

# Conditions Leading to Hypertrophy

Increased functional demand or increased trophic signalling results in adaptive increases in cell or organ size.

**!!!! excess nutrient intake only causes increased fat.**

## Increased Functional Demand

Human skeletal muscle is composed of a mixture of slow-twitch (type I) and fast-twitch (type II) fibers.

Each responds to different types of increased functional demand.

Endurance training with light loads increase the strength of type I fibers functioning with aerobic metabolism mediated by mitochondria.

Weightlifting with large weight loads leads to hypertrophy of type II fibers functioning by anaerobic glycolysis. This is associated with increases muscle mass.

# Hypertrophy

- Increase the size of the cells and consequently the size of the organs.
- Can be physiologic (e.g. increase workload during exercise, uterine myometrium during pregnancy)
  - Increased the synthesis of structural protein and organelles



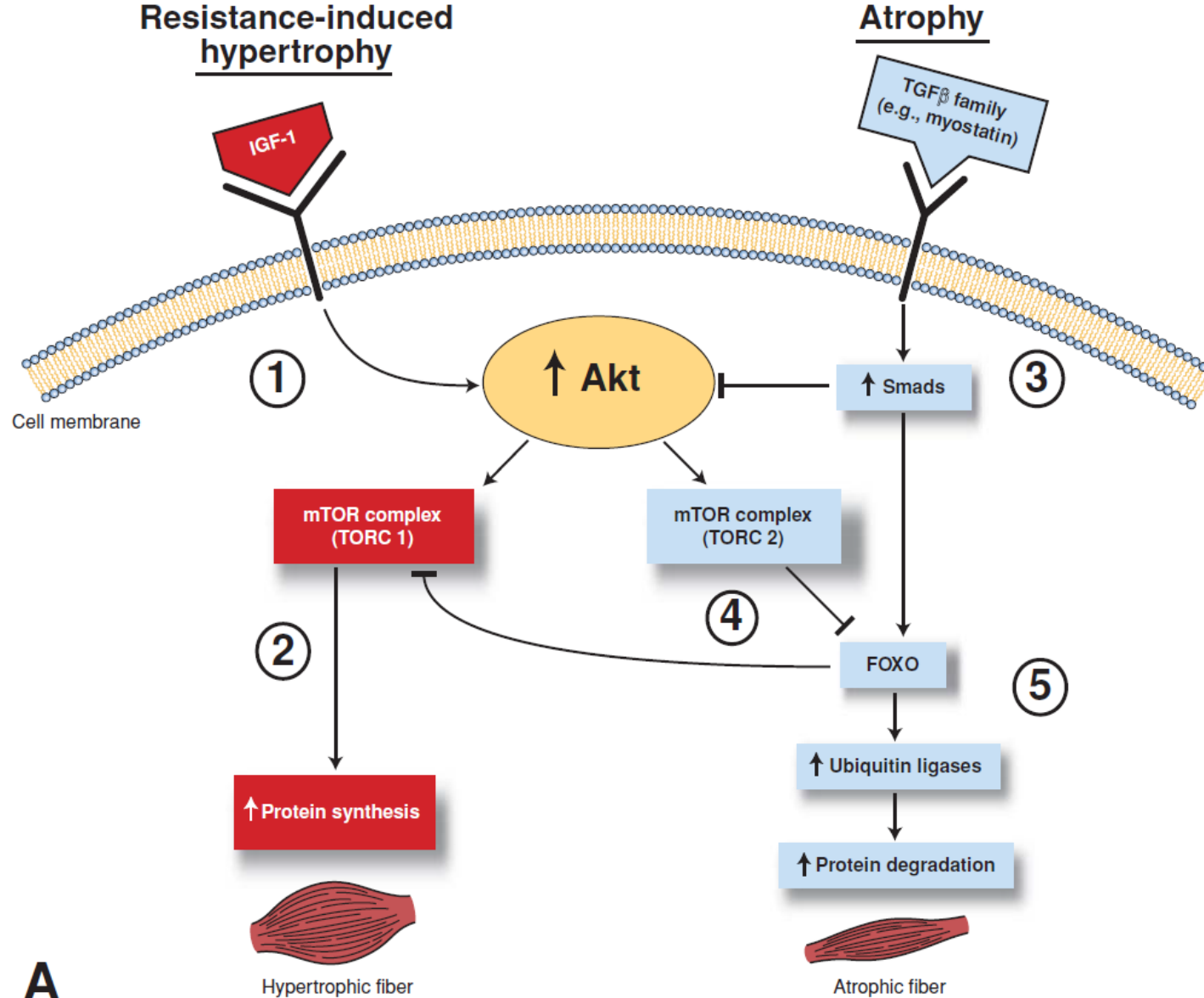
## **Increased trophic signals**

Cells and organs that respond to soluble mediators, such as the thyroid (TSH) or the breast (estrogen and progestins), undergo hypertrophy when levels of trophic hormones increase.

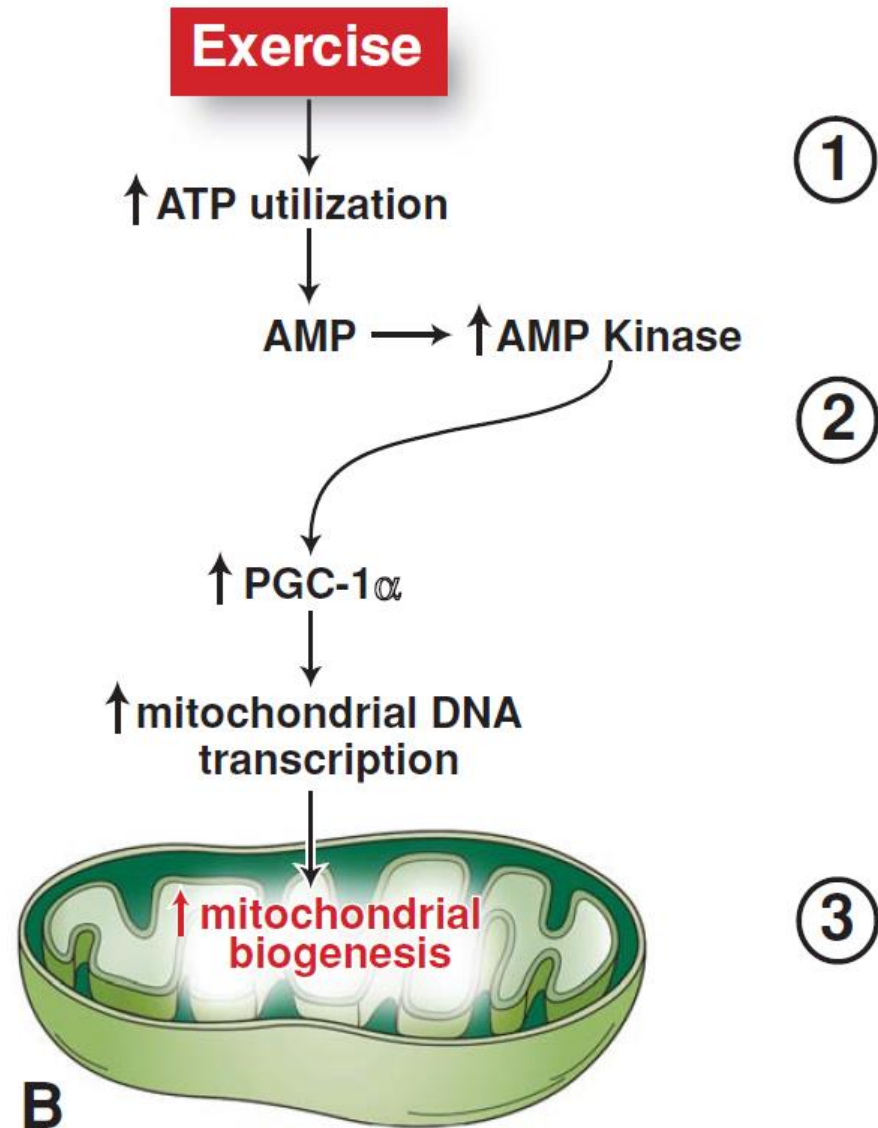
## **Puberty**

The onset of puberty, especially in boys, leads to greater muscle mass. The surge in androgens and growth hormone (GH) raises levels of downstream mediators and consequently increases the mass of muscle and other tissues.

# Interrelationship between muscle atrophy and hypertrophy.



# Akt-independent mechanism of muscle hypertrophy and prevention of atrophy



## **Mechanisms of cellular hypertrophy:**

- Accelerated degradation specifically proteins that do not contribute to the need for hypertrophy.
- Production of proteins that promote hypertrophy tends to increase.

## Signaling mechanisms in hypertrophy

■ Growth factor stimulation: Each tissue responds to different signals.

Certain growth factors are key initiators of hypertrophy.

■ Neuroendocrine stimulation: Especially in the heart, adrenergic signaling may be important in initiating or facilitating hypertrophy.

■ Ion channels: Ion fluxes may activate adaptation to increased demand. Calcium may stimulate a host of downstream enzymes.

## **Mechanisms of cellular hypertrophy (continue):**

- Other chemical mediators: NO•, angiotensin II and bradykinin tend to support hypertrophic responses in some tissues.
- Oxygen supply: Increased functional demand requires increased energy supply. Oxygen deficit will trigger angiogenesis to increase oxygen delivery. Angiogenesis is a key component in adaptive hypertrophy.

## **Effector pathways in hypertrophy**

### **■ Increased protein degradation:**

The ubiquitin–proteasome system (UPS), activation of intracellular proteases and autophagy contribute to hypertrophy.

**■ Increased protein translation:** Production of certain proteins increases via increased translational efficiency without changes in RNA levels.

Activities of translational initiators and elongation factors are often stimulated early in hypertrophy to quickly raise levels of specific proteins needed to meet the increased functional demand.

**■ Increased gene expression:** transcriptional upregulation also contribute to increase the concentrations of key proteins.

Activation of transcription factors and increased transcription of genes encoding growth promoting transcription factors, such as Fos and Myc.



## **Effector pathways in hypertrophy (continue).**

■ **Survival:** During hypertrophy, cell death is inhibited.

Stimulation of specific receptors activates Akt, PI3K that promote cell survival, largely by inhibiting programmed cell death.

■ **Extracellular matrix:** In some situations hypertrophy require the extracellular matrix remodelling to adjust the cell environment.

■ **Recruitment of satellite cells:** Skeletal muscle hypertrophy includes recruiting perimuscular satellite cells (muscle stem cells) that fuse with myocyte syncytia to provide additional nuclei to support the expanded protein synthetic needs of the enlarging muscle.

# Atrophy and Hypertrophy Impact on Similar Signaling Pathways

## Molecular Mechanisms in Atrophy

Atrophy is a cell's reversible restructuring of its activities to facilitate its own survival and adapt to conditions of diminished use.

- Muscle disuse increases extracellular myostatin, a member of the transforming growth factor- $\beta$  (TGF- $\beta$ ) family.
  - Myostatin binding activates its receptor inhibits Akt while unleashing FOXO.
  - FOXO activation increases production of ubiquitin ligases (E3) to mediate the degradation of muscle proteins by proteasomes.
- Protein synthesis: synthesis of certain proteins declines; at the same time, production of other proteins that mediate this adaptation may increase.

## **Molecular Mechanisms in Atrophy (continue).**

### ■ Protein degradation:

Ubiquitin-related specific protein degradation pathways are activated as part of atrophic responses.

Proteasomal degradation of muscle actomyosin is greatly enhanced by prior actomyosin cleavage by caspase-3 or calpain.

If the atrophic state is maintained, cells reach a new equilibrium in which mass remains decreased and rates of protein synthesis and degradation realign.

■ Energy utilization: A selective increase in use of free fatty acids as an energy source for muscle occurs during response to unloading.

**Atrophy is thus an active, specific adaptive response rather than a passive shutdown of cellular processes.** It is also reversible if the environment that existed before atrophy developed is restored, myocytes reassume their prior size and function.

## **Loss of Muscle Mass Commonly Results from Disease**

Loss of 40% of body mass is usually fatal. Even a decrease of 5% in lean body mass can impair function.

A number of conditions are characterized by loss of body mass although the pathways that are implicated may differ.

## **Cancer-related weight loss and cross-talk between adipose tissue and Muscle.**

Over 80% of patients with gastric and pancreatic cancers.

50% of those with lung and colorectal cancers.

Loss of adipose tissue and muscle is seen in cachexia (wasting) occurring in patients with advanced cancers.

Tumor-induced lipolysis and energy utilization from adipose tissue release cytokines that initiate muscle atrophy.

**If such lipolysis is prevented experimentally, muscle mass is preserved.**

## **Loss of Muscle Mass Commonly Results from Disease (continue).**

- **Congestive heart failure (CHF):** In cardiac cachexia, type I (mitochondria-rich) muscle fibers are most affected.
- **Chronic obstructive pulmonary disease (COPD).**
- **AIDS:** Before the introduction of effective antiretroviral therapy, wasting was the initial defining presentation in AIDS in 1/3 of patients. This may reflect the energy needed to mount continuous acute phase inflammatory responses including production of inflammatory mediators and decreased hepatic IGF-I production.
- **Rheumatoid arthritis (RA):** RA, the most common adult autoimmune disease is associated with increased production of many catabolic cytokines ( TNF- $\alpha$ , IL-1 $\beta$ , IL-6).

## Loss of Muscle Mass Commonly Results from Disease (continue).

■ **Aging:** Loss of muscle mass in aging, or **sarcopenia**, is universal and distinct from disease-related cachexia.

Aging-related sarcopenia affects type II (fast-twitch) muscle fibers.

- Reduced protein synthesis.
- Loss of spinal cord motor units.
- Altered production of and response to anabolic hormones and cytokines.

Interestingly, treating elderly patients with

Inhibitors of angiotensin-converting enzyme but not other types of antihypertensive agents tends to preserve muscle strength, suggesting that the angiotensin system may play a role in sarcopenia.

## **Postmitotic cells versus terminal differentiation:**

Neurons and cardiac myocytes are terminally differentiated cells and may not undergo mitosis.

Committed progenitor cells in the brain and heart can proliferate and differentiate in response to cell loss and injury or, in the case of striated muscle, increased functional demand.

There is a natural, albeit low, rate of cell loss and replacement among cells that were once considered irreplaceable.

When the kinetic of such replacement favours cell loss, organ atrophy results, as in the heart, muscle and brain of the very aged.

If progenitor cell activity predominates (the skeletal muscle) hypertrophy may result.

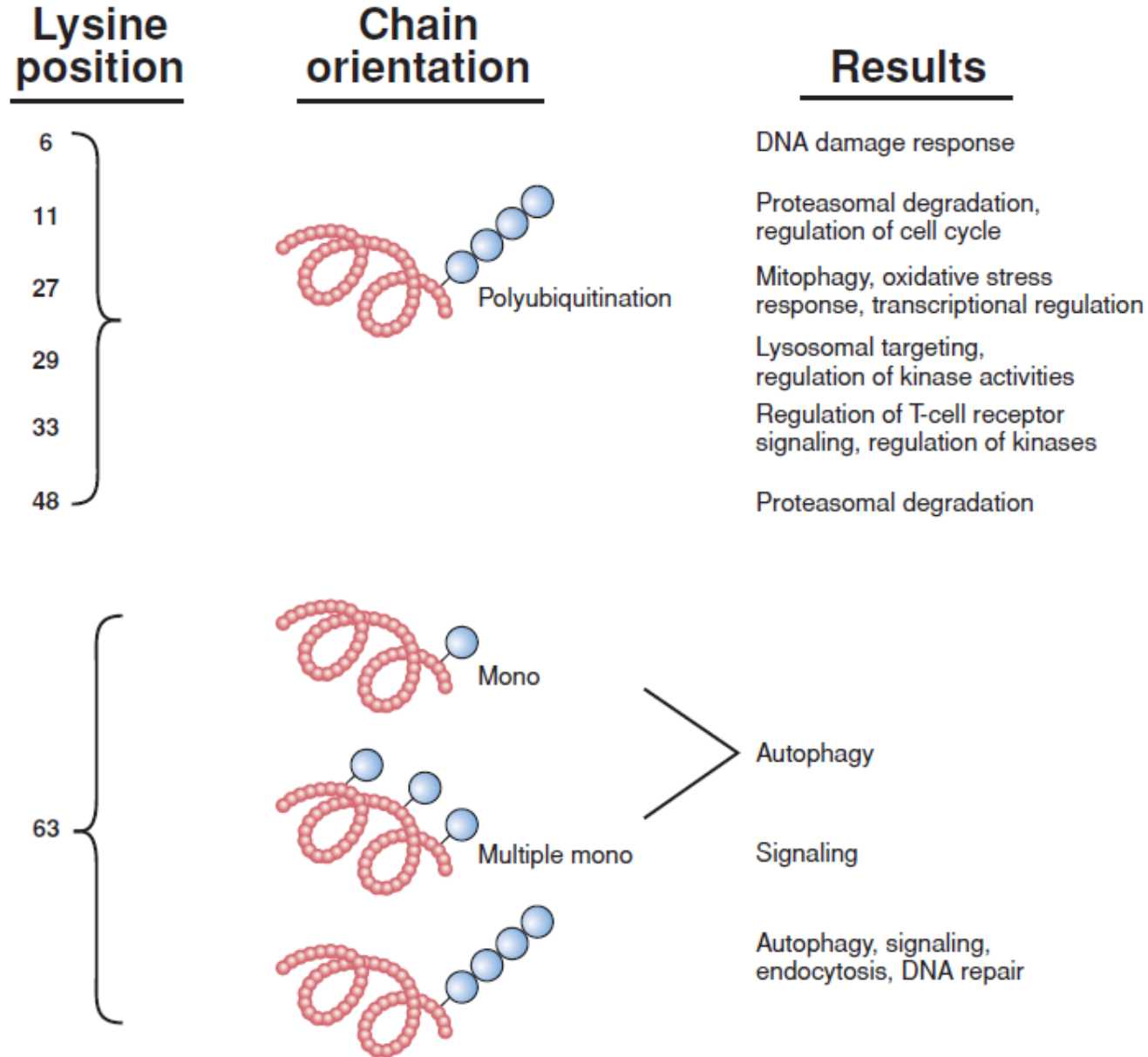
# UBIQUITIN AND THE UBIQUITIN-PROTEASOME SYSTEM



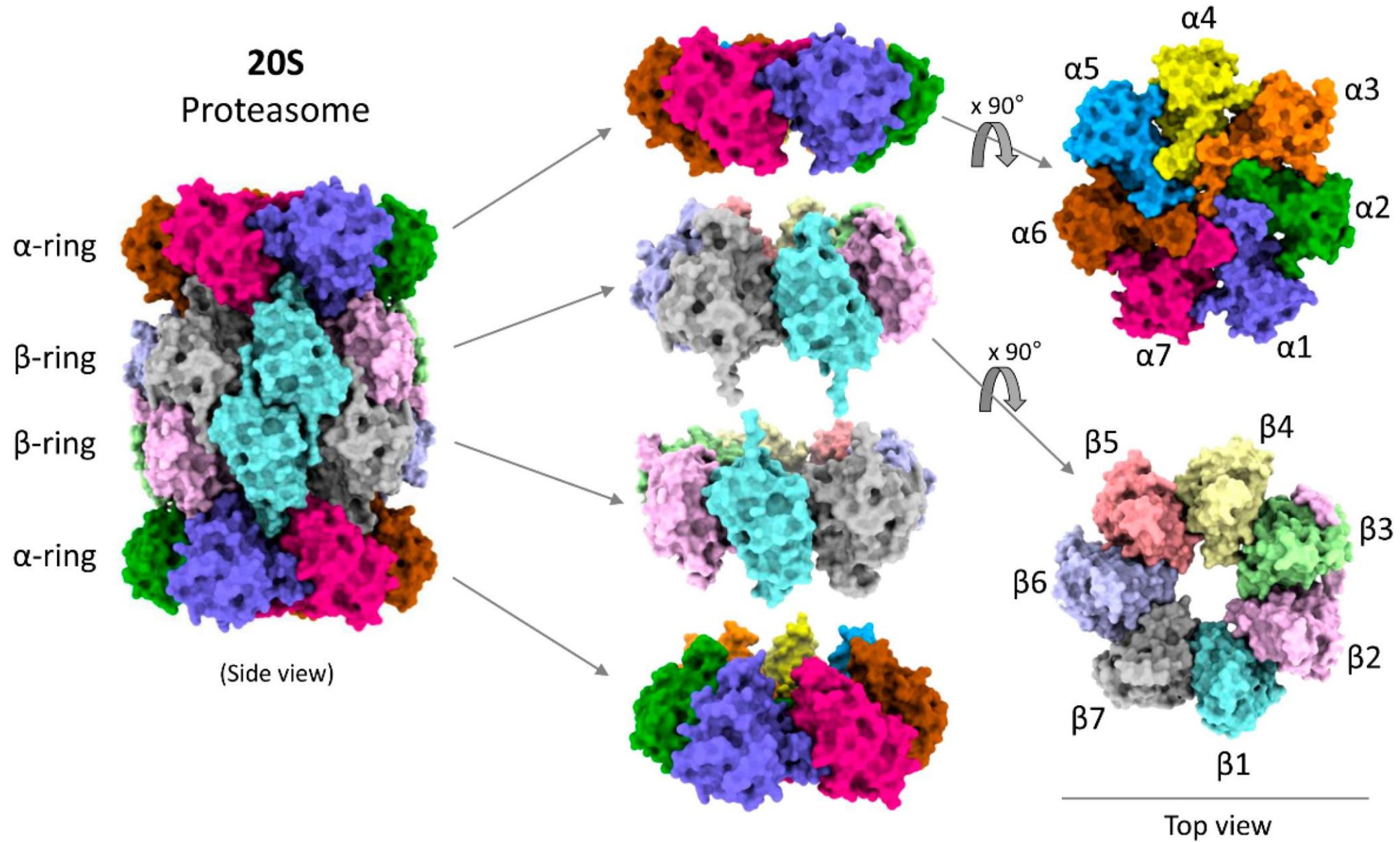
# Mechanisms of Ub conjugation to target proteins

1. Ub-activating enzyme, E1, binds to Ub and transfers it to Ub-conjugating enzymes (E2). There are dozens of E2
2. Ub-E2 conjugate + Ub-ligating enzymes (E3) add Ub to a lysine on target proteins.
3. There are more than 800 E3.

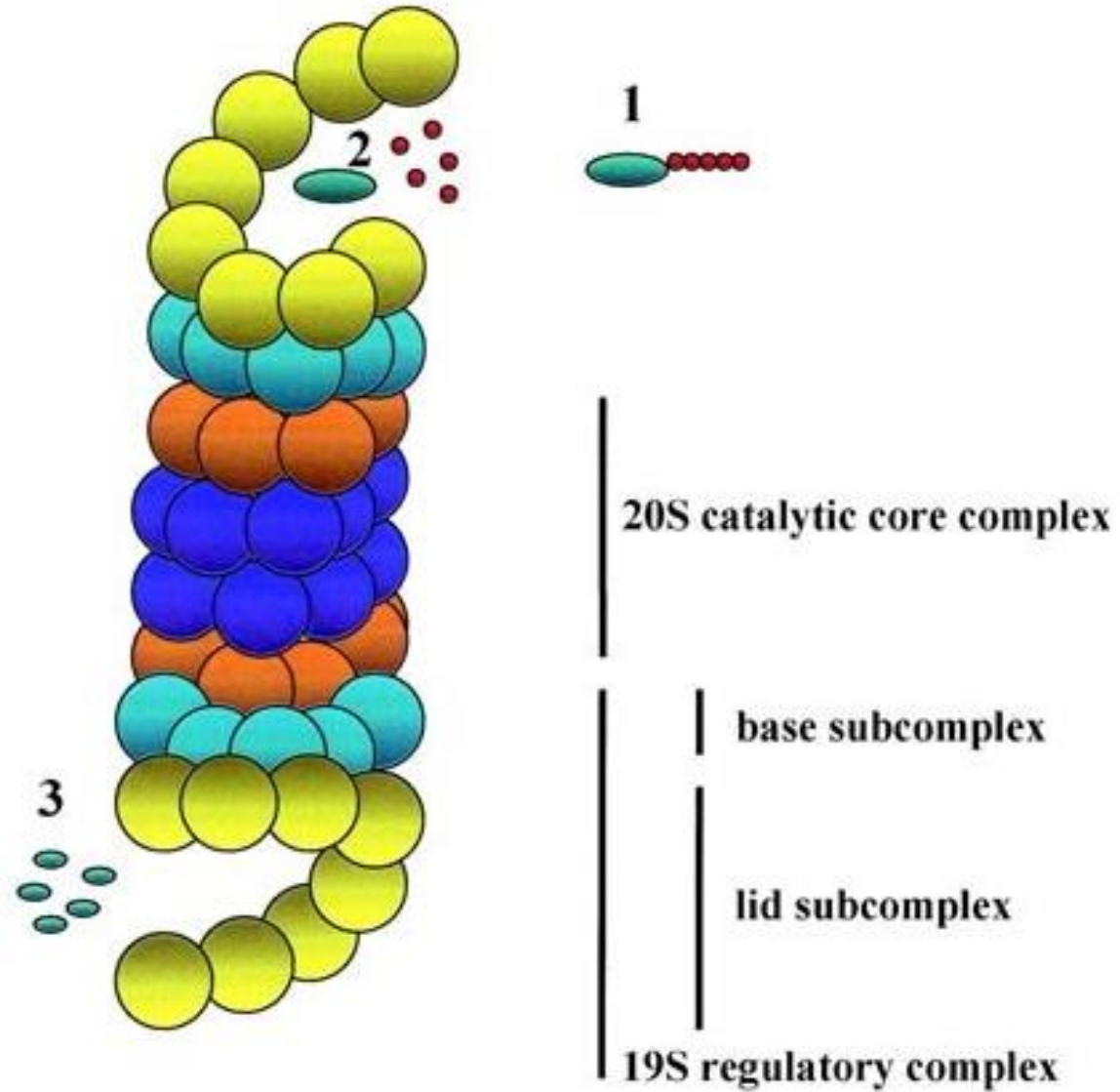
# The diversity of ubiquitination and its consequences.



# 20S Proteasomes



# 26S Proteasomes



# Proteasomes

Proteasomes are highly conserved organelles in the cytoplasm and the nucleus.

They are barrel-shaped complexes whose main function is to digest polyubiquitinated proteins.

Proteasomes 20S and 26S.

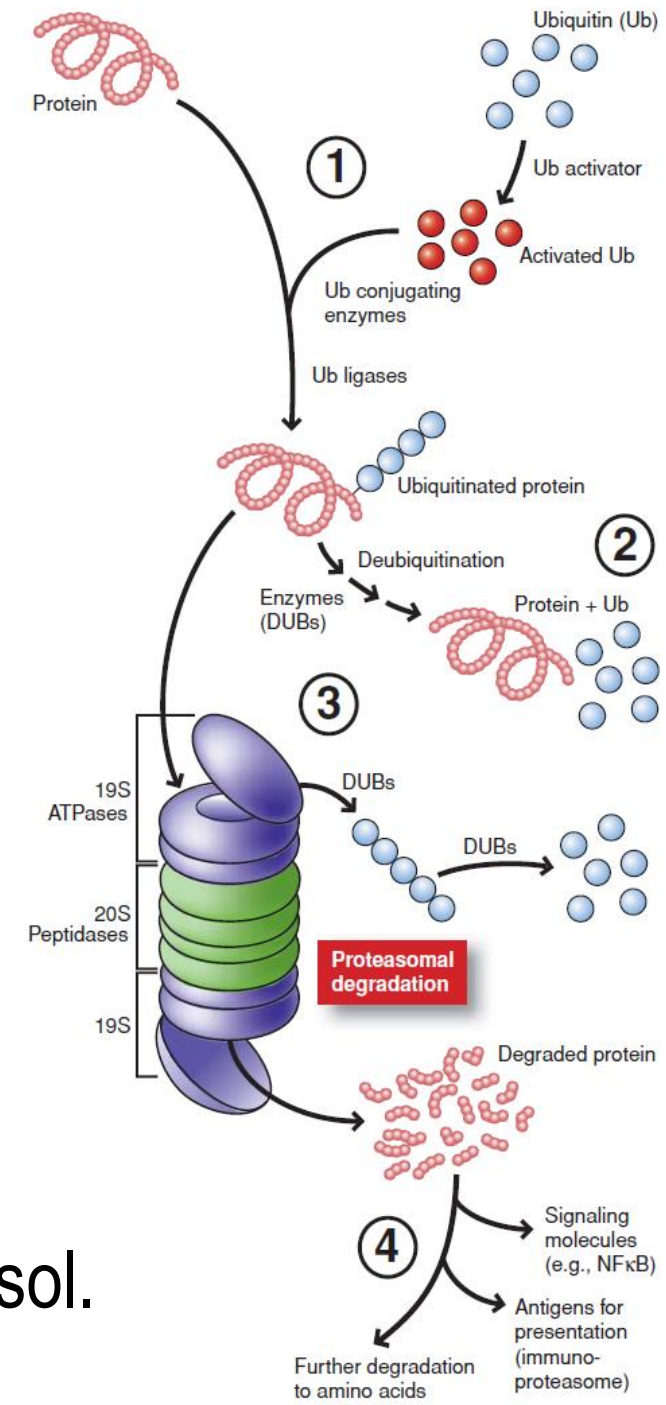
The degradative unit of both is a 20S destruction chamber,

26S proteasome, include the 20S + two 19S “caps”

The caps at the entrance to the proteolytic core regulate entry.

The 20S proteasomes lack these caps.

Produces peptides of 3 to 25 AA, which are released in the cytosol.



**Proteasomes make up 1–2% of the total mass of the cell.**

***Mutations that interfere with normal proteasomal function are lethal.***

The 20S proteasomes are important in degradation of **oxidized proteins**.

In 26S proteasomes is also called the immunoproteasome which is induced by interferon- $\gamma$  (IFN- $\gamma$ ) and degrade **polyubiquitinated proteins**.

Process protein antigens into peptides that attach to major histocompatibility complex (MHC) type I for presentation to the immune System.

- Incorrectly folded.
- Damaged.
- Reached the end of their usefulness.
- Need to be destroyed for some other reason.

Proteasomes specifically eliminate proapoptotic molecules to maintain cellular viability.

# Deubiquitinating Enzymes

Deubiquitinating enzymes (DUBs) are proteases that remove Ubs from poly-Ub chains and their partner proteins.

There are about 100 DUBs known that reverse the effects of ubiquitination.

Some pathogens can control Ub/DUB pathways at multiple points.

Certain bacterial proteins resemble E3 Ub ligases and activate ubiquitination to facilitate invasion and pathogenicity.

*Salmonella typhimurium*, *Chlamydia trachomatis* and herpes simplex virus encode proteins that act as DUBs, suggesting that interference with cellular ubiquitination may confer a selective advantage to these pathogens.

# Autophagy, a form of controlled cellular cannibalism.

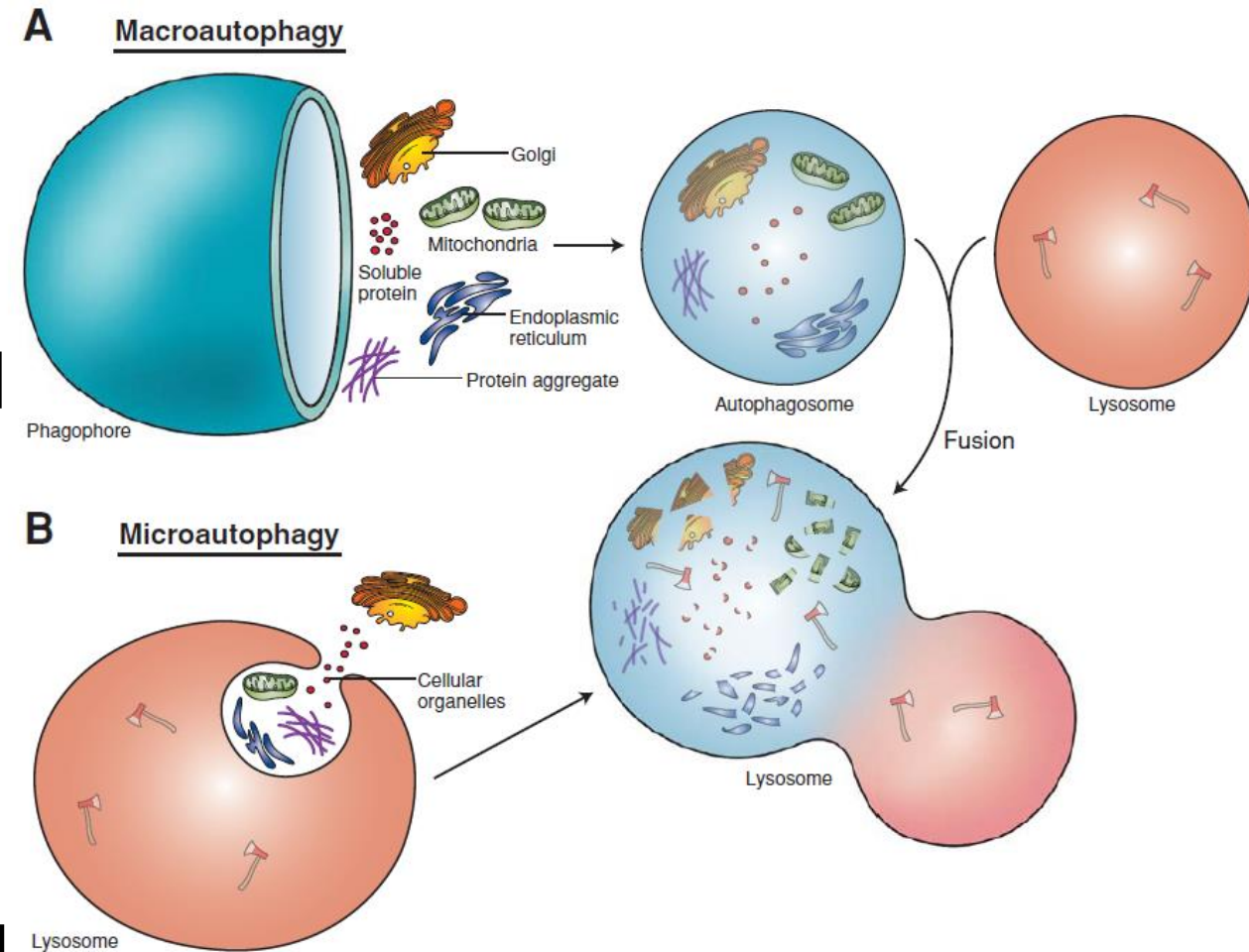
Crucial role in the balance between cell survival, death and adaptation.

**Autophagy** highly conserved catabolic process by which cytoplasmic targets are recognized and delivered to lysosomes for digestion.

Autophagic degradation is generally divided into three categories based on both the cargoes involved and how they arrive at lysosomes.

Macroautophagy hands bulk portions of cytoplasm.

damaged cellular organelles, aggregated, proteins and other injurious materials.



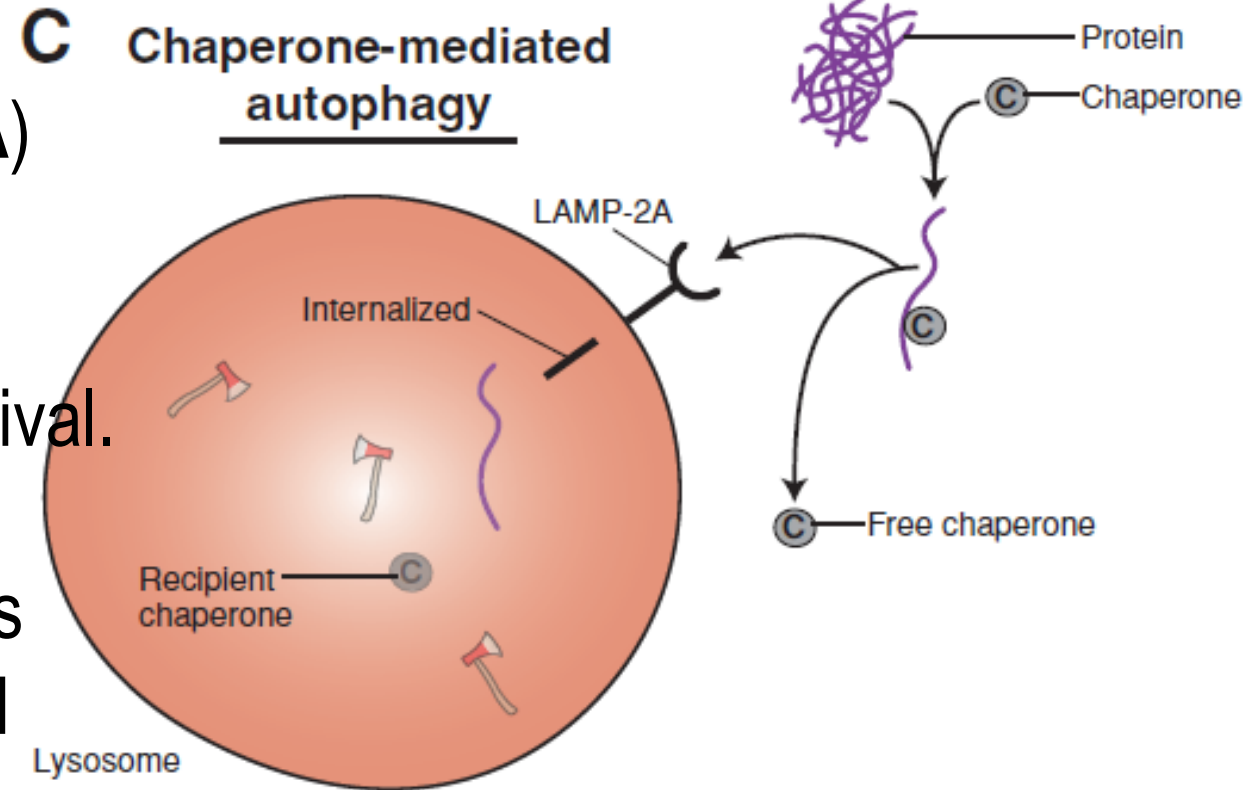


some defective proteins require interaction with molecular chaperones to enter the autophagic system  
via **chaperone-mediated autophagy (CMA)**

Autophagy systems operate continuously.  
Are obligatory for cell homeostasis and survival.

Bulk autophagy protects cells when nutrients are lacking, as in starvation or compromised blood supply.

Other forms of autophagy maintain functional homeostasis among cellular proteins and organelles in normal and in times of stress.



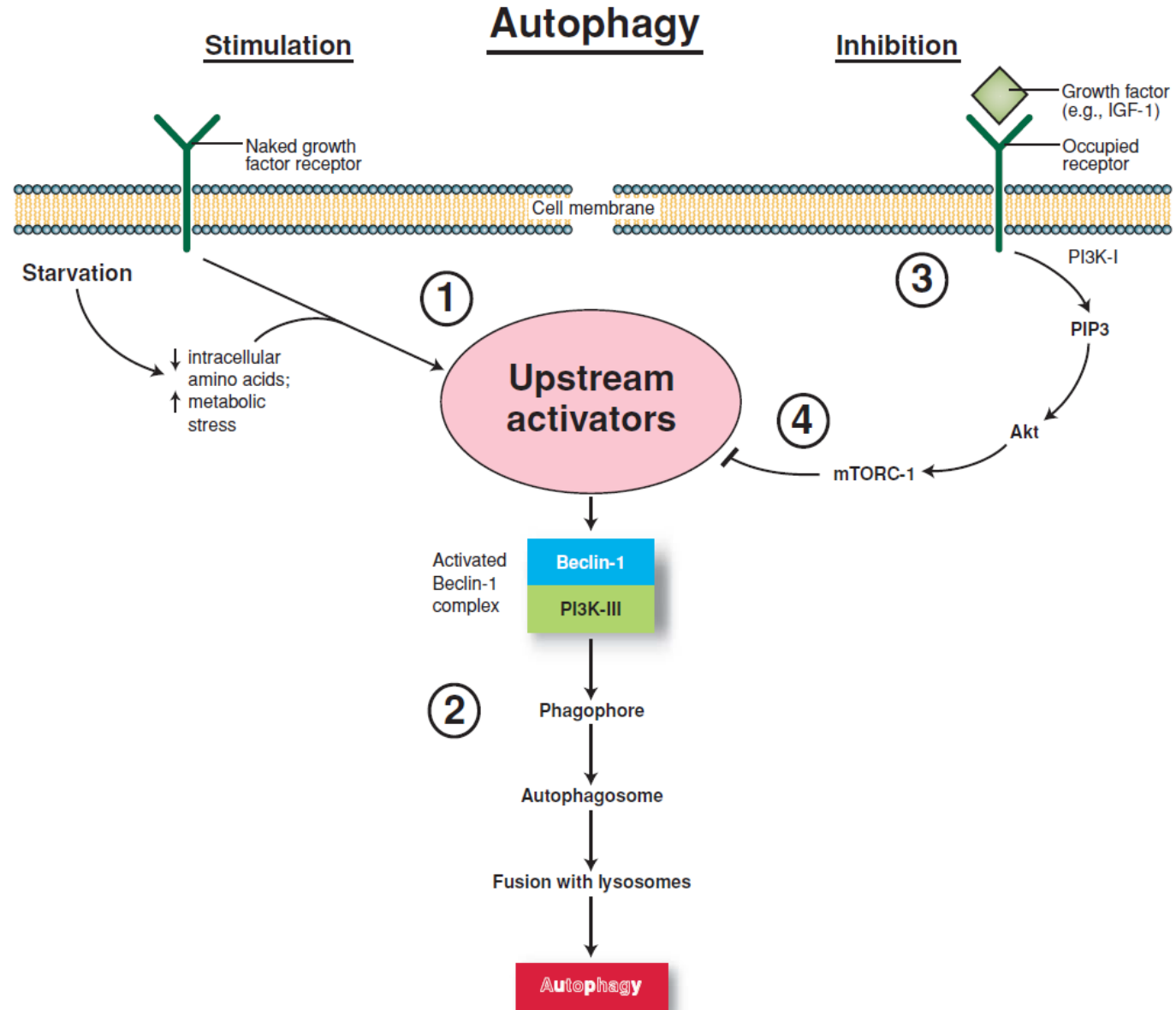
Autophagic pathways is an ongoing physiologic quality control mechanisms.

- Protect from excess production of ROS from damaged mitochondria.
- Essential both for basal cellular physiology and for adaptation to adversity,
  - Starvation
  - Ischemia
  - Recycling nutrients from cellular organelles and macromolecules
  - Clearance of misfolded or damaged proteins and organelles
  - Antigen presentation
  - Protection from tumorigenesis
  - Protection from neurodegeneration

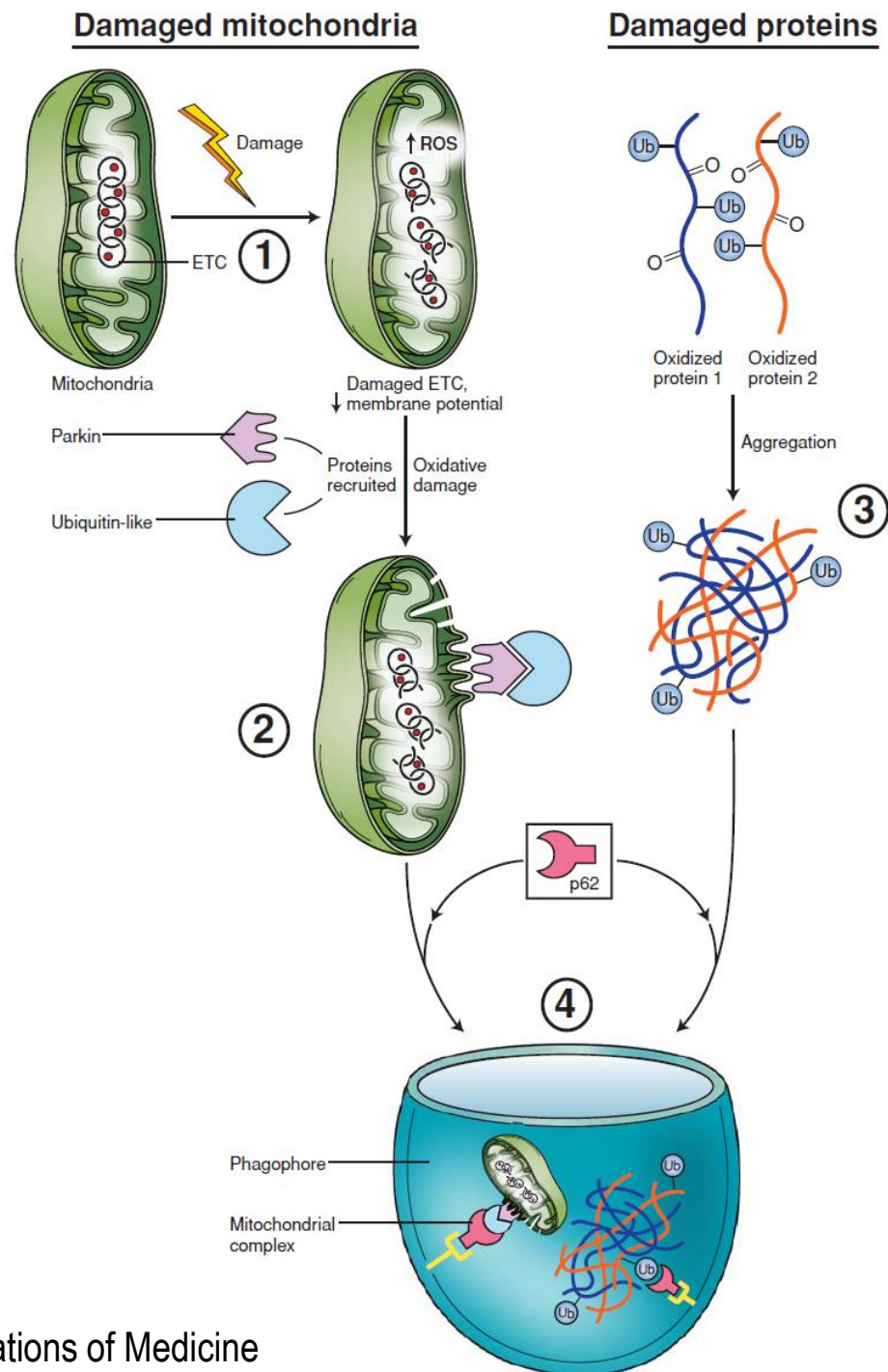
**Impairment of any form of autophagy may lead to accumulation of abnormal proteins and defective organelles. The result may be cell death and disease.**

It is a form of “programmed cell survival.” Under some circumstances, it may give rise to self-cannibalism as a form of cell death.

# Macroautophagy: Mostly non-selective.



# Identifying Targets in Macroautophagy



## **Crosstalk among degradative pathways.**

- Short-lived proteins are generally specifically digested by the UPS.
- Longer-lived proteins are selectively removed by autophagy.

If one system is compromised, the other may compensate, at least in part.

The UPS cannot handle protein aggregates or large cytoplasmic structures, like organelles or endocytosed foreign matter (bacteria).

These systems complement each other.

Impairment of one autophagy pathway lead to compensatory activation of the others.  
*Both the autophagic pathways and the UPS operate continuously, and inhibition of either often has harmful consequences.*

## **Cells invest a great deal of resources to maintain protein homeostasis.**

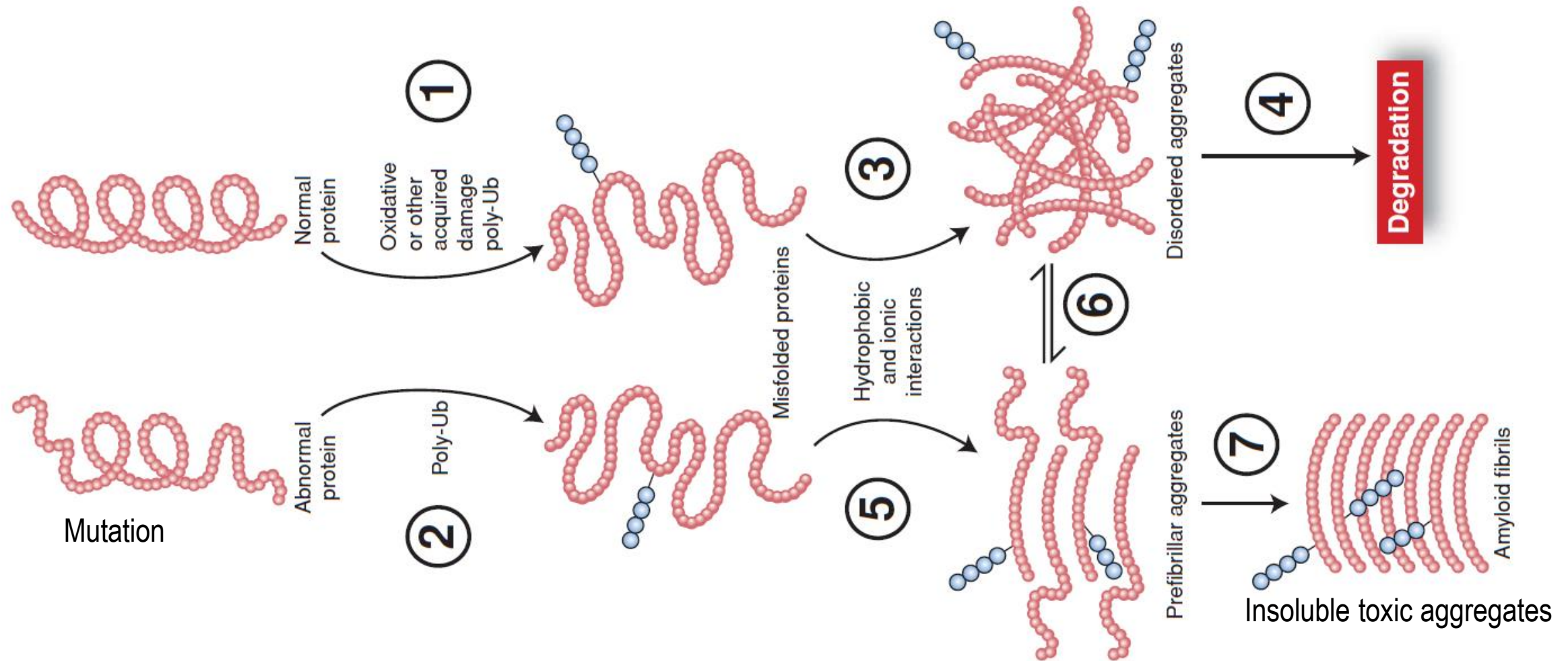
Ribosomes translate messenger RNA (mRNA) into a linear chain of AA without a defined three-dimensional structure.

It is energetically more favorable to produce many foldings, even abnormal ones, and then edit the protein repertoire than to construct only a single correct conformation.

Protein misfolding occurs continuously and are refold by chaperons.

Massive accumulation of misfolded proteins within the cell leads to cell injury. If not resolved to cell death occurs.

# Formation of toxic protein aggregates



- **Neurodegenerative diseases:** Mutations in proteins of the autophagic pathways & proteinopathies.
- **Ageing:** Macroautophagy and CMA decline with age, account for age-related changes in organ systems.
- **Pancreatitis:** Impaired autophagy in this necroinflammatory disease may be responsible for inappropriate conversion of trypsinogen to trypsin.
- **Infectious diseases:** Autophagy contribute to host defense mechanism against pathogens. It is a target of evasion or subversion strategies by pathogens.
- **Crohn disease:** Impair bacterial clearance and promote increased production of proinflammatory mediators.
- **Cancer** (double-edged sword). Beclin-1 and some Atg genes are tumor suppressor, are lost in certain cancers. Autophagy can also protect cancer cells during starvation.



# Cell Death

# Cell Death

- Cell's death is often needed for an organism to live.
- It is crucial for both development and survival of multicellular organisms.

Cell death can represent the consequences of non-physiological and unregulated injury.

This unplanned murder of a cell by external violence, which is called **necrosis**.

Cell death can also be the response of complex intracellular molecular pathways to external and internal triggers to control the size and diversity of many tissue and compartments by eliminating obsolescent cells or cells causing a threat to the organism.

These suicide programs have been identified as **apoptosis, autophagic cell death, necroptosis, NETosis** ....

## **Necrosis is reflected in geographic areas of cell death**

Necrosis occurs when hostile external forces overwhelm cells' adaptive abilities.

Diverse insults can cause necrotic cell death, which typically **affects localized groups of cells.**

**The response** to necrotic cell death is **usually acute inflammation**, which itself may generate further cell injury.

The stimuli leading to necrosis are highly variable and produce diverse and recognizable histologic and cytologic patterns.

# Coagulative Necrosis

*Refers to specific light microscopic appearances of dead or dying cells.*

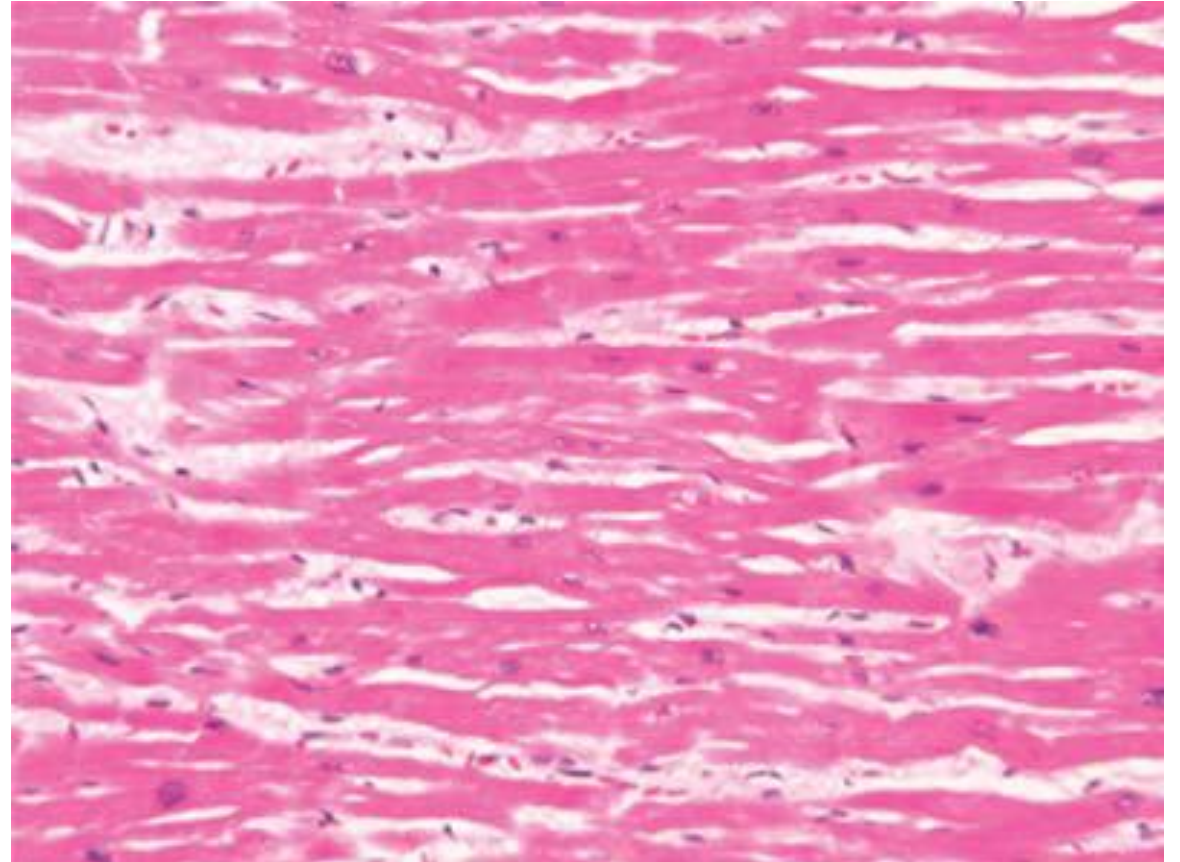
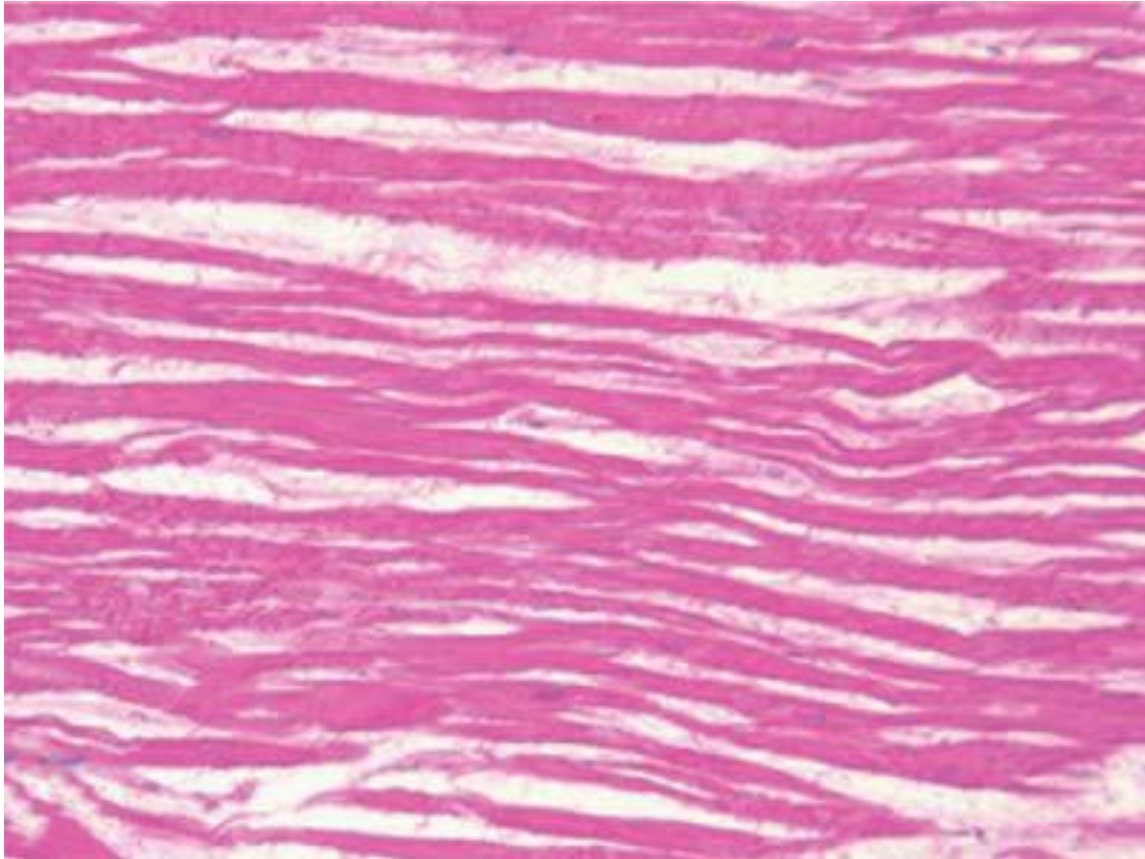
- Outline is maintained.
- Cytoplasm of a necrotic cell is more deeply eosinophilic than usual.
- Nuclear chromatin clumped and redistributes along the nuclear membrane.

■ **Pyknosis:** The nucleus becomes smaller and stains deeply basophilic as chromatin clumping continues.

■ **Karyorrhexis:** The pyknotic nucleus breaks up into many smaller fragments scattered about the cytoplasm.

■ **Karyolysis:** The pyknotic nucleus may be extruded from the cell or it may progressively lose chromatin staining.

# Coagulative necrosis: Normal heart versus Myocardial infarction



## **Liquefactive Necrosis**

Rate at which necrotic cells dissolve greatly exceeds the rate of repair, the resulting appearance is termed **liquefactive necrosis**.

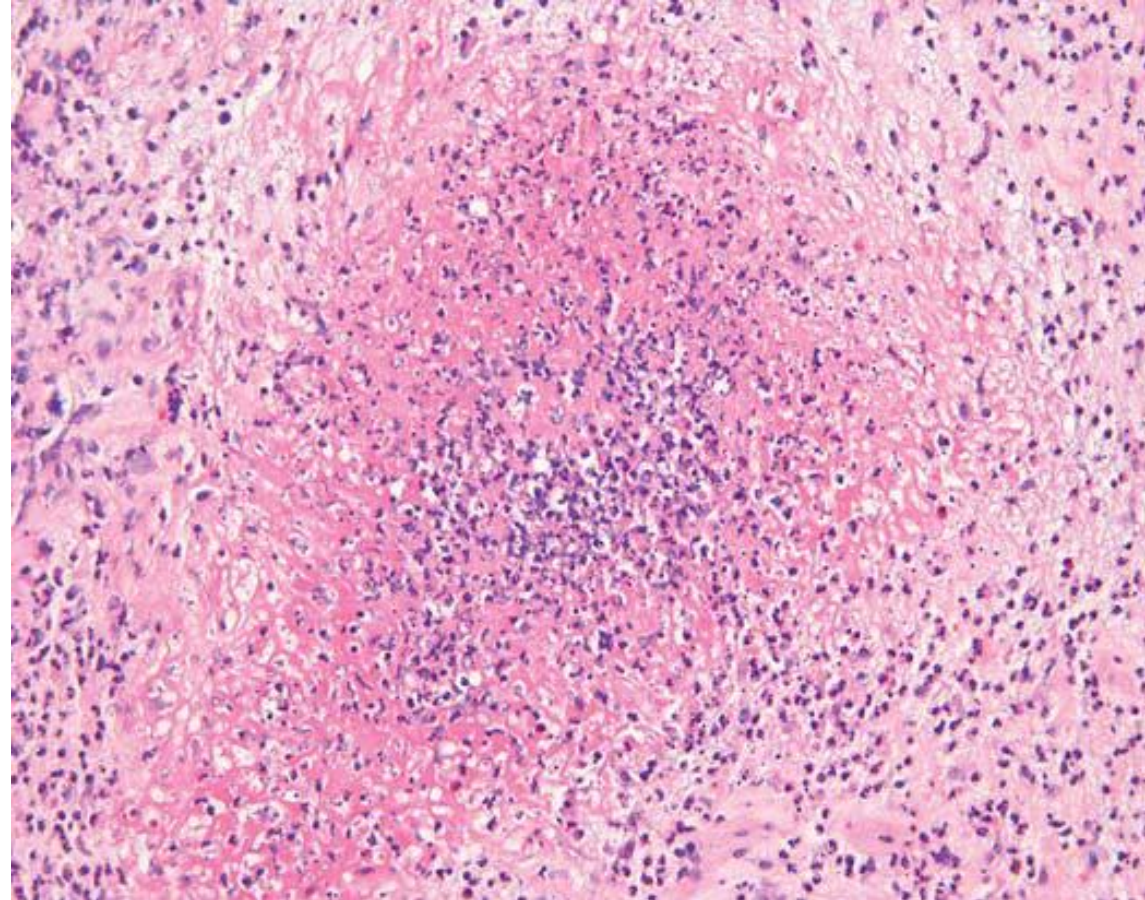
Polymorphonuclear leukocytes of the acute inflammatory reaction contain potent hydrolases capable of digesting dead cells.

Localized collection of these acute inflammatory cells in response to bacterial infection, produces rapid cell death and tissue dissolution resulting in an **abscess**.

The abscess is walled off by a fibrous capsule that contains its contents.

Coagulative necrosis of the brain may occur after cerebral artery occlusion and is often followed by rapid dissolution liquefactive necrosis independently of acute inflammatory response.

# Liquefactive necrosis in an abscess of the skin



# Fat Necrosis

Specifically affects adipose tissue and most commonly results from pancreatitis or trauma.

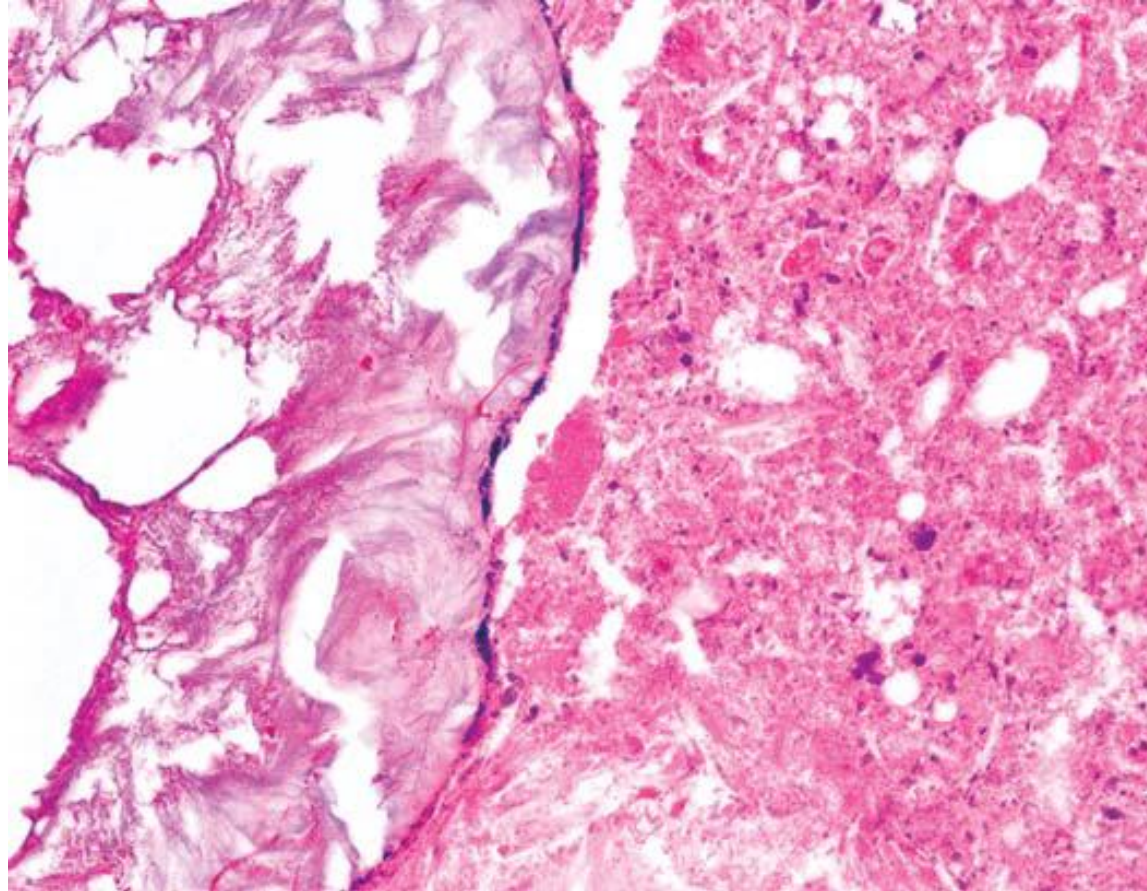
Process begins when digestive enzymes that are normally found only in the pancreatic duct and small intestine lumen are released from injured pancreatic acinar cells and ducts into extracellular spaces.

Upon extracellular activation, these enzymes digest both the pancreas itself and surrounding tissues, including adipocytes.

1. Phospholipases and proteases attack plasma membranes of adipocytes, releasing their stored triglycerides.
2. Pancreatic lipase hydrolyzes the triglycerides releasing free fatty acids.
3. Free fatty acids bind  $\text{Ca}^{2+}$  and precipitate as soaps giving basophilic deposits at the edges of irregular islands of necrotic adipocytes.

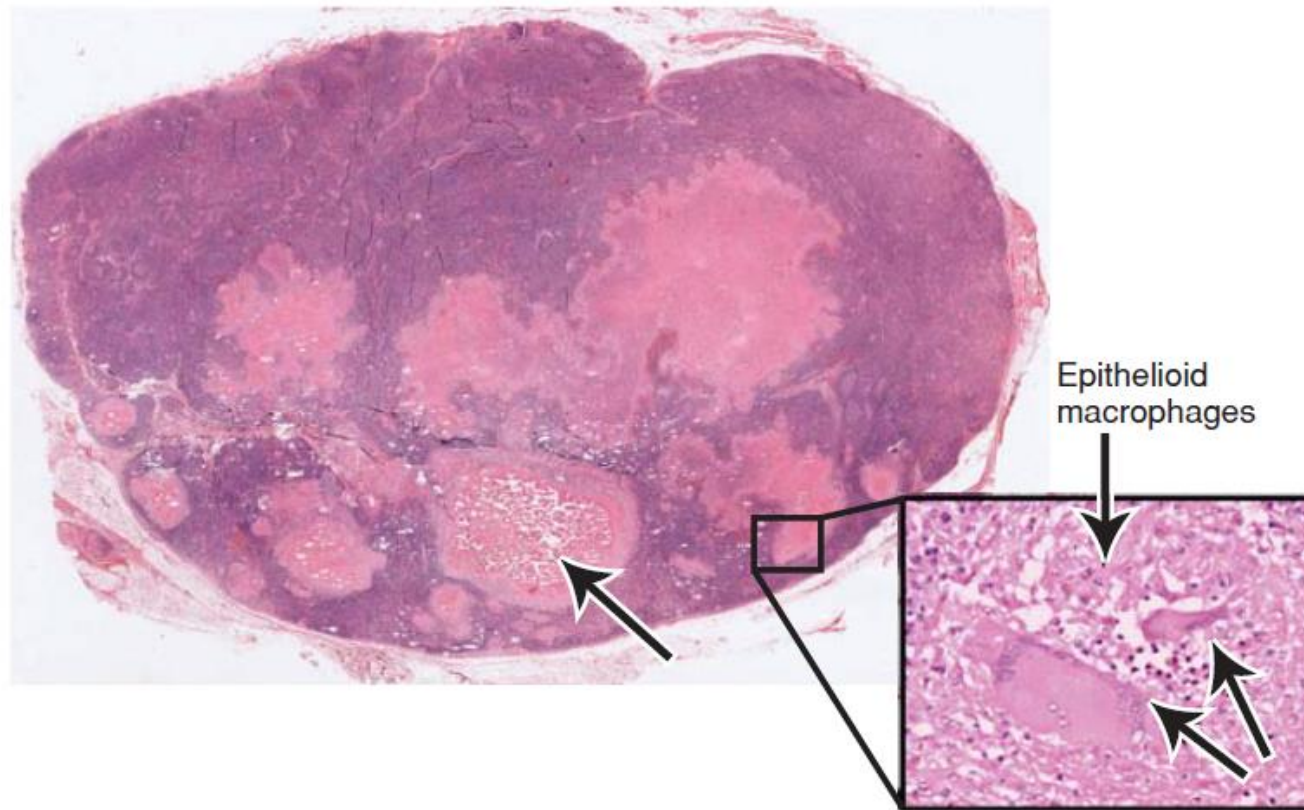


# Fat necrosis.



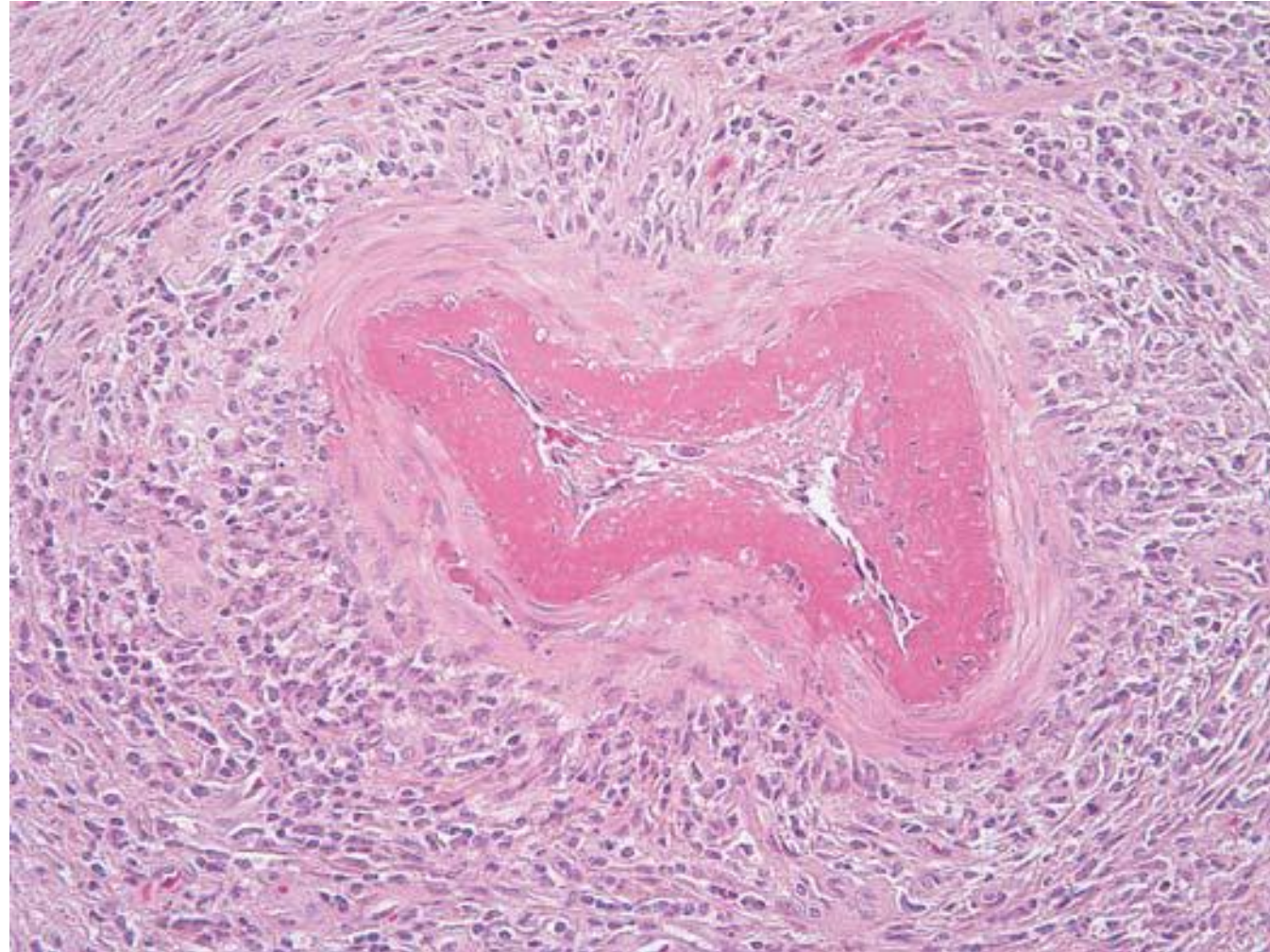
# Caseous Necrosis

**Characteristic of tuberculosis:** The accumulated mononuclear cells mediating the chronic inflammatory reaction to the mycobacteria are killed. The dead cells persist indefinitely as amorphous, coarsely granular, eosinophilic debris.



# Fibrinoid Necrosis

*Fibrinoid necrosis is an alteration of injured blood vessels in which insudation and accumulation of plasma proteins cause the wall to stain intensely with eosin.*



# Ischemic Injury

Ischemia, the interruption of blood flow, triggers decrease  $O_2$  and key nutrients and increased  $CO_2$  in cells.

A number of deleterious events follows:

- Including acidosis
- Generation of ROS,
- Loss of glycogen stores
- Disruption of intracellular  $Ca^{2+}$  homeostasis (increased intracellular  $Ca^{2+}$ )
- Mitochondrial injury
- DNA damage.

**Ischemic cell death takes place by necrosis.**

- Myocardial infarction and stroke are both due to ischemic cell death.
- Together represent the most common cause of mortality in the Western world.

## **The plasma membrane:**

- Separate extracellular fluid from the internal cellular milieu.
- Asymmetric ion distributions.
  - Extracellular  $\text{Na}^+$  and  $\text{Ca}^{2+}$  are orders of magnitude high than intracellular concentrations. The opposite for  $\text{K}^+$ .
  - Required considerable amount of energy to maintain.
  - Structural integrity of the lipid bilayer.
  - Intact ion channel proteins.
  - Normal association of the membrane with cytoskeleton.

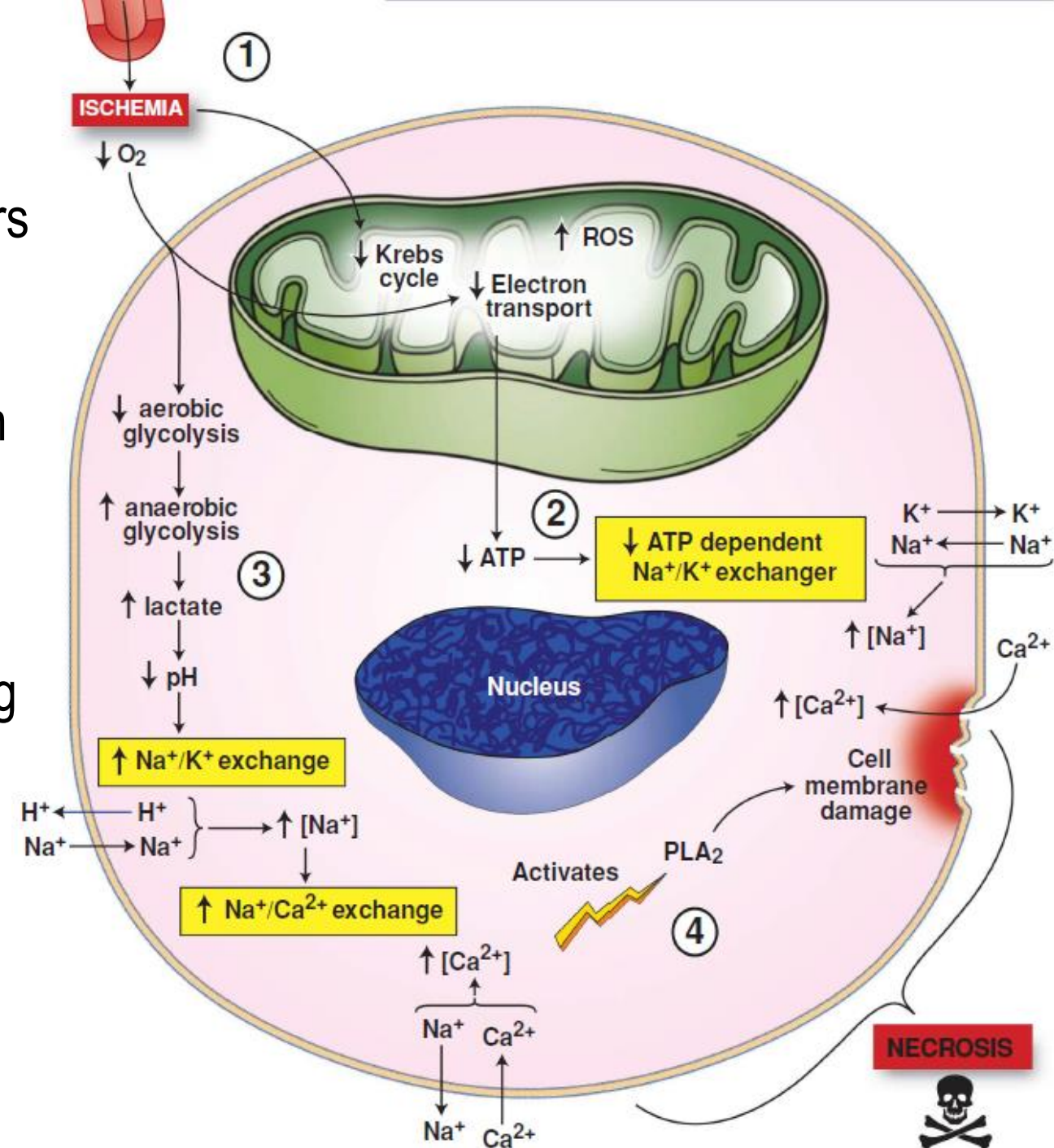
Whatever the lethal insult, cell necrosis is caused by loss of the plasma membrane's integrity with loss of permeability barrier function.

**The loss of ionic balance represents the “point of no return” for the injured cell.**

Massive influx of  $\text{Ca}^{2+}$  through a damaged plasma membrane is key to ischemic cell damage and loss of viability.

# Mechanisms of ischemia induced cell death by necrosis.

1. Loss of  $O_2$  due to vascular occlusion impairs mitochondrial function, resulting in decreased [ATP] production.
2. Decreased ATP impairs ATP-dependent ion exchangers.
3. The loss of aerobic processes causes anaerobic glycolysis to predominate leading to intracellular acidosis, eventually leading to increased cytosolic  $[Ca^{2+}]$ .
4.  $Ca^{2+}$ -dependent phospholipases are activated, causing loss of cell membrane integrity and necrosis.



# Reperfusion Injury

Reperfusion is the restoration of blood flow after a period of ischemia.

The process can cause damage, to which the term “reperfusion injury” is applied. Such injury occurs most often in settings of organ ischemia, such as myocardial infarction, but also in situations of organ transplantation.

Reperfusion injury reflects exposure of damaged tissue to the oxygen that arrives when blood flow is re-established (reperfusion).

Lethal reperfusion injury is significant, account for up to half of the final size of myocardial infarcts.

Ischemic cellular damage leads to generation of ROS. Reperfusion brings additional O<sub>2</sub> to combine with ROS to produce more ROS.

The evolution of reperfusion injury also involves inflammatory mediators, platelet-activating factor (PAF), adhesion molecules, dysregulation of Ca<sup>2+</sup> homeostasis

# Ischemia and Reperfusion Injury

Three different degrees of cell injury, depending on the duration of the ischemia:

- With short periods of ischemia, reperfusion (and, thus, the resupply of  $O_2$ ) completely restores the cell's structural and functional integrity. Cell injury in this case is completely reversible.
- With longer periods of ischemia, reperfusion is associated with cell deterioration and death. In this case, lethal cell injury occurs during the period of reperfusion.
- Lethal cell injury may develop during the period of ischemia itself, in which case reperfusion need not be a factor. A longer period of ischemia is required to produce this third type of cell injury.



# Ischemic Preconditioning

Sudden and complete ischemia may cause cell death before adaptive mechanisms can come into play.

Repeated episodes of ischemia, as in recurrent angina due to coronary artery disease, stimulate adaptive responses. In the heart, these are collectively called **ischemic preconditioning**.

The transcription factor HIF-1 $\alpha$  is the master regulator of transcriptional responses to low O<sub>2</sub> tension. HIF-1 $\alpha$  activates genes whose protein products limit production of ROS, Ca<sup>2+</sup> accumulation and ATP depletion.

HIF-1 $\alpha$  tends to protect against mitochondrial injury, DNA damage and oxidative stress to facilitates survival of the ischemic cell.

# **PROGRAMMED CELL DEATH**

# PROGRAMMED CELL DEATH

Programmed cell death (PCD) refers to processes of cell death regulated by pre-existing signaling pathways.

There are various forms of PCD that are part of the balance between the life and death of cells:

Determines that a cell dies when it is no longer useful or when its survival may be harmful to the organism.

Without programmed cell death to limit the size of bodily compartments a 80 year old would accumulate:

- Two tons of bone marrow and lymph nodes.
- 16 km (10 miles) of intestines.

PCD is also a self defense mechanism: cells that are infected with pathogens or that sustain genomic alterations are destroyed.

# Classification of PCD

There is bewildering variety of mechanisms that eventuate in PCD.

Mutant mice lacking the key elements of the apoptotic machinery develop almost normally. This observation indicated that alternatives to apoptosis exist.

A number of mechanisms of PCD have been identified:

- Apoptosis
- Autophagy-associated cell death
- Necroptosis
- Pyroptosis
- Anoikis
- NETosis
- Pyrosis
- Entosis

**Each mechanism seems to predominate in specific circumstances.** There are interconnections between them.

**APOPTOSIS is a form of PCD that relies exclusively on the caspase cascade.**

It is a highly conserved cell death process that depends on a family of cysteine proteases (caspases) as crucial signaling intermediates and as executioners.

**Apoptosis in development and physiology.**

During development there is sequential appearance and regression of many tissues.

**Some embryonic aortic arches do not persist.**

- The pronephros and mesonephros regress to leave the metanephros.
- Structures required by only one sex disappear in embryos of the other sex.
  - The müllerian duct, the progenitor of the uterus, is deleted in males.
  - The wolffian duct, which forms part of the male genital tract, disappears in females.

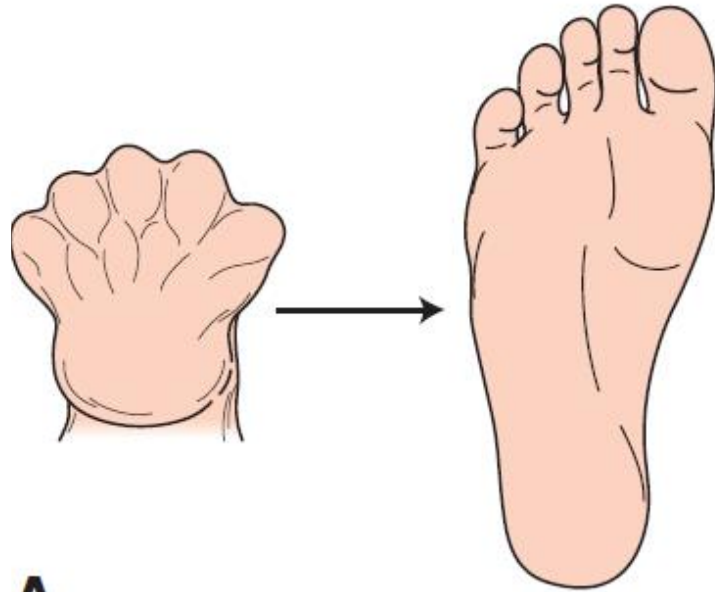
## Apoptosis in development and physiology (continue)

- In the brain and ovaries overproduced cells are then culled.
- Removes interdigital tissues to yield discrete fingers and toes.
- Converts solid primordia to hollow tubes (e.g., gastrointestinal tract),
- Produces the four-chamber heart and mediates other body-sculpting activities.
- Lymphocyte clones that recognize self-antigens are deleted.

Physiologic apoptosis principally affects progeny of stem cells that are constantly dividing (e.g., stem cells of the hematopoietic system, gastrointestinal mucosa and epidermis).

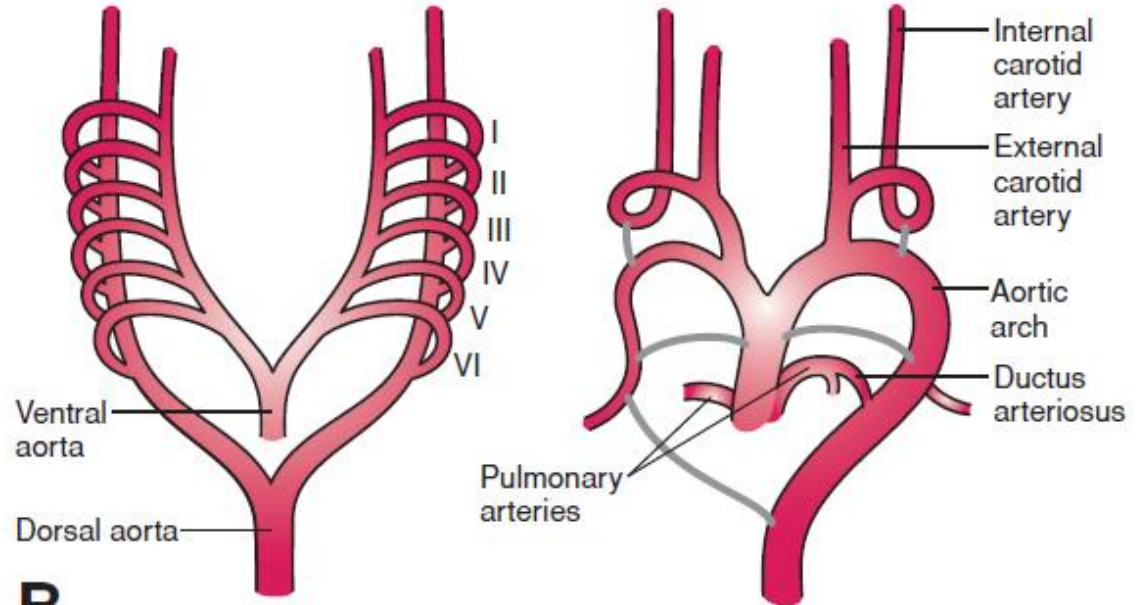
# Activities of apoptosis during embryonic development

## Sculpting



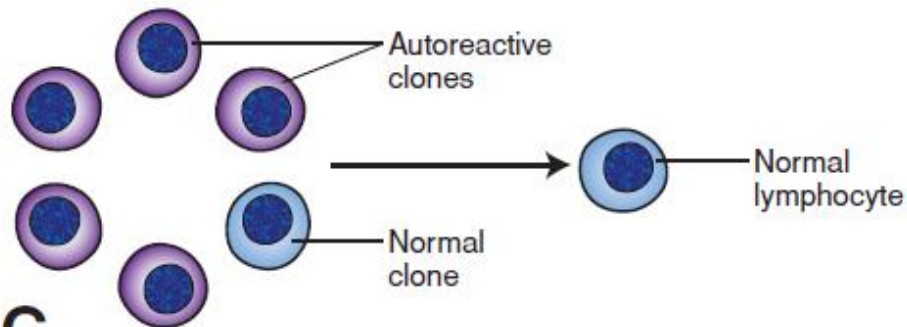
**A**

## Deletion of structures



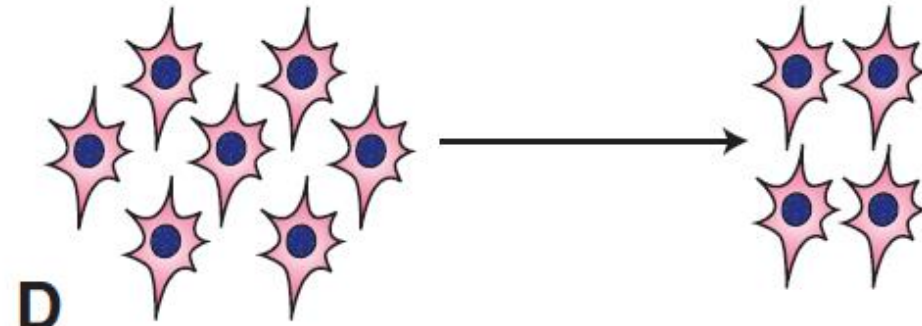
**B**

## Removing dangerous cells



**C**

## Regulating cell number



**D**

## **Apoptosis eliminates obsolescent cells:**

Cell turnover is essential to maintaining the size and function of many organs.

- Older and less functional white blood cells must be eliminated.
- Enterocytes migrate from the depths of the crypts to the tips of the villi, to die by apoptosis.
- The regression of lactational hyperplastic breast in women who have stopped breast-feeding .
- Adult men produce about 1000 new spermatozoa per second of which most undergo apoptosis.

## **Apoptosis deletes mutant cells:**

Environmental stresses such as UV light, ionizing radiation and DNA-binding chemicals.

May alter DNA structure. If the DNA damage is too severe to be repaired, a cascade of events leads to apoptosis. This process protects the organism from cells that cannot control their own proliferation.

## **Apoptosis as a defense against dissemination of infection:**

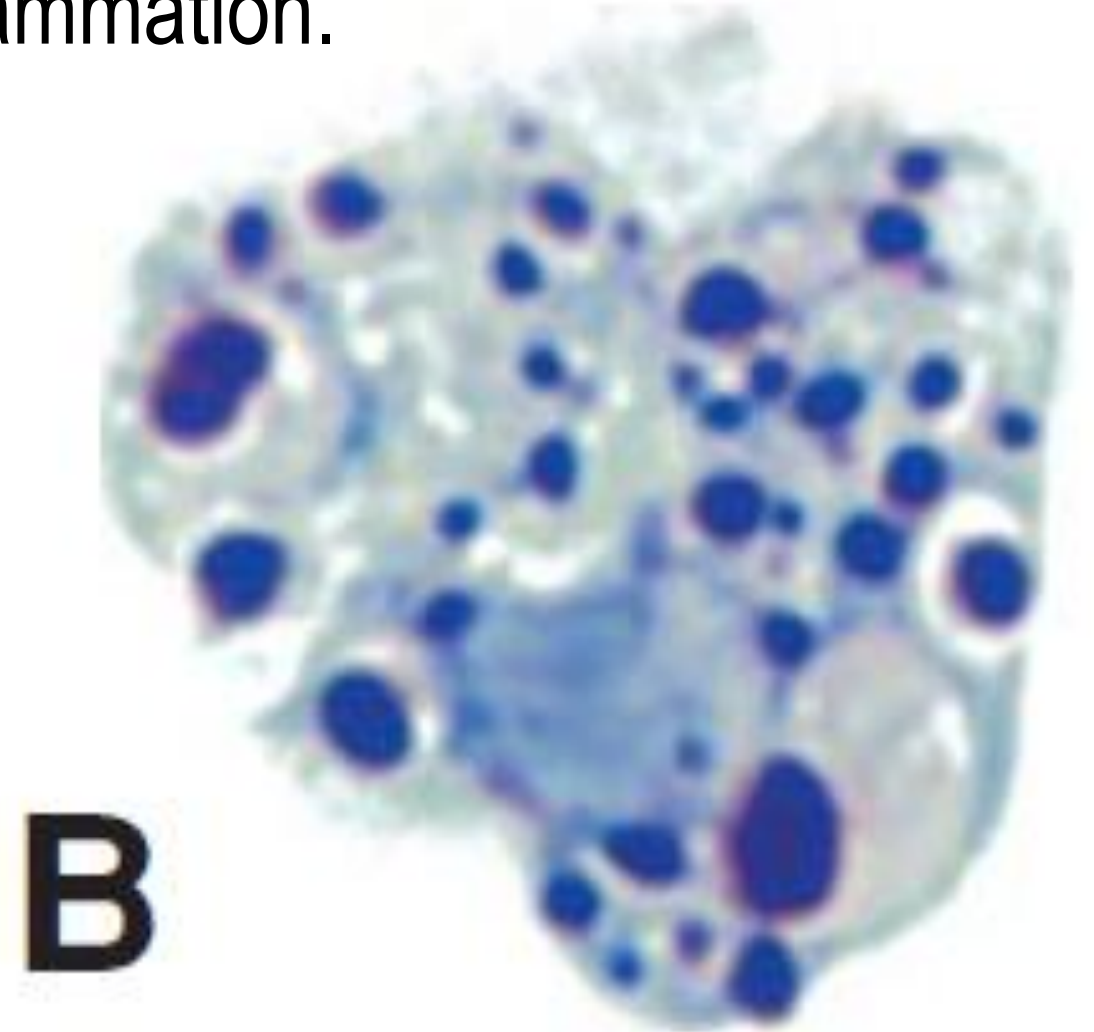
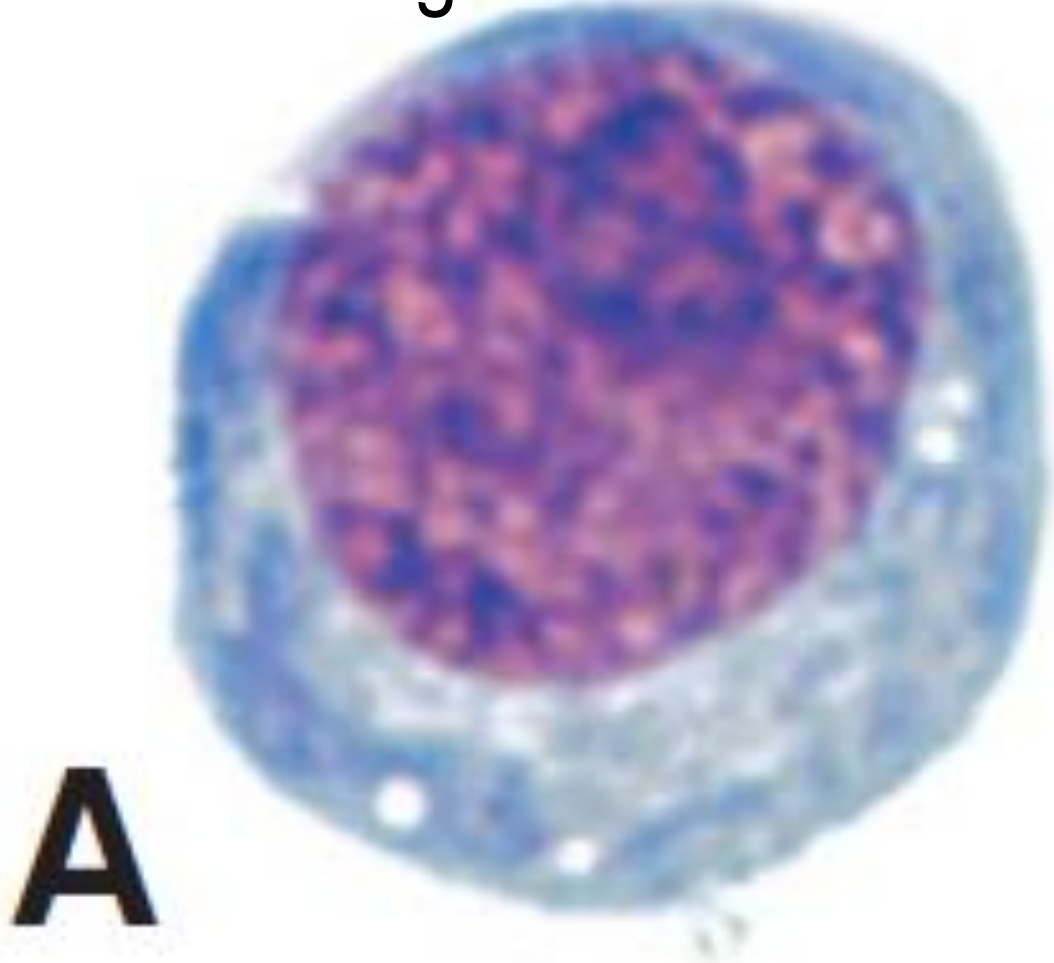
Detection of non-chromosomal DNA replication as in a viral infection, it tends to initiate apoptosis.

**Many viruses have evolved mechanisms that manipulate cellular apoptosis.**



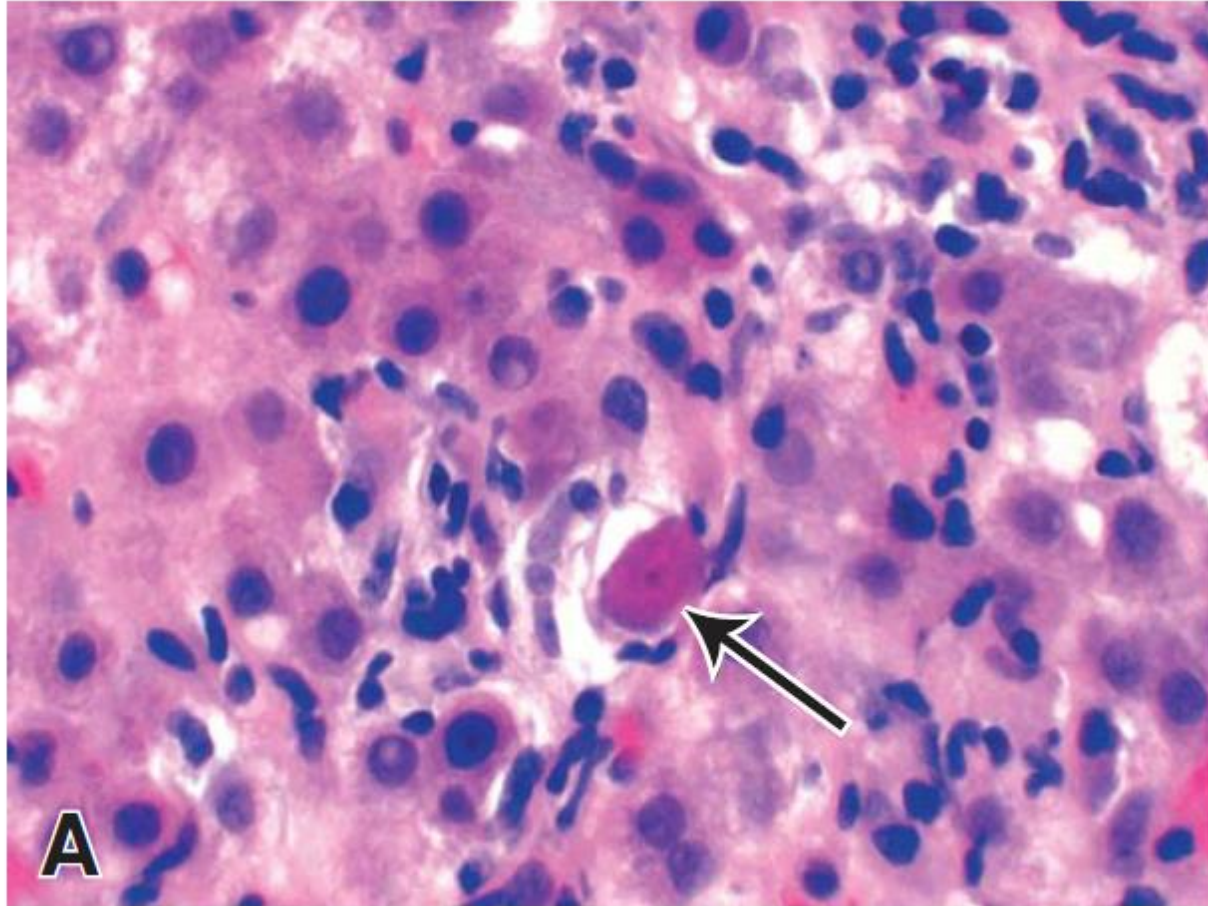
# Morphology of apoptosis

Apoptosis, by contrast, is characterized by plasma membrane blebbing and nuclear fragmentation without inflammation.

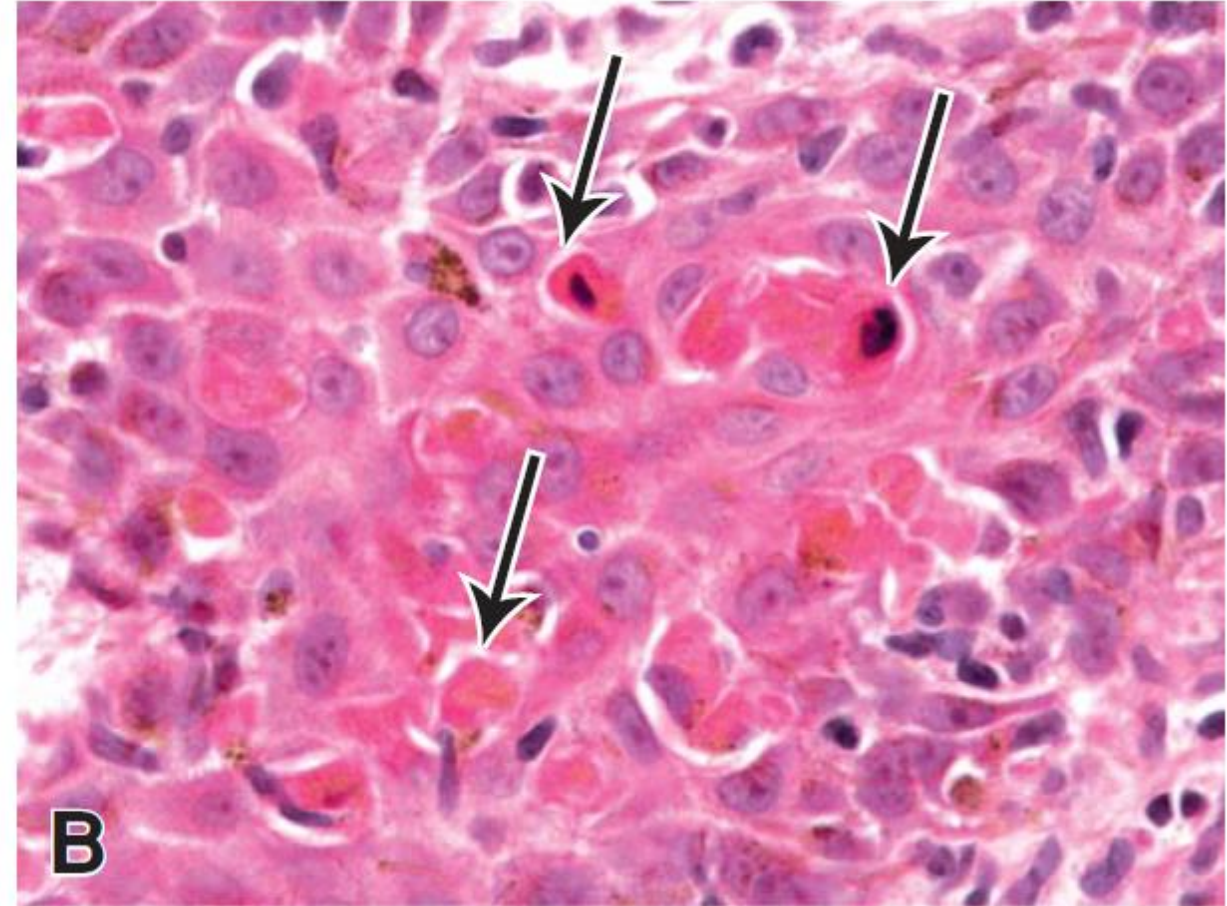


# Morphology of apoptosis

Apoptosis in the liver  
in viral hepatitis



Apoptosis in the skin  
in erythema multiforme



## **MECHANISMS OF APOPTOSIS: Apoptosis comprises several signaling pathways**

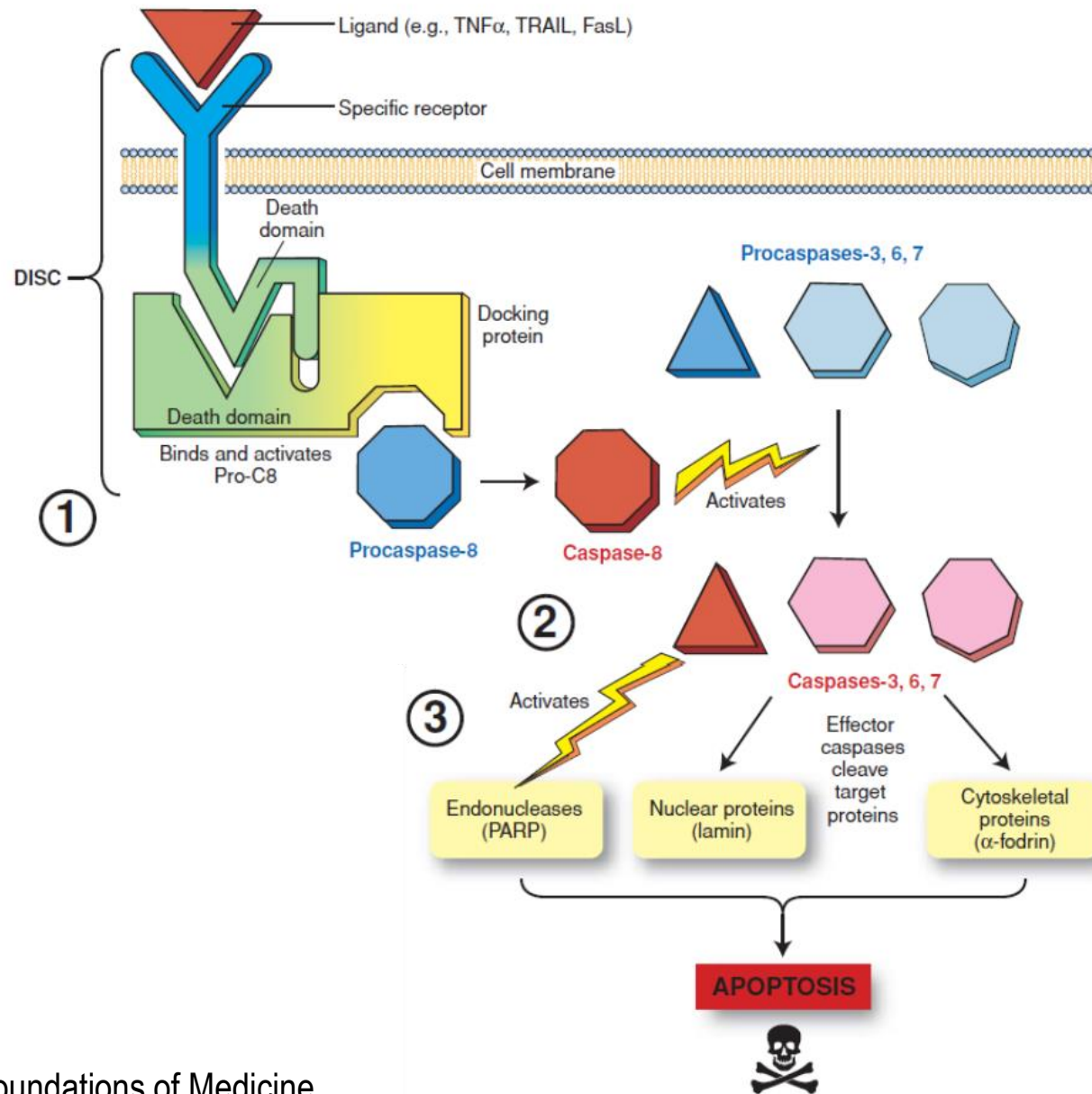
- In ***extrinsic apoptosis***, plasma membrane receptors are activated by their ligands.
- The ***intrinsic pathway*** is initiated by diverse intracellular stresses and is characterized by a central role for mitochondria.
- ***Inflammatory or infectious processes***, Intracellular and extracellular infectious agents both elicit this type of apoptosis, by diverse routes.
- The ***perforin/granzyme pathway*** is triggered when cytotoxic T cells attack their cellular targets, with transfer of granzyme from the killer cell to its intended victim.
- ***p53-activated apoptosis*** occurs in response to cellular stress or DNA damage.
- The ***endoplasmic reticulum*** may elicit apoptosis in which calcium signaling plays a central role.

**Caspases, a family of cysteine proteases, are central to apoptosis.**

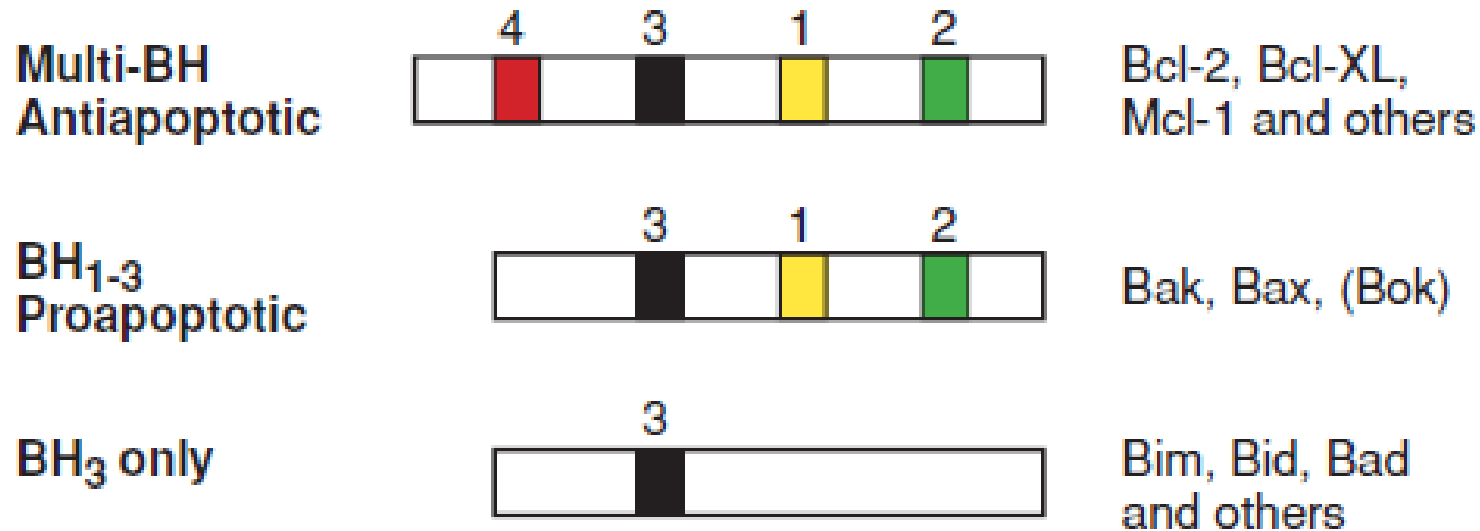
Sequential activation of these enzymes, by conversion from proenzyme forms to catalytically effective enzymes.

Although the various pathways to apoptosis may start differently and signal via different members of this enzyme family, these pathways all generally converge to the executioner caspase-3, -6 and -7.

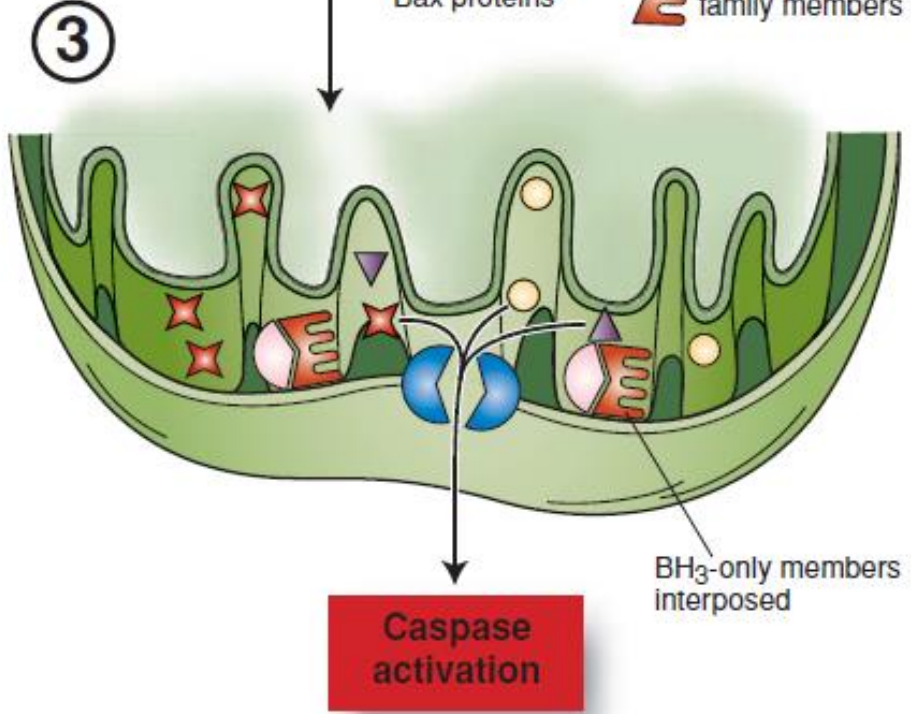
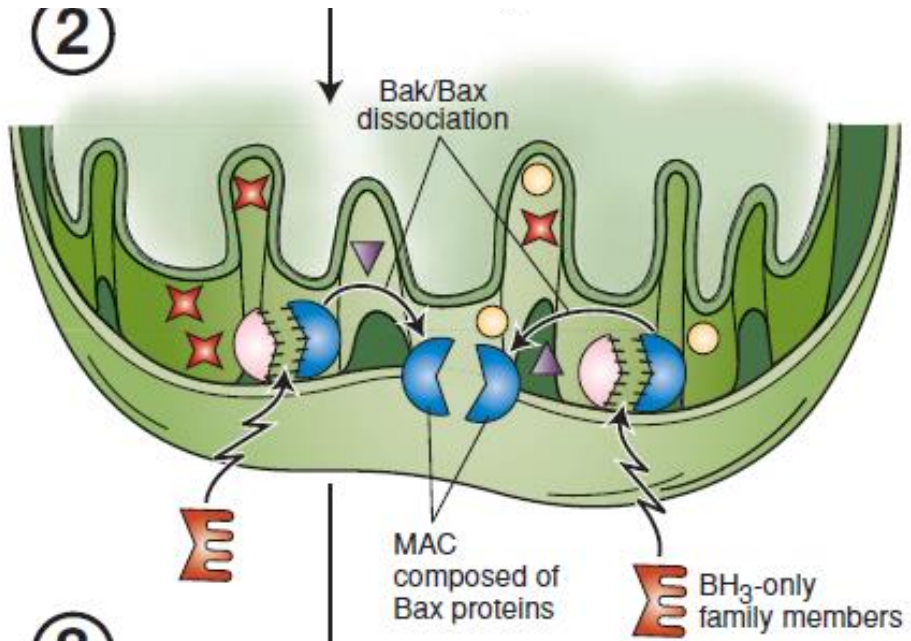
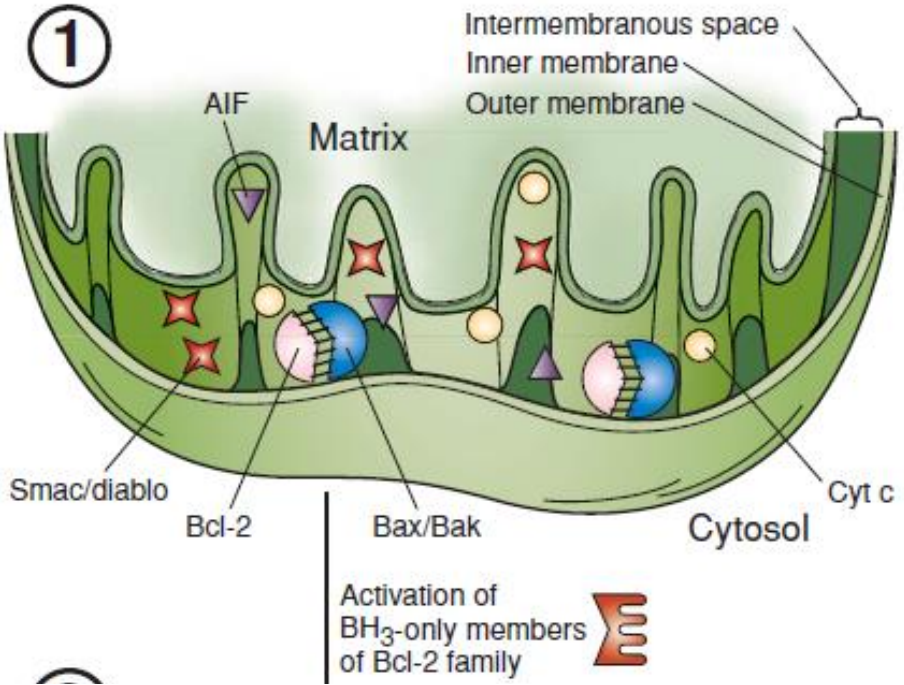
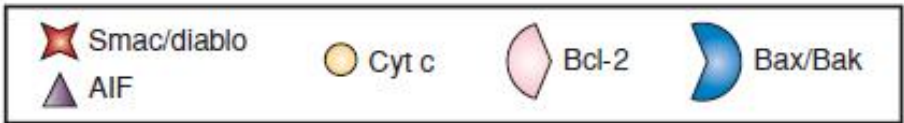
# Extrinsic pathway of apoptosis



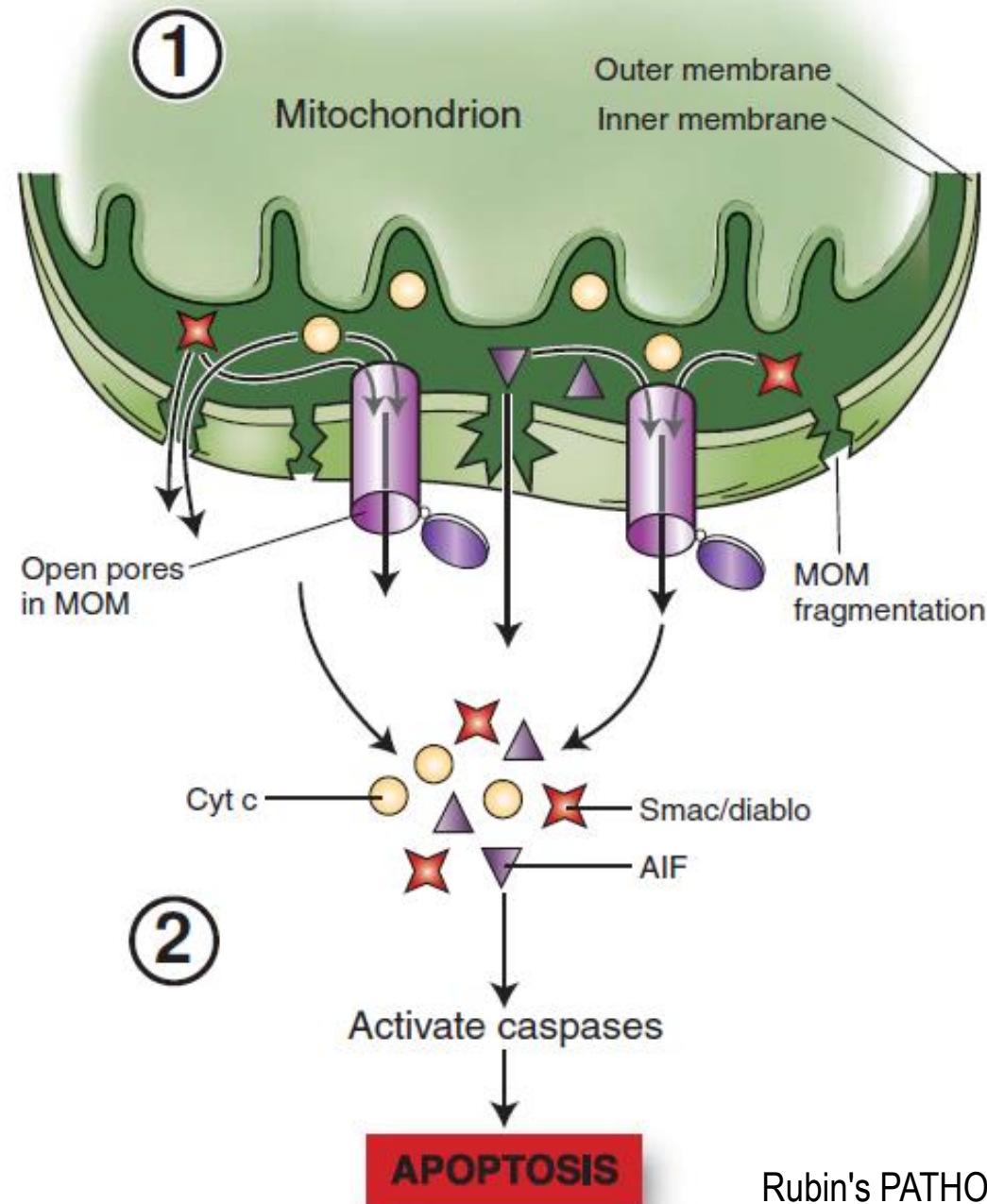
# Bcl-2 protein family are critical for the intrinsic pathway



# Intrinsic pathways of apoptosis

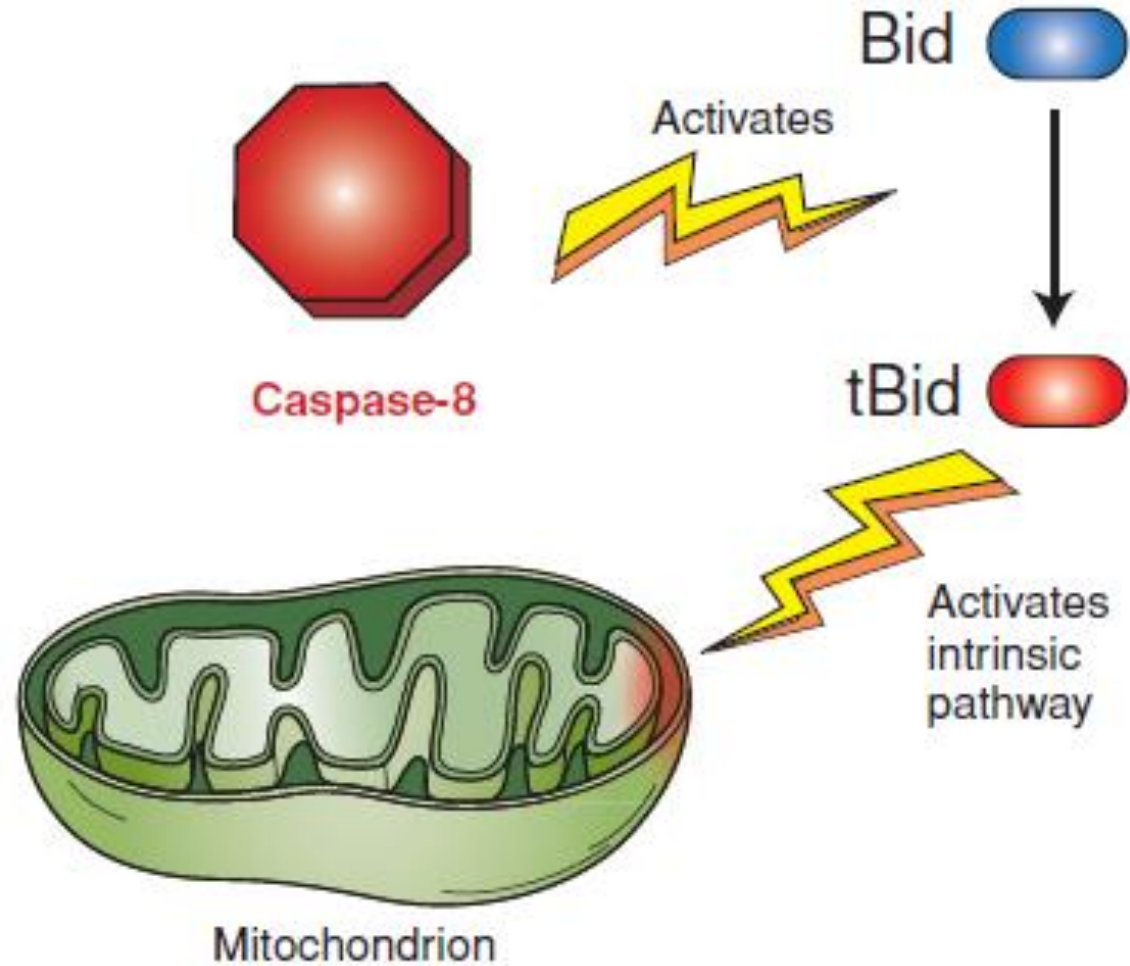


# The MOM in the intrinsic pathway of apoptosis

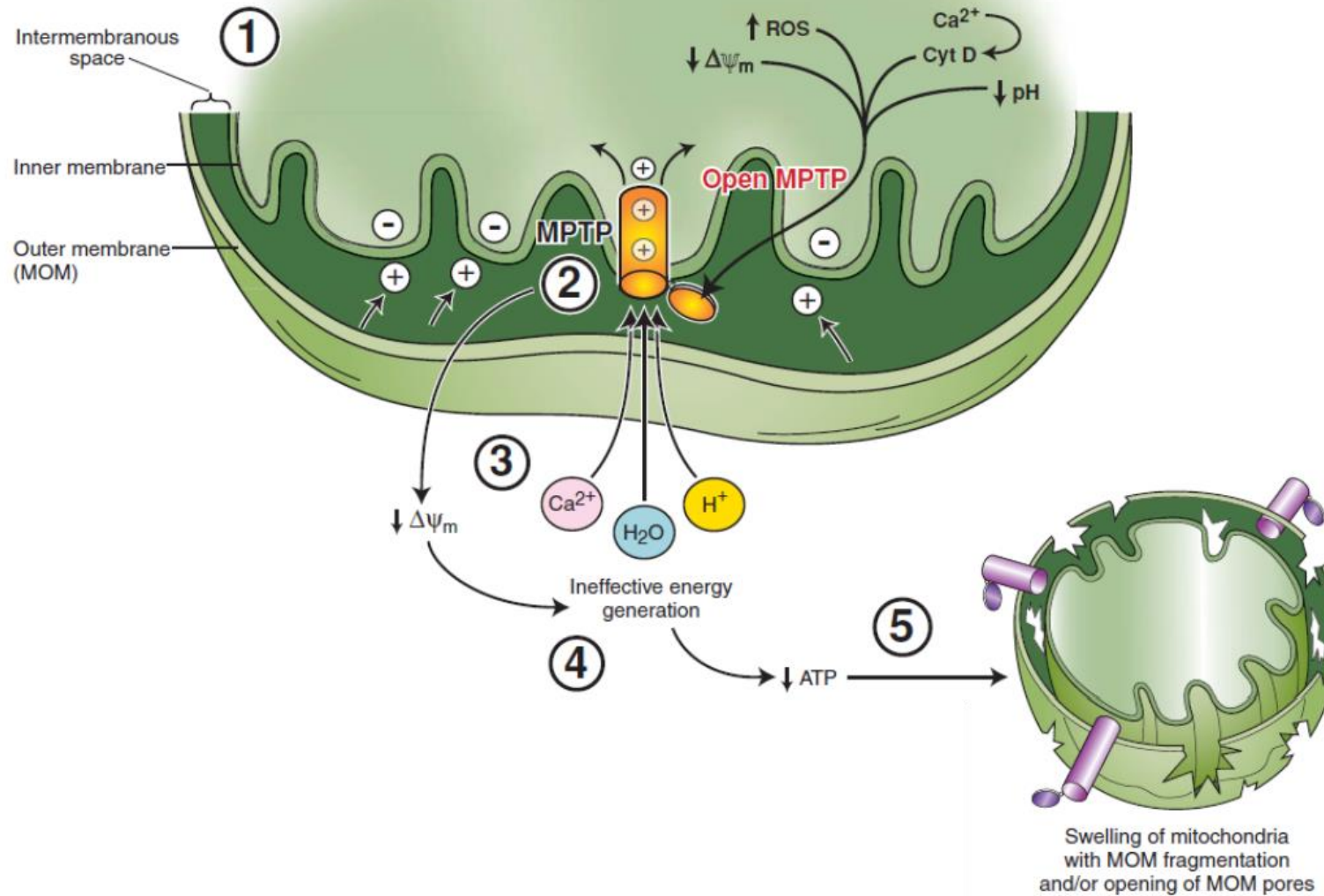




# Intersection of the extrinsic and intrinsic pathways of apoptosis



# Mitochondrial permeability transition pore (MPTP) activation

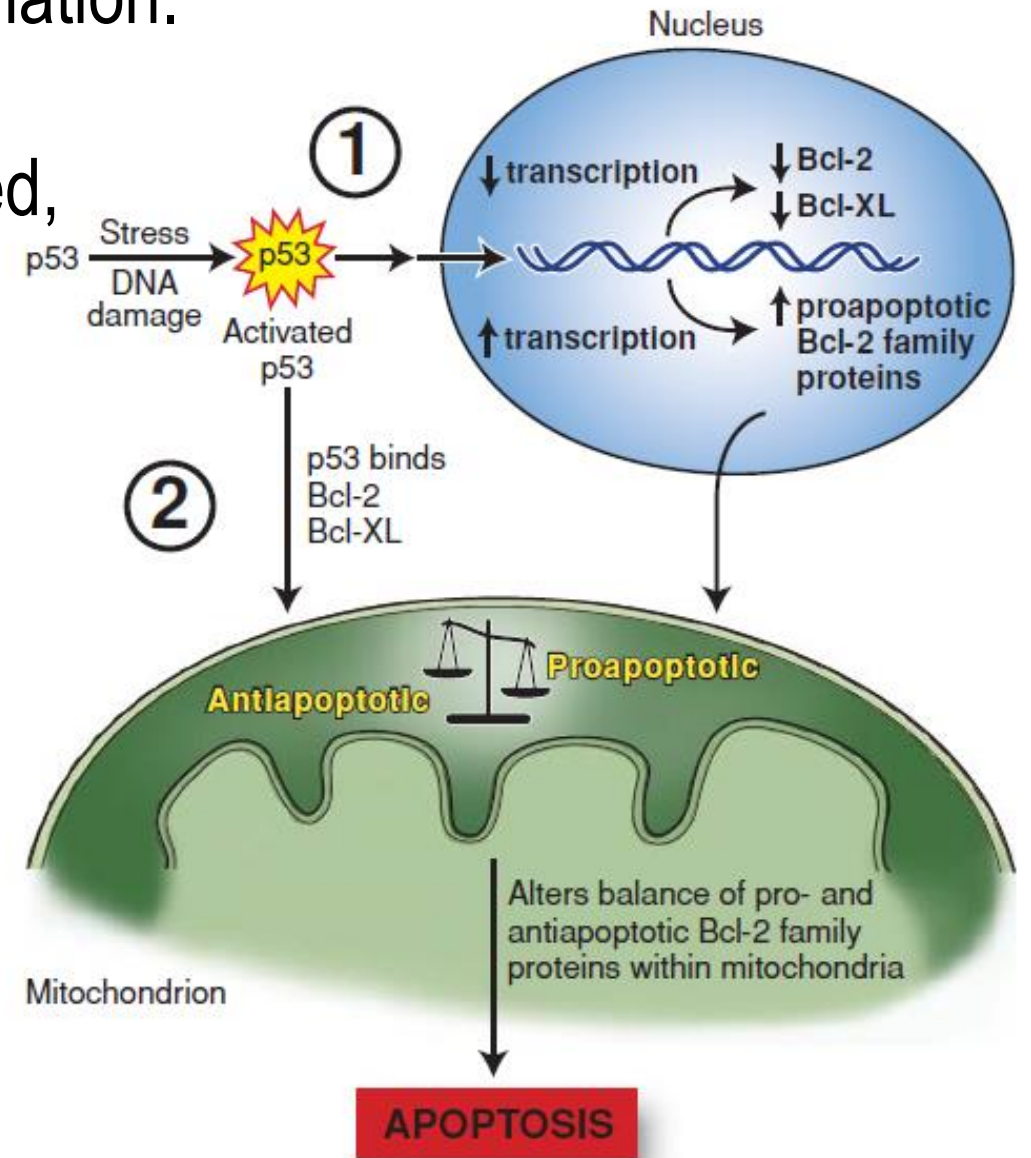


# Activation of p53 and apoptosis

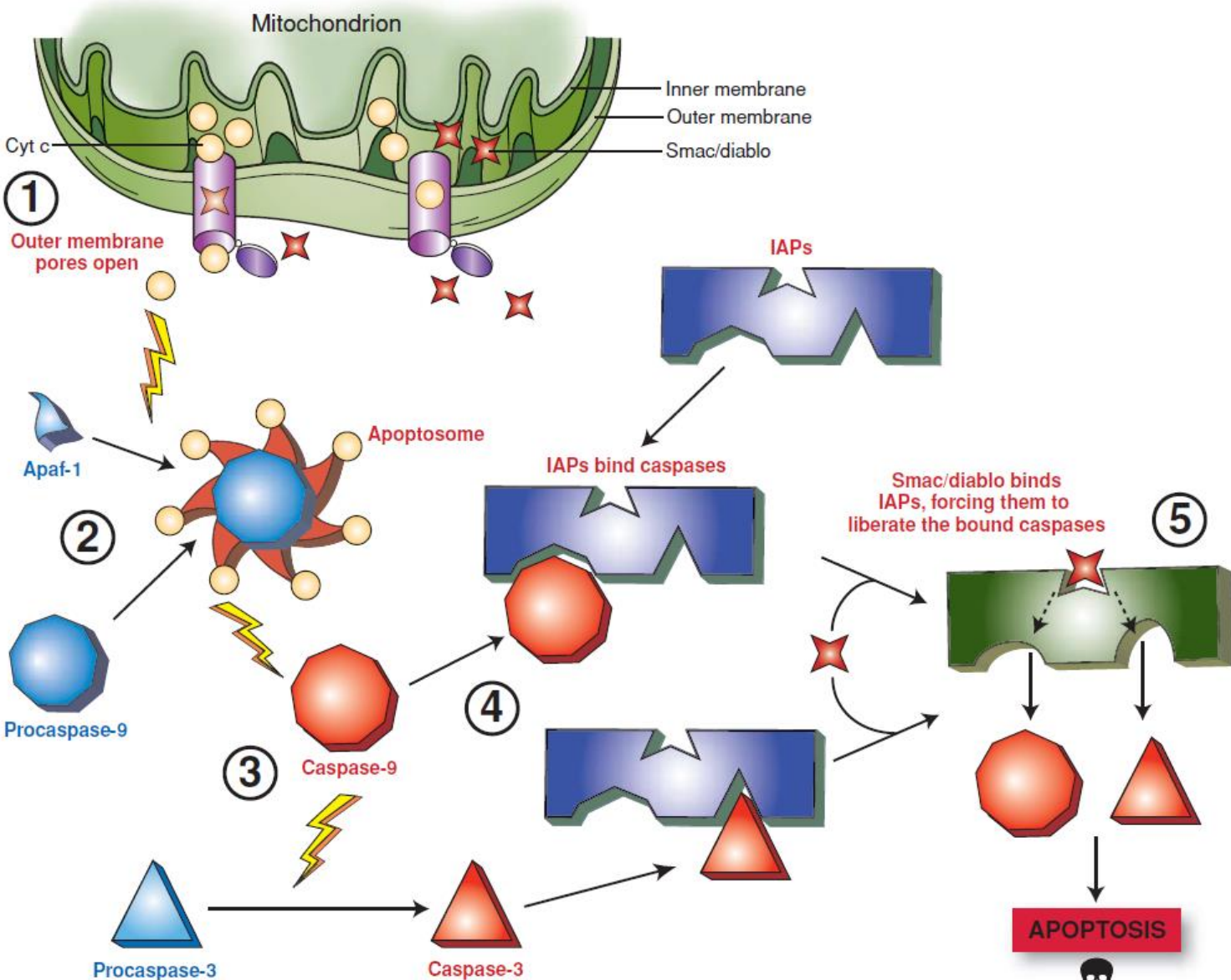
Mdm2 promotes p53 degradation via polyubiquitination.

When a cell is injured or its equilibrium is disturbed, p53 is phosphorylated, monoubiquitinated...

p53 accumulates and enters the mitochondria or nucleus, depending on the specific molecular modification.



# MOMP leading to Apaf-1 activation amplifying the apoptotic cascade



## **Autophagy in PCD:**

Autophagy plays an important prosurvival role in cell adaptation to stress and injury. The function of autophagy as an independent form of cell death is unclear.

Possible mechanisms of autophagic cell death:

Excessive removal of cell organelles irrevocably interfere with vital cellular functions.  
Destruction of proteins that sustain cell survival.

Experimental inhibition of autophagy prevents cell death induced by a variety of agents.

Autophagy can also contribute to apoptosis.

Thus, it is currently unclear whether autophagy is responsible for cell death independently of other forms of PCD and the extent to which such events may occur.

**Necroptosis is a form of PCD morphologically indistinguishable from Necrosis.**

Cell swelling, plasma membrane rupture and nuclear pyknosis, followed by an inflammatory response.

- Necroptosis commonly begins FasL or TNF- $\alpha$  binds to its respective receptors.
- Caspase-8; IAP, RIP1 and RIP3 form a complex.
- Activation of RIP1 and RIP3 leads to cell death by necroptosis.

Increased cytosolic  $\text{Ca}^{2+}$  activates calpain and other degradative enzymes, which attack lysosome membranes and cause release of lysosomal hydrolases.

Calpain also damages mitochondria, precipitating metabolic dysfunction with impaired ATP generation and iron. Increases in ROS damage to proteins, lipids and DNA.

Mitochondria release AIF, which enters the nucleus and activates DNA degradation.

A bioenergetic crisis with the morphologic features of necrosis ensues.

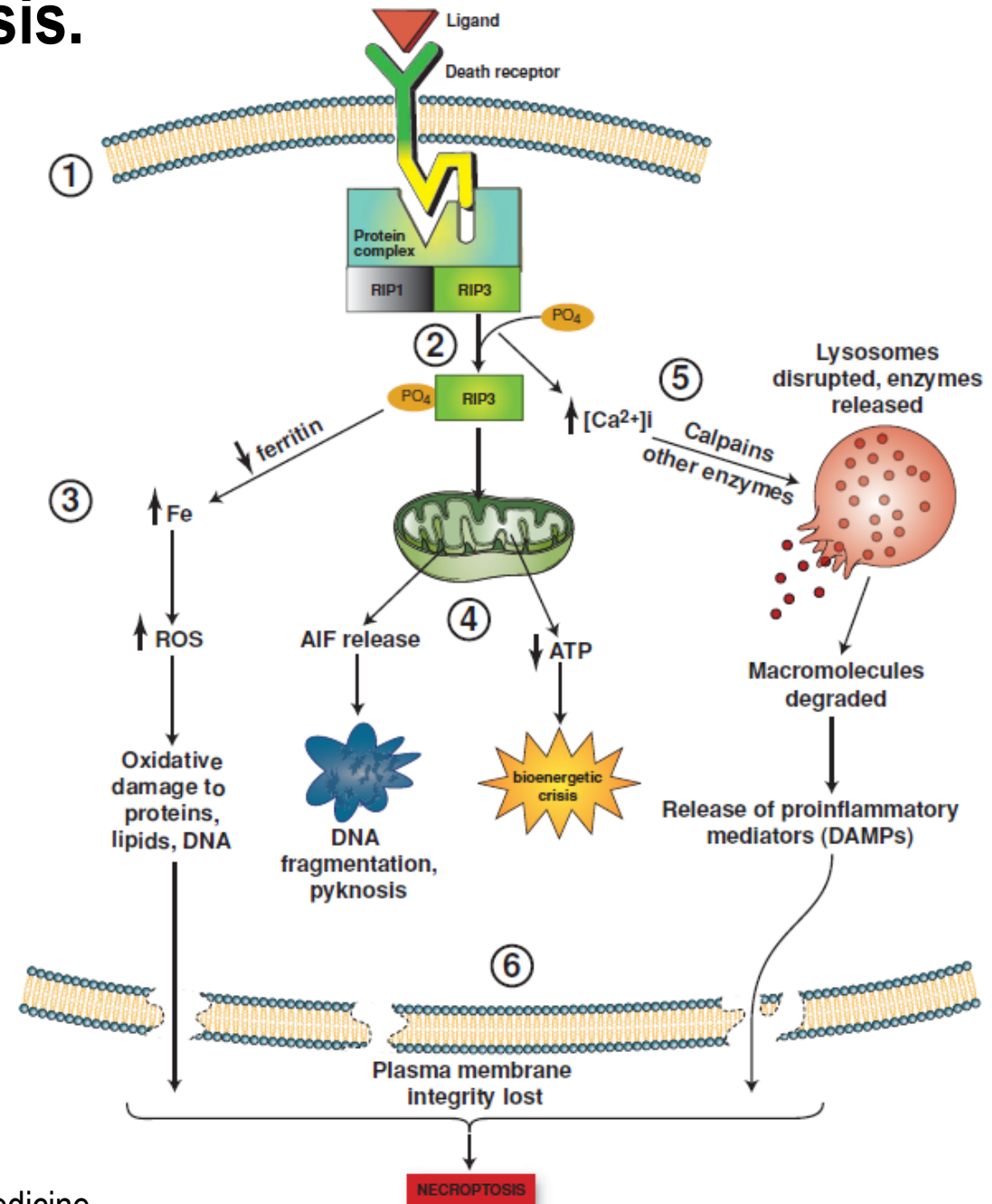
Cells then release **damage-associated molecular patterns** that provoke inflammation.

Under physiologic circumstances, necroptosis participates in development, particularly at the **bone growth plate**. It is also active normally in some adult tissues such as the **lower portion of the intestinal crypts**.

When physiologic apoptosis is unavailable to cells, necroptosis may become the default cell death pathway.

Necroptosis is also important in limiting the spread of certain viral infections.

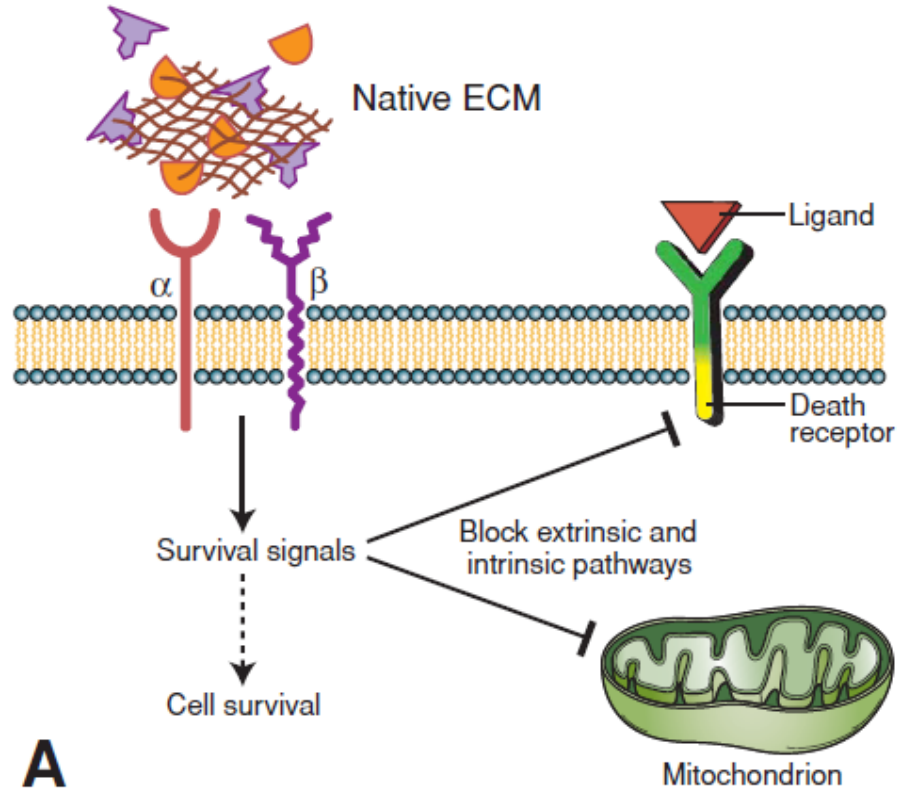
# Pathways leading to necroptosis.





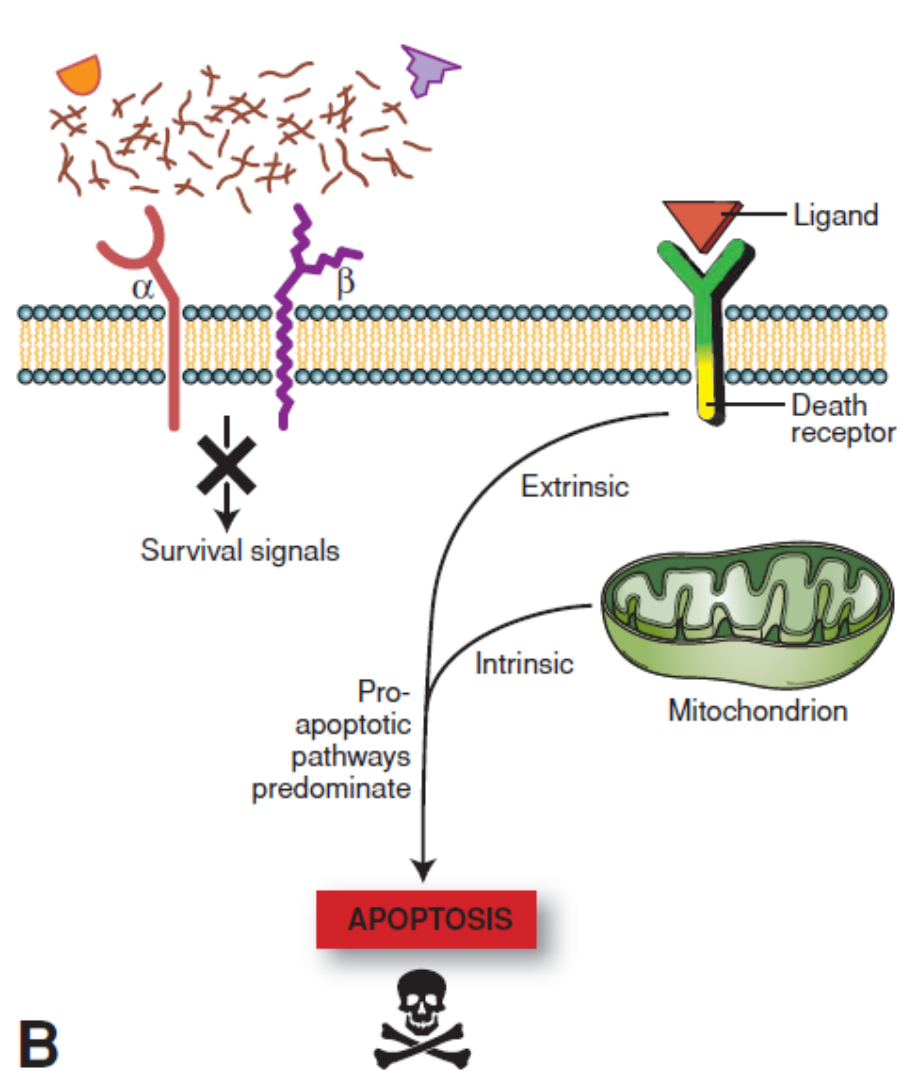
# Mechanisms of anoikis

## Bound integrins



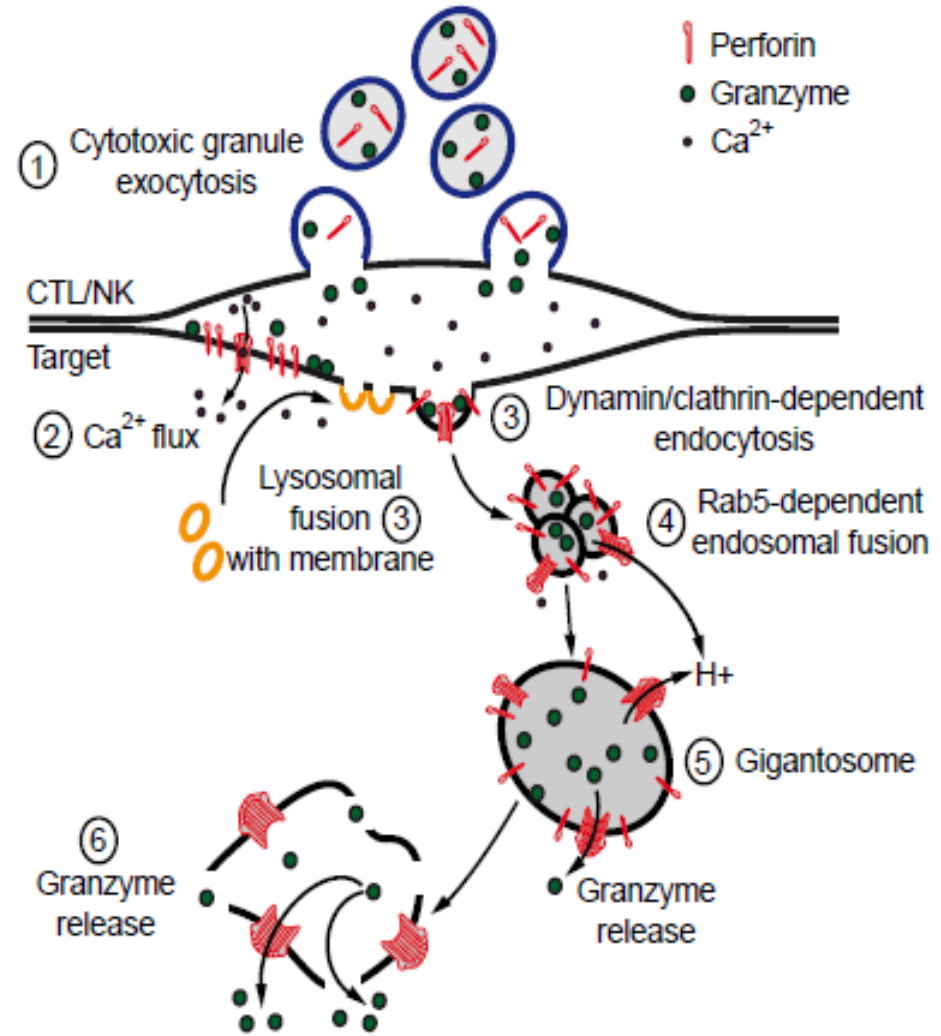
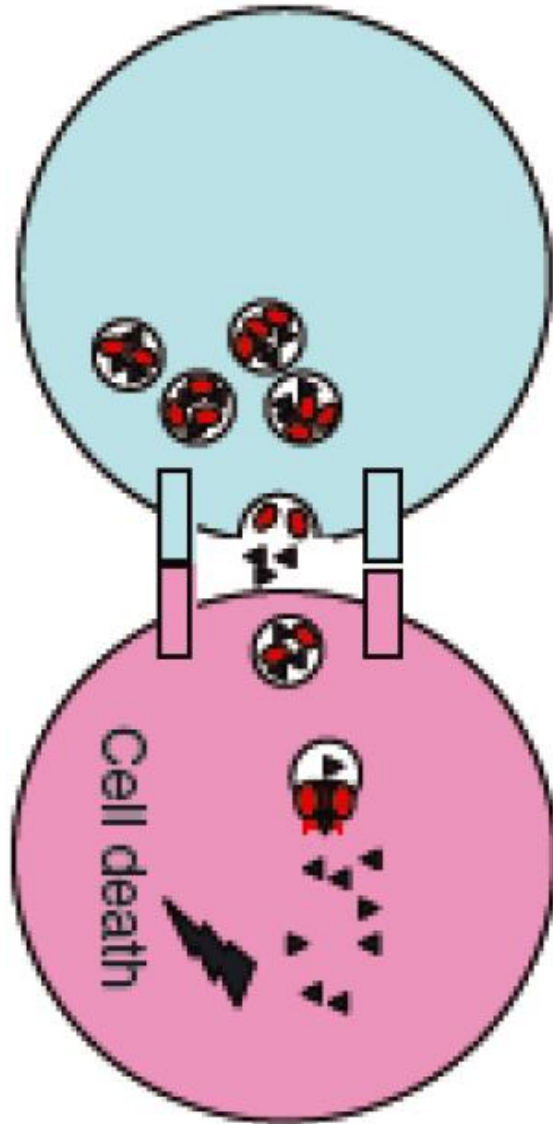
**A**

## Unbound integrins

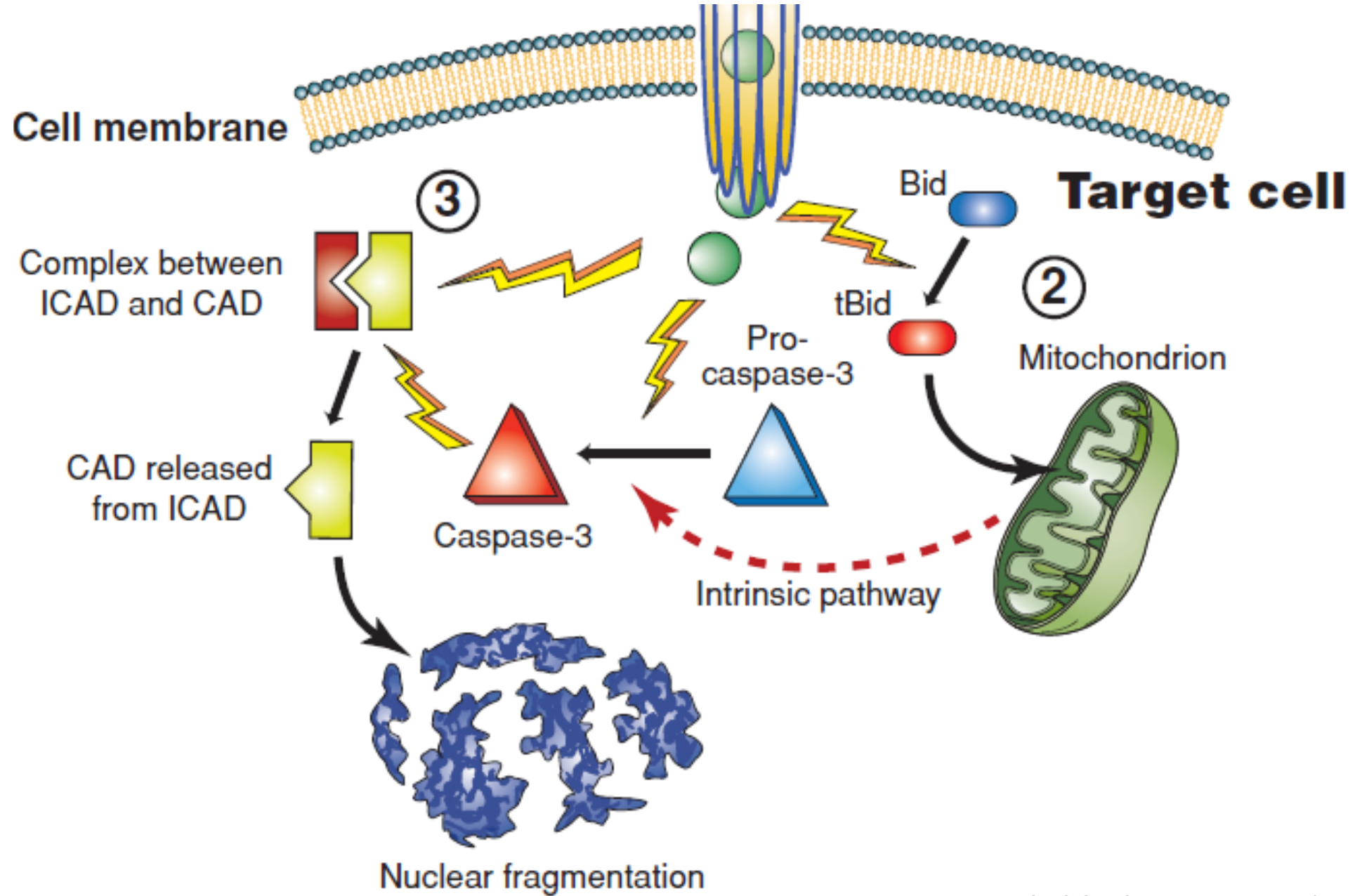


**B**

# Cell death caused by CTLs



# Cell death caused by CTLs



## **Pyroptosis contributes to innate immune defenses.**

Pyroptosis is a cell death program that relies on caspase-1 (IL-1 $\beta$ -converting enzyme).

Although caspase-1 is a cysteine protease involved in PCD, it is independent of apoptotic signaling, and its activation does not bring about apoptosis.

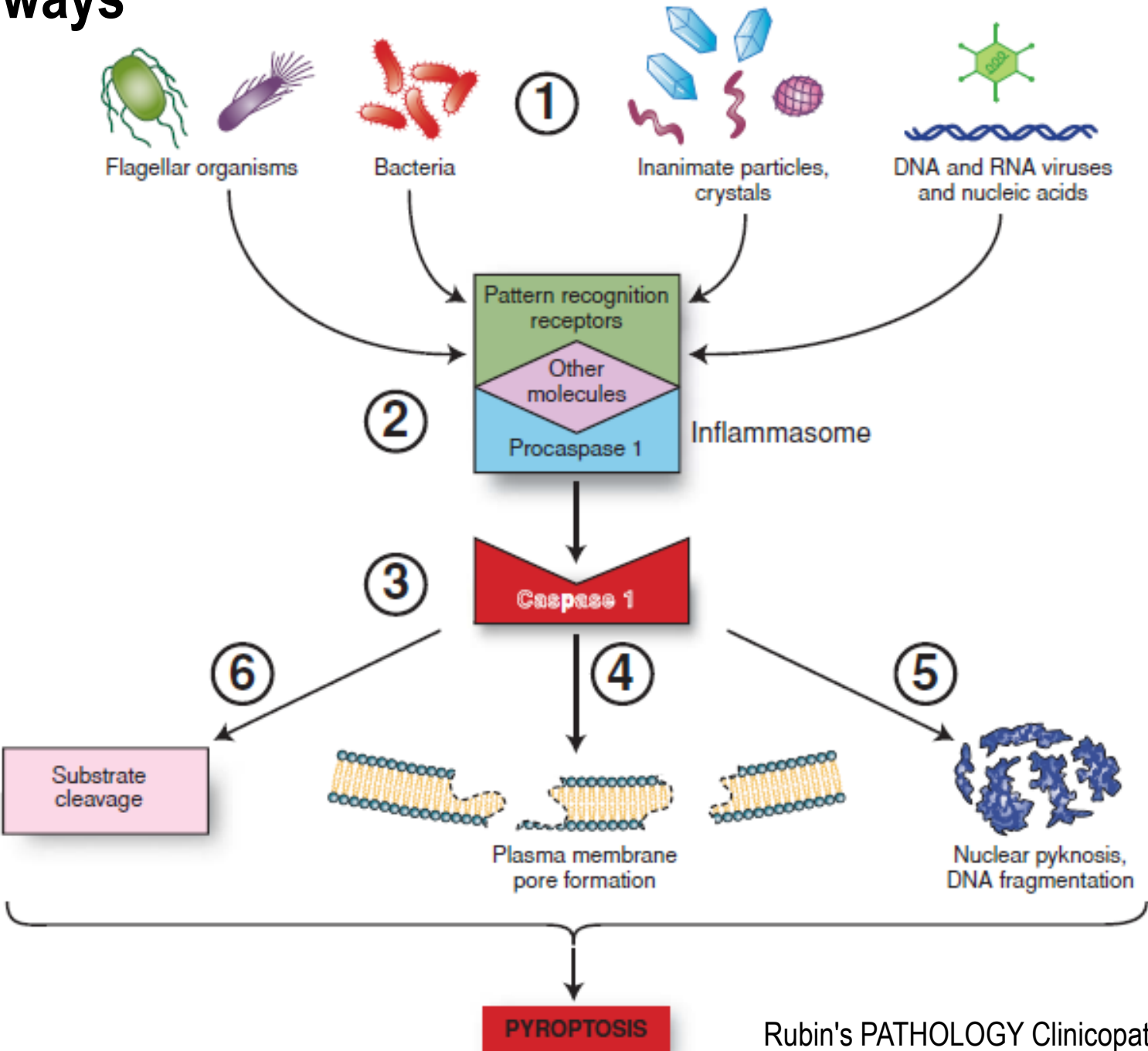
Caspase-1 is a proinflammatory protease activated by the inflammasome.

Activated caspase-1 cleaves select cellular molecules, including enzymes that are important for glycolysis, thereby depleting cellular energy.

It also produces ion-permeable plasma pores, allowing influx of water and solutes to provoke cell swelling and then death.

By activating a number of proinflammatory cytokines, the dead cell elicits inflammation.

# Pyroptosis pathways



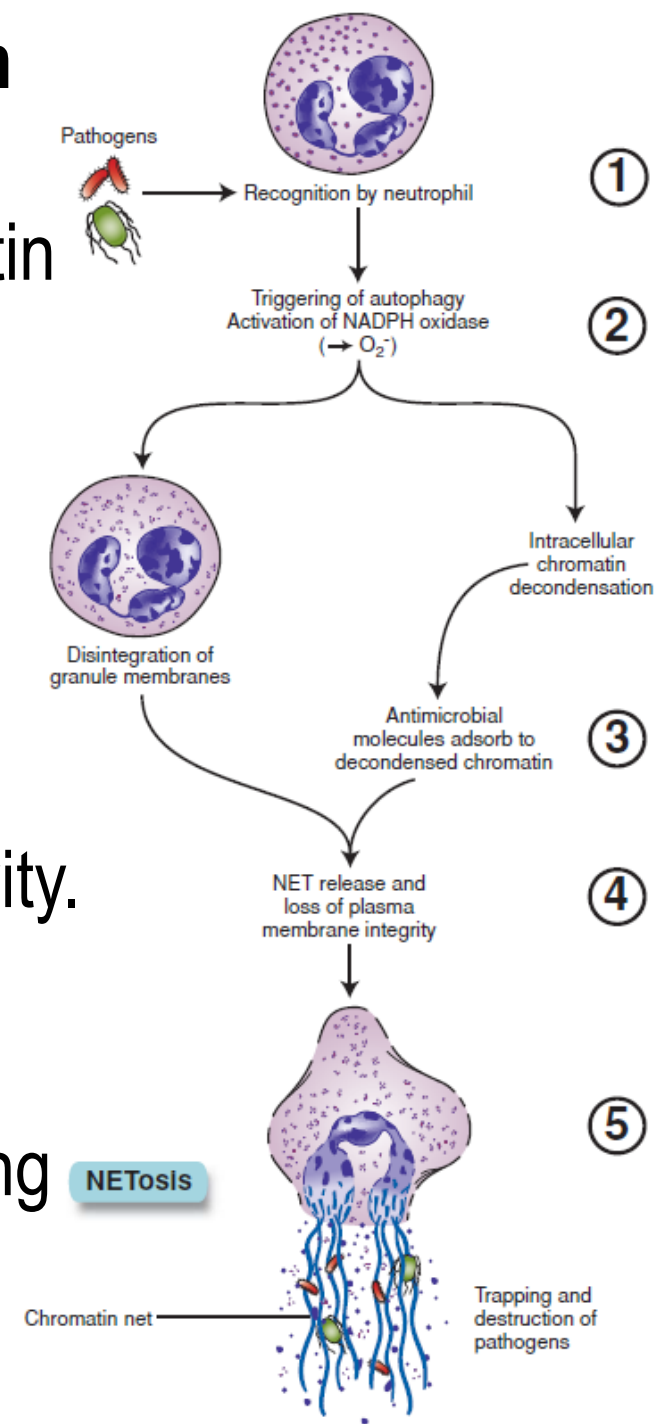
# NETosis a potent antimicrobial defense mechanism

Neutrophil extracellular traps (NETs) are produced by polymorphonuclear granulocytes. Function as chromatin traps for pathogens. Kill bacterial, fungal and protozoal pathogens.

NETosis is activated mainly in neutrophils, but also including eosinophils and mast cells.

NETosis requires autophagy and NADPH oxidase activity. Destruction of the cell's nuclear envelope and the membranes of most cytoplasmic granules.

Chromatin disaggregation is extruded as NET containing both chromatin and strongly microbicidal histones and histone cleavage products. Highly proinflammatory.



**Entosis is a Cell-Eat-Cell form of cell death.**

Type of cellular cannibalism in which cells that are not professional phagocytes engulf nearby living cells.

Aggressor cells may engulf cells of either the same or other lineages.

More often, entosis is seen in tumors.

Vacuoles containing cells undergoing entosis may fuse with lysosomes, in which case target cells usually die.

The cannibalized cell, or parts there of, may survive the process. Its nuclear material may become part of the aggressor cell, leading to multinucleate cells, polyploidy or aneuploidy.

Some engulfed cells actually escape their captors and re-emerge unscratched.

Mechanisms governing entosis are largely obscure.

# **Pathology of Neurodegenerative Diseases**



# Neurodegenerative Diseases

Progressive loss of selectively vulnerable population of neurons.

Contrasts with select static neuronal loss because of metabolic or toxic disorders.

- 1) Clinical features (e.g., dementia, parkinsonism, or motor neuron disease).
- 2) Anatomic distribution of neurodegeneration (e.g., frontotemporal degenerations, extrapyramidal disorders, or spinocerebellar degenerations)
- 3) Principal molecular abnormality.
- 4) The most common neurodegenerative disorders are amyloidoses, tauopathies, a-synucleinopathies, and TDP-43 (TAR DNA-binding protein 43) proteinopathies. The protein abnormalities in these disorders have abnormal conformational properties.
  - Abnormal protein conformers may spread from cell to cell along anatomically connected pathways, which may in part explain the specific anatomical patterns of the disease observed at autopsy.

Few patients have pure, syndromes, with most having mixed clinical features.

"Diagnostic" gold standard is neuropathological evaluation at autopsy.

Although typically defined by specific protein accumulations and anatomic vulnerability, neurodegenerative diseases share many fundamental processes associated with progressive neuronal dysfunction and death

- Proteotoxic stress
  - Abnormalities in ubiquitin–proteasomal and autophagosomal/lysosomal systems,
- Neuroinflammation
- Oxidative stress
- Programmed cell death
  
- Protein abnormalities that define neurodegenerative diseases can be present before the onset of clinical features.
  
- More than one neurodegenerative disease process can be found in an individual.

Protein accumulations within neurons include

- tau in neurofibrillary tangles (NFTs) or Pick bodies
- $\alpha$ -synuclein in Lewy bodies
- Transactivation response DNA binding protein 43 (TDP-43) in cytoplasmic and intranuclear inclusions.

Protein accumulations within astrocytes include

Tau in tufted astrocytes, astrocytic plaques, and thorn-shaped astrocytes.

Protein accumulations within oligodendroglia

- Tau in coiled bodies
- $\alpha$ -synuclein in glial cytoplasmic inclusions.

Diagnostic biomarkers are not available except in rare cases of genetic mutation.

Quest for specific *in vivo* biomarkers are the topic of major research priority:

- Biofluid markers
- Molecular imaging markers

# AMYLOIDOSES

Amyloids are insoluble fibrous proteins (rich in  $\beta$ -sheet-rich secondary structure), amyloid-like filamentous aggregates are mostly cytosolic (in neurons and glia).

The most common amyloidosis is a proteolytic product of the amyloid precursor protein, encoded by a gene on chromosome 21  $\beta$ -amyloid ( $A\beta$ ).

$A\beta$  is a feature of Alzheimer disease (AD), amyloid deposits are found as a comorbid feature of many other neurodegenerative disorders in the elderly, especially in those individuals carrying the major genetic risk factor for AD,

- Apolipoprotein E4

AD is a mixed proteinopathy that includes the presence of both  **$A\beta$**  deposits in the parenchyma as amyloid or senile plaques as well as neuronal **tau** inclusions.

- 

Other types of amyloid are deposited such as prion protein (PrP) or  $A\beta$

Sporadic Creutzfeldt–Jakob disease (CJD) (rapidly progressive dementia little to no brain atrophy).

Gerstmann–Straussler–Scheinker disease (GSS)

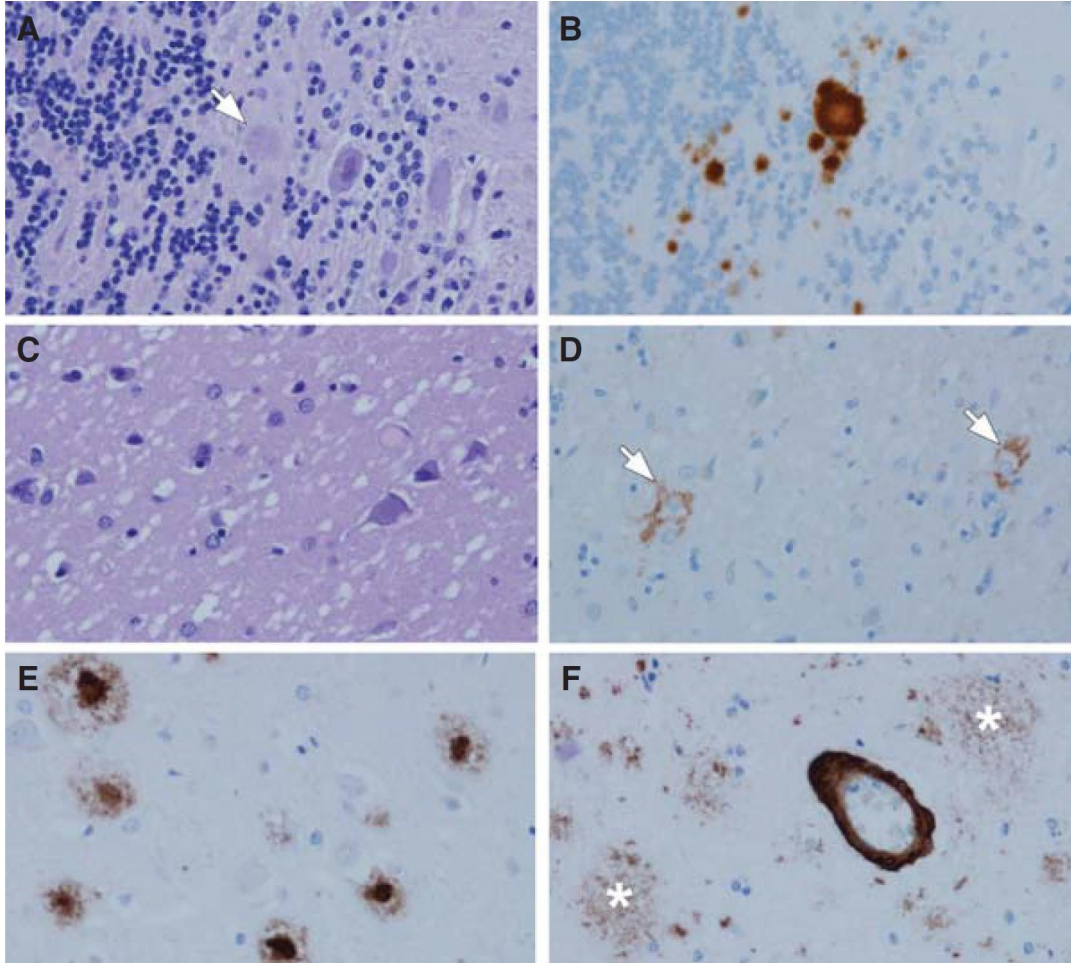
Familial British and Danish dementias

## Prion Diseases

The key molecular event of prion diseases is the conversion of the normal cellular prion protein, PrP<sup>C</sup>, into the pathogenic form, PrP<sup>Sc</sup>. First time that protein was demonstrated as infectious agent.

Neuronal and synaptic loss, microvacuolation (spongiform change), and gliosis.

White matter is relatively spared, most characteristic pathology occurring in gray matter.



**(A,B):** Gerstmann–Straussler–Scheinker disease (GSS) dense-cored amyloid plaques can be detected by hemotoxylin and eosin (H&E) staining. Immunohistochemistry for human PrP reveals more the multicentric nature of the deposits.

**(C,D):** Creutzfeldt–Jakob disease (CJD), One of the hallmark CJD is the spongiform change in affected cortical and subcortical areas with perineuronal synaptic pattern of PrP deposition (arrows) in an adjacent section (D)

**(E,F):** Alzheimer’s disease (AD) amyloid deposits are heterogeneous and include those with dense cores, especially in primary cortices (E), as well as poorly circumscribed and noncompact diffuse plaques in the cortex (asterisks). In addition to parenchymal deposits, most cases of AD also have amyloid angiopathy.

# TAUOPATHIES

Disorders associated with pathological accumulation of tau protein in neurons and glia.

Tau is a microtubule-associated **phosphoprotein** abundant in axons involved in promoting polymerization and stabilization of microtubules.

Other posttranslational modifications have been linked to abnormal

- Ubiquitination,
- Nitration,
- Glycation,
- Acetylation,
- which tau that

Genetic mutations in the gene encoding for tau protein demonstrated that abnormalities in the tau protein could cause neurodegeneration.

- Frontotemporal dementia (MAPT)
- Parkinsonism linked to chromosome 17 (FTDP-17T)

Tau protein exists as six major isoforms produced by alternative mRNA splicing of exons 2, 3, and 10. Four conserved 32-amino-acid repeats (4R tau) or three isoforms have three repeats (3R tau) in the domain critical for binding to microtubules.

## Alzheimer's Disease

The most prevalent tauopathies although considered a secondary tauopathy because mutations that cause AD in the presenilin genes, (PSEN1 and PSEN2) and the amyloid precursor protein gene (APP) are characterized as initial or primary alterations in amyloid metabolism.

**Macroscopic changes include variable atrophy** of multimodal association cortices in the frontal, temporal, and parietal lobes. A subset of cases also showed occipital lobe atrophy.

### **Microscopically, two lesions define AD:**

- Amyloid plaques: A $\beta$  deposits in the parenchyma as amyloid or senile plaques.
- Neurofibrillary tangles (NFTs) composed predominantly of tau protein. As NFTs mature, the neuron bearing the inclusion dies, leaving an extracellular ("ghost") NFT.

**Accumulation of unfolded proteins within the lumen of the endoplasmic reticulum (ER) induces ER stress which if not resolved trigger cell death.**

# Amyotrophic Lateral Sclerosis (ALS)

Affects both upper and lower motor neurons and clinically is associated with weakness, muscle atrophy, fasciculations, as well as spasticity.

- Primary lateral sclerosis: involvement of primarily upper motor neurons.
- Progressive muscle atrophy: involvement of primarily lower motor neurons.

Most ALS is associated with **TDP-43 pathology**.

## **Macroscopic features in ALS are often subtle.**

Mild atrophy of the motor cortex, and spinal cord (atrophy of anterior spinal nerve roots). In patients with dementia: focal atrophy of the temporal and frontal lobes.

## **Microscopic features in ALS are often subtle.**

Neuronal loss and gliosis (lower motor neurons in the spinal cord and brainstem) and upper motor neurons in the motor cortex. Surviving neurons often have **TDP-43 inclusions**. Sparse oligodendroglial inclusions in motor tracts.

Although motor neurons are most vulnerable, there is spread of the pathology to extramotor sites (hippocampus, amygdala and cortex).



# Parkinson's disease

Chronic, progressive, neurodegenerative disease characterized by hallmark signs of bradykinesia, rigidity, tremor, and postural instability.

90% of sporadic cases while 10% have a genetic origin, and at least 11 different linkages with 6 gene mutations (young-onset PD).

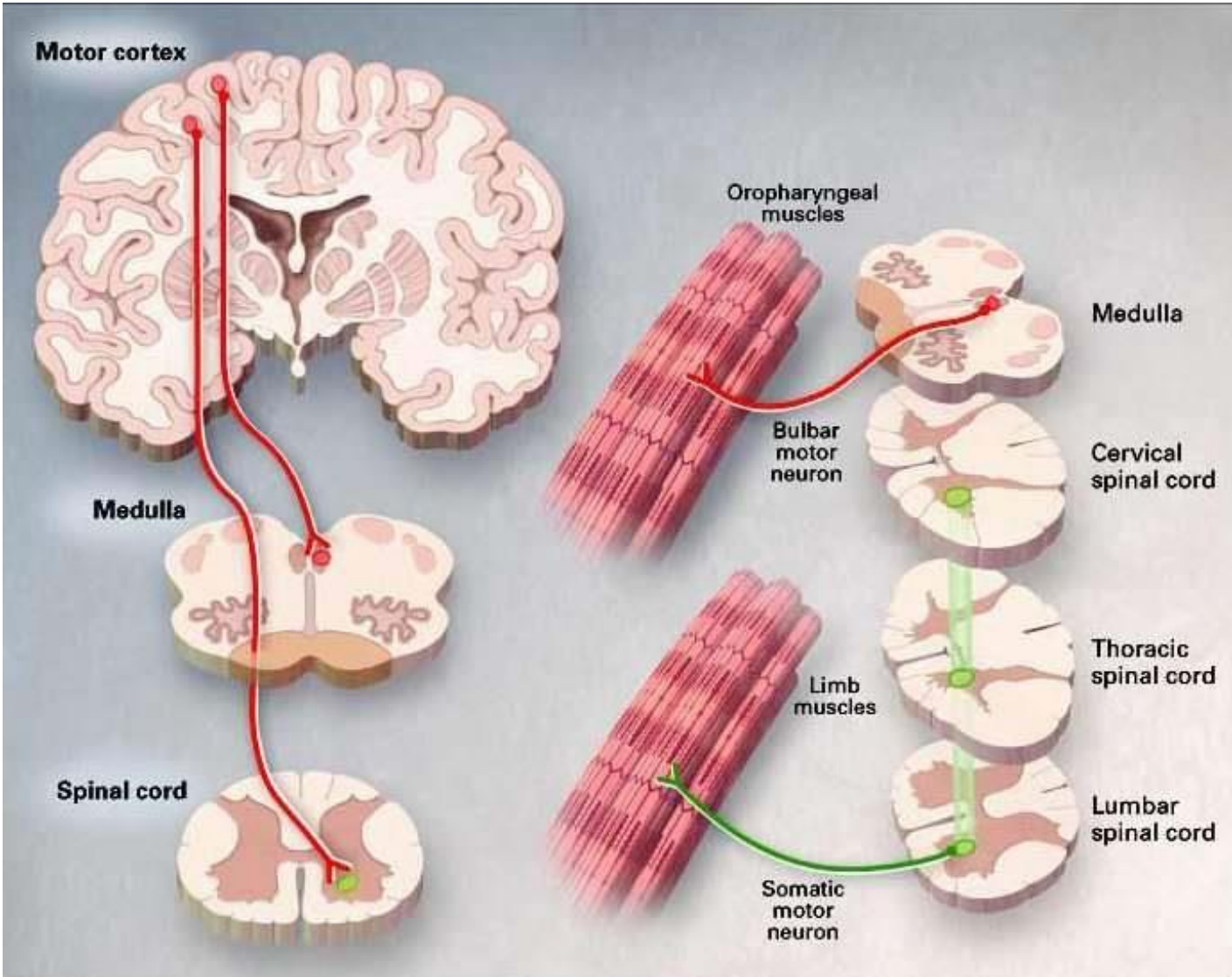
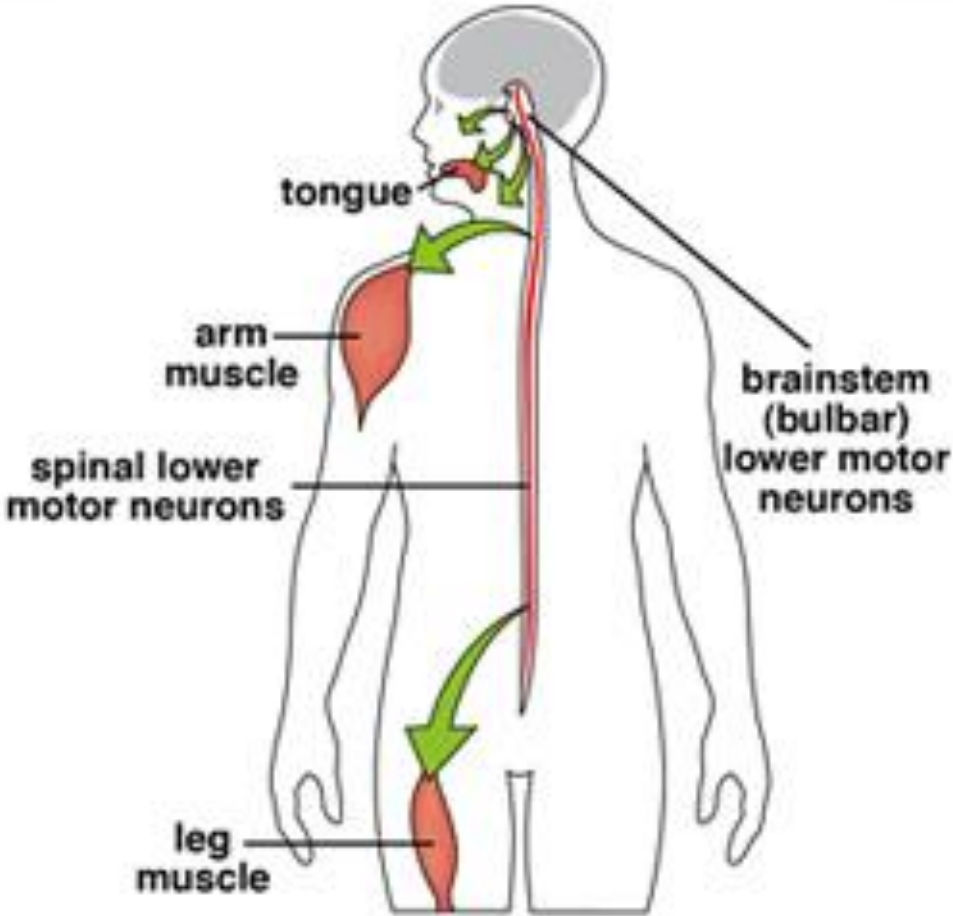
The pathologic hallmark of PD is **degeneration of dopaminergic neurons** in the substantia nigra pars compacta (SNc), resulting in **depletion of striatal dopamine**. This neurotransmitter regulates excitatory and inhibitory outflow of the basal ganglia.

Alpha-synuclein ( $\alpha$ SYN) pathology and mitochondrial dysfunction are implicated in PD pathogenesis in both familial and sporadic PD.

The neurodegenerative process is not limited to the SNc, as neuronal loss with Lewy body formation also occurs in other brain regions accounting for both motor and nonmotor features of the disease.

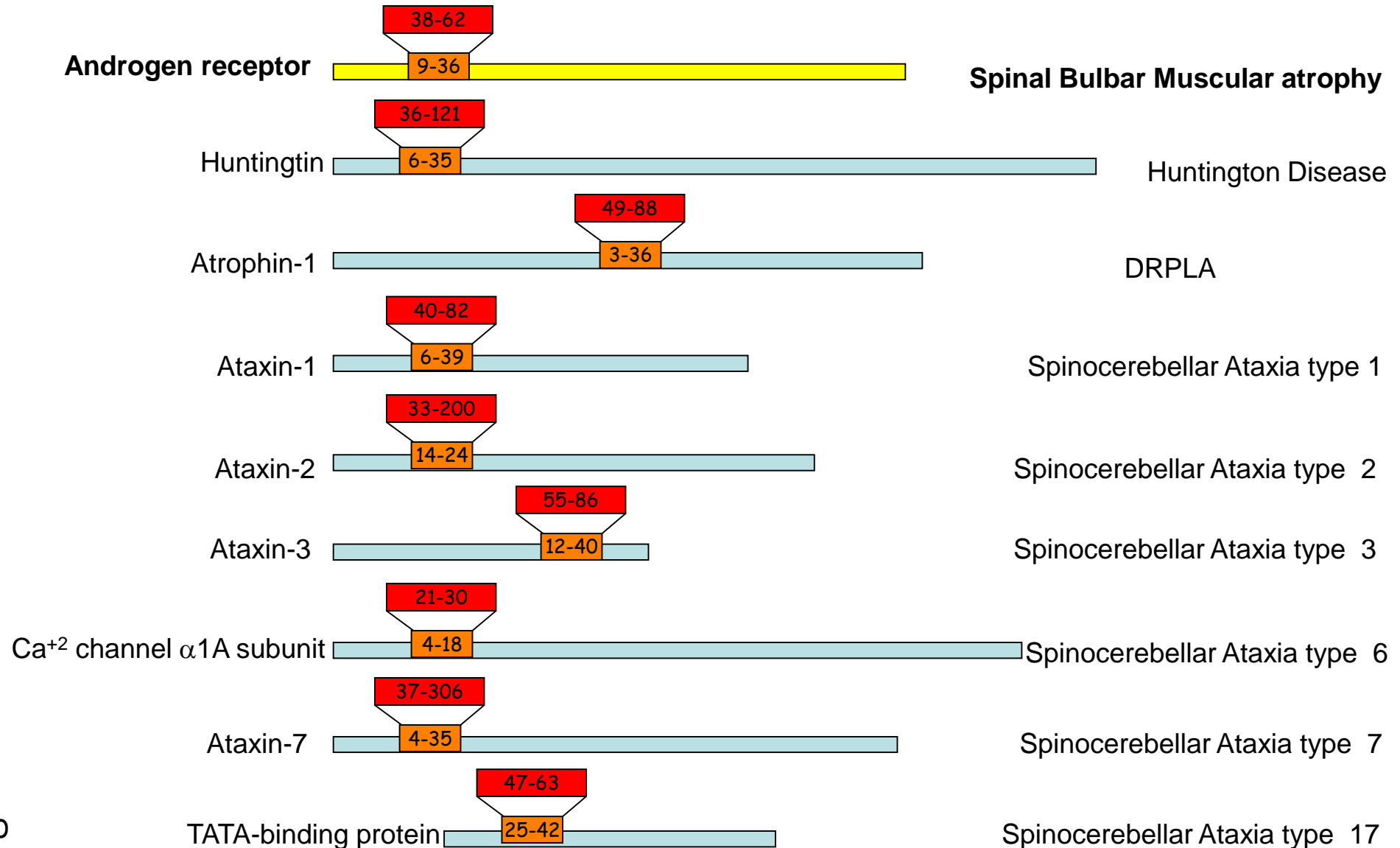
There are also non-motor symptoms such as depression, dementia, and psychosis.

# Spinal and Bulbar Muscular Atrophy

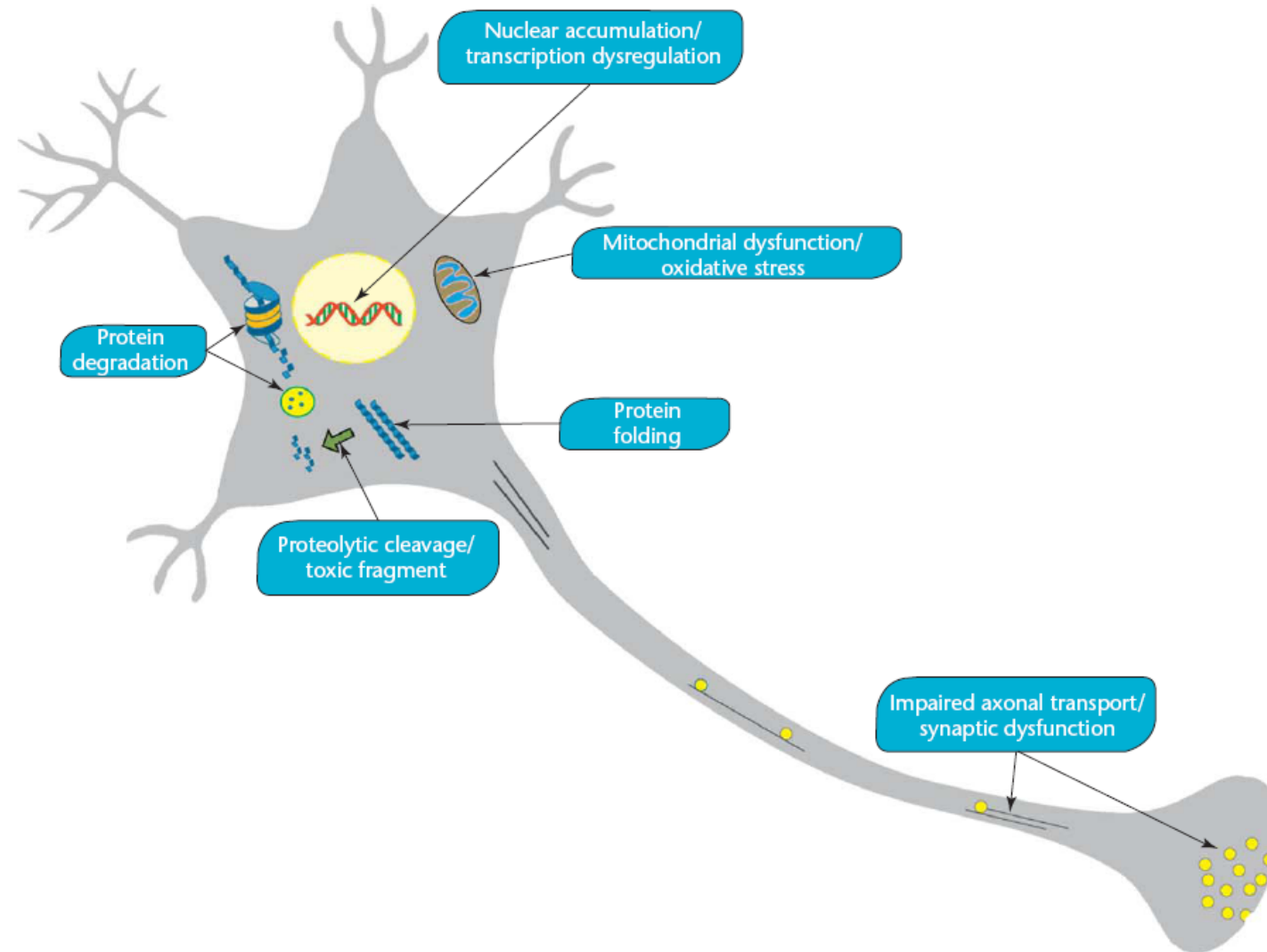




# Polyglutamine diseases



# Polyglutamine proteins: Several toxic events



# Mechanisms of ER stress-induced cell death

