

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ
(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)



Metabolism | Lecture 8

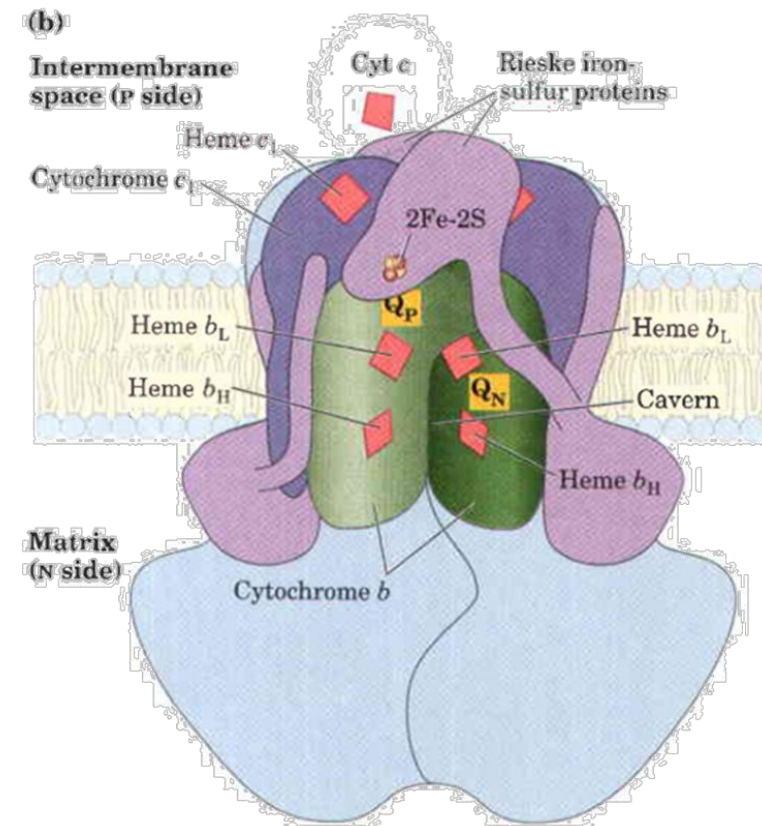
Oxidative (pt.3) Phosphorylation



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OXI-RED COMPONENTS OF THE ETC: “CYTOCHROME BC₁” – COMPLEX III

- Q-cytochrome c Oxidoreductase
- 11 subunits including two cytochrome subunits
- Contains iron sulfur center
- Contain three heme groups in two cytochrome subunits
- b_L and b_H in cytochrome b; c type in cytochrome C₁
- Contain two Co Q binding sites
- 4H⁺



OXI-RED COMPONENTS OF THE ETC: “CYTOCHROME BC ” – COMPLEX III

In Complex III, quinone (Q) can exist in two forms – a reduced form (QH₂) and an oxidized form (Q).

- The reduced form (QH₂) binds at a site near the outer side of the inner mitochondrial membrane (the intermembrane space side).
- The oxidized form (Q) binds at another site close to the matrix side of the membrane.

So, one reduced QH₂ molecule binds at one site, and one oxidized Q molecule binds at another.

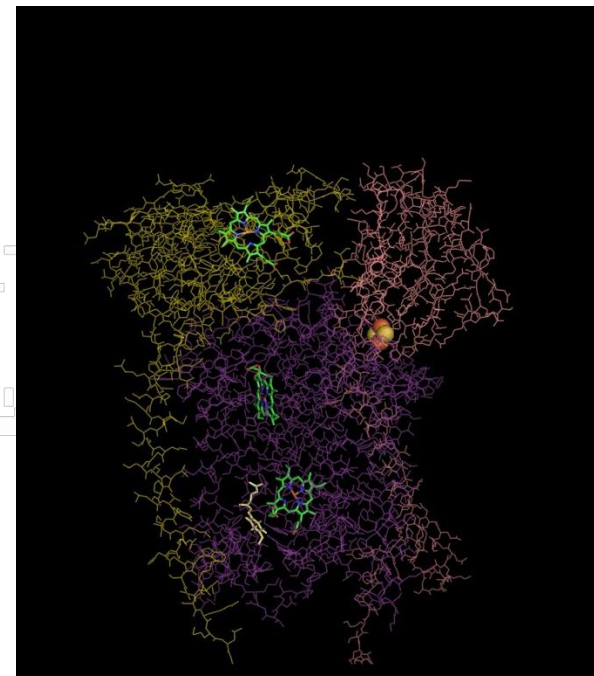
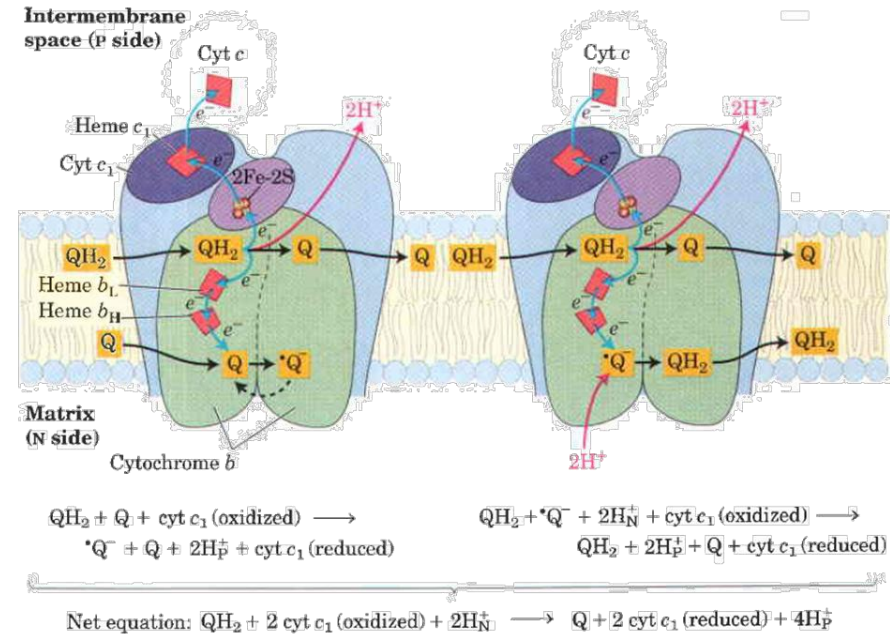
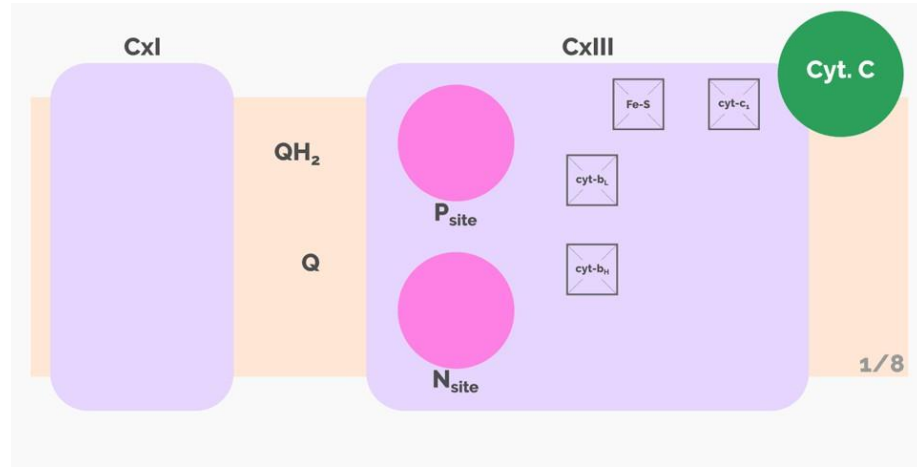
When the reduced QH₂ binds, it donates two electrons:

1. The first electron travels through the iron-sulfur (Fe-S) clusters, then to cytochrome c₁, and finally to cytochrome c, which leaves the complex and carries the electron to Complex IV.
2. The second electron follows a different path – it goes to cytochrome b_L (the low-potential heme), then to cytochrome b_H (the high-potential heme), and finally to the oxidized quinone (Q) at the matrix side, partially reducing it to a semiquinone (also called a semiquinol, semi-reduced, or semi-oxidized form).

Now, a second QH₂ molecule binds to the same site, and the same process repeats:

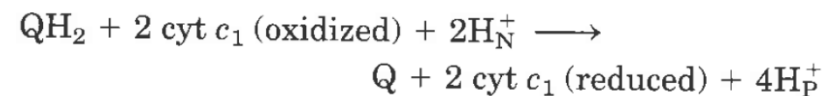
- The first electron again goes through Fe-S → cytochrome c₁ → cytochrome c.
- The second electron again passes through b_L → b_H, but this time it fully reduces the semiquinone to a fully reduced QH₂ molecule.

This newly reduced QH₂ can now re-enter the membrane pool to supply electrons again later.

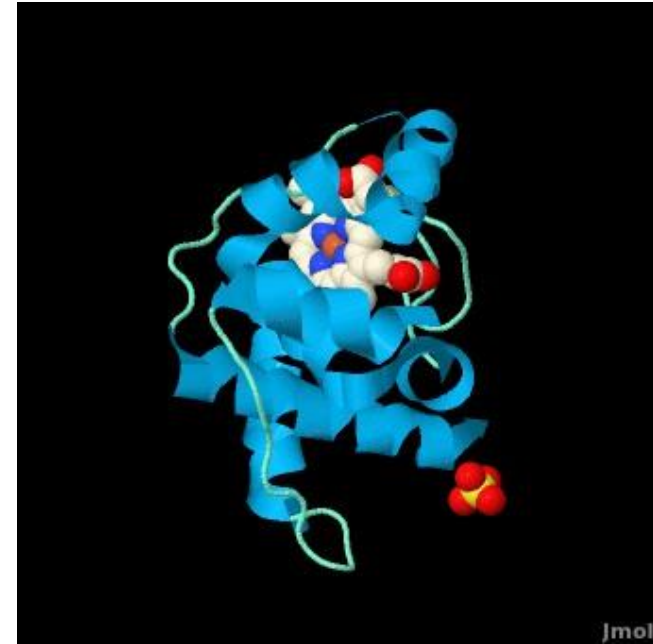
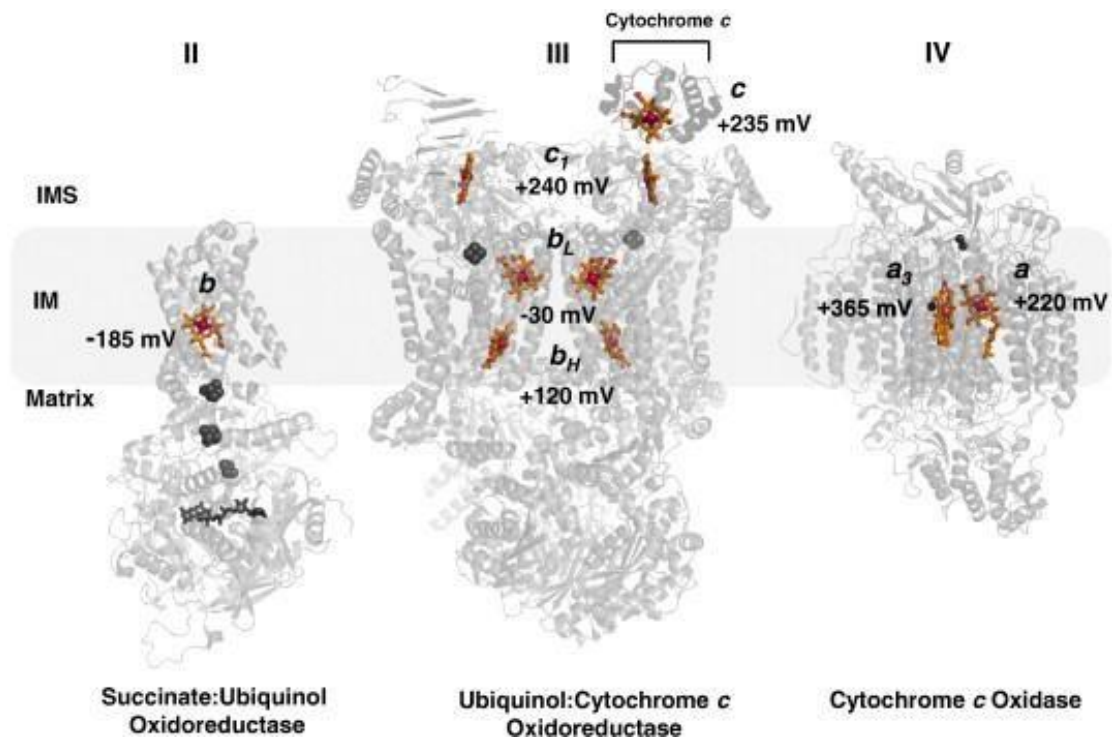


THE Q-CYCLE

- Partial reduction is hazardous
- Accommodates the switch between 2e⁻/1e⁻
- Explains the measured stoichiometry of 4 H⁺/2e⁻



When we look at the structure of cytochrome c, we can compare its small size to the much larger complex III (cytochrome bc₁ complex), which helps us understand how cytochrome c works. The heme group in cytochrome c is positioned very close to the heme c₁ located in complex III. This close proximity allows cytochrome c to easily receive electrons from heme c₁. After picking up the electron, cytochrome c leaves complex III and moves toward complex IV. The heme pocket in cytochrome c is positioned near its surface, which makes it possible for it to accept electrons efficiently from heme c₁.

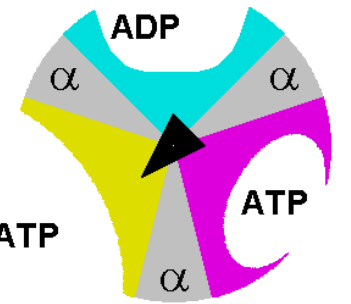
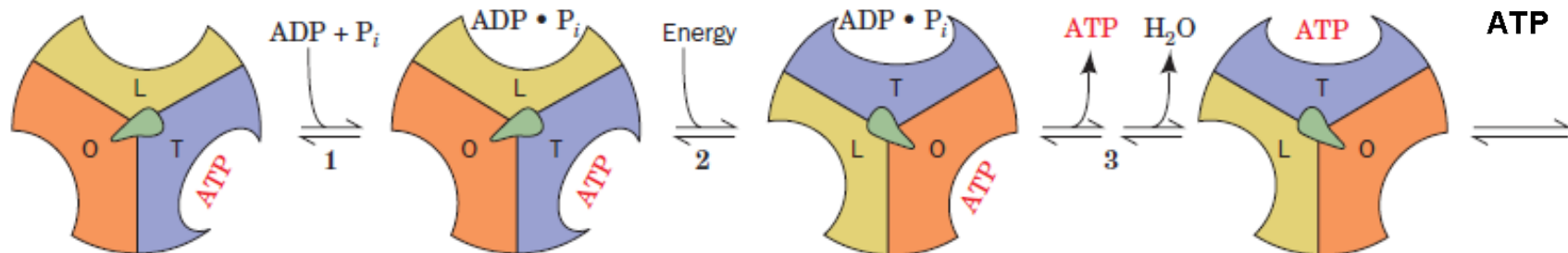
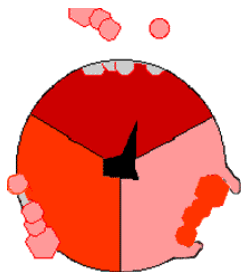
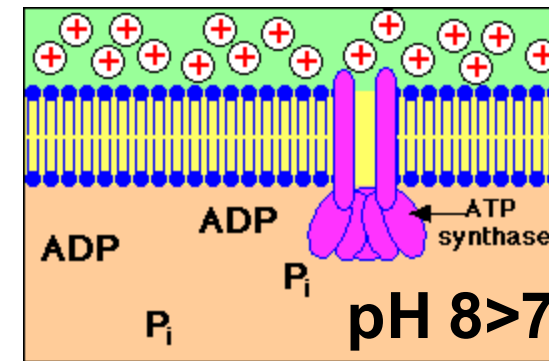
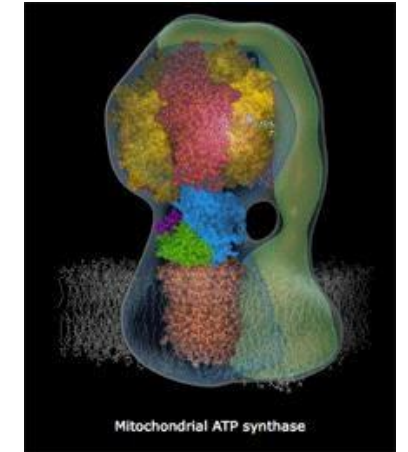
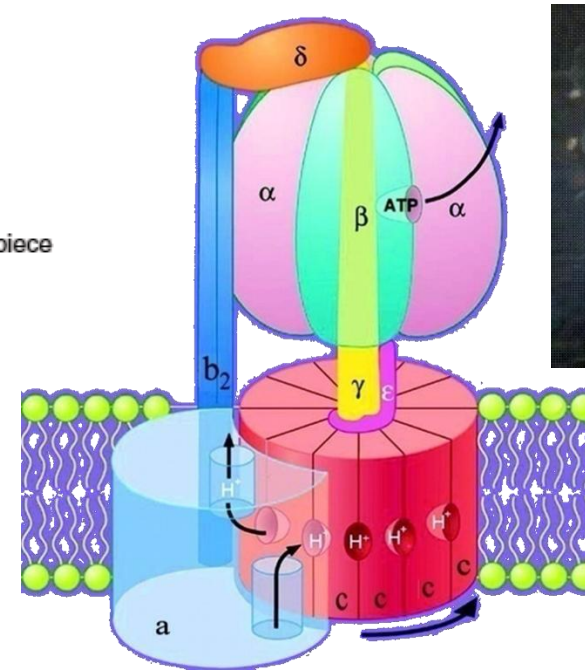
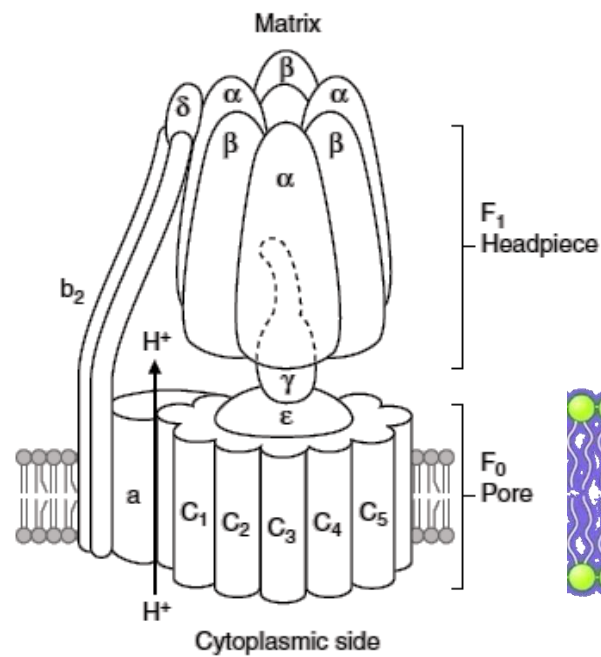


CYTOCHROME C: THE SURFACE MESSENGER



ATP SYNTHASE

- F1:
 - “ γ ” subunit: rotates
 - “ β ” subunit: binds
 - “ α ” subunit: structural
 - 3 conformations: tight (T), loose (L), open (O)
- F0:
 - “a” subunit: point of entry & exit
 - “c” subunit rotates
 - $4H^+/ATP$
- Can run backwards



ATP SYNTHASE

ATP synthase is made up of two main parts: the Fo fragment and the F₁ headpiece.

The Fo fragment is embedded within the inner mitochondrial membrane. It has a cylindrical shape and is composed of c subunits, which are polypeptide chains. Their number can vary between 9 and 15, but in human mitochondria there are 12 c subunits. Together, these subunits form the full cylindrical structure, which is covered on one side by the a subunit.

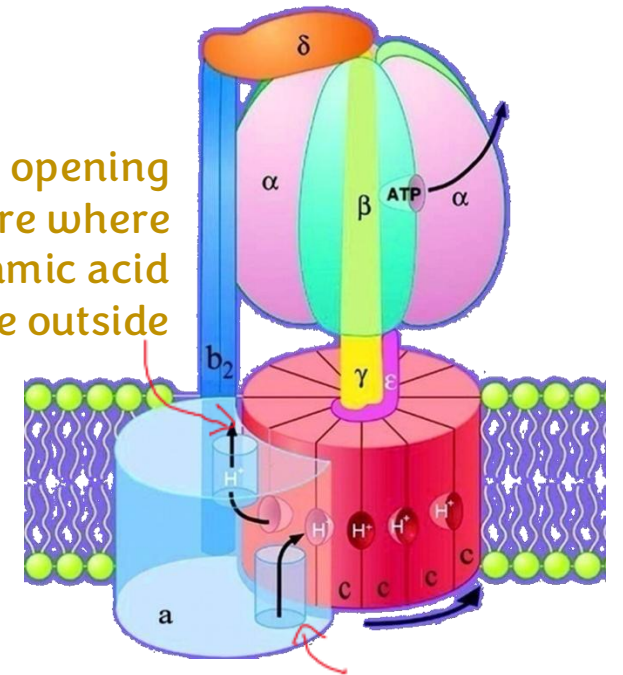
The a subunit acts as the entry and exit point for protons (H⁺).

On the matrix side, the F₁ headpiece projects outward – this is considered the cytoplasmic side of the mitochondrion.

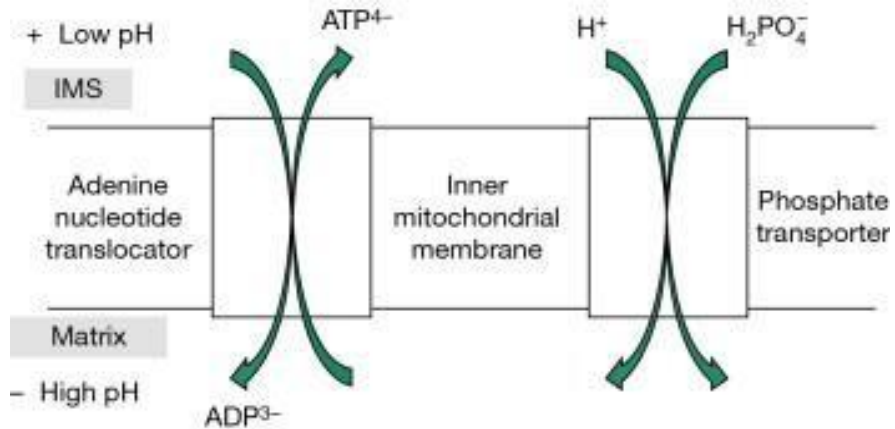
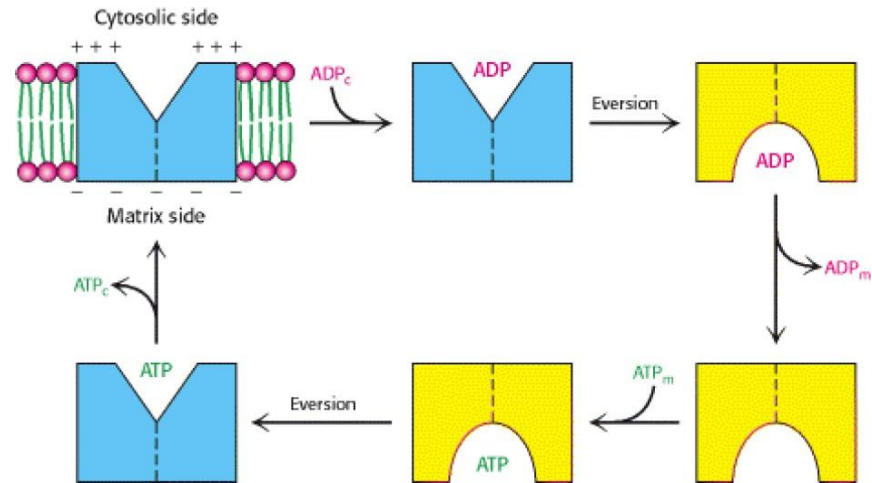
There are many more protons outside the inner mitochondrial membrane than inside – about 10 times more, corresponding to a pH difference of 1 unit.

Because of this gradient, protons from the intermembrane space move through the a subunit toward the c subunits.

another opening
over here where
the glutamic acid
faces the outside



the entry point of protons



MITOCHONDRIAL SHUTTLING SYSTEMS

"ATP/ADP"

- ATP-ADP Translocase (also called adenine nucleotide translocase or ANT)
- The flows of ATP and ADP are coupled (ADP enters only if ATP exits, and vice versa)
- Highly abundant (14% of IMM proteins)
- Contains a single nucleotide-binding site (alternates)
- Similar affinity to ATP and ADP
- Endergonic (25% of ETC)
- Inhibition leads to subsequent inhibition of cellular respiration



ATP-ADP Translocase

After ATP is synthesized inside the mitochondria, it needs to be transported outside to be used for ATP hydrolysis in anabolic (endergonic) reactions. At the same time, the cell must maintain a balanced ratio of ATP to ADP.

This is achieved by an enzyme called ATP-ADP translocase, which is one of the shuttling mechanisms responsible for exporting ATP from the mitochondria and importing ADP into it.

This enzyme has a high affinity for ATP on the matrix side of the inner mitochondrial membrane. When ATP binds to it, the enzyme undergoes a conformational change, which inverts its structure and exposes the ATP-binding site to the cytosolic side.

This inversion reduces the enzyme's affinity for ATP, causing ATP to be released into the intermembrane space and then into the cytosol.

The enzyme remains in this conformation until it binds ADP from the cytosolic side, for which it also has a high affinity. Once ADP binds, another conformational inversion occurs, flipping the enzyme again and exposing the ADP-binding site to the mitochondrial matrix.

In this new position, the enzyme's affinity for ADP becomes low, so ADP is released into the matrix.

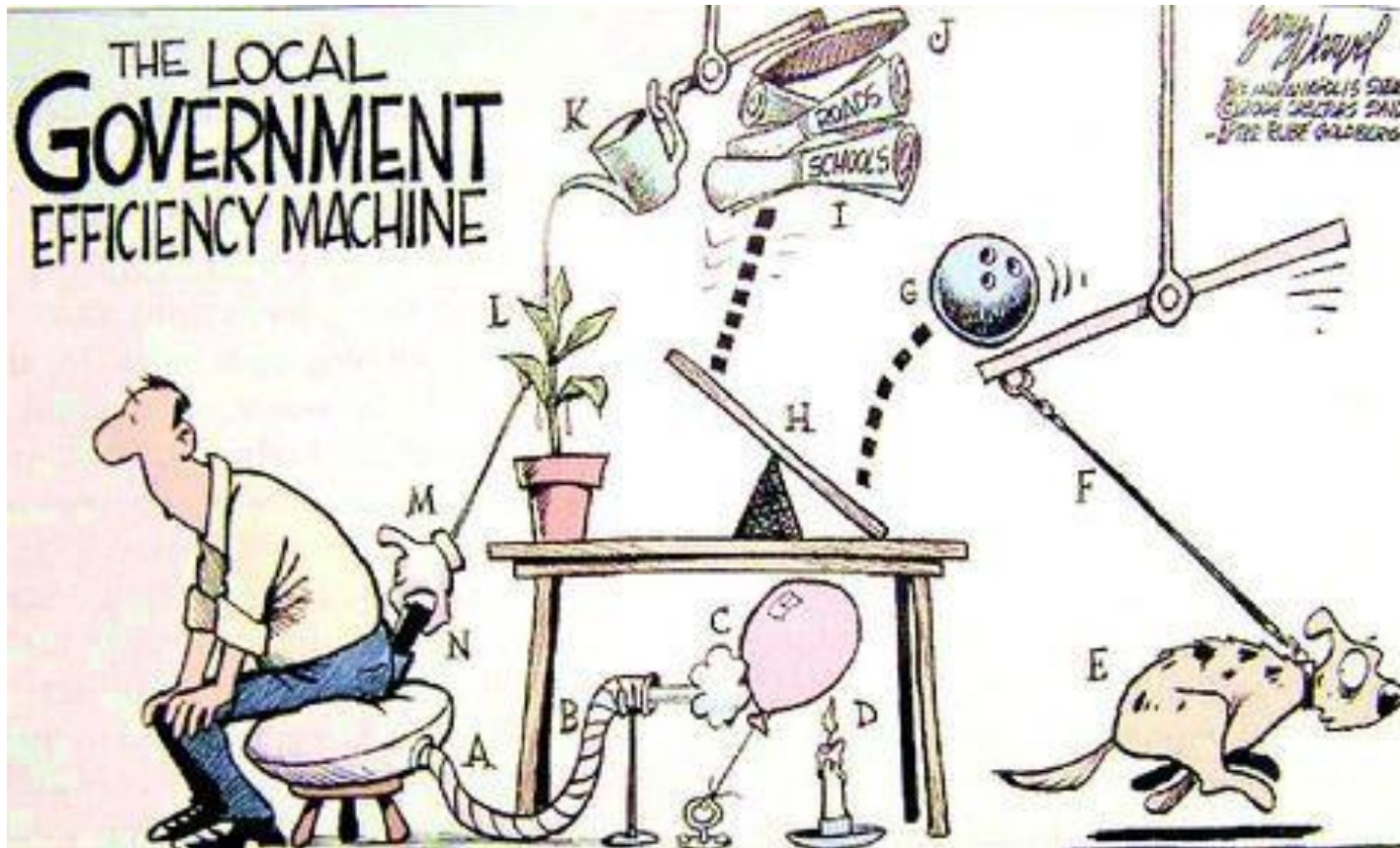
Therefore, for each ATP molecule that exits the mitochondria, one ADP molecule enters. This exchange maintains a nearly constant ATP/ADP ratio inside and outside the mitochondria.

The ATP-ADP translocase is very important, because this exchange process is endergonic – it requires energy to drive the conformational changes. It consumes about 25% of the energy generated by the electron transport chain (ETC).

This enzyme is also highly abundant in the inner mitochondrial membrane, making up roughly 14% of its total protein content, since the export of ATP is essential for cellular energy use.

If this enzyme (or shuttle system) is inhibited, cellular respiration will also be inhibited. This happens because ATP accumulates inside the mitochondria (it cannot exit), while ADP levels decrease (it cannot enter). Since ADP is the main regulator of oxidative phosphorylation, its reduction slows down and eventually stops respiration.

It is important to note that ATP is not being consumed in this case – instead, the process is blocked, so ATP becomes trapped inside and ADP is unavailable, halting the entire cycle.



ENERGY YIELD FROM THE ETC

- NADH, -53 kcal, ATP?
- FADH₂, -41 kcal, ATP?
- $\Delta G^{\circ'}$ is so negative, never reversible
- ATP machine efficiency, (anions, Ca⁺², heat, phosphate, substrates)



The change in free energy ($\Delta G^{\circ'}$) that occurs when two electrons are transferred from NADH to oxygen is about -53 kcal/mol.

Theoretically, one NADH molecule produces 2.5 ATP molecules.

Each ATP molecule stores about 7.3 kcal of energy.

So, $7.3 \times 2.5 = 18.25$ kcal of energy is stored in ATP.

When we compare this to the total available energy ($18.25 \div 53 = 0.344$), we get approximately 35% efficiency.

ENERGY YIELD FROM THE ETC

- NADH, -53 kcal, ATP?
- FADH₂, -41 kcal, ATP?
- $\Delta G^{\circ'}$ is so negative, never reversible
- ATP machine efficiency, (anions, Ca⁺², heat, phosphate, substrates)



From one FADH₂ molecule, we generate 1.5 ATP molecules.

Each ATP stores 7.3 kcal, so $1.5 \times 7.3 = 10.95 \approx 11$ kcal.

Comparing this to the total energy available from FADH₂ ($11 \div 41 = 0.268$), the efficiency is about 27%. Therefore, the energy yield from the ETC is roughly 35% when electrons come from NADH, and 27% when they come from FADH₂.

ENERGY YIELD FROM THE ETC

- NADH, -53 kcal, ATP?
- FADH₂, -41 kcal, ATP?
- ΔG° is so negative, never reversible
- ATP machine efficiency, (anions, Ca⁺², heat, phosphate, substrates)



The $\Delta G^{\circ'}$ value for these reactions is very negative, which means the process is not reversible – it cannot run backward.

Where does the remaining (lost) energy go?

The inner mitochondrial membrane is impermeable to charged molecules, so moving ions such as inorganic phosphate (P_i), calcium (Ca^{2+}), ATP, and ADP across it requires energy.

About one-quarter ($1/4$) of the energy produced by the electron transport chain is used by the ATP-ADP translocase and other transport systems to move these charged molecules.

As a result, the overall efficiency of the process decreases.

Additionally, part of the energy that isn't captured in ATP is released as heat (thermogenesis).

ENERGY YIELD FROM THE ETC

- NADH, -53 kcal, ATP?
- $FADH_2$, -41 kcal, ATP?
- $\Delta G^{\circ'}$ is so negative, never reversible
- ATP machine efficiency, (anions, Ca^{+2} , heat, phosphate, substrates)



REGULATION – THE NEED FOR ATP

- In skeletal muscles, 20% drop in ATP concentration
- In the heart, Ca^{+2} activates TCA enzymes for extra push (NADH, ATP), no drop
- ET is tightly coupled to phosphorylation (simultaneously)
- ADP is the most important factor in determining the rate (respiratory control)



The physiological regulation of the electron transport chain is mainly controlled by ADP.

Because of this, ADP is sometimes referred to as the molecule responsible for respiratory control, since it is the main factor that regulates the rate of the electron transport chain.

In the graph, the y-axis represents oxygen consumption.

At first, oxygen is consumed at a very low rate.

When ADP is added, there is a sharp increase in oxygen consumption, which reflects a rapid increase in the activity of the electron transport chain.

When the ADP supply becomes exhausted, oxygen consumption drops back to its original low level.

Calcium (Ca^{2+}) is also an important regulator (but to a lesser extent than ADP). It activates several enzymes in the citric acid cycle (TCA cycle), including isocitrate dehydrogenase, citrate synthase, and α -ketoglutarate dehydrogenase, as well as some enzymes in the electron transport chain.

Calcium is especially important because its concentration increases when energy demand is high, such as in muscle contraction and in other cells that require more energy.

For example, when hormones bind to their receptors, they can activate phospholipase C, which breaks down phosphatidylinositol bisphosphate (PIP_2) to produce IP_3 (inositol triphosphate).

IP_3 then causes the release of calcium into the cytosol.

This increase in calcium signals that the cell needs more energy, so metabolism must be activated.

Calcium stimulates both the citric acid cycle enzymes and the electron transport chain, ensuring that ATP production increases and energy levels remain stable without any drop.

REGULATION – INHIBITION (COUPLING)

The electron transport chain can be inhibited at different points, and the effect depends on where the block occurs.

- If Complex I is blocked:

- There will be no proton pumping, which means no ATP production from NADH.

- However, electrons coming from Complex II can still pass to coenzyme Q (ubiquinone), so although ATP synthesis is greatly reduced, there is still some electron flow through Complex II or from other flavoproteins that donate electrons to QH₂.

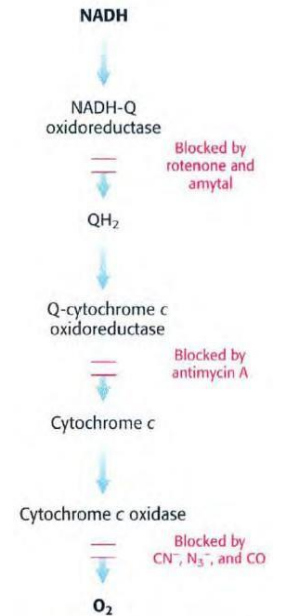
- If Complex II is blocked:

- The main donor of electrons, NADH, can still transfer its electrons through Complex I, so ATP synthesis will continue but at a lower rate compared to a block in Complex I.

- If Complex IV is blocked:

- Everything stops, because Complex IV is the final convergence point for electrons from both Complex III and Complex II.

- As a result, the entire electron transport chain and ATP synthesis are halted.



REGULATION – INHIBITION (COUPLING)

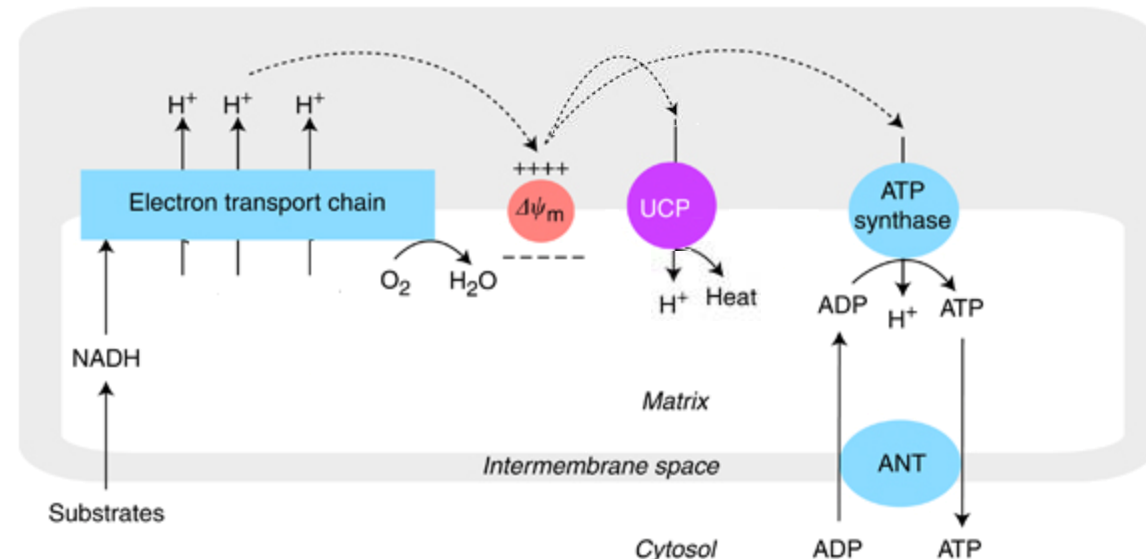
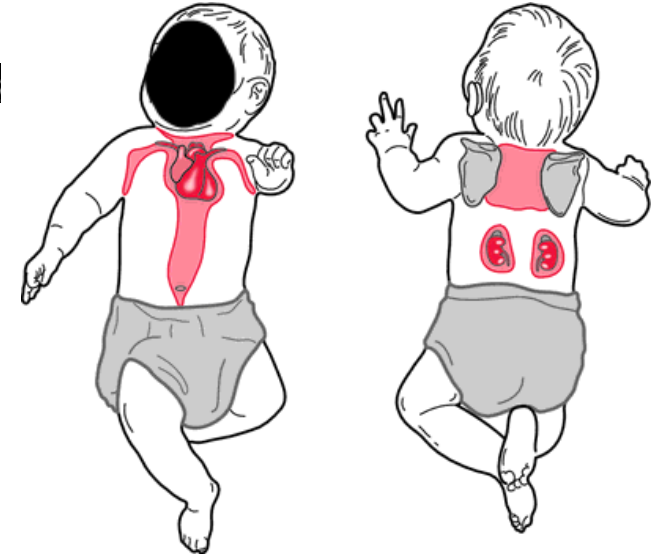
Examples of inhibitors:

- Complex I inhibitors:
- Rotenone (used as an insecticide)
- Amytal (a barbiturate sedative)
- Complex III inhibitor:
- Antimycin A (an antibiotic)
- Complex IV inhibitors:
- Cyanide (CN^-), Azide (N_3^-), and Carbon monoxide (CO)
- These inhibitors bind to the heme group of Complex IV, specifically to the iron ion where oxygen normally binds, thereby blocking oxygen binding and stopping electron transfer.
- Cyanide, for example, is found in high concentrations in the seeds of some edible plants such as apples and peaches, but it does not harm humans because we do not consume these seeds in large amounts.
- ATP synthase (Complex V) inhibitor:
- Oligomycin – it blocks the flow of protons through the F_0 fragment of ATP synthase.
- (In fact, the “ F_0 ” part of ATP synthase is named after this inhibitor, oligomycin.)

Specific inhibitor	Target
Rotenone (insecticide) & Amytal (sedative)	NADH-Q oxidoreductase
Antimycin A (antibiotic)	Q-cytochrome c oxidoreductase
Cyanide (CN^-), Azide (N_3^-), & (CO)	Cytochrome c oxidase
Oligomycin (antibiotic)	ATP synthase

REGULATION – UNCOUPLING REGULATED – UNCOUPLING PROTEINS (UCPS)

- Short-circuiting ATP synthase
- UCP1 (thermogenin):
 - ✓ Brown adipose tissue, non-shivering thermogenesis
 - ✓ Infants: neck, breast, around kidneys
 - ✓ Fatty acids directly activates UCP1
- UCP2 (most cells); UCP3 (skeletal muscle); {UCP4, UCP5} (brain)
- Obesity tendency in some populations



The generation of heat in our bodies can occur through short-term mechanisms such as shivering and non-shivering thermogenesis.

Adaptive thermogenesis refers to the production of heat during ATP synthesis.

The mechanism behind this involves special proteins in our cells called uncoupling proteins (UCPs).

As their name suggests, these proteins uncouple the oxidation-reduction reactions from the phosphorylation process (ATP synthesis).

Here's what happens:

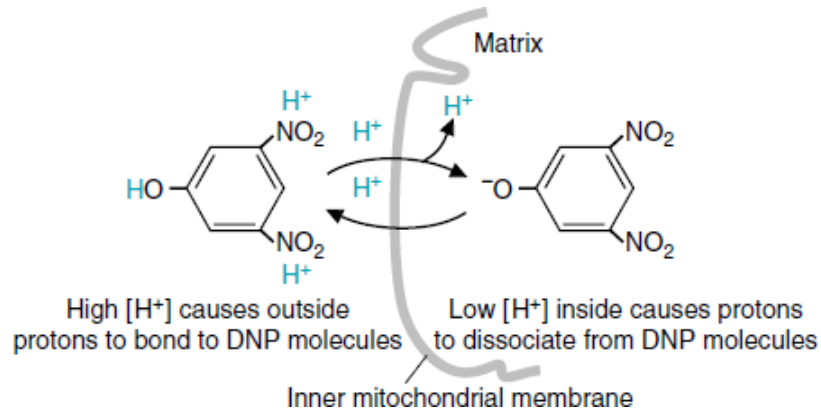
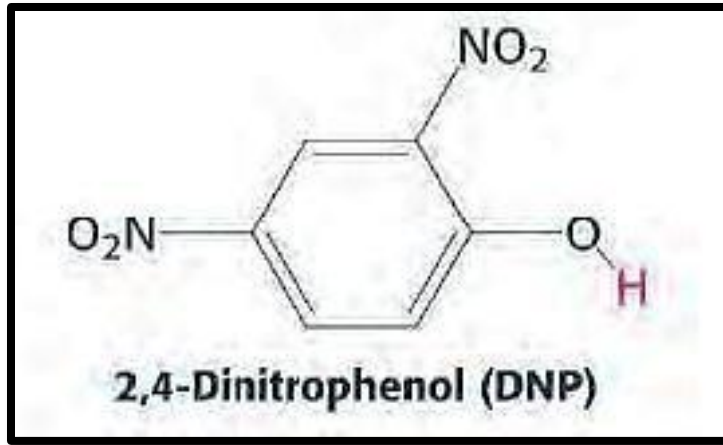
- Electrons move through the electron transport chain, and protons are pumped across the inner mitochondrial membrane.
- Normally, these protons pass through ATP synthase to generate ATP.
- However, if uncoupling proteins are present, some of the protons bypass ATP synthase and return directly to the mitochondrial matrix through these proteins.
- When this happens, the energy difference (from the proton gradient) is released as heat instead of being used to make ATP.
- Meanwhile, the remaining protons can still pass through ATP synthase to produce ATP.

The percentage of protons used by uncoupling proteins varies among individuals – for some people, around 10% of the protons are used this way, while for others it can reach 20%.

This variation depends on factors such as age, type of tissue, environmental conditions, health status, and the body's energy and heat requirements.

REGULATION – UNCOUPLING

UNREGULATED – CHEMICAL UNCOUPLERS



- What is uncoupling?
- How does it occur? Dissipation of PMF
- What is the result?
- Is it physiological or not?
- 2,4-dinitrophenol (DNP) & other acidic aromatic compounds
- What changes happen? $\uparrow$ O_2 consumption, $\uparrow$ NADH oxidation
- Soviet soldiers were given DNP, FDA banned DNP (1938)



Dinitrophenol (DNP) and Its Effect on Metabolism:

There was a research idea suggesting that if we could find a drug capable of moving protons (H^+) from the outside of the inner mitochondrial membrane back to the inside, it could help reduce obesity.

The concept was that this chemical structure would bind H^+ ions where their concentration is high (outside) and then release them inside the mitochondria where H^+ concentration is low.

This process would allow more protons to return inside the mitochondria, releasing energy as heat instead of producing ATP.

As a result, ATP production efficiency would decrease, and less energy would be stored as fat, potentially reducing obesity – meaning a person could theoretically eat more without gaining as much weight.

This idea was proposed a long time ago – around the 1930s (a century ago) – and the drug developed for this purpose was dinitrophenol (DNP).

DNP is a benzene ring containing one hydroxyl group ($-OH$) and two nitro groups ($-NO_2$).

Because of its benzene-like structure, it is lipophilic, allowing it to move through the inner mitochondrial membrane.

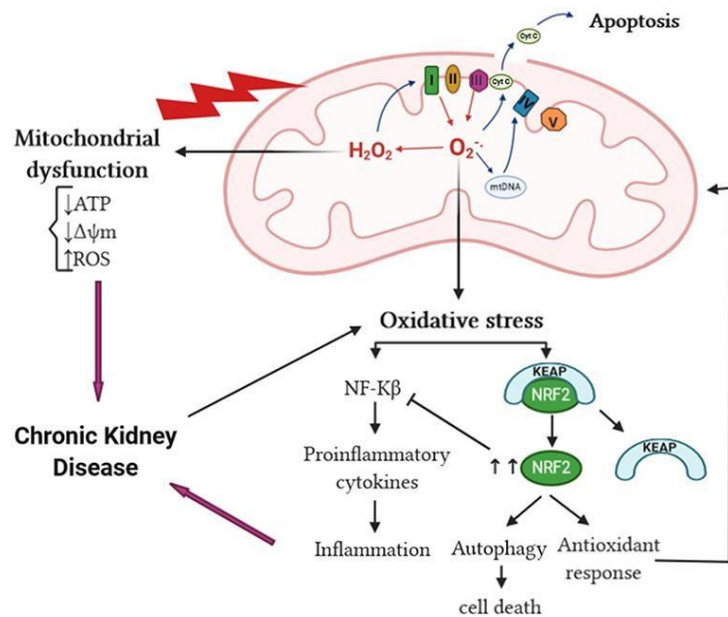
The hydroxyl group enables it to pick up a proton (H^+) on the outer surface of the inner mitochondrial membrane, and then, as it moves toward the matrix side, it releases that proton into the matrix.

This action dissipates the proton motive force, causing energy to be lost as heat instead of being used to make ATP.

However, the use of DNP led to serious side effects and deaths, including eye bleeding and blindness in some individuals. Because of these dangerous effects, the United States banned DNP in 1938.

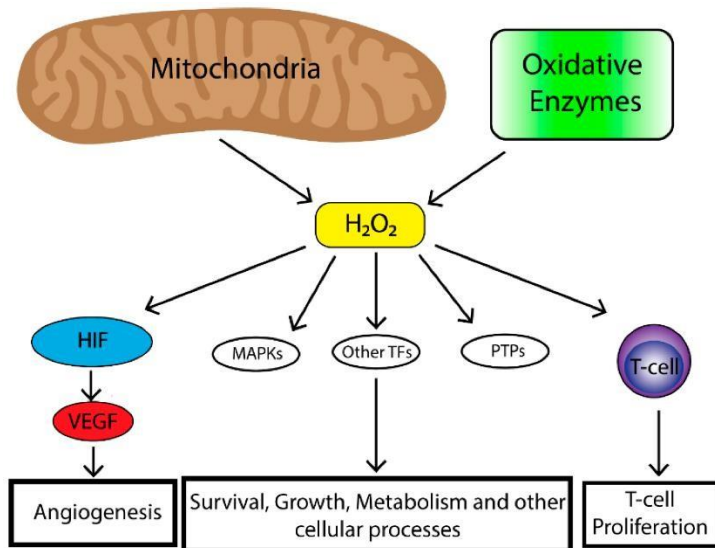
About a year later, during World War II, soldiers used DNP to increase heat production in their bodies and prevent hypothermia in snowy conditions.

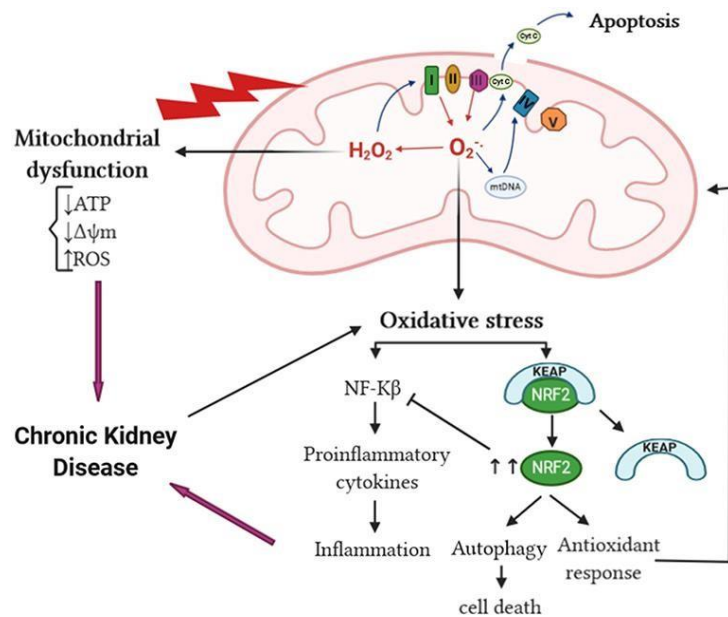
But DNP can cause malignant hyperthermia – a severe, dose-independent reaction that varies between individuals and is uncontrollable even under medical supervision, sometimes leading to death.



THE DARK SIDE: REACTIVE OXYGEN SPECIES (ROS)

- E.g., FMN, semiquinone in Q cycle
- Single electrons can leak and reduce O_2 to superoxide ($O_2^{\cdot-}$)
- **Pathophysiology:**
 - **Primary ROS:** Superoxide ($O_2^{\cdot-}$). Converted by Superoxide Dismutase (SOD) to H_2O_2
 - **Damage:** lipids (peroxidation), proteins (carbonylation), and DNA (strand breaks)
 - **Role in Disease:** Contributes to aging, neurodegenerative diseases (Parkinson's, Alzheimer's), ischemia-reperfusion injury, and inflammatory damage



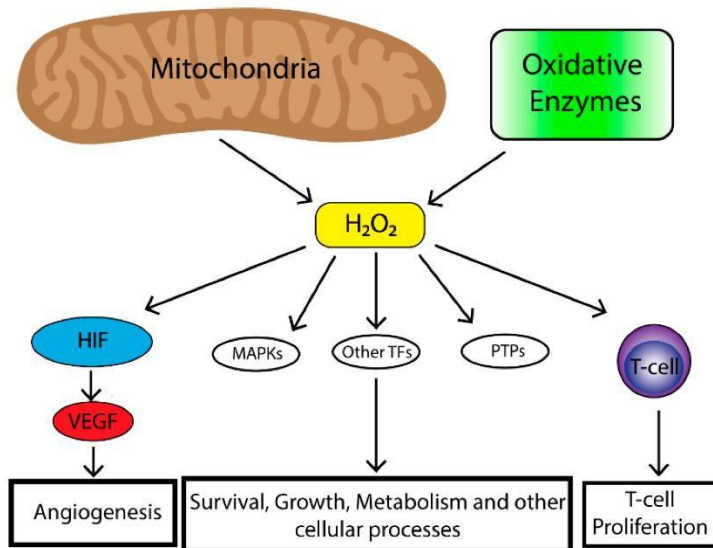


THE DARK SIDE: REACTIVE OXYGEN SPECIES (ROS)

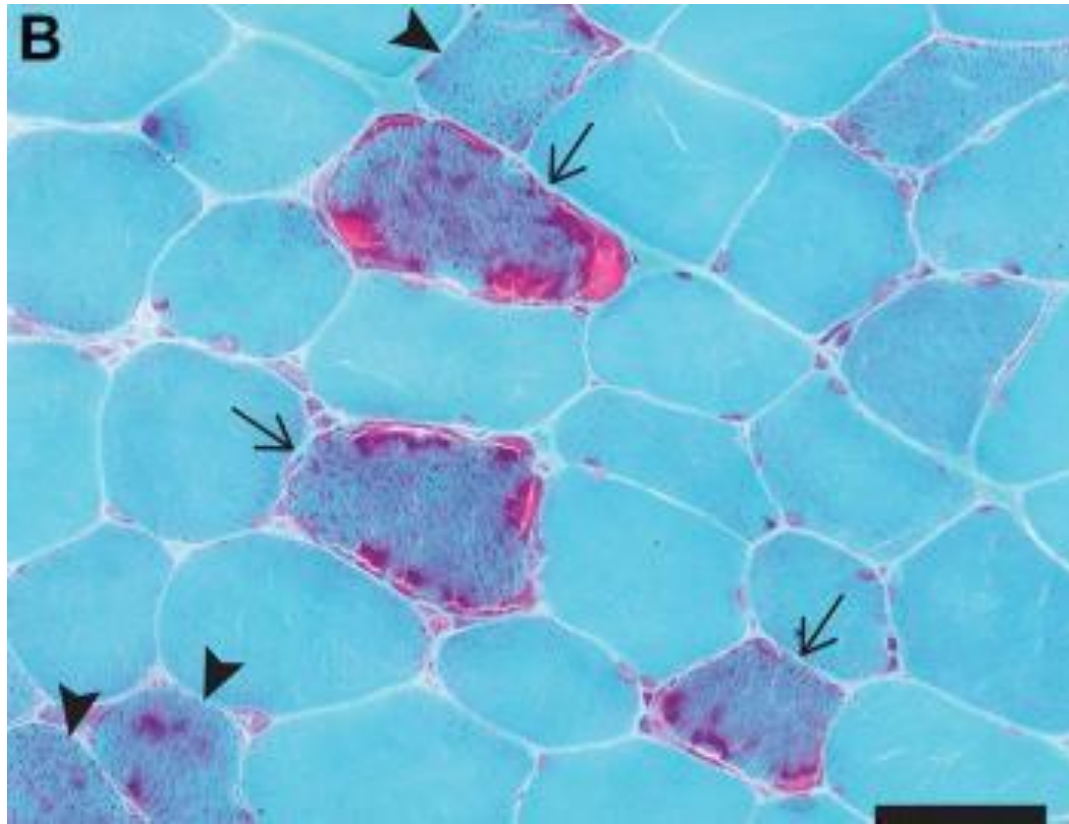
Over time, the production of free radicals and hydrogen peroxide increases, and mitochondria become more damaged.

This leads to enhanced autophagy and cell death – which represents the “dark side” of the mitochondria.

These reactive oxygen species can accumulate throughout life, causing many diseases – either inflammatory in nature or neurodegenerative, such as Alzheimer’s disease and Parkinson’s disease, and they also contribute to the overall aging process.



MITOCHONDRIAL DISEASES: GENETICS AND PRINCIPLES



- A muscle biopsy with "ragged red fibers" (Gomori trichrome stain)
- Etiology:
 - nDNA or mtDNA
 - mtDNA Inheritance: Maternal. High mutation rate, no histones, poor repair.
 - Heteroplasmy: A mixture of wild-type and mutant mtDNA within a cell.
 - Symptoms appear when the mutant load exceeds a threshold.
- Tissues Affected: High-energy demand tissues: CNS, muscle, heart, liver, kidney.



During the electron transport chain, free radicals can form. This happens because some molecules like FMN and FAD can become free radicals while they are being reduced. Similarly, the quinone molecule can exist in a semi-reduced or semi-oxidized state. These free radicals can cause partial reduction of oxygen, producing superoxide free radicals.

Superoxide can then be converted by the enzyme superoxide dismutase into hydrogen peroxide, which is also toxic. Superoxide increases inflammation, leads to cell death, and can signal the mitochondria to start apoptosis. When superoxide becomes hydrogen peroxide, it also causes less ATP production, which leads to the formation of more reactive oxygen species (ROS). This imbalance contributes to diseases such as chronic kidney disease.

High concentrations of hydrogen peroxide can have several effects, including promoting angiogenesis (formation of new blood vessels), stimulating cell growth and proliferation, and affecting immune cells. Over time, this leads to an accumulation of free radicals, more hydrogen peroxide, mitochondrial damage, autophagy, and cell death. This is considered the dark side of the mitochondria, as these effects accumulate through life and can cause many diseases – either inflammatory, neurodegenerative (like Alzheimer's and Parkinson's), or related to aging in general.

To diagnose mitochondrial diseases, a muscle biopsy is performed. The tissue is stained using the Gomori trichrome stain (or a modified version) to detect ragged red fibers, which are a hallmark of mitochondrial myopathy. Under the microscope, affected cells show red fibers around their edges, representing areas rich in abnormal mitochondria.

The causes of mitochondrial diseases can come from mutations in either nuclear DNA or mitochondrial DNA (mtDNA). Mutations happen more frequently in mtDNA because:

1. It is inherited from the mother, not newly synthesized each generation.
2. mtDNA lacks histones, which normally protect DNA.
3. It has a poor DNA repair system.

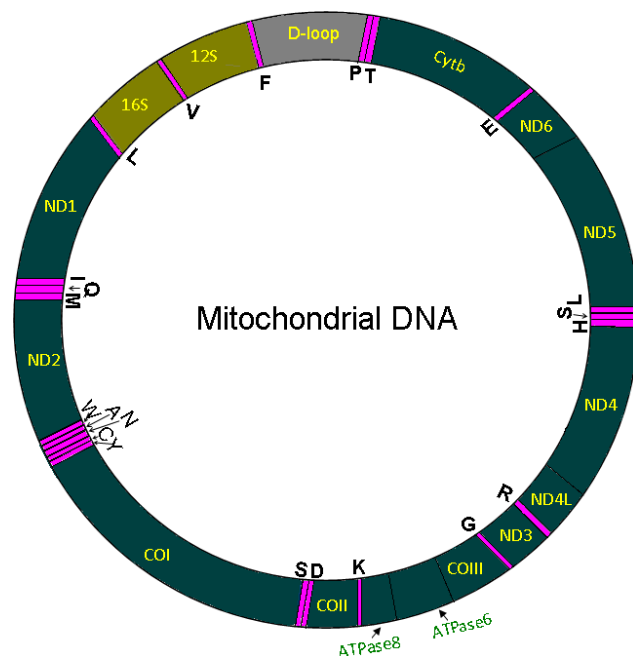
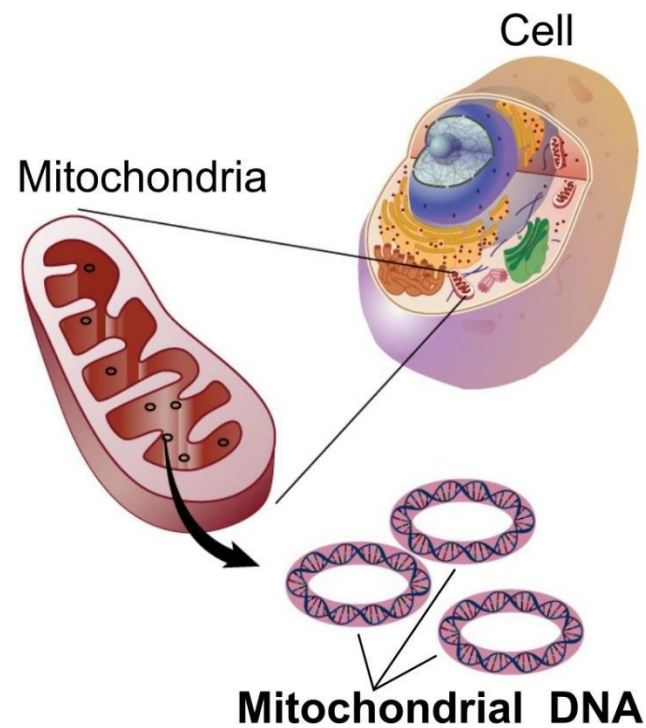
As a result, a single cell can contain a mixture of normal (wild-type) and mutated mitochondria – this condition is called heteroplasmy. Because of heteroplasmy, different tissues (or even the same cell) can have varying levels of healthy and defective mitochondria.

Mitochondrial diseases often appear or worsen with age. Some show symptoms early in life, but many appear in adulthood or later, because mutant mitochondria accumulate over time. When the proportion of defective mitochondria passes a certain threshold, they cannot produce enough ATP, leading to energy deficiency and tissue dysfunction. Tissues with high energy demand – such as the central nervous system, muscles, heart, liver, kidneys, and eyes – are affected the most.

In terms of the electron transport chain complexes:

Complex I is large, made of 25 subunits – 7 are coded by mitochondrial DNA and 18 by nuclear DNA.

Complex III has 11 subunits on each side (22 total) – 1 is coded by mitochondrial DNA, and 10 by nuclear DNA. These subunits combine and become embedded in the mitochondrial membrane.



OXPHOS DISEASES – 1 (GENETIC)

- Mitochondrial DNA and OXPHOS Diseases
 - Small (16,569) base pair, double-stranded, circular DNA
 - Encodes 13 subunits: 7 (I) , 1 (III), 3 (IV), 2 (F0)
 - Also encodes necessary components for translation of its own mRNA: a large and small rRNA and tRNAs
 - Maternal inheritance, replicative segregation and heteroplasmy
 - Accumulation of somatic mutations with age
 - Highest ATP demands: CNS, heart, skeletal muscle, and kidney, liver



These genetic diseases of mitochondrial origin involve the mitochondrial DNA (mtDNA), which is small, circular, and double-stranded.

It codes for 7 subunits of Complex I, 1 subunit of Complex III, 3 subunits of Complex IV, and 2 subunits in the FO portion of ATP synthase. Altogether, the mitochondrial DNA produces 13 subunits that are essential for the oxidative phosphorylation process.

In addition, mtDNA encodes some of the large and small ribosomal RNAs (rRNAs) needed for protein translation, as well as some transfer RNAs (tRNAs).

Mitochondrial DNA is maternally inherited and shows heteroplasmy, which results from a process called replicative segregation. This means that mitochondria replicate and are distributed to daughter cells independently, without strict control by the mother cell.

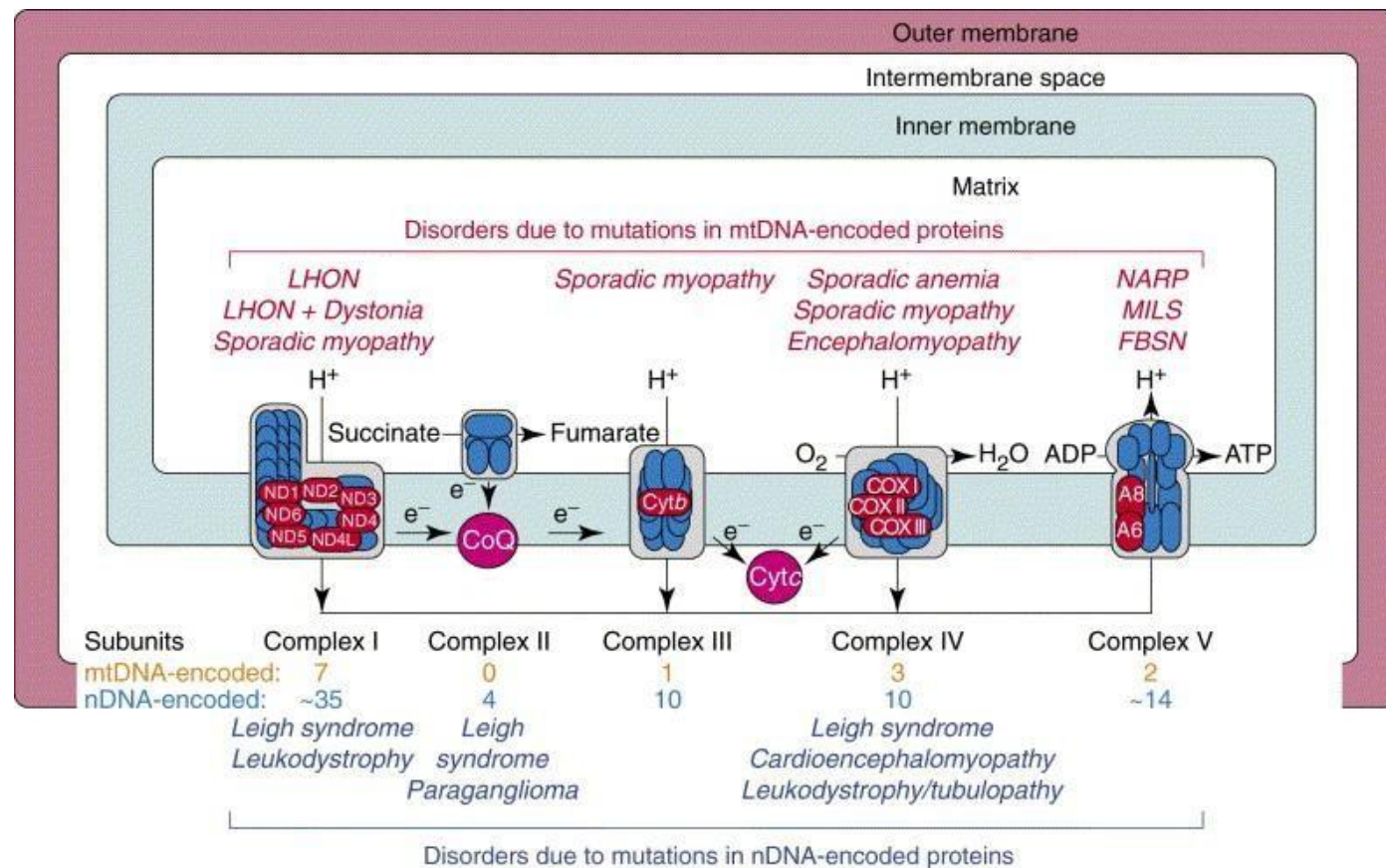
Somatic mutations in mtDNA accumulate with age, which is why mitochondria naturally become more affected over time. If a mutation is already present, its effects become more pronounced as time passes, and the disease phenotype appears most strongly in tissues with high ATP demands.

OXPHOS DISEASES — 2 (GENETIC)

- Nuclear Genetic Disorders of Oxidative Phosphorylation
 - 1,000 proteins
 - Usually autosomal recessive
 - Expressed in all tissues
 - Phenotypic expression with high ATP demand

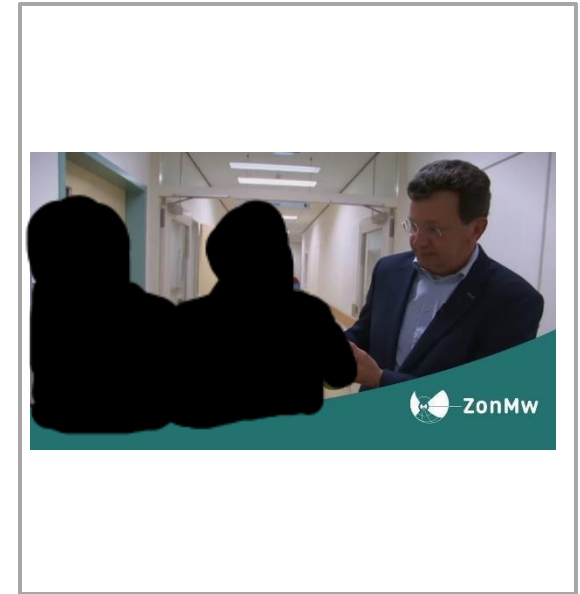
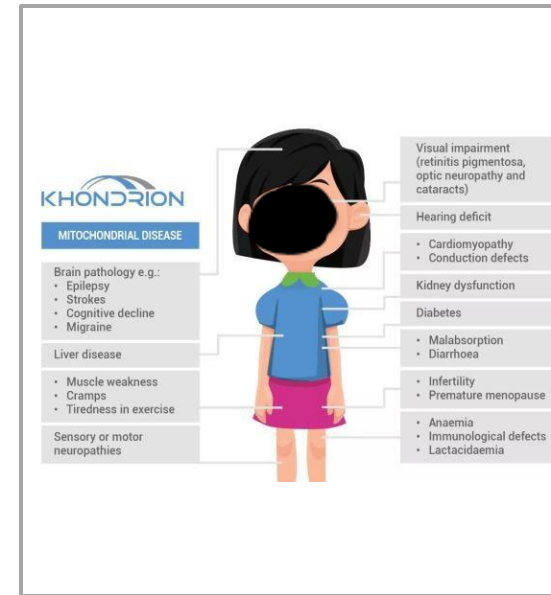
If the problem is of nuclear origin, the nucleus synthesizes more than 1000 proteins. Diseases caused by nuclear DNA mutations are usually expressed as autosomal recessive diseases. They appear in all tissues because of the mitosis process, and the phenotype is especially expressed in tissues with the highest ATP demand.

OXPHOS DISEASES (GENETIC)



MITOCHONDRIAL DISEASES 2: CLINICAL EXAMPLES

- **Examples:**
 - **Leber's Hereditary Optic Neuropathy (LHON):** mutation in Complex I. Causes sudden, painless bilateral vision loss in young adults.
 - **MELAS:** Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes. Caused by mtDNA point mutation.
 - **Chronic Progressive External Ophthalmoplegia (CPEO):** ptosis and inability to move the eyes. Often associated with large-scale mtDNA deletions.



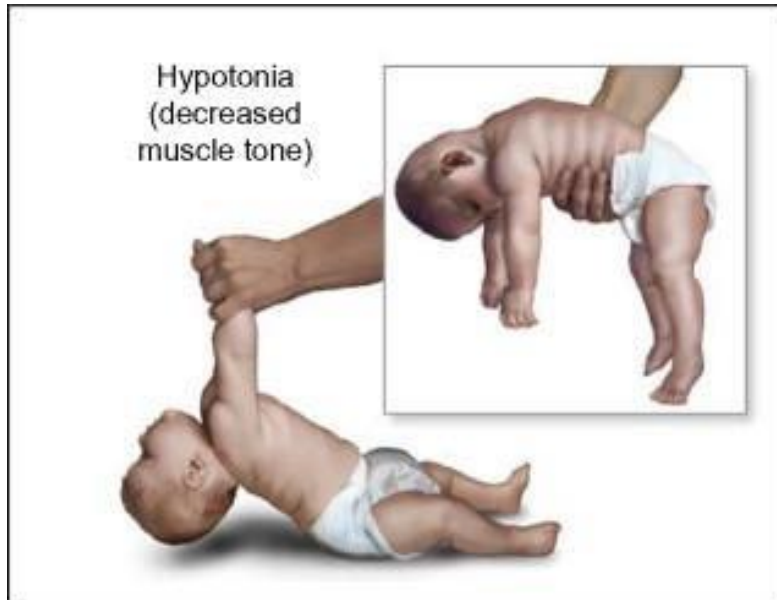
LHON (Leber's Hereditary Optic Neuropathy): This disease mainly affects vision and involves a defect in Complex I of the electron transport chain.

MELAS (Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like Episodes): In MELAS, the brain is affected because energy production in the mitochondria is impaired. When oxidative phosphorylation is disrupted, the body shifts to producing more energy through glycolysis, leading to increased pyruvate and lactate levels. As a result, patients develop lactic acidosis. This lactic acidosis is not exclusive to MELAS – it can also occur in LHON and in any disease that affects mitochondrial energy generation.

CPEO (Chronic Progressive External Ophthalmoplegia): This disease is characterized by ptosis (drooping of the eyelids). The eye muscles lose the energy needed to keep the eyes open because of mitochondrial dysfunction. It is associated with mitochondrial DNA deletions, and over time, the ability of the upper eyelid muscles to lift the eyes gradually decreases. In advanced stages, the patient becomes unable to open the eyes without manual assistance.

INTERACTIVE CASE STUDY

CLINICAL CORRELATION: A DIAGNOSTIC PUZZLE



■ Presentation:

- A 4-year-old boy presents with progressive muscle weakness, exercise intolerance, and developmental delay.
- Lab tests: profound **lactic acidosis**.
- Muscle biopsy: **ragged red fibers**.

■ Questions:

- What is the most likely category of disease?
 - **(Mitochondrial Myopathy)**
- What is the biochemical basis for lactic acidosis?
 - **(Impaired OXPHOS → anaerobic glycolysis)**
- What molecular tests could be ordered?
 - **(mtDNA sequencing, nuclear gene panel for OXPHOS proteins)**



The brain and muscles are affected, which suggests a problem related to the mitochondria.

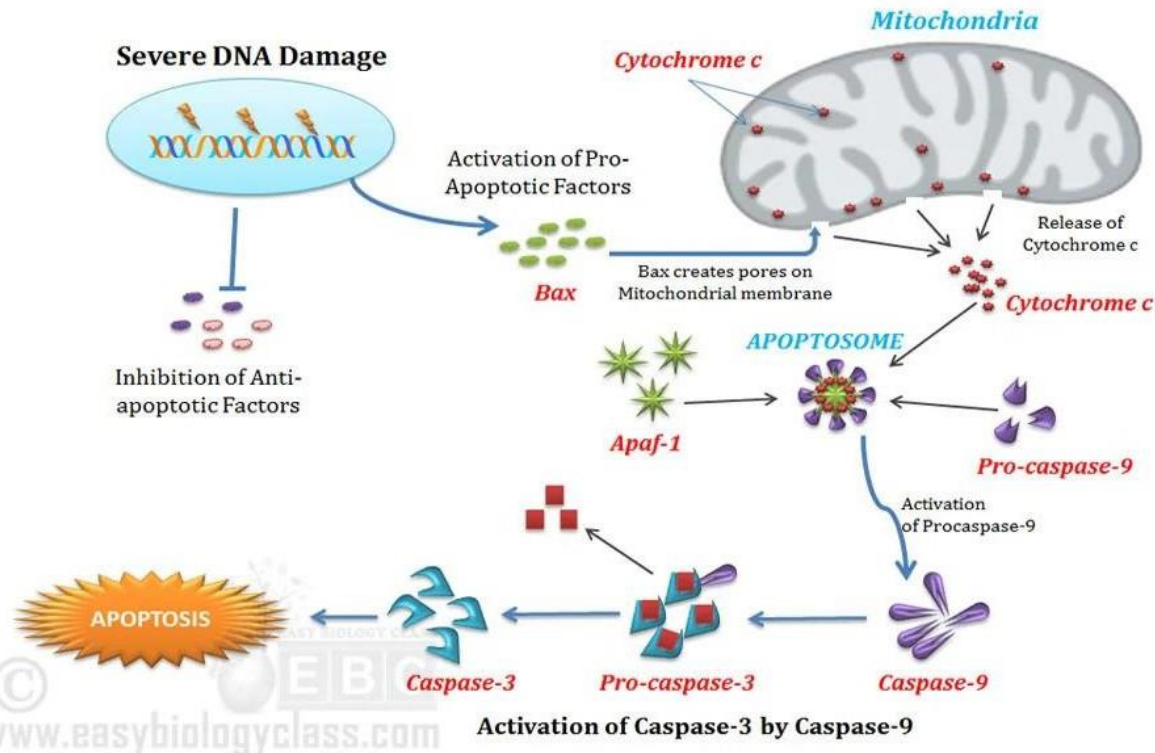
Lab tests show lactic acidosis – why? Because the mitochondria are not producing enough energy, so the body shifts to glycolysis, leading to increased lactic acid production.

A muscle biopsy shows ragged red fibers, confirming that the mitochondria are affected – this indicates a mitochondrial myopathy.

Why does lactic acidosis occur? Because the oxidative phosphorylation process is impaired.

As a physician, you should order mitochondrial DNA sequencing or a nuclear gene panel for oxidative phosphorylation proteins to determine whether the mutation lies in the nuclear genes encoding these enzymes or in the mitochondrial DNA itself. This will help you reach the final diagnosis.

INTRINSIC PATHWAY OF APOPTOSIS (Mitochondria Mediated Programmed Cell Death Pathway)



BEYOND ENERGY: MITOCHONDRIA IN APOPTOSIS (CELL DEATH) AND AGING

- **Apoptosis:** In response to severe stress, mitochondria release Cytochrome c and other proteins, which activate caspases and trigger programmed cell death.
- **The Mitochondrial Theory of Aging:** Accumulation of mtDNA mutations over a lifetime → progressive decline in OXPHOS capacity → increased ROS production → further damage → tissue dysfunction and aging.



1. Apoptosis:

There are two main pathways of apoptosis:

a. The intrinsic pathway:

This pathway is initiated by the mitochondria. It begins when the mitochondria release a molecule called cytochrome c from the intermembrane space through the outer mitochondrial membrane. Normally, cytochrome c cannot leave the mitochondria, but if the cell receives a signal that it should die, cytochrome c is released.

Once released, cytochrome c binds to a protein called apoptotic protease activating factor-1 (Apaf-1). This binding activates Apaf-1, leading to the formation of a wheel-like structure made of seven Apaf-1 molecules, called the apoptosome.

The apoptosome activates procaspase-9 (an inactive enzyme known as the initiator caspase), which then activates procaspase-3 (the executioner caspase). Activation of caspase-3 leads to apoptosis, or programmed cell death.

(The detailed mechanism is not required to memorize.)

The key point is that this pathway is initiated by the mitochondria through the release of cytochrome c.

b. The extrinsic pathway:

(This is another apoptosis pathway that is not initiated by the mitochondria but by external signals to the cell.)

2. Aging:

Over time, mitochondria accumulate mutations because their DNA repair mechanisms are less effective than those in the nucleus. These accumulated mutations affect the ATP production machinery, since mitochondrial DNA encodes some of the subunits of Complexes I, III, IV, and ATP synthase in the electron transport chain.

If any of these subunits are affected, the entire energy production process becomes impaired. As a result, ATP production decreases, reactive oxygen species (ROS) increase, and cellular damage and dysfunction occur.

This gradual decline in mitochondrial efficiency leads to aging, which can be defined as the stage when cells can no longer produce enough ATP to meet the body's energy needs.

رسالة من الفريق العلمي:

واشددْ يديك بحبلِ الله مُعْتَصِمًا فإنه الرُّكنُ إنْ خانتكَ أَرْكانُ
من يتَّقِ اللهَ يُحْمَدُ في عواقبه ويكْفى شرُّ من عزَّوا ومن هانوا
من استعانَ بغيرِ الله في طلبِ
فإنَّ ناصِرَه عَجَزَ وخِذلانُ

لا تنسونا من دُعائكم

For any feedback, scan the code or click on it.



Corrections from previous versions:

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