Programmed Cell Death (apoptosis)

Stereotypic death process includes:

- membrane blebbing
- nuclear fragmentation
- chromatin condensation and DNA framentation
- loss of mitochondrial integrity and
  - release of cytochrome c

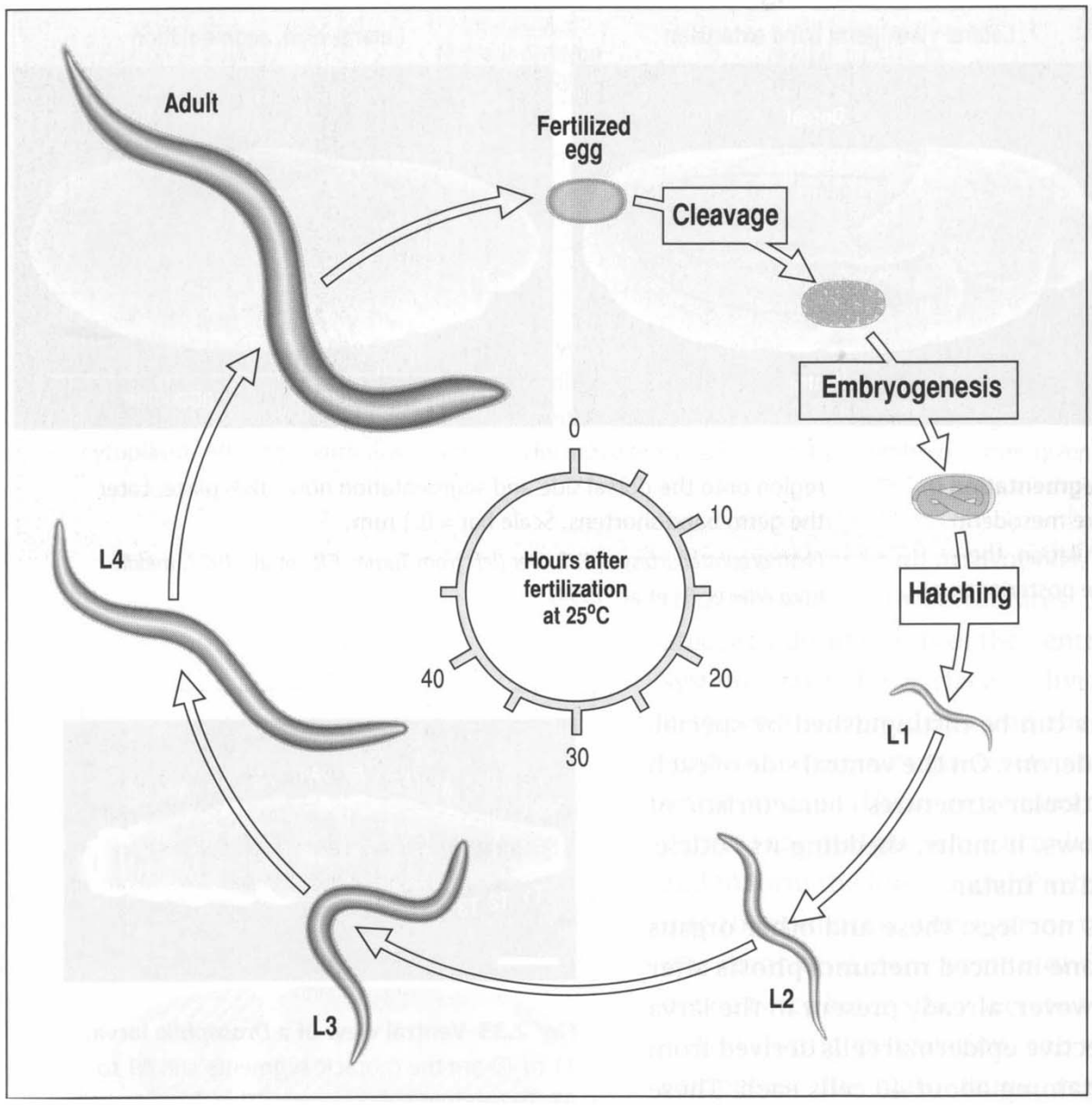
Natural part of development

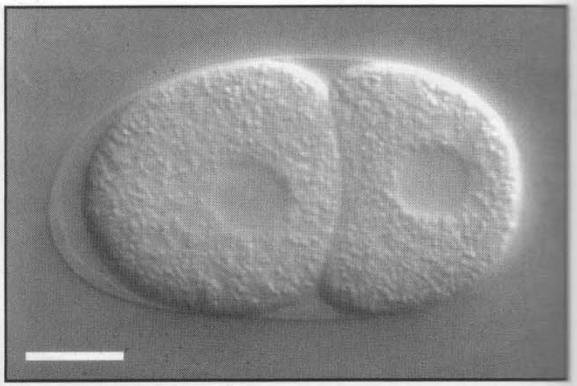
- eg., removal of webbing between digits

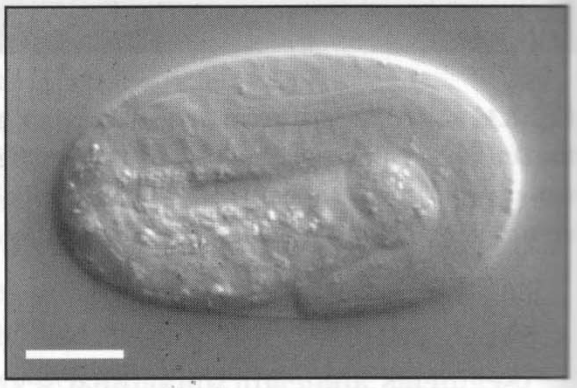
Cancer involves mutations that block apoptosis (p53)

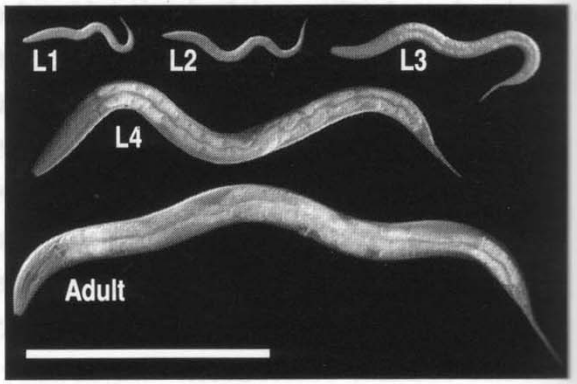
First genes discovered in nematodes

Self-fertile hermaphrodites; rapid life cycle

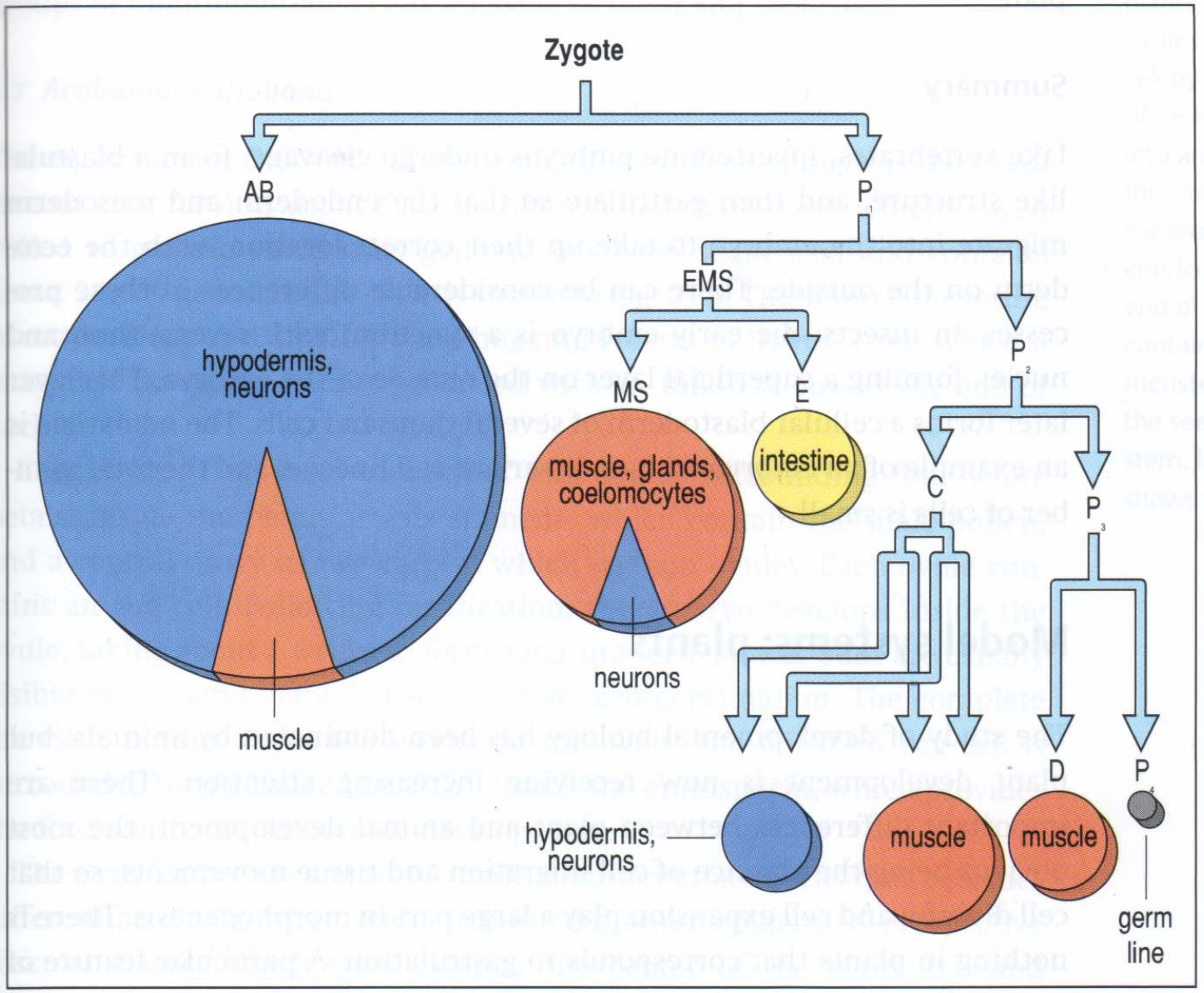




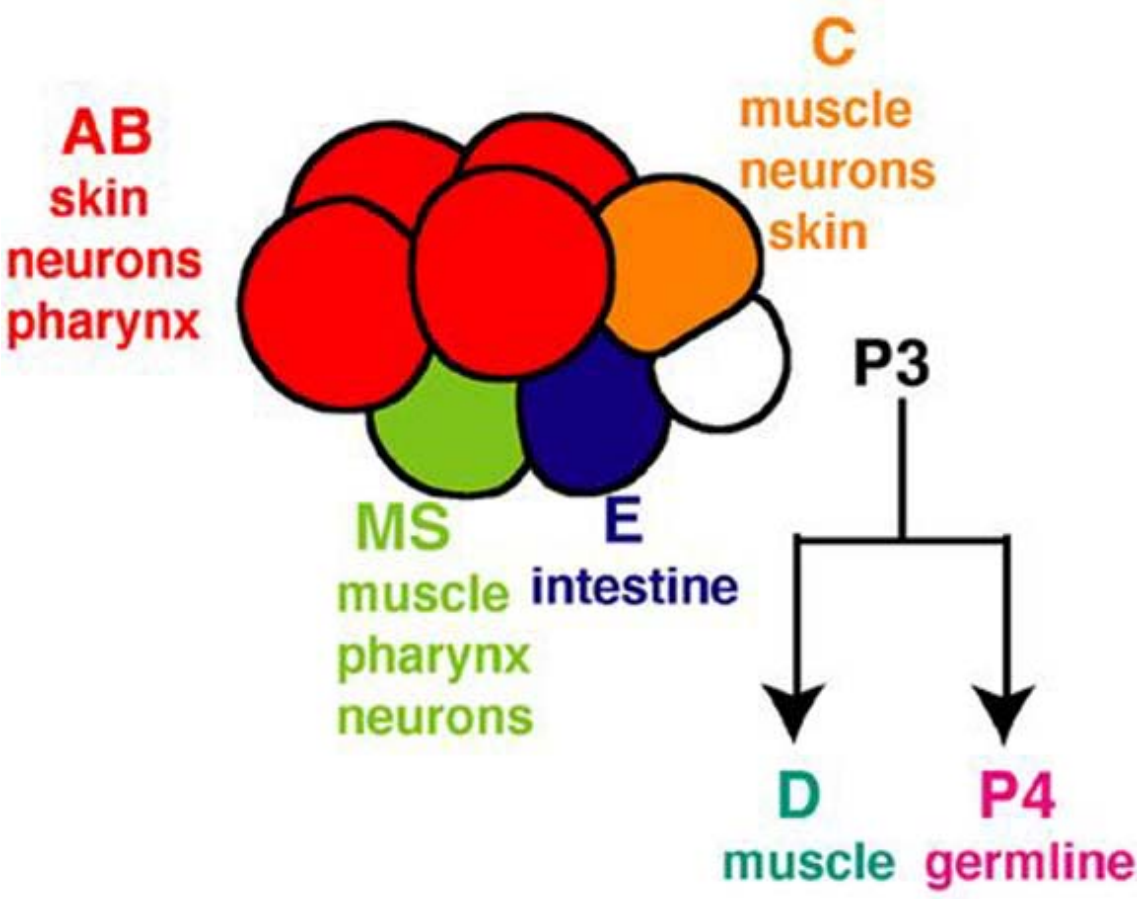




Early Embryogenesis: making founder cells

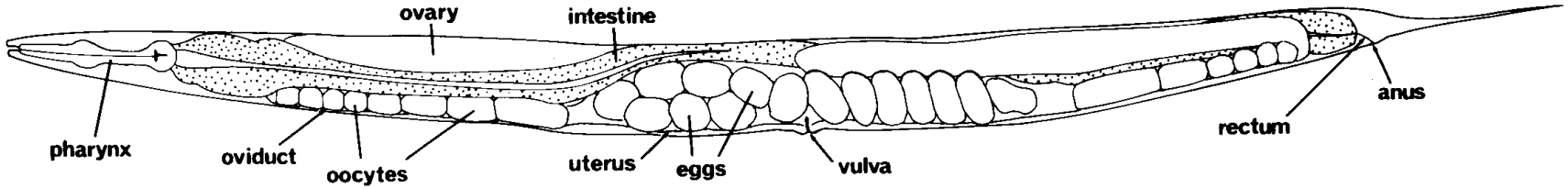


Six Founder Cells
(5 Somatic, 1 Germline)



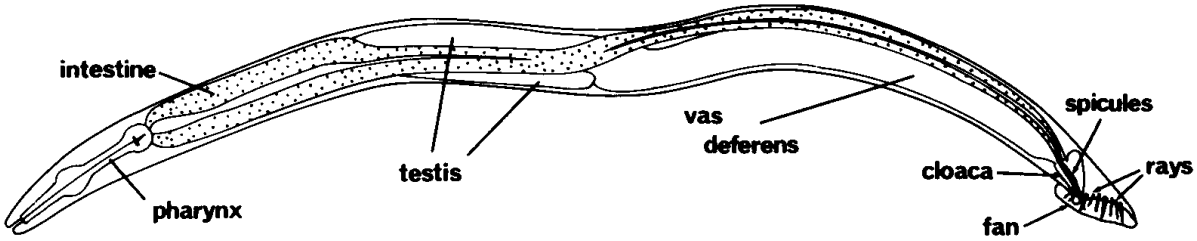
C. elegans reproduces sexually

Hermaphrodite

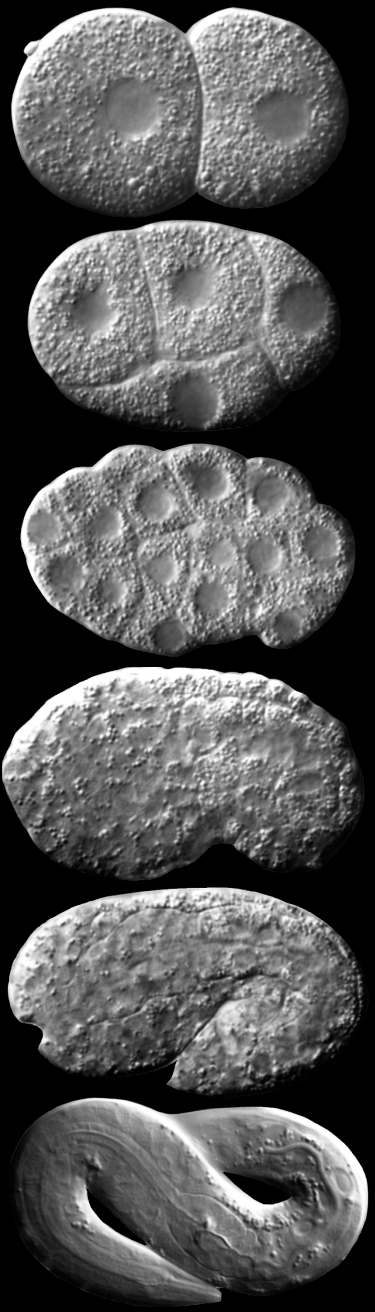


self-fertile: germline makes ~300 sperm then oocytes

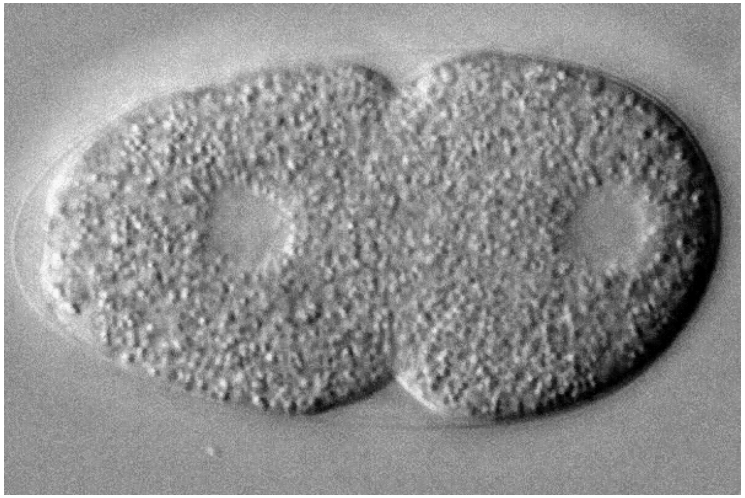
Male

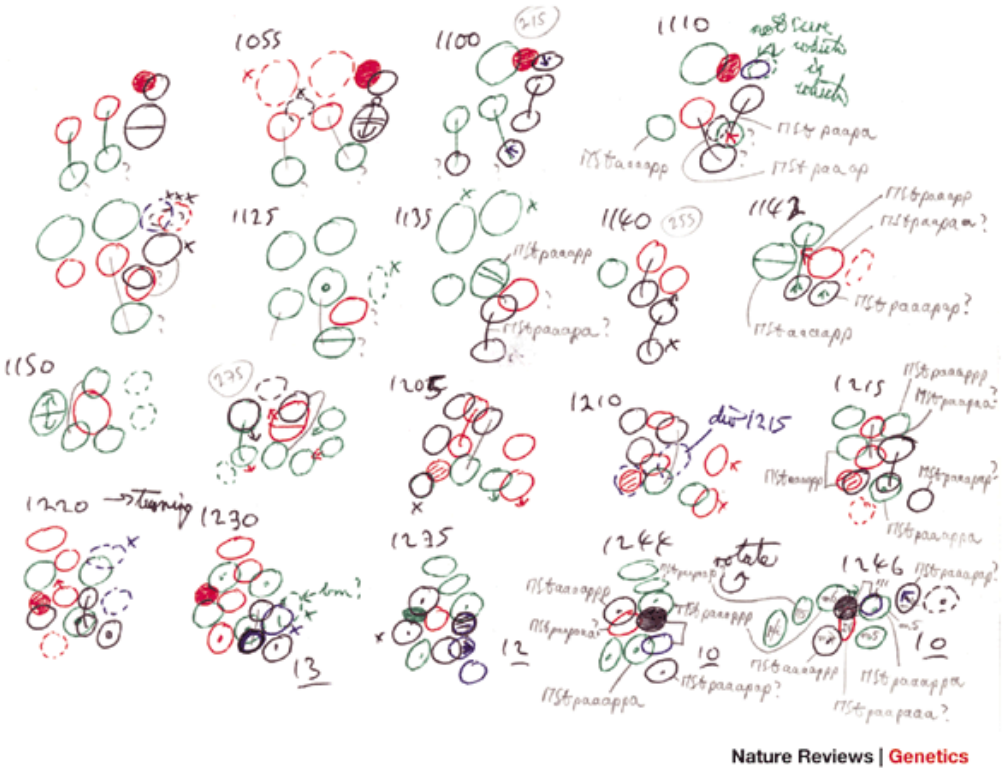


cross-fertile: germline makes sperm only



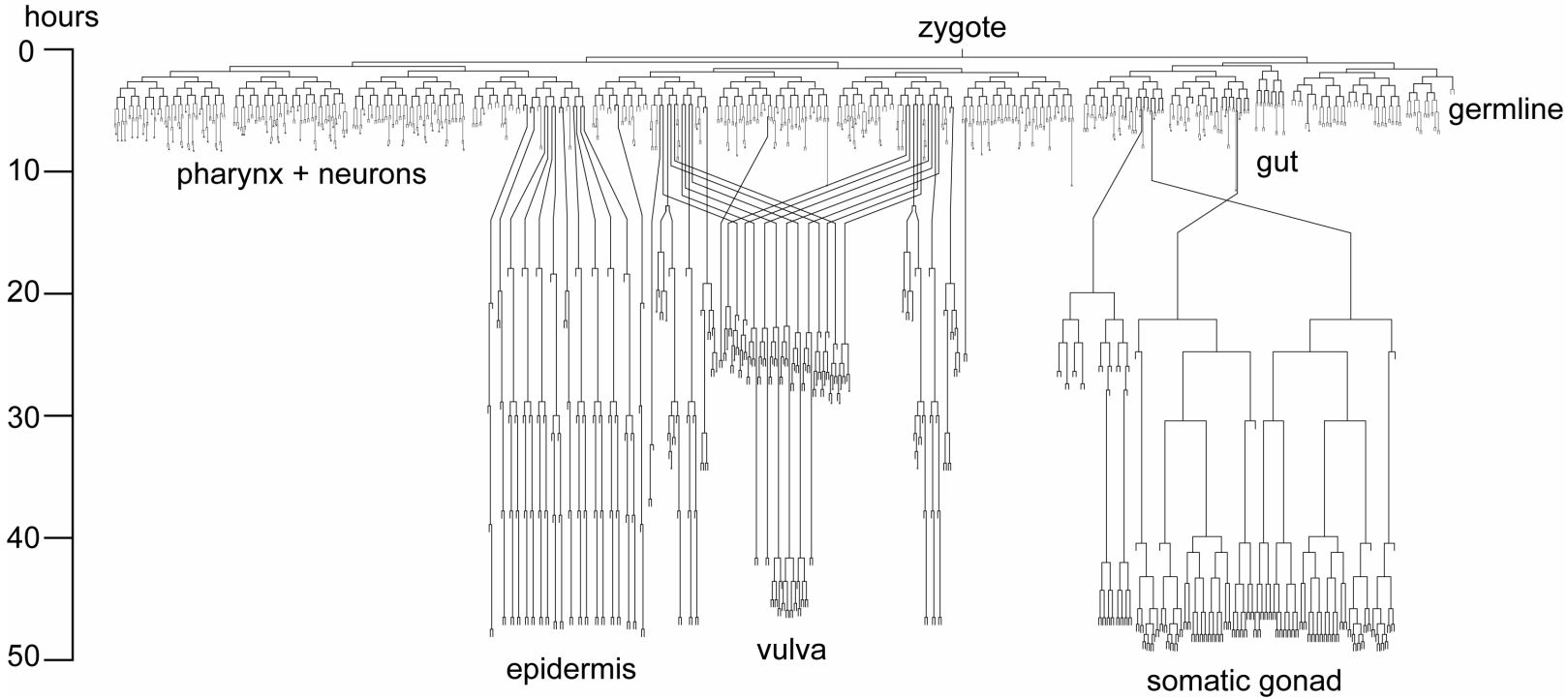
C. elegans development





John Sulston's drawings of nuclear positions (6 May 1980)

An invariant cell lineage



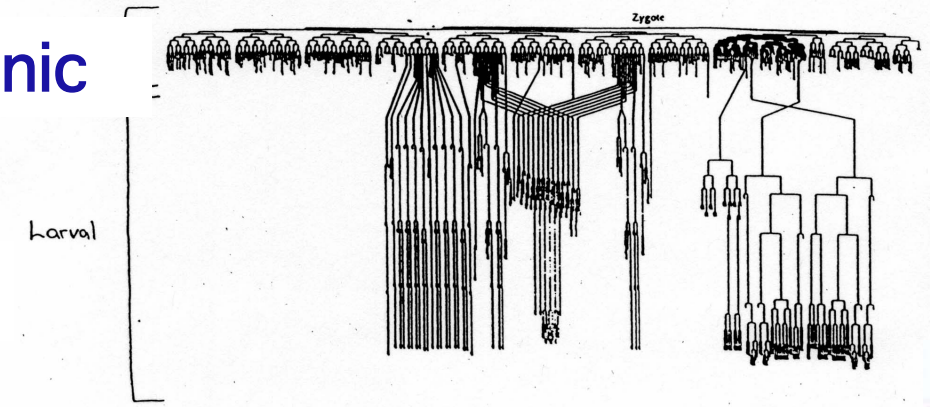
959 somatic cells (adult hermaphrodite)

(John Sulston, Bob Horvitz, Judith Kimble 1977-1983)

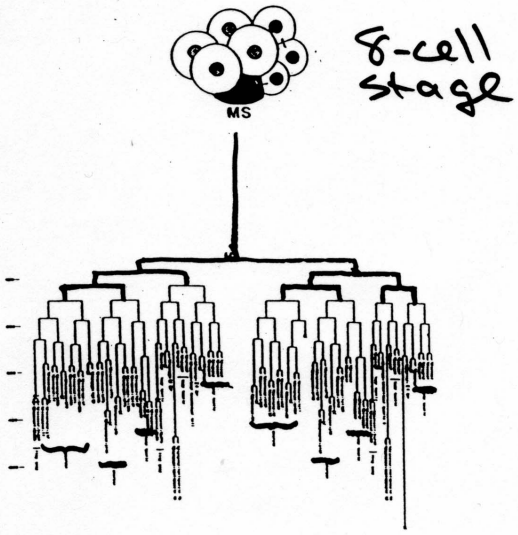
Caenorhabditis elegans Cell Lineage

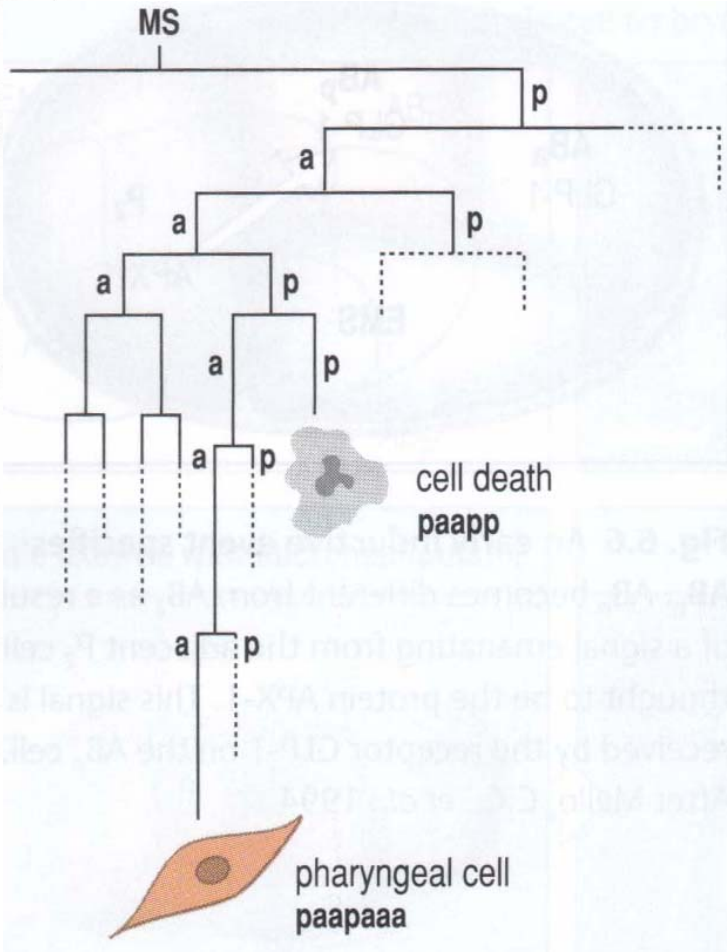
Embryonic

Larval



The MS Lineage:





Programmed Cell Death in C. elegans:

Embryogenesis produces a hatched larva with

--558 living cells

--131 cells eliminated by programmed cell death (shortly after their births).

Additional cell deaths occur during larval development.

Most cell deaths are in neuronal lineages.

Cell death (apoptosis) conserved in most animals.

Cancer connection.

ced mutants: programmed cell death-defective

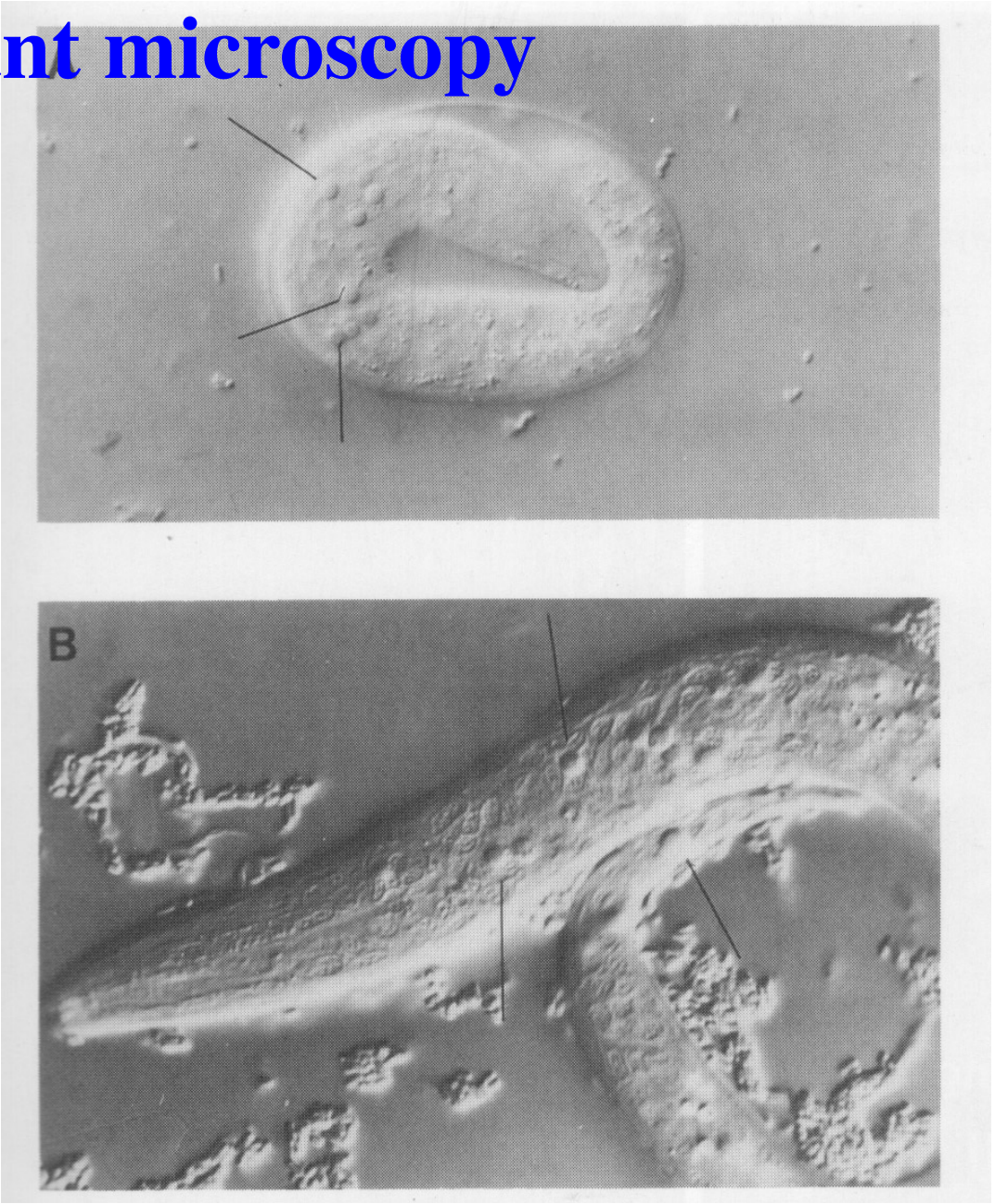
Ed Hedgecock: unbiased Nomarski screen for mutants with defects in cellular anatomy (F2 screens)

Identified two mutants, *ced-1* and *ced-2*, with persistent cell death corpses.

ced mutant microscopy

ced-1(-/-) and ced-2(-/-):

corpses accumulate



Mutants defective for programmed cell death

Cell death corpse accumulation*: an entry point into genetic studies of programmed cell death, *but not what you want to study.

What genes are REQUIRED for programmed cell death???
***Mutants lacking programmed cell death* (not cleaning up the mess).**

Take advantage of *ced-1/2* mutant phenotype: *easy to see that programmed cell death is occurring*.

Screen for mutants in which no corpses are visible (in a *ced-1/2* mutant background)

ced3 mutants fail to accumulate corpses

ced-1(-/-)

Horvitz lab rides again.

Ellis et al, Cell 44, 817-829 (1986)

ced-1(-/-); ced-3(-/-)

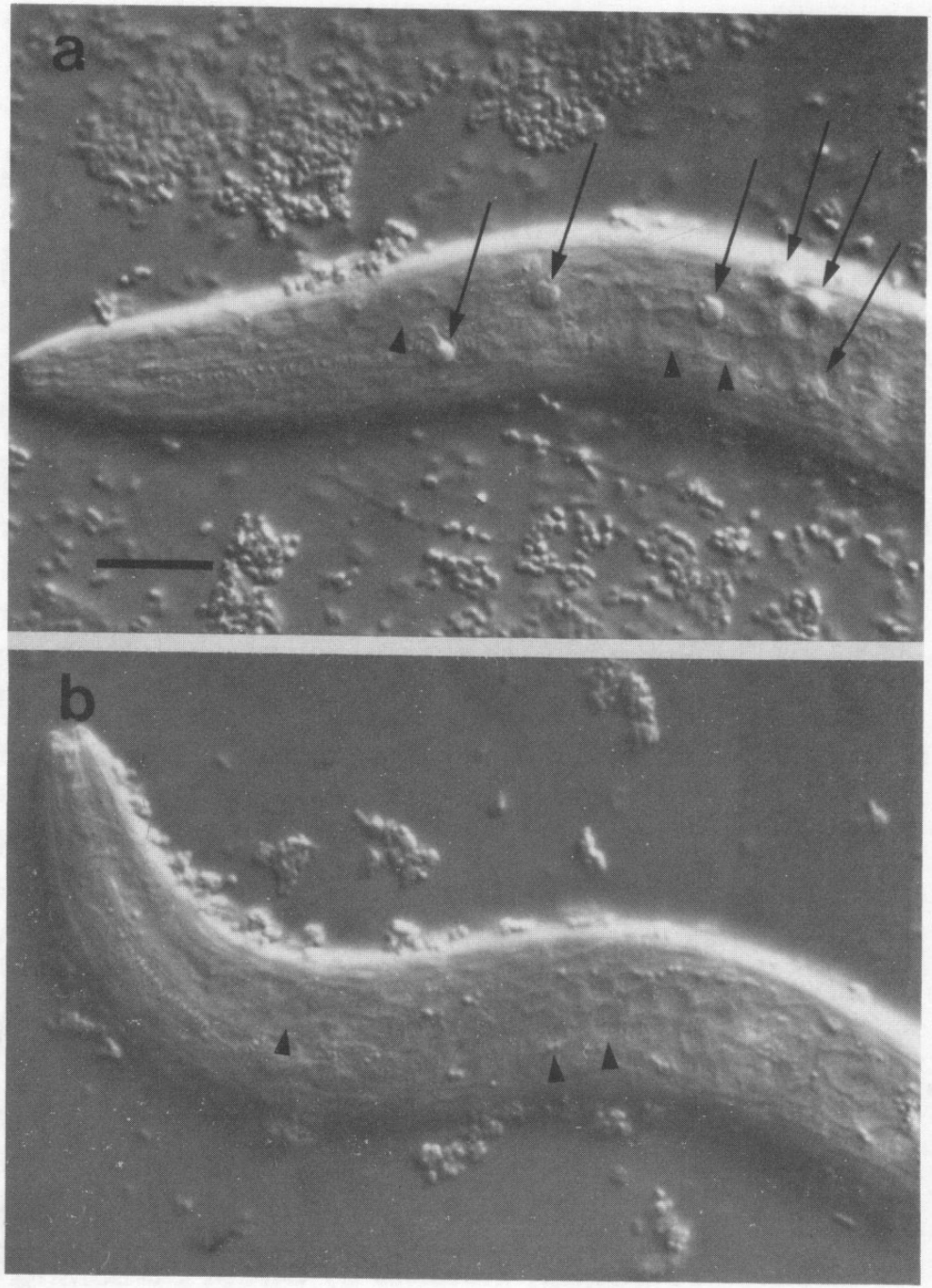


Figure 1. Absence of Cell Deaths in *ced-3* Animals

Screens for mutants identify 2 genes

Screen: 4000 F1 broods examined
2 alleles of *ced-3* identified
1 allele of *ced-4* identified in different screen (Egl sup)

Not an easy screen, but succeeded in identifying two genes required for ALL cell deaths.

First time such genes found in any organism.

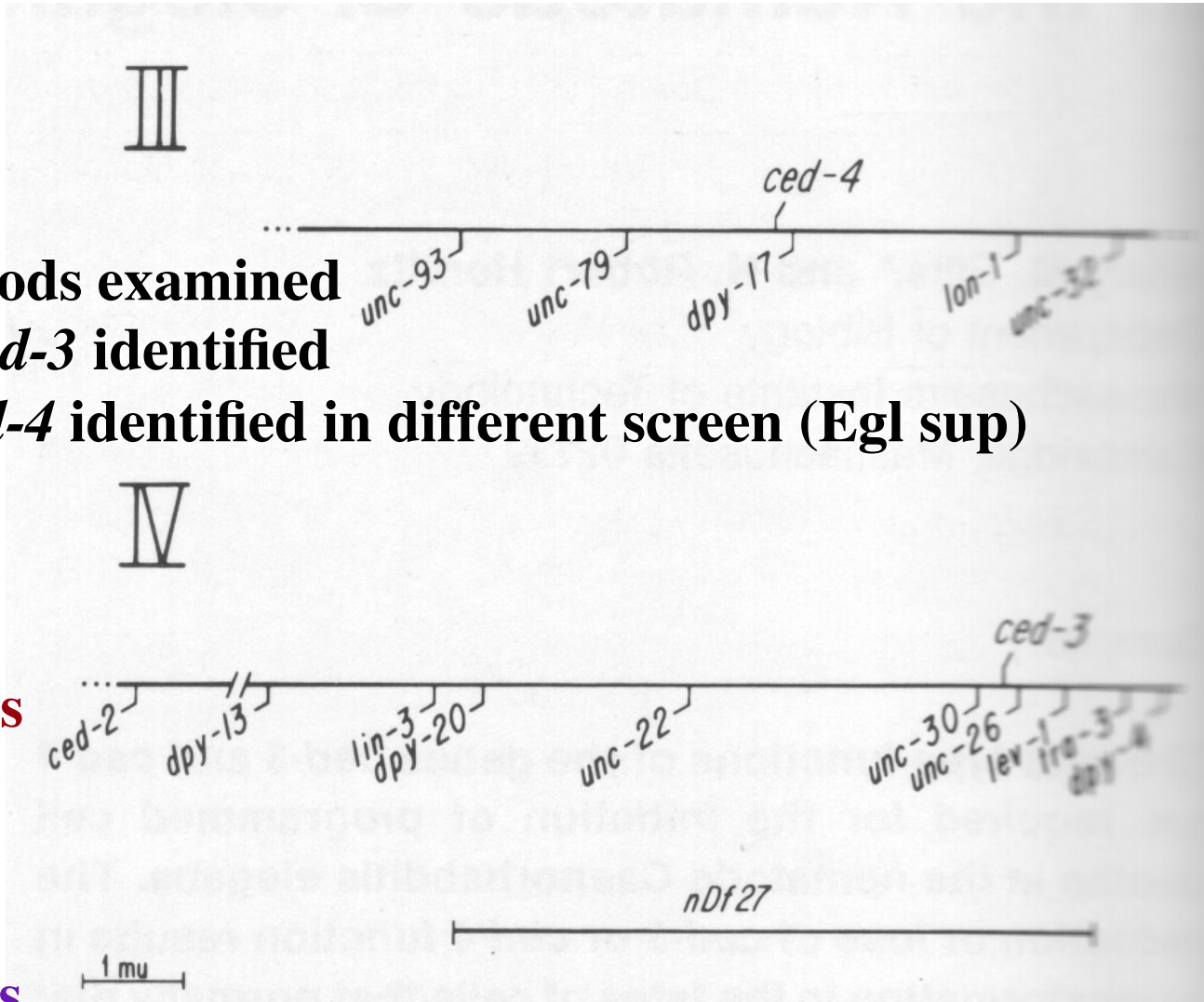


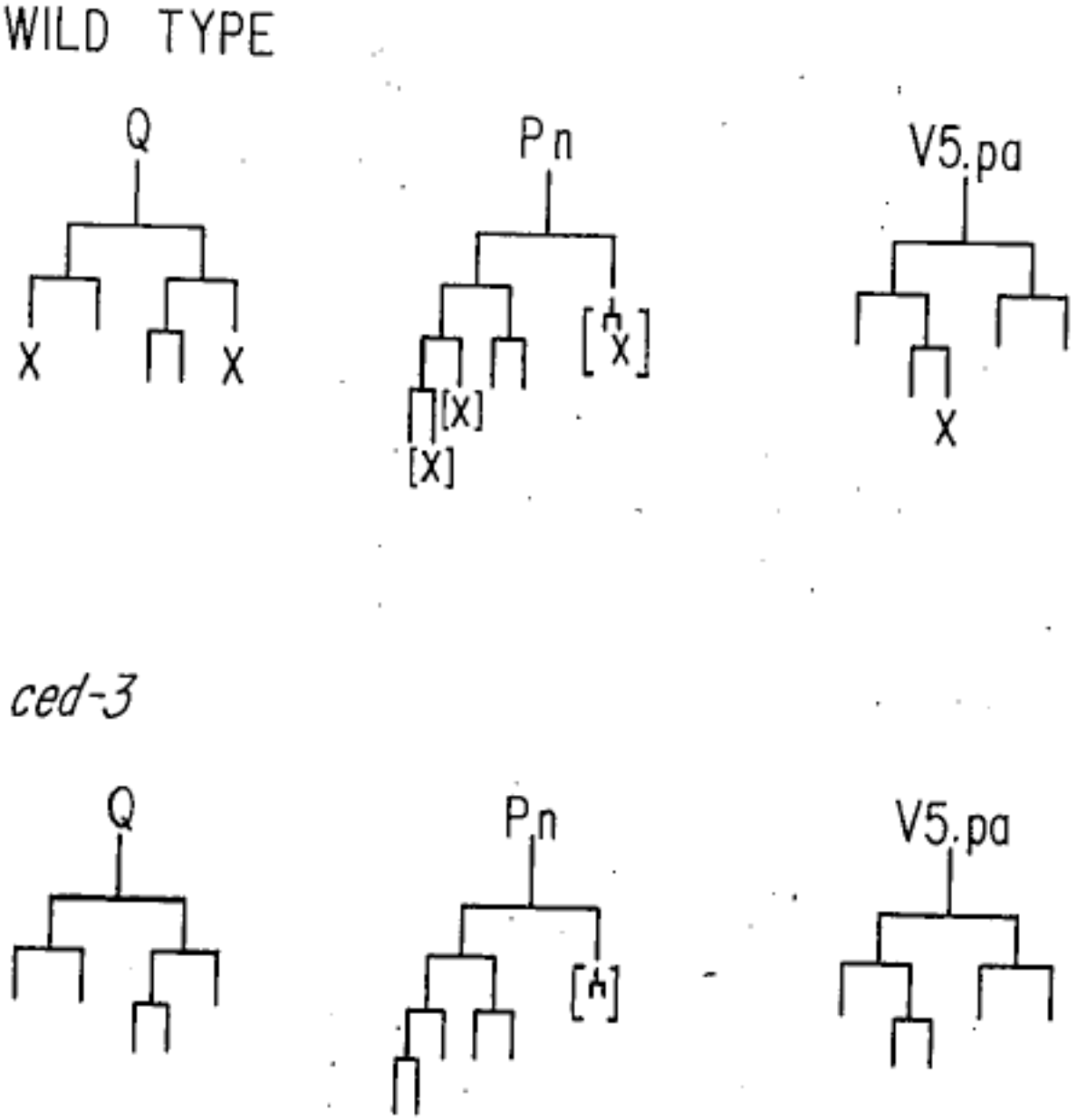
Figure 2. A Partial Genetic Map of Linkage Groups III and IV

Quantitation of cell deaths in *ced-3* and *-4* mutants

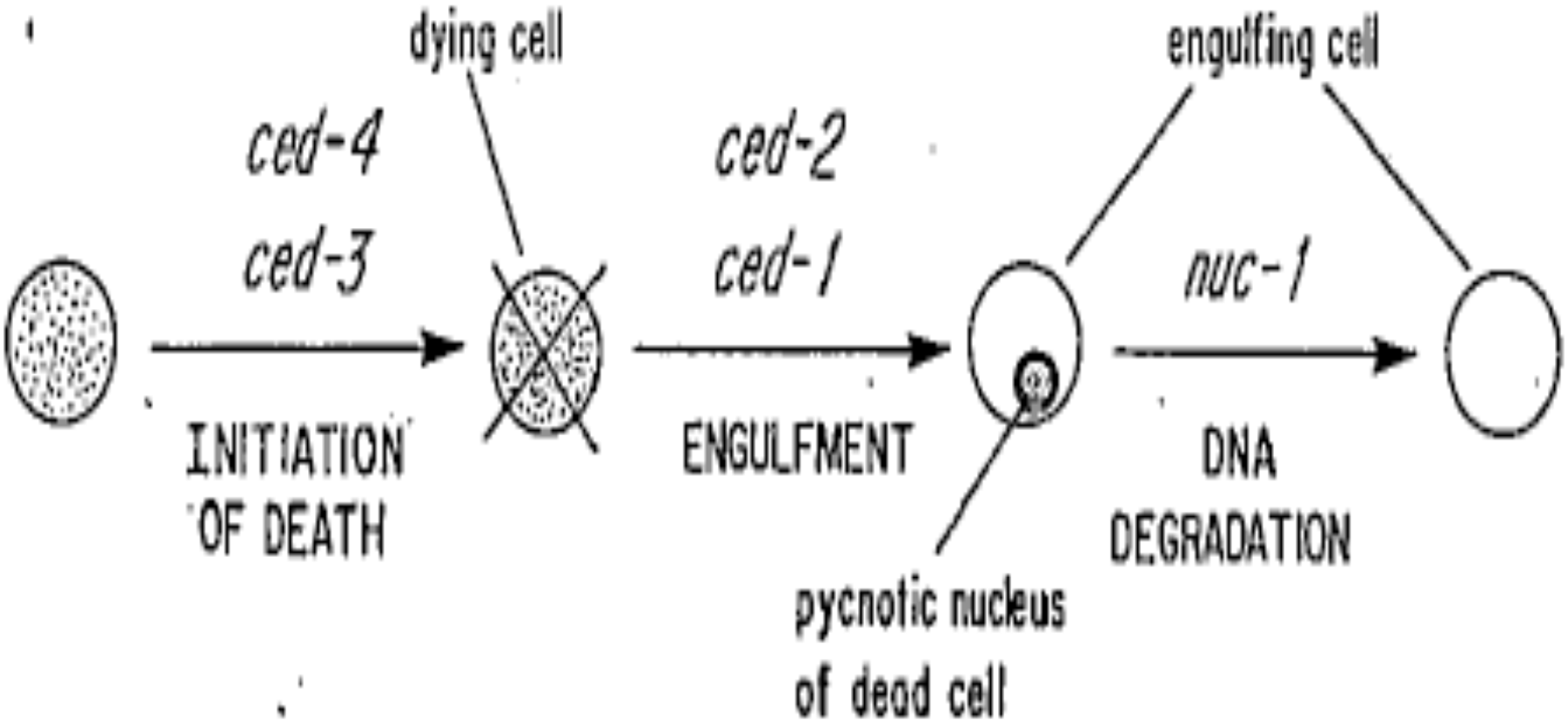
Table 1. Elimination of Cell Death by *ced-3* and *ced-4* Mutations

	Average Number of Deaths Observed			
	Embryonic Deaths	Postembryonic Deaths		
Genotype	Head of L1	Ventral Cord*	Postdeirid†	Q‡
ced-1	28.0 n = 23	8.7 n = 28	0.93 n = 29	1.6 n = 28
ced-1; ced-3 (n717)	0.3 n = 21	0.04 n = 50	0 n = 15	0 n = 24
ced-1; ced-3 (n718)	0.5 n = 26	0.03 n = 35	0 n = 24	0 n = 21
ced-1; ced-3 (n1040)	7.0§ n = 21	0 n = 23	0.05 n = 20	0.06 n = 17
ced-1; ced-3 (n1129)	3.0 n = 22	N.D.	0.13 n = 30	N.D.
ced-1; ced-4 (n1162)	0.6 n = 23	0.04 n = 27	0 n = 21	0 n = 21

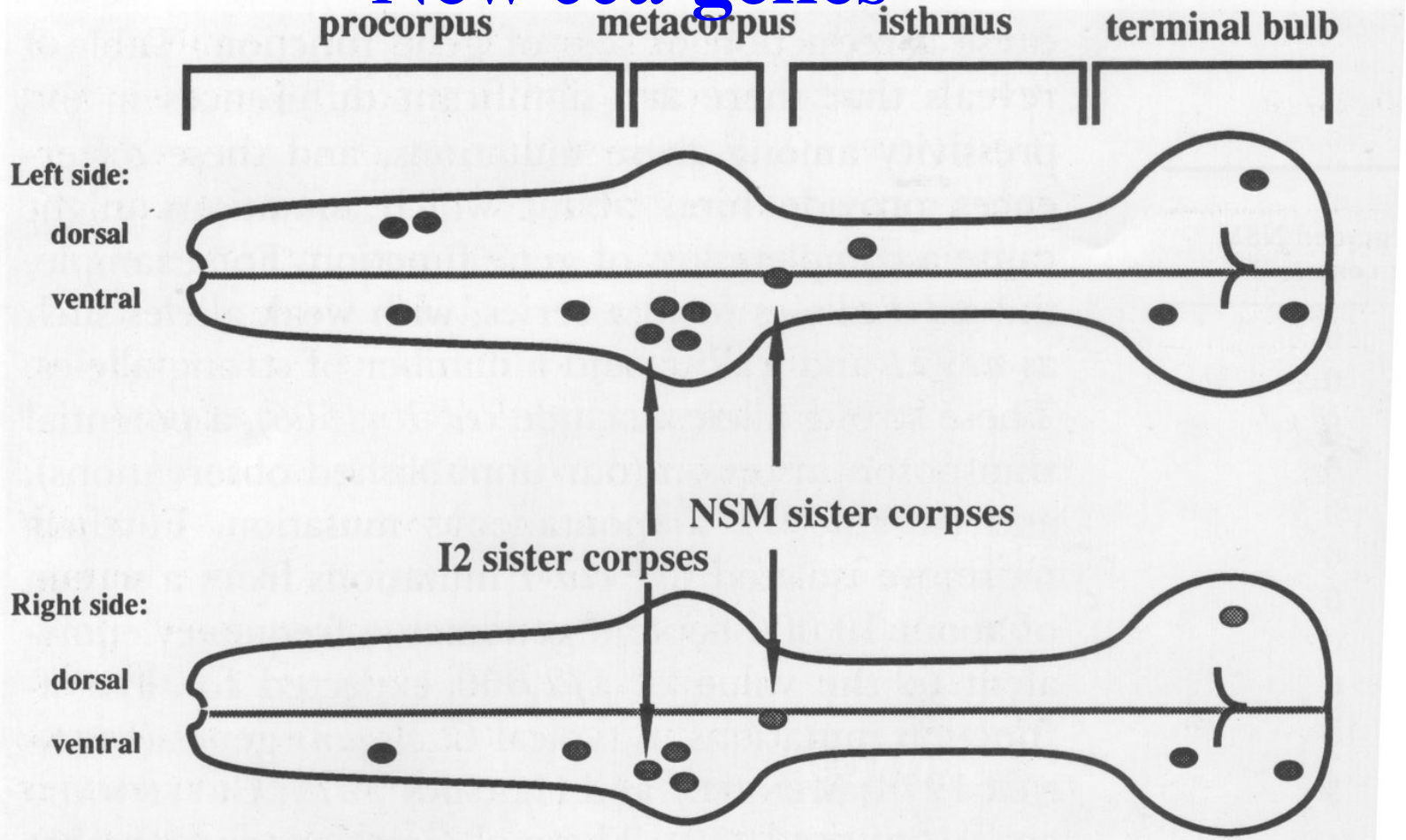
ced-3 (and *-4*) are required for pcd in all lineages



Model for *ced* gene order of action



New *ced* genes



Screened 40,000 F2s for extra pharyngeal cells
(at locations of I2 and NSN corpses)

-- Identified multiple recessive alleles of *ces-2*, required only in pharynx for cell deaths.

--One dominant allele of a gene called *ced-9*, prevents ALL.

ced-9 (n1950) is a dominant gain-of-function allele

TABLE 1 The gain-of-function allele *ced-9(n1950)* prevents programmed cell deaths

(a) *ced-9(n1950)* prevents programmed cell deaths

Maternal genotype	Zygotic genotype	Extra cells in anterior pharynx	No of animals
ced-3/ced-3	*ced-3/ced-3*	12.5±0.7	30
ced-4/ced-4	*ced-4/ced-4*	13.9±0.5	40
ced-9(n1950)/ced-9(n1950)	*ced-9(n1950)/ced-9(n1950)*	13.3±0.6	45

(b) *ced-9(n1950)* is a dominant gain-of-function mutation and shows a maternal effect

+/+	+/+	0.03±0.05	60
	Df/+ *	0.00	50
	ced-9(n1950)/+	5.3±0.8	25
ced-9(n1950)/+	+/+	0.2±0.2	25
	ced-9(n1950)/+	11.4±0.8	30
	ced-9(n1950)/ced-9(n1950)	13.7±0.5	30
ced-9(n1950)/ced-9(n1950)	*ced-9(n1950)*/+	11.8±0.6	30
	ced-9(n1950)/ced-9(n1950)	13.3±0.6	45

(c) *ced-9(n1950)* suppresses the accumulation of cell corpses

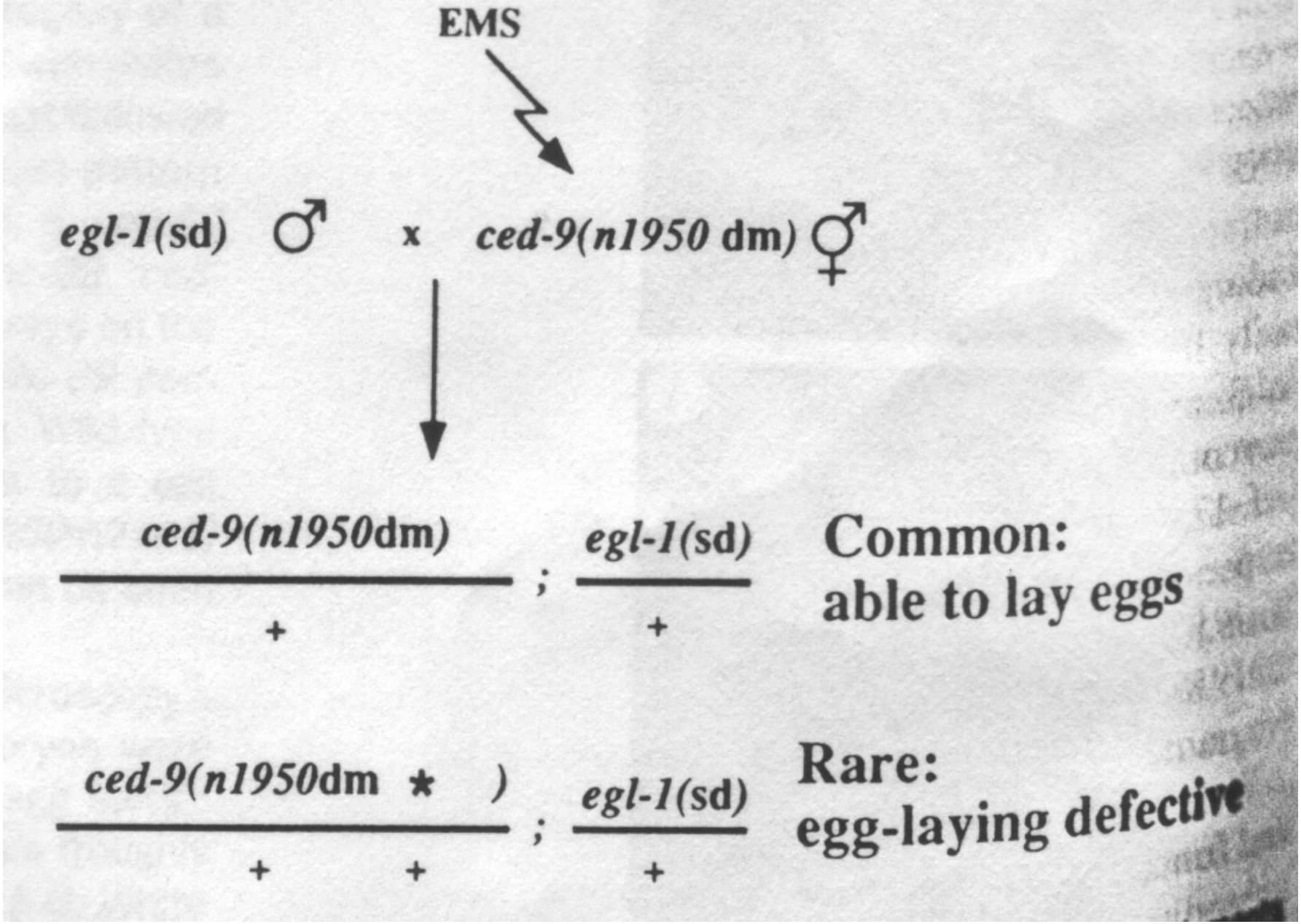
Genotype	Corpses pharynx	*n*	Corpses head	*n*	Corpses P9-P11	Corpses P12	Corpses Tail	Extra cells P9-P11	*n*
Wild type (N2)	0	50	0.0±0.1	50	0	0	0	0	30
ced-1	0.8±0.2	100*	28	10†	3.5±0.3	1.7±0.3	1.7±0.3	0.4±0.3	30
ced-1; ced-3	0.02±0.04	50	0.3±0.1	50	0.03±0.07	0	0.3±0.2	3.9±0.1	30
ced-1; ced-4	0.02±0.04	50	0.7±0.2	50	0.03±0.07	0	0.3±0.2	4.0±0.1	30
ced-1; ced-9(n1950)	0	30	0.5±0.3	30	0	0	0.3±0.2	4.0±0.1	30

ced-9(gof) prevents pcd of HSN neurons also

(d) *ced-9(n1950)* prevents the deaths of the HSN neurons in *egl-1* mutants

Genotype	HSNs missing (%)	No of sides	Egg-laying defective (%)	*n*
Wild type (N2)	1	250	0.4	704
egl-1	99	200	99	447
ced-3; egl-1	0	160	0.2	599
ced-4; egl-1	0	100	0	417
ced-9(n1950); egl-1	0	200	0	417

Isolation of *ced-9* loss-of-function alleles



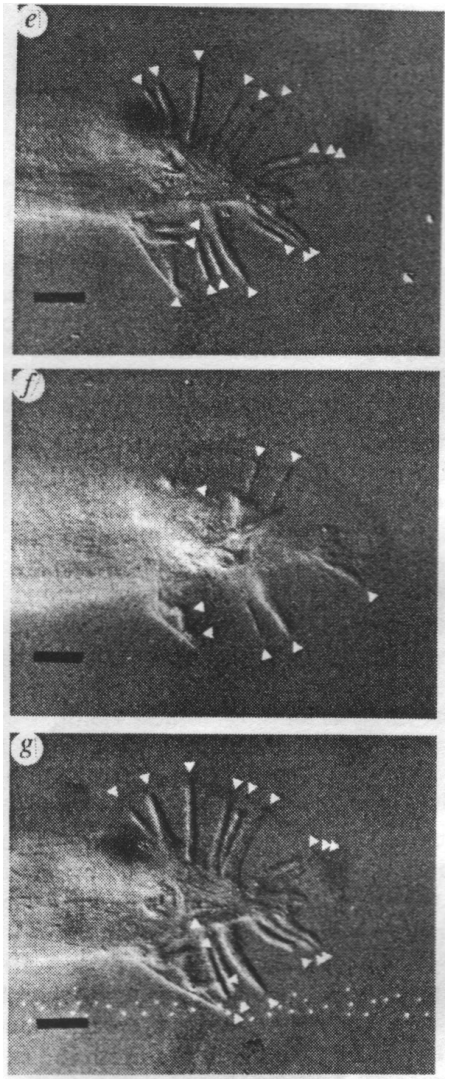
ced-9 lof alleles:

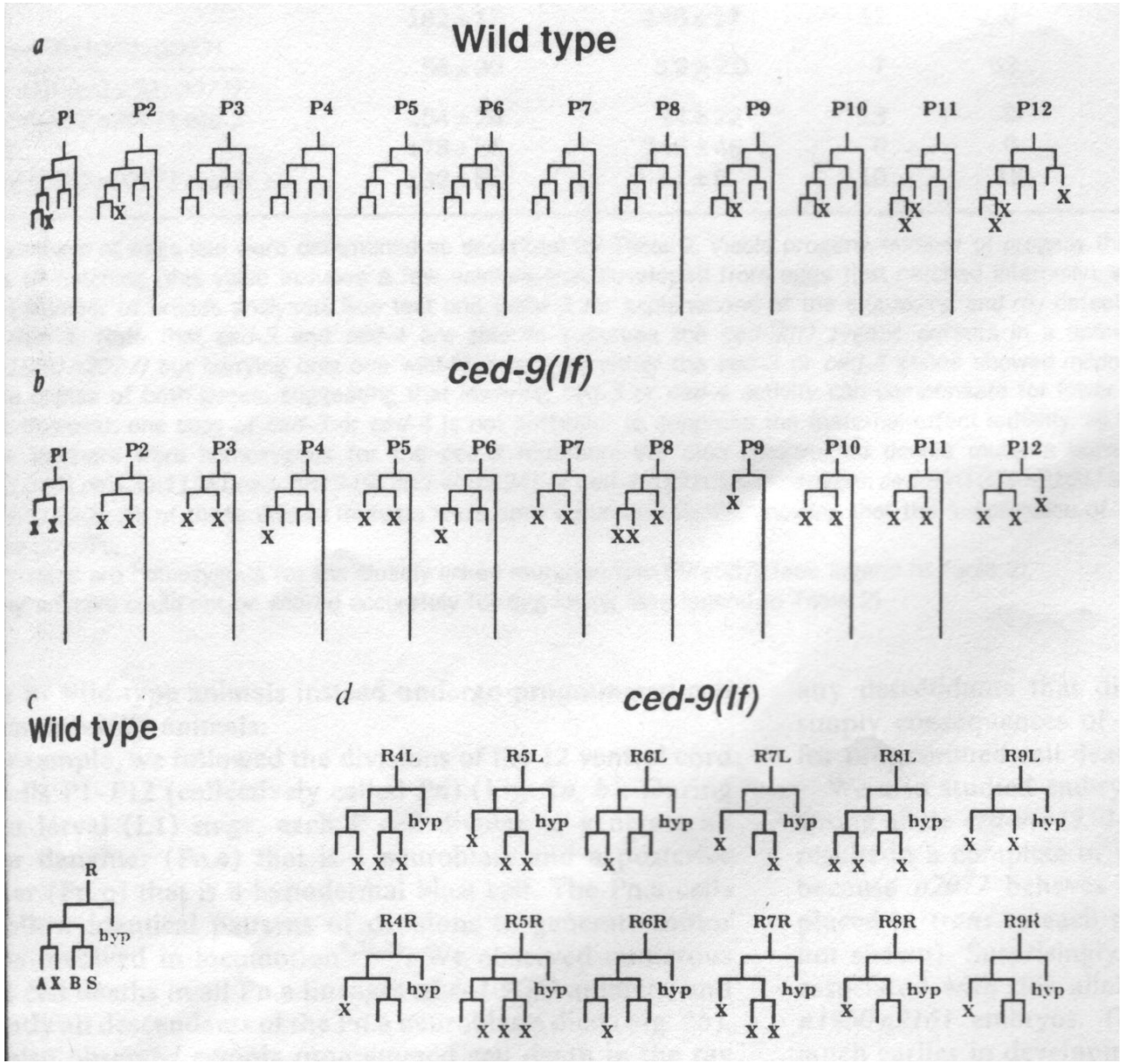
n1950n2077 behaves like a null; *n1950n2161* is a weaker allele

TABLE 2 Phenotypes of *ced-9(lf)* mutants

Genotype*	*ced-9(+)* 20 °C	*ced-9 (+)* Df 20 °C	*n1950 n2161* 15 °C	*n1950 n2161* 20 °C	*n1950 n2161* 23 °C	*n1950 n2161* 25 °C	*n1950 n2161* Df 20 °C	*n1950 n2161* *n1950 n2077* 20 °C	*n1653*ts 25 °C	*n1653*ts Df 25 °C	*n1653*ts *n1950 n2077* 25 °C	*n1950 n2077* 20 °C	*n1950 n2077* Df 20 °C
(*a*) Sterility and maternal-effect lethality													
Eggs laid per animal	209 ± 33	202 ± 60	117 ± 36	97 ± 31	45 ± 22	6.3 ± 4.5	23 ± 14	40 ± 9	2.7 ± 1.1	0	0.3 ± 0.4	1.6 ± 1.4	0.8 ± 1.6
Hatching (%)	99 ± 1	75 ± 2	12 ± 3	2.4 ± 0.7	0.4 ± 0.4	0	0	0	38 ± 24	NA	0.7 ± 1.4	0	0
L1 arrest (%)	0	13 ± 3	100	100	100	NA	NA	NA	40 ± 36	NA	100	NA	NA
	n = 14	*n* = 9	*n* = 23	*n* = 42	*n* = 36	*n* = 60	*n* = 50	*n* = 49	*n* = 26	*n* = 15	*n* = 30	*n* = 20	*n* = 24
(*b*) Egg-laying defect													
Egg-laying defective (%)	0	0	64 ± 16	76 ± 13	94 ± 7	98 ± 3	96 ± 4	96 ± 6	NA†	NA†	NA†	NA†	NA†
	n = 100	*n* = 35	*n* = 23	*n* = 42	*n* = 36	*n* = 60	*n* = 50	*n* = 40					
HSNs missing (%)	0	0	77	87	94	95 *	95	ND	ND	ND	ND	99 *	100
	n = 100	*n* = 48	*n* = 118	*n* = 138	*n* = 100	*n* = 130	*n* = 60					*n* = 220	*n* = 42
(*c*) Absence of rays in male tails													
Rays per side	8.9 ± 0.1	8.6 ± 0.2	8.0 ± 0.3	6.6 ± 0.3	5.9 ± 0.3	5.4 ± 0.4	6.0 ± 0.3	5.9 ± 0.3	8.6 ± 0.2	7.6 ± 0.3	8.1 ± 0.3	4.6 ± 0.3	4.9 ± 0.6
	n = 68	*n* = 34	*n* = 40	*n* = 40	*n* = 58	*n* = 34	*n* = 62	*n* = 38	*n* = 38	*n* = 44	*n* = 52	*n* = 81	*n* = 26

ced-9 (lof) causes ectopic cell deaths





What is the order of action of the *ced* genes?

Loss of CED-9 leads to all cells undergoing programmed cell death (all cells are poised to die, but for CED-9 all would!)

CED-3/4 required for programmed cell deaths

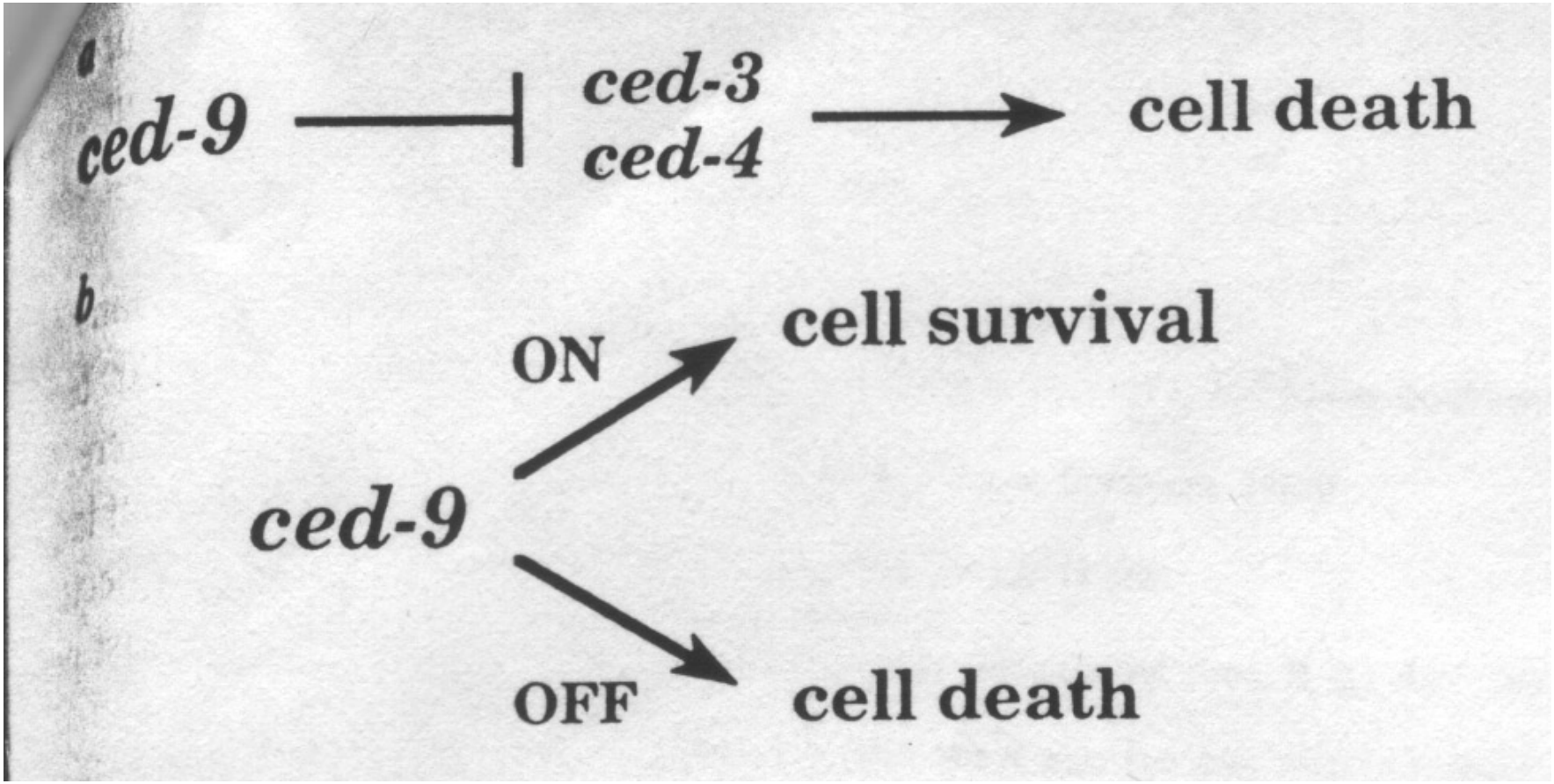
Does CED-9 inhibit CED-3/4 function to prevent programmed cell death?

ced-3 and *-4* mutations suppress *ced-9 (lof)* mutations

TABLE 3 Mutations in *ced-3* and *ced-4* suppress the defects resi

Genotype*	Sterility and maternal-effect lethality		
	Eggs laid per animal	Viable progeny	*n*
ced-9(+)	207 ± 36	207 ± 33	14
ced-9(n1950 n2077)	1.6 ± 1.4	0	20
ced-4 ced-9(n1950 n2077)	200 ± 19	160 ± 20	12
ced-4	182 ± 17	148 ± 17	12

ced function pathway



Adding more relationships to the pathway

Overexpression of Ced-3 and Ced-4 causes ectopic cell death

Enables another genetic test of *ced-9* relationship to *ced-3* and *-4*

Also lets us test the relationship between *ced-3* and *ced-4*

Overexpression of Ced-3 and Ced-4 causes ectopic cell death

Table 1. *Overexpression of* ced-3 *or* ced-4 *can kill the ALM neurons*

	Percent surviving ALMs (no. ALMS/no. sides scored)
	wild-type
—	100 (31/31)
$P_{mec\text{-}7}$*lacZ*	100 (40/40)
$P_{mec\text{-}7}$*ced-3*A	20 (9/46)
$P_{mec\text{-}7}$*ced-3*B	42 (16/38)
$P_{mec\text{-}7}$*ced-3*C	100 (48/48)
$P_{mec\text{-}7}$*ced-4*A	10 (4/39)
$P_{mec\text{-}7}$*ced-4*B	87 (33/38)
$P_{mec\text{-}7}$*ced-4*C	98 (39/40)
$P_{mec\text{-}7}$*ced-4*D	98 (40/41)

Loss of ced-9 enhances ectopic cell death caused by overexpression of Ced-3 and Ced-4

Table 2. *Effects of* ced-9 *on killing by* ced-3 *or* ced-4 *overexpression*

	Percent surviving ALMs (no. ALMs/no. sides scored)	
	ced-9; ced-3	*ced-3*
A. ALM killing by ced-3 *overexpression is better in a* ced-9(lf) *background*[a]		
$P_{mec\text{-}7}$*ced-3A*	0 (0/29)	47 (16/34)
$P_{mec\text{-}7}$*ced-3B*	0 (0/37)	30 (8/27)
$P_{mec\text{-}7}$*ced-3C*	21 (9/43)	100 (34/34)
$P_{mec\text{-}7}$*ced-3/4A*	43 (16/37)	100 (40/40)
$P_{mec\text{-}7}$*ced-3/4B*	67 (18/27)	100 (46/46)
	ced-4; ced-9	*ced-4*
B. ALM killing by ced-4 *overexpression is better in a* ced-9(lf) *background*[b]		
$P_{mec\text{-}7}$*ced-4A*	0 (0/30)	43 (12/28)
$P_{mec\text{-}7}$*ced-4B*	53 (18/34)	94 (32/34)
$P_{mec\text{-}7}$*ced-4C*	42 (15/36)	97 (36/37)
$P_{mec\text{-}7}$*ced-4D*	15 (4/27)	100 (36/36)
$P_{mec\text{-}7}$*ced-3/4A*	70 (35/50)	100 (41/41)
$P_{mec\text{-}7}$*ced-3/4B*	74 (37/50)	100 (30/30)

Ced-3 is required for ectopic killing caused by overexpression of Ced-4, but not vice versa

Table 5. *ALM killing by* ced-4 *overexpression is inhibited by a mutation in the endogenous* ced-3 *gene*

	Percent surviving ALMs (no. ALMs/no. sides scored)			
	wild type	*ced-3*	*ced-4 ced-9*	*ced-4 ced-9; ced-3*
$P_{mec\text{-}7}$*ced-4*A	10 (4/39)	71 (27/38)	0 (0/30)	71 (27/38)
$P_{mec\text{-}7}$*ced-4*B	87 (33/38)	100 (20/20)	53 (18/34)	84 (27/32)
$P_{mec\text{-}7}$*ced-4*C	98 (39/40)	100 (37/37)	42 (15/36)	98 (39/40)
$P_{mec\text{-}7}$*ced-4*D	98 (40/41)	100 (36/36)	15 (4/27)	100 (40/40)

Table 7. *ALM killing by* ced-3 *overexpression can occur in the absence of* ced-4 *function*

	Percent surviving ALMs (no. ALMS/no. sides scored)			
	wild type	*ced-4*	*ced-9; ced-3*	*ced-4 ced-9; ced-3*
$P_{mec\text{-}7}$*ced-3*A	20 (9/46)	43 (24/56)	0 (0/29)	27 (8/30)
$P_{mec\text{-}7}$*ced-3*B	42 (16/38)	30 (18/61)	0 (0/37)	38 (12/32)
$P_{mec\text{-}7}$*ced-3*C	100 (48/48)	90 (35/39)	21 (9/43)	85 (28/33)

Questions Geneticists Ask

Does the (recessive) mutation confer a null phenotype?

Compare phenotype of a diploid homozygous for the mutation to a diploid heterozygous for the mutation and for a deficiency

geneX^{-}/geneX^{-} **geneX^{-}/Df**

Questions Geneticists Ask

Does the (dominant) mutation represent a gain-of-function or an instance of haploinsufficiency?

Compare phenotype of a diploid heterozygous for the mutation to a diploid heterozygous for a deficiency of the region

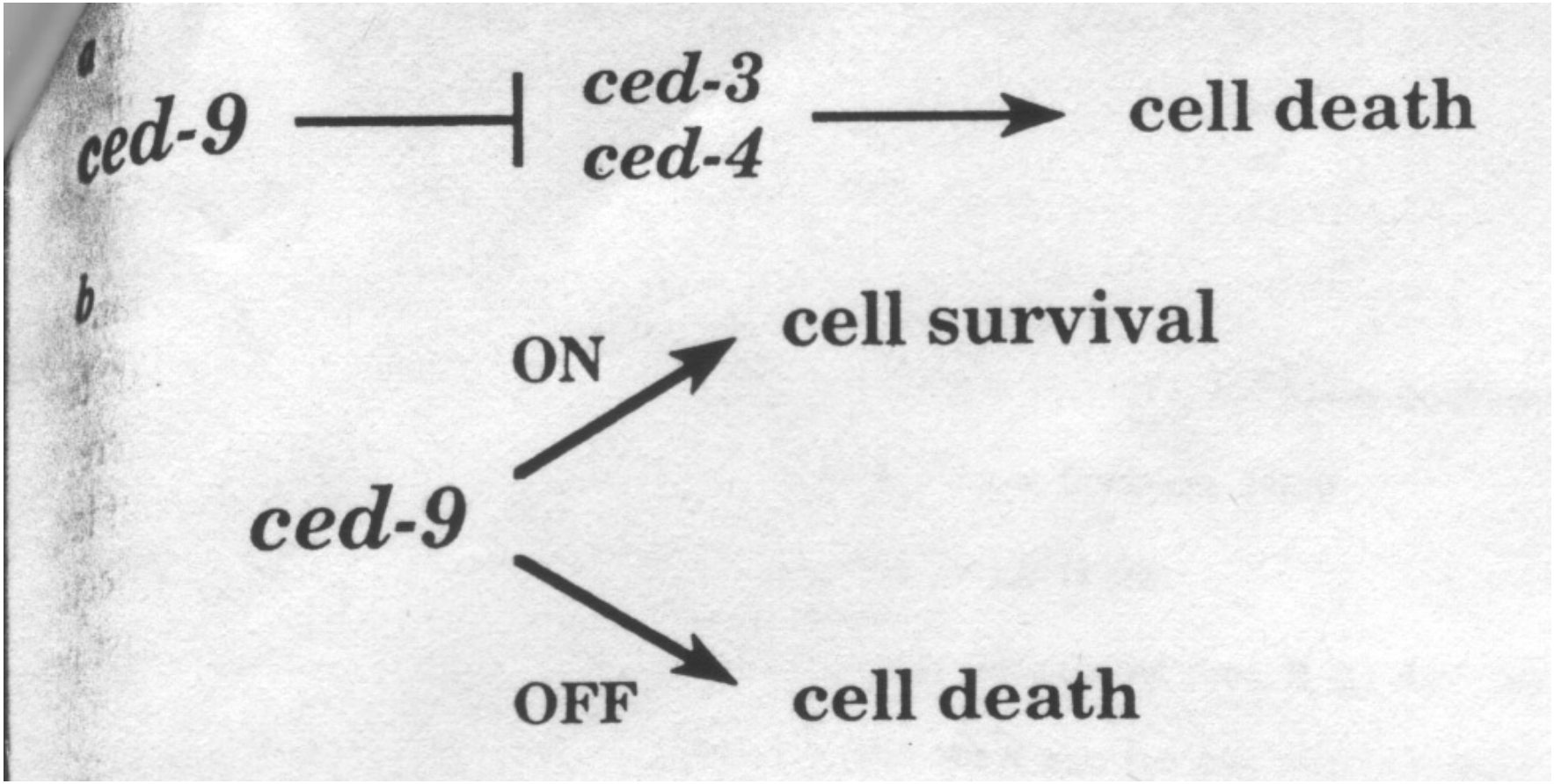
wildtype/mutation **wildtype/Df**

Questions Geneticists Ask

Do the identified genes function in a linear pathway?

Compare phenotypes of two double mutant strains. For each gene, one needs alleles with contrasting phenotypes. In the case of *ced* genes, alleles that confer no cell death (ncd) and alleles that confer ectopic cell death (ecd) are available

ced-3^{ncd} *ced-4*^{ecd} *ced-3*^{ecd} *ced-4*^{ncd}



ced-9 ⊣ *ced-4* → *ced-3* → **cell death**

One more player, Egl-1

Gain-of-function *egl-1* mutations cause HSNs to undergo programmed cell death. (The Horvitz lab used these *egl-1* mutations to isolate some *ced* mutants.)

Loss-of-function *egl-1* mutations, isolated exactly as *ced-9 (lof)* alleles were isolated, prevent programmed cell death.

Epistasis experiments place *egl-1* upstream of all *ced* gene functions

egl-1 induced ectopic killing is suppressed by mutations that block pcd

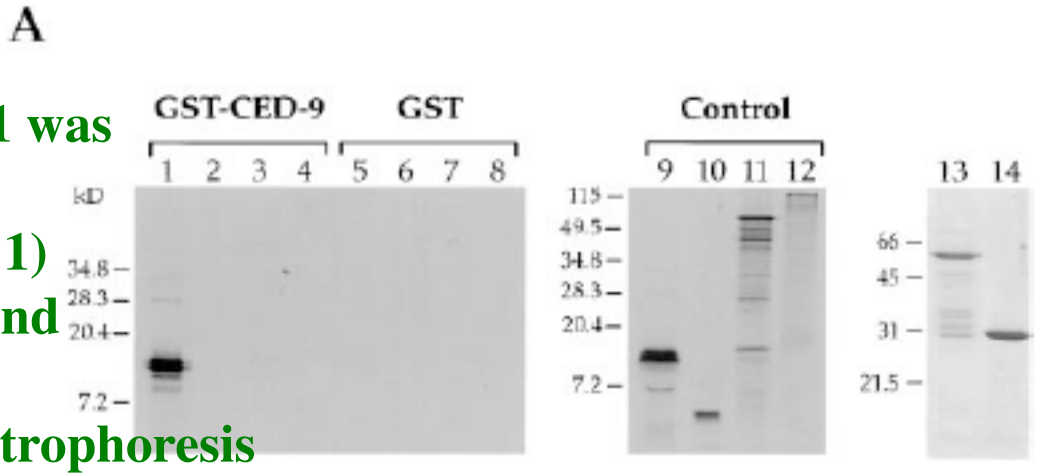
Table 4. *egl-1*–Induced Ectopic Killing Is Suppressed by Mutations that Block Programmed Cell Death

Transgene	% ALMs Surviving (n = 60)
P_{mec-7} A	98
P_{mec-7} B	100
P_{mec-7} *egl-1* A	8
P_{mec-7} *egl-1* B	9
P_{mec-7} *egl-1* C	10
P_{mec-7} *egl-1C/+*	50
P_{mec-7} *egl-1* C; *ced-9* (gf)	98
P_{mec-7} *egl-1* C; *ced-4* (lf)	97
P_{mec-7} *egl-1* C; *ced-3* (lf)	98

Biochemistry and cell biology of Ced proteins

1. Egl-1 and Ced-9 interact (IP experiments)

Radioactive Egl-1 was incubated with GST-Ced-9 (lane 1) or GST (lane 5) and bound proteins separated by electrophoresis



The BH3 domain of Egl-1 is required; compare lanes 1 and 2

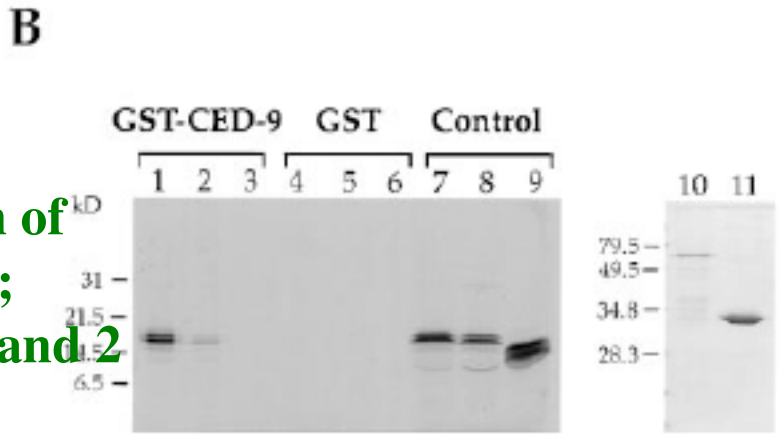


Figure 3. EGL-1 and CED-9 Interactions

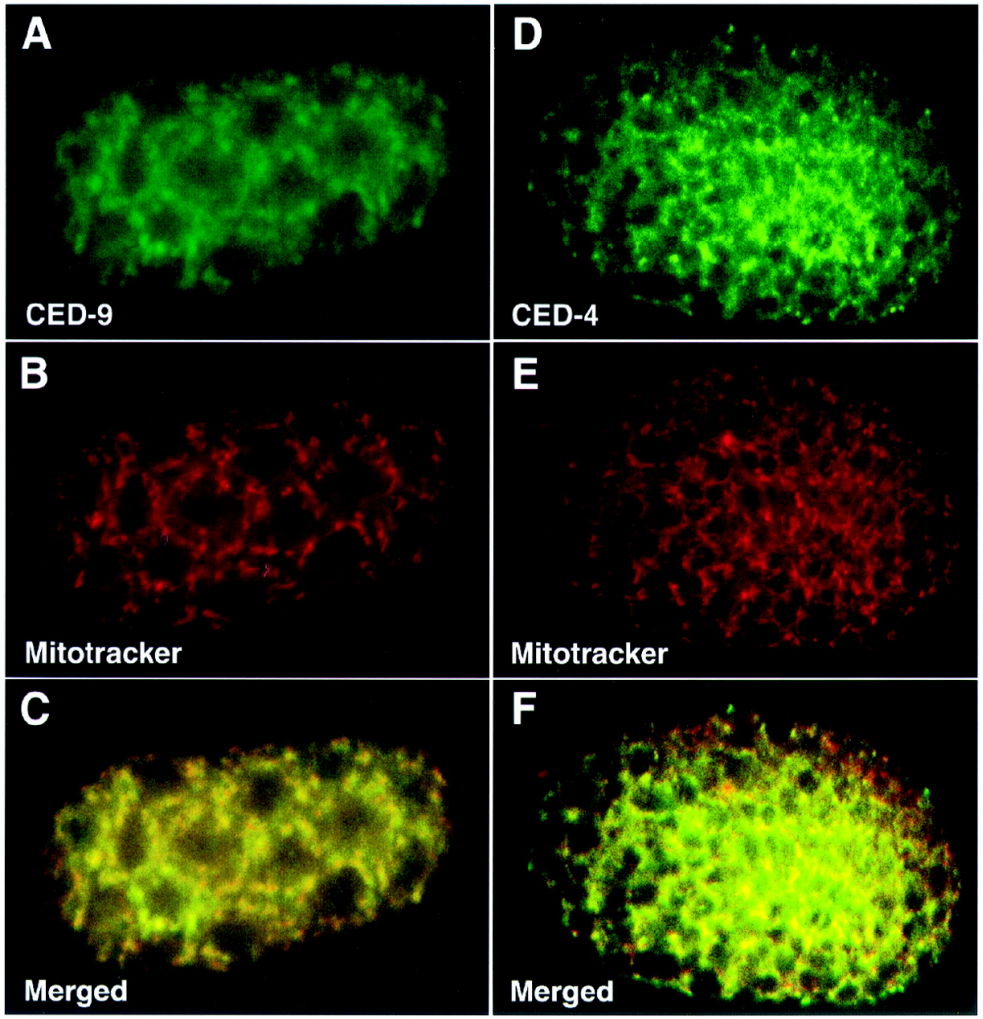
Biochem and cell biology, continued

2. Bunches of IP experiments demonstrate interaction between Egl-1 and Ced-9, between Ced-9 and Ced-4, and between Ced-3 and Ced-4

3. Ced-9 is localized to the mitochondrial outer membrane and recruits Ced-4

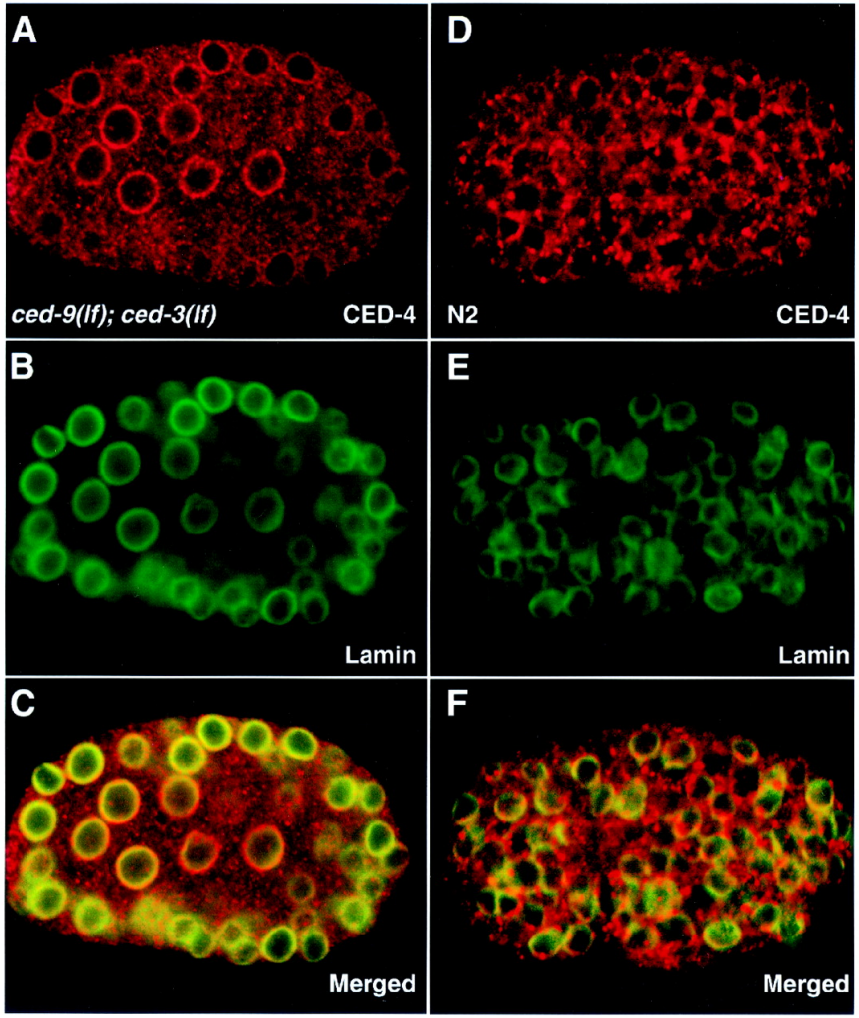
4. Induction of programmed cell death induces Ced-4 translocation to the nuclear membrane but not in gof Ced-9 mutants

Figure 1 CED-9 and CED-4 are localized to mitochondria in WT embryos.



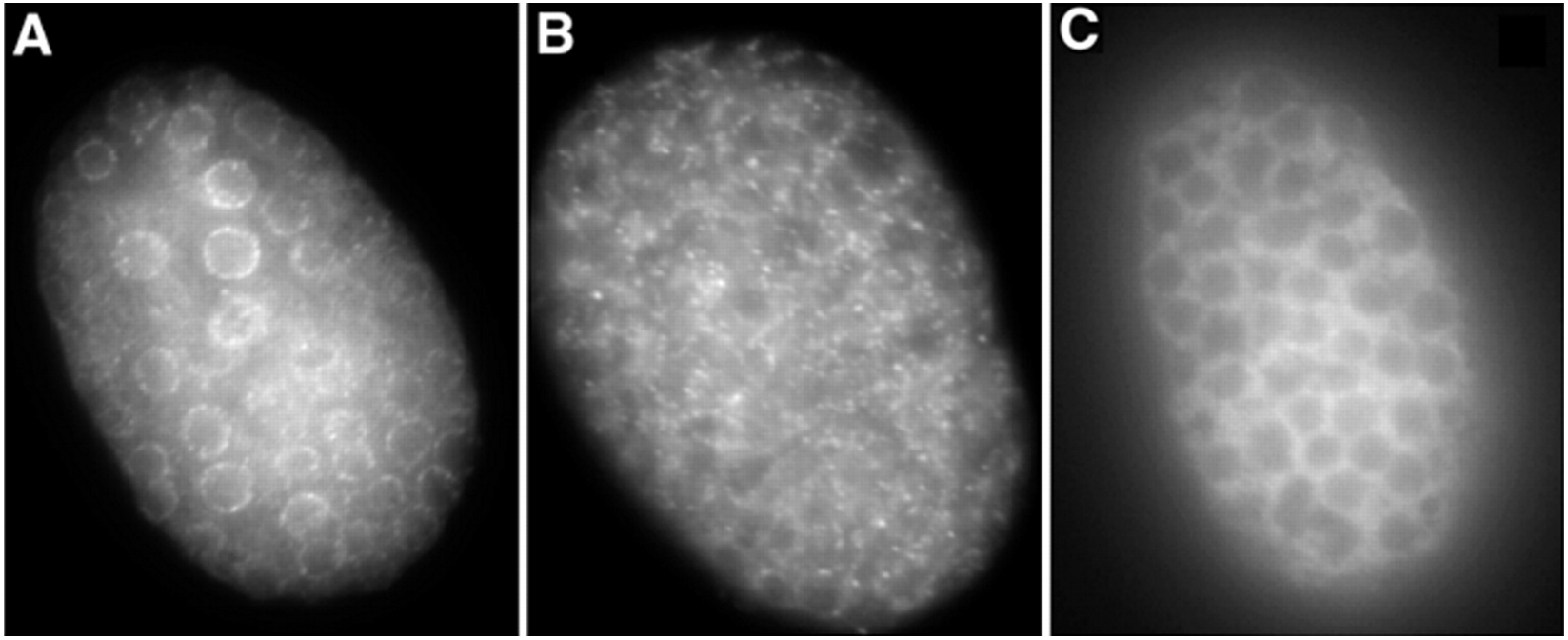
F Chen et al. Science 2000;287:1485-1489

Figure 2 CED-9 is required for the localization of CED-4 to mitochondria.



F Chen et al. Science 2000;287:1485-1489

Figure 4 Overexpression of EGL-1 induces CED-4 translocation from mitochondria to nuclear membranes inced-9(+) embryos but not inced-9(n1950) embryos.

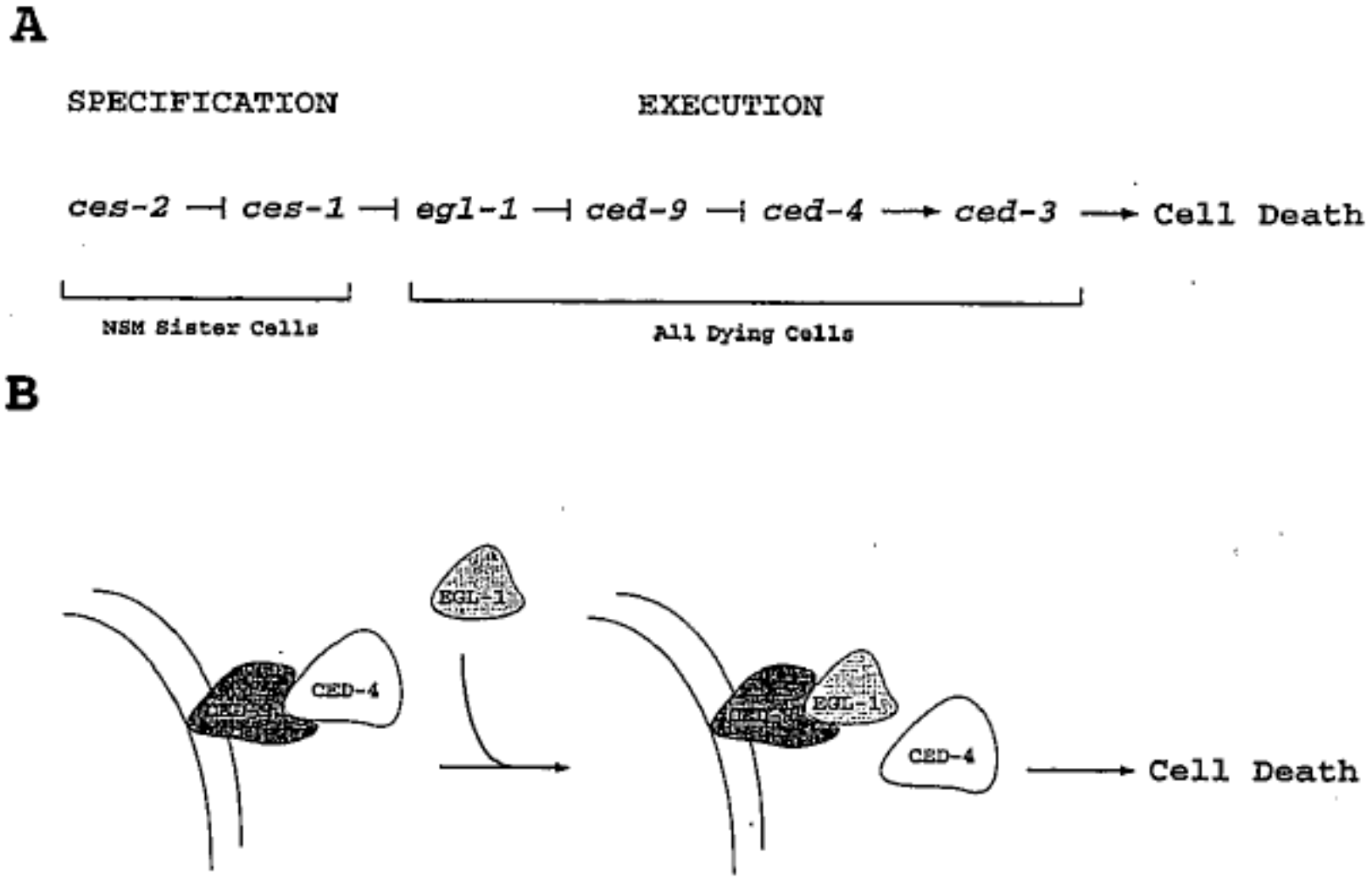


Ced-4 in wt embryos overexpressing Egl-1

Ced-4 in *ced-9(gof)* embryos overexpressing Egl-1

F Chen et al. Science 2000;287:1485-1489

Current model



Model with more detail added

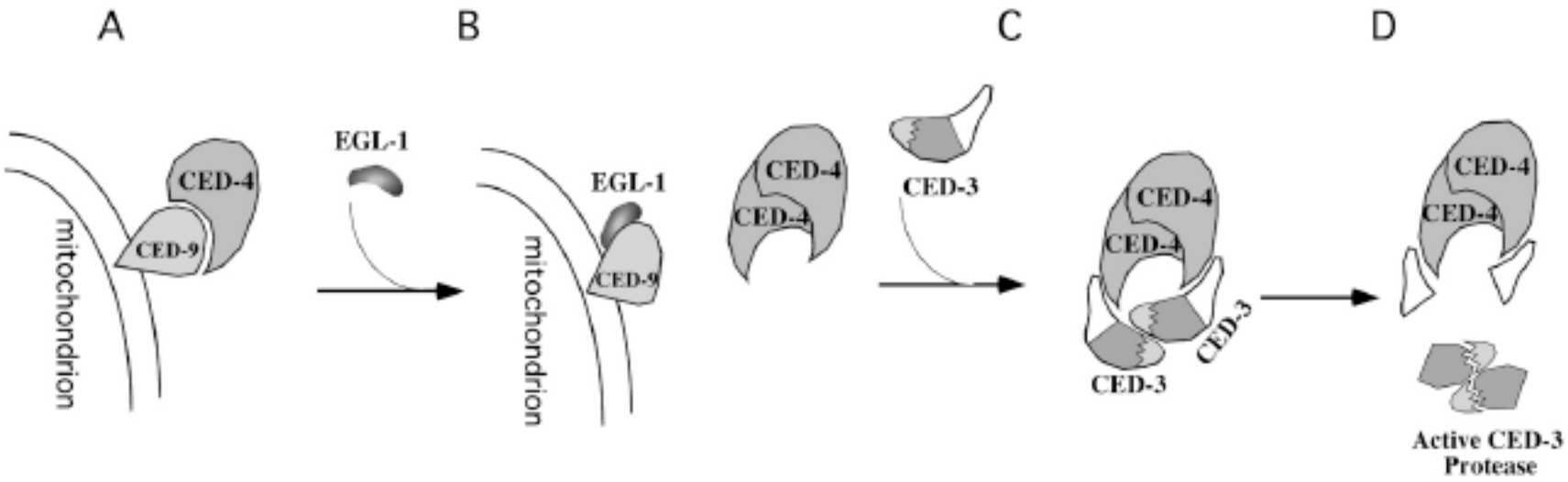


Figure 3. Biochemical model for the activation of programmed cell death. (A) In living cells, CED-4 is tethered to the surface of mitochondria through binding to CED-9. (B) In cells that are doomed to die, the death initiator EGL-1 binds to CED-9, causes a major CED-9 conformational change, and triggers the disassociation of CED-4 from CED-9. (C) Released CED-4 proteins translocate to perinuclear membranes and undergo oligomerization, which brings two CED-3 proenzymes to close proximity. (D) CED-3 proenzymes undergo autoproteolytic activation.

Cloning *ced* genes revealed similarities to mammalian proteins

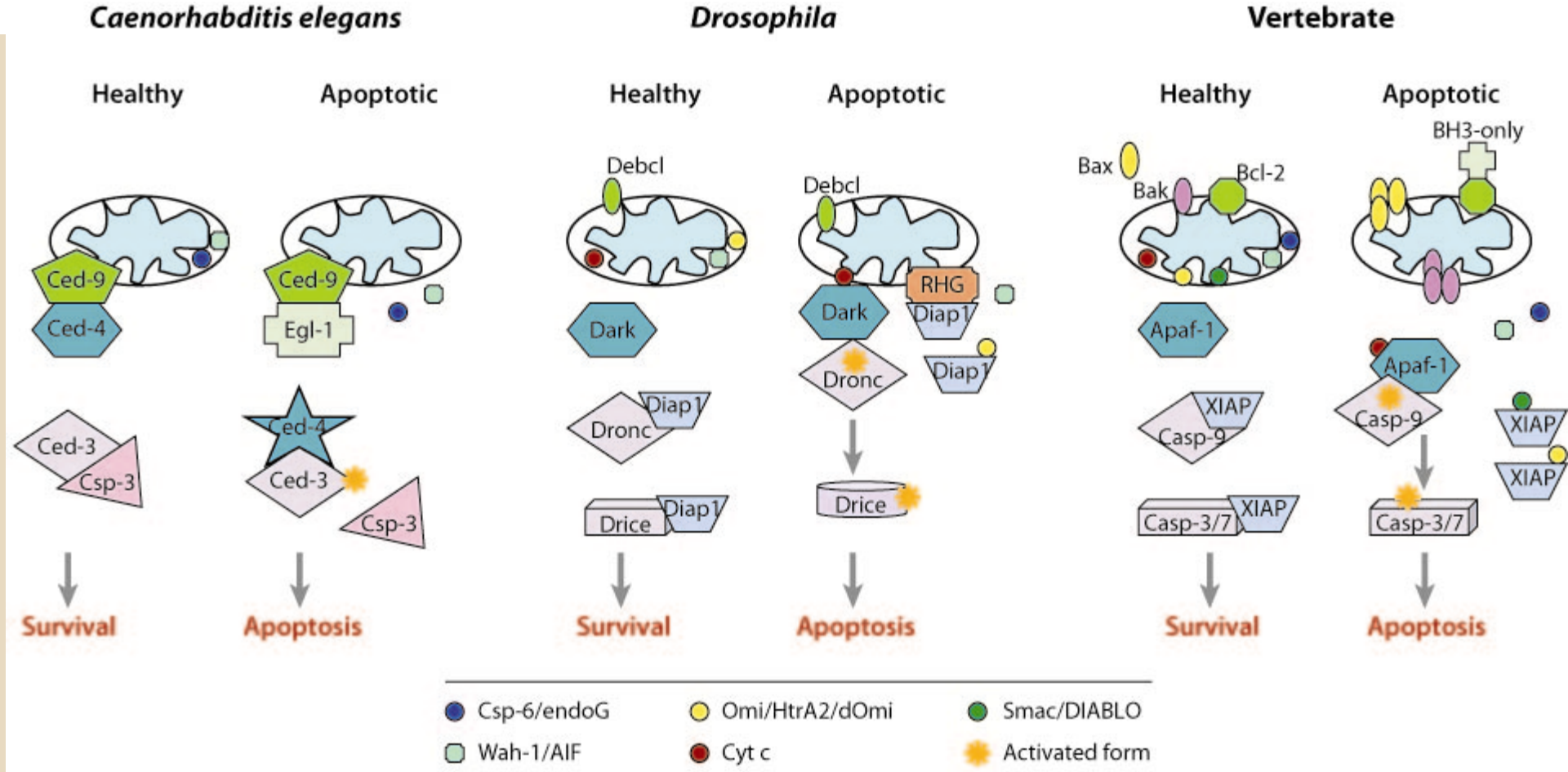
Ced-3 is similar to mammalian interleukin converting enzyme, a cysteine protease

Ced-3 is therefore proposed to be a cysteine protease

In vitro substrates for Ced-3 include actin, tubulin, and proteins involved in ATP synthesis and in DNA synthesis

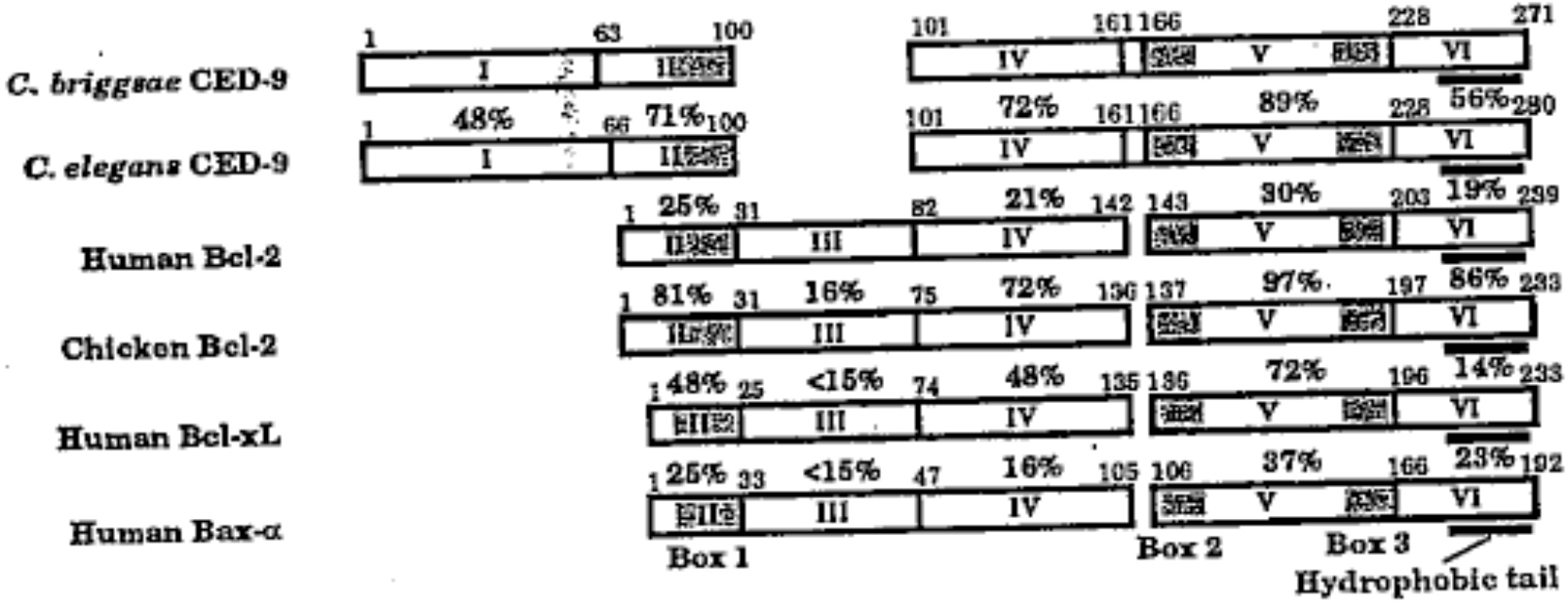
Ced-9 is a homolog of Bcl-2, a mammalian oncogene

Comparison of apoptopic pathways

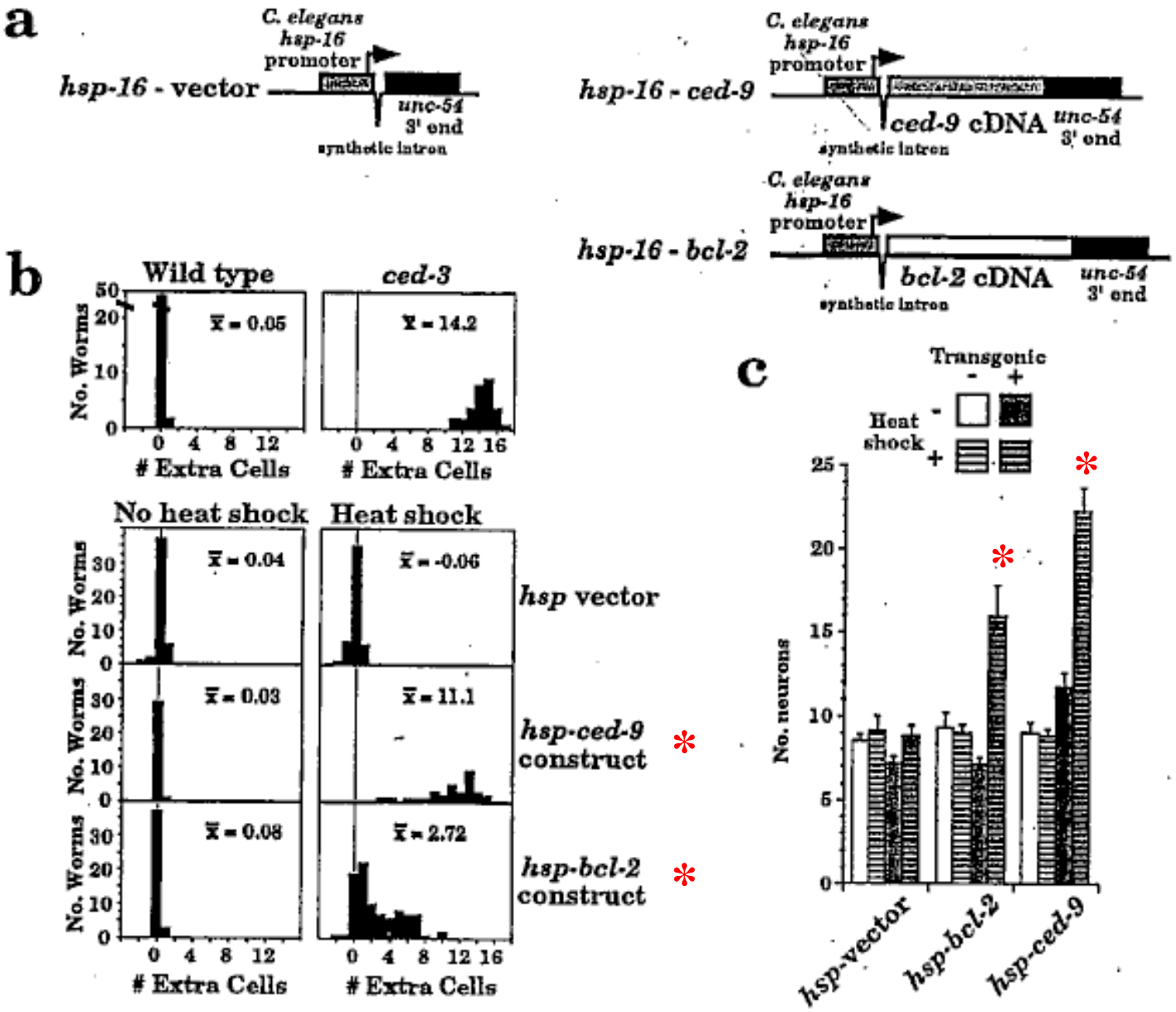


Wang C, Youle RJ. 2009.
Annu. Rev. Genet. 43:95–118

Ced-9 is a functional homolog of mammalian bcl-2



Over-expression of *bcl-2* mimics over-expression of *ced-9*



Parallels of CED-9 in worms and Bcl-2 in humans.

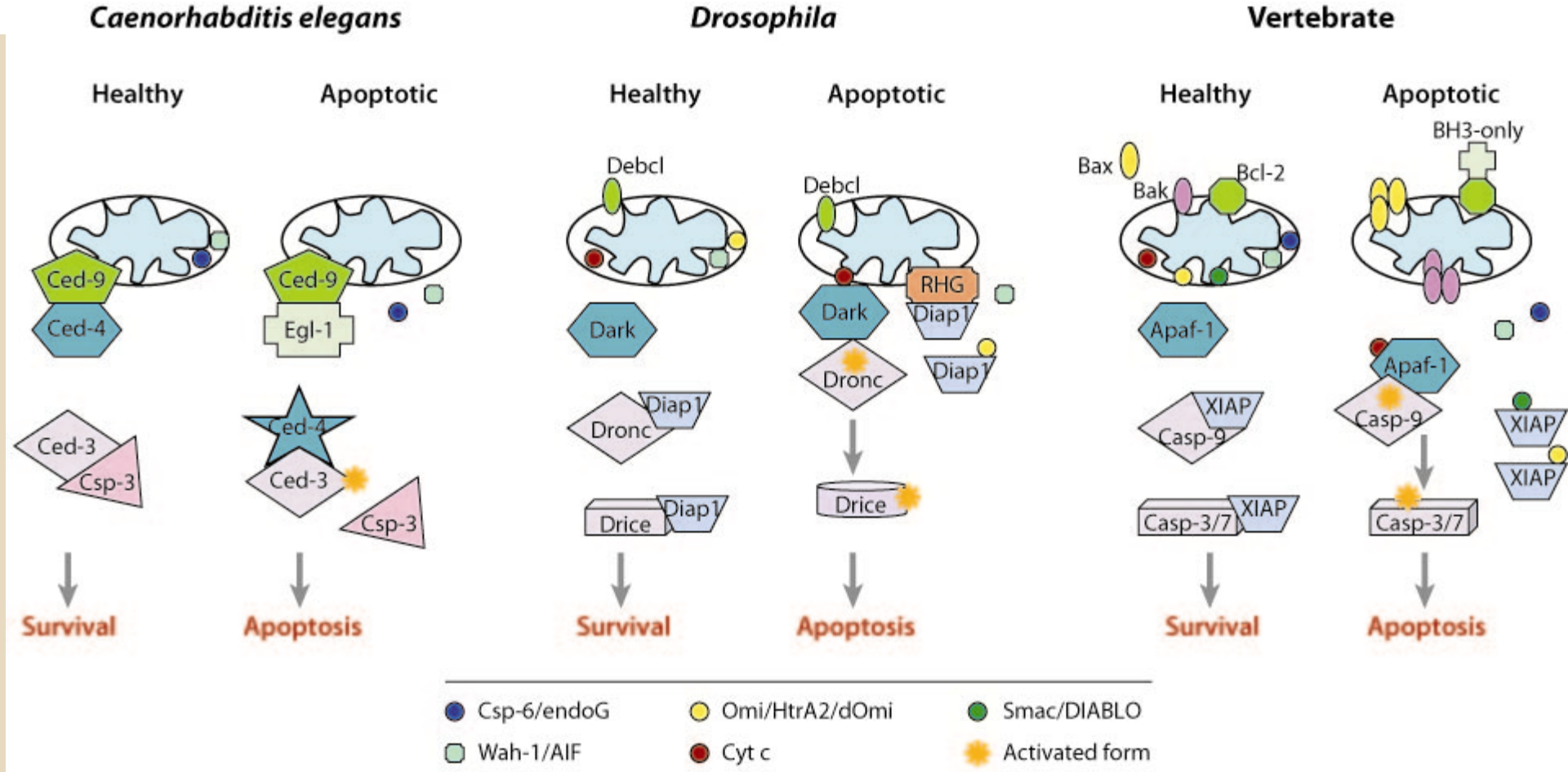
Bcl-2 is a human oncogene with properties similar to CED-9

Over-expression of Bcl-2 prevents or delays cell death in B-cell and T-cell lineages.

Bcl-2 expressed at high levels in blood stem cell lineages; loss of expression correlates with appearance of cell death

In cancer, chromosome translocations activate Bcl-2 expression, preventing cell death in hematopoietic lineages. This results in a leukemia due to over-proliferation of some blood cell lineages. No LOF alleles.

Comparison of apoptopic pathways



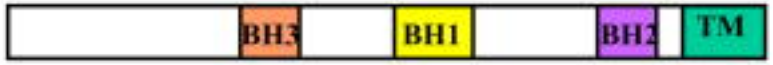
Wang C, Youle RJ. 2009.
Annu. Rev. Genet. 43:95–118

3 subfamilies of Bcl-like proteins

1. Anti-apoptotic proteins, BH1-4 – BCL-2, BCL-xL, MCL-1, A1, BCL-w

BCL-2

2. Pro-apoptotic proteins, BH1-3 – BAX, BAK, BOK



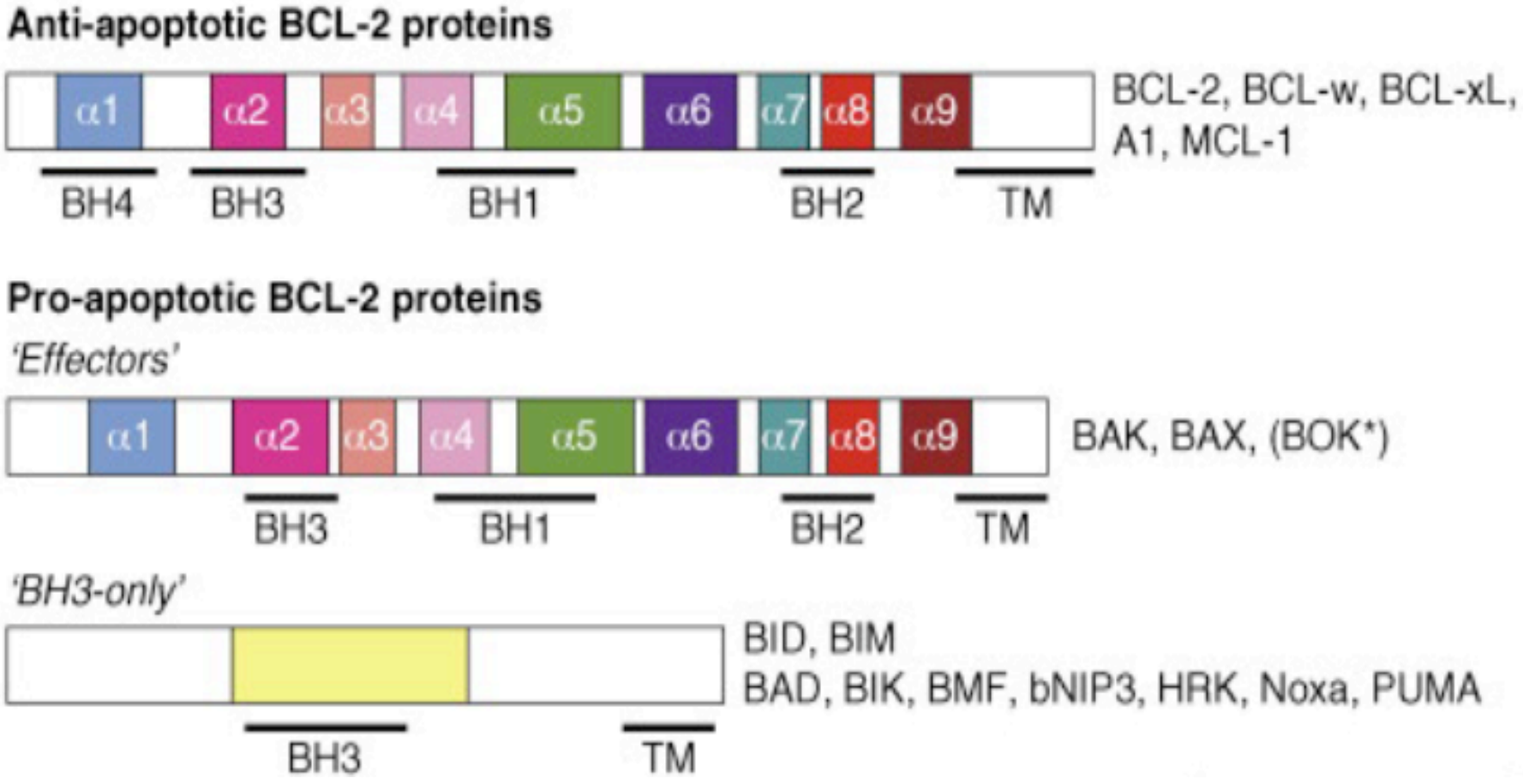
BAX

3. Pro-apoptotic proteins, BH3-only – BIK, BID, BIM, BAD,

PUMA, NOXA, HRK etc

BIK

Another look at the subfamilies

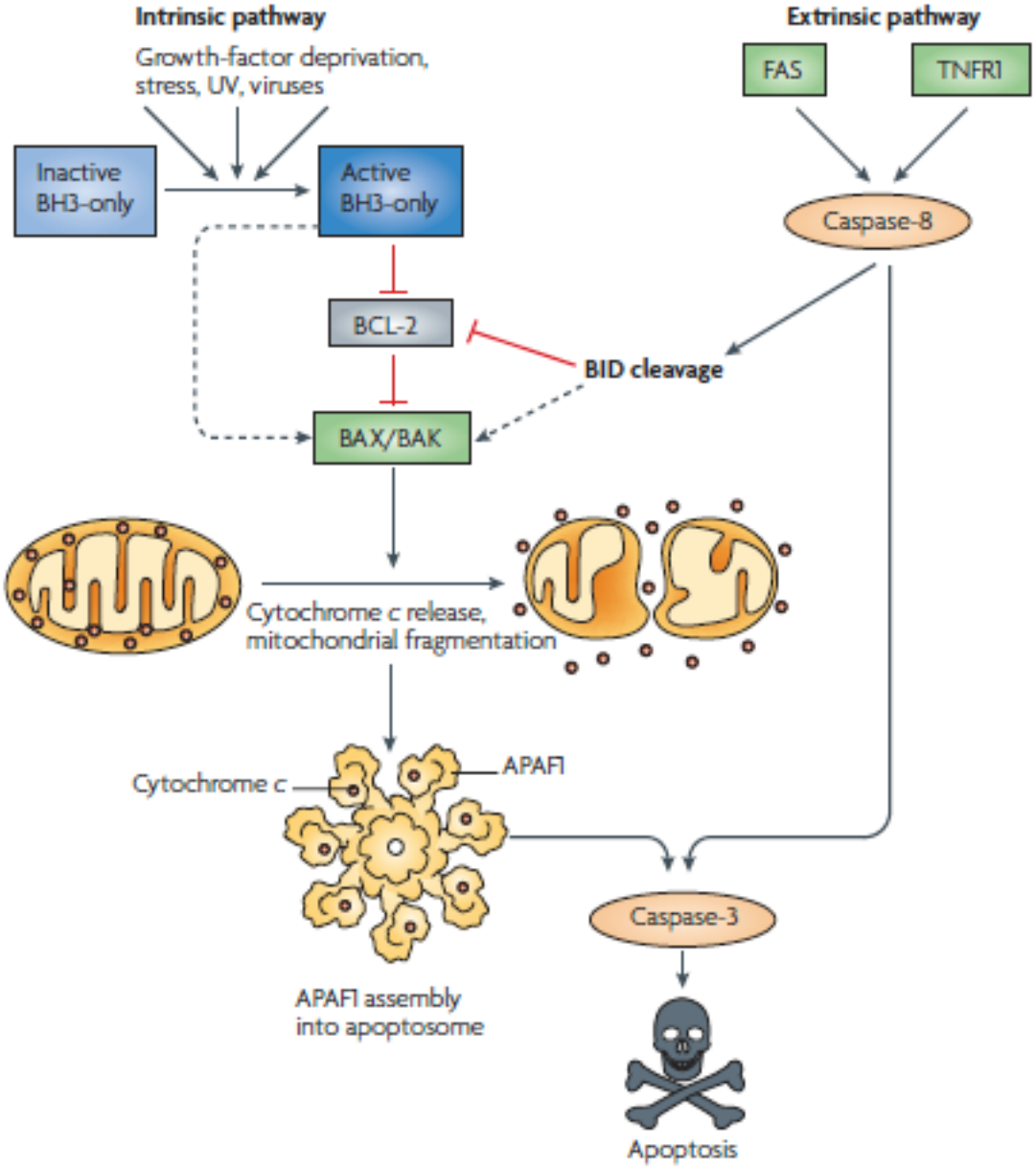


Mouse mutants defective for Bcl-2 family members have altered cell death phenotypes

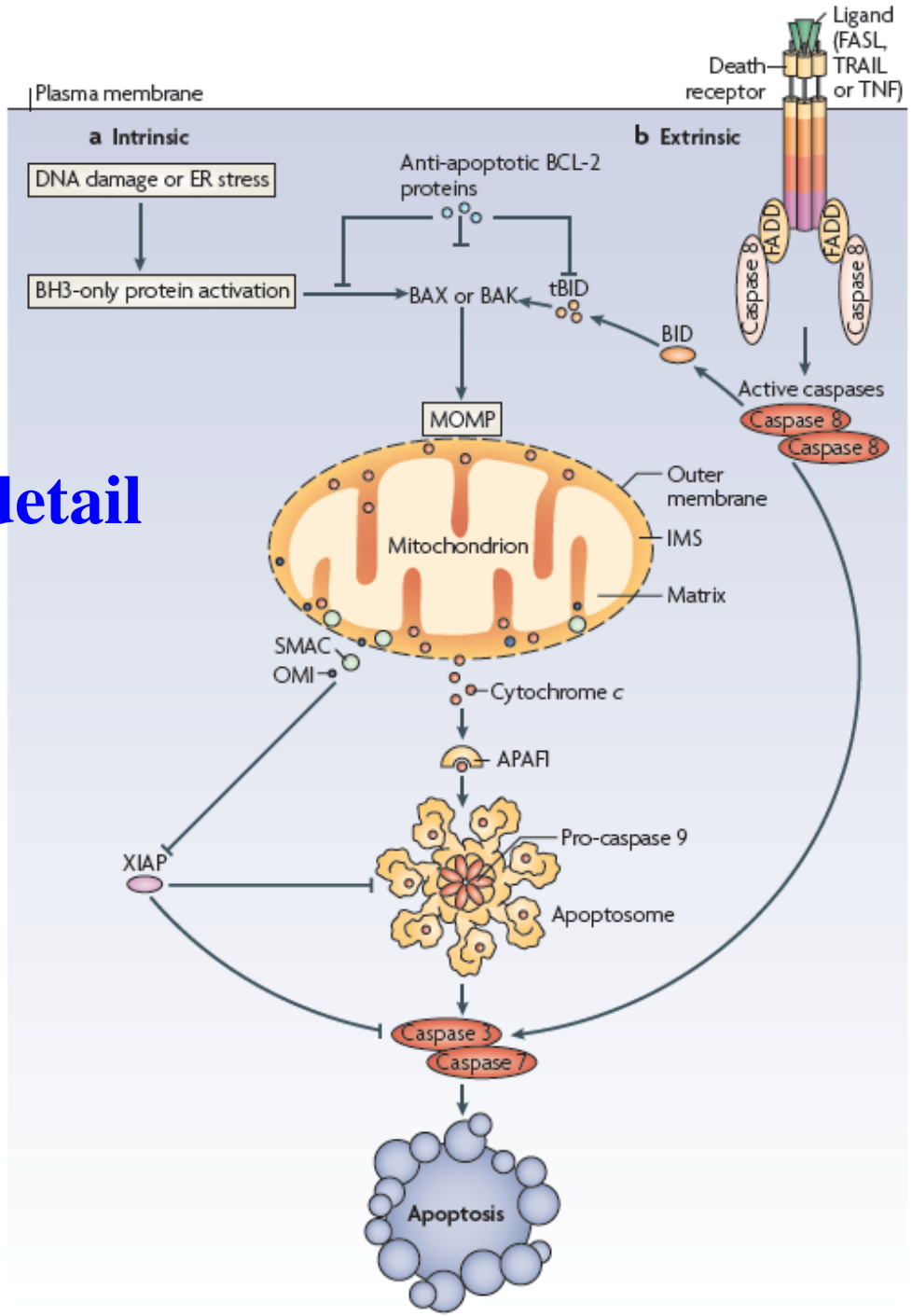
BCL-2 family member	Defects caused by its deletion*	Refs
Pro-survival family members		
BCL-2	Abnormal death of renal epithelial progenitors, melanocyte progenitors and mature B and T lymphocytes. Causes fatal polycystic kidney disease (100% mortality by 6 weeks), premature greying and lymphopoenia (but all of these effects can be rescued by concomitant loss of the BH3-only protein BIM).	130
BCL-XL	Abnormal death of fetal erythroid progenitors and neuronal cells. Causes death around embryonic day 14 (100% mortality).	129
BCL-W	Abnormal death of developing sperm cells. Causes male sterility.	132
A1A	Abnormally accelerated death of granulocytes and mast cells in culture.	133
MCL1	Failure in implantation. Conditional knockout causes premature death of immature and mature B and T lymphoid cells, as well as haemopoietic stem cells.	128
Pro-apoptotic BAX/BAK family members		
BAX	Mild lymphoid hyperplasia, male sterility due to sperm-cell differentiation defect.	135
BAK	No obvious defects detected so far.	136
Pro-apoptotic BH3-only proteins		
BIM	Lymphoid and myeloid cell hyperplasia, fatal SLE-like autoimmune disease (on mixed genetic C57BL/6x129SV background), many cell types are abnormally resistant to cytokine deprivation, deregulated calcium flux and the chemotherapeutic drug taxol; mild but significant resistance of many cell types to DNA damage and glucocorticoids.	143
BID	BID-deficient mice are resistant to Fas-activation-induced hepatocyte killing and fatal hepatitis; however, some cell types (such as lymphoid cells) are normally sensitive to Fas-induced apoptosis.	13, 14
PUMA	Many cell types are profoundly resistant to DNA damage; many are also resistant to cytokine deprivation, glucocorticoids and phorbol ester.	150,151
BAD	Mild resistance of some cell types to deprivation of epidermal growth factor or insulin growth factor.	154
HRK	Abnormal, although relatively mild, resistance of certain neuronal populations to deprivation of nerve growth factor.	155,156
BIK	No obvious defects detected so far.	158
NOXA	Relatively mild resistance of fibroblasts to γ-irradiation or etoposide, but profound resistance of these same cells and keratinocytes in the skin to ultraviolet irradiation.	150

*These are phenotypes found in mice. The roles of these proteins may differ in humans. BAD, BCL-2 antagonist of cell death; BAK, BCL-2-antagonist/killer-1; BAX, BCL-2-associated X protein; BCL-2, B-cell lymphoma-2; A1A, BCL-2-related protein A1A; BCL-W, BCL-2-like-2; BCL-XL, a BCL-2-like protein; BID, BH3-interacting domain death agonist; BIK, BCL-2-interacting killer; BIM, BCL-2-like-11; HRK, harakiri (also known as death protein-5); MCL1, myeloid cell leukaemia sequence-1; PUMA, BCL-2 binding component-3; SLE, systemic lupus erythematosus.

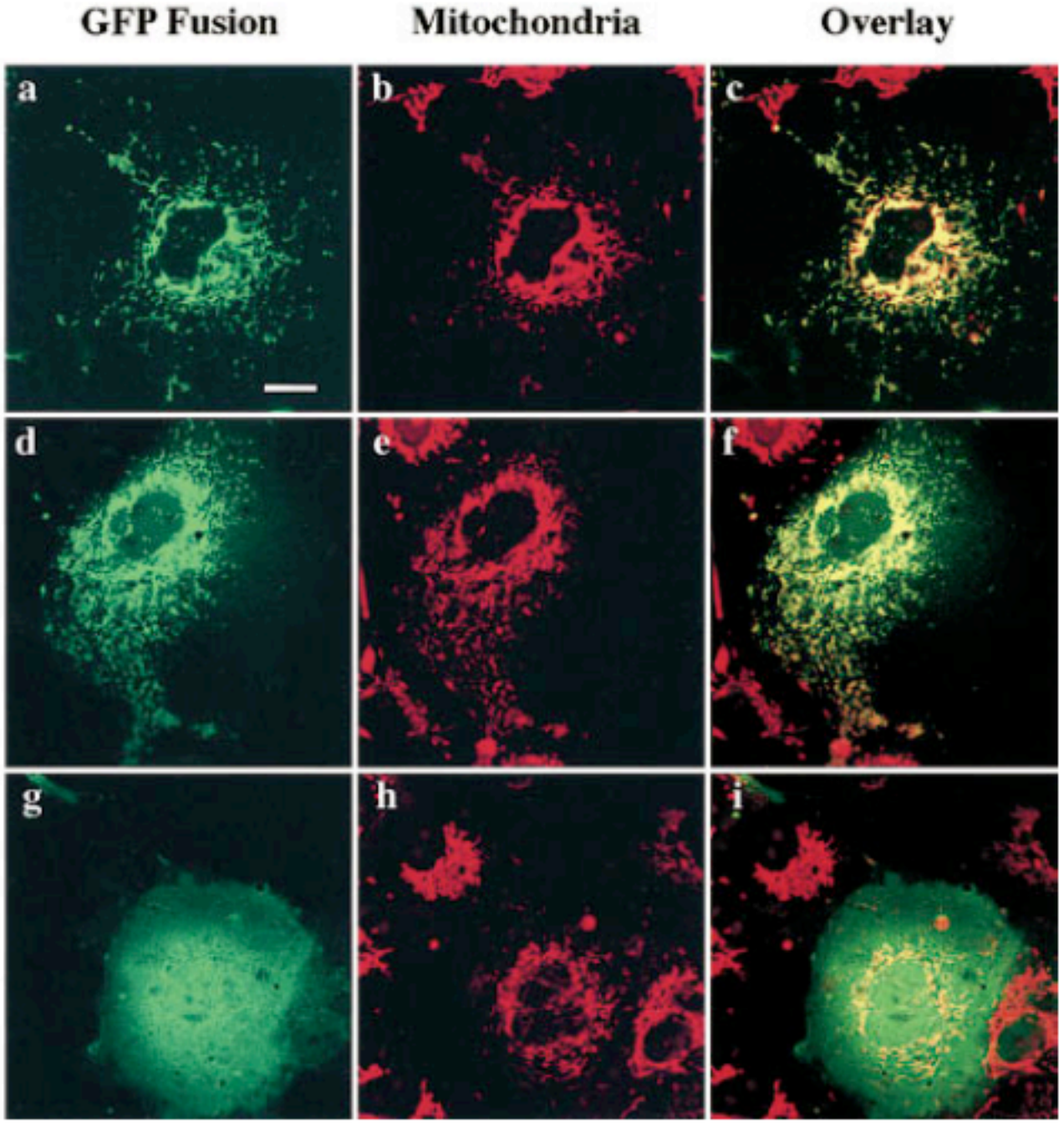
A model for mammalian cells



Same model, a little more detail



Protein localization in dividing cells



Some Bcl is localized to the mitochondrion

Bax is in the cytoplasm

Bax localization after induction of apopotosis

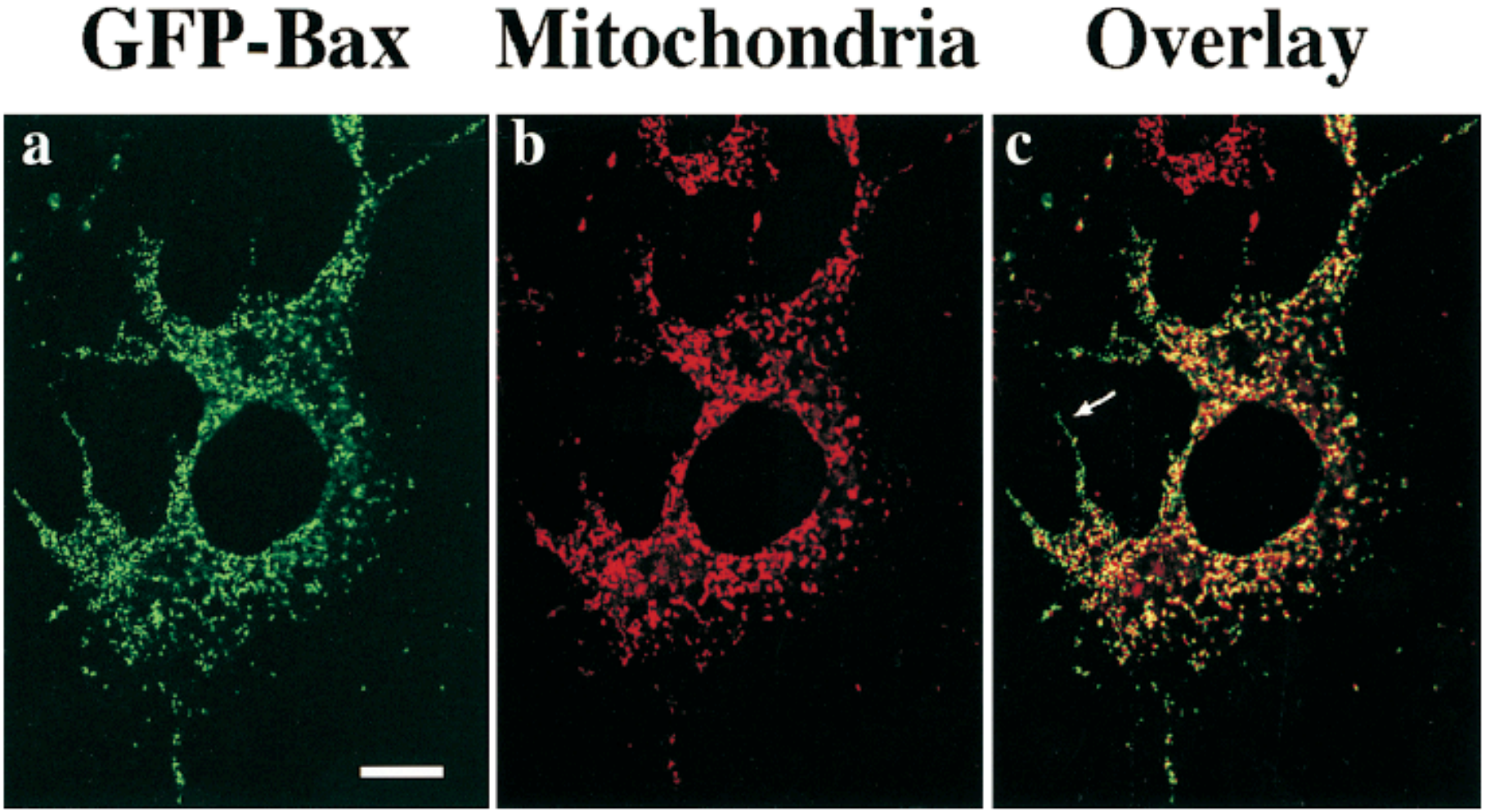
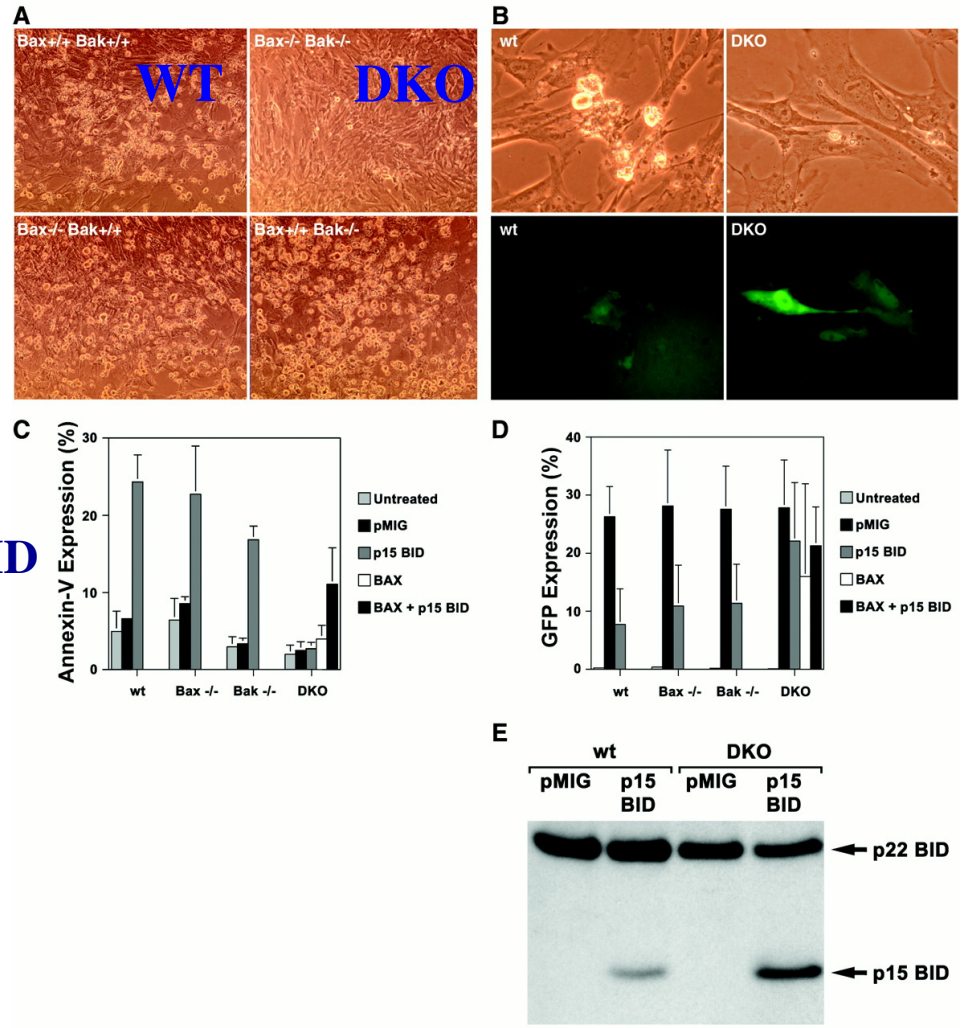


Figure 1 Resistance of Bax, Bak doubly deficient murine embryonic fibroblasts (MEFs) to tBID-induced apoptosis.



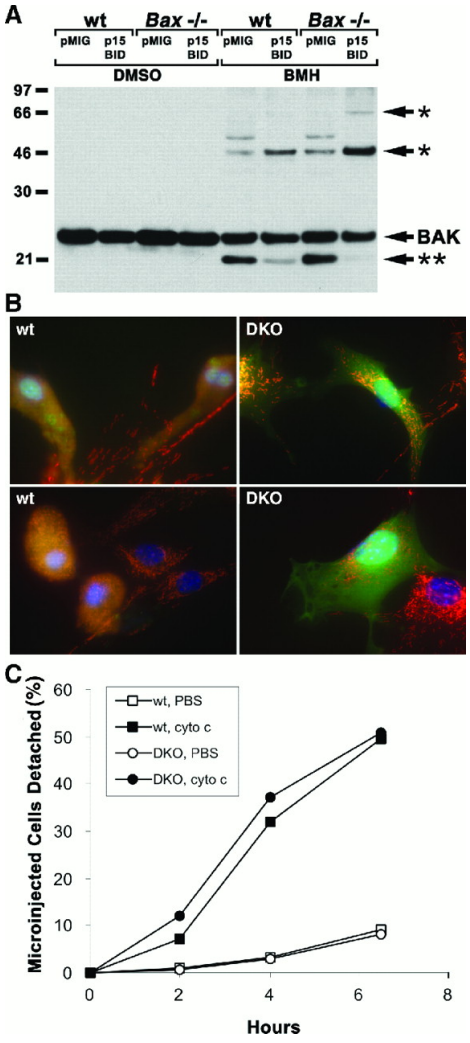
GFP identifies infected cells (expressing tBID)

Quantitation of tBID induced apoptosis

M C Wei et al. Science 2001;292:727-730

Published by AAAS

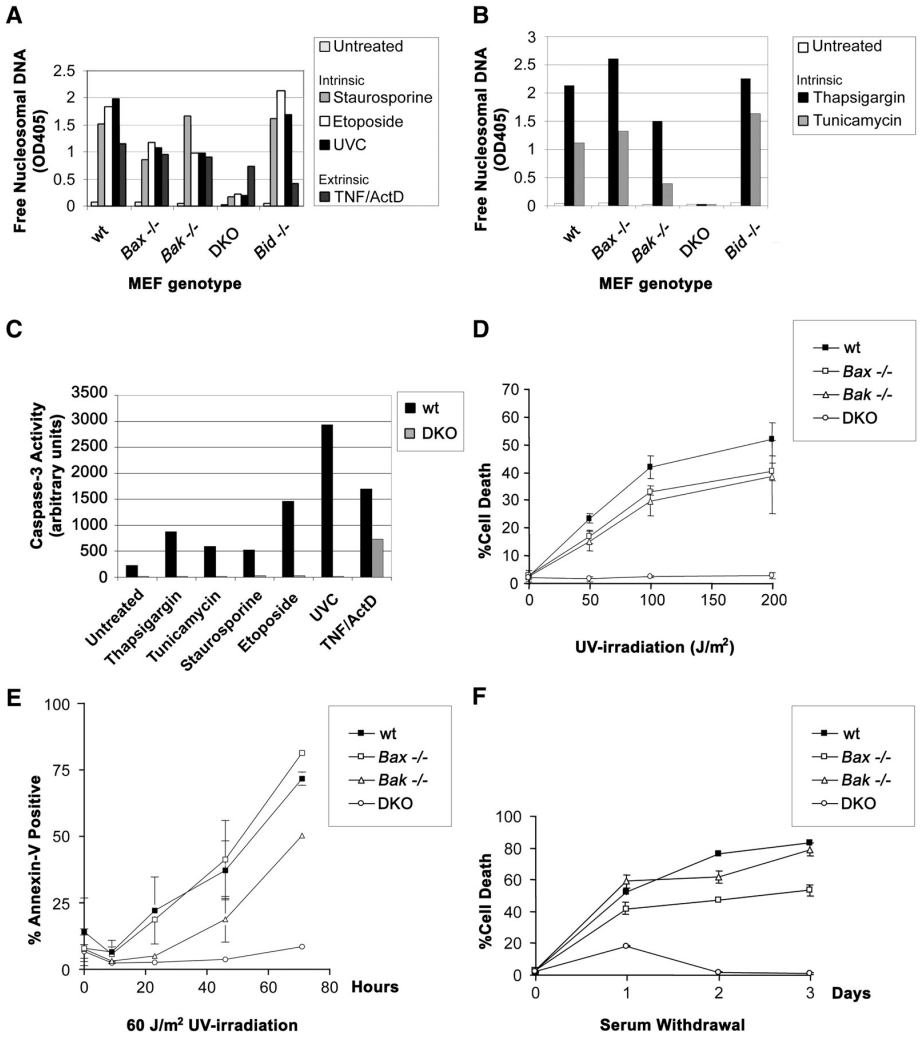
Figure 2 Function of BAX and BAK downstream of tBID and upstream of cytochrome c release.



Cytochrome c microinjection

M C Wei et al. Science 2001;292:727-730

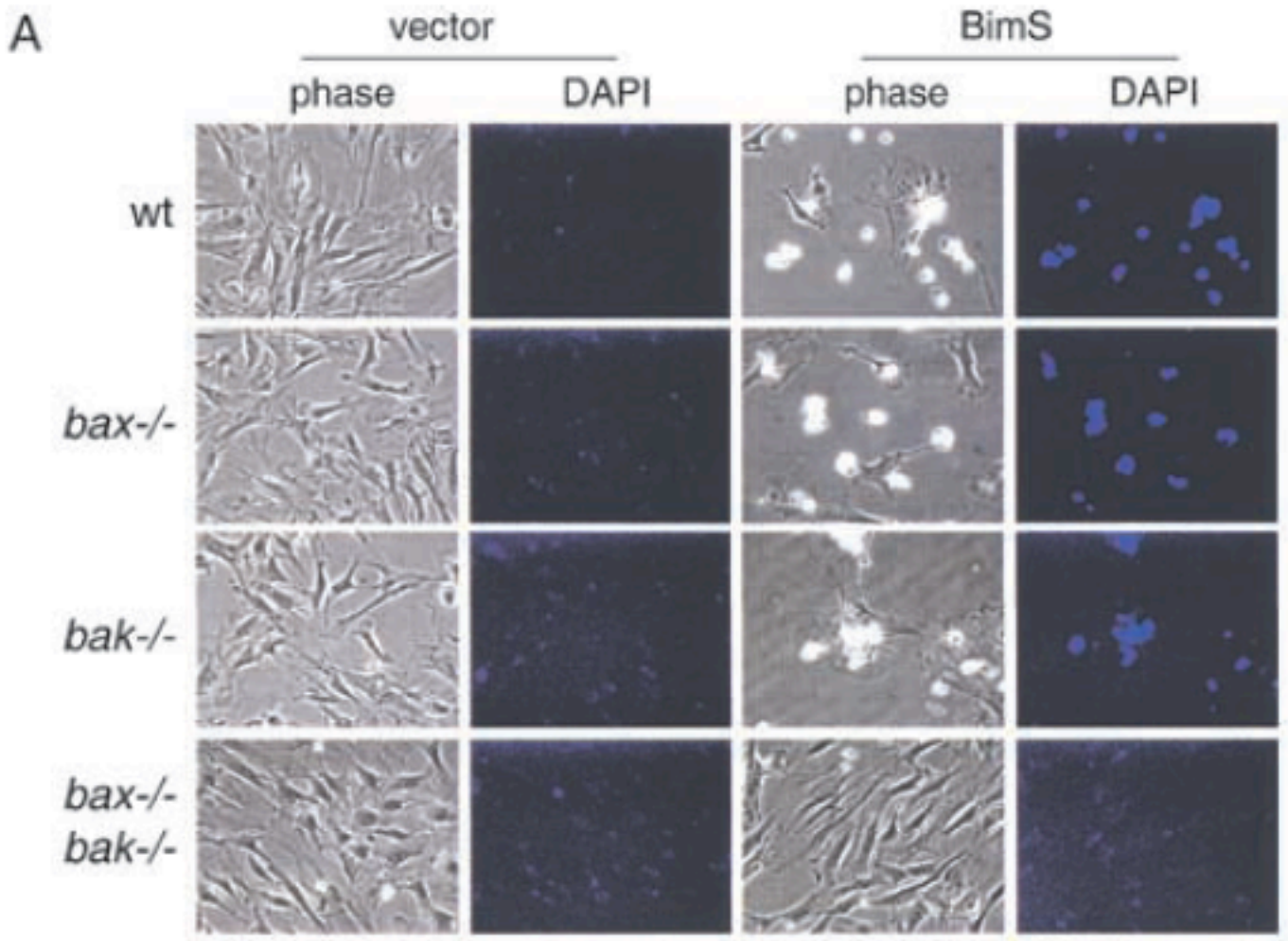
Figure 4 Resistance of Bax, Bak doubly deficient MEFs to multiple intrinsic death signals.



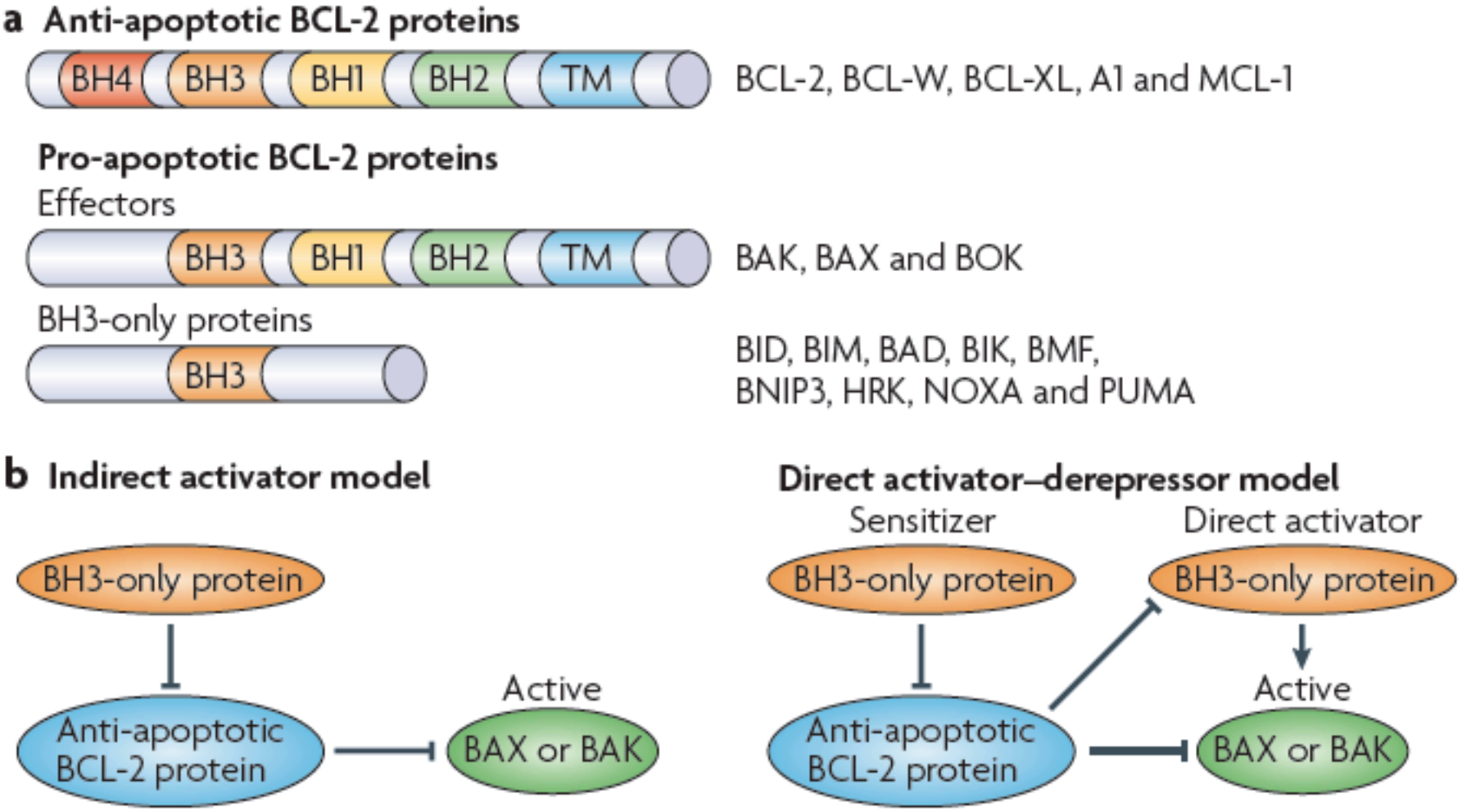
M C Wei et al. Science 2001;292:727-730

Published by AAAS

Bak and Bax are required for Bims-induced apoptosis



BimS is a BH3-only protein


a Anti-apoptotic BCL-2 proteins
BH4
BH3
BH1
BH2
TM
BCL-2, BCL-W, BCL-XL, A1 and MCL-1
Pro-apoptotic BCL-2 proteins
Effectors
BH3
BH1
BH2
TM
BAK, BAX and BOK
BH3-only proteins
BH3
BID, BIM, BAD, BIK, BMF,
BNIP3, HRK, NOXA and PUMA
b Indirect activator model
BH3-only protein
Anti-apoptotic
BCL-2 protein
Active
BAX or BAK
Direct activator–derepressor model
Sensitizer
Direct activator
BH3-only protein
BH3-only protein
Anti-apoptotic
BCL-2 protein
Active
BAX or BAK

Cytochrome c is required for oligomerization of Apaf and for activation of caspase

Caspase

Apaf-1

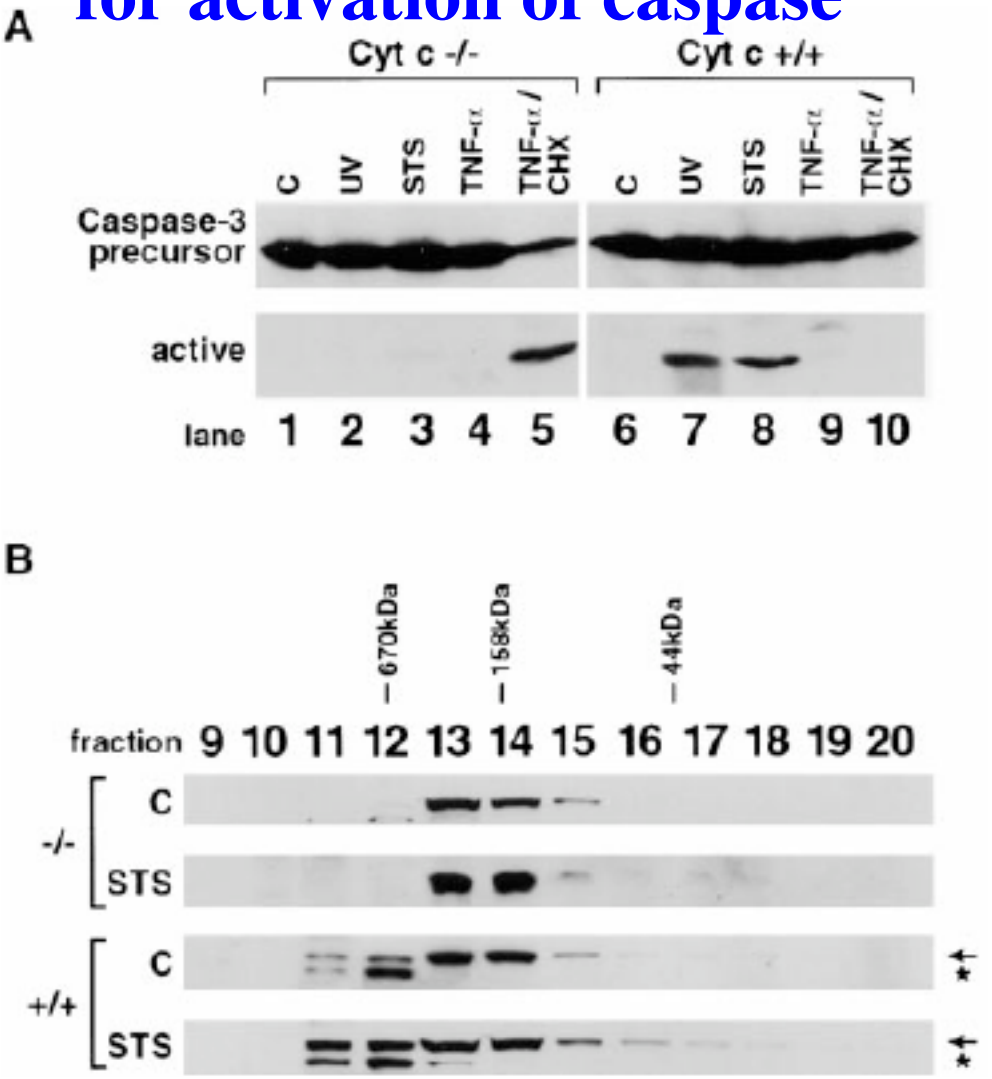


Figure 6. Assays for Active Caspase-3 and Oligomeric Apaf-1 Complexes Induced In Vivo by Proapoptotic Stimuli in *Cyt c*$^{-/-}$ and *Cyt c*$^{+/+}$ Cells

CED-9 is BCL-2 (a negative regulator of Caspases)
Dominant mutation pays off (by way of lof).

CED-3 is a pro-caspase, while CED-4 is related to vertebrate Apaf-1 (caspase activator); both found to be required in all animal cells for apoptosis.

CED-9/BCL-2 are associated with mitochondrial membrane: how they are regulated and role of mitochondria remain subject of active research.

One goal: trigger cancer cells to all enter apoptosis.

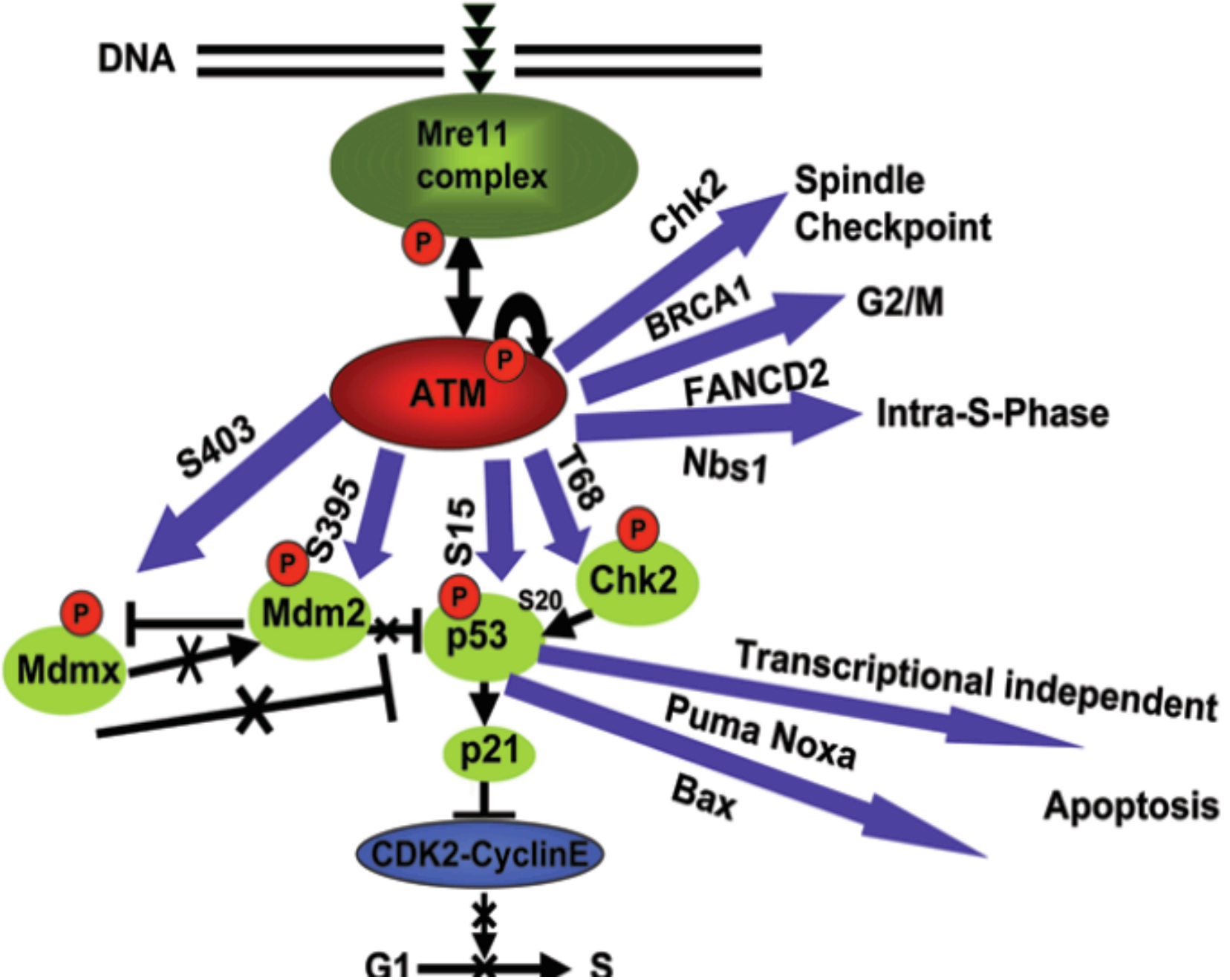
Back to checkpoints

p53 is a target of checkpoint pathways and determines survival versus death

In death mode, p53 interacts with and causes oligomerization of Bak

This interaction causes release of cytochrome c from the mitochondrion

The model is that p53 and the Bcl-2 family member Mcl1 have opposing effects on the death effector, Bak


DNA
Mre11 complex
P
ATM
P
Chk2
Spindle Checkpoint
BRCA1
G2/M
FANCD2
Intra-S-Phase
Nbs1
S403
S395
S15
T68
P
Mdm2
P
Mdmx
P
p53
S20
Chk2
P
Transcriptional independent
Puma Noxa
Bax
Apoptosis
p21
CDK2-CyclinE
G1
S