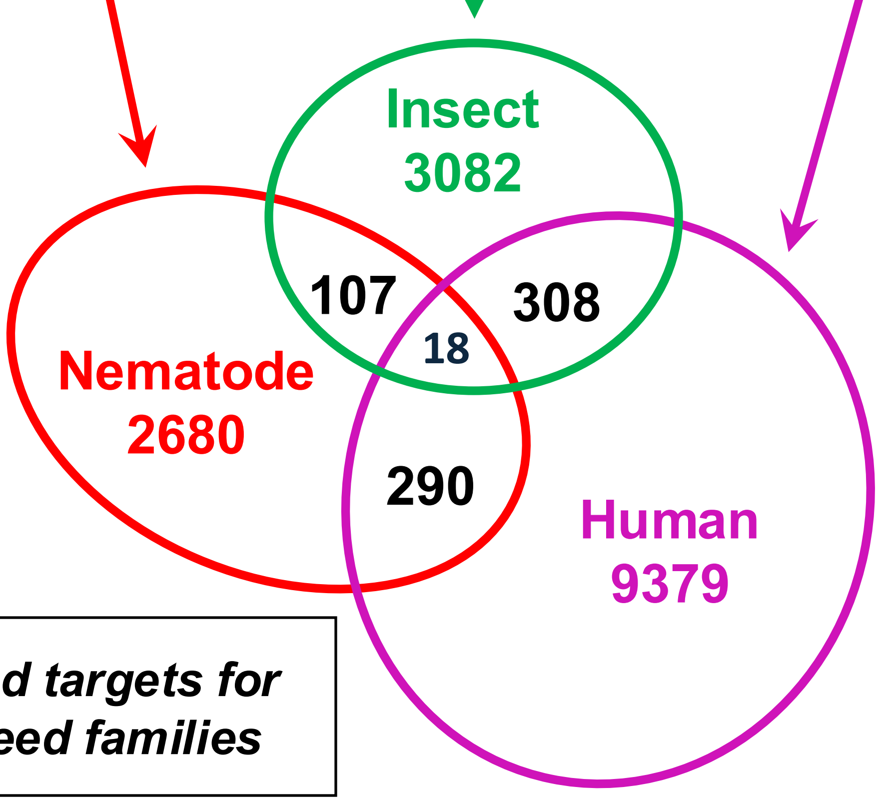
Caenorhabditis elegans

Drosophila melanogaster

Homo sapiens

20 conserved microRNA seed families

>5000 orthologous protein coding genes



Predicted targets for all 20 seed families

From Chen and Rajewsky, 2006

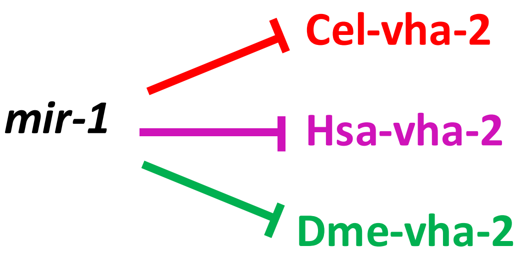
Substantial Evolutionary Fluidity in microRNA targeting

(which microRNAs regulate which genes)

Thought to reflect roles for *loss and gain of microRNA target sites* as evolutionarily adaptive

Also, certain instances of

Deep conservation of targeting

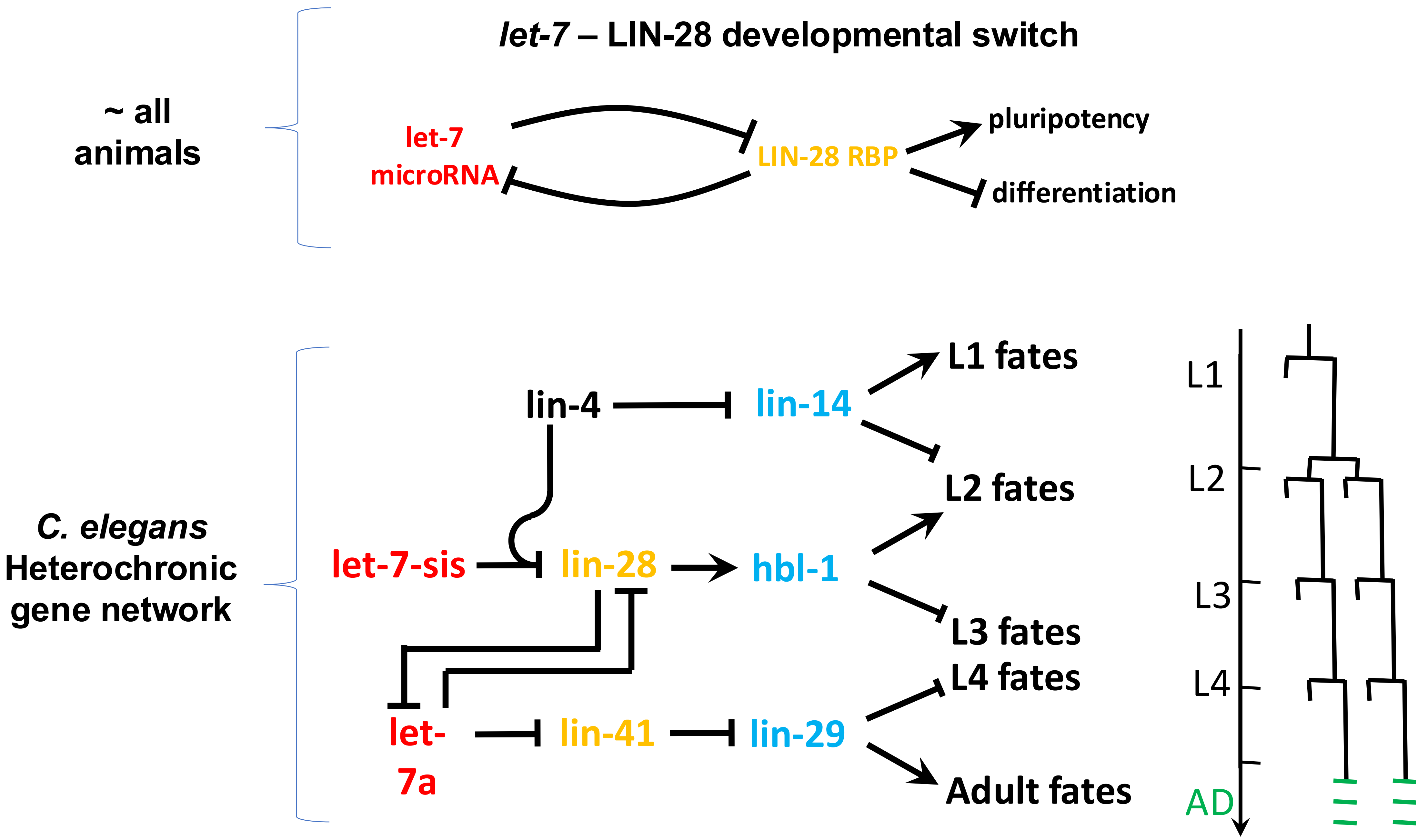


Chen and Rajewsky, 2006

Evolutionarily conserved microRNA -- target regulatory circuits

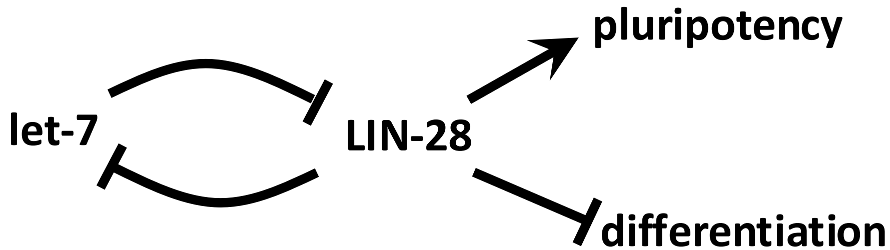
let-7 – LIN-28 developmental switch

Evolutionarily conserved microRNA -- target regulatory circuits



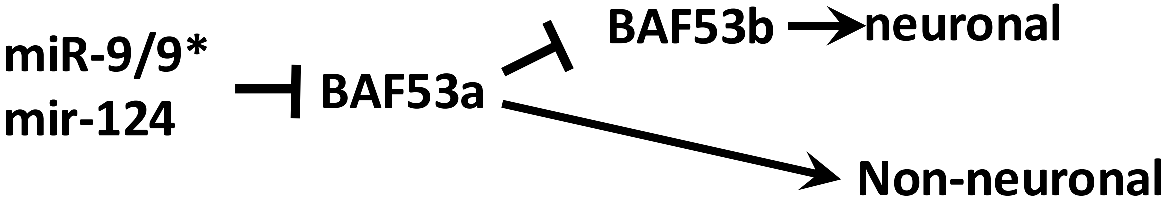
Developmental and Physiological roles of microRNAs in animals

Developmental switches



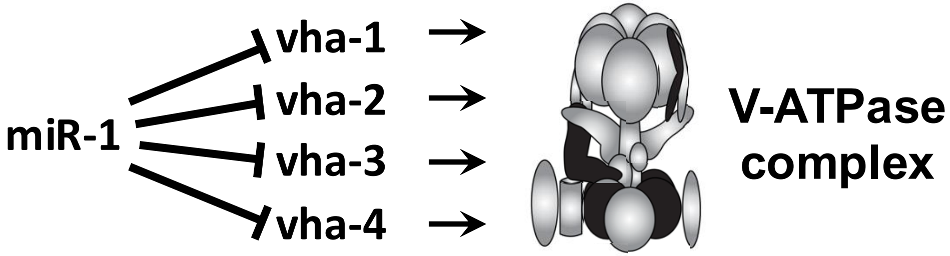
Tsialikas & Romer-Siebert (2015) *Development*

Reinforcing (even reprogramming) cell type



Tang, Yoo, Crabtree (013) *Curr Opin Genet Dev*.

Coordinating components of functional modules



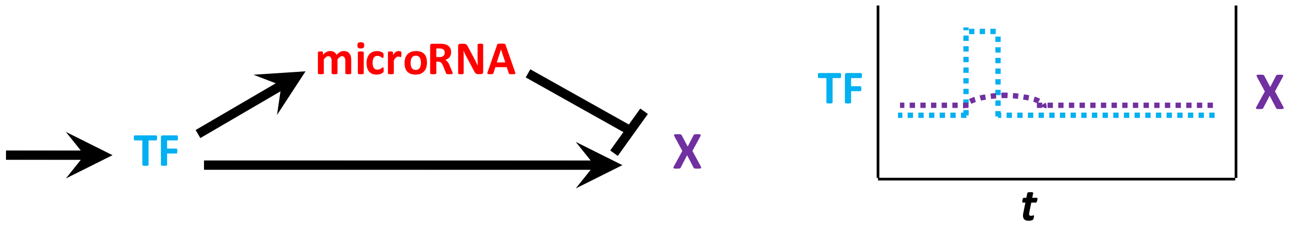
Gutiérrez-Pérez et al, Cochella (2021) *Sci Anv*

Buffering GRN noise

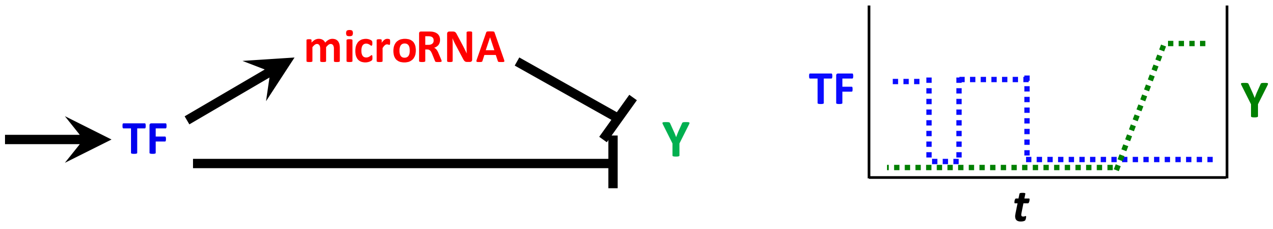
Klein et al Goodman (2007) *Nat Neuro*

Developmental and Physiological roles of microRNAs in animals

Cassidy et al, Carthew (2019) *Cell*



Martinez et al, Walhout (2008) *Genes Dev*.



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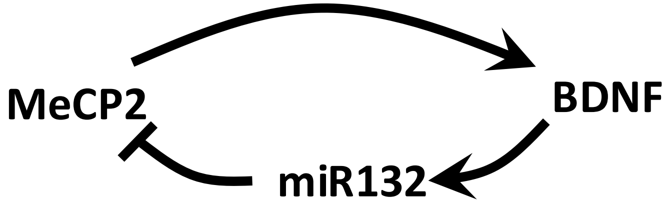

Klein et al Goodman (2007) *Nat Neuro*

Developmental and Physiological roles of microRNAs in animals

What happens to a fish embryo without microRNAs?

It makes a fish!

24 hours **96 hours**

WT

Dicer mutant

Loss of complexity **Loss of robustness**

Giraldez et al, Schier (2005) *Science*

microRNAs: What are they good for?

Promote developmental cell type complexity

Confer physiological and developmental robustness

24 hours **96 hours**

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Giraldez et al, Schier (2005) *Science*

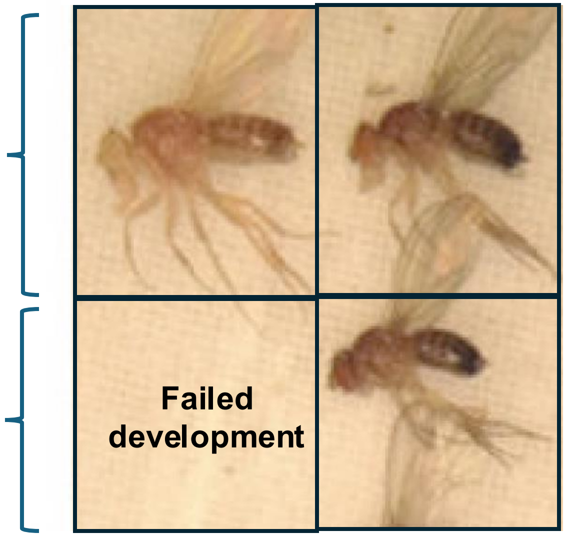
microRNAs: What are they good for?

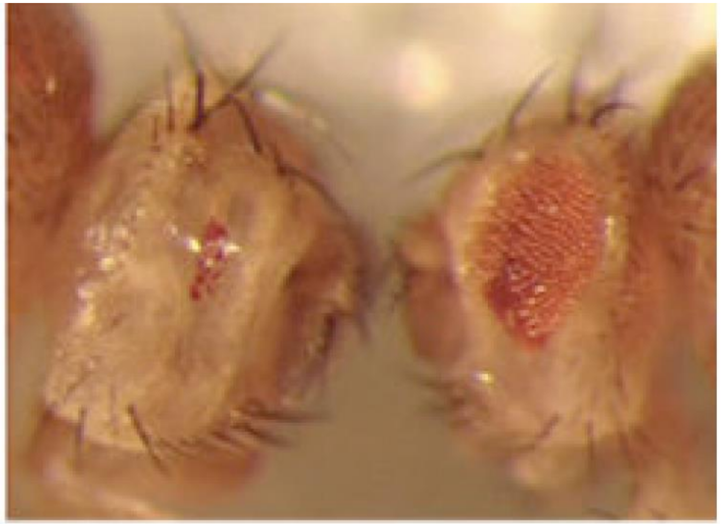
Flies without microRNAs

Promote developmental cell type complexity

Confer physiological and developmental robustness

Enable rapid high-energy growth and development





Cassidy et al, Carthew (2019) *Cell*

MicroRNAs promote organismal complexity and stress-resiliant living

Promote developmental cell type complexity

Confer physiological and developmental robustness

Enable rapid high-energy growth and development

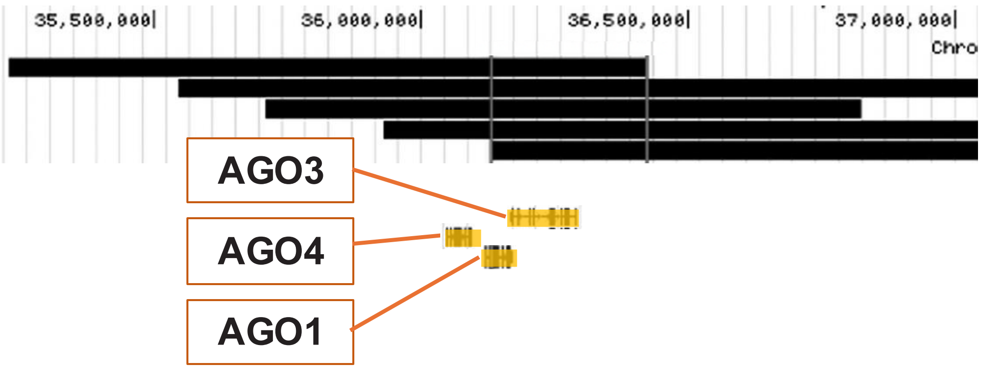
MicroRNAs in Human Disease

Stress Resilience ←→ Disease

Human Argonaute mutations that cause Neurodevelopmental Disorder (NDD)

Tokita et al (2015) Deletions of the AGO1/AGO3/AGO4 gene cluster in NDD patients

Broad disruptions of microRNA regulation by impairing multiple Argonaute proteins



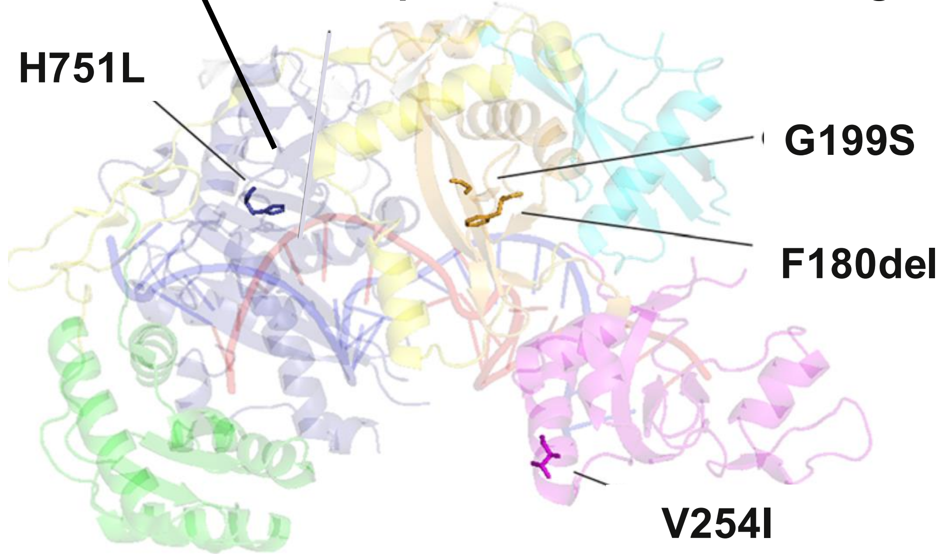
Deletion of <u>multiple microRNA Argonaute genes</u> can cause Neurodevelopmental Disorder (NDD)

Schalk et al. (2021) Human AGO1 variants in NDD

Lessel et al. (2020) Human AGO2 variants in NDD

S750F 'antimorphic' mutation of *C. elegans* ALG-1 Argonaute

Could the single amino acid variants somehow broadly disrupt microRNA regulation?



A *<u>single amino acid change</u>* in *<u>just one microRNA Argonaute</u>* can cause Neurodevelopmental Disorder (NDD)

Zinovyeva etal Ambros (2014) Mutations in conserved residues of *C. elegans* microRNA Argonaute ALG-1

Argonaute Syndrome

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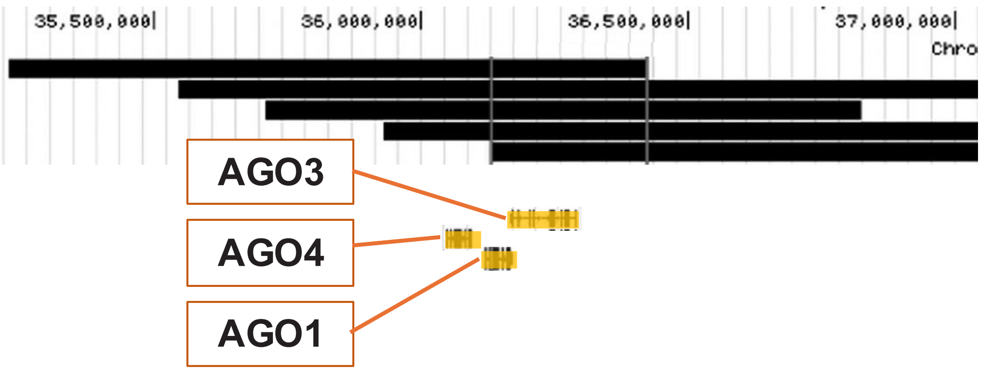

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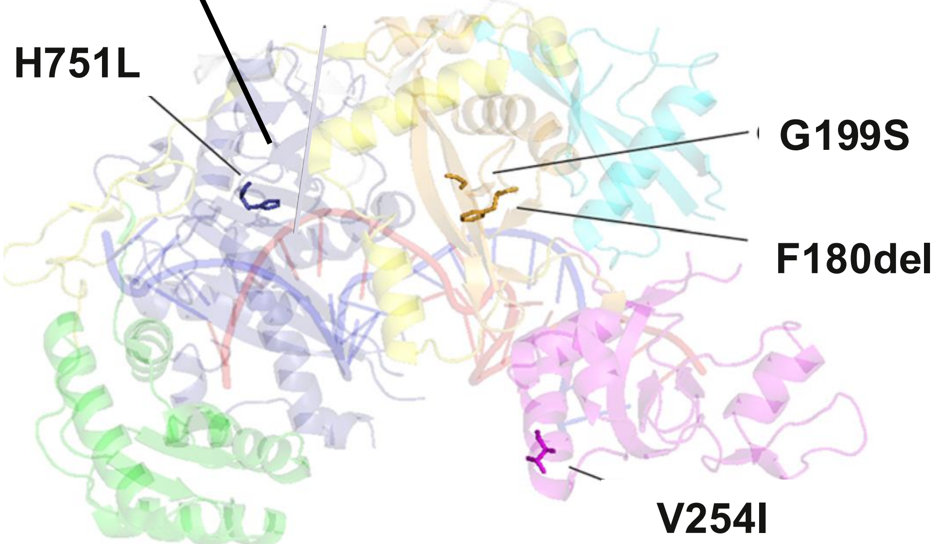

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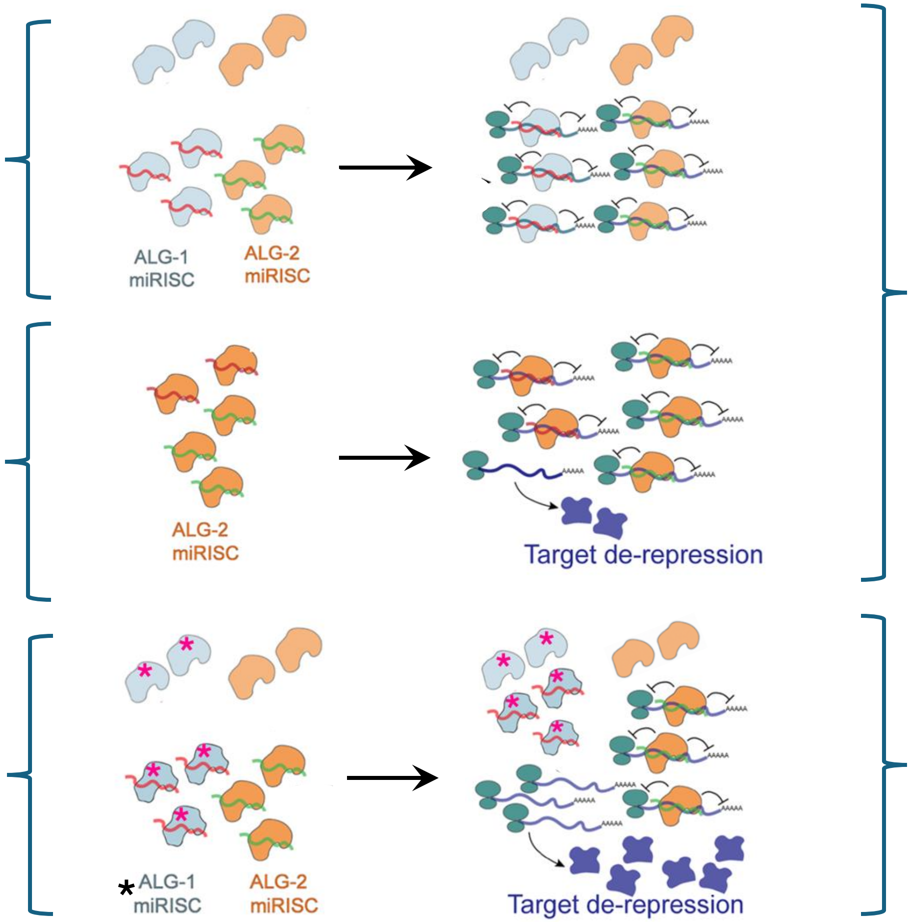
C. elegans ALG-1 ‘antimorphic’ mutations are <u>*worse*</u> than absence of ALG-1

Wild type

alg-1(0)
alg-2(+)

alg-1(anti)
alg-2(+)



redundancy between ALG-1 and ALG-2

disfunctional ALG-1(anti) poisons redundancy of ALG-2

Nematodes have 2 microRNA Argonautes ALG-1 and ALG-2

Zinovyeva etal Ambros (2014) Mutations in conserved residues of *C. elegans* microRNA Argonaute ALG-1

Modeling human microRNA Argonaute NDD mutations in *C. elegans* ALG-1

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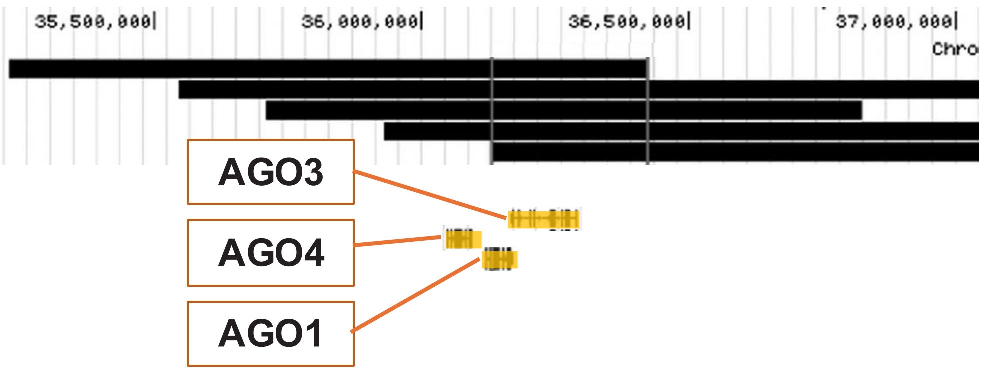
Broad disruptions of microRNA regulation



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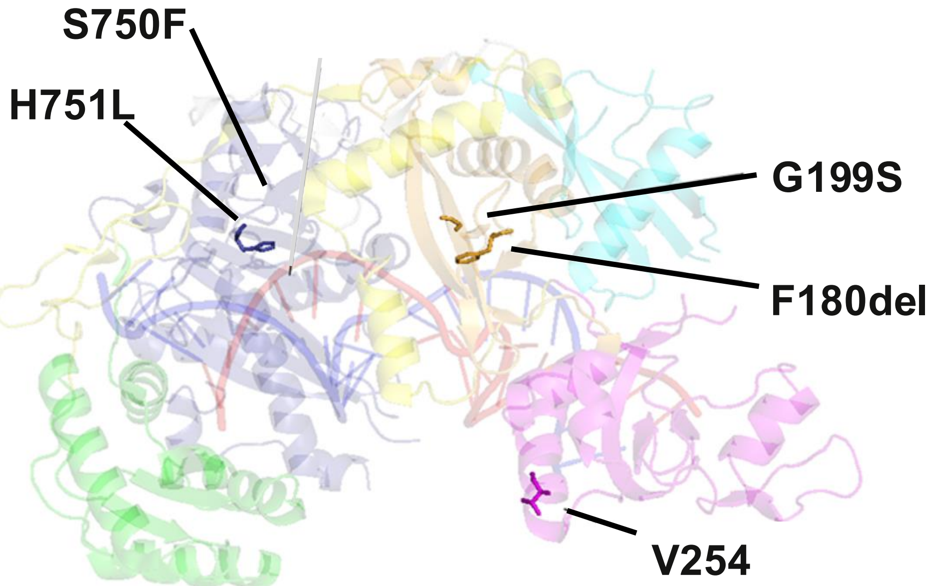
Schalk et al. (2021) Human AGO1 variants in NDD

Lessel et al. (2020) Human AGO2 variants in NDD

Argonaute Syndrome

Could the mutant protein ‘poison’ the redundancy of other human Argonautes?



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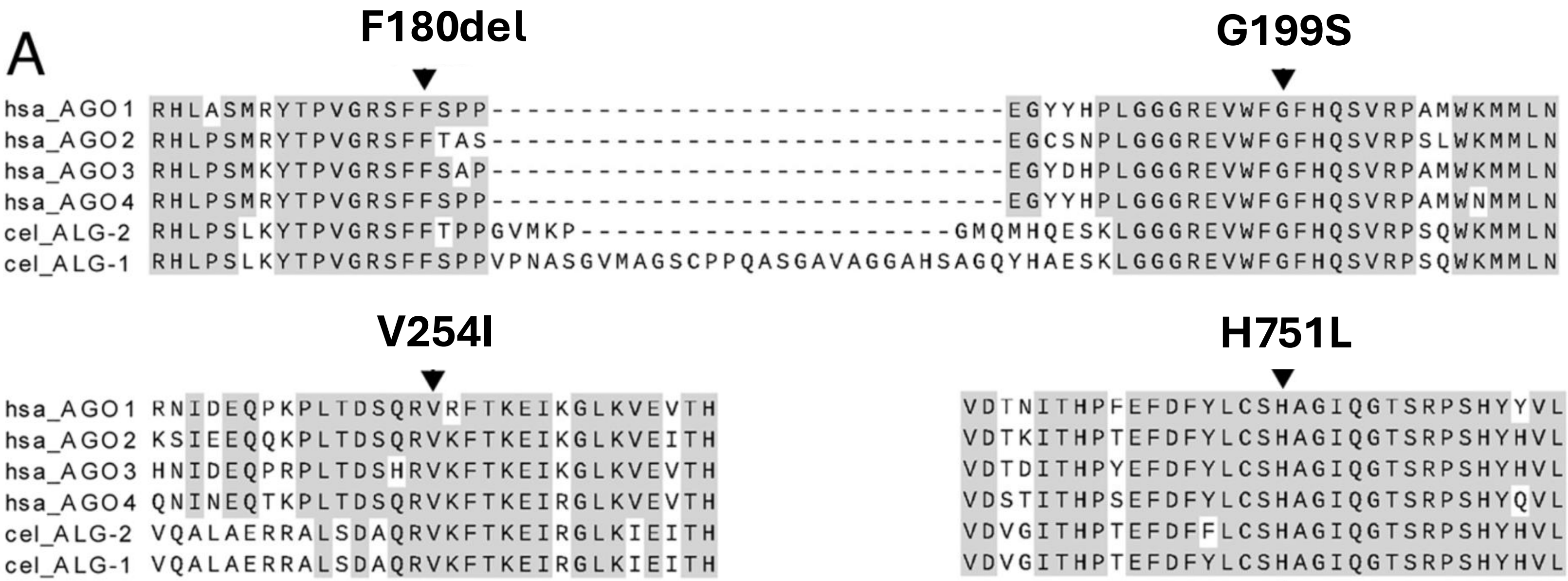
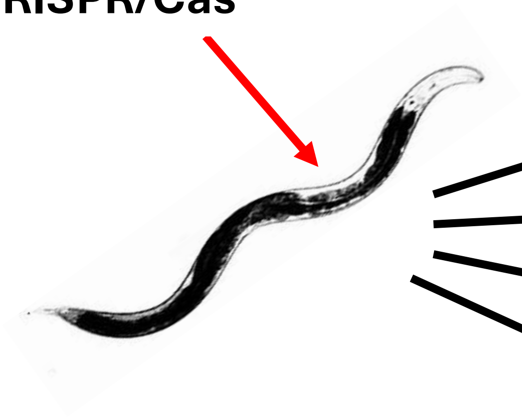
Duan, Li, Panzade, Piton, Zinovyeva, Ambros (2024) *PNAS*



CRISPR/Cas



- *alg-1(H751L)* → antimorphic
- *alg-1(F180del)* → antimorphic
- *alg-1(G199S)* → mild lf
- *alg-1(V254I)* → weak lf

Developmental phenotypes
Heterochronic; viability

Molecular phenotypes
microRNAs; mRNAs

Duan et al 2024

Silverman Endowment

Amelie Piton IGMCB, Strasbourg University
Ann Zinovyeva Kansas State University

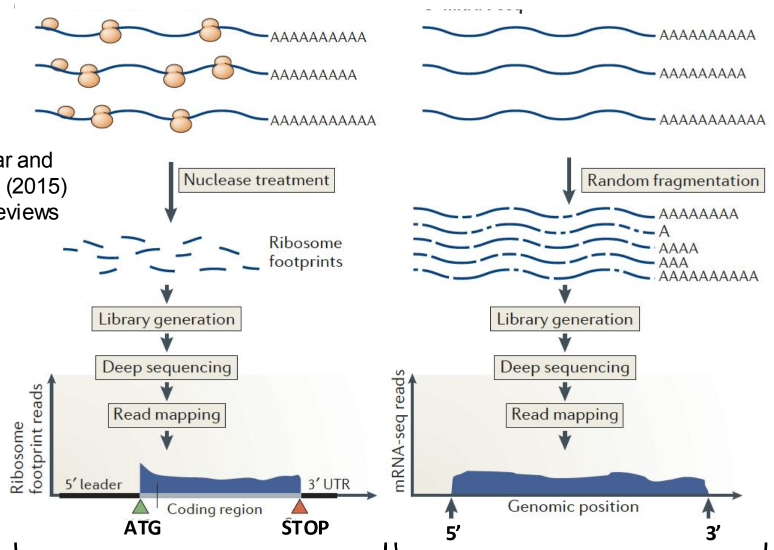
Molecular phenotypes of *C. elegans alg-1(NDD)* mutants

mRNAs ← → microRNAs → Small RNAseq

mRNAs → Ribosome Profiling, mRNAseq

Ribosome Profiling | **mRNAseq**

From Brar and Weissman (2015) Nature Reviews



Genome-wide 'translatome' ← **Ribosome-Protected Fragments (RPF)**

mRNA abundance (mRNAseq) → **Genome-wide transcript abundances**

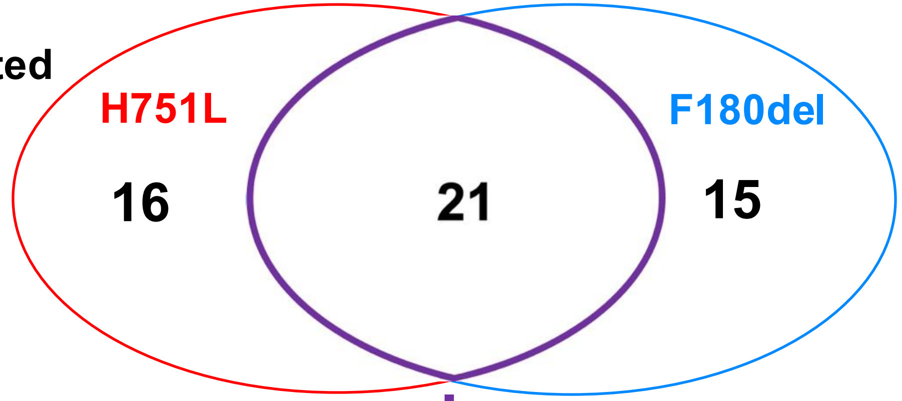
For each mRNA

translational efficiency (TE) $= \frac{\text{RPF}}{\text{mRNAseq}}$

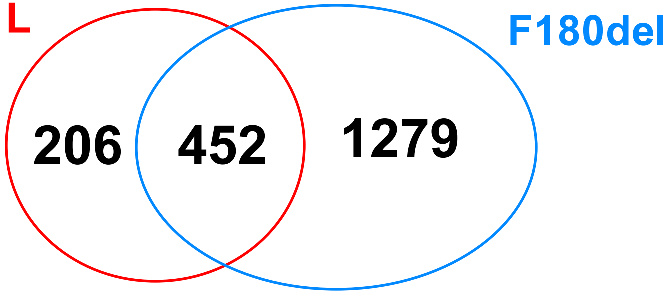
Duan et al 2024

ALG-1(NDD) mutations can have variant-specific downstream consequences

Distinct sets of functionally disrupted microRNAs



Distinct translatome disruptions



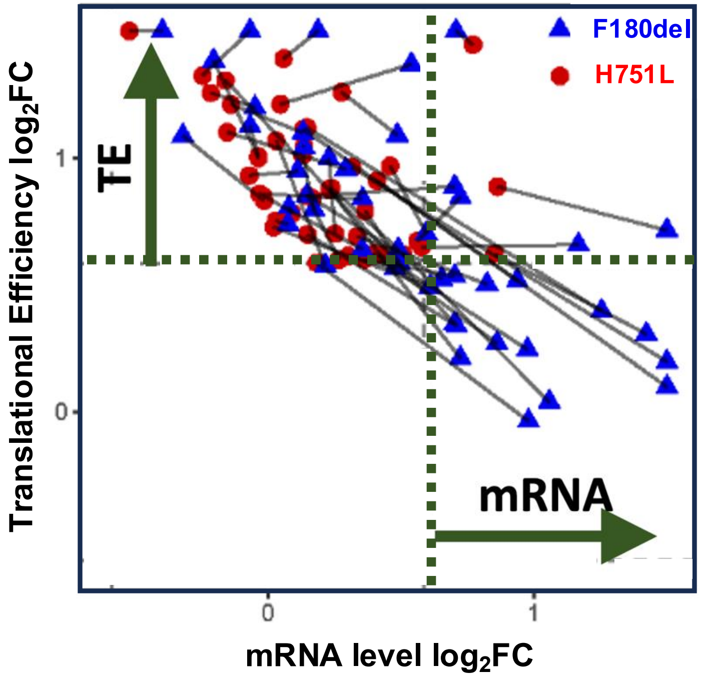
ALL mRNAs disrupted >2-fold

21 miRNAs disabled by both mutations

Seed match mRNA targets that are de-repressed in both mutants

Modes of de-repression?
Translational Efficiency (TE) and/or mRNA stability

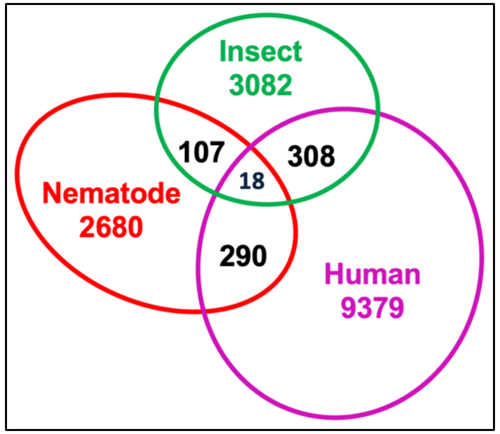
Distinct impairments of mRNA translational repression vs stability

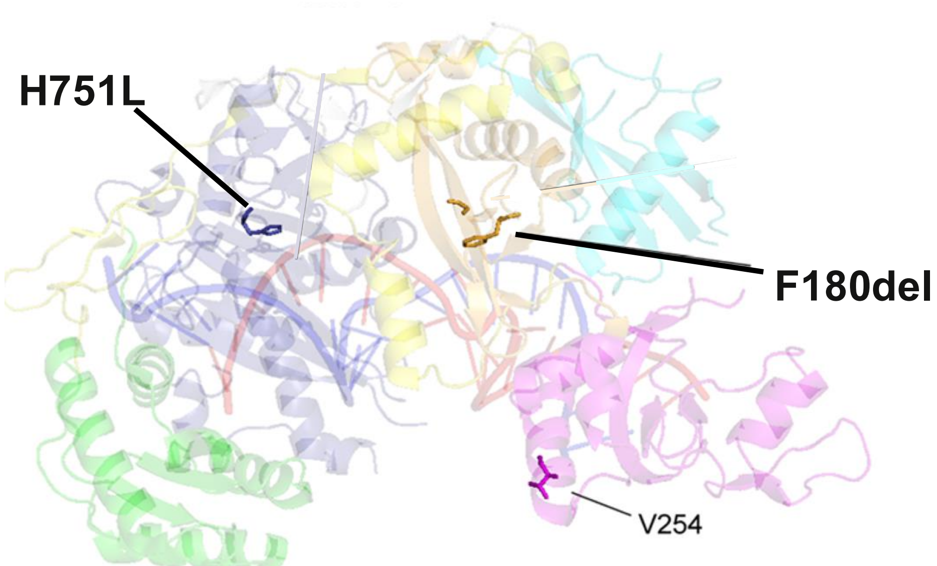


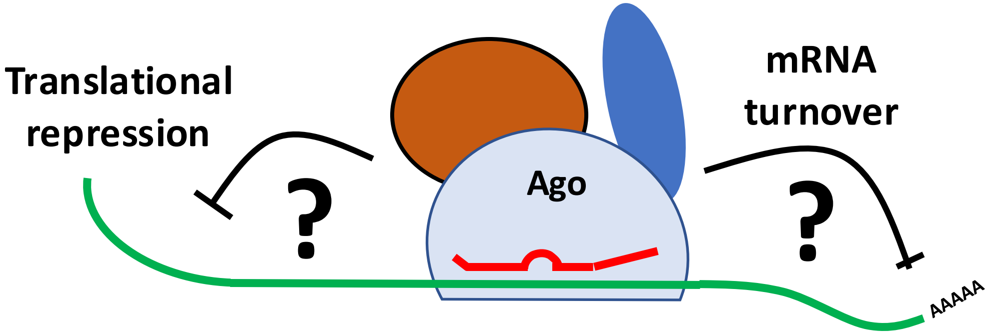
Duan et al 2024

ALG-1(NDD) mutations with distinct variant-specific downstream consequences

	microRNA loci	seed families
H. sapiens	575	273
D. melanogaster	163	101
C. elegans	147	92

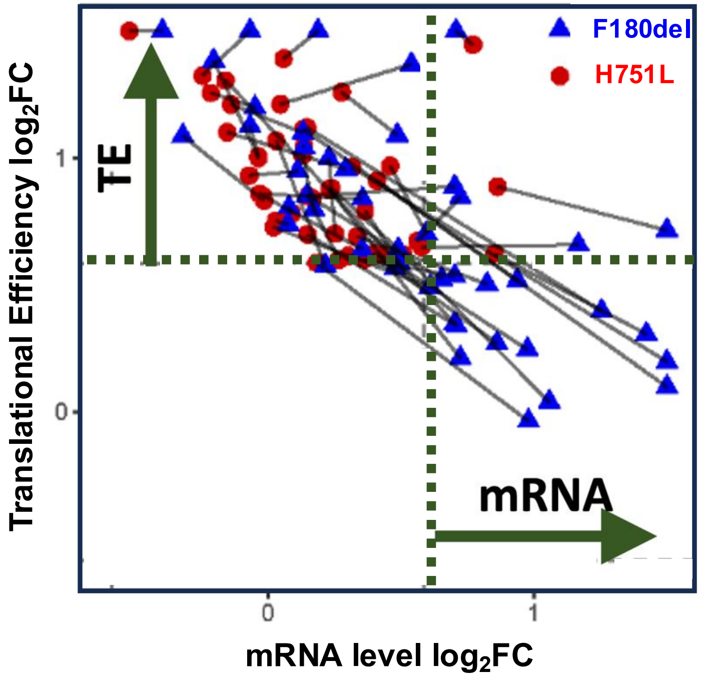






Structural and biochemical basis for functional differences amongst variants

Distinct impairments of mRNA translational repression vs stability



Duan et al 2024

Synergism: Model Organism Genetics ←→ Human Genetics

Basic Science Opportunities: Novel avenues of investigation of microRNA Argonaute mechanisms

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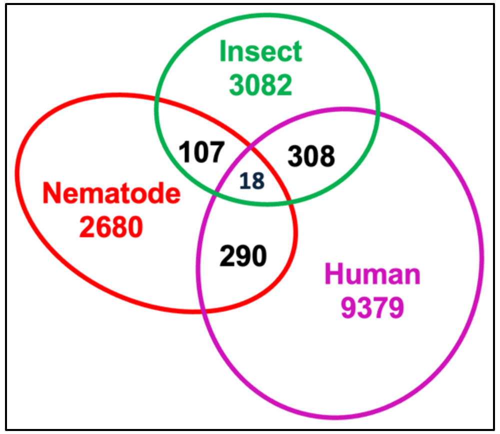

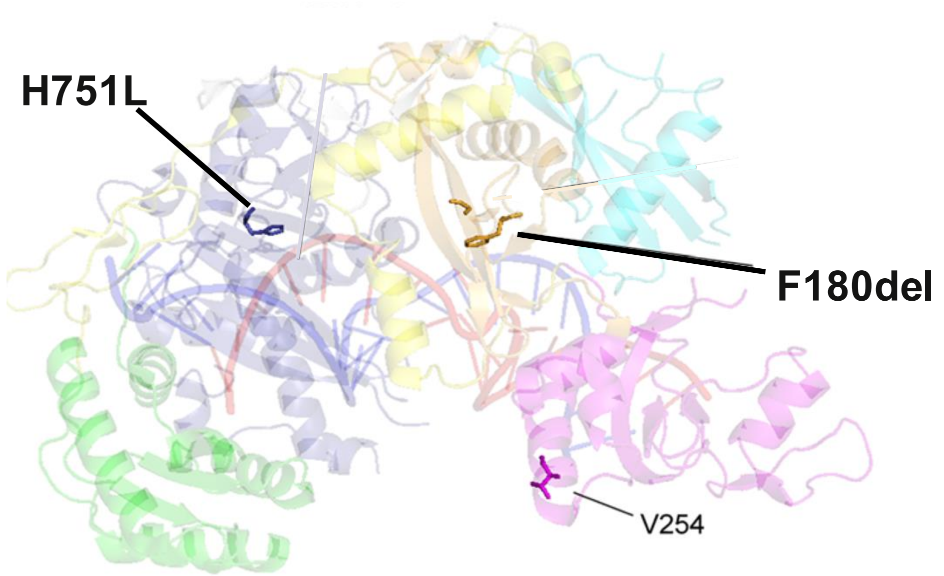

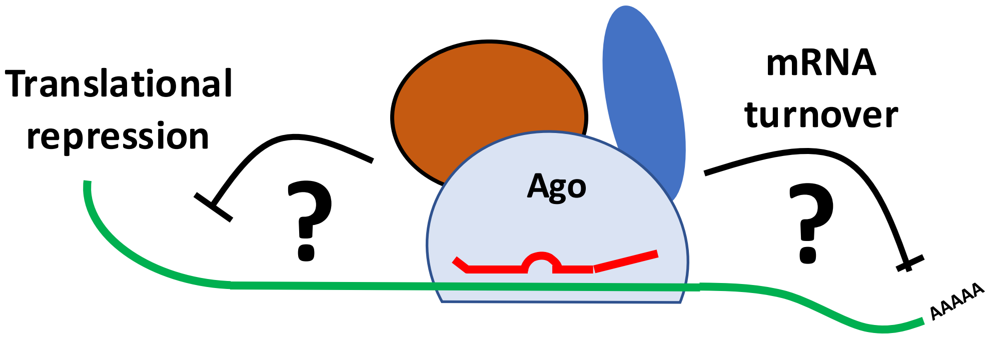

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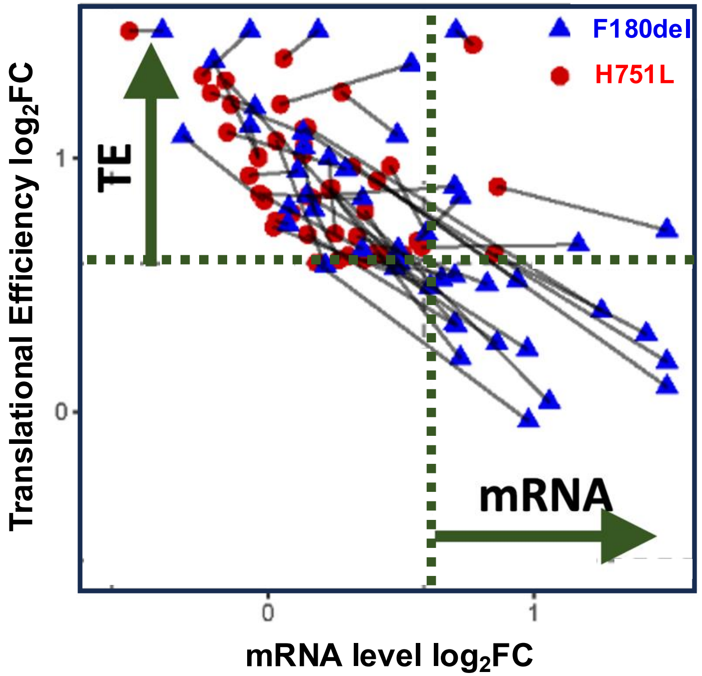
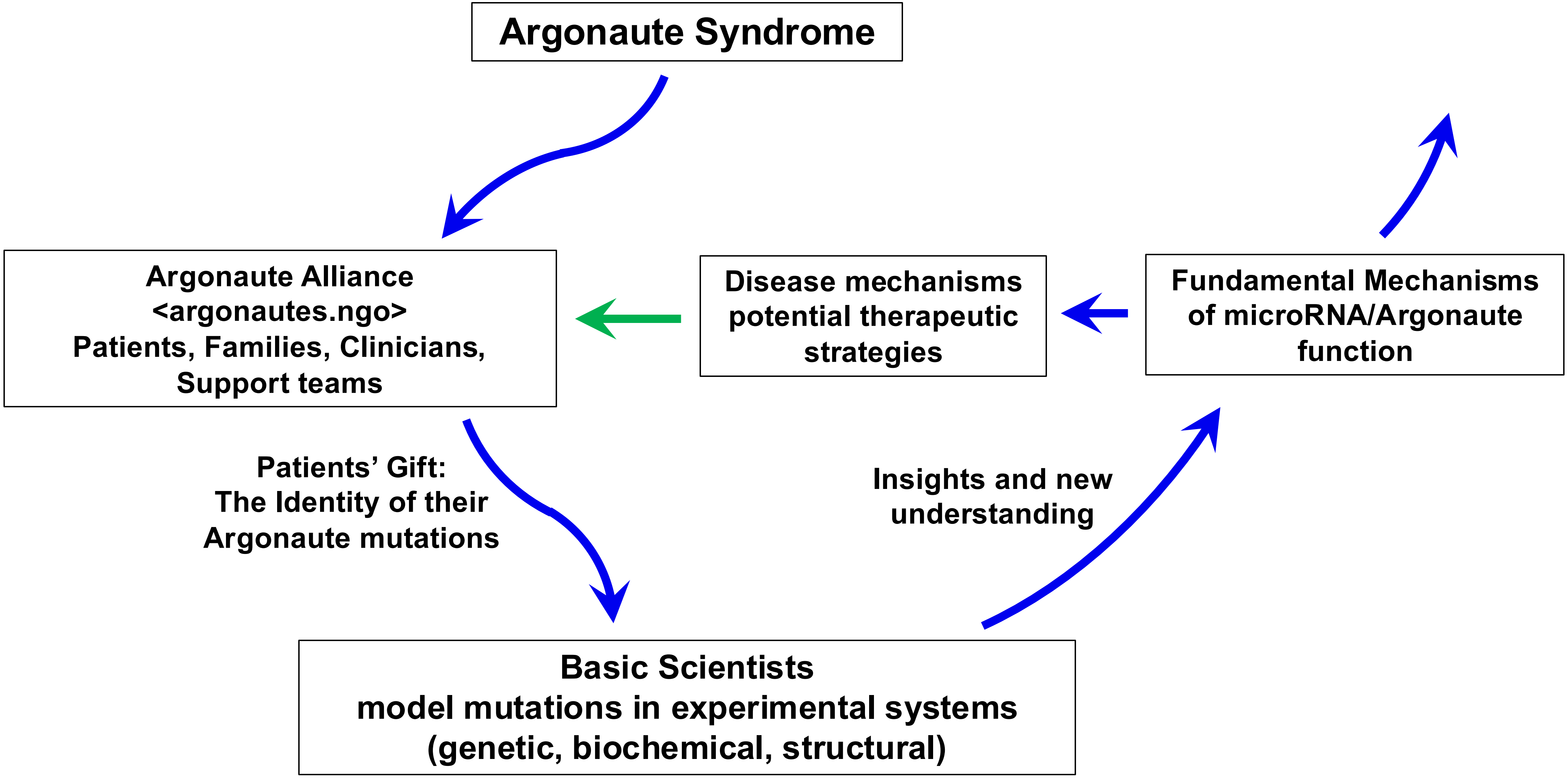


Duan et al 2024

Synergism Among Basic Scientists, Patients, and families



The Marvelous Story of microRNAs Continues to Unfold

THANK YOU!

to YOU ALL
for being here today

to ROSALIND LEE and RHONDA FEINBAUM
for discovering *lin-4* microRNA

to GARY RUVKUN
for sharing struggles,
discoveries, rewards

Most of all ..

to CANDY LEE
for *<u>everything</u>*

UMass Chan
MEDICAL SCHOOL

Morningside
Graduate School of
Biomedical Sciences