Alternative Models (part IV): *C. elegans*

12/06/16

Claudia Scheckel

Special Series on Laboratory Animal Science

The three R's

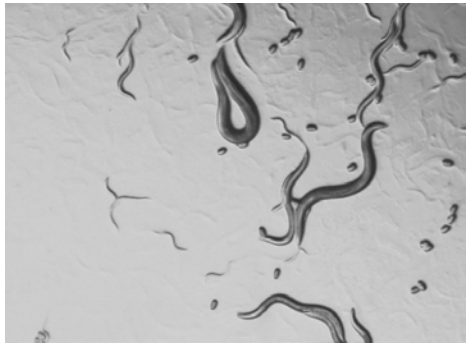
- Replacement
- Reduction
- Refinement

Replace animals that are protected under the animal welfare act with:

- Drosophila
- Zebrafish
- Yeast
- *C. elegans*

The roundworm *Caenorhabditis elegans*

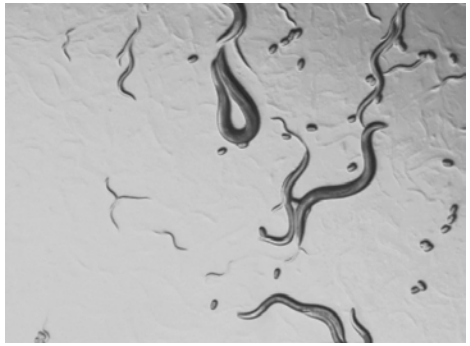
- grow on agar plates with bacteria in 20˚C incubators
  maintenance at 15˚C



The roundworm *Caenorhabditis elegans*

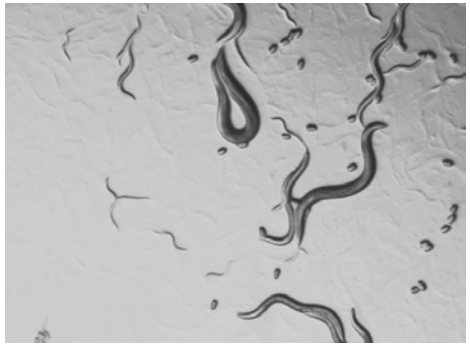
- grow on agar plates with bacteria in 20˚C incubators
  maintenance at 15˚C

- transferred via a “pick” (platinum wire in pasteur pipette) or “junking”



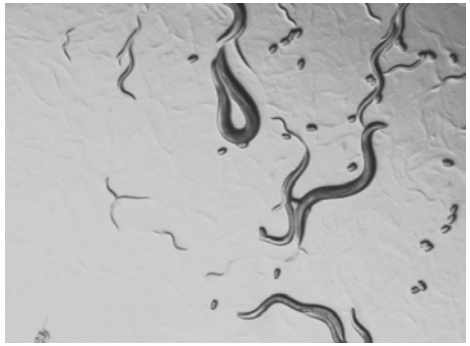
The roundworm *Caenorhabditis elegans*

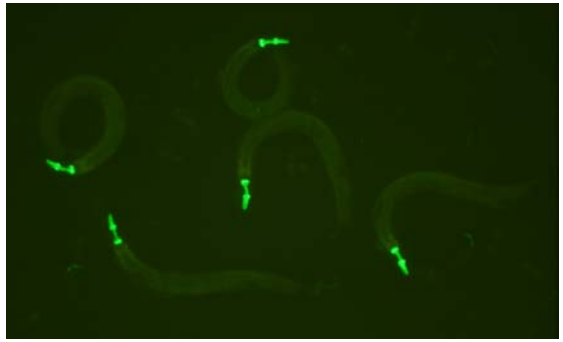
- grow on agar plates with bacteria in 20˚C incubators
  maintenance at 15˚C
- transferred via a “pick” (platinum wire in pasteur pipette)
- can be frozen at -80˚C



The roundworm *Caenorhabditis elegans*

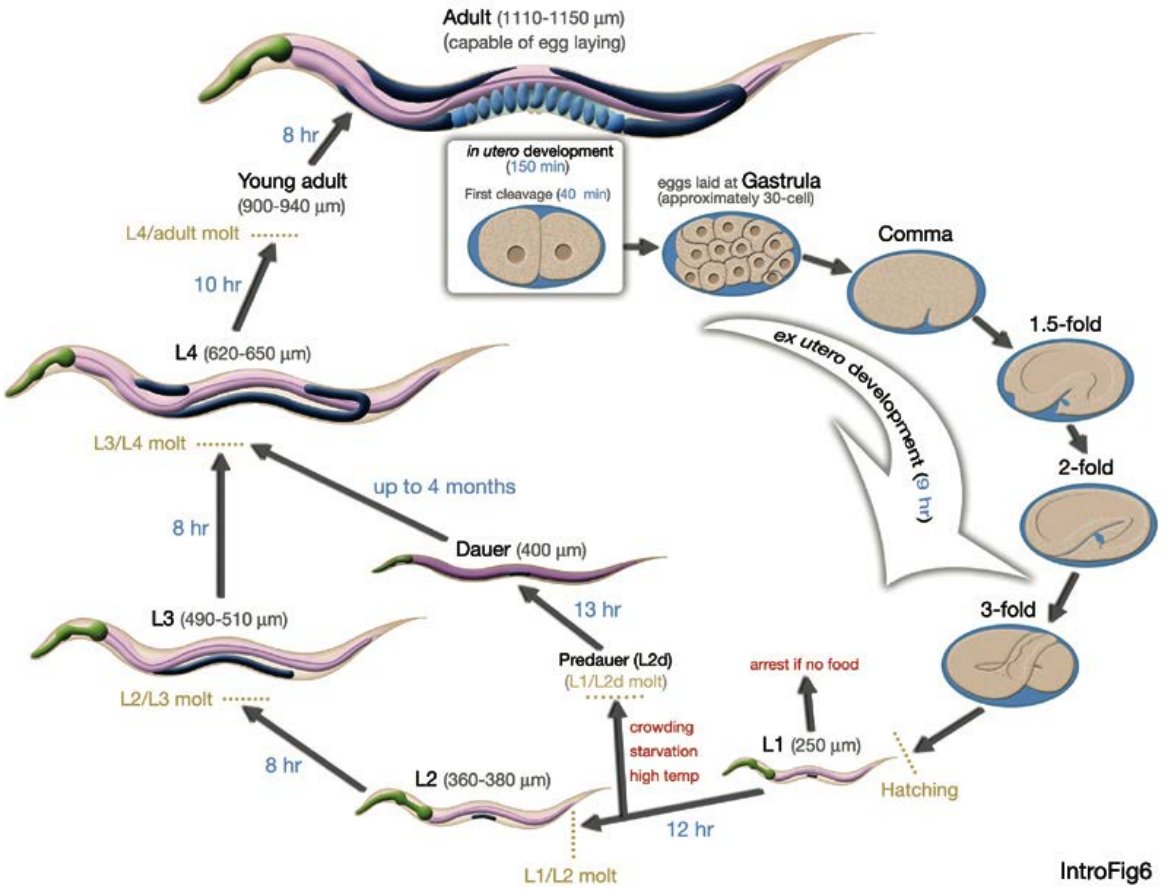
- grow on agar plates with bacteria in 20˚C incubators
  maintenance at 15˚C
- transferred via a “pick” (platinum wire in pasteur pipette)
- can be frozen at -80˚C
- transparent





The roundworm *Caenorhabditis elegans*

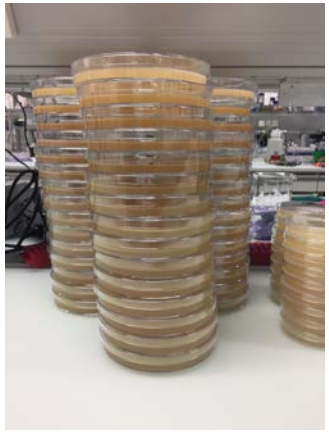
- Short generation cycle:
  Embryogenesis: ~14h
  Four larval stage L1-L4: ~8-12h each
  Adulthood: 300 progenies within 3-4 days



The roundworm *Caenorhabditis elegans*

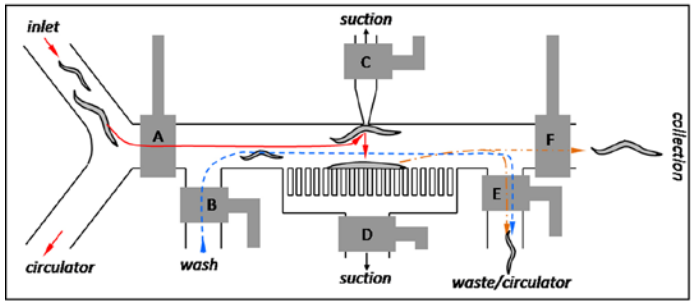
Convenient genetics:

- ~20 000 genes
- temperature-sensitive mutants (inducible at 25˚C)
- balancer chromosomes (maintenance/mutagenesis screens)
- worm sorting



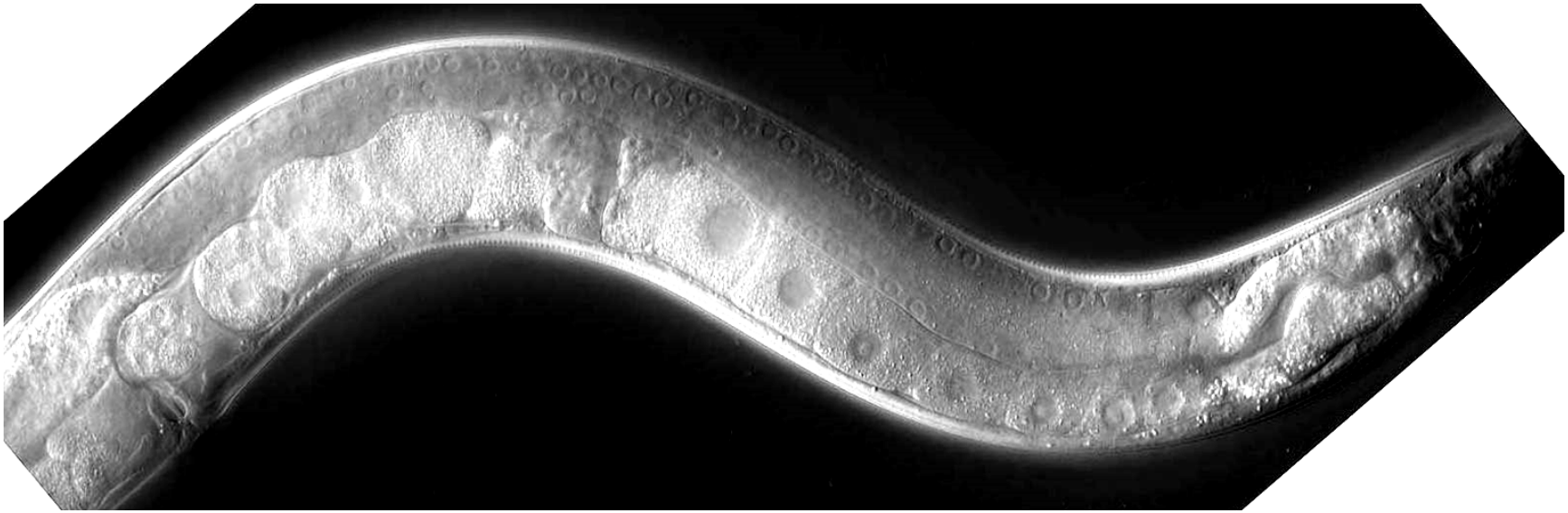
Grow on large ⌀15cm plates
Wash worms off

Worm sorting



The germ line of *C. elegans*

- hermaphrodite (XX)



The germ line of *C. elegans*

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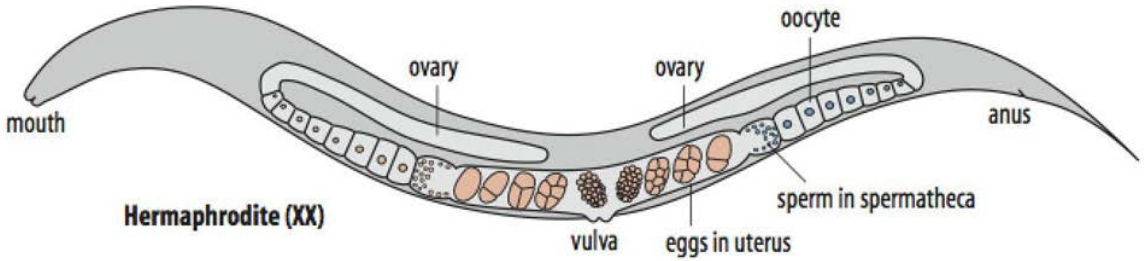
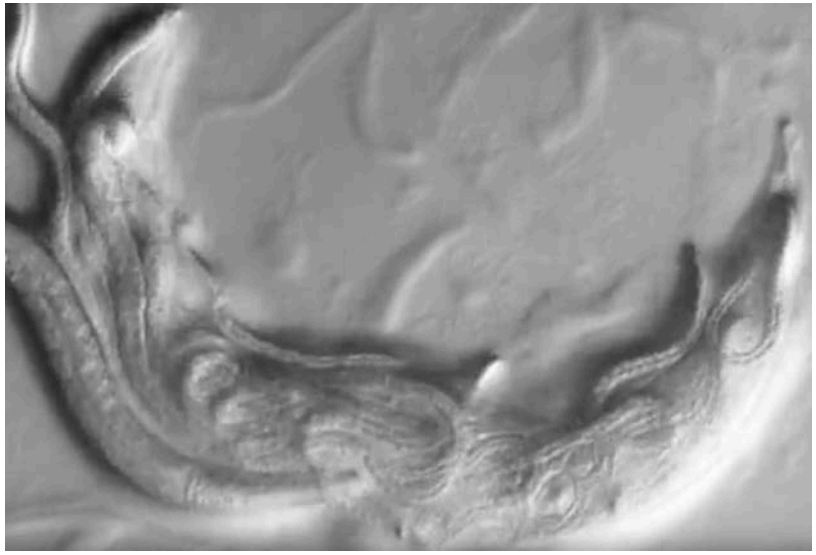
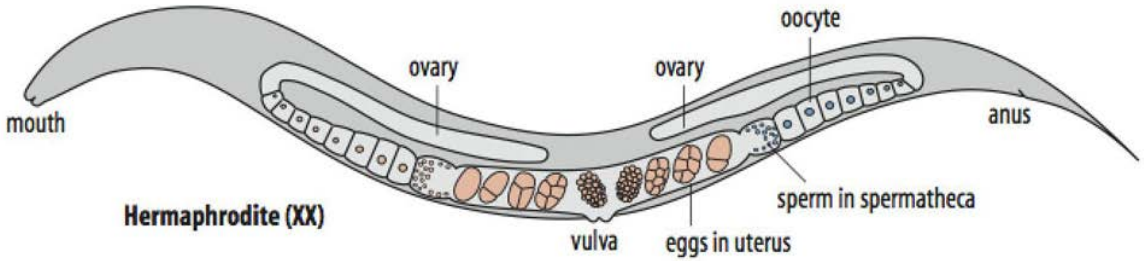


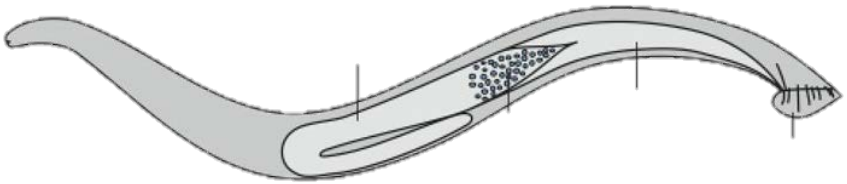


C. elegans breedings

- hermaphrodite (XX)



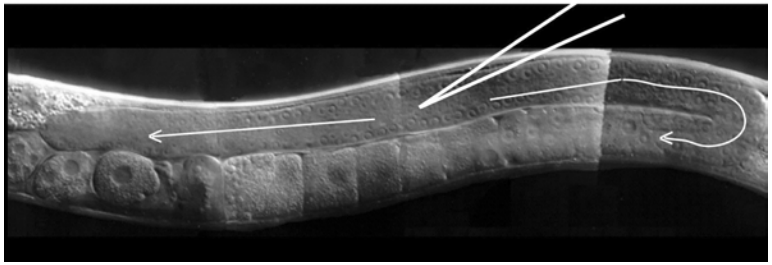
- 0.1% spontaneous males (XO)
  male generation: heatshock for 4-6h at 30˚C



- mating plates (localized spot of bacteria): paralyze female with levamizole

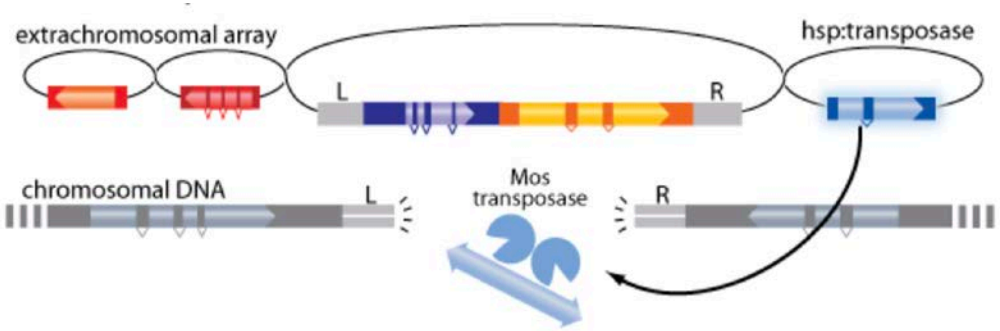
Generation of transgenic *Caenorhabditis elegans*

- Microinjection into the germ line
  extrachromosomal arrays (silenced in the germ line) that could be integrated into the genome



- Microparticle bombardment
  Gold particles coated with DNA were shot at worms (few transgene copies/transformant)

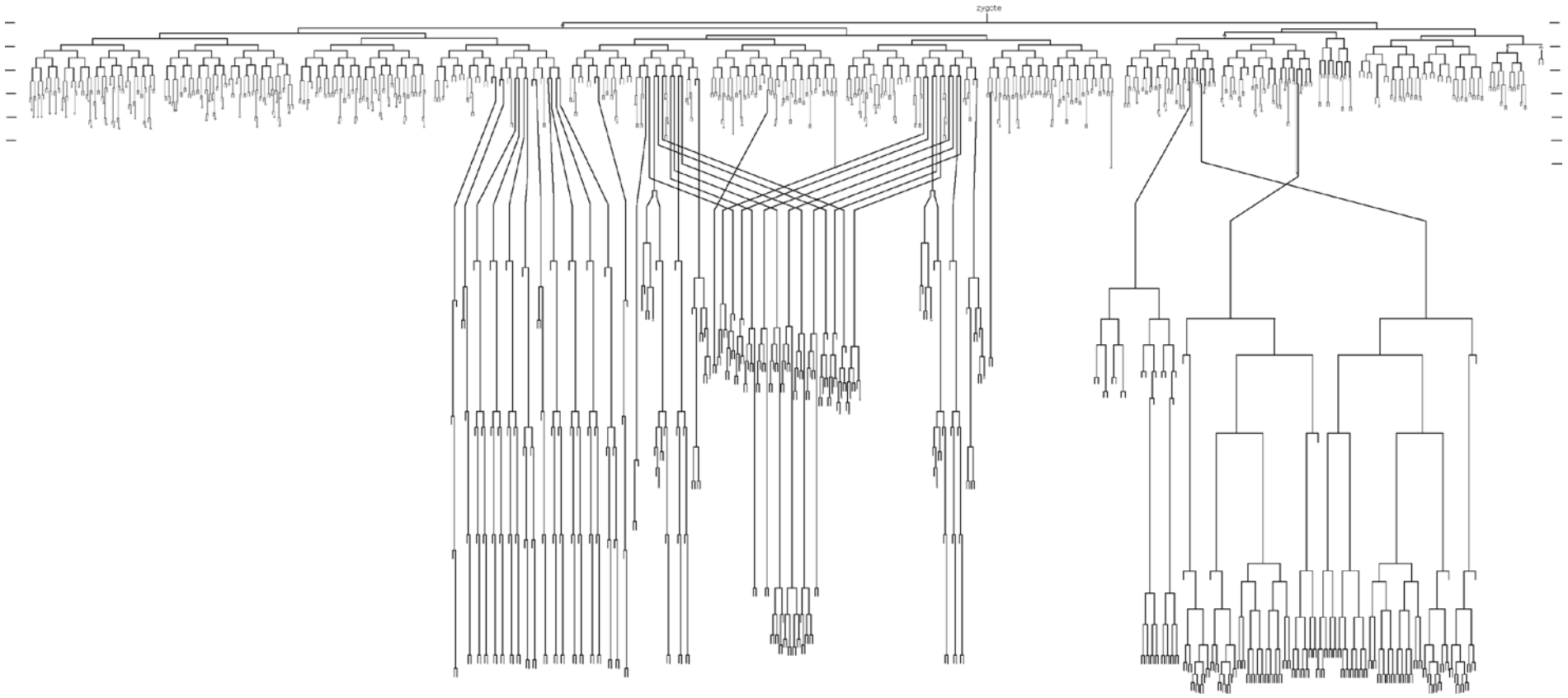
- Mos1-mediated single copy insertion (mosSCI)



- CRISPR

C. elegans has an invariant cell lineage

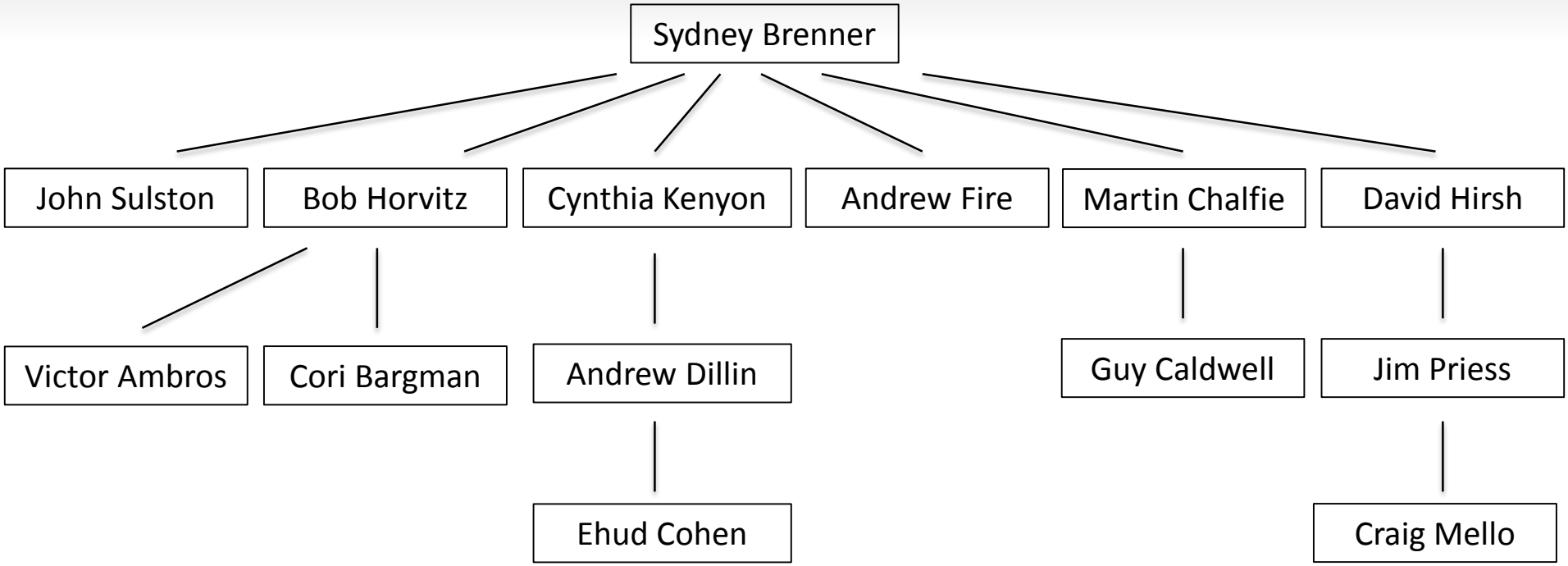
- hermaphrodite: 959 cells
- male: 1031 cells



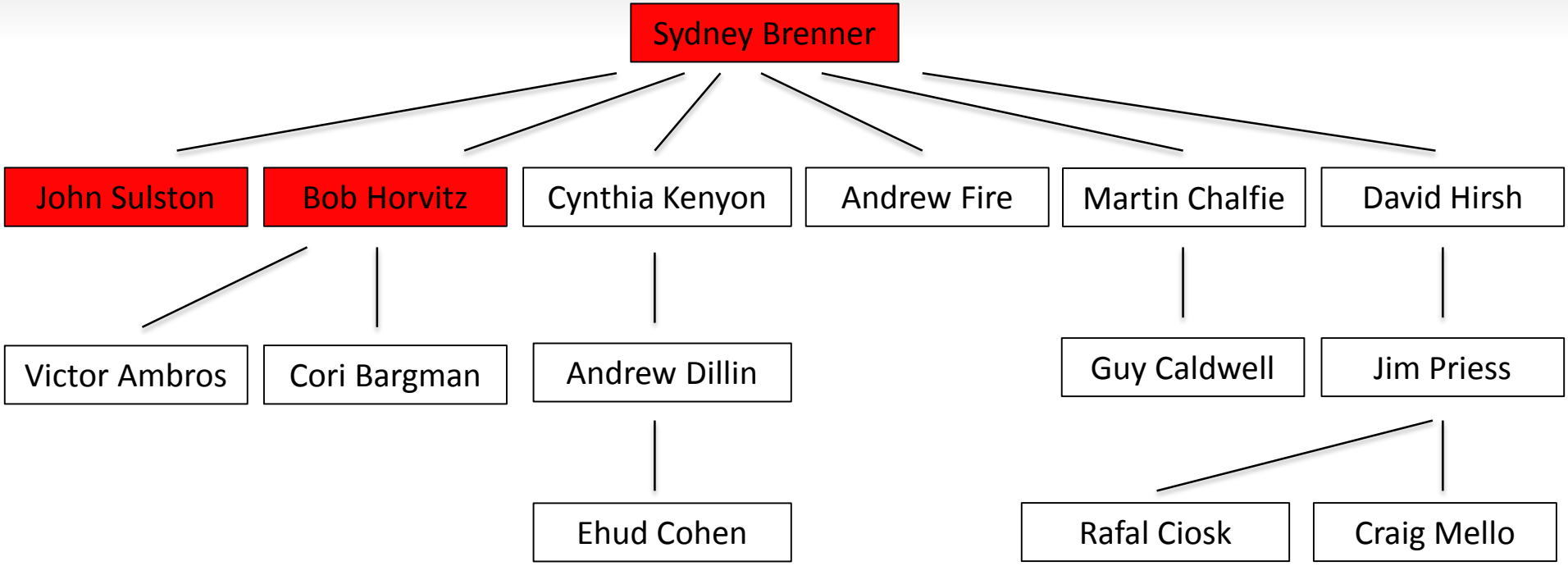
John Sulston:

- followed every division/differentiation event
- apoptosis is an integral part of the differentiation process

Worm Community Cell Lineage



Establishment of *C. elegans* as a model system



Nobel Prize in Physiology or Medicine 2002

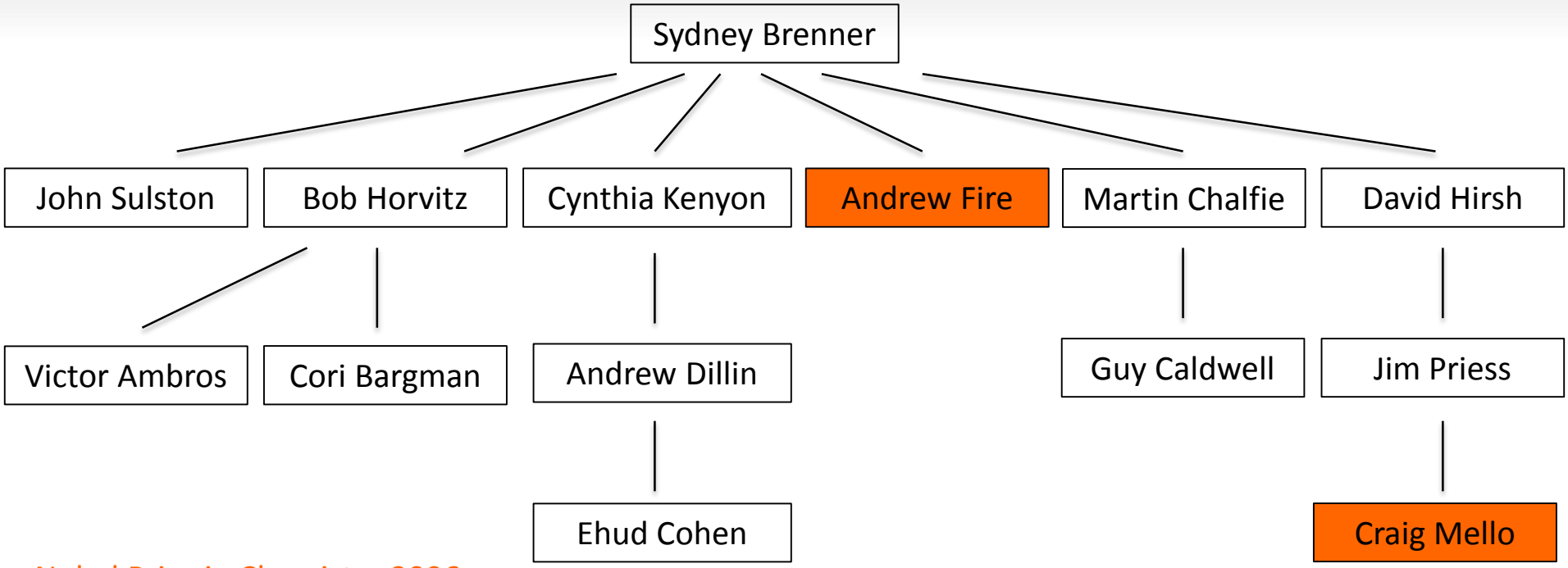
Discoveries on genetic regulation of organ development and programmed cell death

Sydney Brenner: established model system

John Sulston: cell lineage

Bob Horvitz: which genes mediate apoptosis

Discovery of RNA interference



Nobel Prize in Chemistry 2006

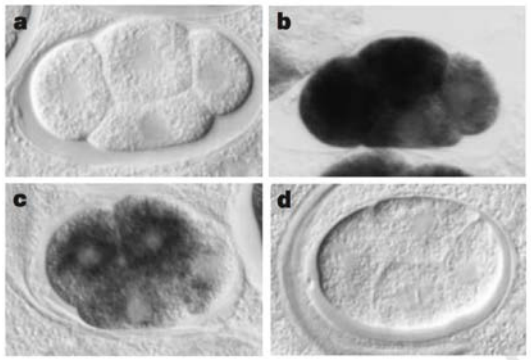
Discovery of RNA interference

Discovery of RNA interference

- sense and antisense RNAs can silence genes

RNAi:

- dsRNA injection: *unc-22, fem-1*, *unc-54*, *gfp*
- Strong twitching/feminizing/paralysis only upon dsRNA injection
- dsRNA injected into the body cavity spreads through the body



Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*

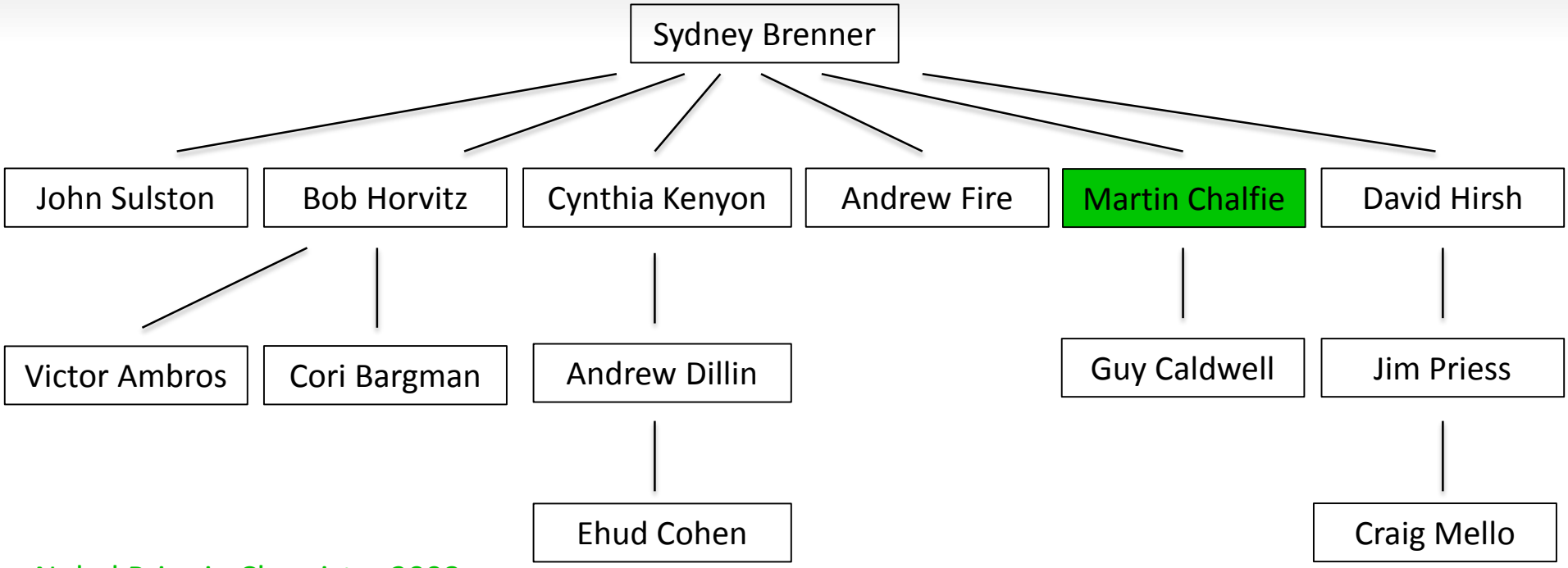
Andrew Fire*, SiQun Xu*, Mary K. Montgomery*, Steven A. Kostas*†, Samuel E. Driver‡ & Craig C. Mello‡

* *Carnegie Institution of Washington, Department of Embryology, 115 West University Parkway, Baltimore, Maryland 21210, USA*
† *Biology Graduate Program, Johns Hopkins University, 3400 North Charles Street, Baltimore, Maryland 21218, USA*
‡ *Program in Molecular Medicine, Department of Cell Biology, University of Massachusetts Cancer Center, Two Biotech Suite 213, 373 Plantation Street, Worcester, Massachusetts 01605, USA*

Fire *et al*, 1998 Nature

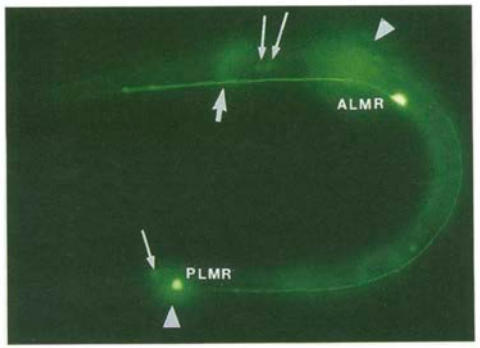
- dsRNA (if targeting exons) decreases endogenous mRNAs across generations

Application of GFP *in vivo*



Nobel Prize in Chemistry 2008

Discovery and development of GFP

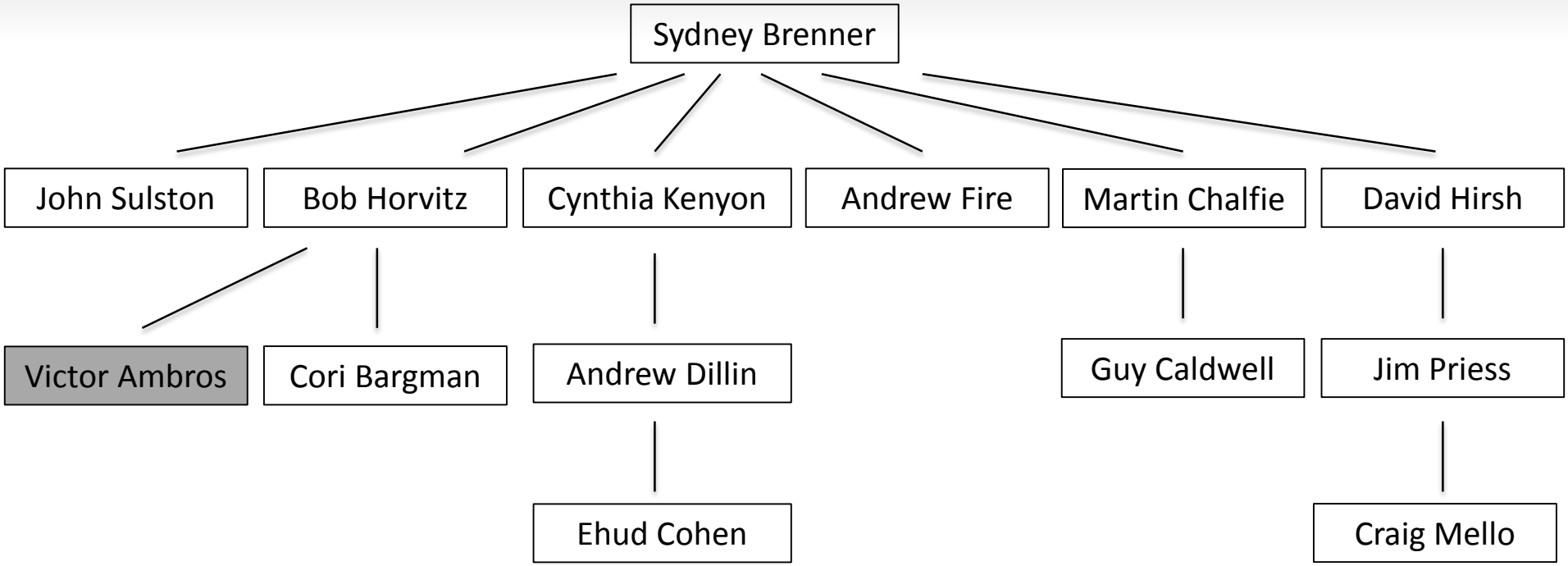


GFP expression in touch receptor neurons

→ monitor gene expression/protein localization

Chalfie *et al*, 1994

Discovery of miRNAs



Discovery of miRNAs

- Heterochronic genes specify temporal fates of cells during larval development
- *lin-14* mutants: skipping of L1-specific events
  lin-4 mutants: reiteration of L1 cell lineage patterns, absence of adult structures

lin-4 ⊣ *lin-14*

- Posttranscriptional regulation of *lin-14*:
  lin-14 mRNA is present
  lin-14 protein is absent
- *lin-14* regulation requires its 3'UTR

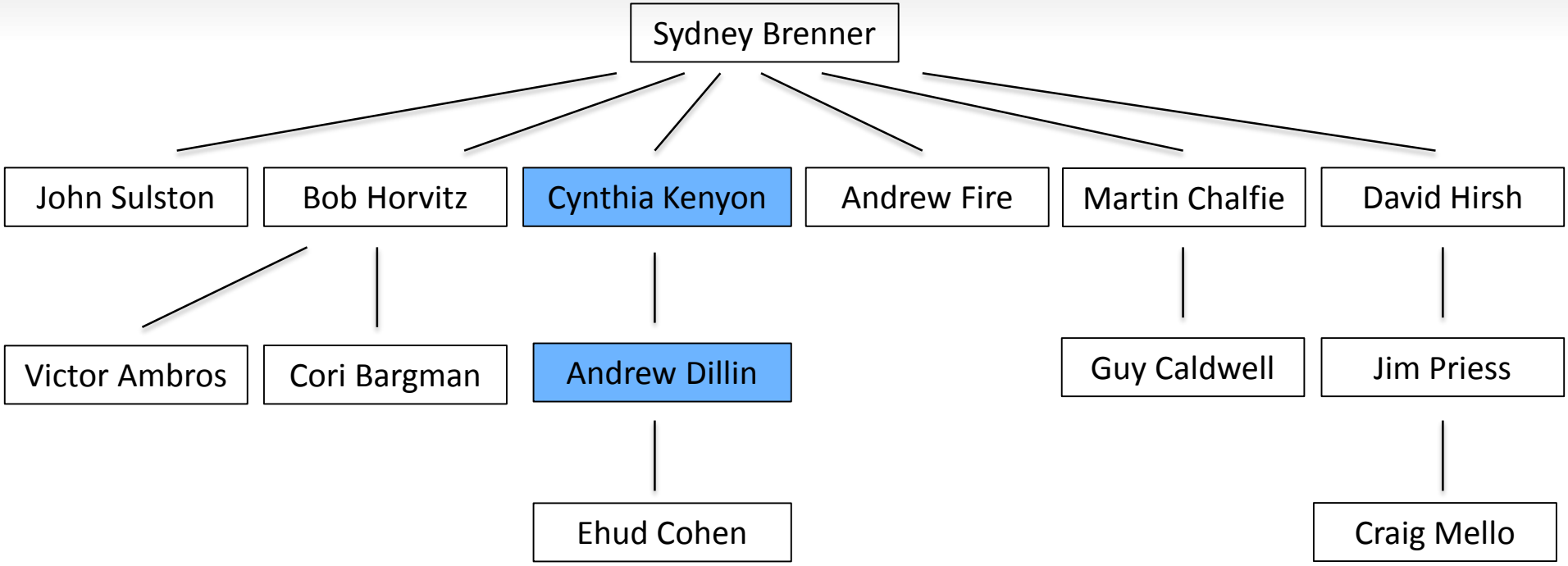
- *lin-4* activity: 100nt, within intron, ATG/ORF-independent
- 61nt/22nt small RNAs (Northern Blot) complementary to *lin-14* 3'UTR

The C. elegans Heterochronic Gene *lin-4* Encodes Small RNAs with Antisense Complementarity to *lin-14*

Rosalind C. Lee,*† Rhonda L. Feinbaum,*‡ and Victor Ambros†
Harvard University
Department of Cellular and Developmental Biology
Cambridge, Massachusetts 02138

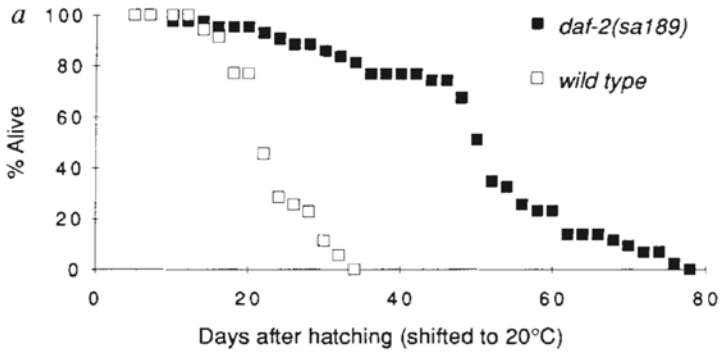
Lee *et al*, 1993 Cell

C. elegans as a model to study aging



Modulation of lifespan in *C. elegans*

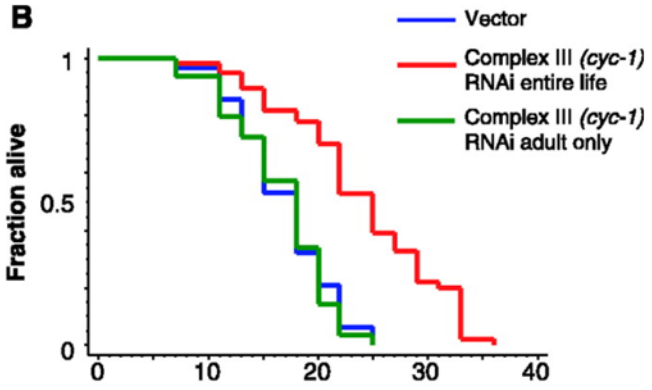
- IGF-1 mutations (*daf-2*) double life in *C. elegans*



IFG-1 signaling influences *C. elegans* lifespan only during adulthood

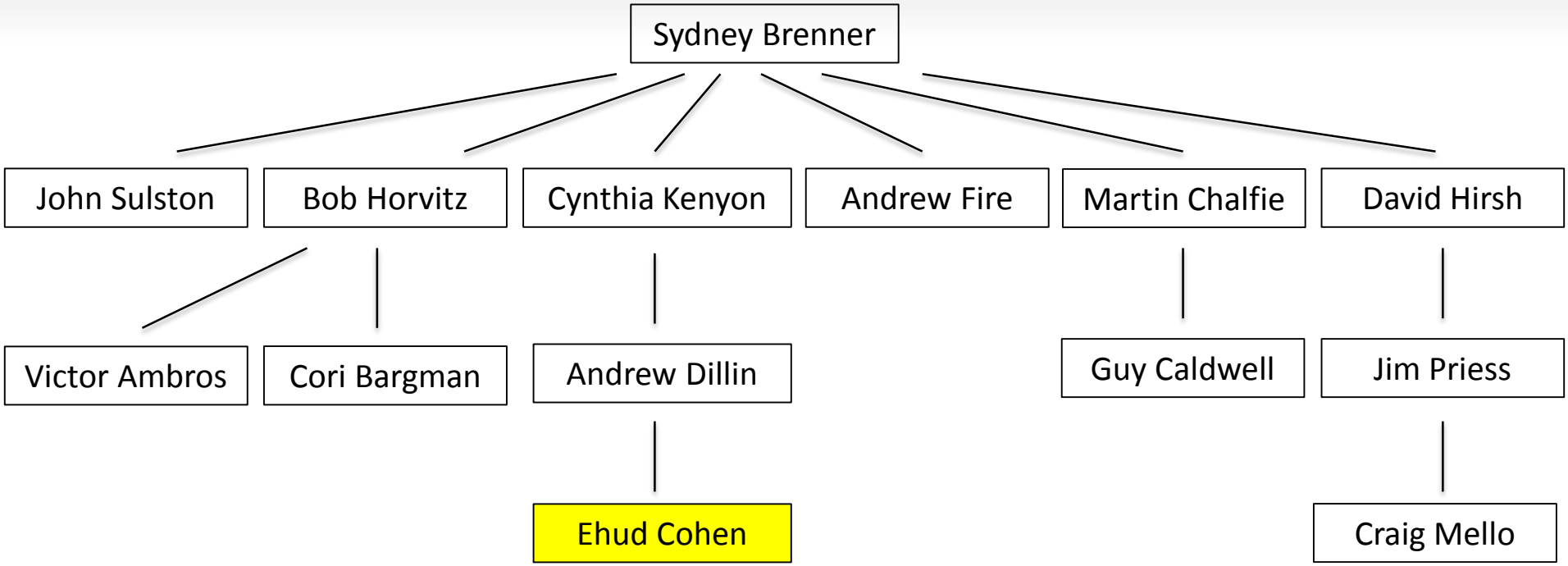
Kenyon *et al*, 1993 Nature

- Reducing respiratory chain (mitochondrial) rate during development reduces lifespan



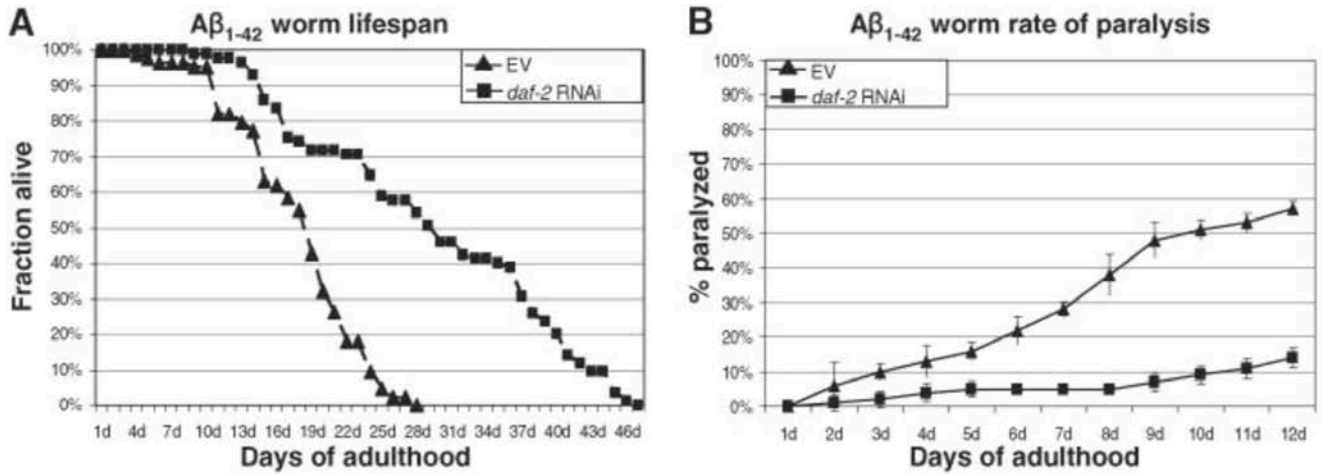
Dillon *et al*, 2002 Science

C. elegans as a model to study aging and protein aggregation



C. elegans as a model to study protein aggregation

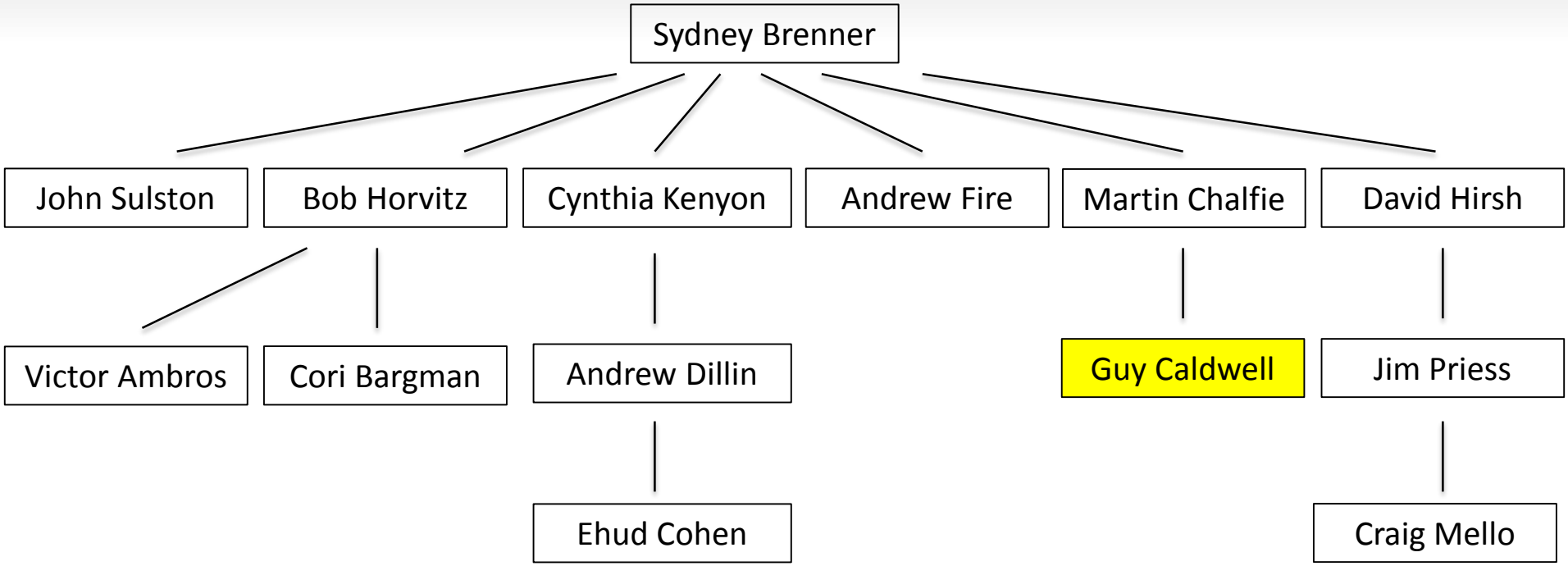
- Lifespan expansion in due to reduced insulin signaling also requires HSF-1
- Integrity of protein folding determines lifespan?
- $A\beta_{1-42}$ expression in body wall muscles induces paralysis (Link, 1995 PNAS)
- Decreased insulin signaling reduces Aβ toxicity



Cohen *et al*, 2006 Science

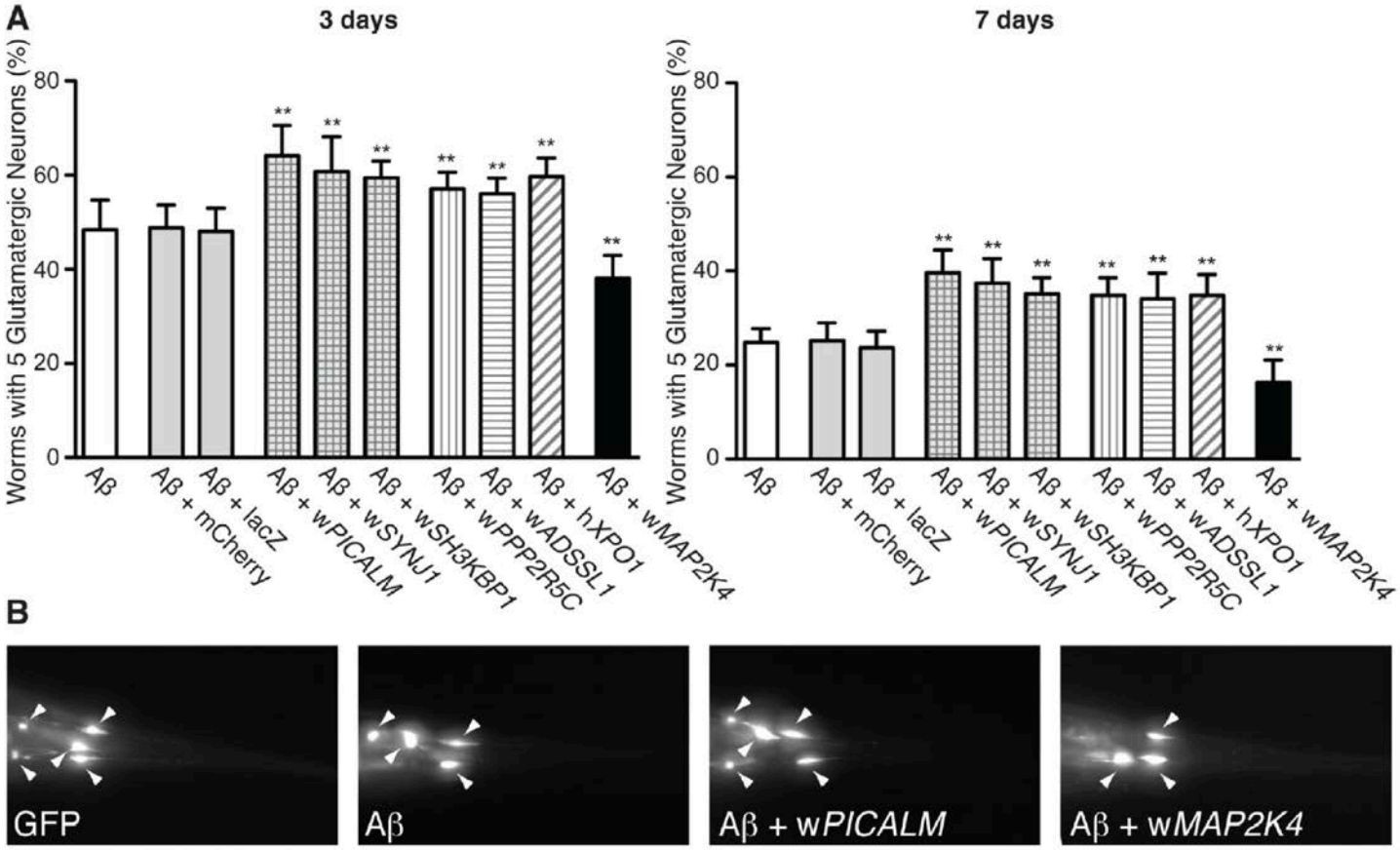
→ Established a link between aging and aggregation-mediated proteotoxicity

C. elegans as a model to study protein aggregation



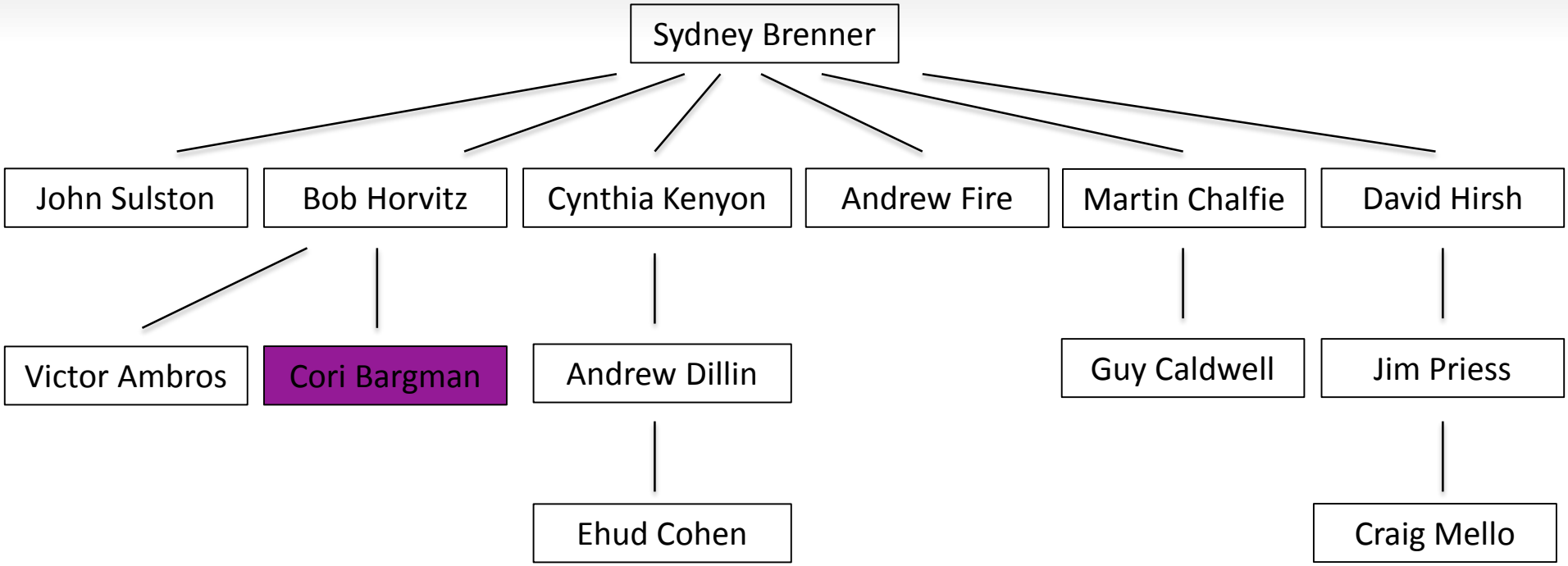
C. elegans as a model to study protein aggregation

- Hit validation of $A\beta_{1-42}$ modifiers identified in yeast
- Worms expressing $A\beta_{1-42}$ in glutamatergic neurons (co-express GFP)



Treusch *et al*, 2011 Science
Caldwell & Lindquist

C. elegans as a model to study behavior



C. elegans as a model to study behavior

Imprinting: exposure to sensory cues in a critical period

- visual: geese attach to a human
- olfactory: salmon will always recognize natal stream

C. elegans:

- 302 neurons
- modifies behavior based on experience:
  single training session (pairing of odor/food): short-term preference
  multiple spaced training sessions: long-term memory (~24h)
  training of newly hatched larvae: imprinting even in following generations
- Neurons/molecules required for pathogen aversion have been identified

Konrad Lorenz
Nobel Prize in Physiology or Medicine 1973

C. elegans as a model to study behavior

Distinct Circuits for the Formation and Retrieval of an Imprinted Olfactory Memory

Xin Jin,[1] Navin Pokala,[1,2] and Cornelia I. Bargmann[1,*]
[1]Howard Hughes Medical Institute (HHMI), Lulu and Anthony Wang Laboratory of Neural Circuits and Behavior, The Rockefeller University, New York, NY 10065, USA
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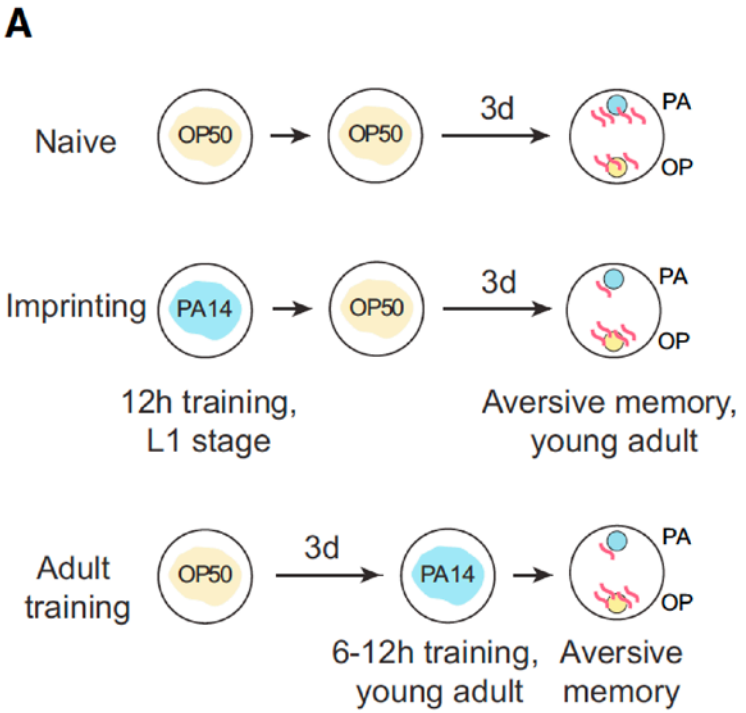
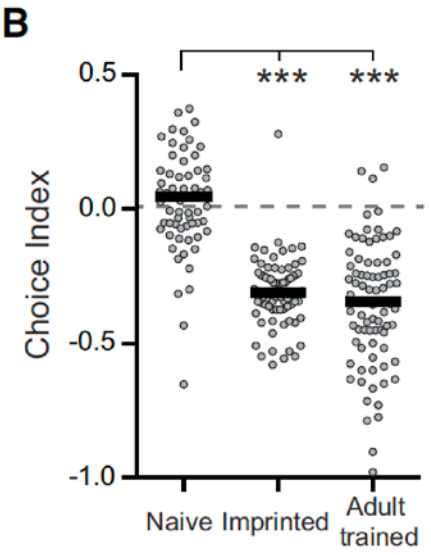
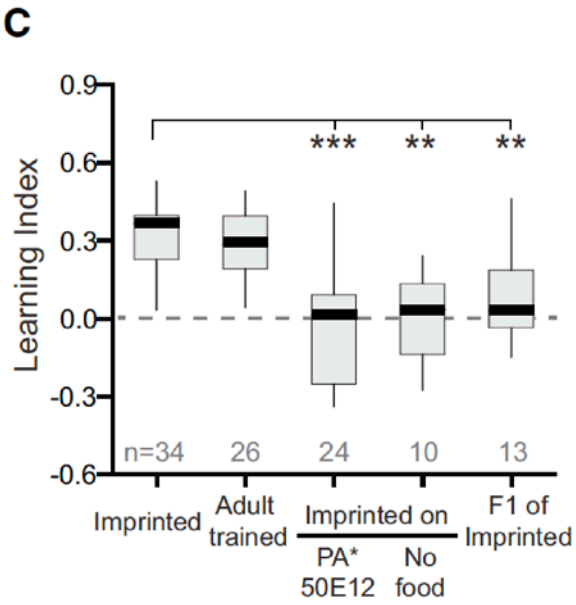
http://dx.doi.org/10.1016/j.cell.2016.01.007

Pathogen imprinting at L1 stage induces long-term aversive memory



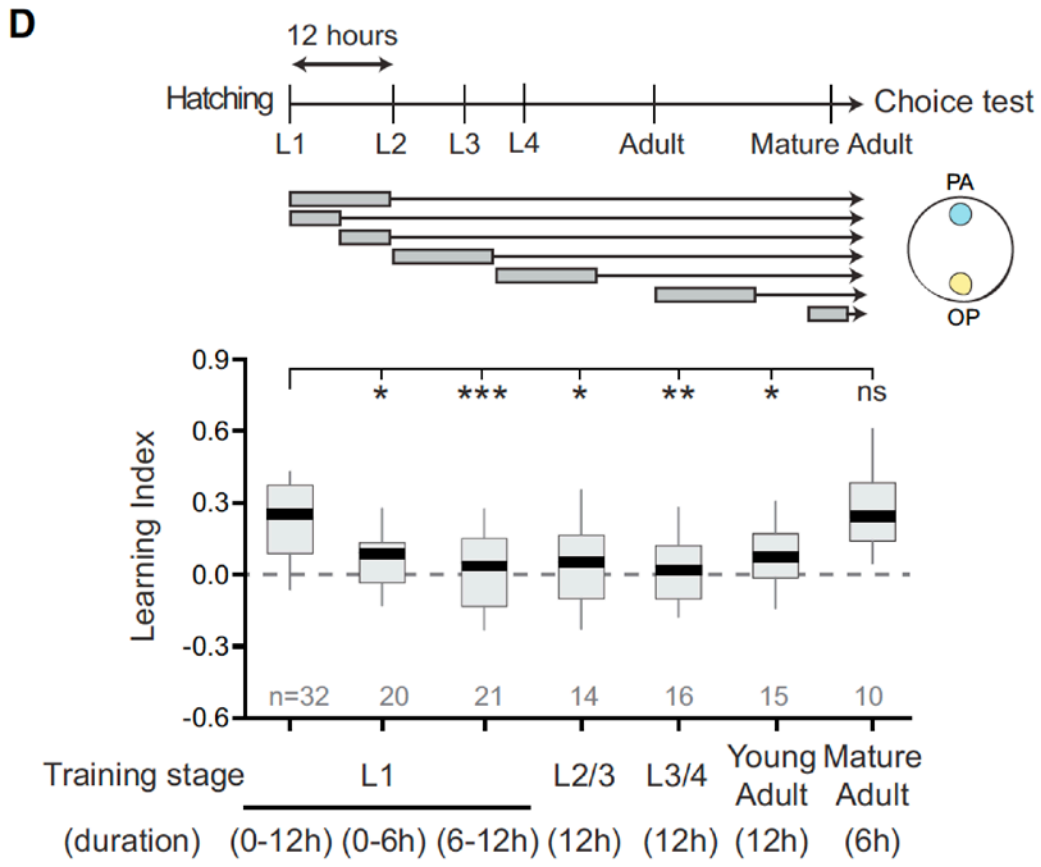


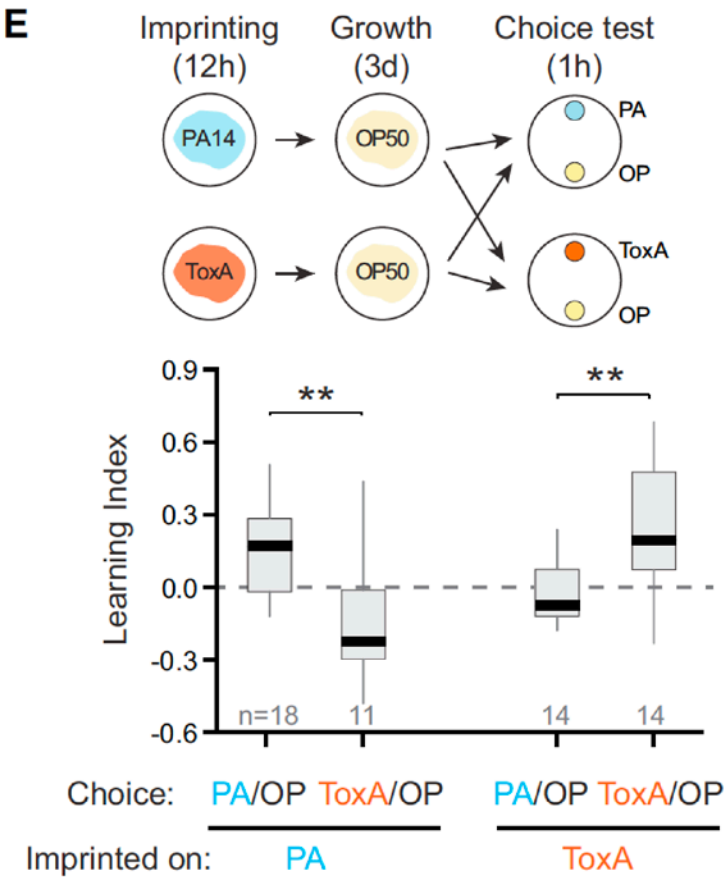


$$\text{Choice Index} = \frac{\#\text{animals in PA} - \#\text{ in OP}}{\#\text{animals in PA} + \#\text{ in OP}}$$

Learning Index = Choice Index (naive) - Choice Index (imprinted)

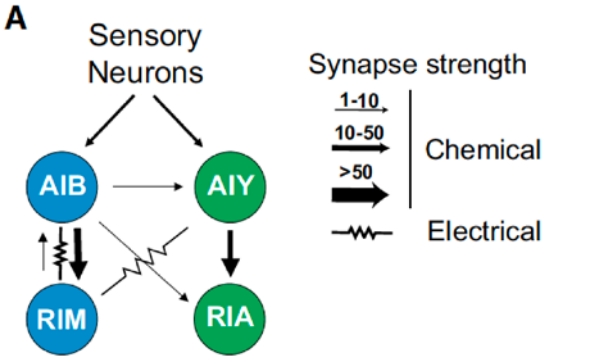
Pathogen imprinting at L1 stage induces long-term aversive memory





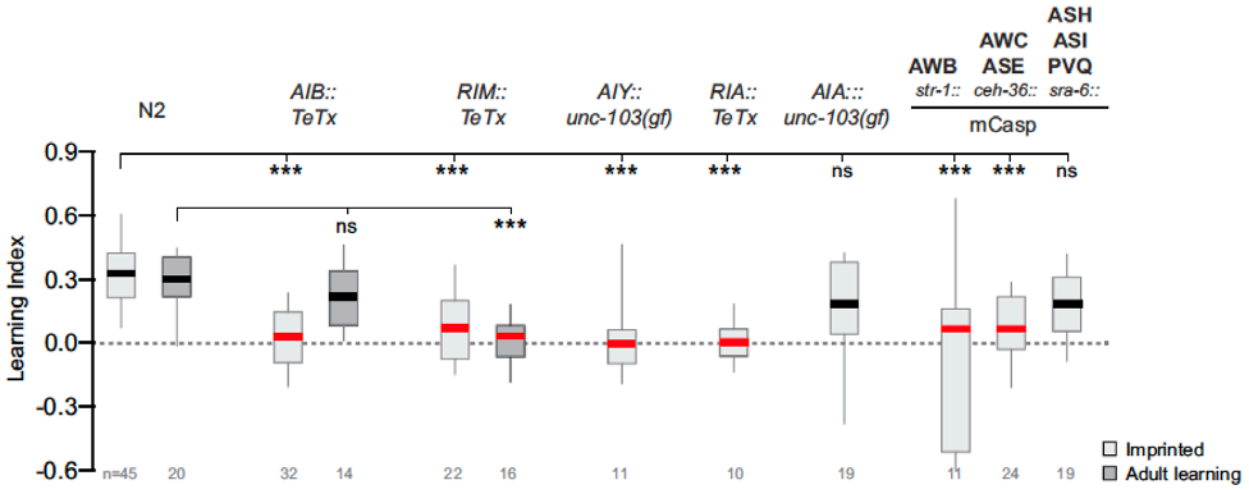
- **12h pathogen** exposure during **L1** induces long-term aversive memory

Similar neurons are required for adult-learned & imprinted aversion

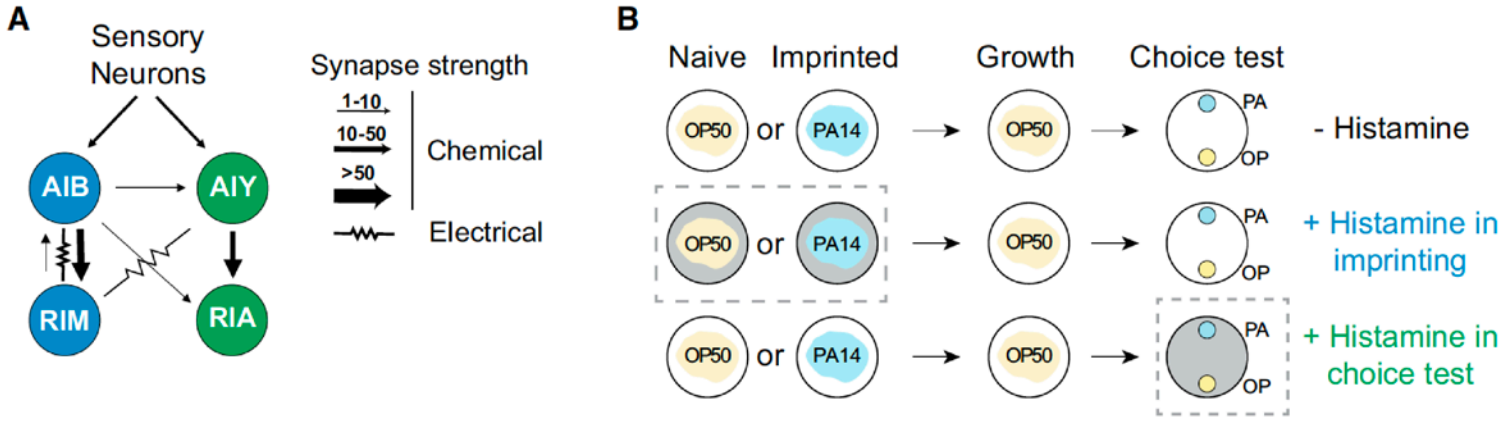


Cell ablation via multicopy transgene expression of:

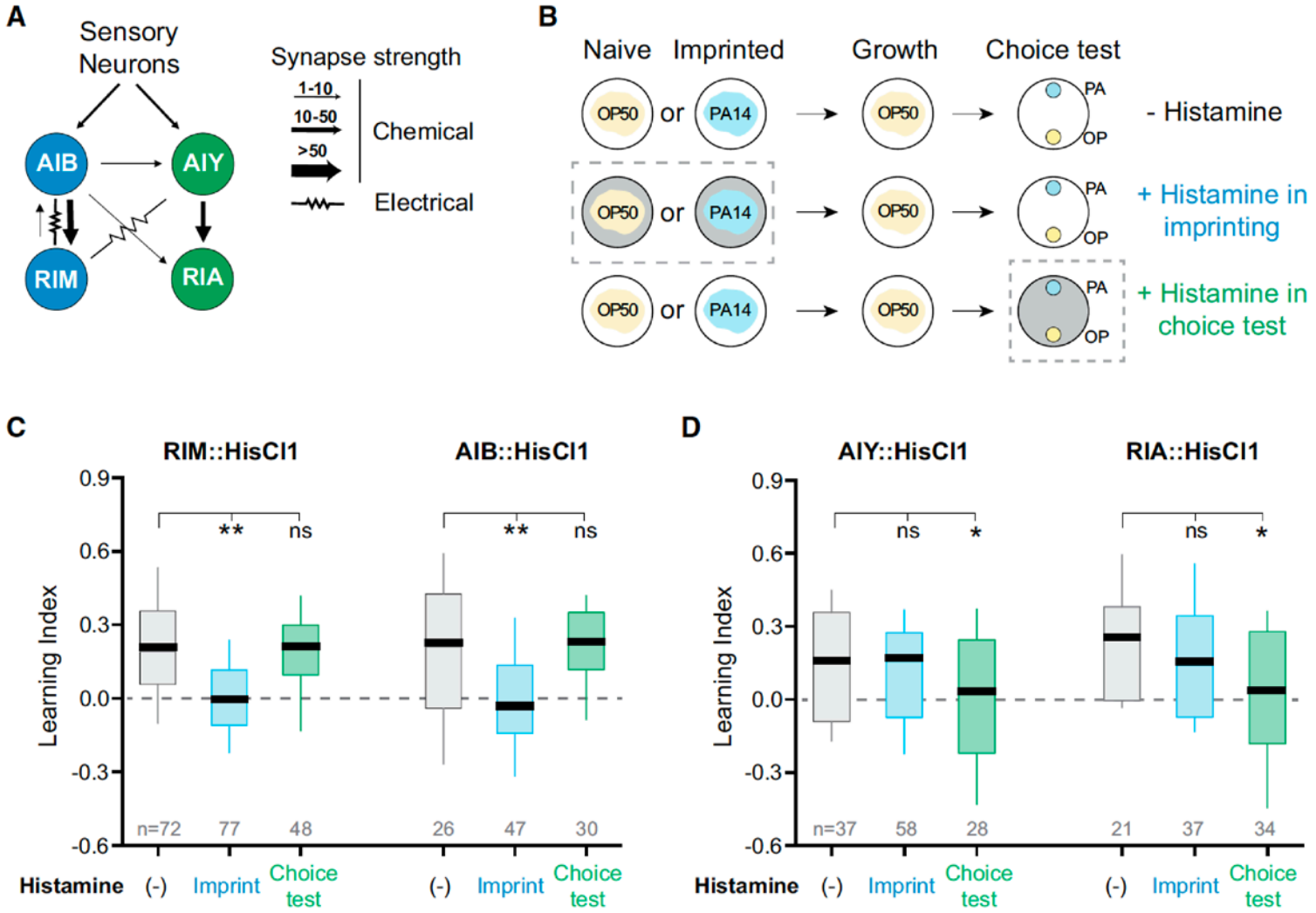
- Tetanus toxin light chain (TeTx)
- Leaky potassium channel (*unc-103*)
- murine caspase (mCasp)



Acute silencing of neurons using a histamine-gated chloride channel

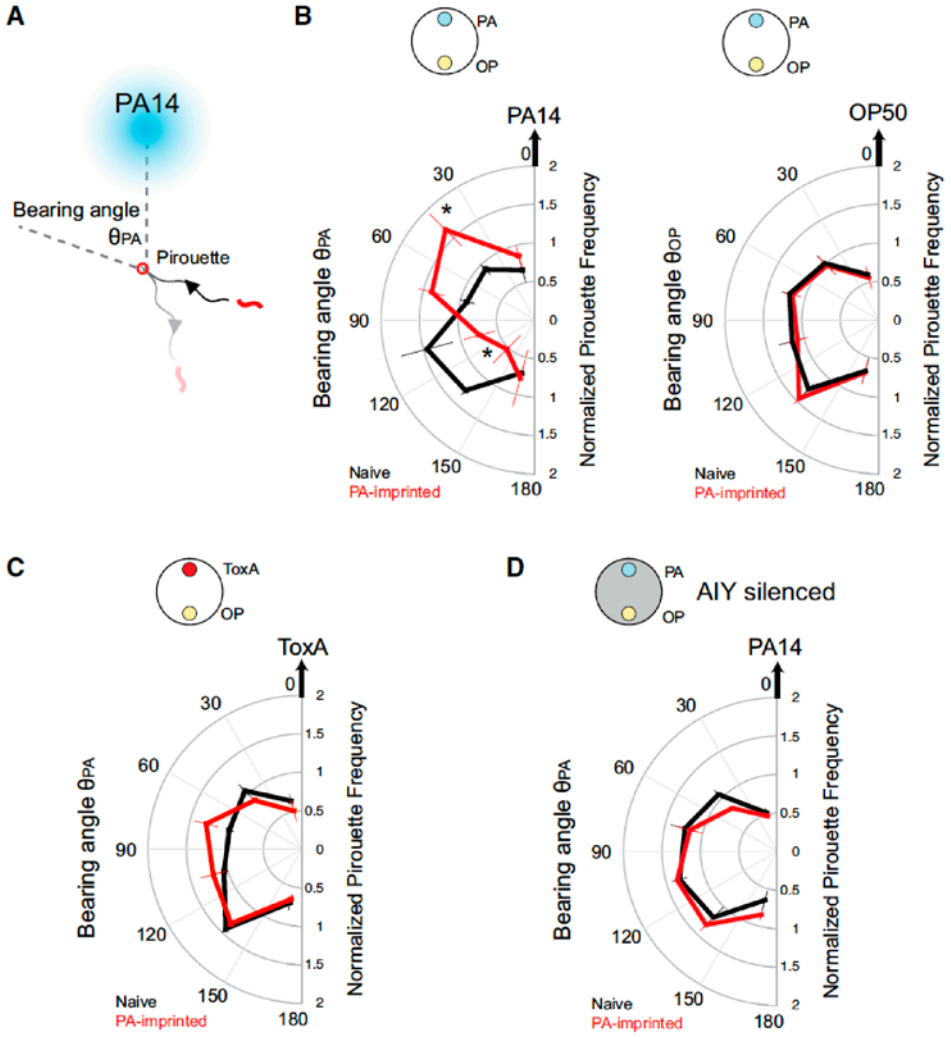


Distinct neurons are required for memory formation & retrieval

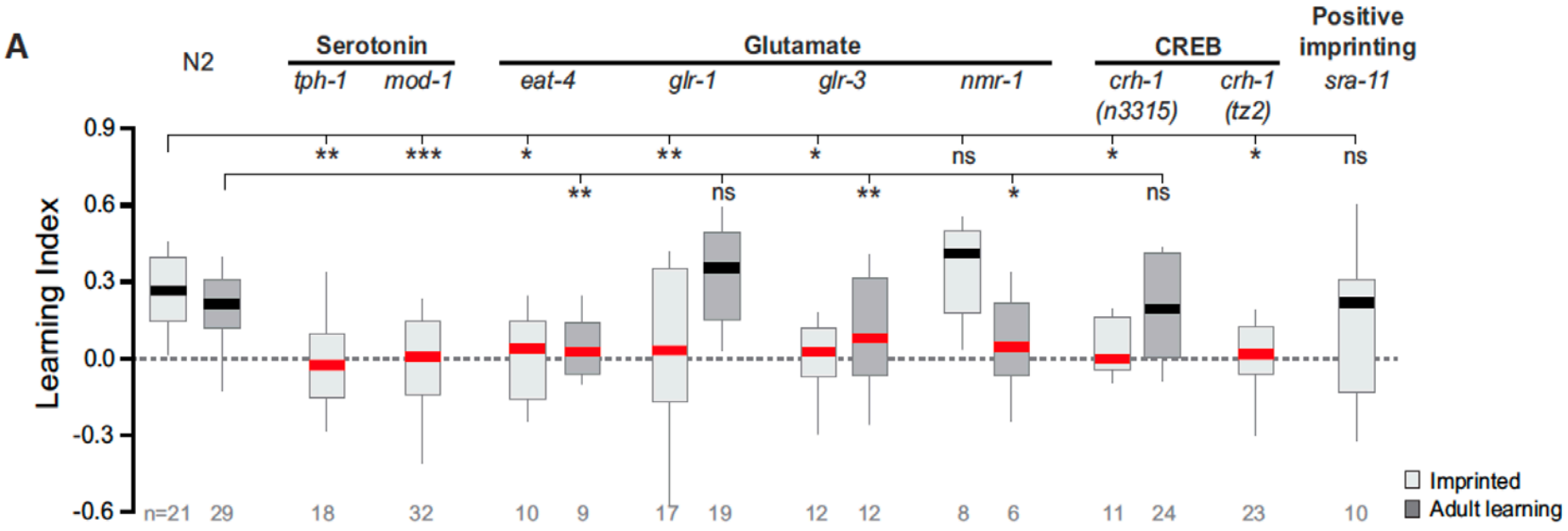


Imprinting alters chemotaxis behaviors

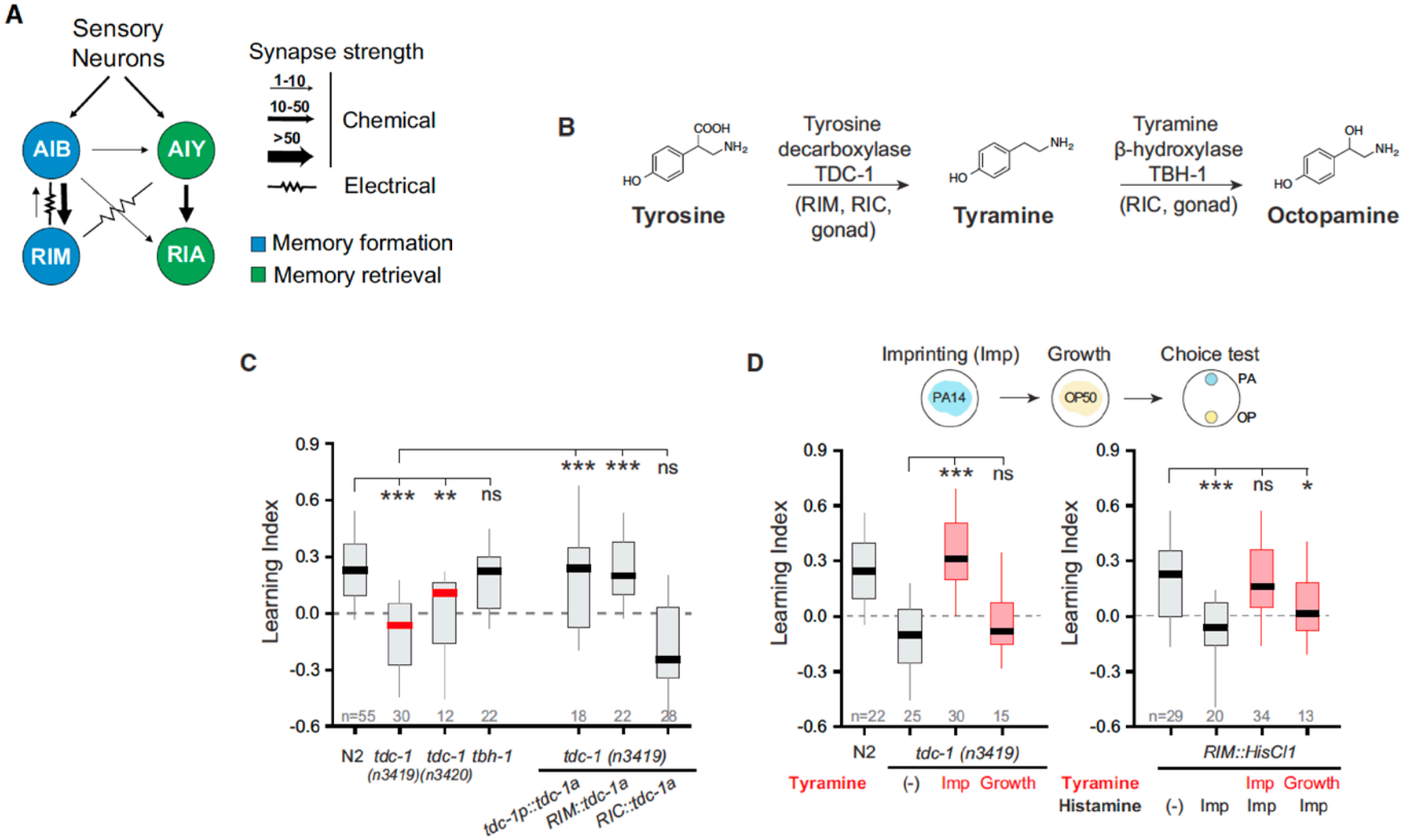
- Attractive chemical:
  random-walk
  the closer the more pirouettes
- Repulsive chemical:
  random-walk
  the closer the less pirouettes
- Imprinted animals show a reversed turning bias (depends on imprinting and memory-retrieval neuron)
- Neurons important for memory retrieval don't change superficially upon imprinting



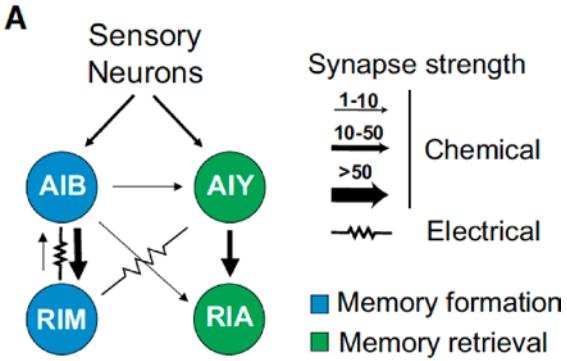
Imprinted aversion shares requirements with other forms of learning/memory



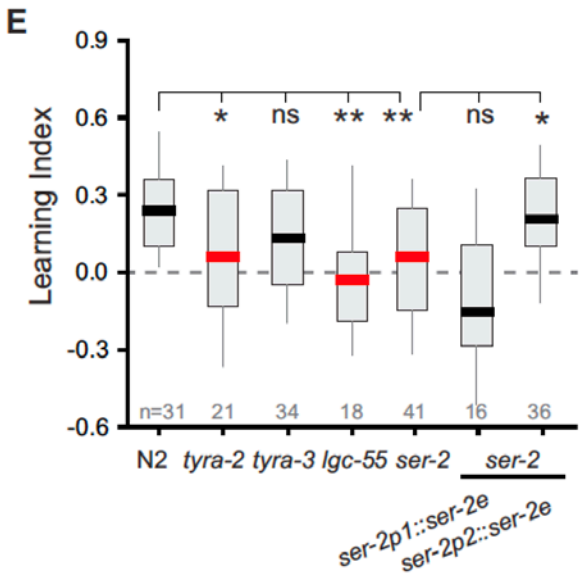
Imprinting requires tyramine in RIM

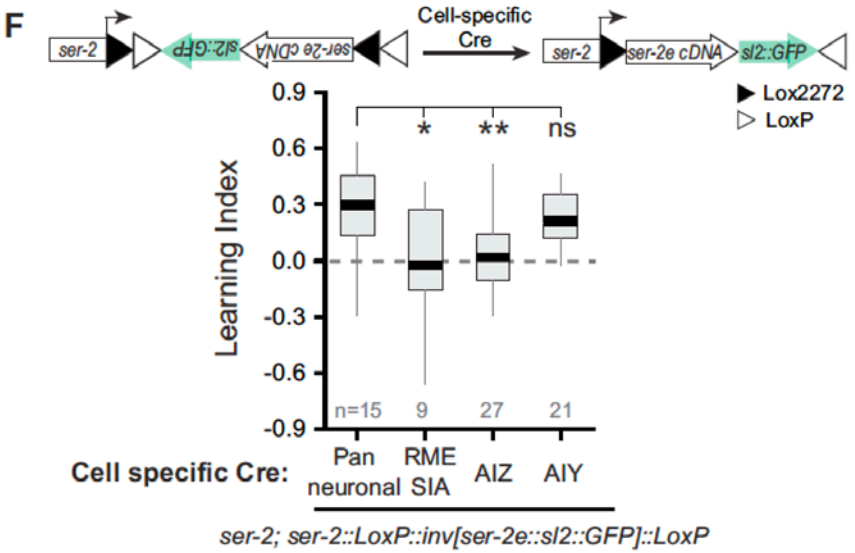


Imprinting requires tyramine receptor SER-2 in AIY neurons

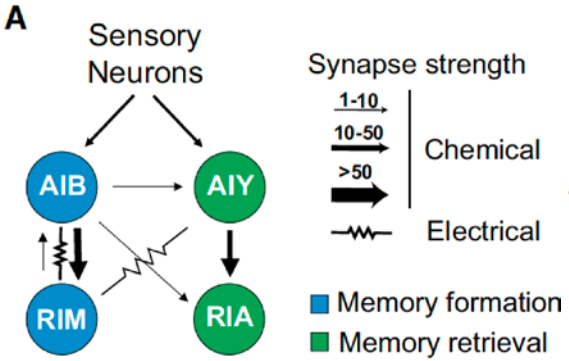


- Tyramine is sensed through GPCRs (TYRA-2/3, SER-2) and tyramine-gated channel LGC-55
- SER-2 is not required for adult-learned aversion
- Distal promotor of SER-2 is required for imprinted aversion
- AIY expression of SER-2 is required for imprinted aversion

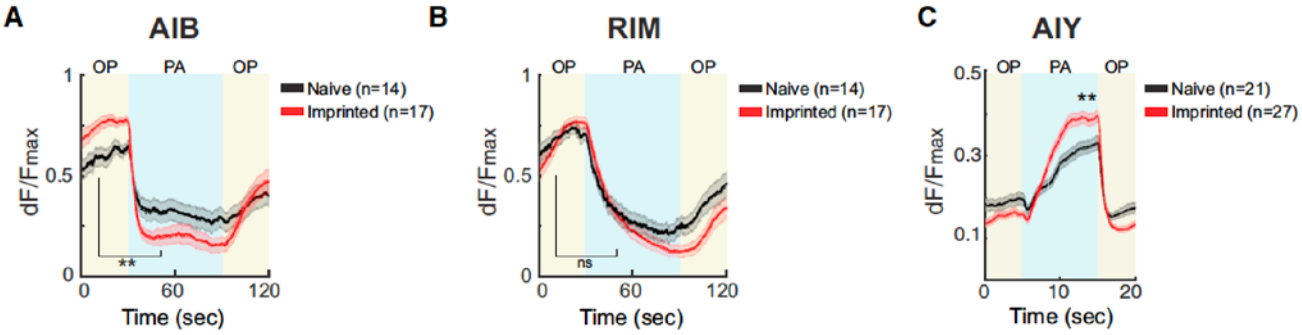




Calcium responses after aversive imprinting

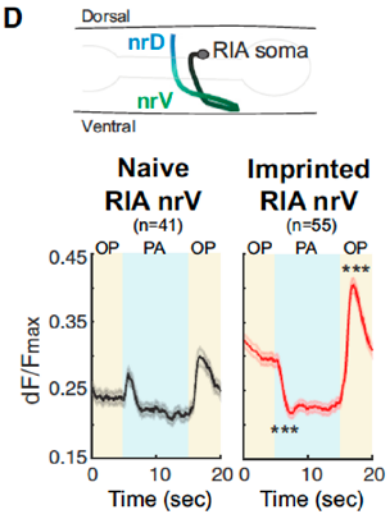


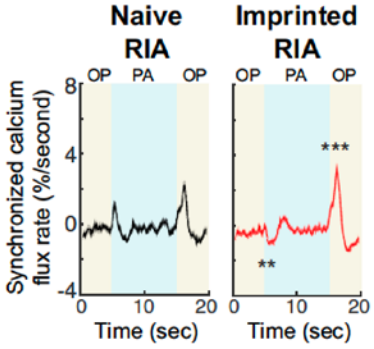
- Genetically encoded Ca^{2+} indicators in neurons of interest to detect Ca^{2+} transients



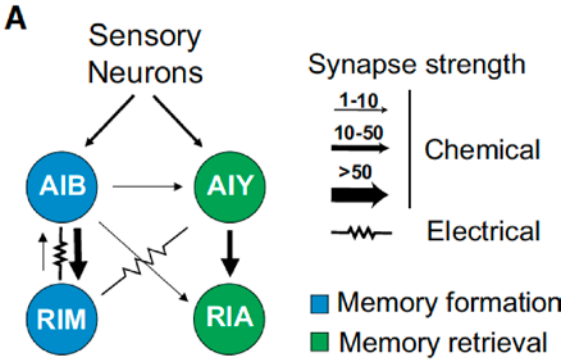
- Memory formation neurons:
  odor-responsive but similar +/- imprinting

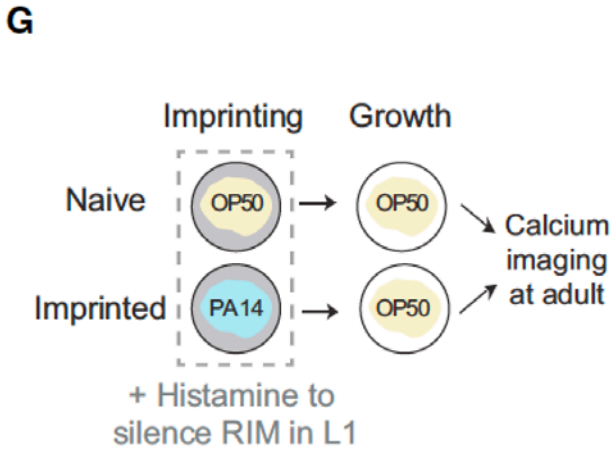
- Memory retrieval neurons:
  AIY: stronger after imprinting
  RIA: switch

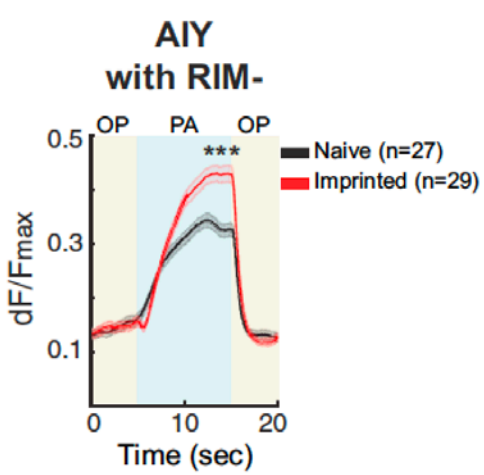


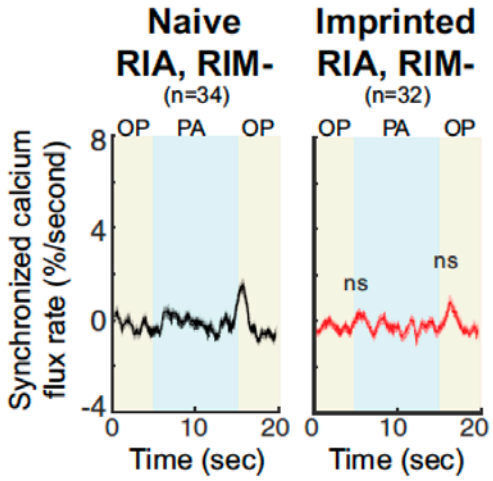


Switch in RIA depends on imprinting

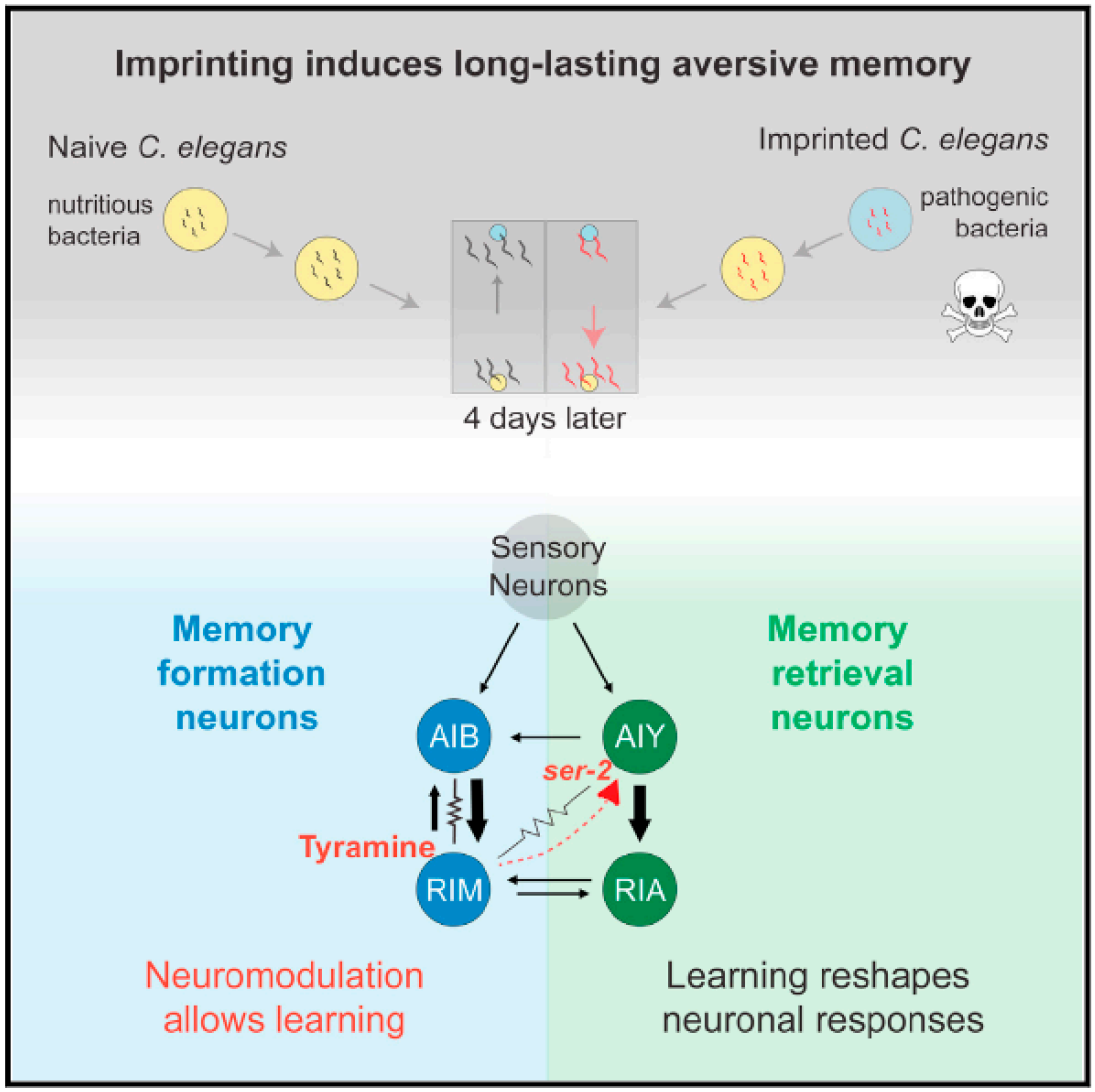








Imprinting induces long-lasting aversive memory



Thank You!