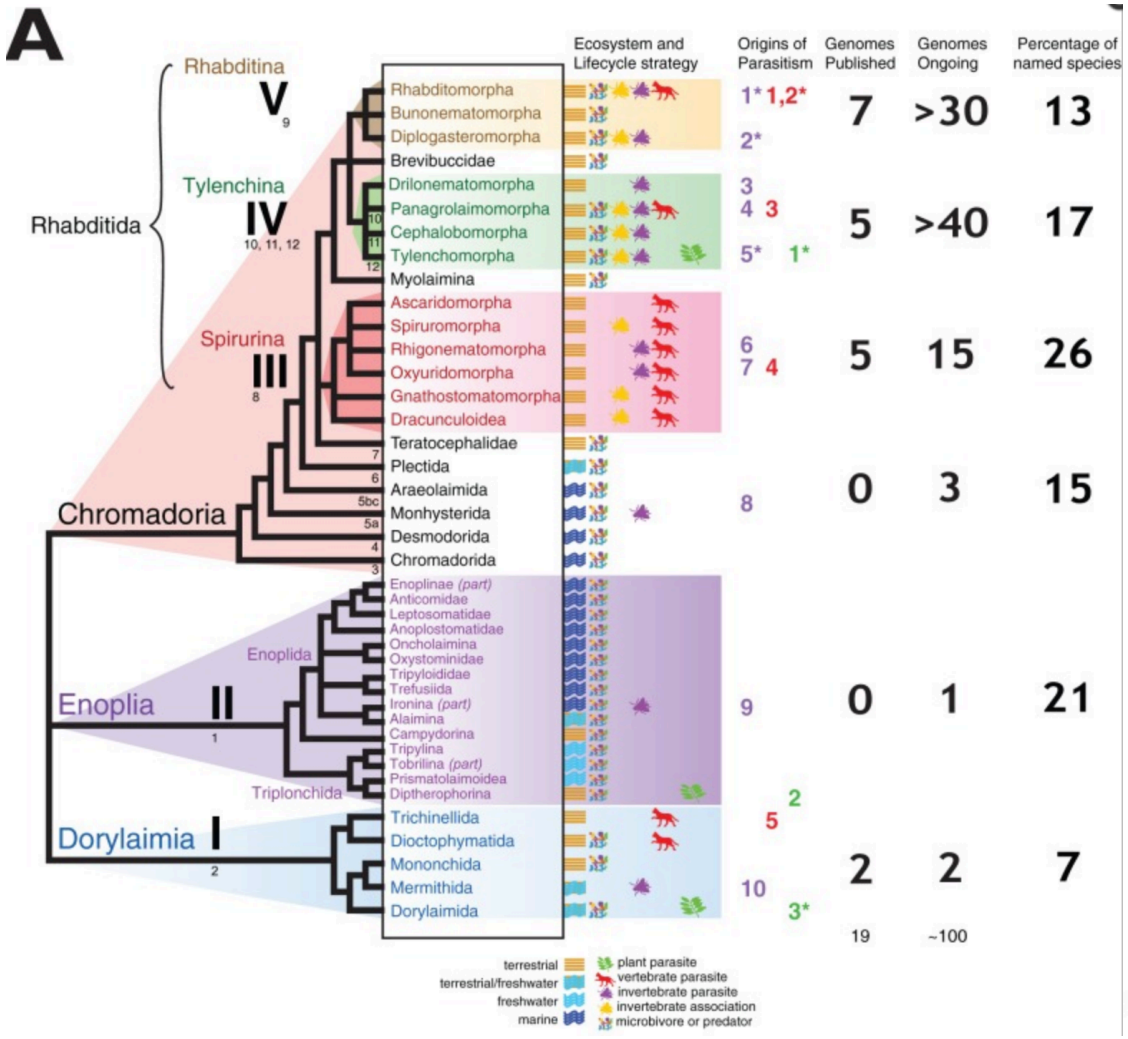
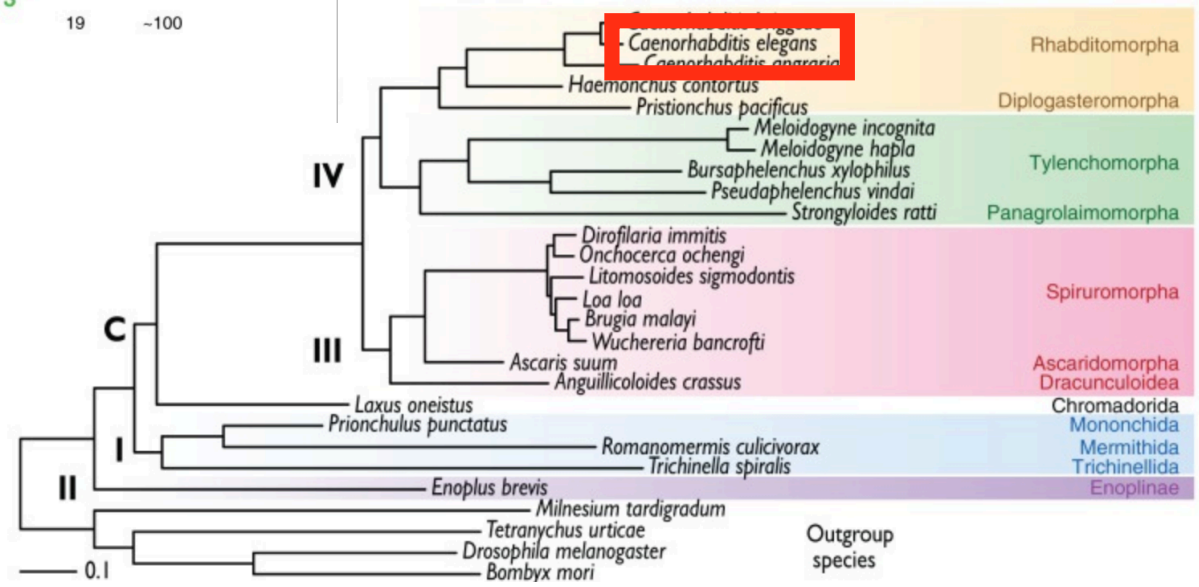
Desenvolvimento de nemátodos
Prática

Principios básicos do desenvolvimento – Nemátoda *C. elegans*



Position of *C. elegans* within the nematodes



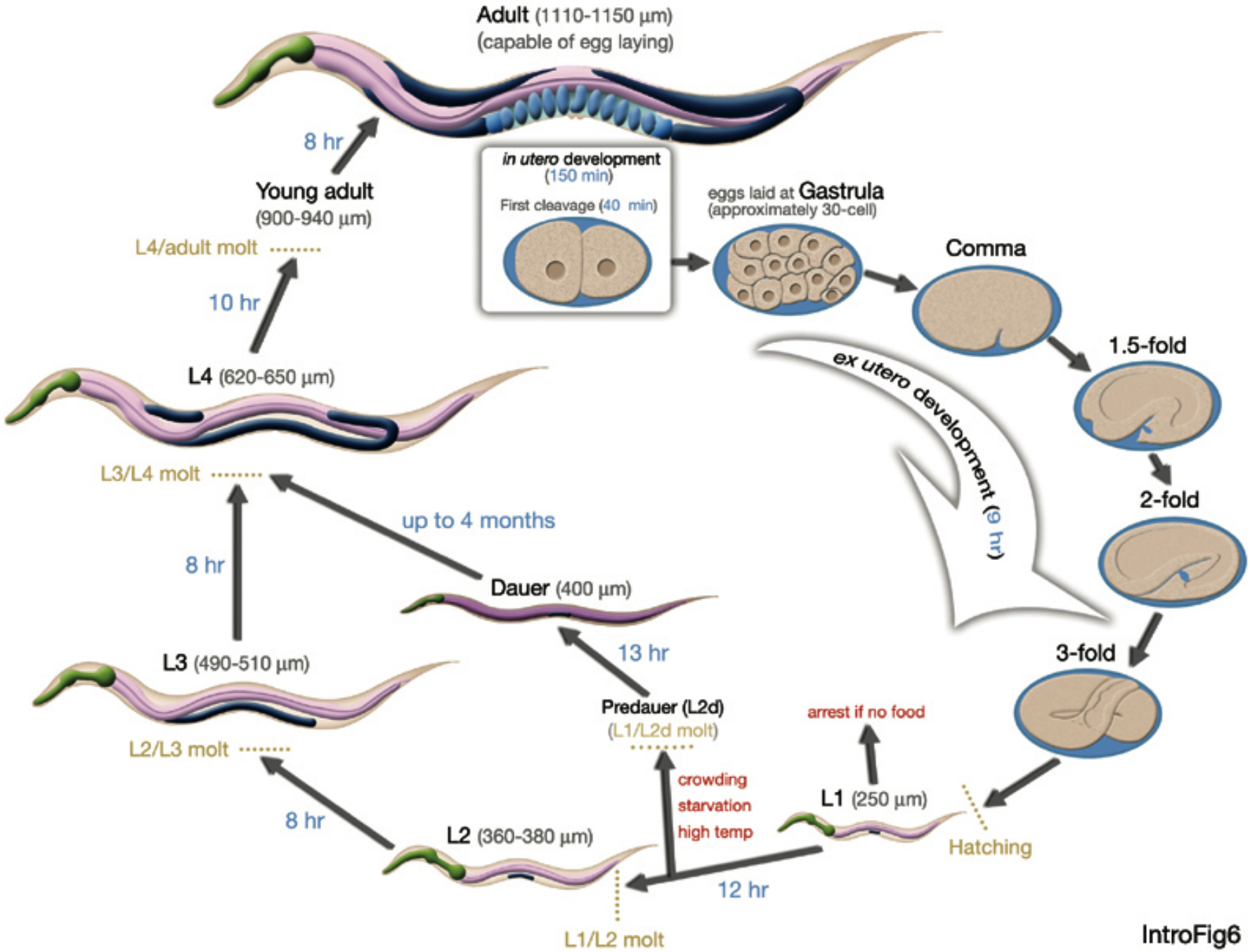
Blaxter & Koutsovoulos, 2015

The Nobel Prize in Physiology or Medicine 2002

Sydney Brenner established the nematode ***Caenorhabditis elegans*** as a novel model organism. This transparent worm is approximately one mm long and consists of 959 somatic cells (1974)

Robert Horvitz identified genes controlling cell death in *C. elegans.* Corresponding genes exist in mammals, including man (1986)

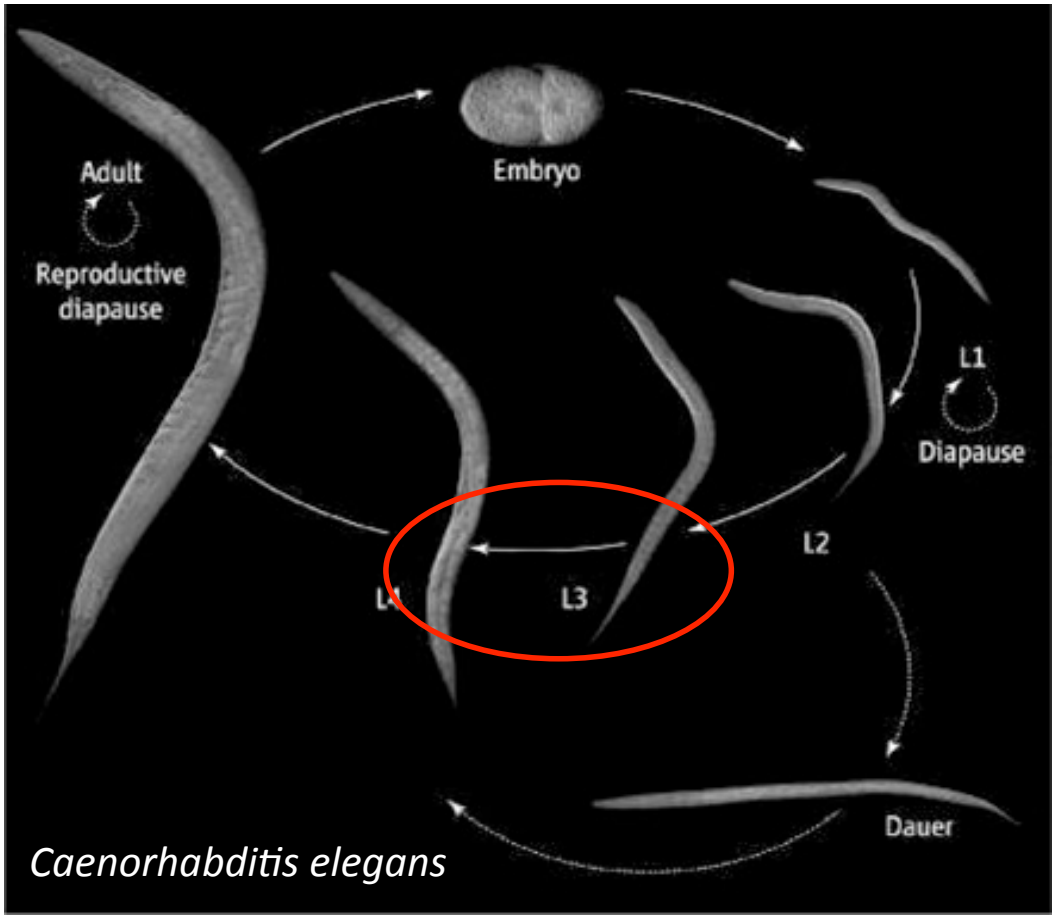
John Sulston mapped a cell lineage in the nematode *C. elegans.* He showed that specific cells undergo programmed cell death during the normal differentiation process (1977)


Adult (1110-1150 µm)
(capable of egg laying)
8 hr
Young adult
(900-940 µm)
L4/adult molt
10 hr
L4 (620-650 µm)
L3/L4 molt
8 hr
L3 (490-510 µm)
L2/L3 molt
8 hr
L2 (360-380 µm)
L1/L2 molt
in utero development
(150 min)
First cleavage (40 min)
eggs laid at Gastrula
(approximately 30-cell)
Comma
1.5-fold
2-fold
3-fold
ex utero development (9 hr)
Hatching
L1 (250 µm)
arrest if no food
crowding
starvation
high temp
12 hr
Predauer (L2d)
(L1/L2d molt)
13 hr
Dauer (400 µm)
up to 4 months

IntroFig6

Introducción: Larva Dauer

The **dauer is a stage juvenile** arrest that occurs during the development of nematodes as a response **to adverse environmental conditions**



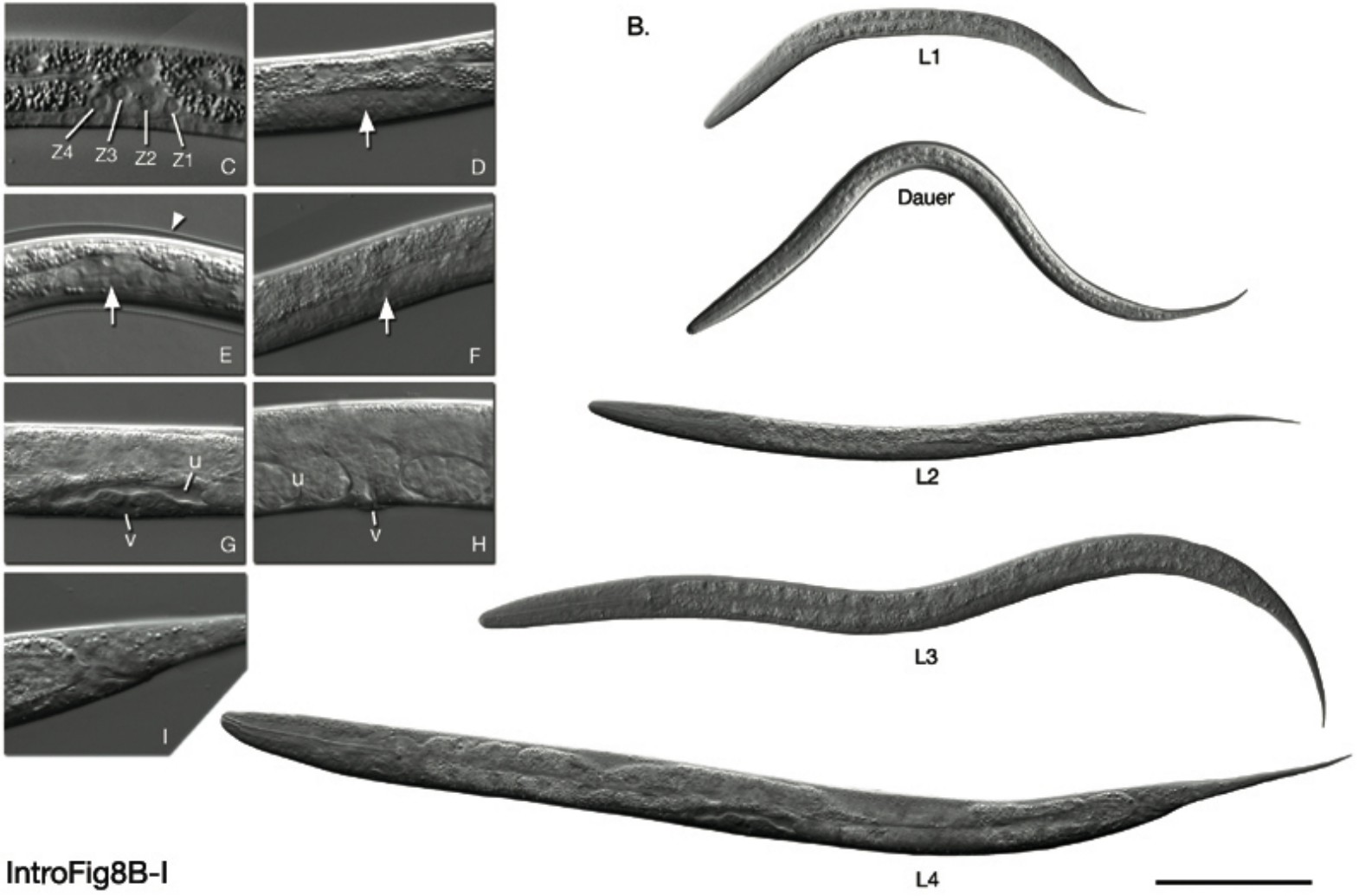
Caenorhabditis elegans

Main features

- Not fed
- Resistant cuticle
- Longevity stadium

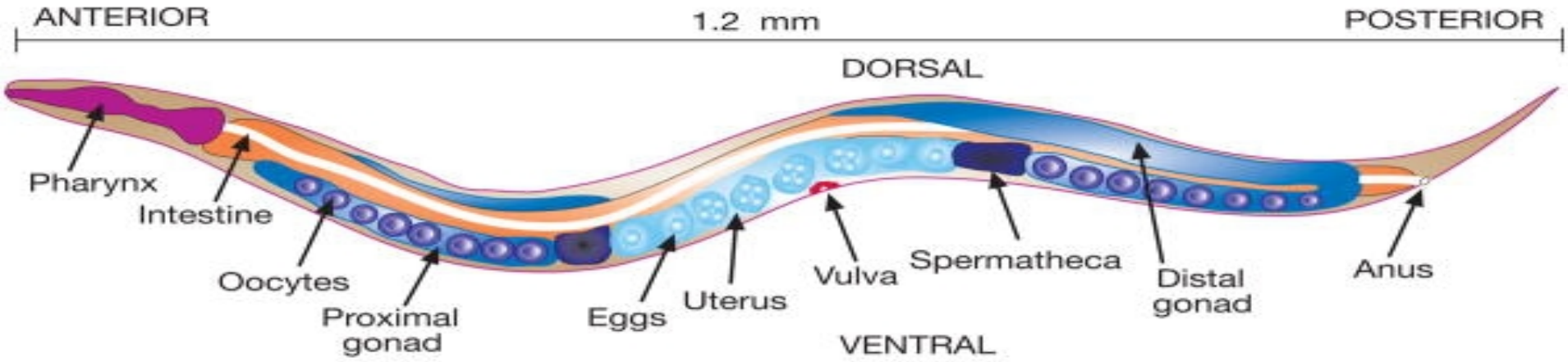
Ogawa, A. *Science*, 2009

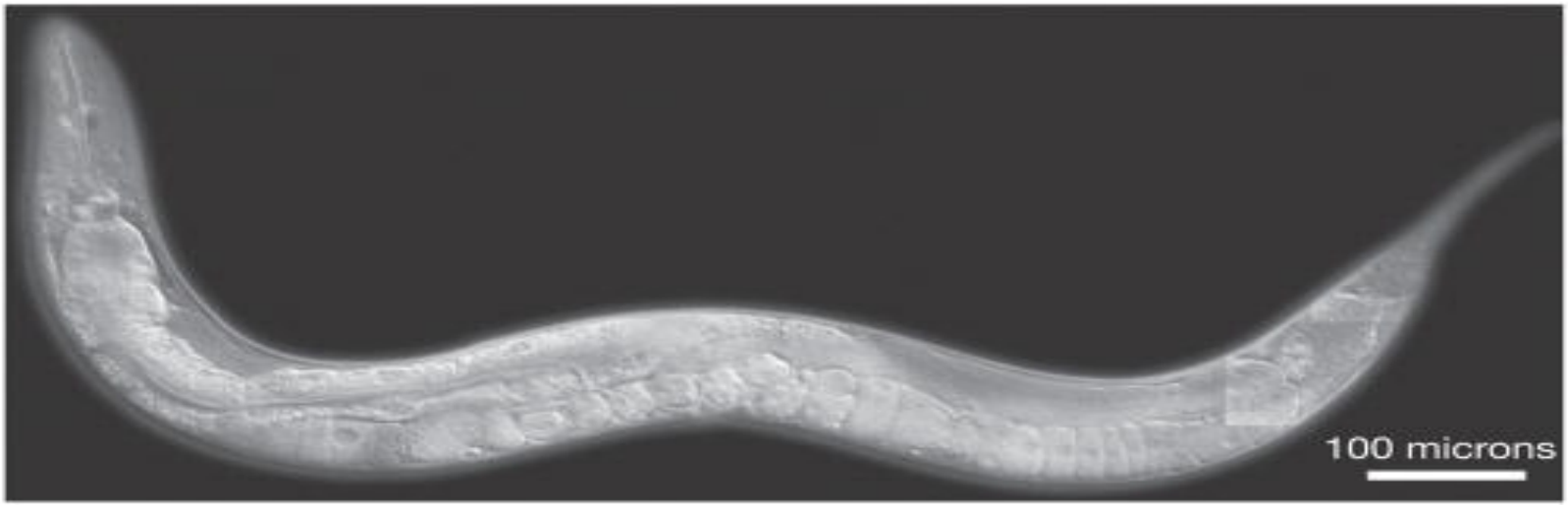
Juveniles



IntroFig8B-I

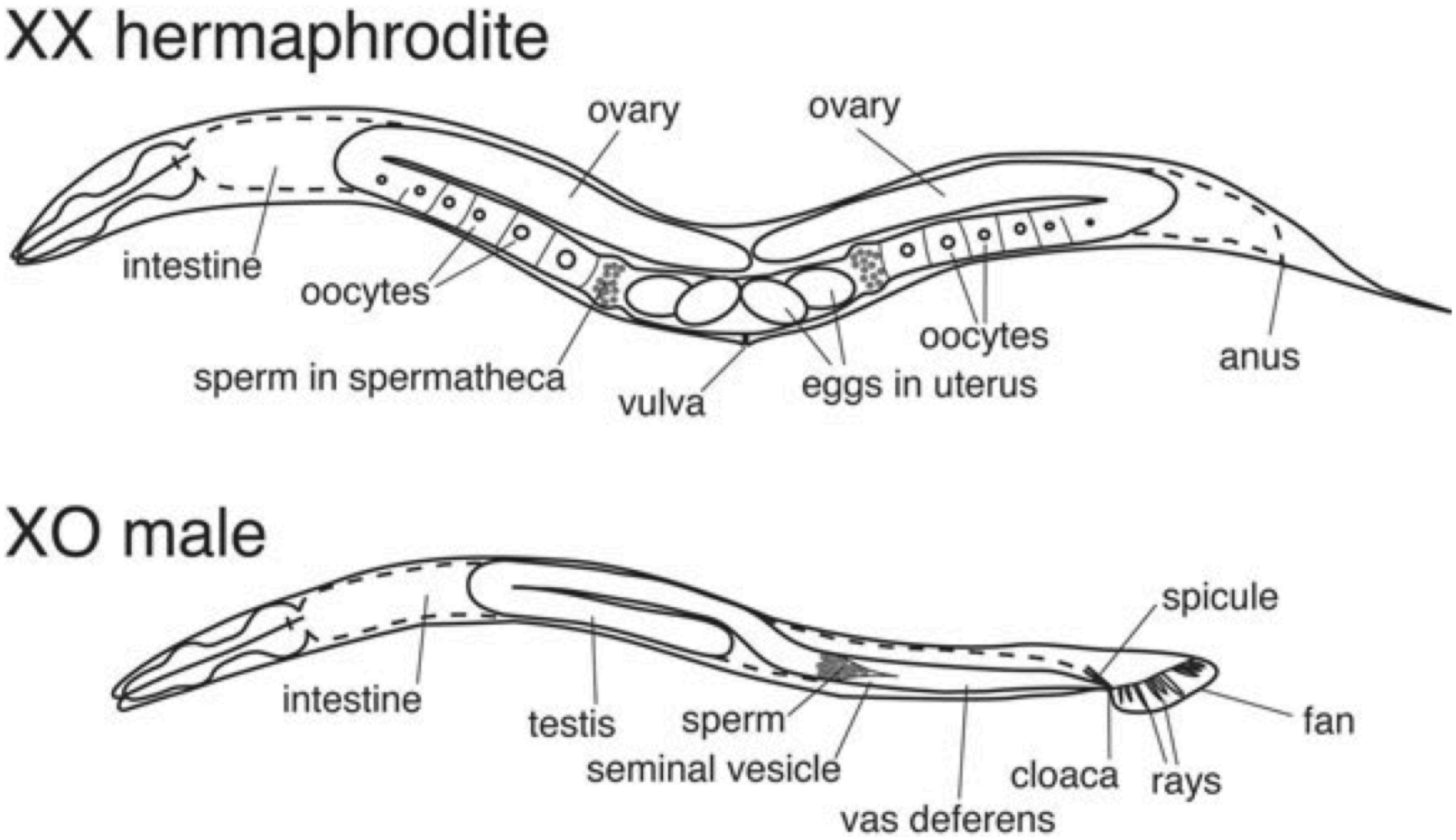
C. elegans adulto





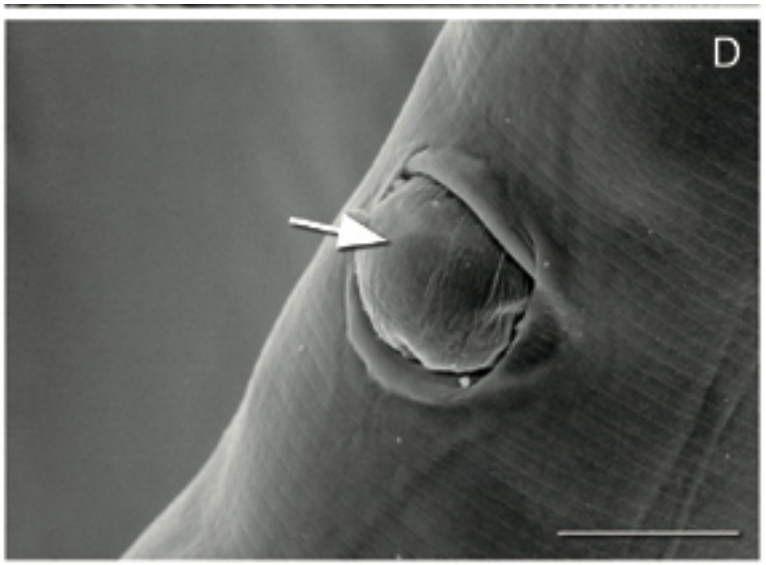
Video adulto

Hermafroditas y machos

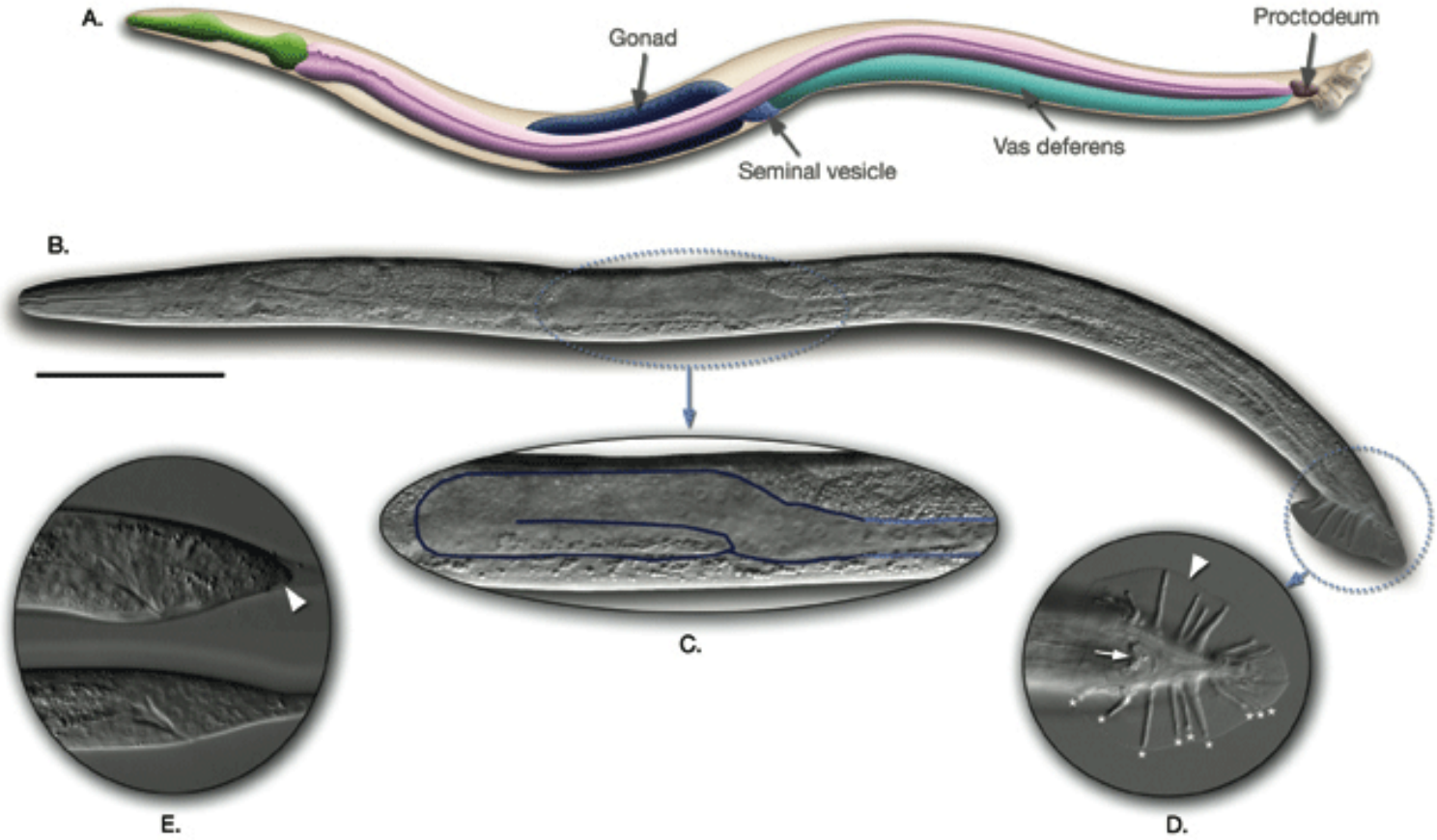


Anatomy of Caenorhabditis Elegans (Zarkower 2012)

Hermafrodita

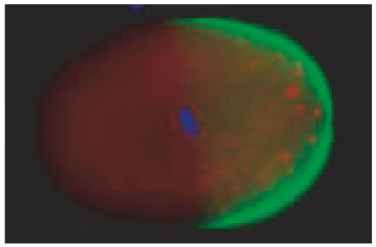


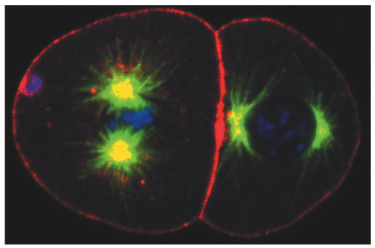
El macho

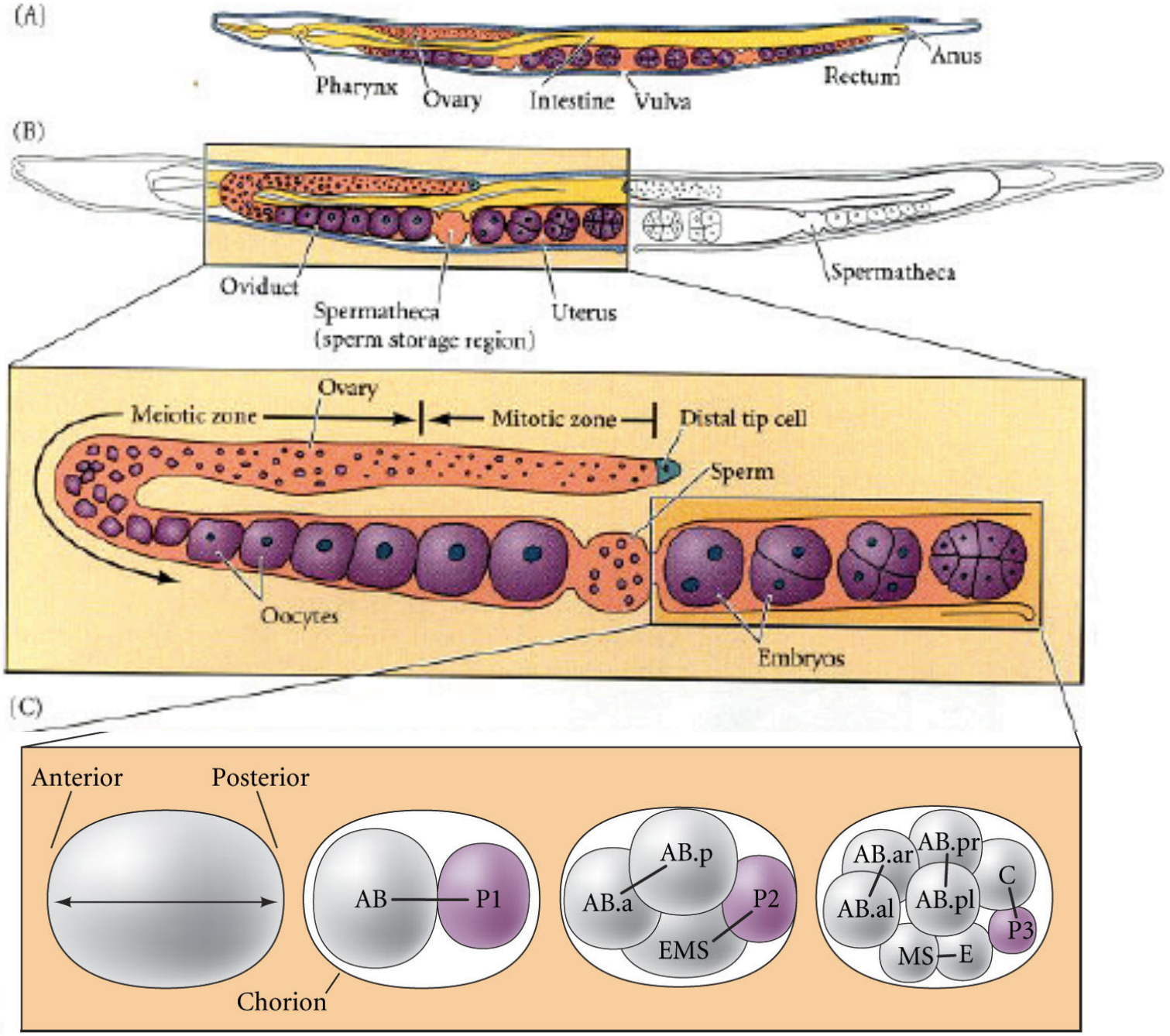


Clivaje holoblástico, asimétrico y rotacional

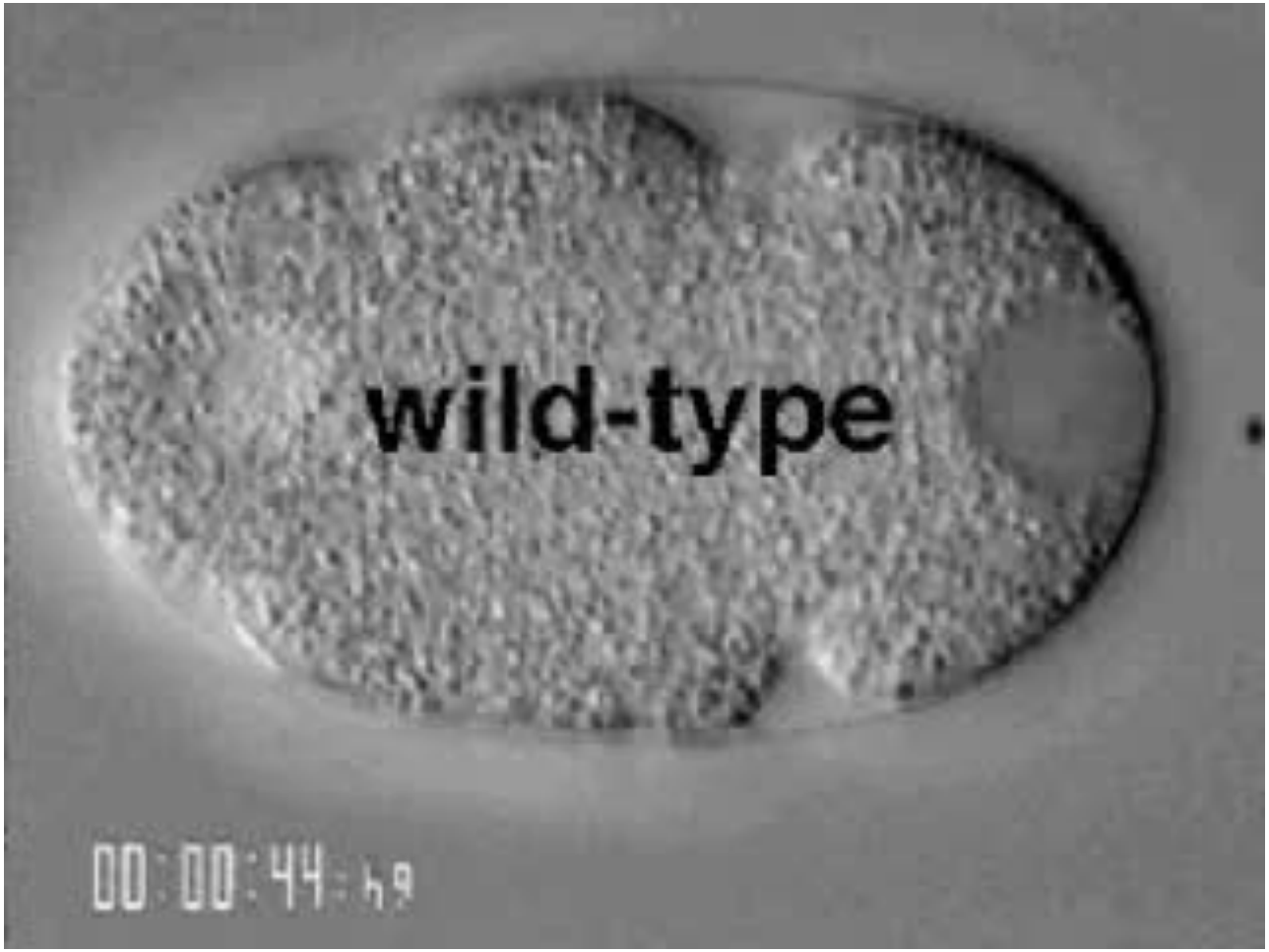
PAR proteins and the establishment of polarity:







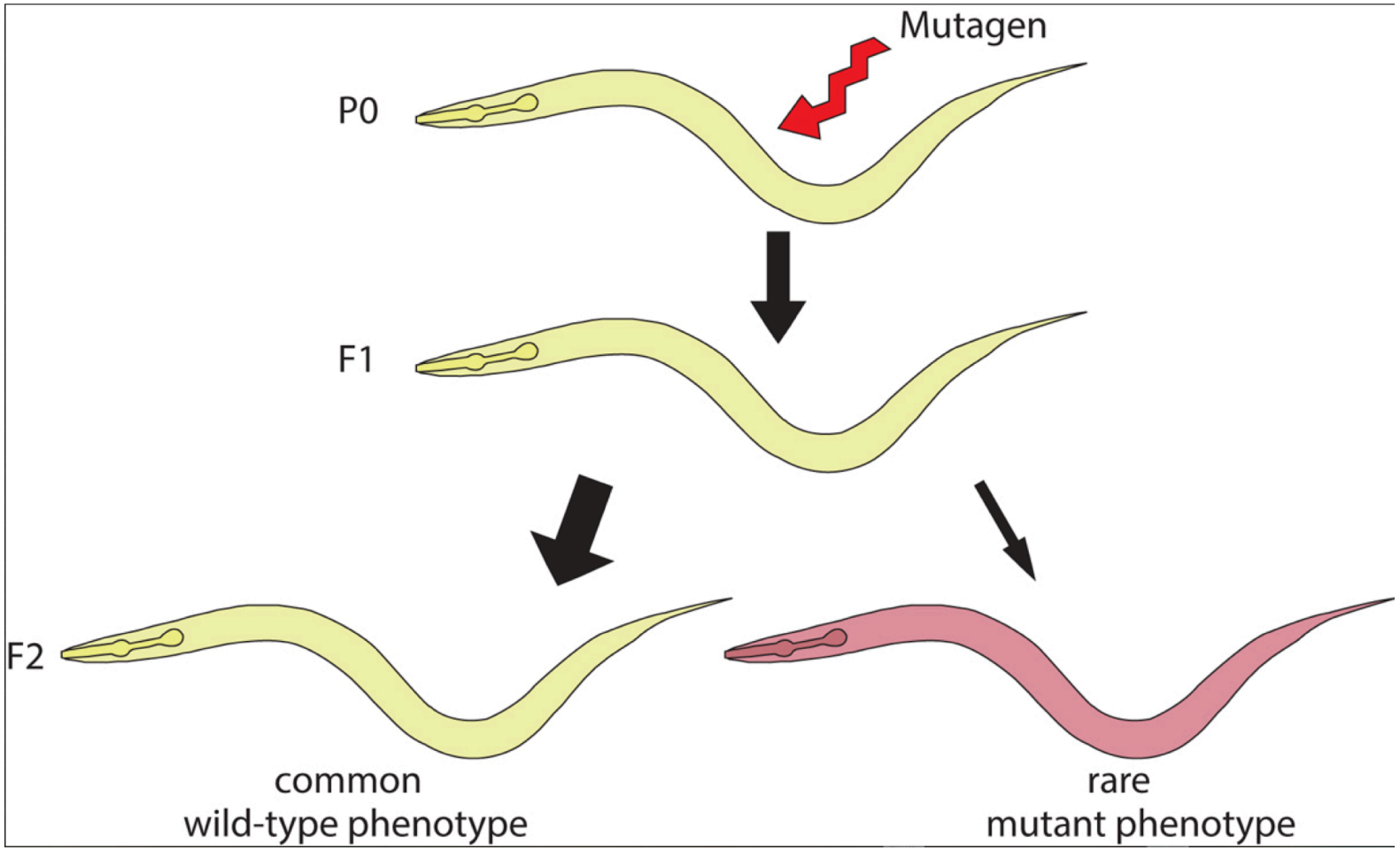
First Cell Cycle in *C. elegans*

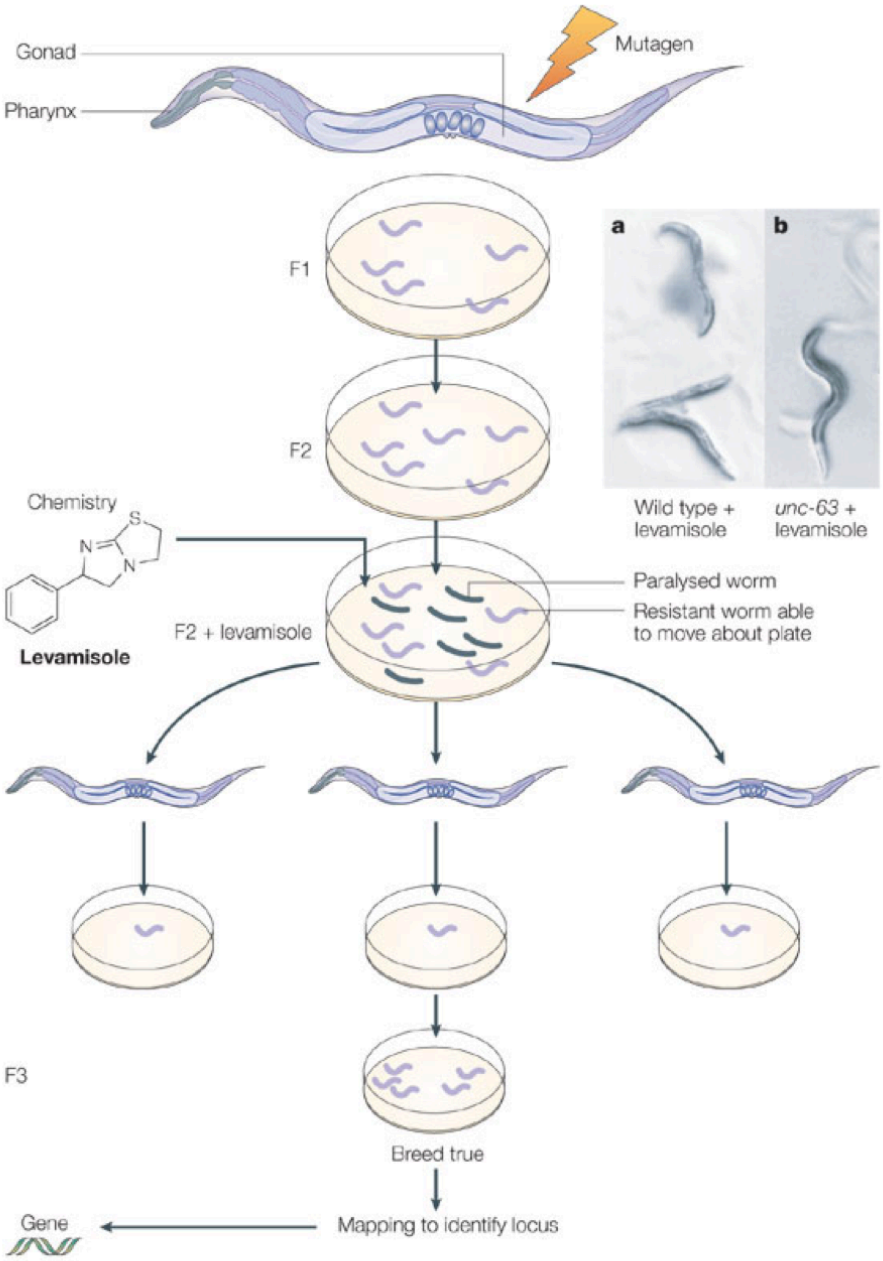



Post-fertilización
Clivaje
Gastrulación
45 min
6 horas
12 horas
Fusión pronúcleos
Estadío 2 celulas
Estadío 24 células
Fase de proliferación
Fase de morfogénesis

Methods

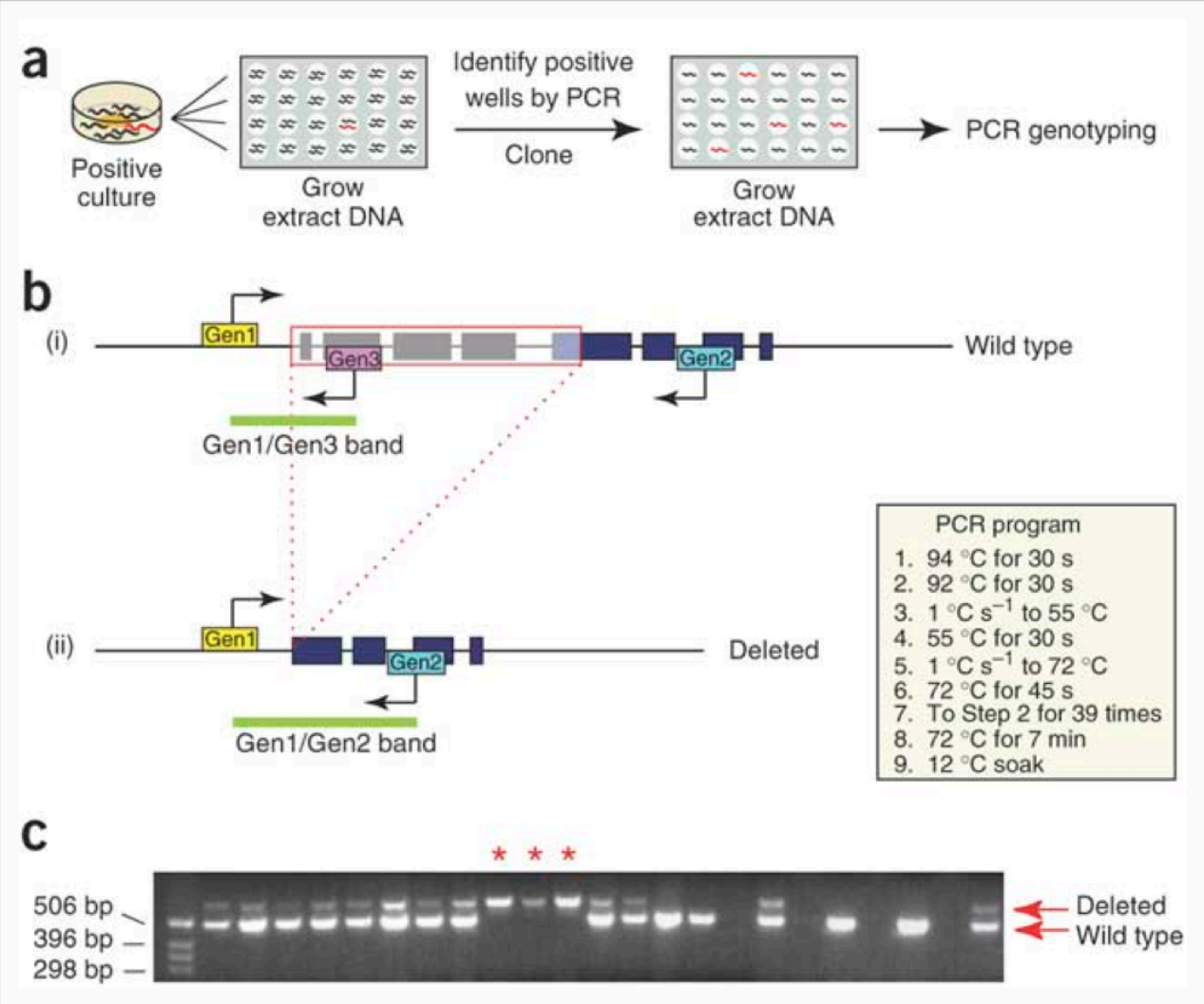
- Forward Genetic Screens:




Gonad
Pharynx
Mutagen
F1
F2
a
b
Wild type + levamisole
unc-63 + levamisole
Chemistry
Levamisole
F2 + levamisole
Paralysed worm
Resistant worm able to move about plate
F3
Breed true
Gene
Mapping to identify locus

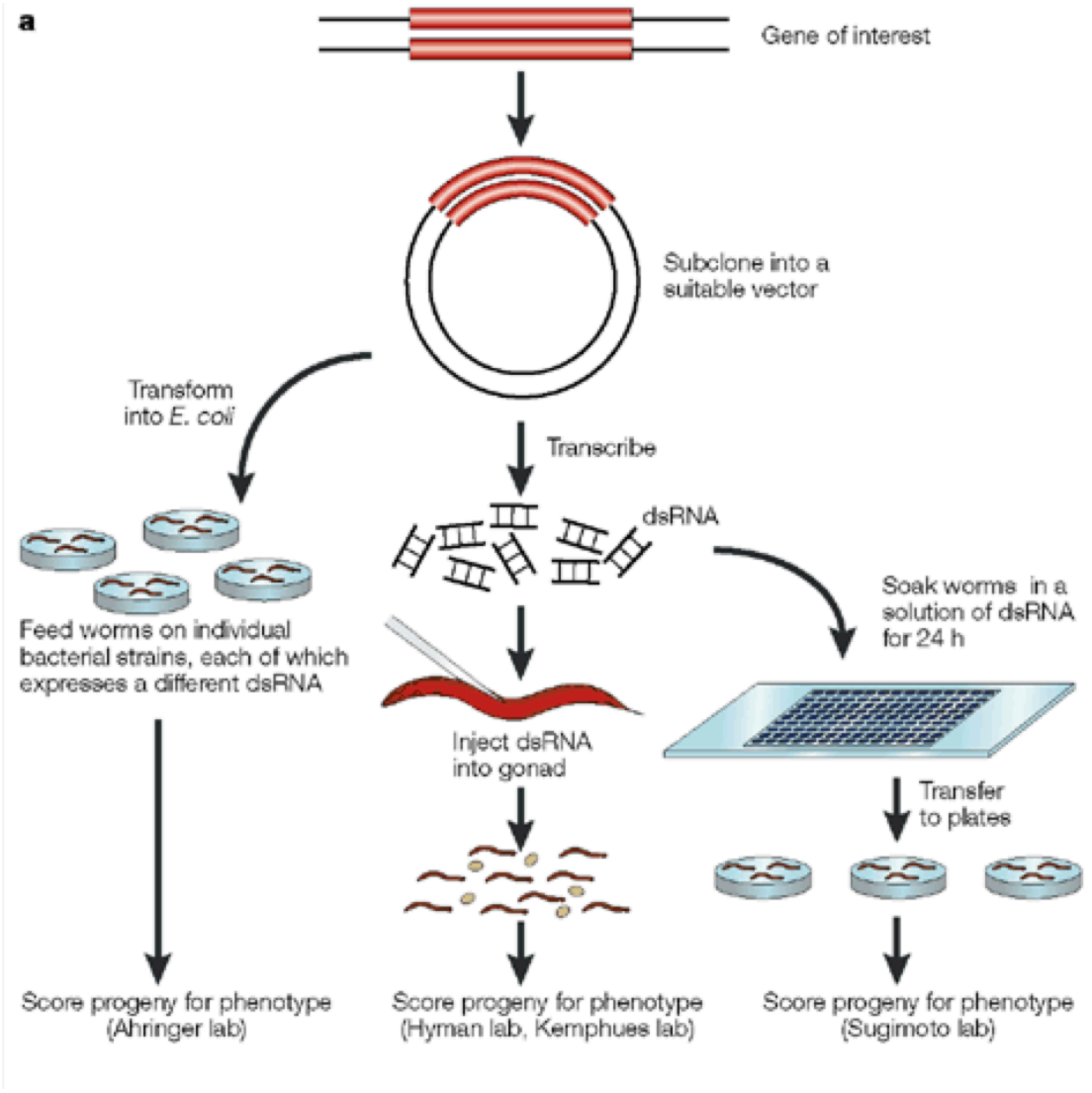
Methods

- Reverse genetic screen (deletion library):



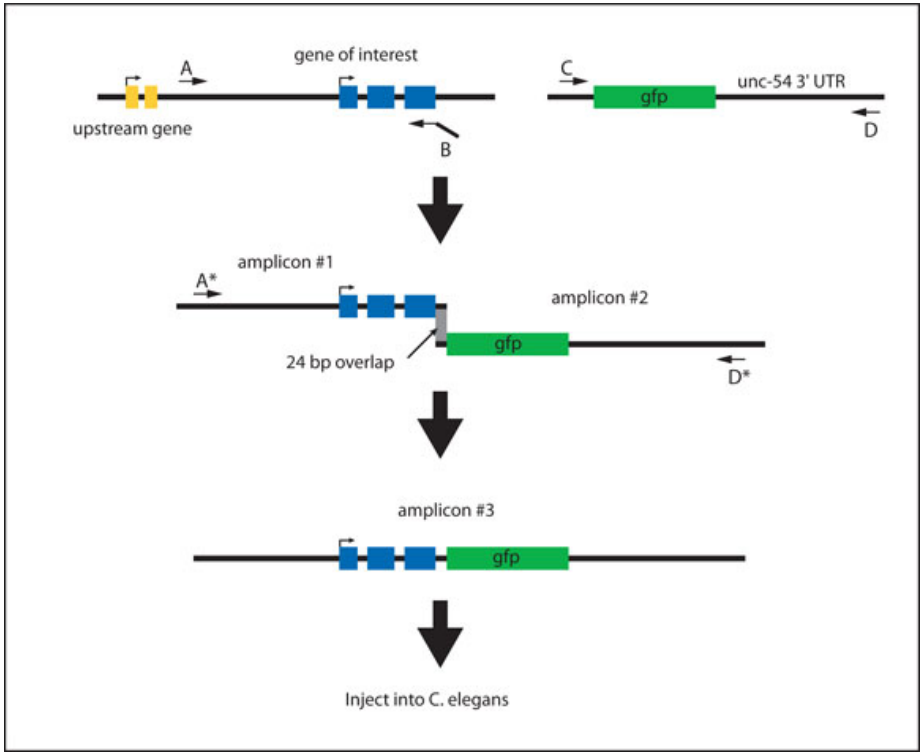
Methods

- RNAi:

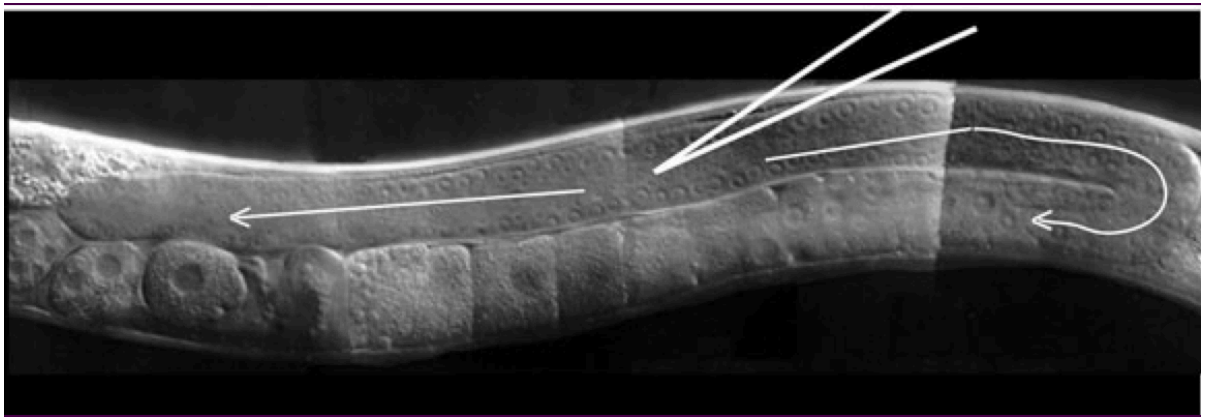


Methods

For transgenic animals, first make your construct by PCR:



For transgenic animals, constructs are injected into the syncytium of the gonad:



C. elegans as a model system

1. Short generation time (3d-1week)
2. Stocks can be frozen
3. Very easy to propagate
4. Cheap to maintain
5. Temperature sensitive
6. Present no biohazard
7. First genome sequenced
8. All cell fates known
9. Easy forward and reverse genetics
10. RNAi
11. Embryos and adults translucid
12. Hermaphroditic and self fertile
13. Large brood size: 300 progeny

Downsides: cultures smell bad and often get contaminated with fungus and other bacteria

Resources

- Wormbase
- Wormbook
- Wormatlas
- Caenorhabditis Genetics Center (CGC) (U. Minnesota)