APOPTOSIS

(Programmed cell death)

Dr Shahla Yasmin
Professor
Department of Zoology
Patna Women's College

Learning objectives

The students will learn:

- The cell death mechanisms
- Significance of apoptosis
- Extrinsic pathway of apoptosis
- Intrinsic pathway of apoptosis
- Role of apoptosis in diseases

Apoptosis (Cell death)

- The word "apoptosis“ is used in Greek to describe the "dropping off" or "falling off" of petals from flowers, or leaves from trees.
- Apoptosis occurs by a programmed sequence of molecular events in which the cell systematically destroys itself from within and is then eaten by other cells.

Historical aspects

- German scientist Carl Vogt - was the first to describe the Principle of apoptosis (1842).

- Walther Flemming described the Process of programmed cell death (1845).

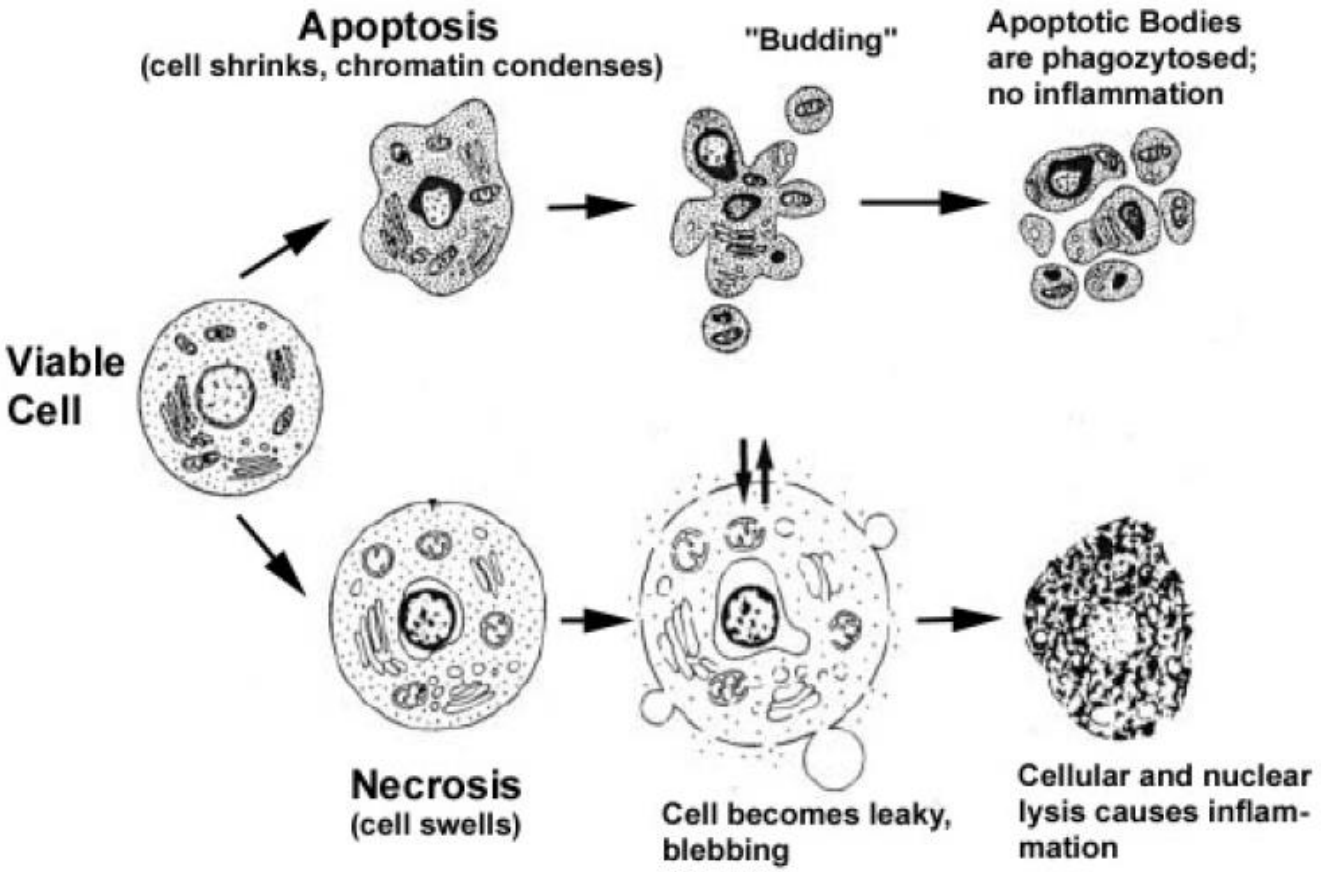
- John Foxton Ross Kerr – Distinguished apoptosis from traumatic cell death (1962).
- ‘Apoptosis’ term coined in 1972 by John Kerr, Andrew Wylie and AR Currie of Univ of Aberdeen

The Nobel Prize in Physiology or Medicine 2002 was awarded jointly to Sydney Brenner, H. Robert Horvitz and John E. Sulston *"for their discoveries concerning genetic regulation of organ development and programmed cell death'"*.

Cell death mechanisms

Death by suicide-apoptosis

Death by injury-necrosis



Apoptosis vs necrosis

- Cells undergoing apoptosis shrink and condense
- The cytoskeleton collapses
- The NE disassembles
- Nuclear chromatin condenses and breaks into fragments
- A large cell breaks up into membrane-enclosed fragments called apoptotic bodies.
- the surface of apoptotic bodies become chemically altered so that a neighbouring cell or macrophage engulf them.
- Thus, the cell dies neatly and is rapidly cleared away.

Apoptosis vs necrosis

- Cells that die in response to trauma or lack of blood supply, do so by a process called cell necrosis.
- Necrotic cells swell and burst, spilling their contents over their neighbours and eliciting an inflammatory response.

	APOPTOSIS	NECROSIS
NATURAL	YES	NO
EFFECTS	BENEFICIAL	DETRIMENTAL
	Physiological or pathological	Always pathological
	Single cells	Sheets of cells
	Energy dependent	Energy independent
	Cell shrinkage	Cell swelling
	Membrane integrity maintained	Membrane integrity lost

APOPTOSIS	NECROSIS
Role for mitochondria and cytochrome C	No role for mitochondria
No leak of lysosomal enzymes	Leak of lysosomal enzymes
Characteristic nuclear changes	Nuclei lost
Apoptotic bodies form	Do not form
DNA cleavage	No DNA cleavage
Activation of specific proteases	No activation
Regulatable process	Not regulated
Evolutionarily conserved	Not conserved
Dead cells ingested by neighboring cells	Dead cells ingested by neutrophils and macrophages

Classic changes during apoptosis

- ✓ Cell shrinkage
- ✓ Nuclear fragmentation
- ✓ Chromatin condensation
- ✓ Chromosomal DNA fragmentation
- ✓ Formation of cytoplasmic blebs& apoptotic bodies
- ✓ Phagocytosis

Significance of apoptosis

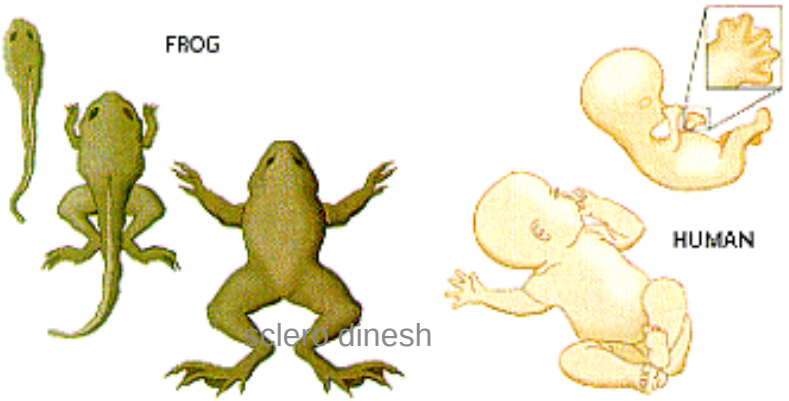
- Helps sculpt hands and feet during embryonic development.
- Cells die by apoptosis when the structure is no longer needed e.g-when tadpole changes into a frog at metamorphosis, the cells in the tail die b'coz tail is not needed by adult frog.
- Helps to eliminate cells that are abnormal, non-functional or potentially dangerous to the animal. In human body about one lakh cells are produced every second by mitosis and a similar number die by apoptosis.
- Animal cells recognize damage in cell organelles and can kill themselves-DNA damage can produce cancer promoting mutations, if not repaired. Cells undergo apoptosis if they cannot repair it.

Why should a cell commit suicide?

- **1. Programmed cell death is as needed for proper normal development as mitosis is.**

Examples:

- The resorption of the tadpole tail in frog .
- The formation of the fingers and toes of the fetus requires the removal, by apoptosis.
- The sloughing off of the endometrium at the start of menstruation.
- The formation of the proper connections (synapses) between neurons in the brain.
- 10^{10}-10^{11} cells in the adult human body die everyday by apoptosis



- **2. Programmed cell death is needed to destroy cells that represent a threat to the integrity of the organism.**

- Examples:
  - Cells infected with viruses

  One of the methods by which **cytotoxic T lymphocytes** (CTLs) kill virus-infected cells is by inducing apoptosis

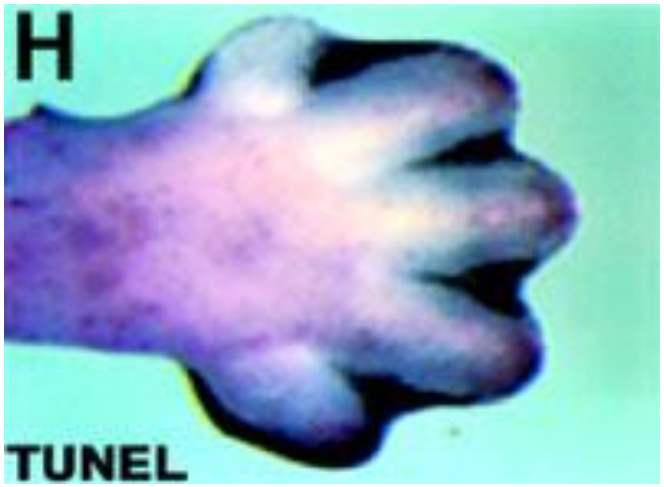
  - Cells of the immune system

  Defects in the apoptotic machinery is associated with autoimmune diseases such as lupus erythematosus (has joint pain and swelling) and rheumatoid arthritis.
  - Cells with DNA damage

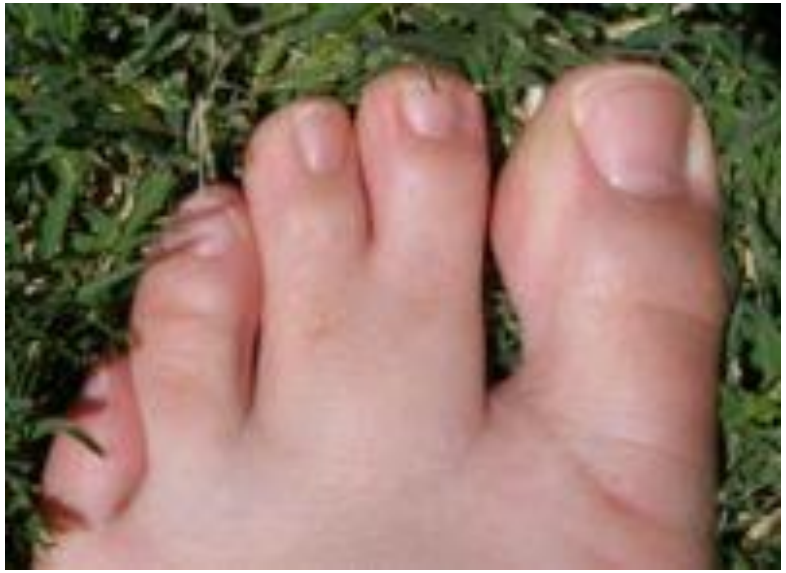
  - Cancer cells (Uncontrolled proliferated cells)

Examples of apoptosis

Apoptosis in bud formation during which many interdigital cells die. They are stained black by a TUNEL method



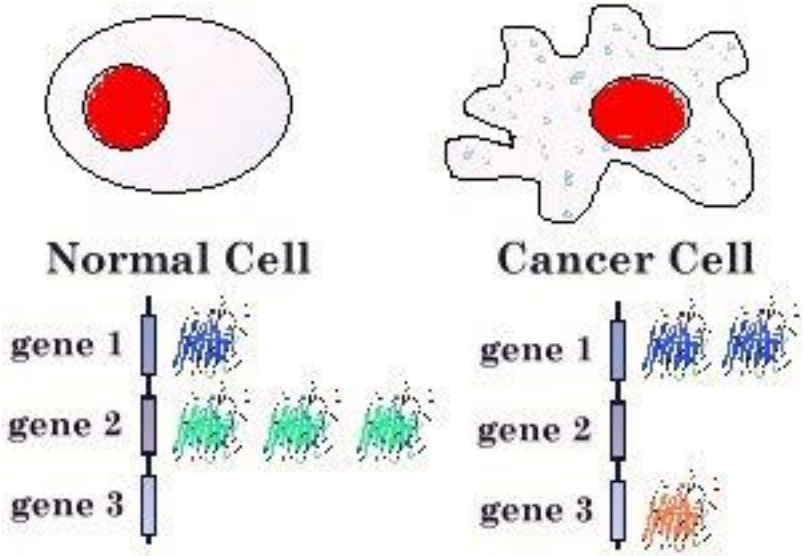
Incomplete differentiation in two toes due to lack of apoptosis



Examples of apoptosis

Cells with DNA damage

- Damage to its genome can cause a cell
  - » to disrupt proper embryonic development leading to birth defects
  - » to become cancerous.
- Cells respond to DNA damage by increasing their production of p53. p53 is a potent **inducer** of apoptosis.



- Radiation and chemicals used in cancer therapy induce apoptosis in some types of cancer cells.

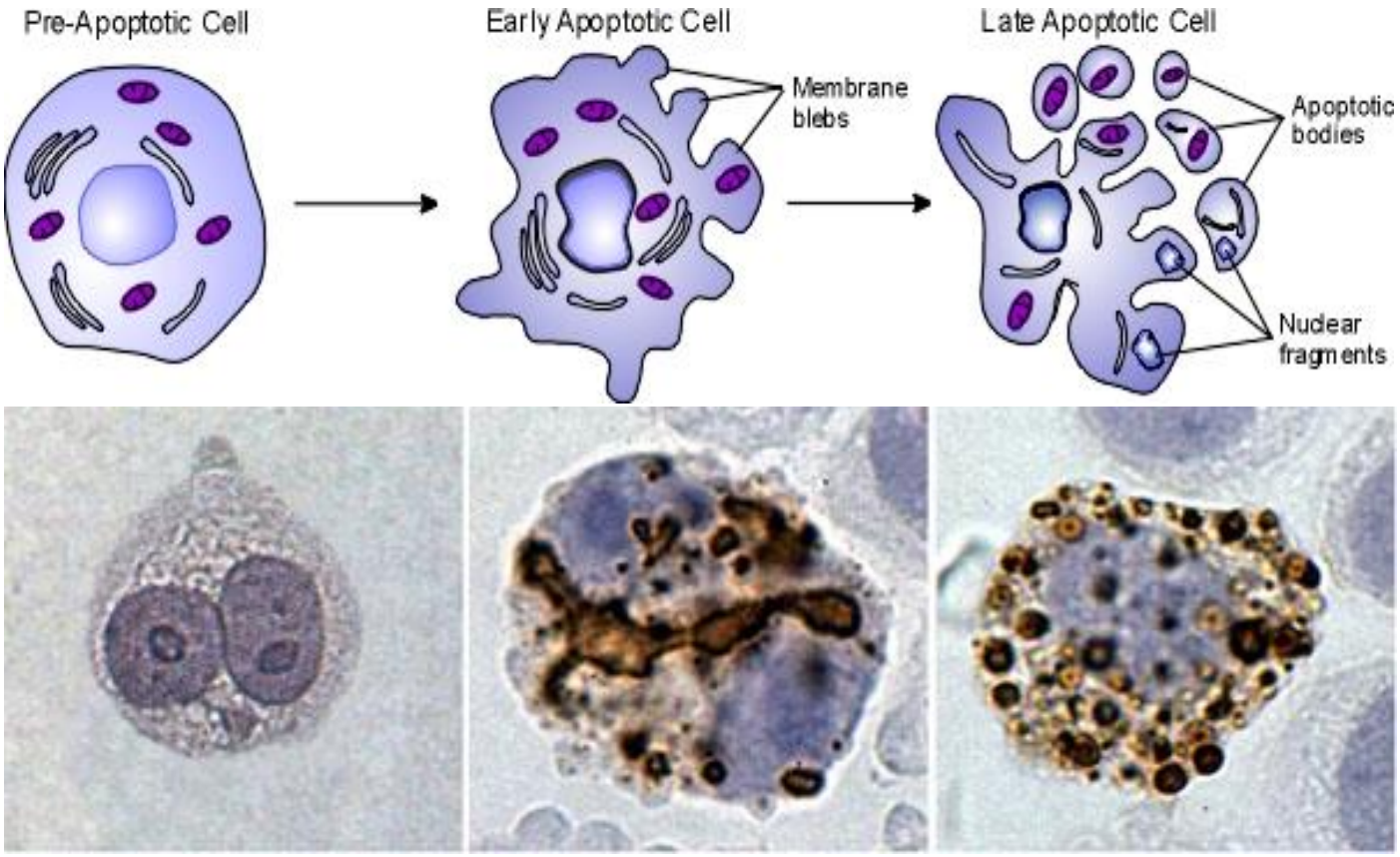


Fig. 1: SC-1 induced apoptosis in stomach carcinoma cells
Left: Before induction
Middle: 24h after induction
Right: 48h after induction

Stimuli for apoptosis

- Triggered by both internal stimuli

e.g Abnormalities in the DNA

- And external stimuli

e.g cytokines (proteins secreted by cells of immune system)

Apoptosis is mediated by Caspases

- Apoptosis is triggered inside the cell by caspases- specialized intracellular proteases synthesized by the cells. Caspases remain inactive – but activated during apoptosis.
- These proteases have a cysteine at their active site and cleave their target proteins at specific aspartic acids.
- Therefore called caspases (c for cysteine and asp for aspartic acid)
- Two types- initiator caspases and executioner caspases

Initiator Caspases

- Initiator caspases begin the apoptotic process. They exist as inactive monomers in the cytosol. An apoptotic signal triggers the formation of large complexes by bringing together multiple initiator caspases.
- Pairs of caspases associate to form dimers.
- Each caspase in the dimer cleaves its partner at specific site in the protease domain. This stabilizes the complex for proper functioning.
- The major function of the initiator caspases is to activate the executioner caspases.
- Caspases specifically cleave their substrates after Aspartate residues.
- Caspase- 8, Caspase- 9 and 10 are initiator caspases

Executioner caspases

- Caspase-3, 6,7 are executioner caspases
- Executioner caspases normally exist as inactive dimers. When cleaved by the initiator caspase at the site in the protease domain, the active site is rearranged from an inactive to an active conformation.
- One initiator caspase complex can activate many executioner caspases resulting in an amplifying proteolytic cascade.
- Once activated, executioner caspases catalyze widespread protein cleavage events that kill the cell.
- The caspase cascade is irreversible-once a cell starts along the path of destruction, it cannot turn back

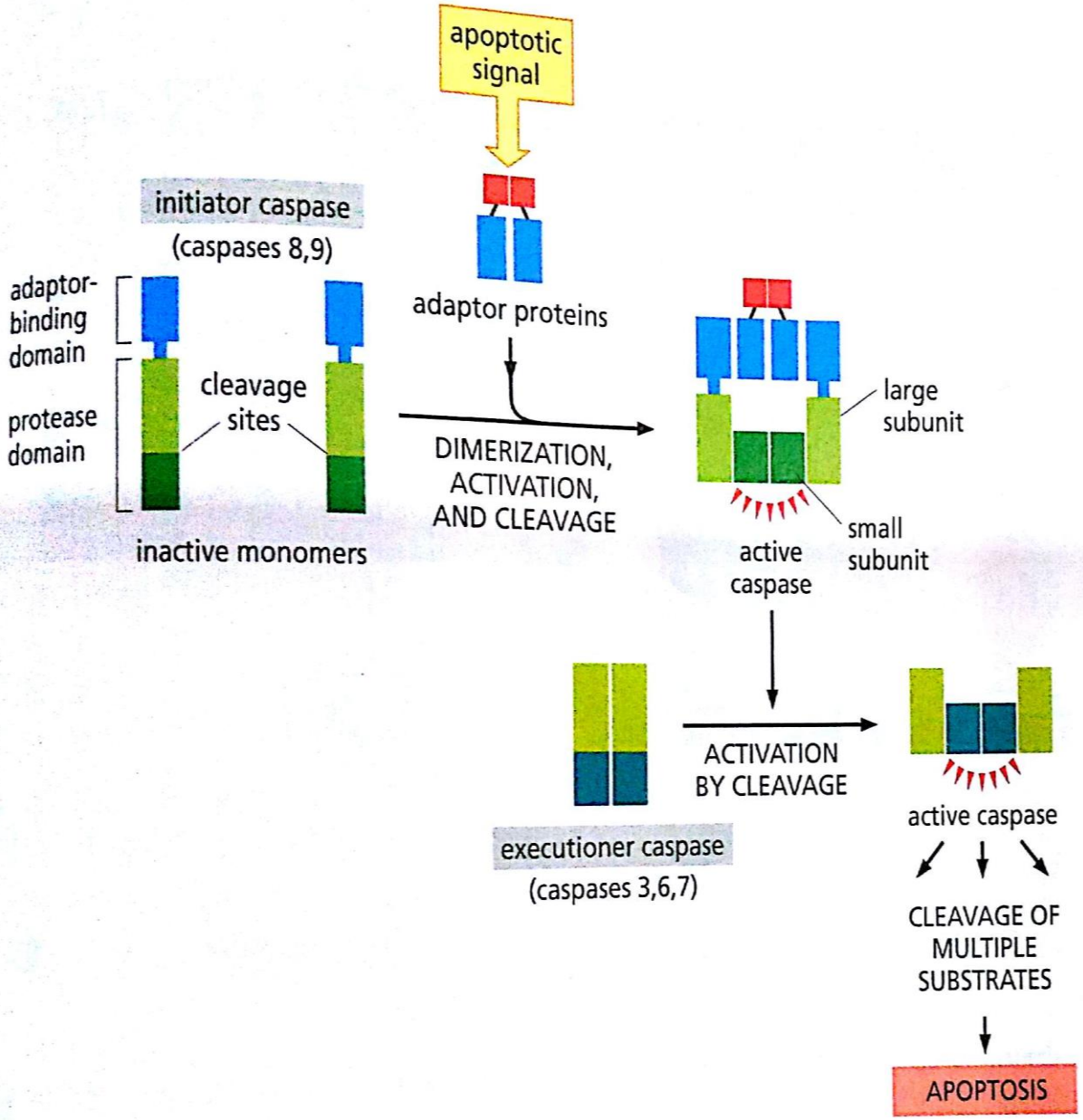
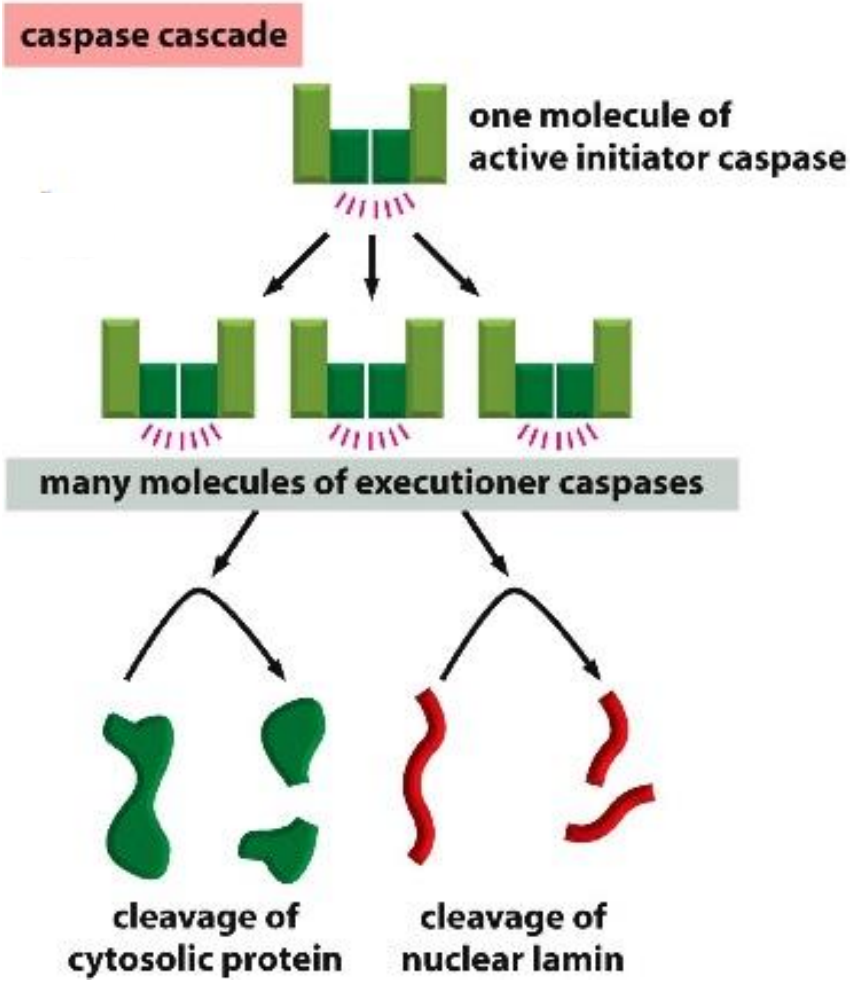


Figure 18–3 Caspase activation during apoptosis. An initiator caspase contains a protease domain in its carboxy-terminal region and a small protein interaction domain near its amino terminus. It is initially made in an inactive, monomeric form, sometimes called procaspase. Apoptotic signals trigger the assembly of adaptor proteins carrying multiple binding sites for the caspase amino-terminal domain. Upon binding to the adaptor proteins, the initiator caspases dimerize and are thereby activated, leading to cleavage of a specific site in their protease domains. Each protease domain is then rearranged into a large and small subunit. In some cases (not shown), the adaptor-binding domain of the initiator caspase is also cleaved (see Figure 18–5). Executioner caspases are initially formed as inactive dimers. Upon cleavage at a site in the protease domain by an initiator caspase, the executioner caspase dimer undergoes an activating conformational change. The executioner caspases then cleave a variety of key proteins, leading to the controlled death of the cell.

Caspase cascade



A small group of **active initiator caspases** can then activate many more caspases called **executioner caspases: amplification cascade.** These executioner caspases act on the intracellular proteins to break them down. *Specific* executioner caspases target *specific* proteins, not every cytosol proteins

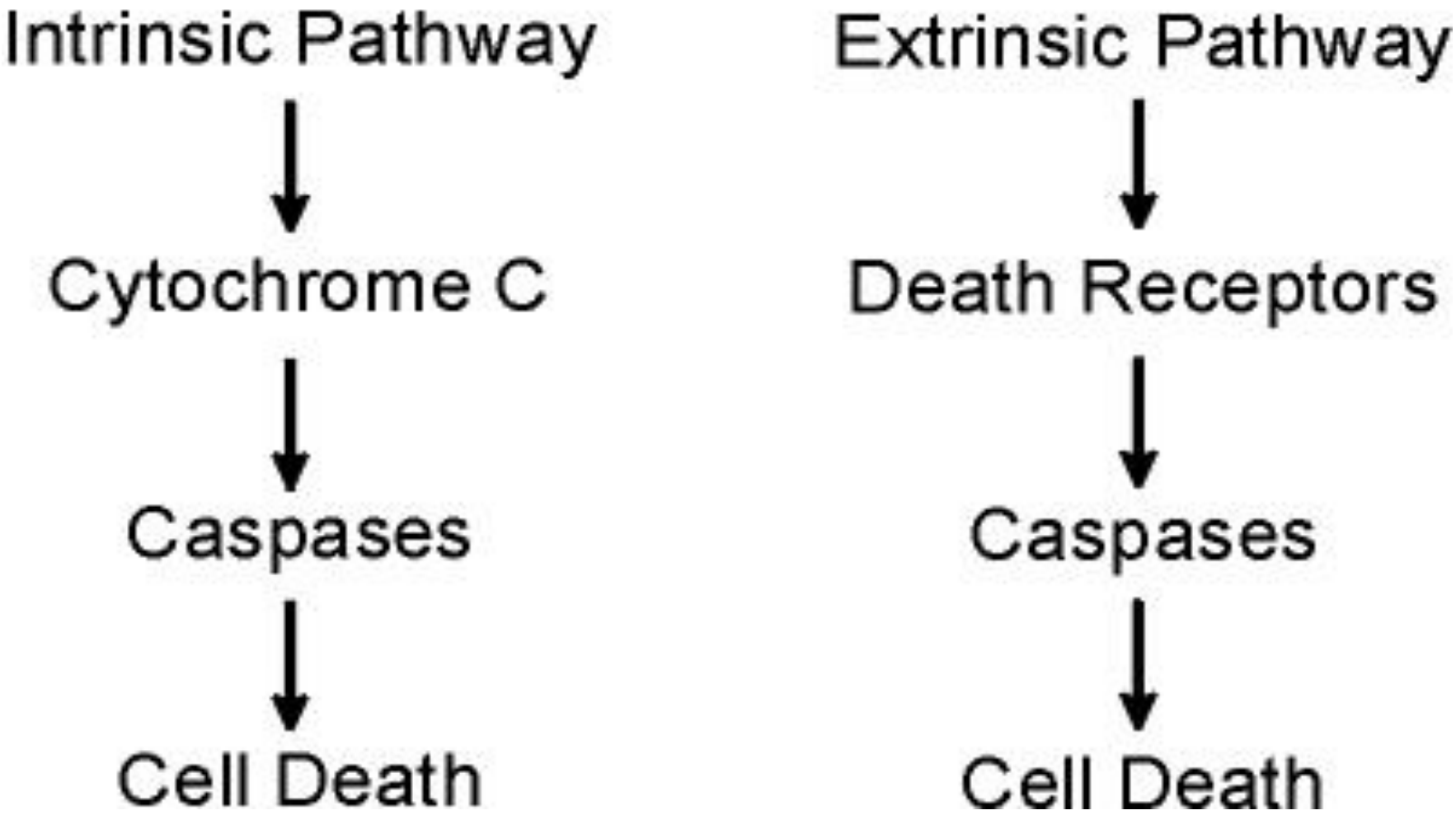
Mechanisms of apoptosis

Pathways of apoptosis

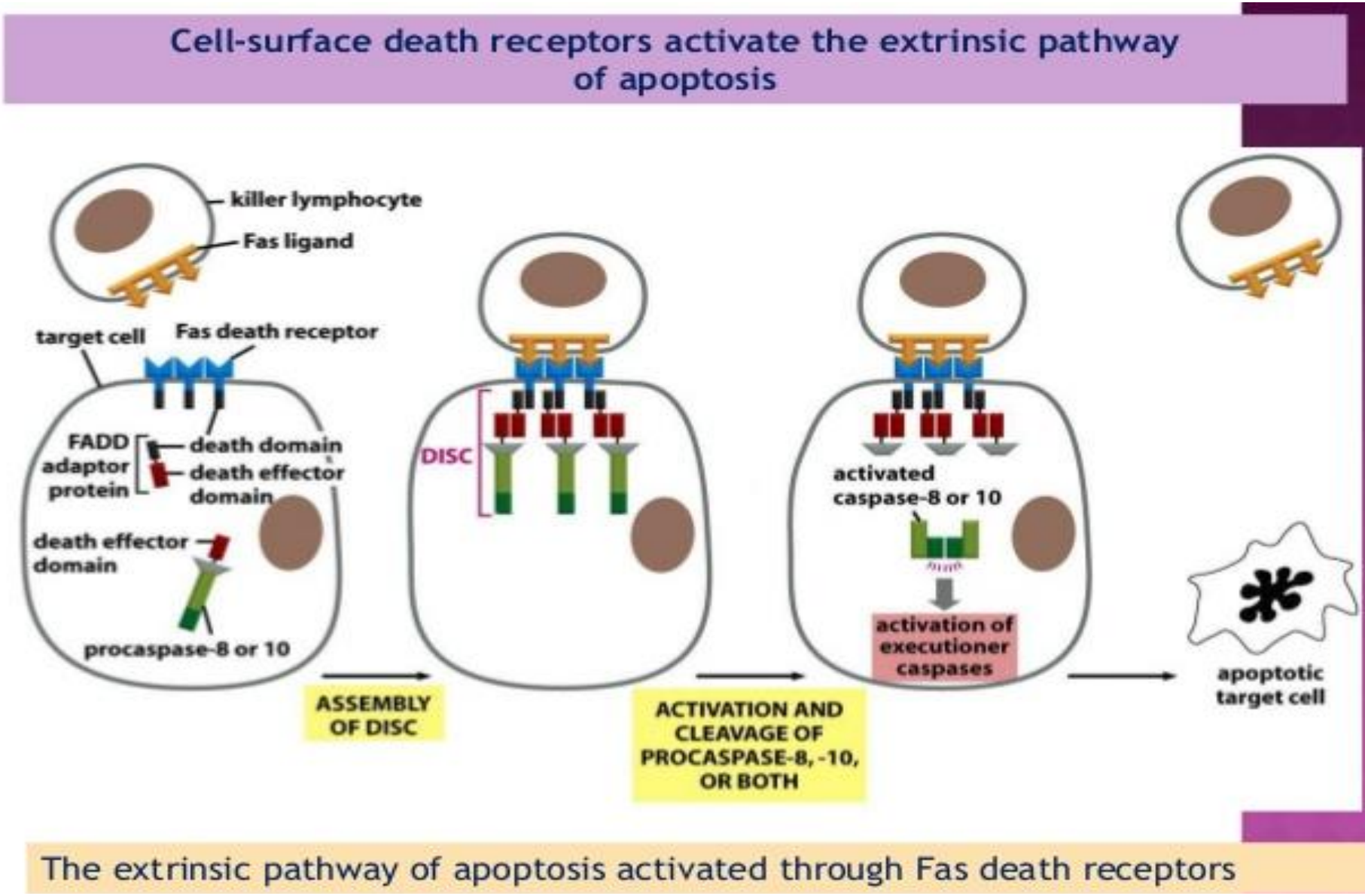
Apoptosis occurs by two activation pathways.

- Extrinsic (death receptor) pathway
- Intrinsic (mitochondrial) pathway

APOPTOSIS


Intrinsic Pathway
Cytochrome C
Caspases
Cell Death
Extrinsic Pathway
Death Receptors
Caspases
Cell Death

Extrinsic pathway of apoptosis through Fas death receptors



The extrinsic pathway of apoptosis activated through Fas death receptors

Extrinsic pathway

- Fas ligand is a transmembrane protein that belongs to the tumor necrosis factor (TNF) family. Its binding with its receptor induces apoptosis. Fas ligand/receptor interactions play an important role in the regulation of the immune system and the progression of cancer.

- Trimeric Fas ligands on the surface of killer lymphocyte (play a major role in defending the host from both tumors and virally infected cells) interact with trimeric Fas receptors on the surface of the target cell. This activates the death domain (FADD) of the receptor that faces the cytosol.
- **FADD adaptor proteins** are present in the cytosol with similar death domain and a death effector domain (DED).
- Activated death domains on the receptor tails interact with similar domains on adaptor protein FADD (Fas-associated death domain).

- Procaspase 8 or 10 in the cytosol also have similar death effector domain which dimerize with DED of **FADD adaptor proteins** .

Extrinsic pathway

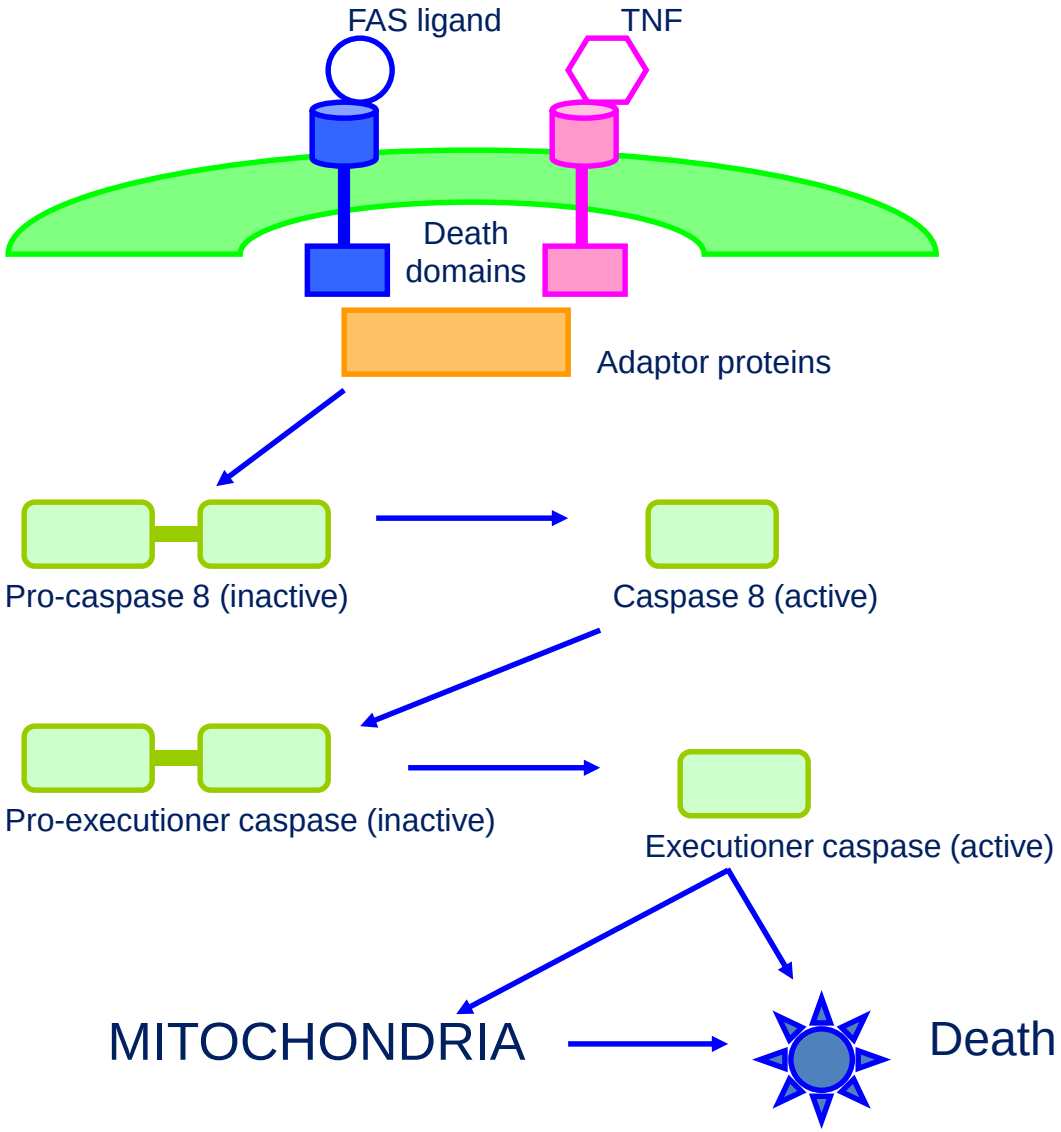
- The complex consisting of Fas ligand, Fas receptor, FADD and procaspase forms a death-inducing signalling complex (DISC).
- The procaspases break off from the DISC at the **prodomain**, **large and small subunits separate** and interact with other similar subunits in the cytoplasm to form an activated protease dimer (active procaspase 8 or 10).
- In the cytoplasm procaspase 8 or 10 activate executioner caspases by cleaving them first at **prodomain** site and then at the site between **large and small subunit**. Due to positive feedback small number of initiator caspases activate many many executioner caspases resulting in a caspase cascade.

Initiation of extrinsic pathway

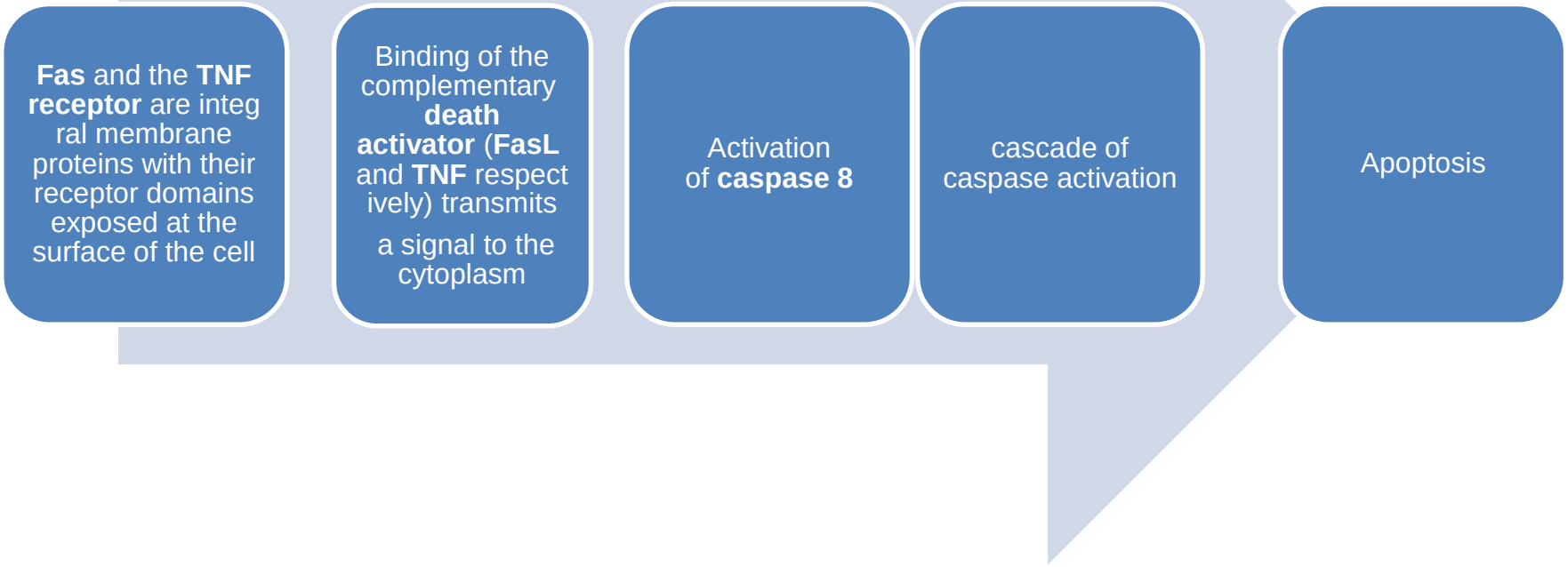
- Absence of stimuli - hormones, growth factors
- Activation of receptors – TNF (tumour necrosis factor) family
- Heat ,radiation, chemicals

Receptor pathway (physiological):

Death receptors:
(FAS, TNF-R, etc)



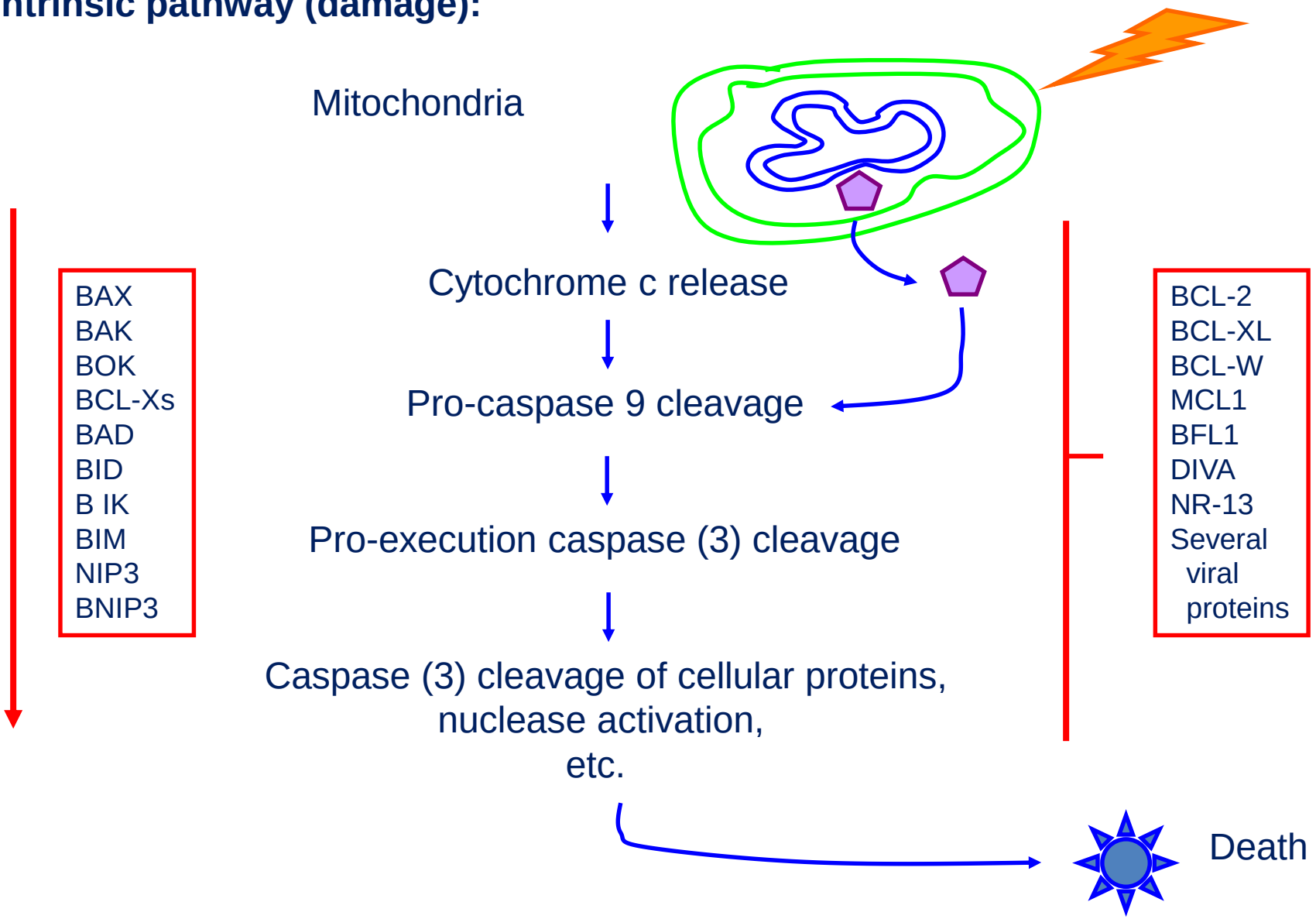
Apoptosis triggered by external signals: the extrinsic or death receptor pathway



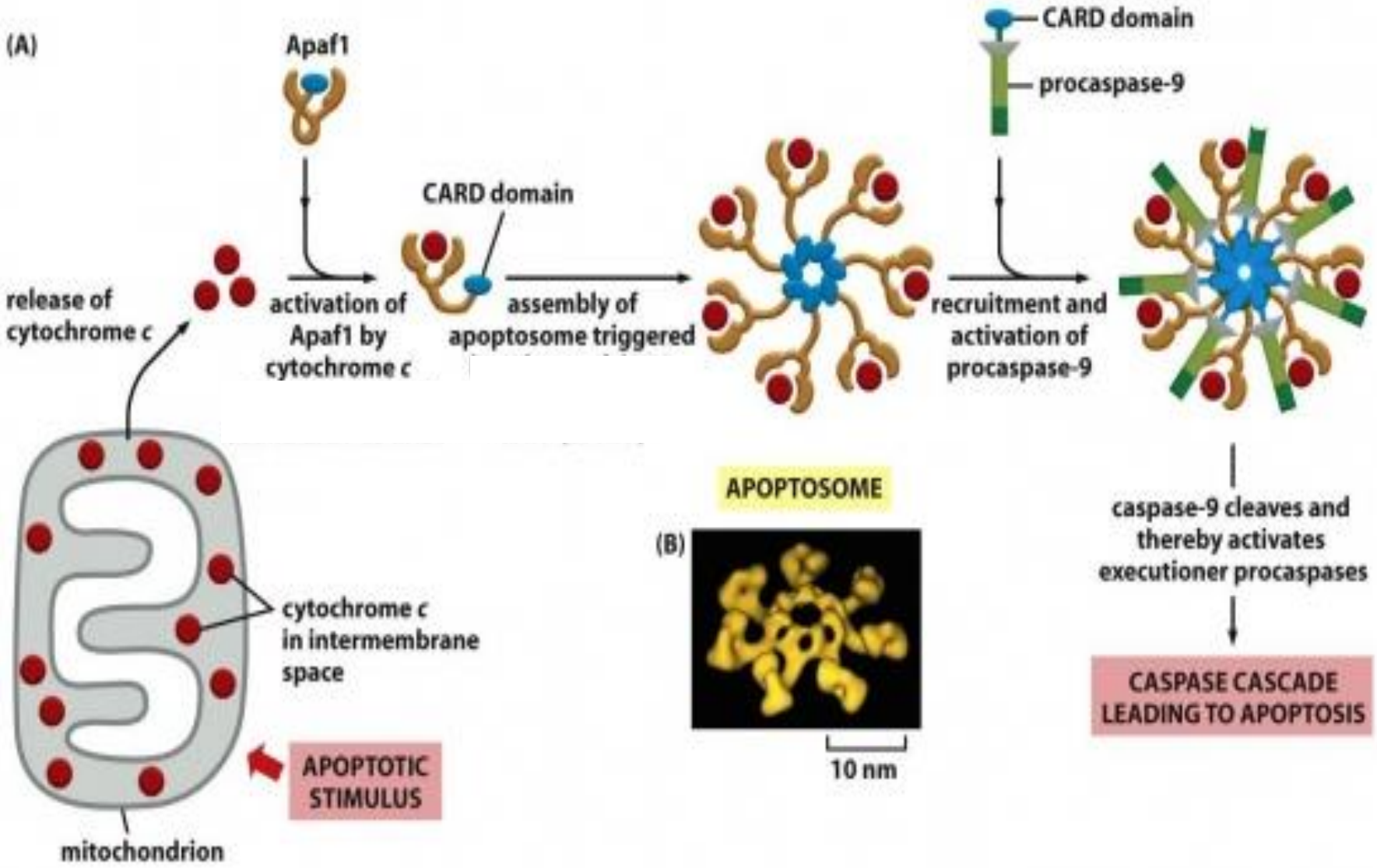
Inhibition of apoptosis

- Many cells produce inhibitory proteins that act to restrain the extrinsic pathway. Some cells produce protein FLIP (**Flice-like Inhibitory Protein)**, which resembles an initiator caspase but has no protease activity b'coz it lacks the key cysteine in its active site. FLIP dimerizes with caspase-8 in the DISC, caspase-8 is not cleaved at the site required for its stable activation and the apoptotic signal is blocked. This prevents inappropriate activation of extrinsic pathway of apoptosis.

Intrinsic pathway (damage):



Intrinsic pathway of apoptosis



Intrinsic pathway

- **Intracellular apoptotic stimuli** cause mitochondria to release cyt c, which interacts with Apaf1 (Apoptotic protease activating factor 1).
- Binding of cyt c causes Apaf1 to unfold partly, exposing a CARD domain that interacts with the similar domain in other activated Apaf1 molecules.
- Seven activated Apaf1 proteins form a large ring complex called **apoptosome.**
- Each Apaf1 protein contains a caspase recruitment domain (CARD) and these are clustered above the central hub of the **apoptosome**.

Intrinsic pathway

- The CARDs bind similar domains in multiple caspase-9 molecules, which are thereby recruited into the apoptosome and activated. caspase-9 activation results from dimerization and cleavage of adjacent caspase-9 proteins at the two cleavage points to separate large and small subunits. Two large and two small subunits form one active caspase-9. Once activated, caspase-9 cleaves and thereby activates downstream executioner caspases.

Signals for intrinsic pathway

- Stresses such as DNA damage

Regulation of intrinsic pathway

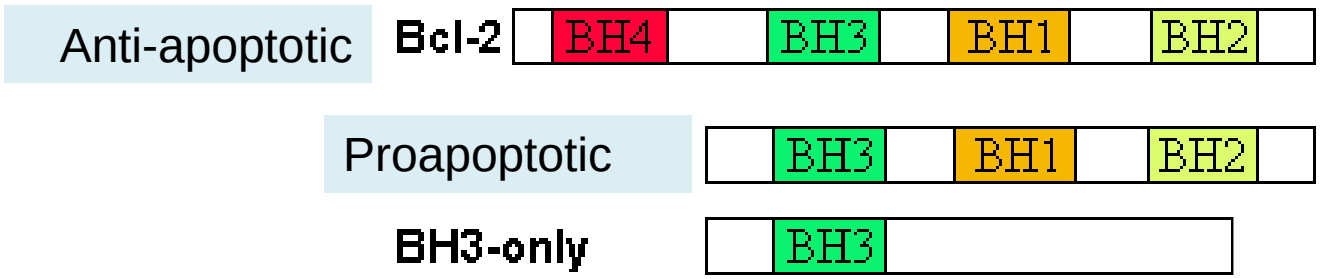
- A major class of intracellular regulators is Bcl_2 family of proteins- conserved in evolution from worms to humans.
- The Bcl_2 family proteins consist of three groups:

1) the proapoptotic Bcl_2 family proteins –which promote apoptosis (Bax and Bak). Also called BH123 (Bcl2 homology domain) proteins.

2) antiapoptotic members that protect cells from apoptosis e.g. Bcl-x_L, Bcl-w & Bcl-2. Bcl2 binds with Bak & Bax & prevent the formation of BH123 channel.

3) the BH3-only proteins (Bid, Bad, Puma, Noxa & Bim) are the largest subclass of Bcl_2 family proteins. They promote apoptosis mainly by inhibiting anti-apoptotic Bcl_2 family proteins. In other cases they promote apoptosis by activating proapoptotic Bax or Bak.

Bcl-2 family proteins



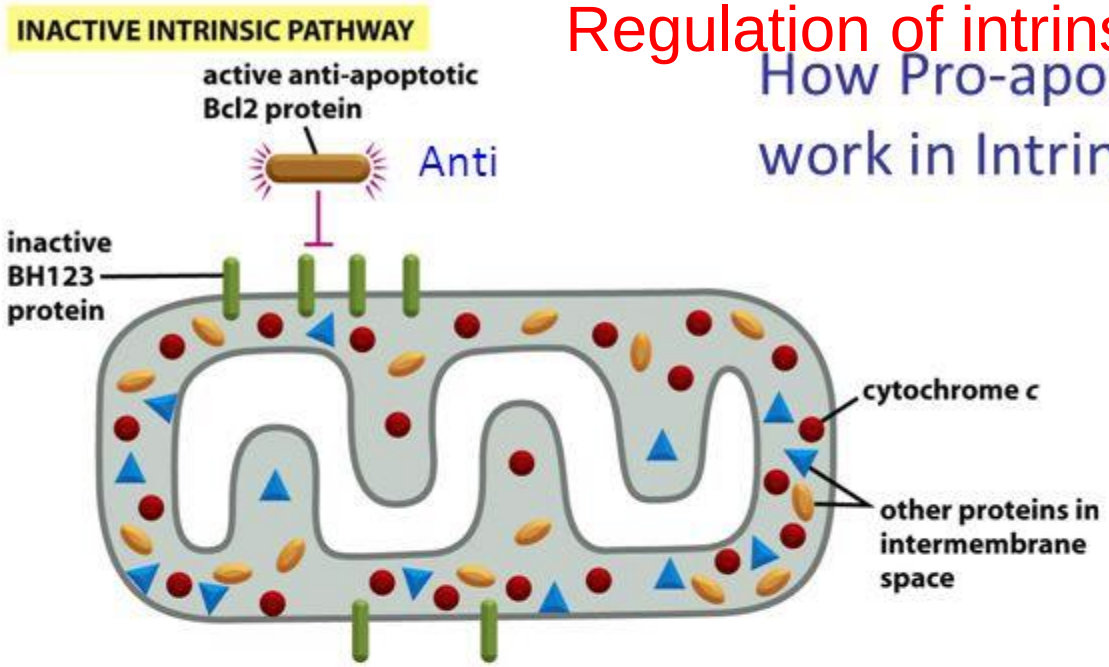
Bcl-2 and its closest relatives **Bcl-X_L**, **Bcl-w** are α-helical proteins having all four BH domains and are **anti-apoptotic** They suppress cytochrome c release, and are oncogenic when overexpressed.

Bak (present on outer mito memb) and **Bax** (present in cytoplasm) lack the BH4 domain, and are **pro-apoptotic** BH123 proteins. Bax expression is stimulated by p53, a mechanism for pro-apoptotic action of p53. Bak & Bax try to aggregate on the outer mito memb to form BH123 channel that allows Cyt c to leave intermemb space of mito into the cytoplasm. Bak & Bax are stopped from aggregating by anti-apoptotic Bcl2 proteins.

The **BH3-only** sub group are **strongly pro-apoptotic**, and include **Bim**, **Bid, Bad, Puma** and **Noxa. They** only have BH3. They inhibit anti-apoptotic Bcl2 to bind with Bak and Bax. Increase in expression of pro-apoptotic BH-3 only proteins can promote apoptosis.

Regulation of intrinsic pathway of apoptosis

How Pro-apoptotic & Anti-apoptotic work in Intrinsic pathway of apoptosis



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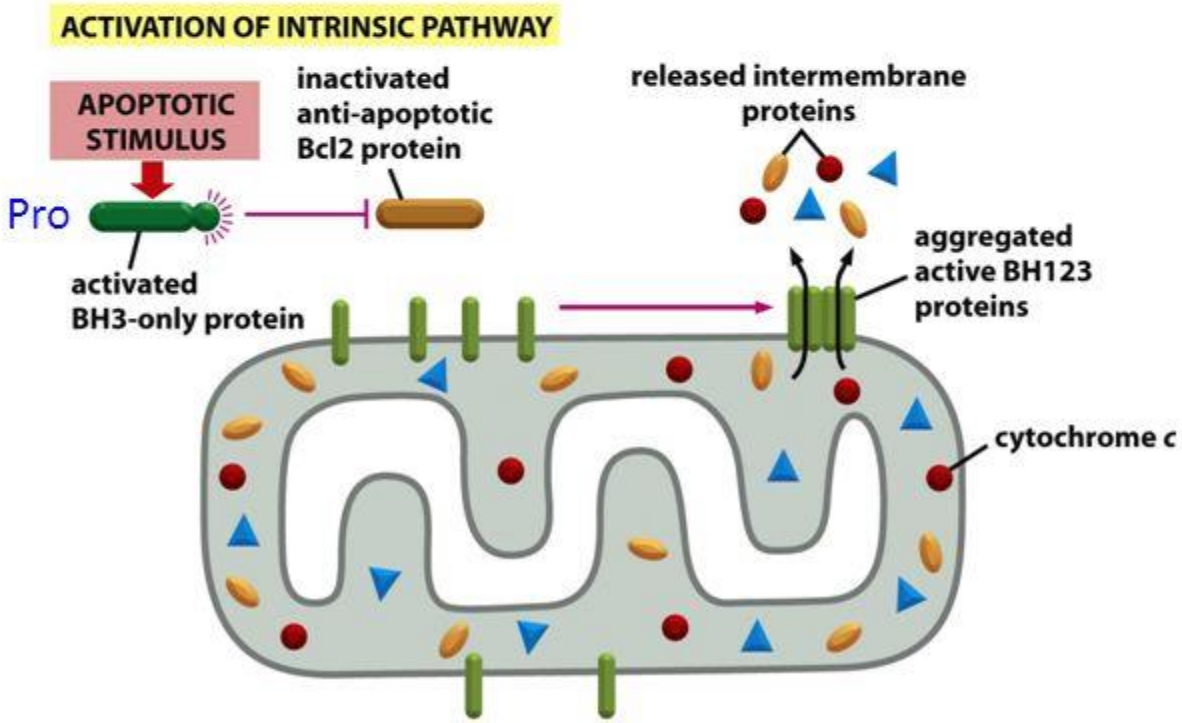
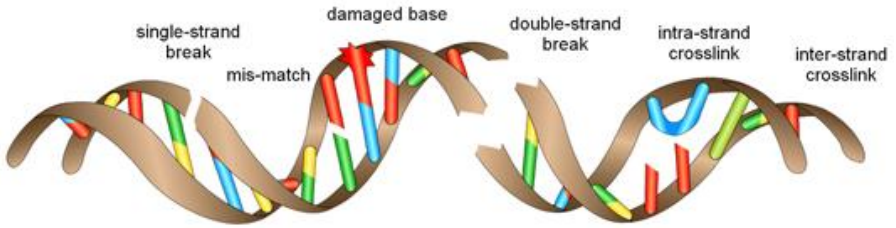


B

Regulation of intrinsic pathway

- In the absence of an apoptotic stimulus, anti-apoptotic Bcl_2 family proteins bind to and inhibit the apoptotic Bcl_2 family proteins on the mitochondrial outer membrane (Bak) and cytosol (Bax).
- In the presence of an apoptotic stimulus, BH3-only proteins are activated and bind to the anti-apoptotic Bcl_2 family proteins, the apoptotic Bcl_2 (Bak, Bax) become activated, aggregate in the outer mito memb, and promote the release of intermemb mito proteins into the cytosol.



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DNA repair

ATM
(Ataxia Telangiectasia mutated)

ATR
(Ataxia Telangiectasia and Rad 3 related protein).

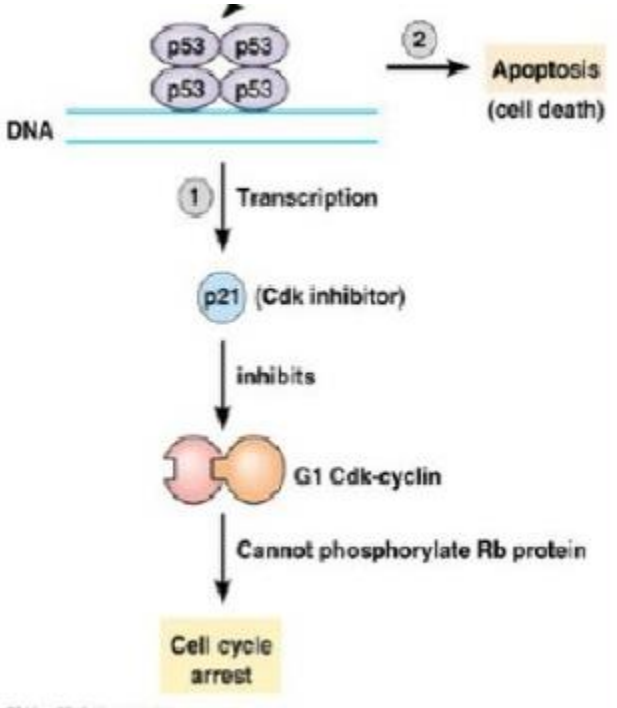
phosphorylation

checkpoint kinase 1 (chk1

checkpoint kinase2 (chk2)

serine/threonine kinases

tetramer



p53 is a Very Important Tumor Suppressor

Most commonly mutated gene in human cancer

"p53 is the Guardian of the Genome"

-Geoff Wahl, the Salk Institute

p53 is Activated in Response to DNA Damage

-p53 is a Transcription Factor for:

- -p21 (CKI)
- -DNA repair genes
- -Apoptotic (Cell Death) genes

In the absence of p53 cells cannot arrest after DNA damage

Intrinsic Pathway

- When DNA is damaged, it has to be repaired. If not repaired, p53 drives the cell towards apoptosis.
- DNA damage is sensed in the cell by two proteins ATM (Ataxia Telangiectasia mutated) and ATR (Ataxia Telangiectasia and Rad 3 related protein). Both these proteins are e.gs of serine/threonine kinases. They sense DNA damage and become activated. The activated ATM/ATR (serine/threonine kinases) will now add phosphate group to specifically two proteins; checkpoint kinase 1 (chk1) and checkpoint kinase2 (chk2). Chk1 and Chk2 are activated. They are also serine/threonine kinases. These proteins activate p53 (tumour suppressing proteins) by phosphorylating the serine/threonine residues on p53. Now, mdm2 (**Mouse double minute 2 homolog**) cannot bind with p53, which survives and forms tetramers.
- p53 is always present . It helps in DNA repair; it can hold cell division; it can causes apoptosis. mdm2 binds to p53 to inactivate it. mdm2 also cause ubiquitination of p53, so that it can be destroyed by proteasome.

Intrinsic Pathway

- p53 tetramer acts as a transcription factor and promote the expression of certain target genes such as: genes involved in DNA repair; p21 protein that arrests cell cycle; if DNA repair fails then it activates pro-apoptotic proteins.
- p53 increases the expression of BH-3 only proteins such as puma/noxa. These bind with and sequester Bcl2 anti-apoptotic proteins. Now Bcl2 cannot bind with Bak and Bax. Bak and Bax can now aggregate to form proapoptotic BH123 aggregates and allow Cyt c to come out into the cytoplasm.
- Cyt c binds with the Cyt c binding domain of the Apaf1 protein (Apoptotic protease activation factor1), freeing the CARD (Caspase recruitment domain). Seven such Apaf1 aggregate to form apoptosome.

Intrinsic Pathway

- Apoptosome recruits another protein--procaspase -9(initiator caspase)- which also specially has a CARD domain at amino terminus . These go and bind with apoptosome with CARD domain. This binding leads to the cleavage of procaspase-9, releasing the two subunits.
- Two large and two small subunits unite to form active Caspase-9, which activates other caspases. There is positive feedback leading to activation of more and more caspases- caspase cascade. Only few caspase 9 are enough to kickstart the apoptosis by activating other caspases.
- This is how intrinsic pathway is triggered in response to DNA damage.

Intracellular signals

Oxidative damage from free radicals, Radiation, Virus infection, Nutrient deprivation, Pro-apoptotic Factors

↓

Damage to the mitochondrial membrane increasing permeability

↓

Entry of Cytochrome C into the cytoplasm

↓

Cytochrome C binds to Apaf-1 forming an apoptosome

↓

Apoptosome activates procaspase-9 to caspase-9

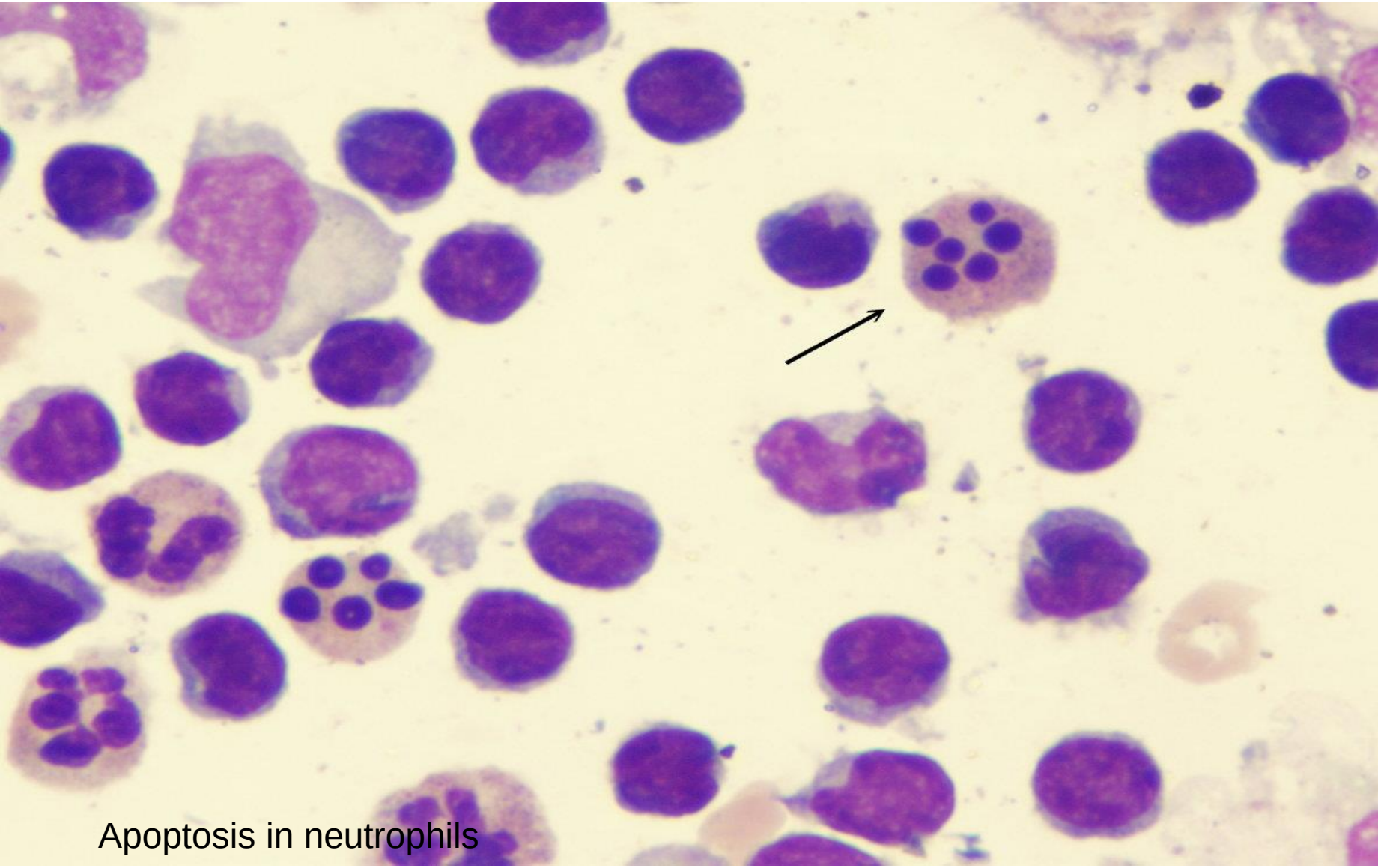
↓

Caspase-9 cleaves and activates caspase-3 and caspase-7.

↓

This executioner caspases activate a cascade of proteolytic activity that leads to: Chromatin condensation, DNA fragmentation, Protein cleavage, Membrane permeability

MICROSCOPY



Apoptosis in neutrophils

Disorders of apoptosis

Apoptosis: Role in Disease

TOO MUCH: Tissue atrophy

Neurodegeneration
Thin skin
etc

TOO LITTLE: Hyperplasia

Cancer
Athersclerosis
Auto-immune diseases
etc

- *High levels of anti-apoptotic proteins*

or

Low levels of pro-apoptotic proteins

===> CANCER

Apoptosis: Role in Disease
Neurodegeneration

- Large number of apoptotic cells that have been detected in the brains of patients with neurodegenerative disorders.

- involving oxidative stress, perturbed calcium homeostasis, mitochondrial dysfunction and activation of cysteine proteases called caspases

- PARKINSON'S DISEASE apoptosis of midbrain neurons

- ALZHEIMER'S DISEASE apoptosis of hippocampal neuron

- HUNTINGTON'S DISEASE apoptosis of neurons in striatum which control body movements
- AMYOTROPHIC LATERAL SCLEROSIS apoptosis of lower motor neurons

Apoptosis: Role in Disease

Cancer

Virus associated cancer

•Several human papilloma viruses (**HPV**) have been implicated in causing cervical cancer. One of them produces a protein (E6) that binds and inactivates the apoptosis promoter **p53**.

•**Epstein-Barr Virus** (**EBV**), the cause of mononucleosis and associated with some lymphomas

- produces a protein similar to Bcl-2
- produces another protein that causes the cell to increase its own production of Bcl-2. Both these actions make the cell more resistant to apoptosis (thus enabling a cancer cell to continue to proliferate).

Apoptosis: Role in Disease
Cancer

- Some **B-cell leukemia** and lymphomas express high levels of **Bcl-2**, thus blocking apoptotic signals they may receive. The high levels result from a translocation of the *BCL-2* gene into an **enhancer** region for antibody production.

- **Melanoma** (the most dangerous type of skin cancer) cells avoid apoptosis by inhibiting the expression of the gene encoding **Apaf-1**.

Apoptosis: Role in Disease

Aging

Aging --> both too much and too little apoptosis (evidence for both)

Too much (accumulated oxidative damage?) ---> tissue degeneration

Too little (defective sensors, signals? ---> dysfunctional cells accumulate hyperplasia (precancerous lesions)

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- Youtube videos