A *C. elegans* model of dopaminergic neuron development and degeneration

What can a tiny nematode teach us about human disease?

Maria Doitsidou

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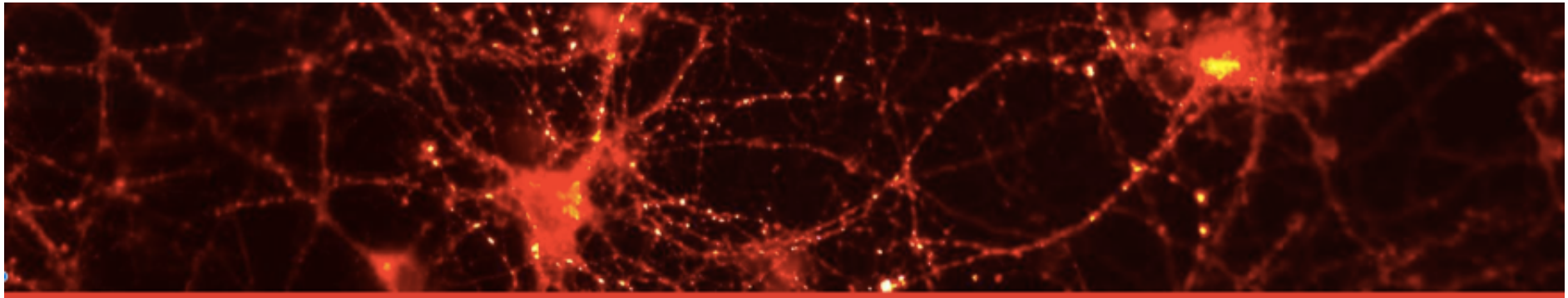
(Undergrad)

Since January 2015

THE UNIVERSITY of EDINBURGH



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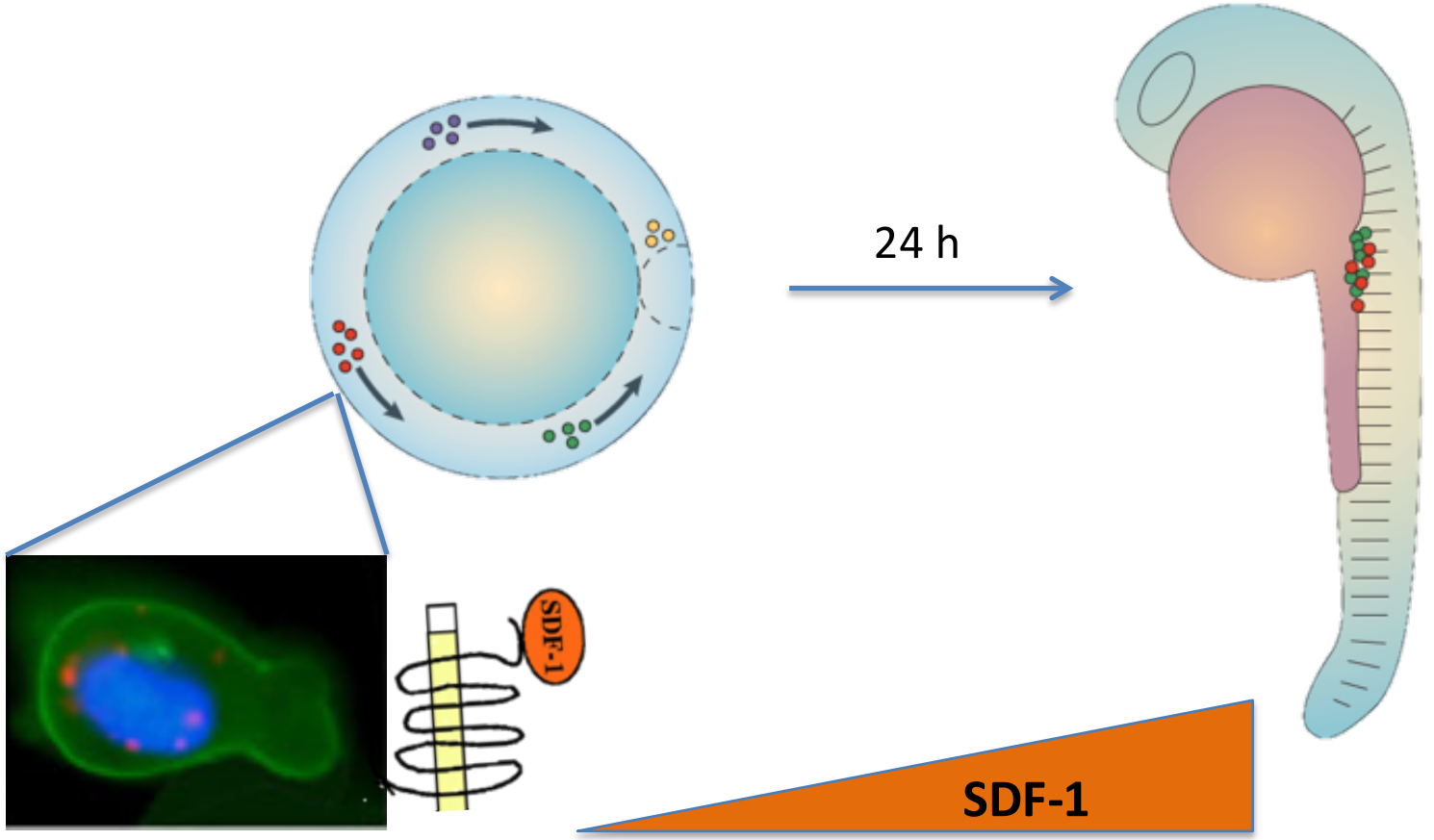
My PhD research: Germ cell migration in zebrafish

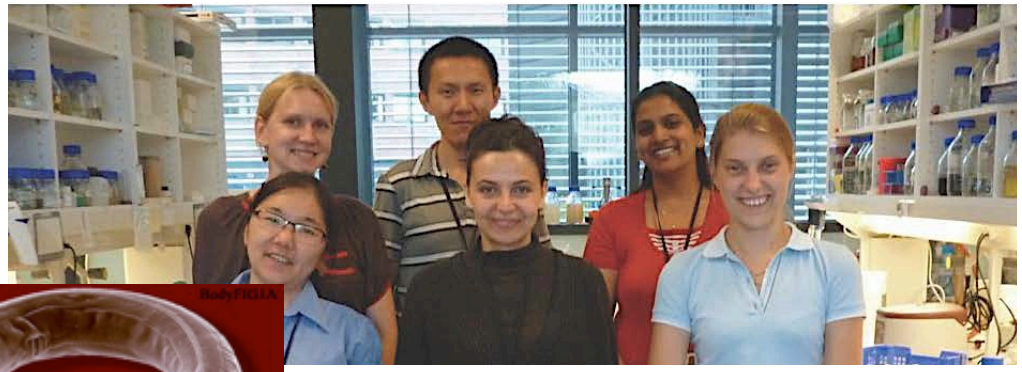


Doitsidou *et al.*, *Cell* 111(5):647-59

Columbia University
Medical Center
New York

Laboratory of Oliver Hobert

Doitsidou lab
University of Stavanger, Norway
University of Edinburgh



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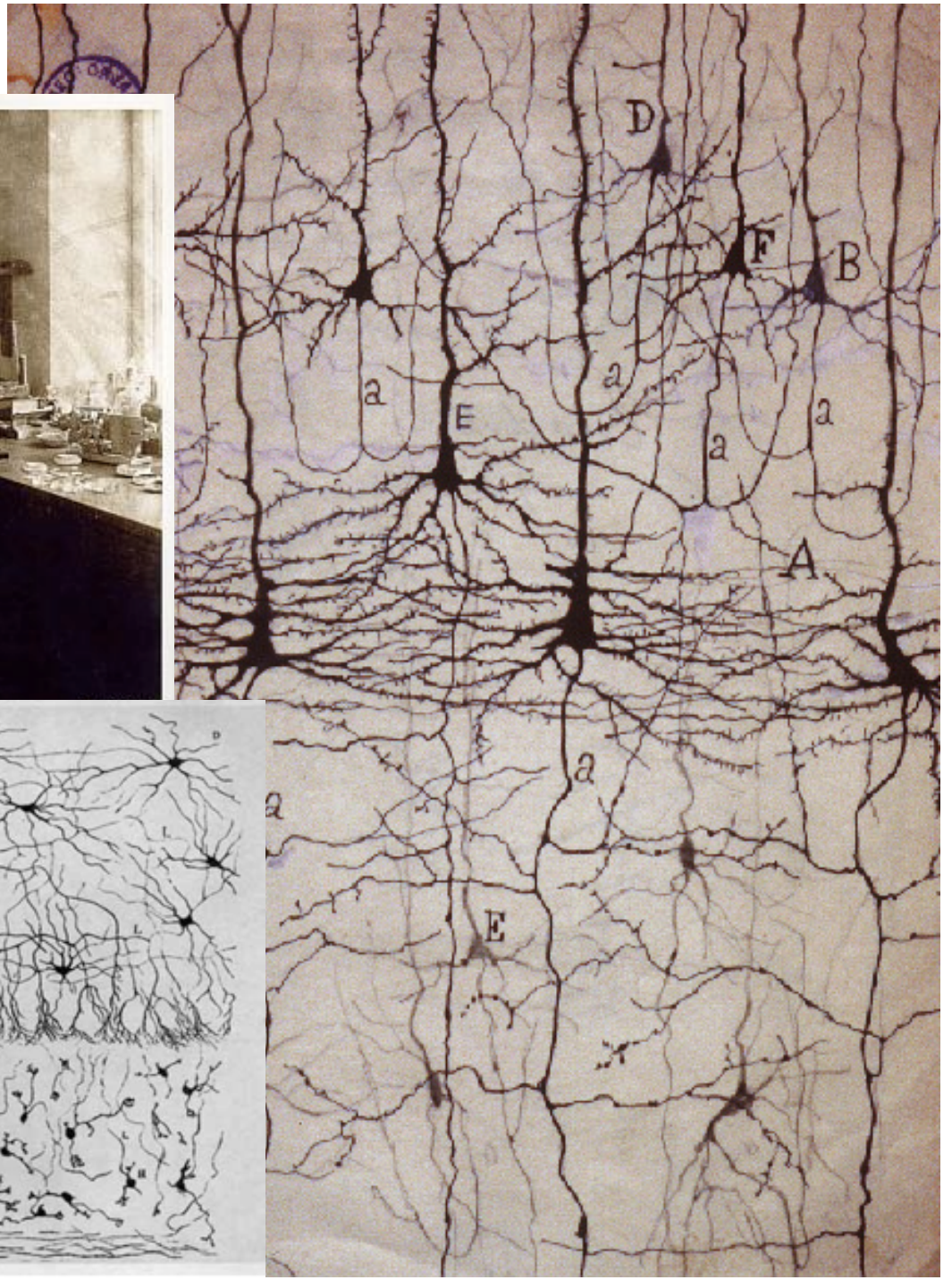
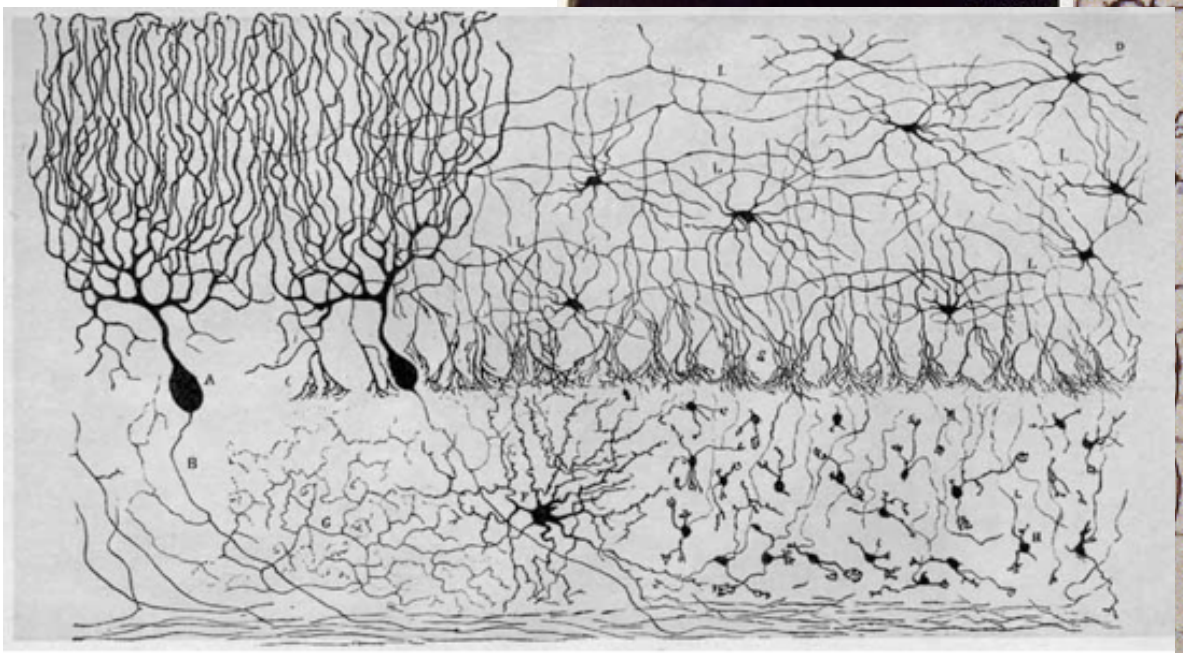
Neuronal diversity





Ramon y Cajal

My talk today

1. A short history of *C. elegans* (and a tribute to basic research)

2. The scientific questions that drive my research

3. Our findings on dopamine neuron development and degeneration. Detour: technological advances that make our research efficient

4. How we bring research findings from worms to humans

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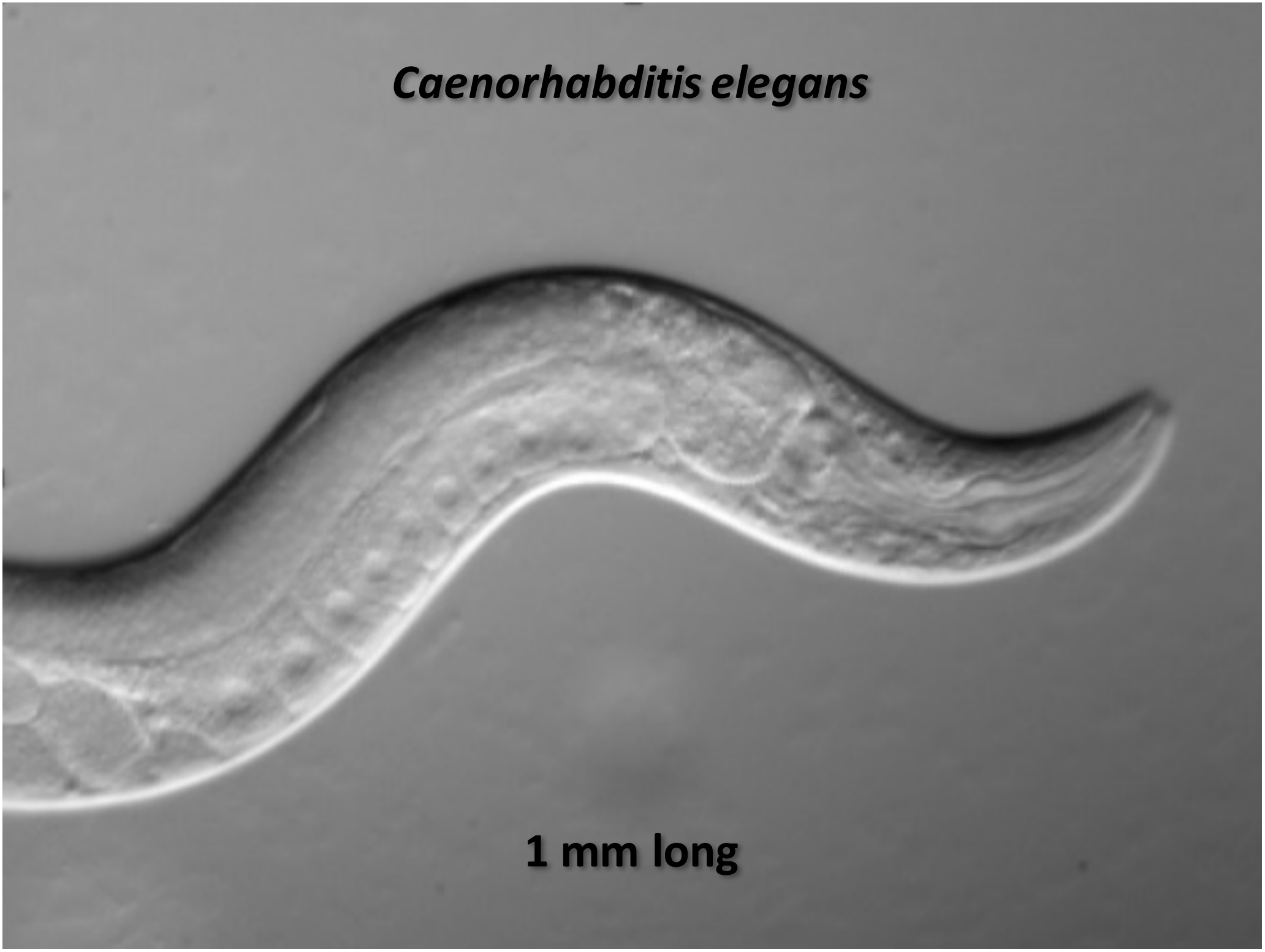
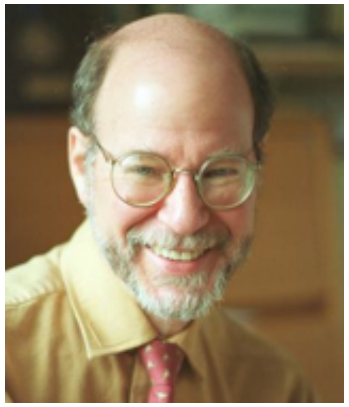

Caenorhabditis elegans
1 mm long

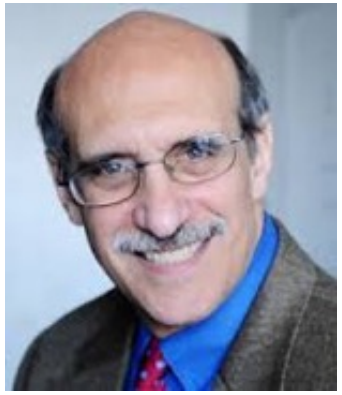
Nobel Prizes for *C. elegans* research





Nobel Prize for Physiology and Medicine
2002
Sydney Brenner
H. Robert Horvitz
John Sulston

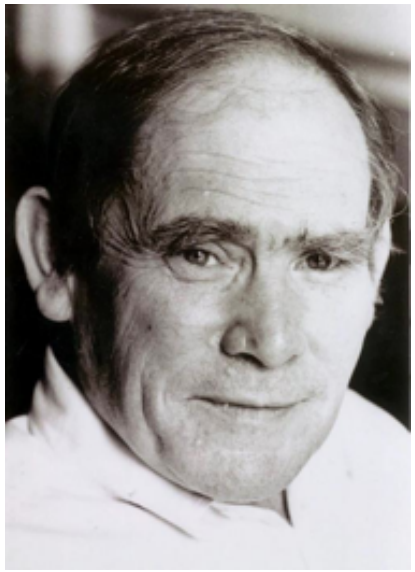
Nobel Prize for Physiology and Medicine
2006
Andrew Fire
Craig Mello



Nobel Prize for Chemistry
2008
Martin Chalfie
(with Osamu Shimamura and Roger Tsien)

But where did it all start?

1963: Sydney Brenner
Laboratory of Molecular Biology, MRC, Cambridge



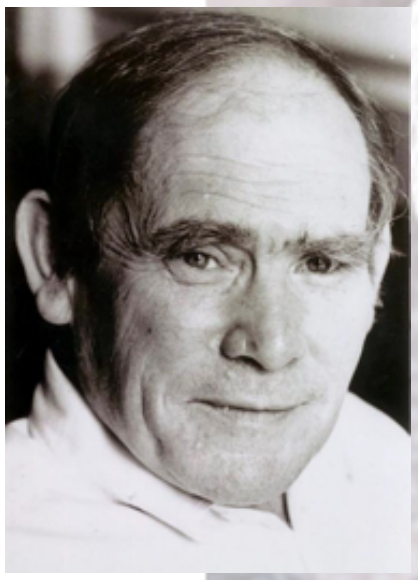
'It is now widely realized that nearly all the "classical" problems of molecular biology have either been solved or will be solved in the next decade… Because of this, I have long felt that the future of molecular biology lies in the extension of research to other fields of biology, notably **development and the nervous system.'**

'I would like **to tame a small metazoan organism** to study development directly. My ideas on this are still fluid and I cannot specify this in greater detail at the present time…'

5 months later…

Letters between Sydney Brenner and Max Perutz in 1963

How genes specify the *complex structures* found in higher organisms?



Sydney Brenner
1963

Mutation

↓

Phenotype

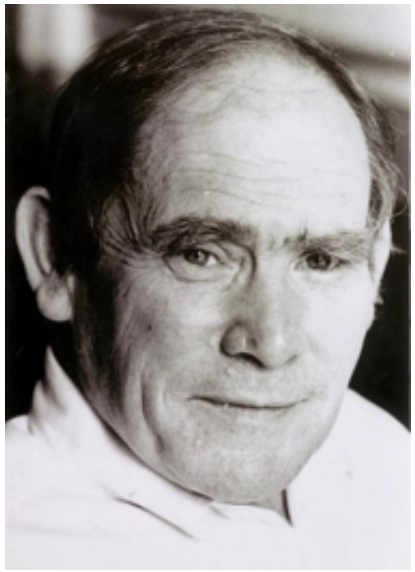
↓

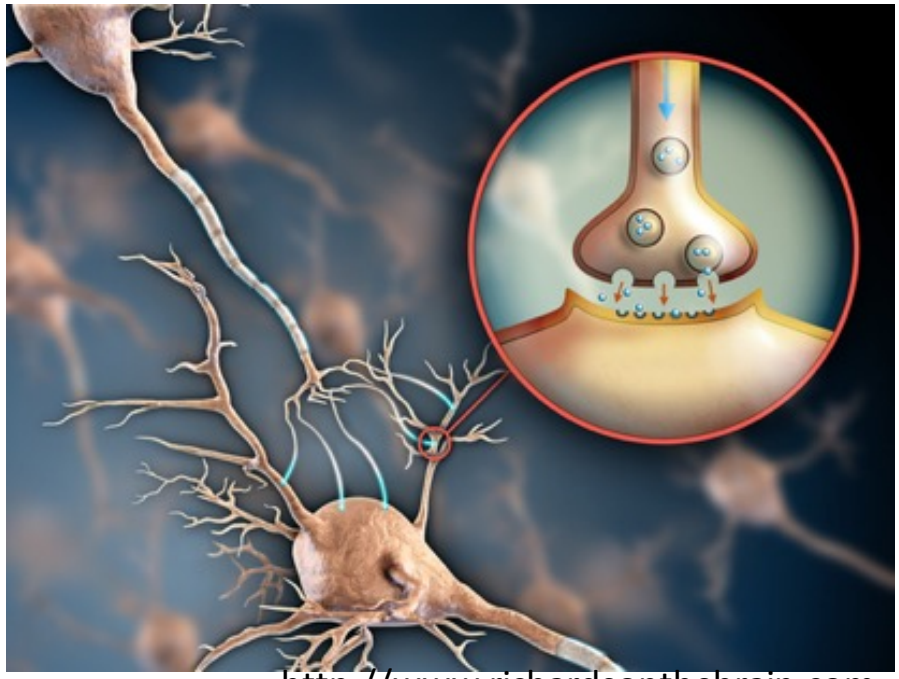
Function

Focus: **Nervous system**

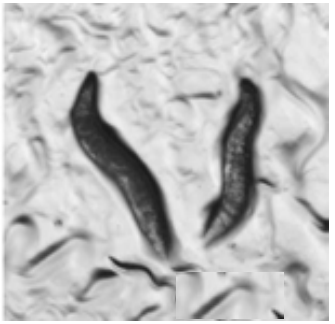
10 Years later: 1973

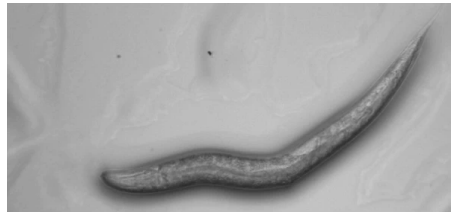
Brenner had identified 619 mutants with visible phenotypes





http://www.richardsonthebrain.com



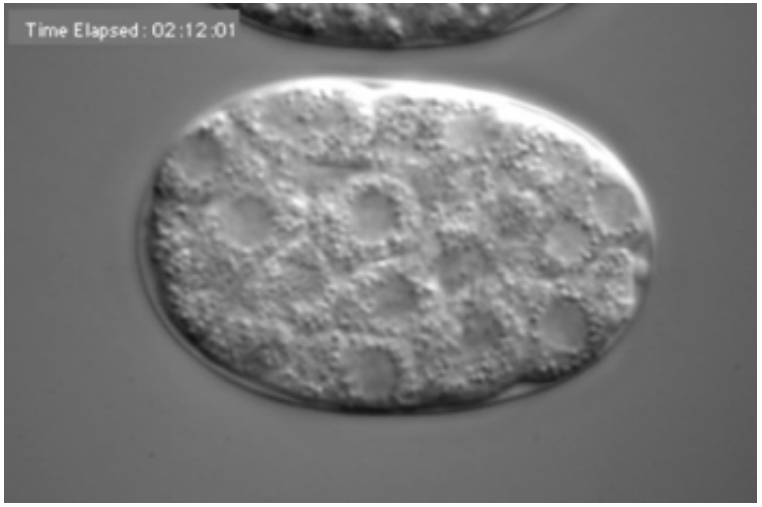


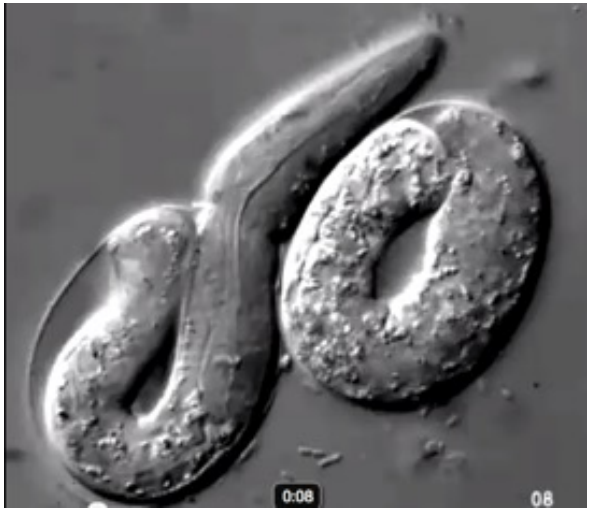
Synaptic transmission
Axon guidance
Neuronal specification

Thus, he set the foundation of developmental Neurobiology

Deciphering the cell lineage

959 cells in the adult hermaphrodite

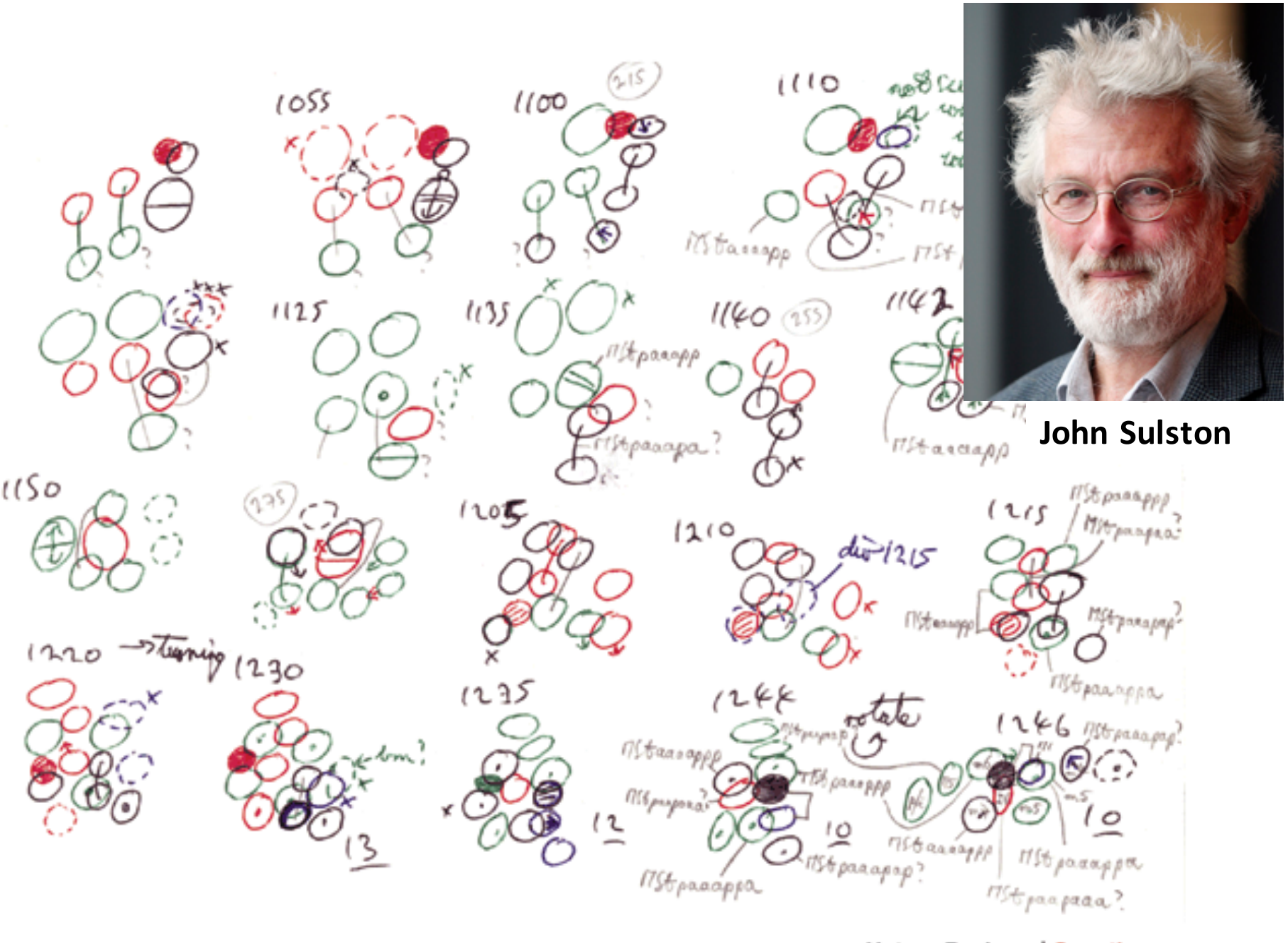




John Sulston

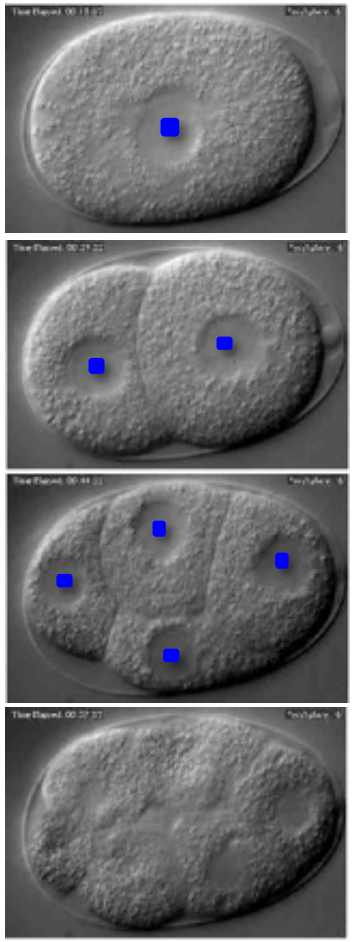
"A simple analogy is to imagine that you are watching a bowl with hundreds of grapes, trying to keep your eye on each grape as it and many others move"

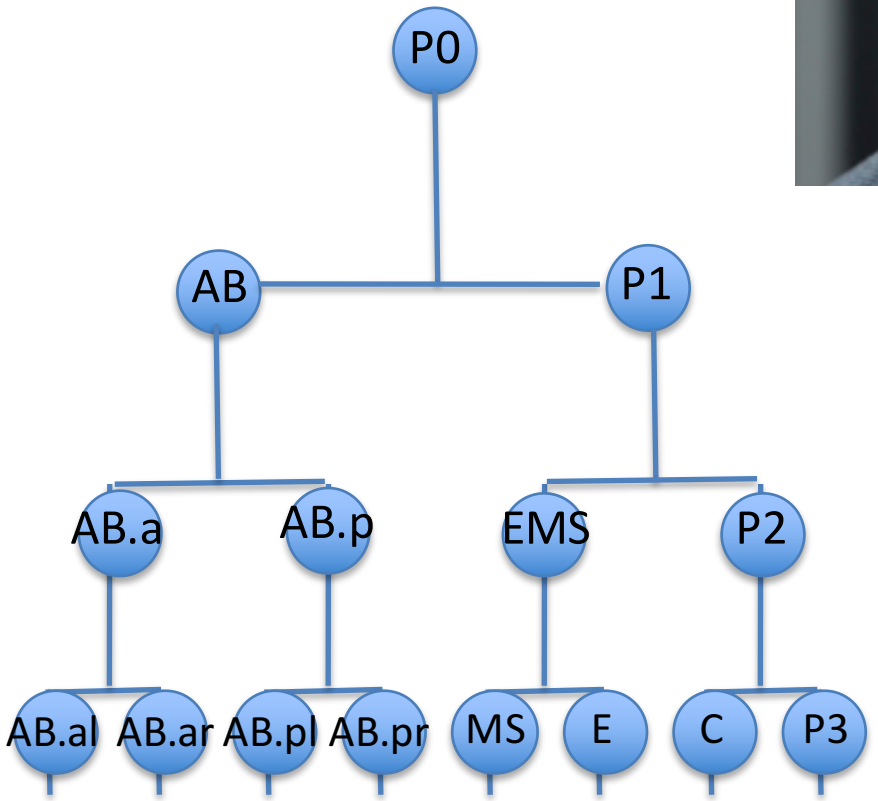
John Sulston



John Sulston

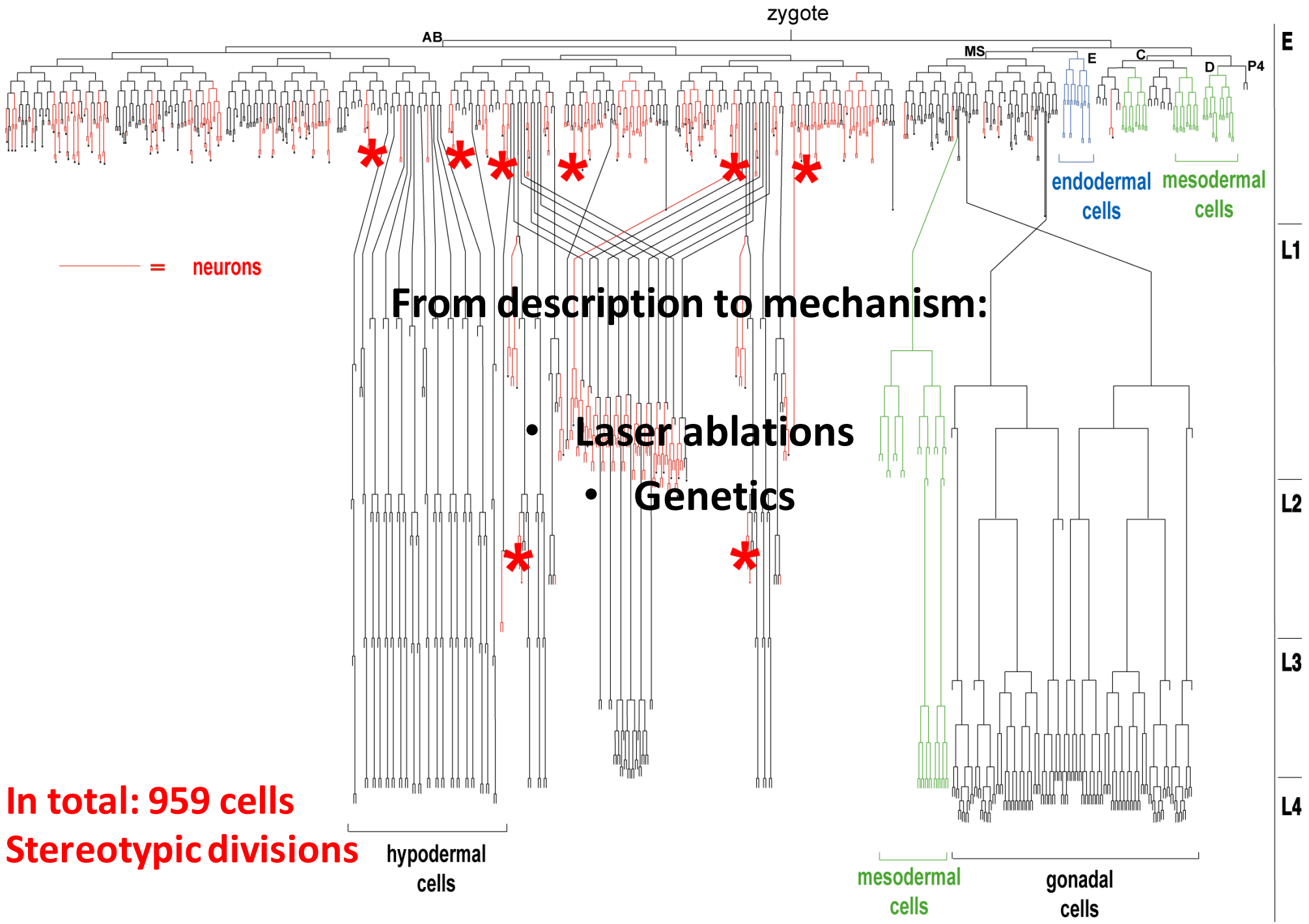
Constructing the lineage



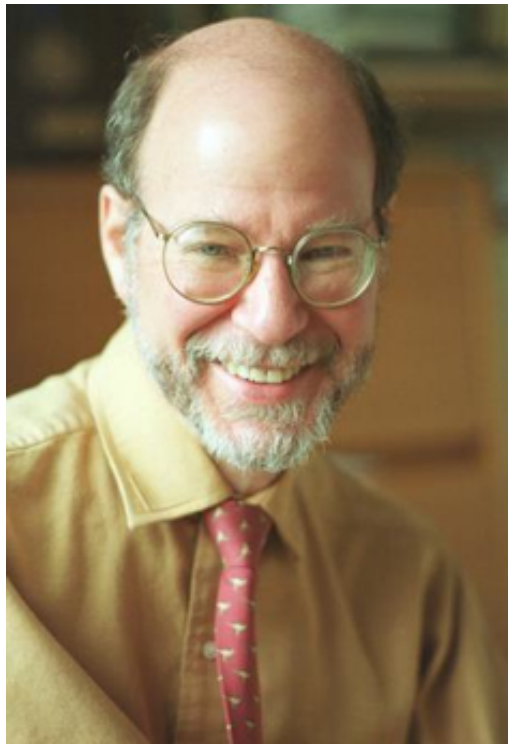


John Sulston

The first complete lineage of an organism



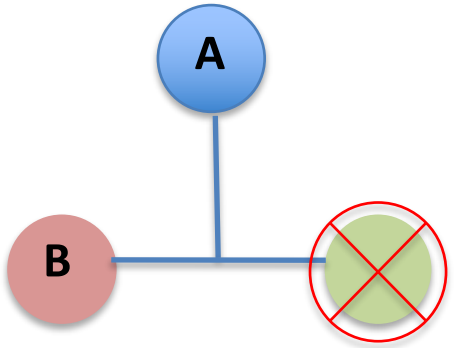
WORMS, LIFE AND DEATH



H. ROBERT HORVITZ

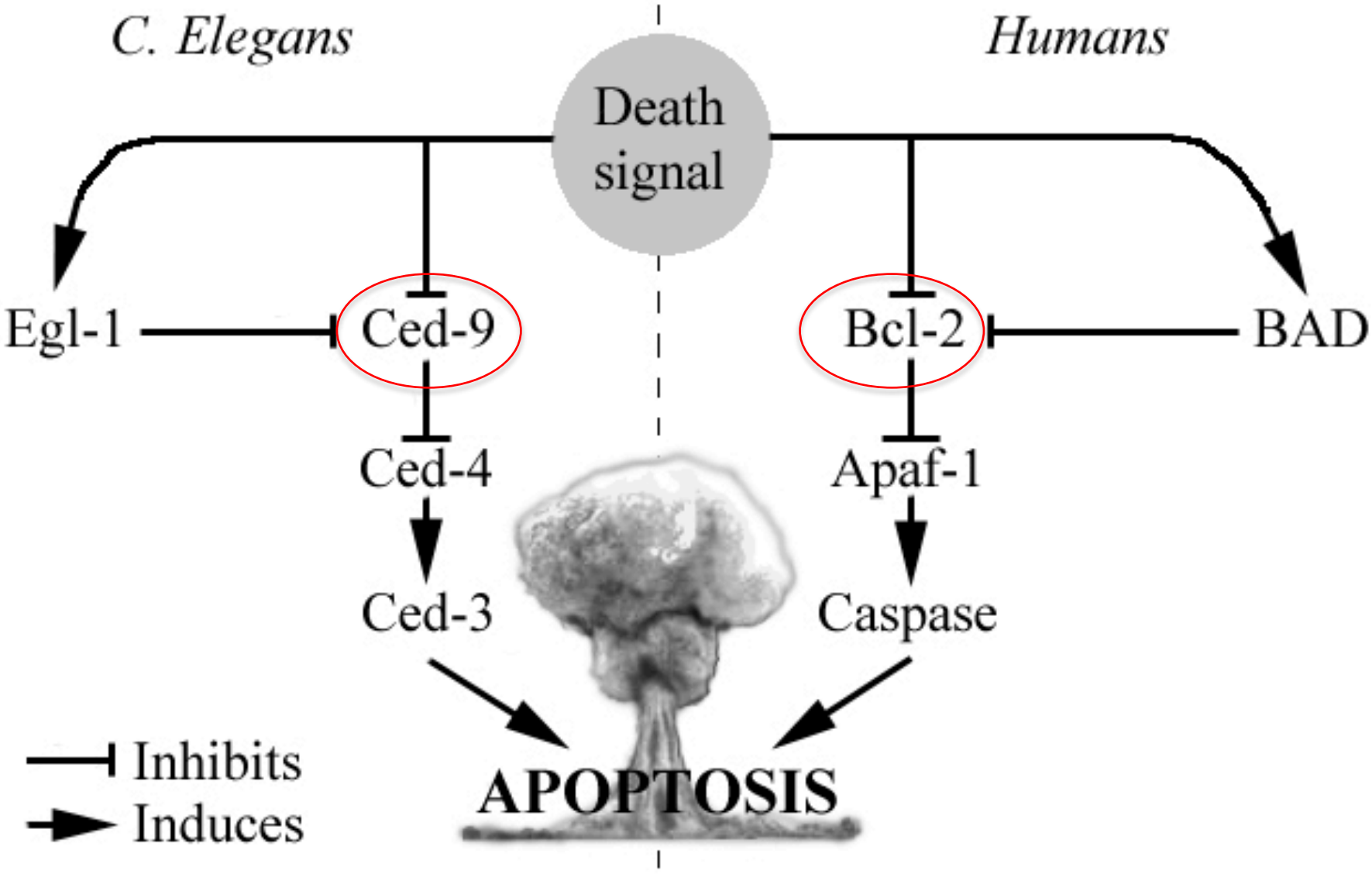
Discovered Apoptosis

Nobel Prize in Medicine

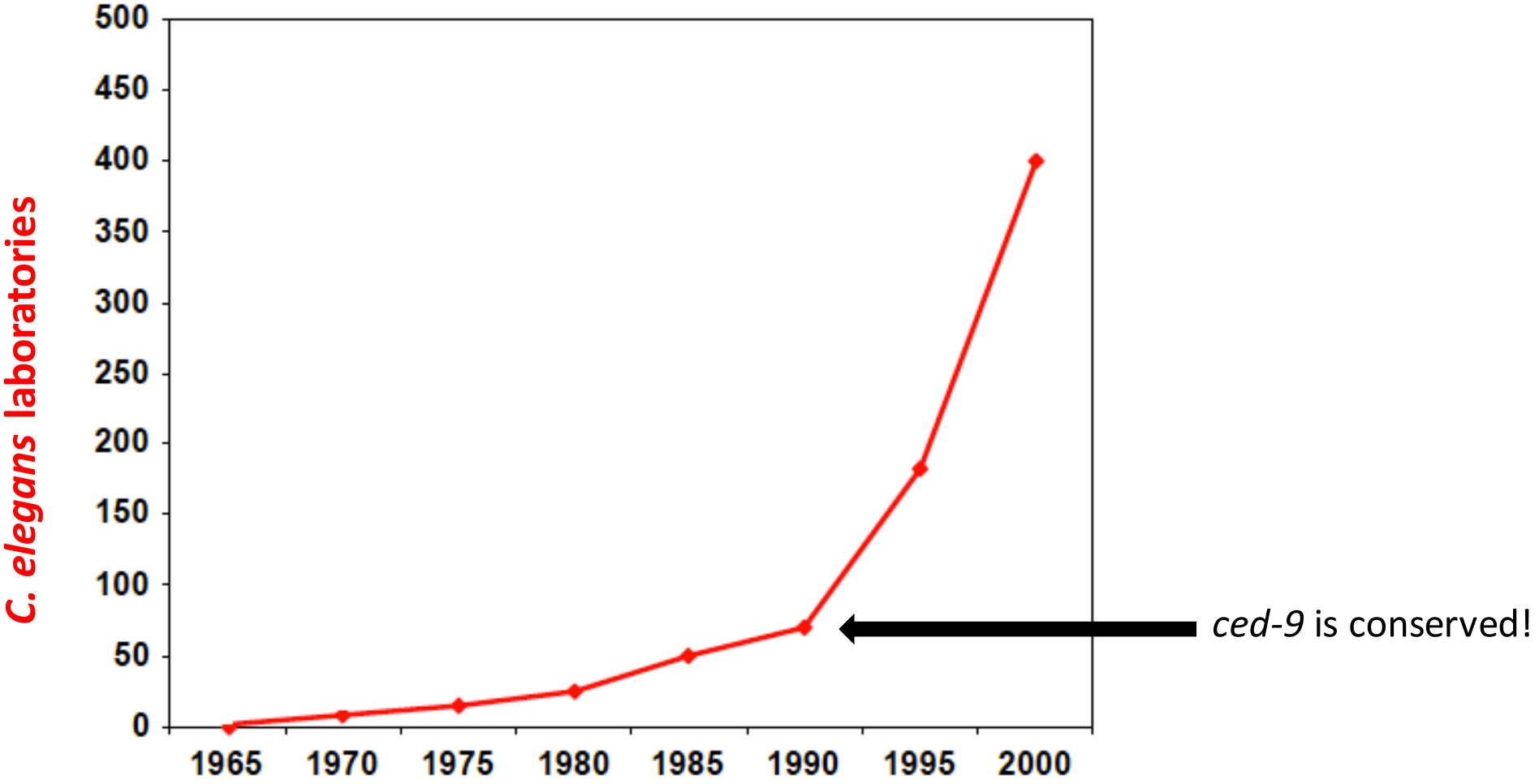


- In addition to the 959 cells found in the adult worm, another 131 cells are generated but are **NOT** present in the adult
- These 131 die in an invariable way: **ALWAYS THE SAME** 131 cells die. As if it is 'programmed'.

Genetics of Programmed Cell Death (=Apoptosis): CED-3, CED-4 and CED-9 have human counterparts.



Exponential growth of *C. elegans* research



Biological universality

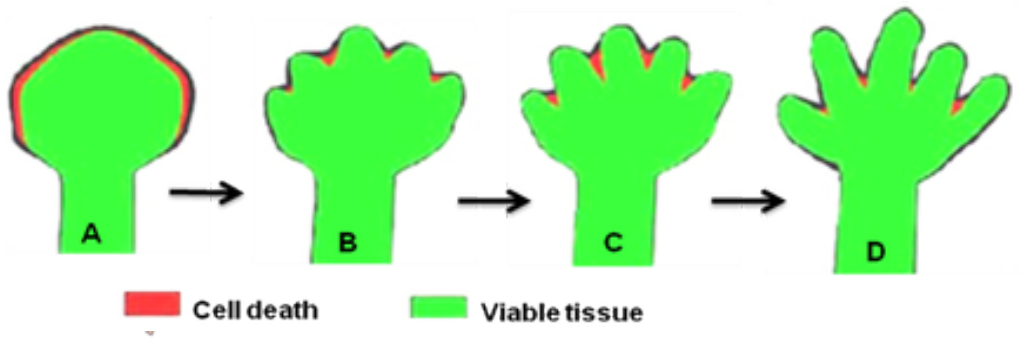
"One point that emerges from the studies of programmed cell death in *C. elegans* and other organisms is the striking similarity of genes and gene pathways among organisms that are as superficially distinct as worms and humans...
I like to refer to this theme as "**The principle of biological universality…**"

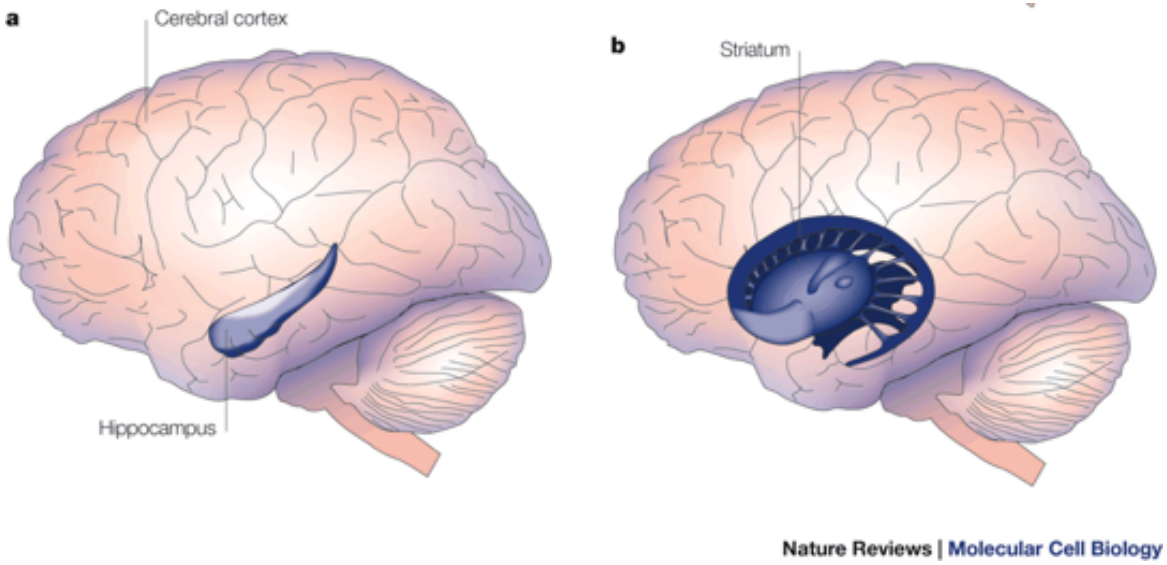
In the words of Nobel Prize winner R. Horvitz

http://nobelprize.org/nobel_prizes/medicine/laureates/2002/horvitz-lecture.html

Examples of apoptosis in humans

In Development: Finger formation





In Disease:

Neurodegenerative Diseases (too much apoptosis)

Parkinson's, Alzheimer's, Huntington's, etc.

Cancer (too little apoptosis) -Tumor cells fail to undergo apoptosis

Sydney Brenner, Bob Horvitz and John Sulston's important discoveries for medicine started...

...with studying how a tiny worm develops

Fundamental research of today fuels medicine of tomorrow

2006 Nobel Prize for Medicine:
Discovery of RNAi (RNA interference) in *C. elegans*

Andy Fire

Craig Mello

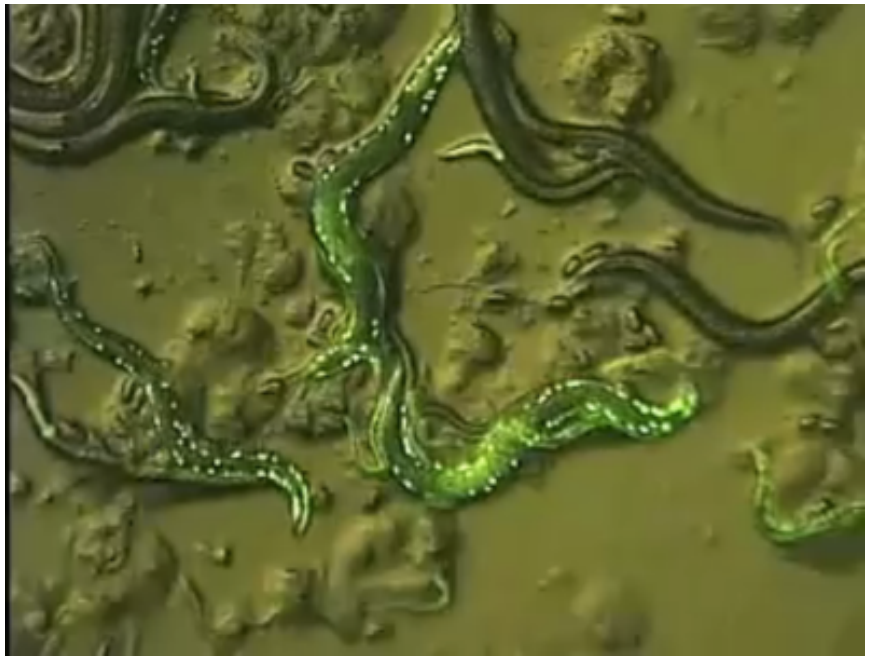
Importance: gene regulation, immunity

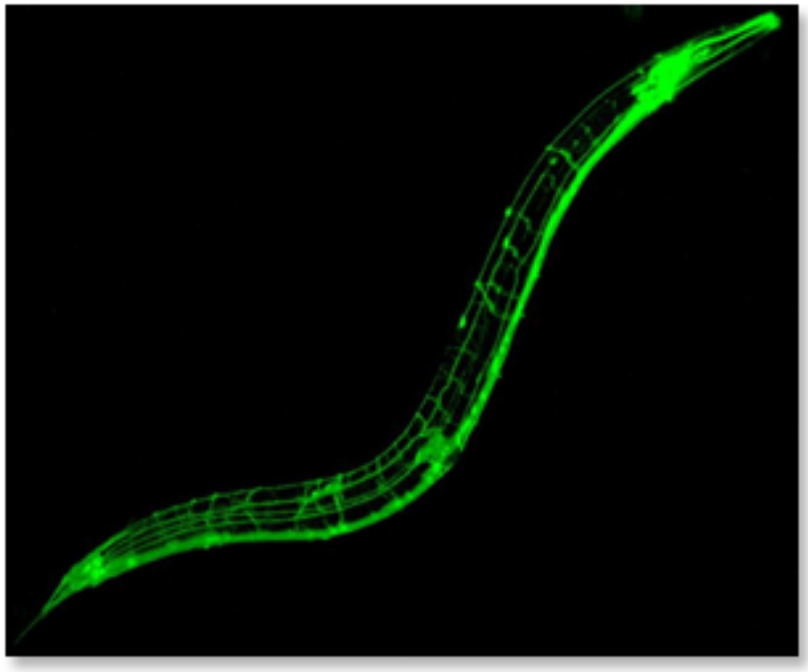
Aequorea victoria

Lighting up life: GFP

Marty Chalfie, Nobel Prize in Chemistry, 2008

C. elegans transparency and use of GFP





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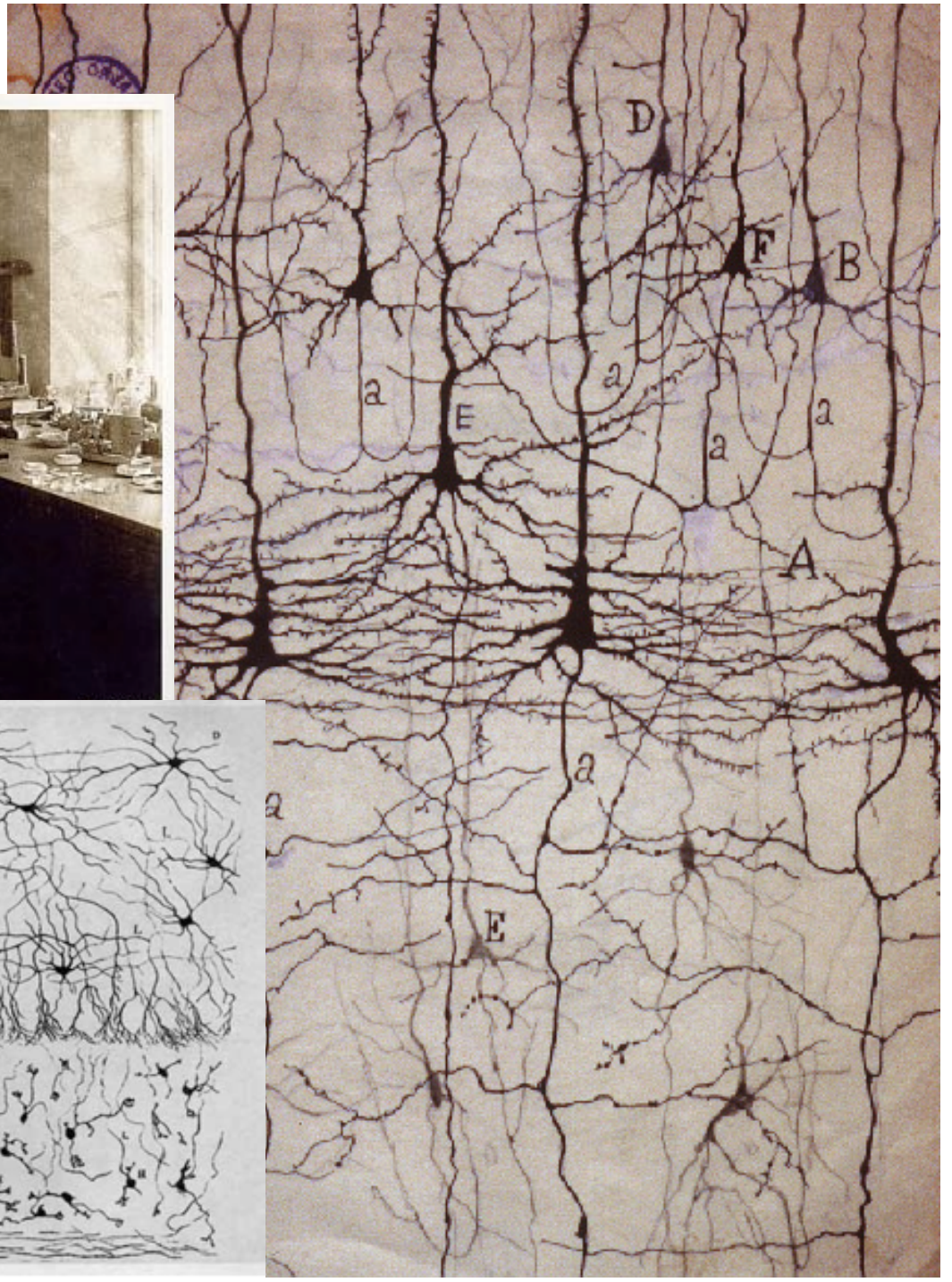
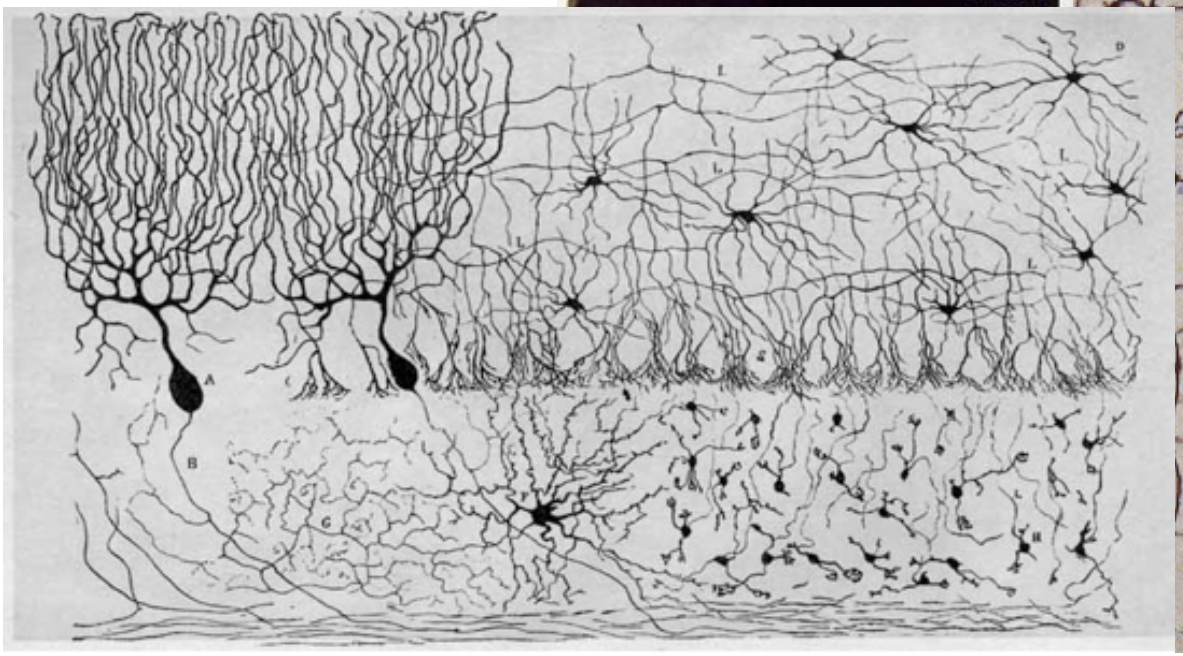
How is neuronal diversity generated?

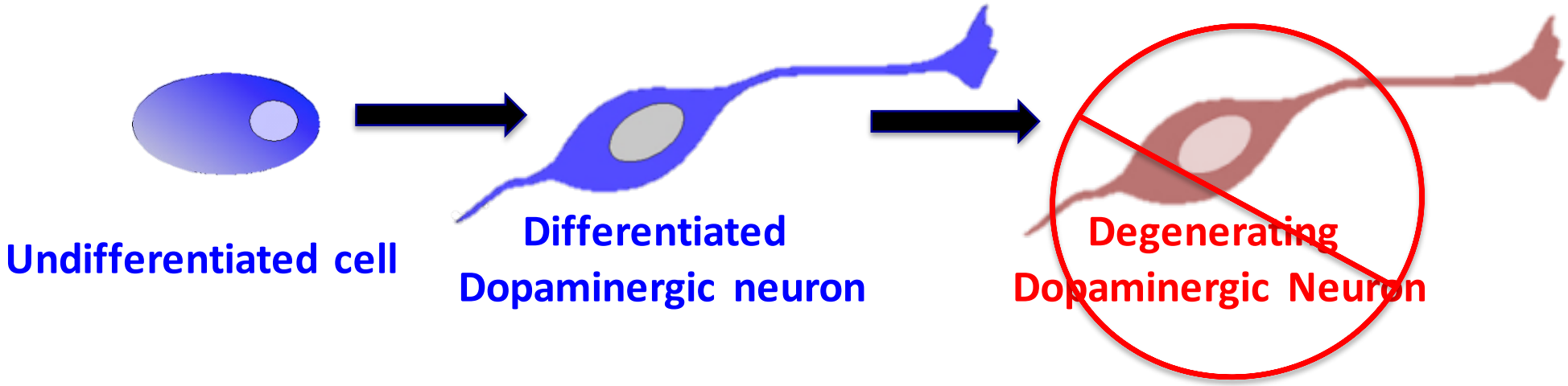




Ramon y Cajal

What cell fate programs coordinate neuronal differentiation?

Which molecular mechanisms govern neuronal degeneration?



Why choose a simple model organism to study these questions?

Complexity of the nervous system

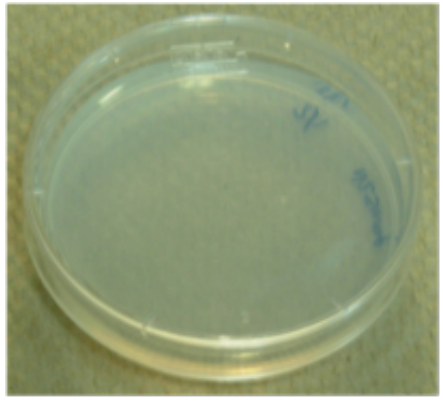
>100 billion neurons

>100 billion stars

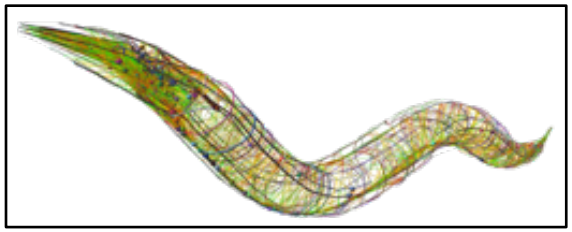
Model organism biology:
Complexity vs. Genetic tractability




Organism	Generation time	# off spring	# organisms/m^3
C. elegans	3 days	300-1000	10.000.000
Drosophila	10 days	500/2	1.000.000
Mouse	3 months	8/2	100
Humans	25 years	2.4/2	0.01



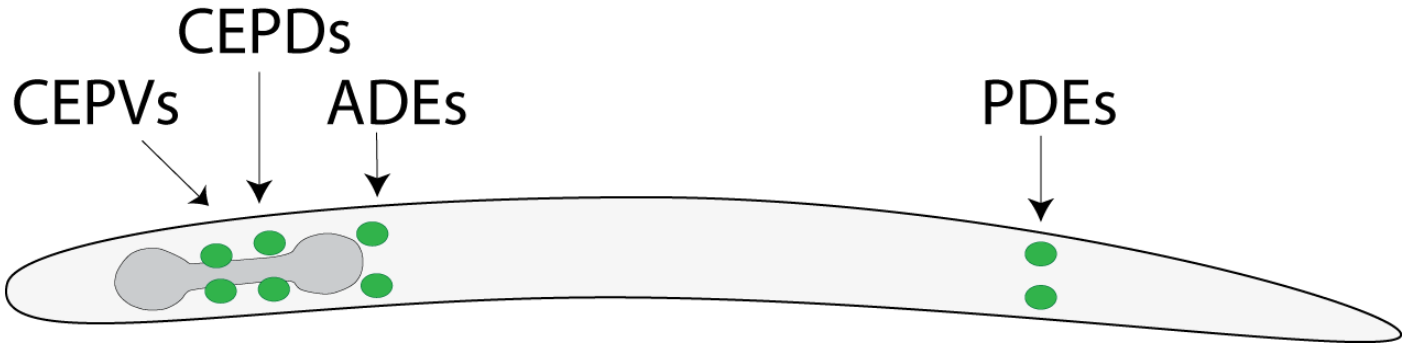

A simple nervous system



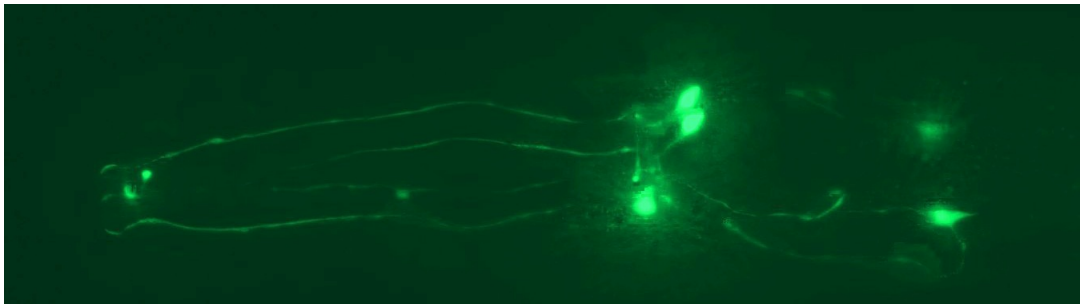
- Exactly 302 neurons

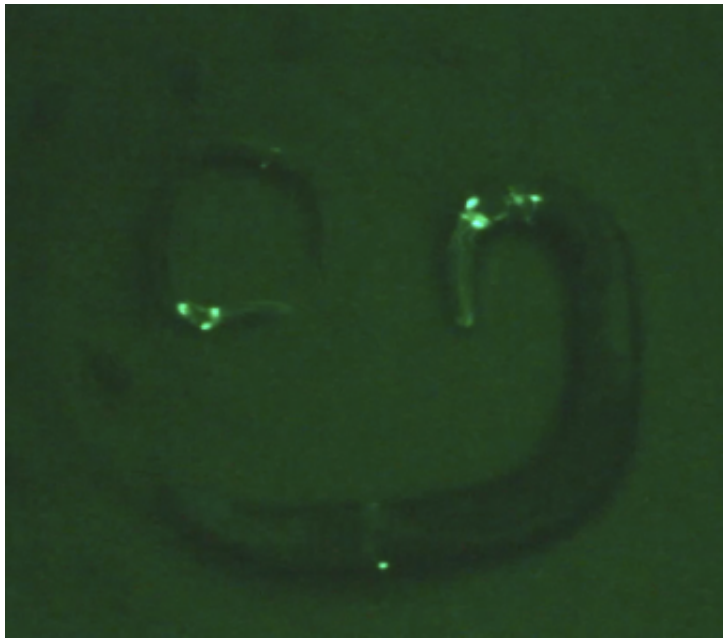
- ~ 7000 synapses, connectome reconstructed
- Tolerates nervous system defects
- Conservation of biological mechanisms

C. elegans dopaminergic system

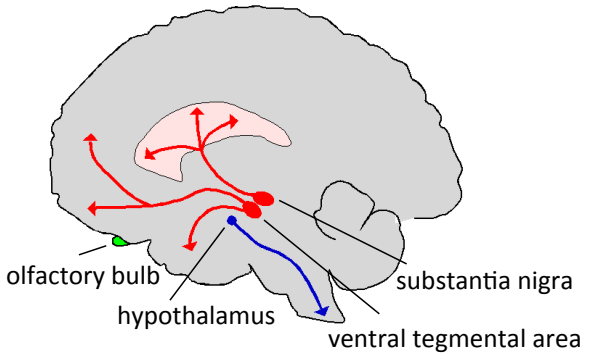


8 dopaminergic neurons





C. elegans dopaminergic system



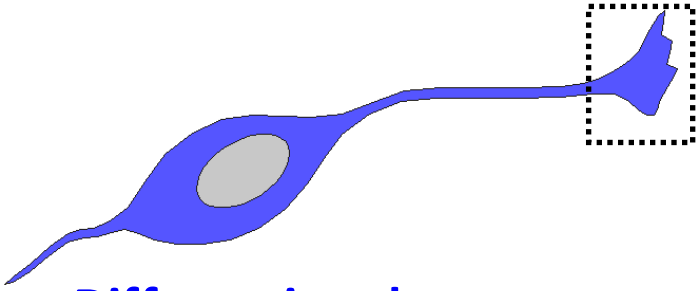
8 dopaminergic neurons

- Locomotion behavior
- Adaptation
- Associative and non-associative learning
- Goal oriented behaviors

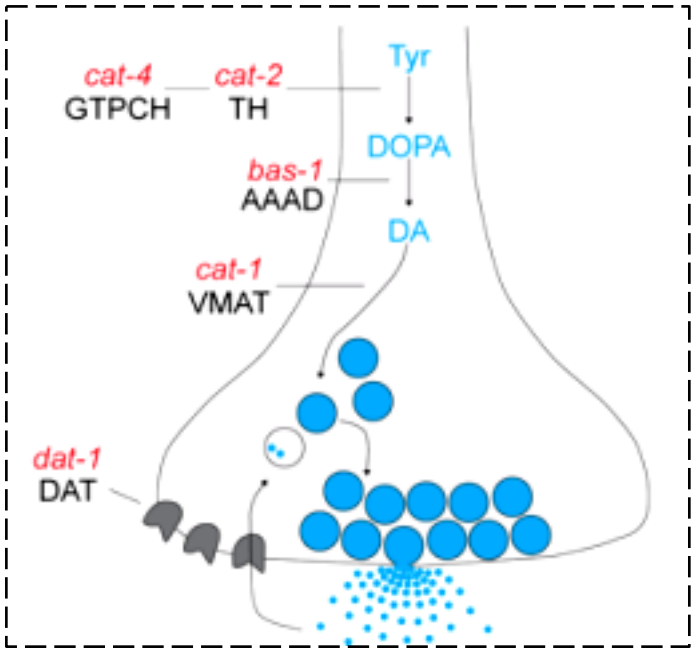
400,000-600,000 dopaminergic neurons

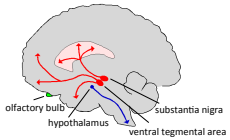
- Coordination of movement
- Memory
- Learning
- Motivation
- Reward

What is a dopaminergic neuron ?



Differentiated dopaminergic neuron



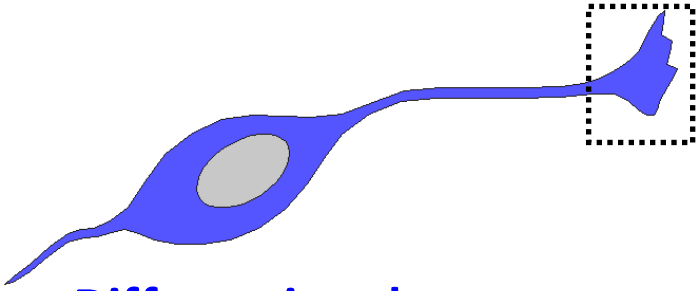


Dopamine pathway genes:

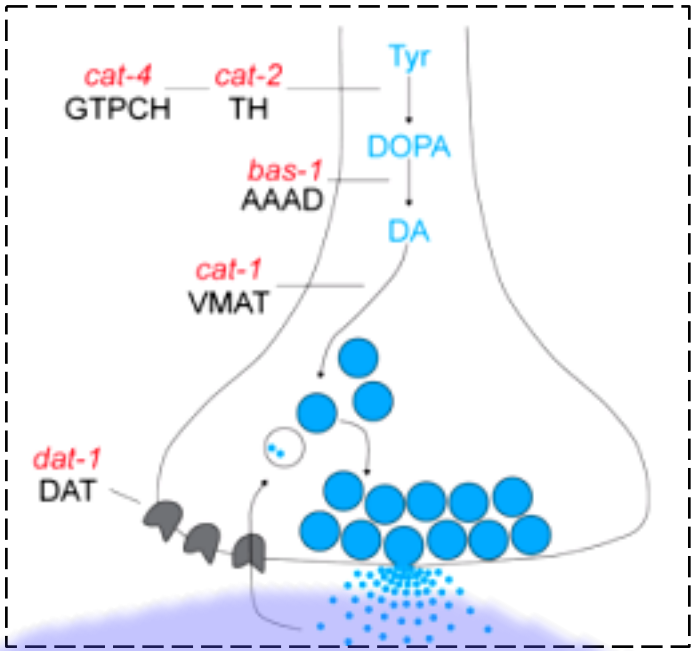
TH: tyrosine hydroxylase
GTPCH: GTP ciclo hydrolase
AAAD: aromatic L-amino acid decarboxylase
VMAT: vesicular monoamine transporter
DAT: dopamine transporter

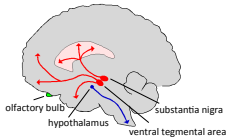
How is the expression of dopamine orchestrated?

What is a dopaminergic neuron ?



Differentiated dopaminergic neuron





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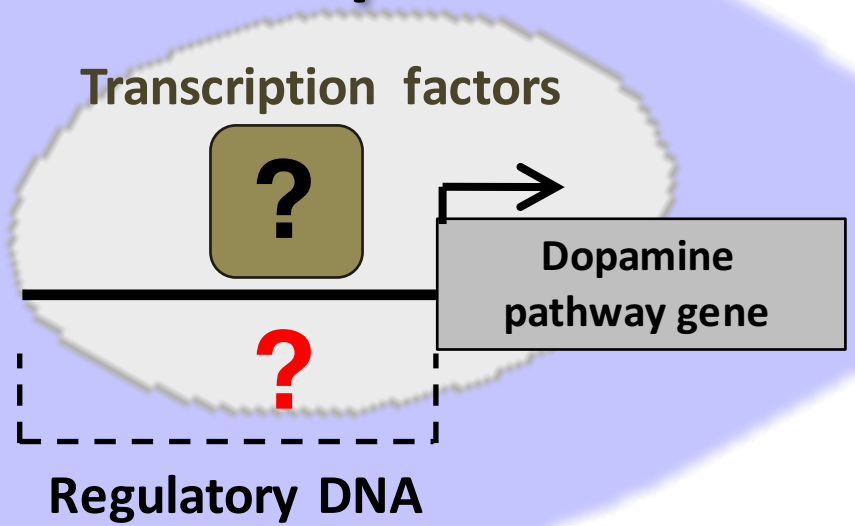
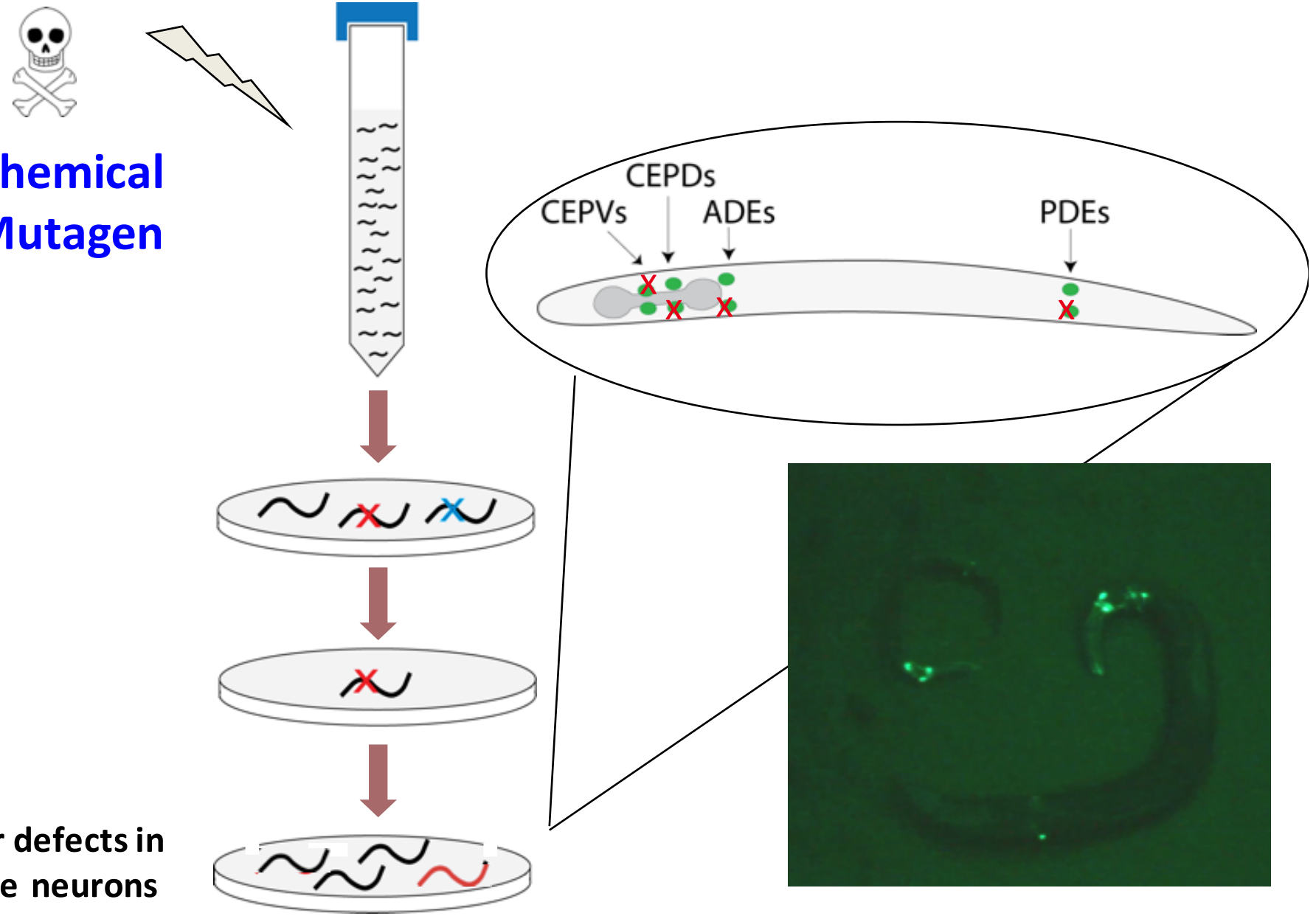
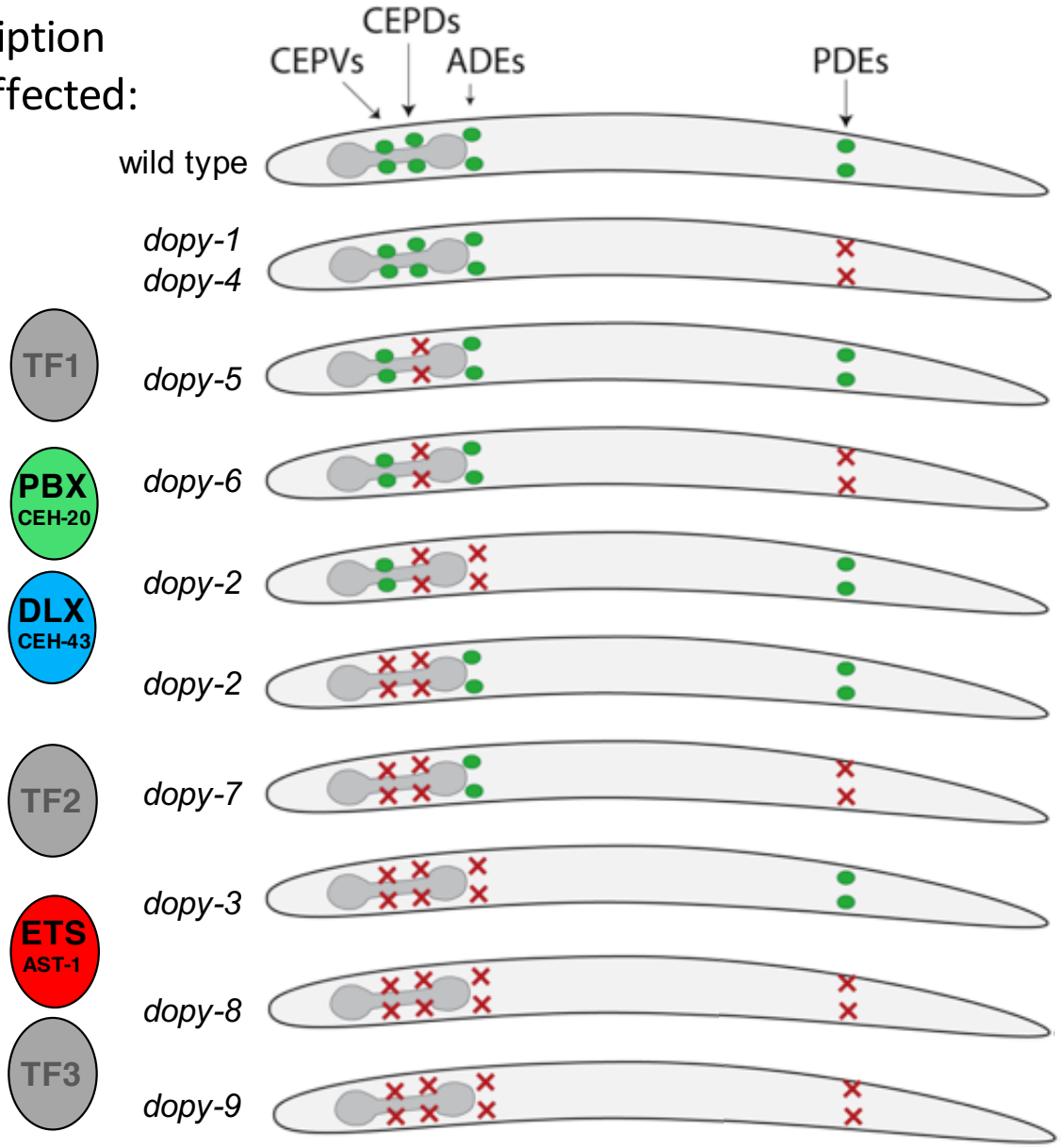


Dopaminergic neuron genetic screen



Mutants with abnormal dopamine neurons (*dopy*)

Transcription factors affected:

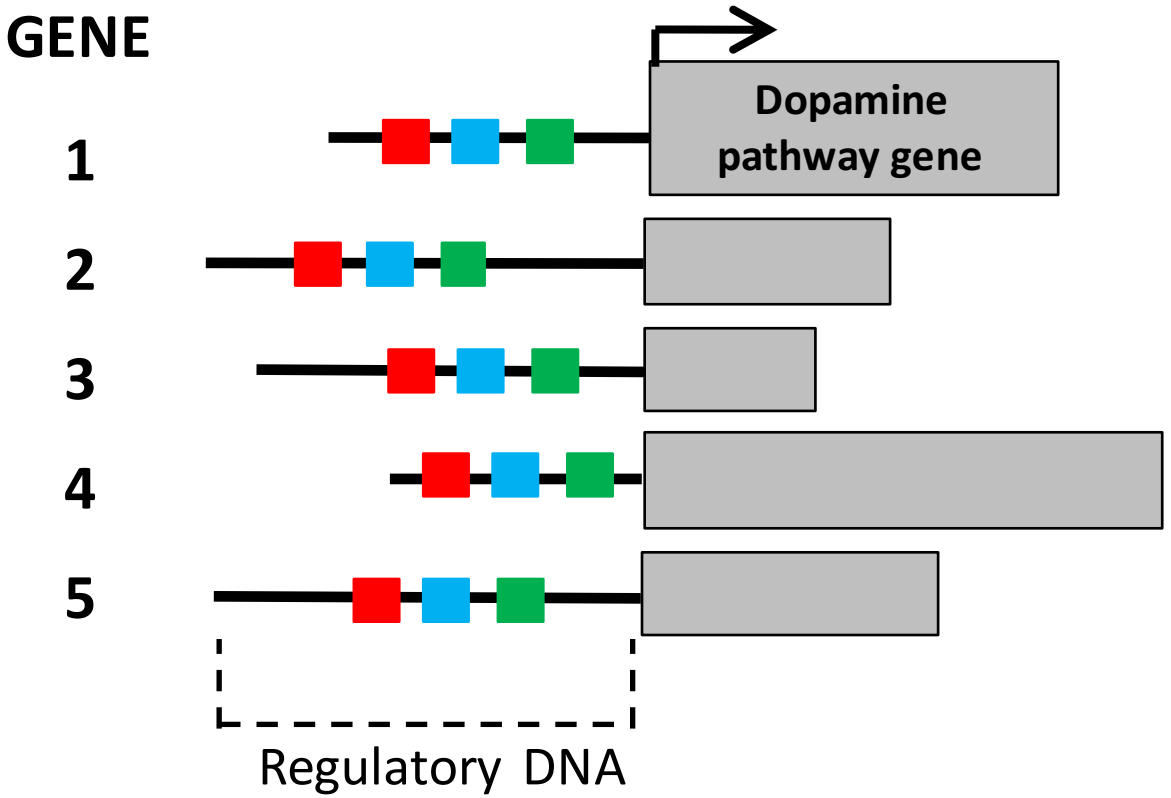


● :dopamine neuron

X :failed to specify

From: **Doitsidou** *et al., Nature Methods, 2008*

Dissecting the regulatory DNA in the dopamine pathway genes

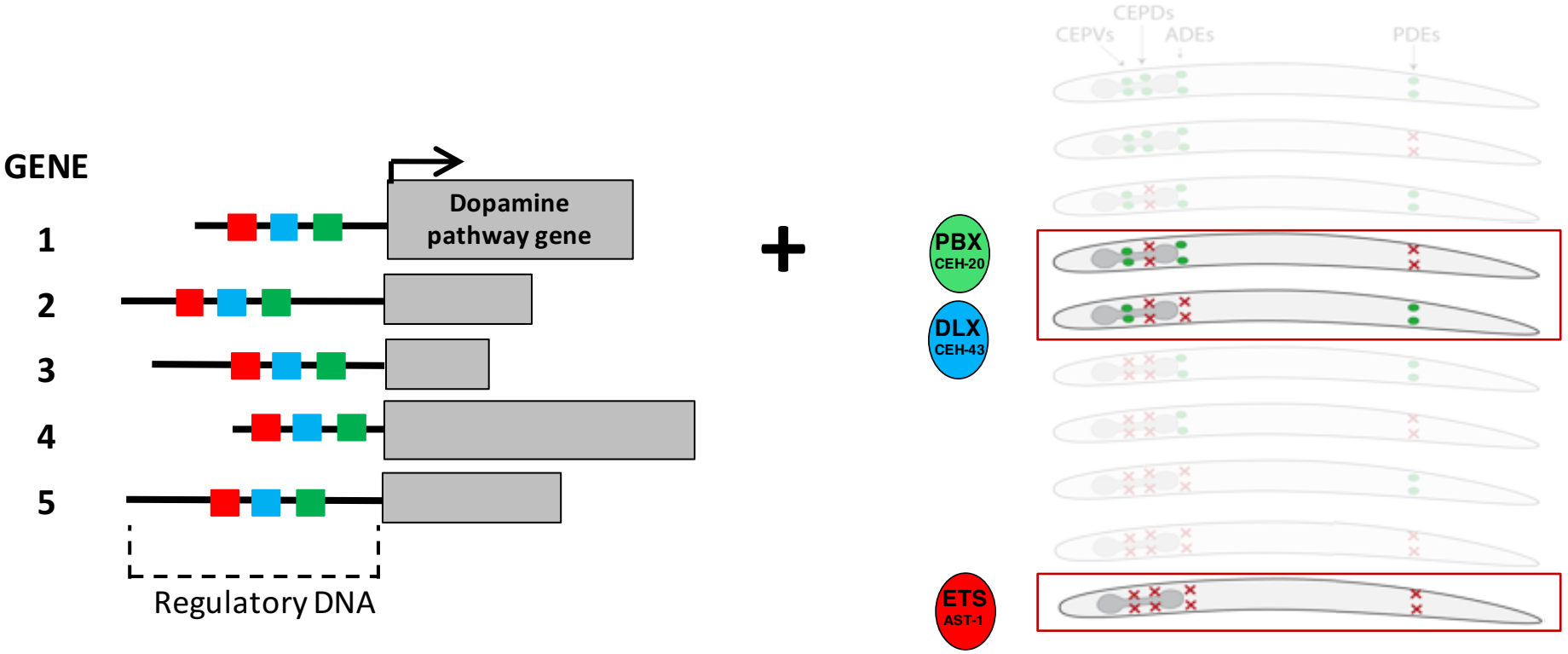


3 Indispensable Motifs: transcription factor binding sites

ETS site Homeodomain site PBX site

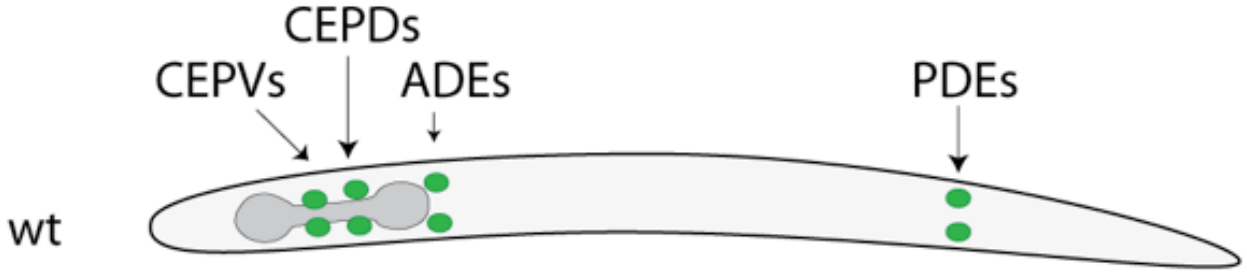
Doitsidou *et al.*, *Genes and Dev, 2013*

Combining the information from the two approaches

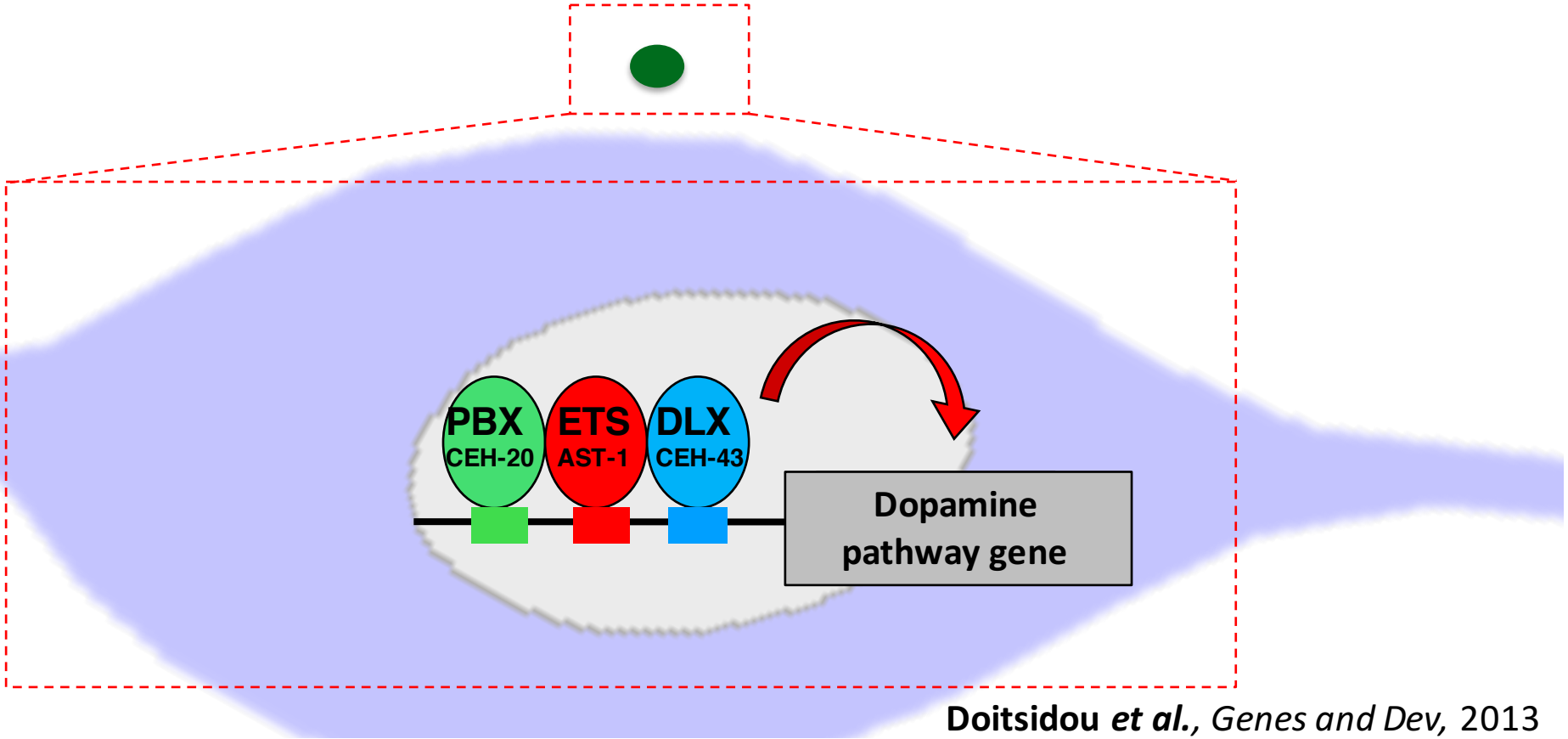


Binding sites	Transcription factors
ETS site Homeodomain site PBX site	ETS AST-1 *ast-1/Ets* DLX CEH-43 *ceh-43/Dxl* PBX CEH-20 *ceh-20/Pbx*

The expression of dopamine is orchestrated through a combinatorial logic

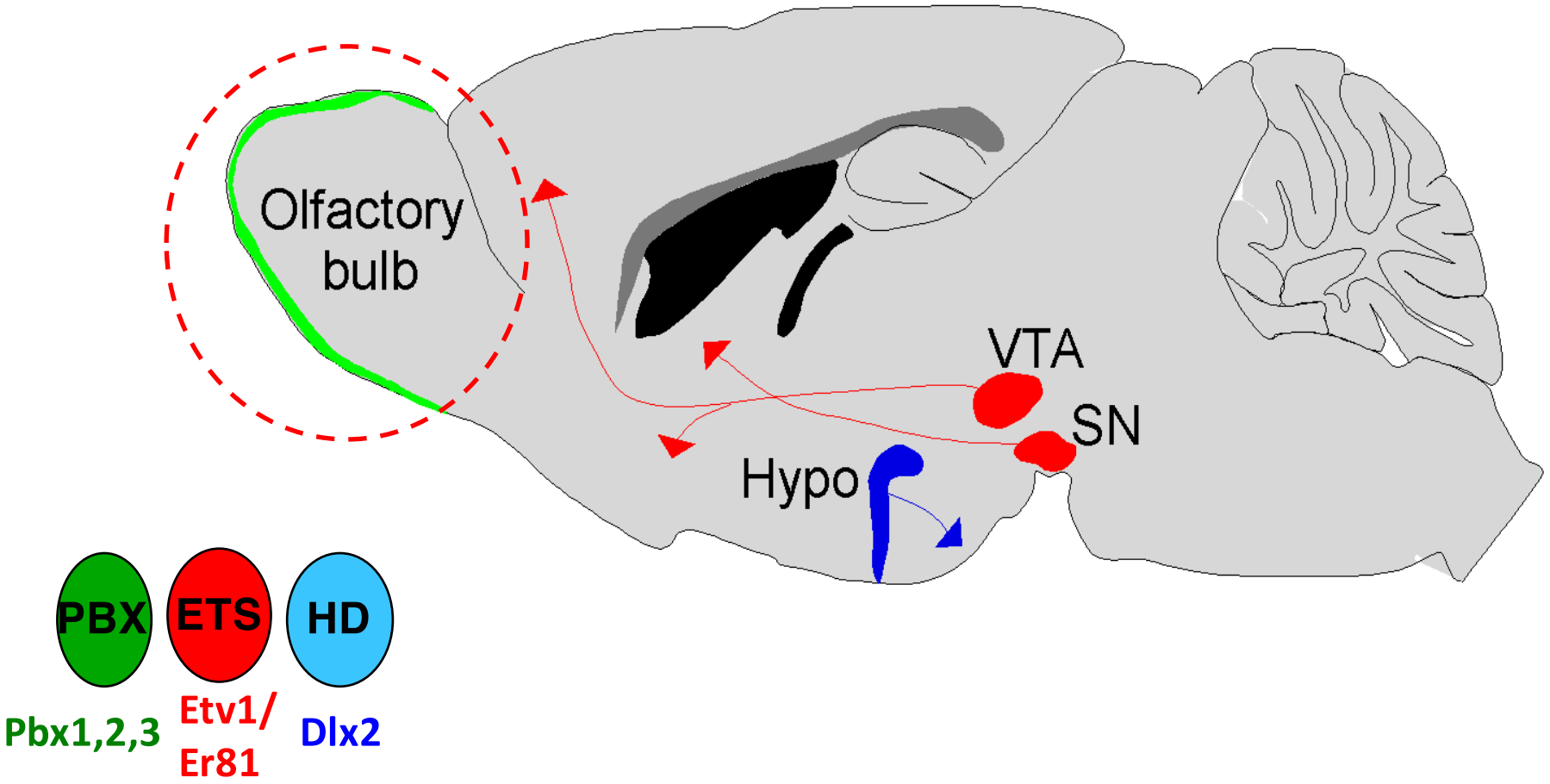


Dopamine neuron



Doitsidou *et al.*, *Genes and Dev,* 2013

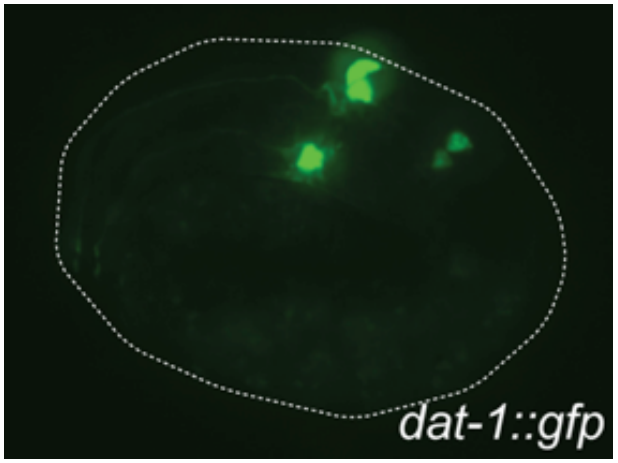
Combinatorial regulation of mouse olfactory bulb dopaminergic neurons?



Qiu *et al.* 1995, Brill *et al.* 2008, Cave *et al.* 2010, Flames *et al.* 2009, Doitsidou *et al.* 2013

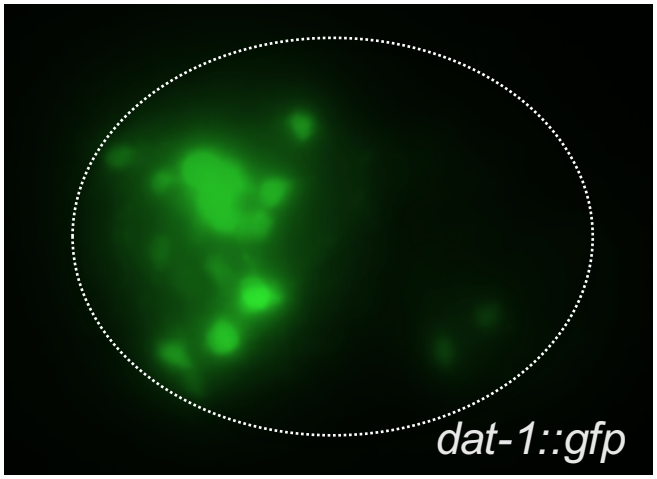
Generating ‘extra’ dopamine neurons

C. elegans embryo

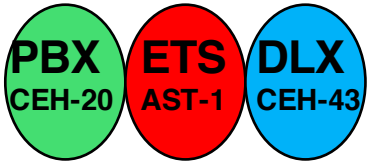


Normal

C. elegans embryo



+ Ectopic



Expressing these transcription factors in cells that would not normally become dopamine neurons, induces dopaminergic fate

Detour

Technology implementation for high-throughput genetics

The power of forward genetics

Unbiased approach

- Unexpected findings
- Hypomorphic mutations
- Gain of function mutations
- Synthetic mutations

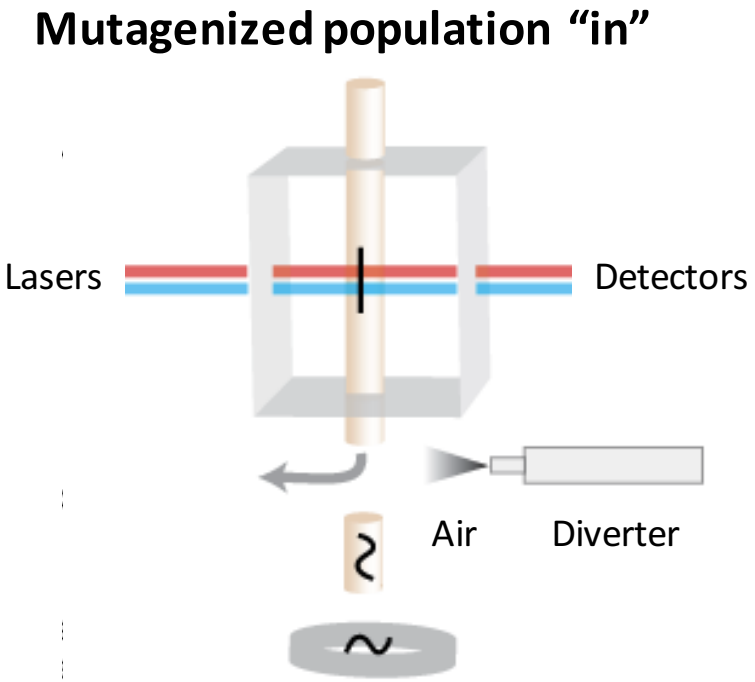
2 Bottlenecks:

Mutant isolation & identification of molecular lesion

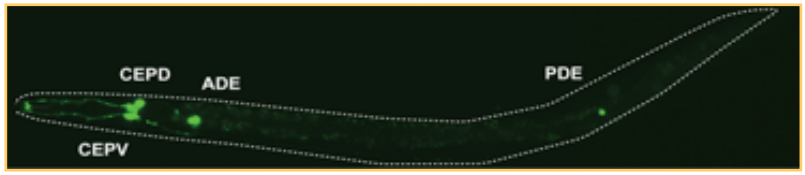
= TIME

The COPAS Biosorter (or ‘worm sorter’)

The COPAS Biosorter (Union Biometrica)



Present at the University of Edinburgh, at CIP



Efficiency of the worm sorter in mutant isolation

VS.

Manual Screen	Worm Sorter Screen
1 mutant / 10 days	1 mutant / day

Doitsidou *et al.*, *Nature Methods 2008*

From mutant to mutation:
whole genome sequencing for every mutant

C. elegans genome sequencing cost: < £ 200

Doitsidou *et al. PLoS ONE 2011*

Sarin S, Bertrand V, Bigelow H, Boyanov A, **Doitsidou M**, Poole R, Narula S and Hobert O. *Genetics, 2010*

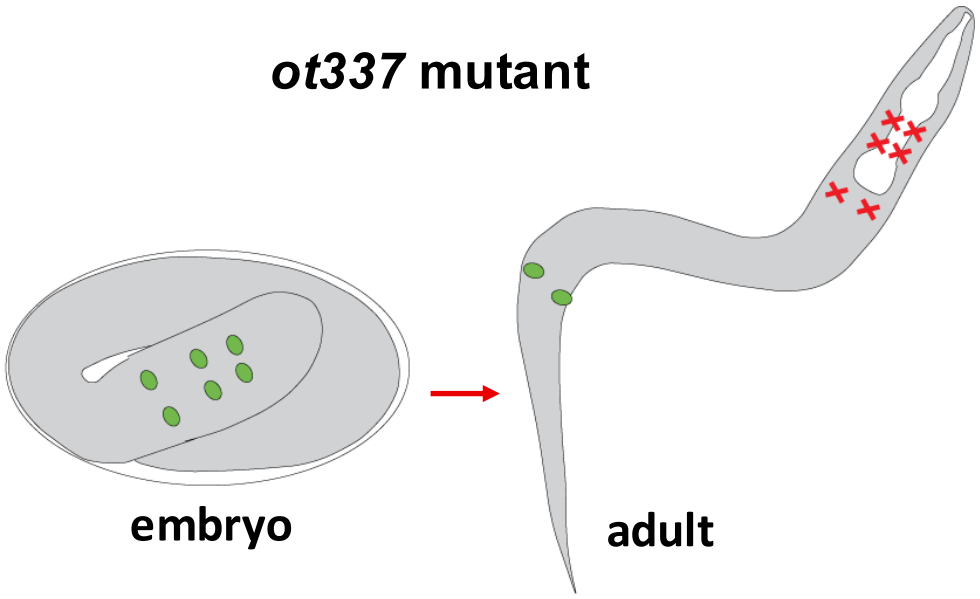
Bigelow H, **Doitsidou M**, Sarin S, Hobert O. *Nature Methods. 2009*

Each mutant, a story

dominant

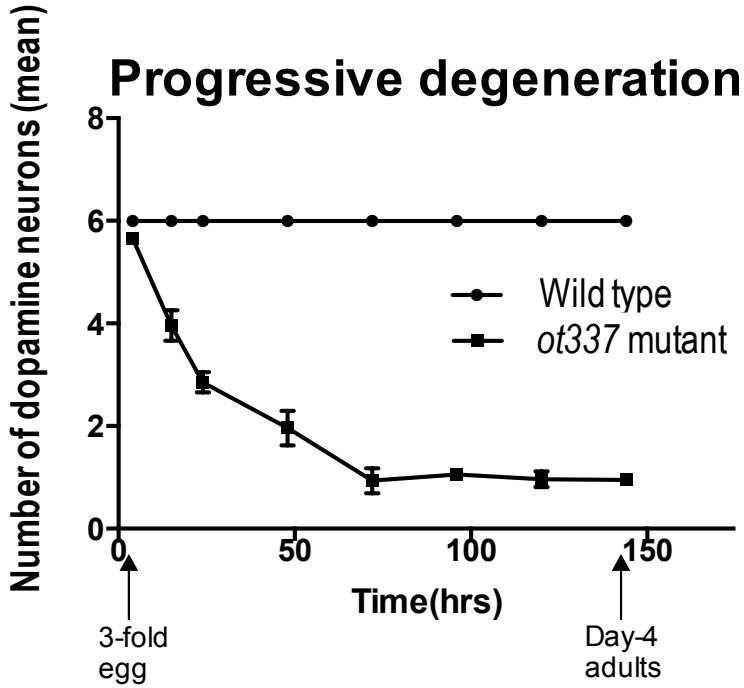
ot337

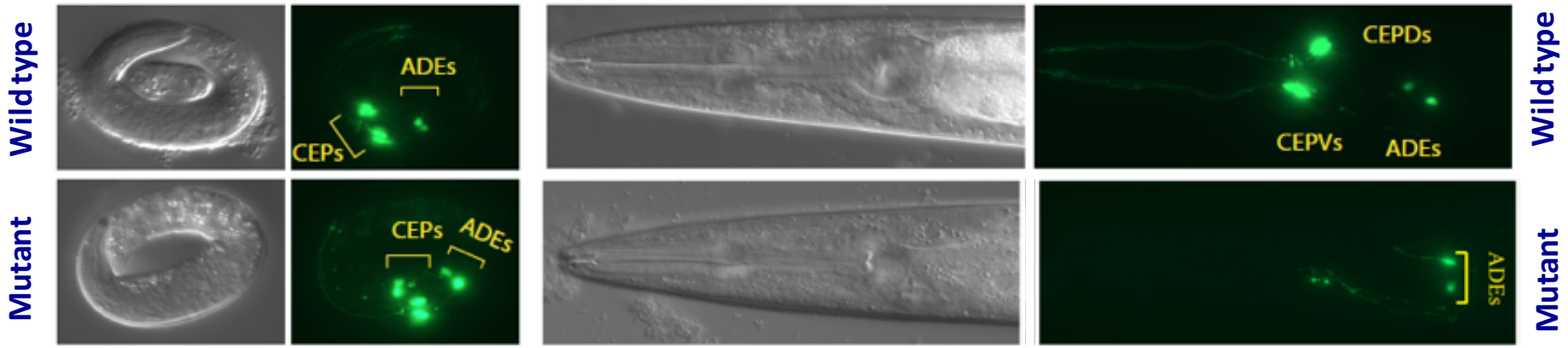
A *C. elegans* mutant with robust dopaminergic neuron degeneration



● :Normal neuron

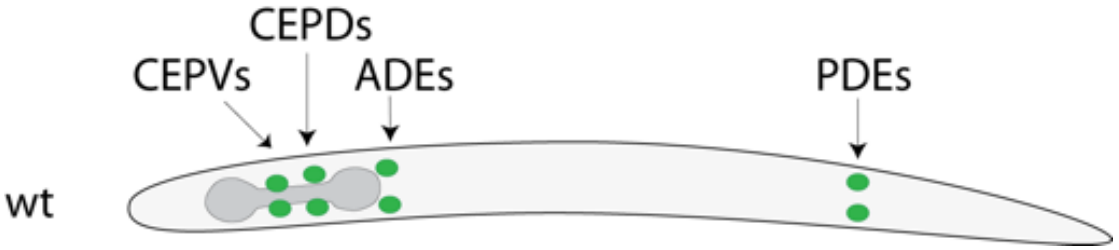
X :Degenerating neuron

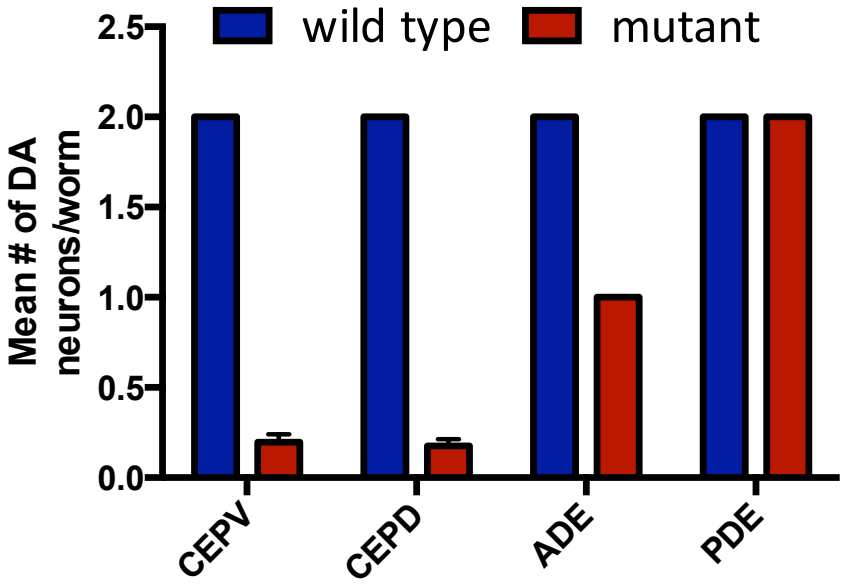




Nagarajan A, Ning Y, Reisner K, Buraei Z, Larsen JP, Hobert O, **Doitsidou M**, *J Neuroscience, 2014*

Differential susceptibility of the various classes of dopaminergic neurons

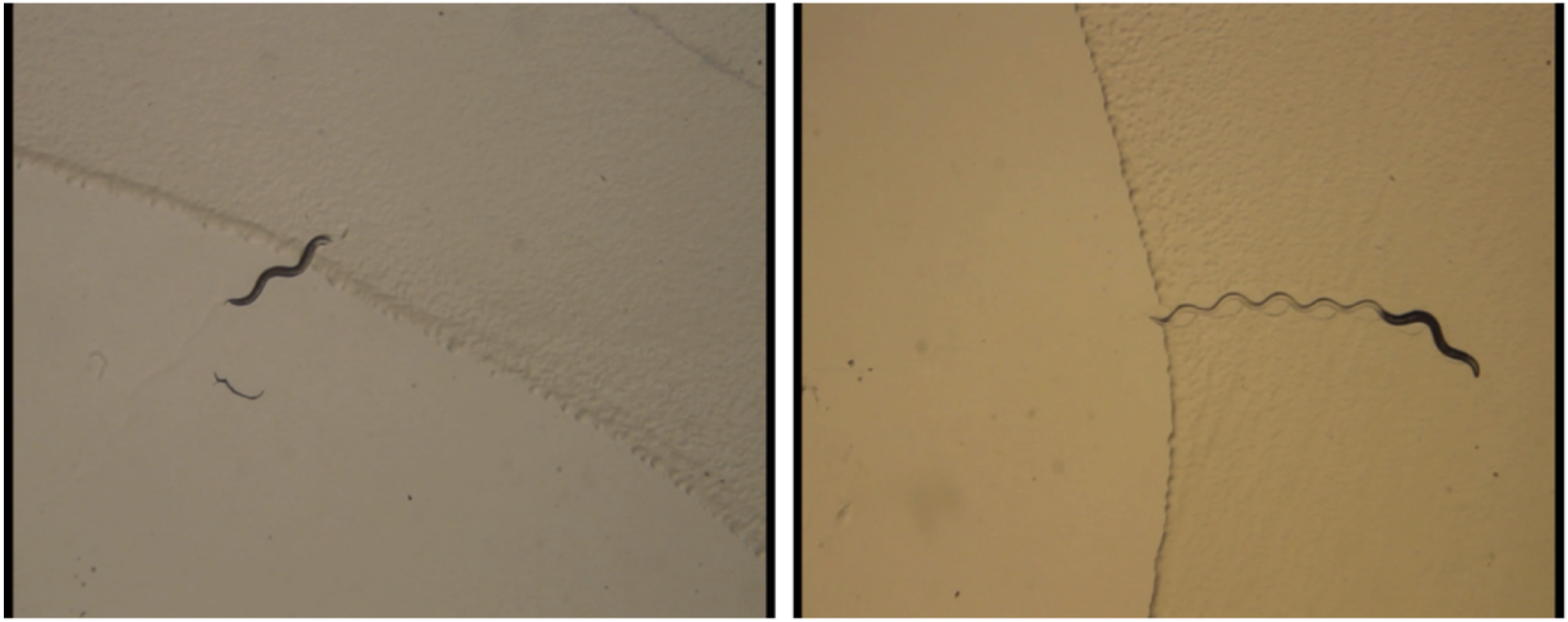




Deregulation of *trp-4* function results in abnormal behaviour

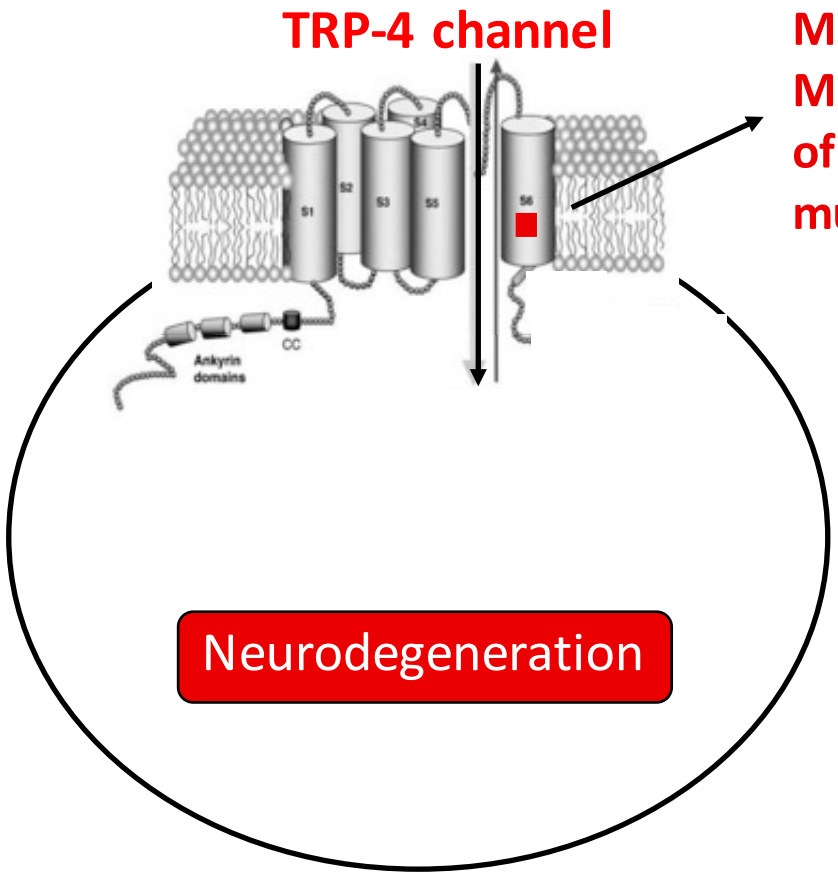
Wild type

Mutant with degeneration

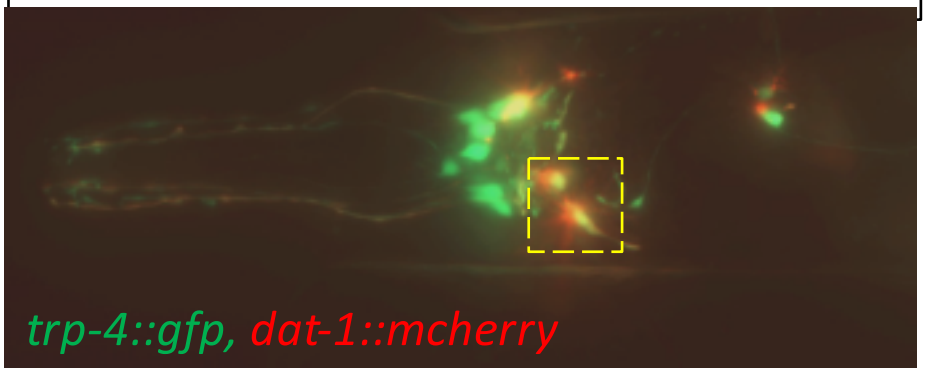


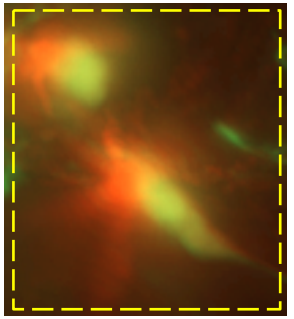
Basal Slowing Response

A gain-of-function mutation in a Transient Receptor Potential (TRP) channel *trp-4* causes degeneration of dopaminergic neurons

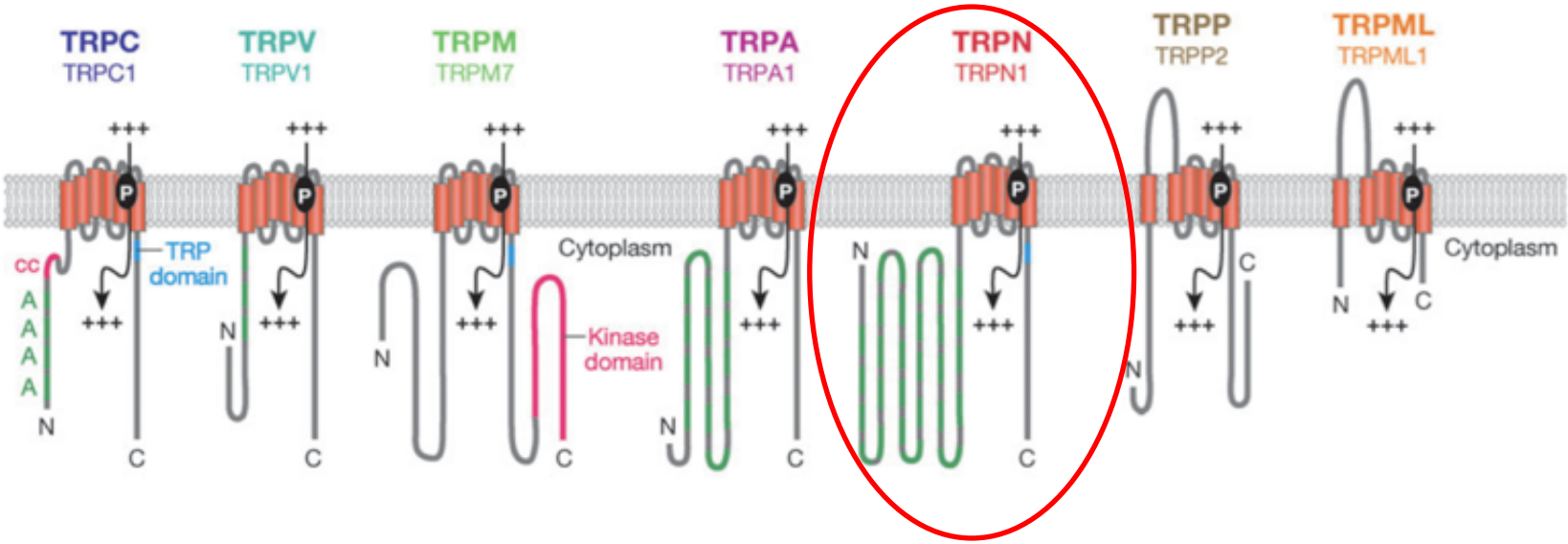


TRP-4 is expressed in dopaminergic and other neurons





Transient Receptor Potential channels



TRP CHANNEL FUNCTION

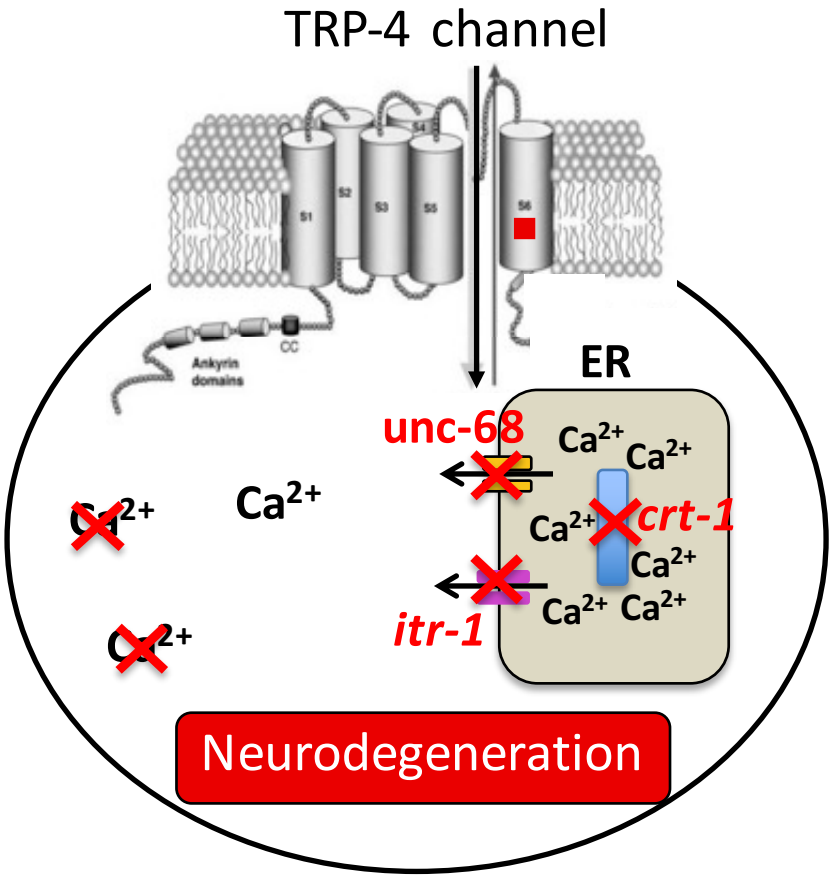
- Conserved across phylogeny
- Are expressed in the brain and in dopamine neurons
- Mediate sensations: taste, touch, pain, temperature

TRP-CHANNELS AND HUMAN DISEASE

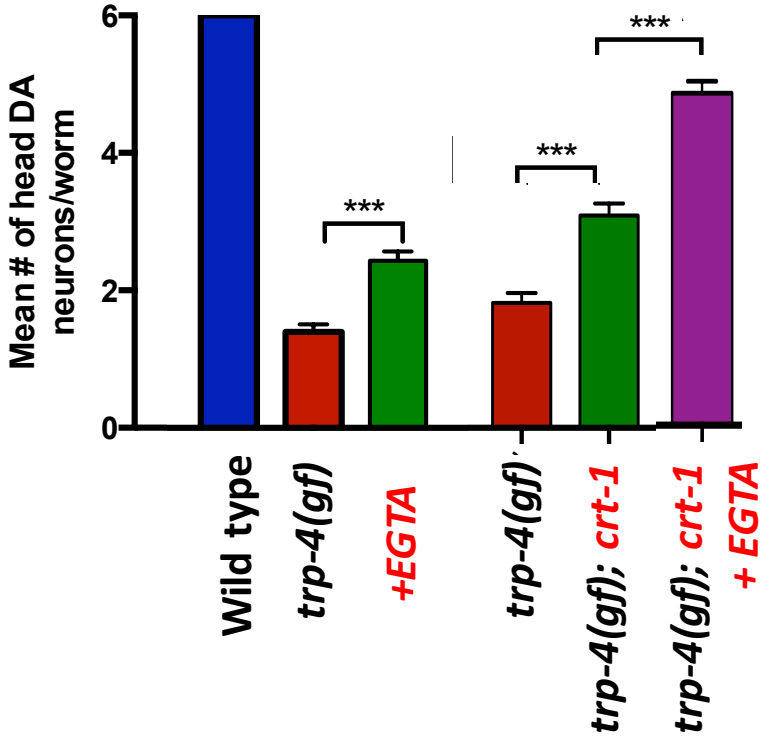
- Hypomagnesia, hypocalcemia (TRPM6)
- Autosomal Dominant Polycystic Kidney Disease (TRPP2)
- Mucolipidosis (TRPML1)
- Scapuloperoneal spinal muscular atrophy
- Charcot-Marie-Tooth disease

From Venkatachalam and Montell, 2007

Reducing intracellular calcium slows down dopaminergic cell death in the mutant *trp-4(gf)*



Dopaminergic neuron

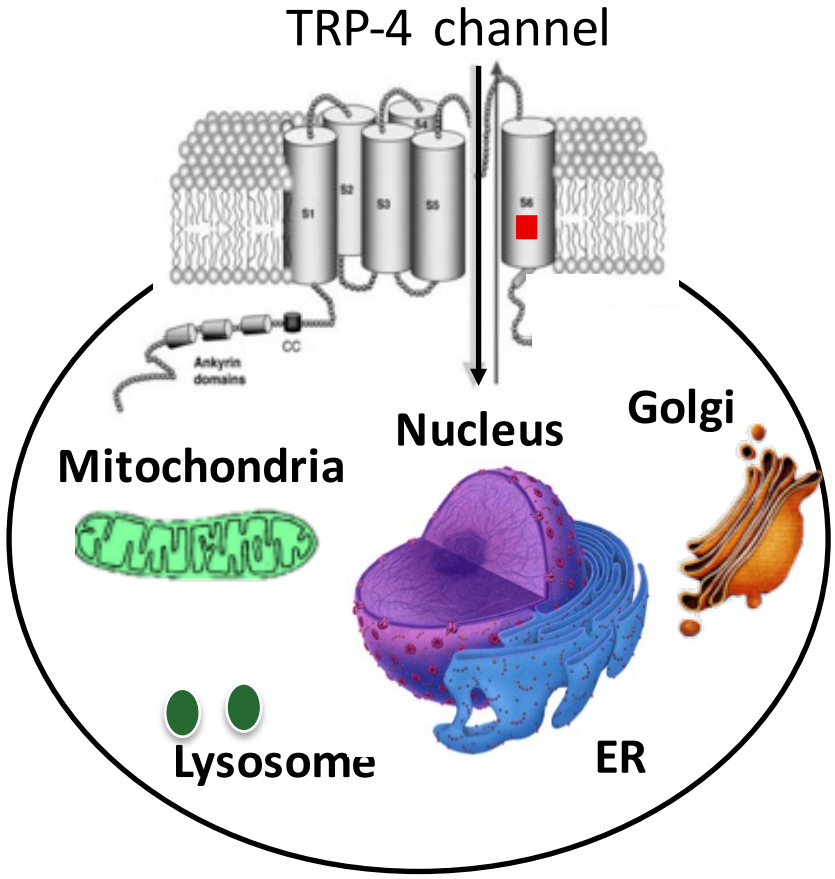


Nagarajan A, Ning Y, Reisner K, Buraei Z, Larsen JP, Hobert O, **Doitsidou M**. *J Neuroscience, 2014*

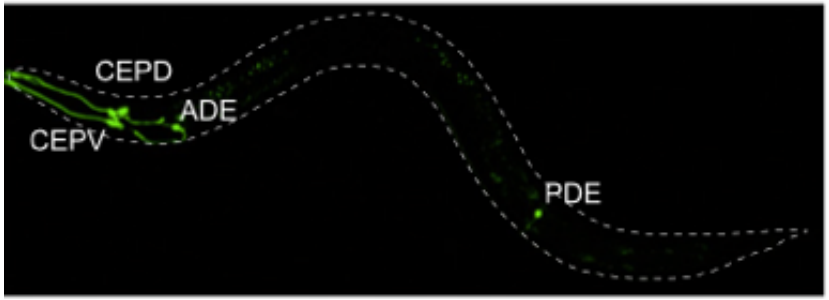
Organelle changes in dying dopaminergic neurons

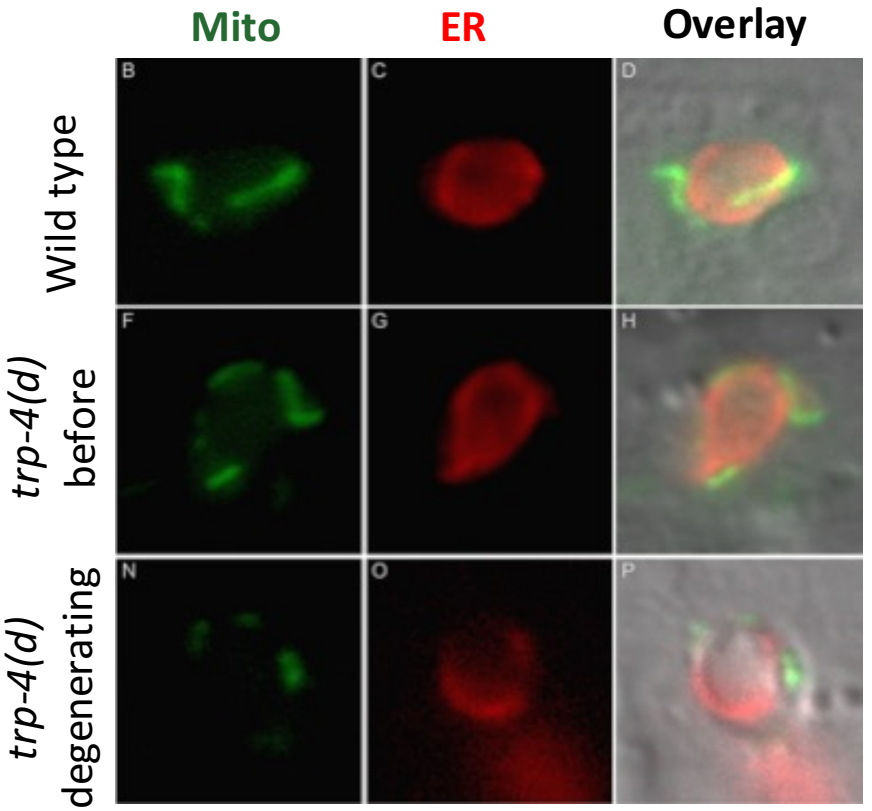
Observe organelle damage in a living organism

Multi-color fluorescence in different organelles

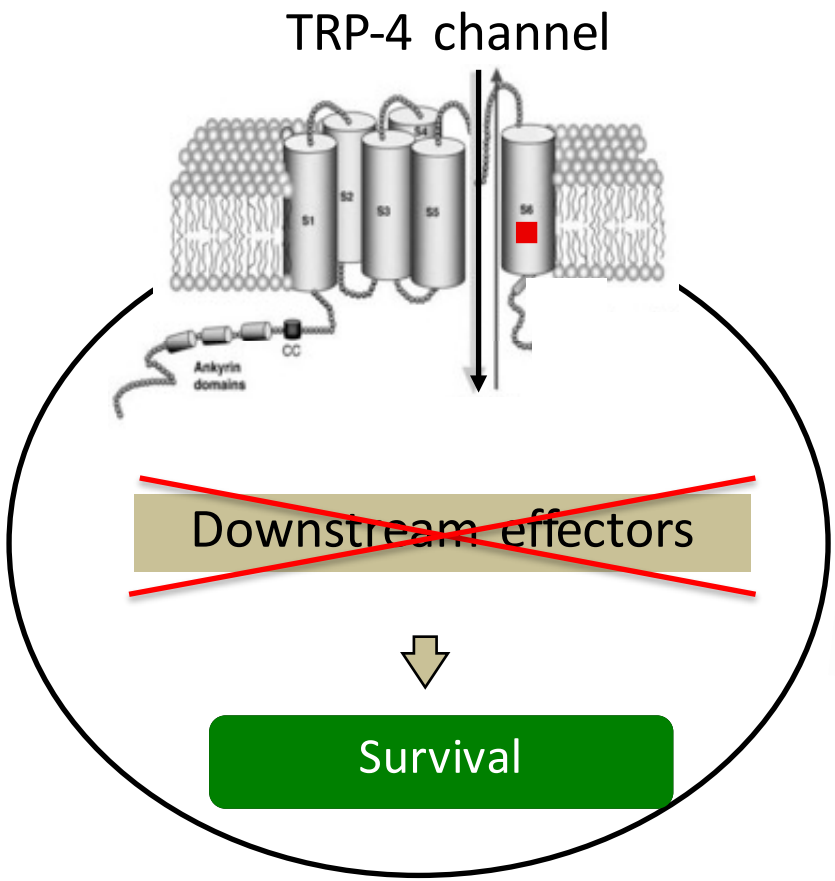


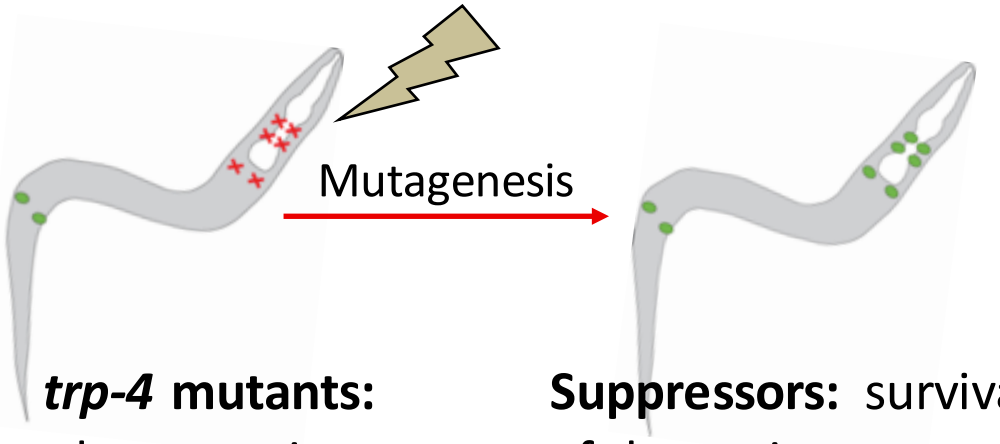
Can we prevent neuronal cell death by manipulating organelle function?





High-throughput screens for mutations that stop neurodegeneration





***trp-4* mutants:** degeneration

Suppressors: survival of dopamine neurons

High-throughput screens for mutations that stop neurodegeneration

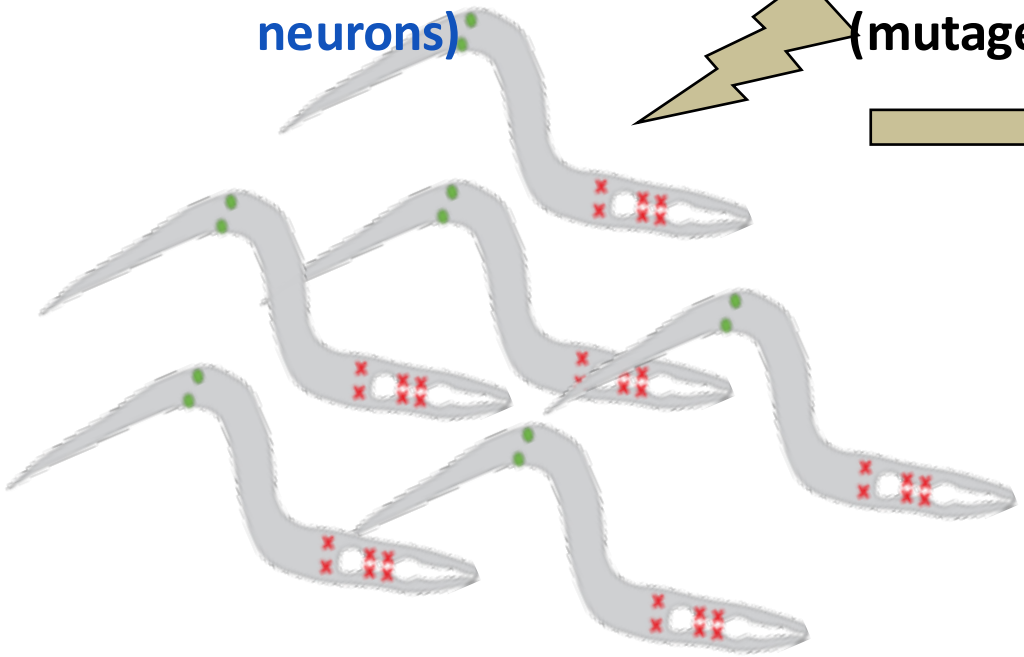
Imagine billions of patients with the same genetic condition:

Some of them might be resistant to the disease

We can do this experiment with our model organism

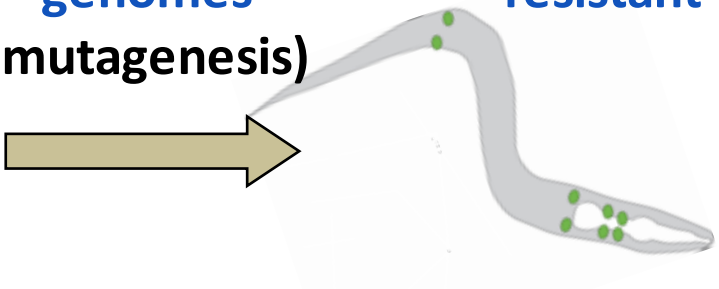
High-throughput screens for mutations that stop neurodegeneration

trp-4 worms (loss of dopamine neurons)



We can generate unlimited number of worms with PD symptoms

Change their genomes (mutagenesis)



Search for worms resistant to disease

Differences in the genome of resistant worm?

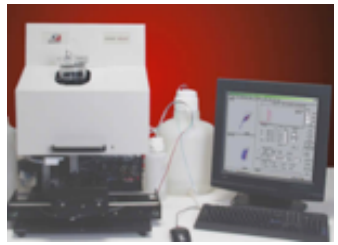
Is the protective mechanism relevant for human disease?

High-throughput screens for mutations that stop neurodegeneration

trp-4 worms (loss of dopamine neurons)

Change their genomes (mutagenesis)

Search for worms resistant to disease



Differences in the genome of resistant worm?

Is the protective mechanism relevant for human disease?

Pilot experiment

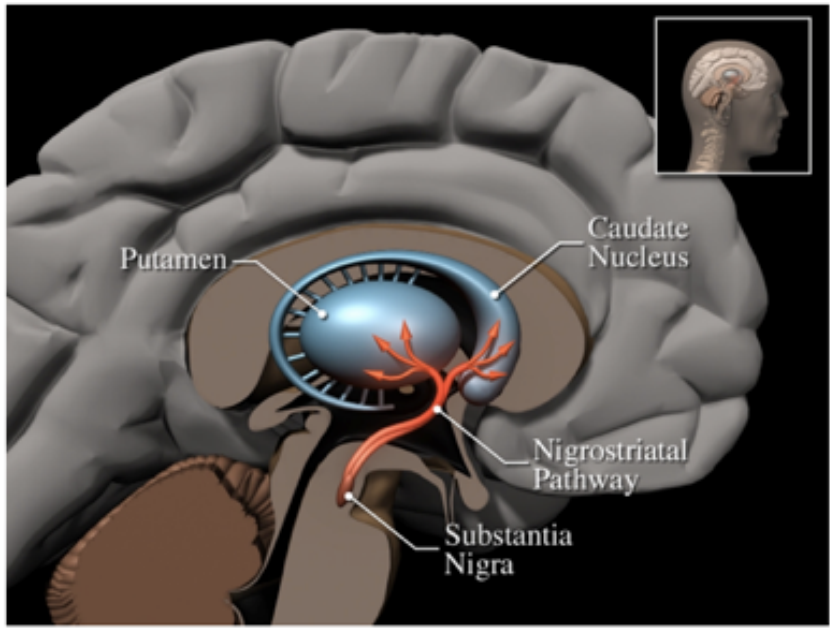
1 round of screen (=1 week): 25 mutants

- delayed degeneration
- very strong protection, most neurons unaffected
- completely immune to degeneration

My talk today

1. A short history of *C. elegans* (and a tribute to basic research)
2. The scientific questions that drive my research
3. Our findings on dopamine neuron development and degeneration: detour on the technological advances that make our research efficient
4. **How we bring research findings from worms to humans**

A role of TRP channels in Parkinson's disease?



The Norwegian Parkwest study

A prospective longitudinal Parkinson's Disease cohort

- **183 patients with PD**
- **192 matched controls**

Jan Petter Larsen
Stavanger University Hospital
Ole-Bjørn Tysnes
Bergen University Hospital

27 TRP channels in humans

➢ **8 in the substantia nigra**

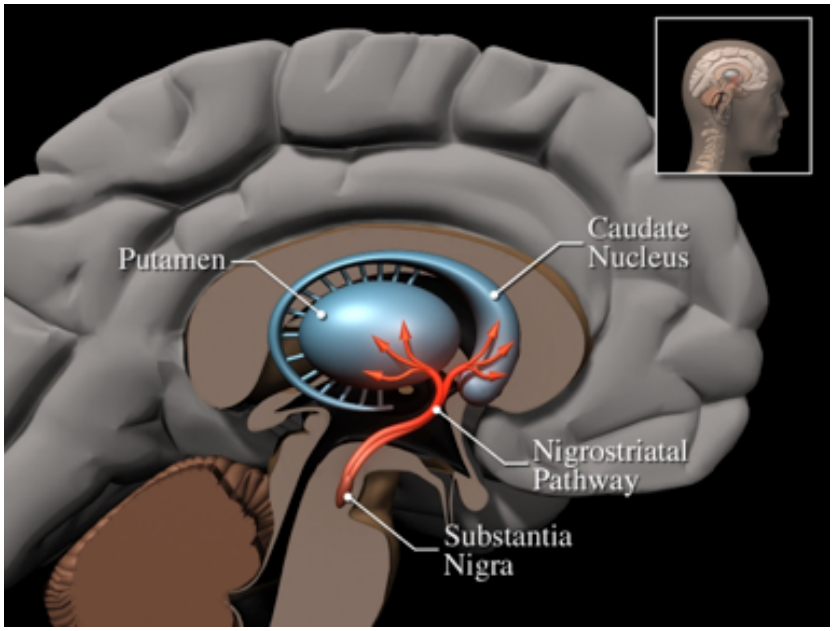
Exome sequencing

Rare protein changing mutations in brain TRP channels

- Genomic data from other cohorts -association

- Introducing human TRP channel variants in the worm

Parkinson's disease

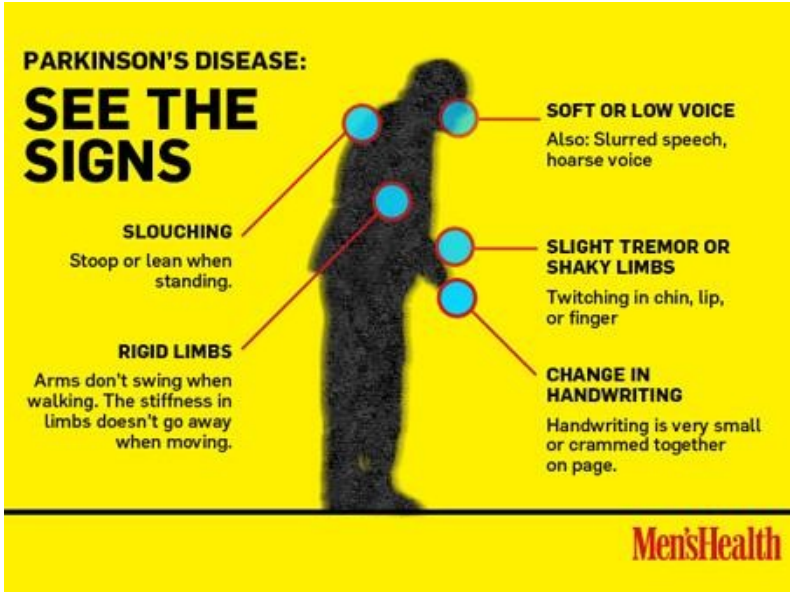


Motor Symptoms:

- Shaking
- Rigidity
- Slowness of movement

Non motor symptoms:

- Depression
- **Dementia**
- Mood disorders



- **No diagnostic test**
- **Cause unknown**
- **No cure that stops neuronal loss**

In need of Biomarkers

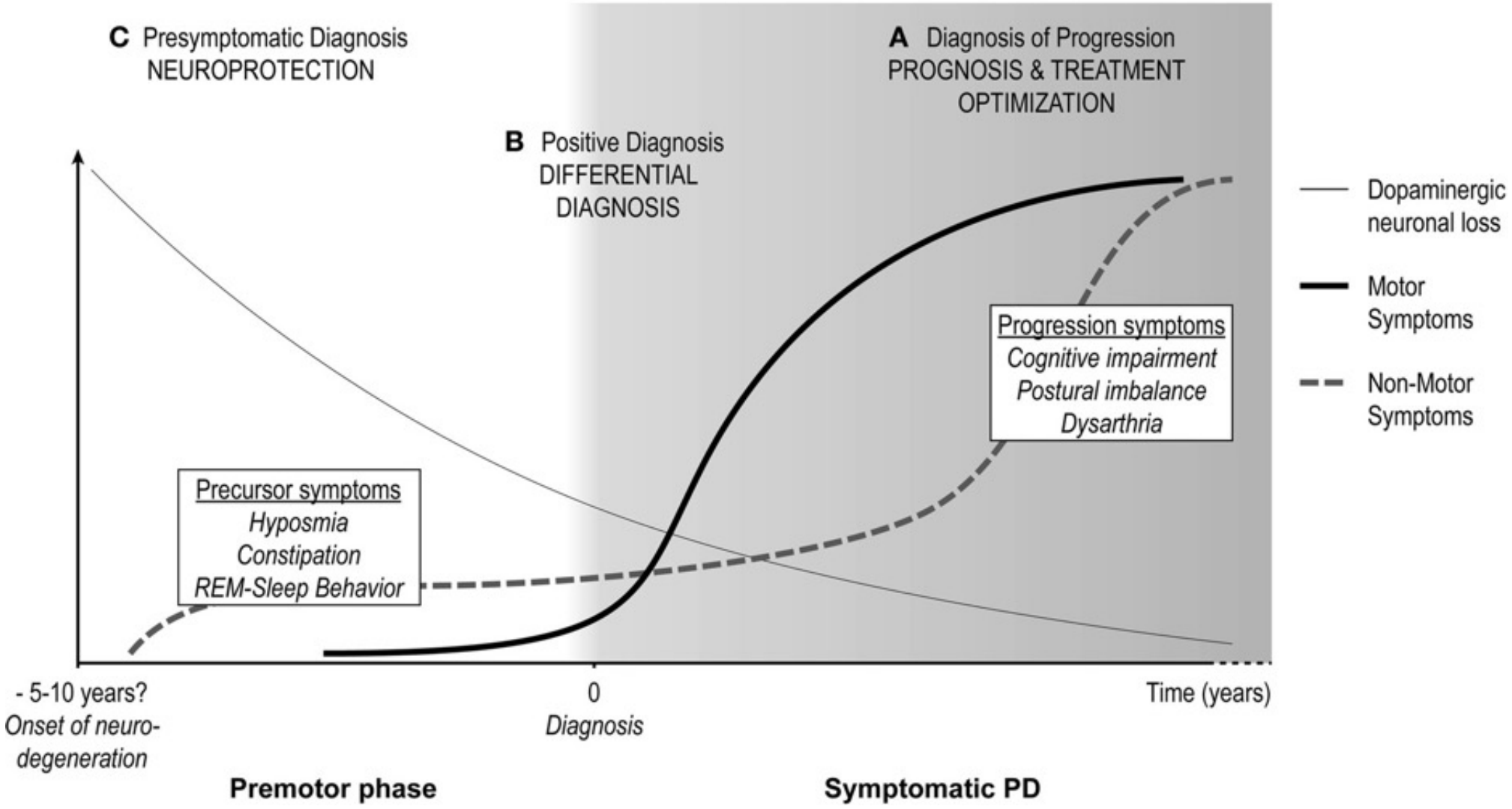


Image: Lebouvier et al., 2010

Differential disease progression

Dementia
soon after diagnosis

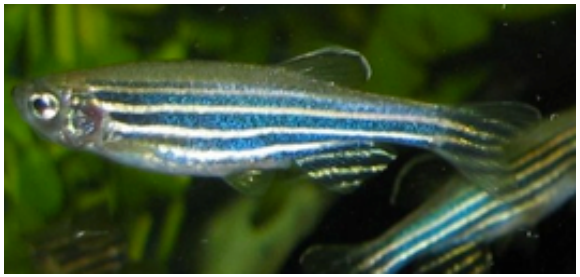
2 EXTREMES

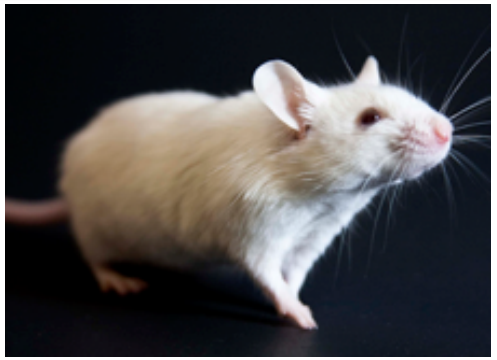
exome sequencing

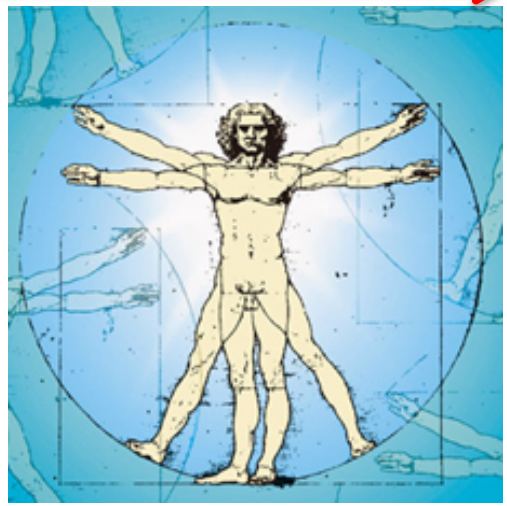
Normal
many years after diagnosis

Is genetic variability contributing to the differential progression?

From worms to the clinic and back







- Functional Validation of exome sequencing findings
- Mechanistic insight

Robin Morgan:

4 Powerful poems about Parkinson’s and growing older.

Many thanks to:

Former lab members (Stavanger)

- Archana Nagarajan
- Kaja Reisner
- Janete Chung
- Ye Ning
- Tatiana Popovitchenko

Current lab members

- Feng Xue (Research Assistant)

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- IRG Marie Curie
- Helse Vest
- Norwegian Parkinson's association

Collaborators

- Oliver Hobert –Columbia University Medical Centre
- Claire Bénard –University of Massachusetts
- Tim Hughes – University of Oslo
- **The Stavanger University Hospital:**
- Jan Petter Larsen
- Alves Guido
- Jodi Maple Grødem