CO C

Lecture 18: C. elegans Development

Read: 789-792
804-808

Fig. C1, C3,C4, C5, C7
C9, C15, C16, C17

Some facts about C. elegans

3 days life cycle

Invariant cell lineage

959 cells in hermaphrodite

1031 cells in male

Small genome 97 MB

19,000 genes (1 gene per 5 kb)

Two sexes: hermaphrodite and male

Six pairs of chromosomes

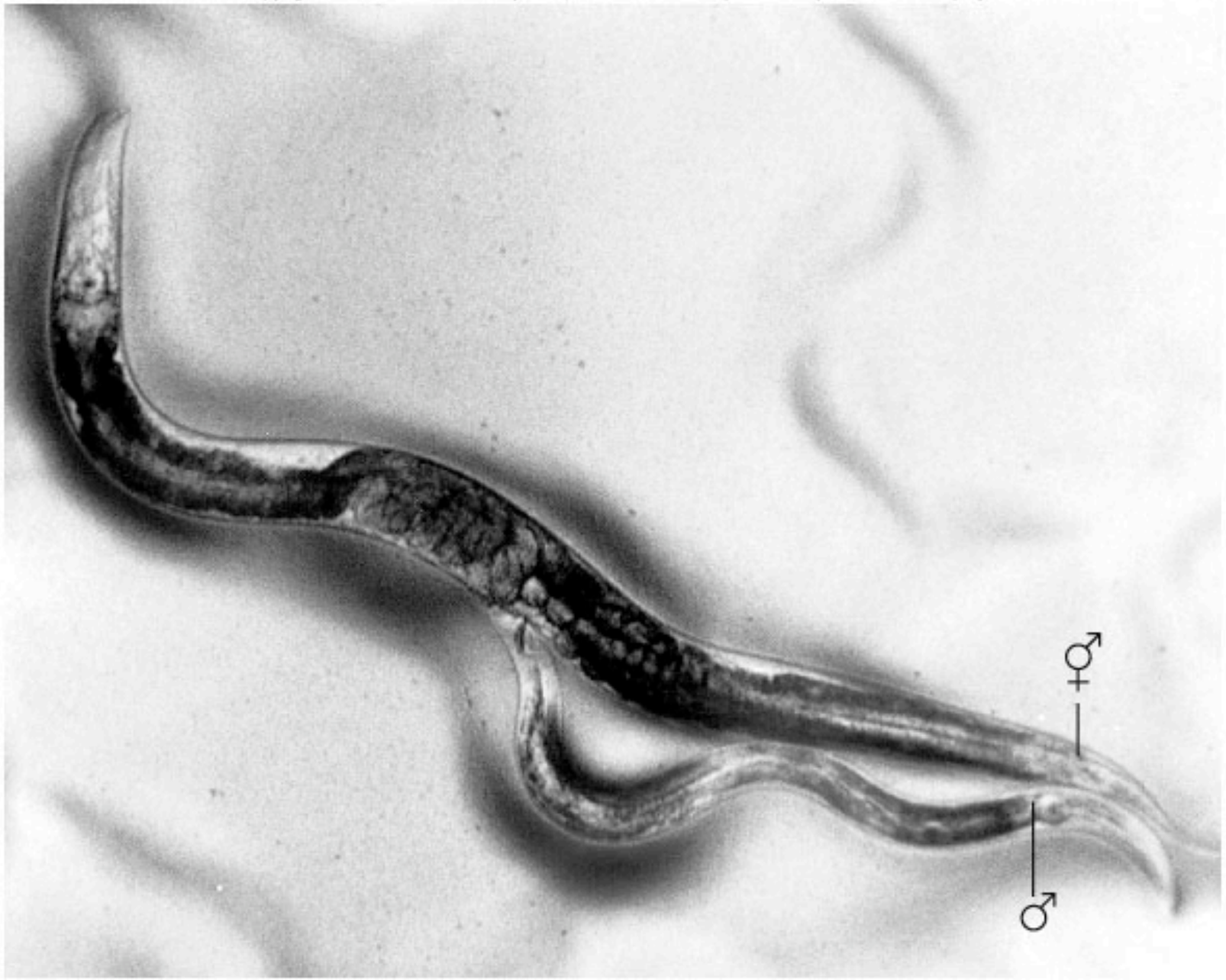
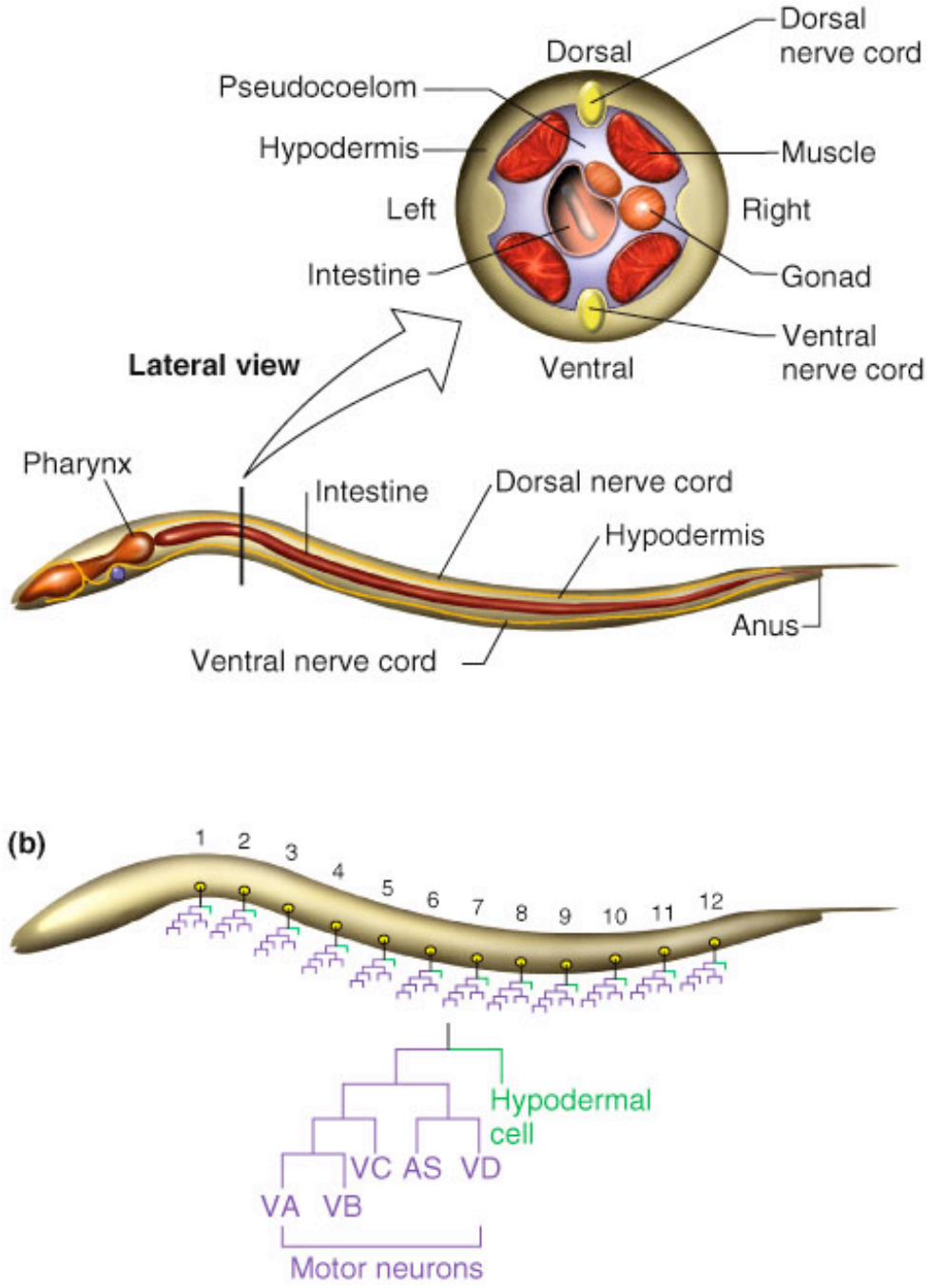
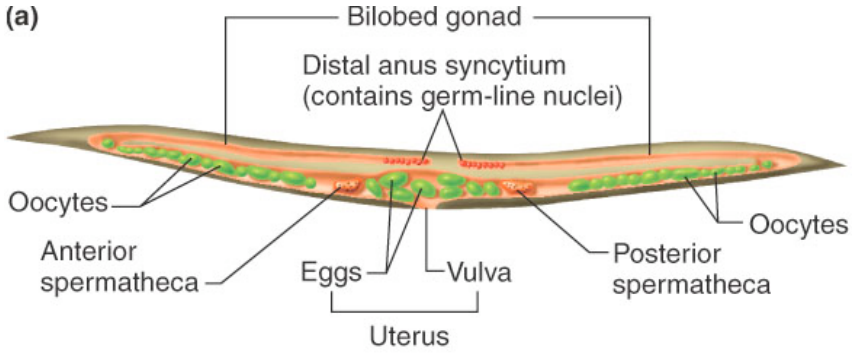


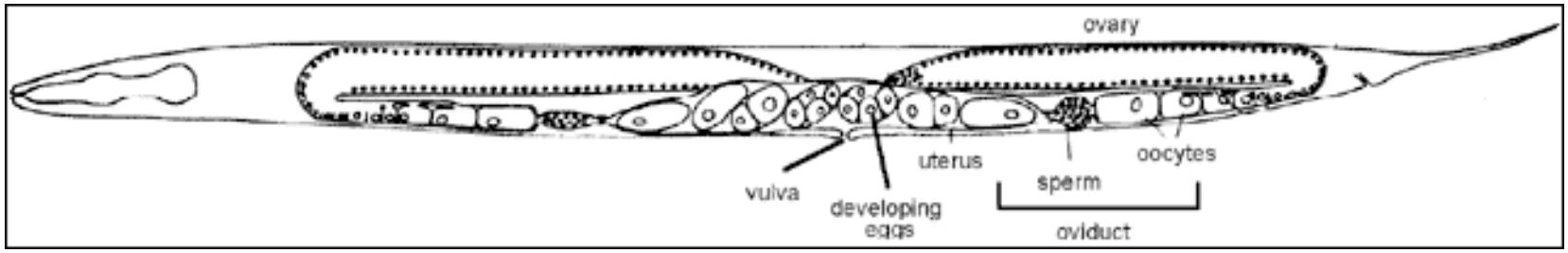
Fig. C.1

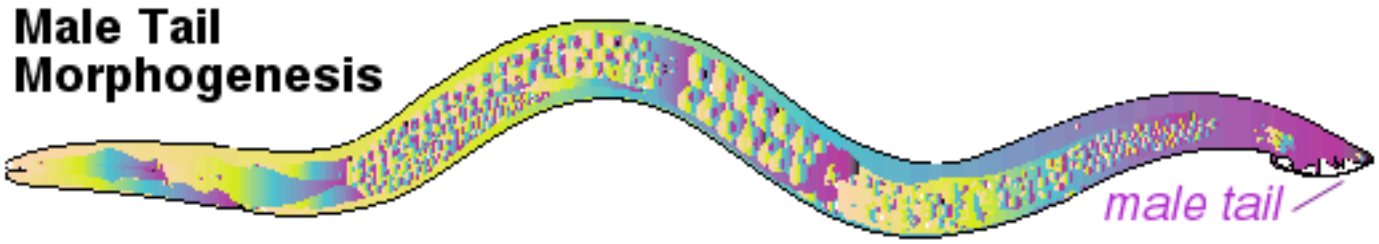
Fig. C.6



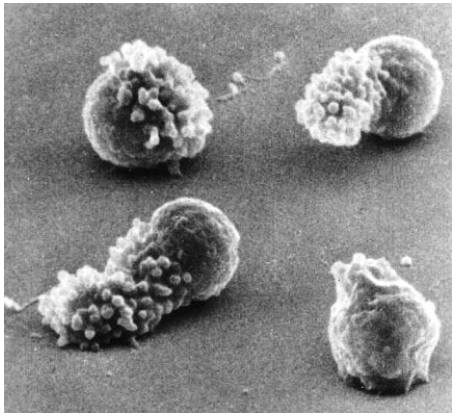


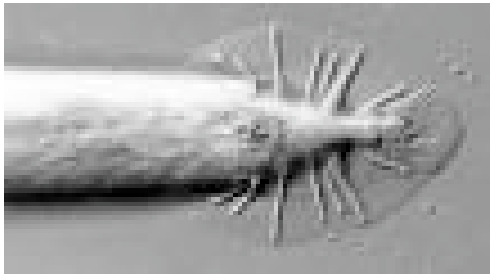
XX: hermaphrodite



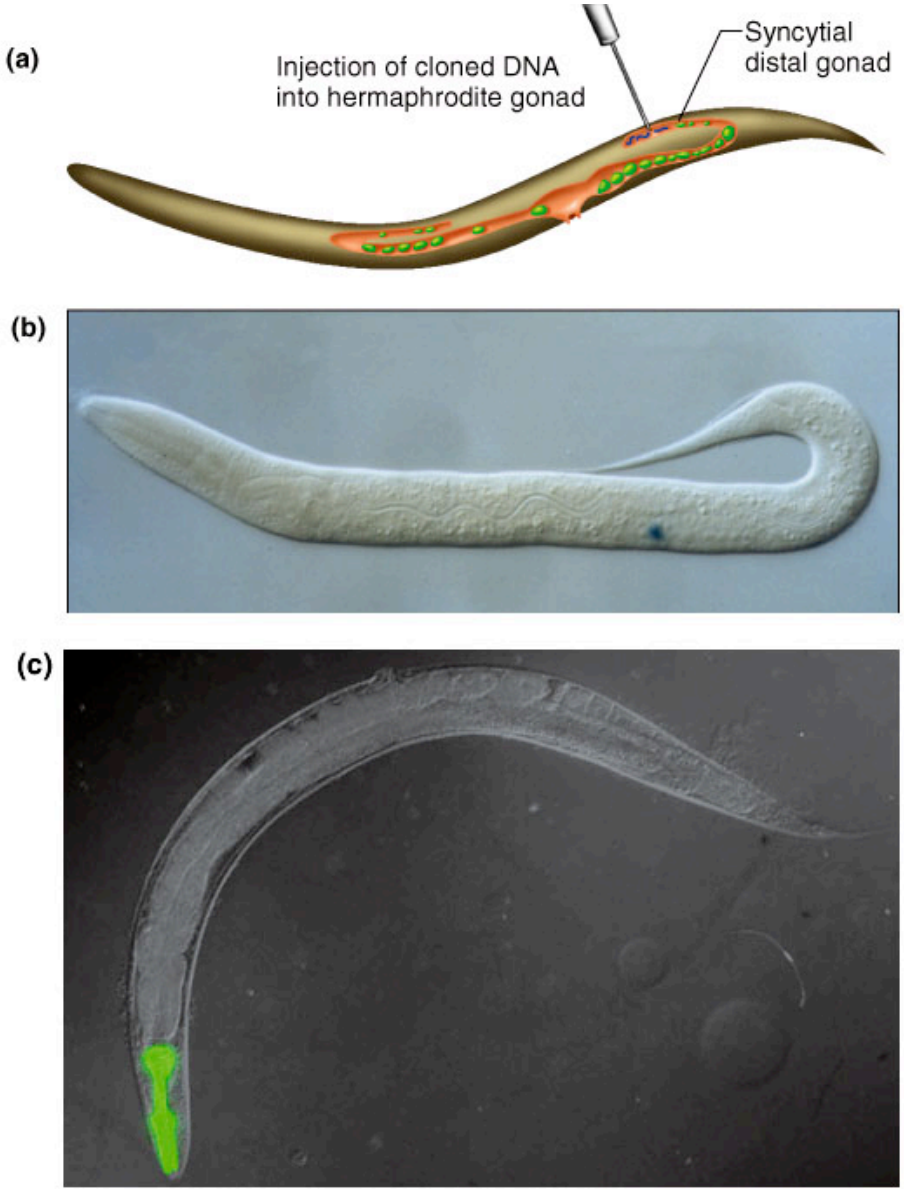


XO: male





DNA transformation



- Inject DNA into distal syncytial gonad of hermaphrodites
- Irradiation promotes integration of transgenes into genome
- Reporter constructs show transgenes

Fig. C.8

Fig. C.3

Trans-splicing and polycistronic operons are prevalent in *c. elegans*

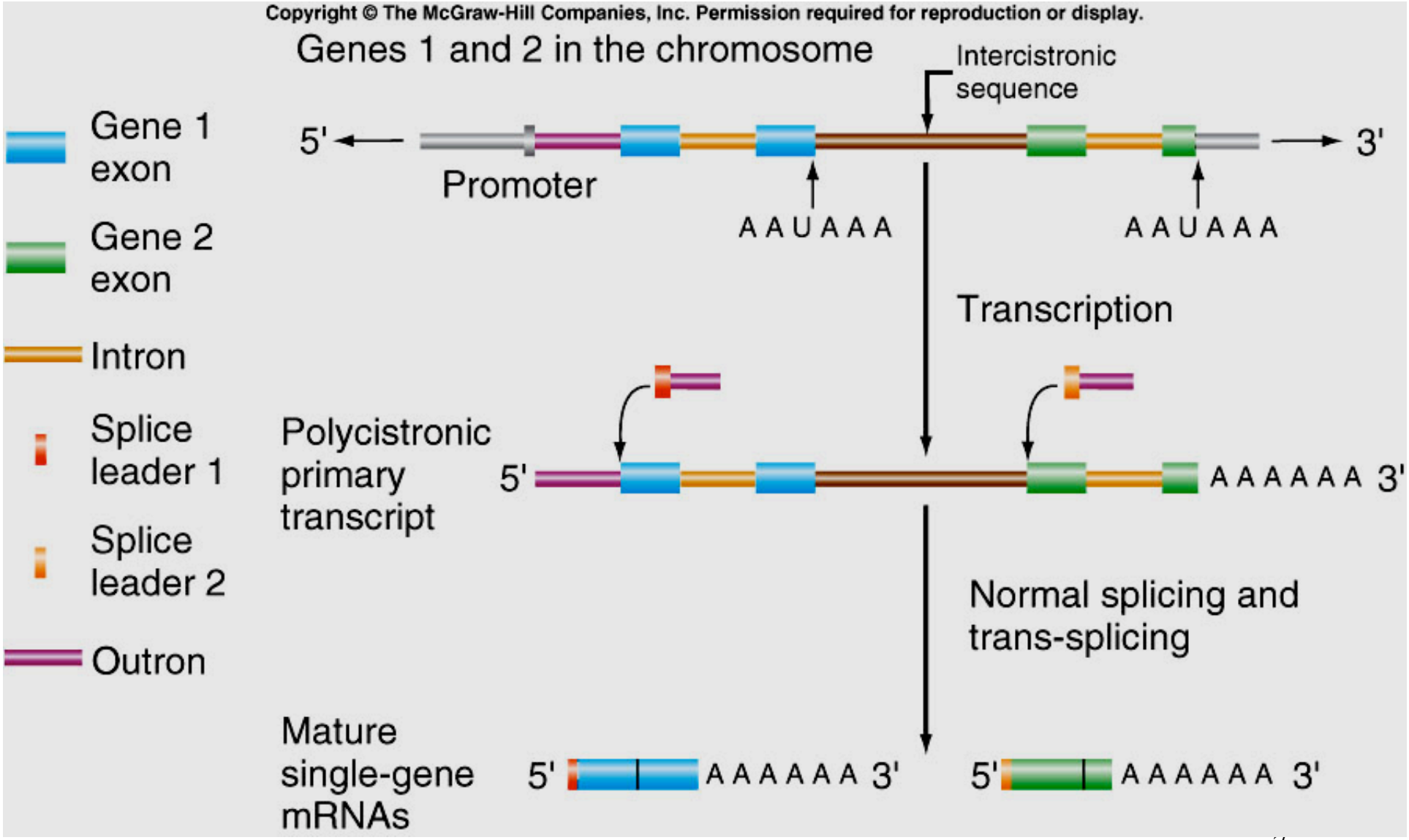
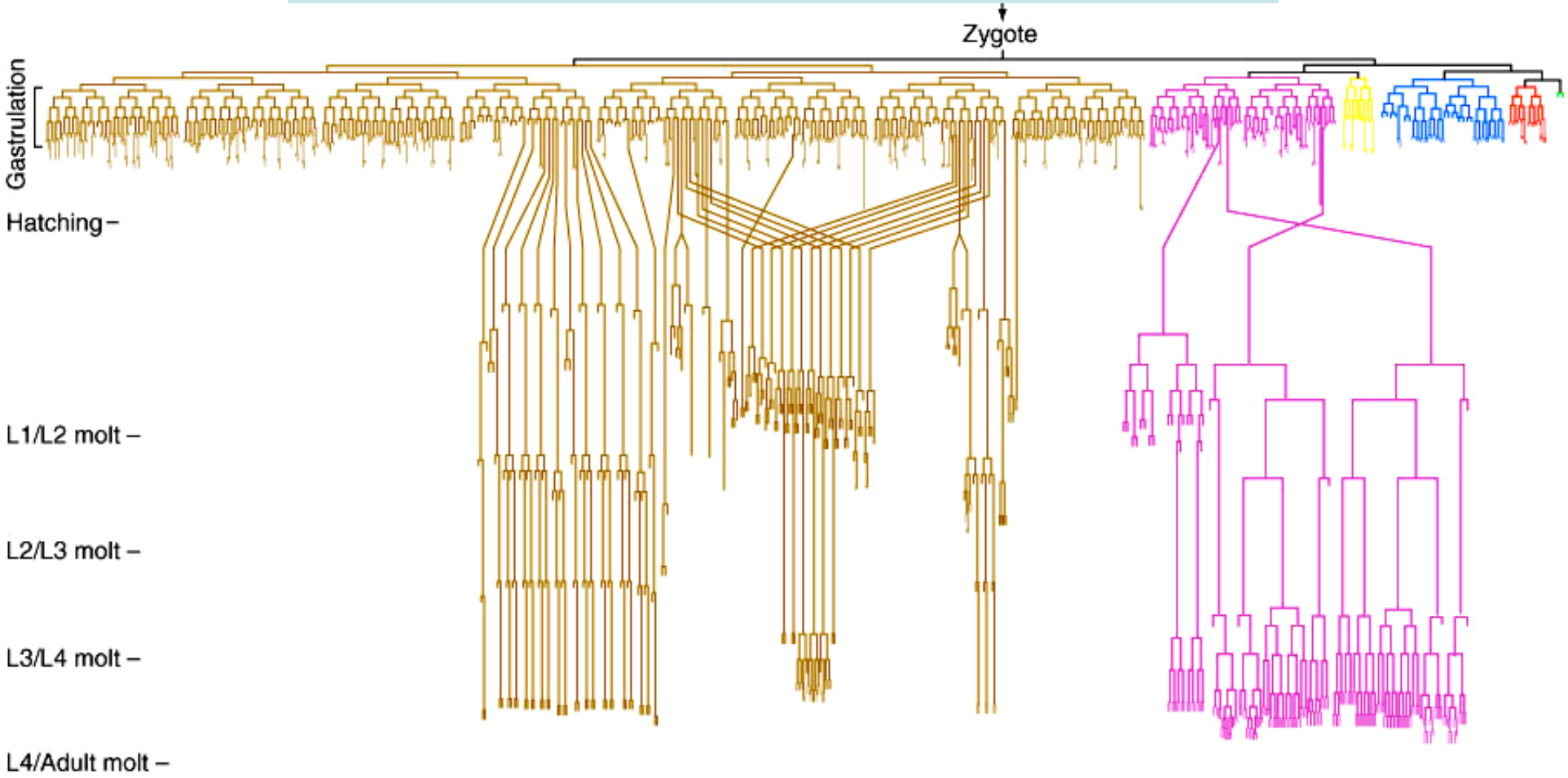


Fig. C.7a

Invariant cell lineages

(959 somatic cells in hermaphrodite)



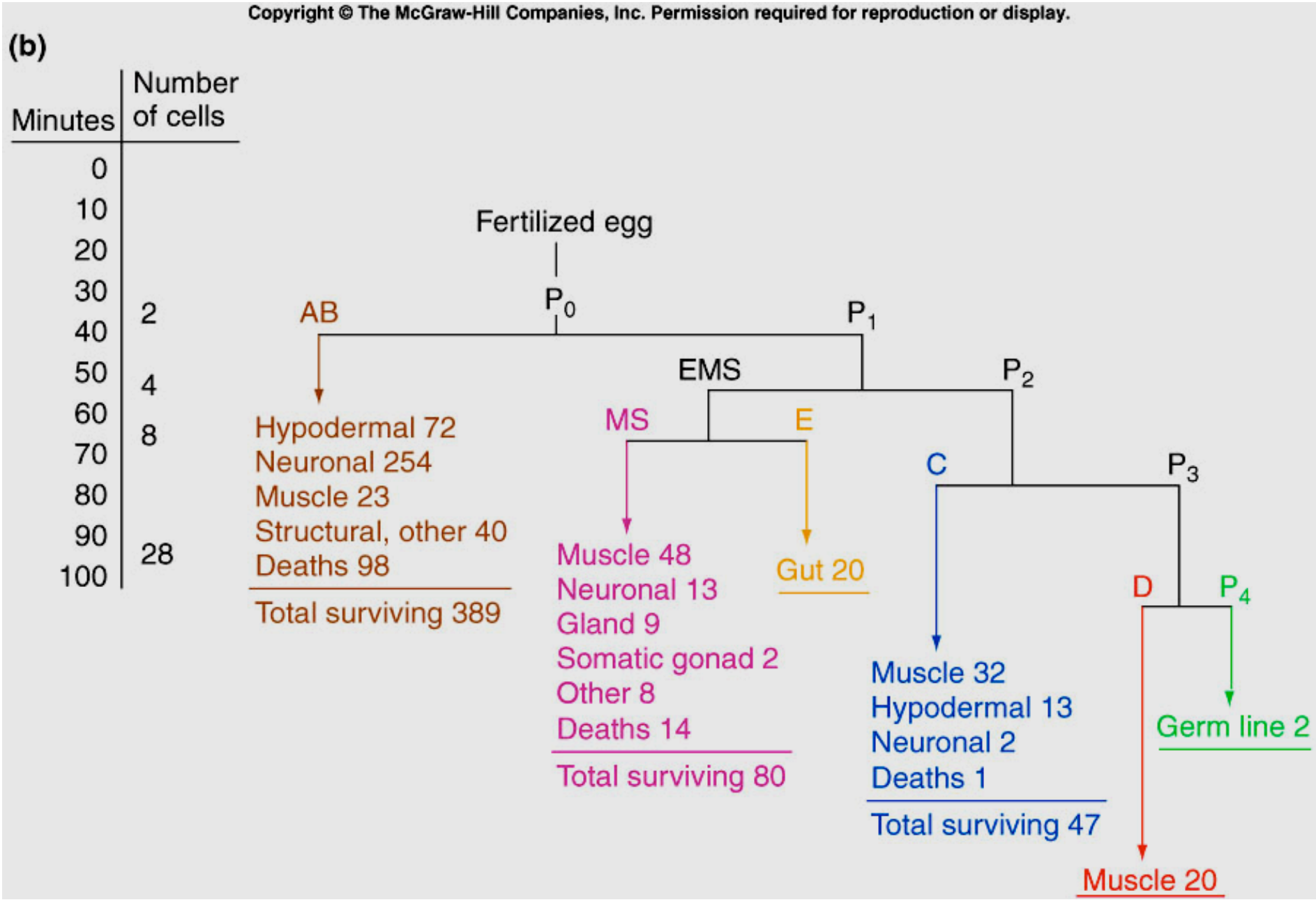


Fig. C.7b

(a)

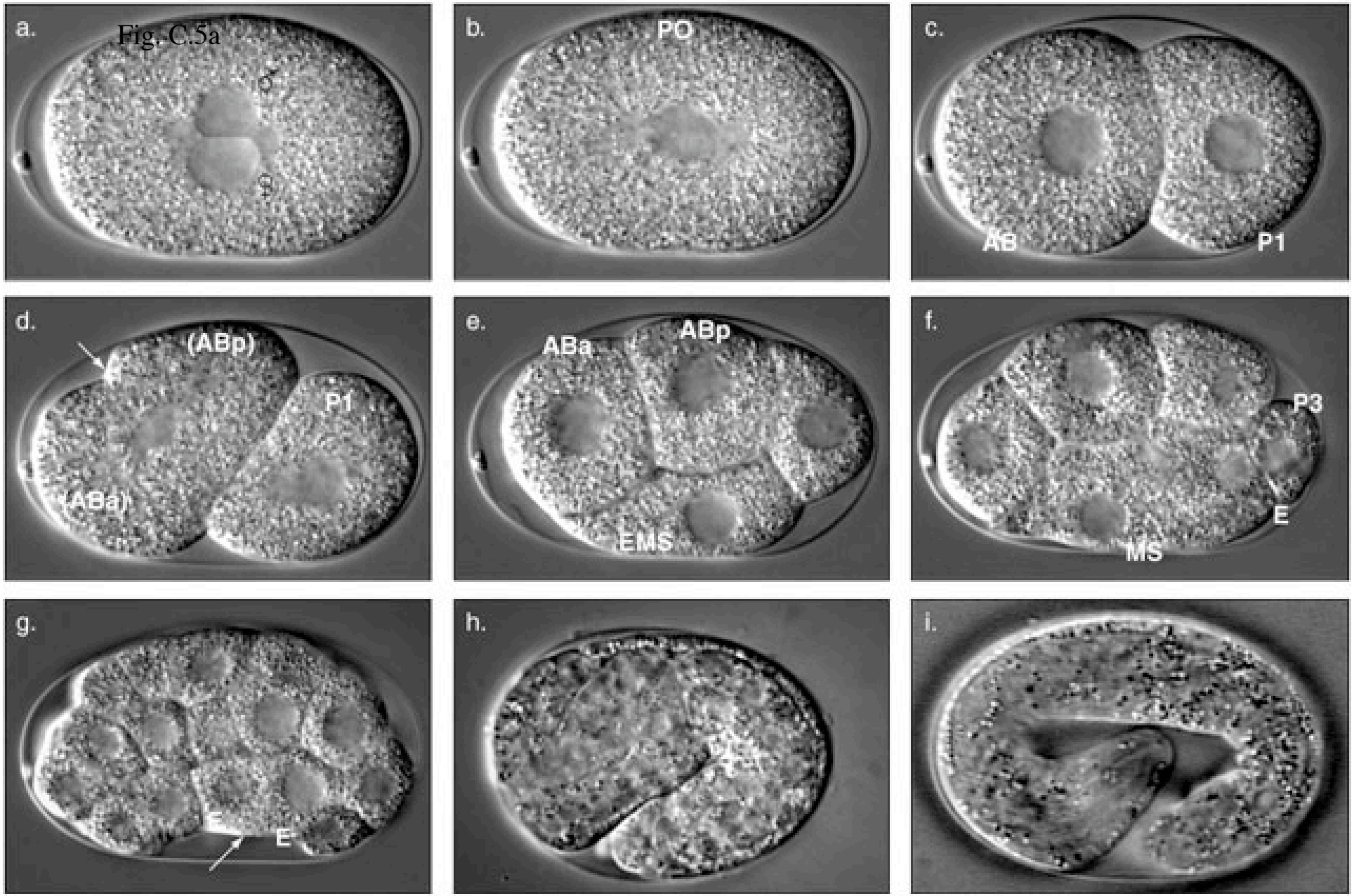
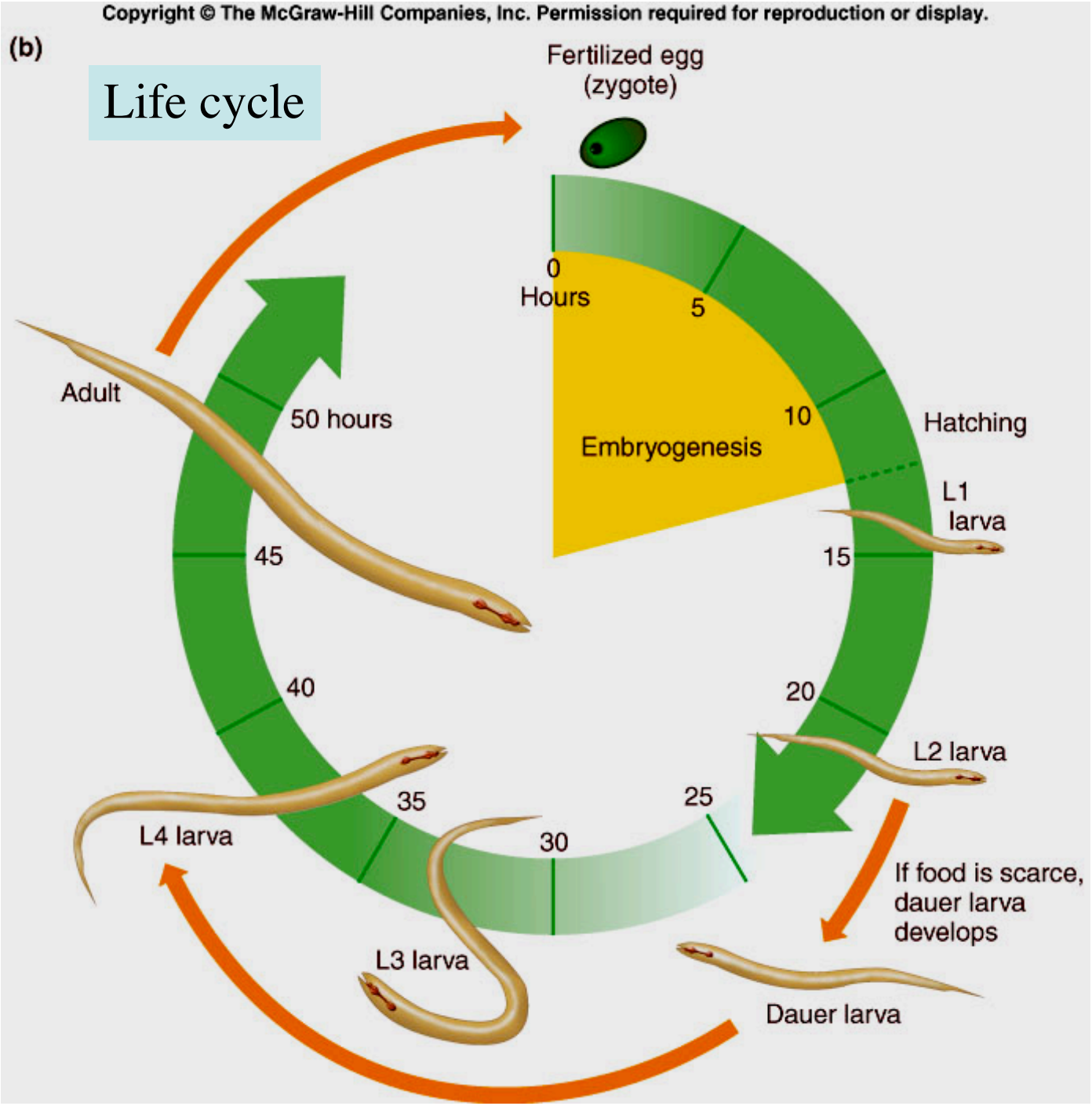


Fig. C.5b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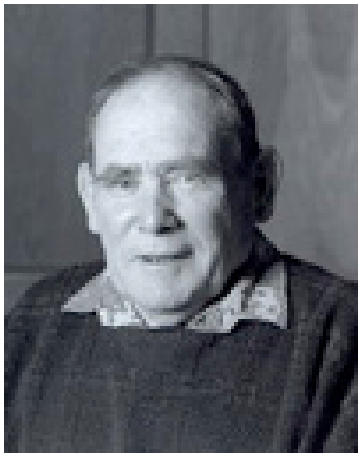
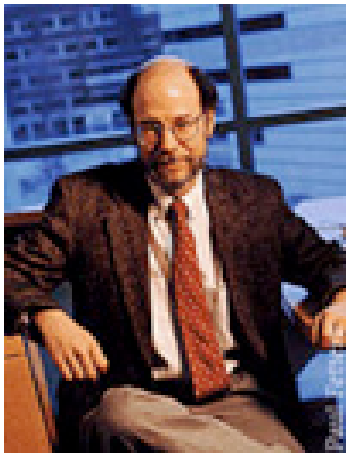
(b)
Life cycle
Fertilized egg
(zygote)
0
Hours
5
10
Hatching
Embryogenesis
L1
larva
15
20
L2 larva
25
If food is scarce,
dauer larva
develops
Dauer larva
30
35
L4 larva
L3 larva
40
45
50 hours
Adult

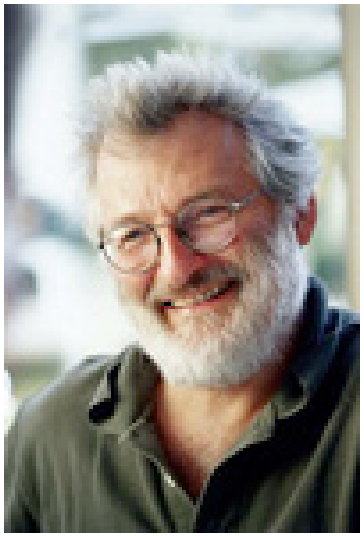
2002 Nobel Prize Winners

Sydney Brenner



Bob Horvits

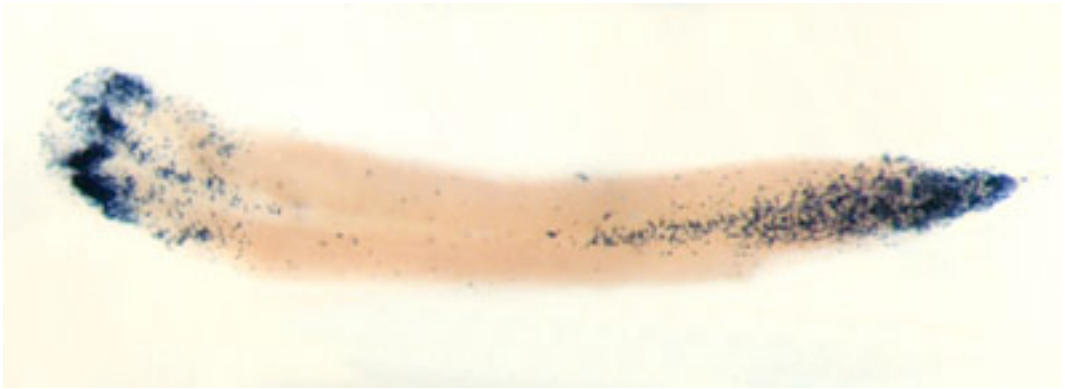




John Sulston

Apoptosis is important in development

Apoptosis patterns in developing *Xenopus* embryos detected via TUNEL



TUNEL: TdT-mediated dUTP digoxigenin nick end labeling

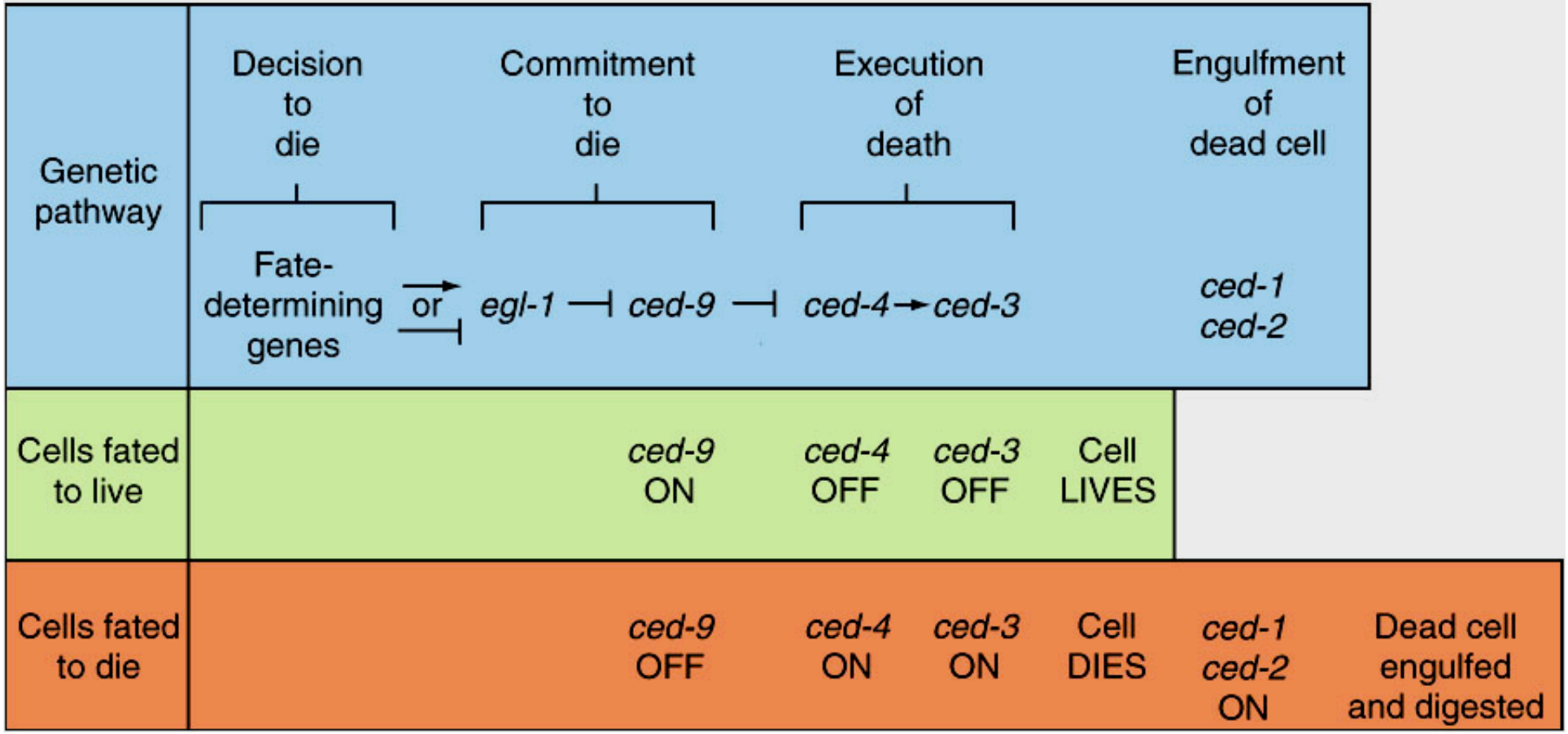
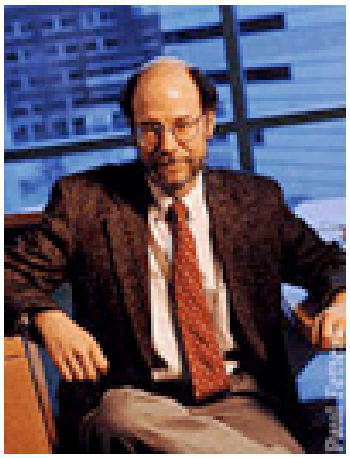


Fig. C.15



Bob Horvitz

Programmed Cell Death Naturally occurring, or programmed, cell death (apoptosis) is common during animal development, and abnormalities in programmed cell death are associated with many human diseases, including certain cancers and neurodegenerative disorders. Our laboratory has defined a molecular genetic pathway for programmed cell death. We have characterized genes that cause cells to die, that protect cells from dying, that function in the engulfment of dying cells by their neighbors, and that are involved in destroying the debris generated by cell corpses. Most of these genes have human counterparts. For example, the killer gene *ced-3* encodes a **caspase (cysteine aspartate protease);** mammalian caspases similarly cause programmed cell death. The action of *ced-3* is facilitated by *ced-4*, which is similar to human APAF1, identified because it promotes caspase activation in a biochemical system. The function of *ced-4* is blocked by *ced-9*, which protects cells against programmed cell death and is similar to the human proto-oncogene *BCL2*, which also protects against cell death. The activity of *ced-9* is inhibited by the worm killer gene *egl-1*, which is similar to a number of mammalian killer genes. The activity of *egl-1* is controlled in a cell-specific fashion by genes that specify which cells are to live and which are to die

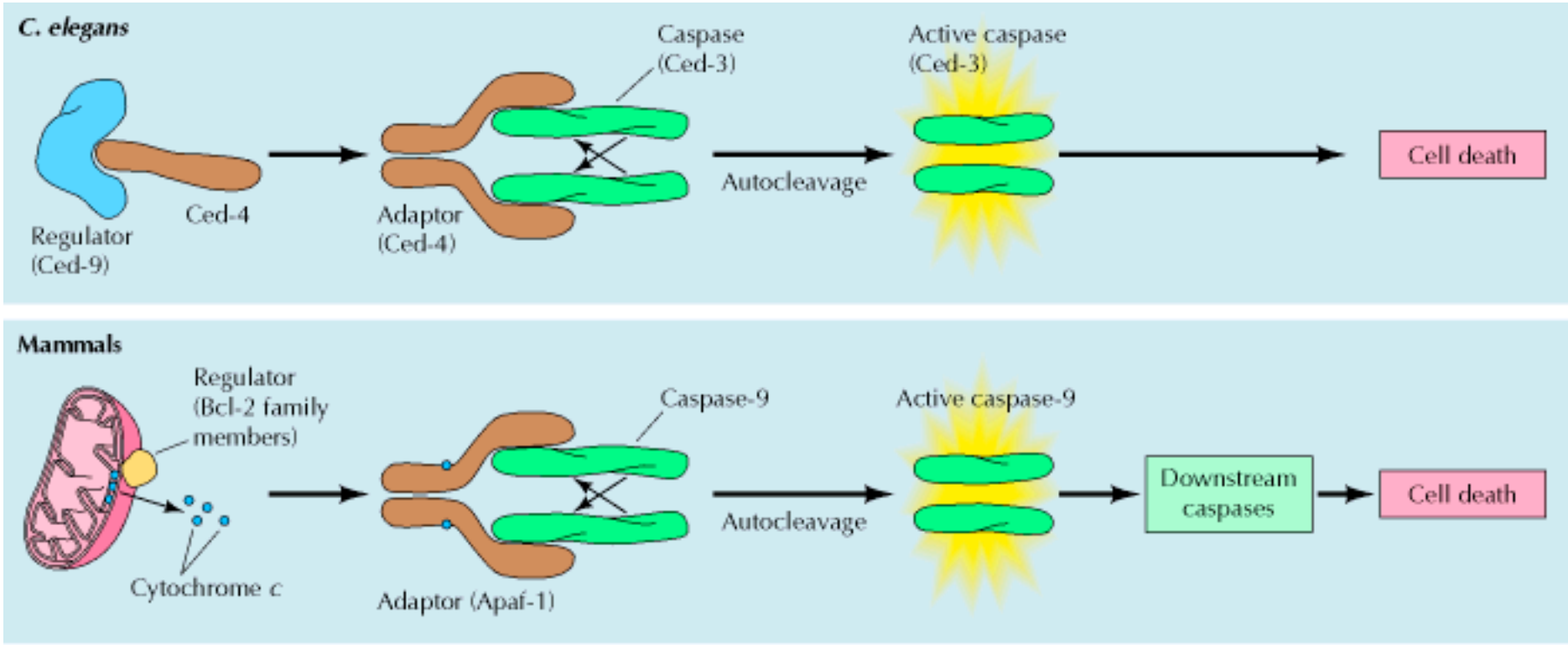


Figure 13.48. Regulators and effectors of apoptosis Many cell death signals induce apoptosis via a conserved pathway of regulators, adaptors, and caspases. In *C. elegans*, the negative regulator Ced-9 inhibits apoptosis by binding to the adaptor Ced-4. In the absence of inhibition by Ced-9, Ced-4 binds two molecules of the caspase Ced-3, resulting in autocleavage and caspase activation. In mammals, regulators of the Bcl-2 family (Ced-9 homologs) act at the mitochondria to control release of cytochrome *c*, which is required for the binding of caspase-9 to the adaptor Apaf-1 (the Ced-4 homolog). Release of cytochrome *c* from mitochondria thus signals the activation of caspase-9, which then activates downstream caspases to induce apoptosis

Programmed cell death in c. elegans

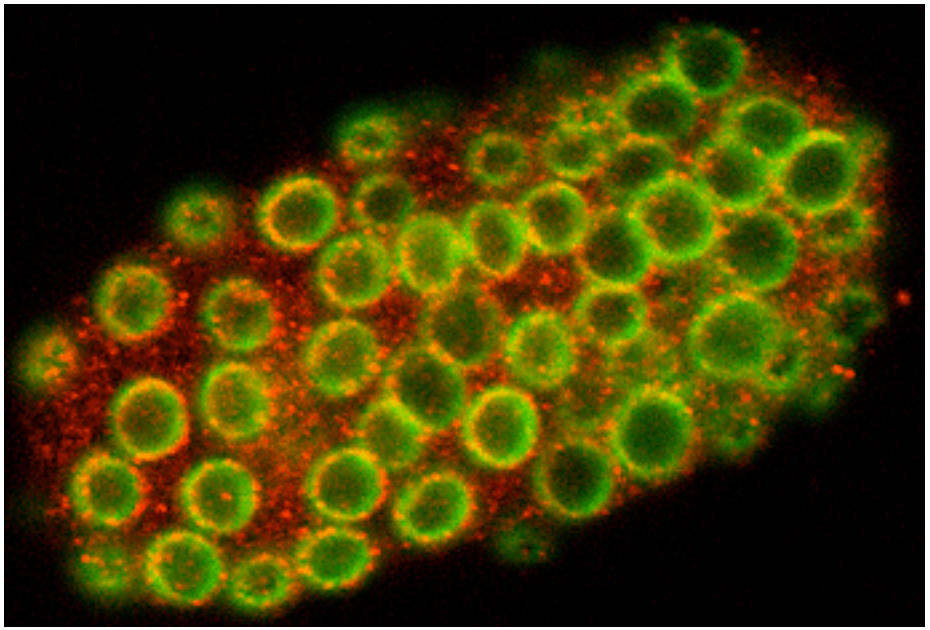


Image using confocal microscopy of a *C. elegans* embryo in which all cells have been caused to initiate programmed cell death (apoptosis). The cell-death killer protein CED-4 (red) and the nuclear envelope protein lamin (green) are both seen at the nuclear envelope (overlap is yellow). By contrast, in normal embryos, CED-4 is instead localized to mitochondria. This experiment helped reveal that CED-4 translocates from mitochondria to the nuclear envelope during programmed cell death.

The C. elegans Lin-4 was the first microRNA discovered

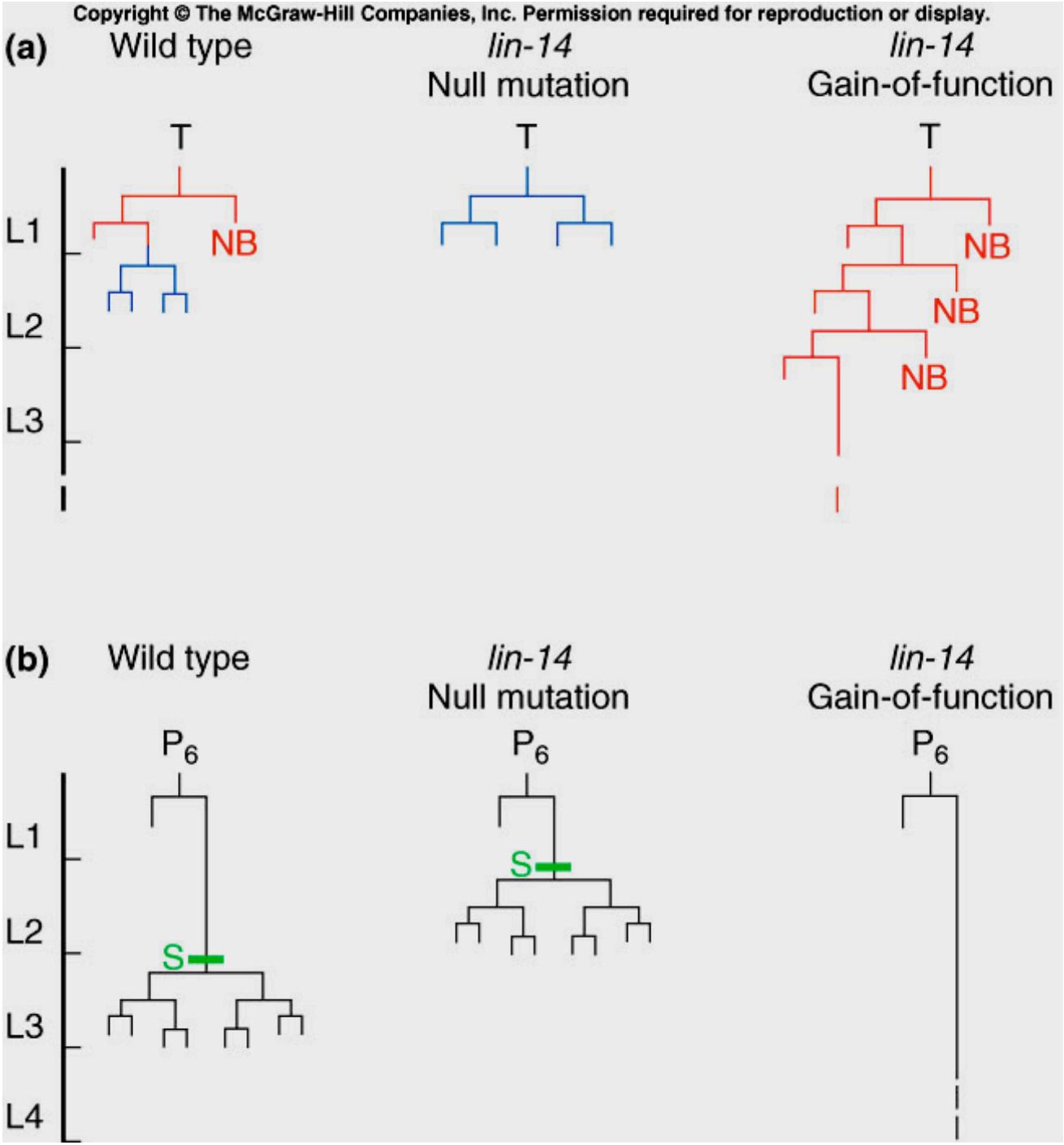
In Dr. Victor Ambros' lab

Lee RC, Feinbaum RL, Ambros V. (1993) The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75(5):843-54.

Lee RC, Ambros V. (2001) An extensive class of small RNAs in Caenorhabditis elegans. Science. 2001 Oct 26;294(5543):862-4.

http://chronic.dartmouth.edu/VRA/ambroslab.html

Fig. C.16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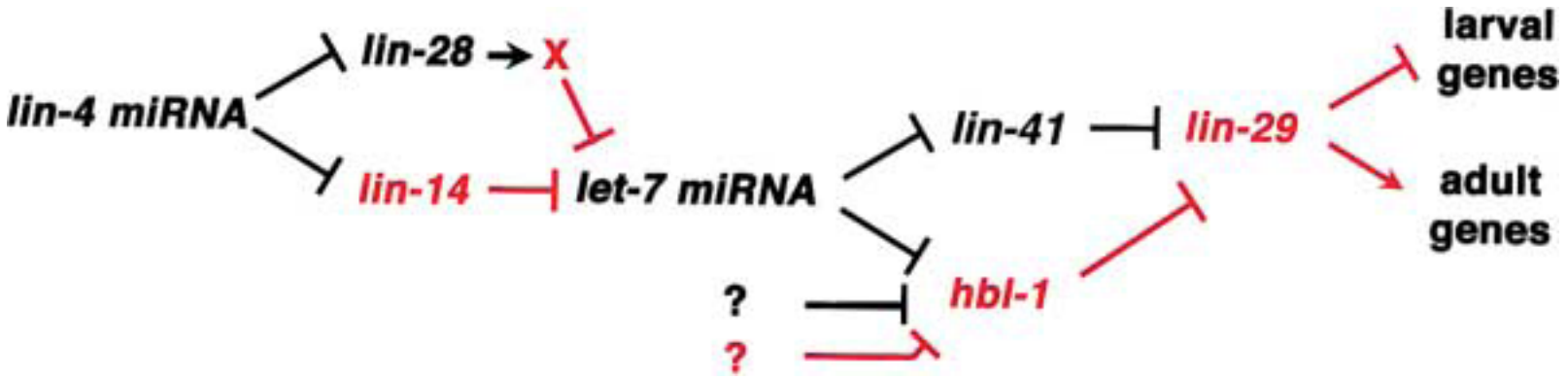
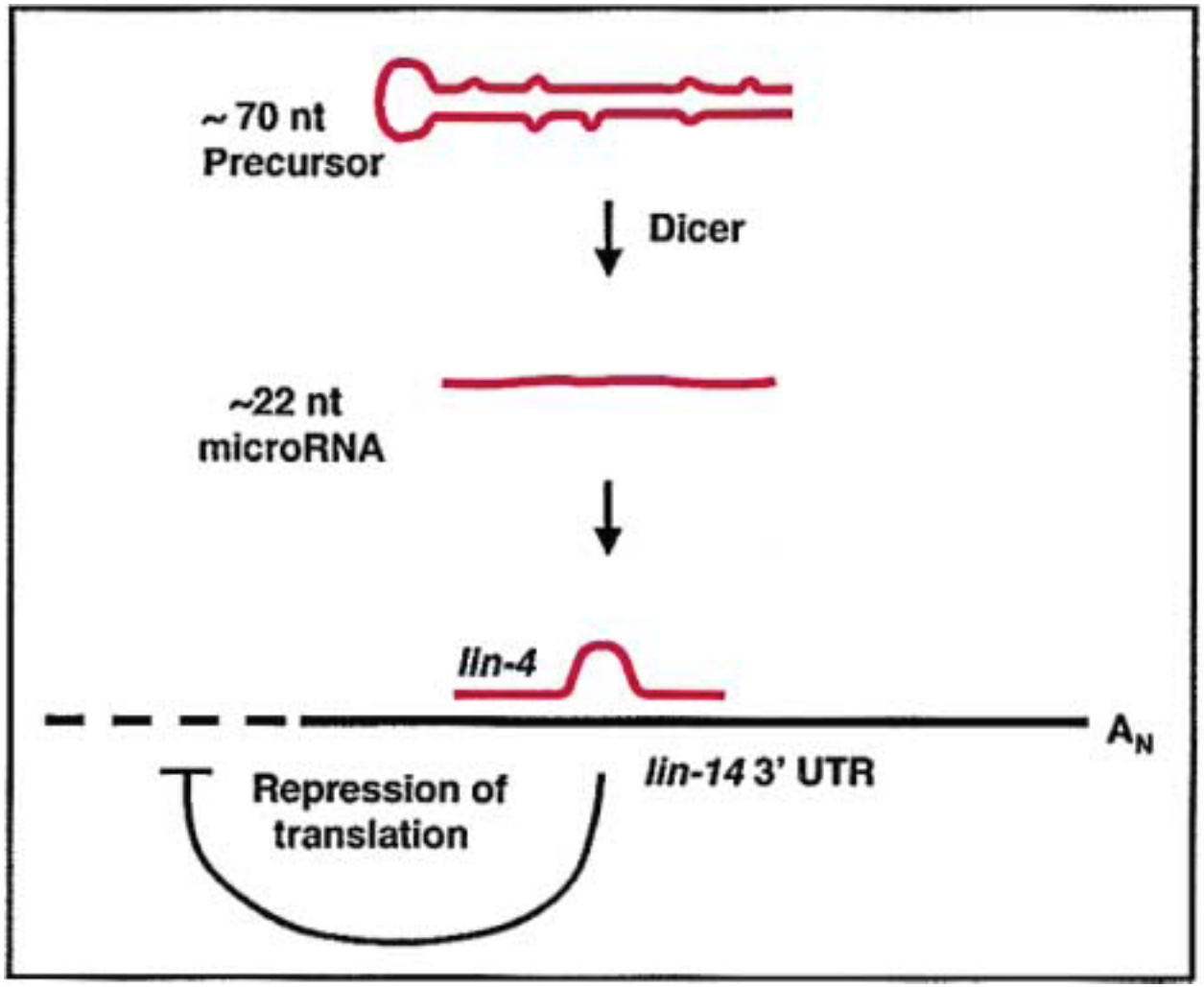
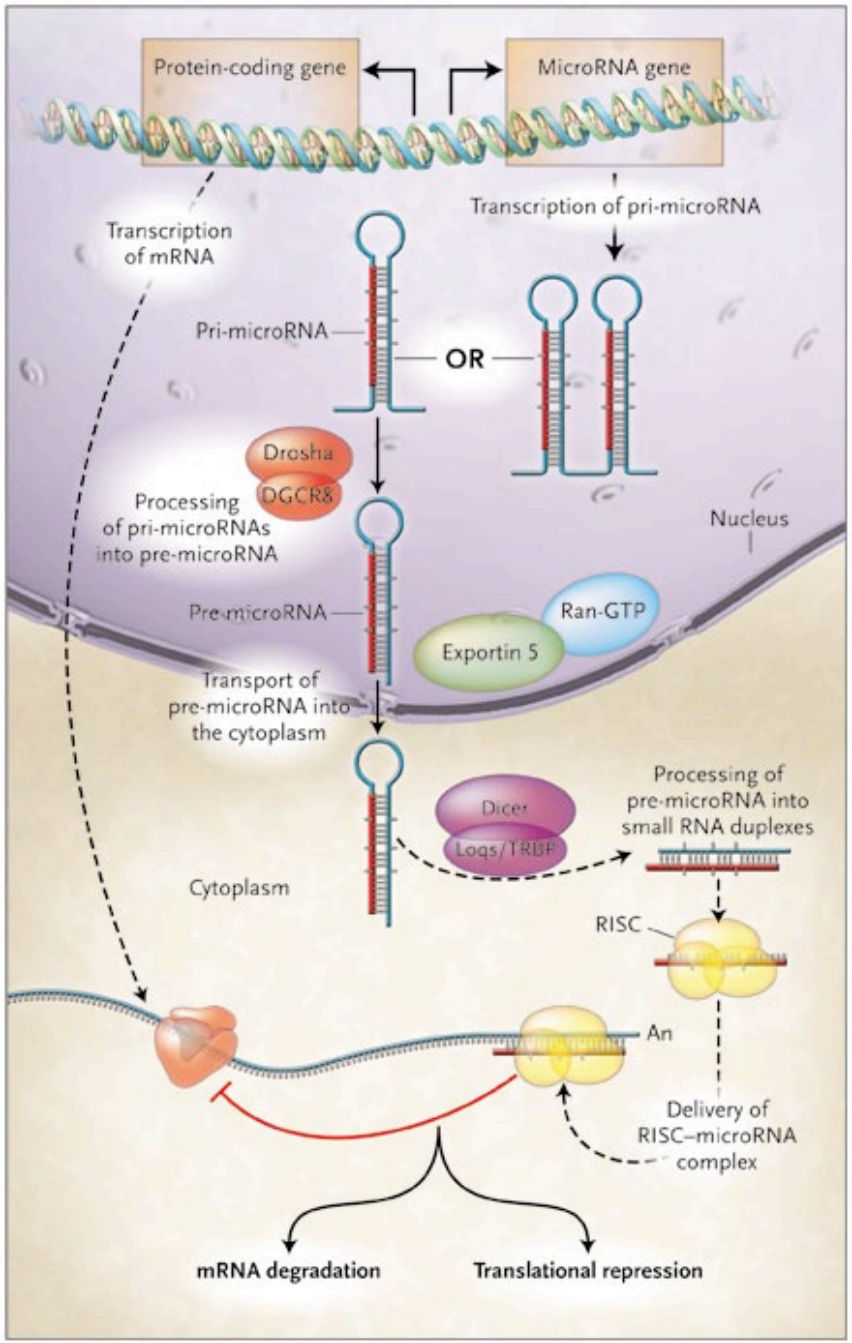
(a)
Wild type
lin-14
Null mutation
lin-14
Gain-of-function
T
T
T
L1
L2
L3
NB
NB
NB
(b)
Wild type
lin-14
Null mutation
lin-14
Gain-of-function
P6
P6
P6
L1
L2
L3
L4
S
S

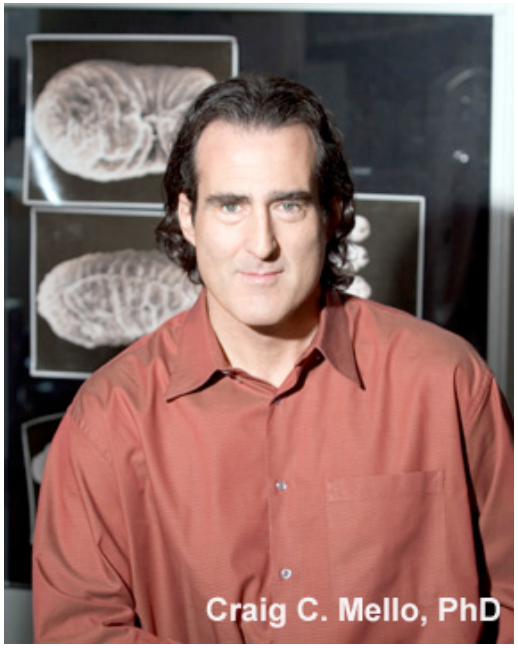
Genetic pathway that regulates developmental timing




~70 nt
Precursor
Dicer
~22 nt
microRNA
lin-4
A_N
lin-14 3' UTR
Repression of
translation

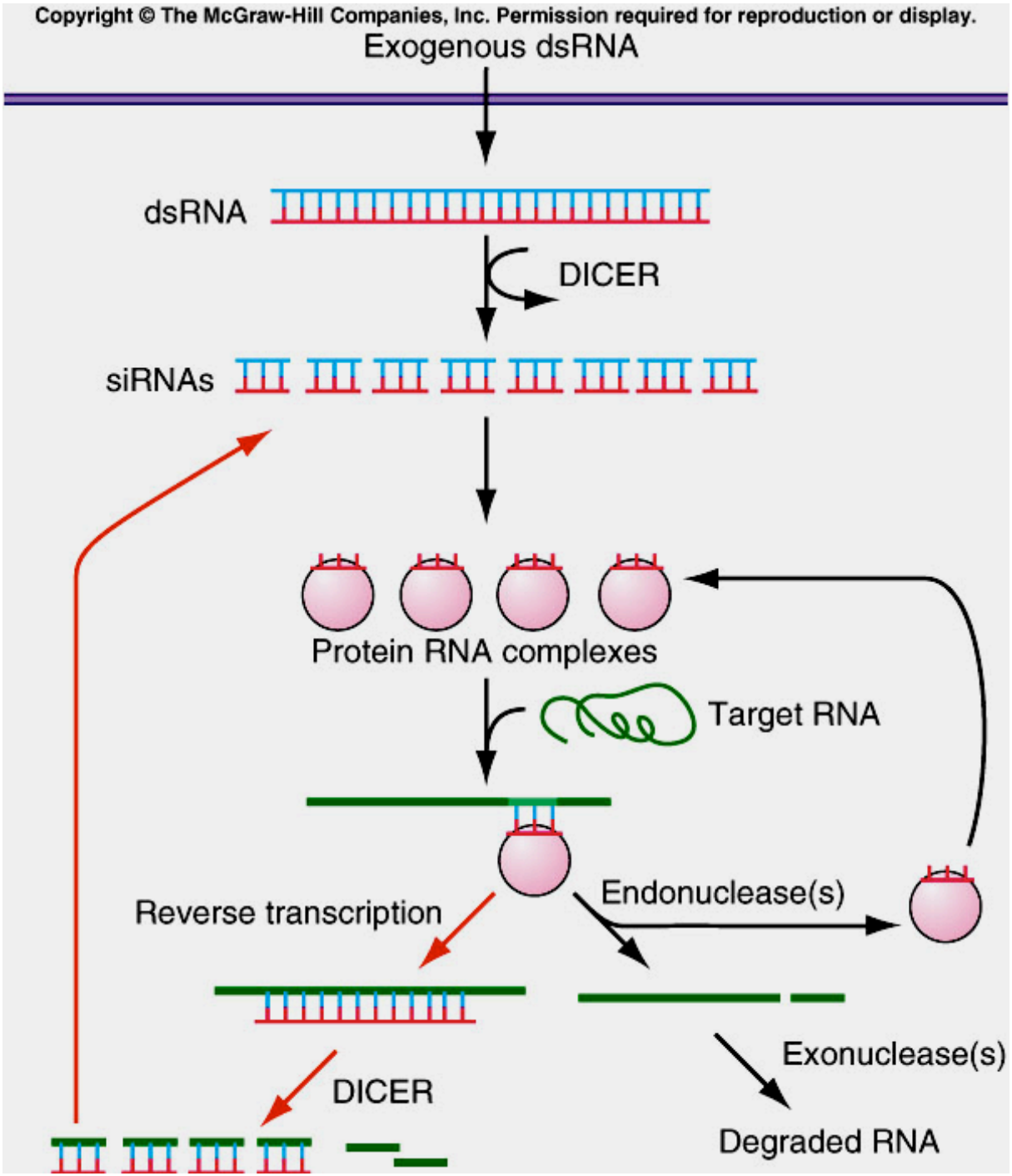

Protein-coding gene
MicroRNA gene
Transcription of pri-microRNA
Transcription of mRNA
Pri-microRNA
OR
Drosha
DGCR8
Processing of pri-microRNAs into pre-microRNA
Nucleus
Pre-microRNA
Ran-GTP
Exportin 5
Transport of pre-microRNA into the cytoplasm
Dicer
Loqs/TRBP
Processing of pre-microRNA into small RNA duplexes
Cytoplasm
RISC
An
Delivery of RISC–microRNA complex
mRNA degradation
Translational repression

RNAi was first discovered in C. elegans



Fire et al., (1998) Potent and specificgenetic interference by double stranded RNA in C. elegans. **Nature** vol 391, 806-810

Fig. C.9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Exogenous dsRNA
dsRNA
DICER
siRNAs
Protein RNA complexes
Target RNA
Reverse transcription
Endonuclease(s)
Exonuclease(s)
DICER
Degraded RNA