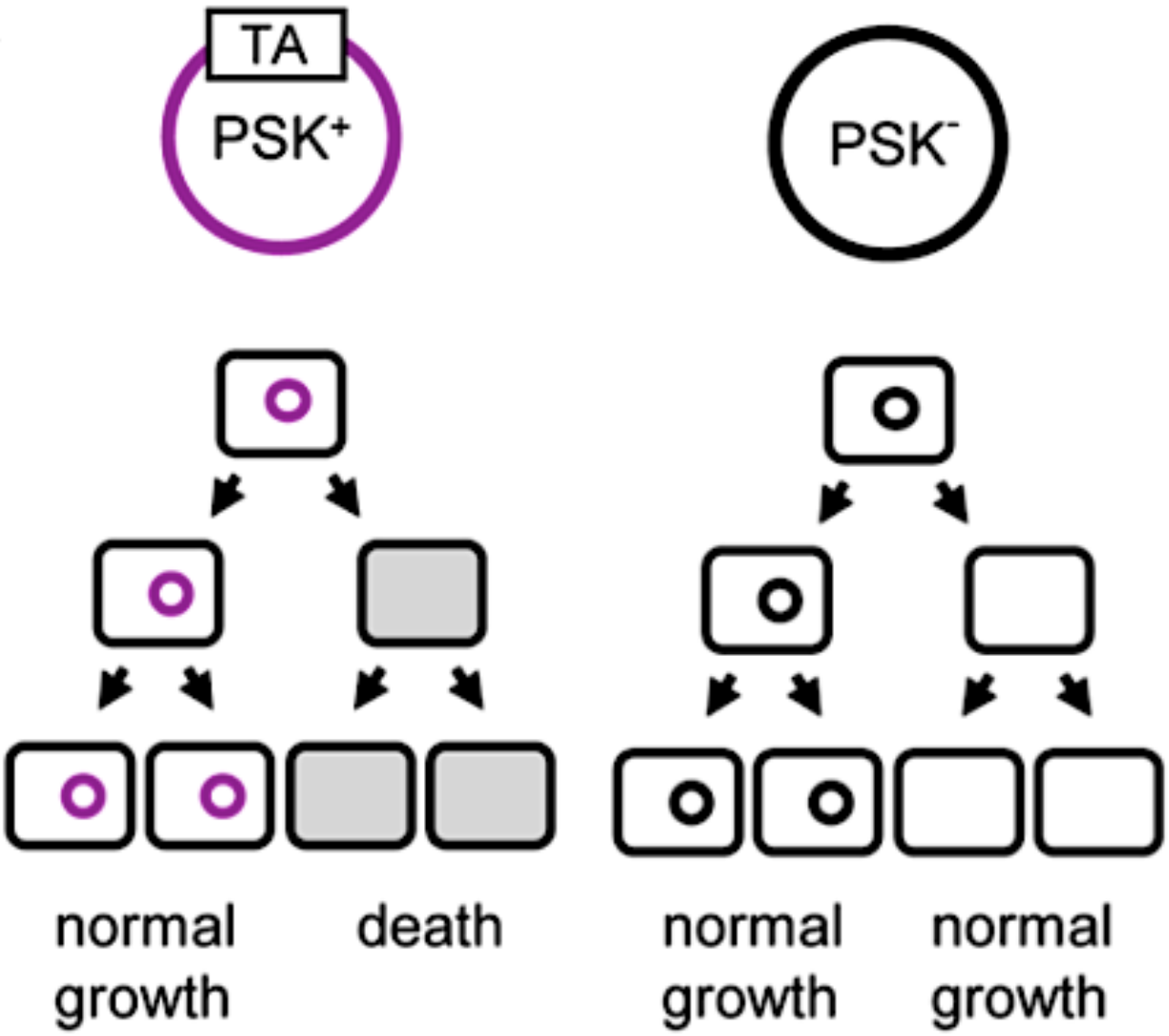
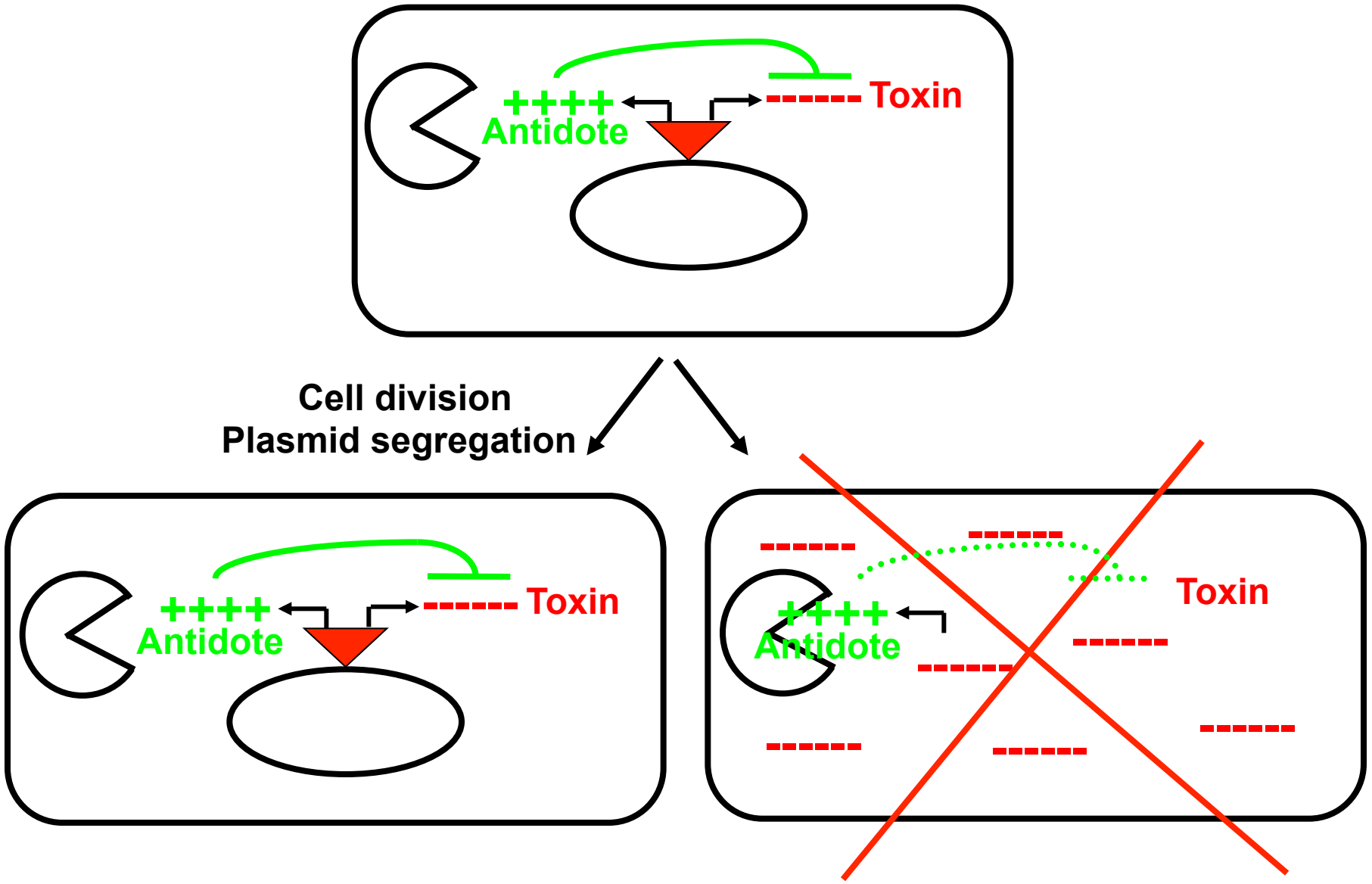
Cell death

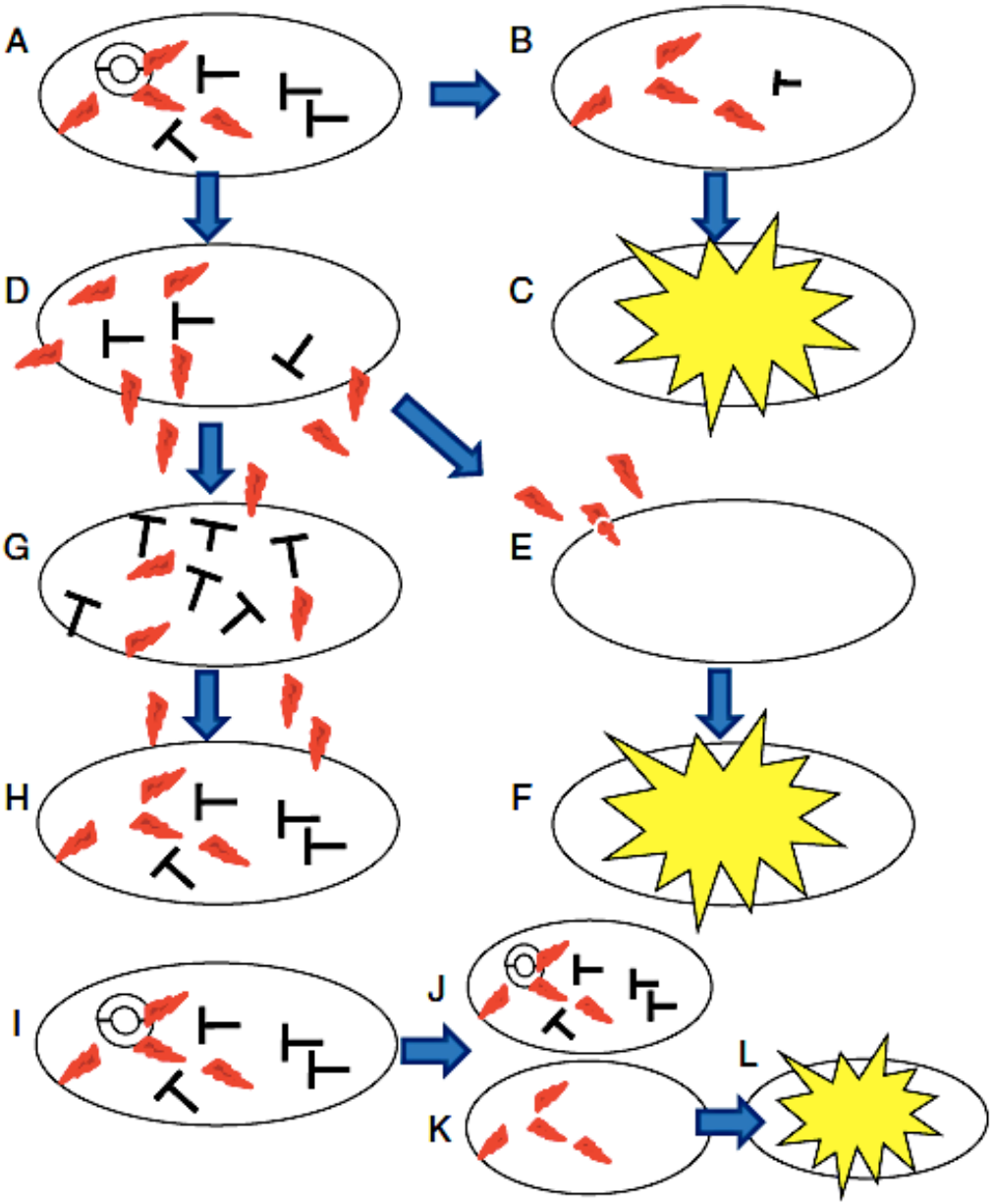
Selfish killing: A toxin-antitoxin system increases the Frequency of the TA-containing plasmid with respect to copies of the Plasmid that lack the element



A prokaryotic addiction module: a plasmid expressing a stable toxin and an unstable antidote behaves as a selfish genetic element



Addiction modules as a simple form of death regulation



Anything that regulates the half-life or expression of the toxin/antidote can influence the fate of self and neighbors.

Addiction modules. A,B,C: A bacterium containing a plasmid secrets a long-lived toxin and a short lived antidote (A). Loss of the plasmid (B) results in loss of the antidote before loss of the toxin and this causes cell death (C); D,E,F,G,H: A bacterium secreting a toxin and producing an antidote (D) will kill a bacterium that does not have the addiction module (E) and (F) whilst being protected itself (G). It is unable to kill a bacterium with the addiction module (H); I,J,K,L: A bacterium housing a plasmid with an addiction module divides (I) and a daughter cell containing the plasmid survives (J) whereas one without the plasmid (K) is killed by remaining toxin (L); a process known as post-segregational killing.

Altruistic cell death: death of some promotes the survival of others

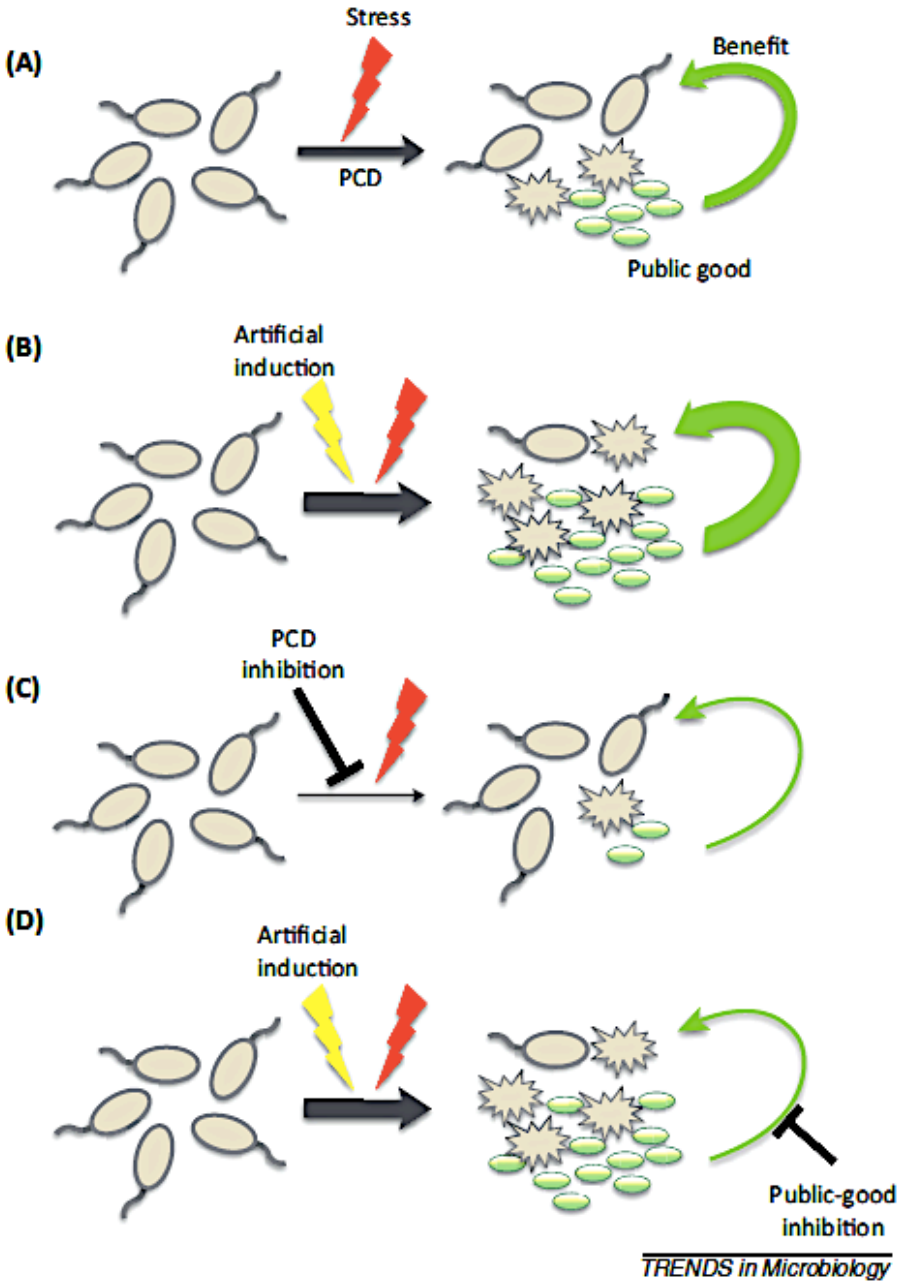
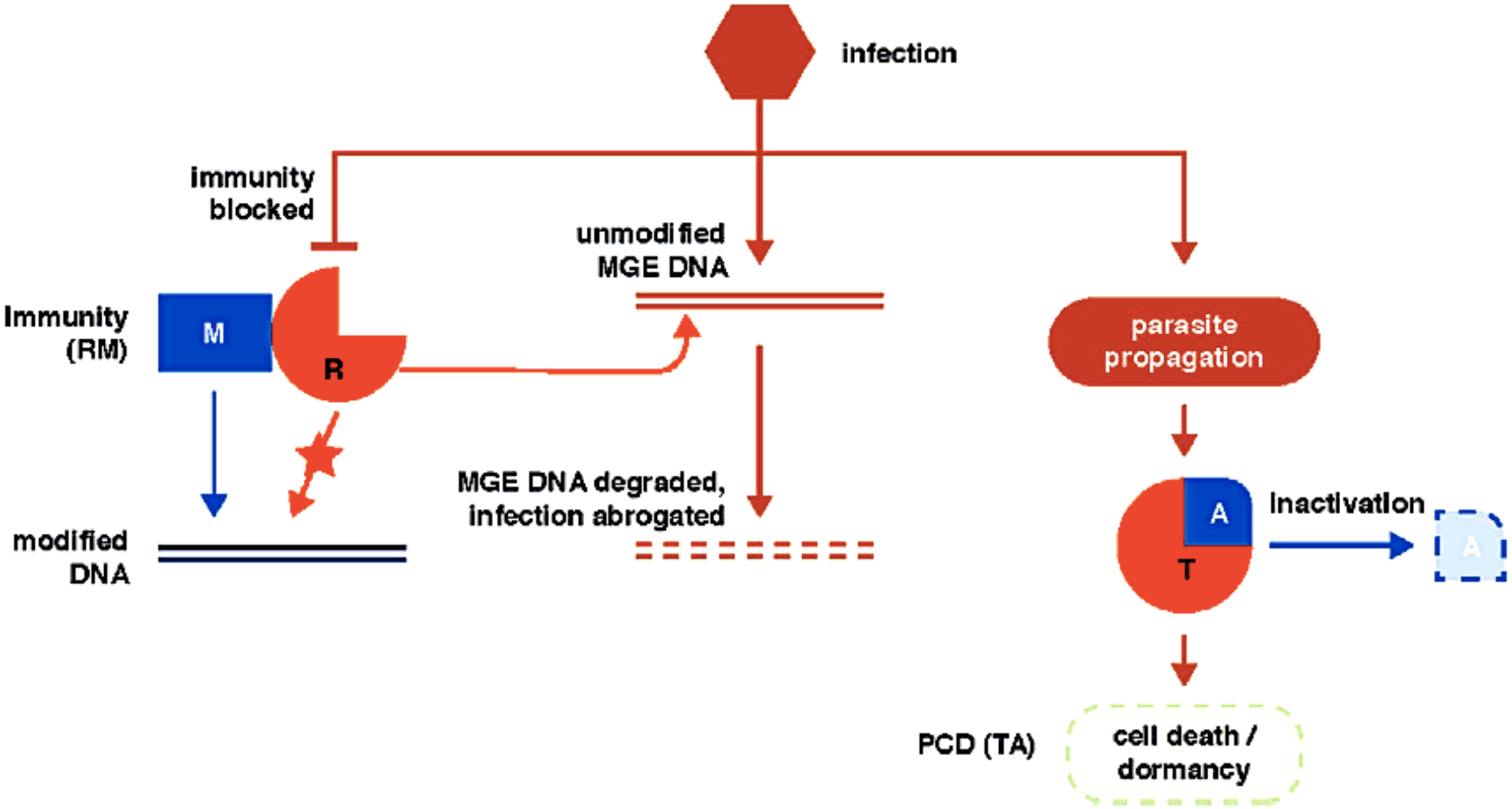


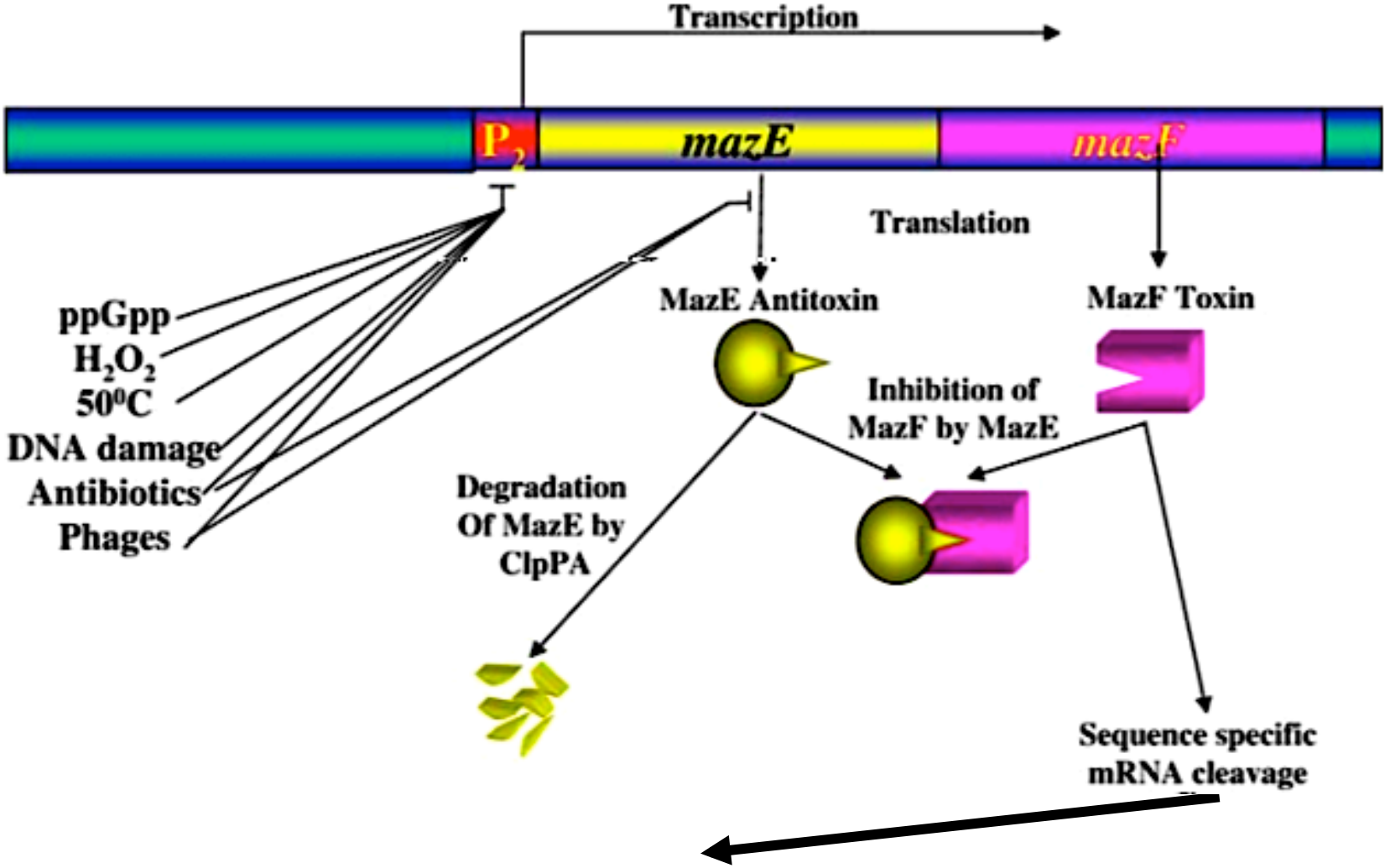
Figure 1. Altruistic programmed cell death (PCD) and its intervention strategies. (A) Induction of PCD by environmental stress may be coupled with release of public goods that provide direct or indirect benefits to the survivors. (B) PCD can be artificially induced as an antibacterial treatment strategy, but this increases the amount of public goods released into the environment (indicated by the larger arrow sizes), which can result in overall better bacterial growth. (C) PCD may be inhibited to decrease the generation of public goods (indicated by the smaller arrow sizes). (D) A combination approach that both induces PCD and inhibits the function or activities of released public goods.

Altruistic death in response to infection



Immunity and programmed cell death: distinct but coupled defense strategies. The host DNA is protected from the action of the restriction enzyme by methylation whereas the invading DNA is sensitive. Innate immunity, in the form of RM systems, can inactivate the parasite DNA and block infection. However, if the innate immunity fails (e.g. due to the activity of the parasite-encoded antidefense system) and the parasite reproduces, the infection induces genotoxic stress which activates proteases cleaving antitoxins. The resulting activation of toxins leads to dormancy or PCD. M, modification enzyme; R, restriction enzyme; T, toxin; A, antitoxin; MGE, mobile genetic element.

A chromosomal Toxin-Antitoxin module lets cells respond to Environmental stimuli through suicide or quiescence (arrest)



Cell death or metabolic arrest
(waiting for a better environment)

A chromosomal Toxin-Antitoxin module protects populations of bacterial cells from spread of a cell-killing phage (virus) infection

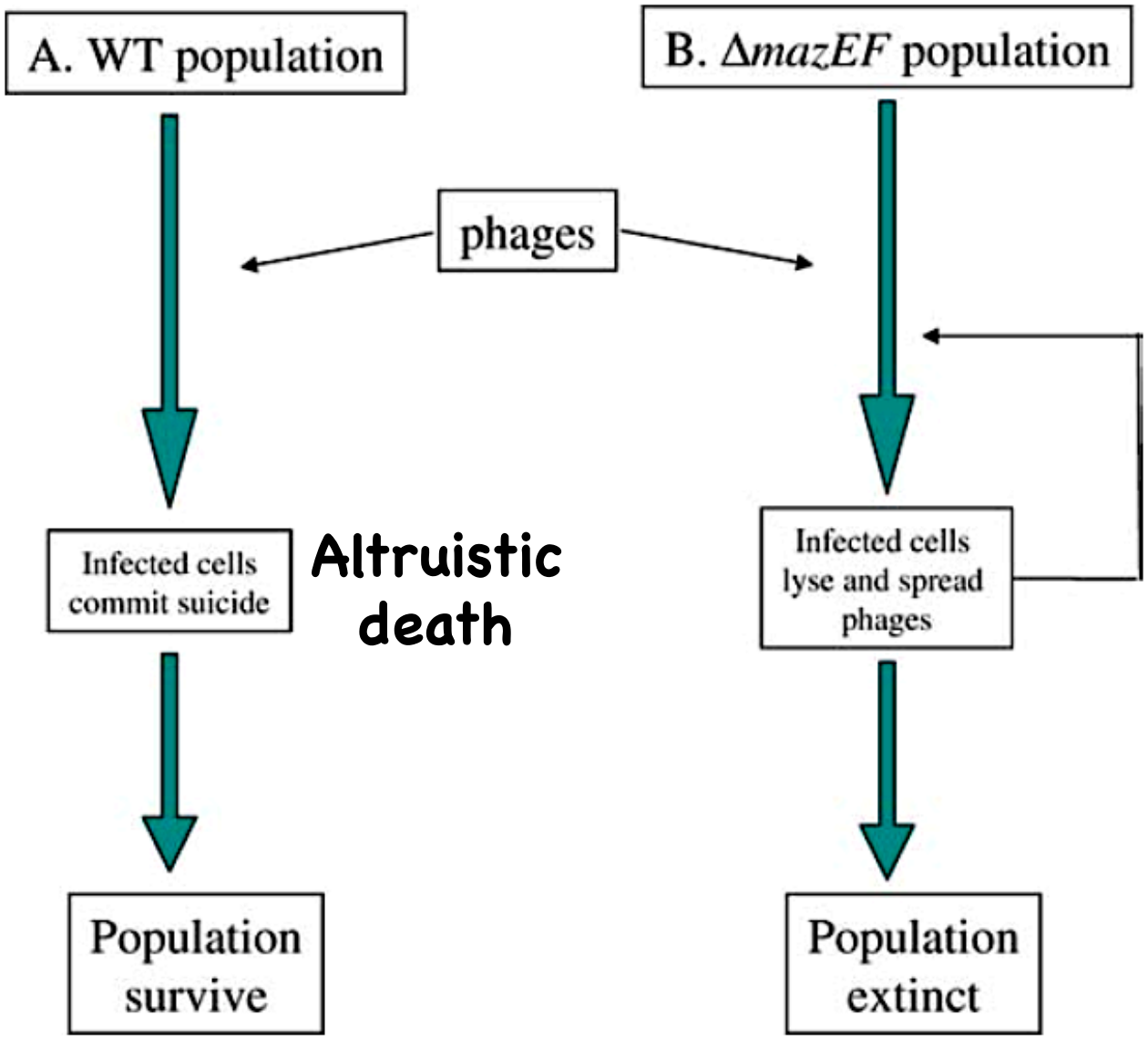


Figure 3. A Model: How Programmed Cell Death Saves a Bacterial Population from Annihilation by Phage P1 Infection

(A) In wild-type cells, P1 infection triggers the action of *mazEF* which mediates the death of the infected cells. Because infected cells die before phage can propagate, few phages are released, the titer of the phages is low, and the population survives.

(B) In Δ*mazEF* cells, nothing interferes with the phage infections: the infected cells lyse, allowing many released particles to spread to the rest of the population. Thus infection by P1 of a Δ*mazEF* population leads to the death of more cells. Though infection by P1 of wild-type populations leads to the loss of some of the cells, more members of the population survive.

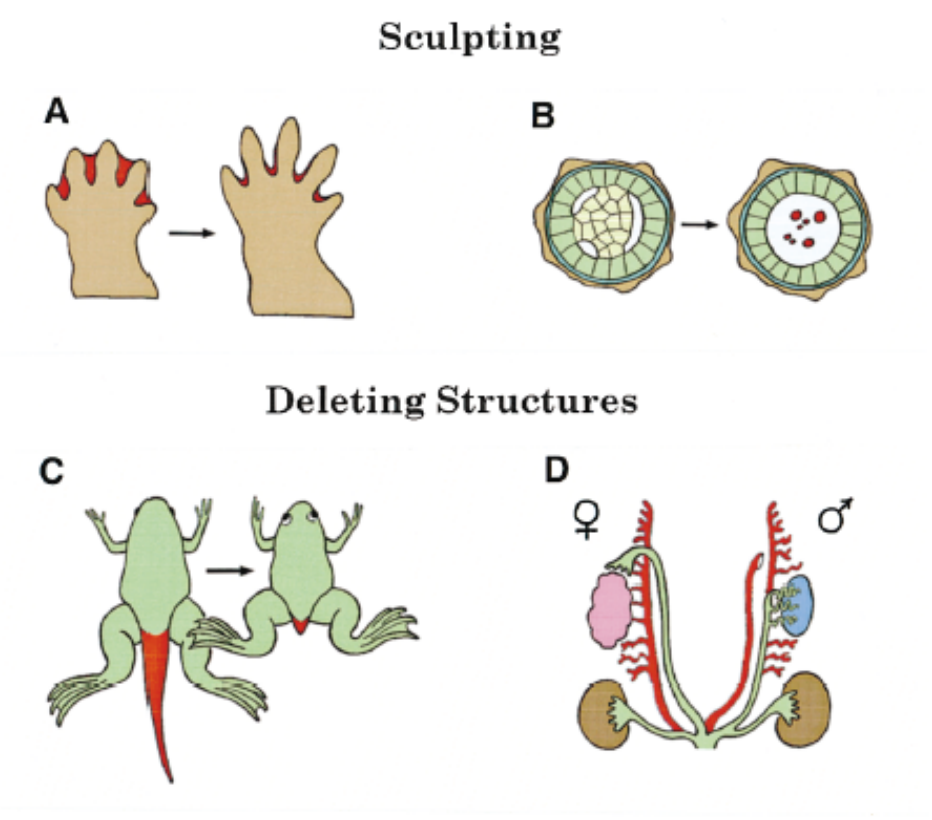
10^{14} cells in your body total

10^{11} cells die each day

Lifetime = about 365 X 50 = 2 X 10^{4} days

2 X 10^{15} total cell deaths

Some functions of programmed cell death



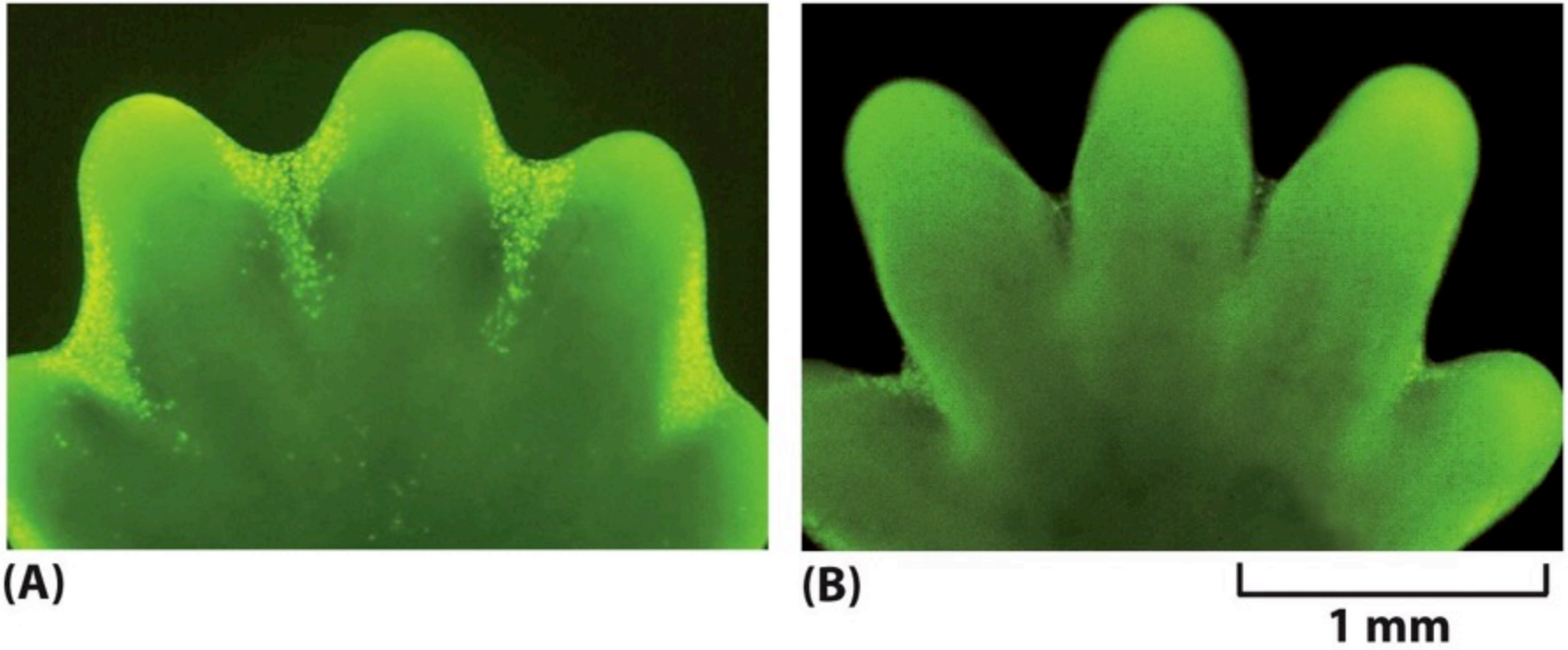
<u>Development</u>:

- **deletion of entire structures**
- **sculpting of tissues**
- **neuron-target matching in the nervous system.**

<u>Surveillance</u>:

remove cells that are damaged or otherwise dangerous to the organism as a whole (cells with mutagenic DNA damage, or viral infections).

Apoptosis removes cells in interdigital "web" tissue



limbs stained with Acridine Orange, a dye specific for apoptotic cells
(A) embryonic day E13.5 (B) embryonic day E14.5.

Figure 18-2 *Molecular Biology of the Cell* (© Garland Science 2008)

Types of Cell Death

Apoptosis (Programmed Cell Death) :

- **Cell-Autonomous**
- **Stereotypic**
- **Rapid**
- **"Clean" (dead cells eaten)**

Necrosis :

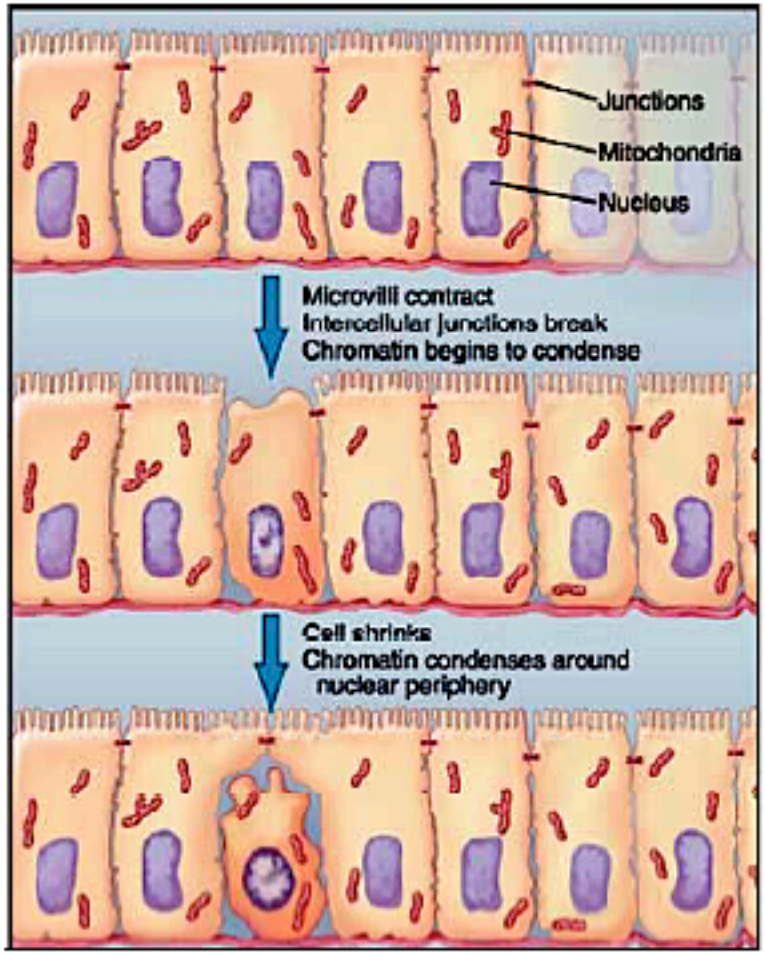
- **Not Self-Initiated**
- **Not Stereotypic**
- **Can Be Slow**
- **"Messy" (injury can spread)**

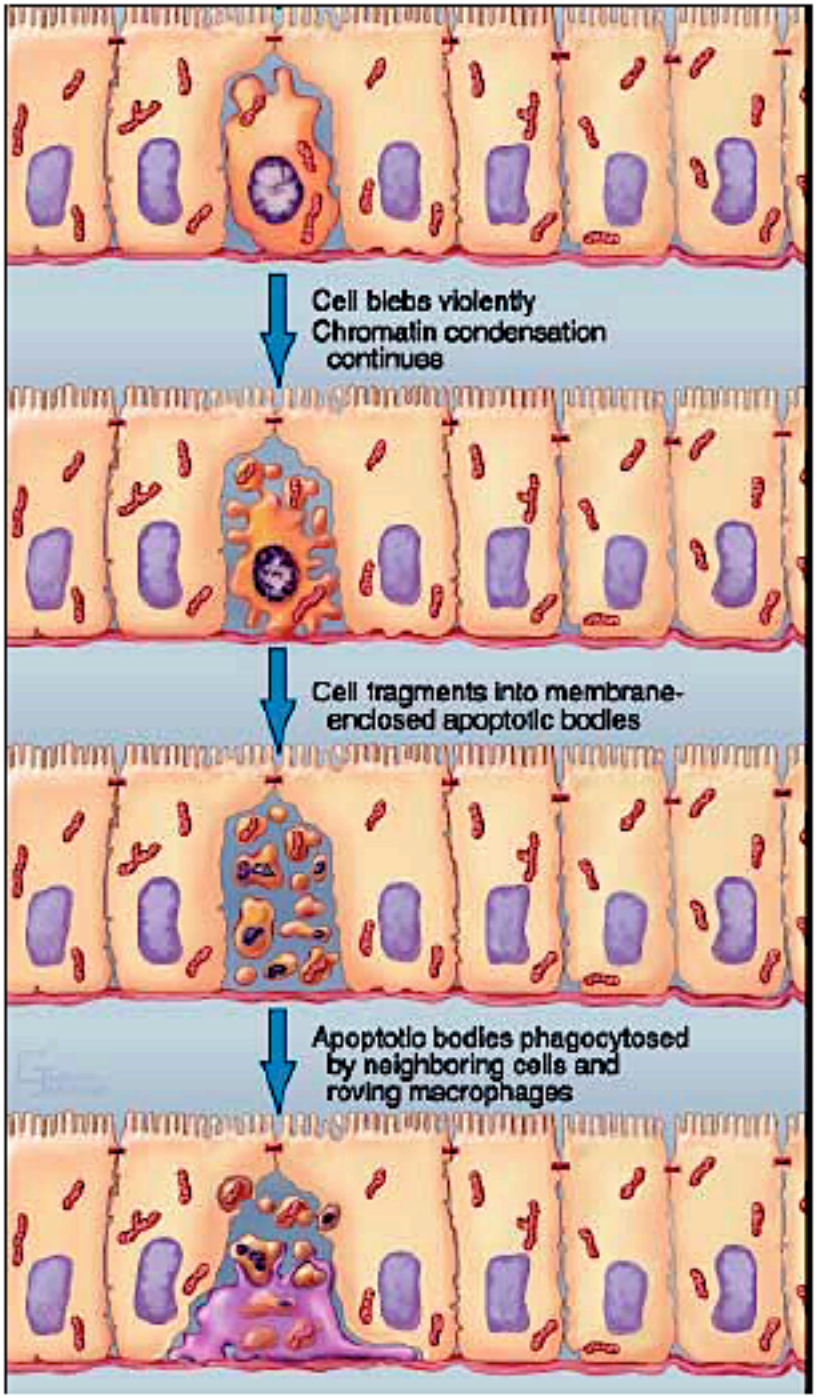
Apoptosis

from Greek
"*apo*" meaning "separation"
&
"*ptosis*" for "falling off"

(Kerr JFR, Wyllie AH & Currie AR, 1972)

Overview: Programmed Cell Death

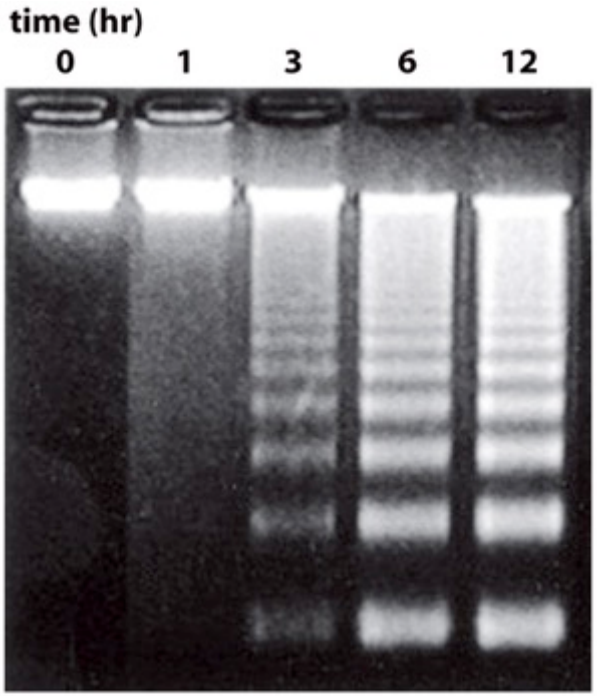




No inflammation—content of cell is never released into environment

Two biochemical hallmarks of apoptosis

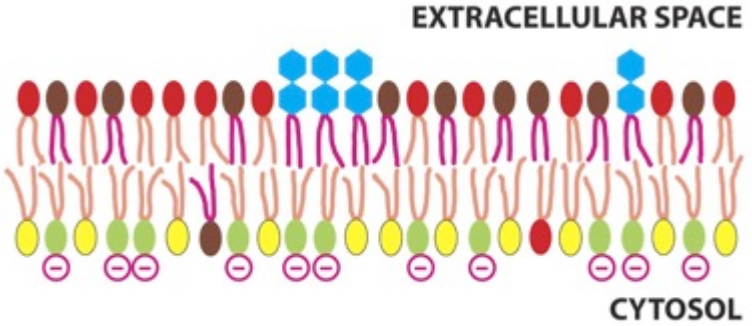
(A)

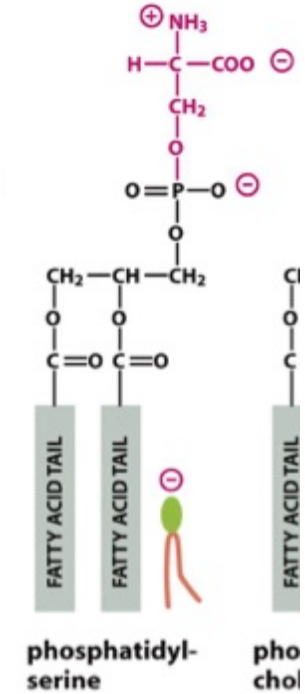


Cells undergoing apoptosis show a ladder of DNA fragments.

Endonuclease cleaves DNA into nucleosomal units.

(B)

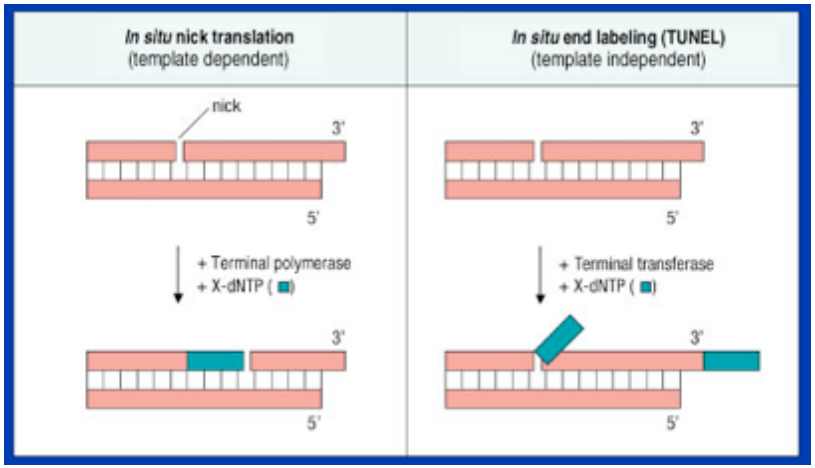


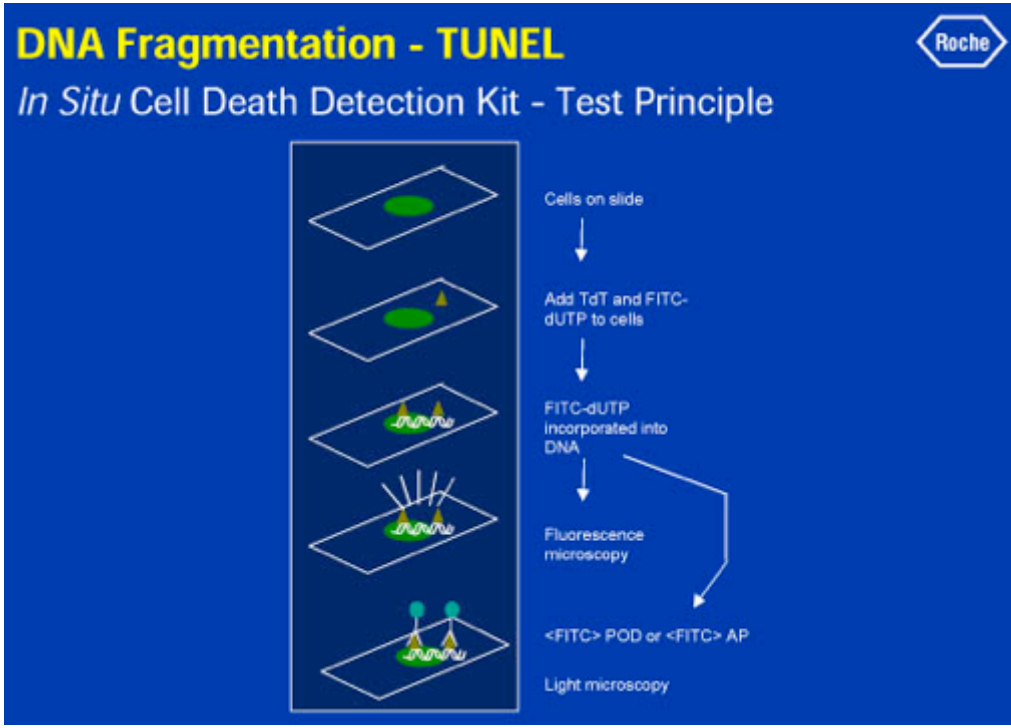


Phosphatidylserine is a negatively charged phospholipid; it is normally restricted to the inner leaflet of the cell membrane.

Phosphatidylserine flips during apoptosis (activation of scramblase by caspase) and signals macrophages to phagocytose the cell.

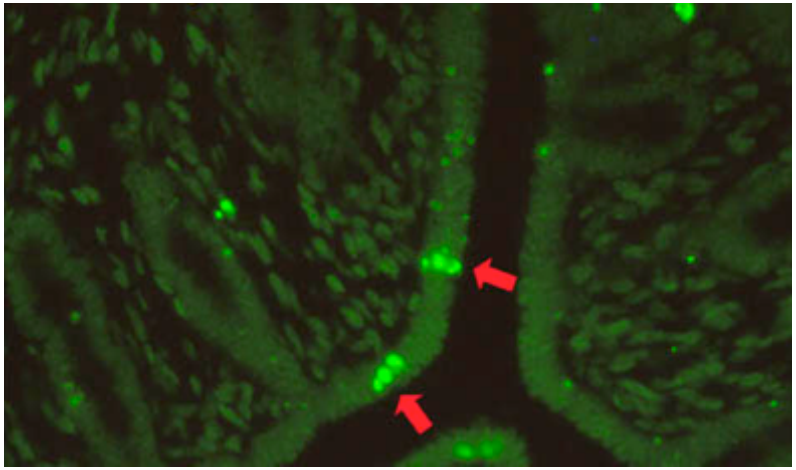
TUNEL assay for apoptosis





TUNEL: Terminal deoxynucleotidyl transferase dUTP nick end labeling

TdT adds nucleotides to 3' end of DNA molecule. Enzyme involved in adding diversity during VDJ recombination in the immune system.

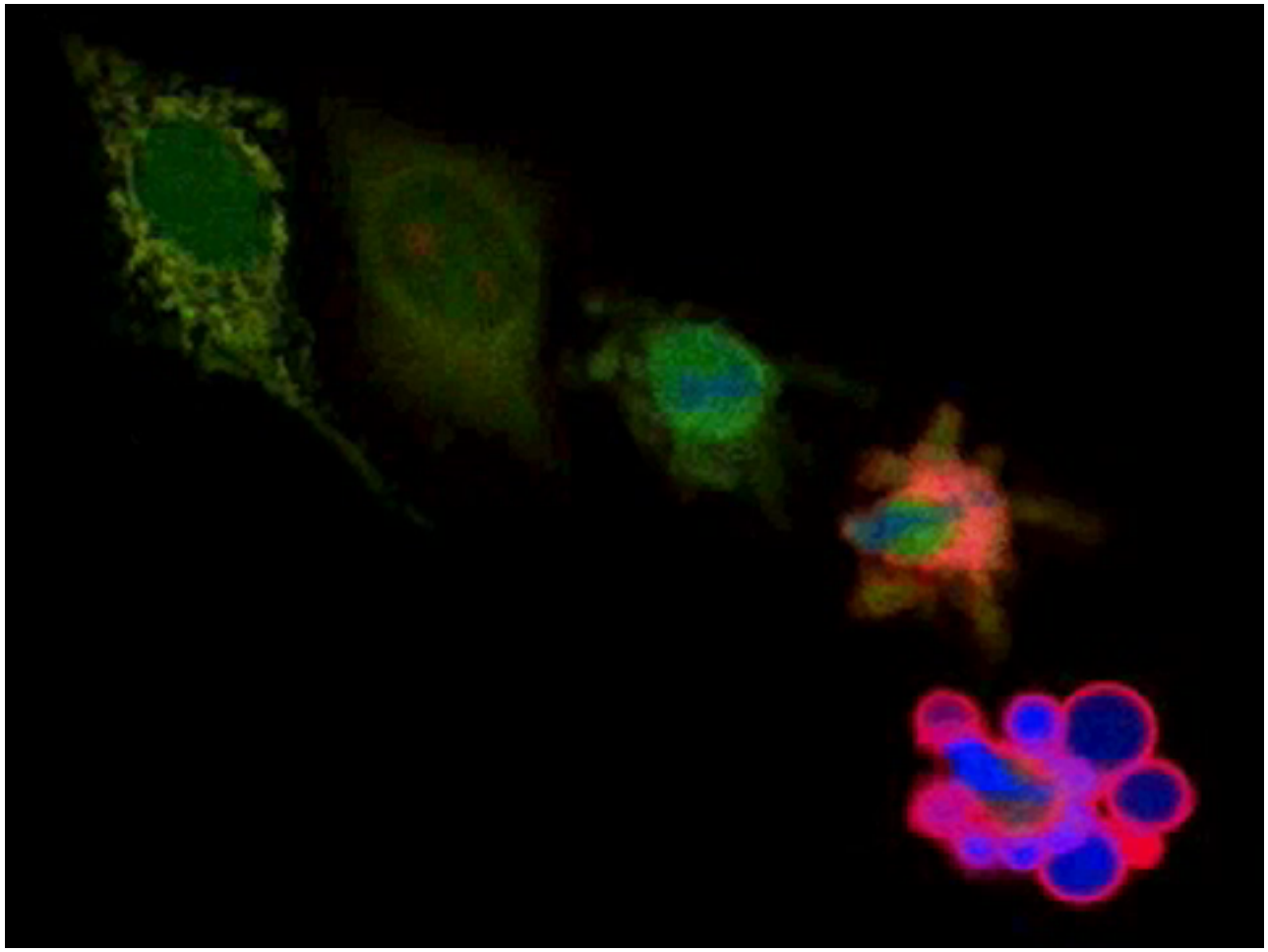


Apoptosis: release of cytochrome c, annexin V staining, and cell permeabilization

cytochrome *c* (green).

Annexin V is a phopholipid binding protein with high affinity for phosphatidylserine (PS).

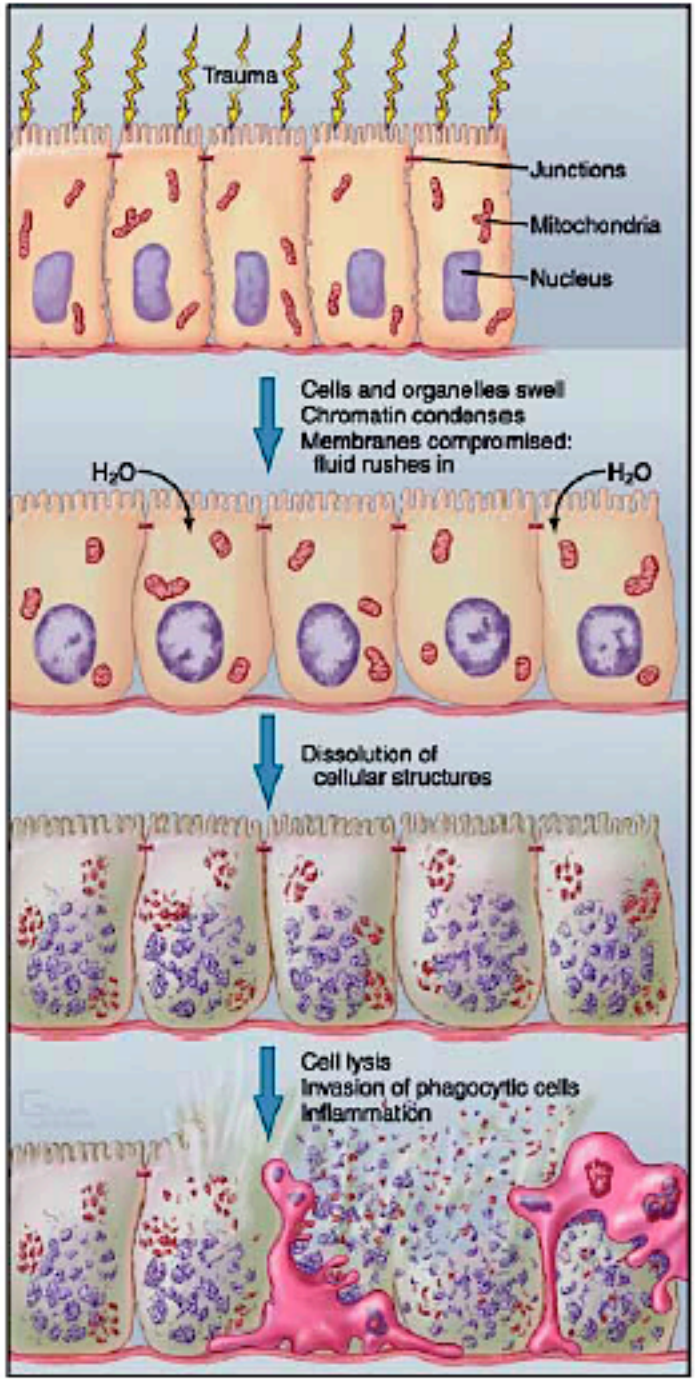
PS (red) is translocated from the inner leaflet to the outer leaflet during cell death.

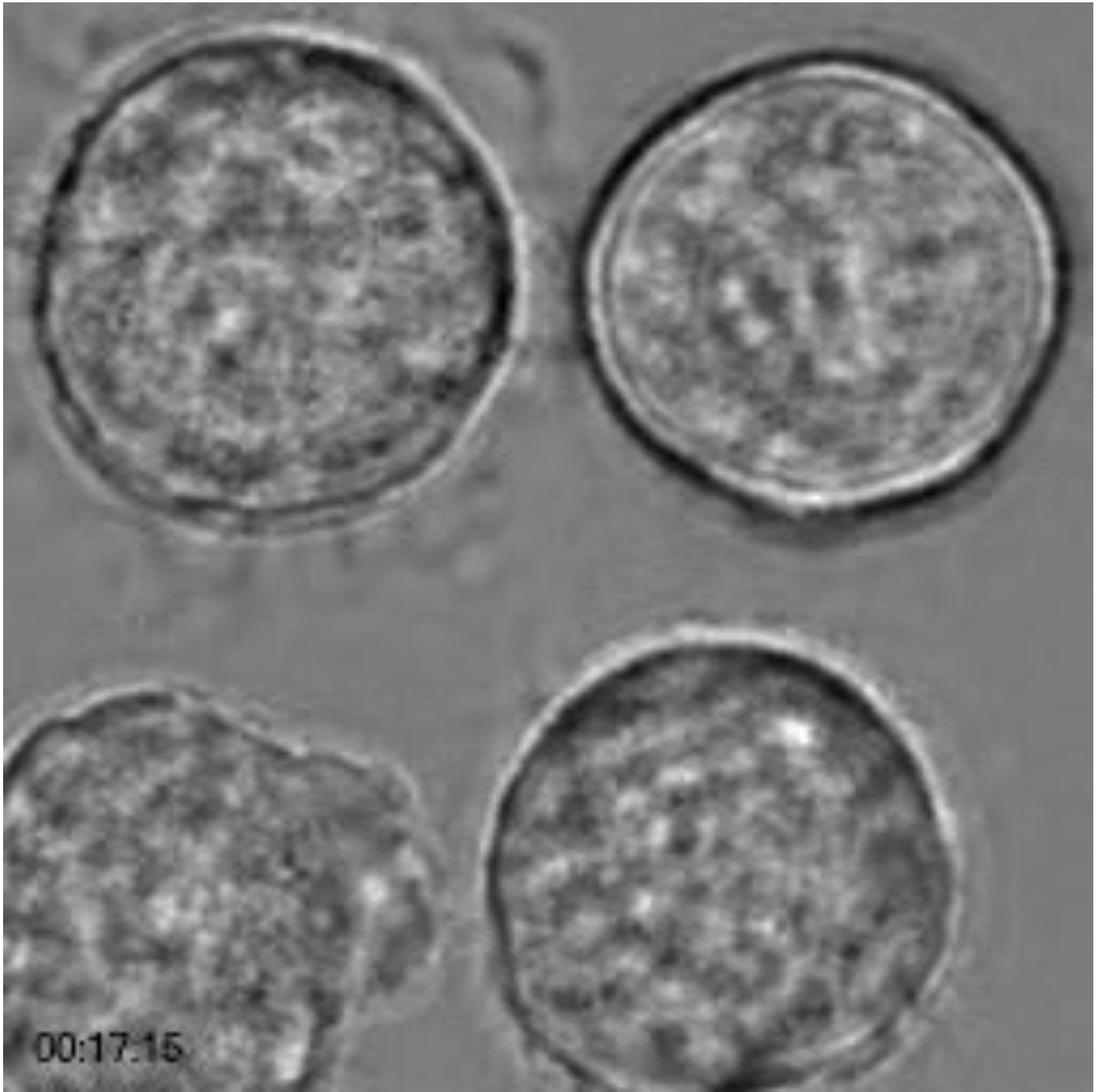


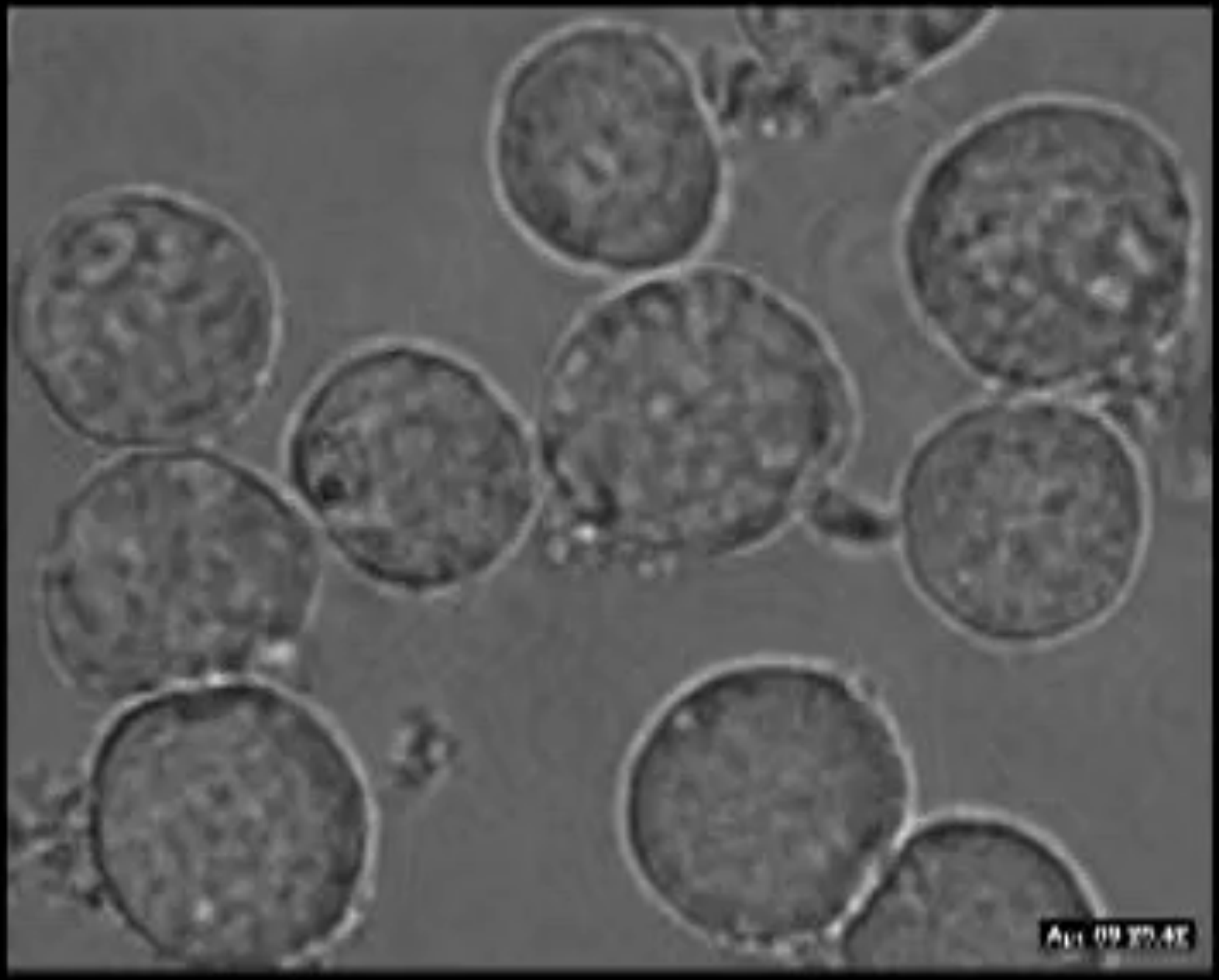
Alberts et al. (2007)

Necrosis

- Cells receives structural or chemical insult—damage!
- Cells and organelles swell
- Release of enzymes from lysome
  - Auto-digestion and dissolution
- Invasion of phagocytic cells and inflammation




00:17:15



Cell death and its regulation

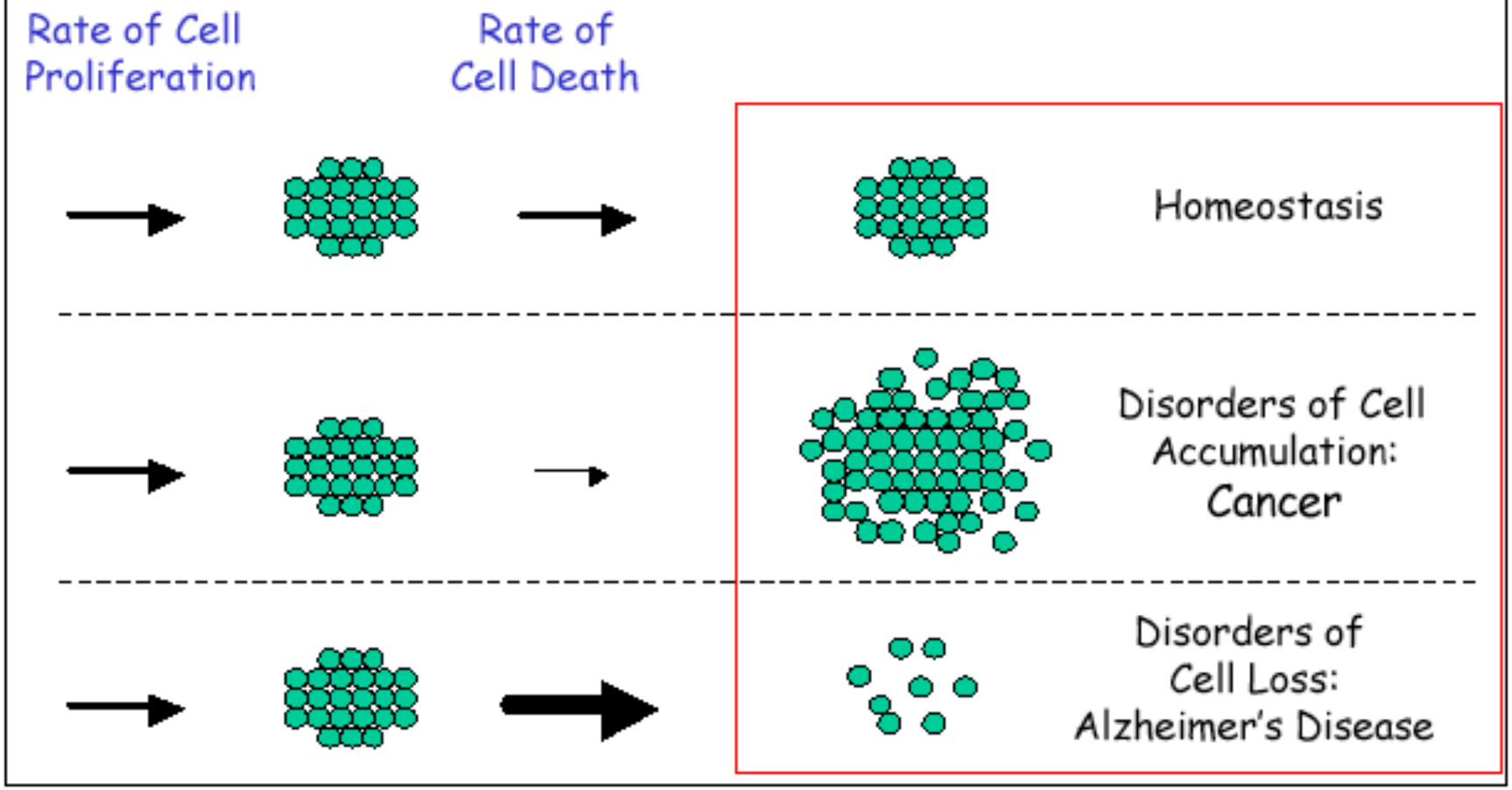
Cellular interactions regulate cell death in two fundamentally different ways

Most cells require signals (trophic factors) to stay alive and will undergo programmed cell death in the absence of these signals. Thus, death is in fact the default pathway

Some cells are triggered to undergo programmed cell death by specific signals

All cells carry the proteins required to bring about their own death

Alteration in the Rate of Cell Death results in Disease



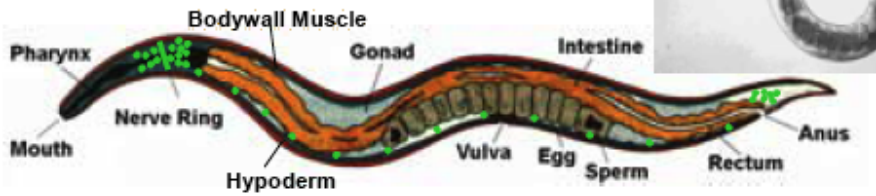
Diseases associated with inhibition of Cell Death

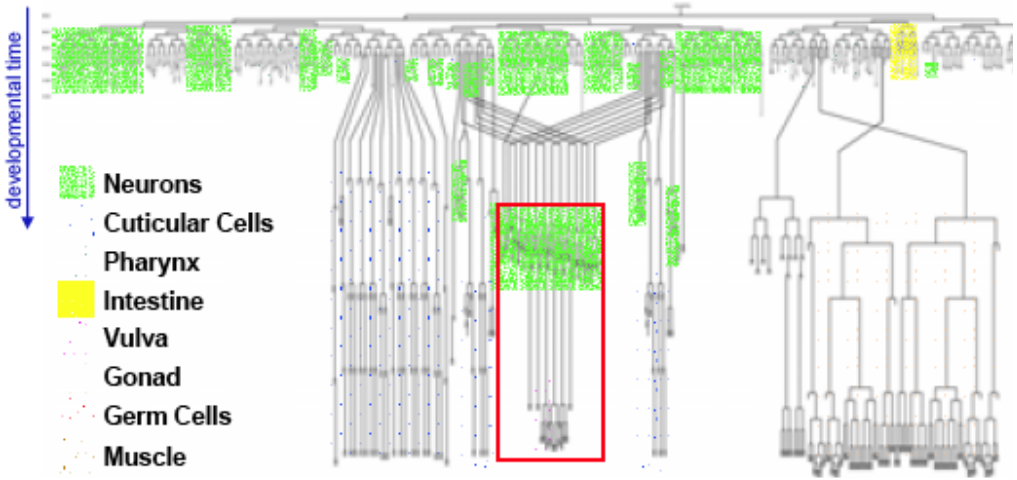
- Tumor formation and metastasis
- Autoimmune disorders
- Viral infections
  - Herpesviruses
  - Poxviruses
  - Adenoviruses

Diseases associated with increased rates of Cell Death

- Neurodegenerative disorders
  - Alzheimer's
  - Parkinson's
  - Cerebellar degeneration
  - Retinitis pigmentosa
- Ischemic Injury
  - Myocardial infarction
  - Stroke
- AIDS
- Toxin-induced liver diseases
  - Alcohol

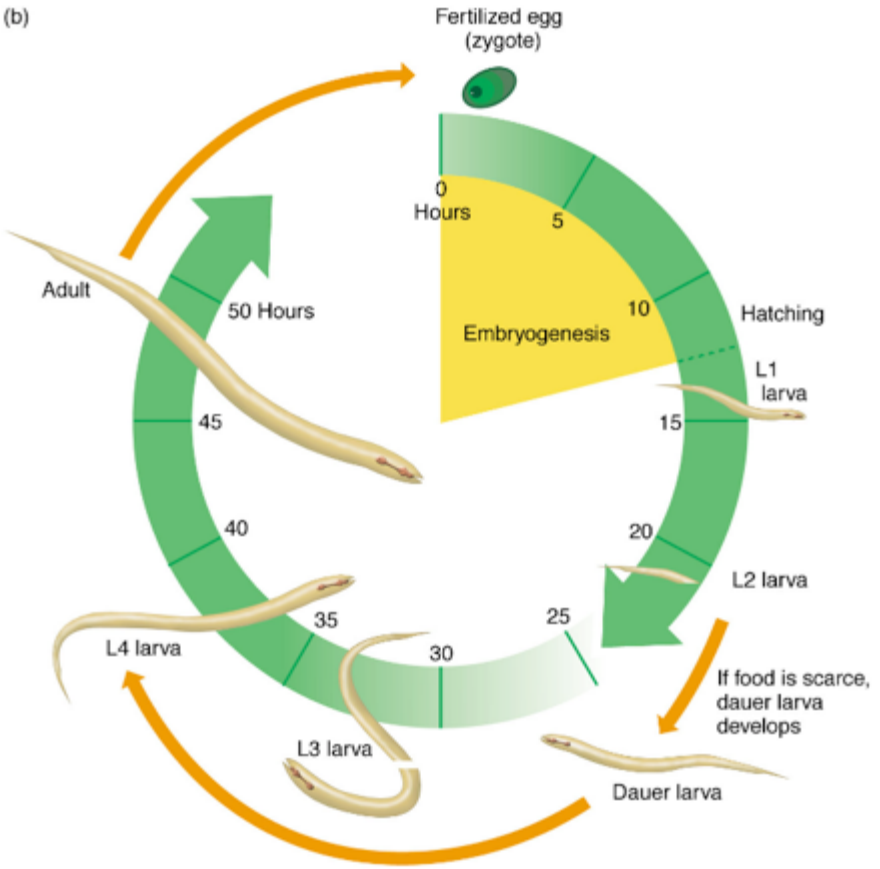
C. elegans is a great model organism for molecular genetic studies of Cell Death





Neuronal Cell Death Lineages

(Brenner, 1973; Sulston and Horvitz, 1977;
White, Horvitz, Sulston, 1982; Sulston, Schierenberg, White, Thomson, 1983)

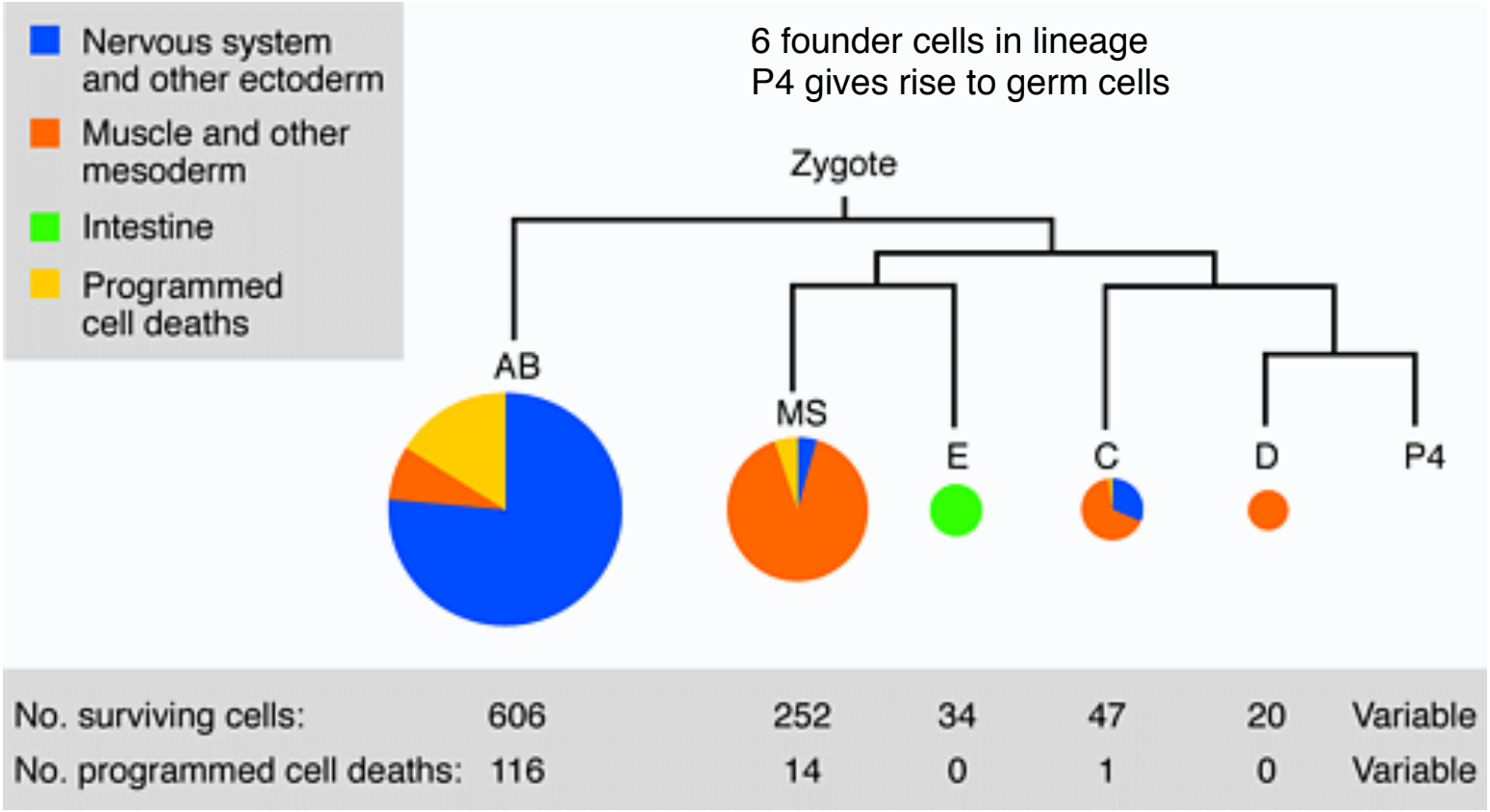


Sydney Brenner

John Sulston

H. Robert Horvitz

Cell deaths in *C. elegans*



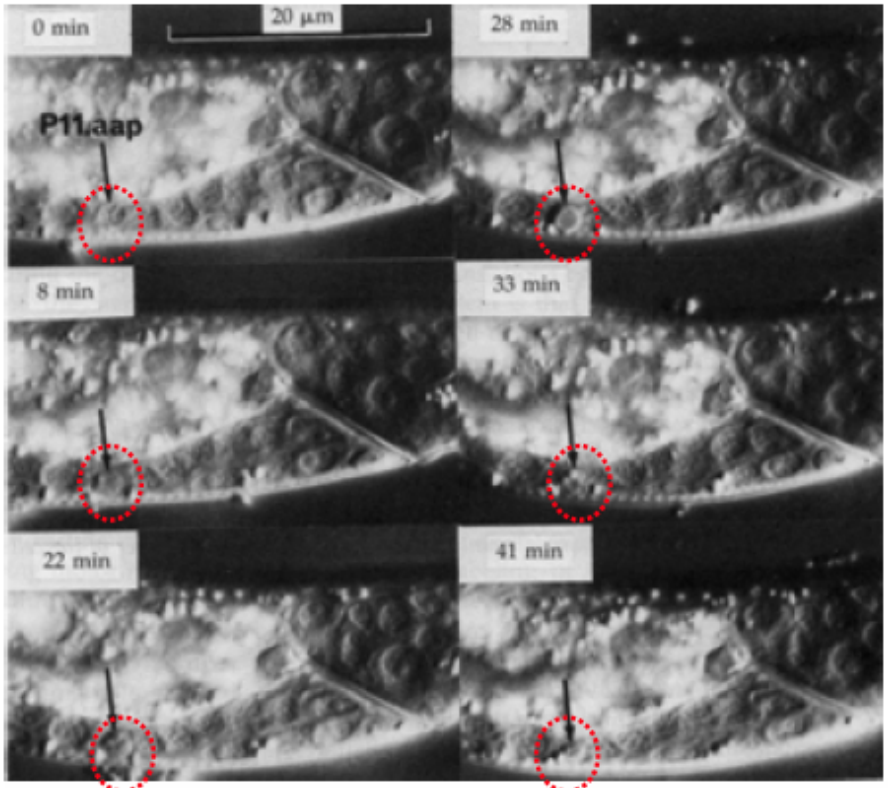
No. surviving cells:	606	252	34	47	20	Variable
No. programmed cell deaths:	116	14	0	1	0	Variable

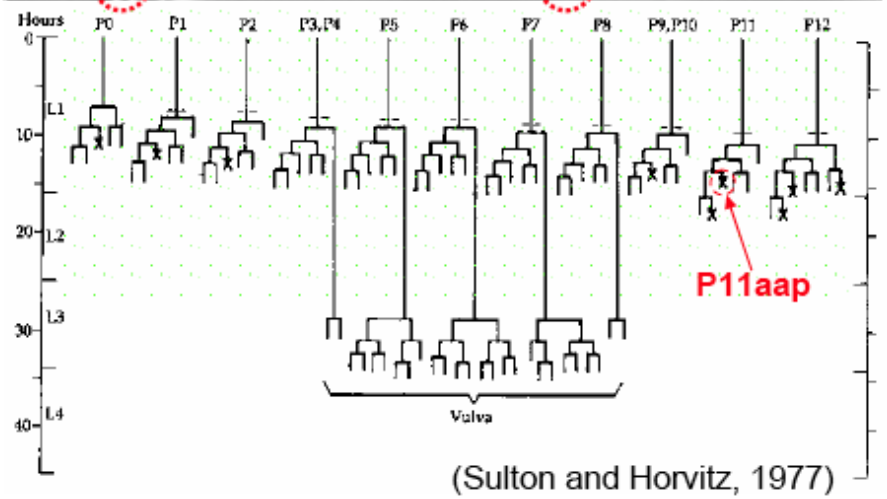
131 cells undergo programmed cell death in a stereotypic manner.

In strong cell death mutants, these 131 cells survive.

Metzstein et al. (1998) TIGS 14:410

Programmed Cell Death of single identified neurons can be followed in live worms



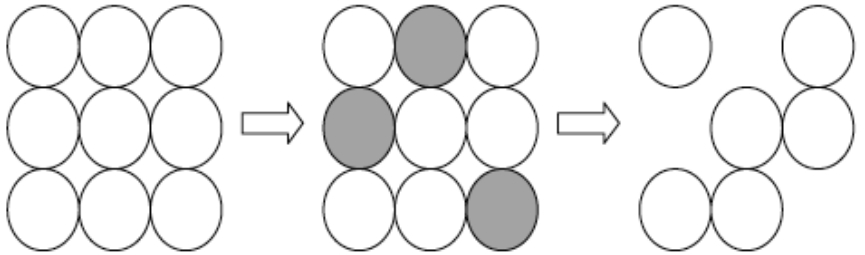


(Sulton and Horvitz, 1977)

2 Classes of *C. elegans* Cell Death Mutants

WT

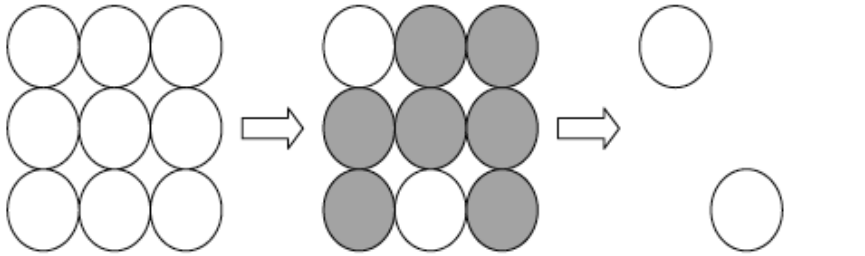
(pro-survival genes + pro-apoptosis genes)



(normal number of cells)

Mutant class I

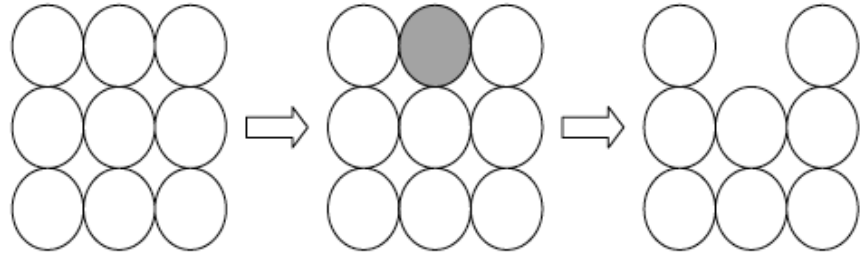
(~~pro-survival genes~~ + pro-apoptosis genes)



(fewer cells)

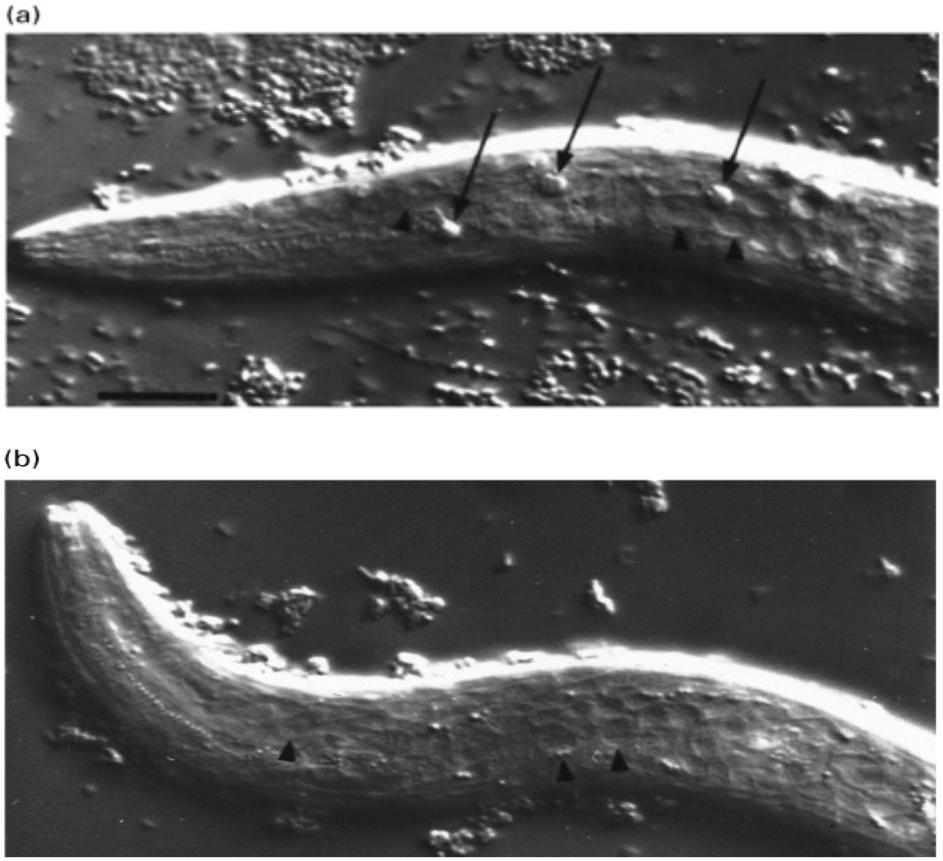
Mutant class II

(pro-survival genes + ~~pro-apoptosis genes~~)



(extra cells)

ced-3 mutants lack programmed cell deaths



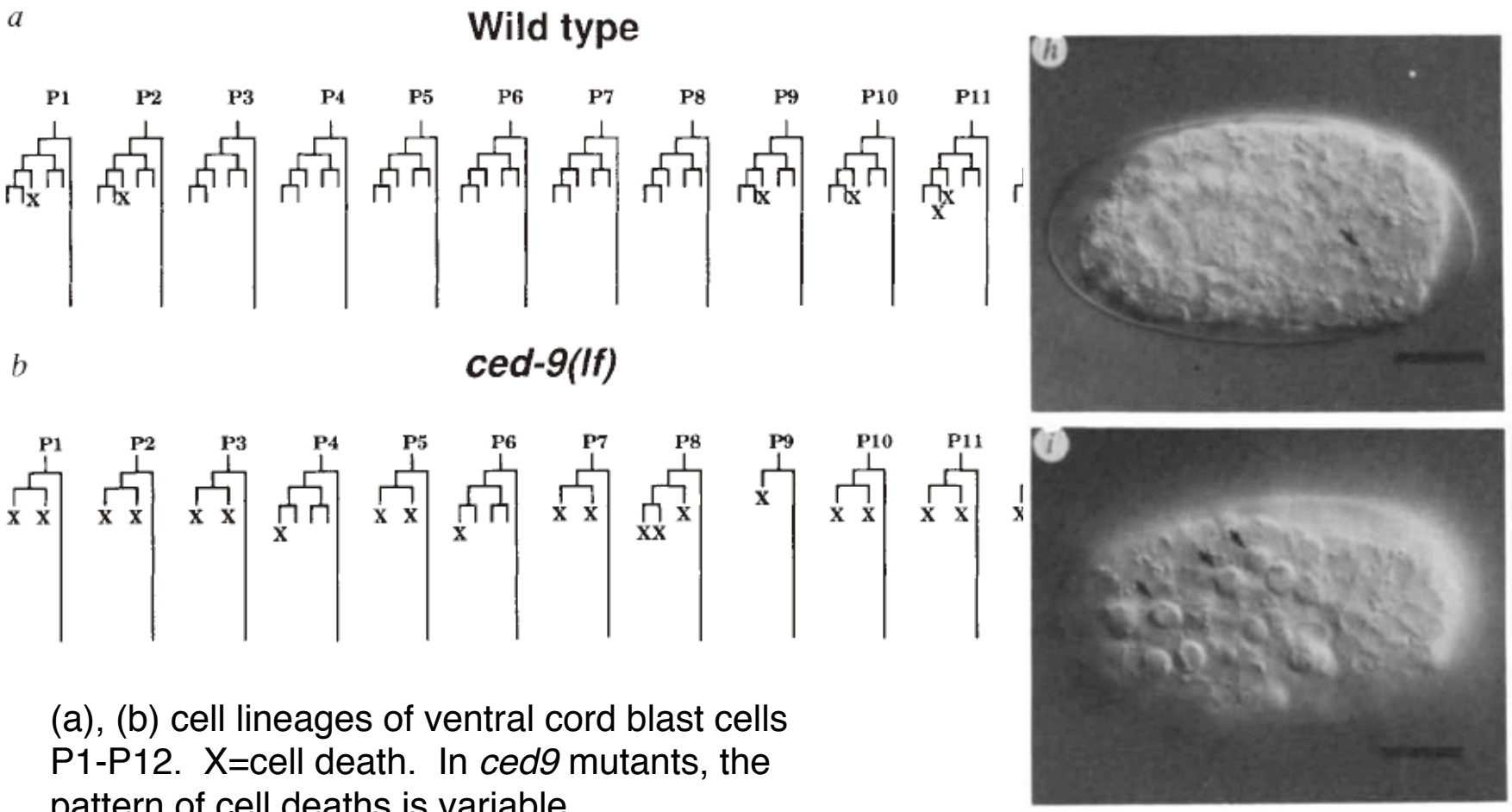
(a) *ced-1* mutant (prevents phagocytosis of dead cells, so that they can be visualized)

(b) c*ed-1/ced-3* mutant does not show dead cells (they are live).

Use of the *ced-1* mutant allows identification of cell death mutants.

Lodish et al. 2004

ced-9 mutants show ectopic cell deaths and embryonic lethality

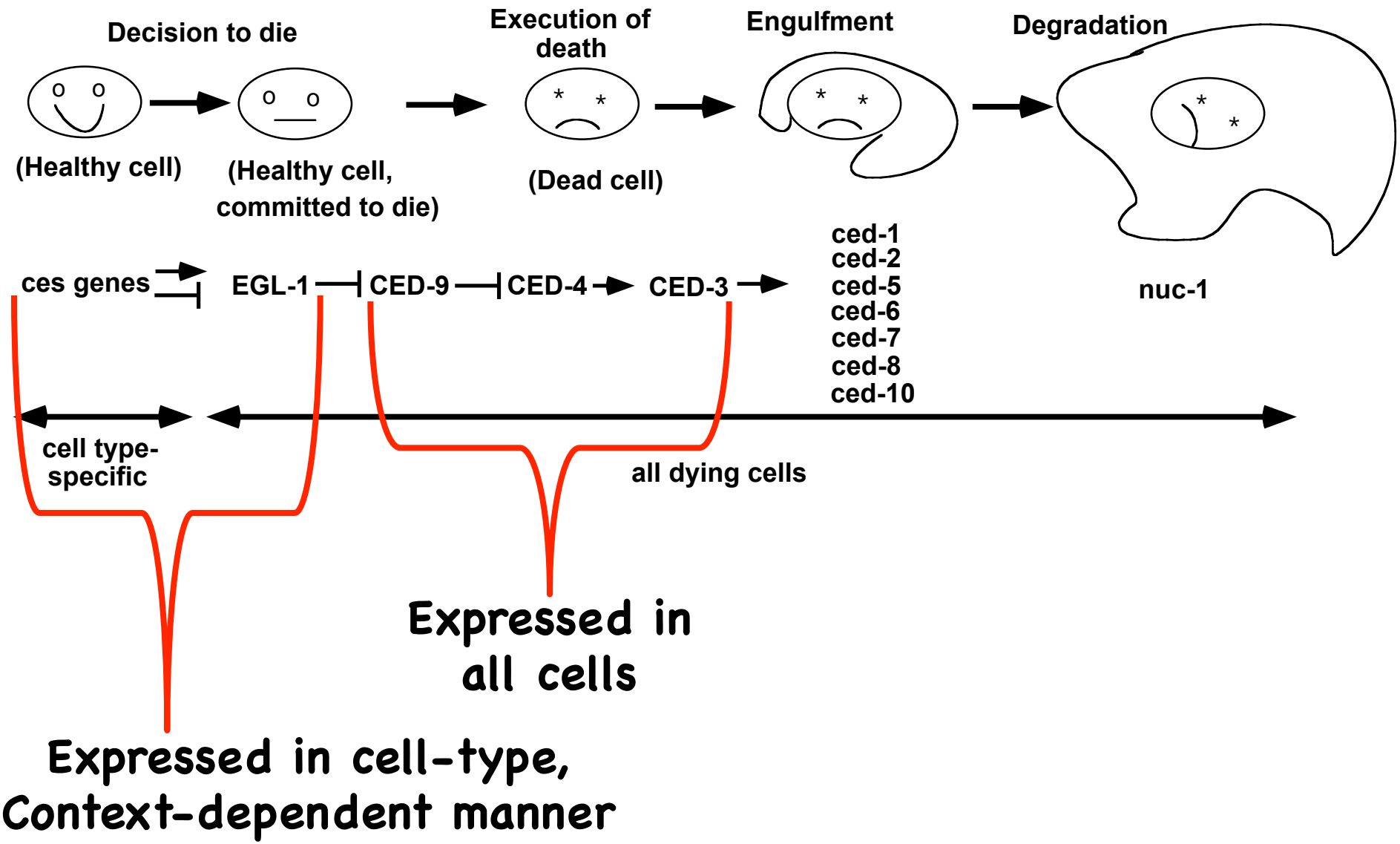


(a), (b) cell lineages of ventral cord blast cells P1-P12. X=cell death. In *ced9* mutants, the pattern of cell deaths is variable

(h) wt embryo, arrow shows cell death
(i) *ced9* mutant, showing ectopic cell death

Hengartner et al. (1992) Nature

Cell death in the worm: the genes



ced-3 – the pioneer caspase

Caspases are Cell Death proteases

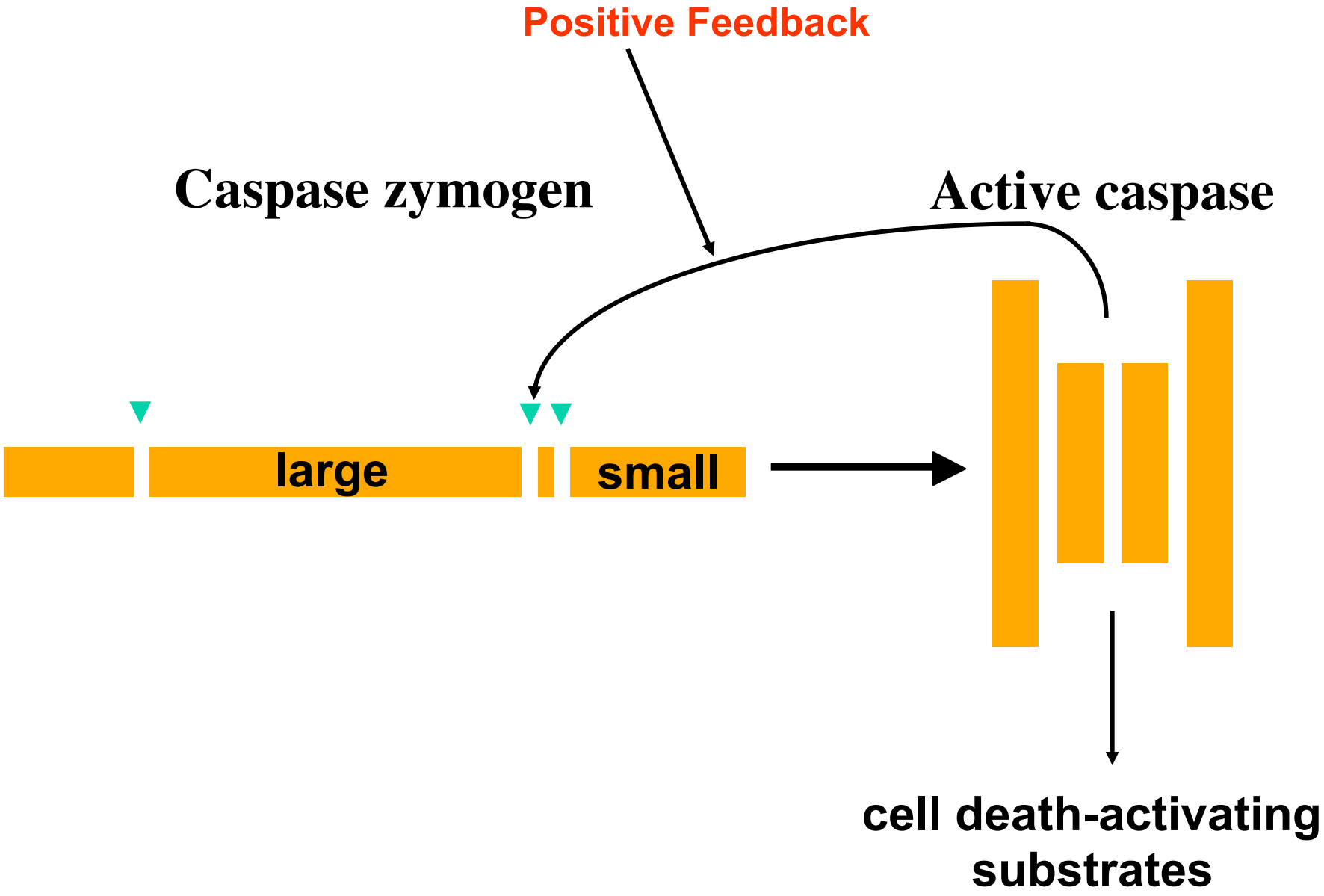
Caspases are a highly specialized class of proteins that cleave (but not necessarily destroy) other proteins

Caspases - contain a Cys residue in the active site (Cys proteases) and they cleave preferentially after Asp residues

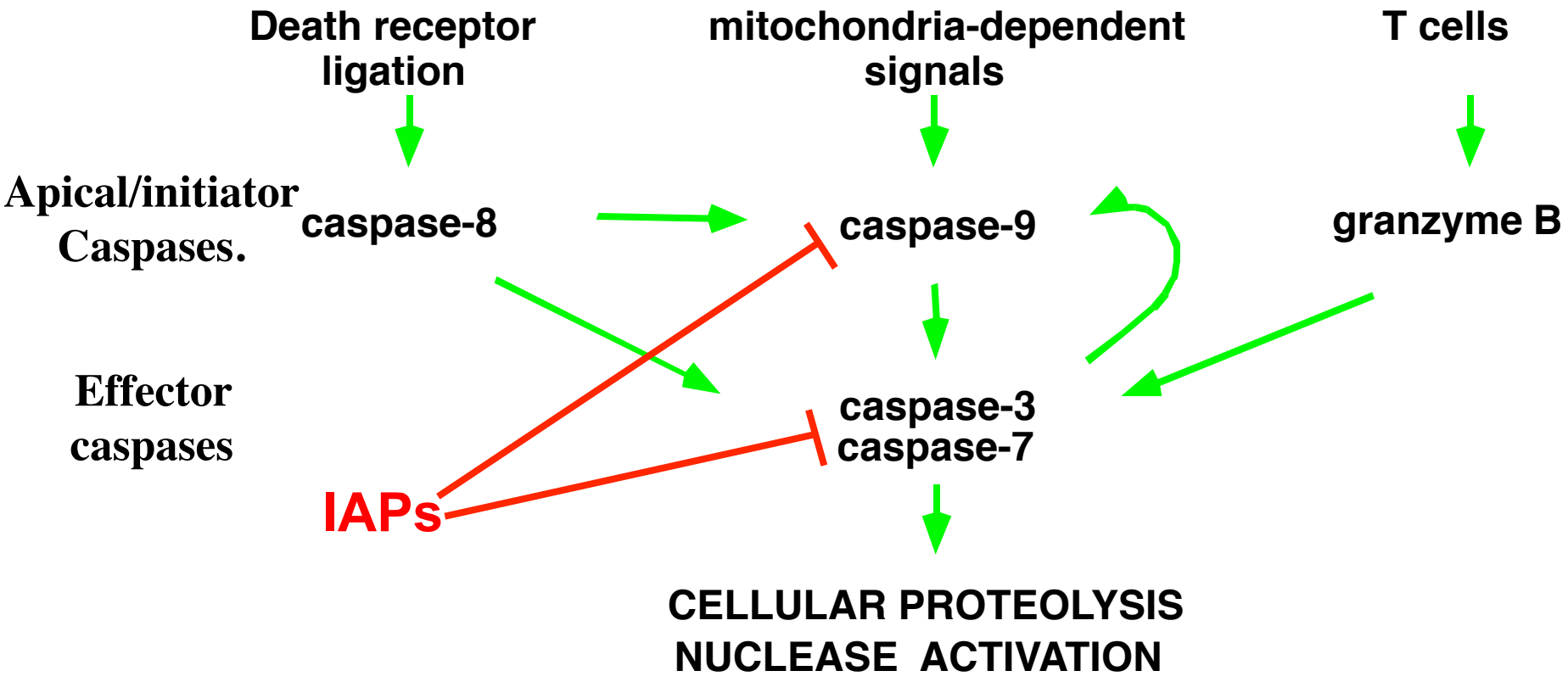
Caspases are present in every cell of our bodies but they are produced as inactive precursor (zymogen)

In response to cell death-inducing stimuli they become activated, cleave other proteins and induce the demise of the cell

Caspases exist in all animals (mammals, insects, worms, etc.)

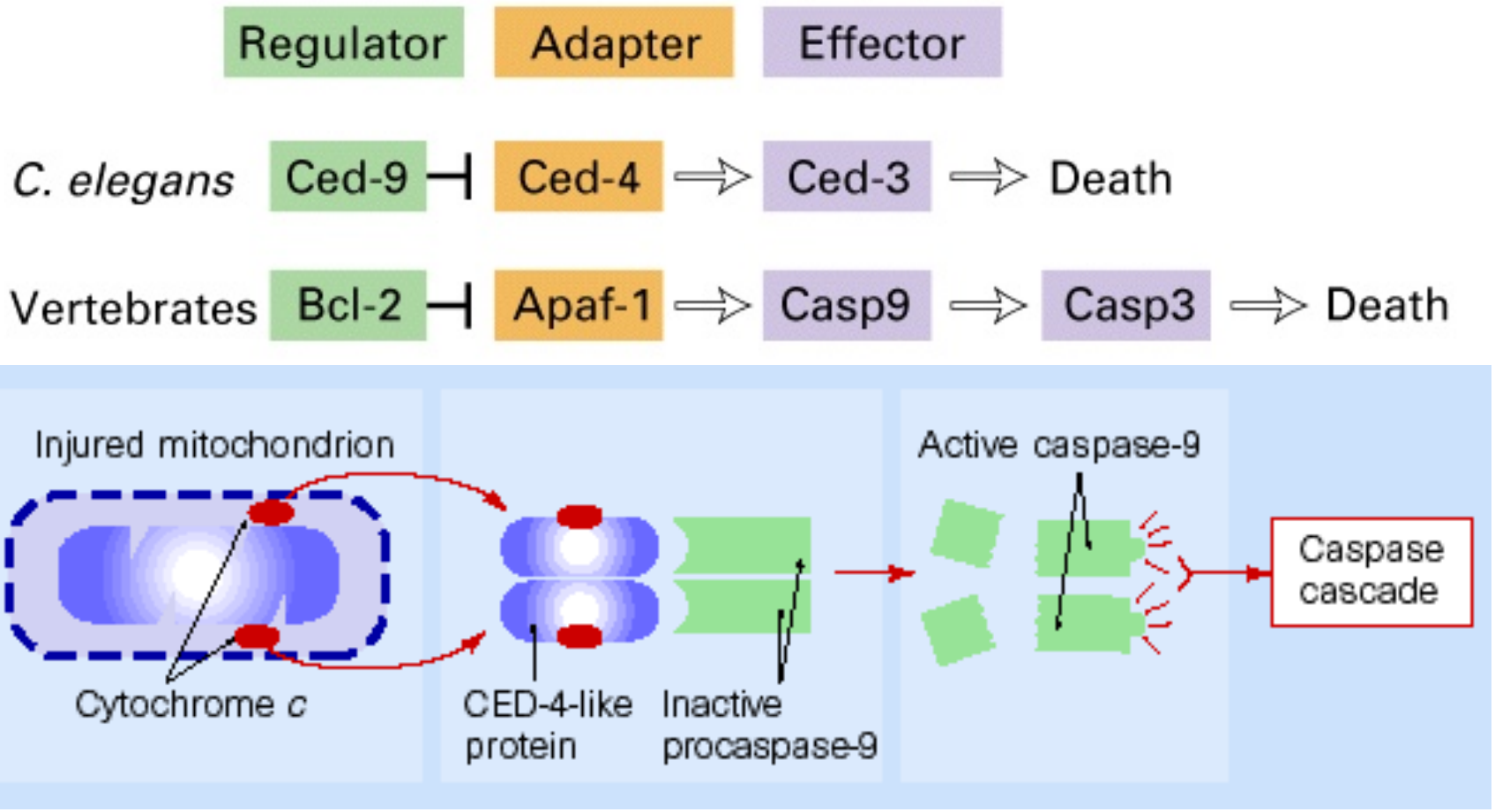

Positive Feedback
Caspase zymogen
Active caspase
large
small
cell death-activating
substrates

Caspases transduce cell death signals and mediate cell destruction



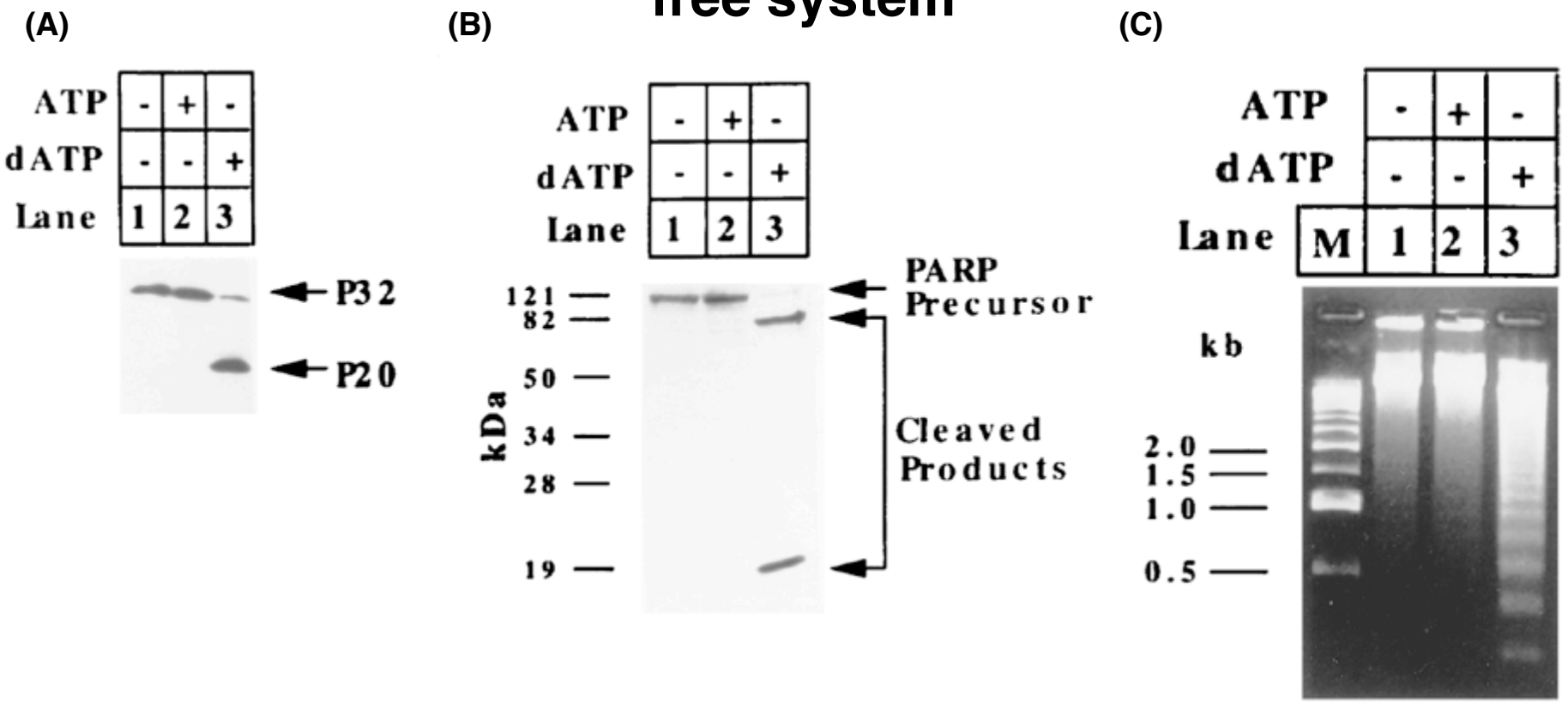
Caspases sufficient to mediate apoptosis are present in all cells

Three classes of proteins function in the intrinsic apoptotic pathway



Bcl-2 family proteins inhibit (or activate) Apaf-1 through an indirect, mitochondria-dependent mechanism

Reconstitution of apoptotic elements in a mammalian cell-free system



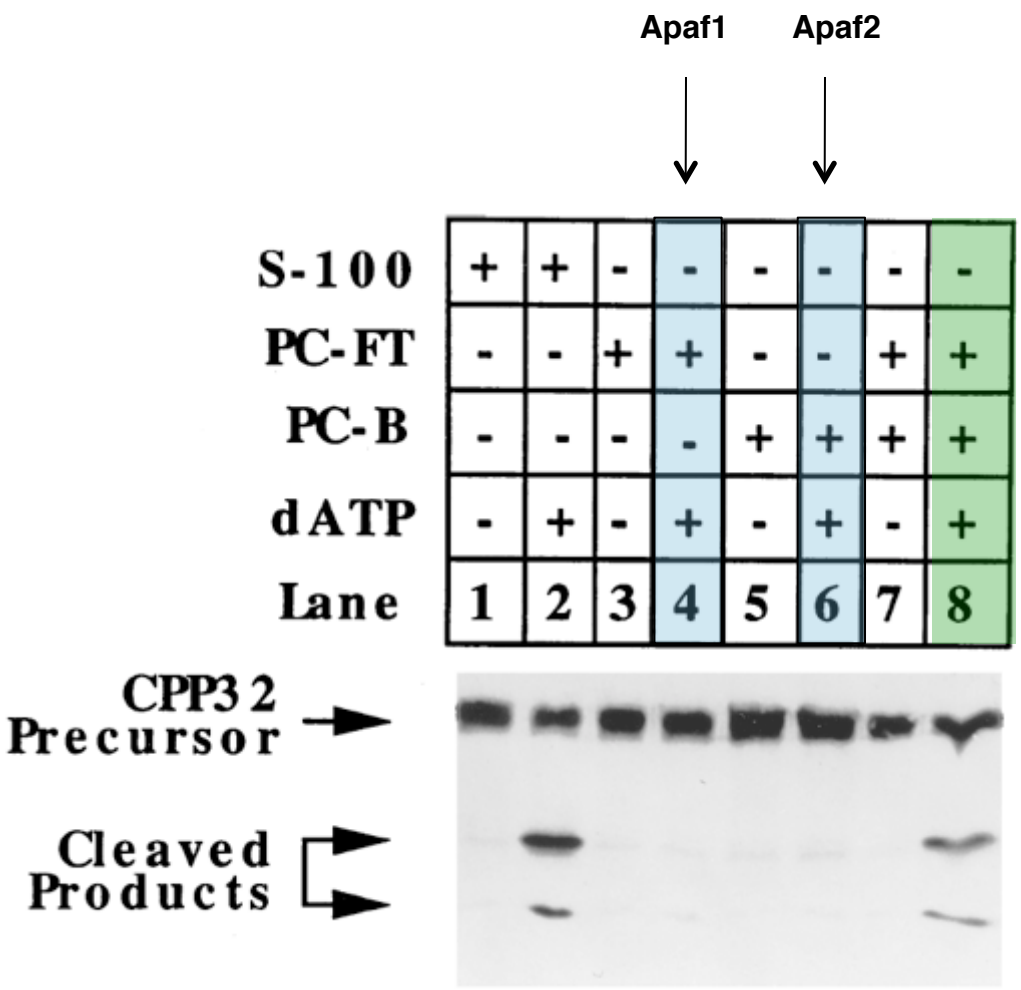
Cleavage of ced3 and DNA fragmentation used as two hallmarks of apoptosis.

(A) Hela (non-apoptotic) cytosolic fraction (S100) incubated with radiolabeled caspase 3 (=CPP32, mammalian ortholog of ced3). dATP required for cleavage.
(B) S-100 could cleave radiolabeled PARP (substrate for caspase CPP32).
(C) S100 incubated with purified nuclei.

Conclusion: S-100 supernatant could be activated by dATP into pro-apoptotic state. In this system, dATP triggers the apoptotic program in healthy cytosol.
dATP: deoxyadenosine-5-triphosphate

Liu et al. (1996) Cell

Separation of 2 factors



S100 loaded onto phosphocellulose column. Neither flow-through (FT) nor bound (B) fractions contained caspase 3 activation activity.

However, mixing the two fractions reconstituted activity.

Factors were named Apaf~apoptotic protease activating factor

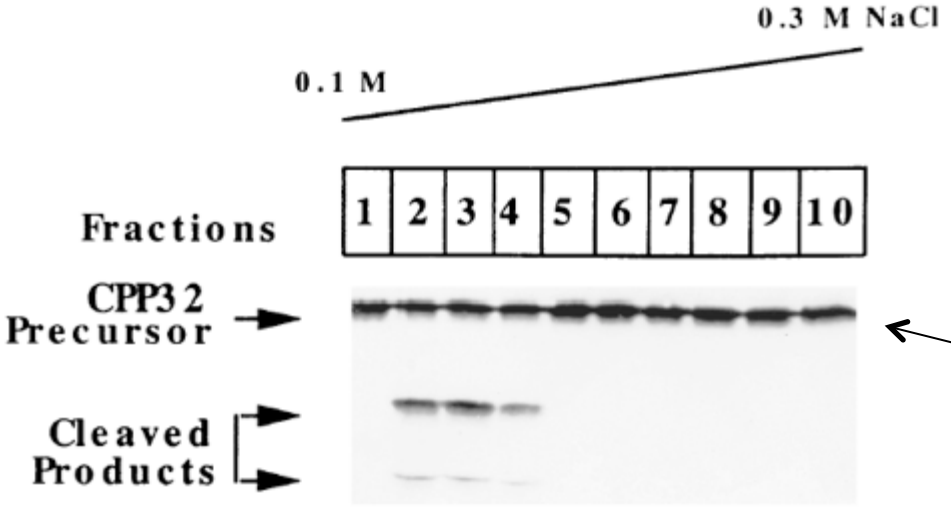
How to purify Apaf2?

Liu et al. (1996) Cell

What is in the PC bound fraction? Purification of Apaf2

Table 1. Purification of Apaf-2 from HeLa Cells

Step	Fraction	Protein	Specific Activity	Total Activity	Purification	Recovery
		mg	units/mg	unit	-fold	%
1	S-100	348.5				
2	Phosphocelluse	104	126.6	13166	1	100
3	50% Ammonium-Sulfate Precipitation	23.8	833.3	19824	6.6	150
4	Phenyl-Sepharose	0.473	42145	19934	333	151
5	Superdex-200	0.460	43367	19950	343	152
6	Mono Q/Mono-S	0.076	263150	20000	2079	152



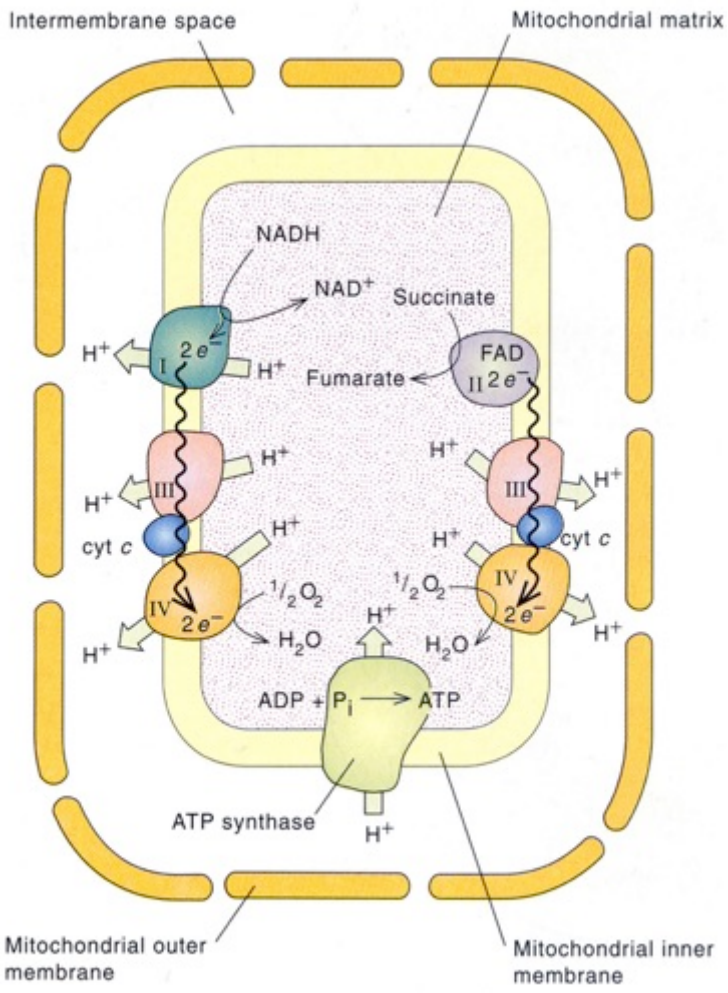
2. Phosphocellulose, bound
3. supernatant
4. bound
5. sizing column
6. Mono Q FT/Mono S (cation column) bound

Mono S fractions (last protein column) were incubated with the phophocellulose flow-through and radiolabeled caspase 3.

Purified Apaf-2 was identified by tryptic digest and peptide sequencing. cytochrome *c*

Liu et al. (1996) Cell

Cytochrome *c* functions in the respiratory chain



<u>Respiratory complexes in the inner membrane generate proton gradient and membrane potential</u>:

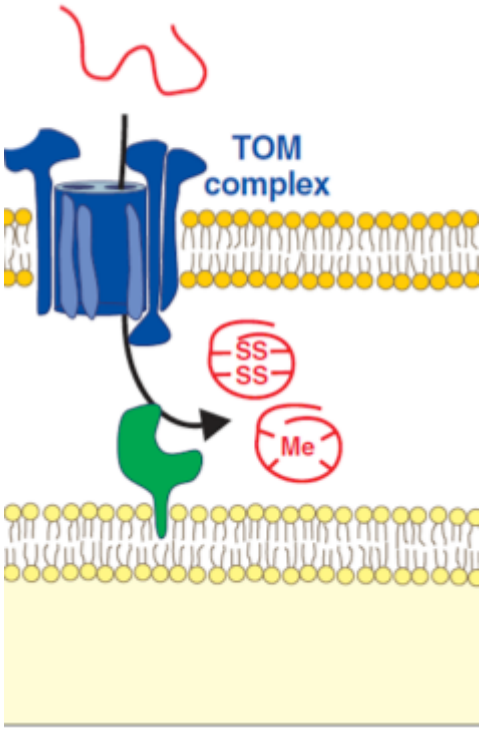
- **Complex I = NADH-CoQ reductase**
  - **Removes 2 electrons from NADH and transfers them to ubiquinone (Q) to make QH2.**
- **Complex III = $CoQH_2$-cytochrome *c* reductase; cytochrome bc_1 complex**
  - **Transfers electrons from QH2 to cytochrome *c*.**
- **Complex IV = Cytochrome c oxidase**
  - **Transfers electrons from cytochrome *c* to molecular oxygen, producing water.**
- **Complex II = Succinate-CoQ reductase; succinate dehydrogenase**
  - **Removes electrons from succinate and transfers them to ubiquinone. No protons pumped.**

Ubiquinone (=Coenzyme Q) and cytochrome *c* are mobile/ soluble electron carriers between the respiratory complexes.

Import of cytochrome *c* to the IMS

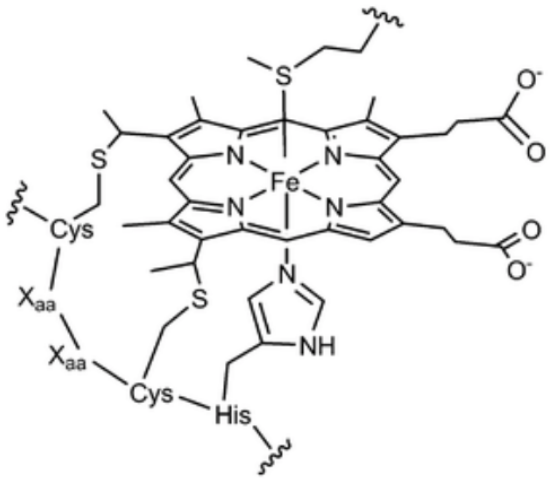
After import into the intermembrane space folding leads to trapping of cytochrome *c*.

Apocytochrome *c* is covalently attached to heme by cytochrome *c* heme lyase (in intermembrane space) to form holocytochrome *c*. Only the latter species is active in promoting apoptosis.



Could apoptosis induction be due to a co-purifying protein?

- **Commercial cytochrome *c* is active.**
- **Immunodepletion of cytochrome *c* removes apoptotic activity of S100.**



Cytochrome *c* contains a CXXCH motif for coordinating heme c. His and Met are axial ligands.

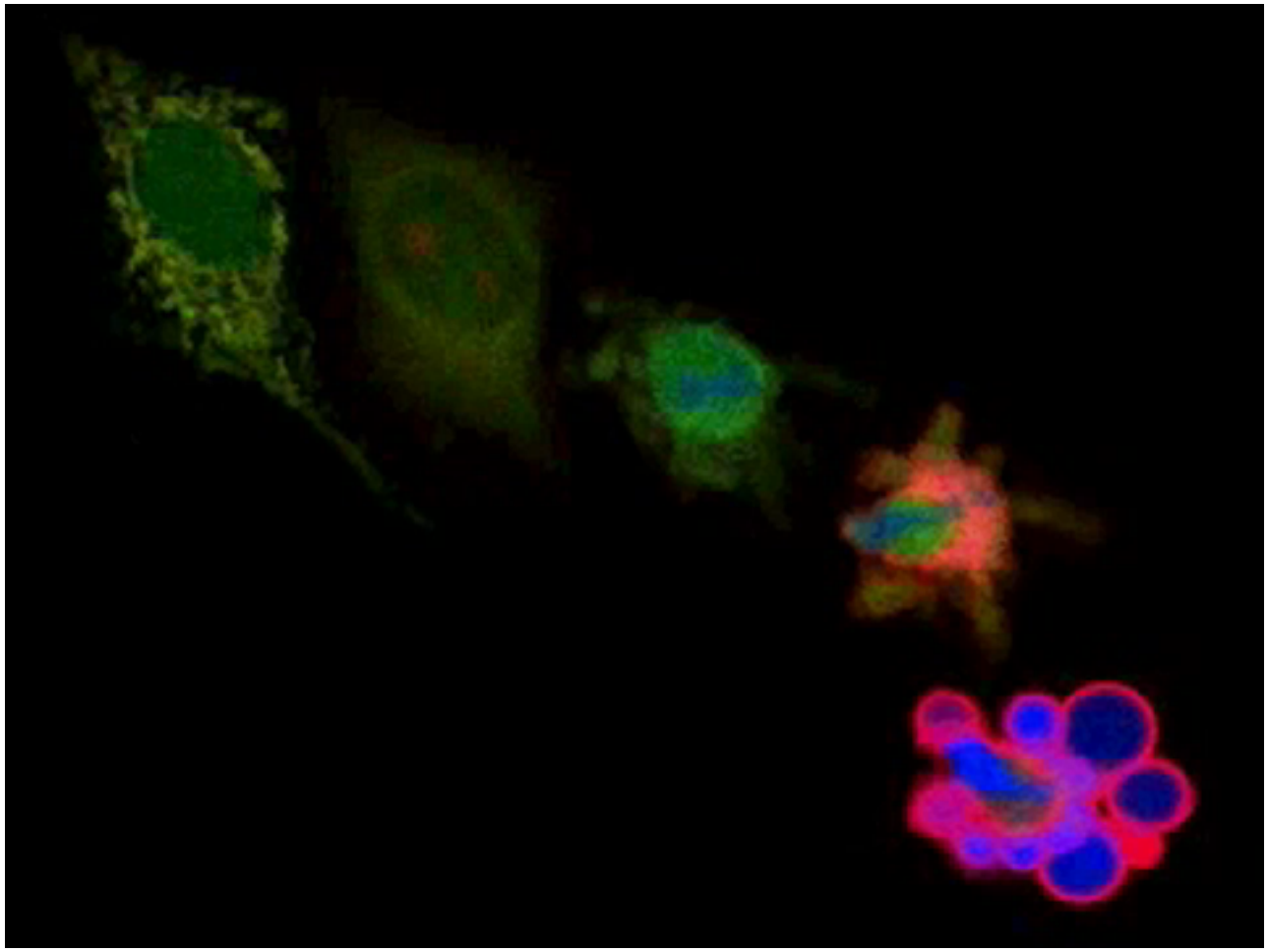
Neupert (2007) Ann Rev Biochem

Apoptosis: release of cytochrome c, annexin V staining, and cell permeabilization

cytochrome *c* (green).

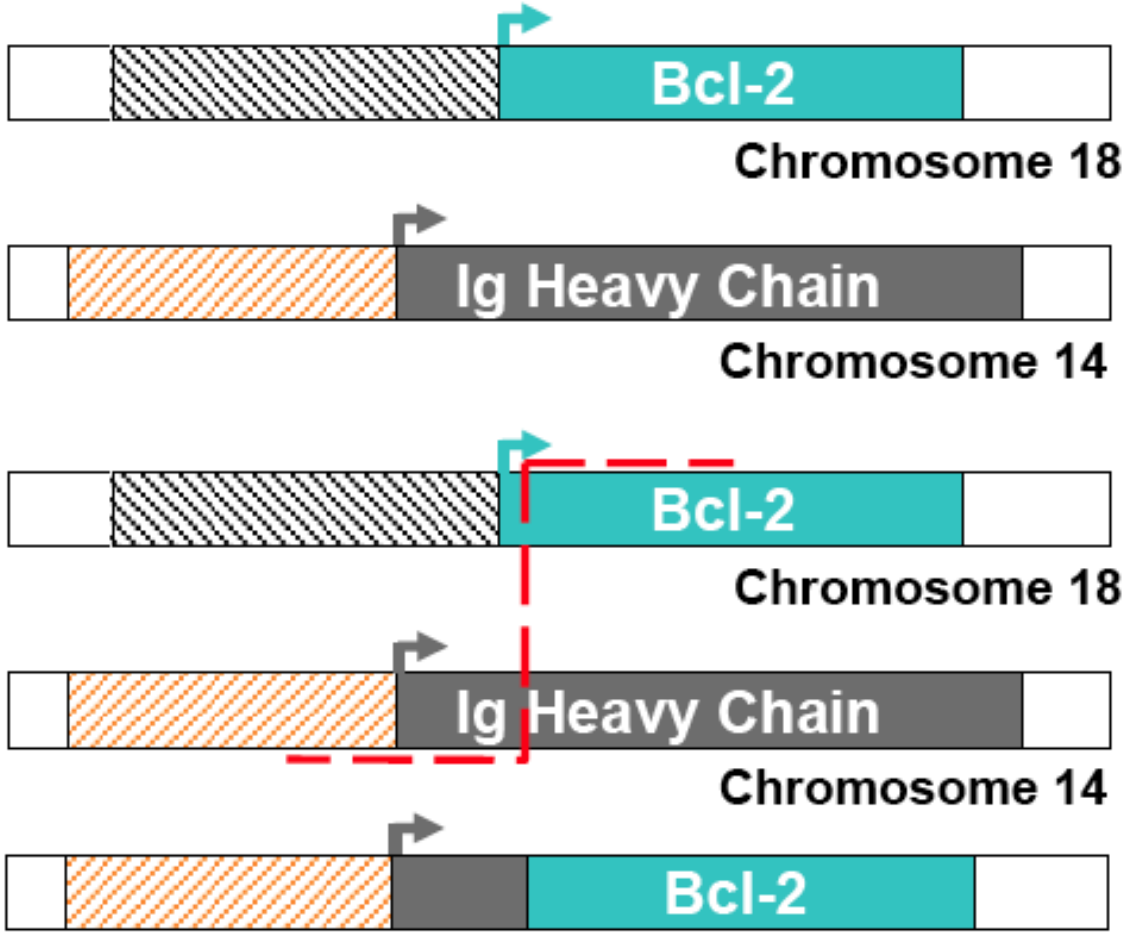
Annexin V is a phopholipid binding protein with high affinity for phosphatidylserine (PS).

PS (red) is translocated from the inner leaflet to the outer leaflet during cell death.



Alberts et al. (2007)

t(14;18) Chromosomal Translocation Causes Human B-Cell Leukemia by Overexpression of Bcl-2

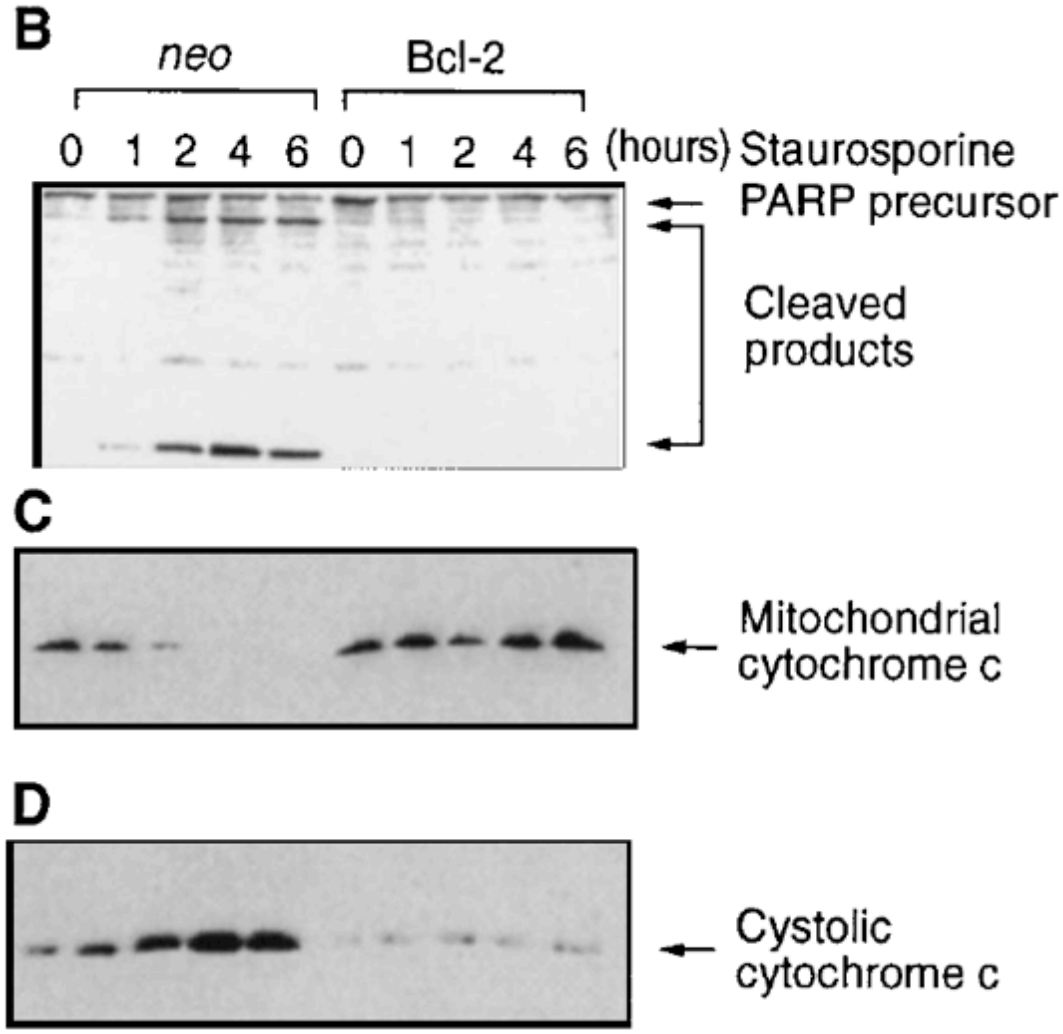


t(14;18) Chromosomal Translocation

(Vaux, Cory & Adams, 1988)

(Korsmeyer, *et al.*; Croce, *et al.*; Sklar, *et al.*; 1985 - 1990)

Bcl2 prevents cytochrome *c* release



Bcl2 (B-cell lymphoma 2) is activated by a chromosomal translocation in follicular lymphomas. Bcl2 is placed under transcriptional regulation by the immunoglobulin locus.

Ced9 is Bcl2 ortholog in worms.

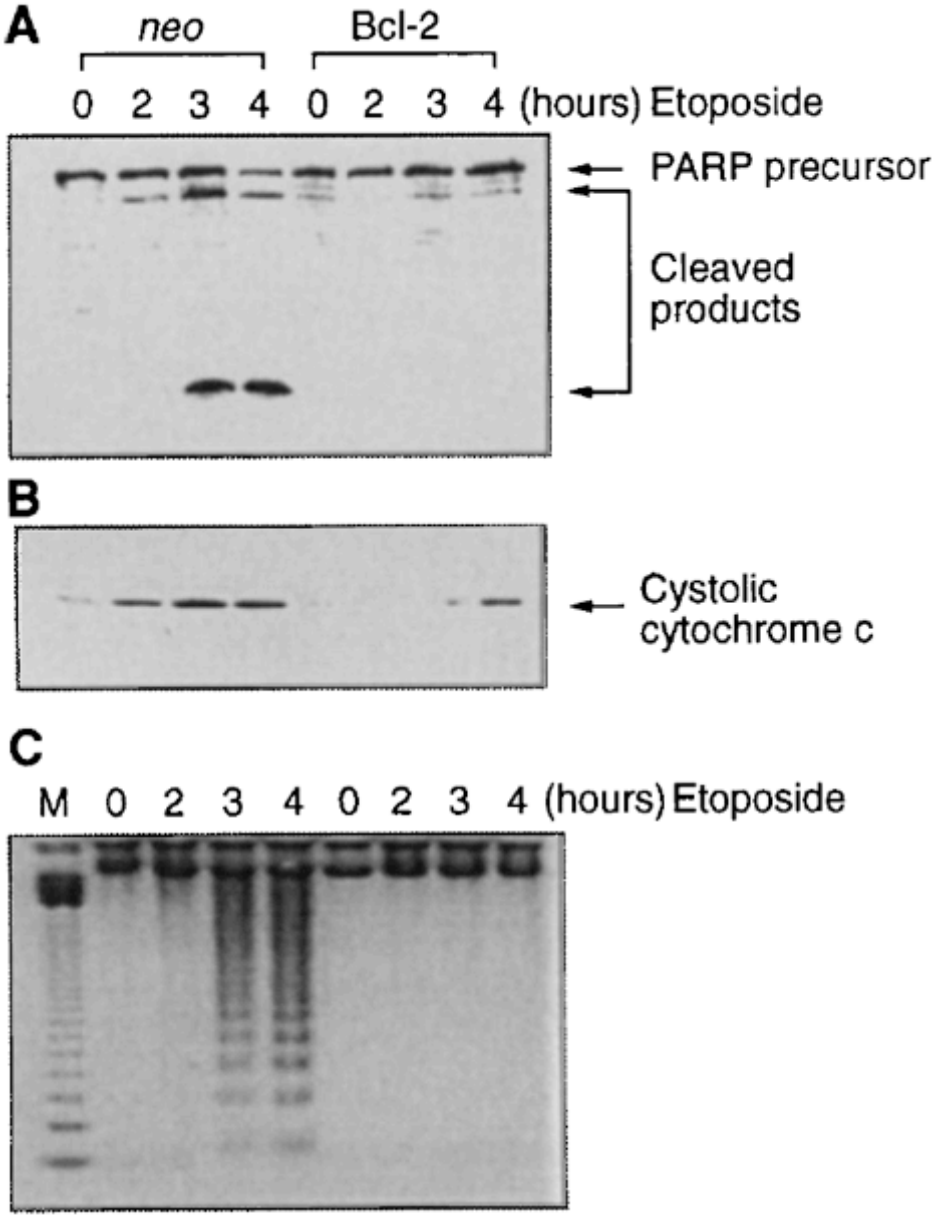
Human leukemia cells transfected with Bcl2 or neomycin control. Treated with 1 μM staurosporin (broad-range protein kinase inhibitor) to induce apoptosis.

(B) PARP [poly(adenosine diphosphate-ribose) polymerase] cleavage used as marker for caspase 3 activity.

(C) and (D) Bcl2 overexpression prevents cytochrome *c* release into cytosol.

Yang et al. (1997) Science

Bcl2 prevents cytochrome *c* release



Cells treated with etoposide (inhibitor of topoisomerase II; causes DNA strand breaks) to induce apoptosis.

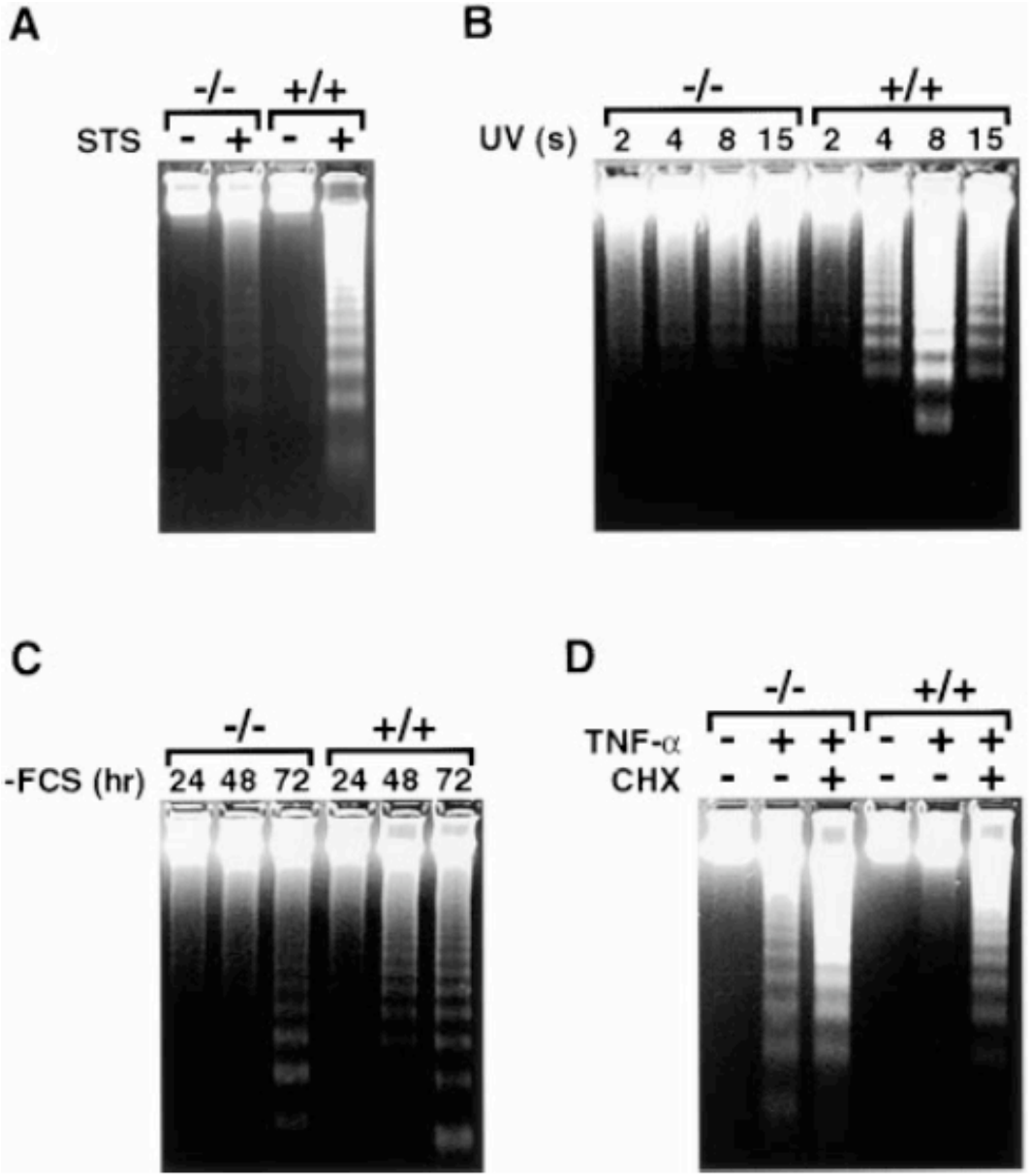
(A) PARP [poly(adenosine diphosphate-ribose) polymerase] cleavage used as marker for caspase 3 activity.

(B) Bcl2 overexpression reduces/slows cytochrome *c* release into cytosol.

(C) DNA fragmentation assay.

Yang et al. (1997) Science

Cells that lack cytochrome c cannot carry out OXPHOS, and are selectively insensitive to some apoptotic stimuli



However, more sensitive to TNF.

Li et al. (2000) Cell

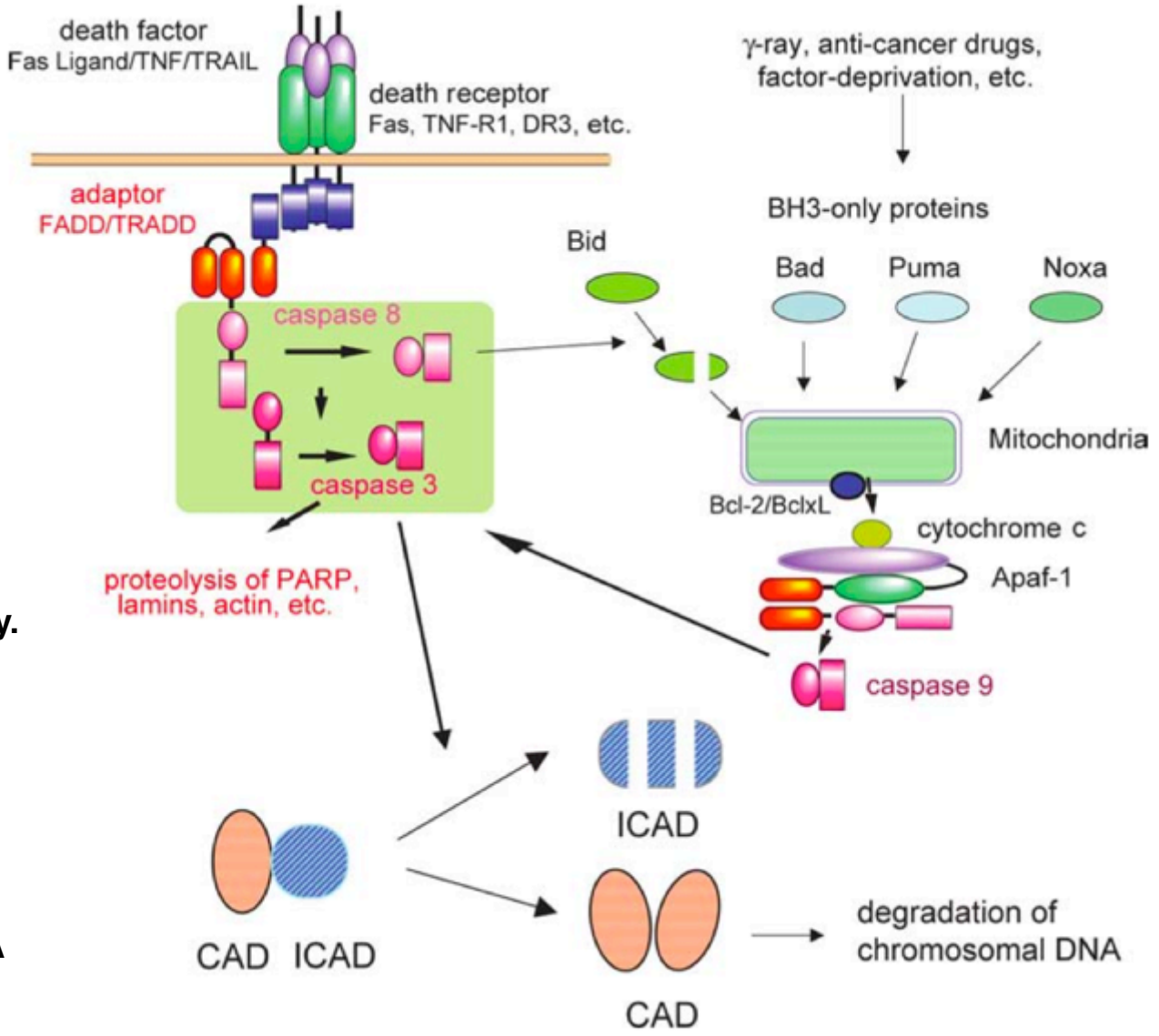
CAD and ICAD act downstream of caspases

caspase 3 cleaves ICAD at 2 positions, ICAD releases CAD, CAD enters nucleus (due to nuclear localization sequence) and cleaves chromosomal DNA between nucleosomes.

However, mice lacking CAD or ICAD deficiency develop normally. Once caspases are activated, cells still die.

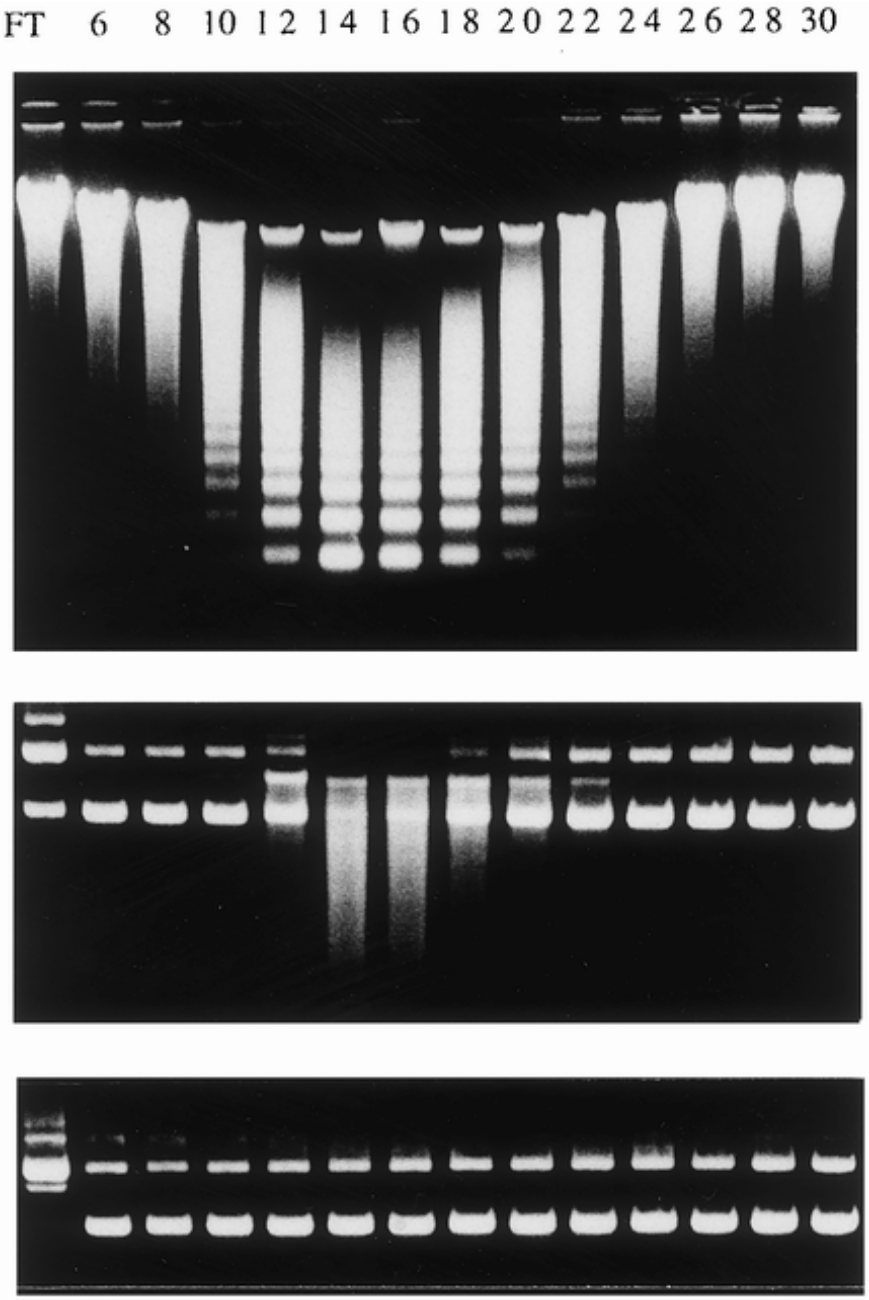
Mitochondrial Endo G and other nucleases also implicated.

DNAse II in macrophages important for degradation of DNA (non-cell autonomous).



Nagata (2009) Annu. Rev. Immunol.

Identification of a caspase-activated DNAse (CAD)



S100 supernatant from mouse T lymphocytes (WR19L cells) were loaded onto DEAE Sepharose column. Fractions eluted, treated with caspase 3.

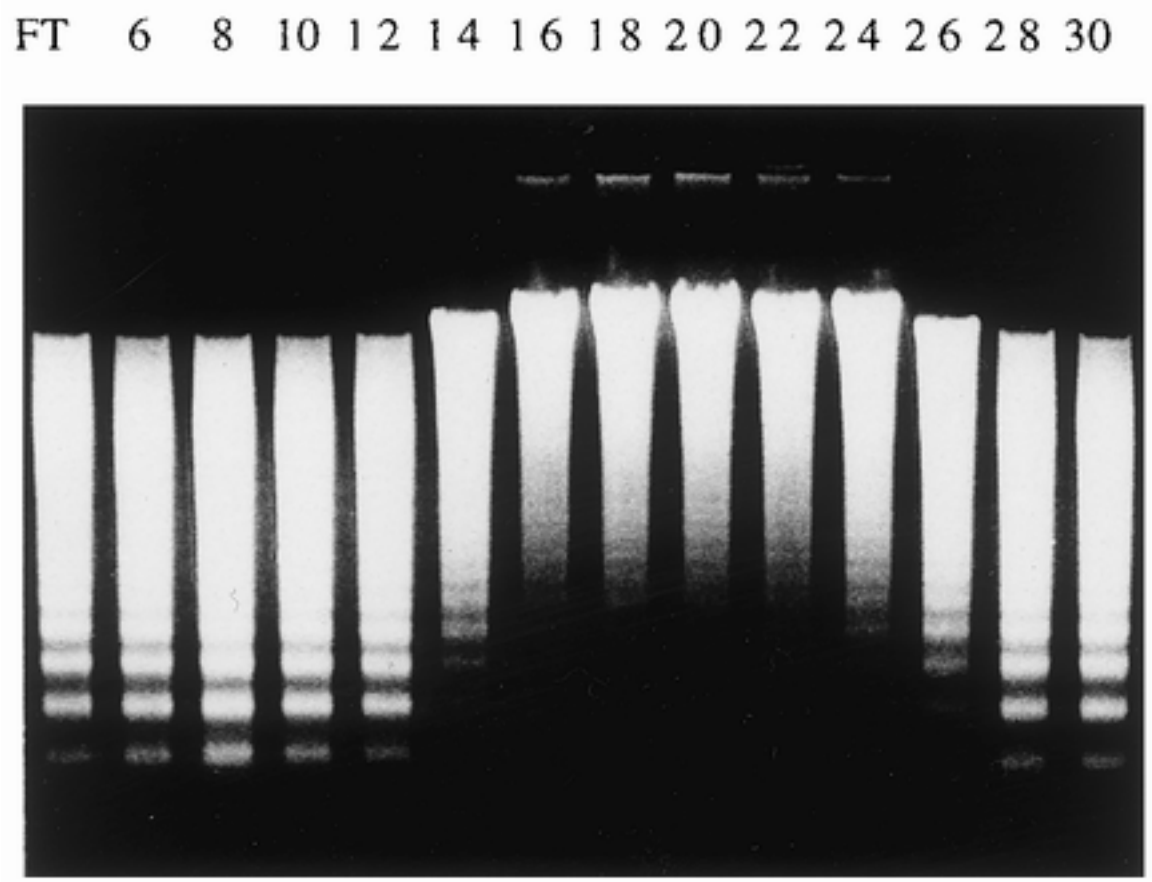
DNA degradation activity tested on genomic DNA (top) and plasmid DNA (middle).

In bottom panel no caspase 3 was added.

<u>Conclusion</u>: caspase 3 activates a nuclease (termed CAD) present in the S100 supernatant.

Enari et al. (1998) Nature

Identification of CAD inhibitor (ICAD)

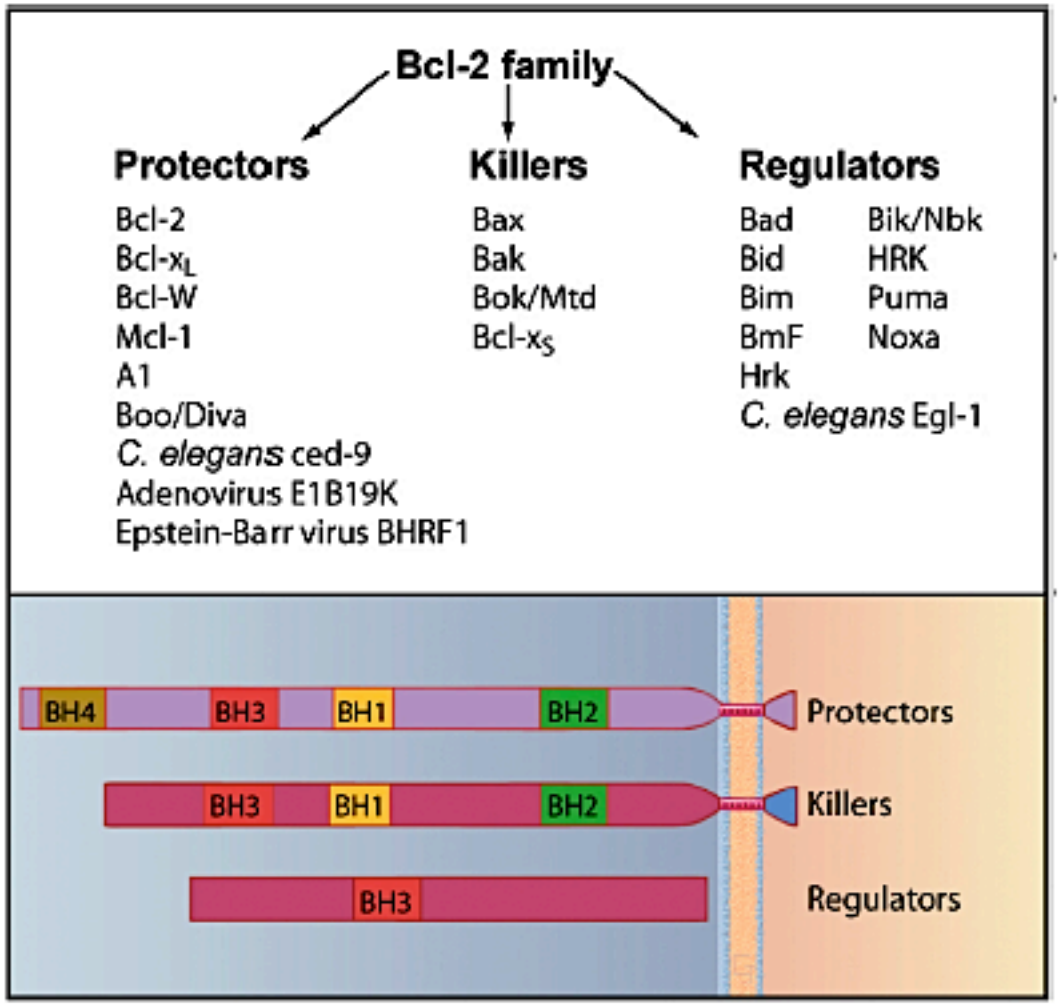


CAD activity of Fas-activated cells was inhibited by S100 fraction of growing cells.

iCAD activity assayed in fractions by inhibition of degradation from active CAD sample.

Enari et al. (1998) Nature

Proteins Regulating Apoptosis: Bcl-2 family



Mechanism of action

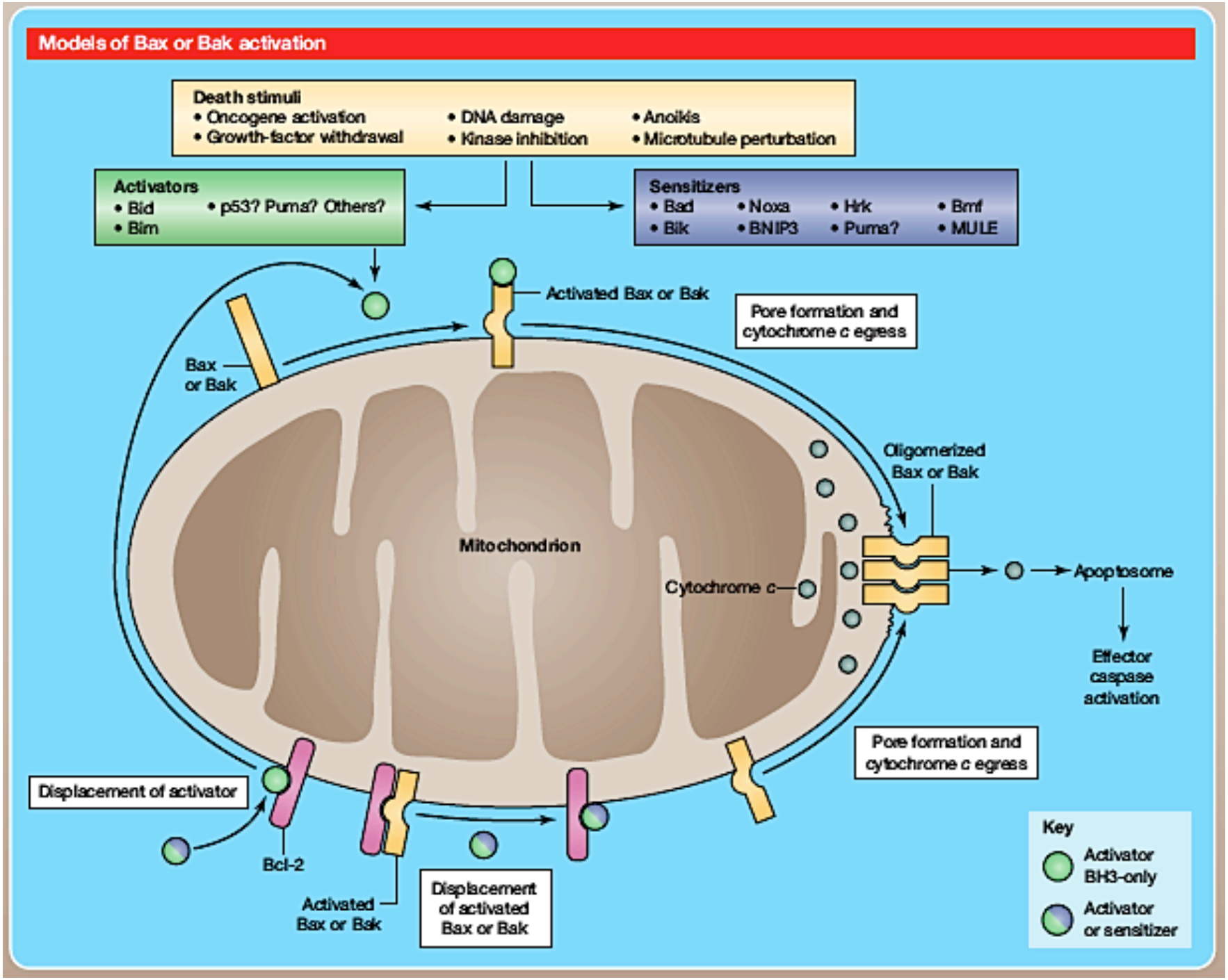
BH domain

Anti-apoptotic Blc-2

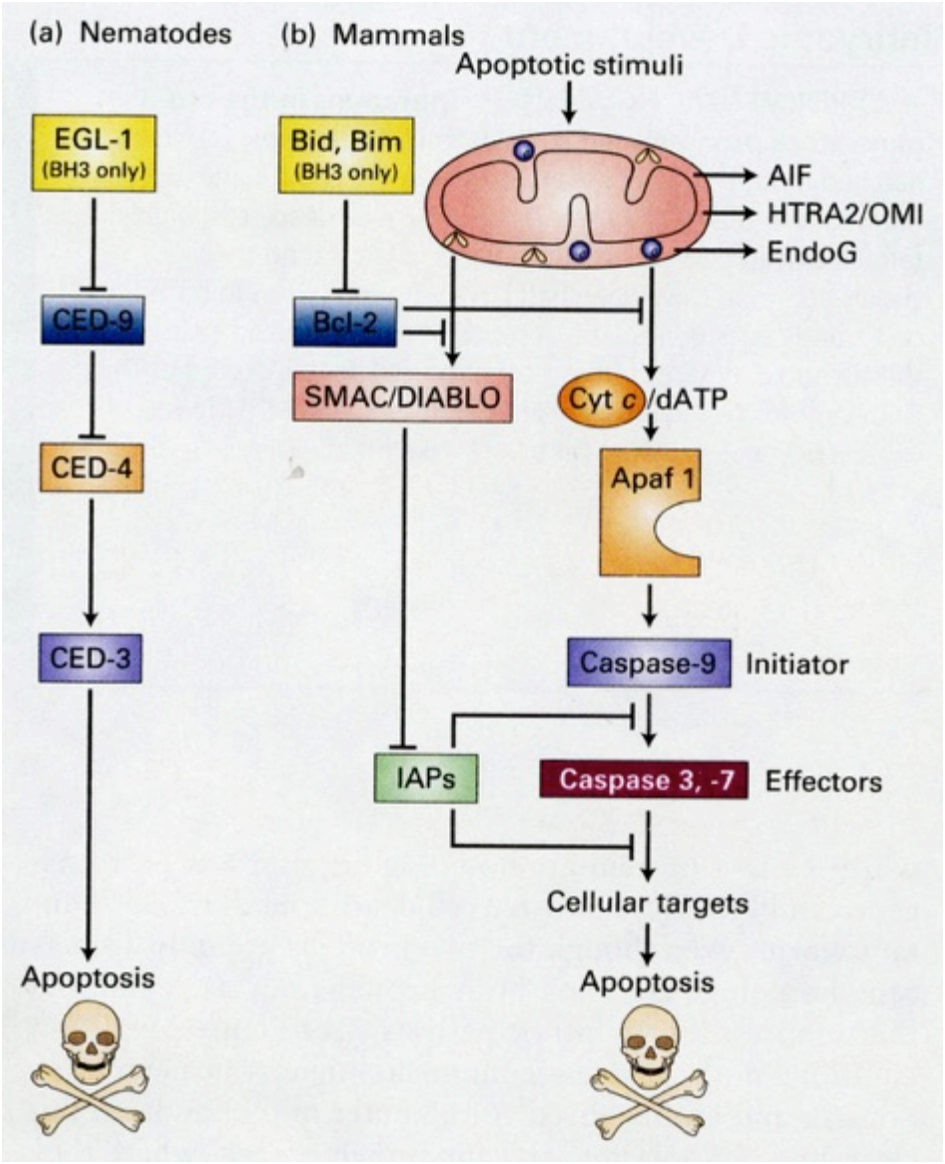
- Have 4 of these domains – BH1, BH2, BH3, BH4
- Block mitochondrial pathway

Pro-apoptotic Bcl-2

- May have 3 of these domains
- BH3 domain
  - Regulate protein-protein interaction that signal cell death


Models of Bax or Bak activation
Death stimuli
• Oncogene activation
• Growth-factor withdrawal
• DNA damage
• Kinase inhibition
• Anoikis
• Microtubule perturbation
Activators
• Bid
• Bim
• p53? Puma? Others?
Sensitizers
• Bad
• Bik
• Noxa
• BNIP3
• Hrk
• Puma?
• Bmf
• MULE
Activated Bax or Bak
Pore formation and cytochrome c egress
Bax or Bak
Oligomerized Bax or Bak
Mitochondrion
Cytochrome c
Apoptosome
Effector caspase activation
Pore formation and cytochrome c egress
Displacement of activator
Bcl-2
Activated Bax or Bak
Displacement of activated Bax or Bak
Key
Activator BH3-only
Activator or sensitizer

Some pathways of apoptosis



Lodish et al. (2008)

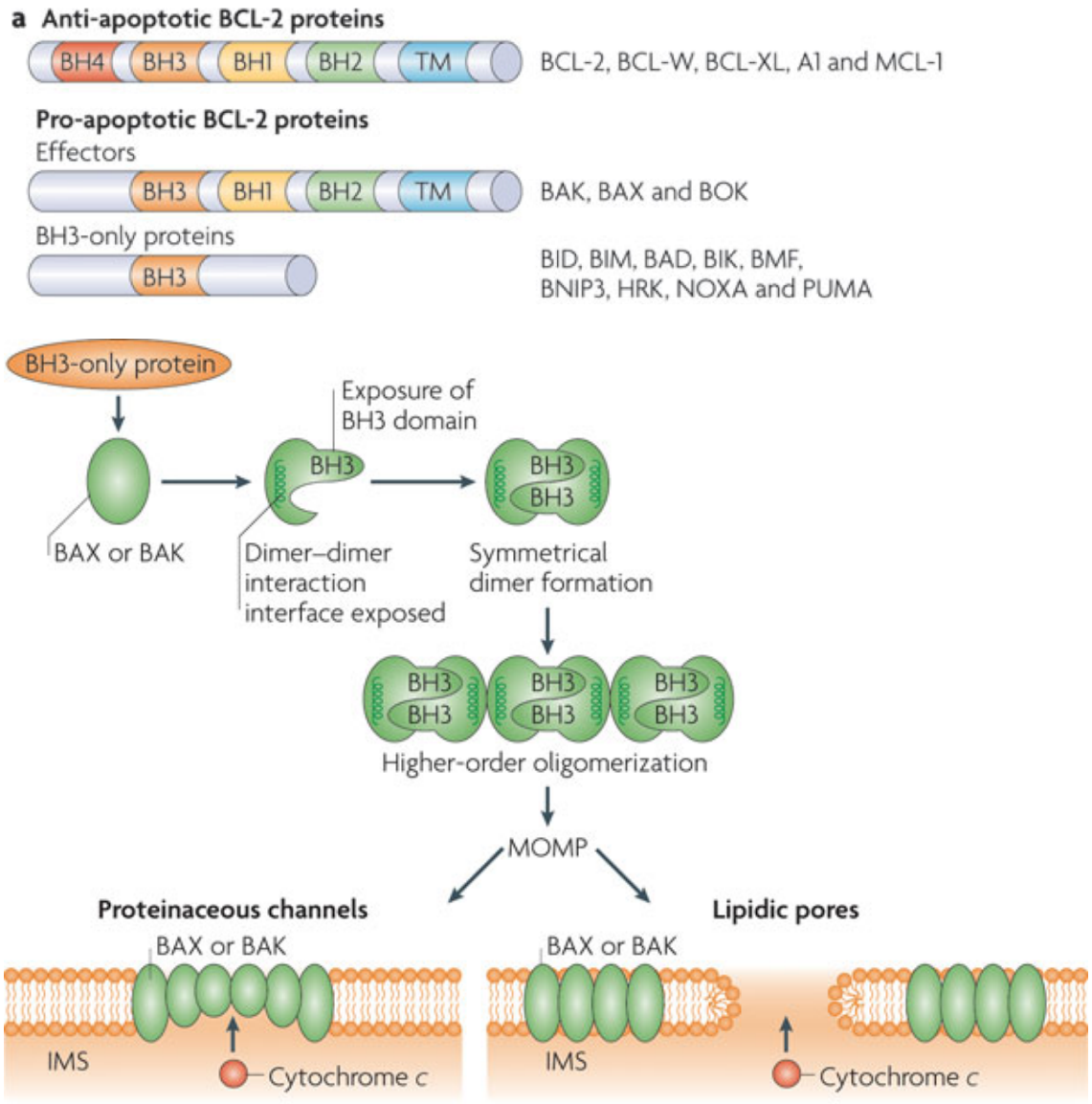
Worms: Ced4 is normally suppressed by its physical interaction with Ced9 on the mitochondrial surface. Eg1-1 transcriptional activation competes for Ced-9 binding, freeing Ced4 to activate Ced3.

Mammals: Release of cytochrome *c* activates Apaf1, resulting in oligomerization into the apoptosome that activates procaspase 9 (by autocleavage). Caspase 9 is an initiator caspase that then activates effector caspases like caspase 3.

Caspases are normally inhibited by binding of inhibitor of apoptosis proteins (IAPs). Upon apoptosis induction, mitochondria release SMAC/DIABLO that release IAP from caspases.

How does mitochondrial outer membrane permeabilization occur?

Bax and Bak cause mitochondrial outer membrane permeabilization (MOMP)



BH domains= Bcl2 homology domains

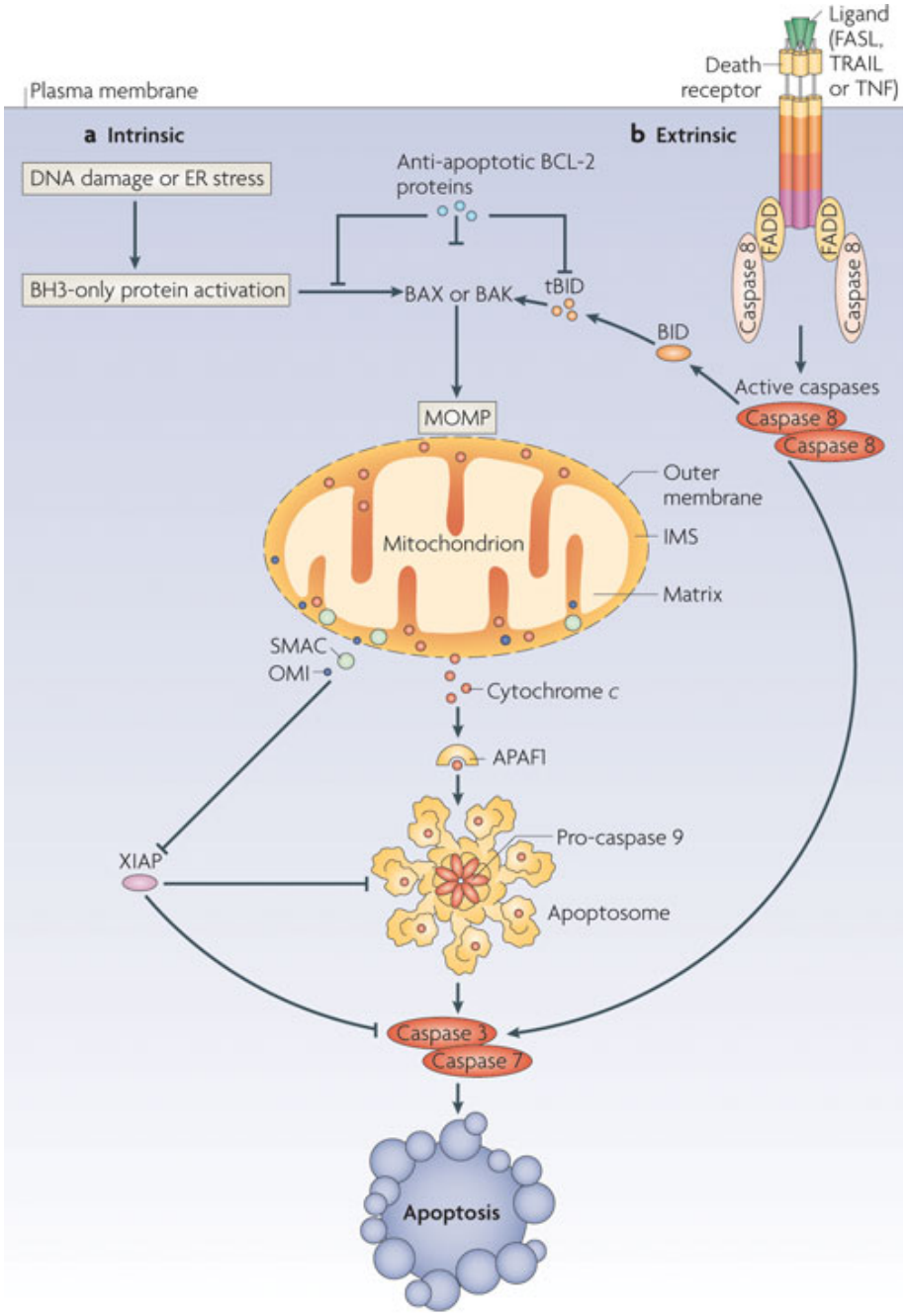
Bid=BH3 interacting domain protein death agonist
tBid=truncated Bid (activated)
Bax=Bcl2 associated X protein
Bak=Bcl2 antagonist or killer

BH3-only proteins (e.g., Bid) activate Bax or Bak, leading to their oligomerization.

These oligomers appears to permeabilize the mitochondrial outer membrane, perhaps by forming pores.

Tait & Green. (2010) Nat Rev MCB

Intrinsic and extrinsic cell death pathways involve MOMP



Intermembrane space proteins implicated in cell death:

Cytochrome *c*: required for caspase activation

SMAC/Diablo: second mitochondria-derived activator of caspase; binds and antagonizes XIAP (X-linked inhibitor of apoptosis protein)

OMI/HtrA2: mitochondrial protease; binds and cleaves XIAP

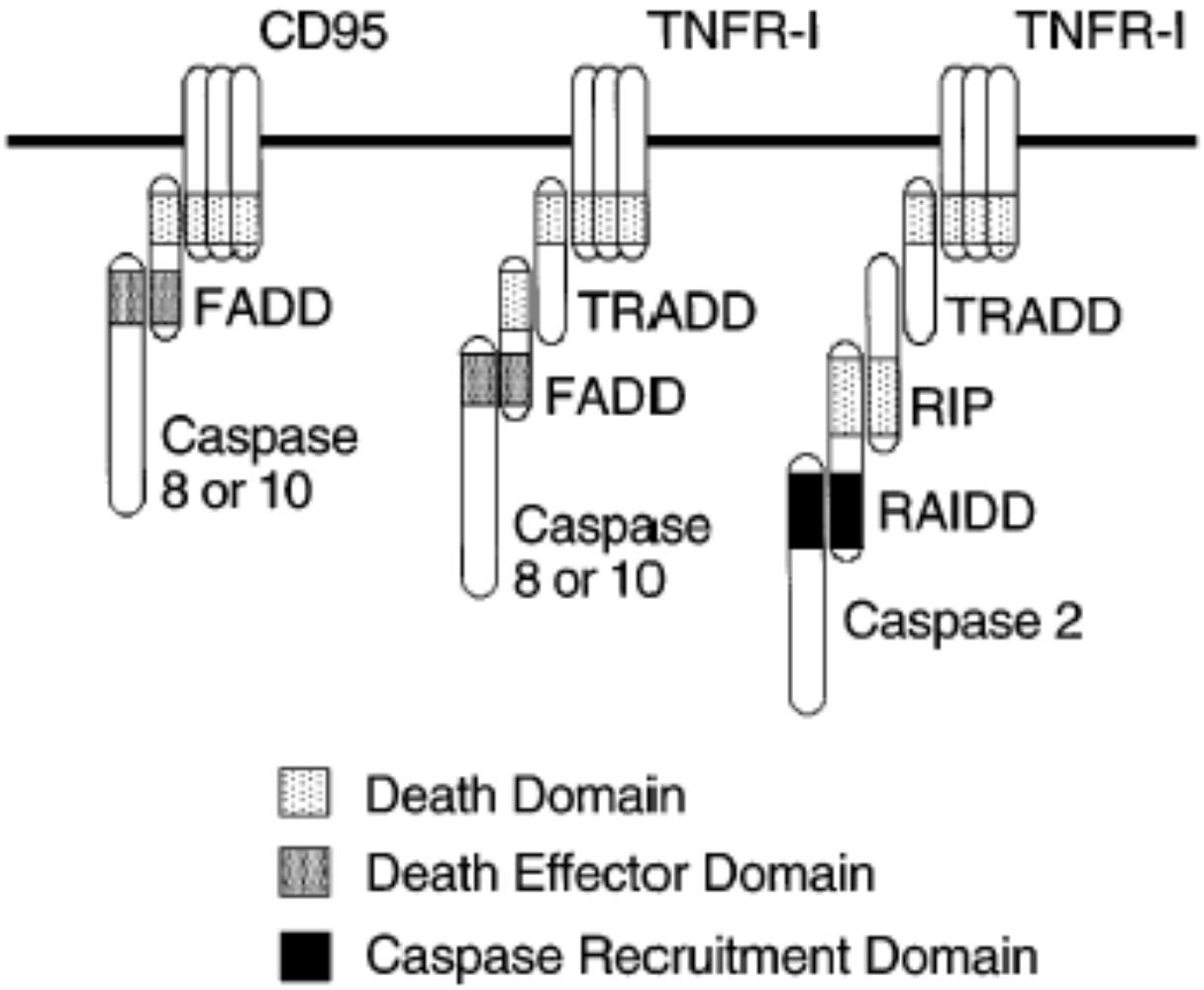
AIF: Apoptosis-inducing factor

Endonuclease G: Nuclease in mitochondria.

However, knockout mice studies indicate that apoptosis can occur in the absence of the latter 4.

Tait & Green. (2010) Nat Rev MCB

Extrinsic signals can signal cell death



Intrinsic versus extrinsic pathways for cell death

Fas= target of 2 monoclonal antibodies that triggered apoptotic cell death in some tumor cells.

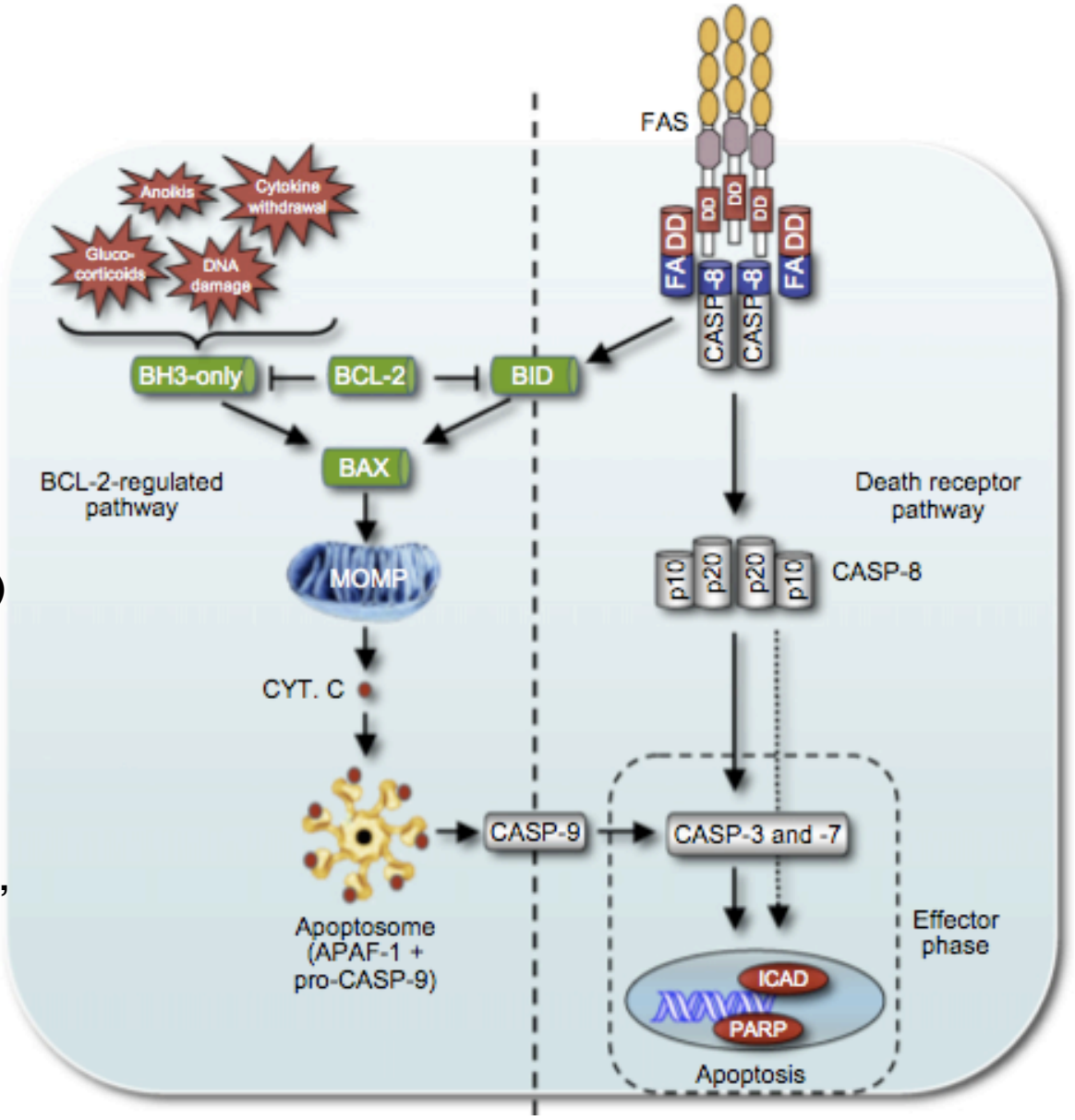
Cloning of Fas showed that it is member of tumor necrosis factor receptor family (class of cytokine receptors).

Led to expression cloning of the Fas ligand (FasL).

Spontaneous mouse mutants in Fas (lpr) or FasL (gld) develop lymphadenopathy and lupus-like autoimmune disease.

Ligation of Fas leads to assembly of DISC (death-inducing signaling complex). Caspase 8 is activated by (1) dimerization and conformational change, and (2) autoproteolytic processing. The activated enzyme leaves the DISC and cleaves substrates within the cell.

CAD=caspase-activated DNAse
iCAD=inhibitor of CAD
BID activated by caspase 8 cleavage.



Nagata et al. (2009) Immunity

Decoy ligands block death signaling from outside the cell; Decoy adapters block death from the inside

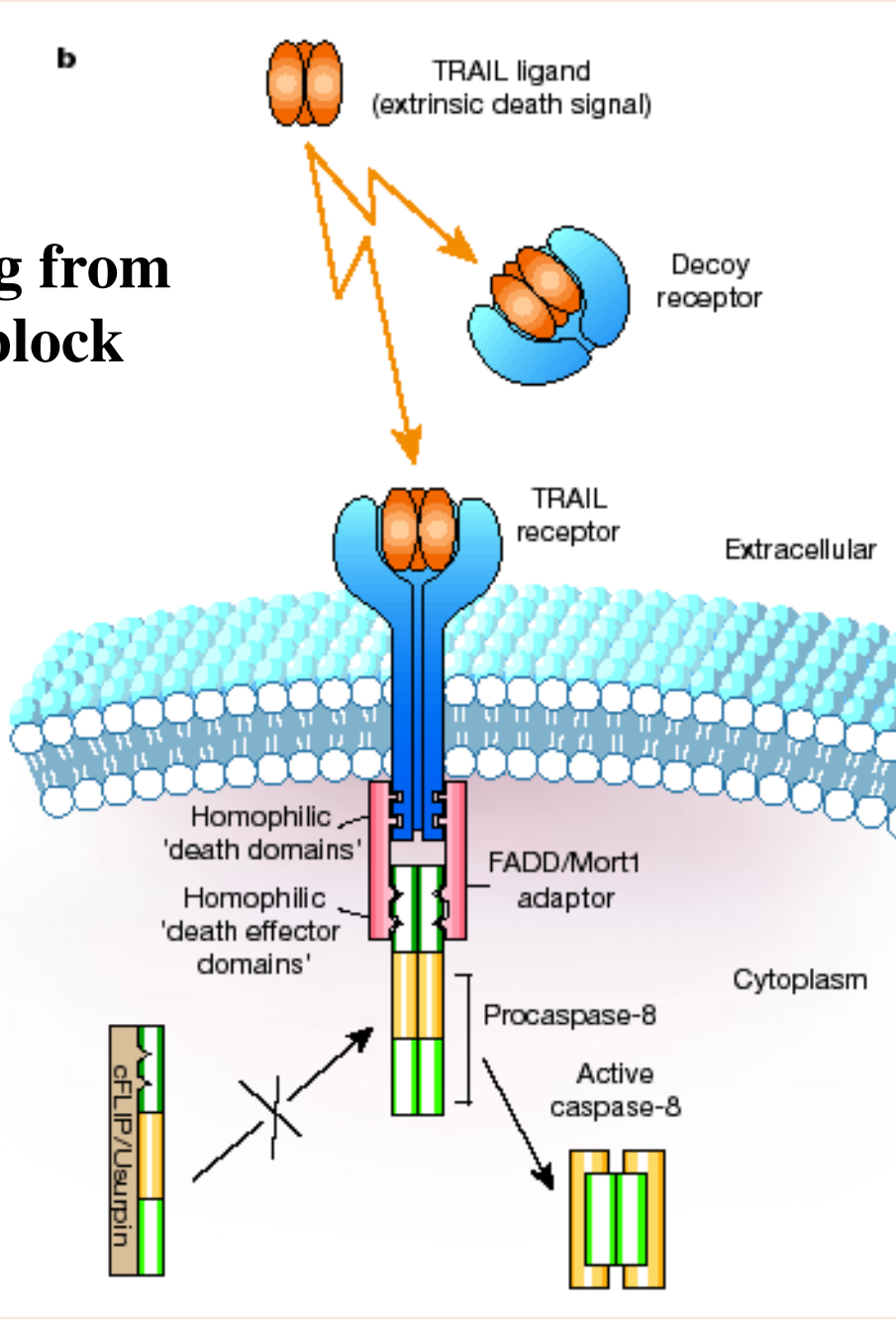


Figure 2 TRAIL. The key elements of the TRAIL signalling pathway are conserved in other members of the TNF 'death receptor' family, such as CD95 (Apo-1/Fas). **a**, The trimerized TRAIL ligand (blue) is shown from a top-down view nestled into a trimer of the DR5/TRAIL-R2 receptor ectodomain (light brown). Coordinated Zn^{2+} seems to have a key role in conferring the appropriate signalling conformation. **b**, The 'extrinsic' cell death pathway is launched by death-receptor ligands that trigger caspase-8 oligomerization and proximity-induced autoproteolytic activation via adapter molecules such as FADD/Mort1. Activation is regulated by decoy receptors, which preclude the binding of TRAIL to the functional receptor, and dominant-negat pseudo-caspases such as c-FLIP/Usurpin, which prevent the recruitment of the caspase-8 proenzyme into the receptor complex.

Survival signals often block preexisting death signals