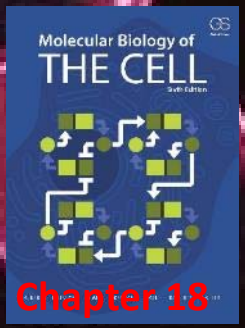
LECTURE 16

Apoptosis

Outline

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

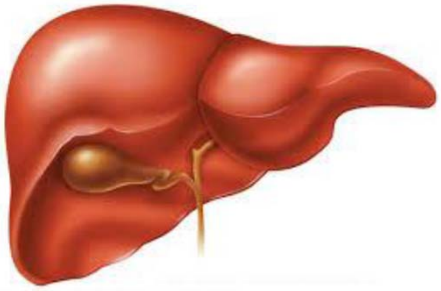


1. Overview of apoptosis (a form of programmed cell death)

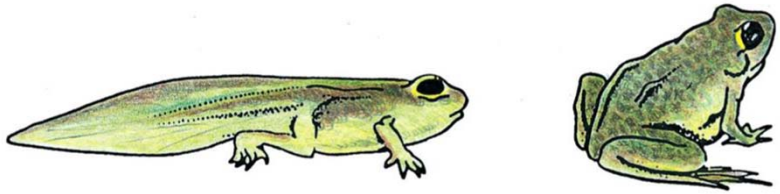
Apoptosis: **eliminates cells** that are abnormal, misplaced, nonfunctional, or potentially dangerous.

It is a natural part of organism development and maintenance.

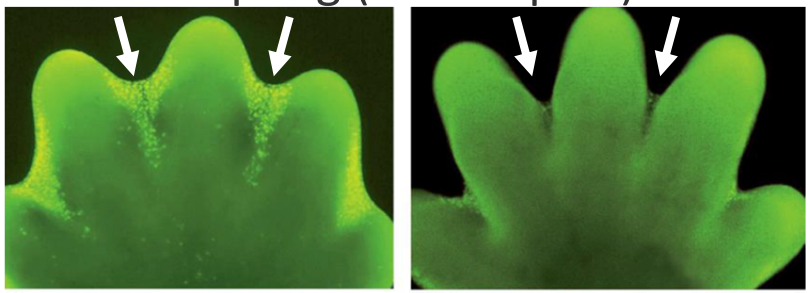
Tissue/organ homeostasis (liver size)



Metamorphosis (tadpole to frog)



Tissue sculpting (mouse paw)

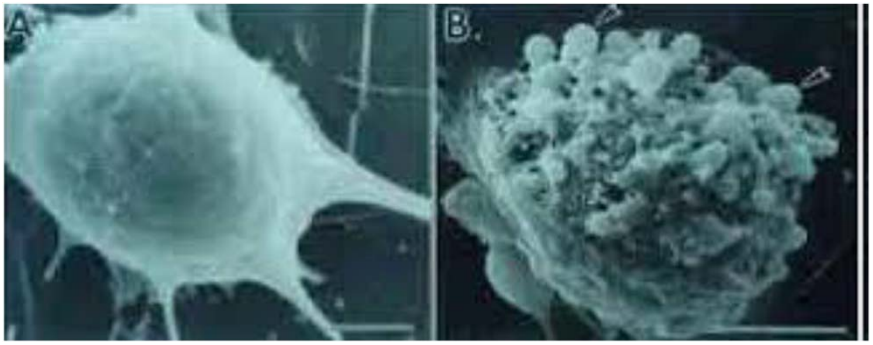


Immune cell apoptosis (short-lived neutrophils)

What is apoptosis?

During apoptosis, cells undergo morphological changes:

Normal cell Apoptotic cell



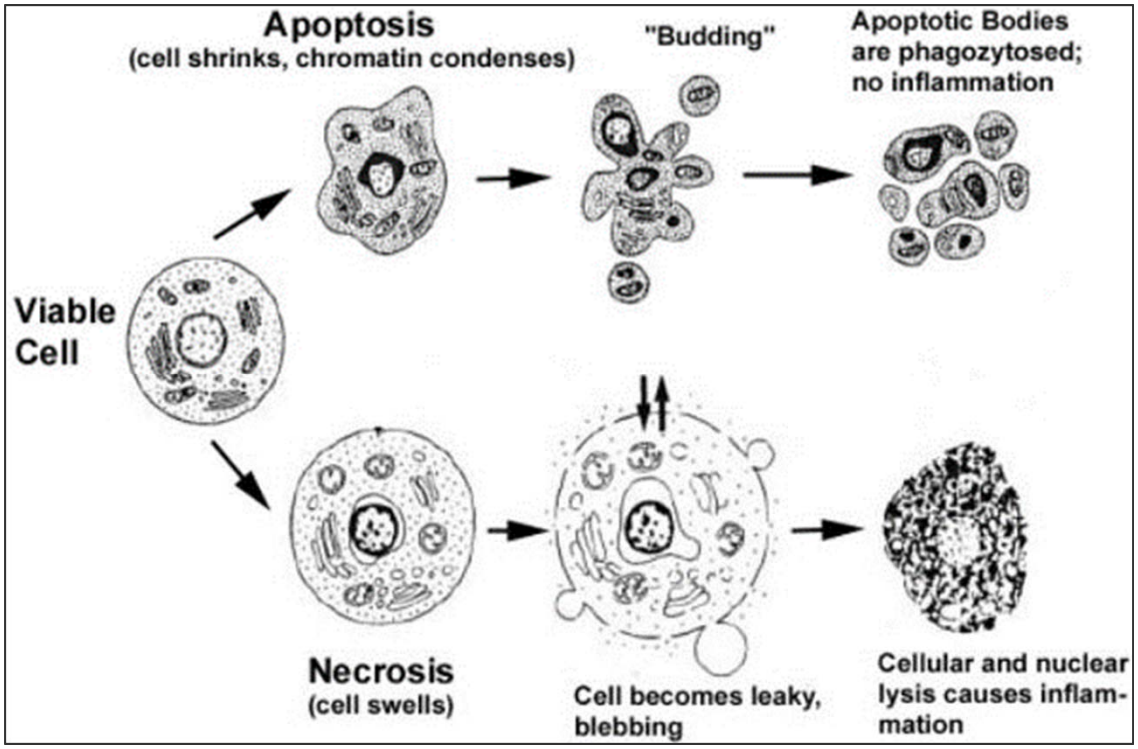
Characteristics of apoptosis :

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)

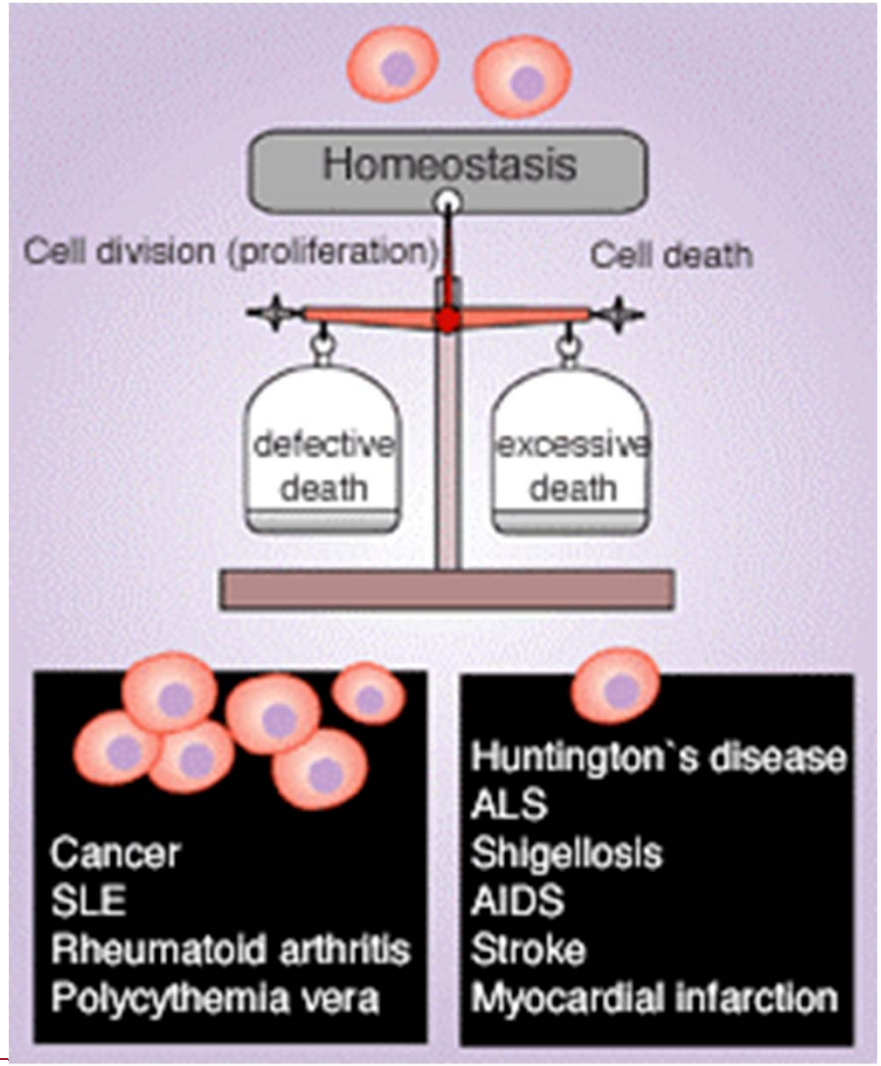
Necrosis: cells swell and burst, content spillage, can cause inflammation, necrosis is usually due to acute insults



Deregulation of apoptosis is involved in diseases

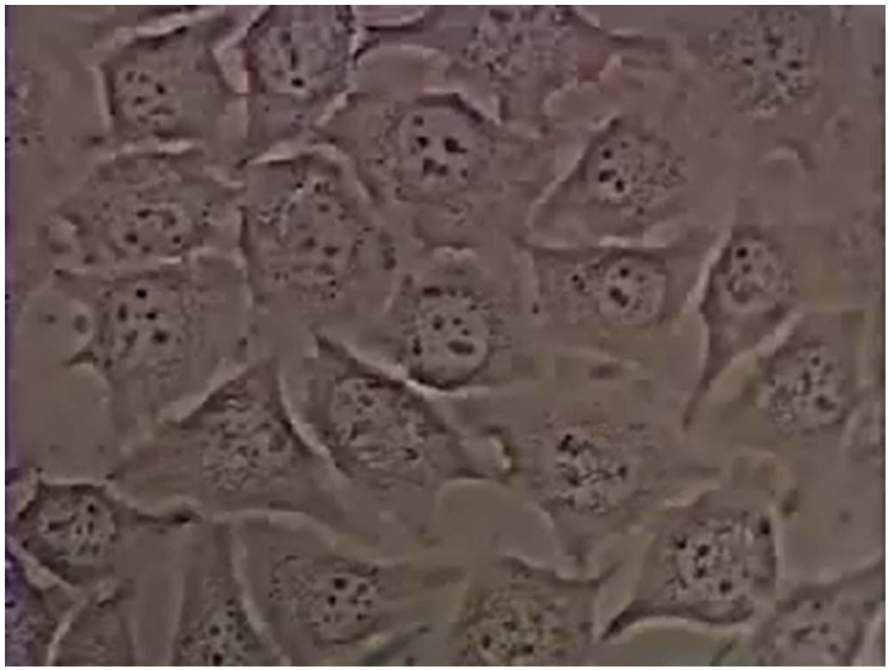
- **High activity of apoptosis**:
  - neurodegenerative disease
  - myocardial infarction
  - radiation injury

- 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

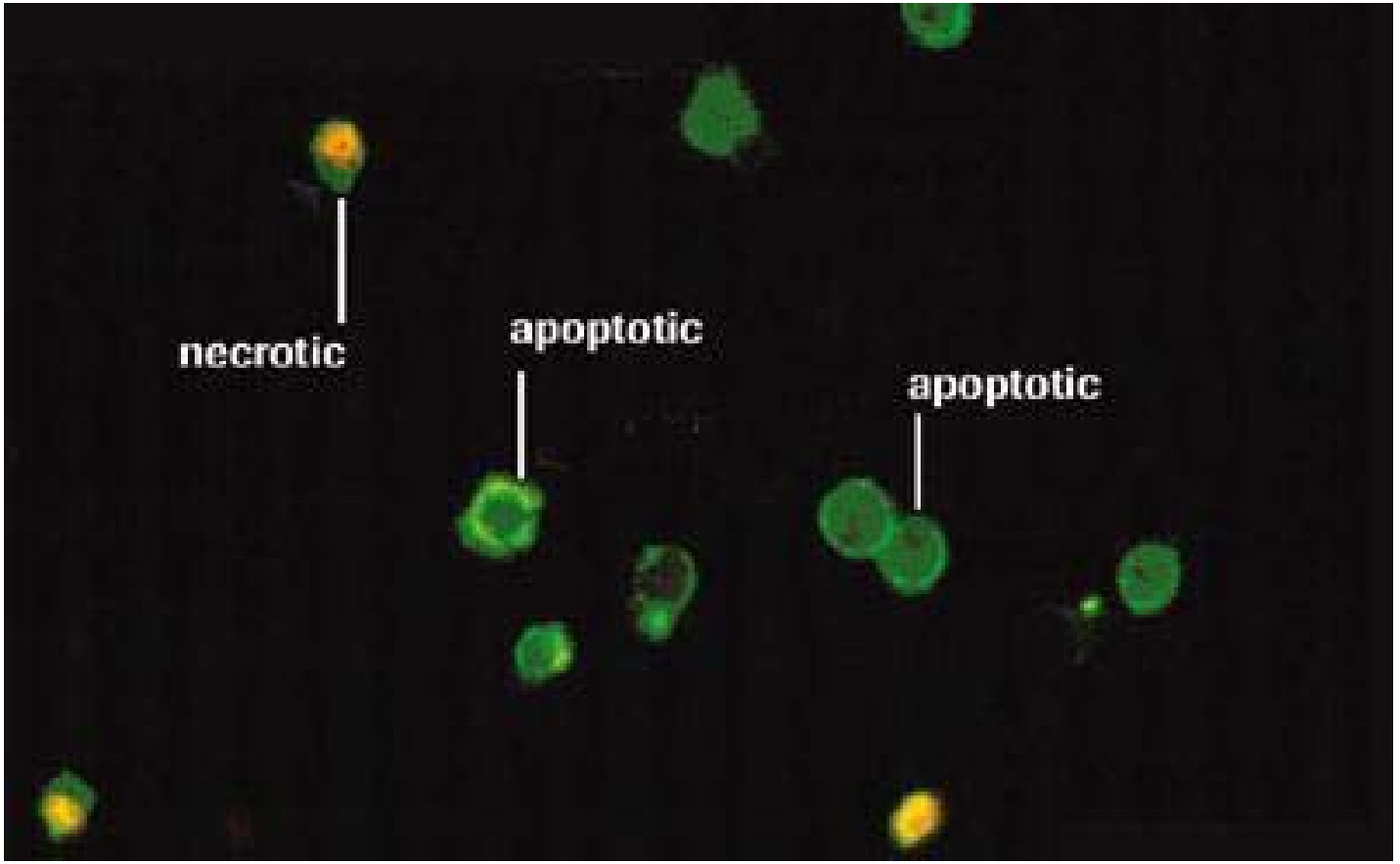
c) DNA fragmentation

d) Tunel assay

e) Western blot for apoptosis markers (Caspase 3, PARP, etc)

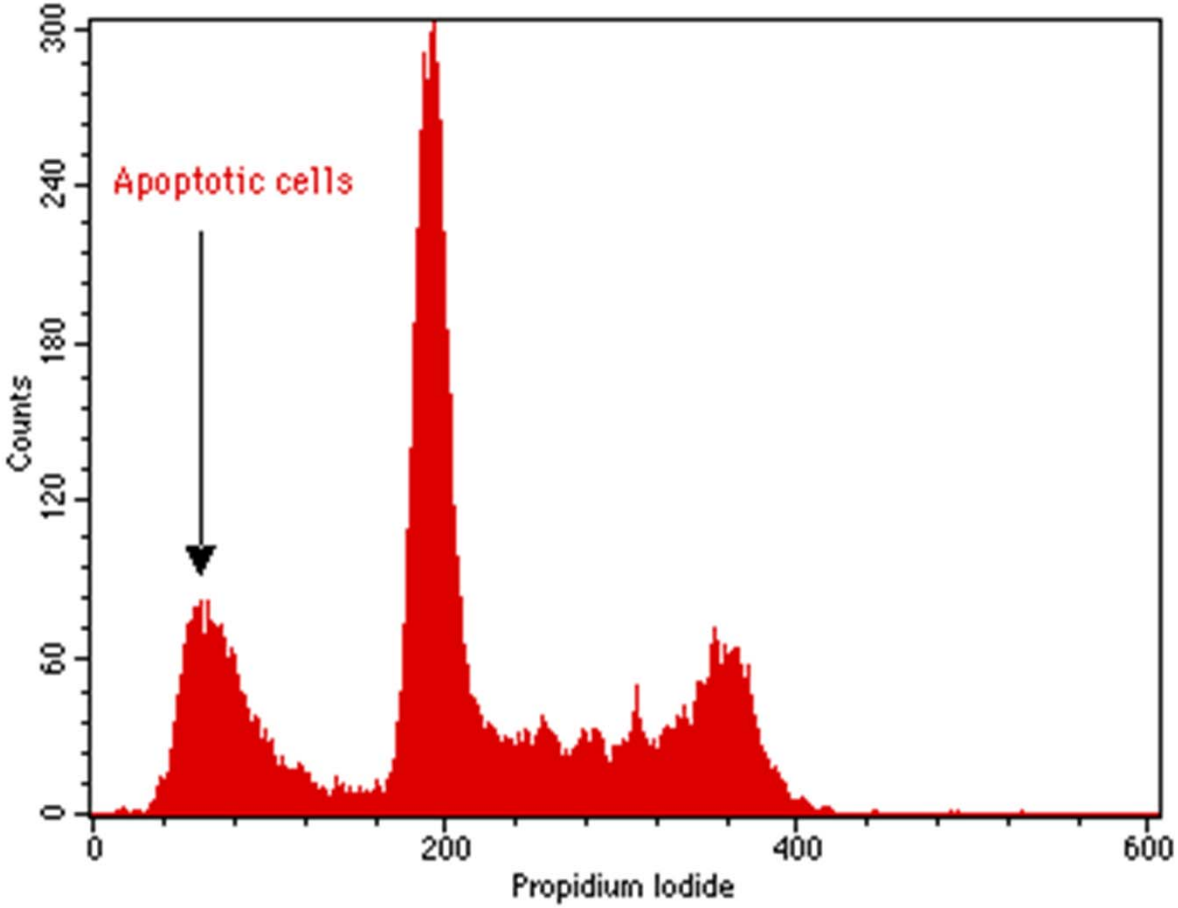
f) Cytochrome c translocation

a) Annexin V staining (early apoptosis)

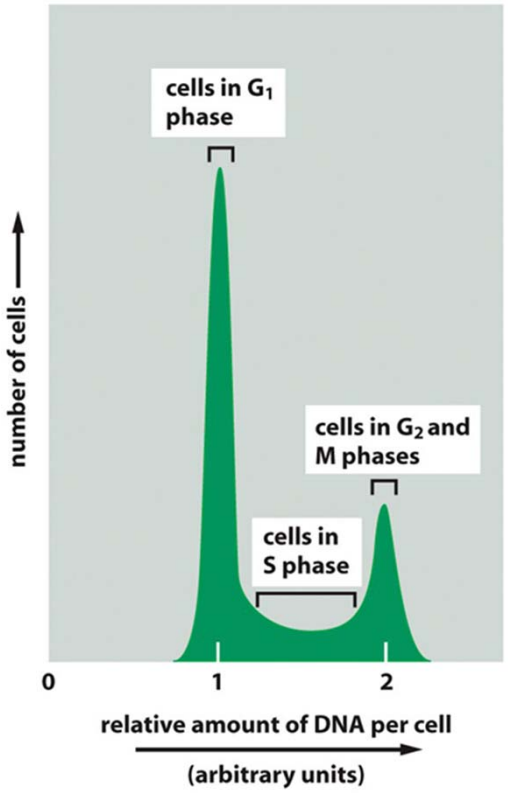


PhosphatidylSerine (PS) is flipped to outer leaflet of plasma membrane and can be detected by annexin V (protein)-conjugate fluorescent dye

b) Cell cycle distribution

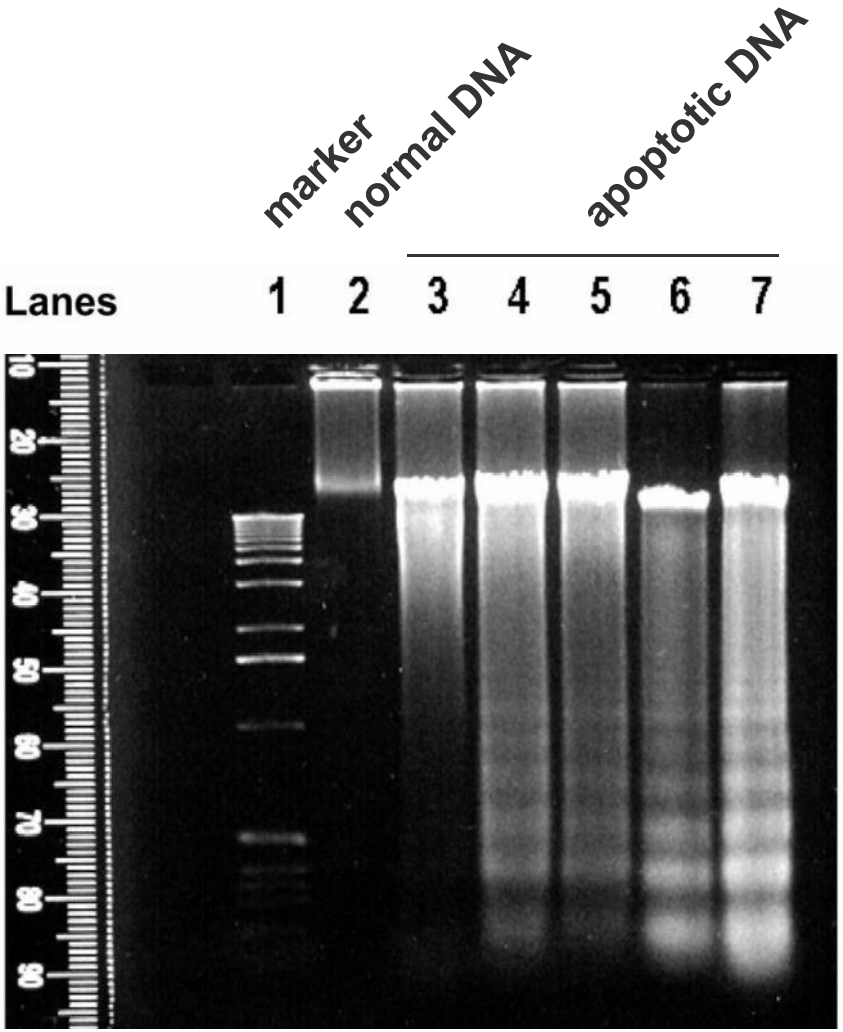


non-apoptotic cells



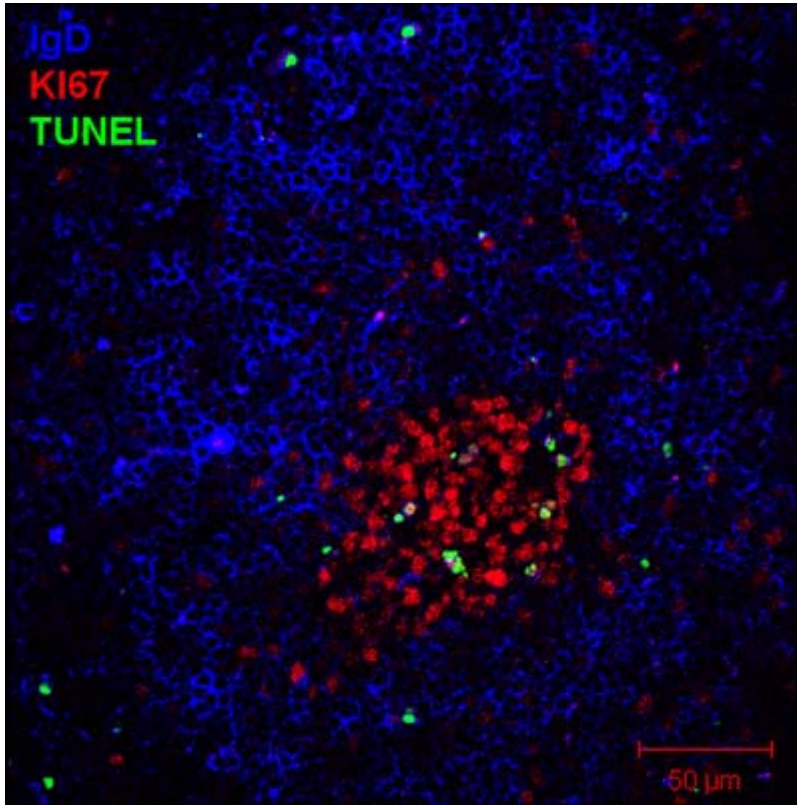
Sub-G1 percentage is an indicator for DNA fragmentation due to apoptosis.

c) DNA fragmentation



d) TUNEL staining -detection of DNA fragmentation (late apoptosis)

Terminal d**U**TP **N**ick **E**nd **L**abelling (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**



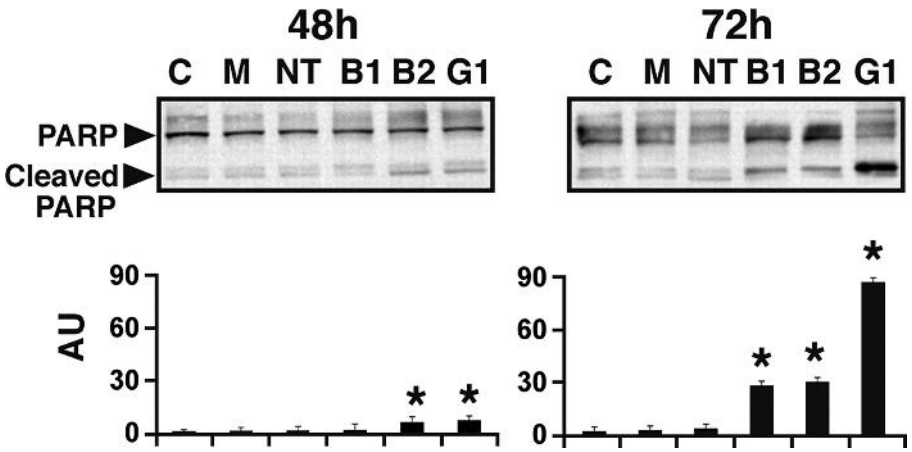
Spleen from NP-KLH immunized mice stained with:

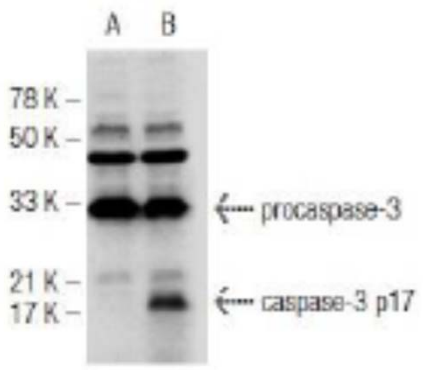
- anti-IgD (blue)
  → B cell follicle
- anti-KI67 (red)
  → germinal center B cells
- TUNEL (green)
  → counter-selected B cells that dye in the germinal center by apoptosis
  (positive for TUNEL stain)

e) Western blotting for apoptotic markers

Commonly used proteins: **cleaved PARP**

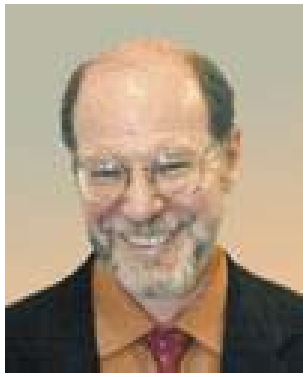
cleaved caspase-3



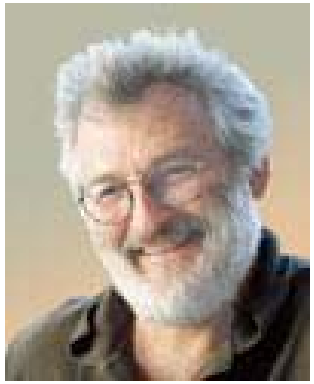


3. Brief History - leading to the Nobel Prize in 2002

Sydney Brenner



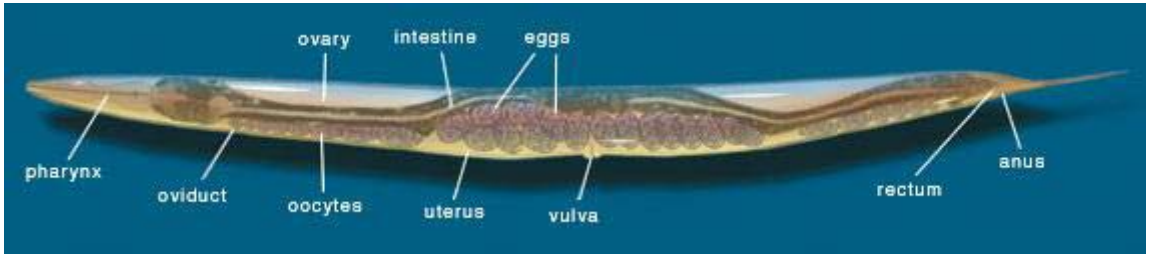
Robert Horvitz



John Sulston

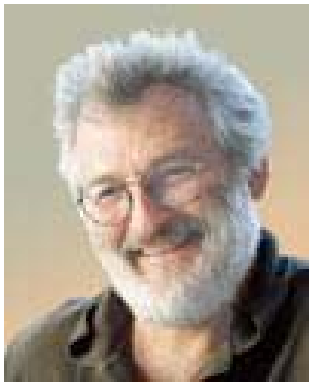
C. elegans as new model organism to analyze organ development

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

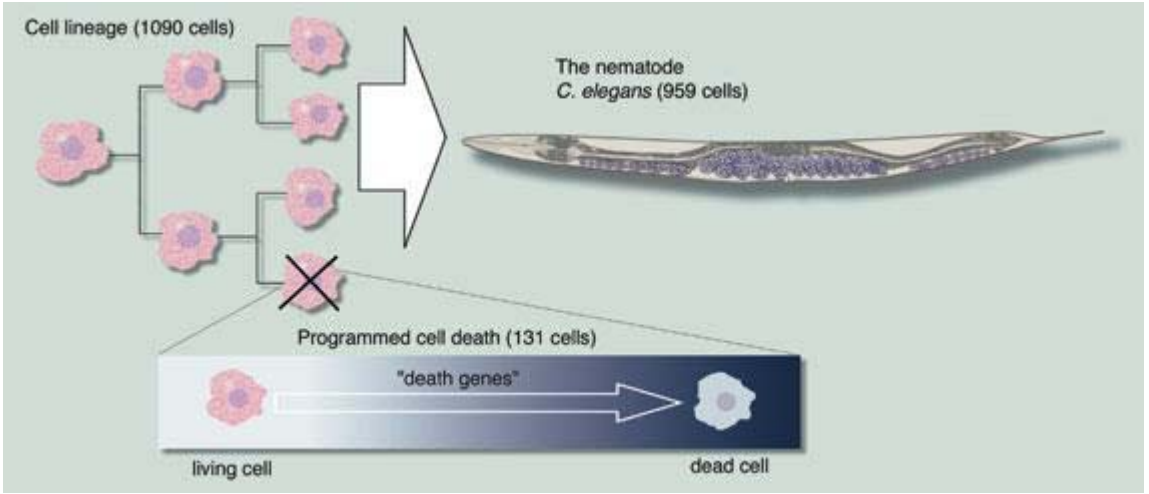


Brenner established *C. elegans* as a novel experimental model organism.
This provided a unique opportunity to link genetic analysis to cell division, differentiation and organ development – and to follow these processes under the microscope.

Cell death is part of the normal differentiation process



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



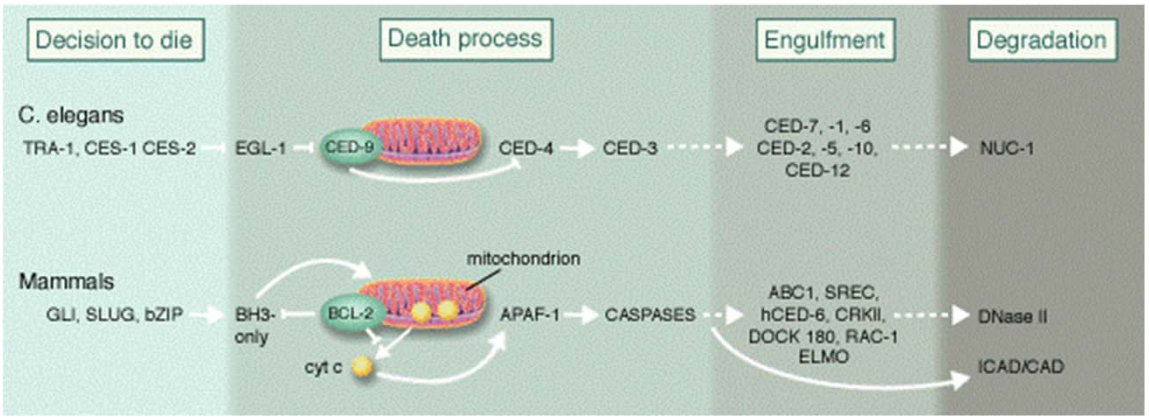
Sulston mapped a cell lineage where every cell division and differentiation could be followed in the development of a tissue in *C. elegans*.

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.*

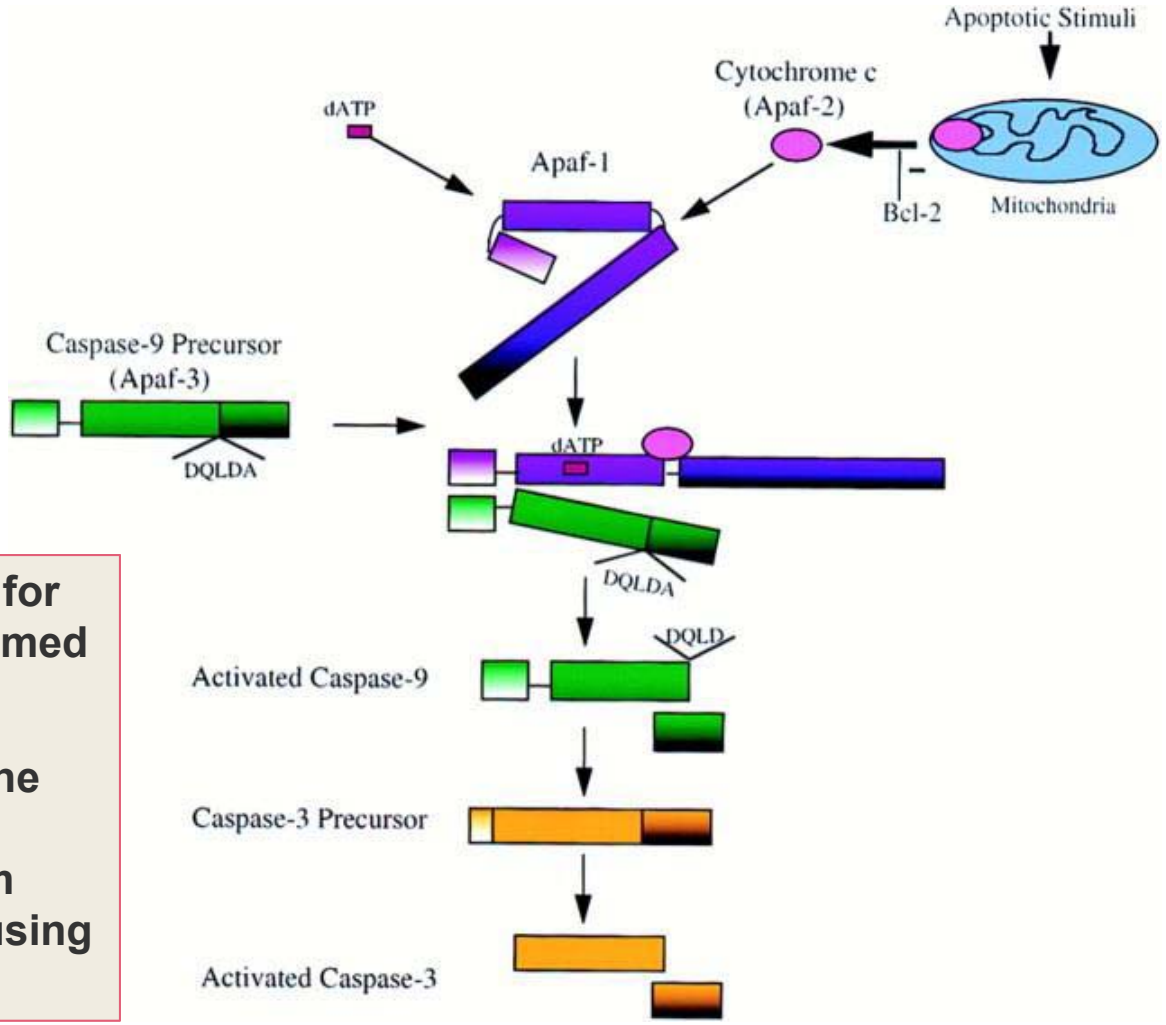
He has shown how these genes interact with each other in the cell death process and that corresponding genes exist in humans.

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:

- **Cas**pases have a **c**ysteine in their active site and cleave their target proteins at specific **asp**artic acids (→ **casp**ase)

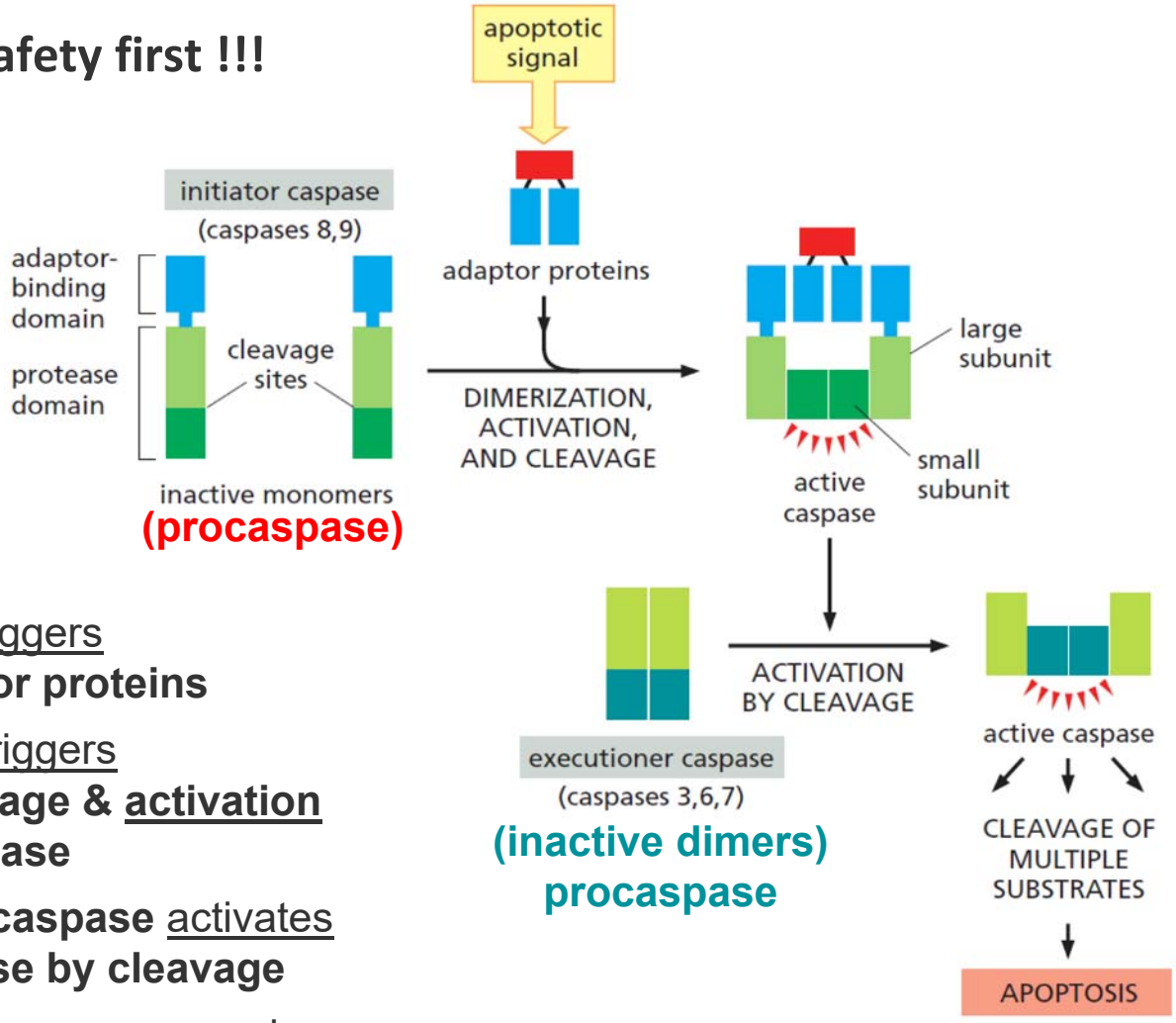
- Caspases are zymogens (synthesized as inactive precursors), →**procaspase**

- **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 !!!

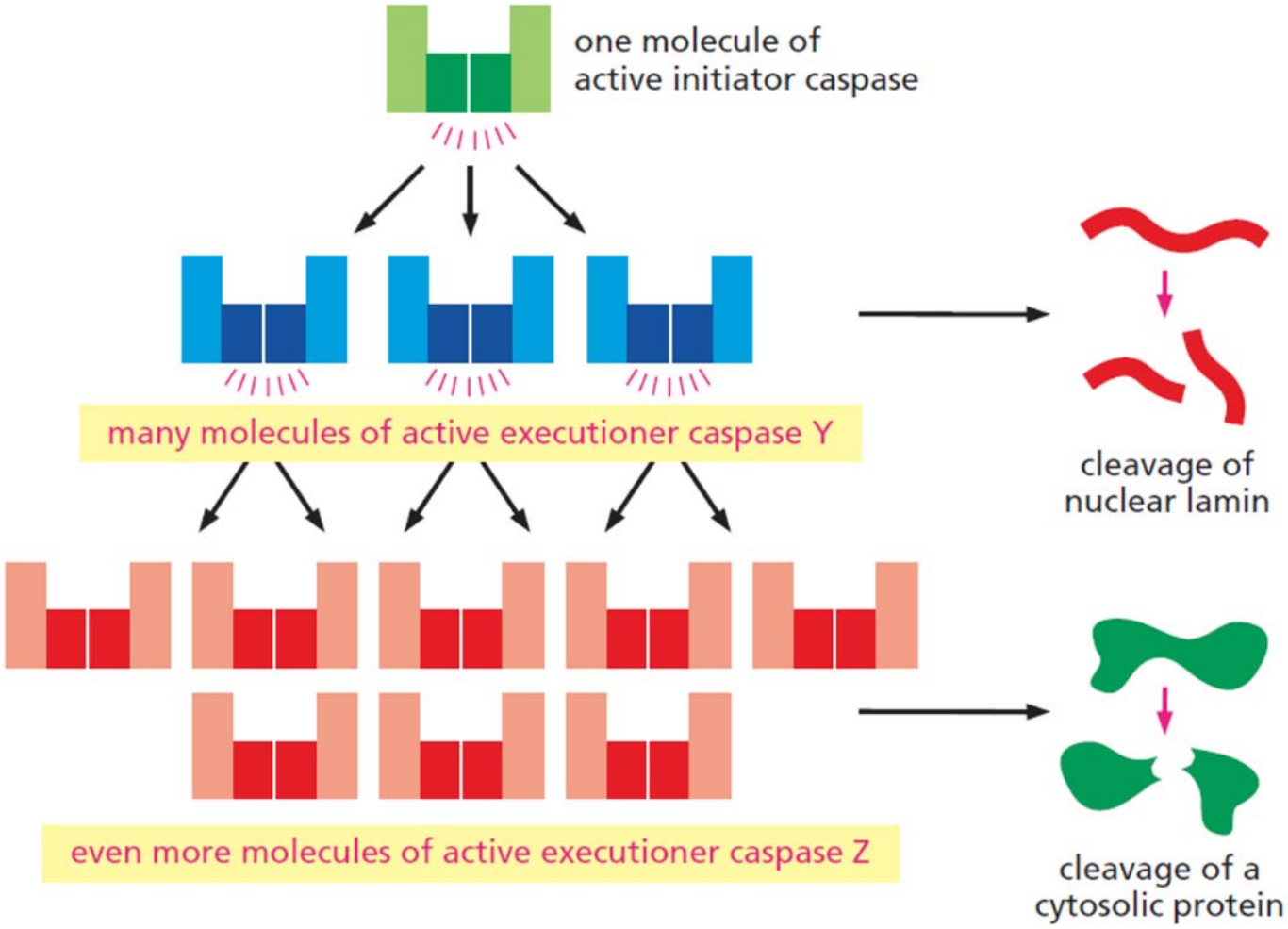


<u>Activation cascade</u>:

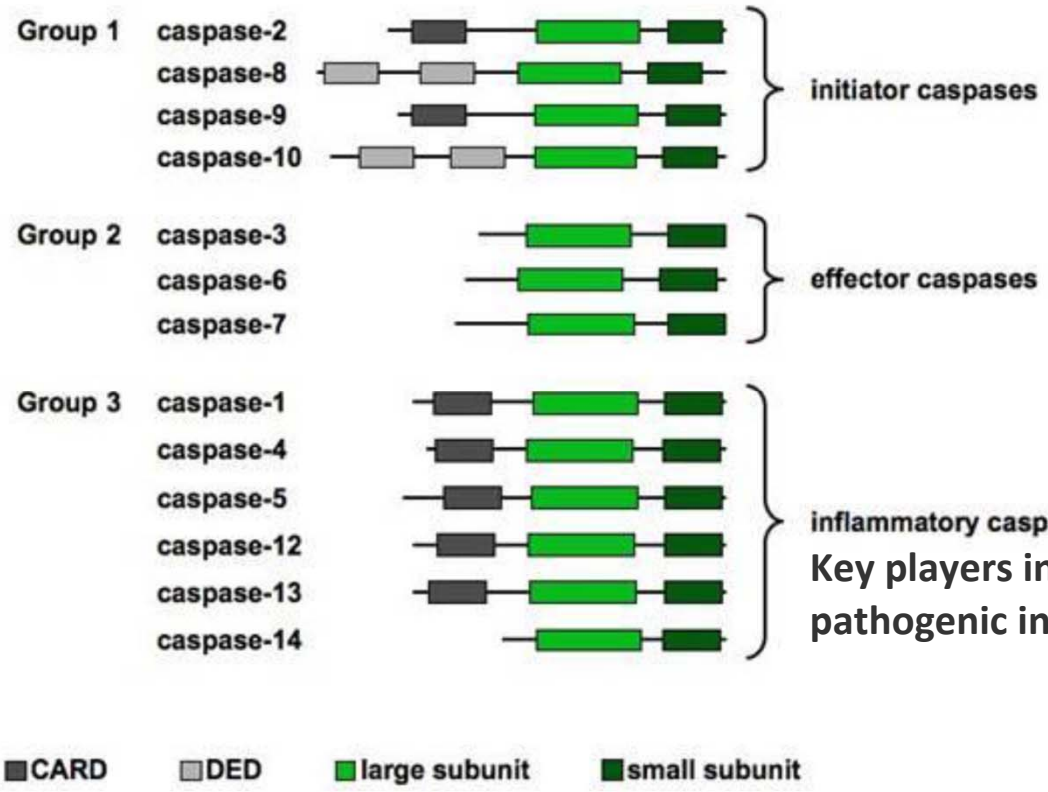
1) **Apoptotic signal** <u>triggers</u> **assembly of adaptor proteins**
2) **Adaptor complex** <u>triggers</u> **dimerization, cleavage &** **<u>activation</u>** of the **initiator caspase**
3) **Activated initiator caspase** <u>activates</u> **executioner caspase by cleavage**
4) **Activated executioner caspase** <u>cleaves</u> **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



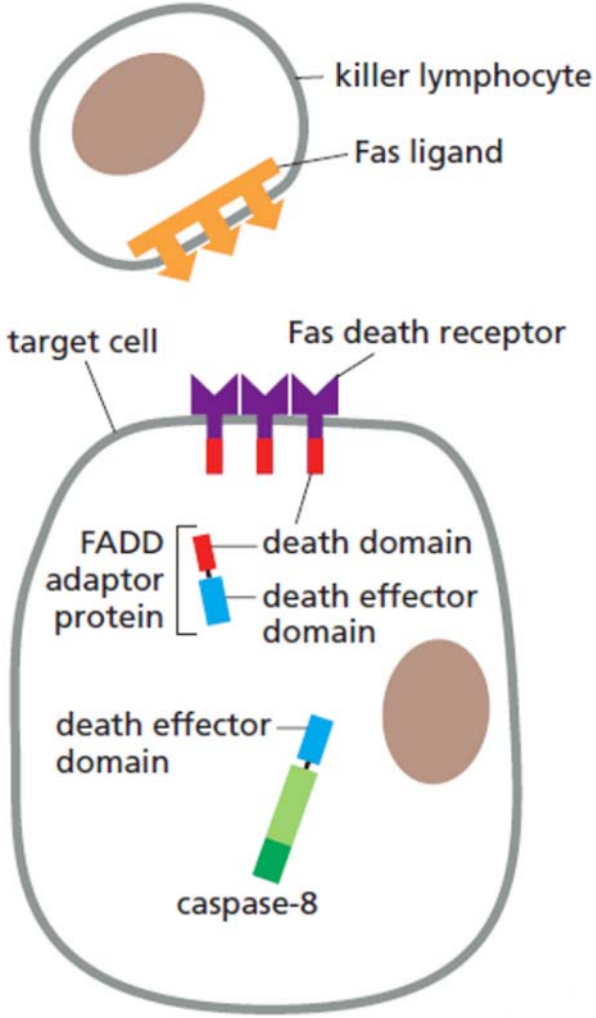
Key players in the host response to pathogenic invasion and sepsis

CARD domain: caspase **r**ecruiting **d**omain
DED domain: death **e**ffector **d**omain

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

Death receptor-triggered extrinsic pathway of apoptosis

Fas (first apoptosis signal) ligand (cytotoxic T cells)-**induced apoptosis**



Fas (first apoptosis signal) receptors (FasR):

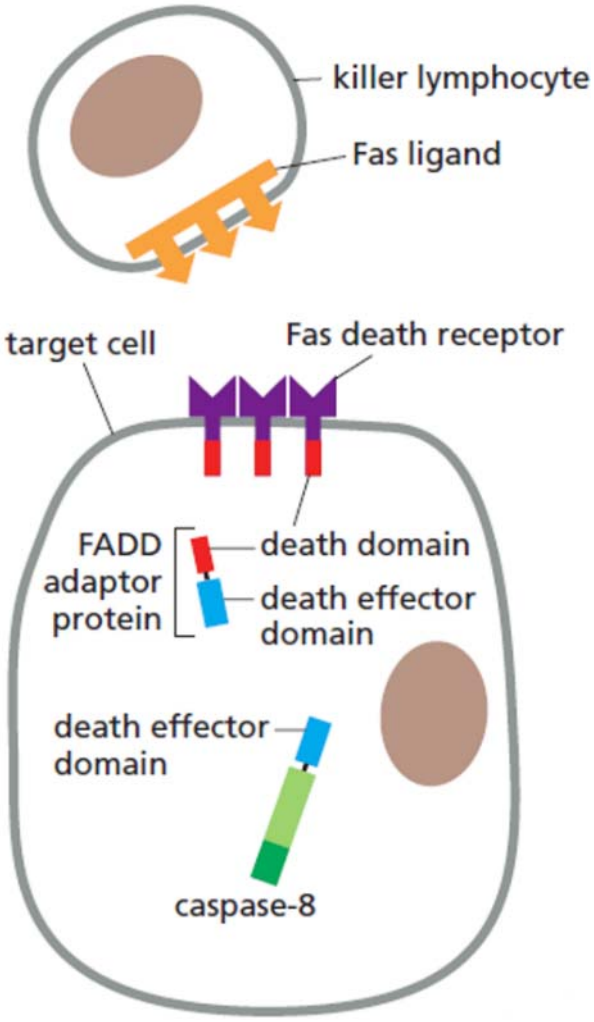
- belongs to the **TNF** (tumor necrosis factor) receptor (**TNFR**) family
- transmembrane proteins
  - extracellular ligand binding domain
  - single transmembrane domain
  - intracellular death domain (to activate the apoptosis program)
  - form homo**trimeric** receptor complexes

Fas receptor interactors:

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

Death receptors trigger the extrinsic pathway of apoptosis

Fas (first apoptosis signal) ligand (cytotoxic T cells)-induced apoptosis



Fas ligands:

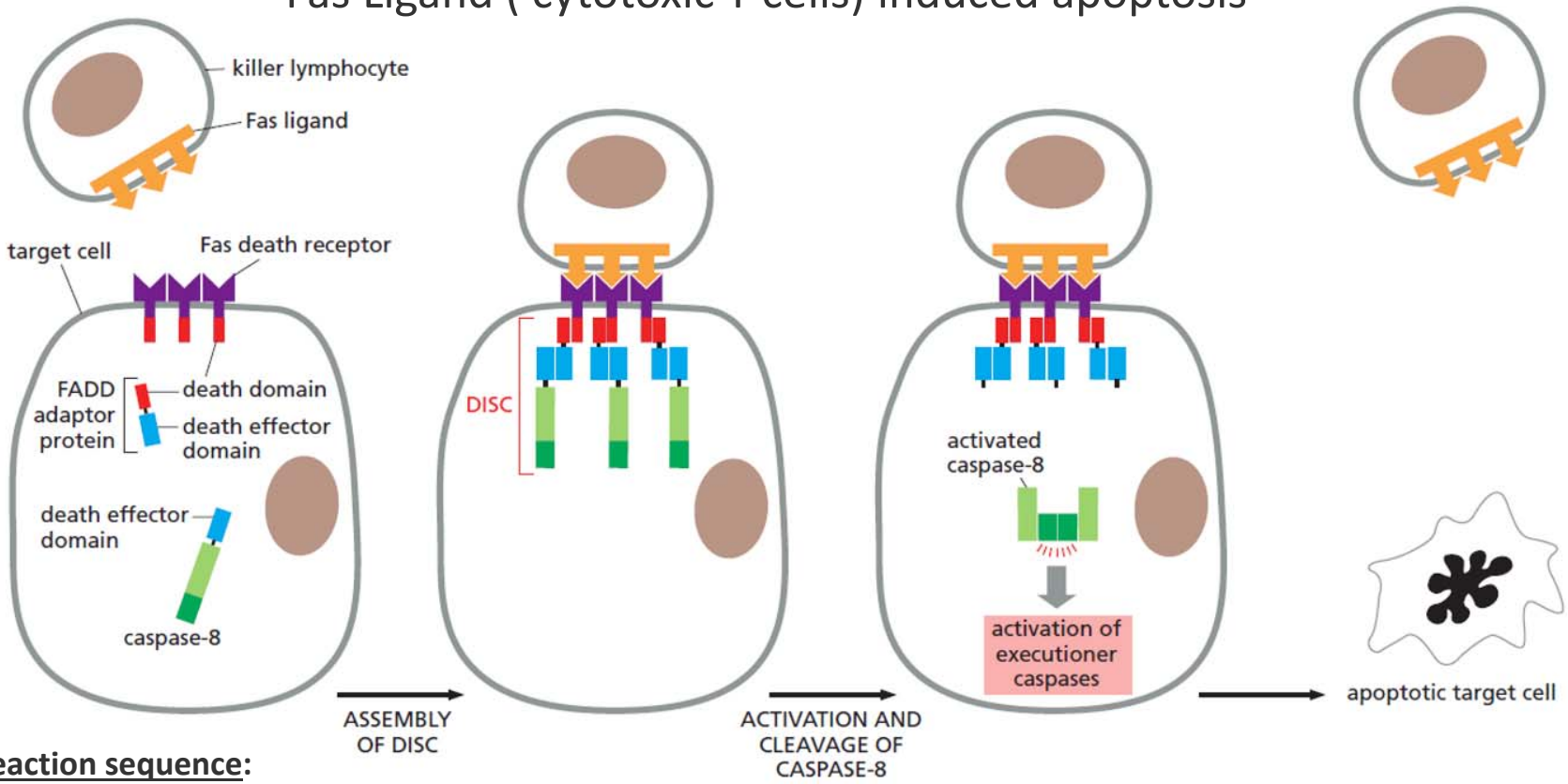
- belong to the TNF family of signal proteins
- form homo**trimers**
- are structurally related to one another

Interactions:

- **activated receptors** recruit **FAAD adaptors**
- **FADD adaptors** recruit an **initiator caspase via DED (death effector domains)**:
  - ➢ **formation** of the **d**eath-**i**nducing **s**ignaling **c**omplex (**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**

Inhibitory proteins prevent accidental activation

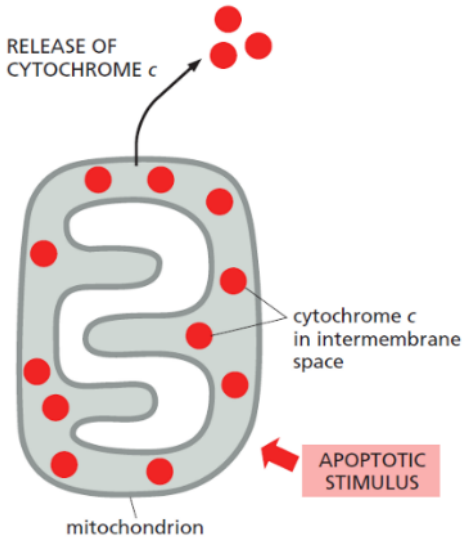
The extrinsic pathway can be restrained by inhibitor proteins:

- **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



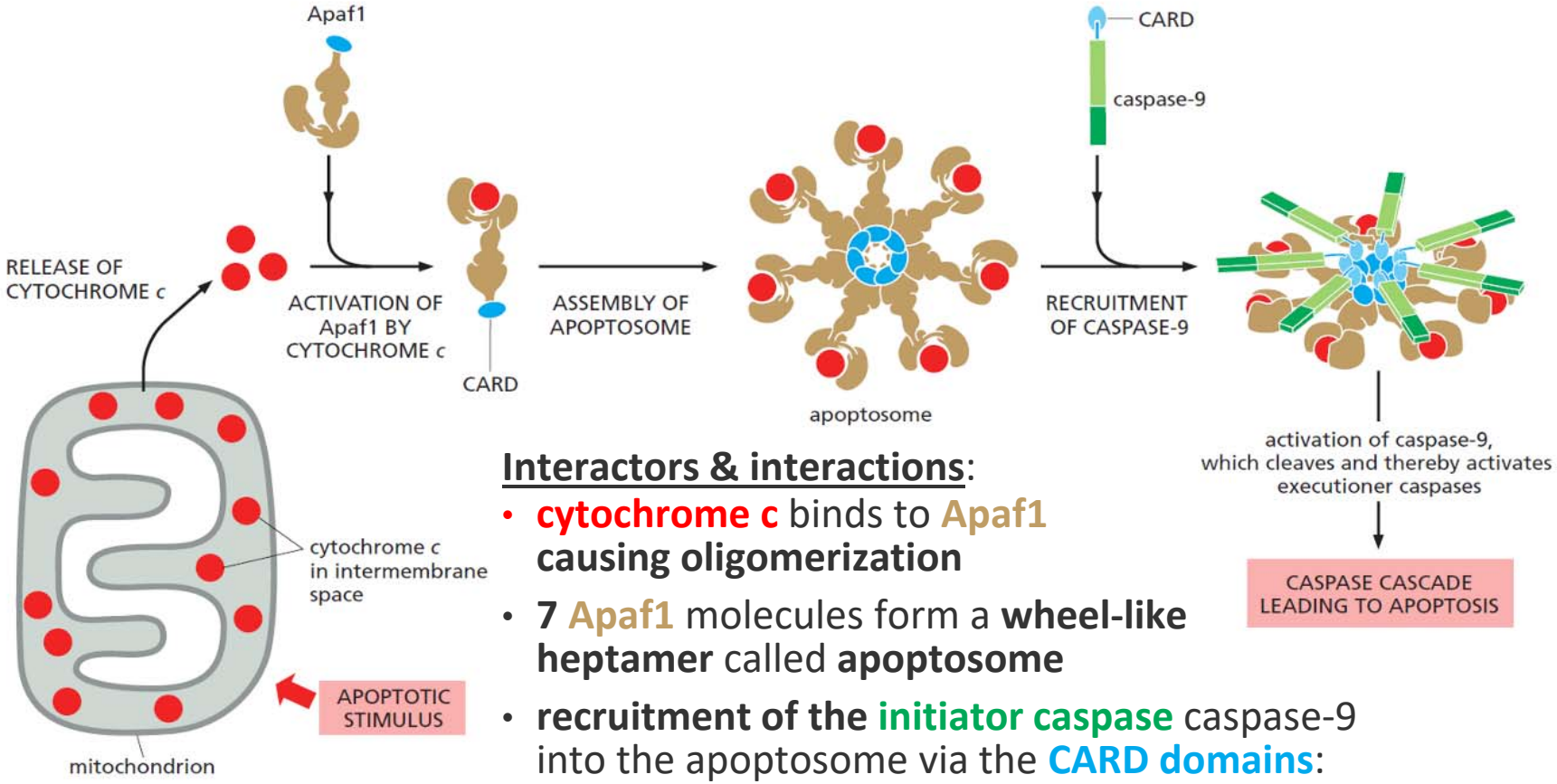
Key players:

- **cytochrome c** (water soluble component of the e- transport chain)
- **Apaf1** (**a**poptotic **p**rotease **a**ctivating **f**actor-**1**) (contains a **CARD** (**ca**spase **r**ecruitment **d**omain) domain
- **caspase-9** initiator caspase

Pathway is triggered in response to:

- **DNA damage**
- **hypoxia**
- **lack of nutrients**
- **lack of extracellular survival signals**

The intrinsic activation pathway

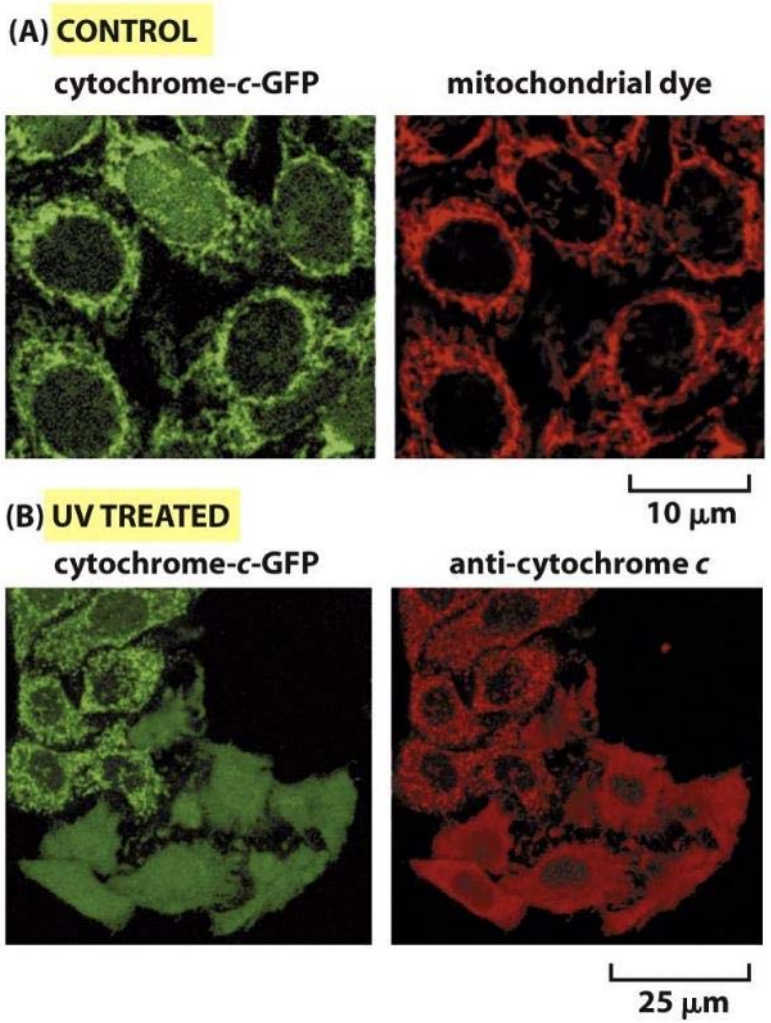


Interactors & interactions:

- **cytochrome c** binds to **Apaf1** **causing oligomerization**
- **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**

Release of mitochondrial cytochrome c during apoptosis

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

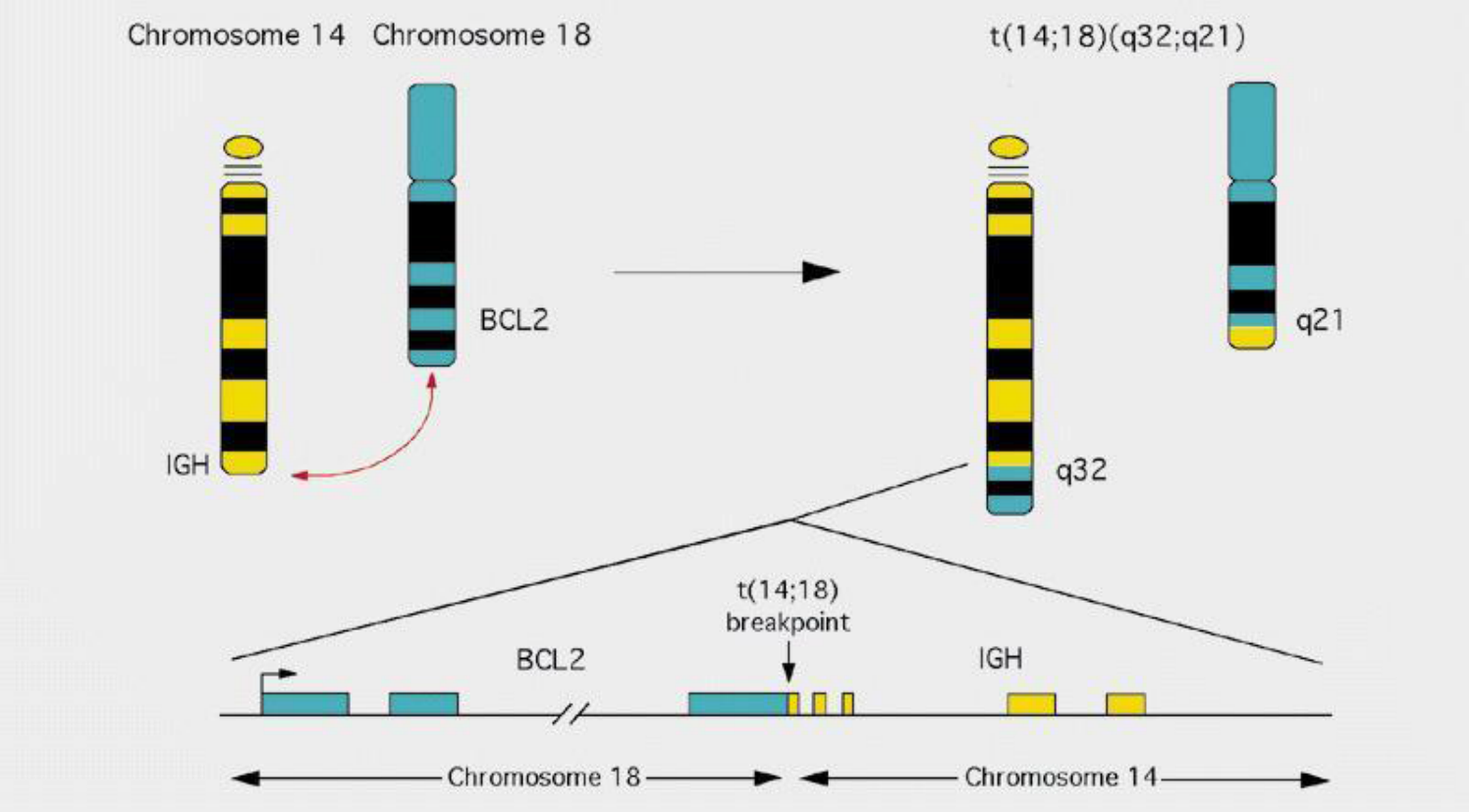


Setup:

- 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

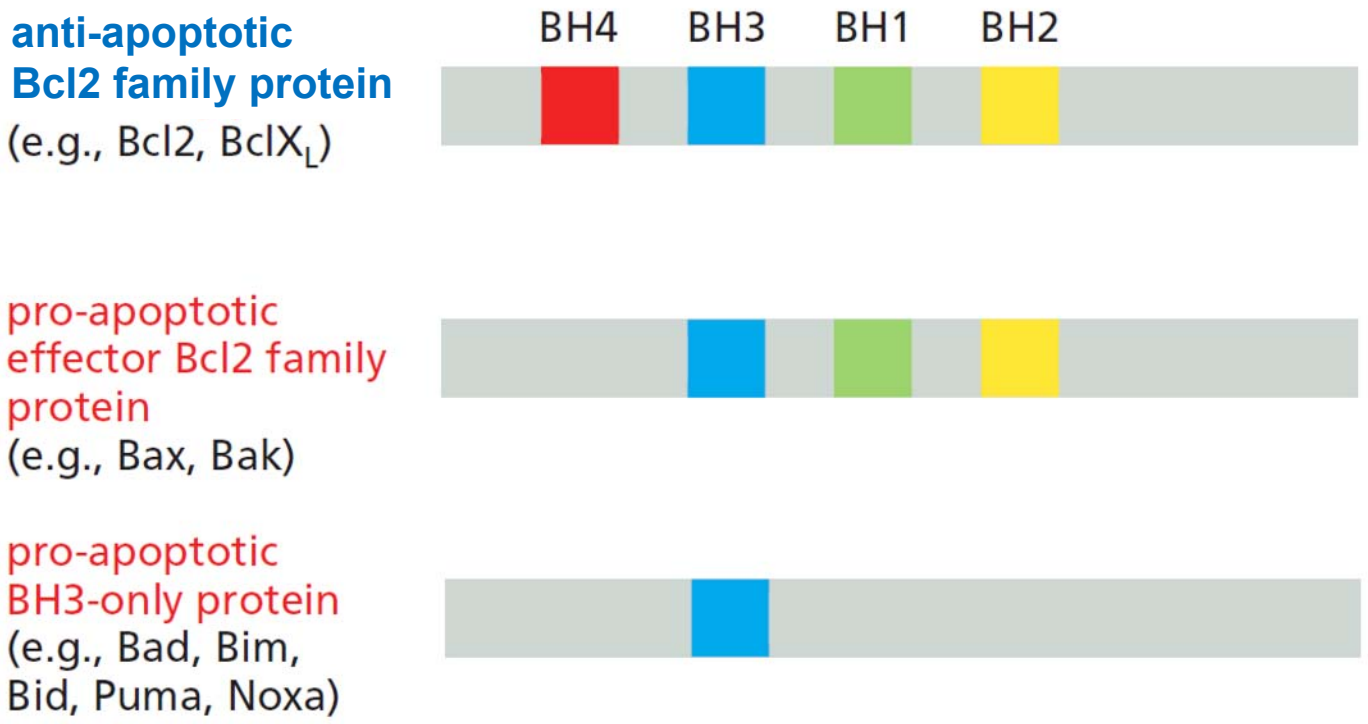
The Bcl2 protein family:

- 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

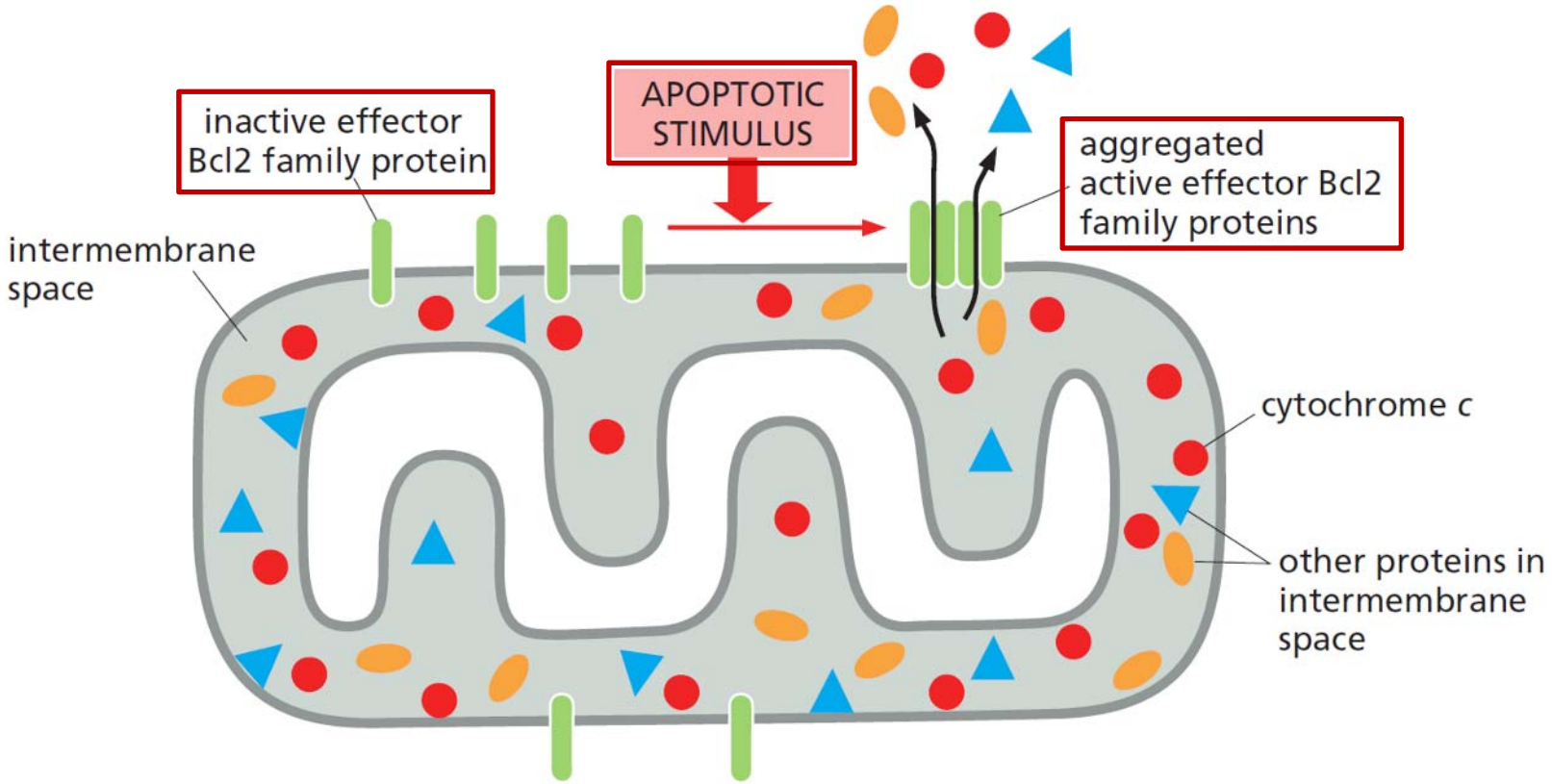
The intrinsic pathway of apoptosis is regulated by Bcl2 proteins

- Domain structures of members of the Bcl2 protein family



How are cytochrome c and IMS proteins released?

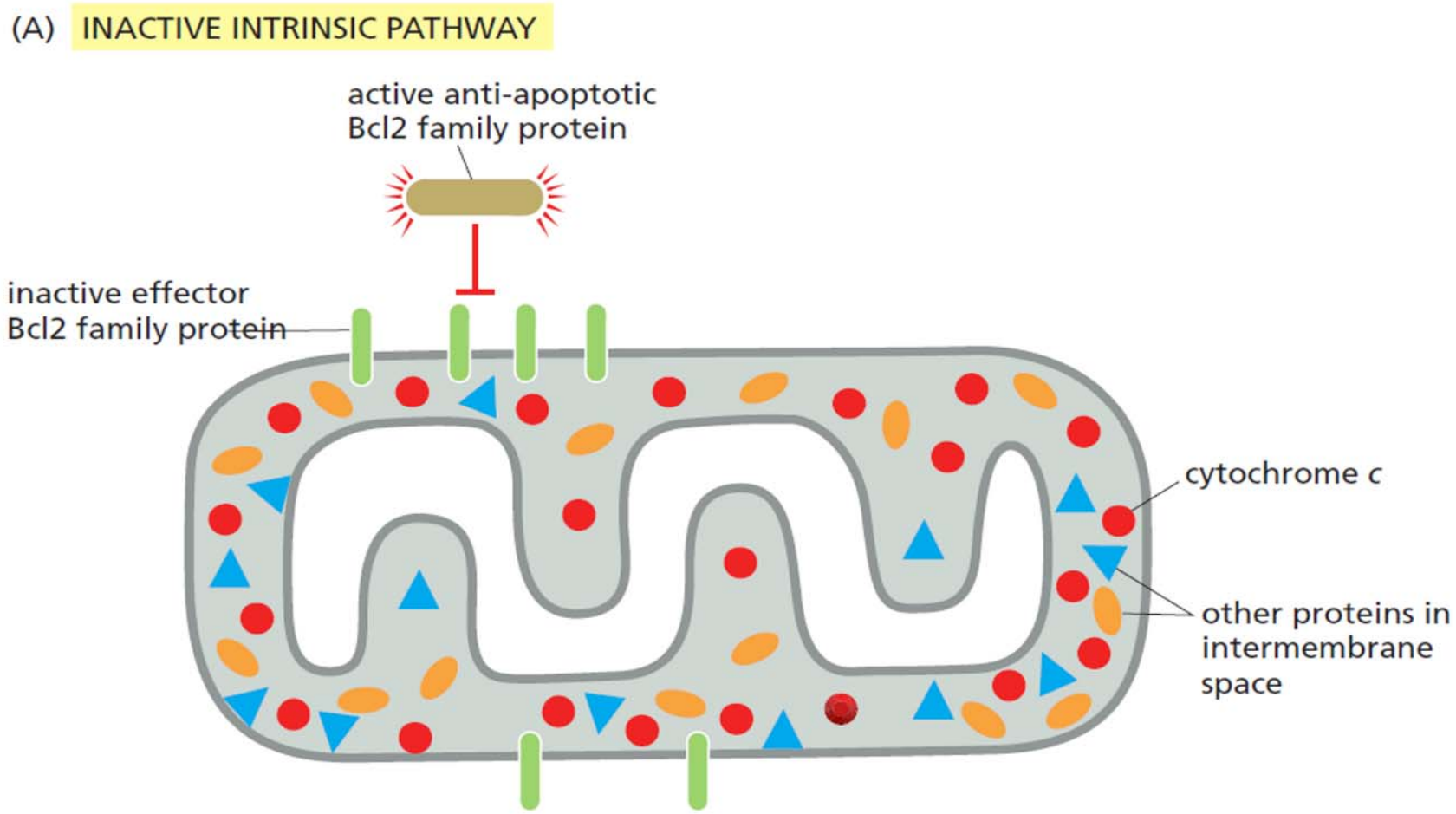
The **pro-apoptotic** effectors **Bax** and **Bak** **aggregate** in the outer membrane of mitochondria to release cytochrome c and IMS proteins



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

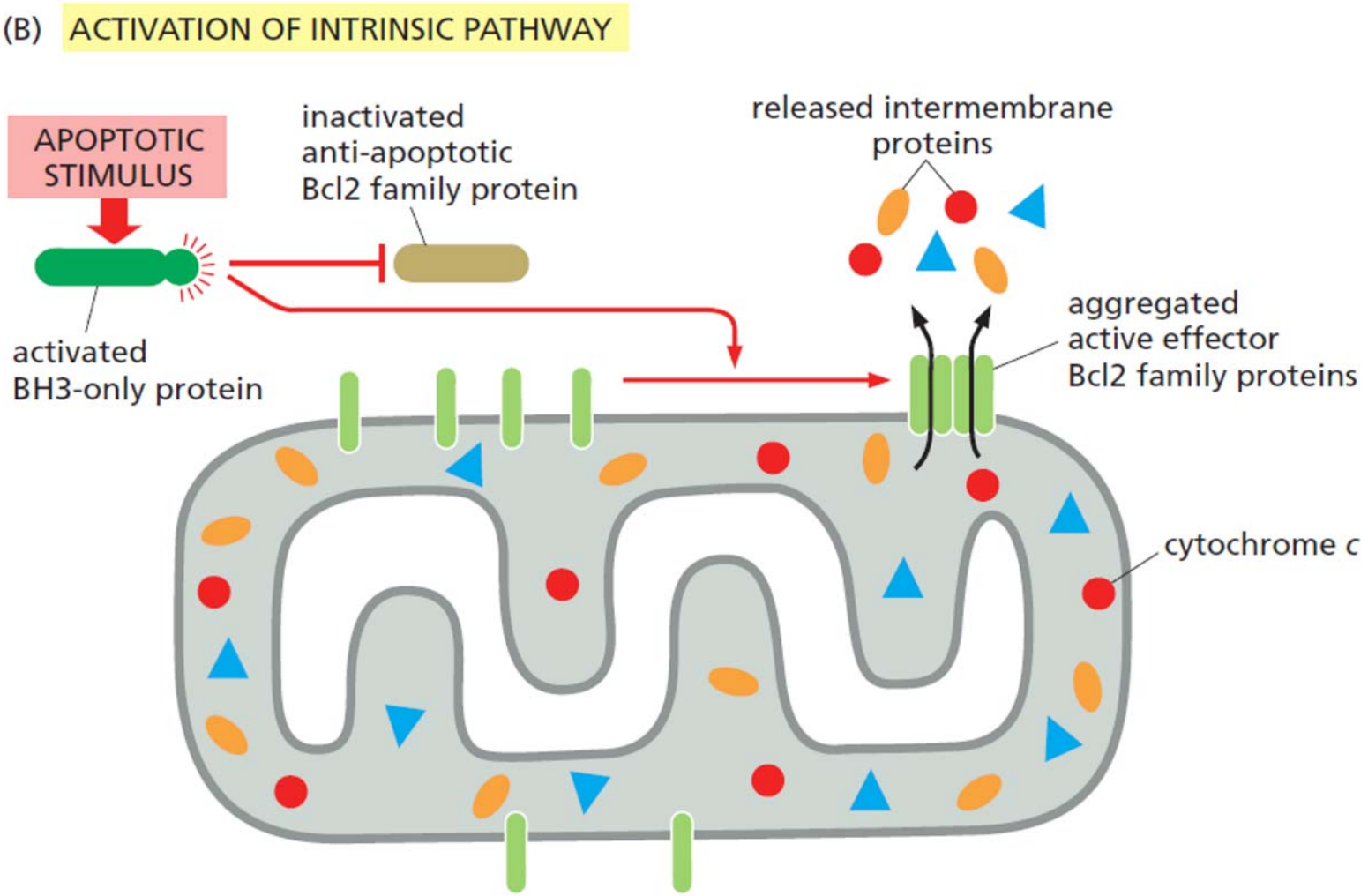
Anti-apoptotic Bcl-2 binds and inhibits Bax and Bak aggregation

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



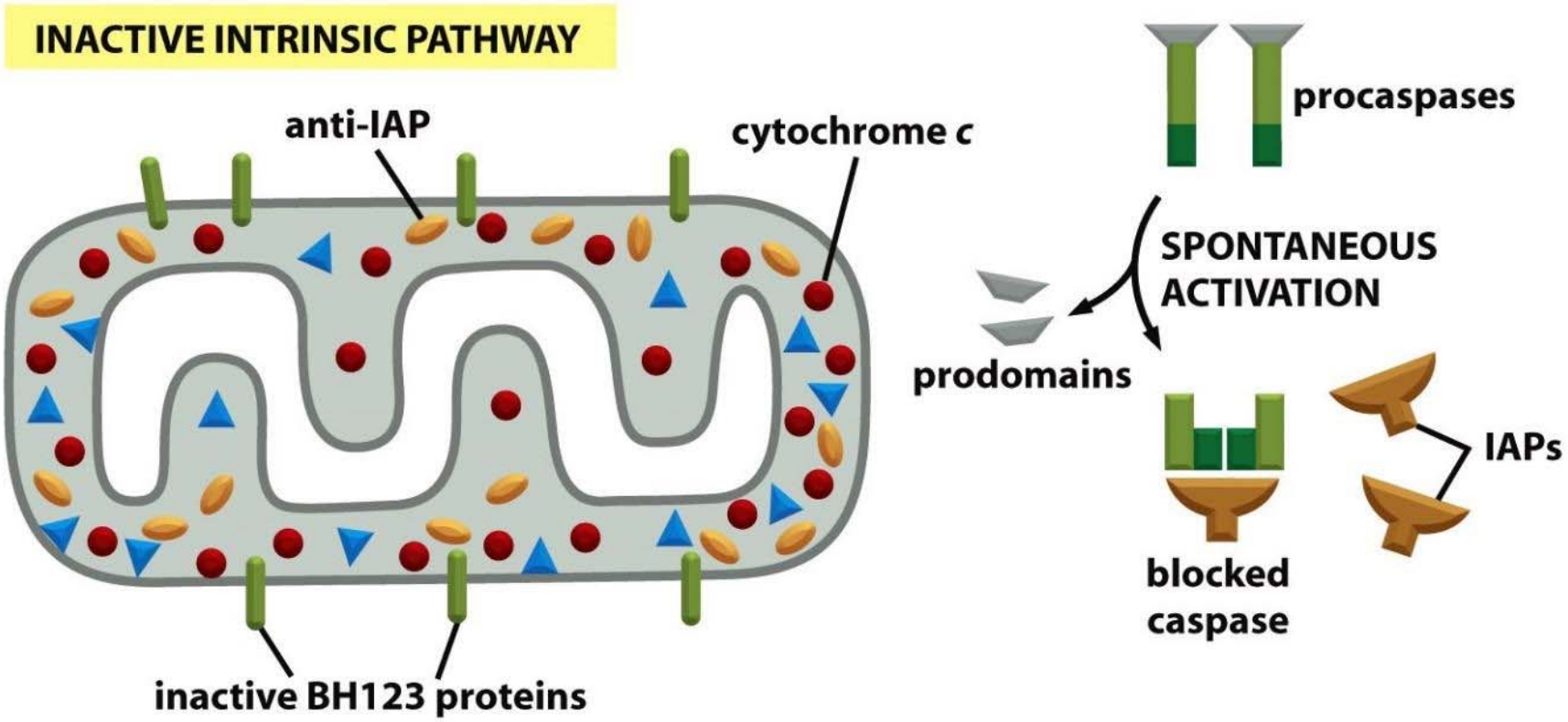
BH3- only proteins inhibit anti-apoptotic Bcl2 family proteins

Inactivation of **anti-apoptotic Bcls2** proteins by **BH3-only** proteins allow for **Bak/Bax aggregation** and thus for **apoptosis**



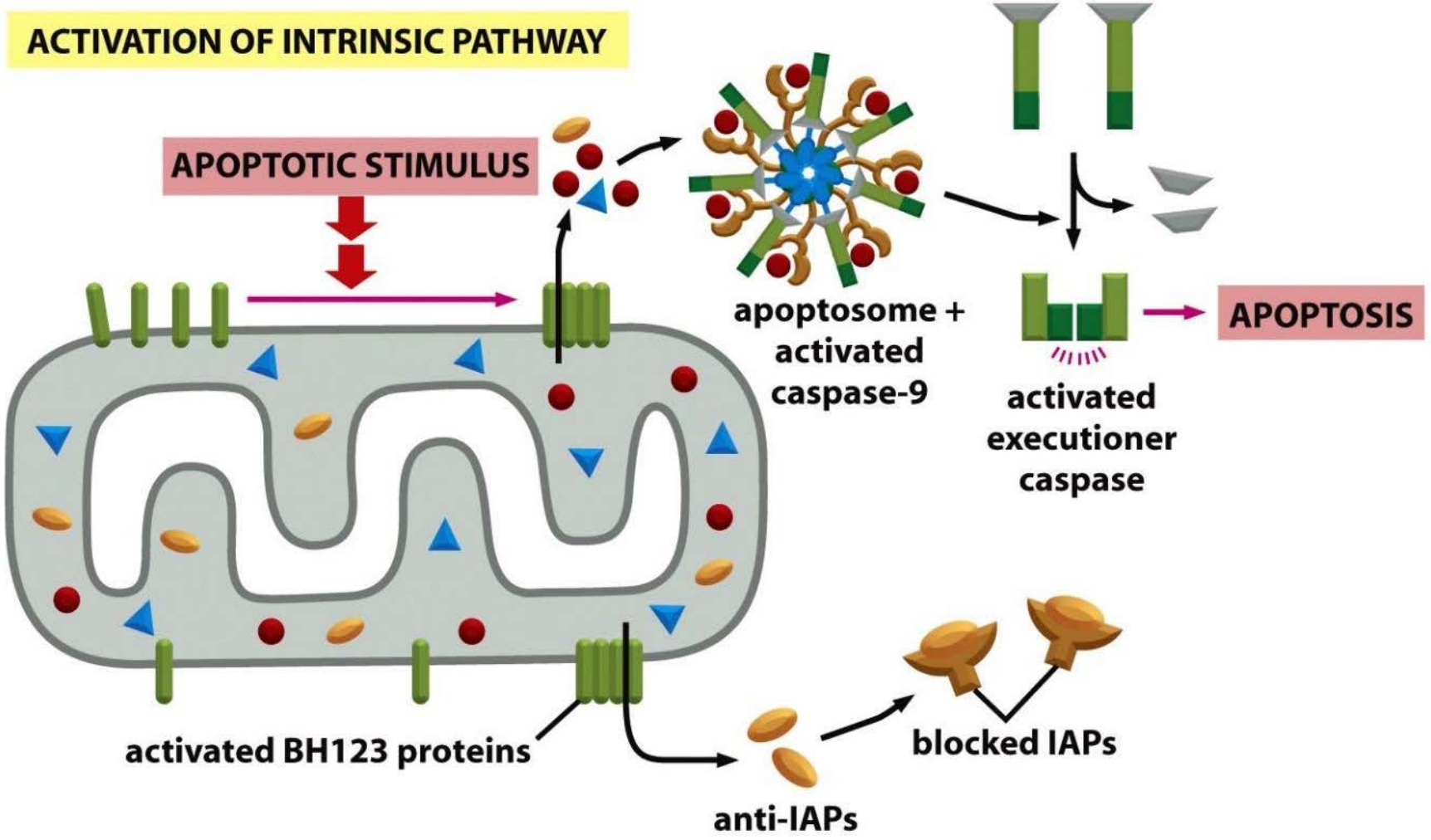
IAPs (inhibitors of apoptosis) inhibit caspases

IAPs prevent the action of "accidentally" activated caspases by binding to them

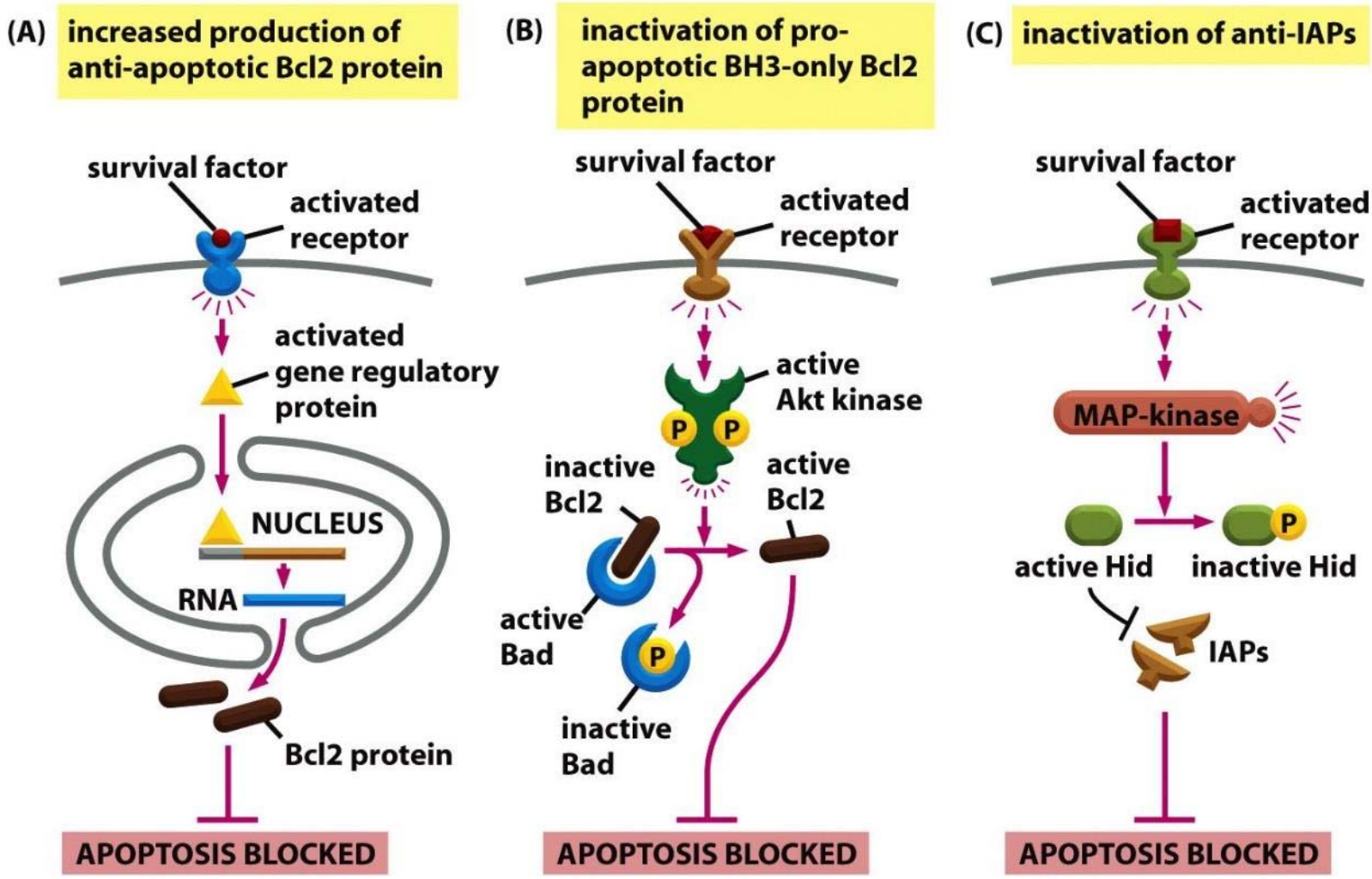


Activation of the intrinsic pathway: ready - aim - fire !!!

Signal: assembly of apoptosome - activation of caspases & inactivation of IAPs



Three ways for survival factors to inhibit apoptosis



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?