

LECTURE 16 - Apoptosis

Outline

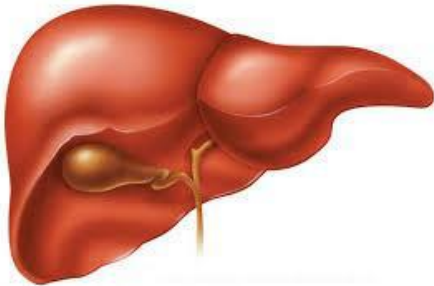
1. Overview of programmed cell death
2. Methods in apoptosis identification
3. Brief history of apoptosis
4. Mechanisms

1. Overview of apoptosis (a form of programmed cell death)

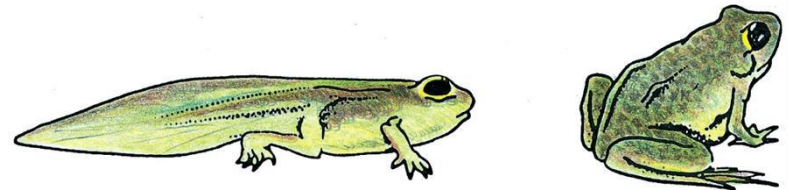
Apoptosis: **eliminates cells** that are abnormal, misplaced, nonfunctional, or potentially dangerous.

It is a natural part of organism development and maintenance.

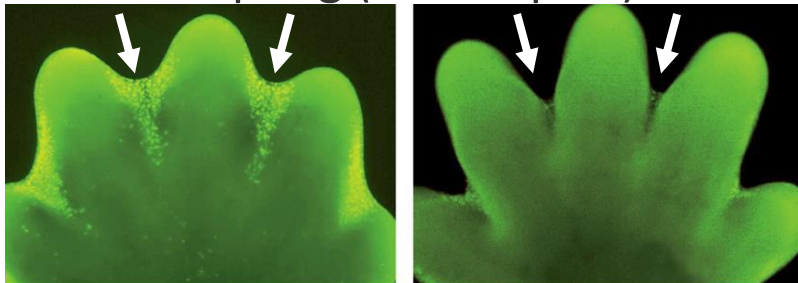
Tissue/organ homeostasis (liver size)



Metamorphosis (tadpole to frog)



Tissue sculpting (mouse paw)



Immune cell apoptosis
(short-lived neutrophils)



What is apoptosis?

During apoptosis, cells undergo morphological changes:

Normal cell

Apoptotic cell



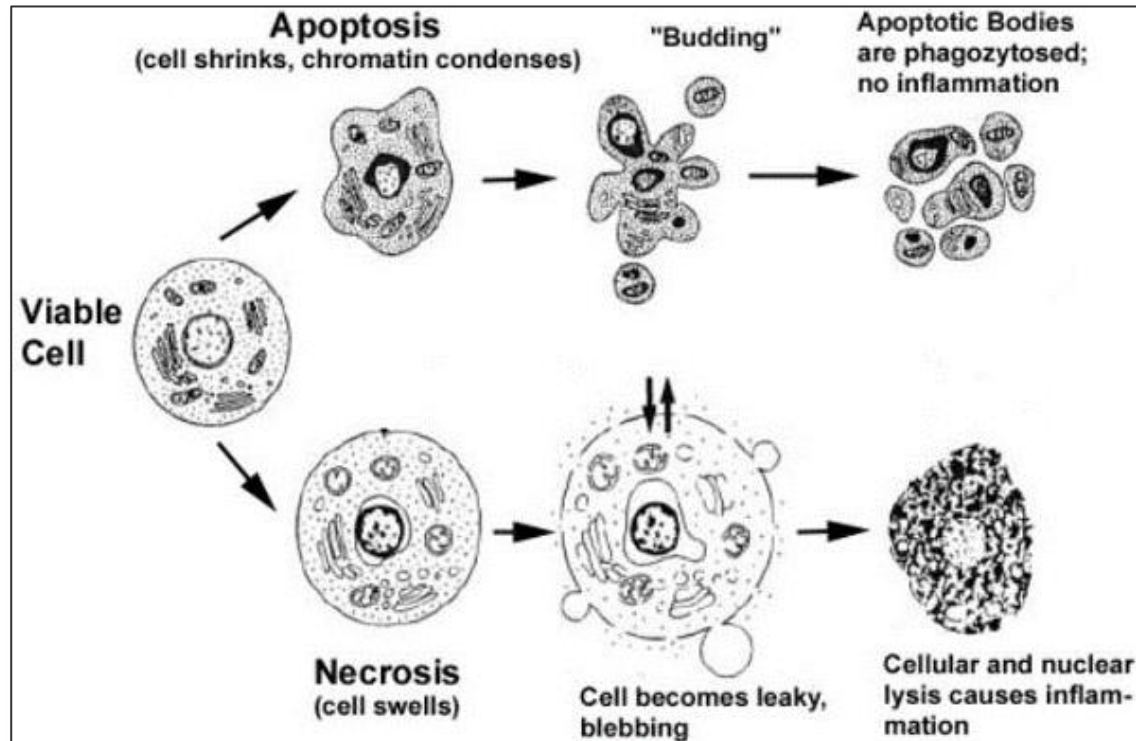
Characteristics of apoptosis :

- 1.Cell shrinkage and chromatin condensation
- 2.PS flipping to outside
- 3.DNA fragmentation
- 4.Nuclear membrane disruption
- 5.Cytoskeleton collapses
- 6.Cell surface blebs---apoptotic bodies

Different types of death: apoptosis versus necrosis

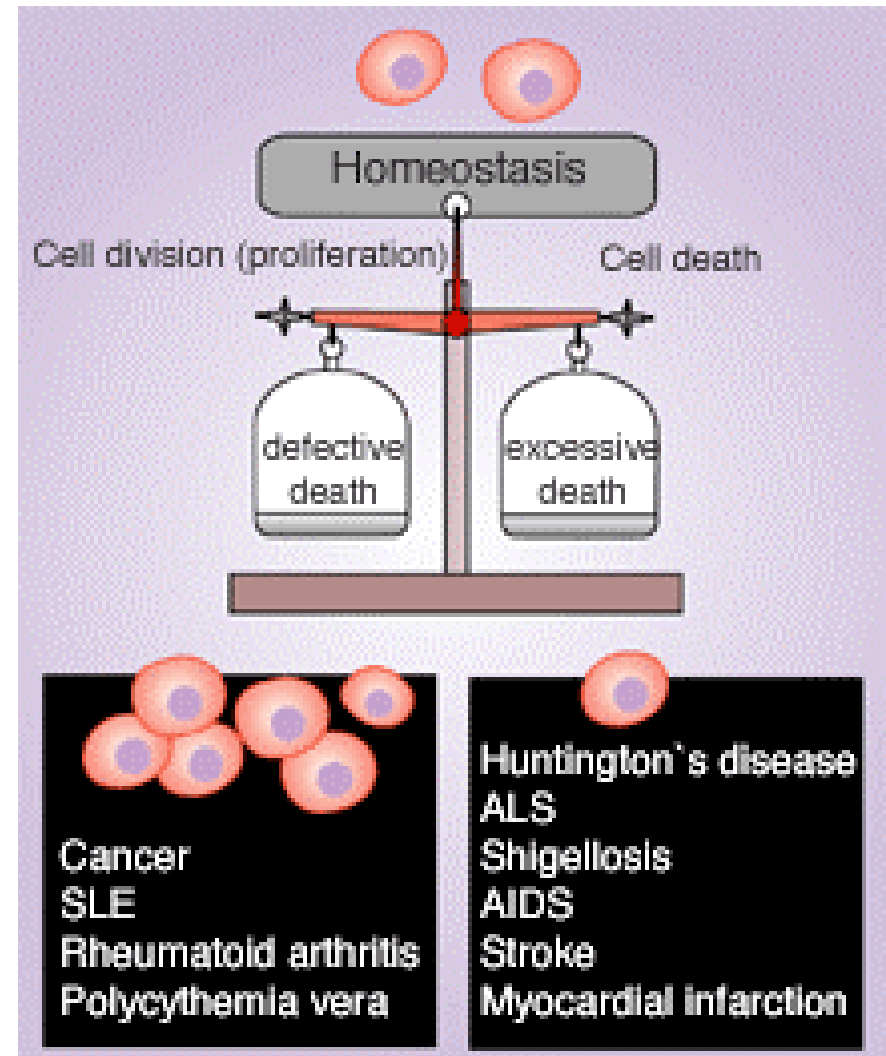
Apoptosis: cells die clean and tidy
(eaten and digested by neighboring cells or macrophages)

Necrosis: cells swell and burst, content spillage, can cause inflammation,
necrosis is usually due to acute insults



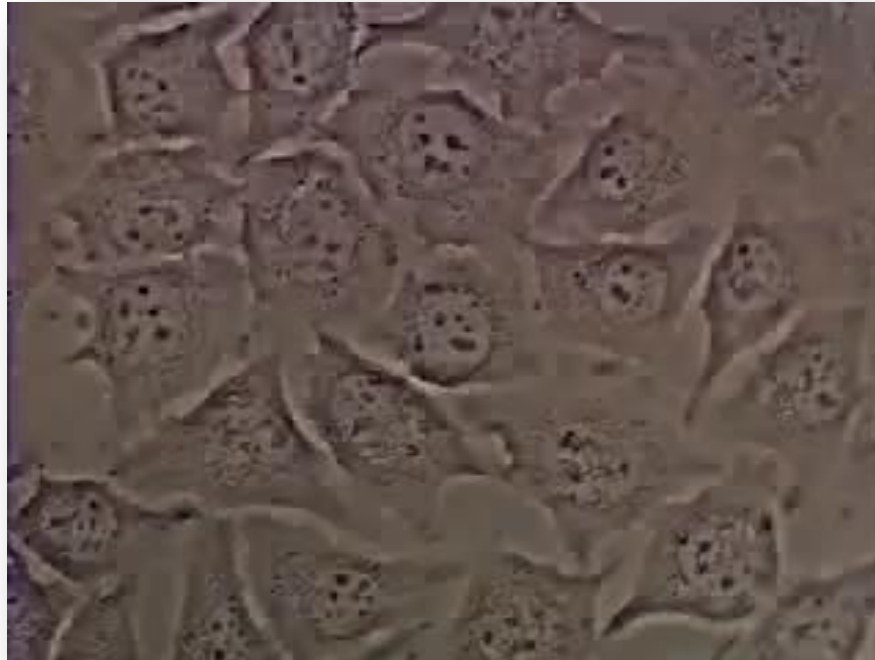
Deregulation of apoptosis is involved in diseases

- High activity of apoptosis:
 - neurodegenerative disease
 - myocardial infarction
 - radiation injury
- Low activity of apoptosis:
 - cancer
 - autoimmune disease



Programmed cell death is not confined to animal cells

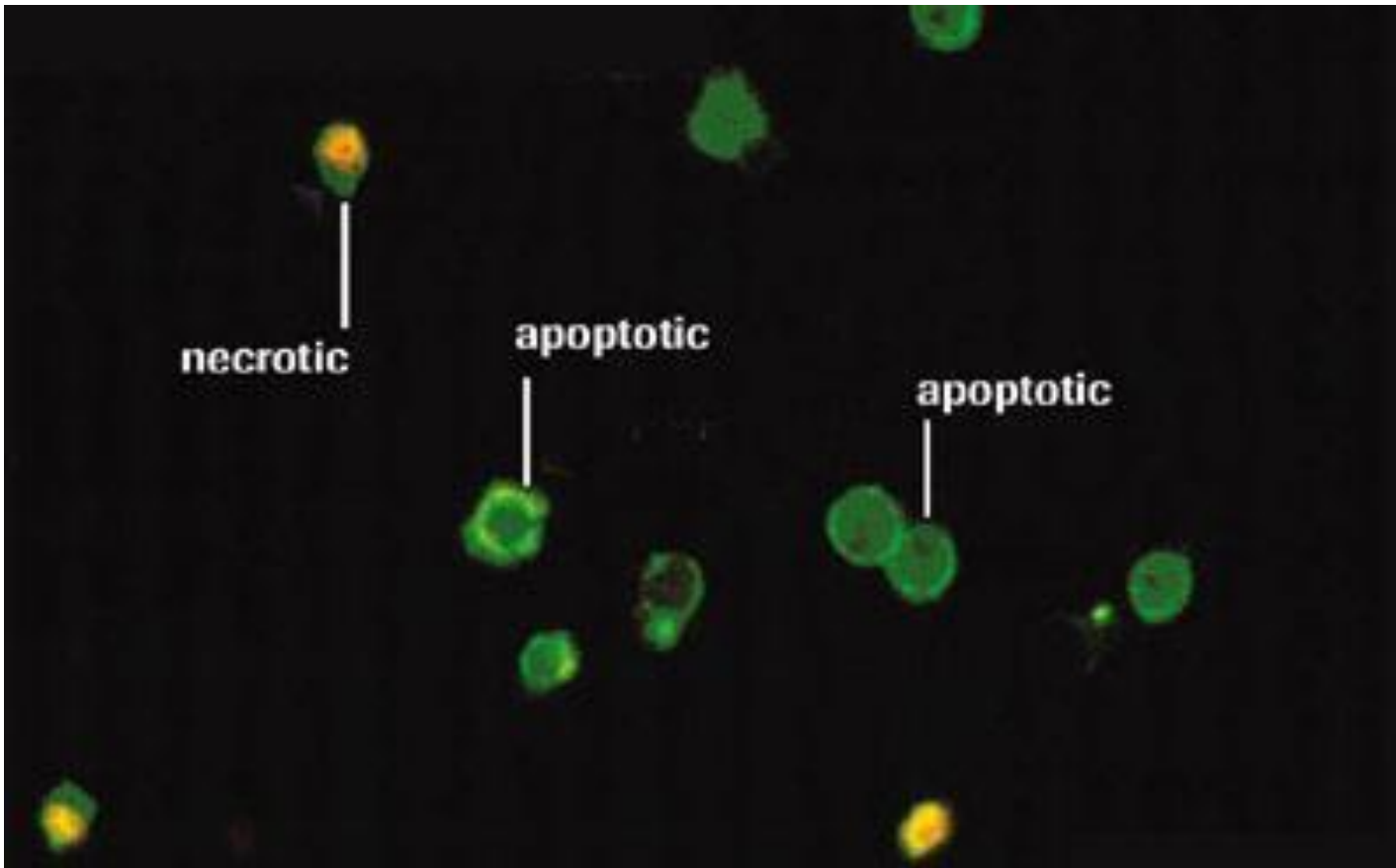
- Plant cells
- Yeast
- Bacteria



2. Methods to identify apoptotic cells

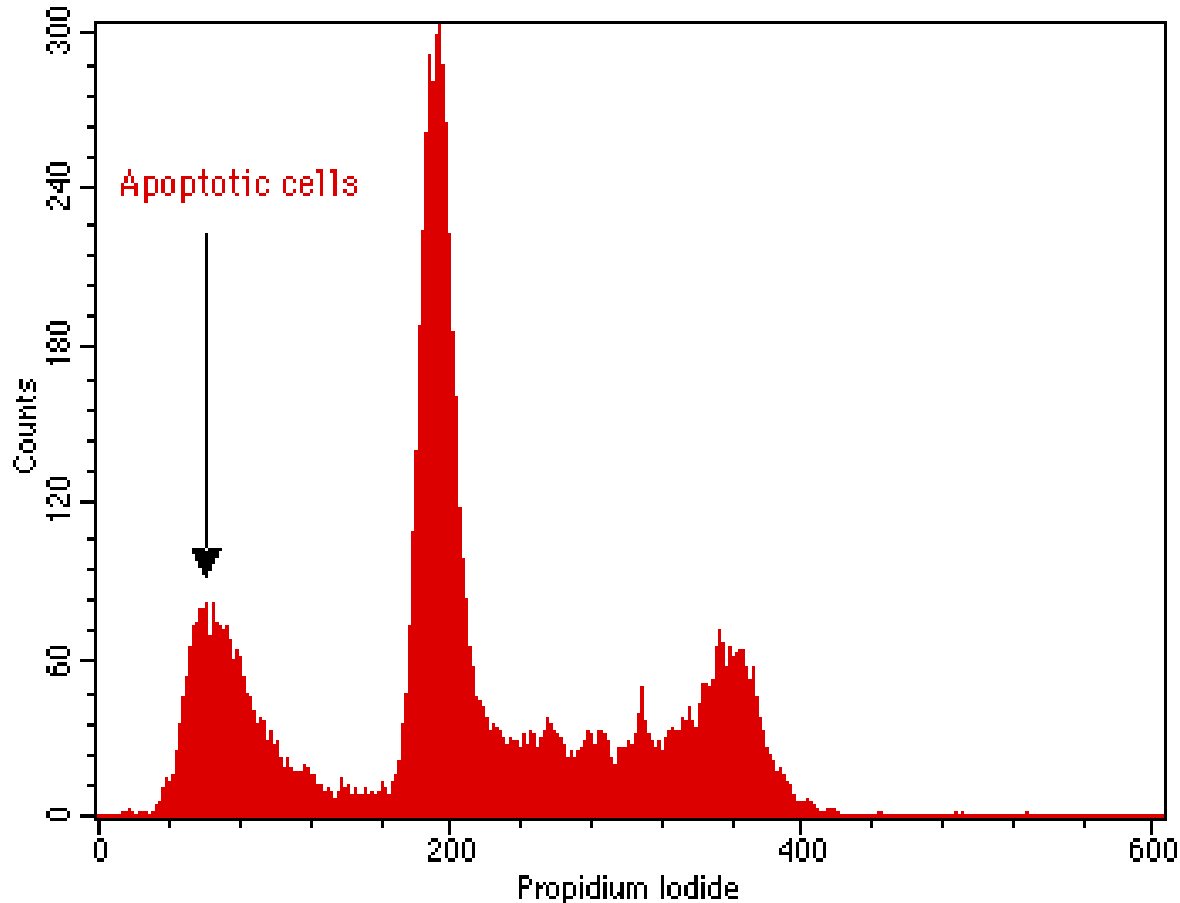
- a) Annexin V staining
- b) Cell cycle distribution
- c) DNA fragmentation
- d) TUNEL assay
- e) Western blot for apoptosis markers (Caspase 3, PARP, etc)
- f) Cytochrome c translocation

a) Annexin V staining (early apoptosis)



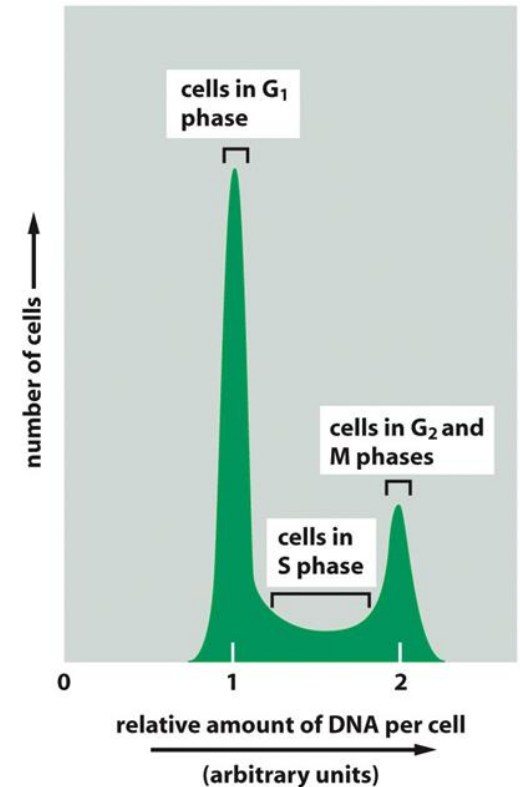
Phosphatidylserine (PS) is flipped to outer leaflet of plasma membrane and can be detected by annexin V (protein)-conjugate fluorescent dye

b) Cell cycle distribution

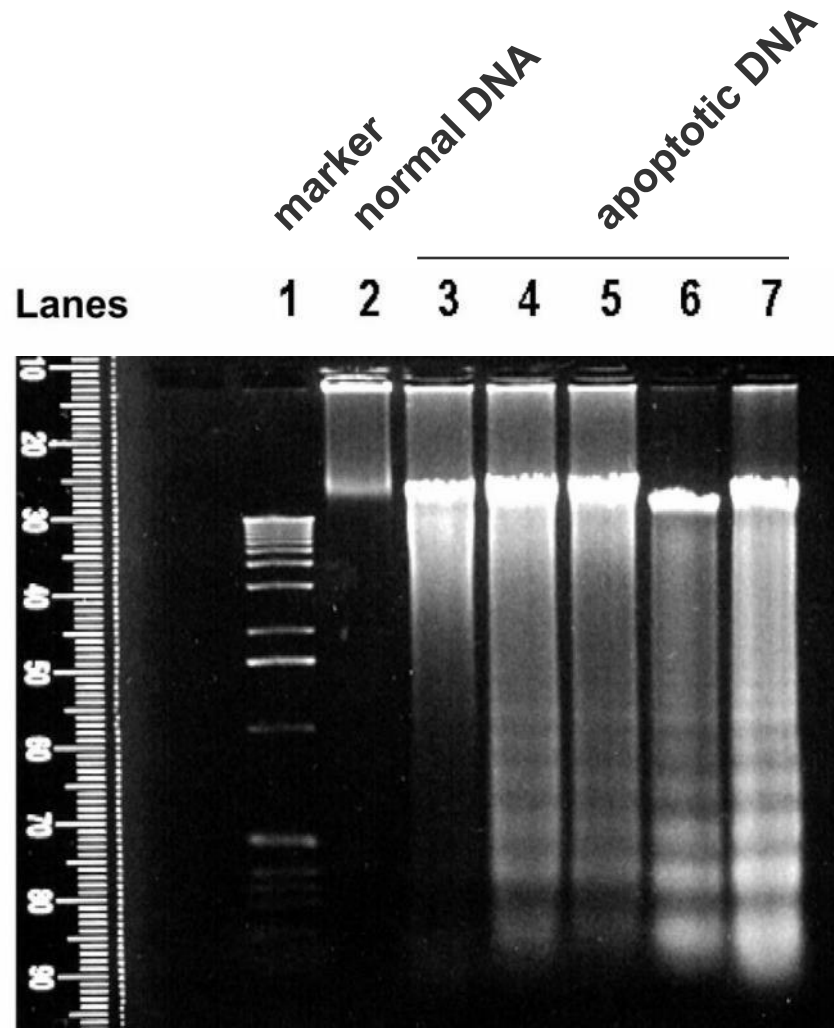


Sub-G1 percentage is an indicator for DNA fragmentation due to apoptosis.

non-apoptotic cells



c) DNA fragmentation

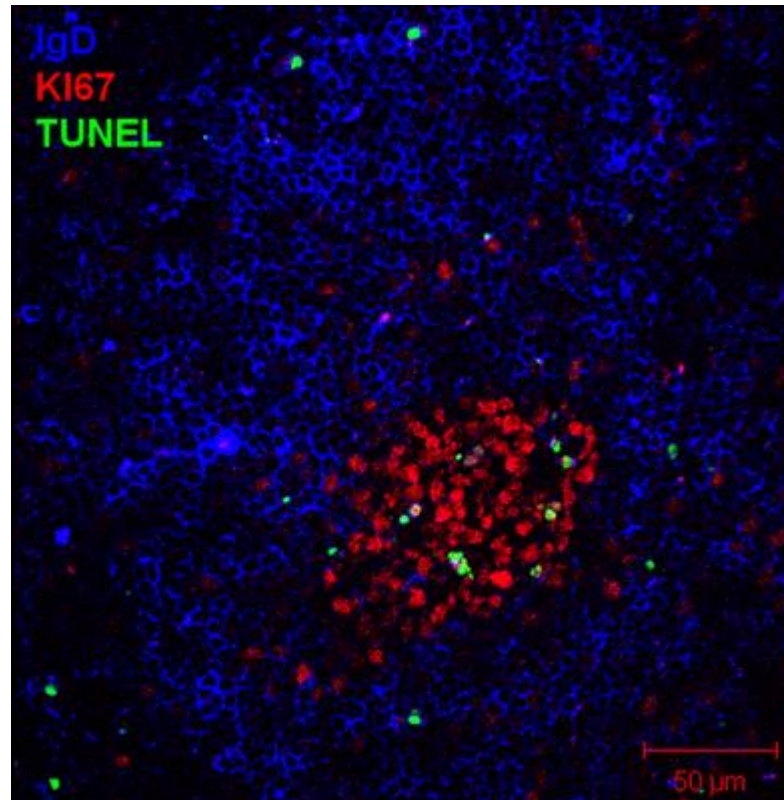


d) TUNEL staining (late apoptosis)

Terminal dUTP Nick End Labelling (TUNEL)

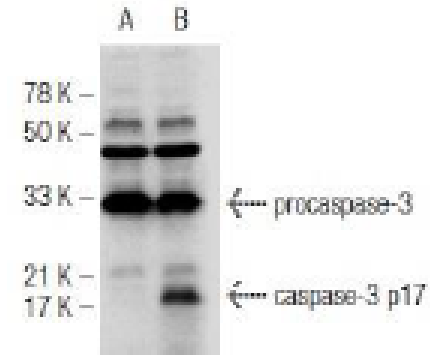
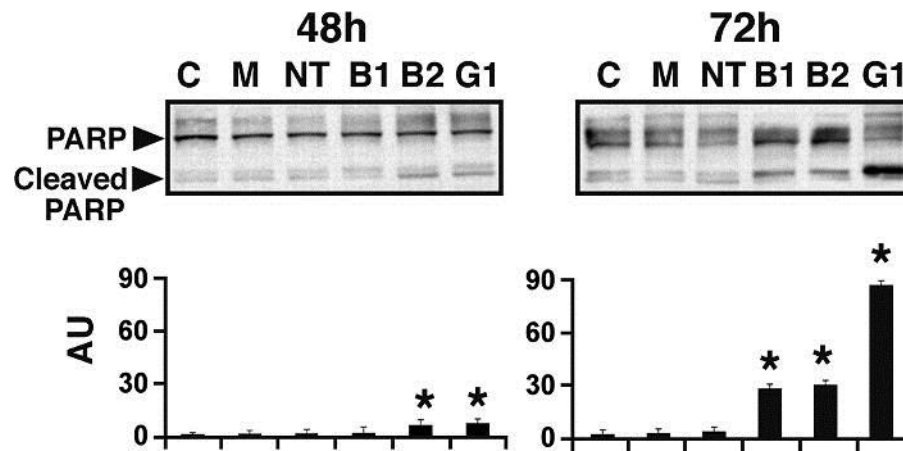
It is another method to detect DNA fragmentation

Terminal **deoxynucleotidyl transferase** catalyzes **dUTP addition on fragmented DNA**,
dUTP is subsequently **labeled with fluorescence dye**



e) Western blotting for apoptotic markers

Commonly used proteins: **cleaved PARP**
cleaved caspase-3



3. Brief History - leading to the Nobel Prize in 2002



Sydney Brenner



Robert Horvitz



John Sulston

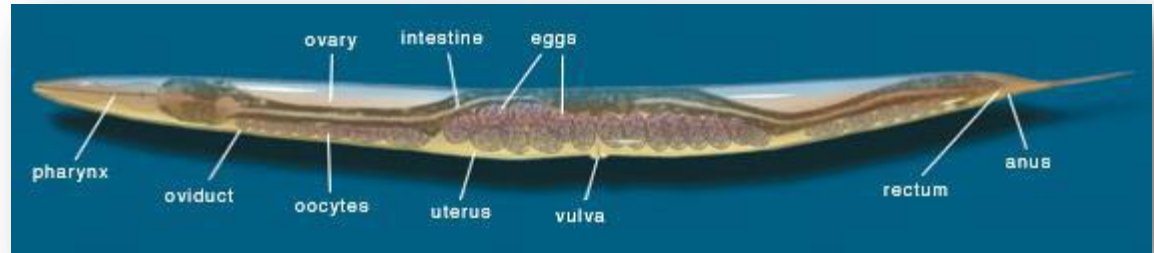


C. elegans as new model organism to analyze organ development



Sydney Brenner

born 1927,
La Jolla, CA, USA. Nobel
Laureate, 2002

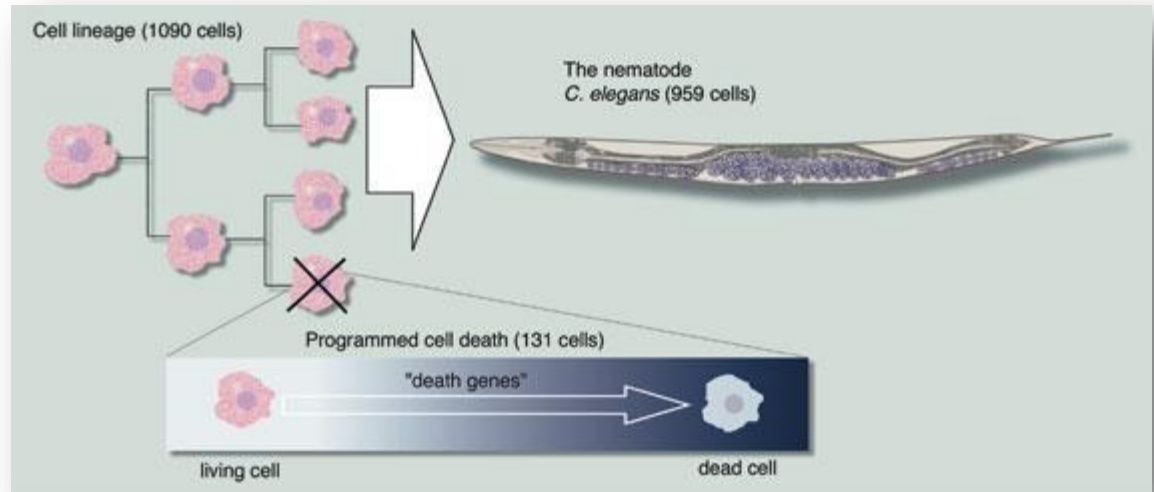


Brenner established *C. elegans* as a novel experimental model organism. This provided a unique opportunity to link genetic analysis to cell division, differentiation and organ development – and to follow these processes under the microscope.

Cell death is part of the normal differentiation process



John Sulston,
born 1942,
Cambridge, England.
Nobel Laureate in 2002



Sulston mapped a cell lineage where every cell division and differentiation could be followed in the development of a tissue in *C. elegans*.

He showed that specific cells undergo programmed cell death as an integral part of the normal differentiation process.

He also identified the first mutation of a gene participating in the cell death process.

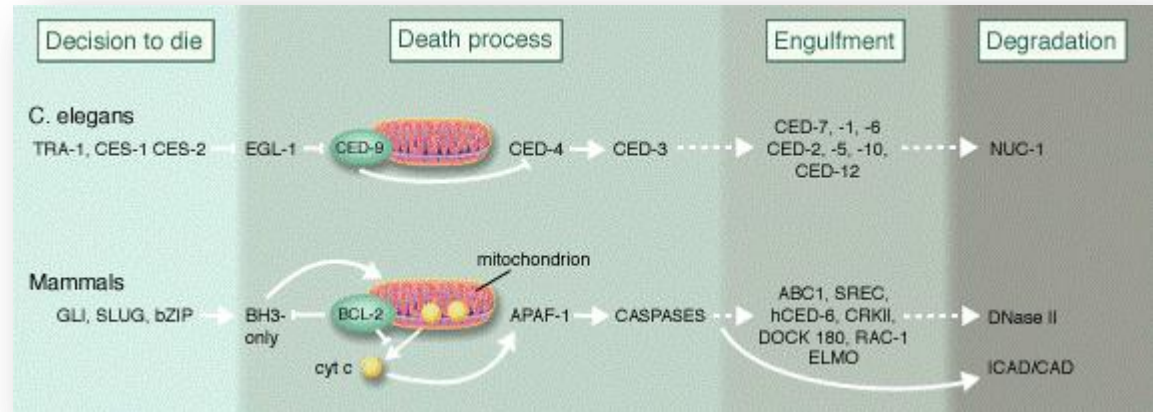
Cell death is controlled by genes



Robert Horvitz

born 1947, Cambridge,
MA, USA.

Nobel laureate, 2002



Horvitz discovered and characterized key genes controlling cell death in *C. elegans*.

He has shown how these genes interact with each other in the cell death process and that corresponding genes exist in humans.

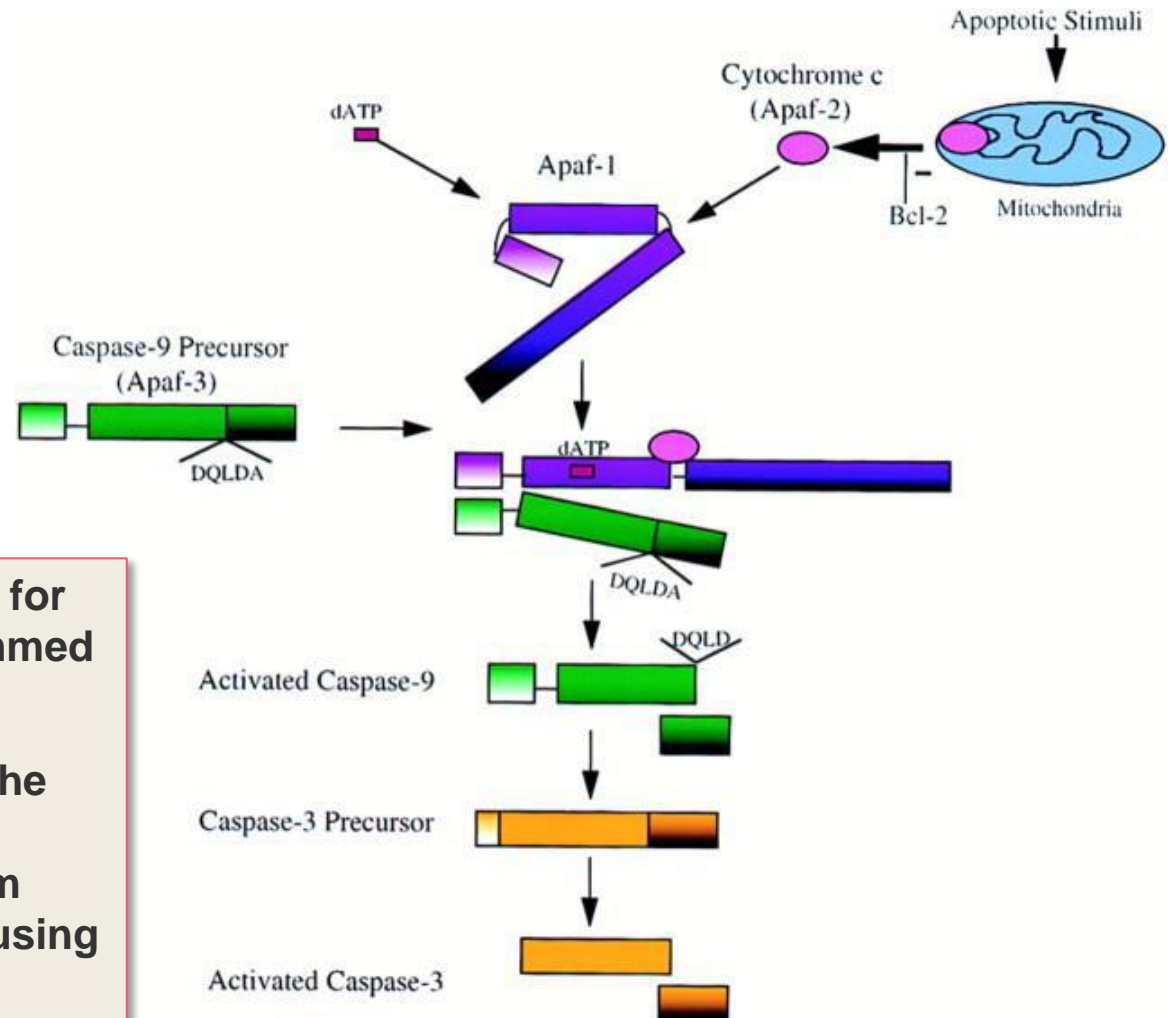
Reveal the mechanism of caspase-3 via apoptosome



Xiaodong Wang

His work revealed a key role for the mitochondria in programmed cell death.

He has extensively studied the interactions triggering the release of cytochrome c from inside this organelle and causing the apoptotic cascade



4. Players and mechanisms

1. Caspases
2. Extrinsic pathway
3. Intrinsic pathway
4. Bcl-2 family
5. IAPs

Apoptosis depends on an intracellular proteolytic cascade

Proteolysis is catalyzed by caspases:

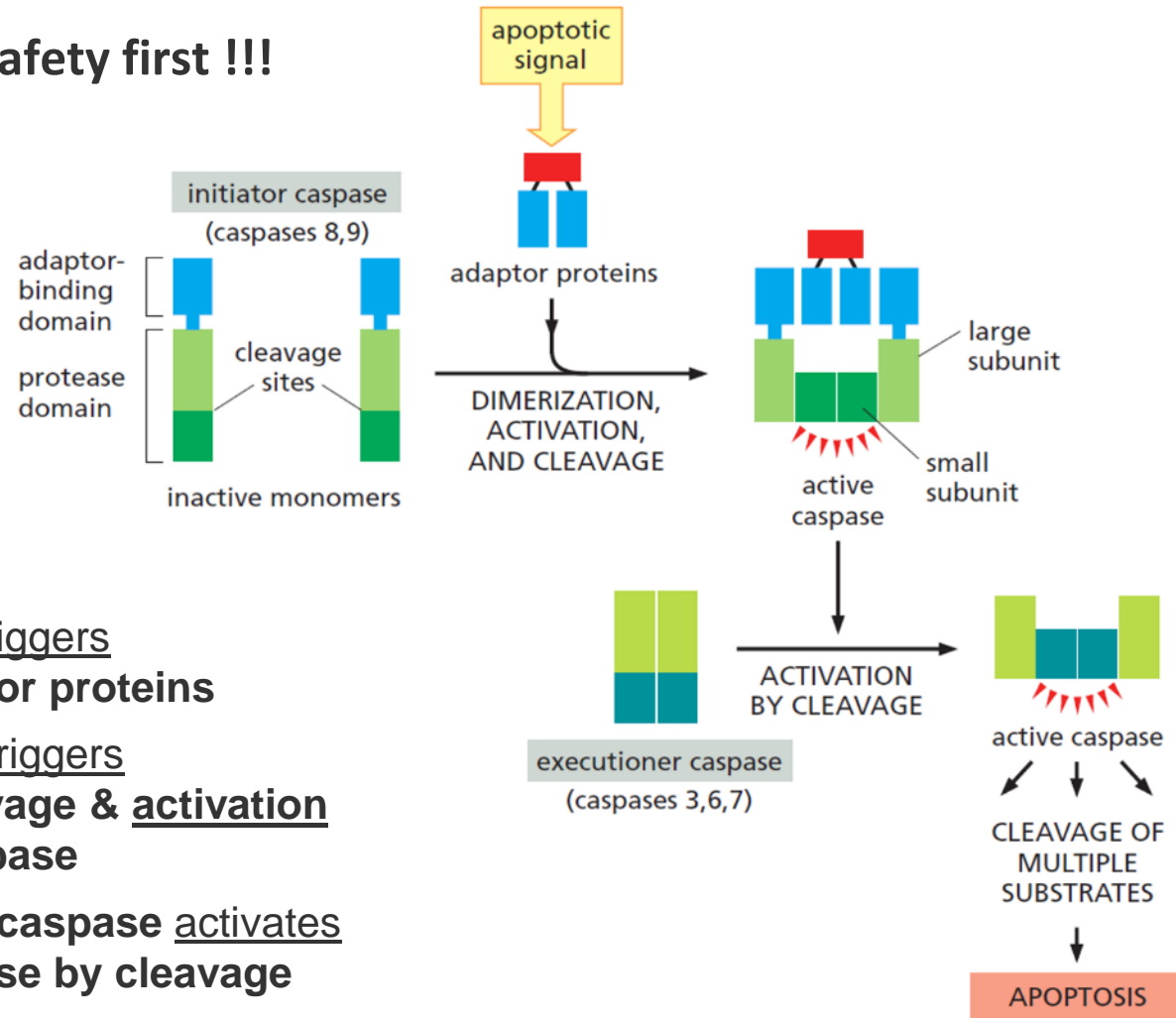
- Caspases have a cysteine in their active site and cleave their target proteins at specific aspartic acids (→ **caspase**)
- Caspases are zymogens (synthesized as inactive precursors), → **procaspase**



- **Two types of caspases:**
 - **initiator caspases**
 - **executioner caspases**
- **Apoptosis is triggered by a cascading reaction of initiator and executioner caspases**

Signal-mediated cascading activation of apoptosis

Death is dangerous: safety first !!!

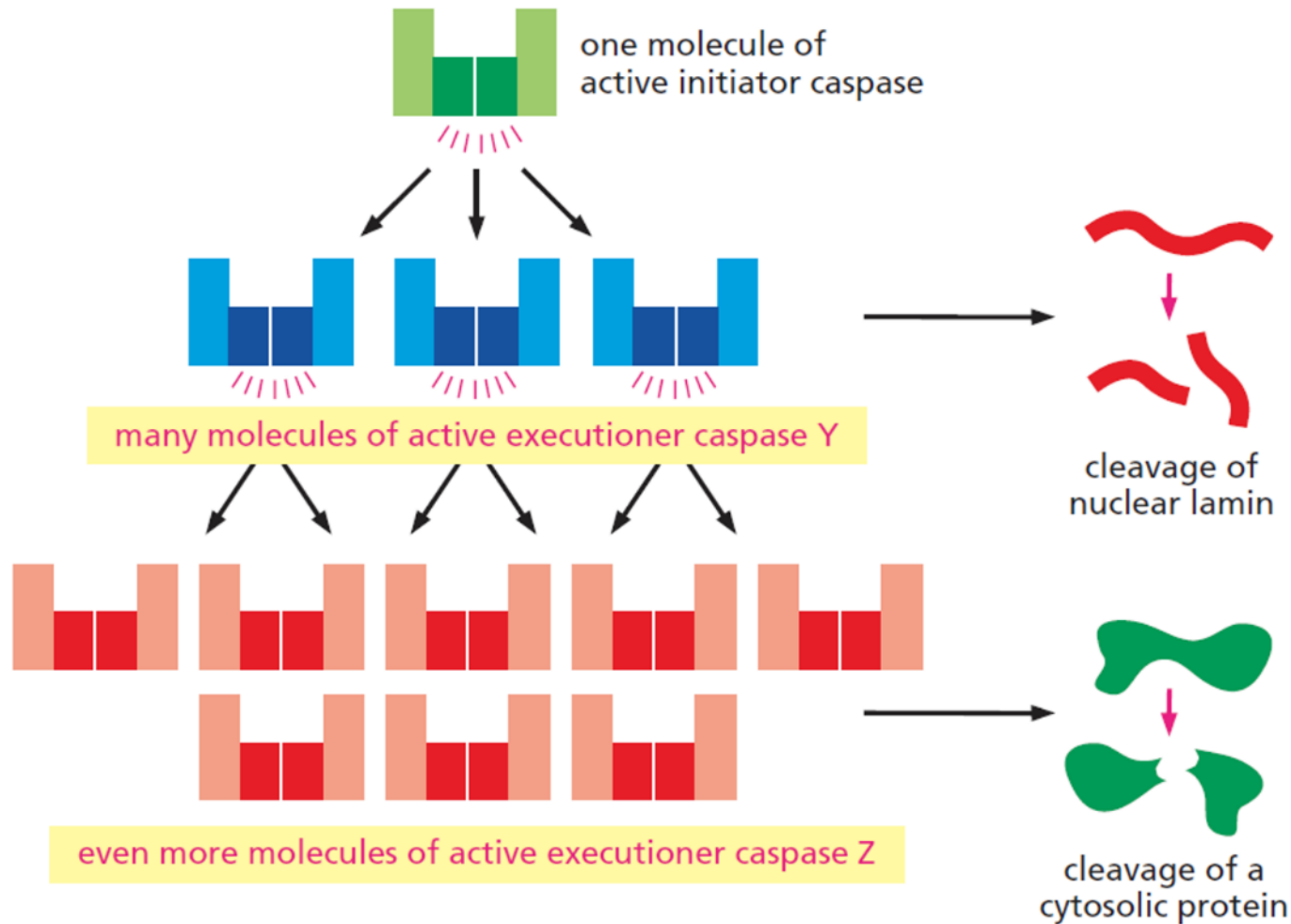


Activation cascade:

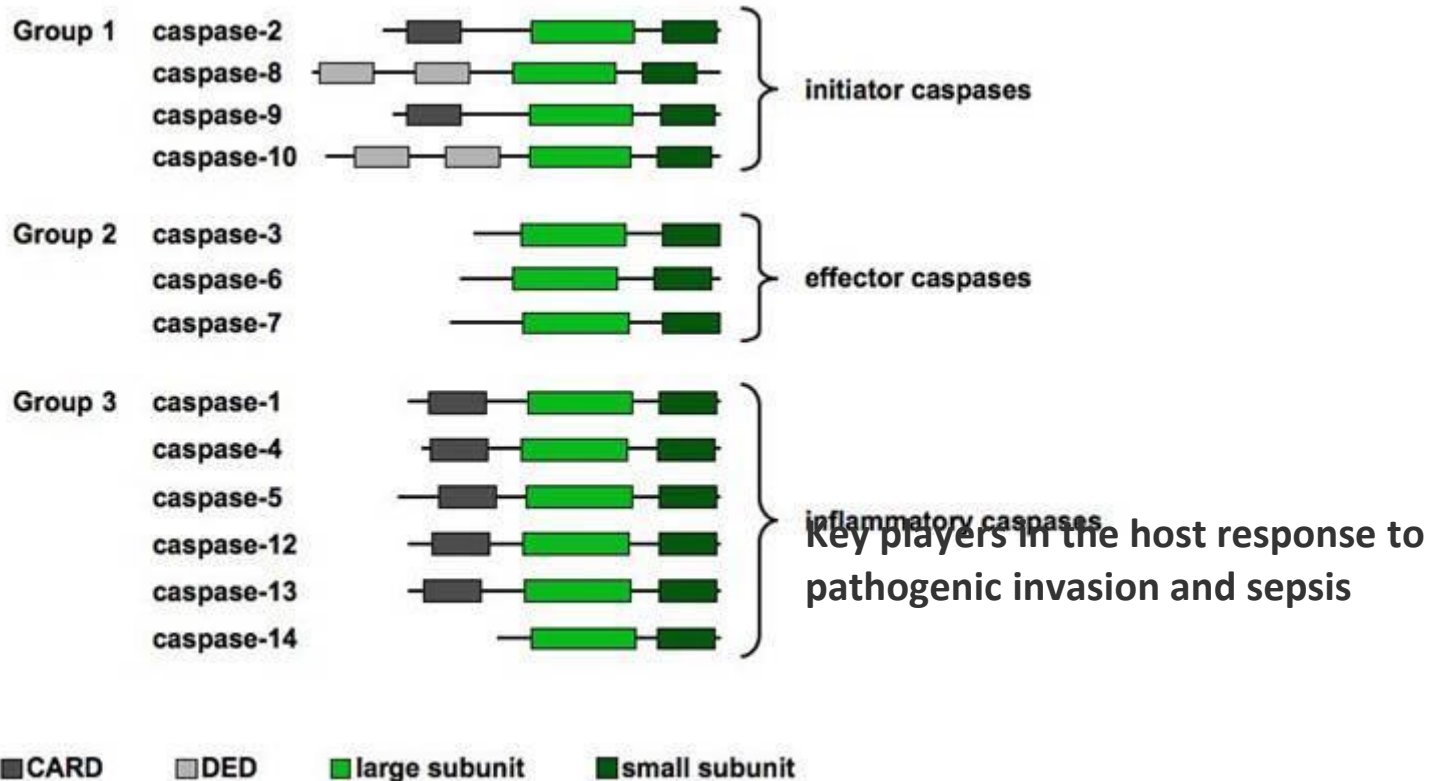
- 1) **Apoptotic signal** triggers **assembly of adaptor proteins**
- 2) **Adaptor complex** triggers **dimerization, cleavage & activation** of the initiator caspase
- 3) **Activated initiator caspase** activates **executioner caspase by cleavage**
- 4) **Activated executioner caspase** cleaves **multiple substrates**, resulting in **cell death**.

The amplifying caspase cascade: the point-of-no-return

A single initiator caspase activates many molecules of executioner caspases



Categories of human caspases



CARD domain: caspase recruiting domain

DED domain: death effector domain

Initiator Caspases recruit adaptor proteins upon stimulations and become activated. Activated initiator caspases cleave each other and cleave effector caspases.

Death receptors trigger the extrinsic pathway of apoptosis

The **Fas** (first apoptosis signal) receptor, **FasR**

Fas receptor:

- belongs to the **TNF** (tumor necrosis factor receptor (**TNFR**) family
- transmembrane proteins
 - extracellular ligand binding domain
 - single transmembrane domain
 - intracellular death domain (to activate the apoptosis program)
 - form homotrimeric receptor complexes

Fas ligands:

- belong to the TNF family of signal proteins
- form homotrimers
- are structurally related to one another

Death receptors trigger the extrinsic pathway of apoptosis

The first apoptosis signal (Fas) receptor, FasR

Fas receptor interactors:

- homotrimeric ligands (extracellular)
- **FAAD** (fas-associated death domain) adaptor proteins

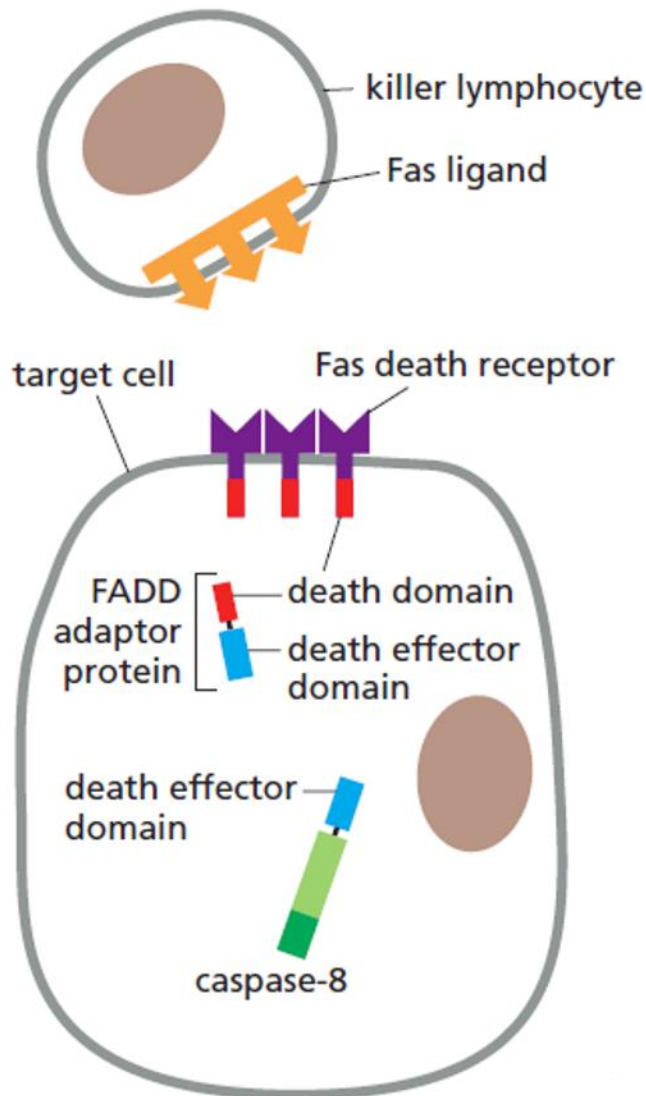
Interactions:

- activated receptors recruit FAAD adaptors
- FADD adaptors recruit an initiator caspase via **DED** (death effector domains):
 - **formation** of the death-inducing signaling complex (**DISC**)
 - **cleavage, activation & release of the initiator caspase**
 - activation of executioner caspases

• .

Death receptors trigger the extrinsic pathway of apoptosis

Fas Ligand (cytotoxic T cells) induced apoptosis

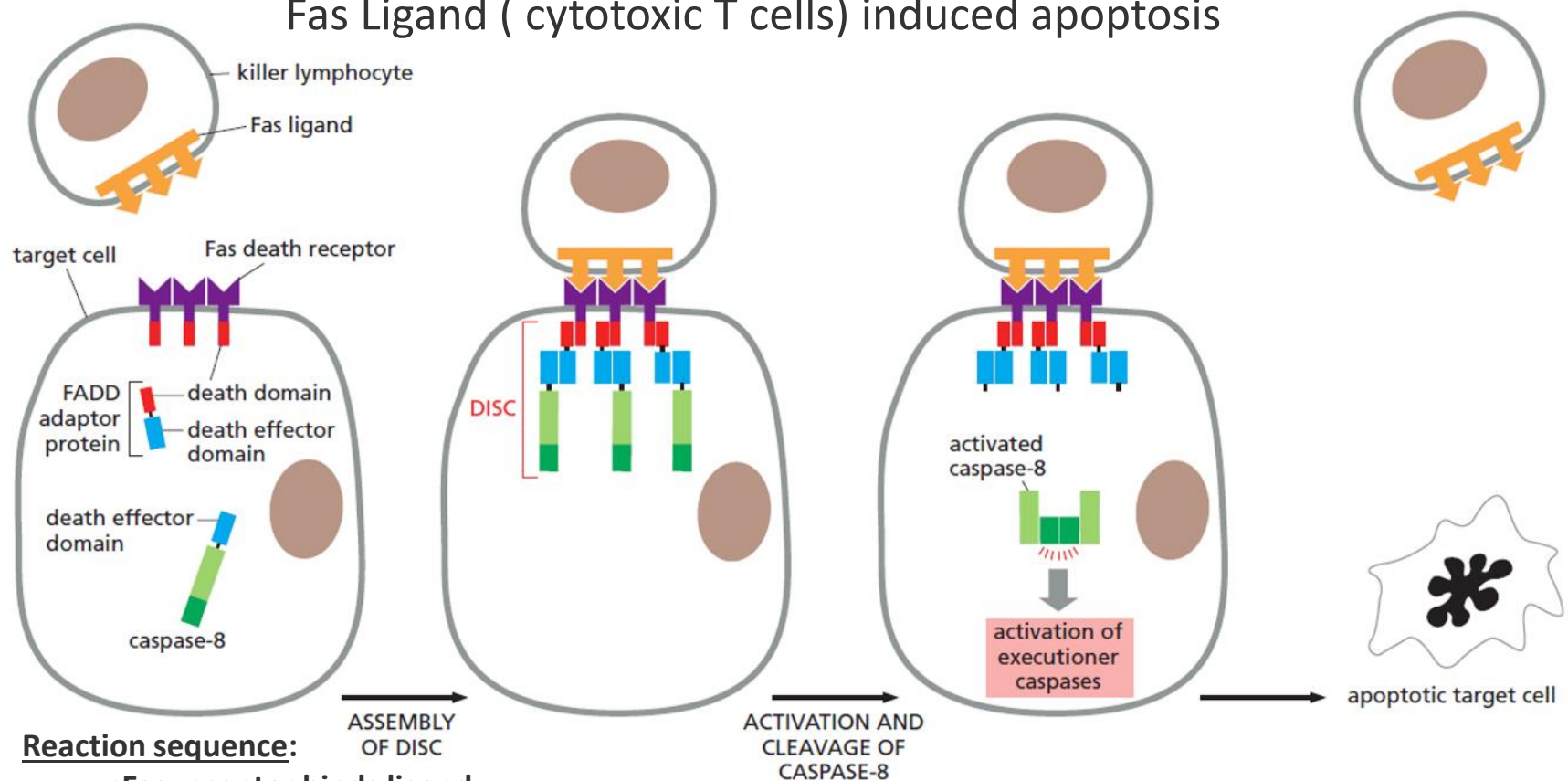


Reaction sequence:

- Fas receptor binds ligand
- recruitment of Fas-associated death domain (FADD) adaptor proteins
- FADD adaptors recruit an initiator caspase via death effector domains (DED)
 - formation of the death-inducing signaling complex (DISC)
 - cleavage, activation & release of the initiator caspase as activated dimers
 - activation of executioner caspases

Death receptors trigger the extrinsic pathway of apoptosis

Fas Ligand (cytotoxic T cells) induced apoptosis



Inhibitory proteins prevent accidental activation

The extrinsic pathway can be restrained by inhibitor proteins:

- **Inhibitor proteins** e.g. FLIP, resemble **initiator caspases** but they have no **protease activity**.
- Inhibitor proteins lack the crucial cysteine in their active site.
 - Inhibitor proteins **dimerize** with caspase-8 in DISC but are not cleaved and activated
 - the signaling chain is interrupted

The intrinsic or mitochondrial apoptosis activation pathway

The intrinsic apoptosis activation pathway:

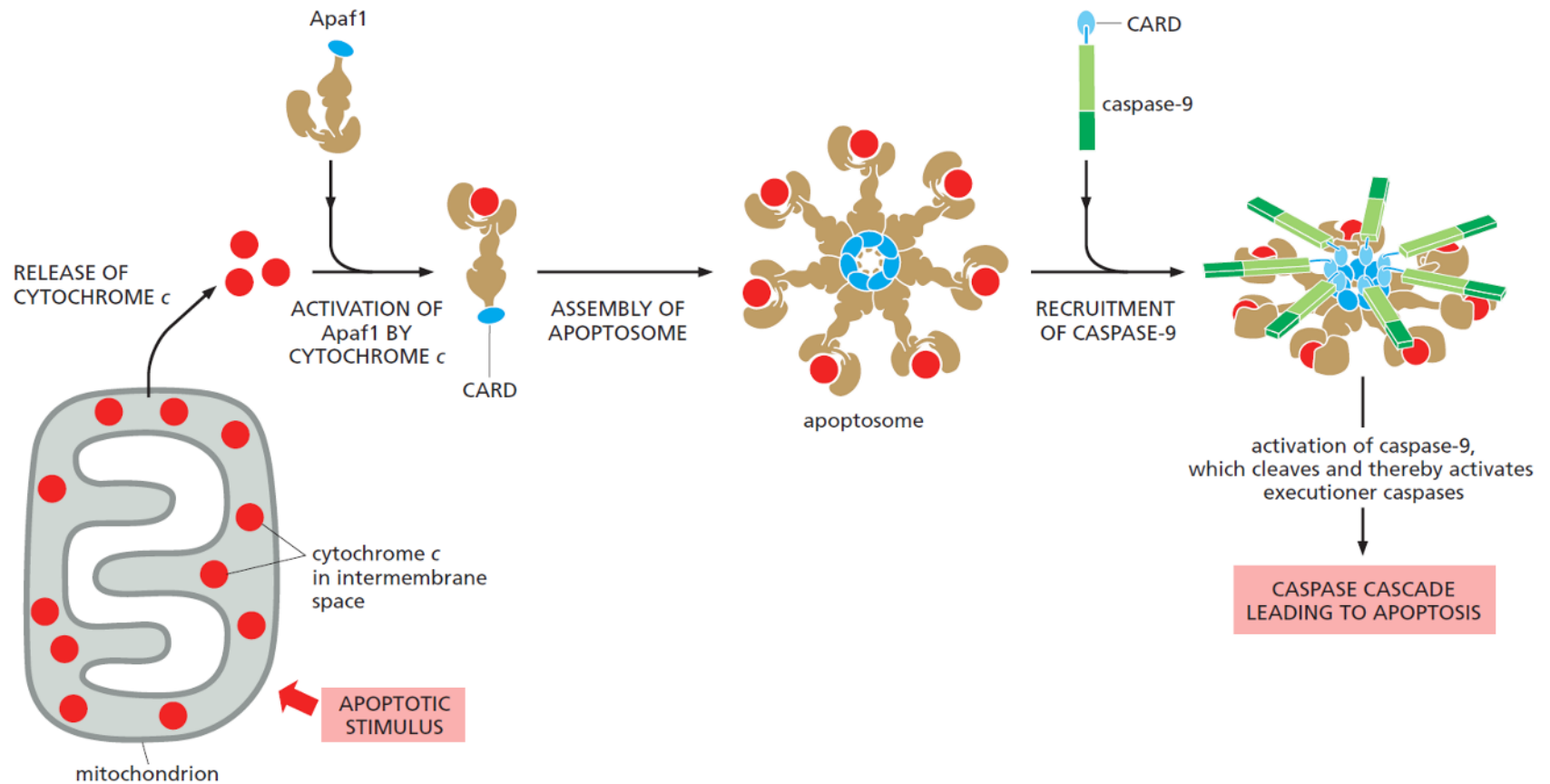
- mediated by **mitochondria**
- triggered by protein release from the mitochondrial intermembrane space (IMS):
 - key player: - **cytochrome c**
(water soluble component of the e^- transport chain)
 - **Apaf1** (apoptotic protease activating factor-1)
(contains a **CARD** (caspase recruitment domain) domain)
 - **caspase-9** initiator caspase
- pathway is triggered in response to:
 - **DNA damage,**
 - **hypoxia,**
 - **lack of nutrients,**
 - **lack of extracellular survival signals**

The intrinsic or mitochondrial apoptosis activation pathway

Interactors & interactions:

- **cytochrome c** binds to **Apaf1** causing the **Apaf1** to **oligomerize**.
- **7 Apaf1** molecules form a wheel-like heptamer called **apoptosome**
- **recruitment of the initiator caspase** caspase-9 into the apoptosome via the CARD domains
 - **cleavage & activation of the initiator caspase caspase-9**
 - **activation** of executioner caspases

The intrinsic activation pathway

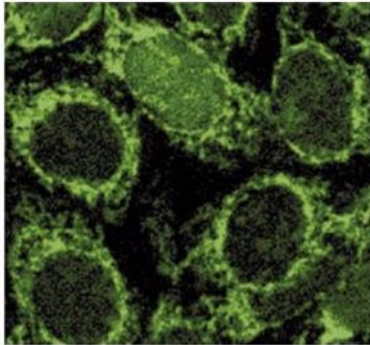


Release of mitochondrial cytochrome c during apoptosis

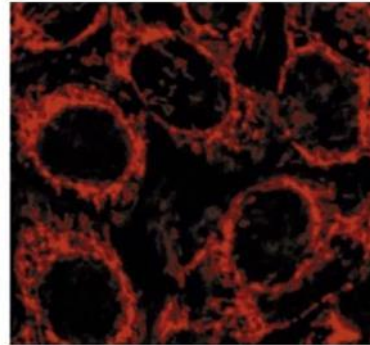
UV-light induced DNA damage triggers release of mitochondrial cytochrome c

(A) CONTROL

cytochrome-c-GFP



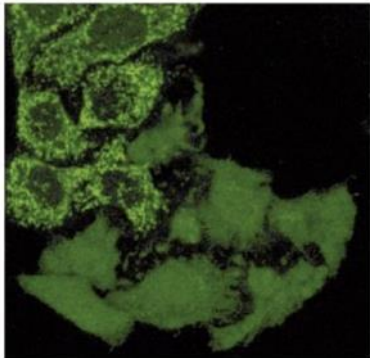
mitochondrial dye



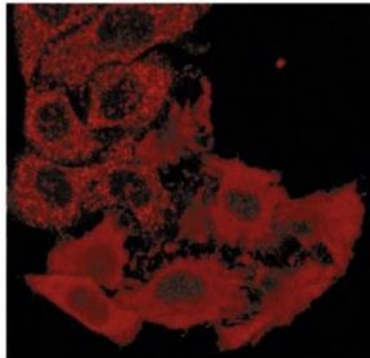
10 μ m

(B) UV TREATED

cytochrome-c-GFP



anti-cytochrome c



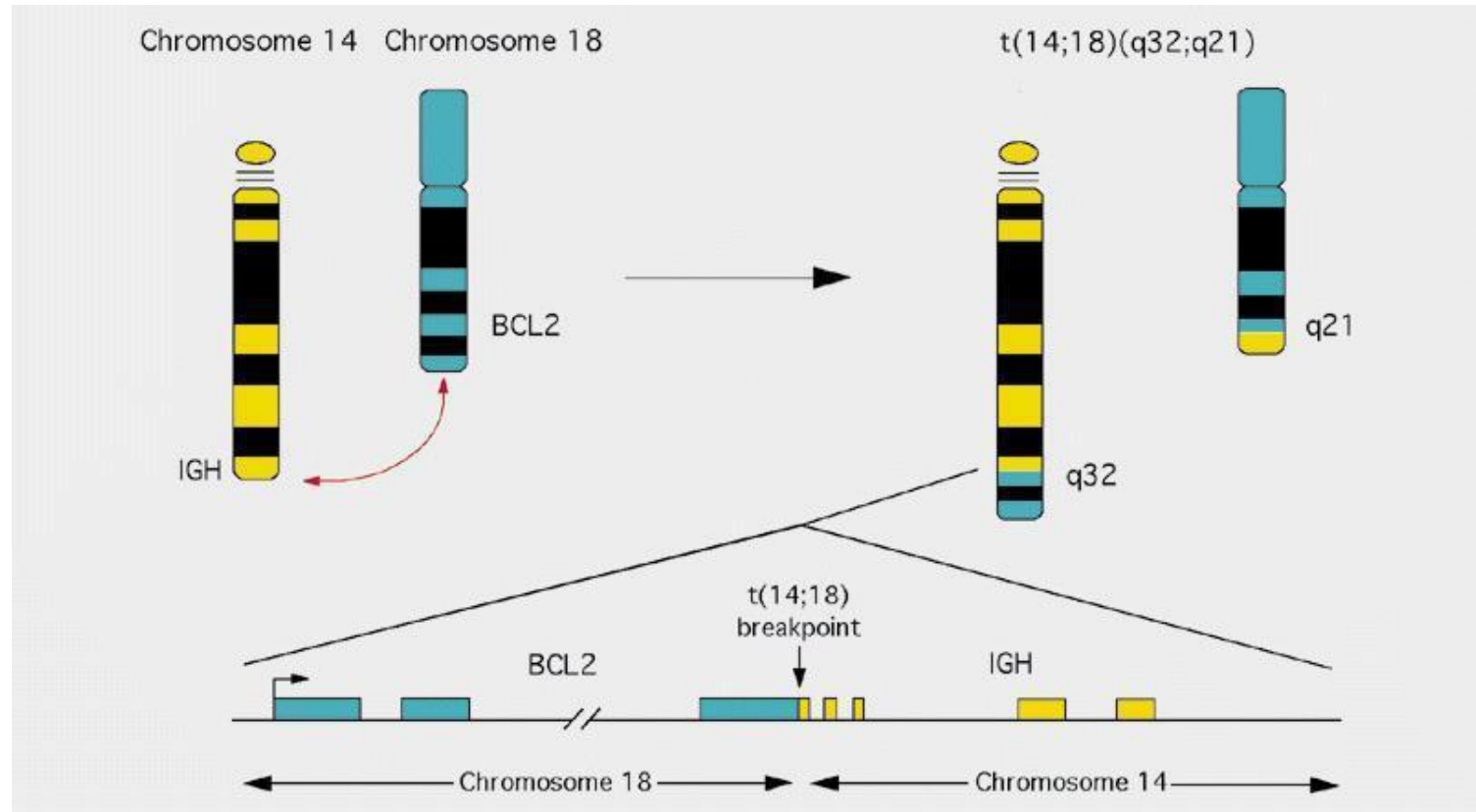
25 μ m

Setup:

- Human cancer cells expressing a mitochondrial cytochrome c -GFP fusion protein (green)
- Mitochondria are labeled with fluorescent dye (red)
- UV-light treatment triggers release of mitochondrial proteins into the cytosol and triggers apoptosis (lower 6 cells, 5h after treatment)

The intrinsic pathway of apoptosis is regulated by Bcl2 proteins

Discovery of Bcl-2 **during chromosome translocation** in follicular lymphoma



**Bcl-2 is under control of IGH promoter
and this results in higher Levels of Bcl-2, which causes cancer.**

The intrinsic pathway of apoptosis is regulated by Bcl2 proteins

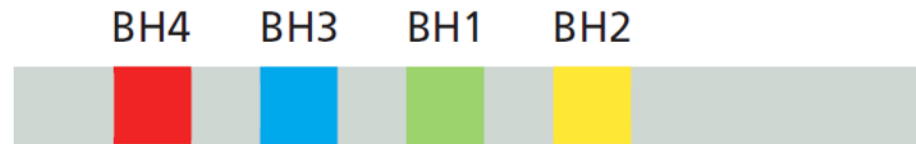
The Bcl2 protein family:

- Bcl2 proteins control the release of cytochrome c and other IMS proteins from the IMS into the cytosol
- Bcl2 proteins are highly conserved
- common feature: BH (**B**cl2 **h**omology) domain
- grouped in anti-apoptotic and pro-apoptotic proteins:
 - **anti-apoptotic: inhibit apoptosis by blocking protein release**
 - **pro-apoptotic: promote apoptosis by enhancing protein release**
 - **Bcl2 proteins can form heterodimers:**
 - **balance** between inhibitory/promoting activities decides about life and death of the cell

The intrinsic pathway of apoptosis is regulated by Bcl2 proteins

- Bcl2 () protein family

anti-apoptotic
Bcl2 family protein
(e.g., Bcl2, BclX_L)



pro-apoptotic
effector Bcl2 family
protein
(e.g., Bax, Bak)

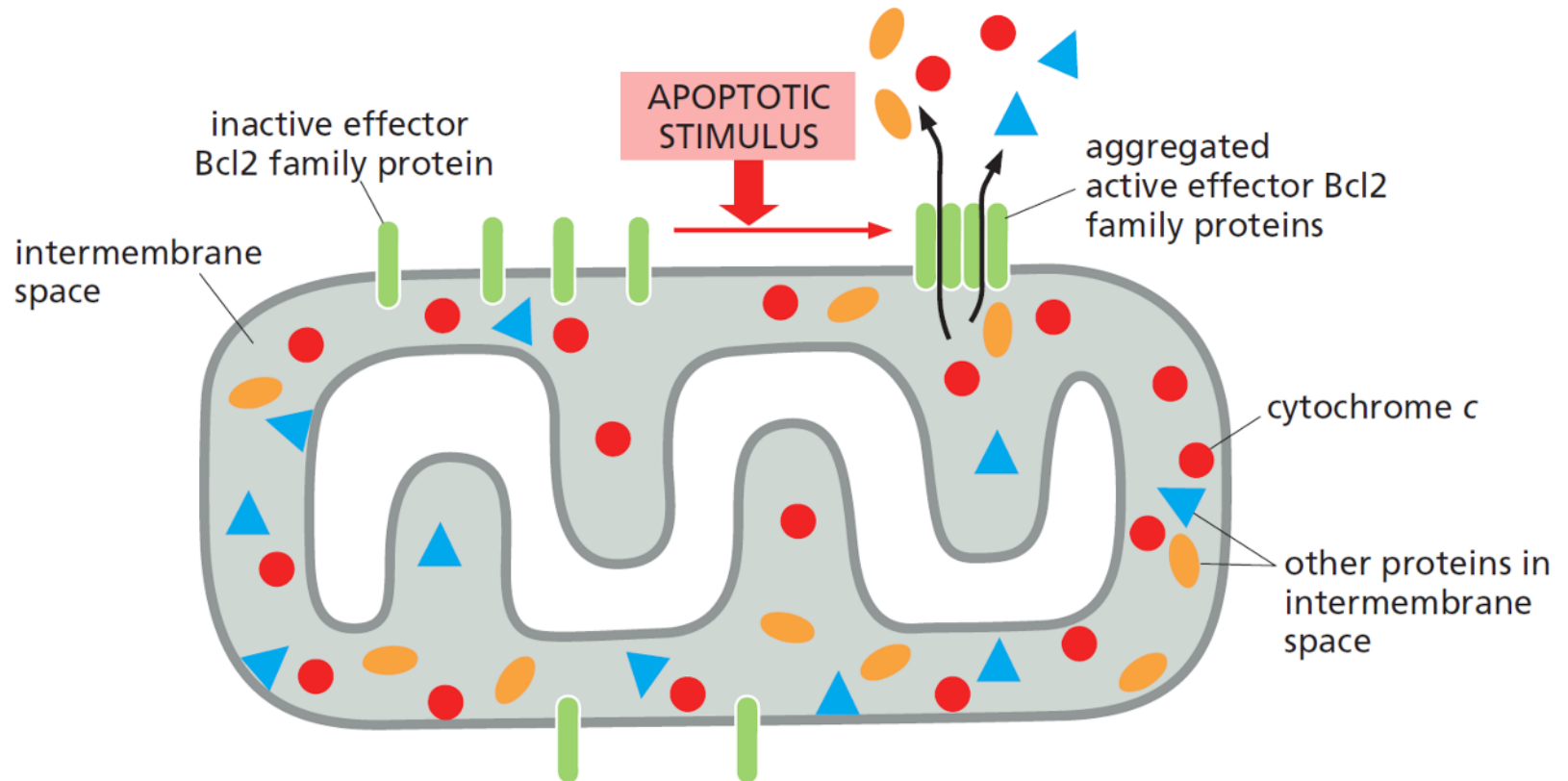


pro-apoptotic
BH3-only protein
(e.g., Bad, Bim,
Bid, Puma, Noxa)



How are cytochrome c and IMS proteins released?

the pro-apoptotic effectors Bax and Bak aggregate in the outer membrane of mitochondria to release cytochrome c and IMS proteins

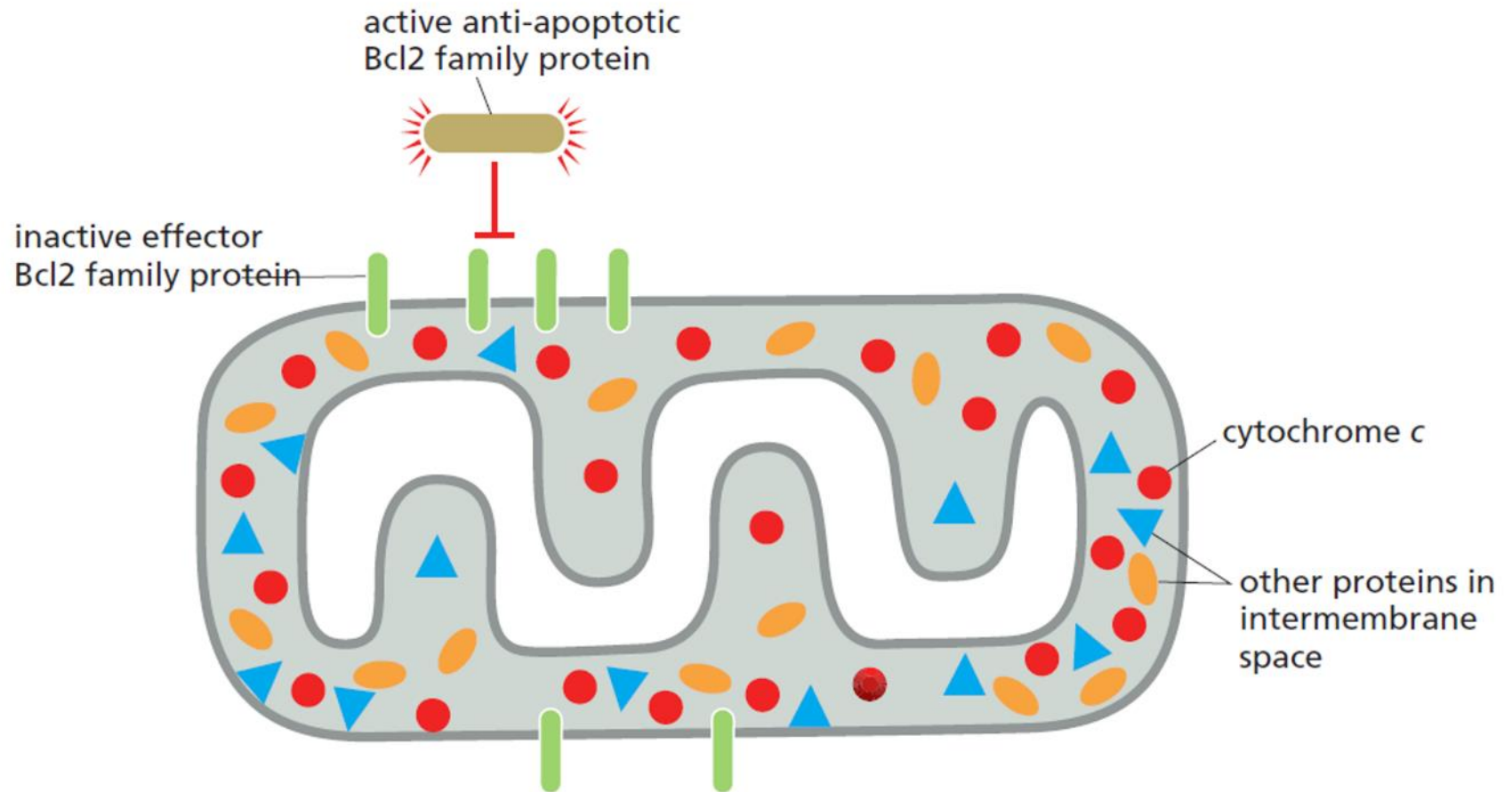


Absence of signal: Bak is mainly in the outer membrane, whilst Bax is cytosolic. Upon signaling: Bax relocates to the outer membrane and interacts with BAK to trigger the efflux from the IMS

Anti-apoptotic Bcl-2 binds and inhibits Bax and Bak aggregation

active anti-apoptotic Bcl2 family proteins prevent aggregation of receptors at the outer mitochondrial membrane

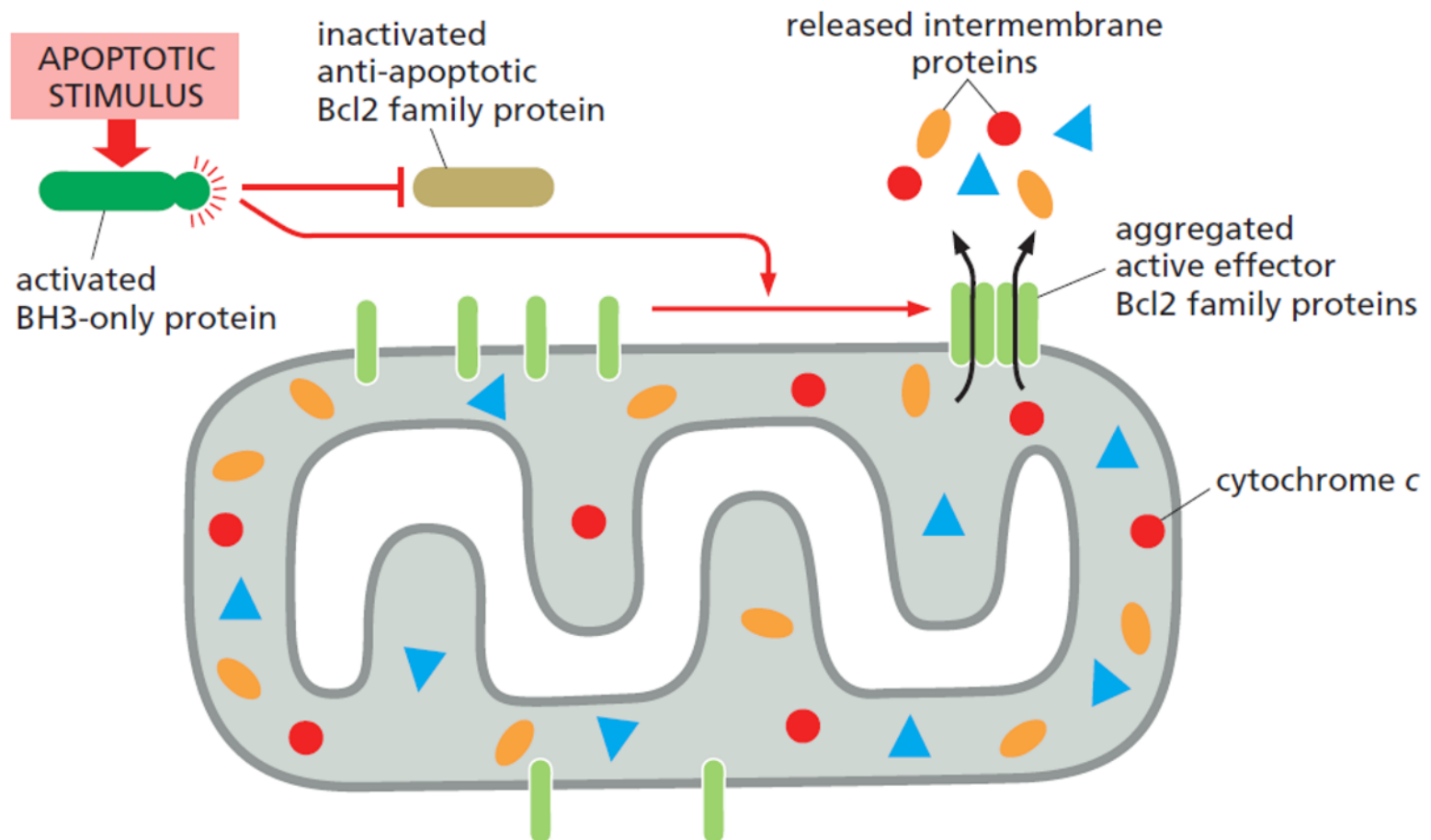
(A) INACTIVE INTRINSIC PATHWAY



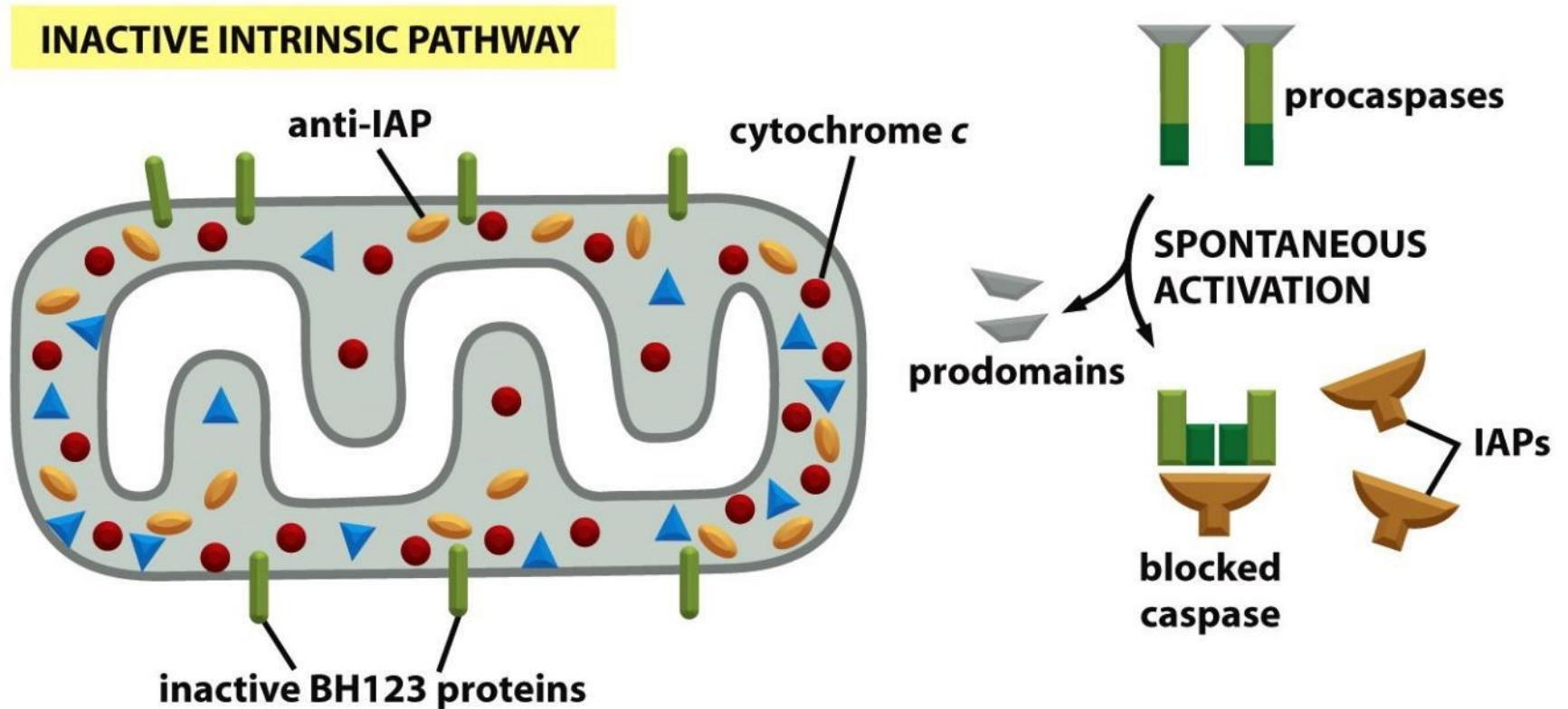
BH3- only proteins inhibit anti-apoptotic Bcl2 family proteins

Inactivation of anti-apoptotic Bcl2 proteins by BH3-only proteins allow for Bak/Bax aggregation and thus for apoptosis

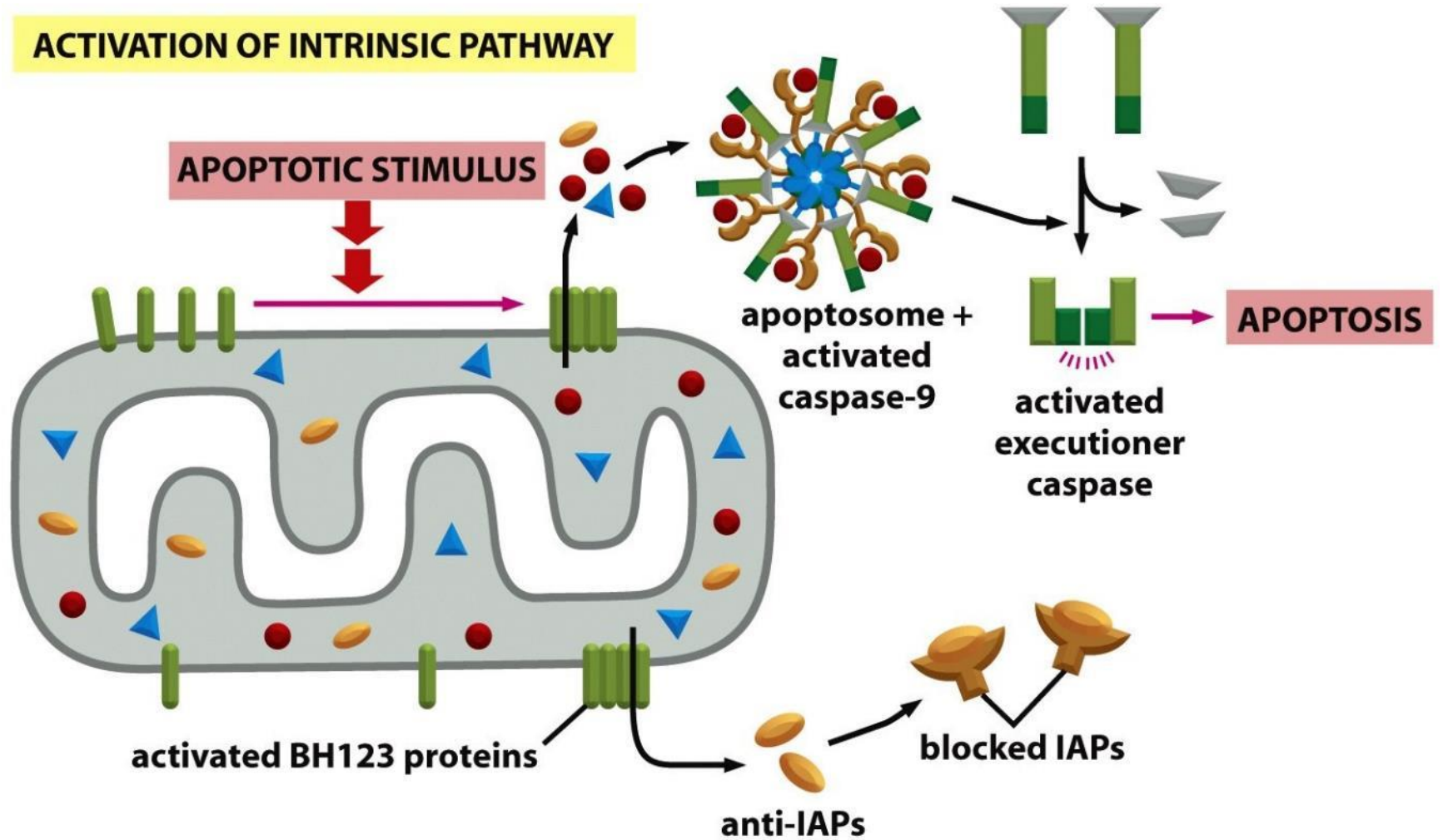
(B) ACTIVATION OF INTRINSIC PATHWAY



IAPs (inhibitors of apoptosis) inhibit caspases

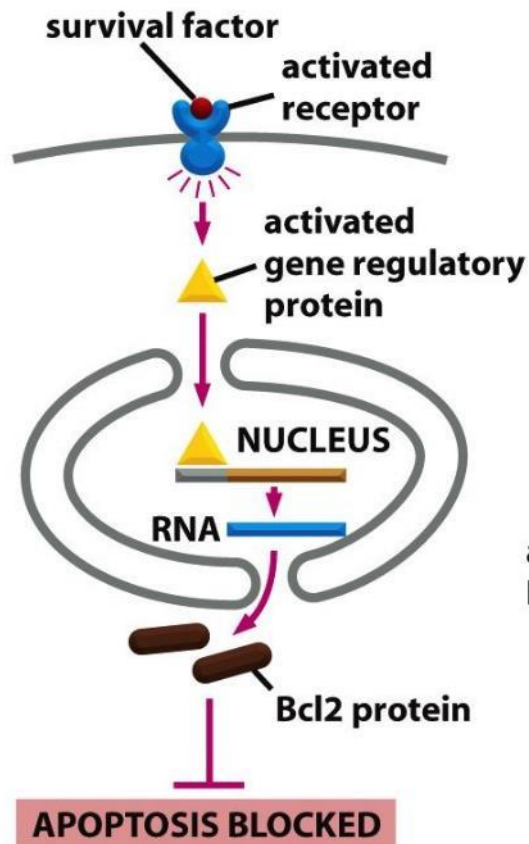


ACTIVATION OF INTRINSIC PATHWAY

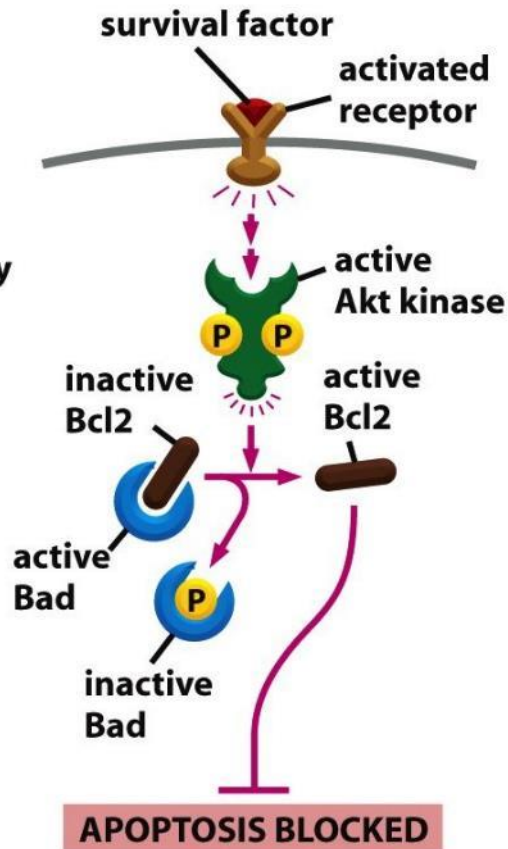


Three ways for survival factors to inhibit apoptosis

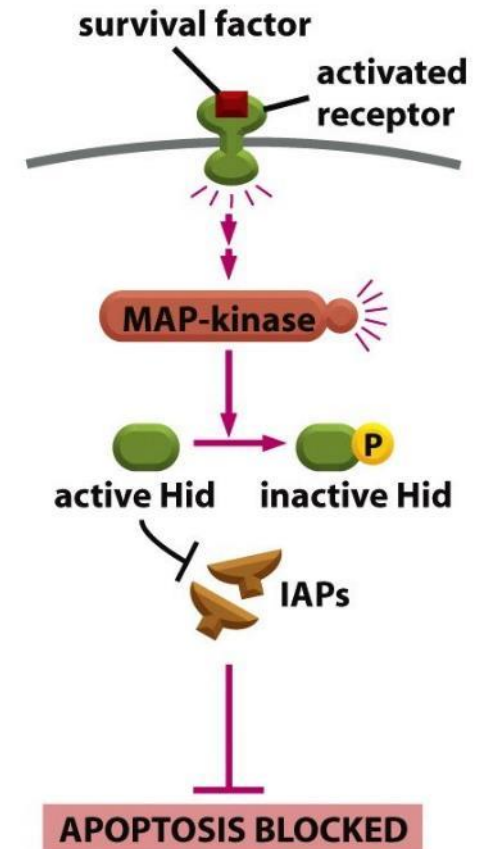
(A) increased production of anti-apoptotic Bcl2 protein



(B) inactivation of pro-apoptotic BH3-only Bcl2 protein



(C) inactivation of anti-IAPs



What we don' t know:

- How many forms of programmed cell death exist? What are the underlying mechanisms and benefits of each?
- Thousands of caspase substrates have been identified. Which ones are the critical proteins that must be cleaved to trigger the major cell remodeling events underlying apoptosis?
- How did the intrinsic pathway of apoptosis evolve, and what is the advantage of having mitochondria play such a central role in regulating apoptosis?
- How are “don' t eat me” signals eliminated or inactivated during apoptosis to allow the cells to be phagocytosed?