# The model organism *C. elegans*

## Part I: Anatomy and Genetics

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# Overview of the *C. elegans* part

Week 1
- Introduction
- *C. elegans* Anatomy
- *C. elegans* Genetics

Week 2
- *C. elegans* Neurobiology
- Examples from own *C. elegans* research

Week 3
**Laboratory course** (Handling and Maintaining *C. elegans*, Observation of Mutant Phenotypes, Genotyping, Chemotaxis Experiments, GFP Expression in *C. elegans*)
=> Come to the 5$^{th}$ floor of Life Science Building I (ONE) / Room 509

# Today's topics

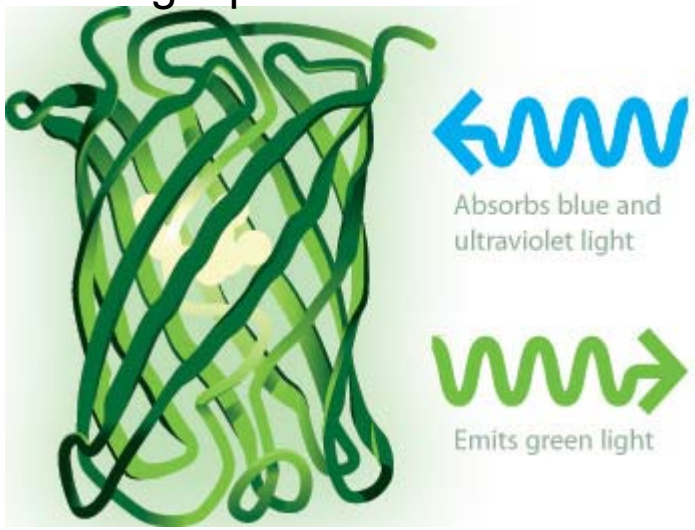
- Introduction to *C. elegans*
- Basic and Specific Anatomy of *C. elegans*
- *C. elegans* Genetics

# Short introduction to *C. elegans*

• In 1965 Sydney Brenner looked around for **a "minimal animal"** nearly as simple as *E. coli* to study genetics and molecular biology
• He has chosen *C. elegans* due to its small size (1 mm) and light-thru features allowing visualization and **mapping of each cell** in the living animal
• Exactly 959 cells form a working **nervous system**, **muscles**, **sexual organs** and **intestine** with **many features similar to humans**
• More than 10,000 worms can grow on a single petri dish **reproducing rapidly** (from egg to mature animal in 3.5 days)
• Used for studying (for example) **apoptosis**: 15 genes control apoptosis and exact **131 cells** of the 1090 cells in the embryo **die** within one hour **after hatching**
• **Nobel Prize 2002** to Drs. Brenner, Horvitz and Sulston on their work of organ development and apoptosis in *C. elegans*
• **Nobel Prize 2006** to Drs. Fire and Mellow on their discovery of RNAi in *C. elegans*
• **Nobel Prize 2008** to, e.g., Dr. Chalfie for expressing GFP in specific *C. elegans* cells

# What is GFP?

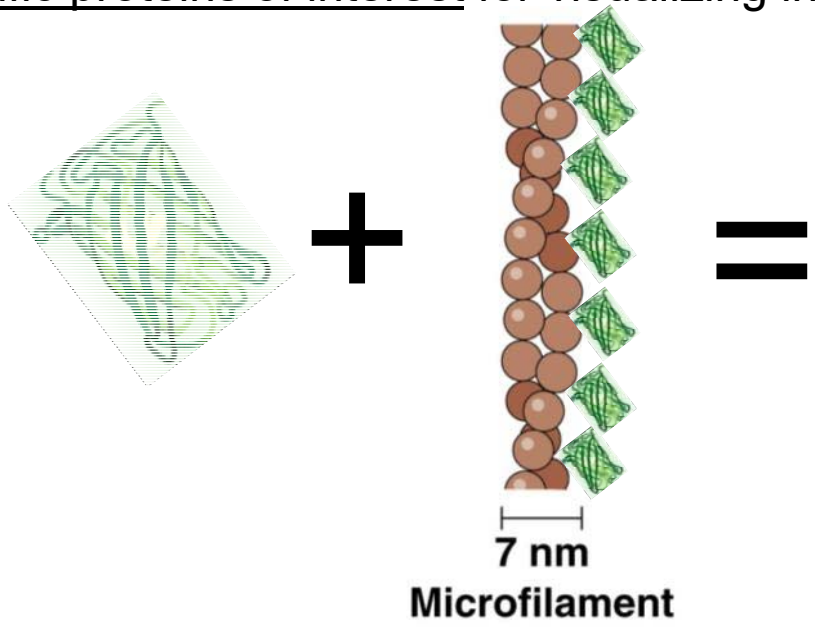
Single protein

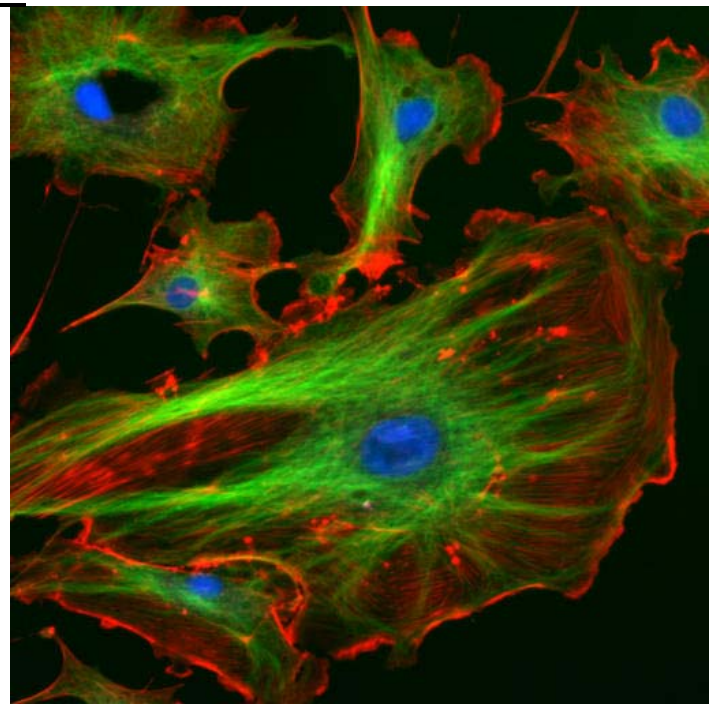



Excitation/Emissio n

**Green fluorescent protein** isolated from jellyfish *Aequorea victoria*

Engineering genes that express GFP fused to specific proteins of interest for visualizing in cells



# The Nobel Prize in Chemistry 2008

"for the discovery and development of the green fluorescent protein, GFP"

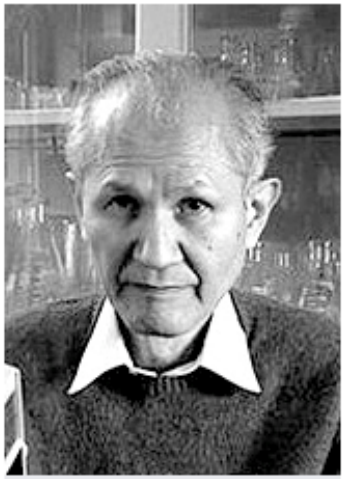


Photo: J. Henriksson/SCANPIX

**Osamu Shimomura**

1/3 of the prize

USA

Marine Biological Laboratory (MBL)
Woods Hole, MA, USA

b. 1928

Photo: J. Henriksson/SCANPIX

**Martin Chalfie**

1/3 of the prize

USA

Columbia University
New York, NY, USA

b. 1947

Photo: UCSD

**Roger Y. Tsien**

1/3 of the prize

USA

University of California
San Diego, CA, USA

b. 1952

NTHU May 2012

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**27,000 Worm Publications**

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3. C. **elegans** and Arabidopsis thaliana Show Similar Behavior: ROS Induce Escape Tropisms Both in Illuminated Nematodes and Roots.
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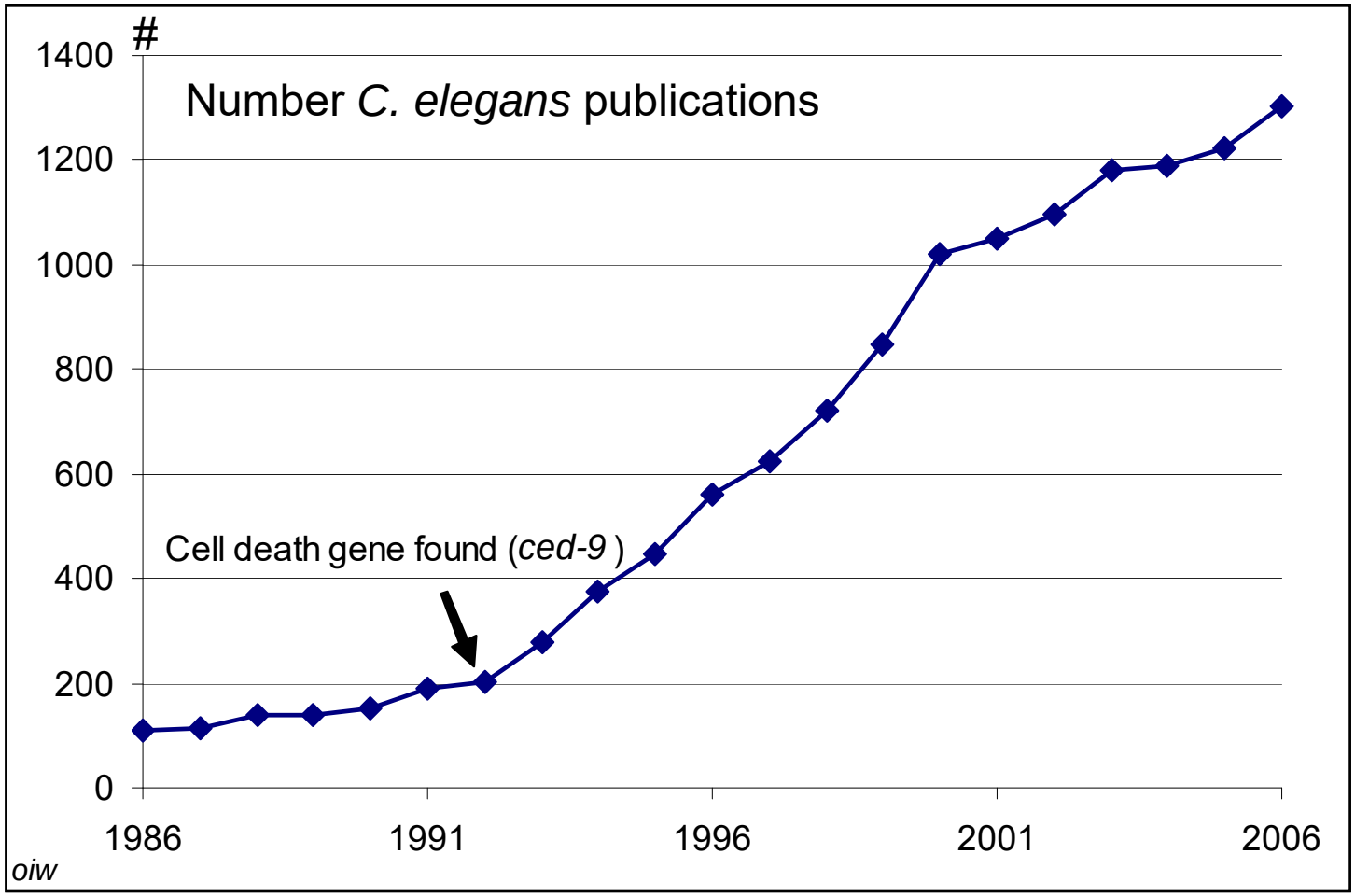
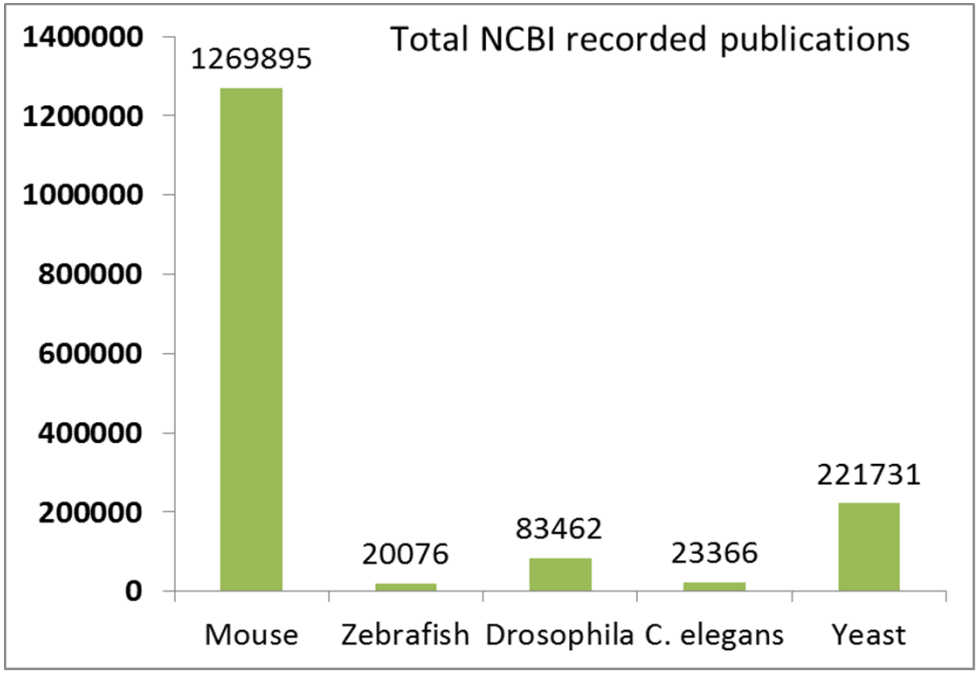
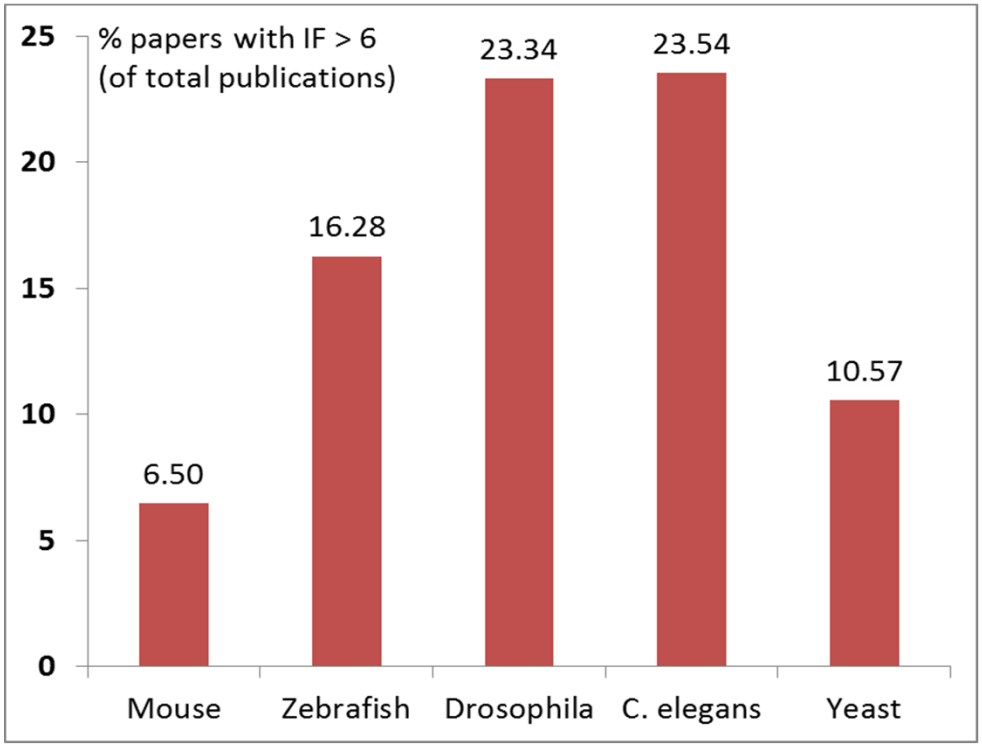
4. Integrated interactions database: tissue-specific view of the human and model organism interactomes.

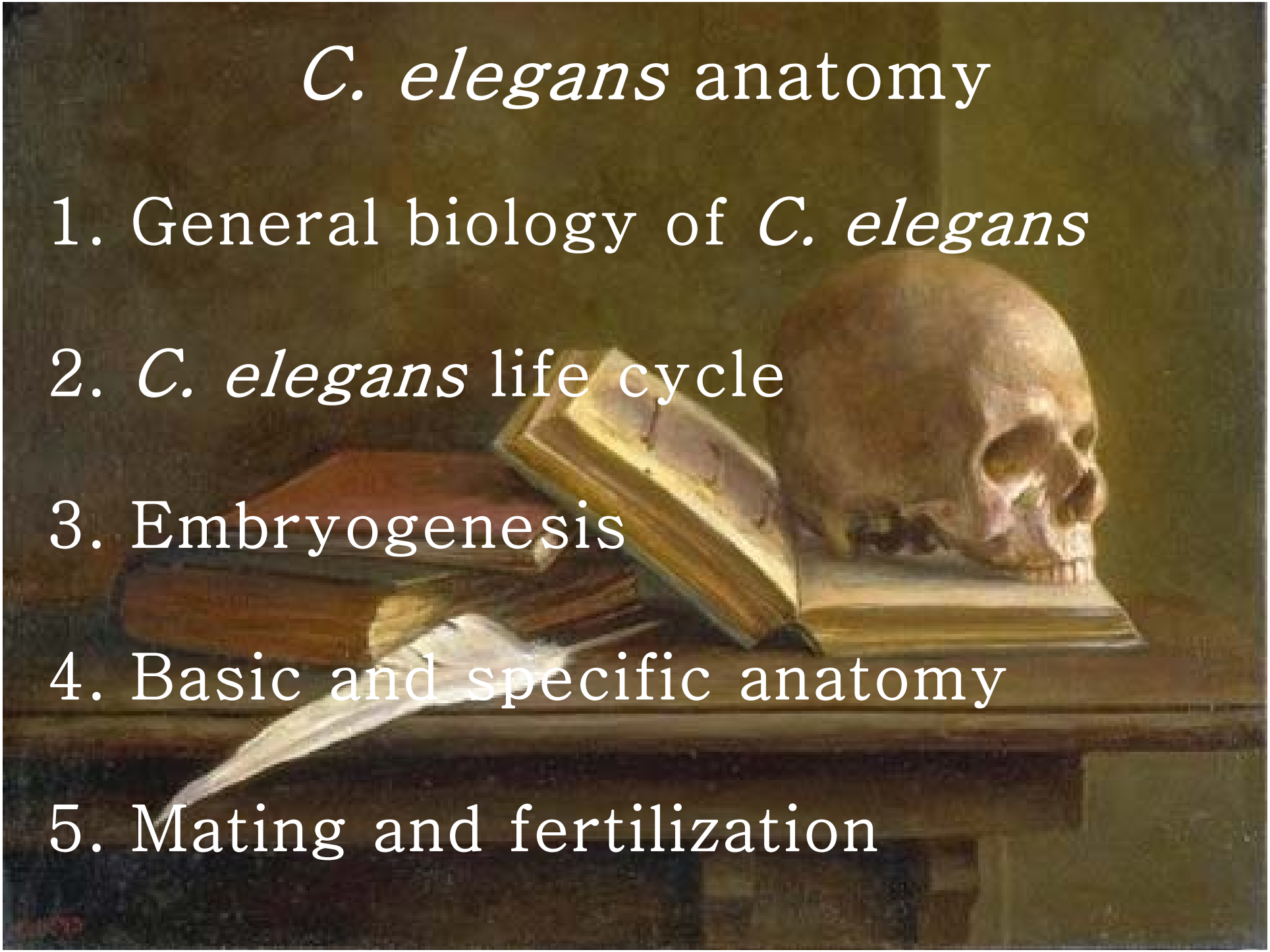
• In 1992, Drs. Hengartner, Ellis and Horvitz published in *Nature* that the gene ***ced-9*** **protects** against programmed cell death
• After 1992, the rate of *C. elegans* publications increased significantly
• Drug companies are now looking for small molecules that protect against cell death (**longevity research**!)






Relation between quantity and quality of model organism publications



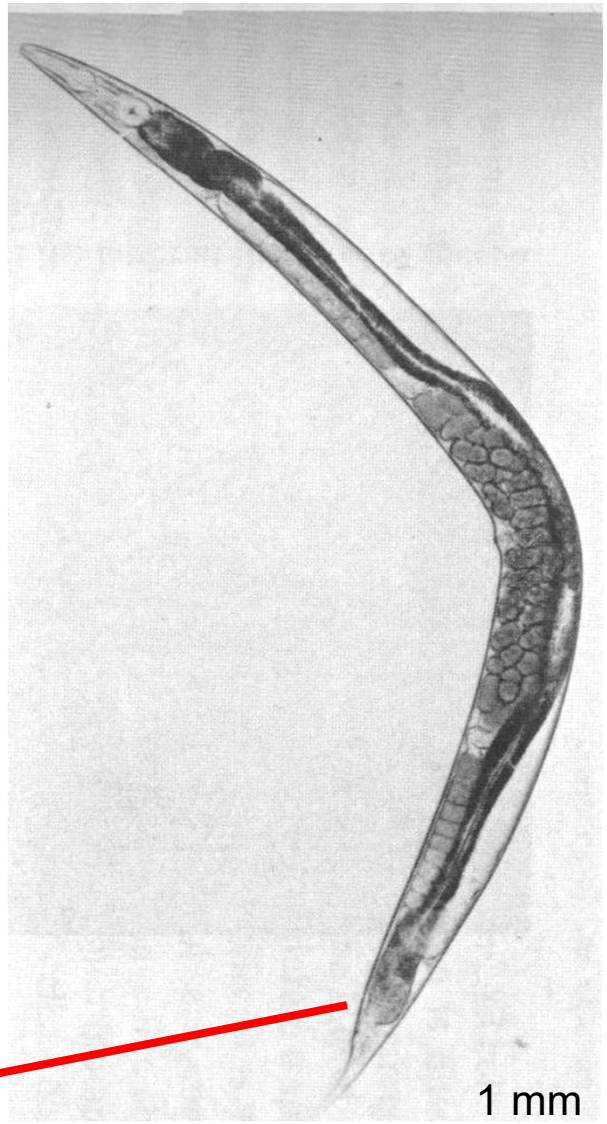
# *C. elegans* anatomy

1. General biology of *C. elegans*
2. *C. elegans* life cycle
3. Embryogenesis
4. Basic and specific anatomy
5. Mating and fertilization

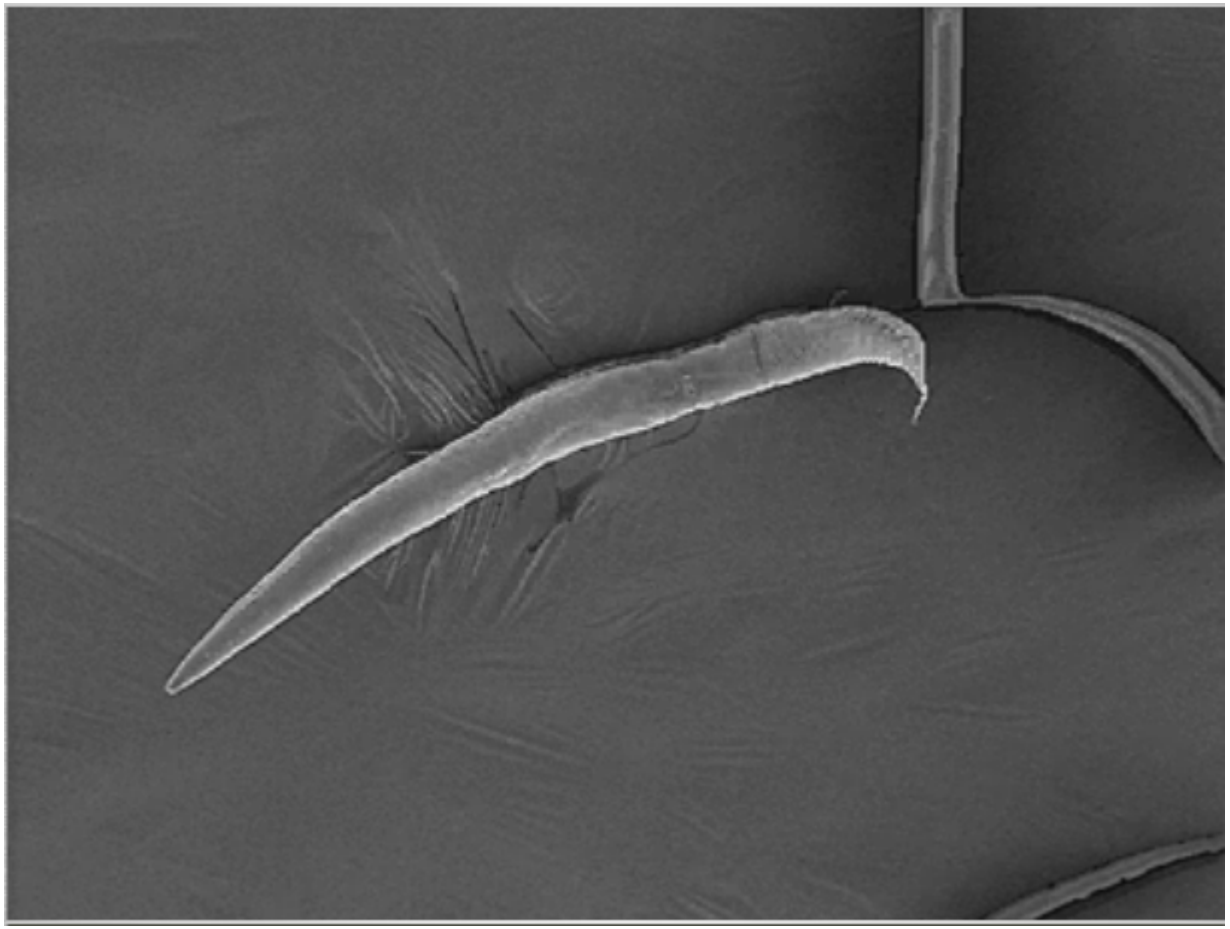
# Introduction to *Caenorhabditis elegans*

- ***Caeno* = recent, *rhabditis* = rod, *elegans* = nice**
- *C. elegans* is a member of the family Rhabditidae, a large and diverse group of **nematodes**
- It is 1 mm long, bacteriovorous (eat bacteria) and is transparent (suitable for GFP expression)
- In the lab, *C. elegans* is fed by ***E. coli* mutants** (OP50) with an uracil biosynthesis defect to ensure that the bacteria **do not overgrow inside the worm**
- Some rhabditids are pathogenic or parasitic on animals, but *C. elegans* does not harm humans
- *C. elegans* can be easily found in decomposing fruits; it has been isolated from **mild regions** world-wide
- In the soil, *C. elegans* associates with **woodlice** and is using it as a transport host:

1-1.5 cm



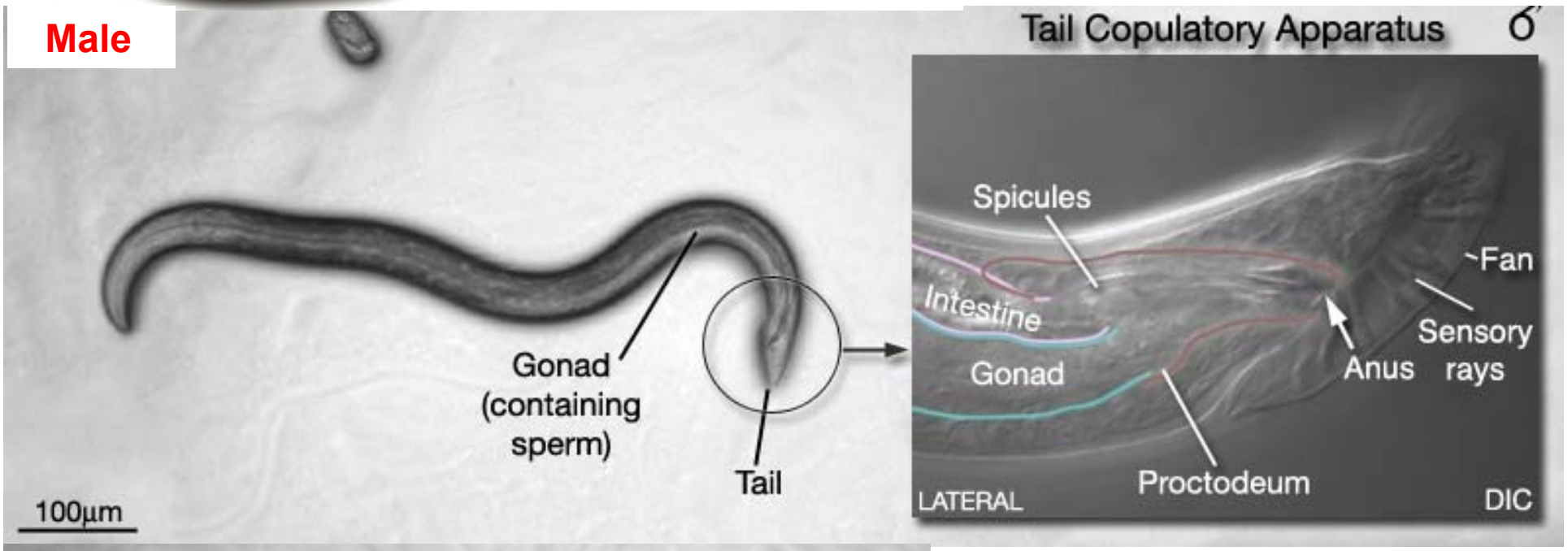
# A bird’s eye view of *C. elegans*

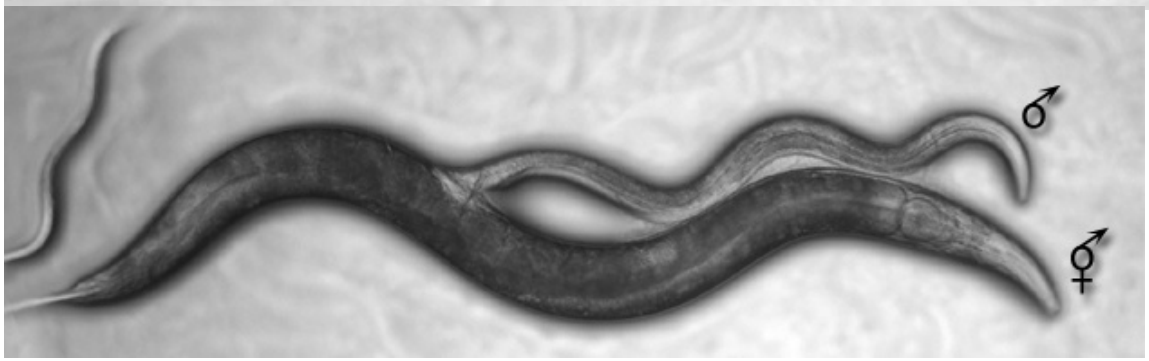


## Hermaphrodite

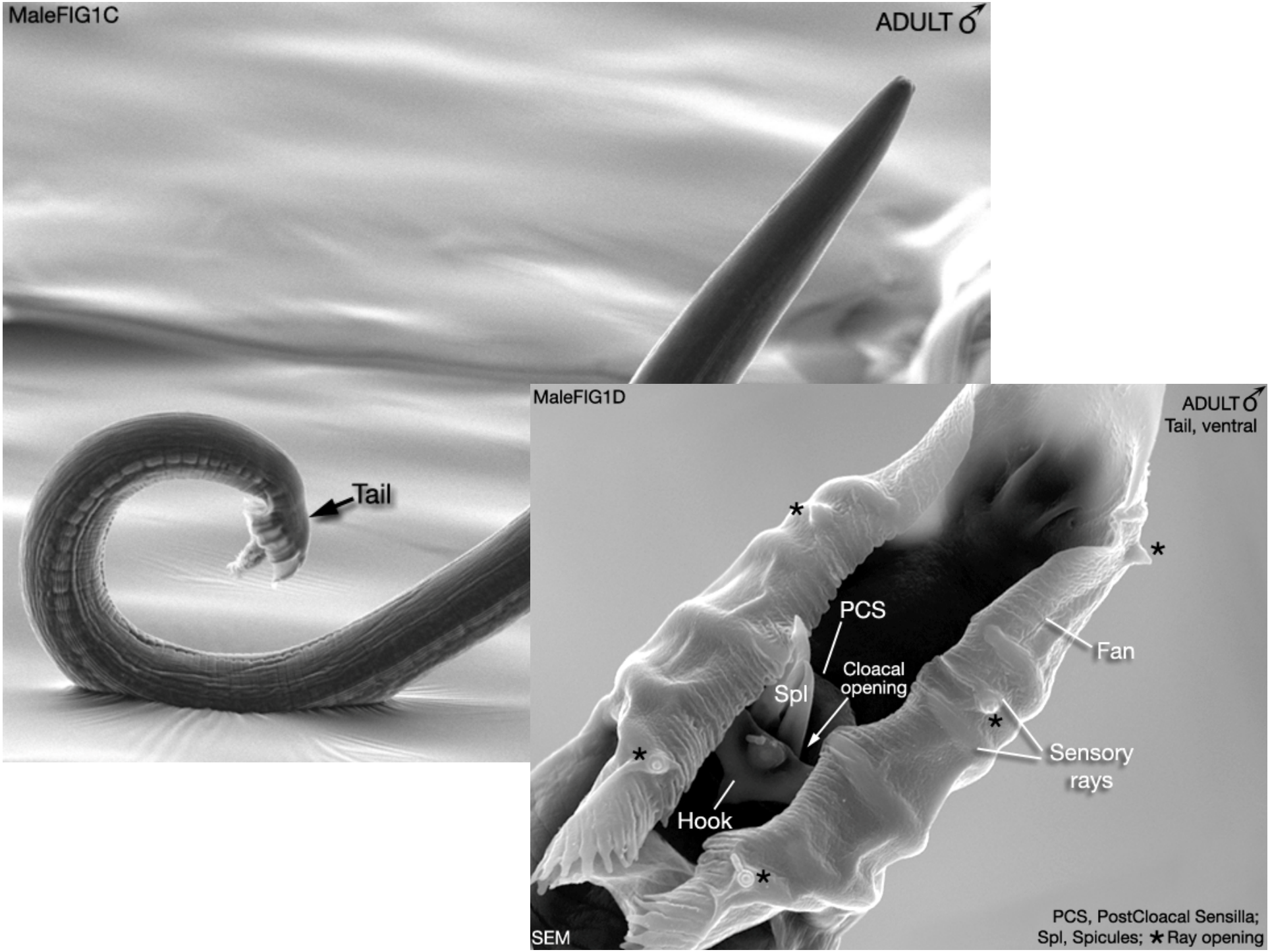
Male worm

## Male




When hermaphrodites mate with males, 50% of the progeny will be males, however, self-fertilization produces only 0.1% males


MaleFIG1C
ADULT ♂
Tail
MaleFIG1D
ADULT ♂
Tail, ventral
PCS
Cloacal opening
Spl
Fan
Sensory rays
Hook
SEM
PCS, PostCloacal Sensilla;
Spl, Spicules; ✱ Ray opening

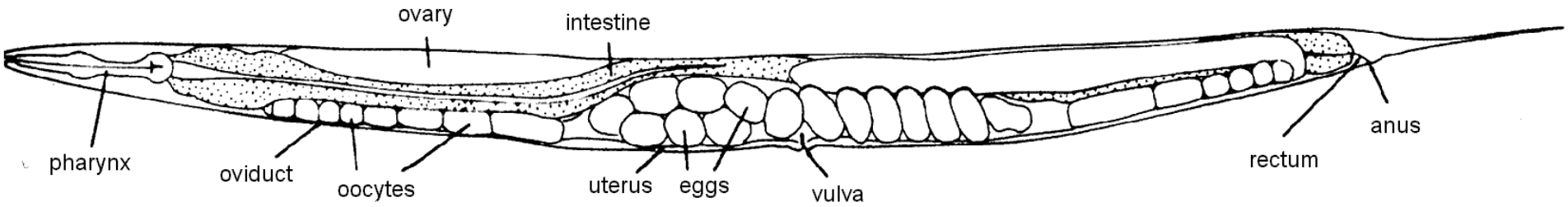
# General biology of *C. elegans*

• The anatomy of *C. elegans* has been fully described using electron microscopy: by stacking 200,000 EM sections together a full 3D worm has been constructed

• Development of all **959 somatic cells** has been traced back from their appearance in the embryo until their localization in the adult ("**wiring diagram**" or **cell lineage**)

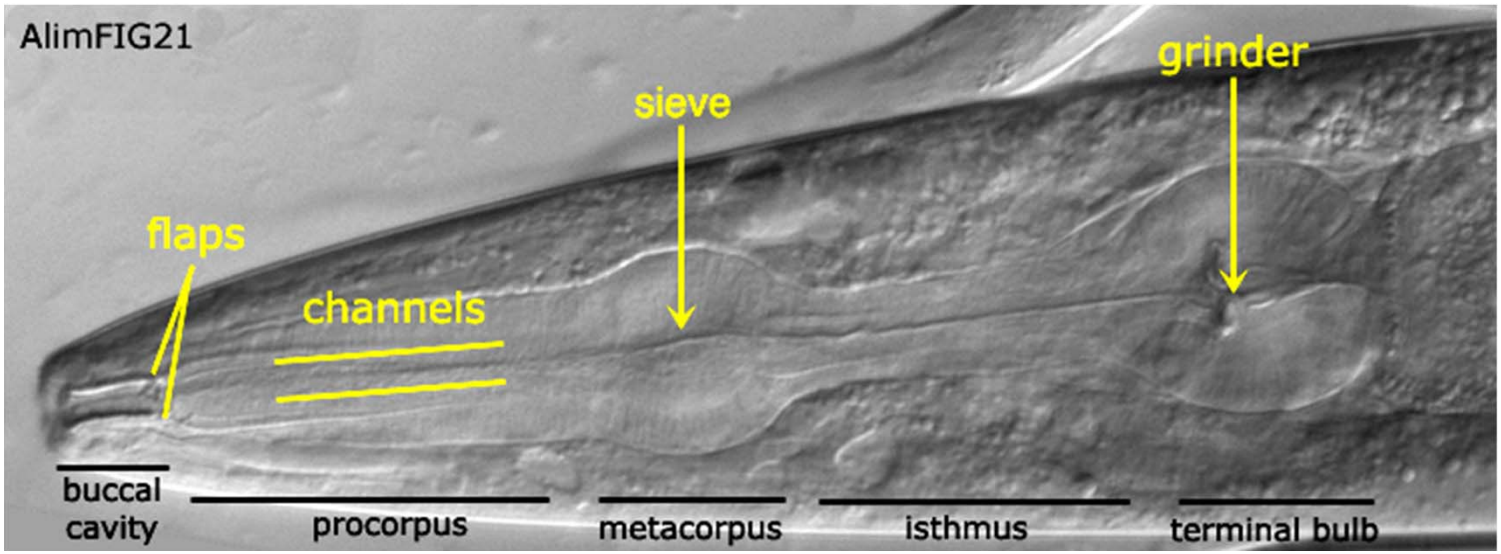
• All synaptic connections made by the **302 neurons** are known

**Basic feature of the hermaphrodite**:

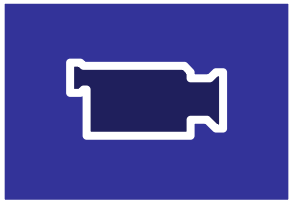



• Via coordinated muscular contraction, the two bulbed-**pharynxes** assist to suction-in bacteria which are then crushed in the grinder

• The pharynx is a nearly autonomous organ with its own nervous and muscle system



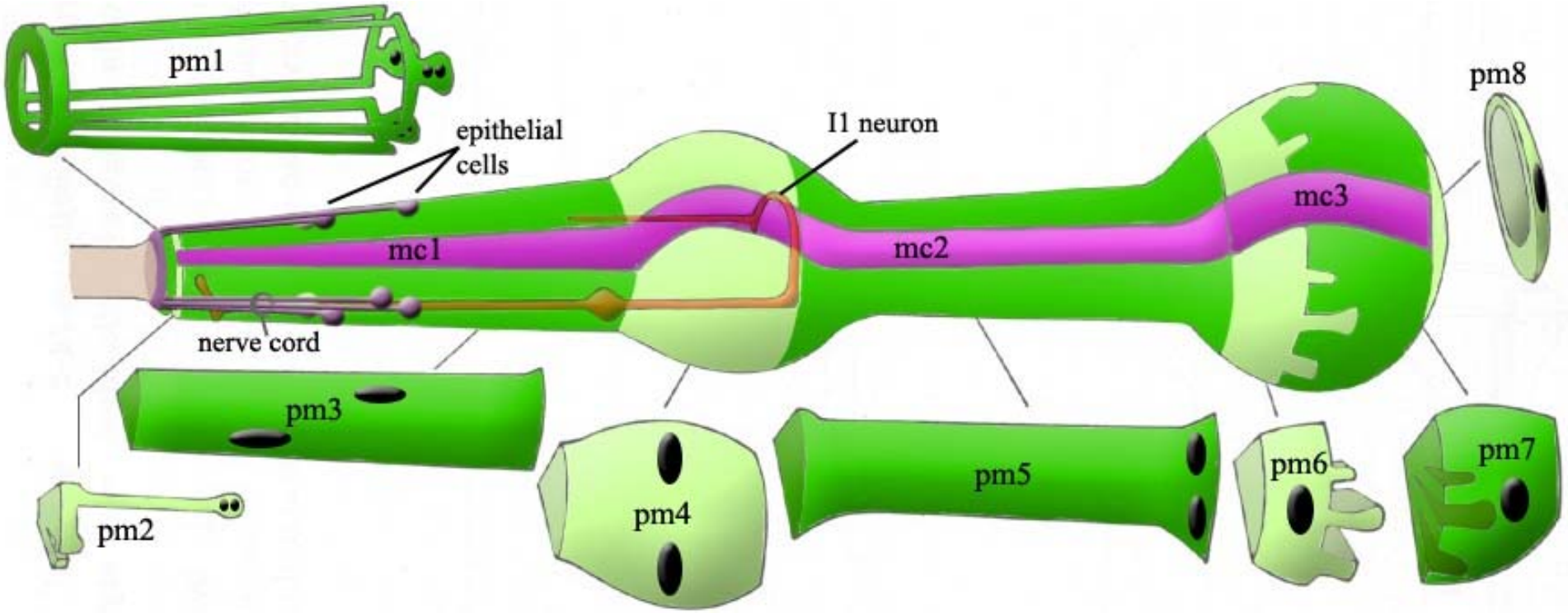

The pumping of the grinder can be observed by DIC microscopy



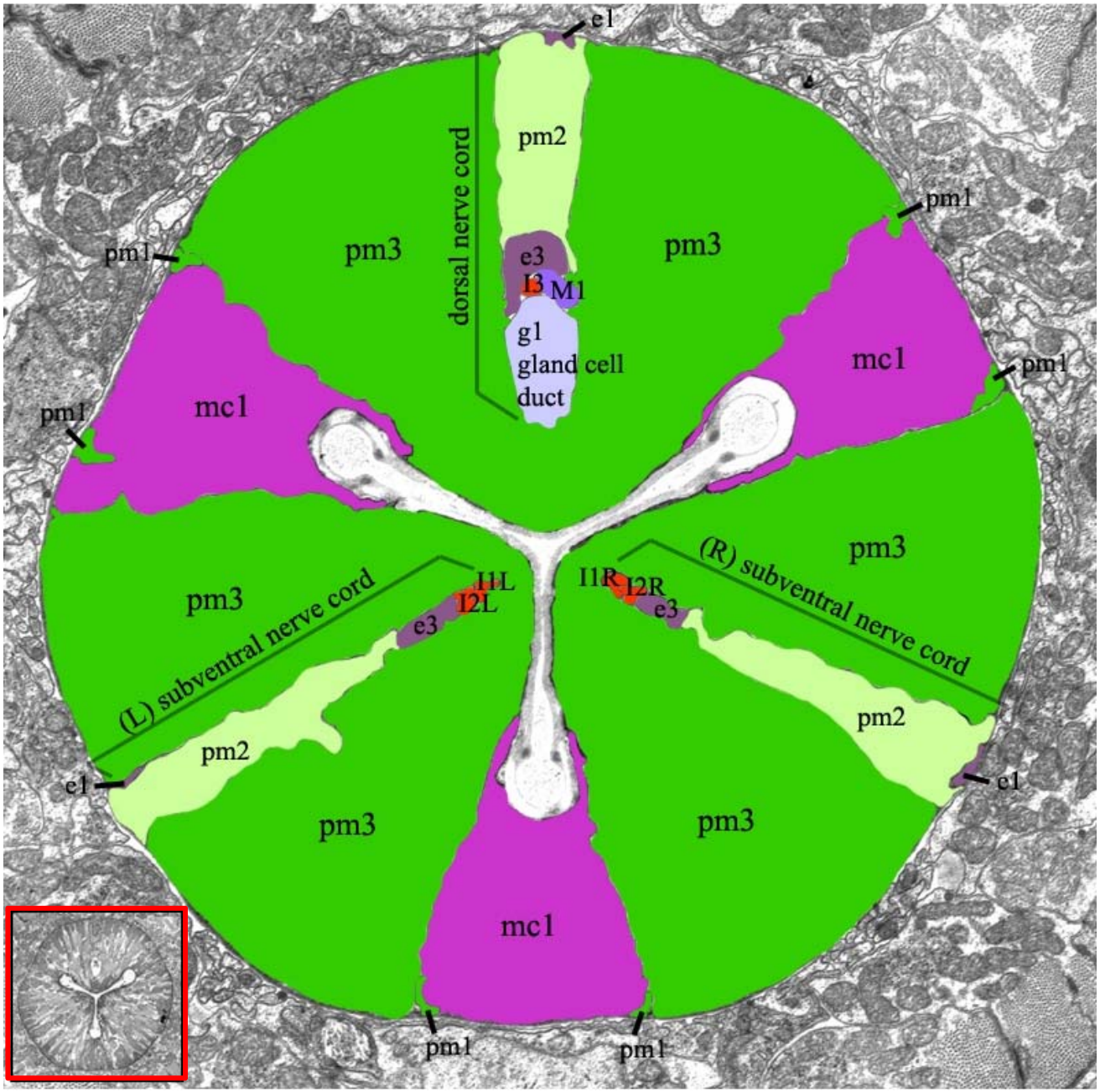
# Pharynx muscles

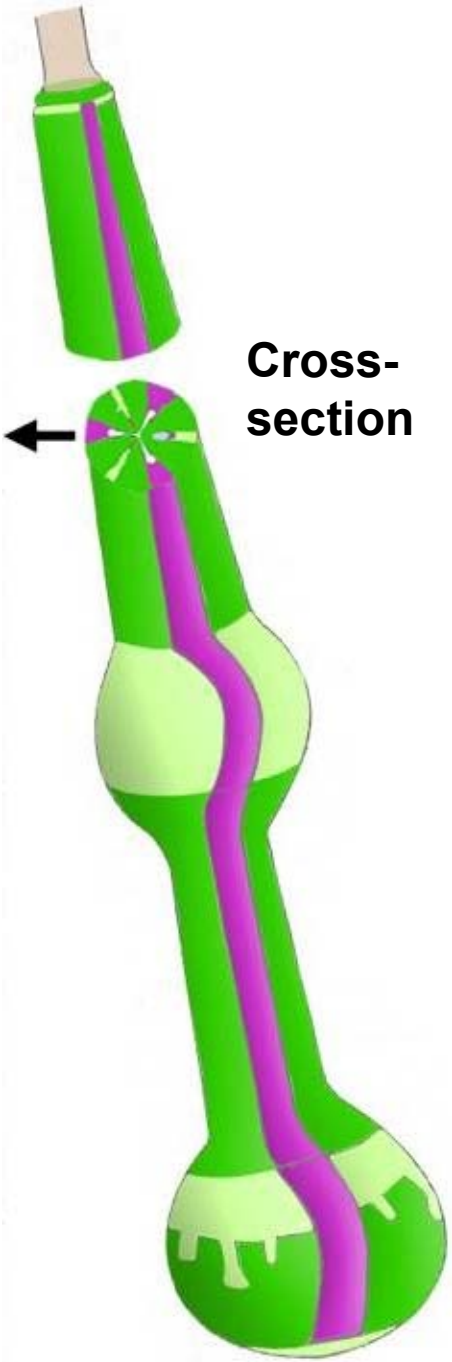
- **Pharyngeal muscles** are grouped into eight separate segments (pm1-8)
- They form 8 consecutive rings of **radial musculature** encircling the pharynx
- Most of these muscle rings are **made up of (only) 3 cells**
- Some of them are **syncytial cells** containing up to 6 nuclei
- **Marginal cells** (mc1-3) separate and strengthen the three main muscle segments

**Sophisticated cell architecture of the pharynx**

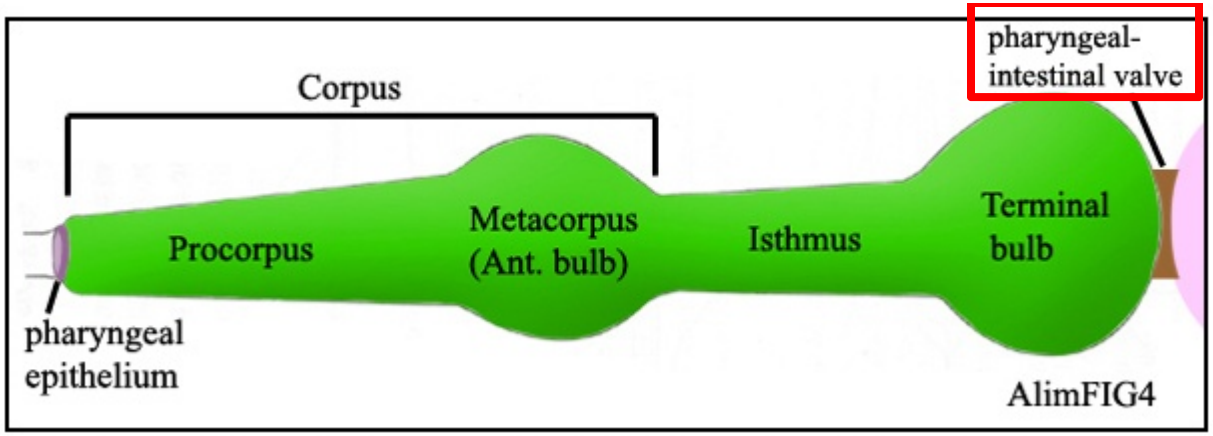


# Cross section of the pharynx in TEM

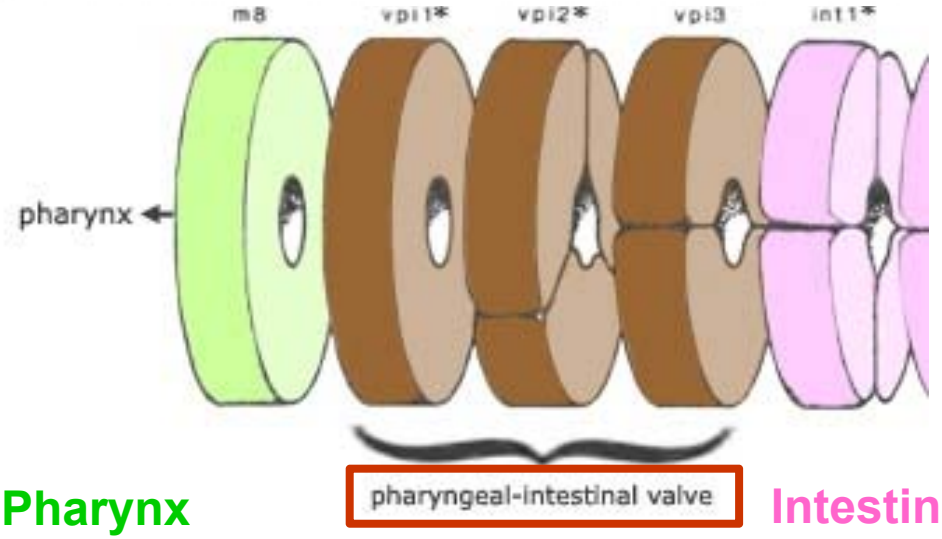


**Cross-section**

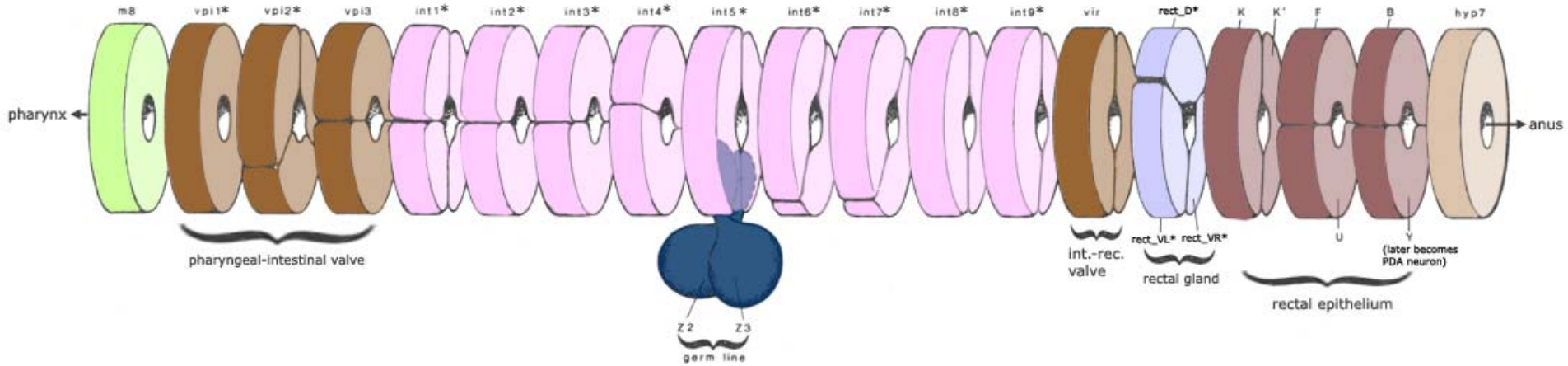



The pharyngeal intestinal valve connects the pharynx to the intestine

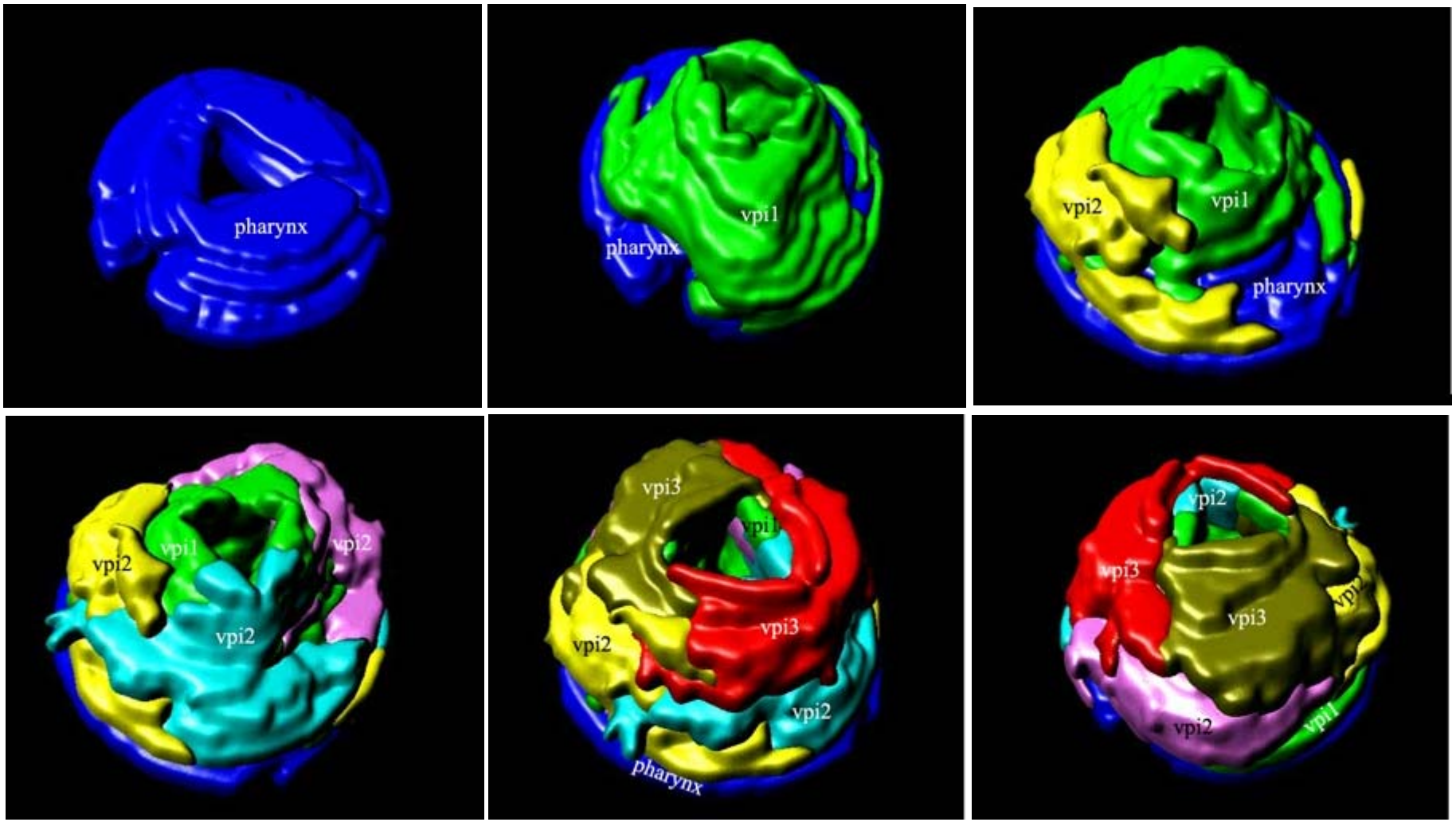


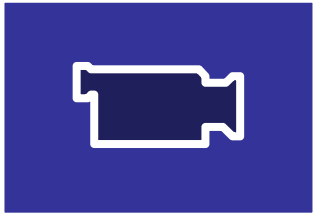

- A group of 6 cells form the **pharyngeal intestinal valve** => to **link** the narrow **terminal bulb** to the wider **intestine**

**Pharynx** **Intestine** **Anus**

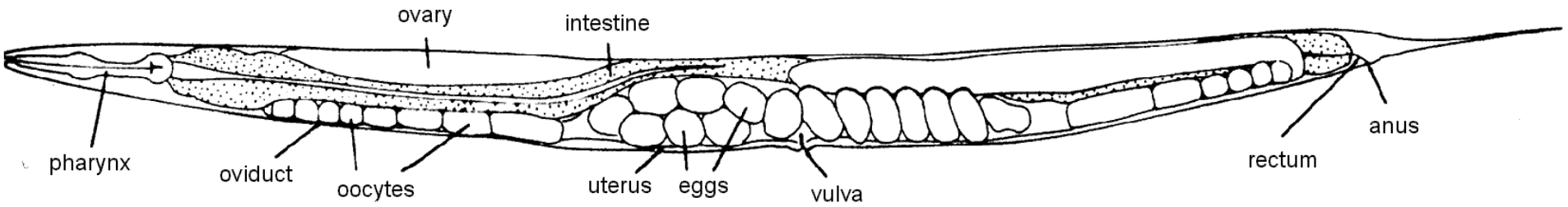


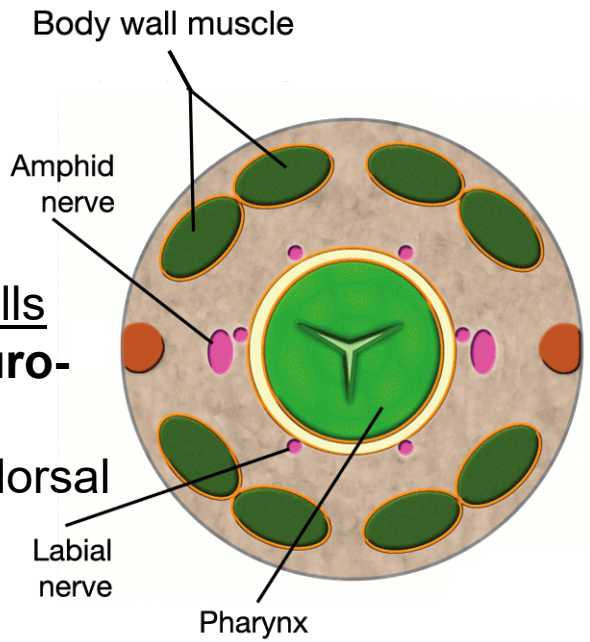
**6** pharyngeal-intestinal valve **cells form a small epithelial channel** linking the *narrow* lumen of the pharynx to the *wider* lumen of the intestine



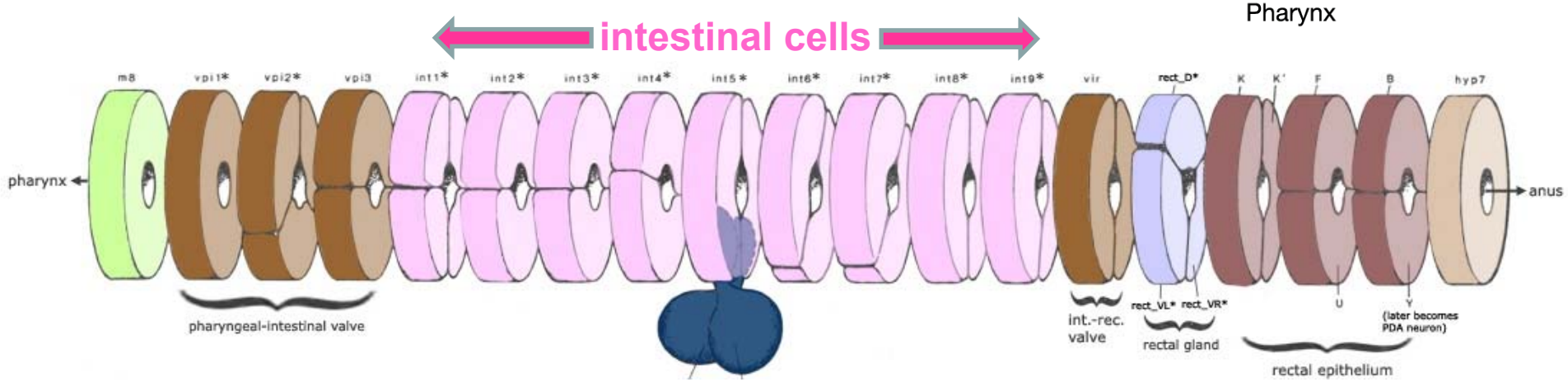



# Intestine and body wall muscles



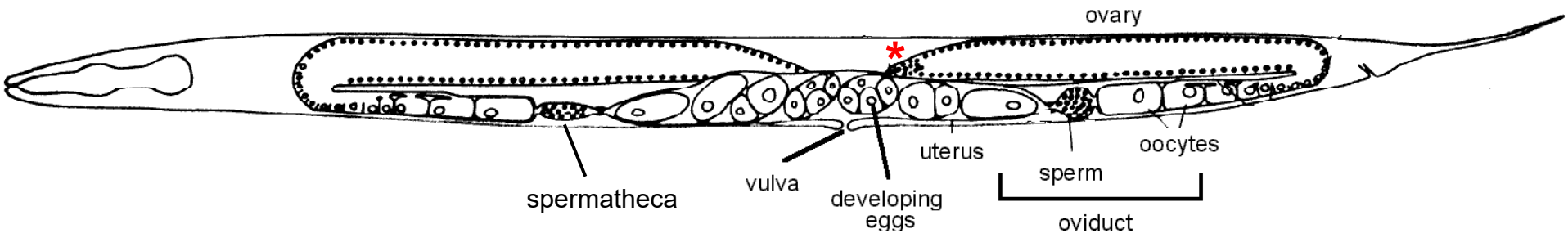




- The **intestine** is a tube composed of only 20 cells (arranged in 9 rings)
- Below the hypodermis, **body-wall muscles** are organized in **4 longitudinal bands** and each band is composed of 2 cells
- The muscle cells send processes to nerve cells to form **neuro-muscular junctions** (NMJs) => different from mammals!
- Coordinated contraction of the ventral muscles versus the dorsal muscles generates the “elegant(s)” waves of the body

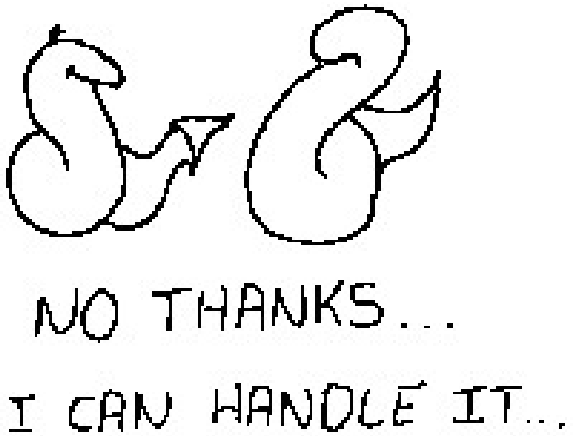


# Reproduction

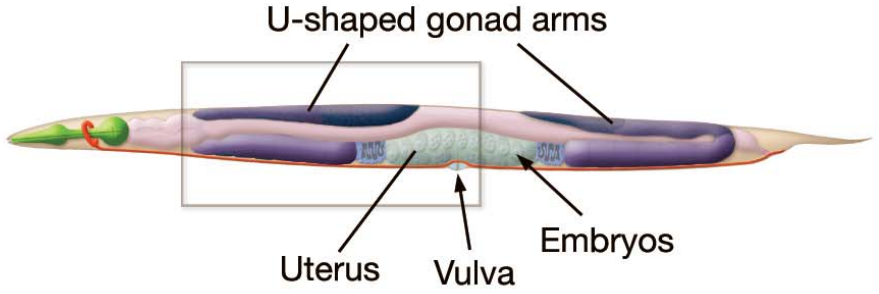
• 99% of adult *C. elegans* are **self-fertilizing hermaphrodites**
• This feature enables scientists to easily generate homozygous mutants
• Hermaphrodites are **protandrous**: the gonads produce **germ cells** which differentiate to both, sperm cells *and* eggs
• *C. elegans* produce males to about 0.1% that produce sperm cells only
• Sperm of males can be transferred to hermaphrodites during **mating** => feature enables scientists to **transfer plasmids** (as extrachromosomal arrays)

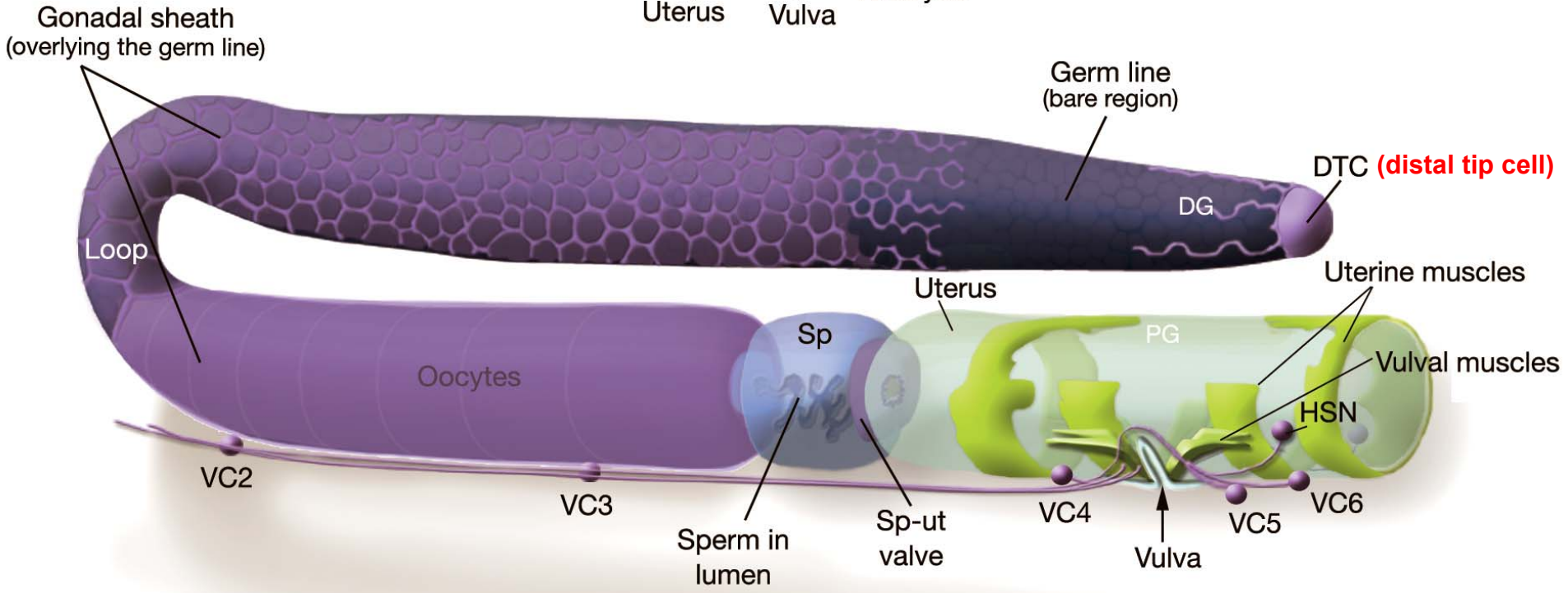



• **Oocyte nuclei** are produced by meiosis at the distal end* of the gonad and grow in a syncytium
• Just before fertilization the single nuclei are enclosed by a separate plasma membrane
• Produced sperm is stored in the **spermatheca**
• After fertilization the **chitin egg-shell is added:**
=> self-fertilization produces up to 300 eggs within 4 days

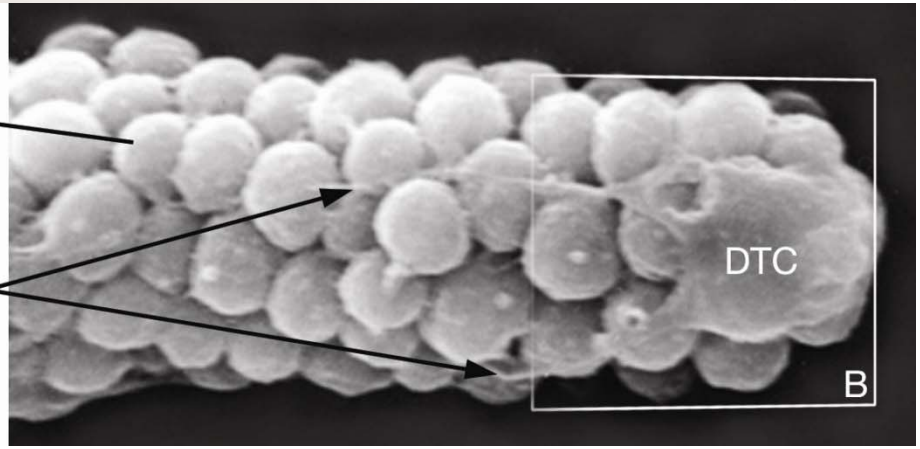


# Reproduction



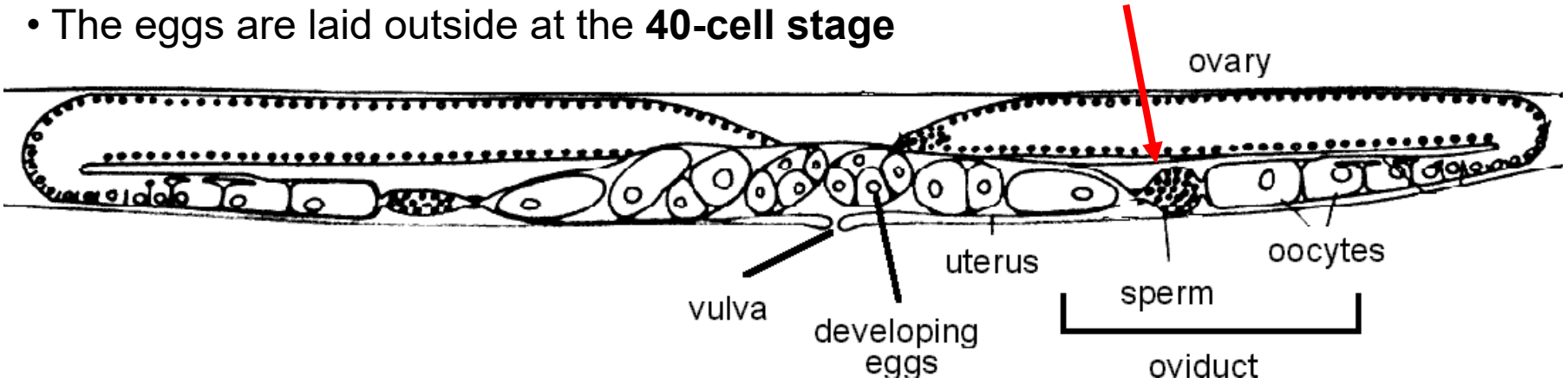




- Germ “cells” (nuclei surrounded by a membrane) emerge from a single distal tip cell (DTC); here, germ cells are mitotic only and highly undifferentiated
- As germ cells **move proximally**, they pass through the stages of **meiosis**



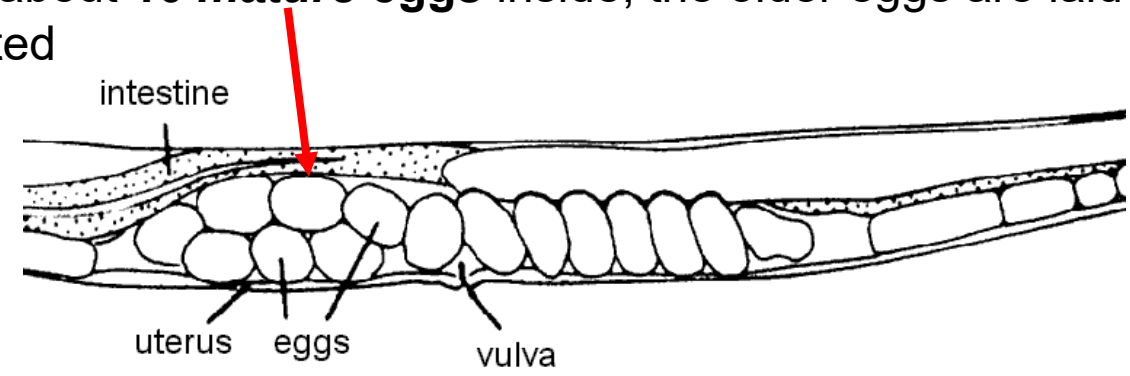
# Reproduction

• Fertilization takes place by squeezing mature oocytes through the spermatheca
• The eggs are laid outside at the **40-cell stage**



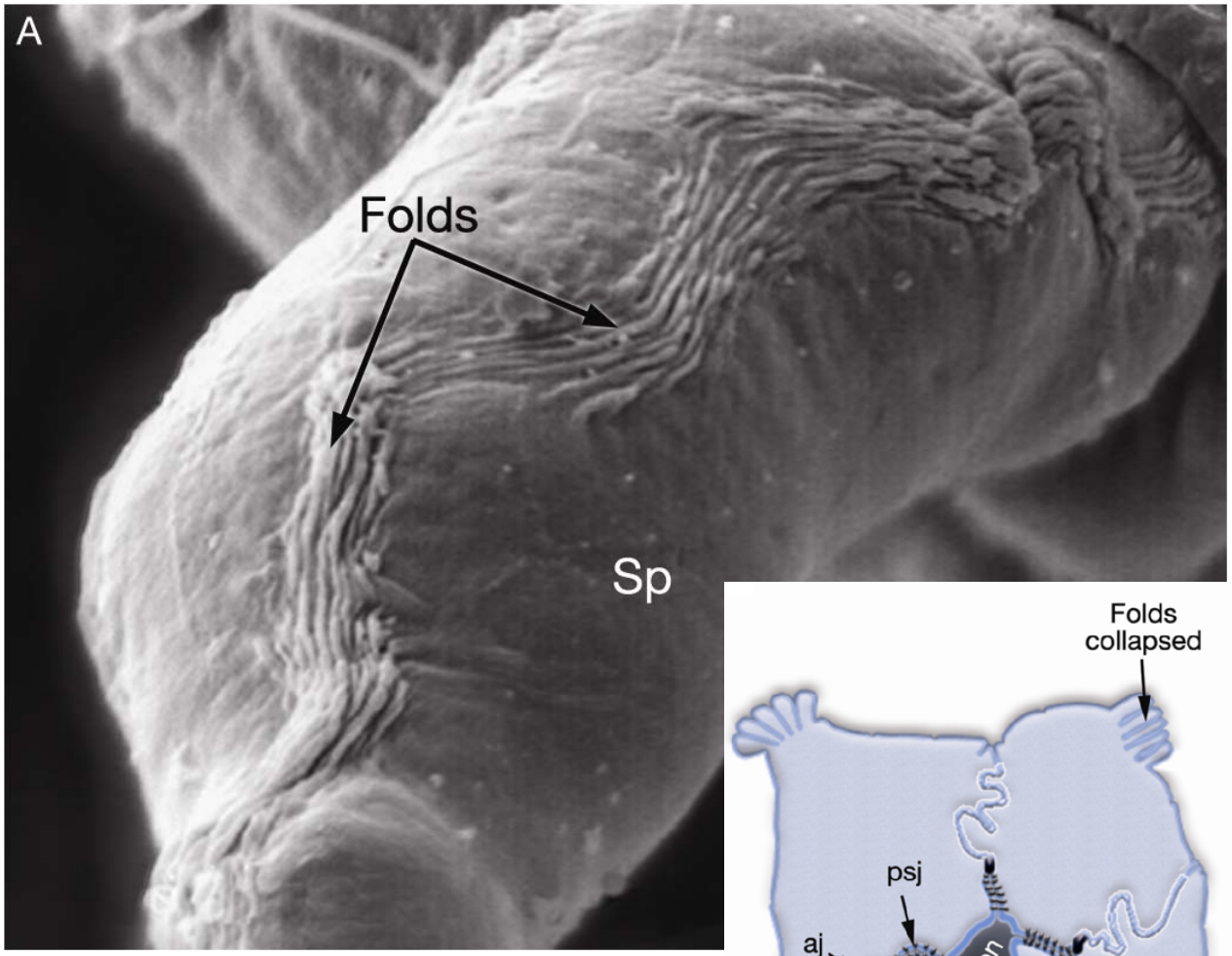

• Adult hermaphrodites have about **10 *mature* eggs** inside; the older eggs are laid as fast as new eggs are generated

A hermaphrodite produces up to 1300 eggs during its lifetime




• In the case of mating, the male sperm **outcompetes** the hermaphrodite's sperm
• Males have XO gonosomes: spontaneous loss of X chromosome: XX => XO

# Spermatheca is highly extendable

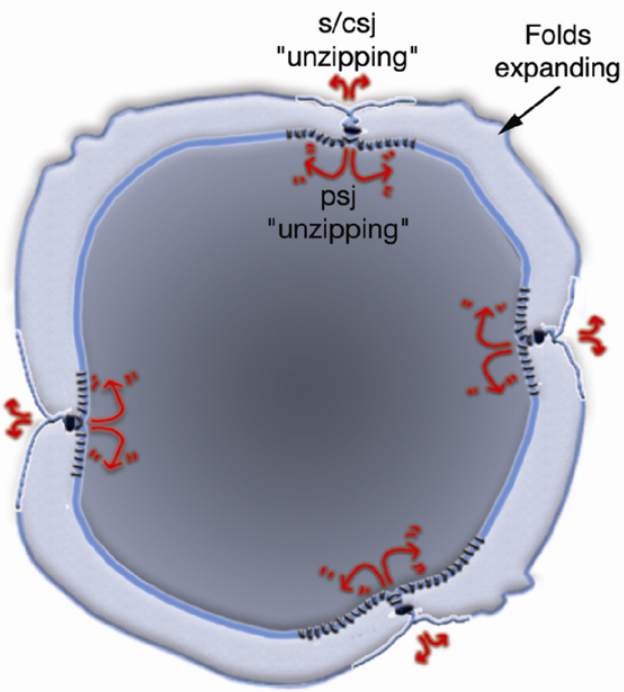



- Spermatheca is an <u>accordion-like tube</u> (containing the sperm)
- <u>In the absence of an oocyte</u>, spermatheca lumen is **narrow**
- Numerous **longitudinal folds** allow for the <u>radial expansion during oocyte passage</u>



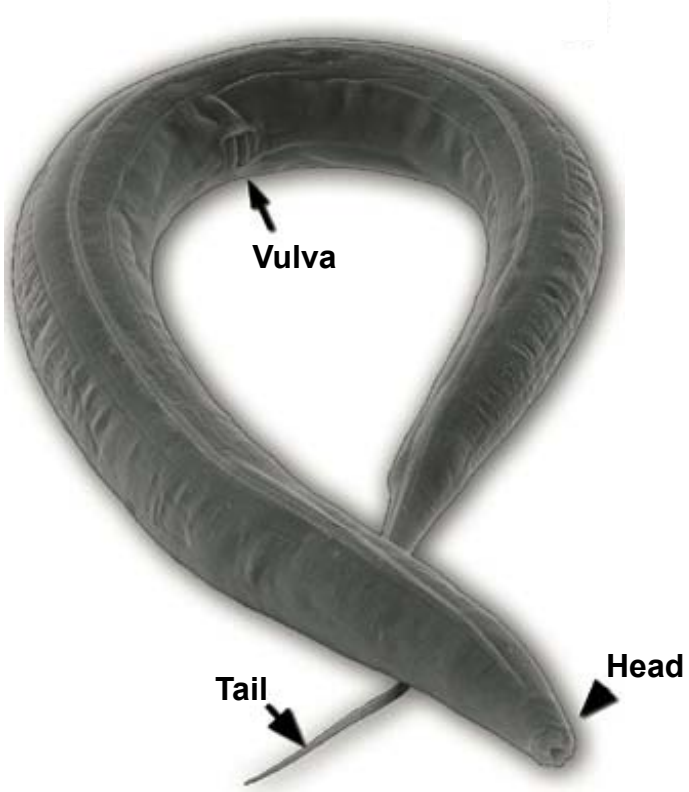


EMPTY

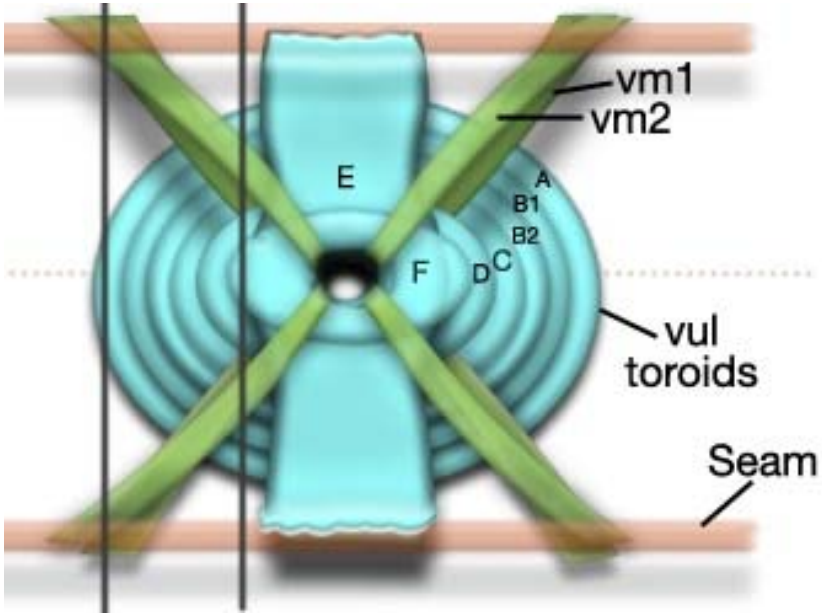


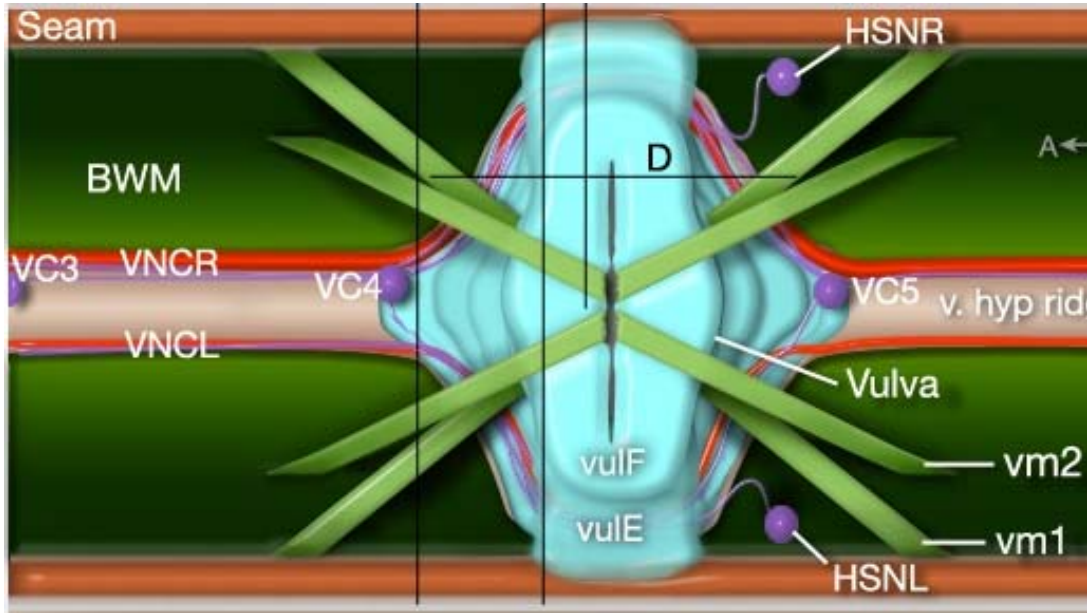

INFLATED

# Egg release




- The vulva is formed from a **stack of 7** epithelial **toroids** (ring-type cells)
- Coordinated shortening of the **vulval muscles** pulls the lips apart allowing eggs to pass through the lumen (out into the environment)
- Vulva muscles receive inputs from two groups of motor neurons: VC and **HSN** neurons

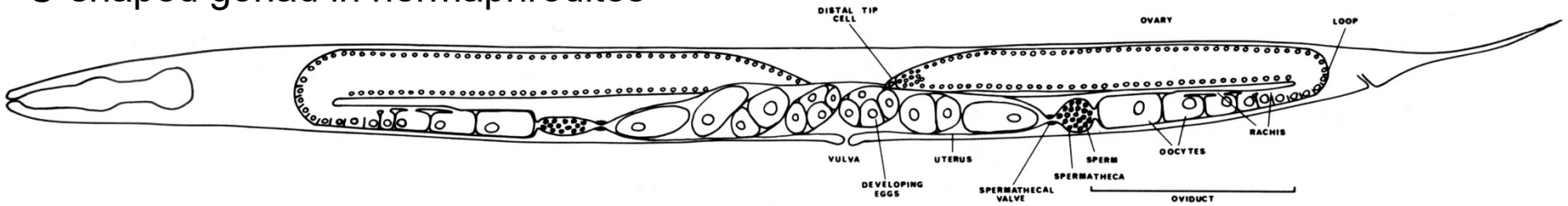


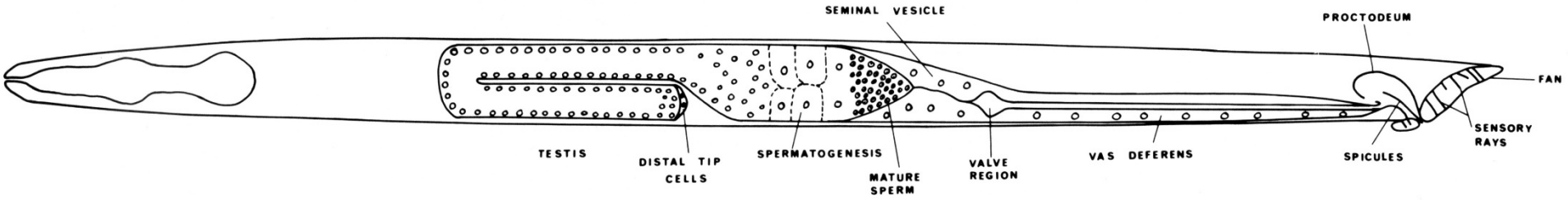
# Special features of male anatomy

- Only one X-chromosome (XO)
- Different gross morphology and **behaviors** from hermaphrodites
- Slimmer than hermaphrodites (no eggs) and a clear (white) ventral gonad
- The hermaphrodite gonad is **U-shaped** while the male gonad is **J-shaped**

U-shaped gonad in hermaphrodites




J-shaped gonad in males



# Stress induces throwing of males

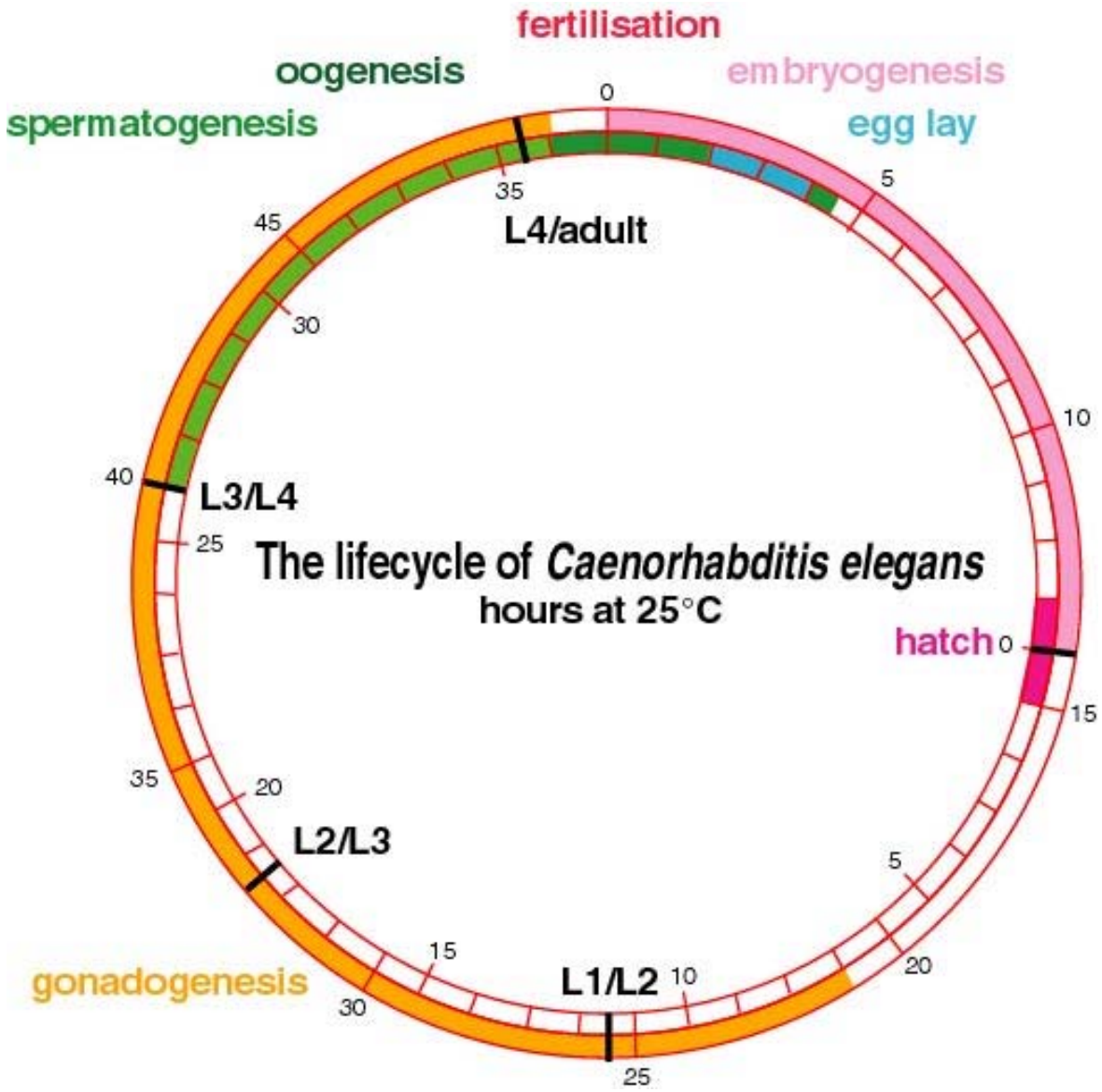
- Males arise from fusion of **nullo-X gametes** and **normal** X-carrying **gametes**
- Nullo-X gametes are generated by *spontaneous* non-disjunction of the X chromosome during meiosis in the germ line

Ways to increase the number of males:

- Using ***him*** mutations (*him* = high incidence of males) => these mutations increase the frequency of X-non-disjunction => up to 30% males
- **Male mating**: mating hermaphrodites with males increases the number of males up to 50%
- **Heat-shock**: exposure of hermaphrodites to 30°C for several hours increases the number of males
- Also exposure to **ethanol** increases the number of males

# The *C. elegans* lifecycle

- The **4 larval stages** ("juveniles") are common features of nematodes
- Note that **embryogenesis** occurs *inside* **and** *outside* the worm (laid eggs at 40-cell stage)



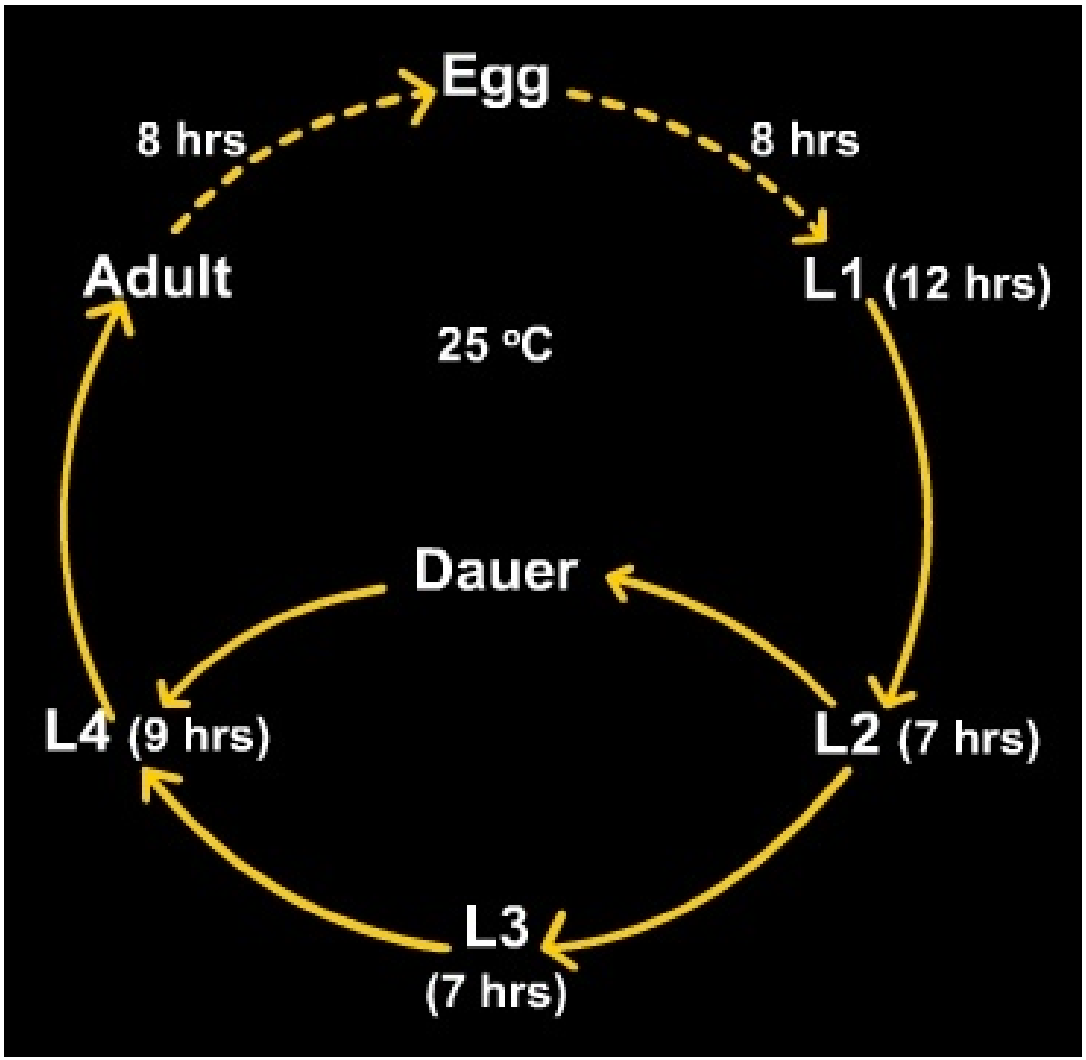

**Outer ring**: **gonadogenesis**, **spermatogenesis**, **oogenesis**, **embryogenesis**

**Inner ring**: Hatching, L1, L2, L3, L4 and adult

Speed of lifecycle is temperature dependent:
2.5 days at 25°C
3.5 days at 20°C
5.5 days at 15°C

# The *C. elegans* lifecycle

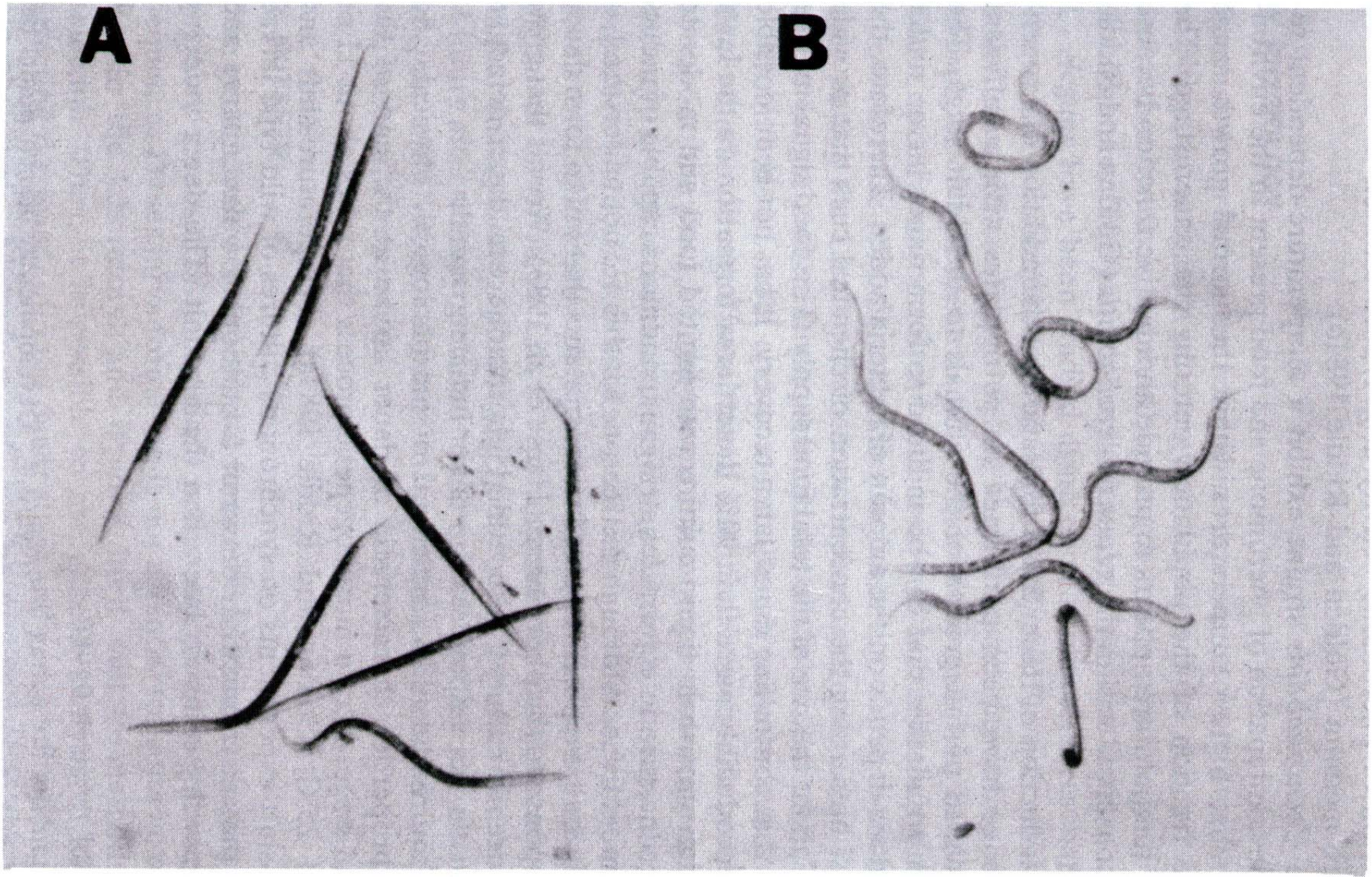
- *C. elegans* has an alternative L3 stage known as **dauer** ("enduring") **stage**
- The dauer stage is a **metabolic diapause** to survive extreme conditions (mainly lack of food = starvation)




- The **entry into the dauer** stage is determined by worm-crowding, high temperature and lack of food
- As a dauer, *C. elegans* can survive for **up to 3 month**, highly extending its lifespan (from usually only 2-3 weeks)
- When **conditions improve** the L3 dauer exits and development resumes
- Parasitic nematodes use the dauer to **infect hosts**

Dauer larvae usually appear dark, thin, rigid and motionless

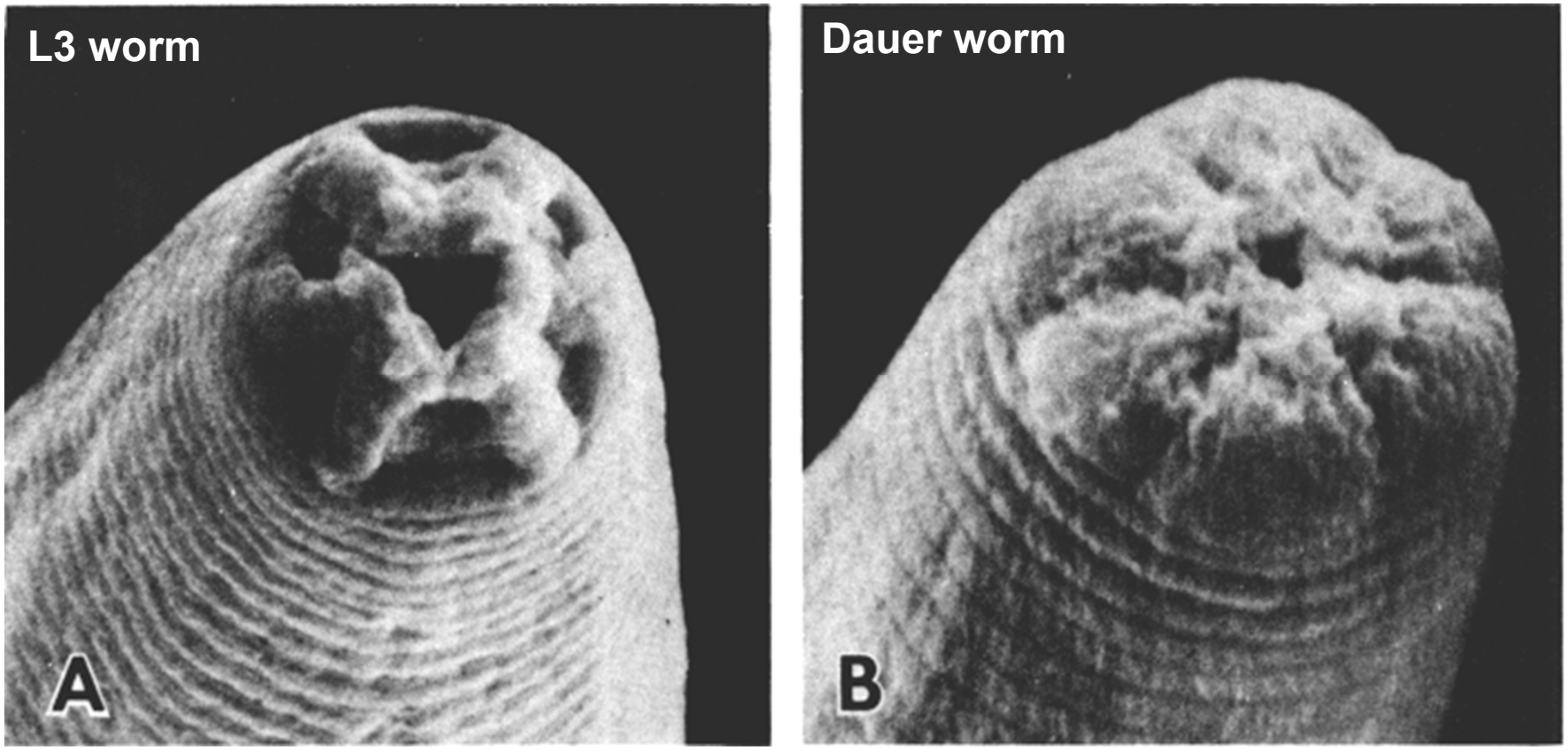
Recovered dauer larvae retain their transparent appearance and begin feeding with increasing motion



# During dauer-formation the mouth closes

• There is **no aging** at dauer state! Due to the dauer stage worms can live up to **10 times longer** than their normal lifespan!

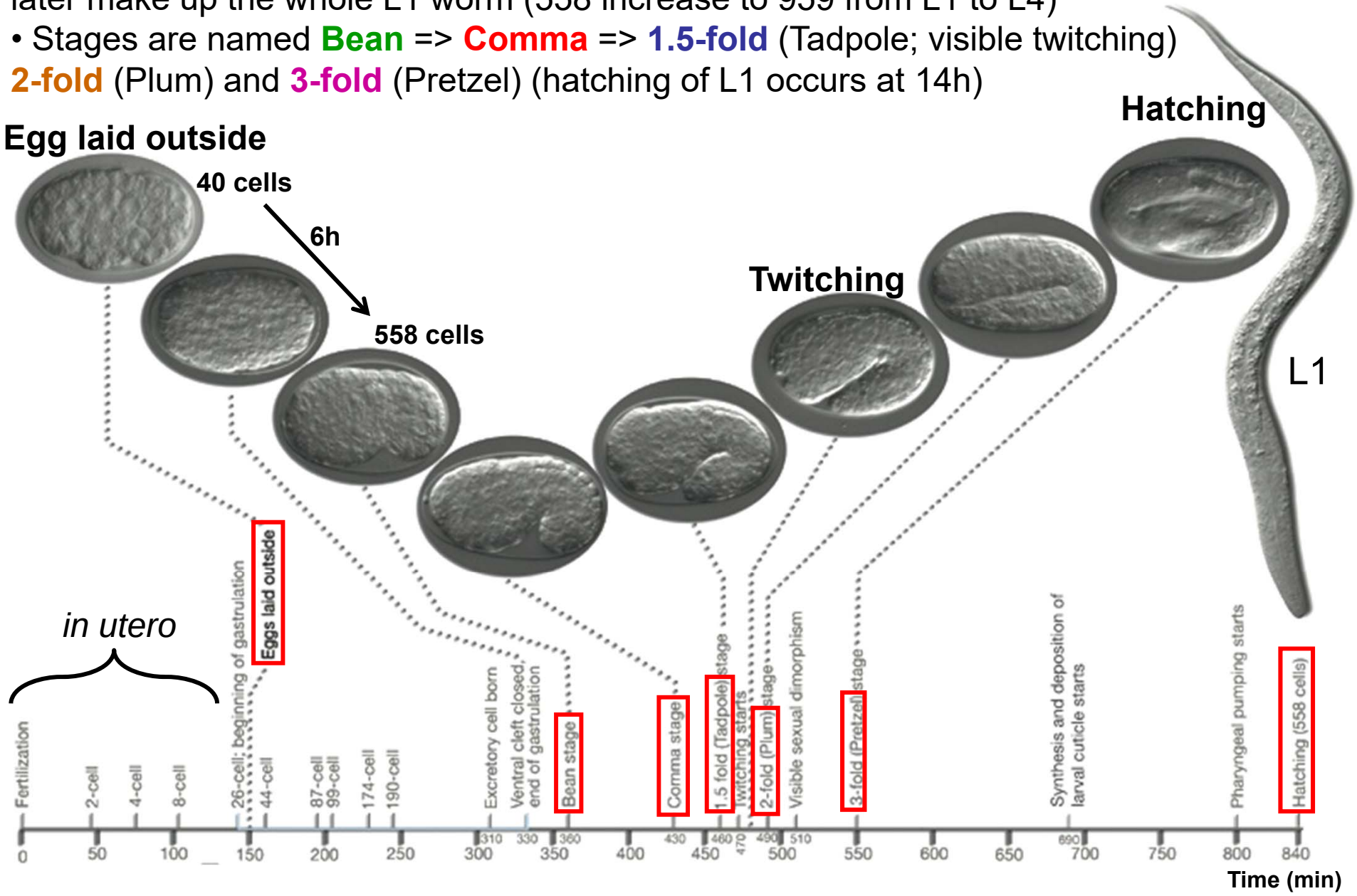
• Due to the physical **mouth closure** (by overgrowing tissue) the worms are restricted from eating



# Introduction to embryogenesis

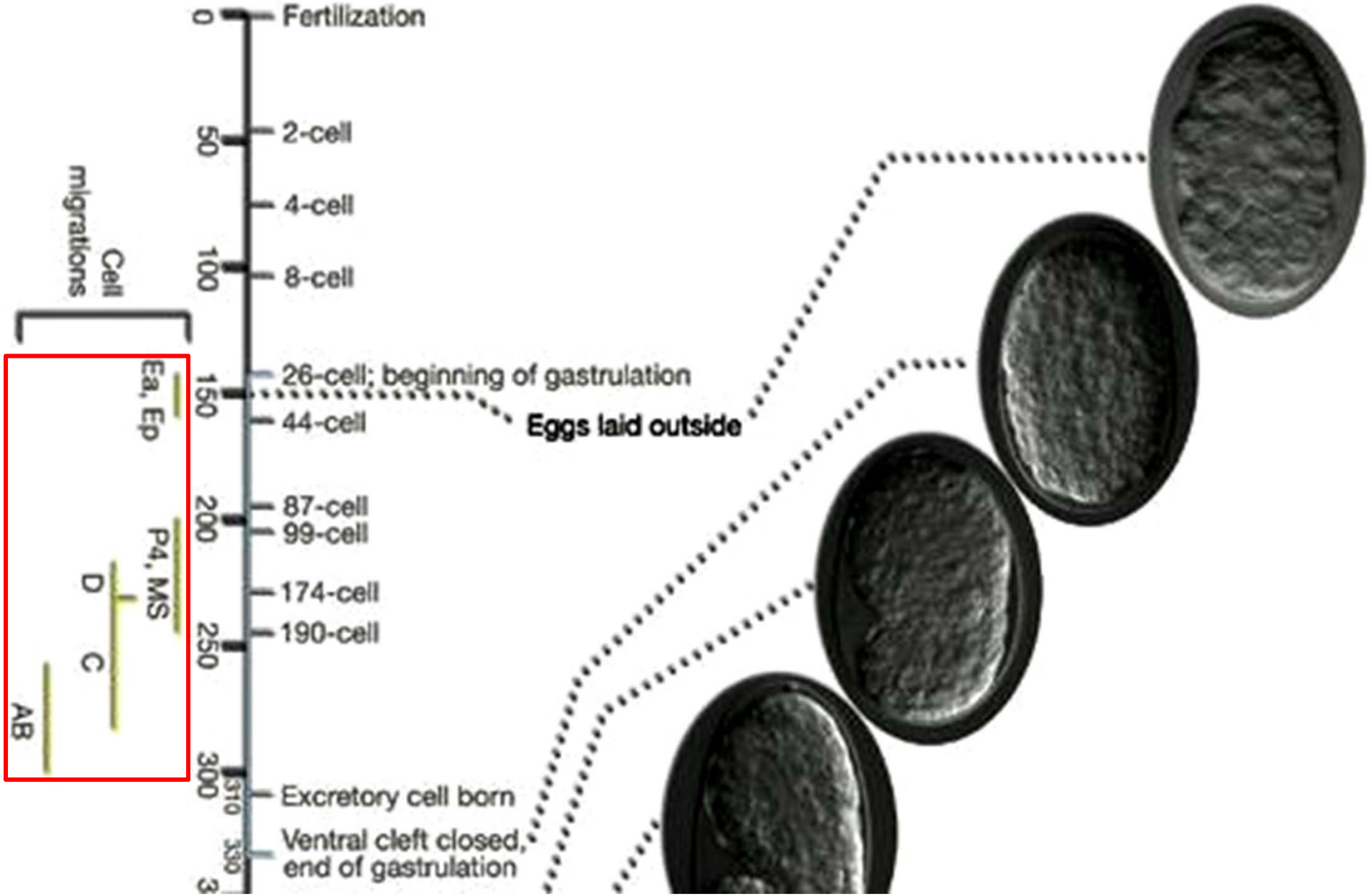
• During the first 360 min (6h), cell division produces all 558 cells (**bean stage**) that later make up the whole L1 worm (558 increase to 959 from L1 to L4)

• Stages are named **Bean** => **Comma** => **1.5-fold** (Tadpole; visible twitching) **2-fold** (Plum) and **3-fold** (Pretzel) (hatching of L1 occurs at 14h)

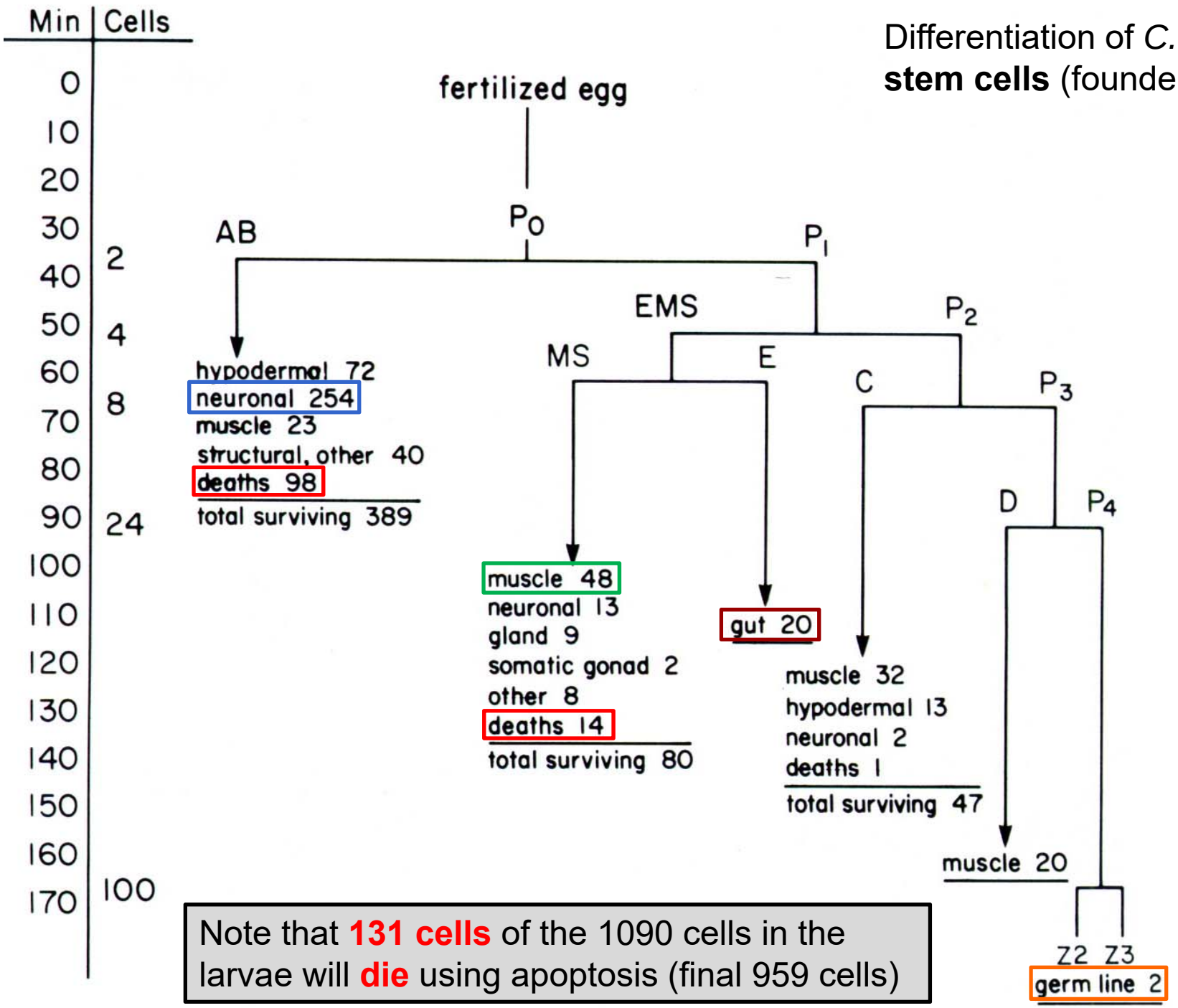


Embryogenesis in a time-lapse movie

After egg has been laid outside, **gastrulation** follows by **generating six** so called **founder cells** ("similar to stem cells"): **E, P4, MS, D, C, AB**



Differentiation of *C. elegans*
**stem cells** (founder cells)

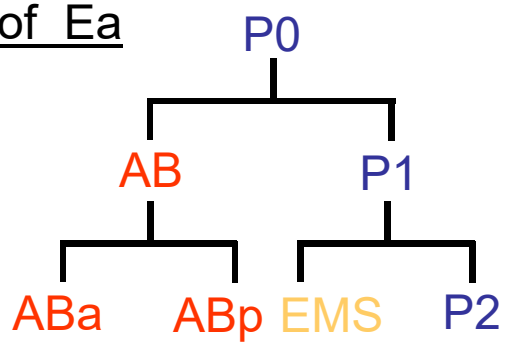


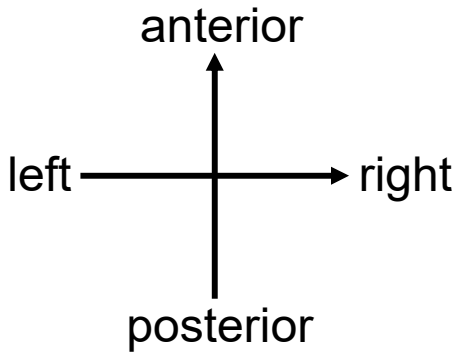

Note that **131 cells** of the 1090 cells in the larvae will **die** using apoptosis (final 959 cells)

# The cell lineage diagram and cell nomenclature

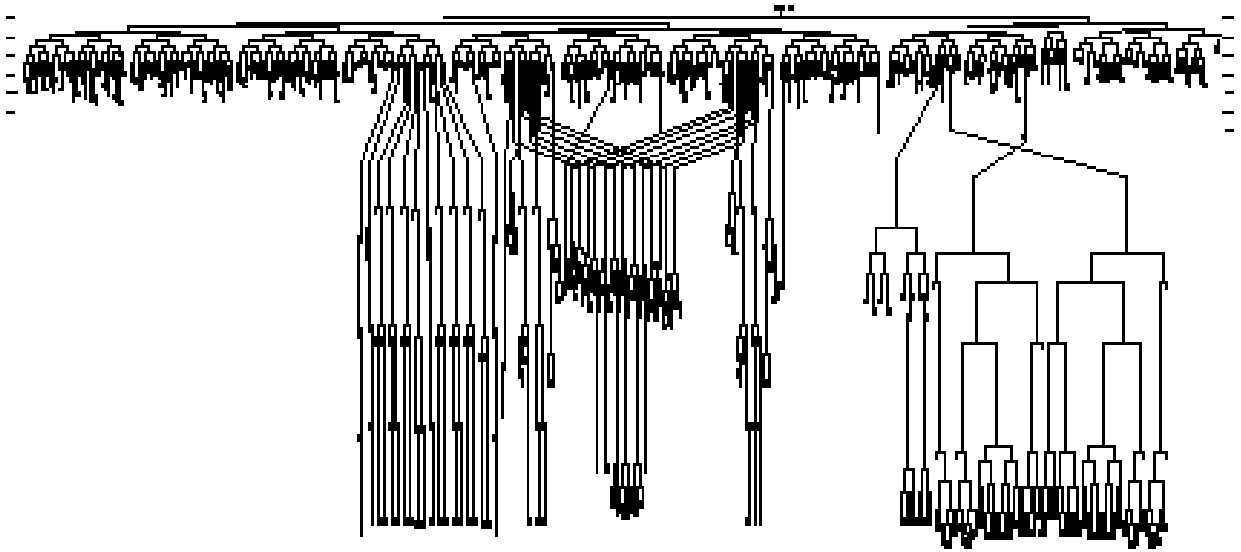
- **Key blastomere cells** are designated with an upper case letter
- Blast cell **progeny** have an additional lower case letter according to the pattern of cell division (by which they were generated):

**Ea** is the ***a***nterior daughter of the founder cell E; **Eap** is the ***p***osterior daughter cell of Ea

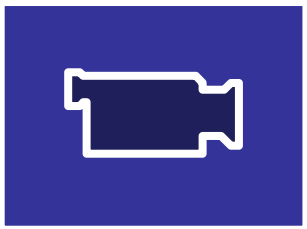


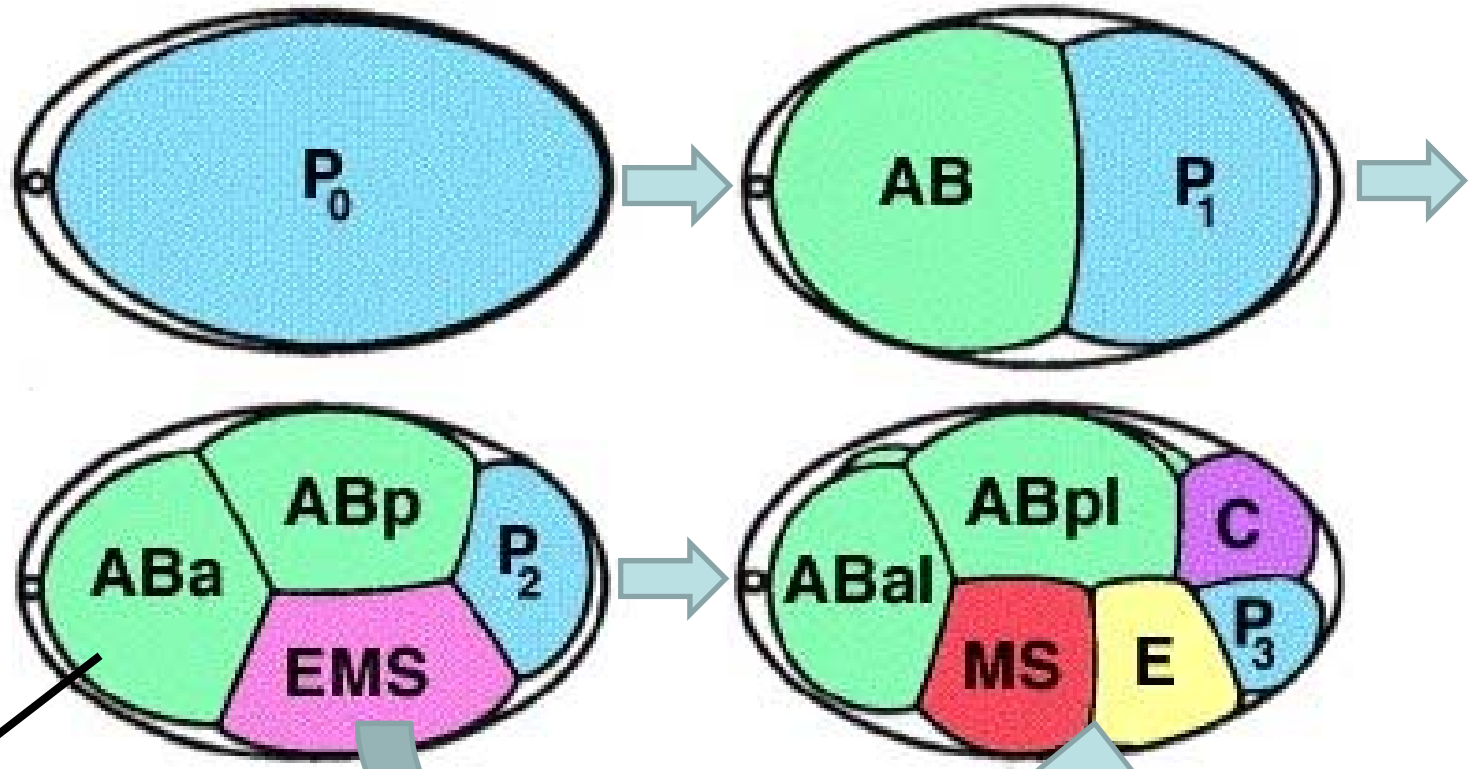

- Dr. J. Sulston spent 1.5 years in the dark to generate the cell lineage wire diagram
- He did not use any staining (antibody staining or GFP expression)
- He followed each cell by hand using DIC microscopy (received Nobel prize for this)



Complete cell lineage



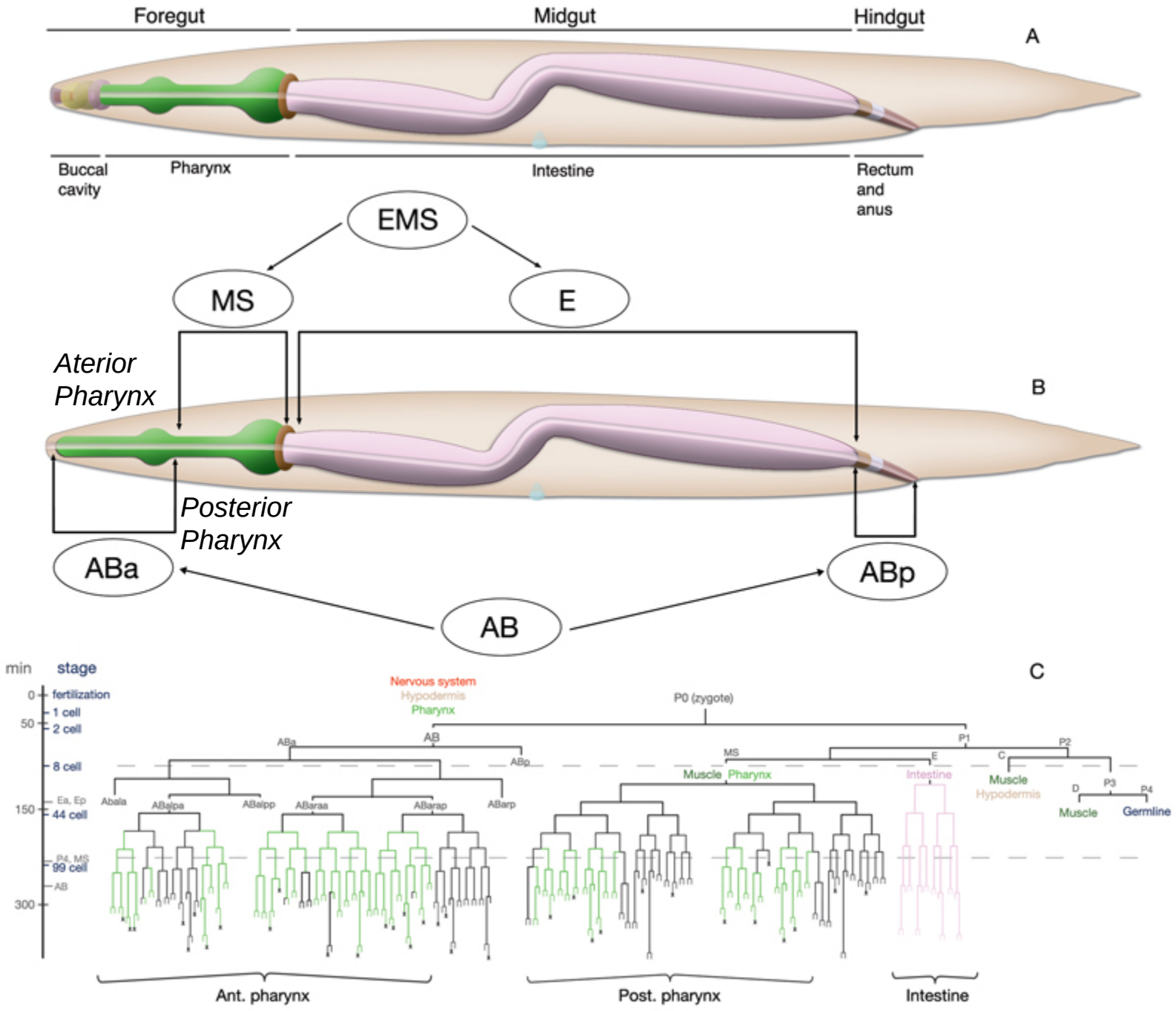
The **EMS founder cell** forms two major germ layers:
**endoderm** (intestine) and **mesoderm** (muscle)



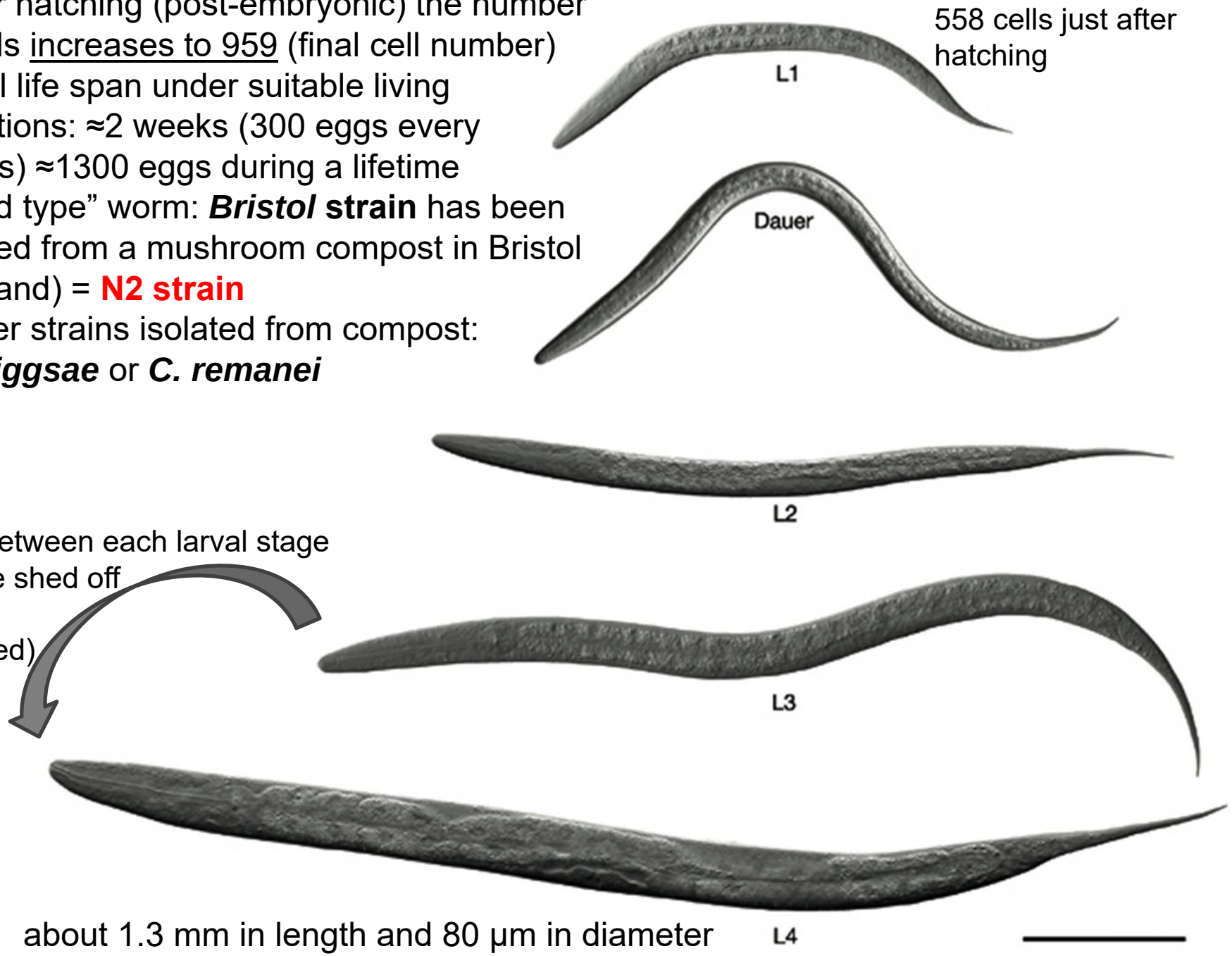

**ABa** = founder cell of
**epidermis** and **pharynx**

EMS makes MS + E

**MS** = founder cell
of mesoderm (**muscle**
and **pharynx**)
**E** = founder cell of
endoderm (**intestine**)


Foregut
Midgut
Hindgut
A
Buccal cavity
Pharynx
Intestine
Rectum and anus
EMS
MS
E
Aterior Pharynx
B
Posterior Pharynx
ABa
ABp
AB
min
stage
0
fertilization
50
1 cell
2 cell
8 cell
150
Ea, Ep
44 cell
P4, MS
99 cell
AB
300
Nervous system
Hypodermis
Pharynx
P0 (zygote)
ABa
AB
ABp
P1
P2
MS
E
C
Muscle
Pharynx
Intestine
Muscle
Hypodermis
D
P3
P4
Muscle
Germline
Abala
ABalpa
ABalpp
ABaraa
ABarap
ABarp
Ant. pharynx
Post. pharynx
Intestine

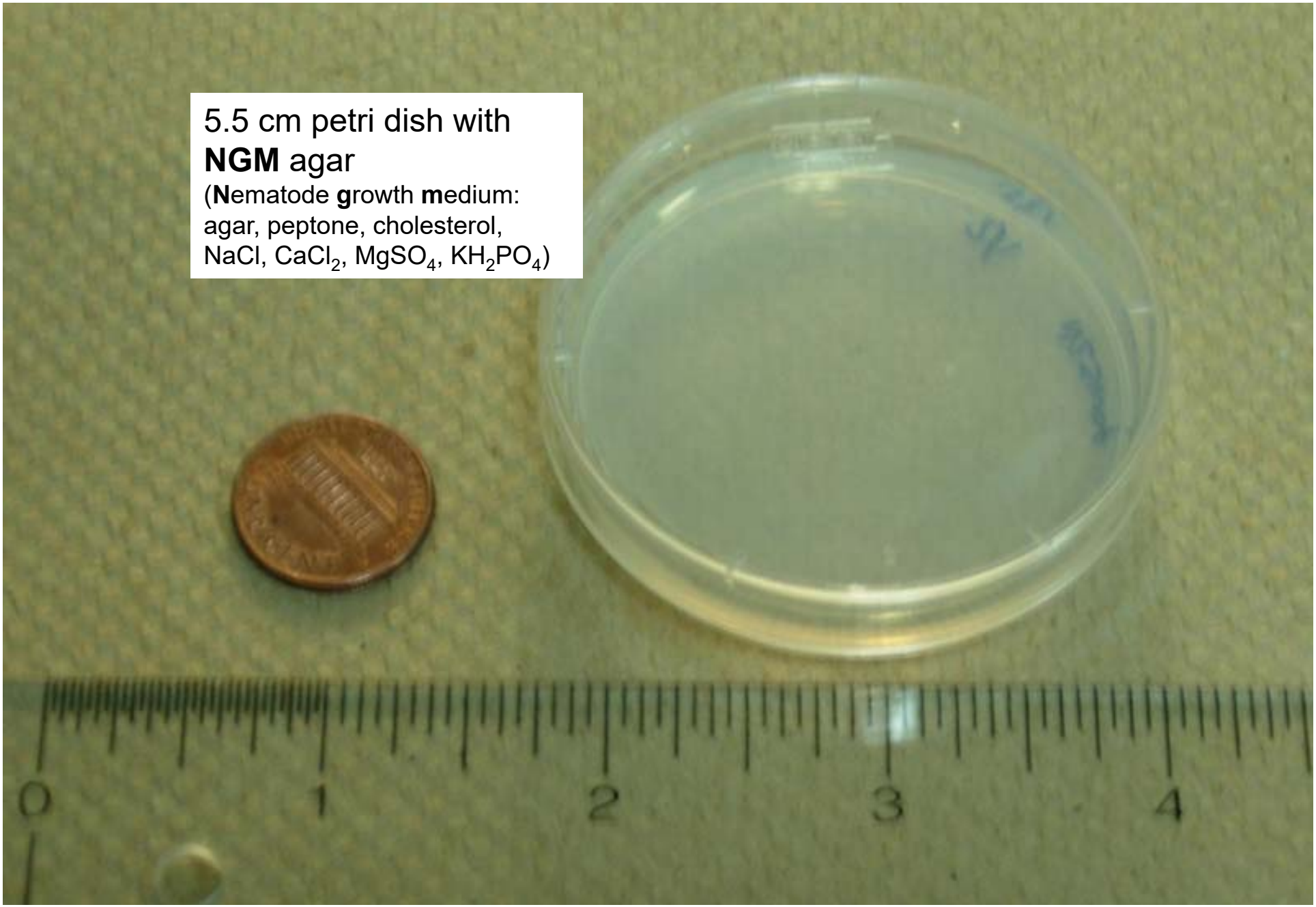
• After hatching (post-embryonic) the number of cells <u>increases to 959</u> (final cell number)
• Total life span under suitable living conditions: ≈2 weeks (300 eggs every 4 days) ≈1300 eggs during a lifetime
• "Wild type" worm: ***Bristol* strain** has been isolated from a mushroom compost in Bristol (England) = **N2 strain**
• Other strains isolated from compost: ***C. briggsae*** or ***C. remanei***

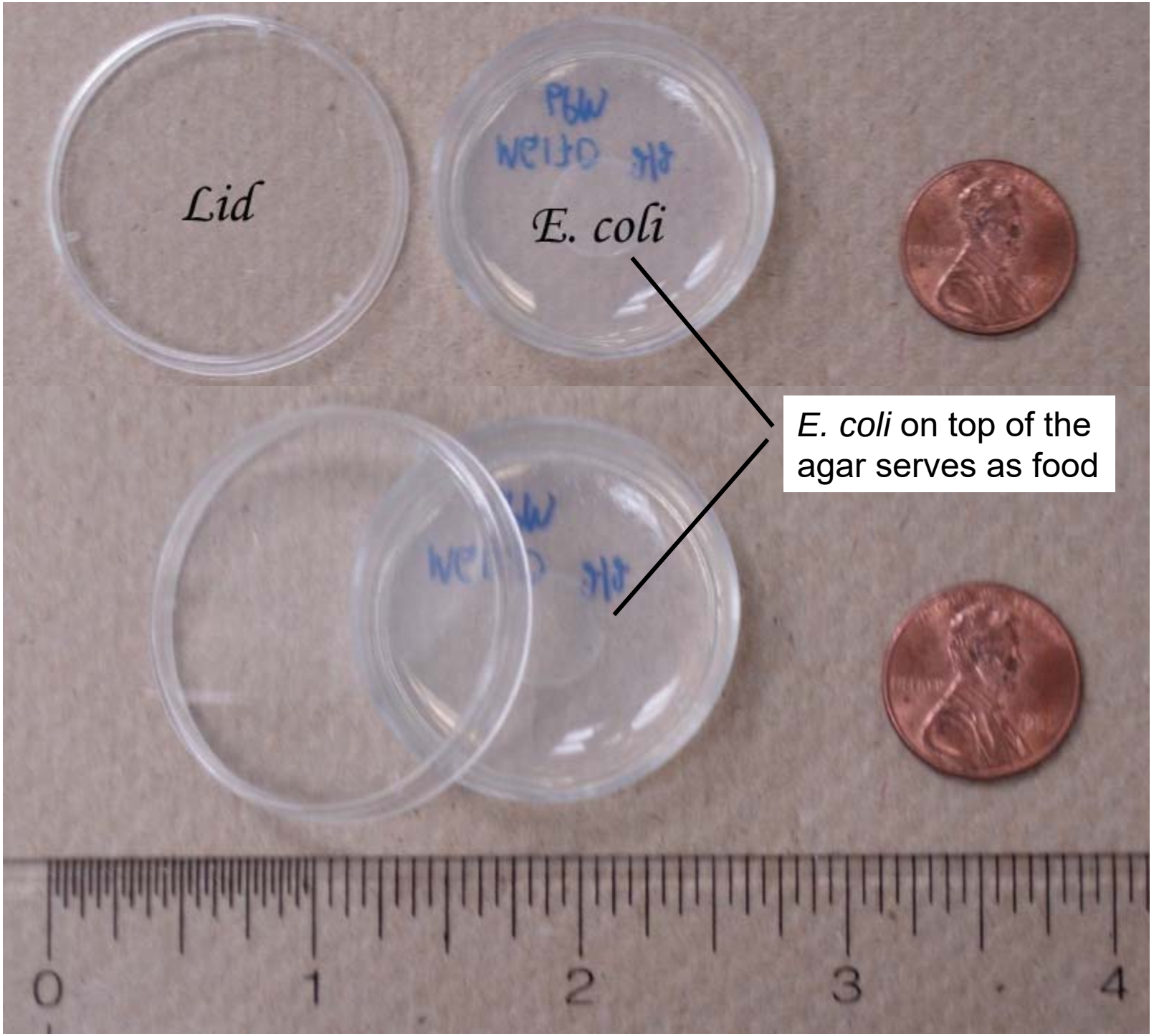



**Molt** between each larval stage (cuticle shed off and renewed)

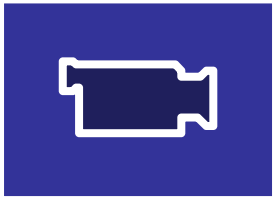
about 1.3 mm in length and 80 µm in diameter

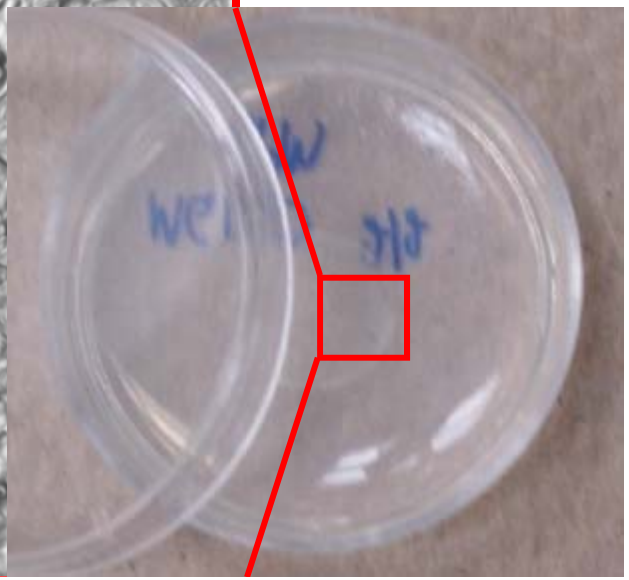
# How to cultivate *C. elegans*?

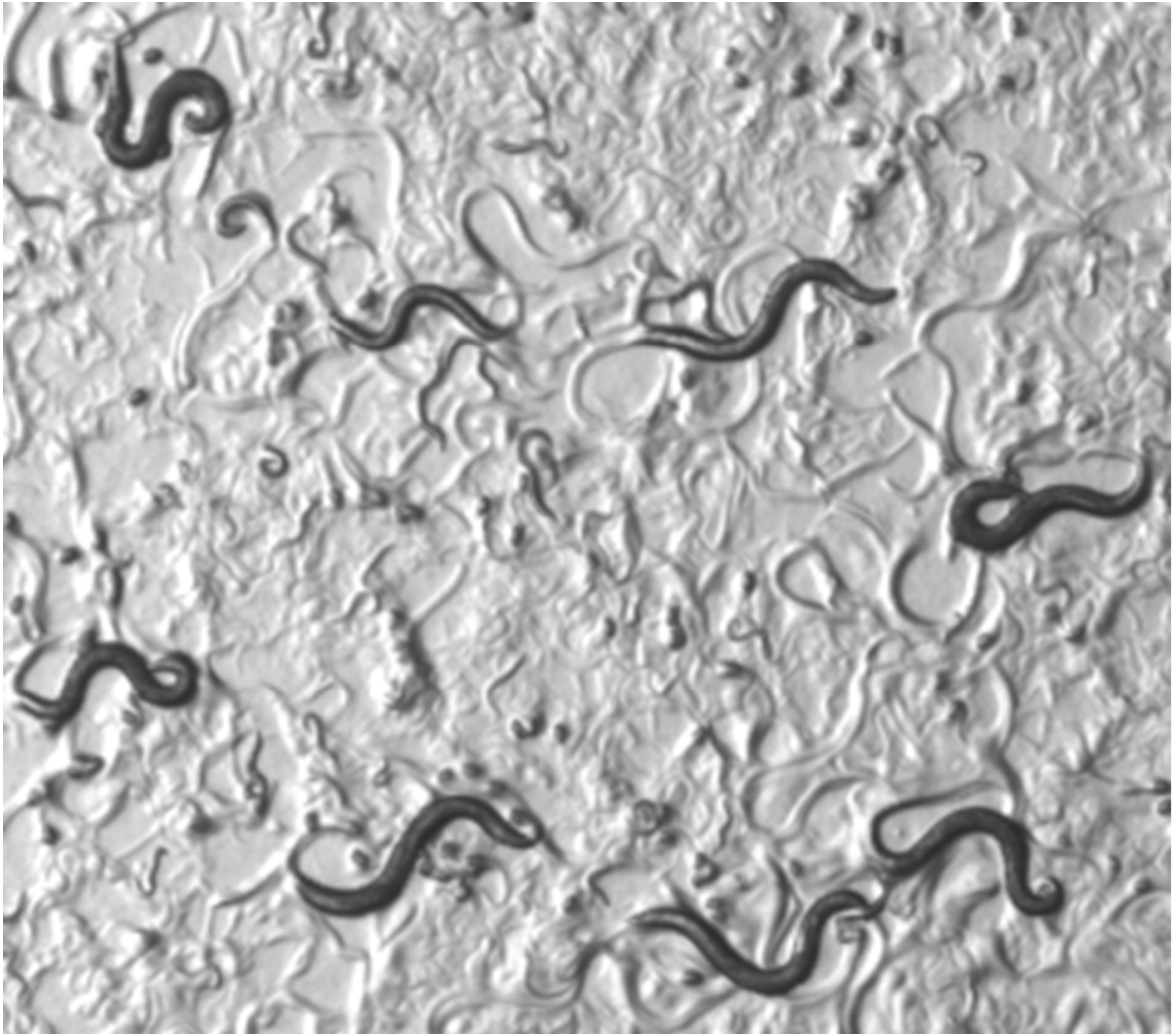



Lid
E. coli
*E. coli* on top of the agar serves as food
0
1
2
3
4

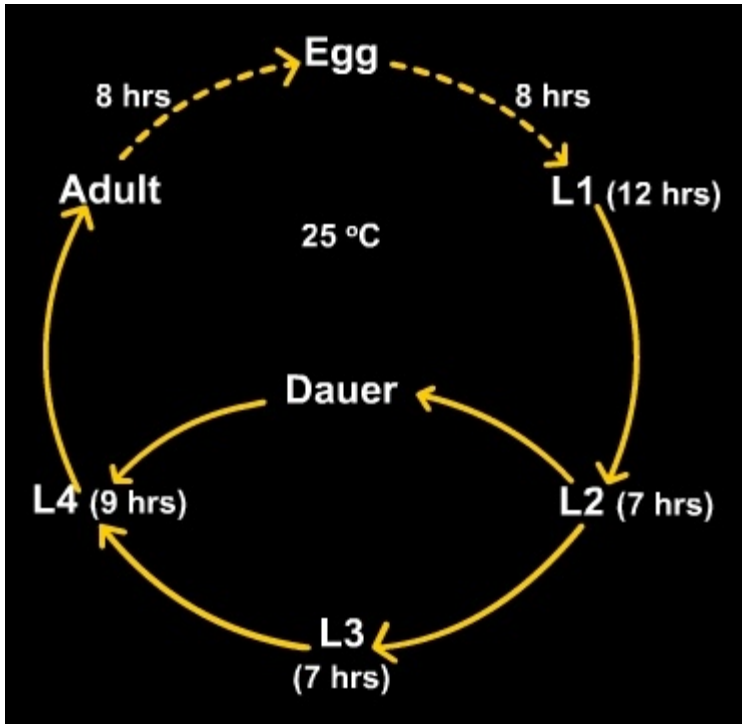
- Bacterial lawn (smear)
- Eggs
- Larvae
- Adults

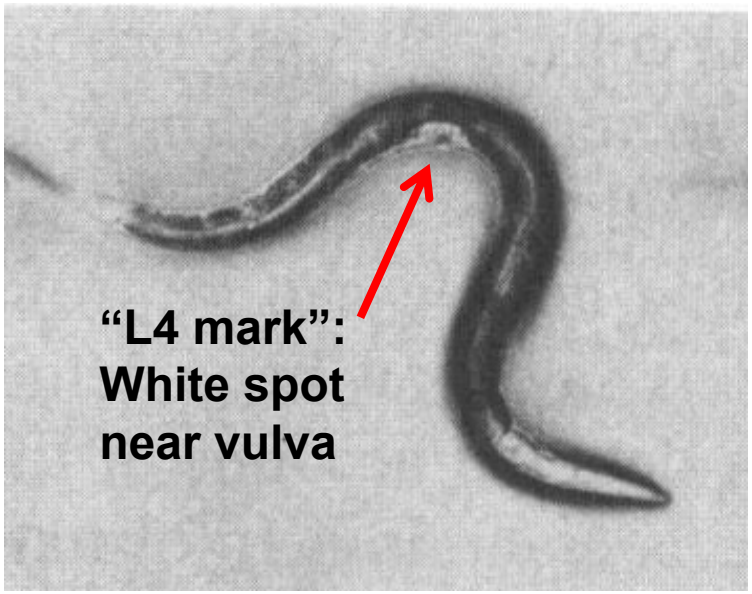


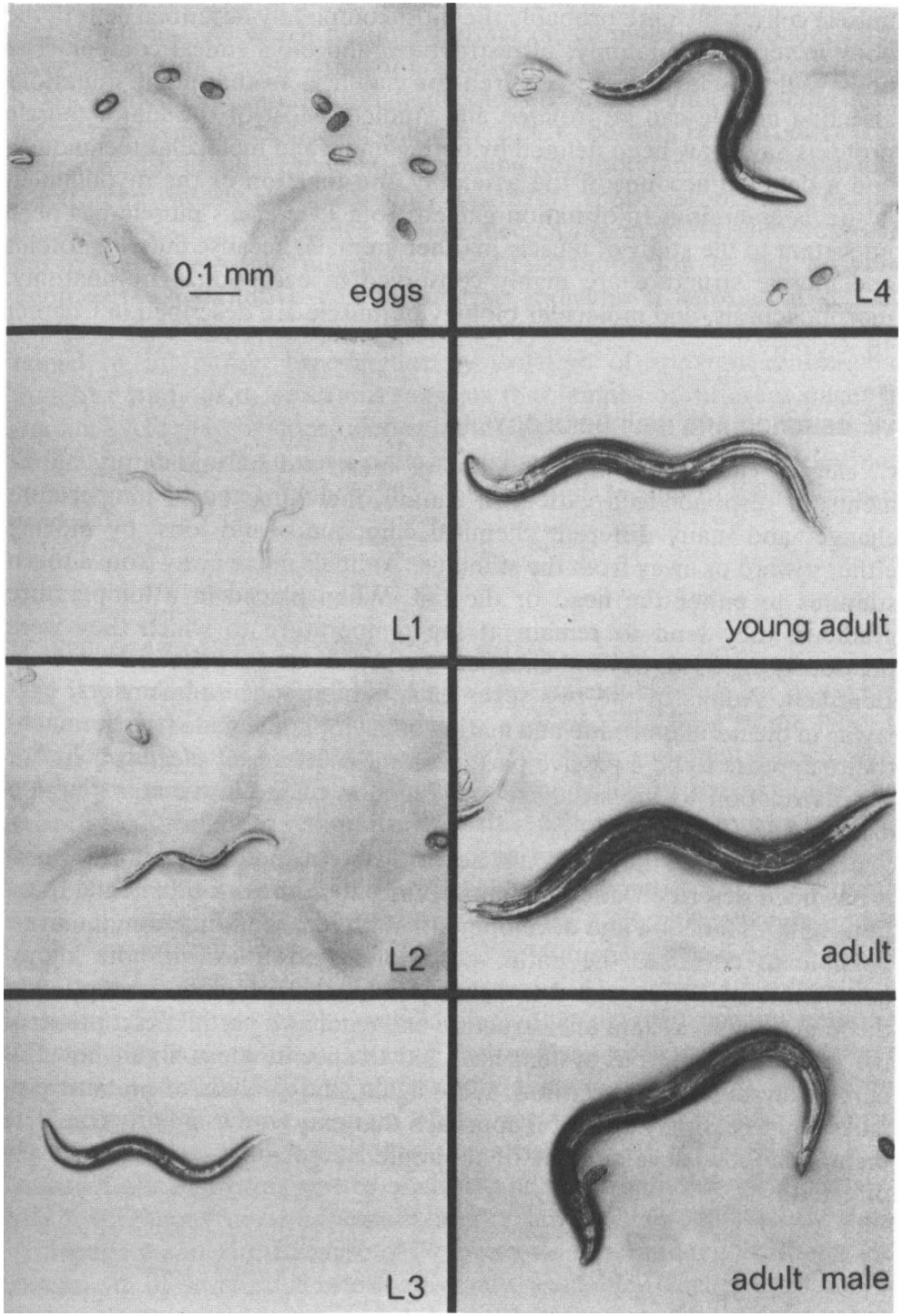




# 4 larval stages (“juveniles”)

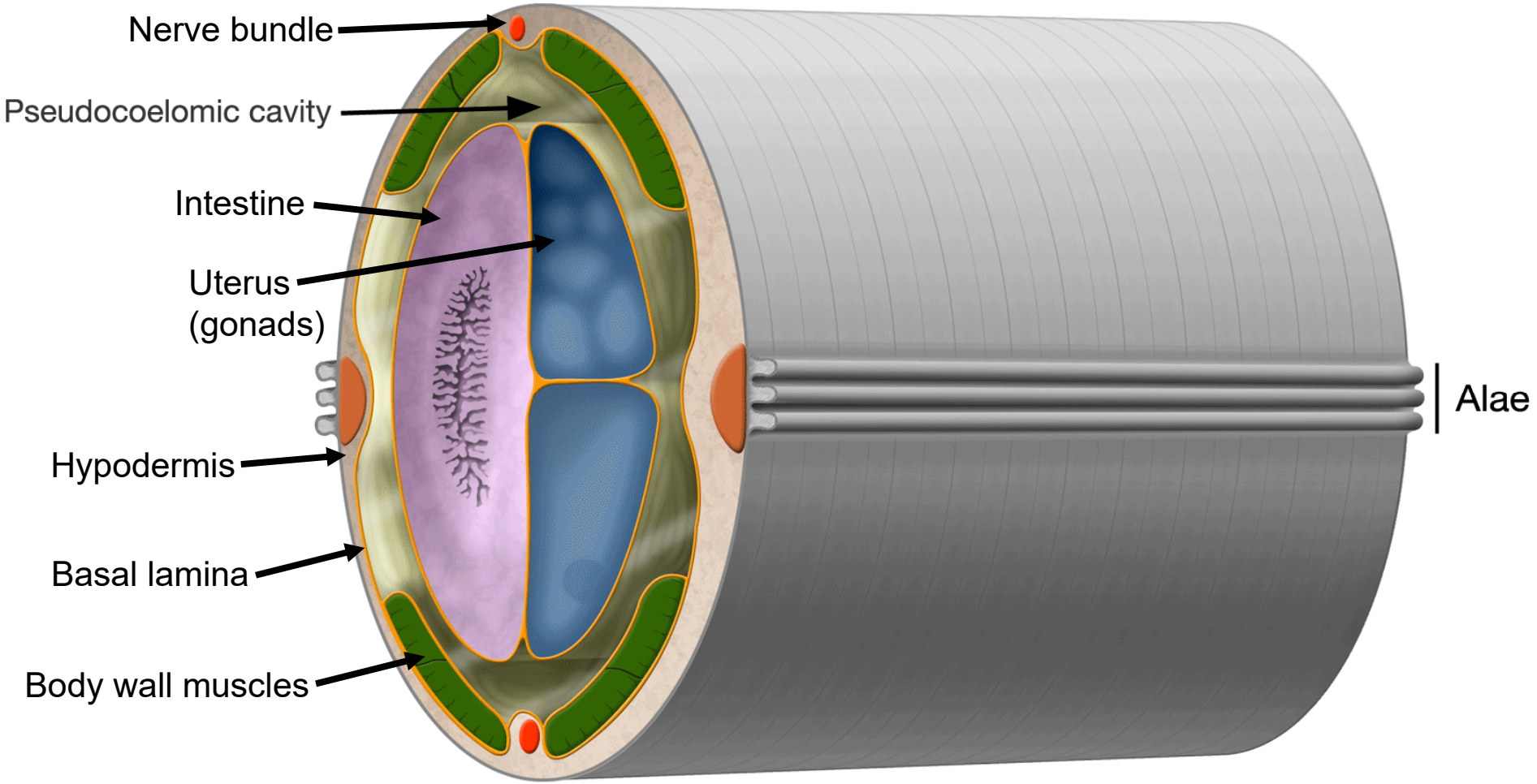


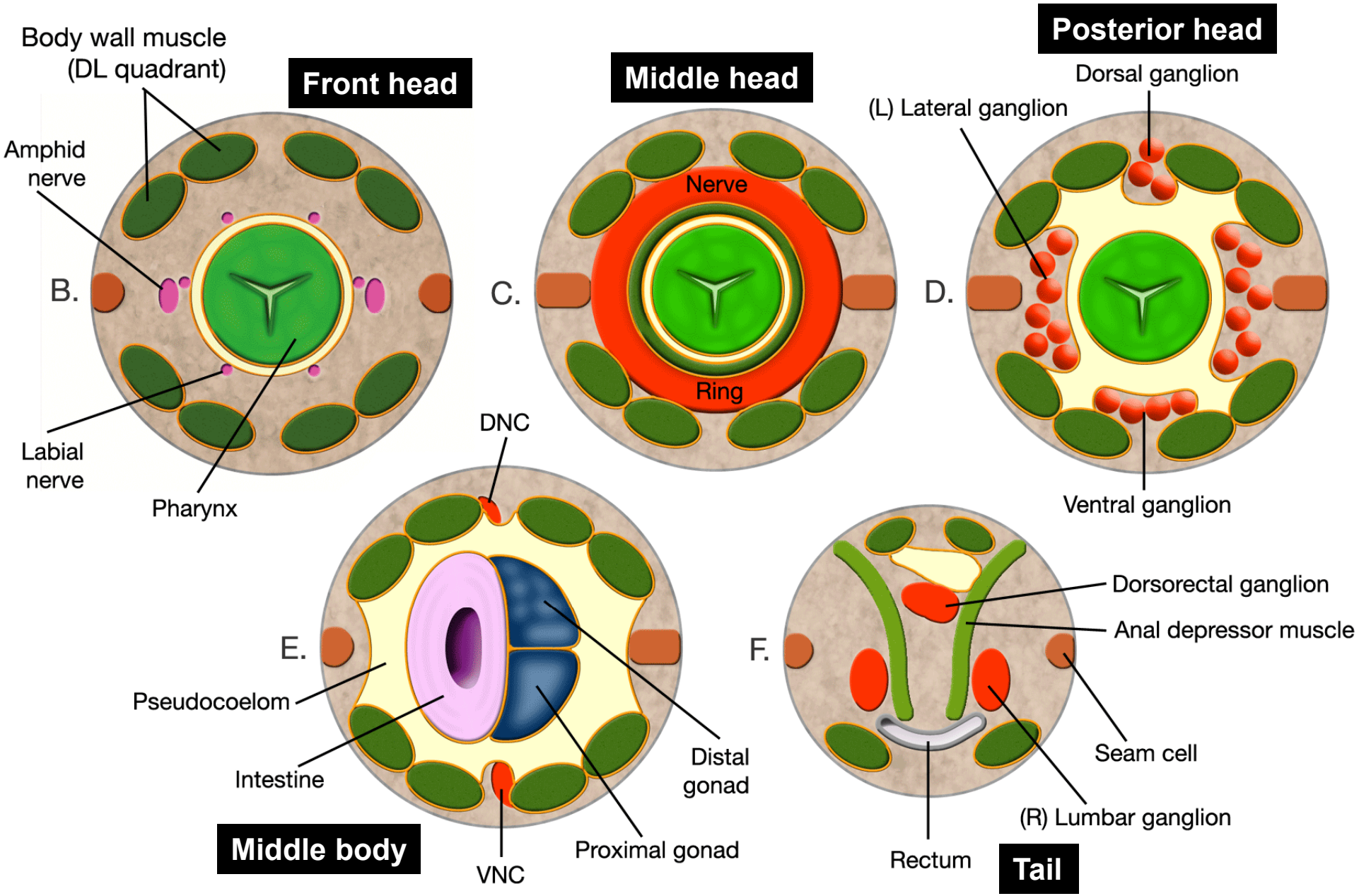



# Basic Anatomy

• As a common feature of nematodes, *C. elegans* has an unsegmented, cylindrical body shape with two openings at the end (mouth and anus)
• It reflects the typical nematode body plan: an **outer tube** separated from an **inner tube** by an **pseudocoelomic space**
• Outer tube consists of the cuticle, hypodermis, excretory system, neurons and muscles
• Inner tube consists of the pharynx, intestine and the gonad/uterus

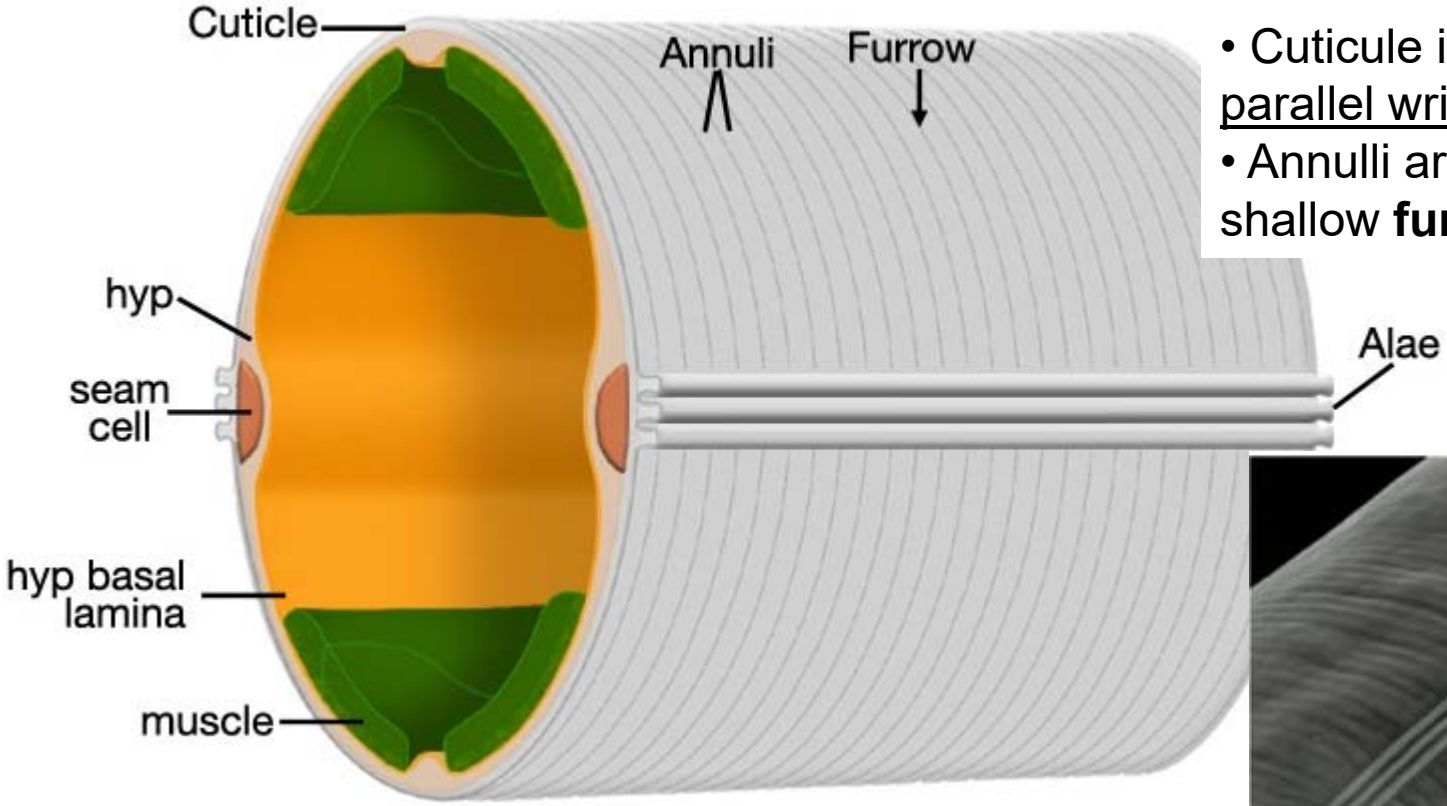


# Basic Anatomy: Follow the inner and outer tubes

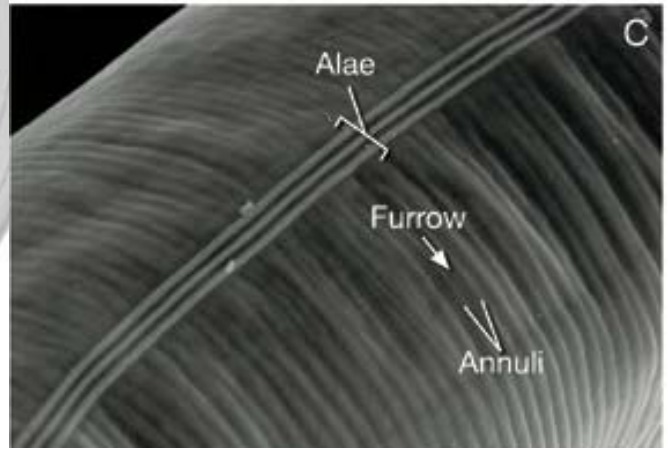


# Anatomy of the outer tube

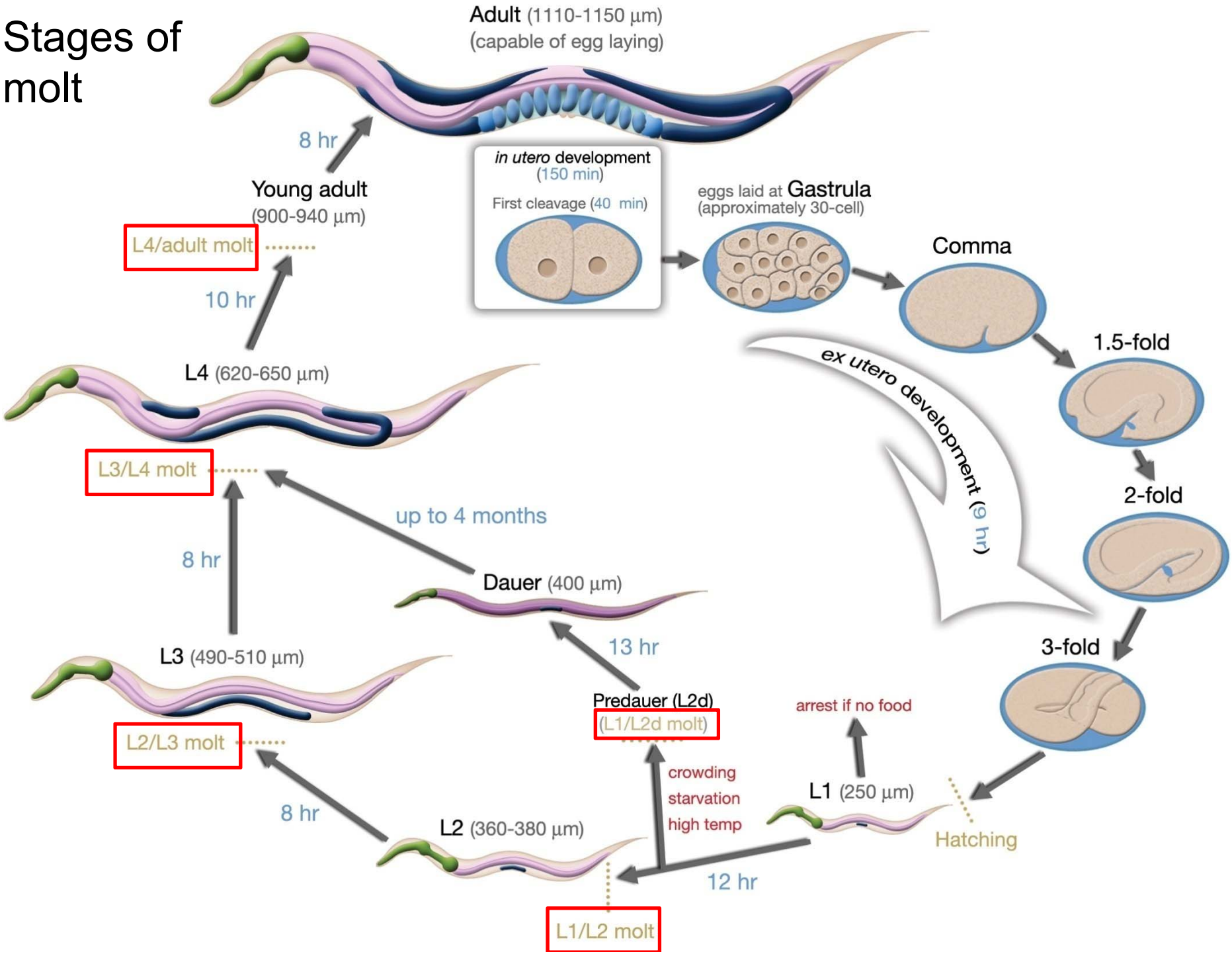
• **Cuticle** protects the animal from the environment and acts as an external skeleton
• The elastic, collagenous **cuticle** is secreted by underlying epithelial cells: **hypodermis** and **seam cells**
• **Seam cells** are postembryonic stem cells which produce the **alae**
• Alae are linear ridges supposedly providing better adhesion during movement
• Cuticle surface is covered by a surface coat (**glycocalyx**) secreted by gland cells
• At each larval stage an entirely new cuticle is generated while the old cuticle is shed off allowing for growth (**molt**)




• Cuticule is segmented by parallel wrinkles called **annuli**
• Annulli are separated by shallow **furrows**

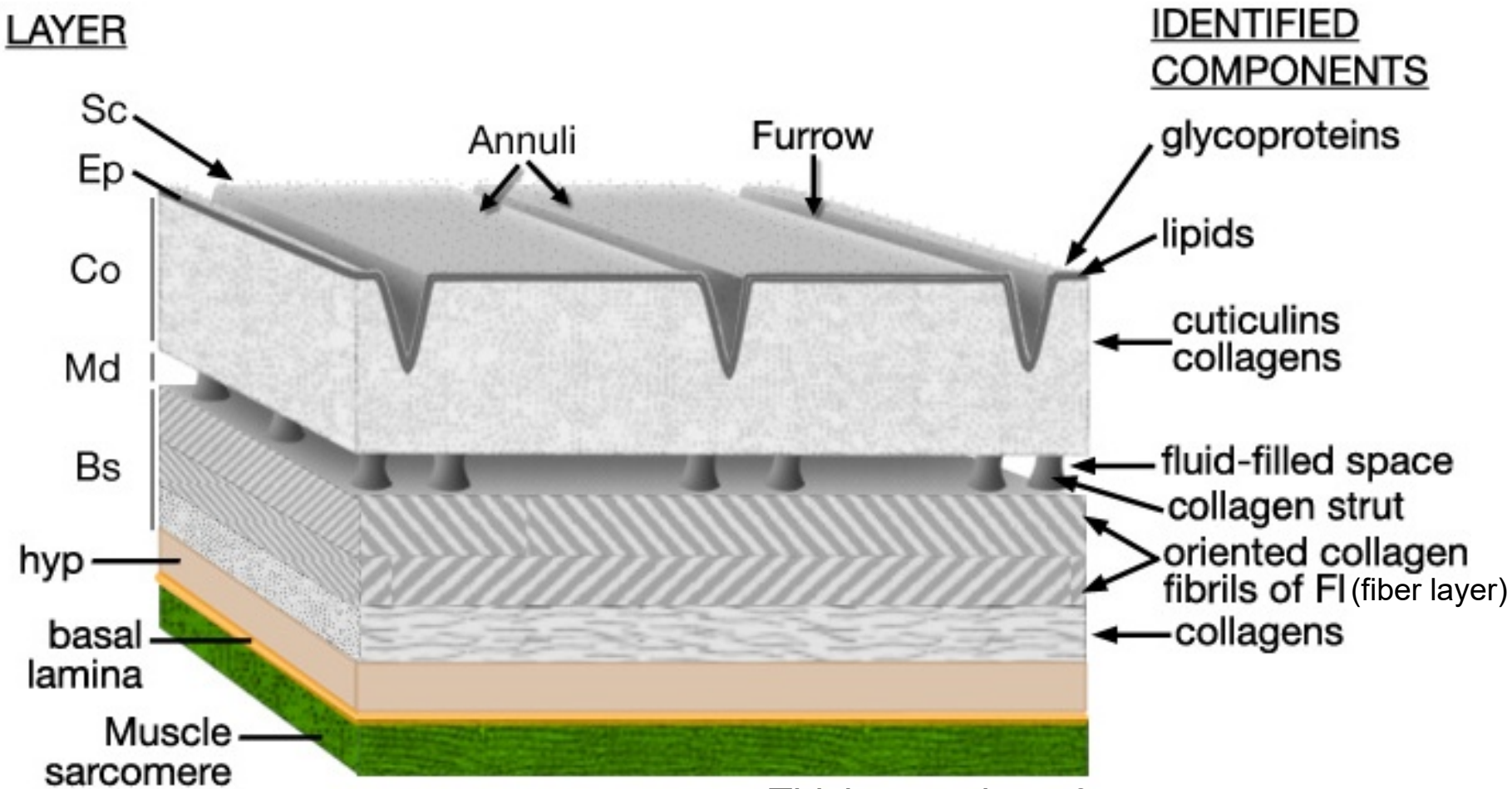


# Stages of molt

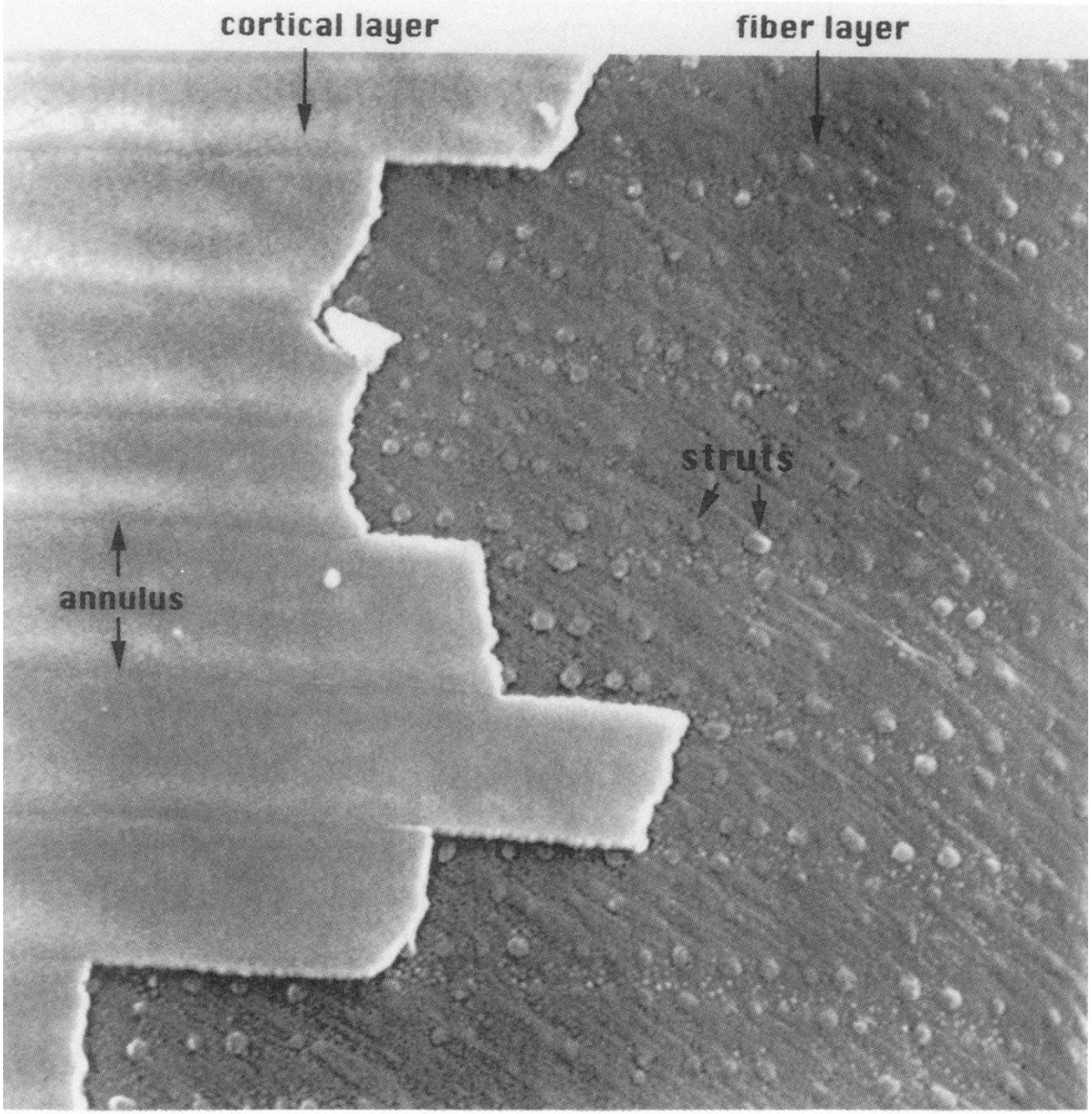


# The cuticle

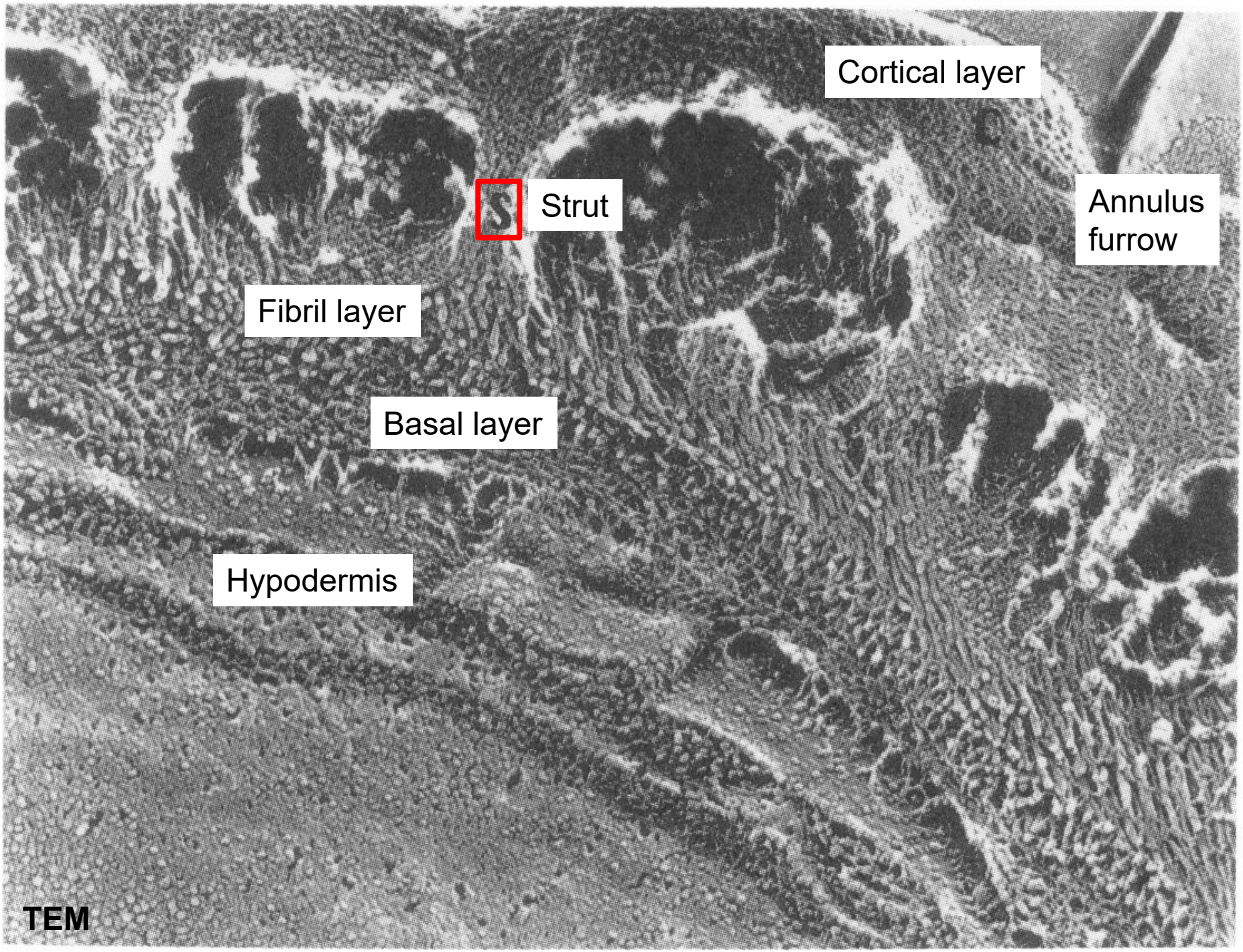
The cuticle is organized in 5 major layers:
**s**urface **c**oat (glycocalyx ), **ep**icuticle, **co**rtical zone, **m**e**d**ial zone and **b**a**s**al zones




Thickness about 0.5 µm

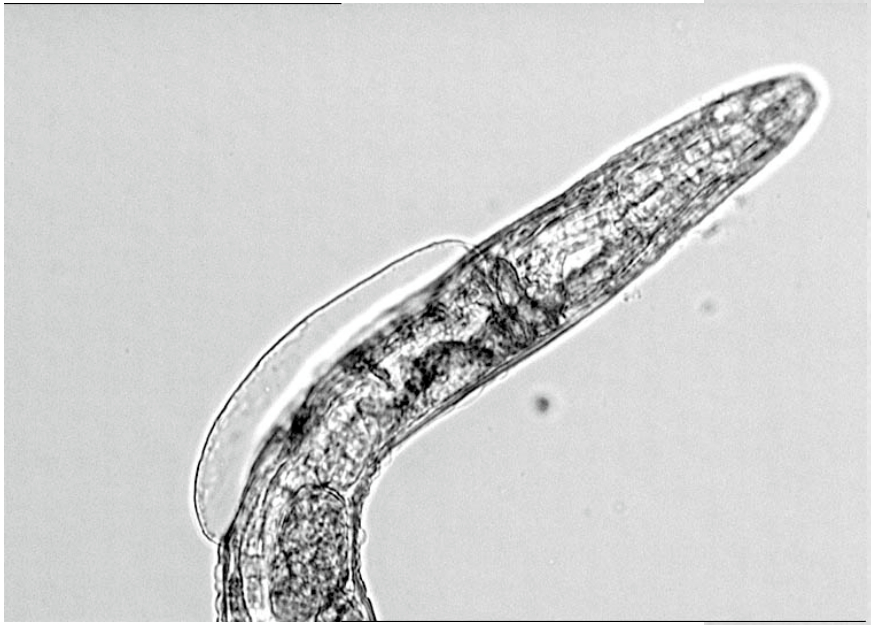


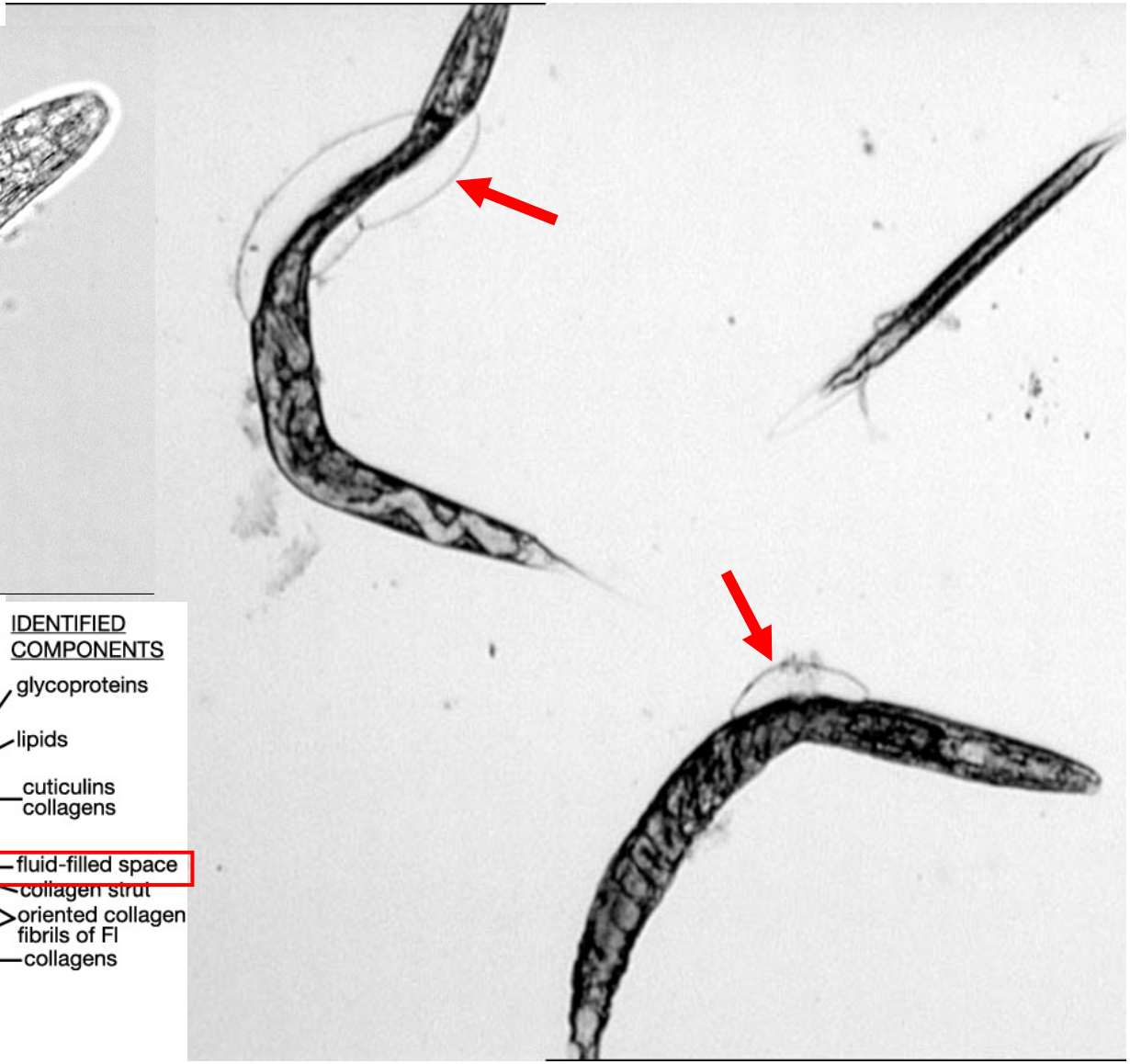

The cuticle in freeze-fracturing EM

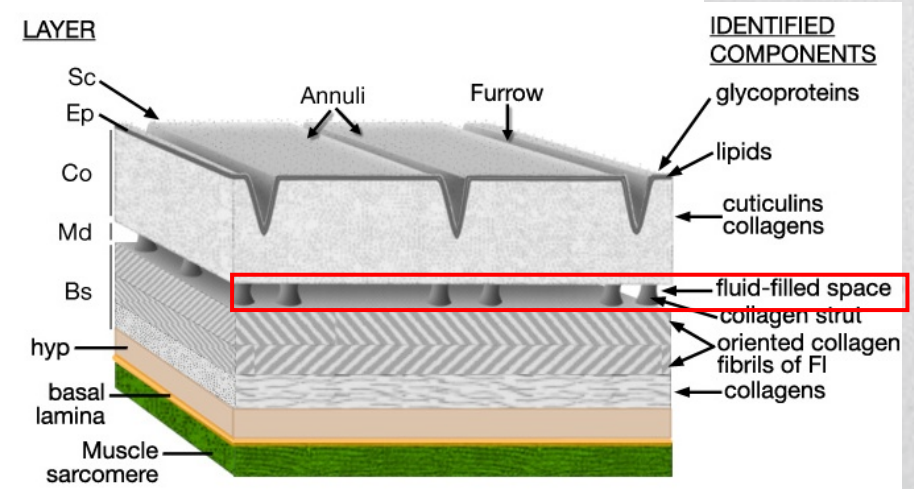

Cortical layer
S
Strut
Annulus furrow
Fibril layer
Basal layer
Hypodermis
TEM

# The cuticle in blister mutants

- The cortical and the basal zones are separated by a **fluid-filled space**
- In **blister mutants** (*bli*) this space is largely expanded resulting in a bubble-like epidermis

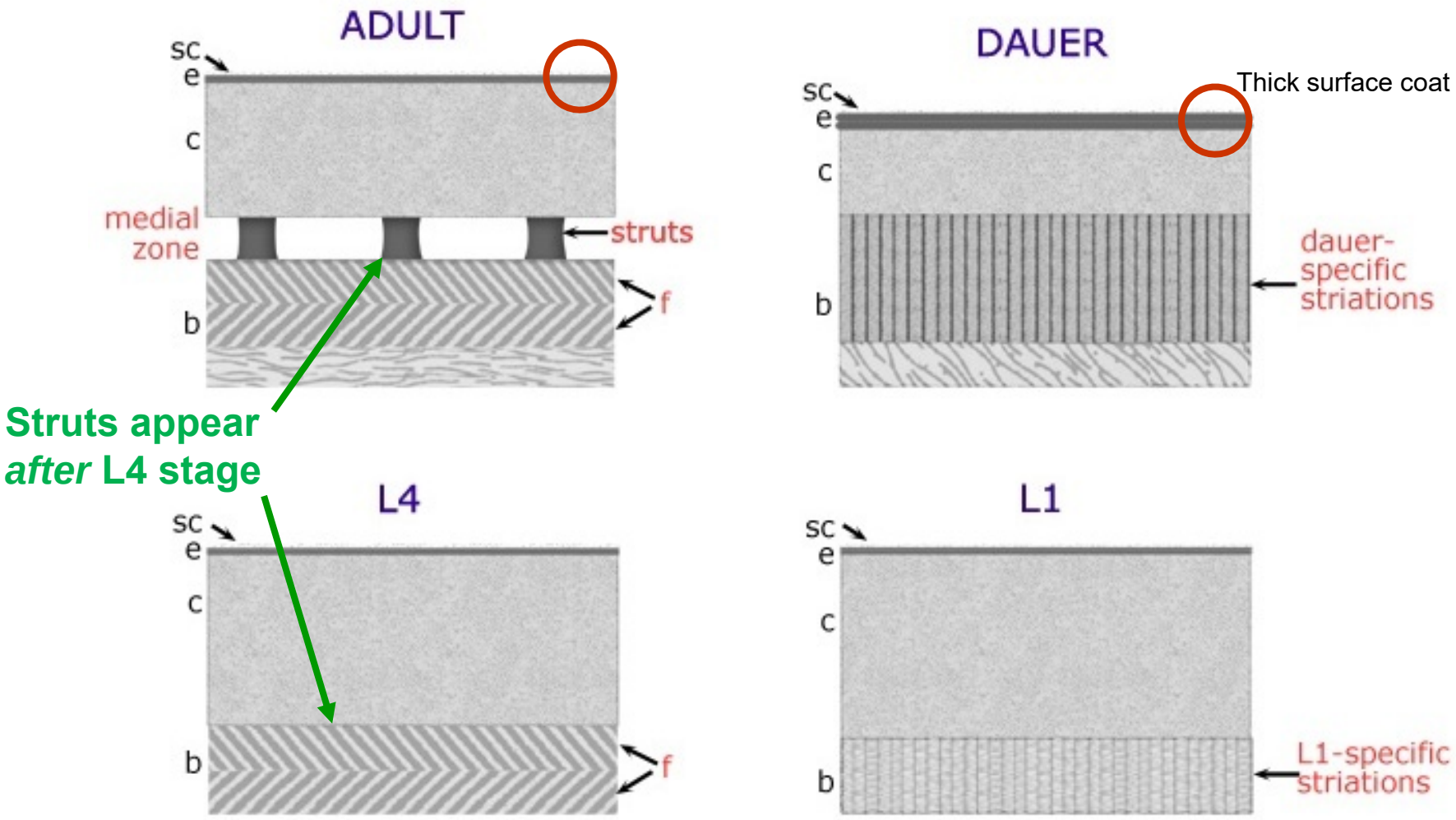






# The cuticle during different life stages

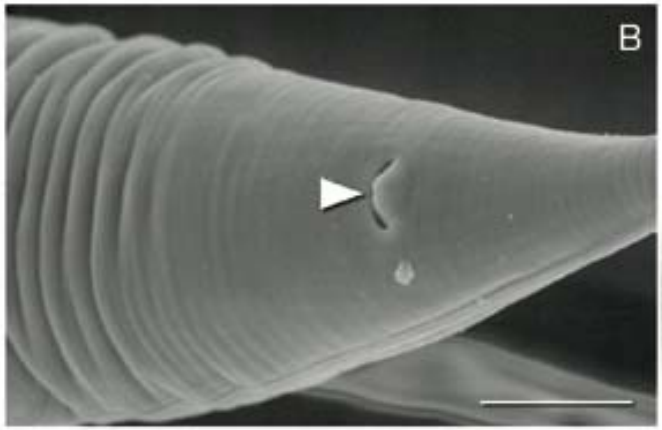
- The dauer cuticle is **less permeable** and the surface coat is thicker
- In addition, the dauer cuticle is thicker compared to the reduced body diameter



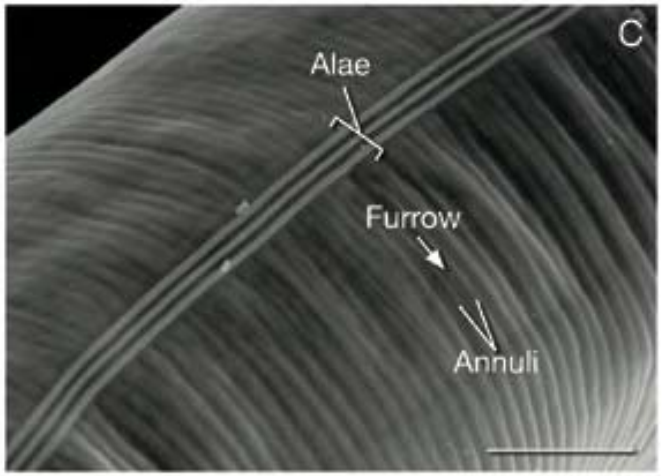

surface coat (sc); epicuticle (e); cortical (c) and basal (b) zones; fiber layer (f)

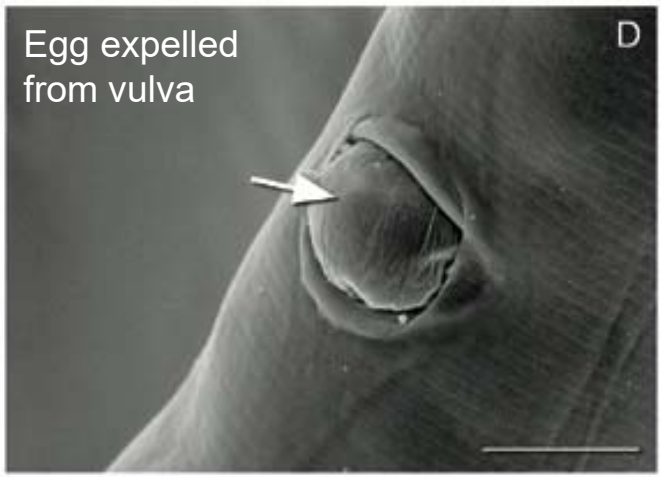
# Openings of the cuticle

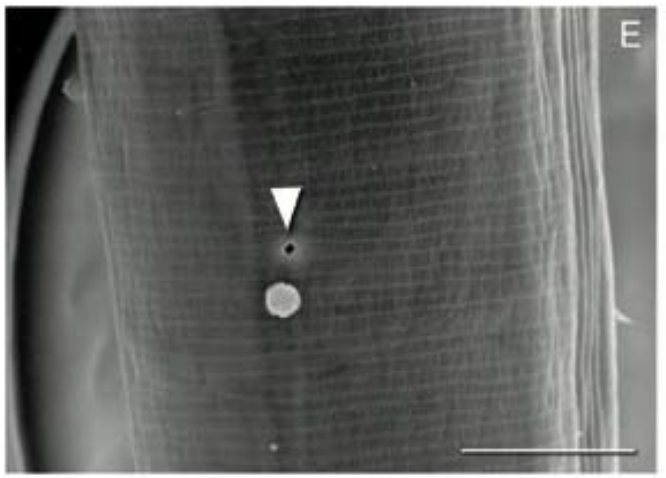
The cuticle contains **4 openings**: the mouth (A), the anus (B), the vulva (D), and the excretory pore (E)







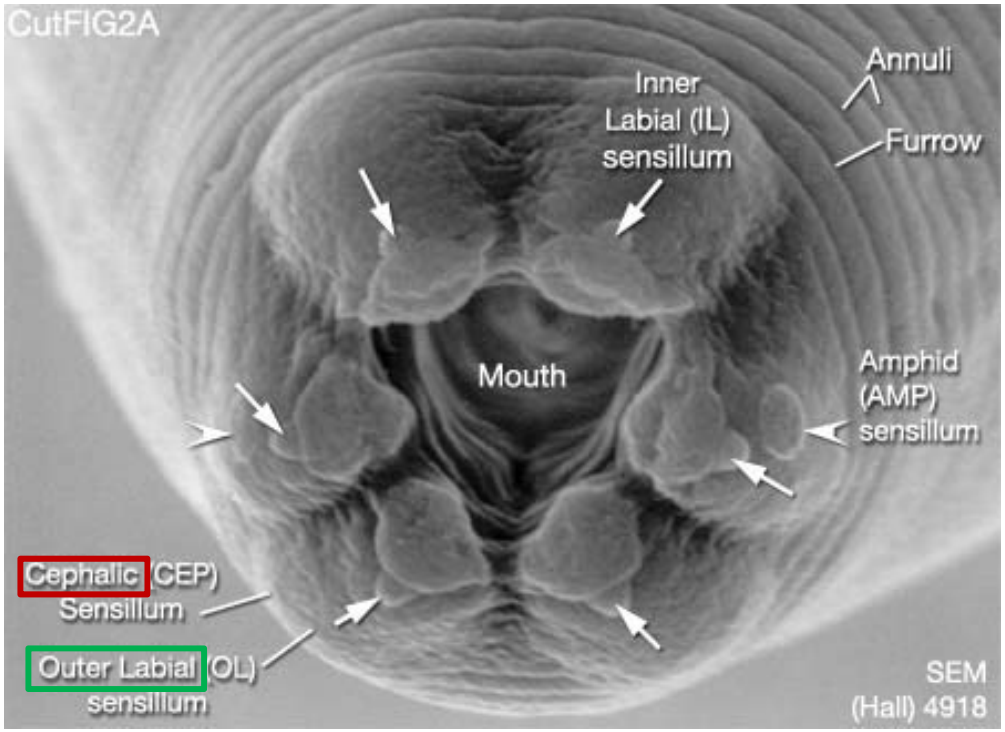



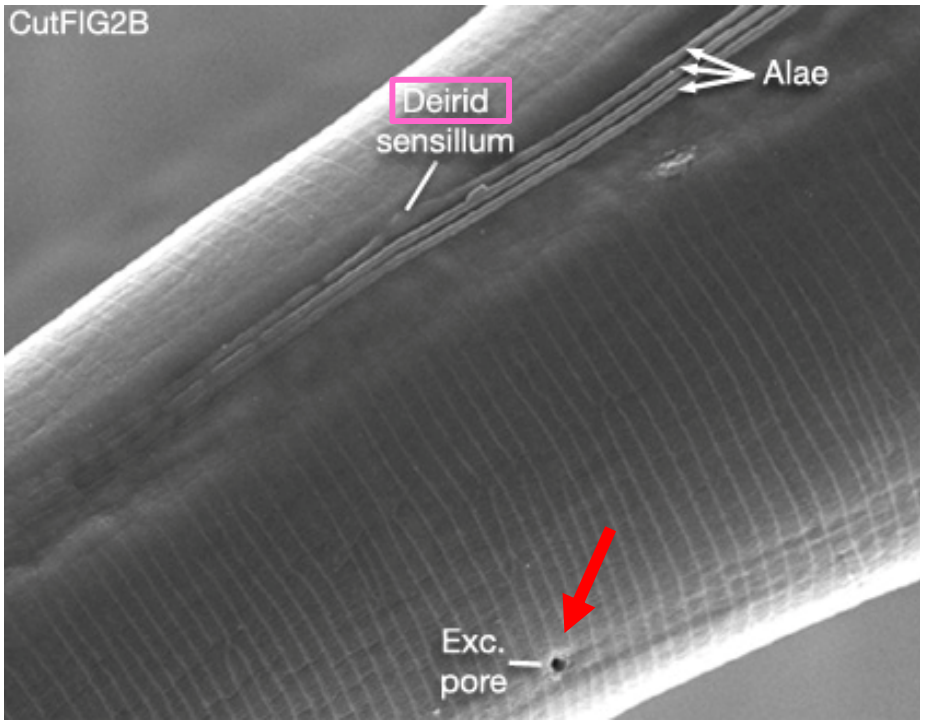



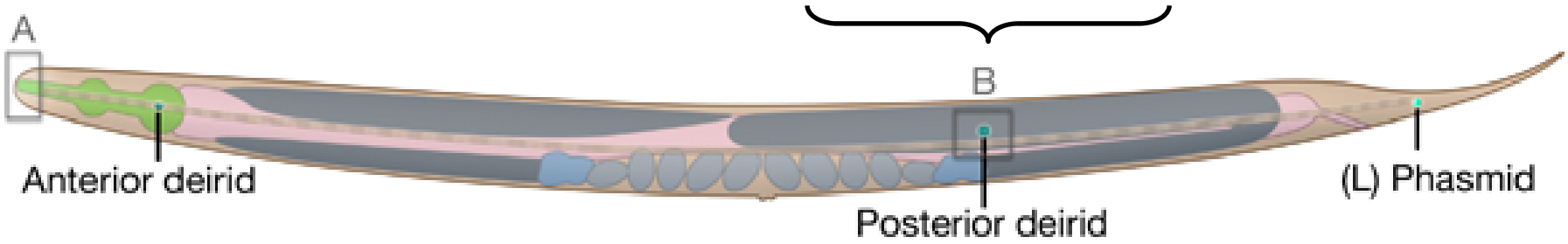

Scale bars: 10 μm

# Sensory organs of the cuticle

- Cuticle surface is marked by swellings (***papillae***) where a number of neuronal cilia are exposed to the exterior: **amphid** and **inner labial** **sensillum**
- Some sensory organs lie directly beneath the surface (***nubbin***): **outer labial**, **cephalic** and **deirid** **sensillum**

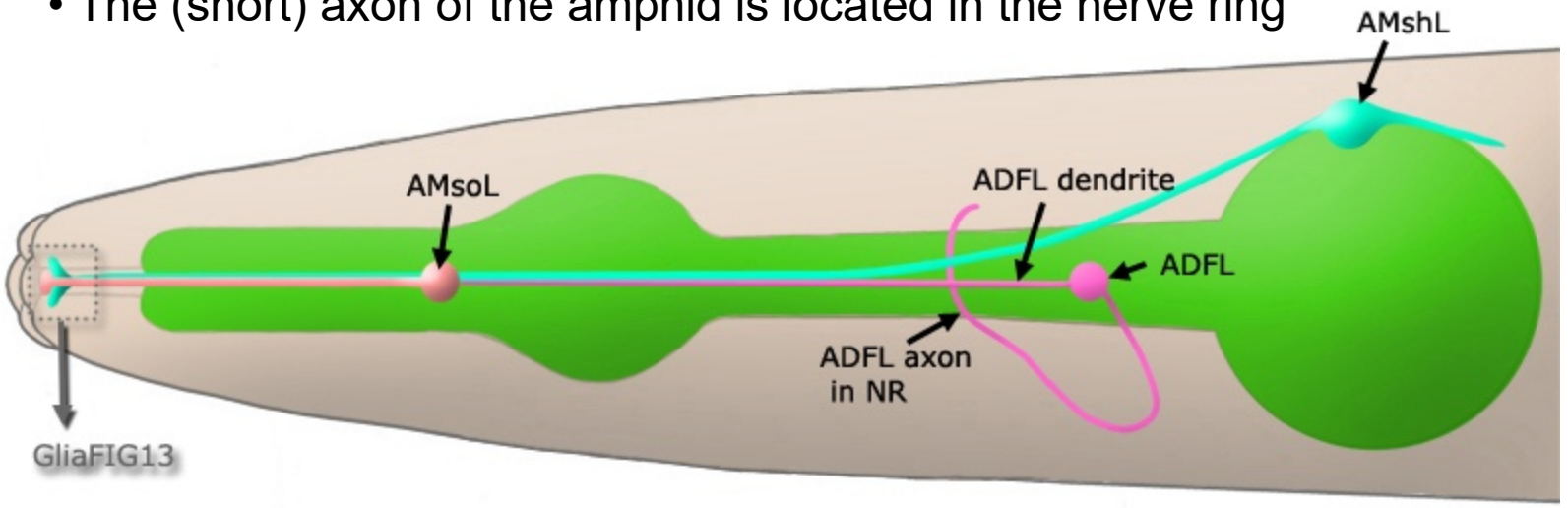


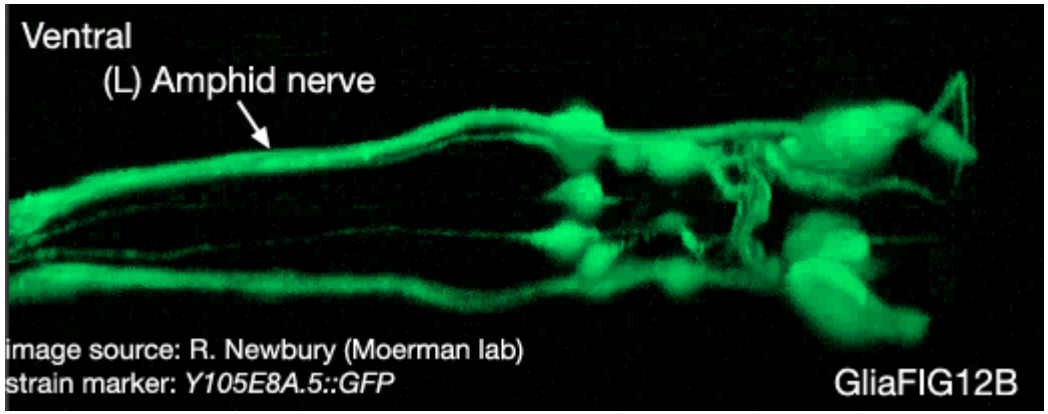



# Sensory organs of the cuticle

- **Amphids**: are a pair of laterally located sensilla in the head which are open to the outside at the base of the lips
- These chemosensory organs can be **stained with FITC or DiI** (fluorescent dyes)
- Some mutants fail to be stained => *dyf* = dye filling (mutant)
- Each amphid is made up of 12 sensory neurons (ADF, ADL, ASH…) with **ciliated dendrites** as well as one ***sh*eath** and one ***so*cket cell**
- The (short) axon of the amphid is located in the nerve ring



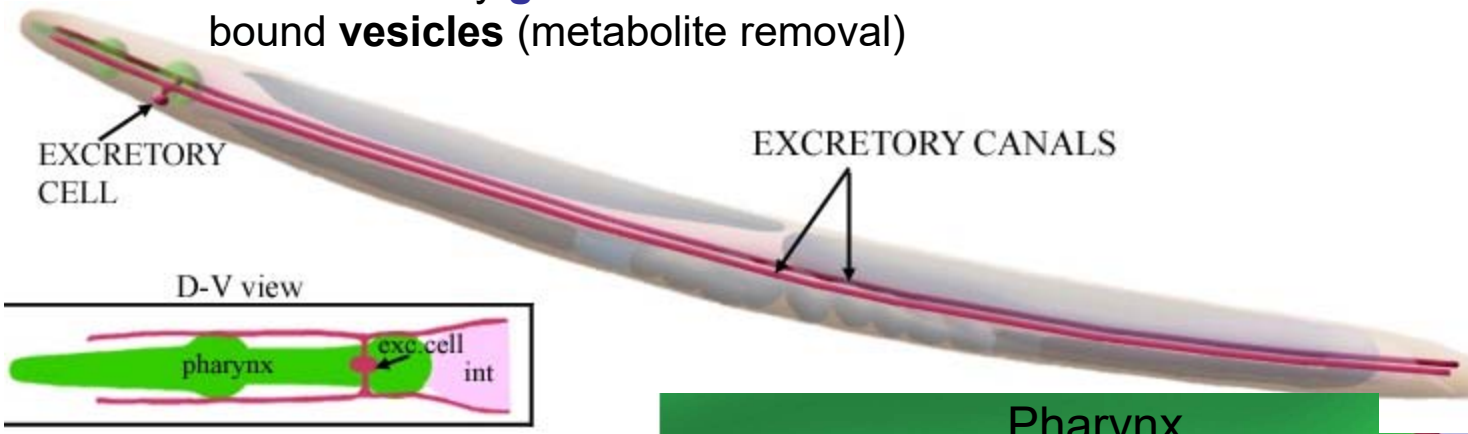



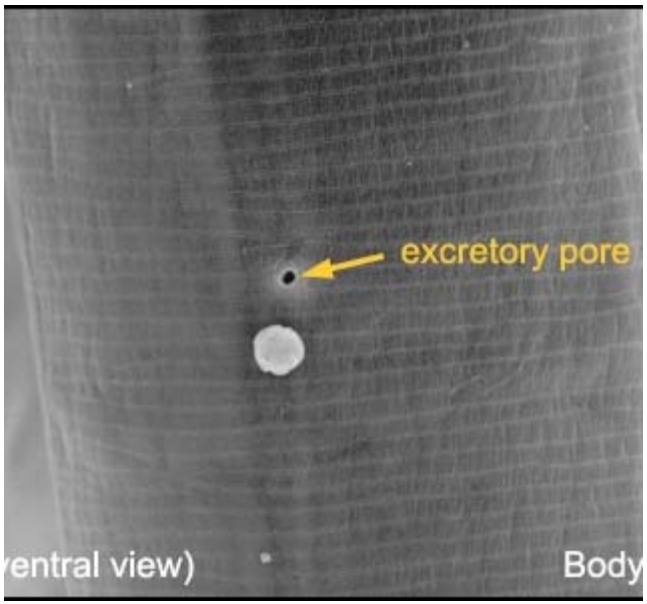
# The excretory and secretory system

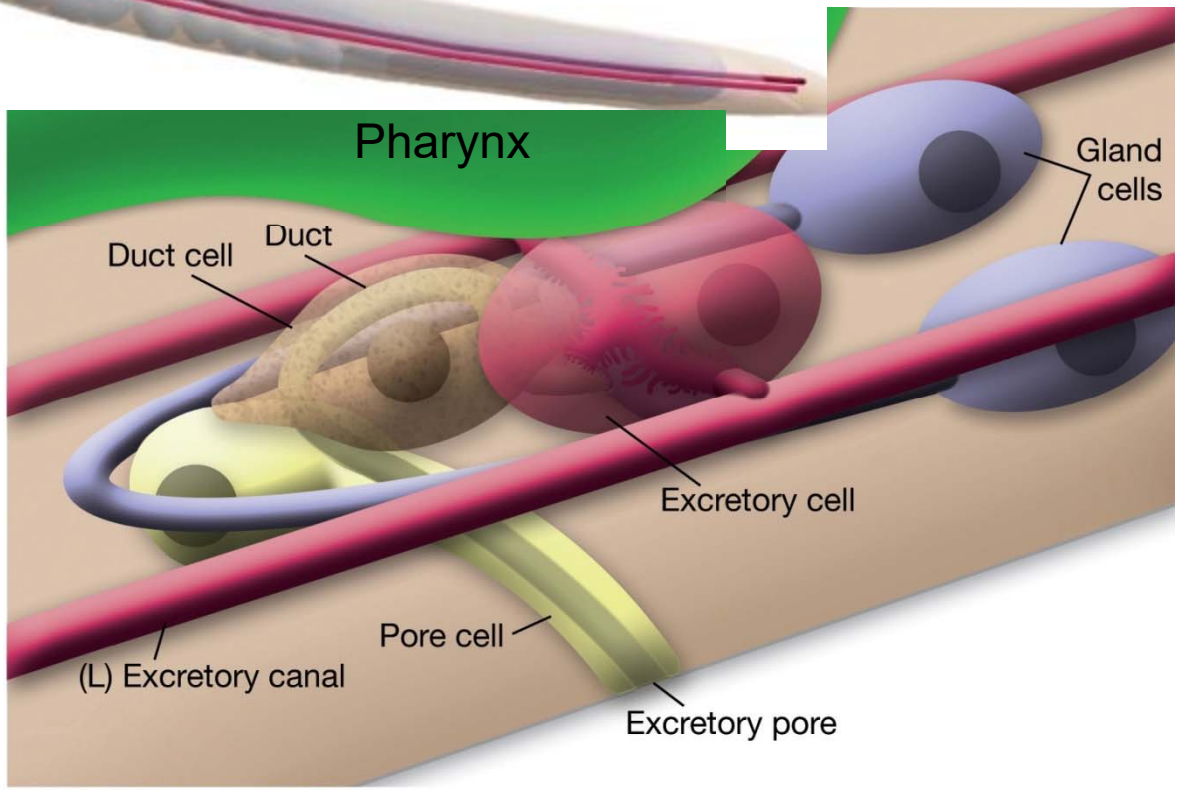
• The excretory/secretory system is composed of one **excretory pore cell**, one **duct cell**, one **canal cell** and a fused pair of **gland cells**

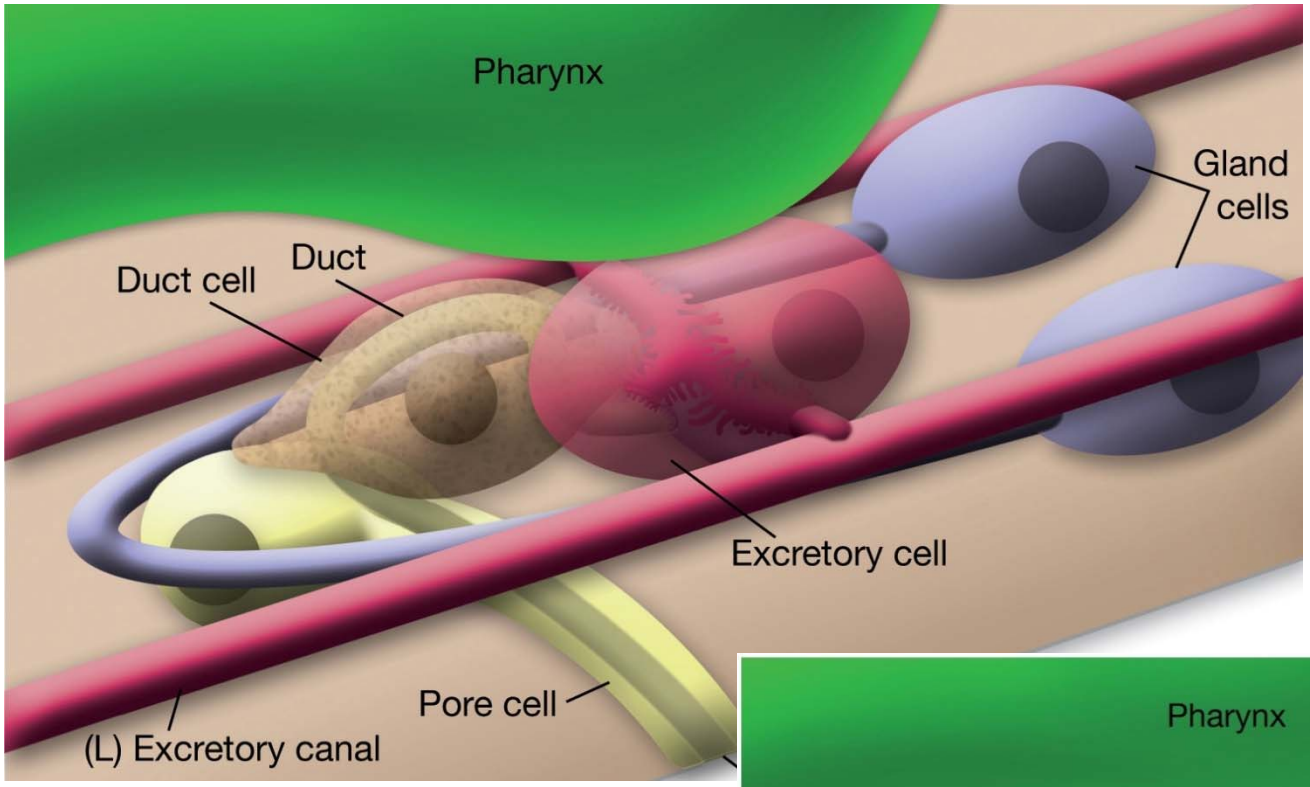
• The excretory **canal cell** functions as a "kidney" secreting saline fluid via the duct and the pore to maintain the animals salt balance (**osmoregulation**)

• The excretory **gland** is also connected to the canals and **secretes large** membrane bound **vesicles** (metabolite removal)



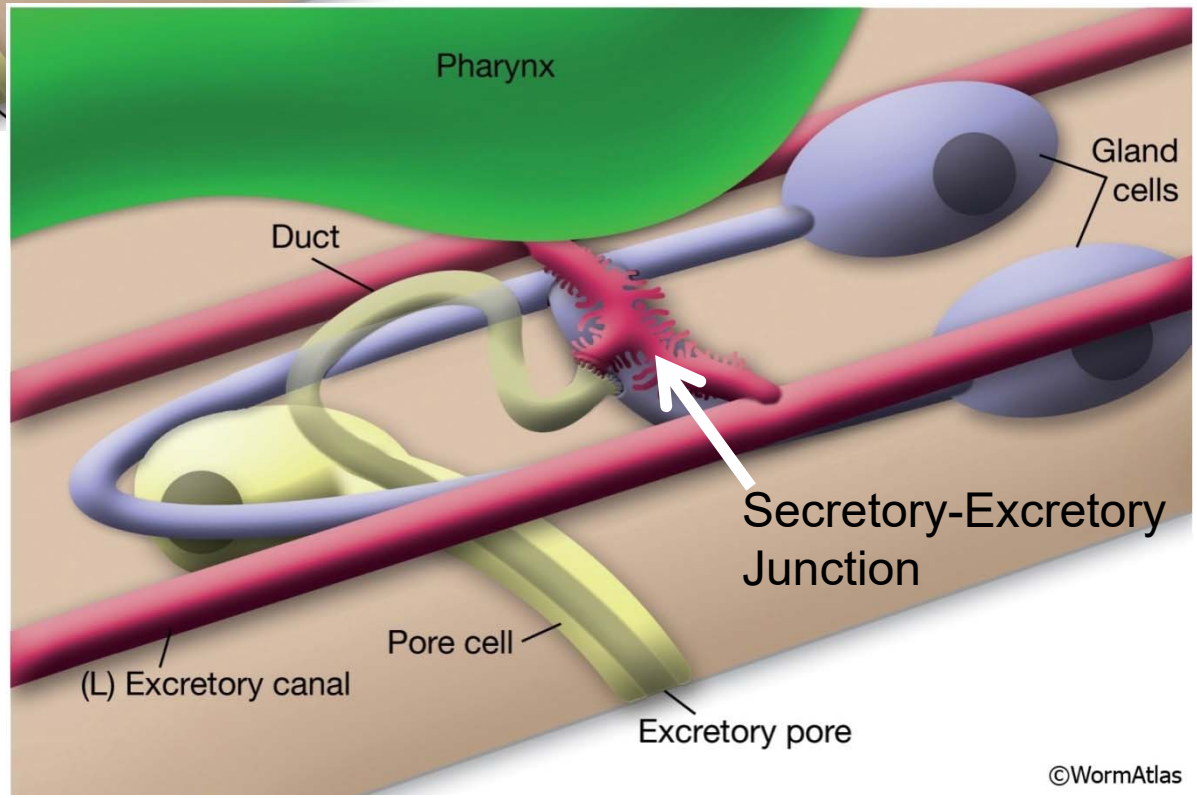





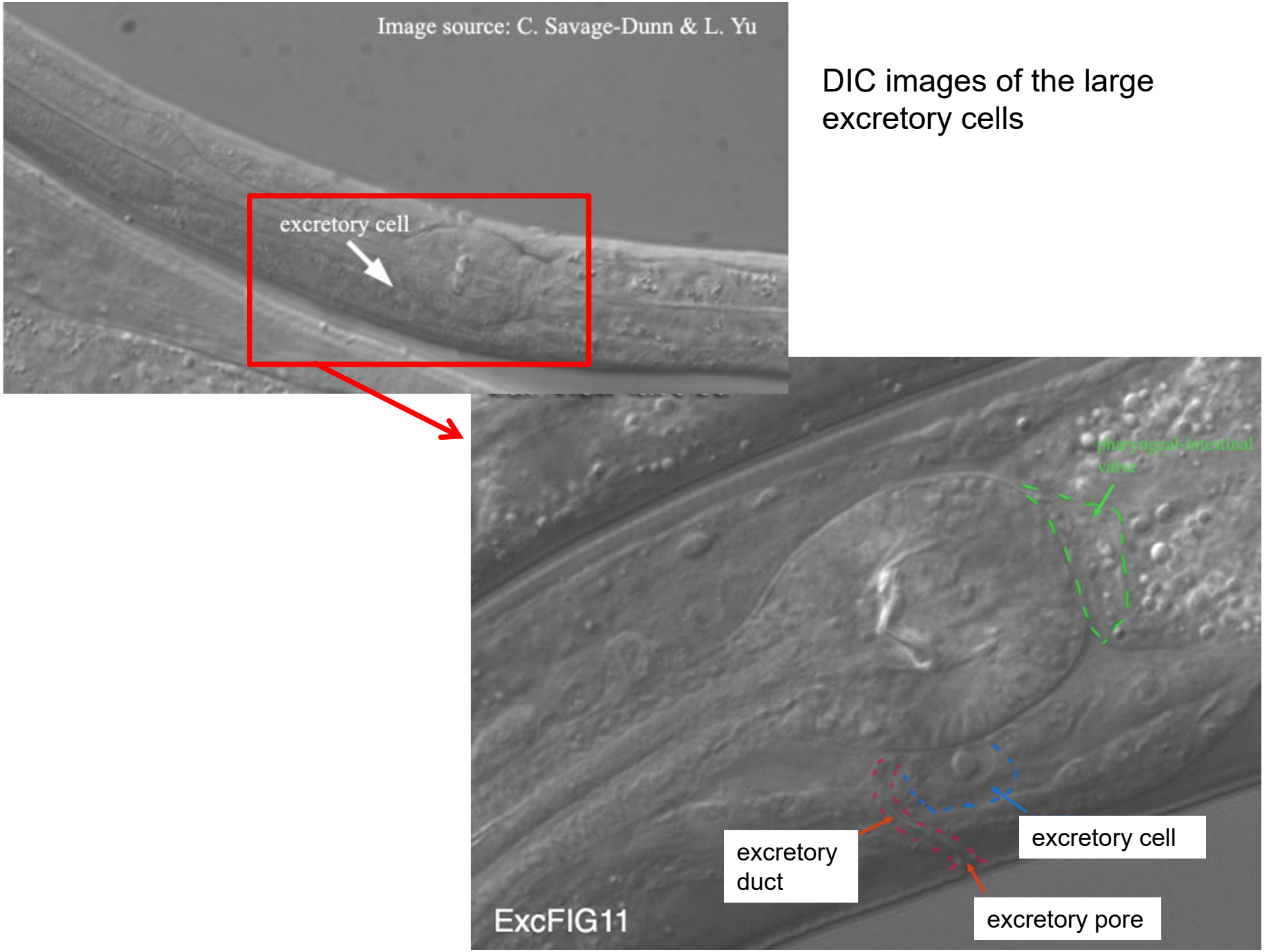

All excretory cells are connected with each other

Position of **duct cell** and **gland cells** near the **terminal bulb** of the pharynx



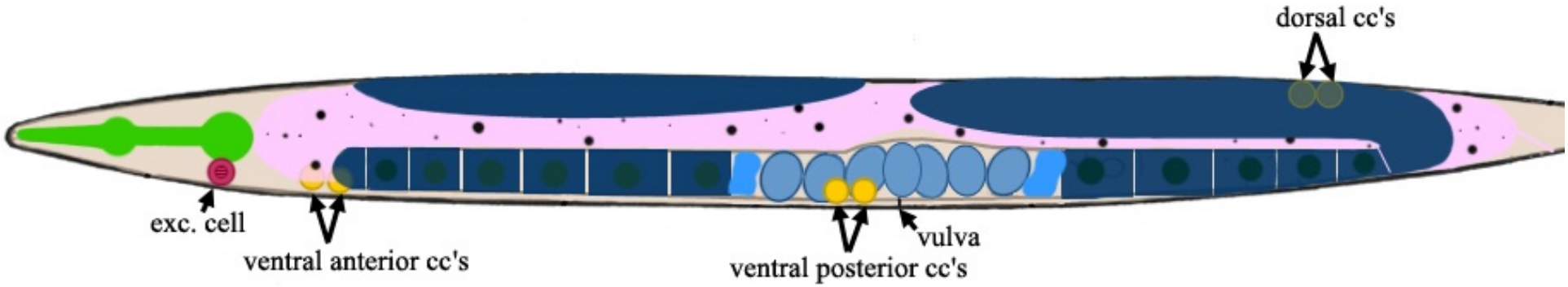

- The outflow of **the fused gland cells** ends at a specialized <u>permeable junctional complex</u> (secretory membrane)
- Thru this complex the <u>contents</u> of the gland cells is <u>dumped into the duct lumen</u>

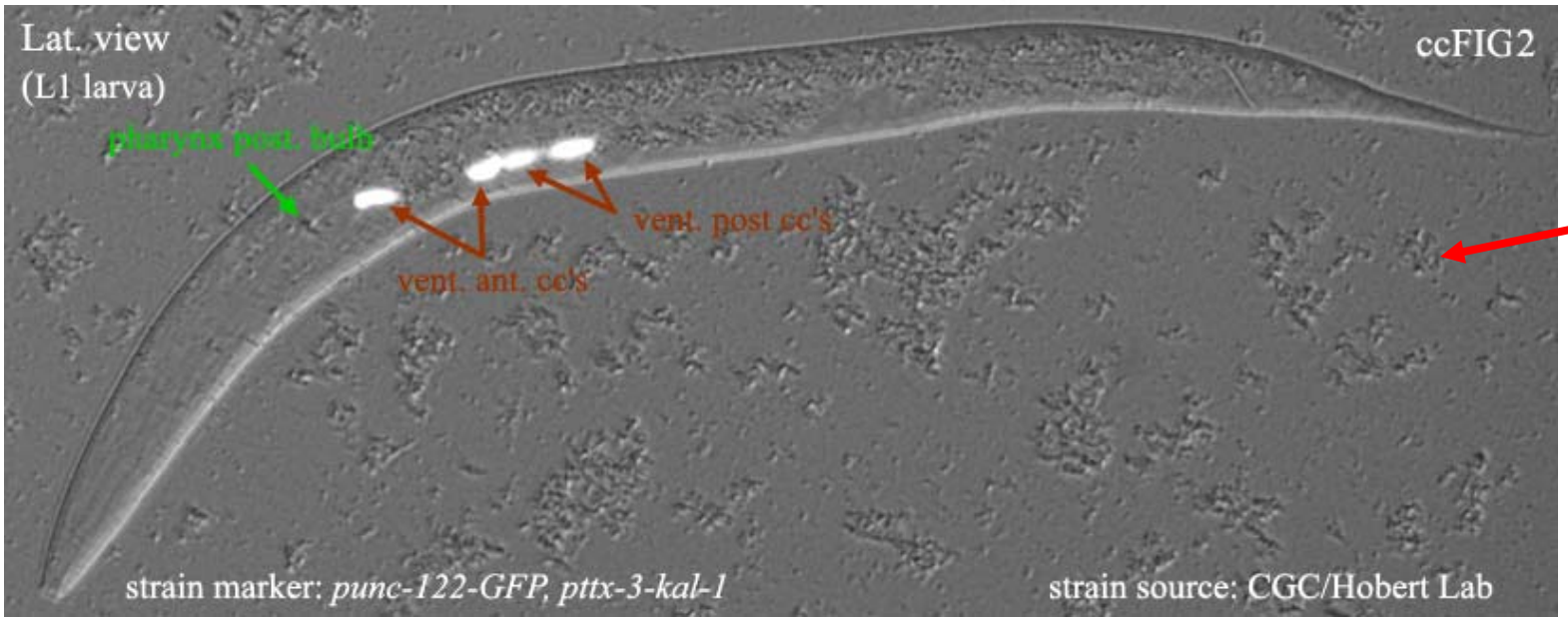
DIC images of the large excretory cells



# The “immune system” (**innate immunity**)

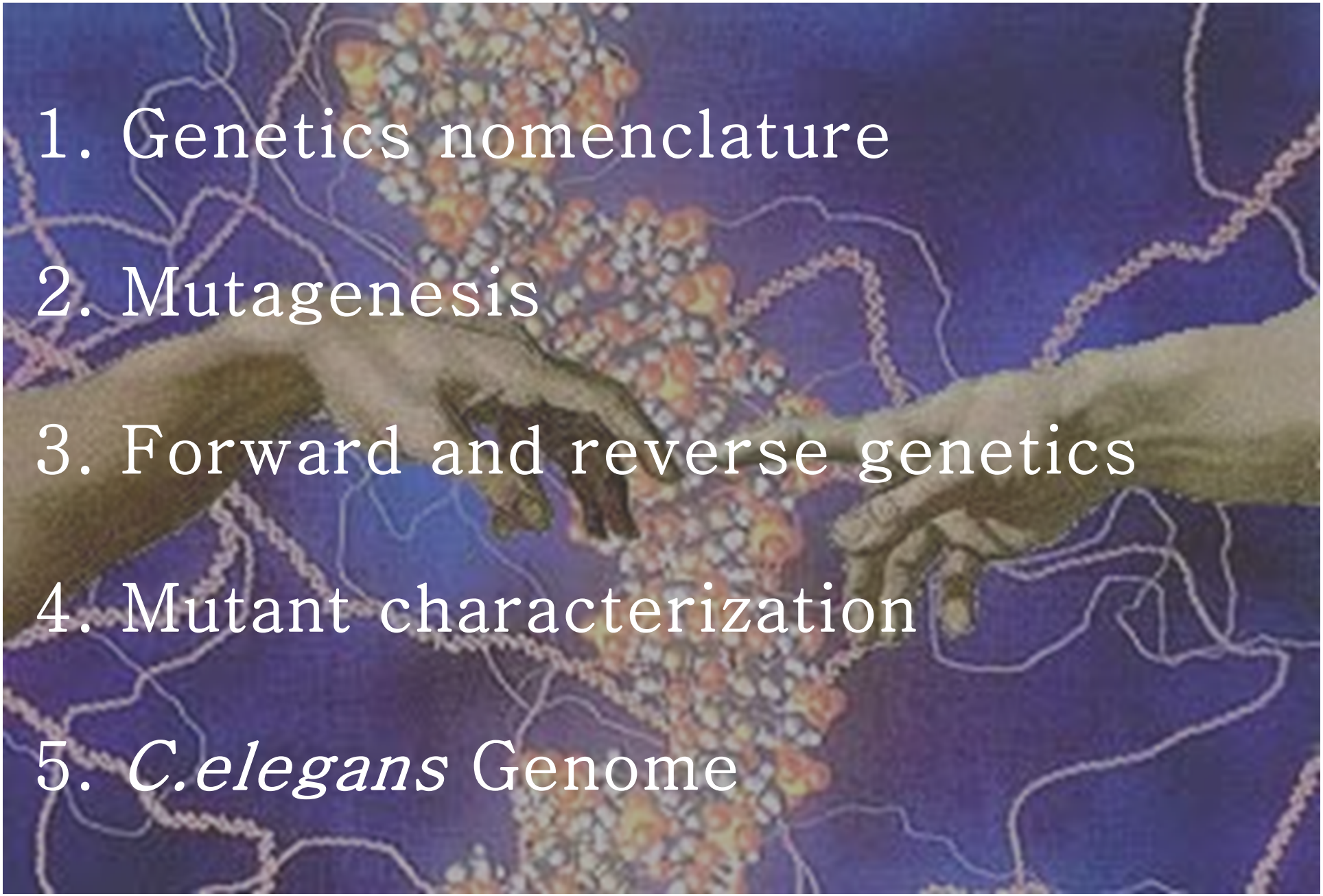
• Non-specific immune system: Cells recognize and respond to pathogens in a non-adaptive way (no long-lasting protection)
• Three pairs of **coelomocytes** (cc’s) form pseudocoelomic organs which function as “trash cans” (scavenger cells)
• These cells can endocytose fluid that has been secreted into the pseudocoelom




?

# *C. elegans* genetics



1. Genetics nomenclature
2. Mutagenesis
3. Forward and reverse genetics
4. Mutant characterization
5. *C.elegans* Genome

# Genetics nomenclature

• *C. elegans* is **diploid** and has **5** pairs of **autosomal** chromosomes (I, II, III, IV, V) and **one pair of gonosomal** chromosomes (XX for hermaphrodites, XO for males)
• The genome size is **100.2 Mb** with 21,000 protein coding genes => even the genome size is 30 times smaller than that of humans it encodes only slightly fewer proteins
• 35% of genes have human homology: possible to express and study human proteins in worms
• *C. elegans* genetics nomenclature is different from other model species, for historical reasons; it is carefully controlled, and thus easy to follow:
• The **loci** have a "**3-letter dash number**" designation; the locus is *italicized*
    • Letter describe (usually) a phenotype observed (with consecutive numbering)
    • Because *C. elegans* is a self-fertilizing organism, all alleles we look at are homozygous

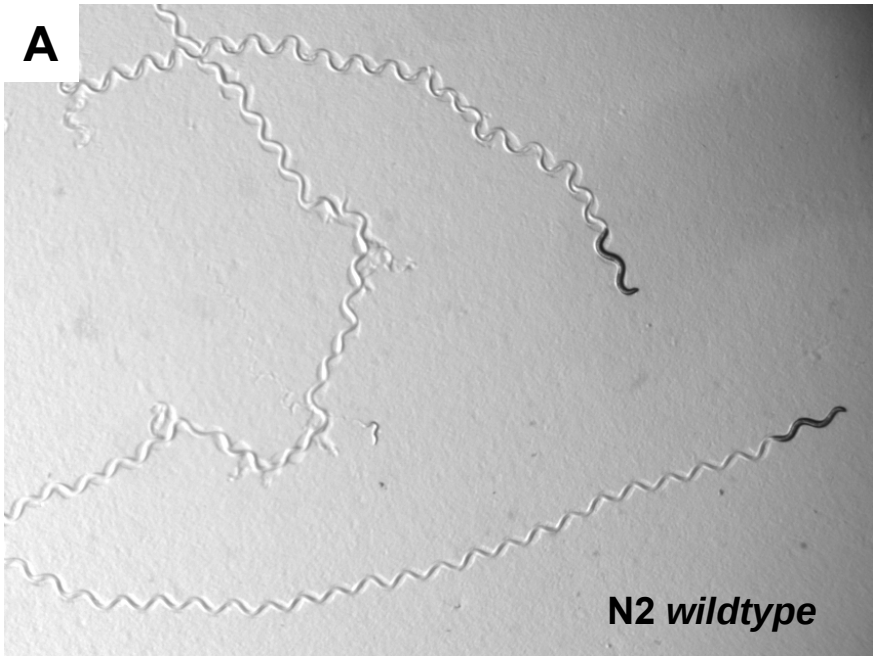
***unc-10*** = uncoordinated 10 => the worm exhibits uncoordinated movements

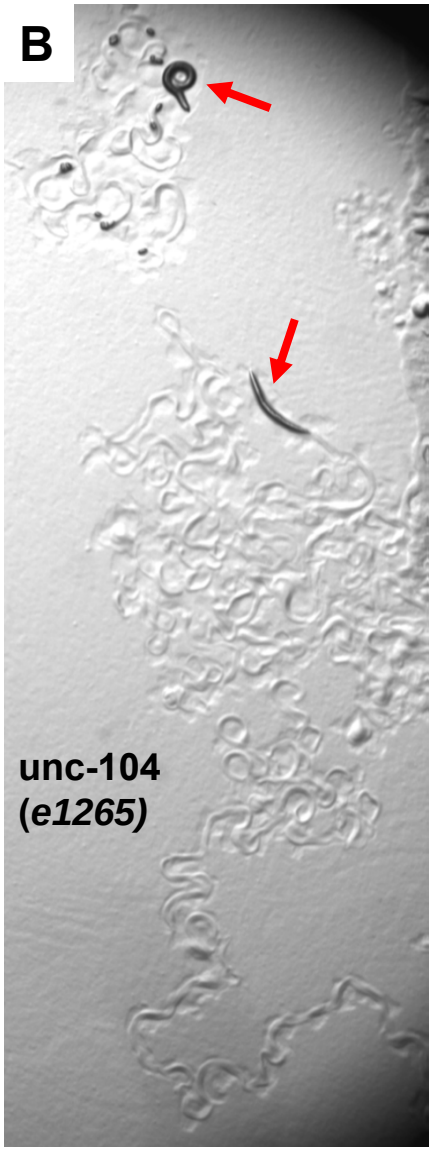
***unc-10*(*e102*)** = unc-10 gene is localized on allele e102 (the letter identifies the isolating laboratory)

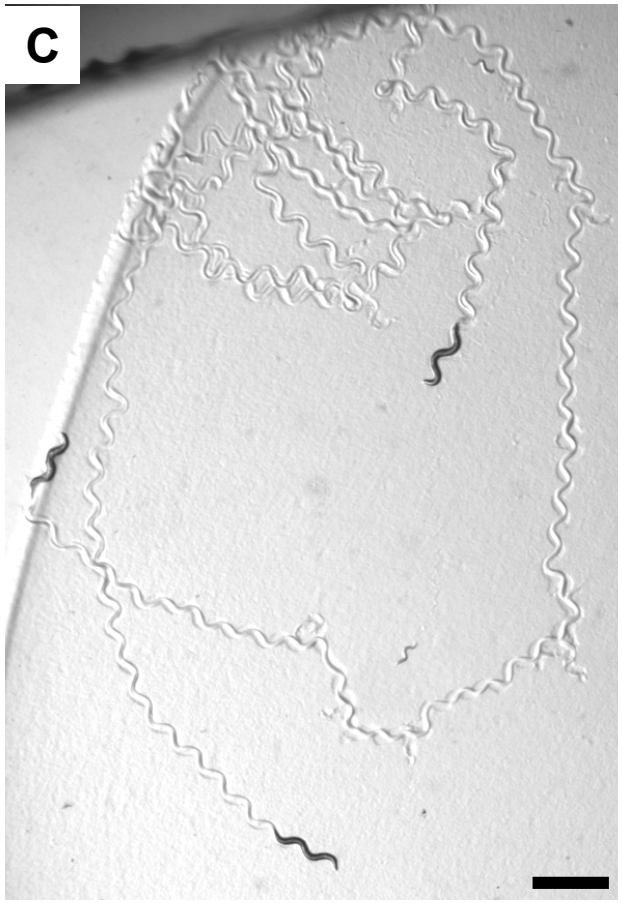
***unc-10*(*e102*)X** = e102 allele is localized on chromosome X

**CB102 *unc-10*(*e102*)X** = The strain has an inventory code (**strain name**) which usually identifies the lab PI (CB = Hodgkin J, Oxford Univ. England)

# What is an “rescue experiment”?




**UNC-104::GFP**
***(e1265)***

- Worms are moving very slow and uncoordinated
- Worms are paralyzed and coiled up

**unc-104 mutant**

**Rescue of the unc-104 phenotype: the worms move again like wildtype**

***unc-104*(*e1265*);Ex[UNC-104::GFP]**

# Genetics nomenclature

• Multiple mutant alleles carried in one strain are organized by chromosomes while the chromosomes are separated by semicolons:

ZM588 *fsn-1*(*hp1*) III; *juIs1* IV; *scd-2*(*ok565*) V

• **Rearrangements**: Chromosomal duplications and deficiencies carry a letter prefix (indicating the isolating lab) a **Dp** (pronounced "dupe" for duplication) or **Df** (pronounced "dif" for deficiency) and a number:

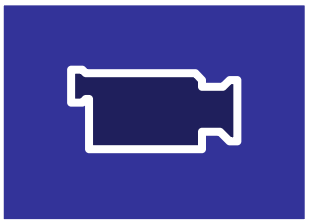
KR1440 *dpy-5*(*e61*) *vps-34*(*h797*) *unc-13*(*e450*) I; sDp2 (I;f)

• **Transgenes** (plasmid) as free extrachromosomal arrays are designated in brackets:

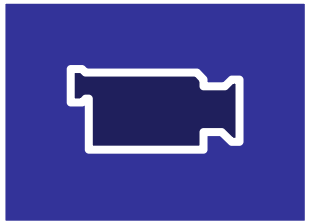
RK1 *unc-13*(*e323*) I; *jsIs1*[pSB120(snb-1::GFP)+pRF4(*rol-6*(su1006))]

Transgenes frequently derive from injecting a selection marker (**co-injection marker**):

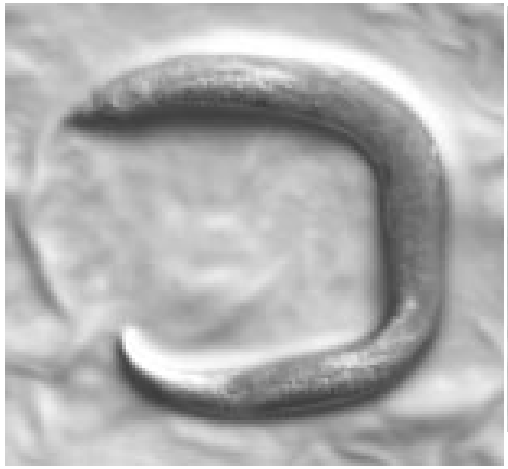
odr-1::RFP = RFP expressed in odorant (sensory) neurons in the head
*rol-6* = inducing roller phenotype
*dpy* = dumpy phenotype
*him* = throwing increased males
*bli* = blister phenotype



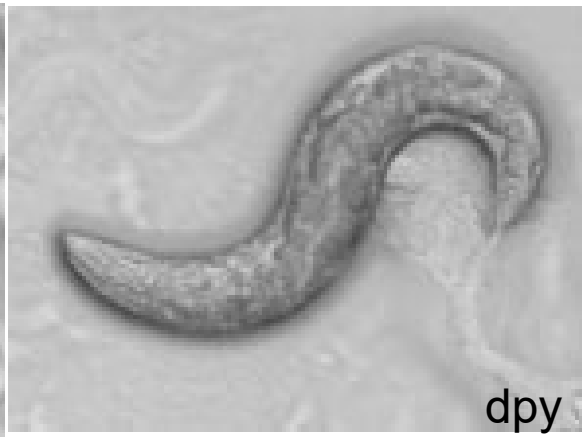
Dumpy worm



Roller worm

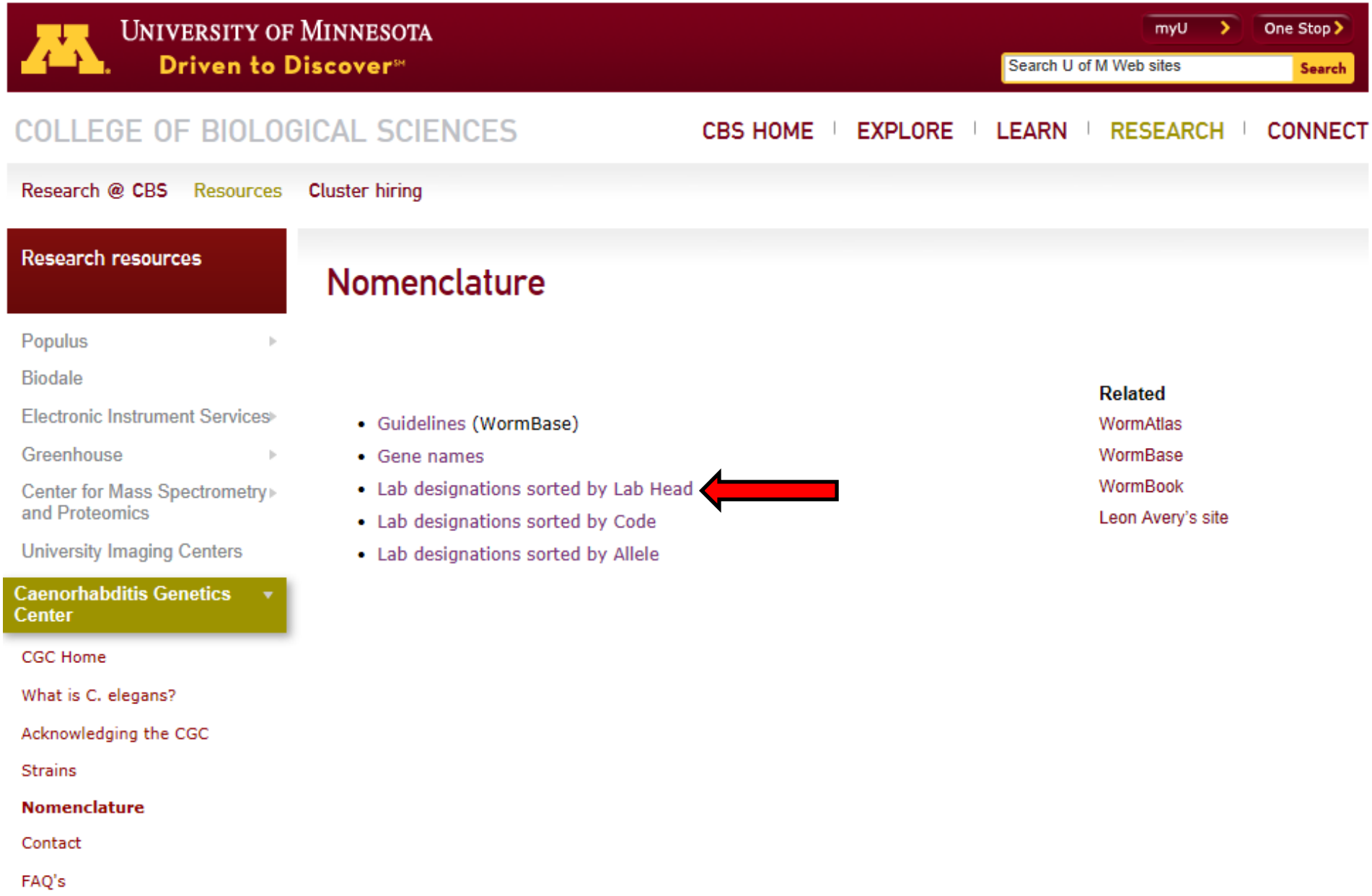


rol: cuticle collagen defect



# Genetics nomenclature

https://www.cbs.umn.edu/research/resources/cgc/nomenclature



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- FAQ's

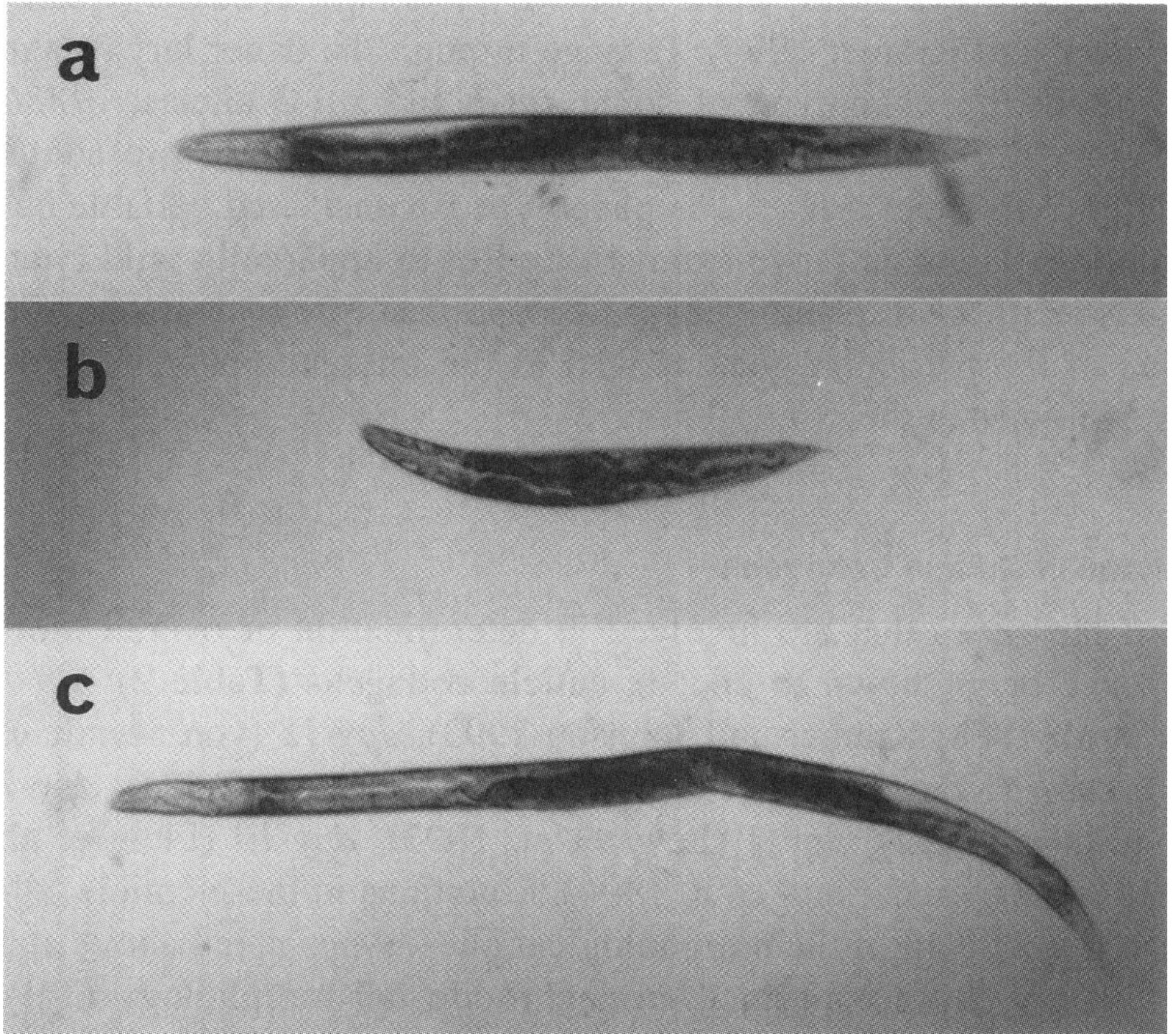
## Labs sorted by lab head

| Strain | Allele | Investigator/Location |
|---|---|---|
| EA | ls | Aamodt E, Louisiana State University Medical Center, Shreveport |
| PAB | -- | Abad P, INRA LBA, Antibes, France |
| AY | ac | Aballay A, Duke University, Durham, NC |
| RF | xw | Abbott A, Marquette University, Milwaukee, WI |
| EVL | lh | Ackley B, University of Kansas, Lawrence, KS |
| ZE | ze | Adam S, Northwestern University Medical School, Chicago, IL |
| BAD | -- | Adams B, Brigham Young University, Provo, UT |
| AEB | eth | Aebi M, ETH Zurich, Zurich, Switzerland |
| HGA | lyn | Aguilaniu H, Ecole Normale Superieure, Lyon, France |
| YA | yp | Ahmed S, University of North Carolina, Chapel Hill, NC |
| KJ | jh | Ahnn J, Hanyang University, Seoul, Korea |
| JA | we | Ahringer J, University of Cambridge, Cambridge, England |
| XZ | yak | Ailion M, University of Washington, Seattle, WA |
| NZ | drj | Albrecht D, Worcester Polytechnic Institute, Worcester MA |
| QZ | jx | Alcedo J, Friedrich Meischer Institute, Basel, Switzerland |

# Genetics nomenclature

https://www.cbs.umn.edu/research/resources/cgc/nomenclature

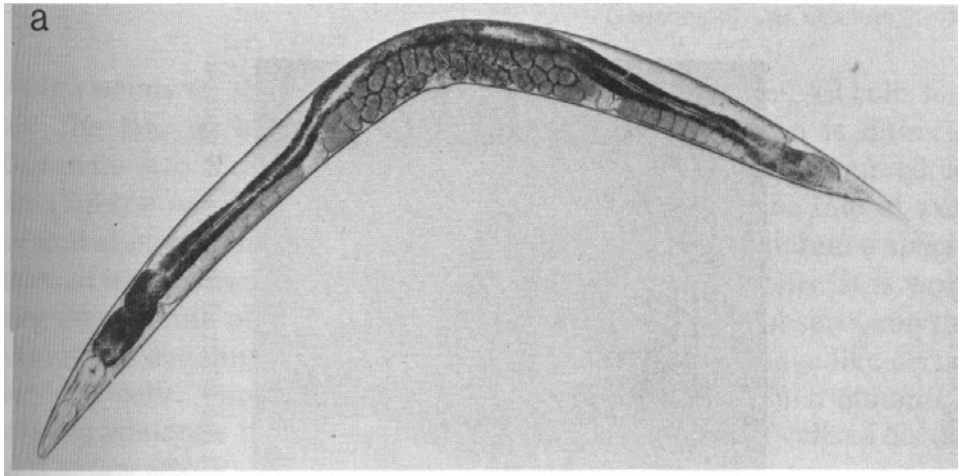
| | | |
|---|---|---|
| MV | vm | Vidal M, Harvard Medical School, Boston, MA |
| AV | me | Villeneuve A, Stanford University Medical School, Stanford, CA |
| MEV | | Viney M, University of Bristol, UK |
| BT | em | Vogel B, University of Maryland, Baltimore, MD |
| GG | g | von Ehrenstein G, Max-Planck Institute, Gottingen, Germany |
| UMT | mnt | Voronina E, University of Montana, Missoula, MT |
| IN | dt | Waddle J, UTSW Medical Center, Dallas, TX |
| IM | ur | Wadsworth W, UMDNJ, Piscataway, NJ |
| OIW | nth | Wagner O, National Tsing Hua University, Hsinchu, Taiwan |
| VL | ww | Walhout M, University of Massachusetts, Worcester, MA |
| WAL | ker | Walker A, Umass Medical School, Worcester, MA |
| YU | uw | Walston T, Truman State University, Kirksville, MO |
| KMW | | Walstrom K, New College of USF, Sarasota, FL |
| ER | jd | Walthall B, Georgia State University, Atlanta |
| WDY | nds | Wang D, Southeast University Medical School, Nanjing, China |
| GXW | gxw | Wang G-X, Huazhong Normal University, Wuhan, China |
| IW | iw | Wang J, Johns Hopkins University, Baltimore, MD |
| BRC | ant | Wang J, Academic Sinica, Taipei, Taiwan |
| LWA | wle | Wang L, The Salk Institute, La Jolla, CA |
| XW | qx | Wang X, NIBS, Beijing, China |
| YMW | xmu | Wang Y, Xiamen University, Xiamen, Fujian, China |




wt

*dpy*

*lon*

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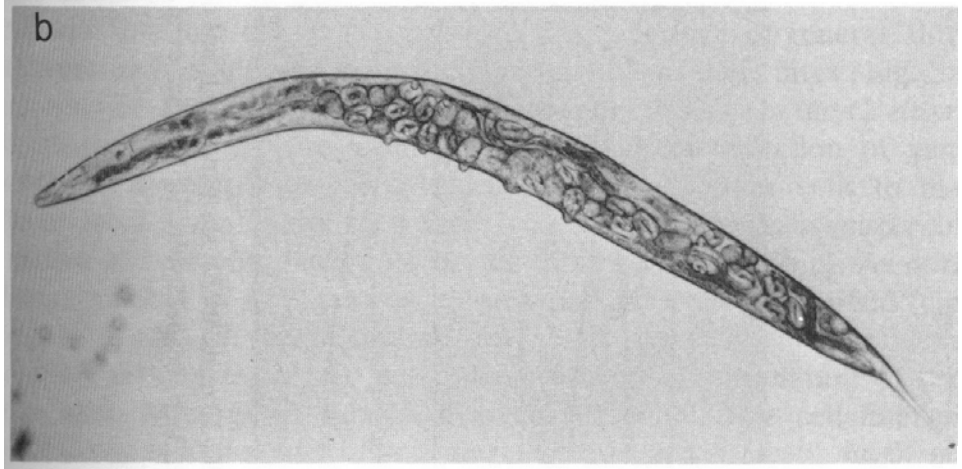
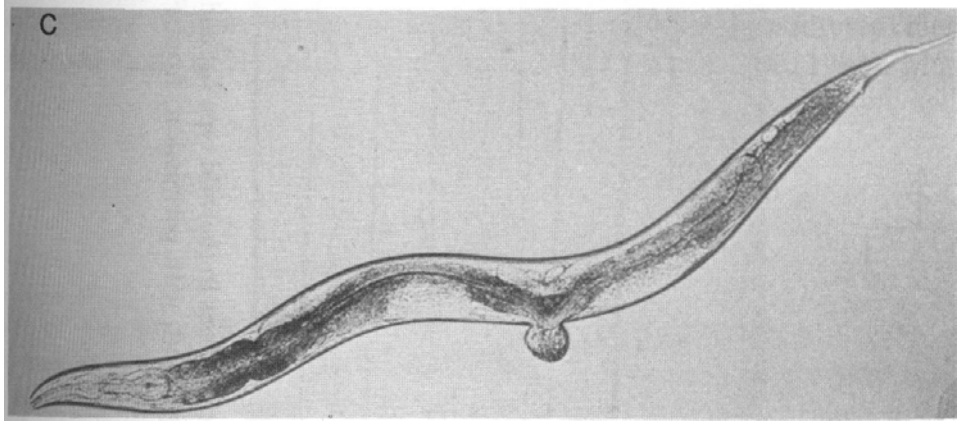



multi vulva




vulvaless (with hatchbag)

# Genetics nomenclature

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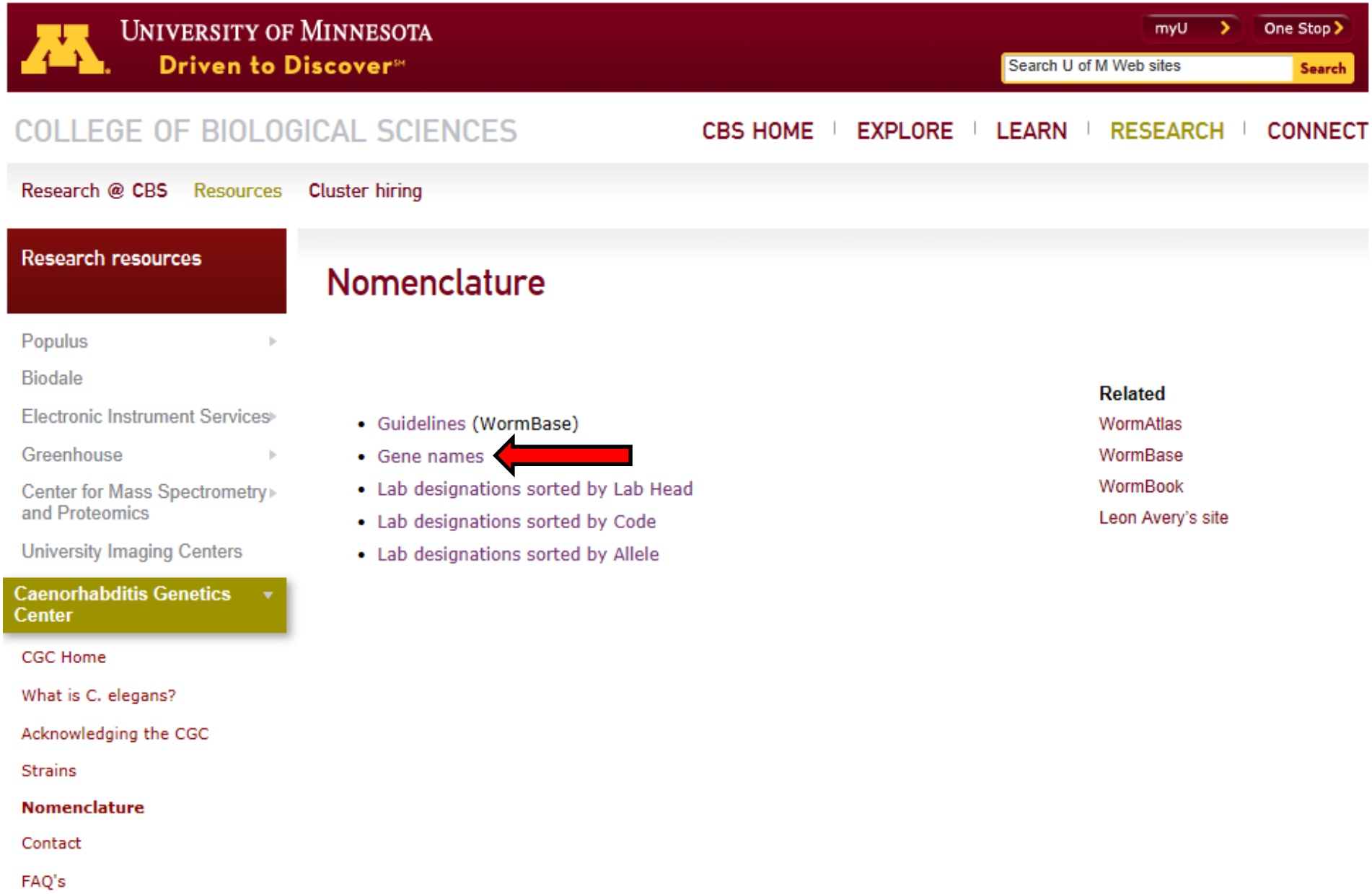

# Genetics nomenclature

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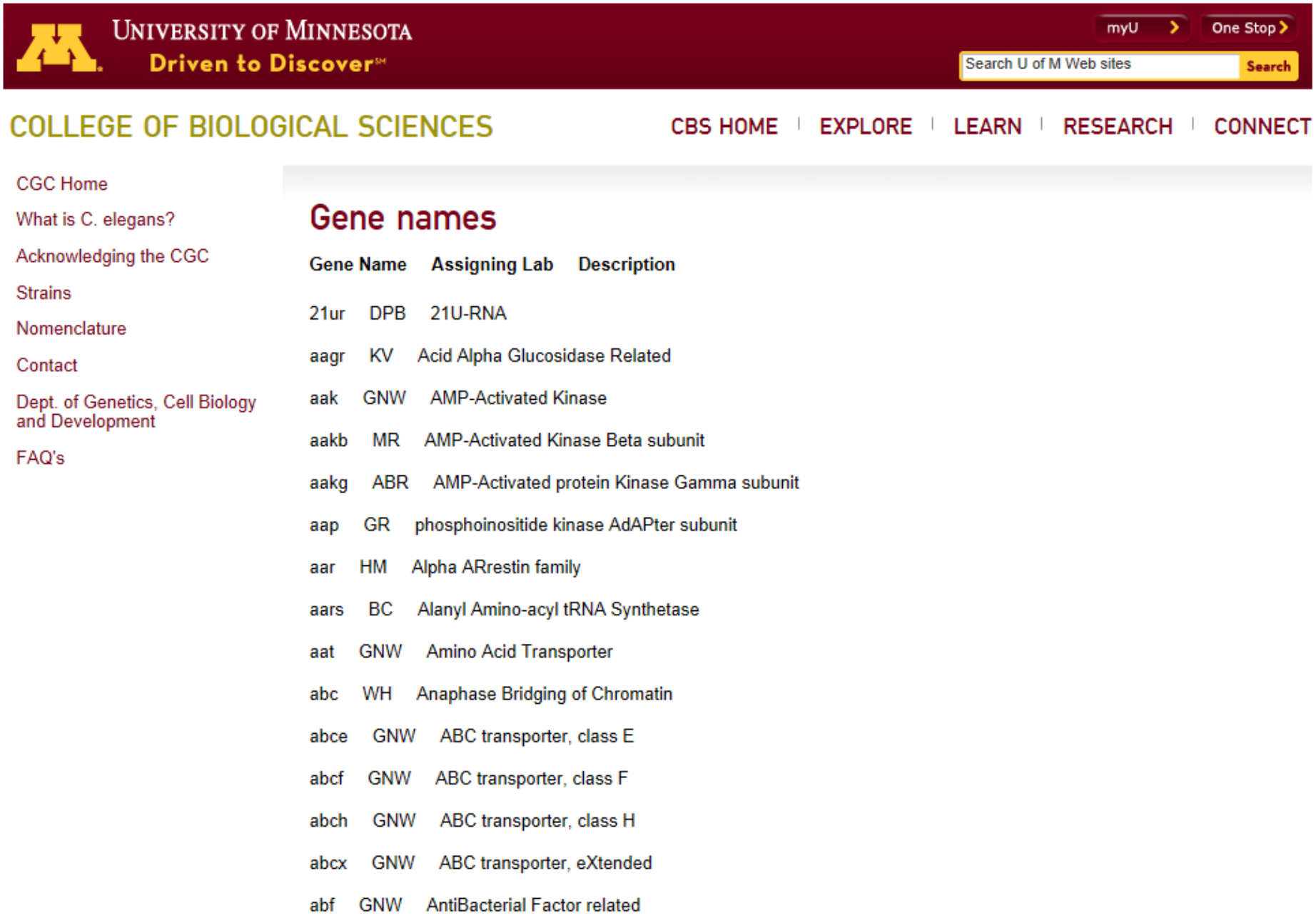

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- Dept. of Genetics, Cell Biology and Development
- FAQ's

## Gene names

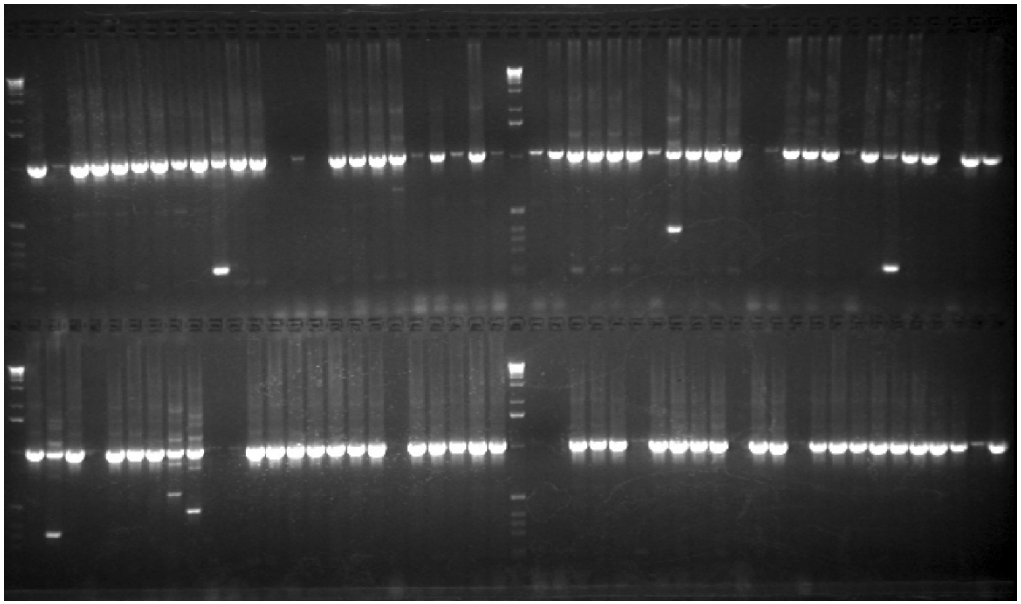
| Gene Name | Assigning Lab | Description |
|---|---|---|
| 21ur | DPB | 21U-RNA |
| aagr | KV | Acid Alpha Glucosidase Related |
| aak | GNW | AMP-Activated Kinase |
| aakb | MR | AMP-Activated Kinase Beta subunit |
| aakg | ABR | AMP-Activated protein Kinase Gamma subunit |
| aap | GR | phosphoinositide kinase AdAPter subunit |
| aar | HM | Alpha ARrestin family |
| aars | BC | Alanyl Amino-acyl tRNA Synthetase |
| aat | GNW | Amino Acid Transporter |
| abc | WH | Anaphase Bridging of Chromatin |
| abce | GNW | ABC transporter, class E |
| abcf | GNW | ABC transporter, class F |
| abch | GNW | ABC transporter, class H |
| abcx | GNW | ABC transporter, eXtended |
| abf | GNW | AntiBacterial Factor related |

# Genetics nomenclature

Examples of some important gene names:

**aex** = Anterior contraction and EXpulsion defect in defecation
**age** = AGEing alteration
**bli** = BLIstered cuticle
**ced** = CEll Death abnormality
**daf** = abnormal DAuer Formation
**dpy** = DumPY: shorter than wild-type
**dyf** = abnormal DYe Filling (fails to stain amphid neurons with FITC)
**eat** = EATing: abnormal pharyngeal pumping
**egl** = EGg Laying defective
**him** = High Incidence of Males (increased X chromosome loss)
**let** = LEThal
**lin** = abnormal cell LINeage
**osm** = OSMotic avoidance abnormal
**rol** = ROLler: helically twisted body, animals roll when moving
**sle** = SLow embryonic development
**sma** = SMAll (body size)
**syd** = SYnapse Defective
**unc** = UNCoordinated
**vab** = Variable ABnormal morphology
**zyg** = ZYGote defective : embryonic lethal

# Mutagenesis



PCR screening gel

# The genome sequencing was a team effort: authors of the 1998 Science paper

Rachael Ainscough, Simon Bardill, Karen Barlow, Victoria Basham, Caroline Baynes, Lisa Beard, Alastair Beasley, Mary Berks, James Bonfield, Jacqueline Brown, Christine Burrows, John Burton, Connie Chui, Emma Clark, Louise Clark, Gerard Colville, Theresa Copsey, Amanda Cottage, Alan Coulson, Molly Craxton, Auli Cummings, Paul Cummings, Simon Dear, Thomas Dibling, Richard Dobson, Jonathan Doggett, Richard Durbin, Jillian Durham, Andrew Ellington, David Evans, Kerry Fleming, John Fowler, Debbie Frame, Audrey Fraser, Alison Gardner, Jane Garnett, Iain Gray, Jane Gregory, Mark Griffiths, Sarah Hall, Barbara Harris, Trevor Hawkins, Cathy Hembry, Sarah Holmes, Bijay Jassal, Matt Jones, Steve Jones, Ann Joy, Paul Kelly, Joanna Kershaw, Andrew Kimberley, Yuji Kohara, Neil Laister, Dan Lawson, Nicola Lennard, Julia Lightning, Simon Limbrey, Sarah Lindsay, Christine Lloyd, Simon Margerison, Anna Marrone, Lucy Matthews, Paul Matthews, Rebecca Mayes, Kirsten McLay, Amanda McMurray, Mark Metzstein, 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Cynthia Geisel, Steve Geisel, Alicia Gibson, Candi Giddings, Barbara Gillam, Warren Gish, Danielle Glossip, Jennifer Godfrey, Deepa Goela, Norma Goins, Tina Graves, Tracie Greco, Phil Green, Serena Gregory, William Haakenson, Priscilla Hale, Charles Harkins, Gwen Harmon, Mark Harper, Anthony Harris, Michelle Harrison, James Hawkins, Maria Hawkins, Clay Hawryszko, Chuck Heidbrink, John Henkhaus, LaDeana Hillier, Kurt Hinds, Michael Holman, Andrea Holmes, Donna Hopson, Melisa Hotic, Monica Hultman, Ann Jacobs, Craig Jenkins, Mohamed Jier, Doug Johnson, Mark Johnston, Brenda Jones, Kimberly Jones, Paula Kassos, Kimberly Keen, Jennifer Kellen, Kimberley Kemp, Deana Keppler, Amy Kerstetter, Melissa Ketterman, Kyung Kim, Mark King, Jennifer Kirsten, Bill Klinke, Jeremy Kock, Sara Kohlberg, Ian Korf, Amy Kozlowicz, Jason Kramer, Rebecca Krauss, Tamara Kucaba, Michelle Lacy, Thomas Lakanen, Betty Lamar, Yvonne Langston, Yvonne LaPlant, John Latreille, Daniel Layman, Thomas Le, Thuy-Tien Le, Tri-Tin Le, John Ledwith, Lynn Lehnert, Darcy Leimbach, Sarah Lennox, Shawn Leonard, Lili Li, Paul Lowery, Terrie Lynch, Chris Macri, Len Maggi, Maggie Maher, Elaine Mardis, Marco Marra, Gabor Marth, John Martin, Rachel Maupin, Ken McDonald, Ramonna McDonald, Rebecca McGrane, Kelly Mead, Becky Meininger, Sandra Menezes, Brian Merry, Rebecca Miko, Kevin Miller, Nancy Miller, Walt Miller, Brian Minges, Patrick Minx, Tonya Modde, Bradley Moore, Matthew Morris, Garrett Mullen, Molly Mullen, Jennifer Murray, Diane Nelson, Joanne Nelson, Amy Nguyen, Christine Nguyen, Nham Nhan, Susan Nichols, Laura Niemann, David O'Brien, Darla O'Neal, Ben Oberkfell, Amy Ozanich, Philip Ozersky, Dimitrios Panussis, Kimberly Pape, Jeremy Parsons, Adele Pauley, Charlene Pearman, Dale Peluso, Kymberlie Pepin, Denise Peterson, Amy Phillips, Craig Pohl, Faye Prevedell, Tim Raichle, Jennifer Randall, Mary Reynolds, Carrie Rhine, Lorrie Rice, Joanne Rieff, Lisa Rifkin, Linda Riles, Judy Robertson, Kerry Robinson, 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Wohldmann, Cliff Wollam, Kimberly Woods, Xiaoyun Wu, Shiaw-Pyng Yang, Martin Yoakum, Xiao Zheng, Hui Zhu, Michael Zidanic

# Mutagenesis: To analyze the function of genes

- A sequenced genome allows for the **identification of all proteins** in an organism
- But this **does not provide** sufficient information to identify the pathways and structures in which these proteins function
- To integrate the sequence information into cellular and developmental processes, functional analysis of as many genes as possible is necessary
- The **easiest way** to study the function of genes is **by mutation**
- Three types of mutations:
    - **Target-selected mutagenesis** (specific mutations): Transposon or CRISPR
    - **Spontaneous mutagenesis** (non-specific mutations): Uncommon approach
    - **Induced mutagenesis** (non-specific mutations): Very common => inexpensive + can unravel novel genetic pathways and protein interrelationships
- **Mutant phenotypes** can be divided into three categories:
    - **Visible**: *unc, sma, dpy, bli*…
    - **Lethal**: *let, emb, mel, zyg*…
    - **Conditional**: temperature sensitive defects in protein products

## Lethal and non-lethal gene classes

| Name | Phenotype | Number in class | Number with lethal alleles |
|---|---|---|---|
| *let* | lethality | 464 | 464 |
| *unc* | uncoordinated | 114 | 13 |
| *lin* | lineage-defective | 48 | 14 |
| *egl* | egg-laying-defective | 46 | 3 |
| *sup* | suppressor | 37 | 6 |
| *emb* | embryonic arrest | 34 | 34 |
| *daf* | dauer-defective or -constitutive | 31 | 12 |
| *mel* | maternal-effect lethal | 29 | 29 |
| *dpy* | dumpy | 26 | 2 |
| *evl* | eversion of vulva | 24 | 24 |
| *che* | homeobox | 21 | 2 |
| *mab* | male abnormal | 21 | 2 |
| *spe* | sperm-defective | 19 | 12 |
| *eat* | eating abnormal | 17 | 0 |
| *mec* | mechanosensory abnormal | 15 | 1 |
| *him* | high-incidence male | 14 | 0 |
| *dyf* | dye-filling | 13 | 0 |
| *ced* | cell death | 11 | 1 |
| *zyg* | zygotic-arrest | 11 | 11 |
| *pat* | twofold arrest | 9 | 9 |

# Non-specific mutations

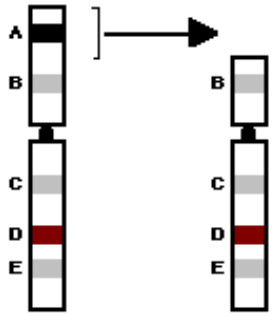
1) Spontaneous mutations: production of mutations without using any mutagenic agent
   - These mutations are based on **replication error**, **background irradiation** damage or **environmental chemical** mutagenesis
   - Spontaneous mutation occur in N2 wildtype at a rate of 1 per 2000-3000 animals
   - In so called mutator strains the high frequency of spontaneous mutations is based on increased **transposable element** activity (increased transposase activity)

2) Induced mutagenesis:
   - **EMS** (ethylmethanesulfonate)
   - **UV/TMP** (ultra violet light/tetramethylpsoralen)
   - **DES** (diethyl sulfate)
   - **ENU** (N-nitroso-N-ethylurea)
   - **Formaldehyde**
   - **Irradiation**: X-rays, $\gamma$-rays, UV-light
   - **Crossing with a mutator strain** (e.g., *mut-2* activates transposon movements)

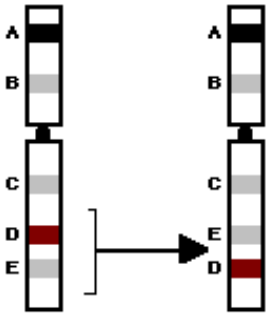
   => **Chemical mutagenesis** basically induces **point mutations** and **small deletions** while **irradiations** can induce **large deletions** and **chromosomal rearrangements**

# Non-specific mutations

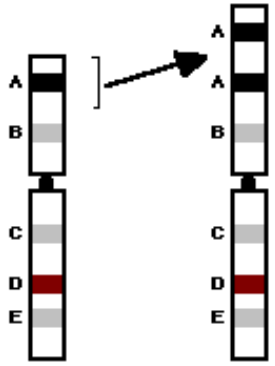
- **Point mutations** are defined by localized sequence changes:
  - Transitions
  - Transversions
  - Nucleotide additions
  - Nucleotide deletions
- **Chromosomal rearrangements** include:
  - **Deletions (Deficiencies)**
  - **Inversions**
  - **Duplications**
  - **Translocations**
  - Combinations of above



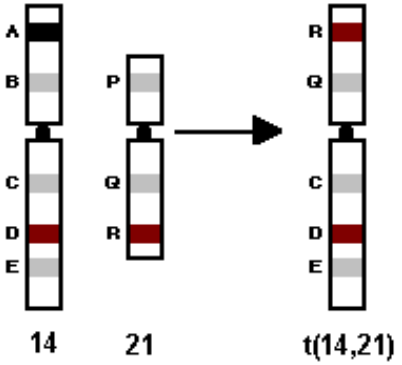

**Deletion**




**Inversion**




**Duplication**




**Translocation**

# Non-specific mutations

• Point mutations are generally used to obtain effective **loss-of-function** or **gain-of-function** mutations
• **EMS** is widely used to introduce point mutations (and small deletions): it usually causes G/C-A/T transitions
• **ENU** produces transitions and transversions and small deletions
• **Formaldehyde** as well as **UV/TMP** can induce large deletions (up to 15 kb), duplications, inversions and translocations and can disrupt one or more genes
• **Irradiation**-induced chromosomal rearrangements can be used to map genes or as genetic balancers (to avoid recombination events in let/+ mutants)
• **Mutators**: *mut-2* activates several families of **transposons** (including **Tc1**) inducing large deficiencies

# Forward and reverse genetics


Genome-wide automated high-throughput RNAi screening

# Forward genetics

Two main strategies for mutagenesis: **forward genetics** and **reverse genetics**

- **Forward genetics**: Which mutants show the phenotype of interest (for example short tails)? Relate phenotype to genotype => **EMS for example**
- **Reverse genetics**: Does downregulation of a gene cause the phenotype of interest? Relate genotype to phenotype => **RNAi for example**

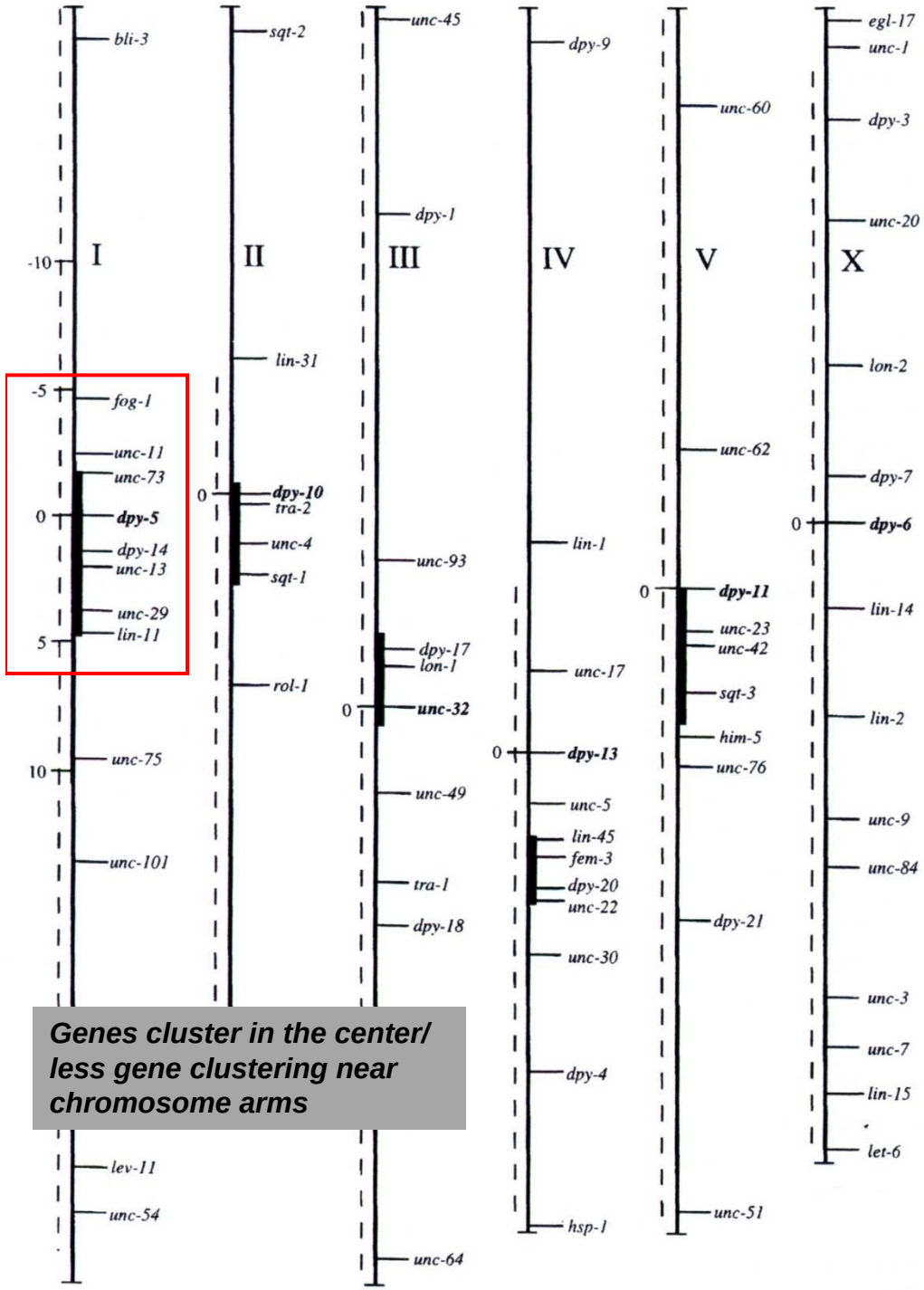
Linkage groups analysis in forward genetics:

- Some **phenotypes** are **genetically linked** to other phenotypes
- For example *dpy-5* is linked to 3 different unc genes: unc-13, unc-29 and unc-73
- If a “dpy worm” is “unc” after mutagenesis then either of these three unc genes is mutated

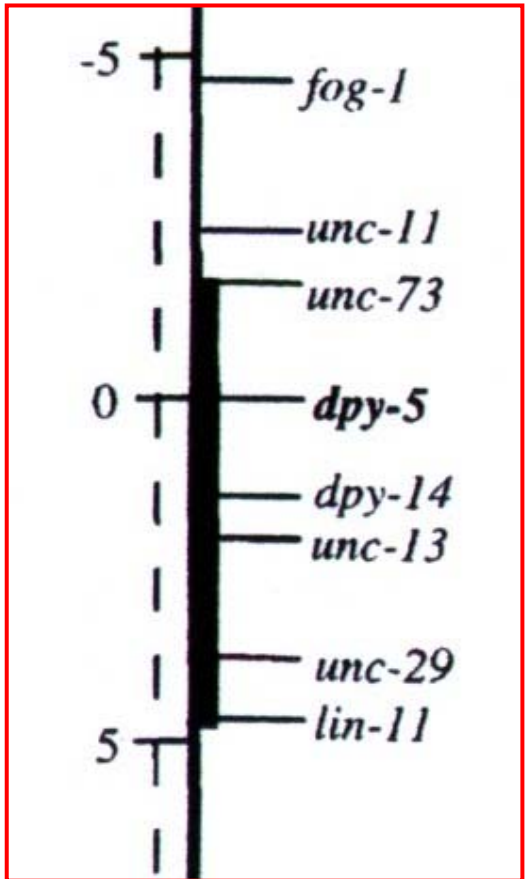
# Forward genetics

*C. elegans* chromosomes look like a rod and are called **linkage group** (LGI, LGII… LGX)

If *dpy-5* worms are uncoordinated you may have induced a *unc-13*, *unc-29* or *unc-73* mutation!




***Genes cluster in the center/ less gene clustering near chromosome arms***



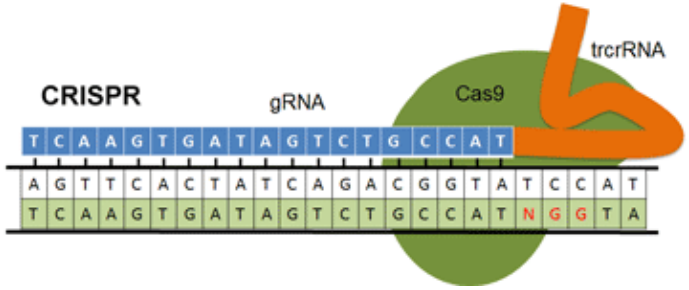
# Genetic balancers

• 1/3 of *C. elegans* genes are essential, so it frequently happen that homozygous **mutations** of a gene of interest **are lethal**
• These mutants must be maintained as heterozygous using a *wt* allele (let/+)
• A balancer is a (visible) genetic marker in *trans* (on the opposite chromosome of a homologous pair) to the lethal mutation: e.g., $\frac{\text{let} \ +}{+ \ \text{unc}}$

example for *trans* marker

# Reverse genetics

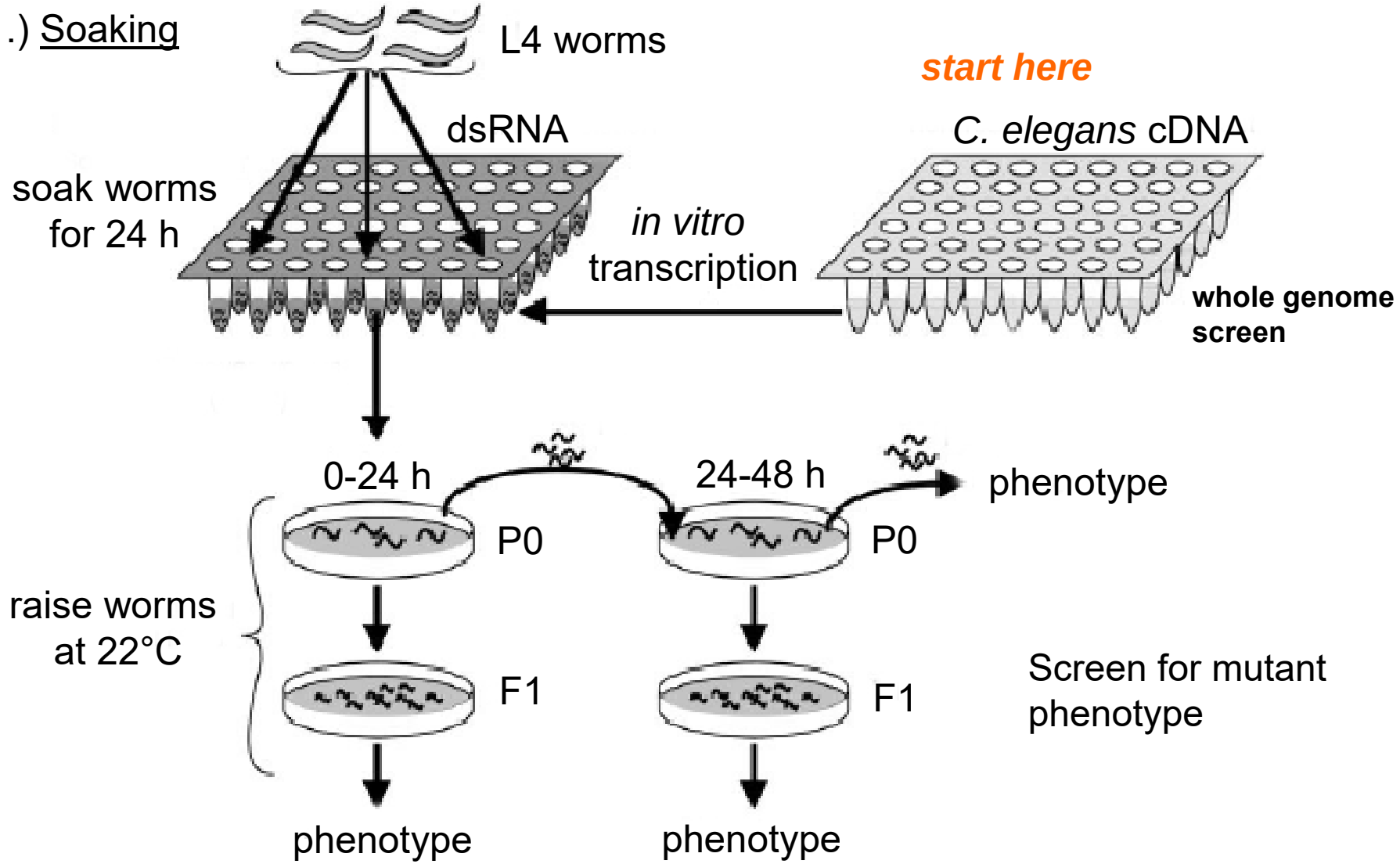
Most popular:
• **RNA interference**
• **Transposon (Tc1) screen** (cross in mutator strain, PCR screen)
• **Induced mutagenesis** (PCR screen to isolate deletions after mutagenesis)
• **New Method**: **CRISPR/Cas9** for generating knockout animals => a bacterial nuclease Cas9 binds to a gRNA (guide RNA) sequence that also contains the target sequence on the genomic (double stranded) DNA; the gRNA pairs with the area to be deleted on the genomic DNA and the **nuclease removes the targeted area**




Clustered regularly interspaced short palindromic repeats (CRISPR)

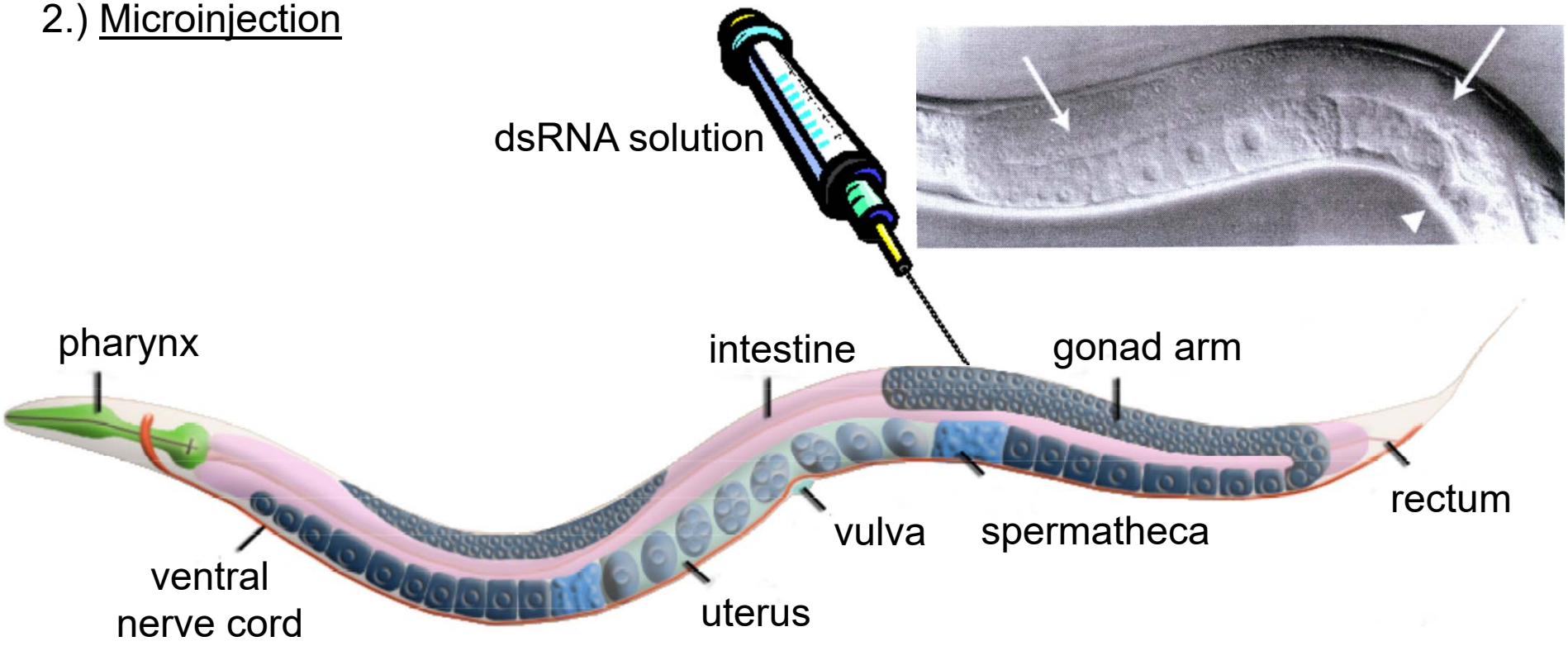
# RNA interference

Double-stranded RNA (**dsRNA**) introduced in worms is cleaved into short interfering RNAs (**siRNAs**) => can hybridize with homologous **mRNAs** and induce their degradation => three methods: **soaking**, **feeding** and **microinjection**



# RNA interference

2.) Microinjection

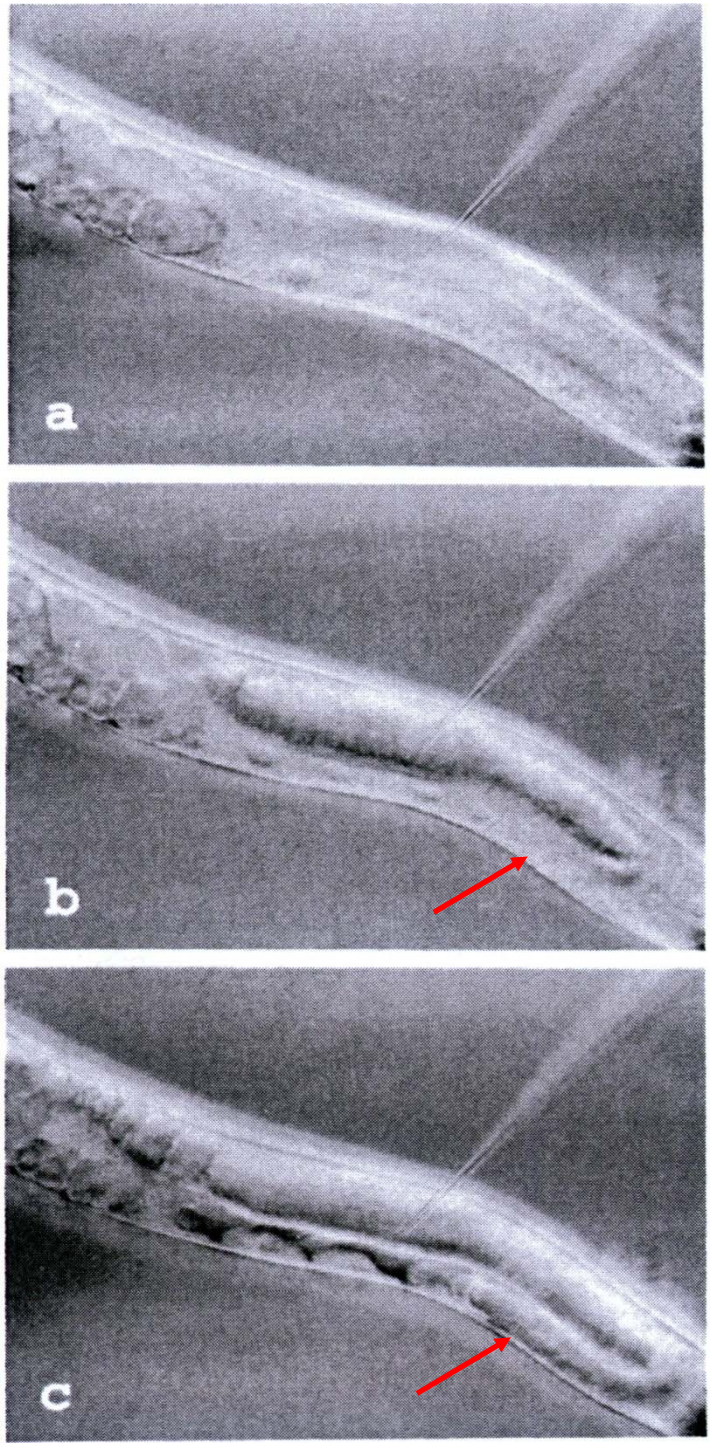



dsRNA made from cDNA using **T7 RNA polymerase**

# RNA interference

2.) Microinjection

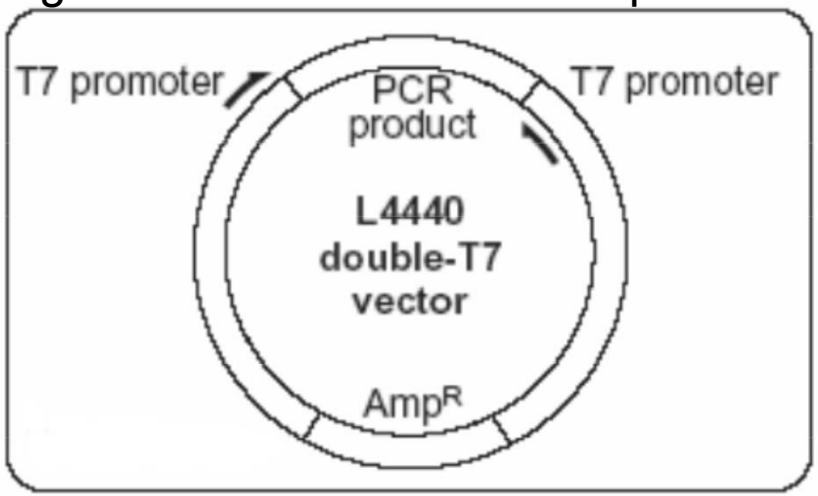
Injection of DNA lets the gonad swell up
(sausage-like appearance)



# RNA interference

3.) Feeding (worms with bacteria producing dsDNA)

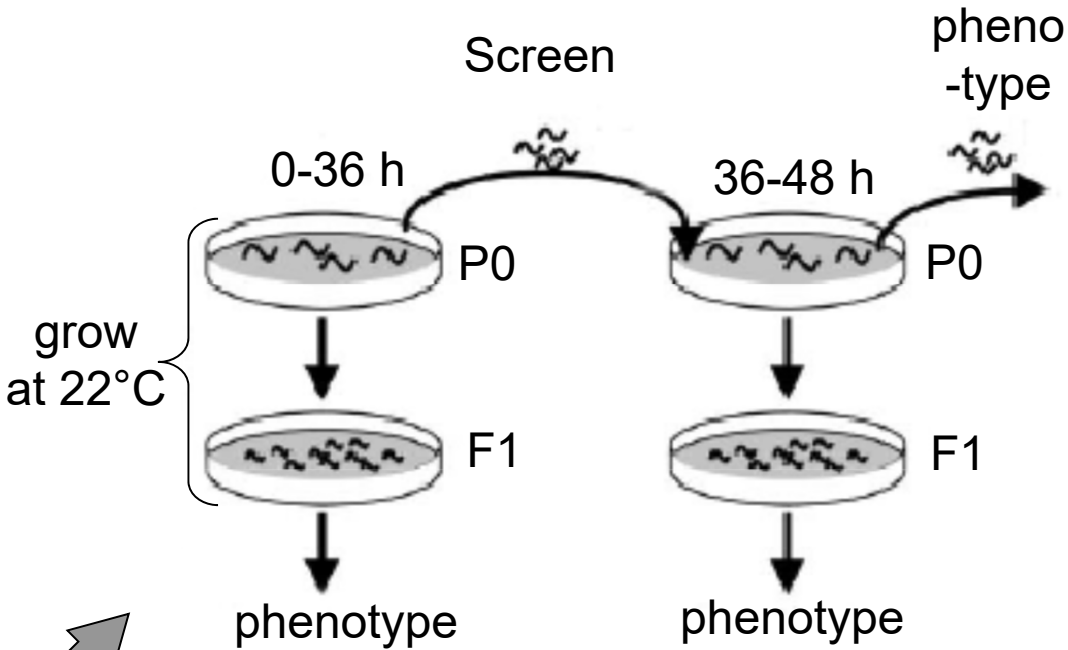
Two T7 RNA polymerase promoter generate dsRNA from PCR product




transform into
*E. coli HT115(DE3)*

(RNase III deficient strain expressing T7 RNA polymerase from an IPTG-inducible promoter)

Feed worms (put *E. coli* on agar together with IPTG)




20,000 bacteria exist expressing dsDNA targeting 20,000 genes

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## *C. elegans* RNAi library

# RNAi feeding library

This *Caenorhabditis elegans* RNAi feeding library provided by Geneservice was constructed by Julie Ahringer's group at the The Wellcome CRC Institute, University of Cambridge, Cambridge, England. .

*C. elegans* genomic fragments were PCR amplified using Research Genetics GenePairs, cloned into the EcoRV site of vector L4440 from Timmons and Fire (Nature, 395, 854) and transformed into bacterial strain HT115 (Gene, 263, 103-112) as described (Nature 408, 325-330). The whole genome library consists of 16,757 bacterial strains, which cover 87% of *C. elegans* genes.

Bacterial strains carry the GenePairs name, which usually, but does not always correspond to a predicted *C. elegans* gene name. A current mapping of GenePair to gene can be found in WormBase (http://www.wormbase.org). GenePairs primer sequences are available at http://cmgm.stanford.edu/~kimlab/primers.12-22-99.html.

There is also a "*C.elegans* Finder" tool available which allows you to find the primer sequences for any given GenePair Name in addition to the location of that bacterial strain in Geneservice 384-well plates. The complete *C.elegans* RNAi database can be downloaded by right clicking here . Note that the mapping of some GenePairs has changed since WS56 when this database was built. A correlation table between WS56 and WS152 can be accessed here.

The libraries is available by individual chromosome sets (I, II, III, IV, V and X) from Geneservice as frozen glycerol stocks of bacterial strains arrayed in 384 well plates or as individual bacterial strains (clones).

We are also supplying re-arrayed sub-sets of the libraries in the following areas:

1. Chromatin (257 clones)
2. Phosphatase (166 clones)
3. Transcription factors (387 clones).

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(technical information as supplie

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# miRNA / RNAi Resources

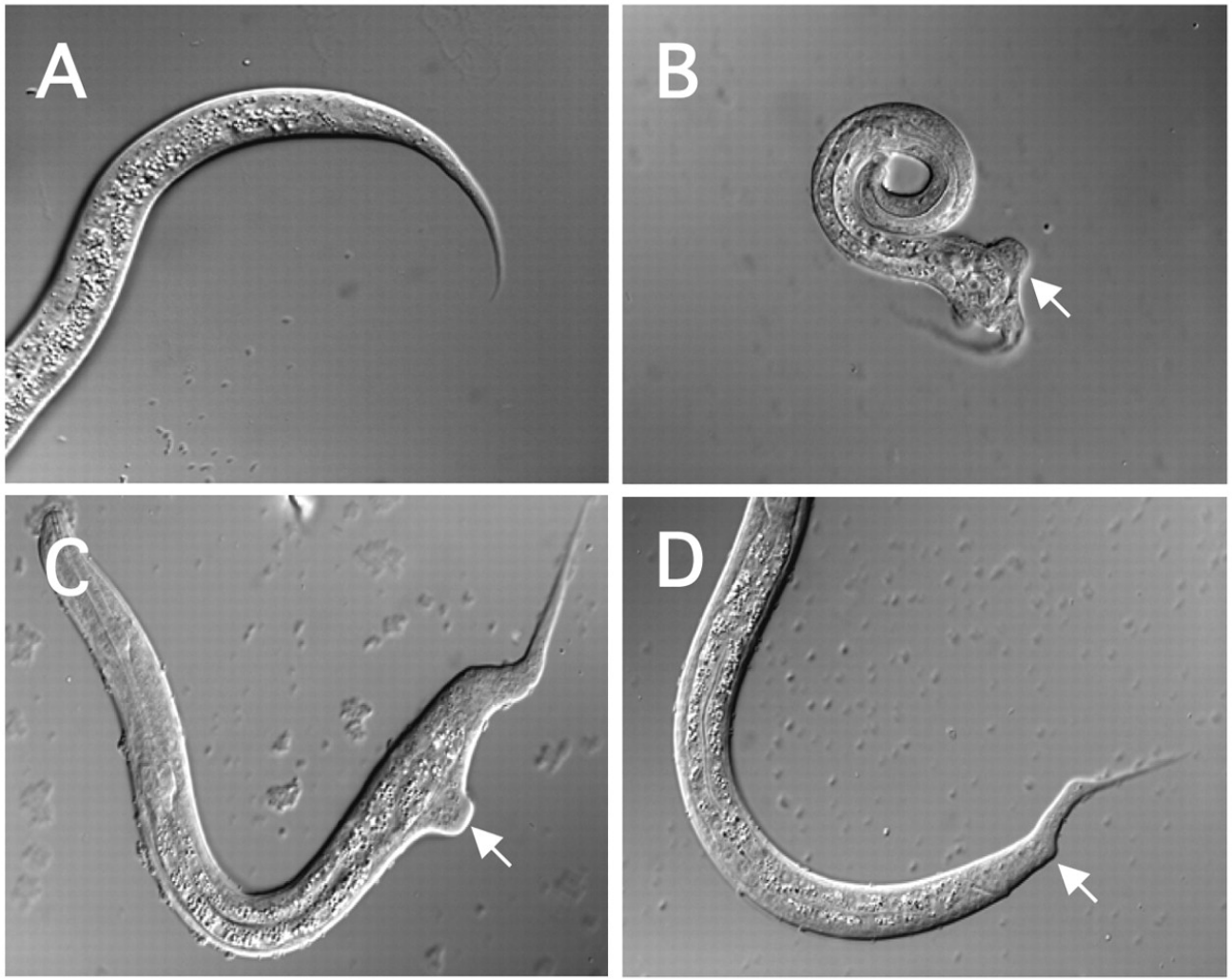
| RNAi Resources | | | |
|---|---|---|---|
| **Resource**(click links for more information on each resource) | **Format**(click links to get information about ordering and access the order forms) | | PRICE PER CHROMOSOME SET |
| *C.elegans* RNAi (more info) | Order Set of Library Plates # * Price is for each individual Chromosome set: (I,II,III,IV,V,X) | Academic Users | £ 992.25 |
| | | Non-Academic Users | £ 3307.50 |

**170,420 * 6 = 1,022,520 NT**

| RNAi Resources | | | |
|---|---|---|---|
| **Resource**(click links for more information on each resource) | **Format**(click links to get information about ordering and access the order forms) | | PRICE PER SET |
| *C.elegans* RNAi Chromatin subset (more info) | Order plate sets # Chromatin Phosphatase Transcription factor | Academic Users | £ 435.75 |
| | | Non-Academic Users | £ 2625.00 |
| *C.elegans* RNAi Phosphatase subset (more info) | Order plate sets # C.elegans RNAi Phosphatase subset | Academic Users | £ 288.75 |
| | | Non-Academic Users | £ 1680.00 |
| *C.elegans* RNAi Transcription Factors subset (more info) | Order plate sets # | Academic Users | £ 724.50 |
| | | Non-Academic Users | £ 4305.00 |

| RNAi Resources | | | | | |
|---|---|---|---|---|---|
| **Resource**(click links for more information on each resource) | **Format**(click links to get information about ordering and access the order forms) | | PRICE 1-5 | PRICE 6-24 | PRICE 24+ |
| Sequence Verified *C.elegans* RNAi (more info) | Order Bacterial Strain (Individual Clone) | Academic Users | £ 98.70 | £ 88.83 | £ 83.90 |
| | | Non-Academic Users | £ 514.50 | | |
| *C.elegans* RNAi (more info) | Order Bacterial Strain (Individual Clone) | Academic Users | £ 67.20 | £ 67.20 | £ 57.12 |
| | | Non-Academic Users | £ 498.75 | | |
| *C.elegans* ORF-RNAi library (more info) | Order Clone(s) | | £ 67.20 | £ 67.20 | £ 67.20 |
| | Order full plate set# | | £ 13650.00 | | |

# Mutant characterization



# Mutant characterization

• Initial step is an **outcross**: cross *wildtype male* with *mutant hermaphrodite*
⇒ if mutant phenotype disappears, mutation is probably **recessive** (m/m) OR is **X chromosomal linked**
⇒ if mutant phenotype does not disappear, mutation is probably **dominant** (M/+)
• **Anatomical** characterization (*unc, sma, dpy, bli…*)
• **Developmental** description (lack of dauer)
• **Lineage** analysis: **Mosaic screen**: Does mutation affect only specific cells? Is the mutation cell autonomous or non-cell autonomous? Meaning, does the mutation affect neighboring cells or not?
• **Cellular and subcelluar** analysis (enlarged cells, misexpressing of fluorescent proteins)
• **Biochemical / Mol. biol. analysis** (RT-PCR, Immunohistochemistry…)

# Mutant characterization

Other useful tests:

**Penetrance and expressivity**

- It is hard to work with highly variable mutants: **test for penetrance**
    - Is the mutant 100% penetrant or is it difficult to distinguish homozygotes from wildtypes?
    - Is the phenotype of homozygotes constant or does it vary? (constant expressivity)

**Hermaphrodite fertility**

- Counting progeny/eggs: reduced brood size could be based on **egg-laying defects**
- Counting unhatched eggs: increased unhatched eggs indicate **embryonic developmental defects**
- Counting males: increase in male frequency indicates a **meiotic defect**

# Mutant characterization

## Maternal effects

- Phenotype might significantly weaken if the progeny receives a **wild-type gene**
    - Is the mutant phenotype derived from a homozygous or from a heterozygous parent?

## Expression during the life-cycle

- **Behavioral phenotypes** can be different during the life-cycle => a L1 worm can behave very different from the adult (effects on neuronal circuity)
    - Is the phenotype visible throughout the life-cycle or does it vary in strength?

## Temperature effects

- Some **conditional mutations** are based on temperature shifts
- Some mutants are lethal at higher temperatures (**restrictive temperature**) but survive at lower temperatures (**permissive temperature**)
    - Is the mutant phenotype the same at low (15°C) and high (25°C) as compared to the standard growth temperature (20-22°C)?

## Starvation effects

- If a worm dies easily by starvation a mutated gene might be involved in specific **metabolic pathways**

# Mutant characterization

**Aldicarb resistance**

• Worms exposed to the **insecticide aldicarb** usually become highly paralyzed

• Aldicarb is an acetylcholinesterase inhibitor and worms resistance to aldicarb might have mutated genes **related to the nervous system**

**Serotonin resistance**

• Serotonin induces egg-laying based on the action of the **serotonergic HSN neuron**

• Worms exposed to serotonin and do not throw eggs might have an **egg-laying defect**

**Dye filling**

• **FITC** or **DiI** stains sensory neurons (amphids); worms that fail to stain might have mutations in genes affecting the **sensory neuronal system** (gene: *dyf*)

**Osmotic resistance**

• Worms avoiding high gradients of, e.g., NaCl, fructose, caffeine; worms resistant to those gradients might have genes mutated involved in **osmoregulation**

# *Thanks for your attention*

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Professor

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Institute of Molecular & Cellular Biology

Department of Life Science