Morte celular: necrose e apoptose

Manuela Gomes da Silva

Biologia Celular

Imagens:
Molecular Biology of the Cell. Garland Publishing, Inc.

Cell death definition

Nomenclature Committee on Cell Death (NCCD, 2009)

Classification according to:

- **Morphology**: necrotic, apoptotic…
- **Enzymology**: nucleases or distinct classes of proteases
- **Function**: programmed or accidental
  physiological or pathological.

Point-of-no-return

Any one of the following morphological criteria

- Integrity loss of plasma **membrane**
  (incorporation of vital dyes *in vitro*)
- Cell and nucleus **fragmentation** into discrete bodies
  ('apoptotic bodies')
- Cell (or its fragments) **engulfment** by adjacent cell

Kroemer et al 2009. Cell Death and Differentiation. 16:3

Distinct types of cell death

CELL DEATH MODE	MORPHOLOGICAL FEATURES
Necrosis	• **Cytoplasmic swelling** • **Swelling** of cytoplasmic **organelles** • **Rupture** of plasma membrane
Apoptosis	• **Rounding-up** of the cell • **Reduction** of cellular and nuclear **volume** • Chromatin **condensation** • **DNA fragmentation** • Minor modification of cytoplasmic organelles • Plasma **membrane blebbing**

Kroemer et al 2009. Cell Death and Differentiation. 16:3

The road to necrosis

Homeostatic 'steady state'

⇩

Cellular **adaptations**

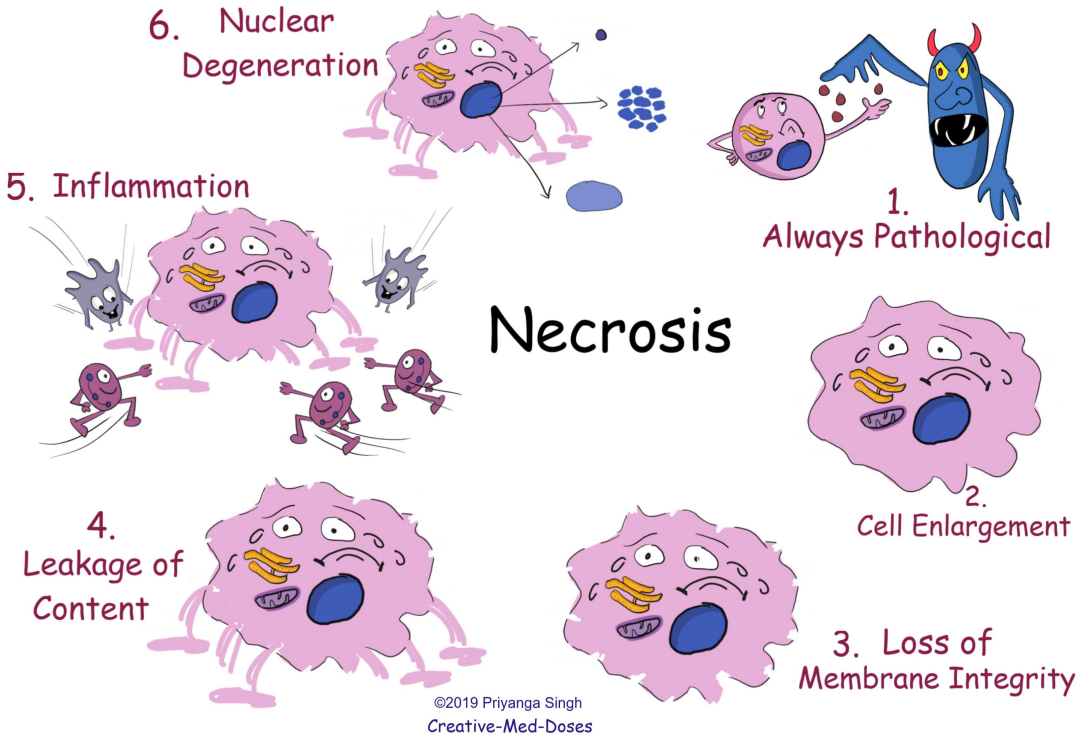
⇩

Reversible cell injury

⇩

Irreversible cell injury

Cell death ⟲ **Necrosis**



Homeostasis is the property of a system within the body of a living organism in which a variable is actively regulated to remain nearly constant.

Programmed Cell Death

Programmed cell death (PCD) is defined as
a sequence of events that lead to
controlled and organized destruction of the cell.

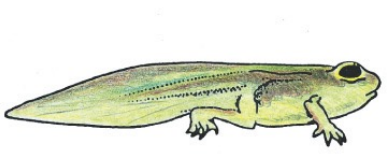
(Lockshin and Zakeri, 2004)

The **growth**, **development**, and **maintenance** of multicellular organisms depend not only on the production of cells but also on mechanisms to destroy them.

- During development, **carefully orchestrated patterns of cell death** help determine the **size and shape** of tissues and organs.
- Cells that become **damaged** or **infected**, are removed before they threaten the health of the organism.

→ PCD is not a random process
but occurs by a **programmed sequence of molecular events**

PCD is a process **conserved** in **animals and plants?**



PCD in **plants** displays <u>common features</u> with PCD in **animals**
But with a number some <u>differences</u>.

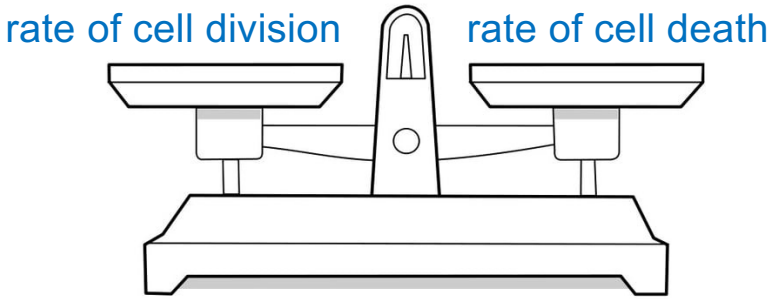
Conservation of function
but some **proteolytic enzymes** involved in PCD

→ are <u>different</u> between animals and plants
→ <u>localized in different compartments</u> of plant cells

'**Apoptotic like-pathways**' are **conserved** between plants and animals

Adult complex multicellular organisms

controlling the rate of cell division



Multicellular organism

- highly organized community of cells

→ **PCD regulates cell number**

Cells no longer needed → activating an intracellular suicide program (**PCD**)

Examples:

- In a healthy adult human, billions of cells in the **bone marrow** and **intestine** die every hour.

- If part of the **liver** is removed in an adult rat, for example, liver cells proliferate to make up the loss.

Treatment with phenobarbital → stimulates liver cell division → liver enlarges.

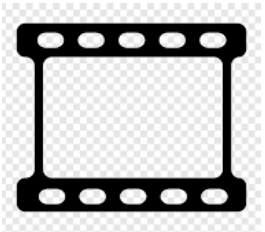
Treatment stop → apoptosis in the liver increases → liver returns to its original size

→ the liver is kept at a constant size through regulation of both the rate of cell division and death.

Actividade – visualização de vídeos em grupo

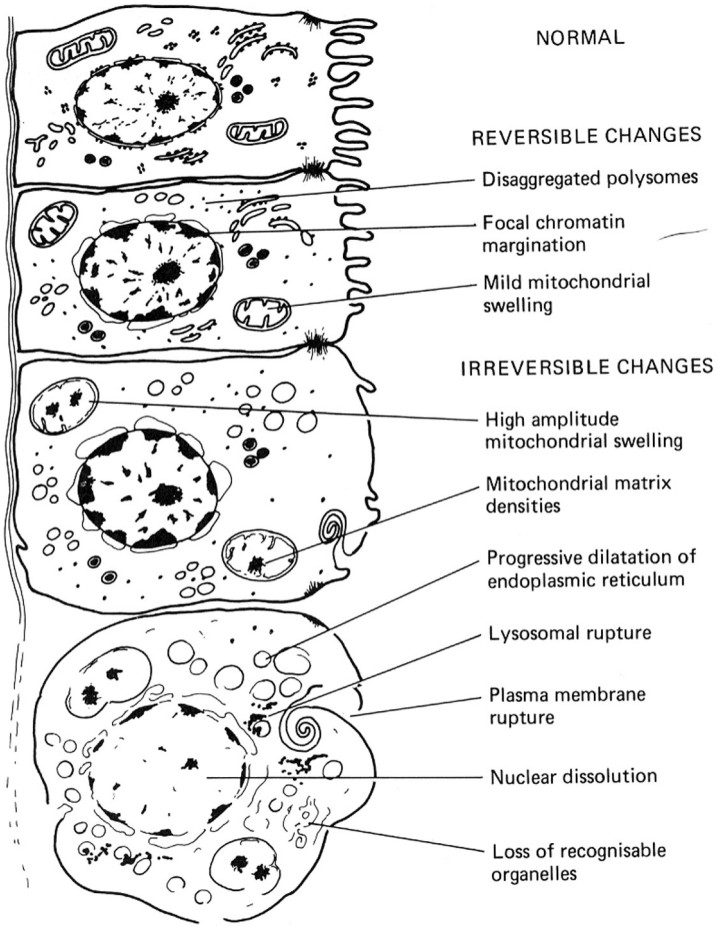
- Grupos de 4/5 alunos
- Cada grupo vê um **vídeo** (15 min)
- Cada grupo discute o conteúdo do vídeo e elabora um pequeno **resumo** (15 min)
- Cada grupo elege um porta-voz
- No fim, **apresentação** do resumo sobre cada um dos vídeos (5 min)

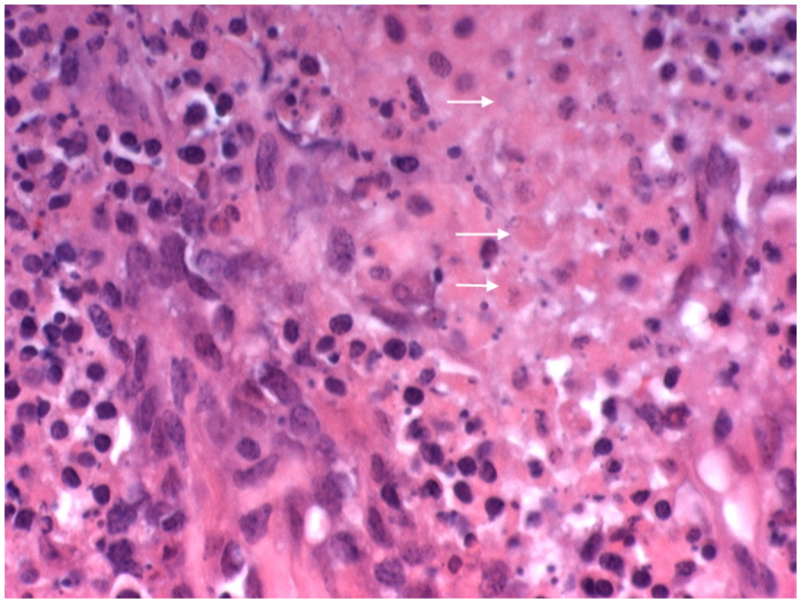
VÍDEOS



1. Cell Death Explained: Necrosis vs. Apoptosis – YouTube
https://www.youtube.com/watch?v=iKWVSgMmteI&ab_channel=MichaelPost
2. Necrosis vs. Apoptosis: Cell Death - YouTube
https://www.youtube.com/watch?v=zFrBwGfOQs0&ab_channel=AMBOSS%3AMedicalKnowledgeDistilled
3. Apoptosis: Introduction - YouTube
https://www.youtube.com/watch?v=Vf7hOX2DvDE&ab_channel=JoeDeMasi
4. H. Robert Horvitz (MIT/HHMI): Discovering Programmed Cell Death - YouTube
https://www.youtube.com/watch?v=F4lUnOY0U5w&ab_channel=iBiologyScienceStories
5. Apoptosis: The Extrinsic Pathway - YouTube
https://www.youtube.com/watch?v=mR3yE0Tc64E&ab_channel=JoeDeMasi
6. Apoptosis: The Intrinsic Pathway, part 1 - YouTube
https://www.youtube.com/watch?v=s7ixxiv6FZM&ab_channel=JoeDeMasi
7. Apoptosis: The Intrinsic Pathway, part 2 - YouTube
https://www.youtube.com/watch?v=c-DVmv4v8Ks&ab_channel=JoeDeMasi
8. Apoptosis assays: DNA fragmentation, TUNEL, DAPI - YouTube
https://www.youtube.com/watch?v=dVSVO39-Ok8&ab_channel=JoeDeMasi
9. Apoptosis assay - AnnexinV PI - YouTube
https://www.youtube.com/watch?v=z-9ksbAm4H0&ab_channel=JoeDeMasi
10. PLANTS Licensed to kill: mitochondria, chloroplasts and cell death
https://www.youtube.com/watch?v=7v9DHt4peGU&t=20s&ab_channel=CellPress

Necrosis: consequences of irreversible cell injury



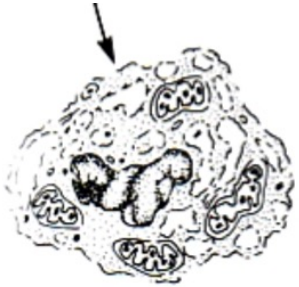


'Necrotic cell death' or 'necrosis'

- **increasing** cell **volume**
- **swelling** of organelles
- plasma **membrane rupture**

  → loss of intracellular contents

Necrosis is different from PCD



Necrosis is defined as cell death that results from:

- Interference with the cell's supply (energy, oxygen, etc.).
- Highly toxic compounds
- Enzymatic degradation
- Severe cold or heat stress
- Traumatic injury that leads to immediate damage to membrane or cellular organelles

→ **Necrosis is not programmed**

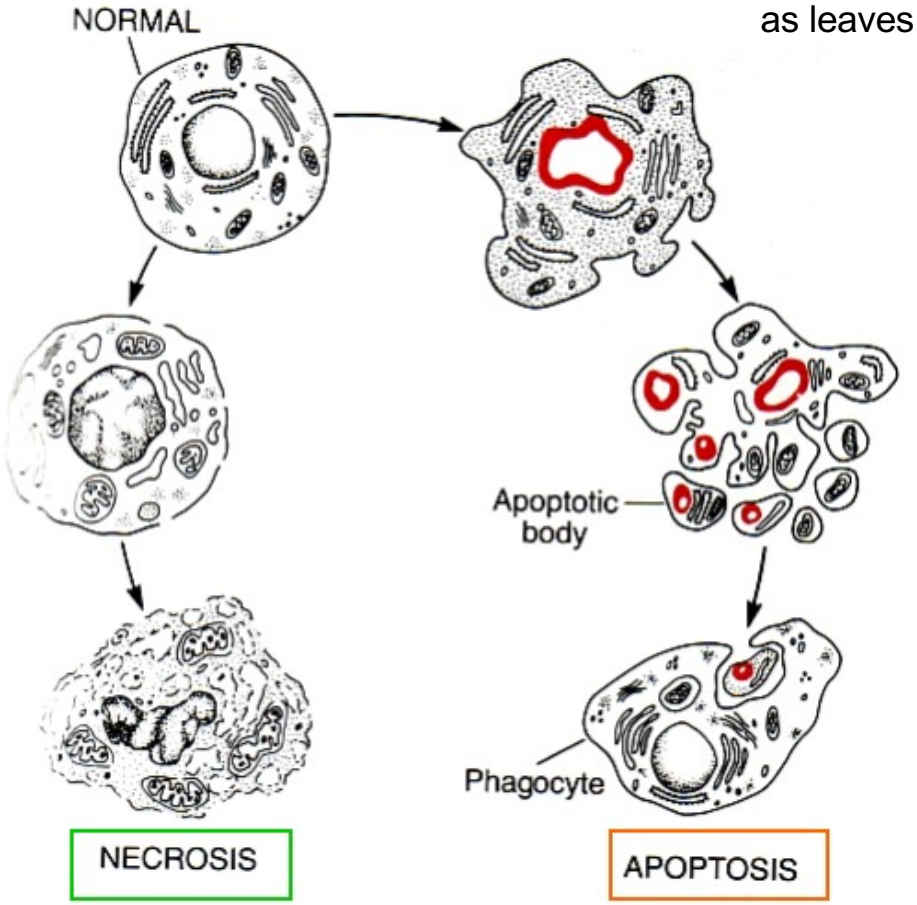
Necrosis and Apoptosis

Apoptose / Morte Celular Programada

From the Greek word meaning “falling off,” as leaves from a tree

Necrosis

“nekrosis” – mortificação



	NECROSIS	APOPTOSIS
	Premature death of cells and living tissue. "Unprogrammed" cell death process.	Programmed cell death, is a form of cell death that is generally triggered by normal, healthy processes in the body.
Cause	Caused by factors external to the cell or tissue, such as infection, toxins, or trauma.	Natural
Effects	Always detrimental	Usually beneficial. Only abnormal when too many or too few cell deaths.
Process	Membrane disruption, respiratory poisons and hypoxia which cause ATP depletion, metabolic collapse, cell swelling and rupture leading to inflammation.	Membrane blebbing, shrinkage of cell, nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation, apoptopic body formation, engulfment by white blood cells.
Symptoms	Inflammation, decreasing blood flow, tissue death.	Usually, no symptoms noticeable in the organism.

Necrosis: a **pathological response** to cellular injury

Chromatin clumps

Mitochondria swell and rupture

Plasma membrane lyses

Cell contents spill out

General inflammatory response is triggered

Apoptosis: a **physiological response** to
- specific suicide signals
- lack of survival signals

Chromatin condenses and migrates to nuclear membrane. Internucleosomal cleavage leads to laddering of DNA at the nucleosomal repeat length, ca. 200 bp.

Cytoplasm shrinks without membrane rupture

Blebbing (bolhas) of plasma and nuclear membranes

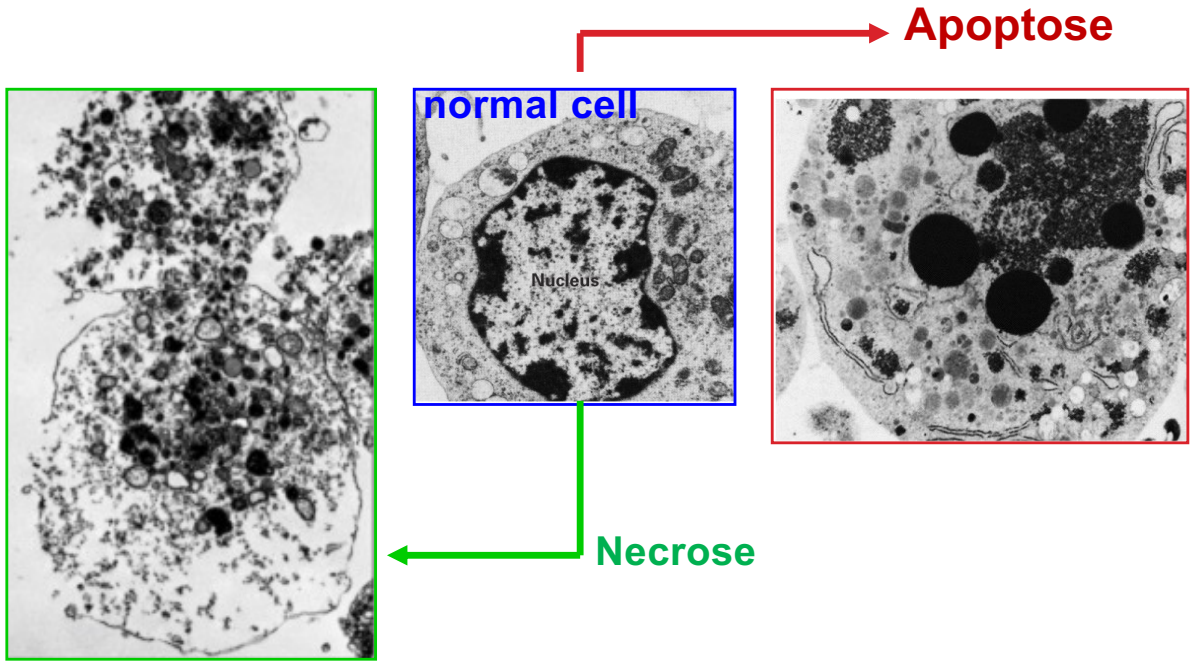
Cell contents are packaged in membrane bounded bodies, internal organelles still functioning, to be engulfed by neighbours.

Epitopes appear on plasma membrane marking cell as a phagocytic target.

No spillage, no inflammation

Necrose vs Apoptose

Características celulares comparadas



Necrose – a **perda de estanquicidade** da membrana plasmática com extravasamento de componentes citoplásmicos resulta em **alterações tecidulares**

(Ex. morte de tecidos, cicatrizes, respostas imunológicas, etc)

Apoptosis Purposes

Developmental PCD

- Regulates the rate of cell division
- Essential for the successful development and growth of complex multicellular organisms
- Shaping of tissues and organisms

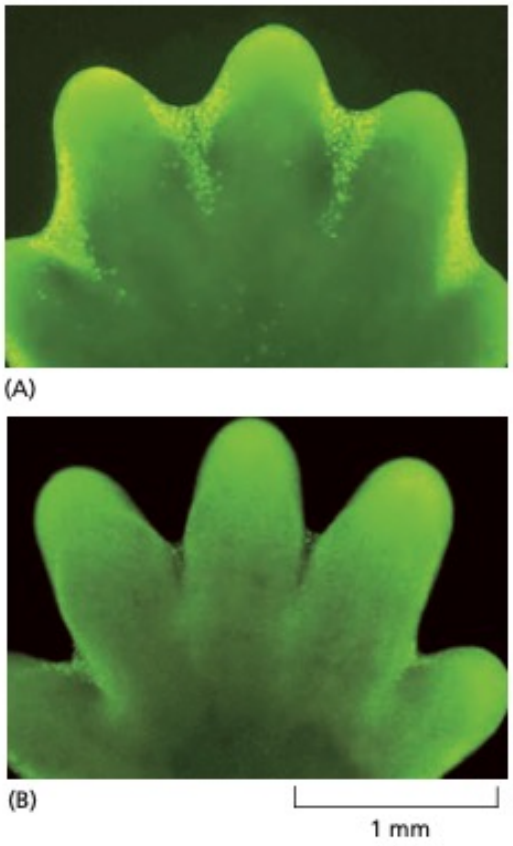
  (adult tissues neither growing nor shrinking - cell death and cell division must be tightly balanced)

Defense PCD

- Control of cell population
- Defense against invading microbes
- Needed to destroy the cells that represent a threat to the integrity of the organism

Development and growth of complex multicellular organisms

Sculpting the digits in the developing mouse paw by apoptosis



(**A**) the paw in this **mouse fetus** has been stained with a dye that specifically labels cells that have undergone apoptosis.

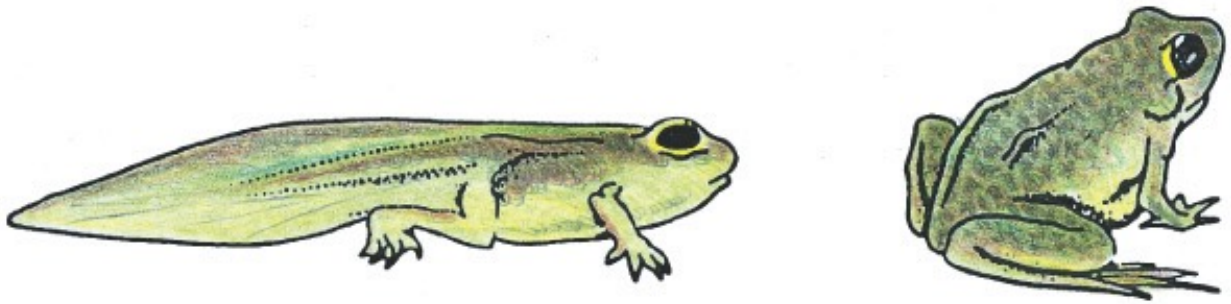
The apoptotic cells appear as bright green dots between the developing digits.

(**B**) the interdigital cell death has eliminated the tissue between the developing digits, as seen one day later, when there are very few apoptotic cells.

(Wood et al., Development 127:5245–5252, 2000)

Development and growth of complex multicellular organisms

The tail of the tadpole is absorbed via apoptosis



In this case, cells die when the structure they form is no longer needed.

When a **tadpole** changes into a frog at metamorphosis, the cells in the tail die, and the tail, which is not needed in the frog, disappears.

Humans EACH HOUR lose many BILLIONS of cells via apoptosis.

Most of these are healthy cells which have no defects.

In **adult multicellular organisms** cell death through apoptosis occur regularly.

Importância fisiológica da apoptose

→ Desenvolvimento **embrionário e fetal**:
ex. - programas de formação embrionária
- organização do sistema nervoso
- células auto-reativas do sistema imunitário

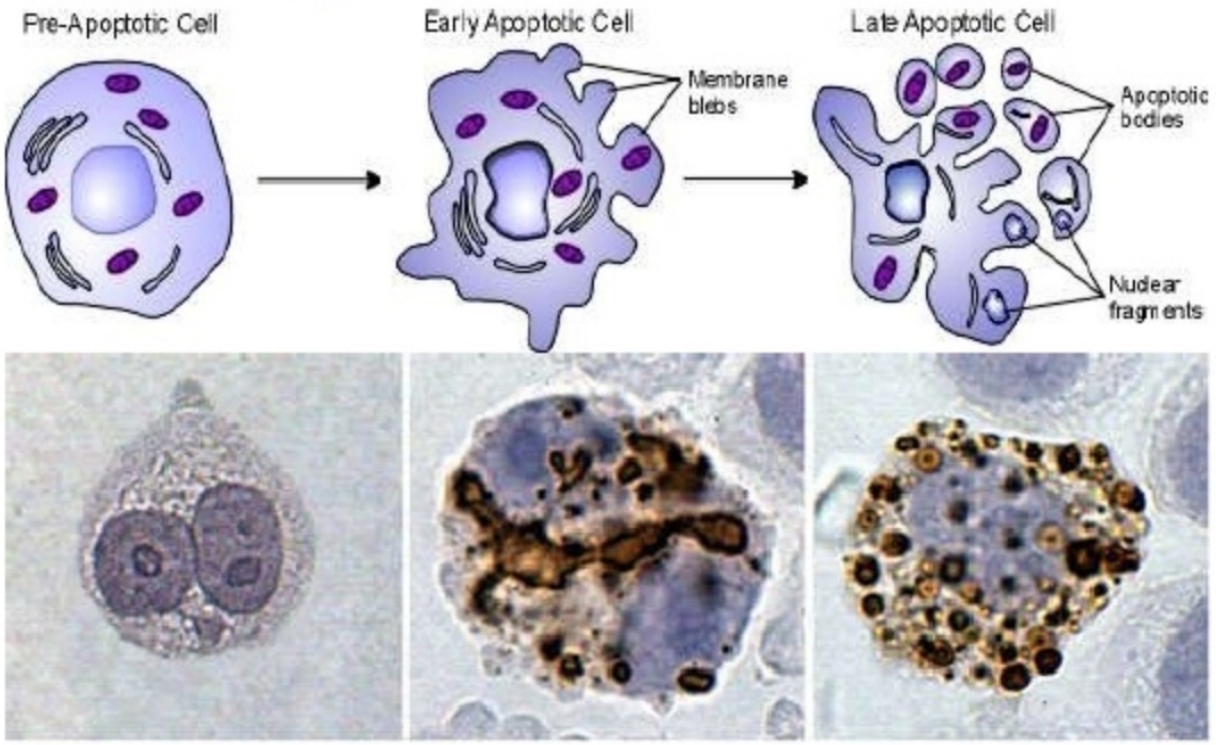
→ Estado **adulto**:
ex. - em resposta a danos do DNA consequentes de radiações, infeções virais, etc
- nalguns órgãos e tecidos por ausência de estimulo hormonal

Erros de activação em humanos

Activação indevida – doenças neurodegenerativas
Ativação deficiente – doenças autoimunes, processos oncogénicos

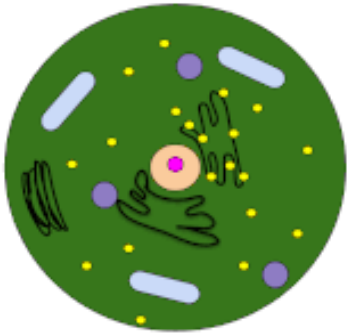
Apoptosis results – chromatin condensation

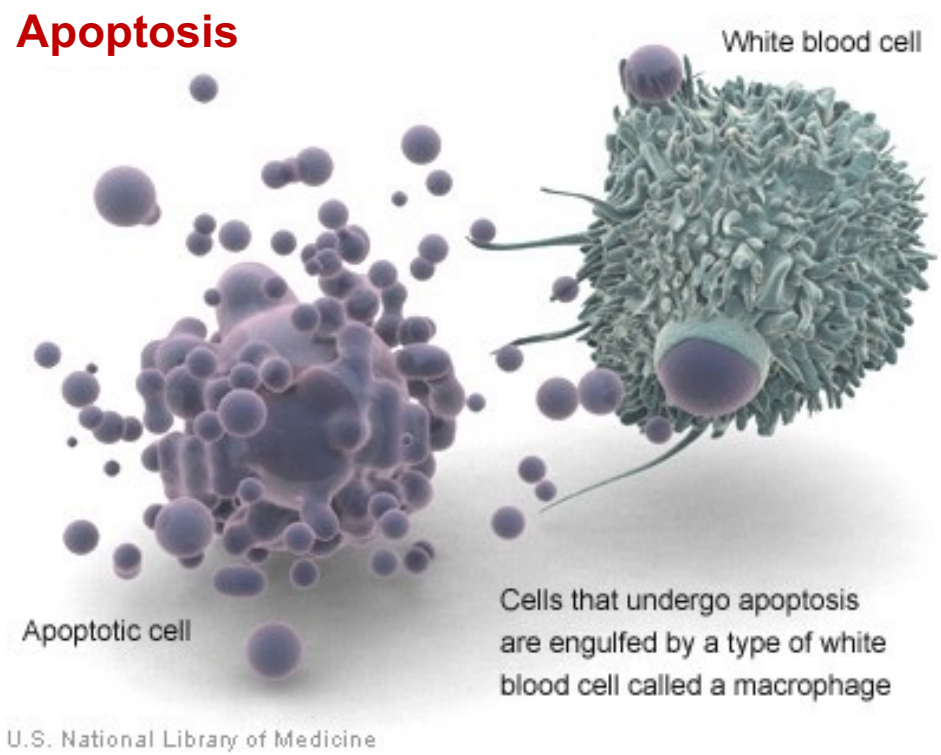
Radiation and chemical are used to **induce apoptosis** in cancer therapy in some types of cancers



Apoptosis in stomach carcinoma cells chemicaly induced (before, 24h and 48h after)

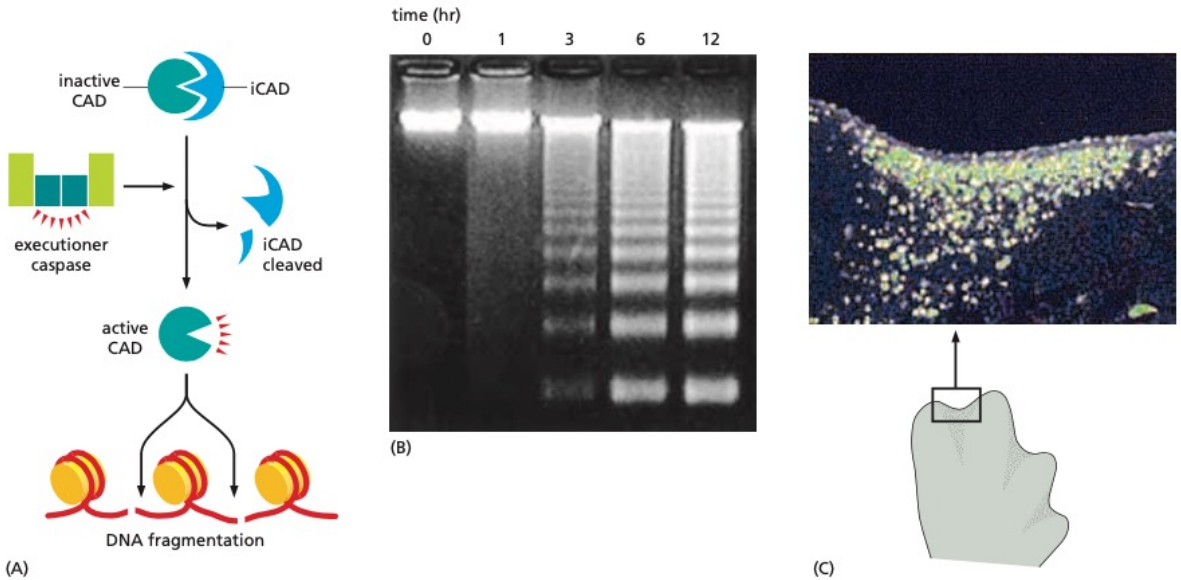
Formação de corpos apoptoticos





Cells dying by apoptosis undergo **characteristic morphological changes**
→ **Apoptotic bodies**

DNA fragmentation during apoptosis

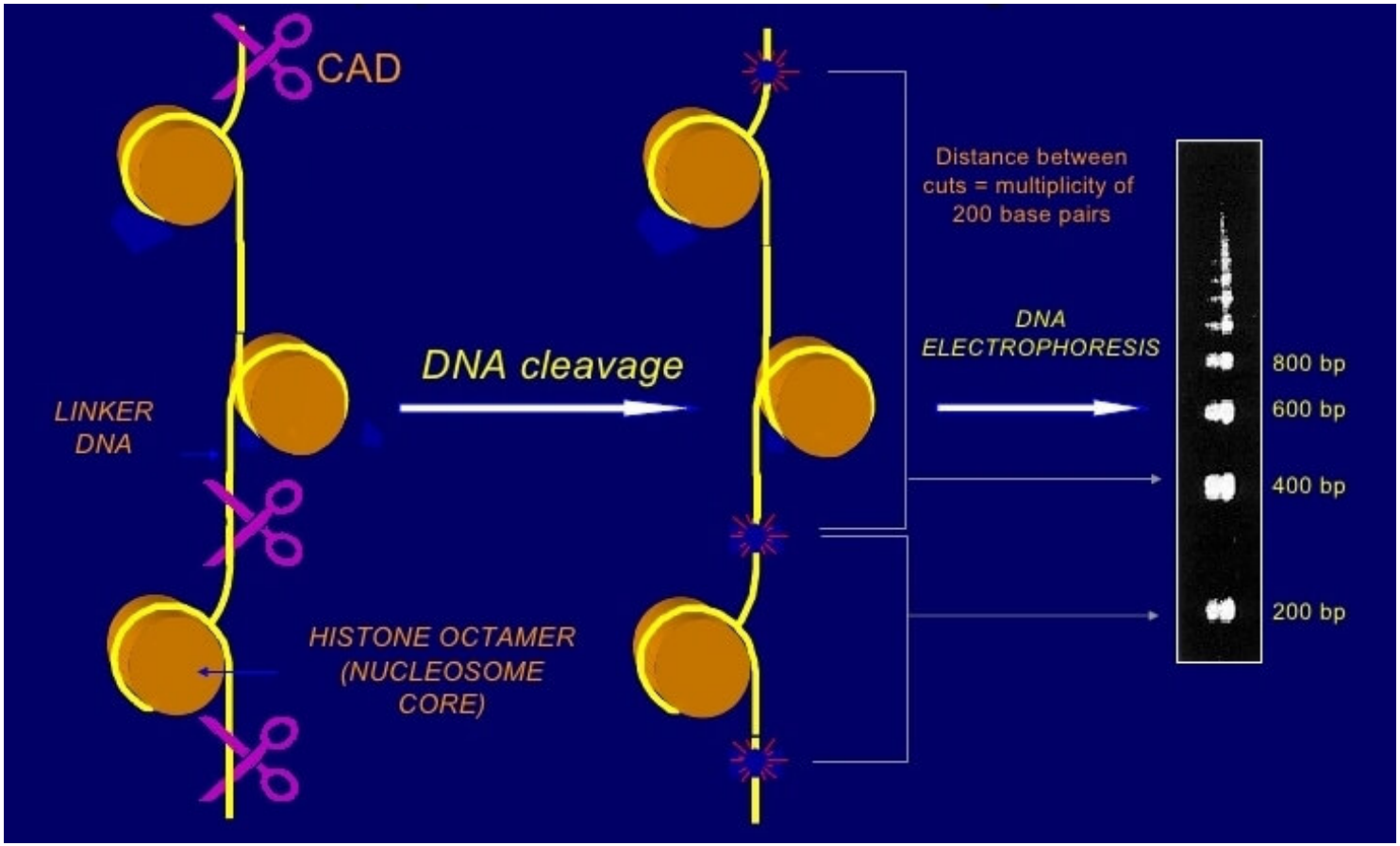


- **Nuclear lamina cleavage** → irreversible breakdown of the nuclear lamina

- Cleaves a protein that normally holds an endonuclease (**CAD**) in an inactive form

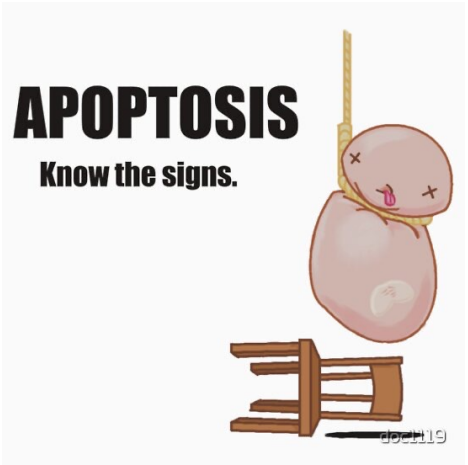
  → frees and activates the **endonuclease** to cut DNA in the linker regions between nucleosomes
  → DNA fragments that form a **ladder pattern**.

DNA Ladder assay

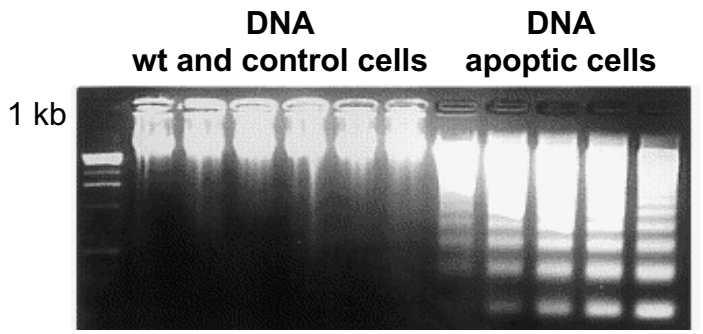


DNA cleavage by a specific nuclease Caspase-Activated DNase (CAD) during apoptosis

Detection of programmed cell death



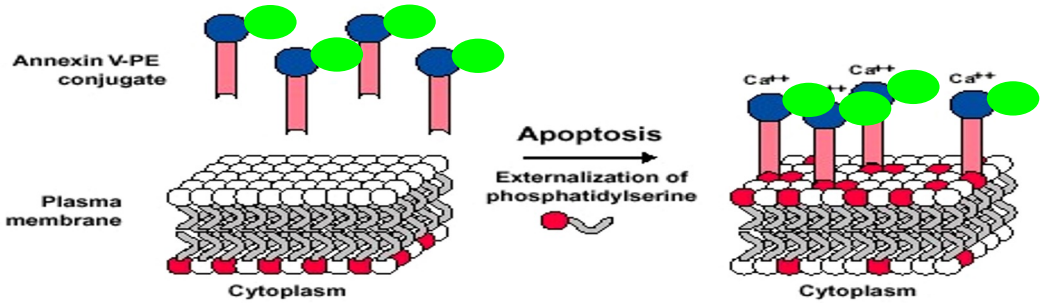
Efeitos na cromatina



Externalização da fosfotidilserina (PT)

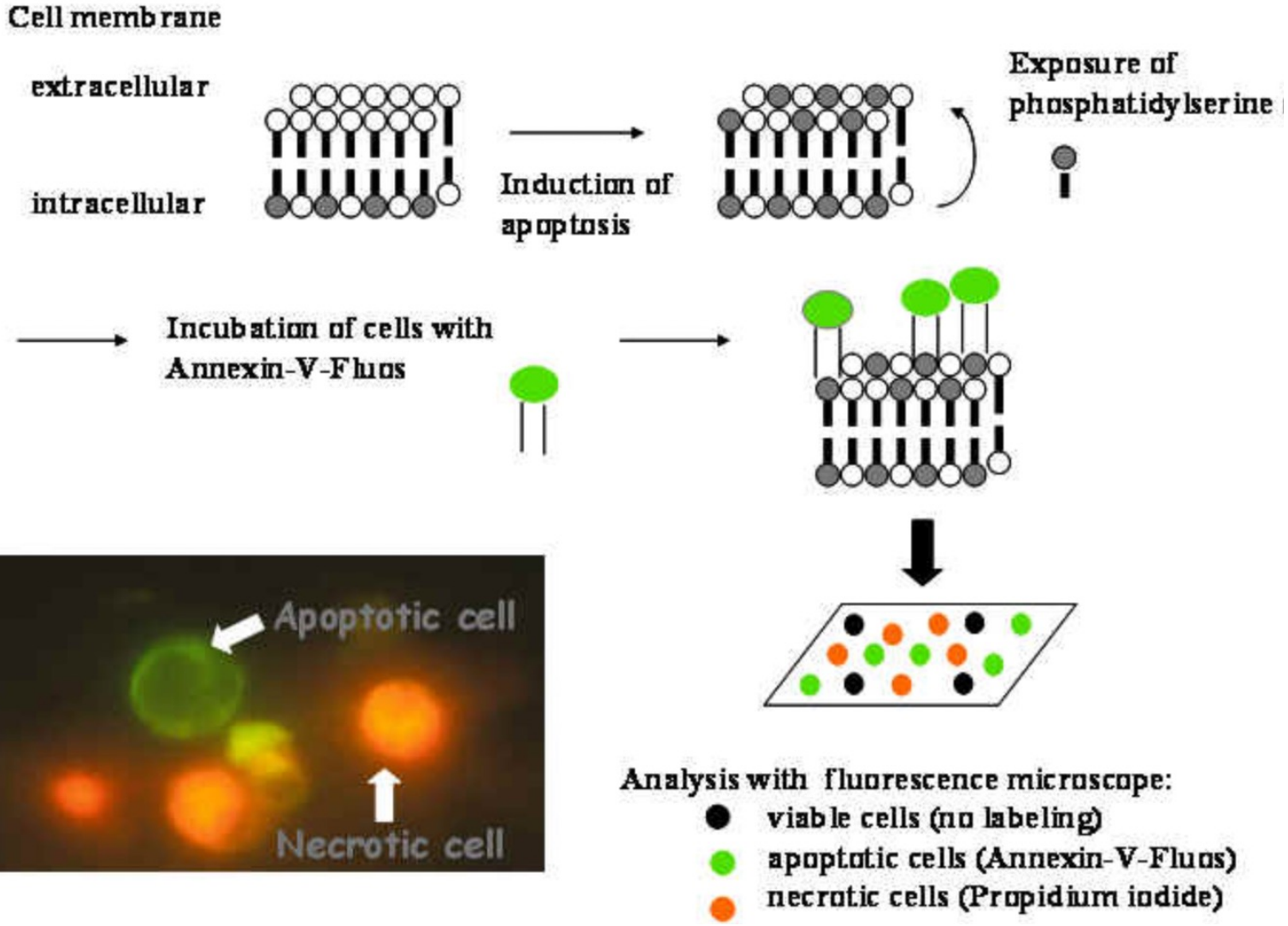
→ <u>ausência da normal assimetria</u> de distribuição de fosfolipidos nas mono-camadas da membrana plasmática

→ **Annexin V** liga-se a fosfotidilserina
Só em células apoptóticas
é detetável na face externa

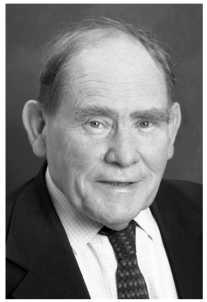


Schematic representation of the Annexin V assay.

Annexin V assay

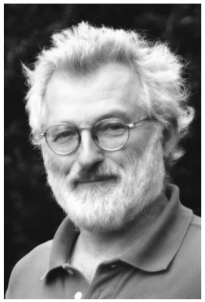


Prémio Nobel 2002



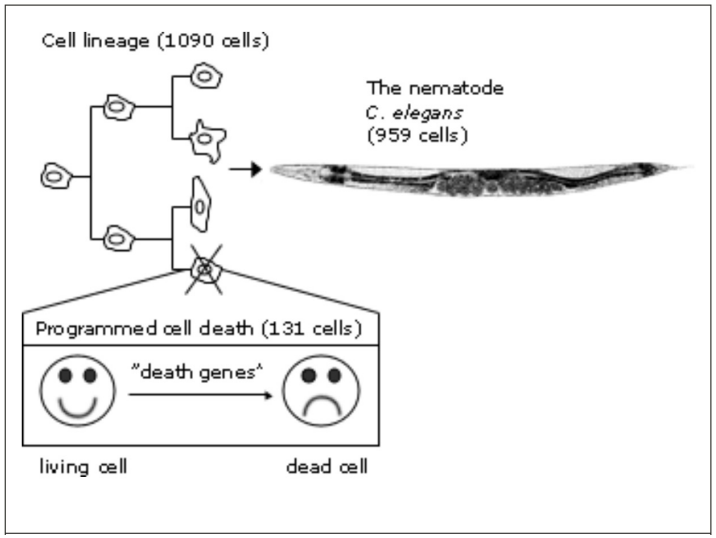
Sydney Brenner

H. Robert Horvitz



John E. Sulston

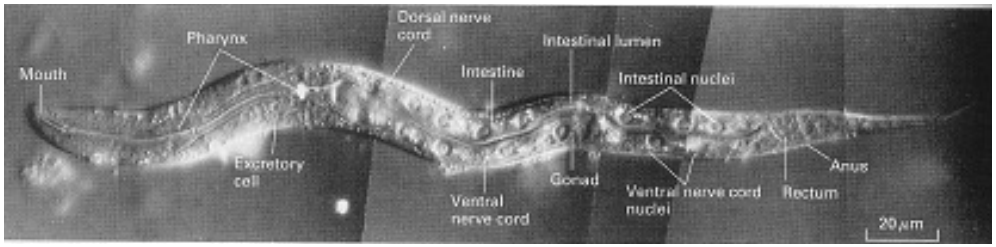
1099 – 131 PCD
→ 959 cell in adult *C. elegans*



"For their discoveries concerning the **genetic regulation** of organ development and **programmed cell death (PCD)**"

→ identified key conserved genes regulating programmed cell death and demonstrated that corresponding genes exist also in higher animals, including man.

Discovery of programmed cell death in *C. elegans*



***C. elegans* genome:** 19099 genes

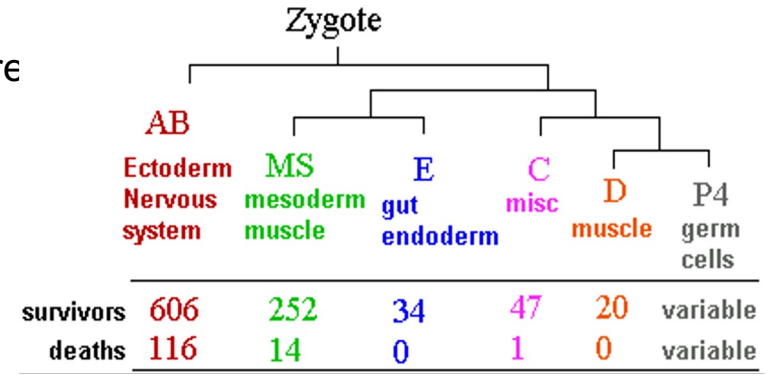
C. elegans Sequencing Consortium, 1998 - the first multicellular organism to be sequenced

The **adult** hermaphrodite consists of exactly **959 somatic cells** of precisely determined lineage and function.
Individual cells are named and their relationships to their neighbors are known.

The **959 somatic cells** of adult *C.elegans* arise from **1090 original cells**

→ exactly **131** somatic cells undergo

Programmed Cell Death (PCD)



www.chembio.uoguelph.ca

How are procaspases activated?

Caspase - **C**istein-dependente **asp**artic acid recognizer prote**ase**
(proteinas que degradam outras proteinas)

General principle
Activation triggered by adaptor proteins

→**Aggregation** of multiple copies of specific procaspases (initiator procaspases)

→ **Active** caspases

→ **Reinforcement** of procaspases activation

CASPASE CASCADE

CASPASES INICIADORAS
-8 (extrinsic) e -9 (intrinsic)

CASPASES EFECTORAS
-3, -6 e -7

Execução por via proteolítica: caspases efectoras

As **caspases** têm numerosos **alvos** (mais de 400 proteínas),

- degradação de vários factores de transcrição
- degradação de factores associados à tradução
- fragmentação dos organelos
- disrupção do citoesqueleto

Via proteolítica

EFEITOS CELULARES CUMULATIVOS

Anulam a homeostasia e processos de reparação

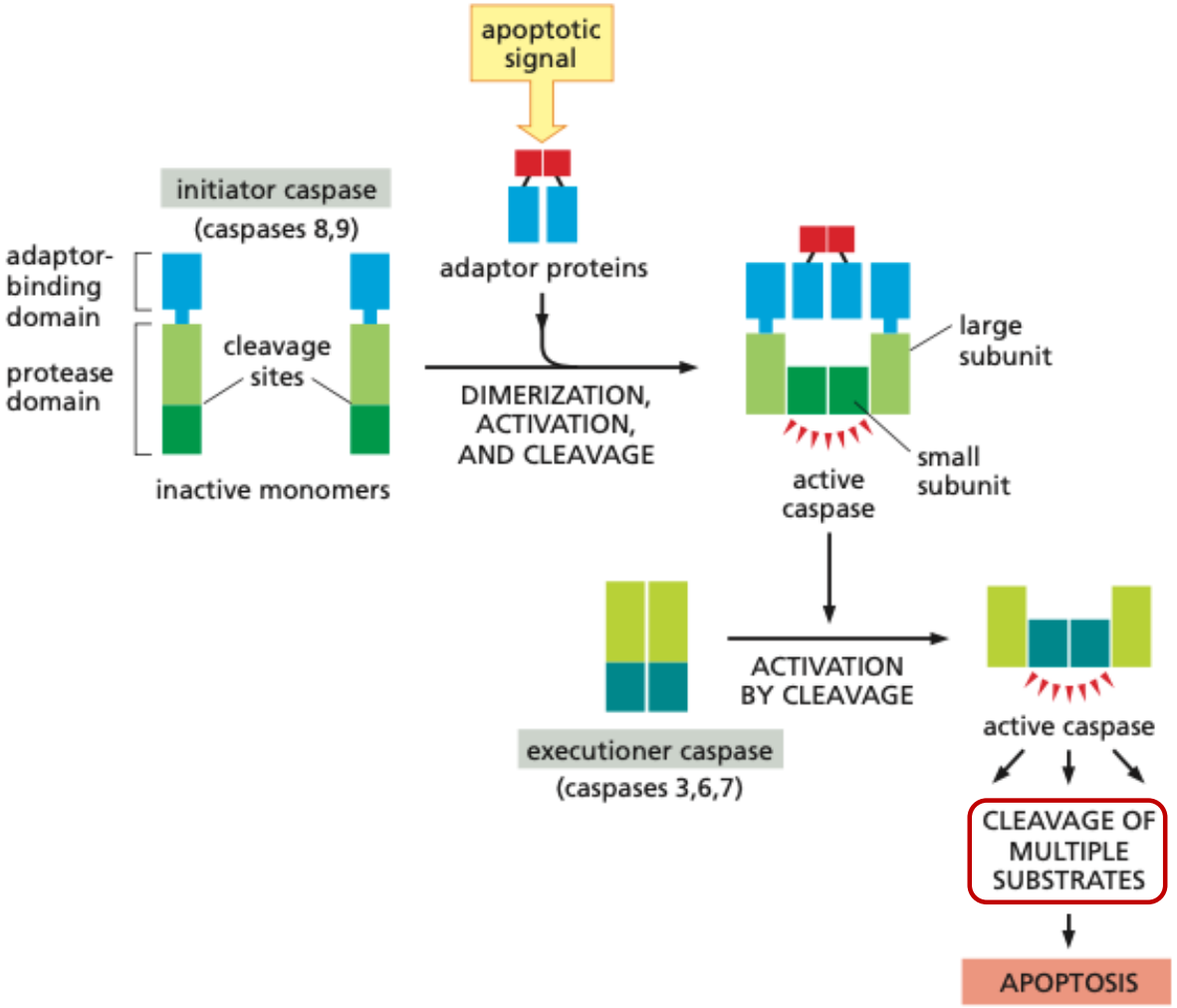
Paragem da progressão no **ciclo celular**

Inactivação de **inibidores** da apoptose

Alterações estruturais e morfológicas

Marcação das células para **fagocitose**

Apoptosis depends on intracellular proteolytic cascade mediated by caspases



Apoptotic signal → activation of **initiator caspases** - assembling pairs of caspases associate to form dimers

→ protease activation

Each caspase in the **dimer** then **cleaves** its partner at a specific site in the protease domain, stabilizing the **active complex**.

Active initiator caspases → **executioner caspases activation** (normally exist as inactive dimers)

→ **cleave** at a site in the protease domin

→ active conformation that catalyze protein cleavage events that kill the cell.

One initiator caspase can activate many executioner caspases

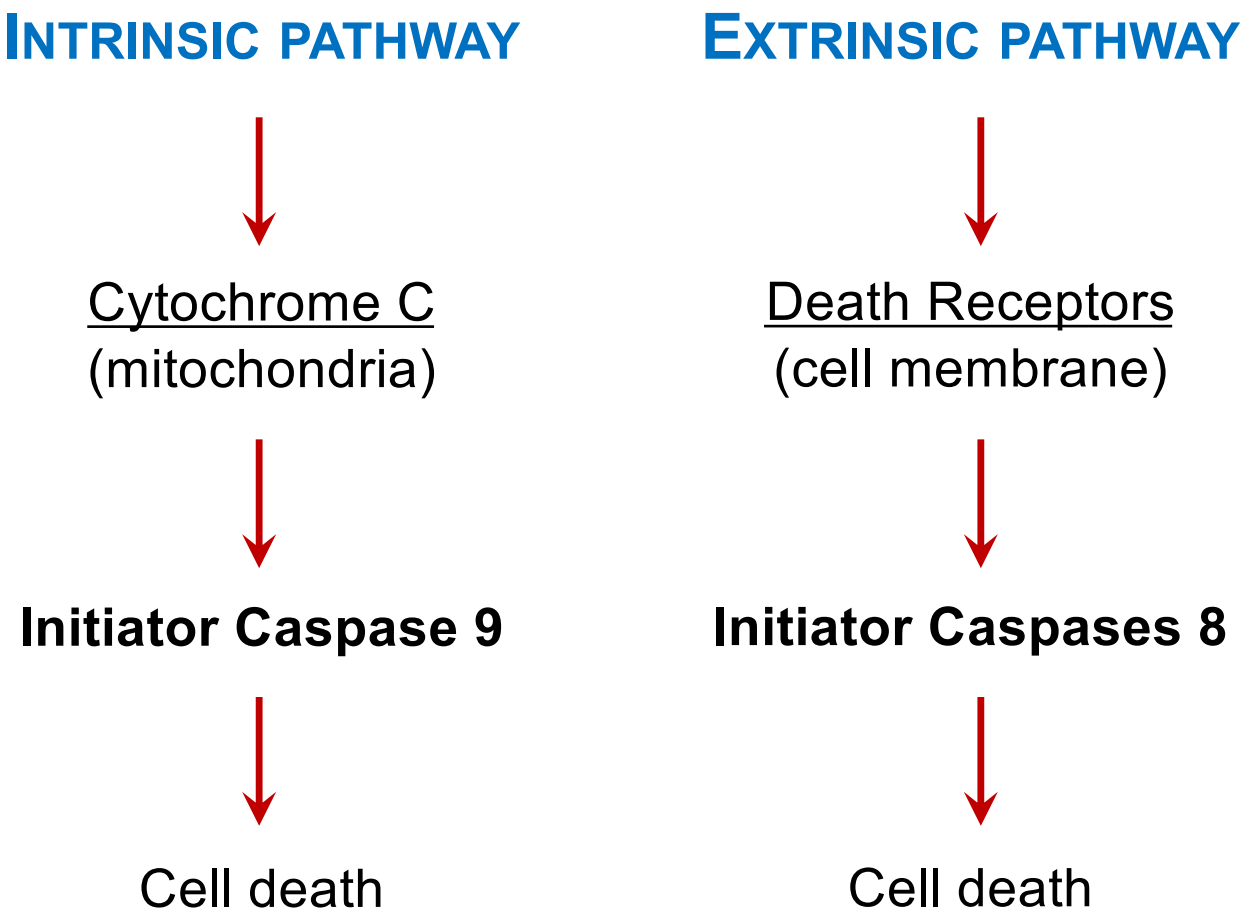
→ **proteolytic cascade**.

Destructive

Self-amplifying

Irreversible

Apoptosis pathways

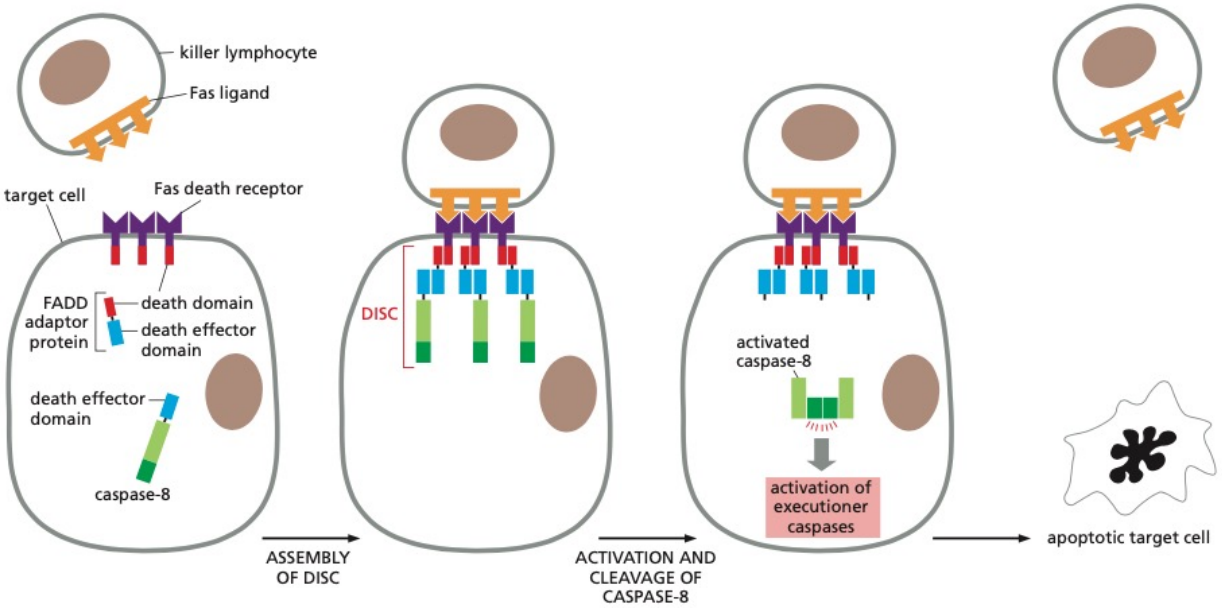


Cell-surface death receptors activate the **Extrinsic pathway of apoptosis**

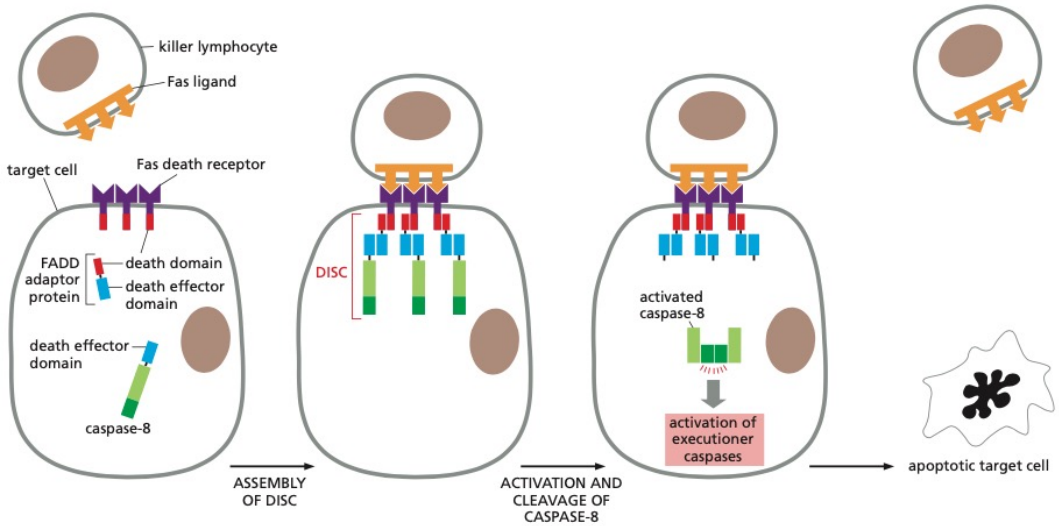
- A **via extrínseca** é activada por ligantes extracelulares que se ligam a vários receptores 'death'

Fas ligand – Fas death receptors

→ activação de caspases (efectoras)
→ **MORTE CELULAR**



Cell-surface death receptors activate the **Extrinsic pathway of apoptosis**



Trimeric **Fas ligands** on the surface of a killer lymphocyte interact with
trimeric **Fas receptors** on the surface of the target cell
(clustering of several ligand-bound receptor trimers)
→ activates death domains on the receptor tails, which interact with similar domains on the adaptor **protein FADD** (**FADD Fas-associated death domain**).

Each FADD protein then recruits a **pro-caspase** (**caspase-8**) via a
death effector domain on both FADD and the caspase → death-inducing signaling complex (**DISC**).
Within the DISC, two adjacent initiator caspases interact and cleave one another → **activated protease dimer**,

→ stabilizes and releases the active caspase dimer into the cytosol
→ Activates by cleavage **executioner (or effector) caspases**

Via apoptótica intrínseca

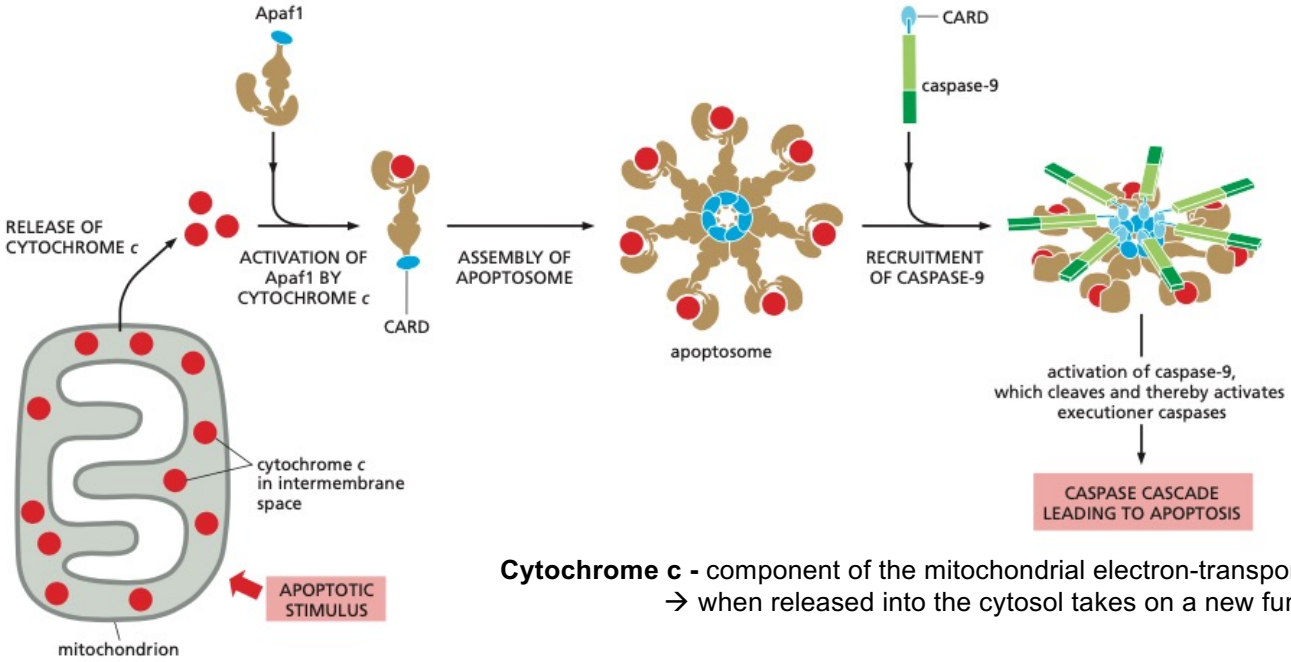
- Activada no caso de **defeitos internos da célula**
  - alterações do **DNA**
  - vários **stresses**
  - agentes citotóxicos

- A **mitocôndria** é essencial no processo apoptótico
  → → → libertação de **Citocromo c**
  liga-se à adaptor protein **Apaf-1**

- Regulação da via **intrínseca ou mitocondrial** envolve
  membros da **família de proteínas Bcl2**
  - proteínas pro a anti apoptoticas

que funcionam como:
- sensores de estímulos
- protetores ou agressores mitocondriais

Intrinsic pathway of apoptosis



Cytochrome c - component of the mitochondrial electron-transport chain
→ when released into the cytosol takes on a new function

Intracellular apoptotic stimuli → mitochondria release **cytochrome c**
Cytochrome c binds to **Apaf1** → Apaf1 unfolds a domain that interacts with the same domain in other activated Apaf1
→ Seven activated **Apaf1** proteins form a large ring complex called the **APOPTOSOME**.

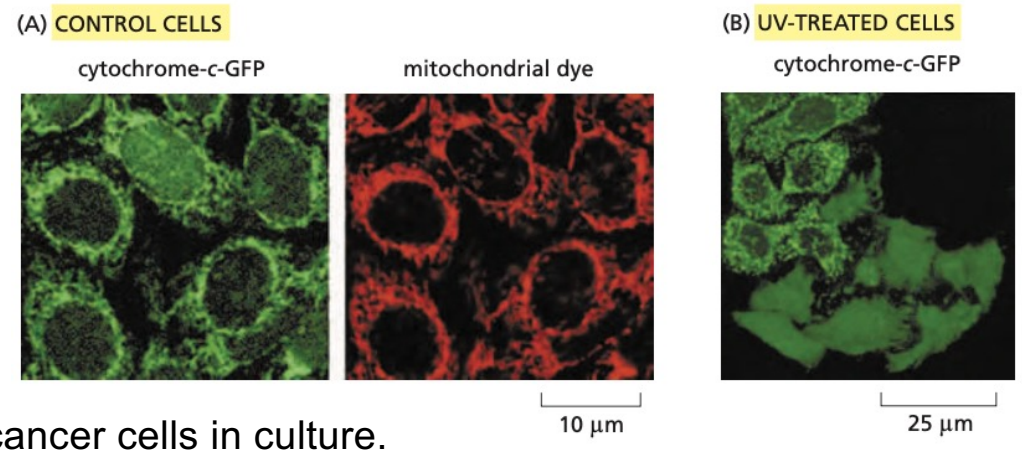
Apaf1 proteins contains caspase recruitment domains (**CARD**) that bind similar domains in **caspase-9** molecules
→ **APOPTOSOME ACTIVATION**
Caspase-9 cleaves → activates downstream executioner caspases

(**CARD** is related in structure and function to the **death effector domain** of caspase-8)

Intrinsic pathway of apoptosis - release of cytochrome c from **mitochondria**

Cells can also activate their apoptosis program from inside the cell, often in response to **stresses**, such as DNA damage, or in response to **developmental signals**.

Mitochondia
(Cytochrome C release)



Fluorescence micrographs of human cancer cells in culture.

(**A**) The **control cells** were transfected with a gene encoding a fusion protein consisting of **cytochrome c** linked to **GFP** and **mitochondria** red staining.

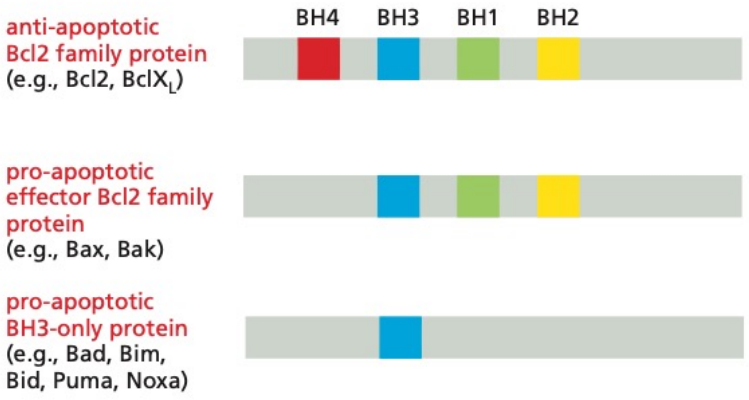
Signals overlapping → **cytochrome-*c*-GFP is located in mitochondria** (in the intermembrane space)

(**B**) UV irradiated cells → induce the **intrinsic pathway of apoptosis**

(photographed after 5 hours).

The six cells in the bottom half of this micrograph have released their cytochrome c from mitochondria into the cytosol → activation a caspase proteolytic cascade in the cytoplasm (J.C. Goldstein et al., Nat. Cell Biol. 2:156–162, 2000)

Bcl2 Proteins regulate the intrinsic pathway of apoptosis



Three classes of **Bcl2 family proteins**:

Anti-apoptotic Bcl2 family proteins: **Bcl2** and **BclXL**, four distinctive Bcl2 homology (BH) domains (BH1–4)
(cytosol)

Two subfamilies of **Pro-apoptotic** Bcl2 proteins
— **Effector** Bcl2 family proteins (**Bax** and **Bak**)
(mitochondria membrane)
— **BH3-only** proteins (**Bad, Bim, Bid, Puma, Noxa**)
(cytosol)

Sharing distinct BCL2 HOMOLOGY (BH) DOMAINS

Major class of intracellular regulators Bcl2 family of proteins ensure that cells kill themselves only when it is appropriate
(conserved in evolution from worms to humans)

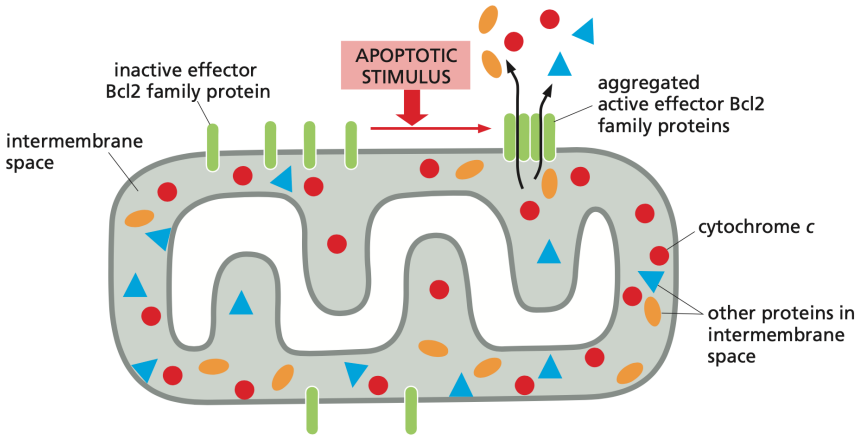
→ **controlling the release of cytochrome c** and other intermembrane mitochondrial proteins into the cytosol

Anti-apoptotic - inhibit apoptosis by blocking the release
Pro-apoptotic - promote apoptosis by enhancing the release
Bind to each other in various combinations to form heterodimers in which the two proteins inhibit each other's function

→ Balance between their activities **control intrinsic pathway of apoptosis**

Pro-apoptotic EFFECTOR BCL2 family proteins

Pro-apoptotic Bcl2 proteins
EFFECTOR BCL2 family proteins (**Bax** and **Bak**)
(mitochondria membrane)



In **mammalian** cells,
Bax and **Bak** are the main EFFECTOR BCL2 family proteins, and at least one of them is required for the intrinsic pathway of apoptosis to operate

When activated by an **apoptotic stimulus**,
→ **effector Bcl2** family proteins (**Bax** and **Bak**) aggregate on the outer mitochondrial membrane

→ **Release cytochrome c** and other proteins from the intermembrane space into the cytosol

The activation of Bax and Bak usually depends on activated **pro-apoptotic BH3-only** proteins.

Anti-apoptotic BCL2 FAMILY proteins

Anti-apoptotic **BCL2 FAMILY** proteins:
Bcl2 and **BclXL**
four distinctive Bcl2 homology (BH) domains (BH1–4)
(cytosol)

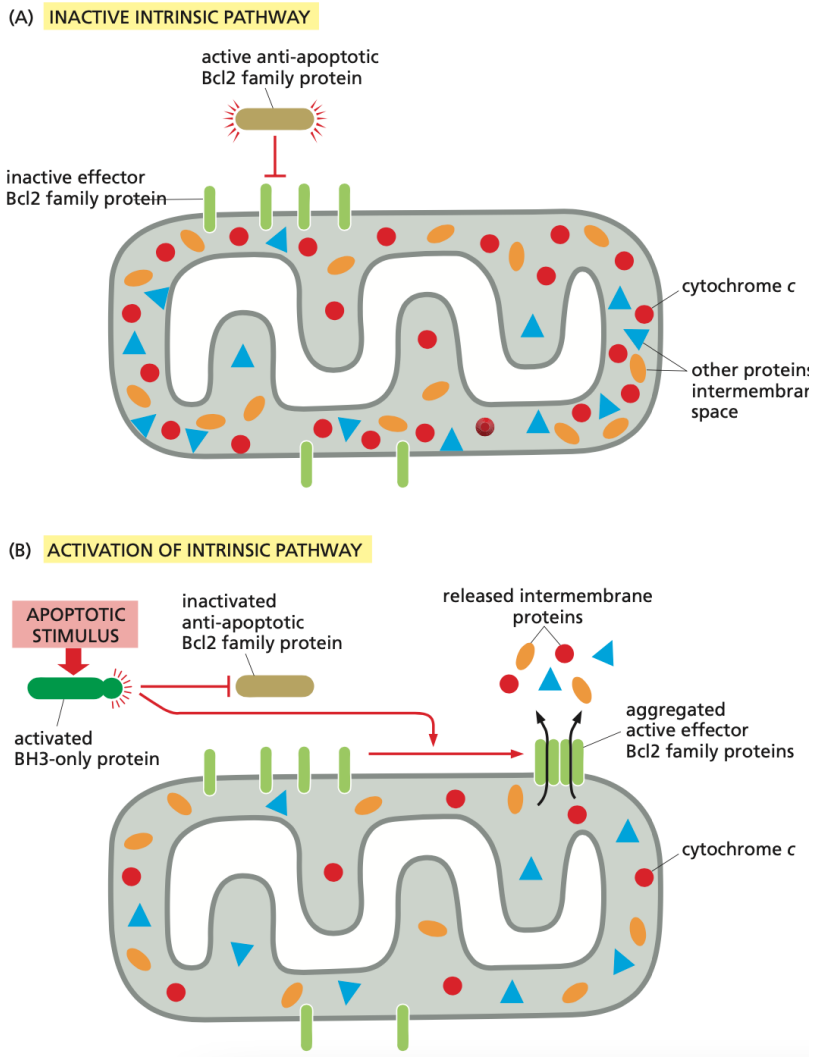
Anti-apoptotic **BCL2 FAMILY** proteins (**Bcl2** and **BclXL**) are also located on the cytosolic surface of the outer mitochondrial membrane

→ Help **prevent inappropriate release** of intermembrane proteins.

(binding to and inhibiting pro-apoptotic Bcl2 family proteins
on the mitochondrial membrane or in the cytosol)

There are at least five mammalian anti-apoptotic Bcl2 family proteins,
cell requires at least one to survive.
These proteins must be inhibited for the intrinsic pathway to
induce apoptosis

Pro-apoptotic BH3-ONLY and Anti-apoptotic Bcl2 family proteins regulate the intrinsic pathway of apoptosis



(**A**) In the **absence of an apoptotic stimuli**
→ **Anti-apoptotic BCL2 FAMILY** proteins bind to and inhibit the effector Bcl2 family proteins

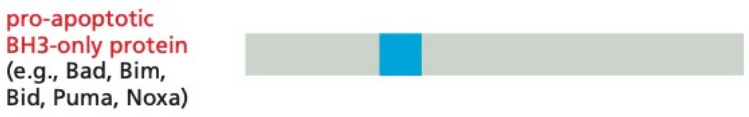
(**B**) In the **presence of an apoptotic stimulus**

→ **Pro-apoptotic BH3-ONLY** proteins are activated and bind to the anti-apoptotic Bcl2 family proteins
(no longer inhibit the effector Bcl2 family proteins)

→ **EFFECTOR BCL2 proteins** become activated, **aggregate** in the outer mitochondrial membrane
→ promote the release of intermembrane mitochondrial proteins into the cytosol

Pro-apoptotic BH3-only proteins

link between apoptotic stimuli and the intrinsic pathway of apoptosis



Pro-apoptotic BH3-ONLY proteins
(**Bad, Bim, Bid, Puma, Noxa**)
(cytosol)

Pro-apoptotic BH3-ONLY proteins (**Bad, Bim, Bid, Puma** and **Noxa**) are the largest subclass of Bcl2 family proteins.

Apoptotic stimulus → produces or activates Pro-apoptotic BH3-only proteins
(inhibiting anti-apoptotic Bcl2 family proteins)

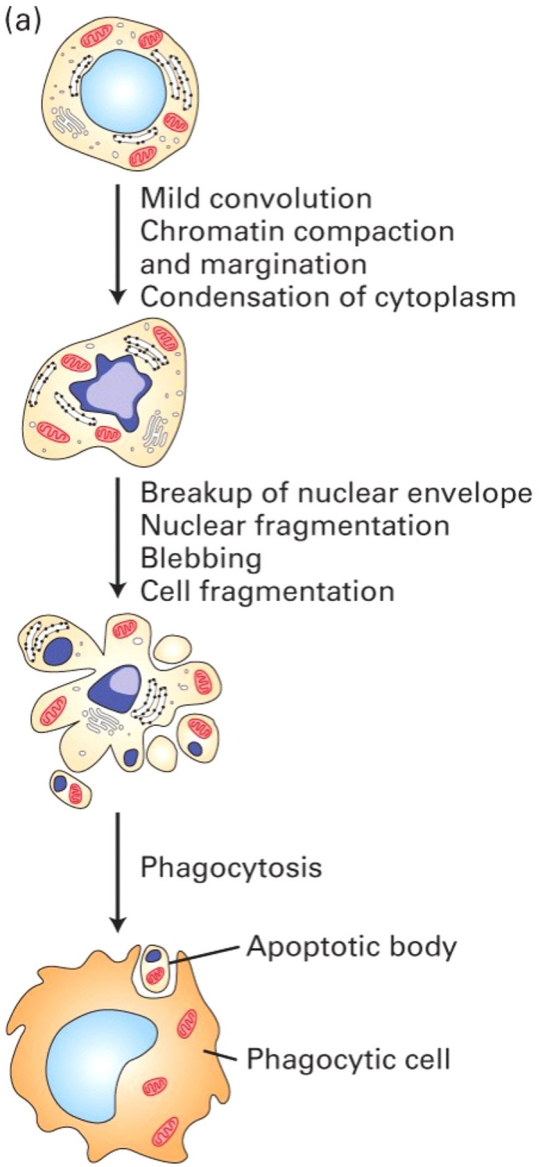
→ Aggregation of **Pro-apoptotic Bcl2 proteins (**Bax, Bak) on the surface of mitochondria
→ release of the intermembrane mitochondrial proteins
→ **Apoptosis**

Phagocytosis: the end of apoptotic cells

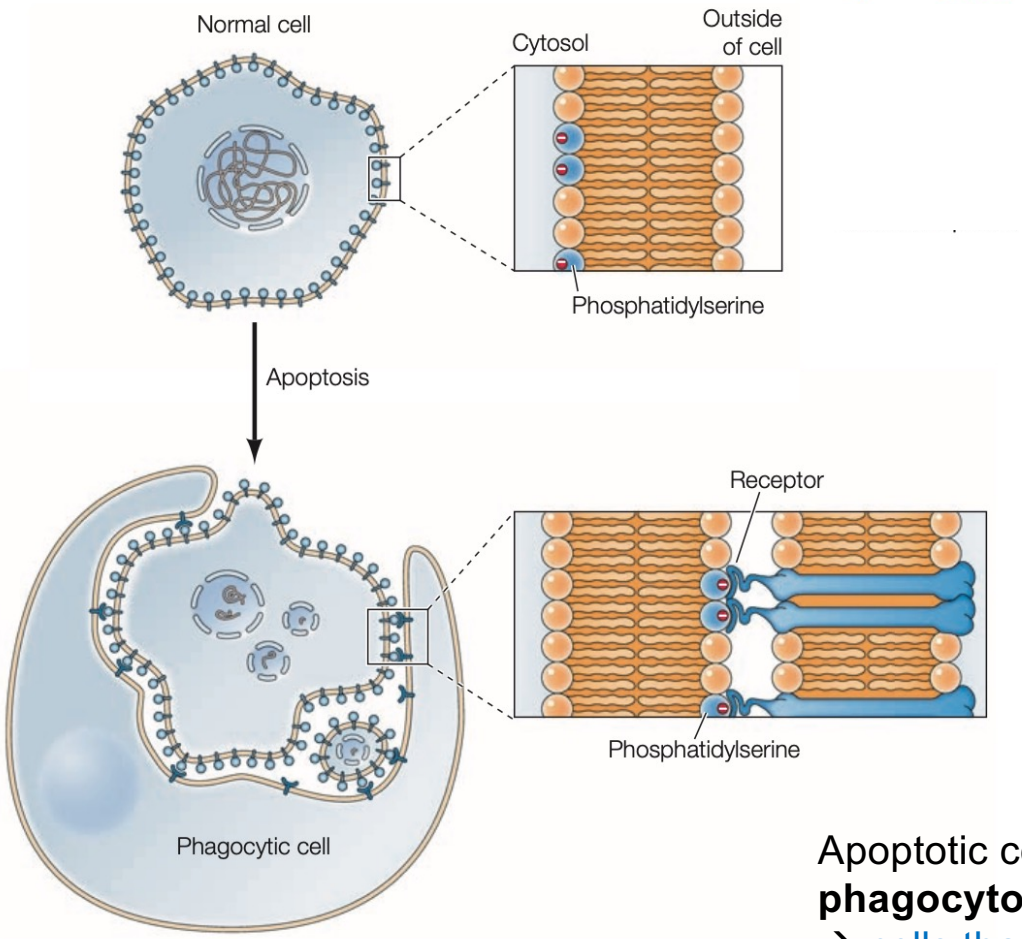
Although apoptotic cell death is widespread, dying cells are rarely seen *in situ* because of their rapid **clearance by neighboring PHAGOCYTES**.

Phagocytic recognition of apoptotic cells is less well understood than the death program itself, but an increasing number of recent studies are highlighting its importance.

- **Signals / Receptors** for apoptotic cells **phagocytosis**
- **Mechanisms of uptake**



Phagocytosis of apoptotic cells



Apoptotic cells and cell fragments are recognized and engulfed by
PHAGOCYTIC CELLS

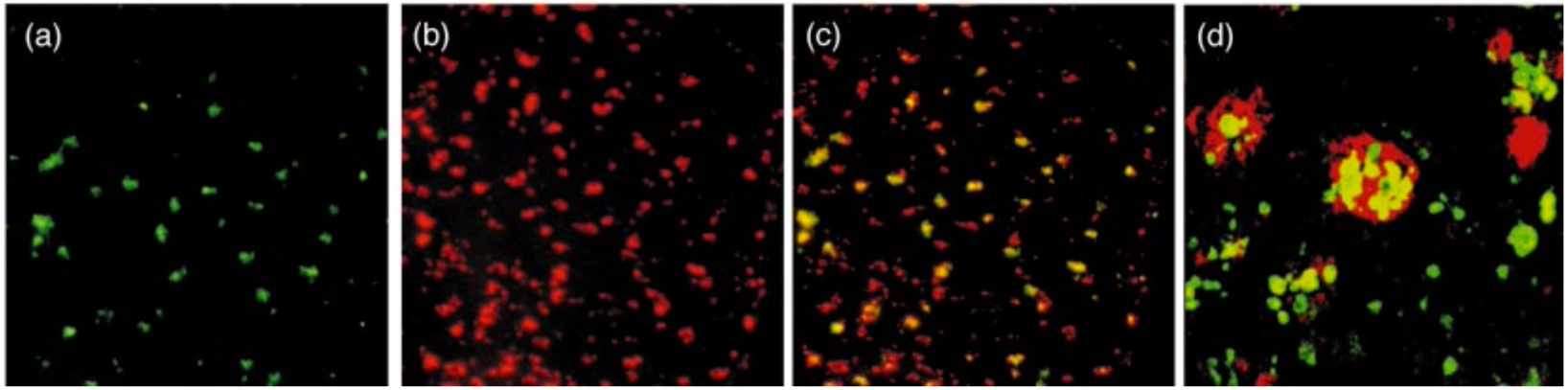
Recognized by **phosphatidylserine (PT)** on the cell surface.
In normal cells, phosphatidylserine is restricted to the inner leaflet of the plasma membrane, but is externalized on the cell surface during apoptosis.

PT - "eat me" signals

Apoptotic cells and cell fragments are efficiently recognized and **phagocytosed by macrophages**
→ cells that die by apoptosis are rapidly removed from tissues.

Phagocytosis of apoptotic cells

Distribution of apoptotic cells and macrophages



(**a**) **Apoptotic** cells in the murine thymus detected by TUNEL assay (green).

(**b**) Distribution of **macrophages** in the same region (red), identified by immunostaining with a monoclonal antibody.

(**c**) Double exposure demonstrates that essentially all TUNEL-positive thymocytes are associated with macrophages (**yellow**). (Macrophages that have not recently phagocytosed dying cells appear red.)

(**d**) Detail of multiple **apoptotic nuclei** detected by TUNEL (yellow) **inside** a single **macrophage** (red).

1998 Gordon - Recognizing death: the phagocytosis of apoptotic cells

Animal apoptosis - Summary

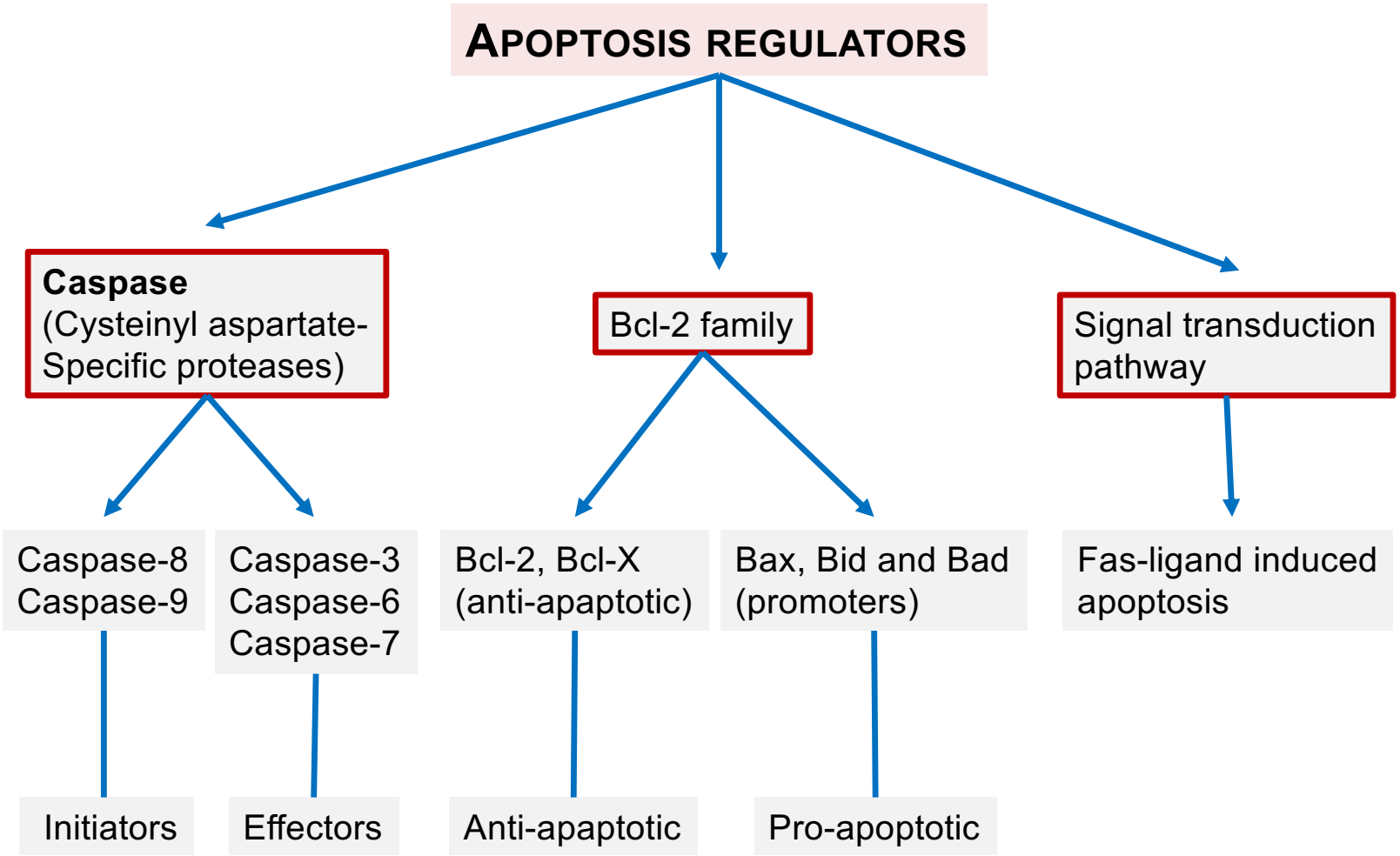
Animal cells can activate an intracellular death program and kill themselves in a controlled way when they are:
- irreversibly damaged,
- no longer needed,
- are a threat to the organism.

→ **Apoptosis**: the cells shrink, condense, and frequently fragment, and neighboring cells or macrophages rapidly phagocytose the cells or fragments before there is any leakage of cytoplasmic contents.

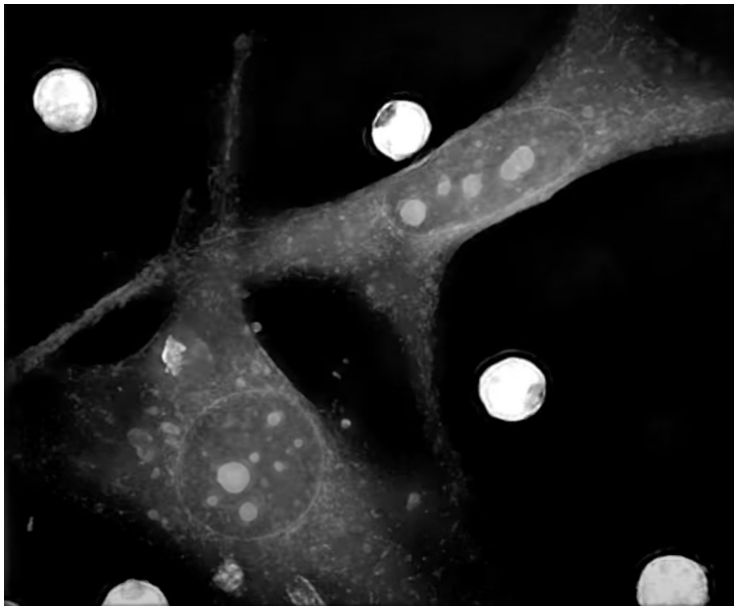
Mediated by proteolytic enzymes called **caspases**, which cleave specific intracellular proteins to help kill the cell.
Inactive precursors are **activated** when brought into proximity in activation complexes.
→ **pro-caspases** cleave and thereby activate downstream **executioner caspases**
that cleave various target proteins in the cell, producing an amplifying, irreversible proteolytic cascade.

Extrinsic pathway - activated by extracellular ligands binding to cell-surface death receptors.
Death receptors recruit caspase-8 via adaptor proteins to form the DISC
Intrinsic pathway - activated by intracellular signals generated when cells are stressed
Cytochrome c released from the intermembrane space of mitochondria activates Apaf1, which assembles into an apoptosome and recruits and activates caspase-9.

Intracellular Bcl2 family proteins (anti-apoptotic and pro-apoptotic) and IAP proteins tightly regulate the apoptotic program.


APOPTOSIS REGULATORS
Caspase
(Cysteinyl aspartate-
Specific proteases)
Bcl-2 family
Signal transduction
pathway
Caspase-8
Caspase-9
Caspase-3
Caspase-6
Caspase-7
Bcl-2, Bcl-X
(anti-apaptotic)
Bax, Bid and Bad
(promoters)
Fas-ligand induced
apoptosis
Initiators
Effectors
Anti-apaptotic
Pro-apoptotic

Necrosis
live cell imaging

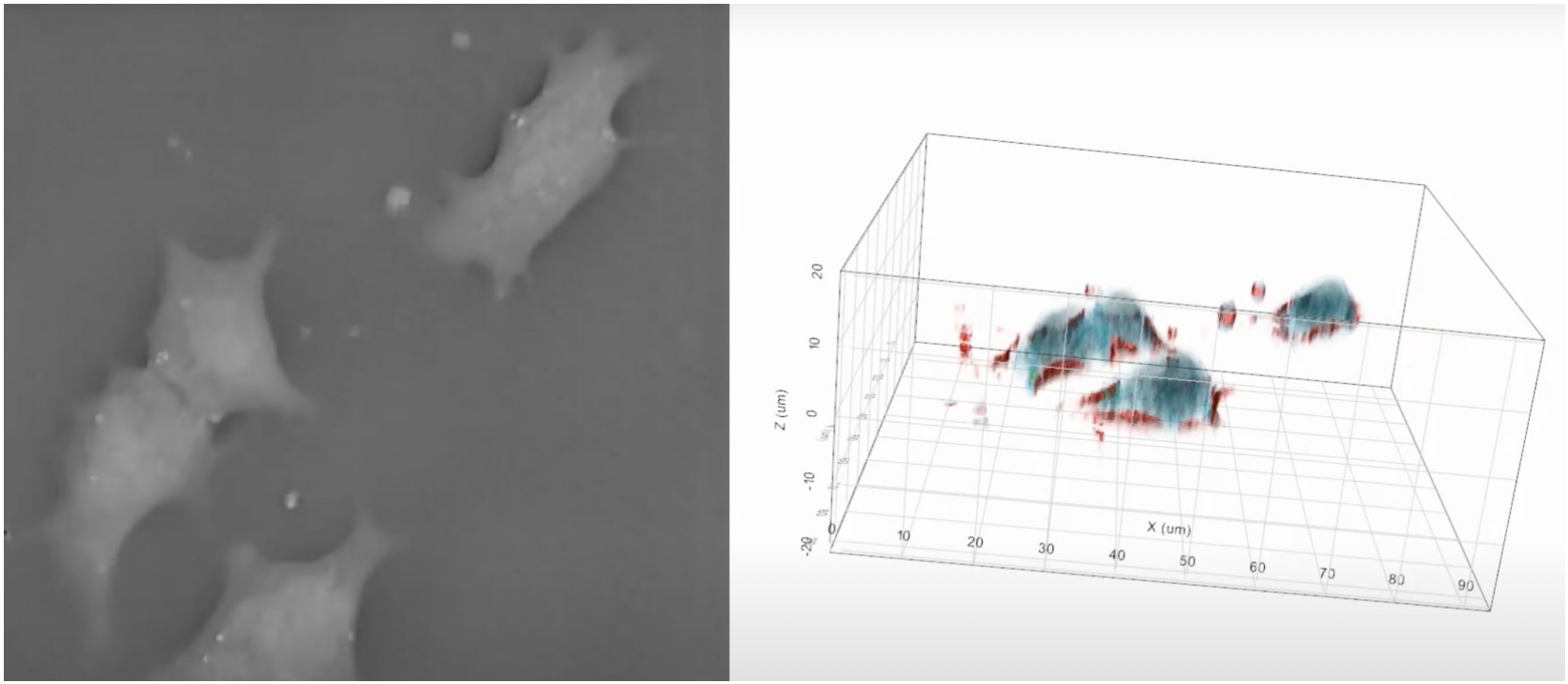


Label-free live cell imaging of simultaneous, massive mammalian cell necrosis

https://www.youtube.com/watch?v=MYJfPWhiTUE&ab_channel=Nanolive%2CLookinginsidelife

Comparison between apoptosis and necrosis

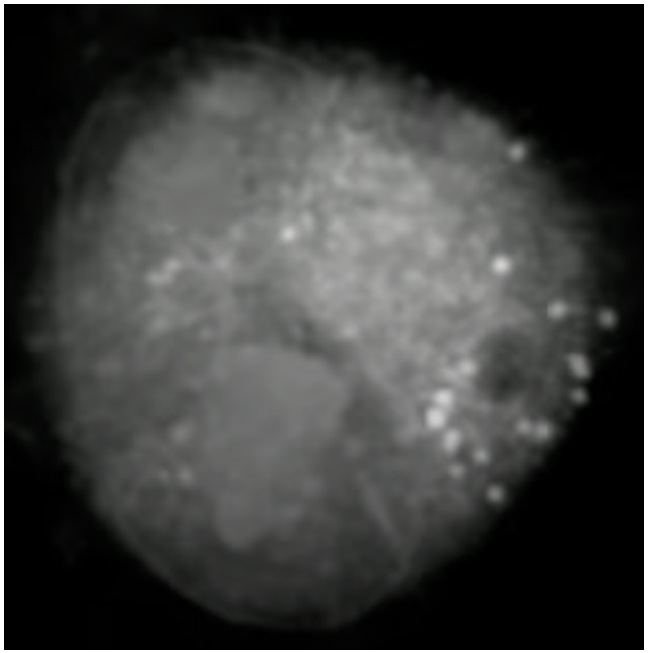
live cell imaging



https://www.youtube.com/watch?v=nh-Wn3DBONg&ab_channel=Nanolive%2CLookinginsidelife

Apoptosis

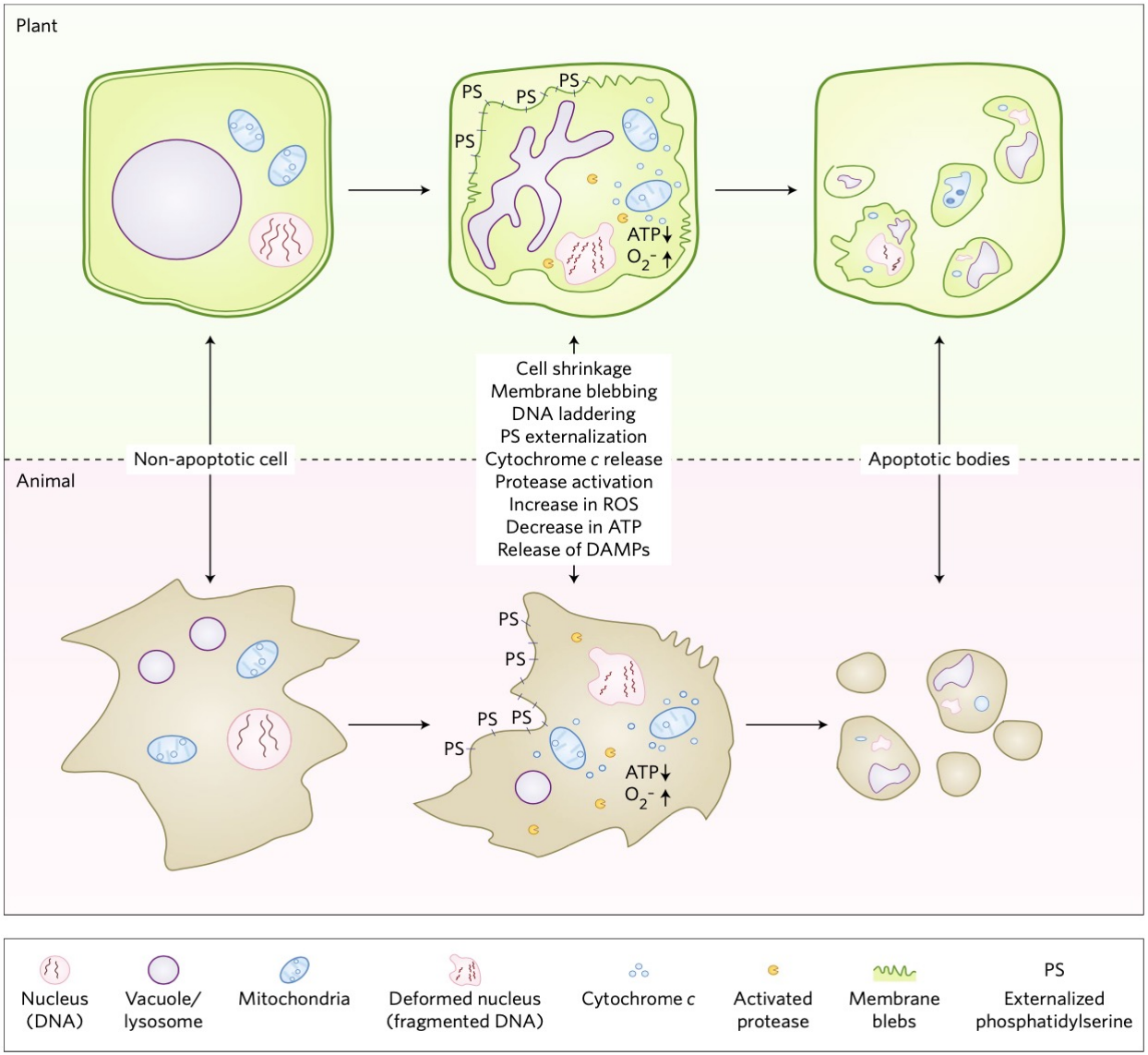
live cell imaging



Label-free live cell imaging of apoptosis of T685A human melanoma cancer cell

https://www.youtube.com/watch?v=FO7EgQ5zA14&ab_channel=NikonInstrumentsComp

Comparison of apoptotic-like cell death in animal and plant cells



Mechanisms of PCD in Plants

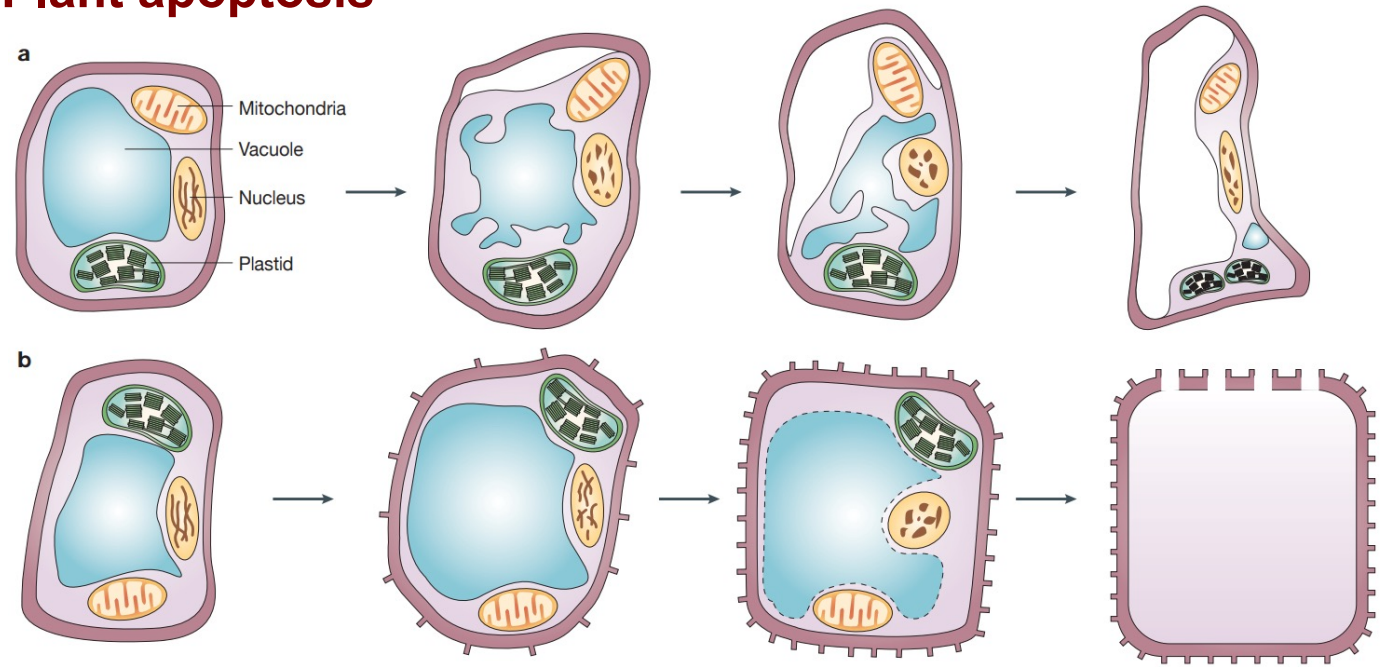
Programmed cell death (PCD) is an integral and essential part of the lifecycle of multicellular organisms, in **plants** as well as in animals.

In plants, PCD occurs during **development** as well as in response to **environmental** and biotic stimuli.
Understanding of the regulation of plant PCD has advanced.
However, the molecular machinery remains elusive.

Based on morphological criteria, at least **two types of PCD** can be distinguished in plants:

- '**vacuolar**' (autolytic) - **developmental**
- '**necrotic**' (non-autolytic) - **environmental**

Plant apoptosis



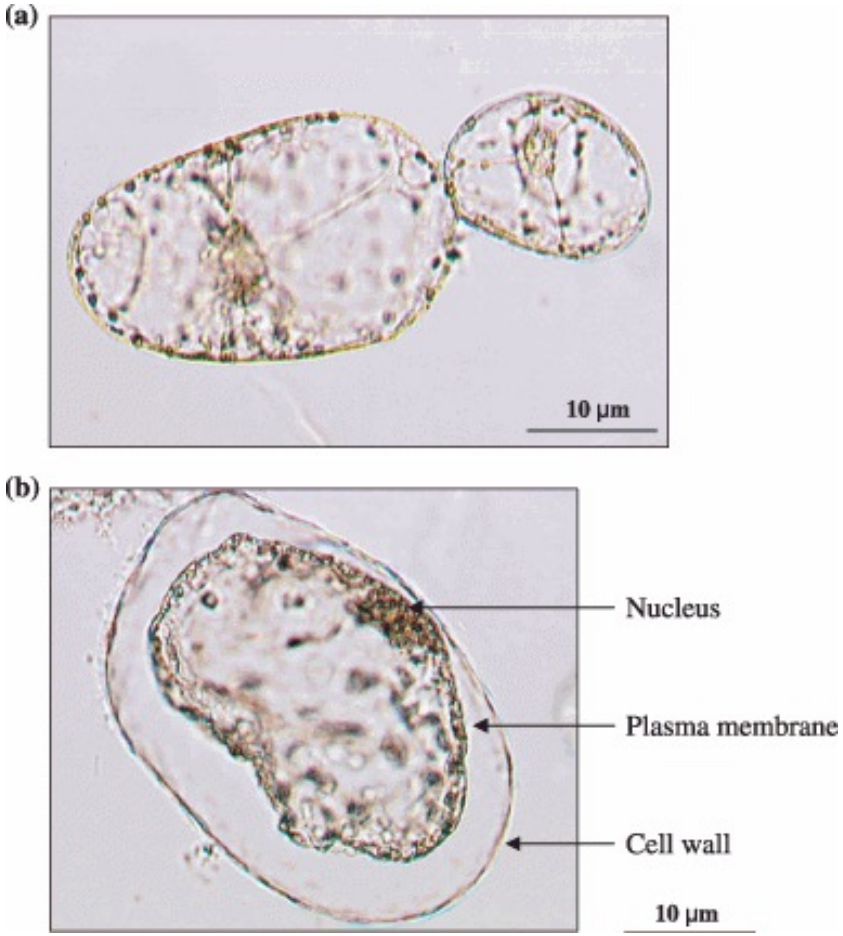
(a) Necrotic Non-autolytic

In the **hypersensitive response**, chromatin condensation and DNA cleavage, blebbing of the vacuole and plasma membranes, destruction of organelles.
Final stage → plasma membrane collapses and separates from the cell wall
→ leakage of the dead cell's content into the apoplast.

(b) Vacuolar Autolytic

During the **differentiation** of **tracheary elements**, vacuole swelling and rupture, thickening and restructuring of the cell wall.
Final collapse of the vacuole precedes nuclear DNA fragmentation, final autolysis of the cell.
(Broken areas in the cell wall of terminally differentiated tracheary elements)

Environmental induced apoptotic-like programmed cell death in plants → hypersensitive response



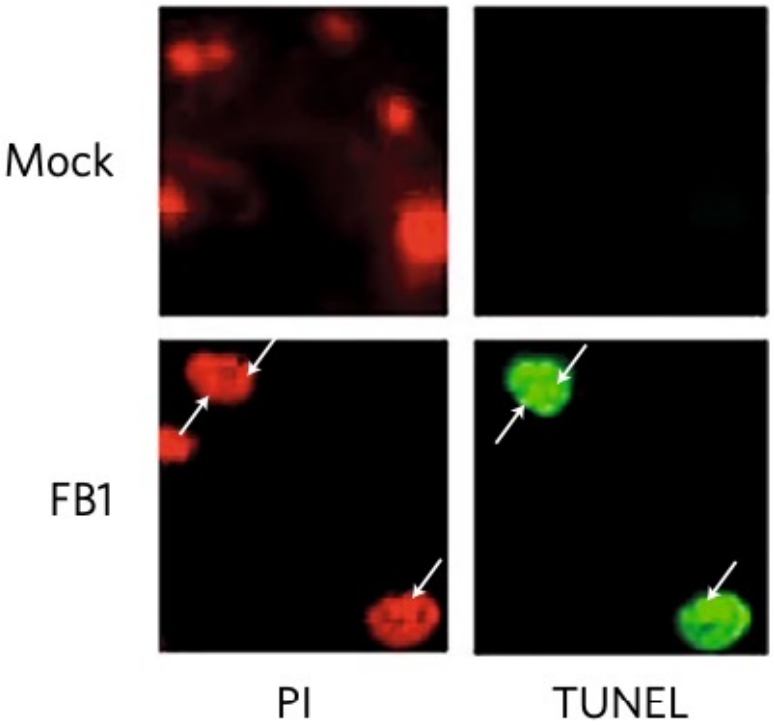
Carrot cells

Treatment of cells with **temperatures 55°C**
→ Death cell with a specific cellular morphology

The most obvious feature of this morphology consisted of a <u>retraction of the protoplast away from the cell wall</u>

Plant apoptotic-like bodies

Rigid **cell wall** surrounding plant cells → no necessity for breakdown of the cell into apoptotic-like bodies.
Further, plant have cell walls and do **not have phagocytes**
→ no engulfment and removal of apoptotic cells by adjacent cells.

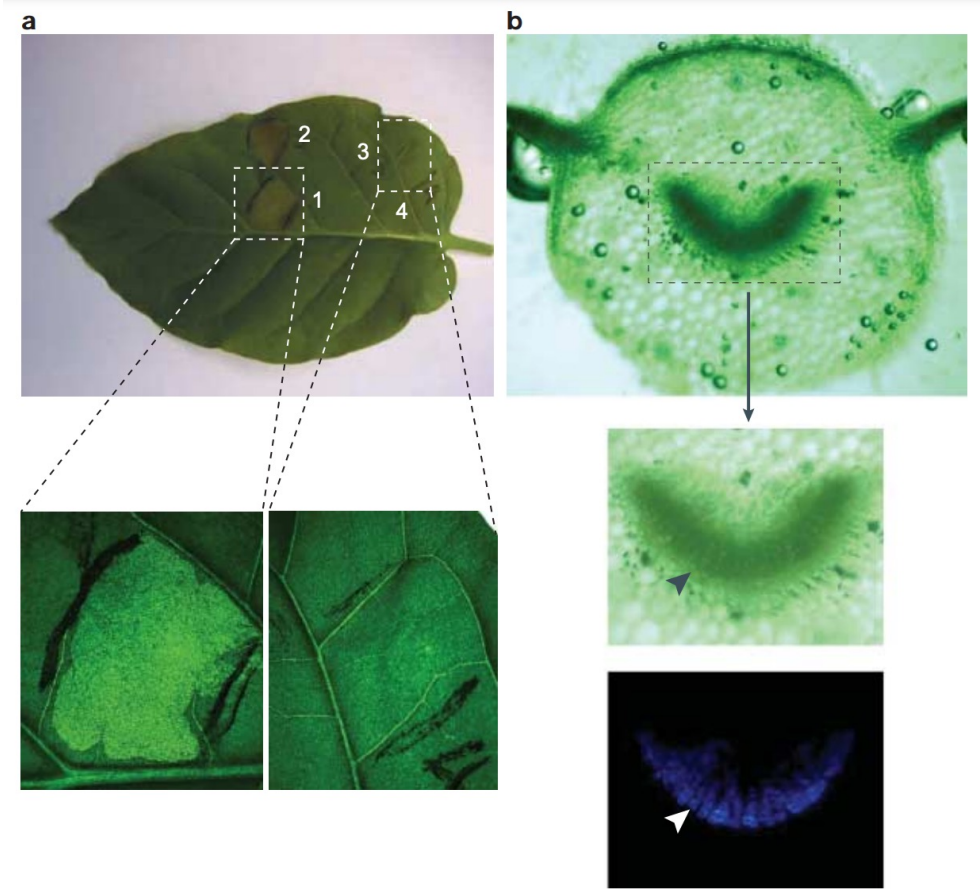


However, **apoptotic-like bodies** have been observed in plant cells (protoplasts) induced by abiotic and biotic stress

Apoptotic bodies, recognized by nuclear changes, propidium iodide (**PI**) staining (left) and **TUNEL** assay (right) in tomato protoplasts treated with the mycotoxin Fumonisin B1 (FB1).

Dickman et al. Reassessing apoptosis in plants. *Nature plants* 3 (2017): 773

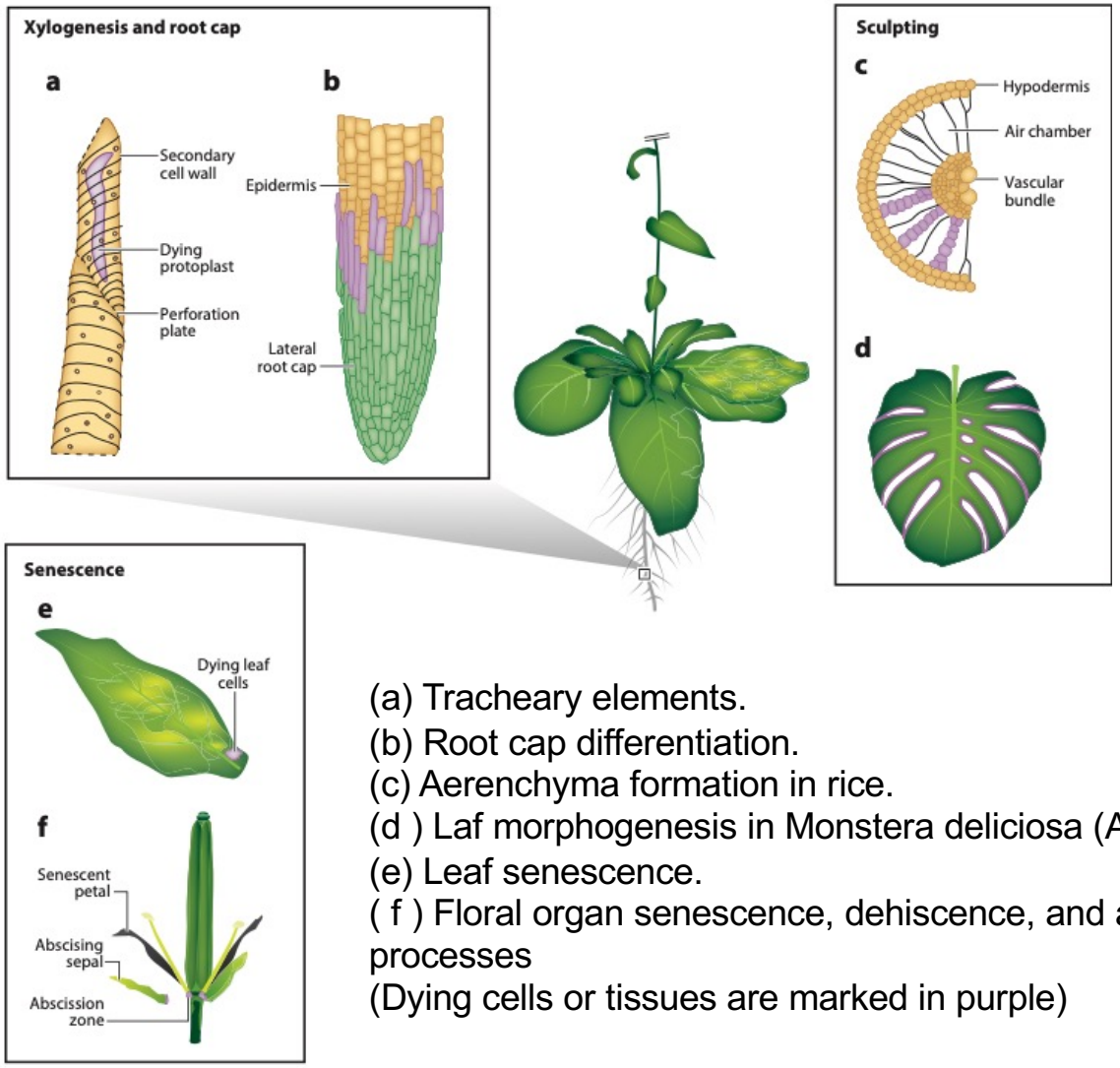
Examples of programmed cell death in plants



(a) Hypersensitive response: tobacco leaf infiltrated with *Pseudomonas syringae*. Visible cell death of 1 and 2 inoculated regions. Enlarged views show cleared cells with little chlorophyll in zones 1 and 2.

(b) Developmental death:
formation of the xylem.
The top panel shows a cross-section of a tobacco leaf, boxed region showing the central tracheary elements that undergone programmed cell death.
Reinforced secondary cell walls are highly autofluorescent (shown by arrowheads in enlarged views).

Cell death during vegetative development



(a) Tracheary elements.
(b) Root cap differentiation.
(c) Aerenchyma formation in rice.
(d) Laf morphogenesis in Monstera deliciosa (Araceae).
(e) Leaf senescence.
(f) Floral organ senescence, dehiscence, and abscission processes
(Dying cells or tissues are marked in purple)

Daneva et al. Functions and regulation of programmed cell death in plant development. *Annual Review of Cell and Developmental Biology* 32 (2016): 441.