بسم الله الرحمٰن الرحيم

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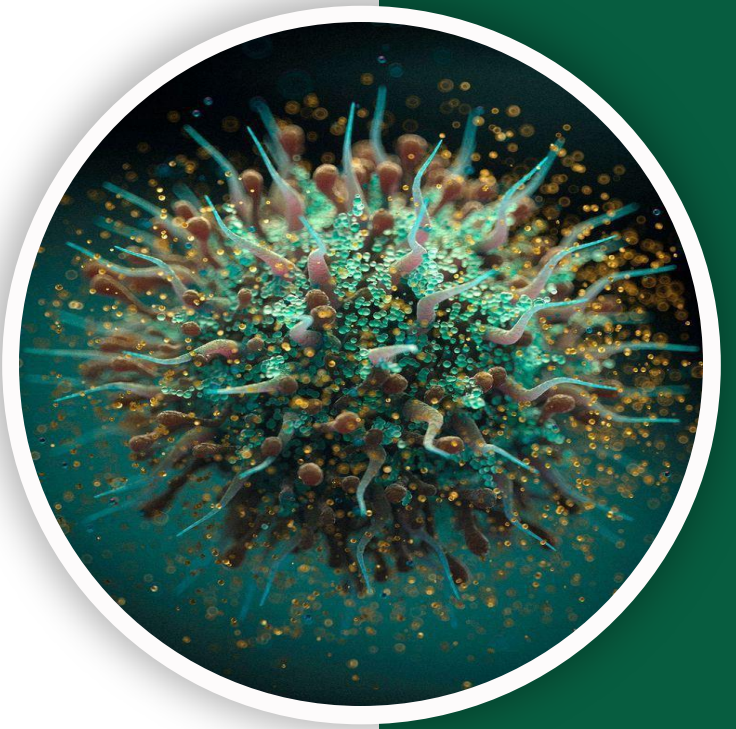
Pathology | Lecture 3

Mechanisms Of cell injury



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MECHANISMS OF CELL INJURY

cell injury and adaptations

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MECHANISMS OF CELL INJURY
Principles

- The cellular response to injury depends on:

type of injury

Hypoxia, Ischemia, infections and other types of injuries each one has its own consequences

duration

For example ischemia to the heart (cardiac muscle) :for 1-5 minutes causes angina (chest pain or pressure) (reversible) which is different from ischemia for 20 minutes that causes myocardial infraction (irreversible)

The same for brain cells and skeletal muscles; ischemia for half an hour to a skeletal muscle doesn't cause death to it but if ischemia continues for 3 hours it causes necrosis (death)

severity

I. Hypoxia (decrease oxygen supply) is different from ischemia (cut of blood and nutrients supply) with regards to severity
II. Decreasing the blood supply is different from cutting the blood supply (complete occlusion of blood vessels which is more severe and leads to necrosis)

MECHANISMS OF CELL INJURY
Principles

- The consequences of injury also depend on:

type,

For example, myocardial infarction happens after 20 minutes of ischemia in the heart whereas the skeletal muscles withstand ischemia for 3 hours because they have higher adaptability

status,

The status of the cell before the injury affects the consequences; the same injury will cause different consequences on healthy cells from previously diseased cells (a cell in a reversible injury might undergo necrosis if it is affected by another injury)

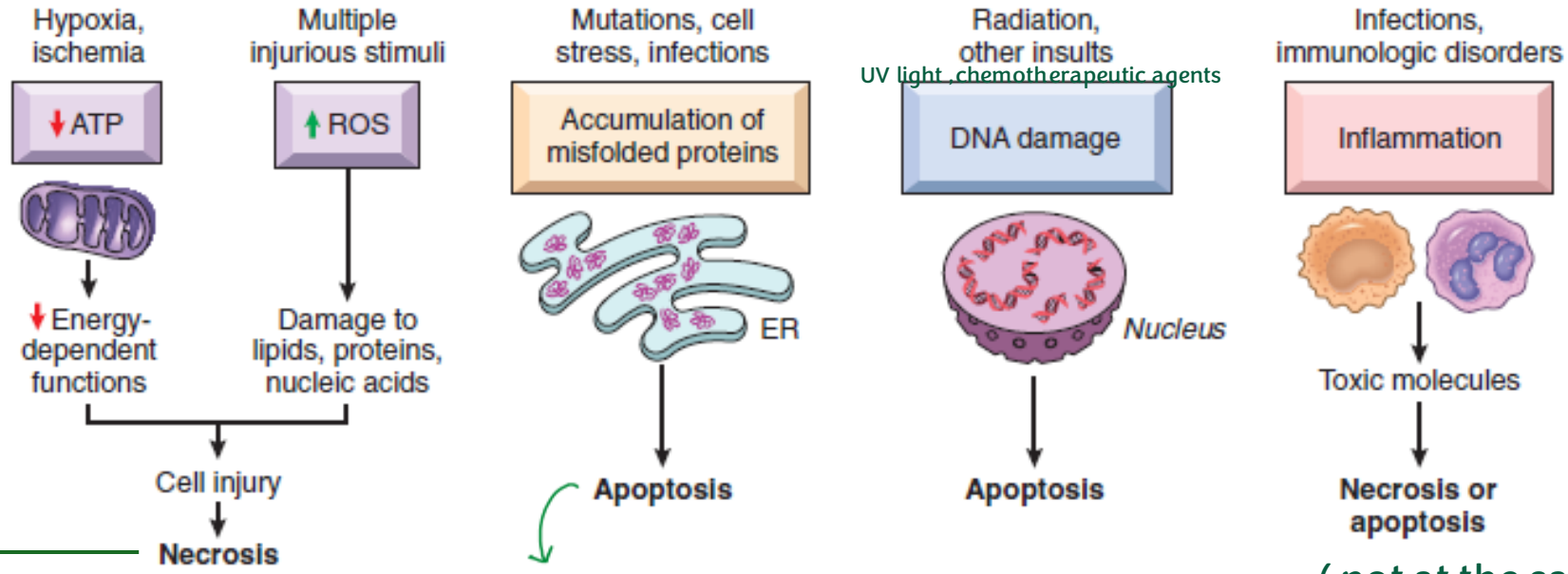
adaptability, and genetic makeup of the injured cell (precision medicine concept)

The genetic makeup differs from one person to another, so the response to the same injury differs between individuals One patient may require a higher dose than another, and some patients' cells may respond to certain drugs while others' cells do not. (The genetic makeup : the complete set of genes an organism has, inherited from both parents, which contains the instructions for building and maintaining the body)

- Cell injury results from functional and biochemical abnormalities in one or more of several essential cellular components.
- Same injury may trigger more than one mechanism.

The same injury can activate more than one mechanism, the same cell can undergo more than one mechanism, the same mechanism can be triggered by more than one type of injury.

The principal biochemical mechanisms and sites of damage in cell injury



(The end result of irreversible cell injury)

peculiar Form of cell death that has certain circumstances and triggers

(not at the same cell but at the same tissue)

* Necrosis can take place by more than one mechanism

Reactive Oxygen Species (ROS) – free radicals –: particles have one extra single unpaired electron in the outer shel which give them very high damaging energy (by convert the molecules they interact with into free radicals, initiating a chain of reactions that ultimately result in complete tissue damage)

Hypoxia and Ischemia

- One of the most frequent causes of injury.
- Defective oxidative phosphorylation (in the mitochondria)>>Failure of ATP generation>>>depletion of ATP in cells
- Failure of energy dependent pathways (membrane transport, protein synthesis, lipogenesis and phospholipid turnover)

Like the Na^+/K^+ pump which needs ATP to pump three sodium ions (Na+) out of the cell and two potassium ions (K+) in so failure with it leads to accumulation of Na+ inside and attraction of water –> swelling of the cell and organ

- Anaerobic glycolysis. (not in all cells, generation a little amount of ATP)
- Liver cells and skeletal muscle cells Vs brain and heart.

↓ Can do anaerobic glycolysis

↓ Can not do anaerobic glycolysis

Hypoxia effects:

- Reduced activity of membrane ATP dependent sodium pumps>> cell swelling>> organ swelling
- Lactic acid accumulation >> decreased PH>> failure of enzymes. (Acidosis)
- Disruption of the ribosomes>> decreased protein synthesis.
- Accumulation of ROS (inefficient redox reactions produce ROS)
- Damage to mitochondrial and lysosomal membranes. (lysosomal enzymes will get out to the cytoplasm then to the outside of the cell (auto digestion) – greater damage and destruction)
- Necrosis is the end result.
- Apoptosis can contribute. (according to severity; in severe injuries death mainly happens by necrosis but sometimes in some cells by apoptosis)

Any cellular injury makes the cell non functional , regardless of whether the damage is reversible or irreversible."

blood supply returns to tissue after a period of ischemia or lack of oxygen

Ischemia-Reperfusion Injury

(Although reperfusion is expected to restore cell viability after ischemia, it paradoxically increases injury, mainly due to free radical generation from dysfunctional mitochondrial redox reactions during the reversible stage of injury.)

- Paradoxical cell injury after restoration of blood flow to ischemic but viable tissues.
- After myocardial and cerebral ischemia.

- **Increased generation of ROS from:**
- Injured cells with damaged mitochondria & defective antioxidant mechanisms.
- Infiltrating new leukocytes.

- **Inflammation induced by influx of leukocytes, plasma proteins and complement** (Inflammatory cells in the bloodstream (white blood cells) clean the area and generate additional ROS during reperfusion.)

Oxidative Stress

Oxidative stress occurs when ROS accumulate in the cell. The cell is exposed to high stress, resulting in a lot of damage to every single component of the cell

- Cellular abnormalities induced by ROS (free radicals)
- Chemical species with single unpaired electron (extremely unstable)

- **ROS generated in:**
- Chemical injury (CCL4)
- Radiation injury (UV, Xray)
- Hypoxia
- Cellular aging
- Inflammation
- Ischemia-reperfusion injury.

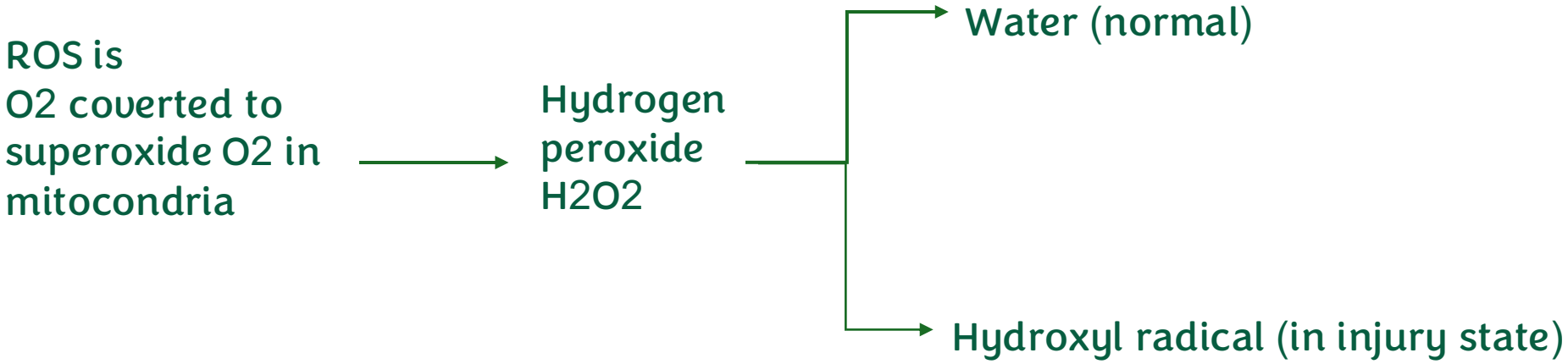
Aging

ROS Once they are produced in large number, they enter a closed chain and expose the cell to oxidative stress, which leads to damage in every single component of the cell: the membrane, proteins, and DNA. These are the three components targeted by ROS

Generation and Removal of Reactive Oxygen Species

In a way that prevents damage to the cells.

- **1-Normally produced in small amounts in all cells during the redox reactions.**
- Oxygen is reduced to produce water.
- Small amounts of highly reactive but short-lived toxic intermediates are generated.
- Superoxide (O2 •), hydrogen peroxide (H2O2), hydroxyl radical •OH.



Phagocytes are white blood cells that
phagocytose microbes or foreign substances.

- **2-Produced in phagocytic leukocytes (neutrophils and macrophages) during inflammation.**
- In phagosomes and phagolysosomes to kill microbes.
- O2 >> superoxide >> H2O2 >> hypochlorite.
- Myeloperoxidase (H2O2 into hypochlorite).

Is produced by myeloperoxidase
موجود بكلور المطبخ وهوbactericidal

Found normally

in phagocytes

To confirm under the microscope whether these are phagocytes or macrophages, we can use myeloperoxidase immunostaining to highlight these cells

- It is interesting that ROS are normally produced in the cell and removed. ROS are produced during inflammation and microbial infection, but there is always a removal mechanism. They undergo spontaneous decay; although they have high energy, they are short-lived, and the cell contains certain enzymes to manage ROS

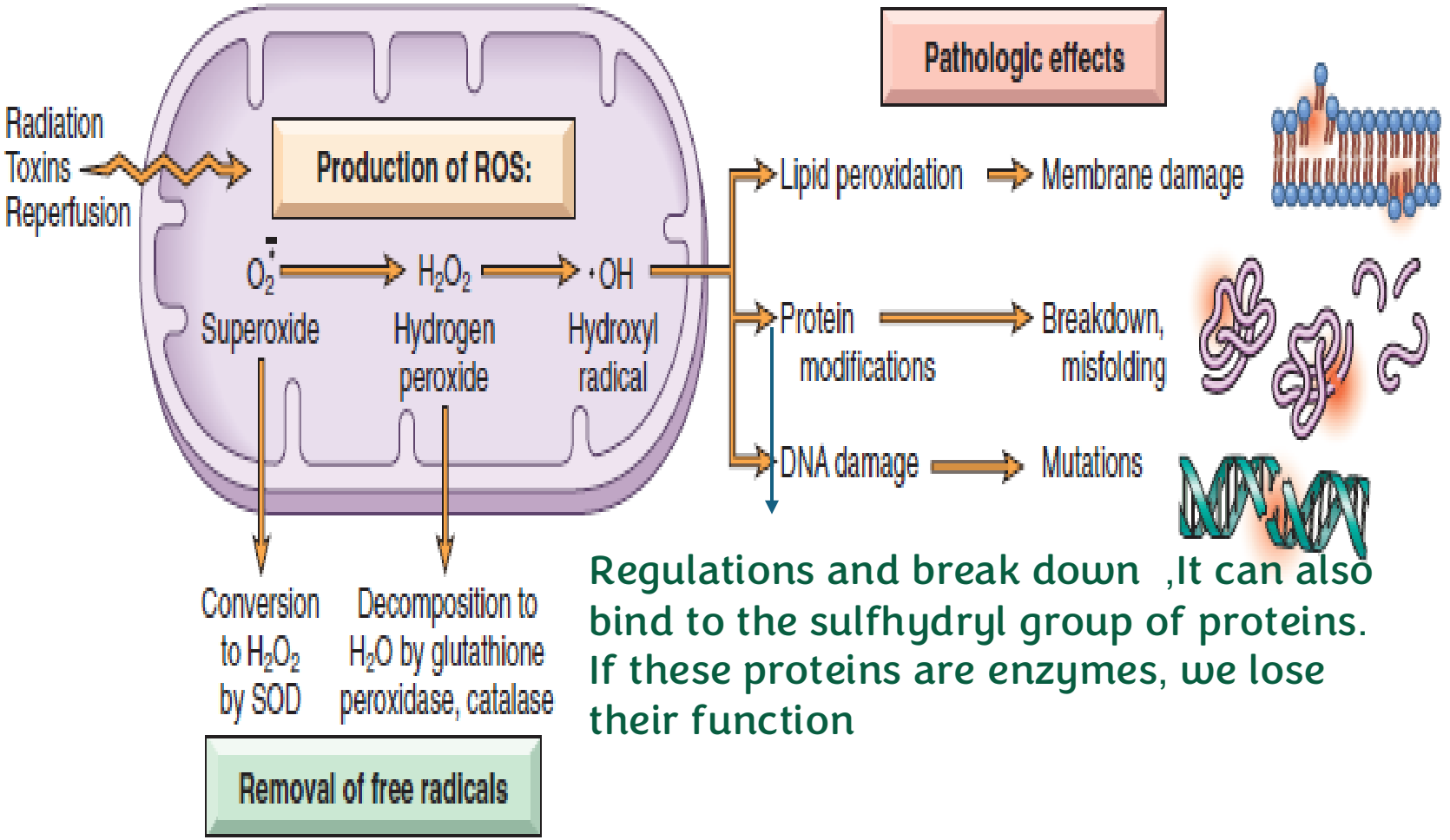
2 مادة حارقة H2O

Removal of free radicals

- Decay spontaneously
- Superoxide dismutase (SOD). Convert superoxide to H2O2 which is a potent ROS
- Glutathione (GSH) peroxidases.
- Catalase (one of most active enzymes known)
- Endogenous or exogenous anti-oxidants (e.g., vitamins E, A, and C and β-carotene)

H_2O_2 must be reduced to water by glutathione peroxidase and catalase. Catalase is one of the most important enzymes known ever

Protective for the cell because they always can protect the cell from the damaging effects of free radical



Regulations and break down ,It can also bind to the sulfhydryl group of proteins. If these proteins are enzymes, we lose their function

Not only the cell membrane, but also the mitochondrial membrane and lysosomal membrane are affected and all cell membrane

Effects or ROS:

- **1-Lipid peroxidation of membranes.**
- (plasma, lysosomal & mitochondrial membranes)
- **2-Crosslinking and other changes in proteins.**
- (degradation, fragmentation, loss of enzymatic activity & misfolding).
- **3-DNA damage.**
- Single strand breaks, mediate: apoptosis, aging, malignant transformation
- **4-Killing of microbes.**

Continuous
DNA damage

Necrosis

When a person is exposed to UV or X-rays, the continuous exposure and continuous DNA damage can cause malignant transformation. The end result is not always cell death or apoptosis. Sometimes, if the effect is minor but continues for a long period of time, it causes mutations that lead to malignant transformation

Cell Injury Caused by Toxins

- Environmental chemicals & substances produced by infectious pathogens.

- **Direct-acting toxins**
- **Latent toxins.** Indirect-acting
- inactive substances that must be converted to active metabolites in the liver by cytochrome P450 enzymes. These reactive metabolites exert the cellular damage

Direct-acting toxins

They mediate the damage immediately

- Act directly by combining with a critical molecular component or cellular organelle.

- **Mercuric chloride poisoning**
- Contaminated seafood
- Mercury binds to sulfhydryl groups of membrane proteins>>inhibit ATP-dependent transport and increase permeability.
- **Chemotherapeutic agents**
- **Toxins from microorganisms.**

• Which is transporter protein

The chemotherapeutic agent directly targets and damages malignant cells –this is a desirable effect.

Toxin of the cholera toxin, of certain bacteria

Latent toxins

• These mechanisms do not work in isolation; they all occur in parallel. More than one mechanism can be activated at the same time, and one mechanism can mainly influence the other mechanisms

- Not intrinsically active
- Must be converted to reactive metabolites, then act on target cells.
- Via cytochrome P-450 in SER of the liver.
- Damage mainly by formation of free radicals>>membrane phospholipid peroxidation.

Paracetamol

- **CCl4 and acetaminophen**. (in over dose)
- Membrane peroxidation>>>>damage
- ER membranes >> detachment of ribosomes>>decline in synthesis of enzymes and proteins +decreased synthesis of apoproteins >> fatty liver
- Mitochondrial membranes>> decreased ATP >> cell swelling >> cell death.

ATP- dependent

CCL4 toxicity

When protein synthesis in the liver decreases, what happens to lipids? They remain in the liver because lipids need to bind to proteins to be transported in the blood. Decrease synthesis of proteins lead to accumulate the lipid in the liver (fatty change)

- CCl4

Free radical

CCl4 —(sER)→ $CCl_3^{\cdot}$ —($+O_2$)→ Lipid peroxidation

Lipid peroxidation → rER damage → Polysome detachemnt → ↓ Apoprotein Synthesis → Fatty liver

لو طلعت على الكبد رح يكون yellowish نتيجة الدهون

Lipid peroxidation → Plasma membrane damage → Δ membrane permeability, Cell swelling, Ca2+ influx, Mitochondrial ...

Endoplasmic Reticulum Stress

- Chaperones in ER control proper protein folding
- Misfolded proteins >> ubiquinated >> targeted to proteolysis
- **Unfolded protein response (adaptive response):** increase chaperones production, decrease protein translation and increase destruction.
- If failed >> proapoptotic sensor activation (BH3-only family) + direct activation of caspases >>**apoptosis by the mitochondrial pathway.**

When ribosomes synthesize proteins, they are transferred to the endoplasmic reticulum (ER), where they undergo a process called protein folding.

This folding is essential because it allows the protein to acquire its proper three-dimensional conformation and become functionally active.

Proper folding enables the protein to be transported from the ER to its final destination, whether within the cytoplasm or outside the cell.

If proper folding fails, misfolded proteins accumulate in the ER, leading to a condition known as ER stress.

To cope with this stress, the cell activates a protective mechanism called the unfolded protein response (UPR).

The UPR increases the synthesis of molecular chaperones (which assist in refolding misfolded proteins), reduces overall protein synthesis, and enhances the degradation of abnormal proteins.

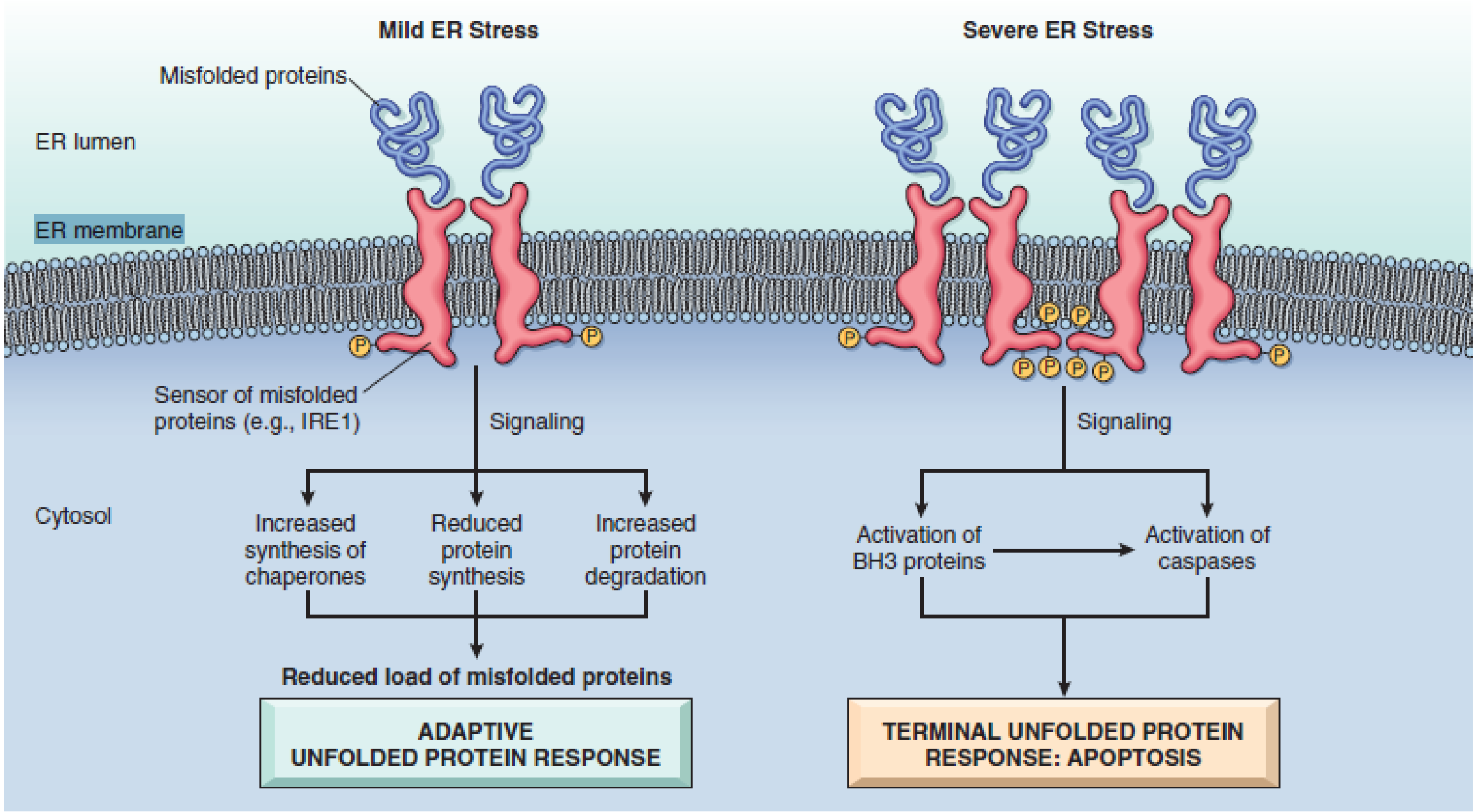
These combined actions decrease the burden on the ER and help restore cellular homeostasis.

If the adaptive response is successful, the cell survives.

However, if misfolded proteins continue to accumulate and exceed the cell's capacity to adapt, the response fails.

In this situation, the pro-apoptotic pathways are activated through members of the BH3-only family, leading to the activation of caspases—the enzymes responsible for apoptosis.

This triggers the mitochondrial (intrinsic) pathway of apoptosis, resulting in programmed cell death.


Mild ER Stress
Severe ER Stress
Misfolded proteins
ER lumen
ER membrane
Sensor of misfolded proteins (e.g., IRE1)
Signaling
Signaling
Cytosol
Increased synthesis of chaperones
Reduced protein synthesis
Increased protein degradation
Activation of BH3 proteins
Activation of caspases
Reduced load of misfolded proteins
ADAPTIVE UNFOLDED PROTEIN RESPONSE
TERMINAL UNFOLDED PROTEIN RESPONSE: APOPTOSIS

Causes of misfolding

Aging not just for humen butt also for cells for example our cells in the skin aging cells the most superficial they will replace by new cells and those old cell will die How ???? they accomadate misfolded protein and this will target to apoptosis

- Gene mutations
- Aging (decreased capacity to correct misfolding)
- Infections, especially viral infections (microbial proteins) As prion disease
- Increased demand for secretory proteins such as insulin in insulin-resistant states(**NEXT SLIDE**)
- Changes in intracellular pH Enzymes will be non –functional
- Neurodegenerative diseases
- Deprivation of glucose and oxygen in ischemia and hypoxia.

- **Increased demand for secretory proteins such as insulin in insulin-resistant states :** Insulin resistance occurs when insulin is present, but receptors fail to respond. Consequently, the pancreas increases insulin synthesis, leading to insulin accumulation in cells. Over time. individuals with type 2 diabetes may experience decreased insulin production due to misfolded protein accumulation in pancreatic cells, triggering apoptosis. This cell death necessitates insulin injections for diabetic patients
- **Neurodegenerative diseases :** As Alzheimer's and Huntington's diseases that lead to the deathof neurons due to the accumulation of misfolded proteins within fnese cells. Vitimately. Inis process results in orain atropny

Protein misfolding causes disease by:

- **Deficiency of an essential protein due to degradation**
- Cystic fibrosis
- **Inducing apoptosis of the affected cells**
- Neurodegenerative disorders (Alzheimer disease, Huntington disease & Parkinson disease), type 2 diabetes and prions disease.
- **Inducing both:**
- Alpha 1 antitrypsin deficiency.
- **Improperly folded proteins accumulation in extracellular tissues**
- Amyloidosis

Sometimes, misfolded proteins maybecome inactive, preventing them frombeing secreted outside the cell andhindering their proper function.

DNA Damage

- Radiation As UV light
- Chemotherapeutic agents
- Intracellular generation of ROS
- Mutations

Tumor suppressor gene

- DNA damage >> p53 activation >>arrest cell cycle at G1 phase for repair >> if repair is impossible >> apoptosis.
- In P53 mutations >> mutated cells replicate >> neoplastic change.

This mechanism will protect us from tumors

Inflammation

- Pathogens As viruses, bacteria, and protozoa.
- Necrotic cells,
- Dysregulated immune responses (autoimmune diseases and allergies)

- Inflammatory cells (neutrophils, macrophages, lymphocytes) secrete products that destroy microbes and damage host tissues.

Inflammation is beneficial for resisting microbes, removing dead cells, andmediating certain immune responses. However, when inflammation exceedswhat is necessary, it can lead to necrotic changes and damage to host cells.

Common Events in Cell Injury From Diverse Causes

All mechanisms of cell injury end up with one of the 2 following damages:

- Mitochondrial Dysfunction
- Defects in Membrane Permeability

Whether the plasma membrane,mitochondrial or lysosomal membrane

Mitochondrial Dysfunction

- Energy factory
- Hypoxia, toxins, radiation.
- In necrosis and apoptosis.

All decrease the efficiency of the mitochondria → decrease ATP productionleading to either apoptosis or necrosis

- **Consequences:**
- Failure of oxidative phosphorylation, ATP depletion.
- Abnormal oxidative phosphorylation, formation of ROS
- Mitochondrial permeability transition pores, loss of membrane potential.
- Release of cytochrome c >> apoptosis

Which leads to the leak of cytochrome c to the cytoplasm, activating caspases andinitiating apoptosis

Mitochondrial Damage and Dysfunction

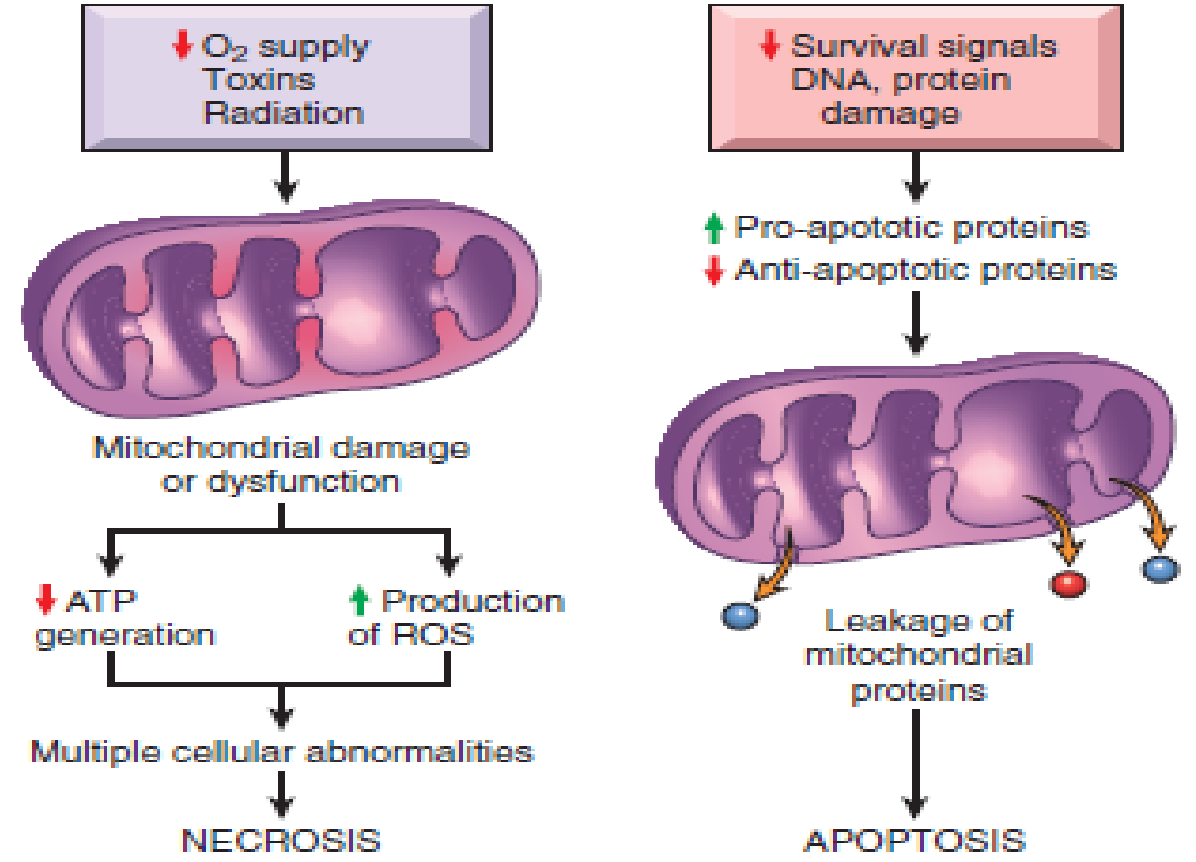


Figure 1–16 Role of mitochondria in cell injury and death. Mitochondria are affected by a variety of injurious stimuli and their abnormalities lead to necrosis or apoptosis. This pathway of apoptosis is described in more detail later. ATP, adenosine triphosphate; ROS, reactive oxygen species.

Depletion of ATP

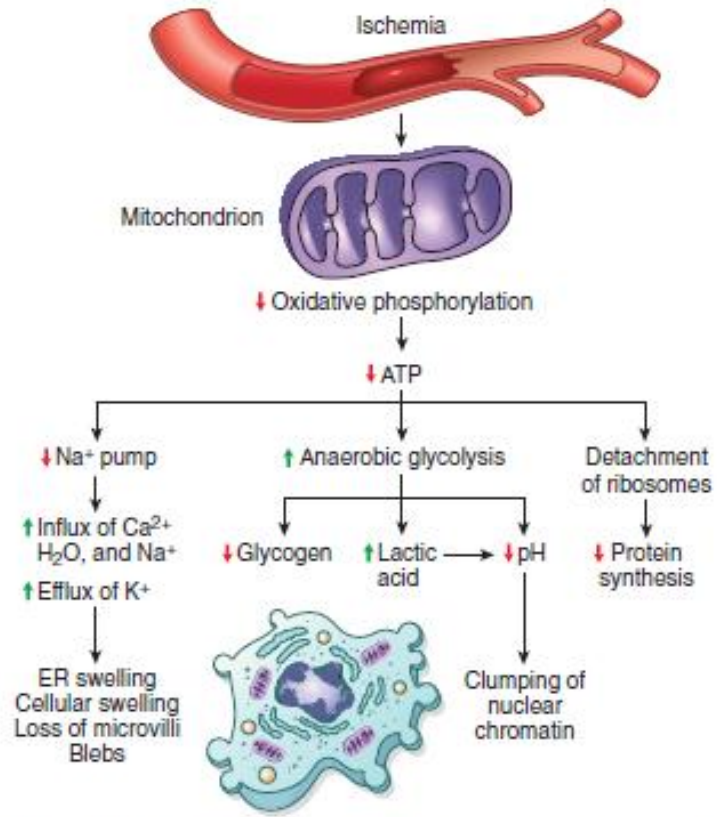


Figure 1–15 The functional and morphologic consequences of depletion of intracellular adenosine triphosphate (ATP). ER, endoplasmic reticulum.

Defects in Membrane Permeability

- Mitochondrial membrane damage: decreased ATP
  in addition to the release of cytochrome c to the cytoplasm because of the increased permeability
- Plasma membrane damage: loss of osmotic balance, influx of fluids, leak of contents
- Lysosomal membranes: leakage of enzymes >> cellular digestion.

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Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			