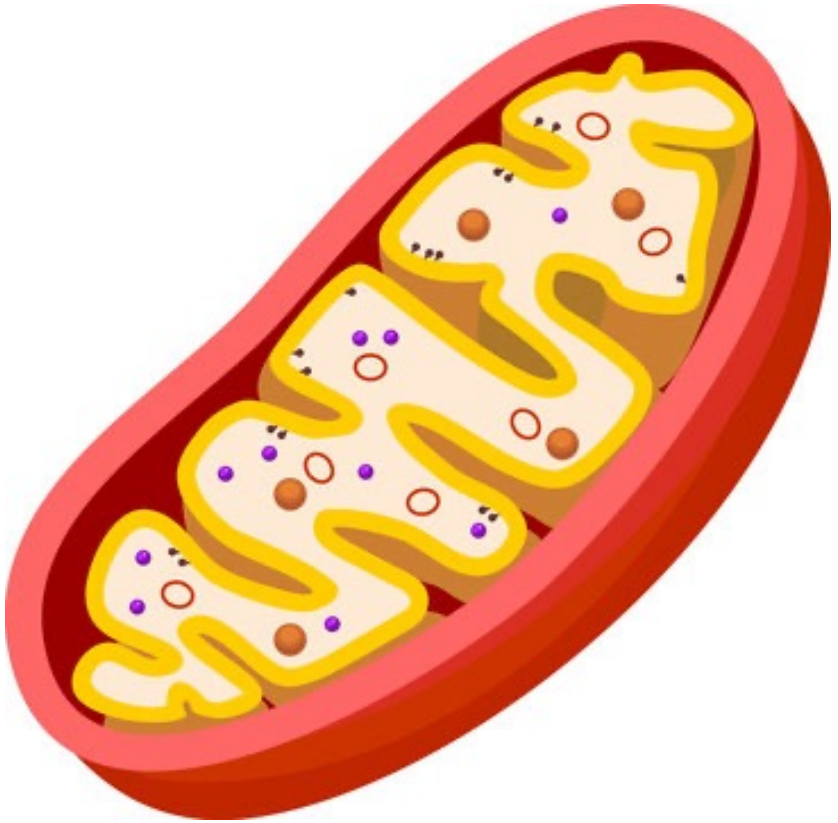
Unit 7

The Metabolic Role of Cellular Mitochondria

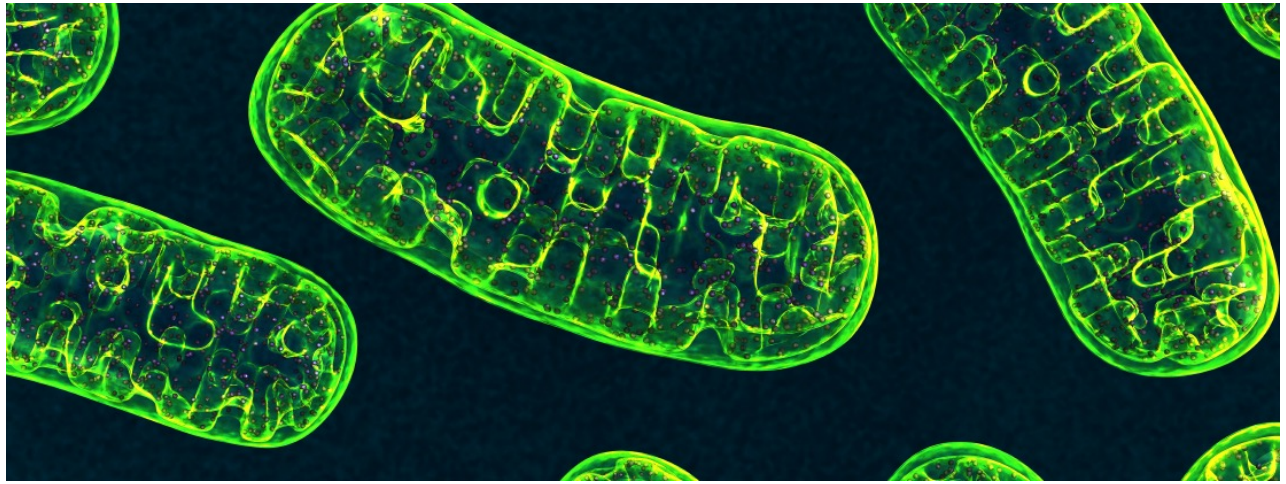


Bowen Li, Ph.D.

Oct-19-2023

What are mitochondria?

- These are cytoplasmic double-membraned organelles that carry the task of oxidation of the final products of metabolism using the oxygen breathed in.

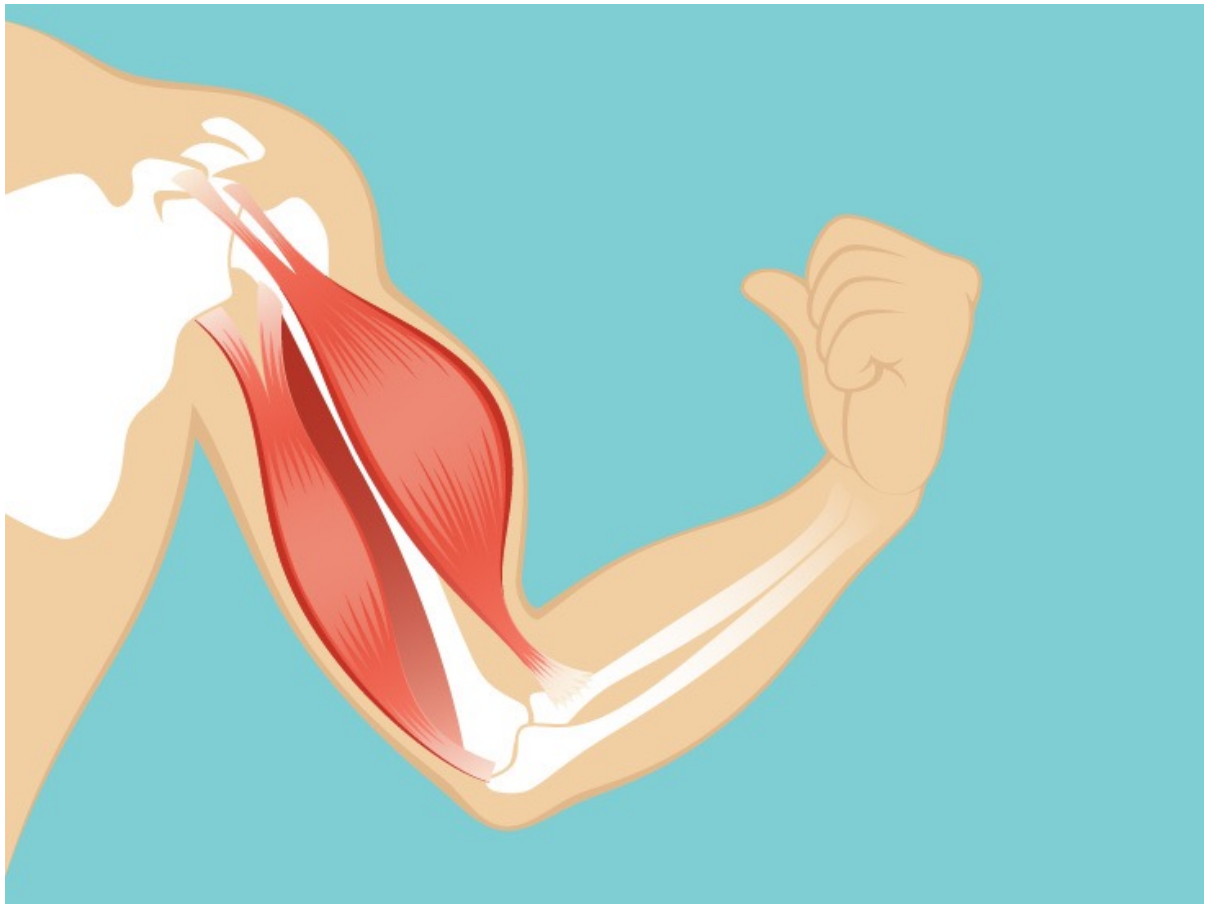


- Able to generate adenosine triphosphate, or ATP
- Own genetic material – mitochondrial DNA (mtDNA)

https://www.vai.org/article/explainer-what-are-mitochondria/

How many mitochondria are there in a cell?

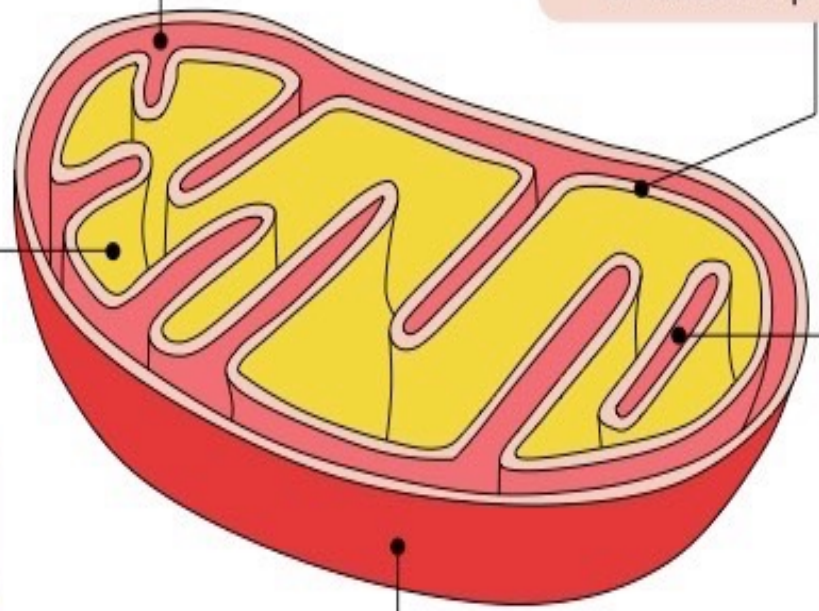
- The number depends on what the cell needs to do: they are found in large numbers in the heart and skeletal muscle.
- Red blood cells and skin cells have very few as well.
- Up to 10% of the weight of the human body is mitochondria.



General Structure of Mitochondria

Intermembrane space
Small space to quickly accumulate protons

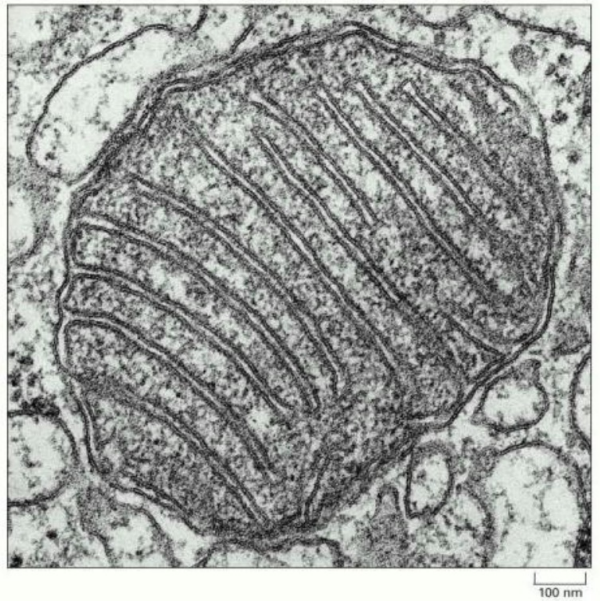
Inner membrane
Contains ETC and ATP synthase for oxidative phosphorylation



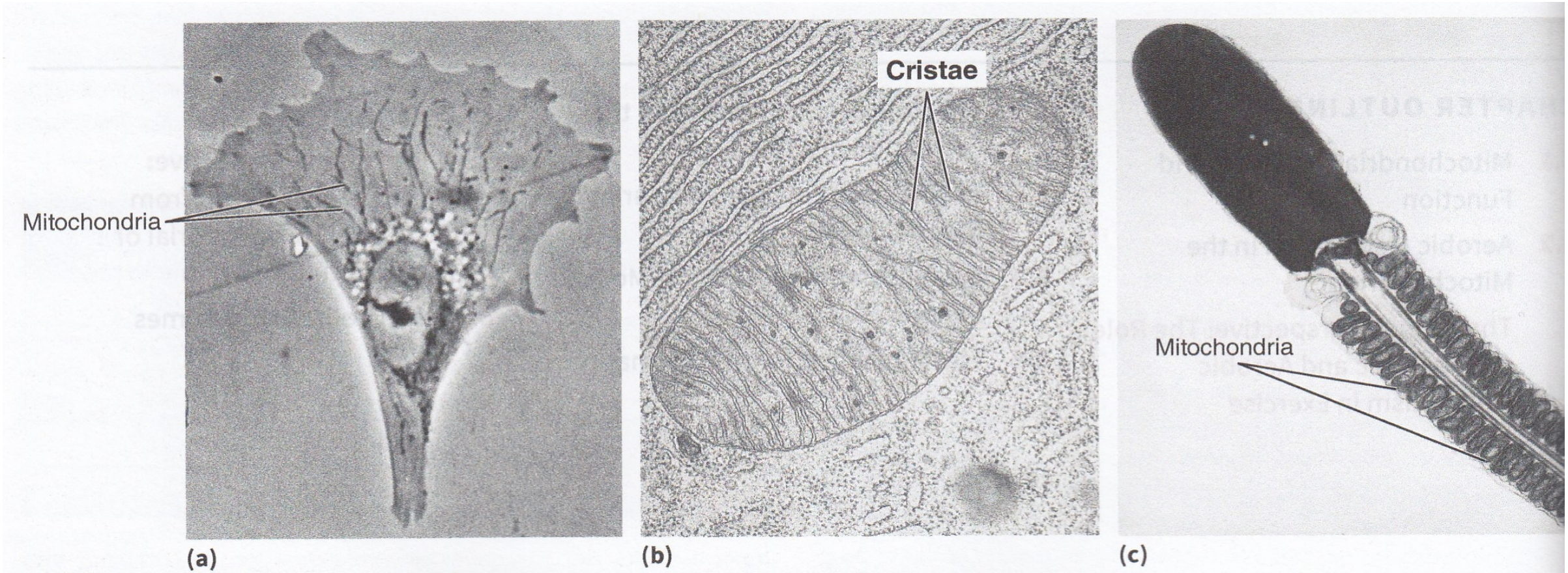
Matrix
Has appropriate enzymes and a suitable pH for the Krebs cycle

Cristae
Highly folded so as to increase SA:Vol ratio

Outer membrane
Contains transport proteins for shuttling pyruvate into mitochondrion



Increased surface area (SA) allows for a greater number of protein complexes, enzymes, and electron transport chain components to be embedded in the inner membrane.



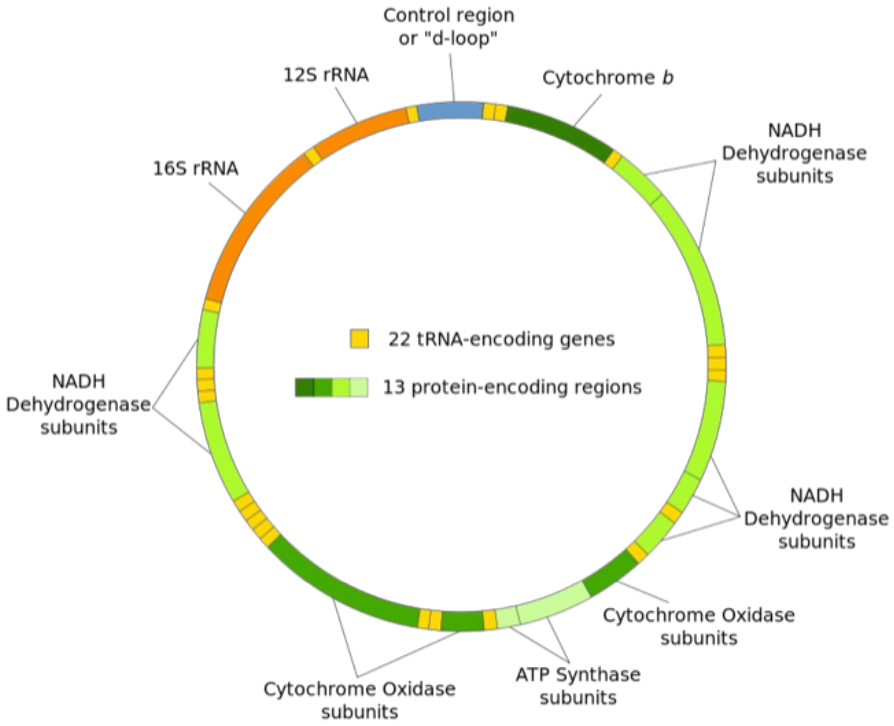
Mitochondria. (a). A living fibroblast with a phase contrast microscope where mitochondria are seen as elongated dark bodies (b). TEM of a thin section through a mitochondrion revealing the internal structure of the organelle. (c) localization of mitochondria in the midpiece surrounding the proximal portion of the flagellum of a bat sperm. (Karp's Cell and Molecular Biology, 9th Ed.)

mtDNA - Small but Essential

- In terms of size, mtDNA is relatively compact compared to nuclear DNA. It encodes a limited number of genes—typically 37 genes in humans. These genes are crucial for the production of proteins that are directly involved in mitochondrial function.

- These mtDNA-encoded proteins are integral to the **electron transport chain (ETC)** and **oxidative phosphorylation**, which are the very processes that enable mitochondria to generate ATP, the cell's primary energy source.

Genes of Mitochondrial DNA (mtDNA)



Human mitochondrial DNA "chromosome" showing the arrangement of regions that code for proteins and RNA molecules.

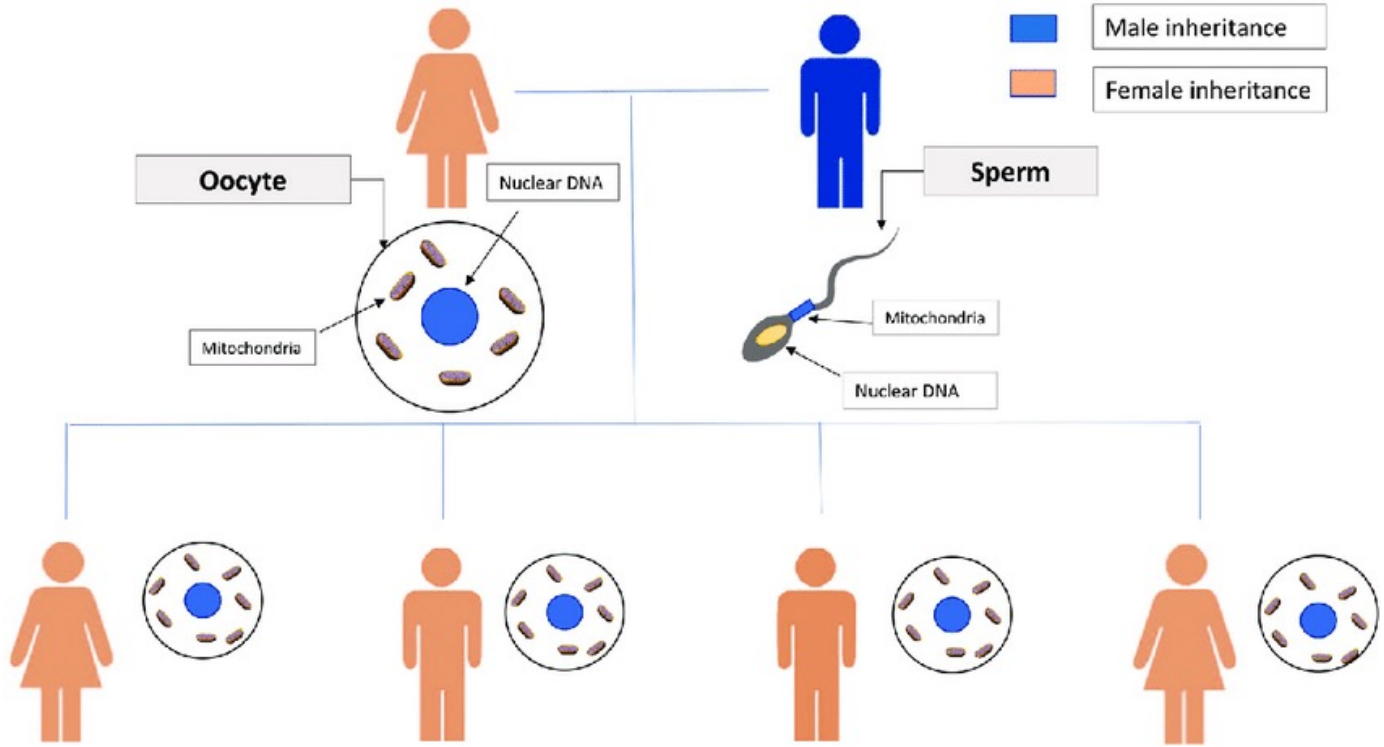
Key Differences Between Nuclear DNA and Mitochondrial DNA	
Nuclear DNA	Mitochondrial DNA
Inherited from both parents	Inherited from mother only
Linear	Circular
3.2 billion base pairs	16,569 base pairs
20,000 genes	37 genes
23 pairs in each cell [chromosomal DNA]	Hundreds to thousands in each cell
Paired or diploid	Not paired or haploid
Varied by recombination	No recombination
Repair and proof reading mechanisms	No repair or proof reading mechanisms
Lower mutation rate	Higher mutation rate **10 times higher**
Genetic code differs	Genetic code differs
Has introns	No introns
Monocistronic	Polycistronic

e.g. in mitochondria, the triplet codon AUA codes for the amino acid methionine whereas the same codon in nuclear DNA codes for isoleucine.

https://en.wikipedia.org/wiki/Mitochondrial_DNA

Maternal Inheritance of mtDNA

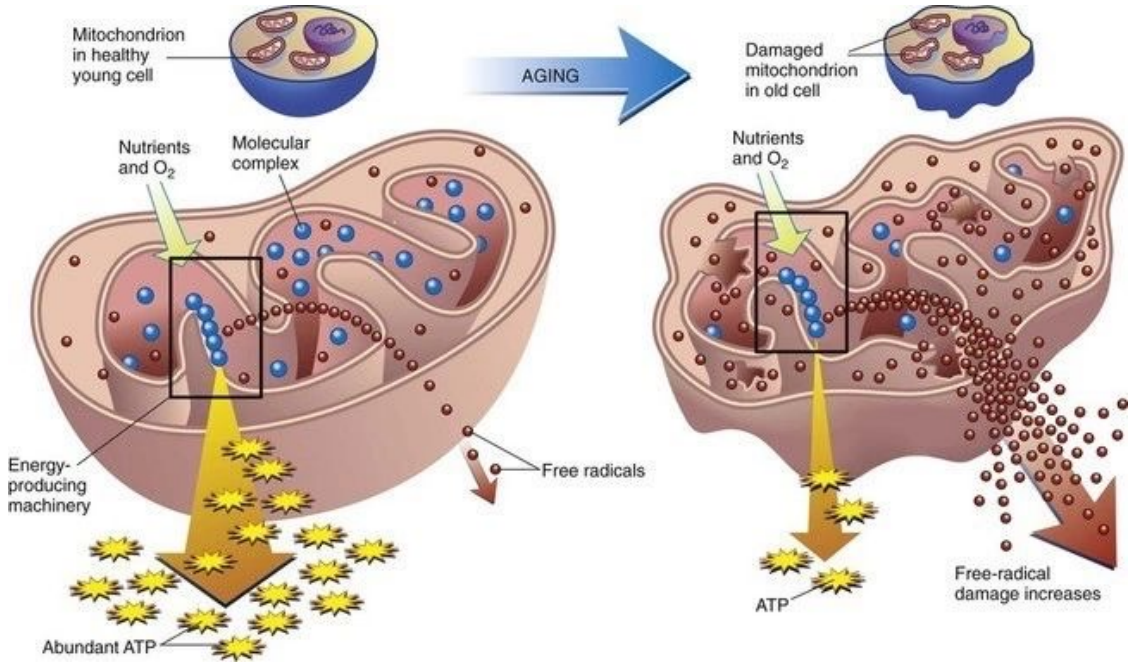
This peculiarity exists because ova contain numerous mitochondria within their abundant cytoplasm, whereas **spermatozoa contain few**, if any. Hence the mitochondrial DNA complement of the zygote is derived entirely from the ovum. Besides, **sperm mitochondria are degraded by ooplasmic proteasomes**



Ortiz, G. G.. Mitochondrial Aging and Metabolism: The Importance of a Good Relationship in the Central Nervous System. in (2018).

The house of power or the house of weakness

- While mtDNA is essential for mitochondrial function, its compact size and proximity to the site of reactive oxygen species (ROS) production within mitochondria make it susceptible to mutations. mtDNA also doesn't have the protective histone proteins that nuclear DNA does.
- Mutations in mtDNA can lead to mitochondrial diseases, which can affect various tissues and organs, leading to a range of health issues.



https://bodycheckphysio.ca/mitochondria

A few key concepts to help you understand the process of cellular respiration

- Redox reactions
- Electron carriers

Redox reactions in cellular respiration

- Cellular respiration involves **redox reactions** (short for "oxidation-reduction"), where electrons move between molecules. To remember this, think "LEO goes GER": Lose Electrons, Oxidized (LEO); Gain Electrons, Reduced (GER).
- Redox reactions alter electron density in atoms. In biology, H and O changes suggest redox. For instance, in glucose breakdown:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

- Redox reactions release energy as electrons transition to lower energy states. This energy is captured, leading to **ATP (Adenosine Triphosphate)** production.

Electron carriers in cellular respiration

- There are two types of electron carriers that are particularly important in cellular respiration: **NAD+** (nicotinamide adenine dinucleotide) and **FAD** (flavin adenine dinucleotide).

Pick up electrons

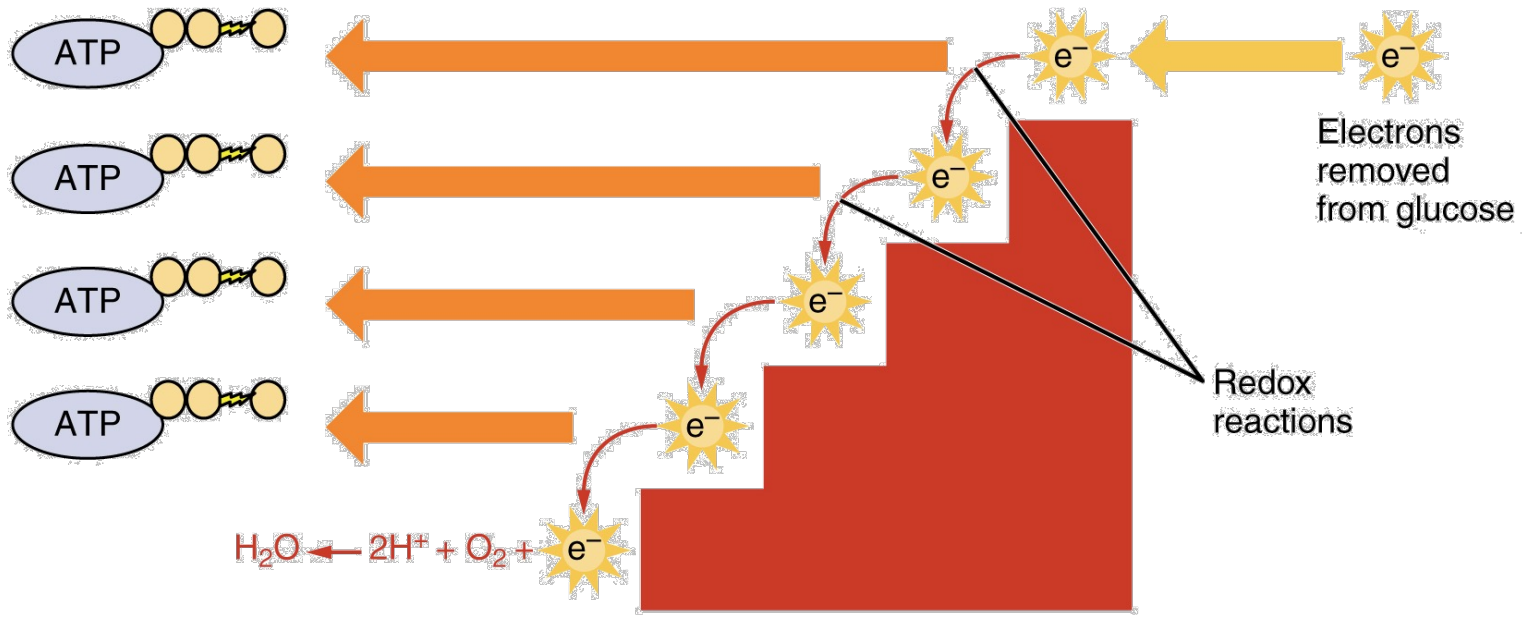
$$NAD^+ + 2e^- + 2H^+ \rightarrow NADH + H^+$$

$$FAD + 2e^- + 2\,H^+ \rightarrow FADH_2$$

Drop electrons off

$$NADH \rightarrow NAD^+ + 2e^- + H^+$$

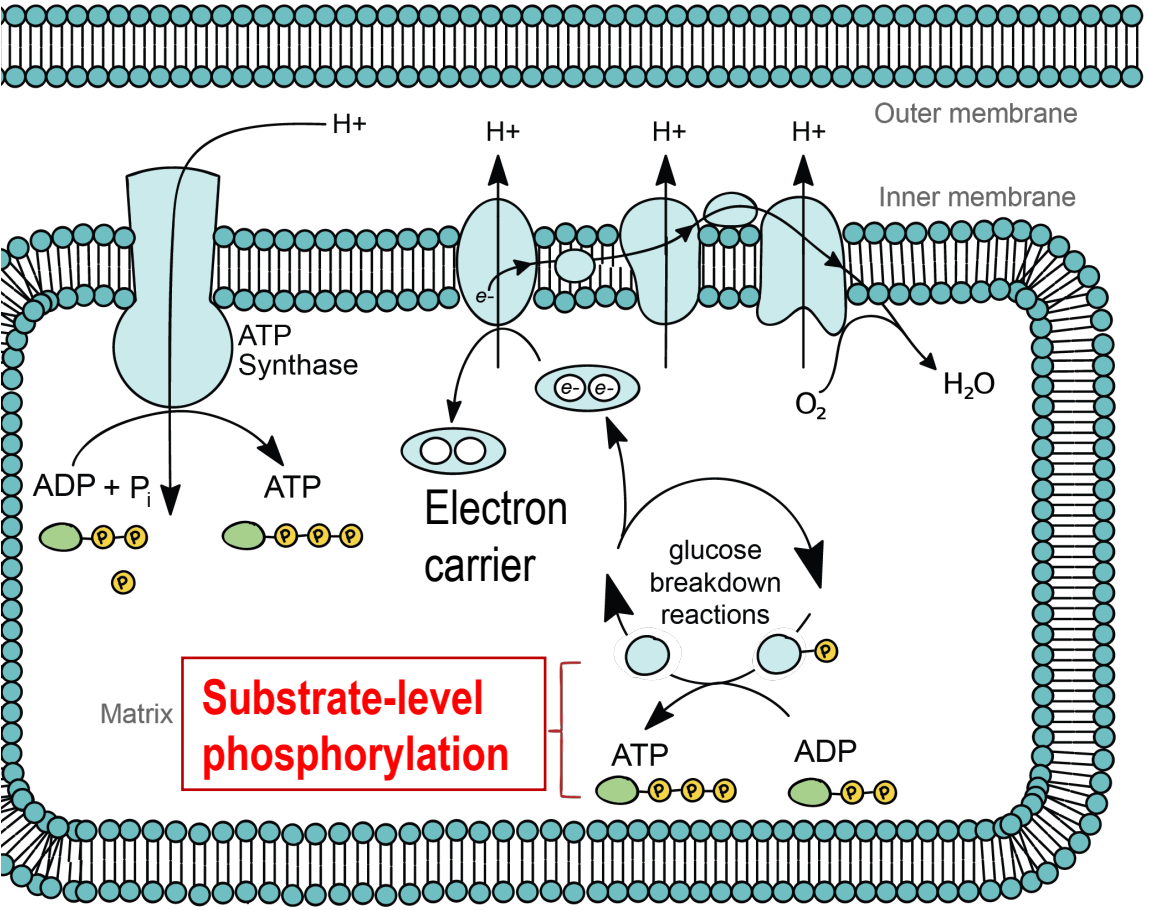
$$FADH_2 \rightarrow FAD + 2e^- + 2\,H^+$$



The reactions in which NAD+ and FAD gain or lose electrons are typical examples of **redox reactions.**

Substrate-level phosphorylation

The reactions that extract energy from molecules like glucose are called **catabolic reactions**. Energy from glucose bonds is released, with some captured as ATP, while the rest is dissipated as heat.

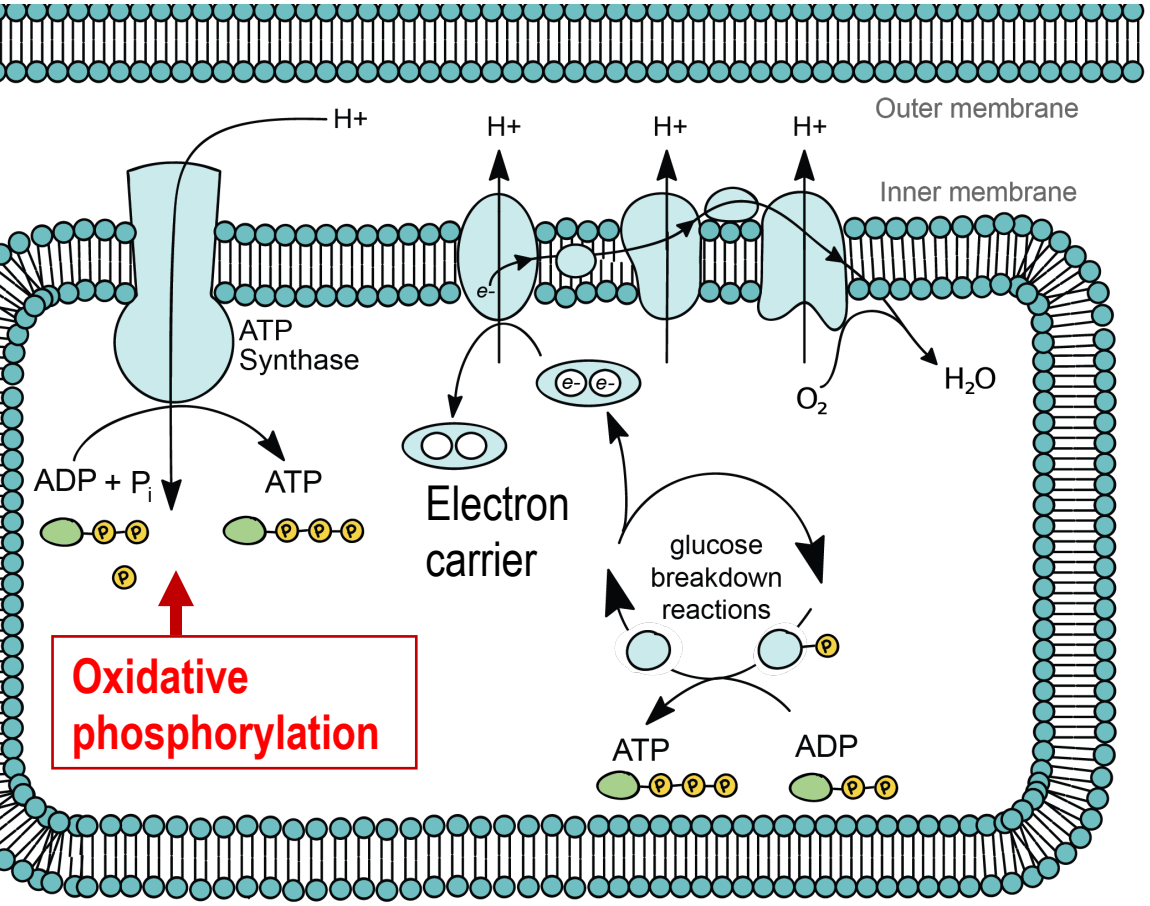


As a glucose molecule is gradually broken down, some of the breakdown steps release energy that is captured directly as ATP. In these steps, a phosphate group is transferred from a pathway intermediate straight to ADP, a process known as **substrate-level phosphorylation**. It is a direct method of ATP synthesis, **rapid but less efficient**.

Image modified from "Etc4(Opens in a new window)" by Fvasconcellos

Oxidative Phosphorylation

Oxidative Phosphorylation is the **primary but indirect method** of ATP production in the mitochondria. It's a **highly efficient but relatively slower way** of generating **a large amount** of ATP.

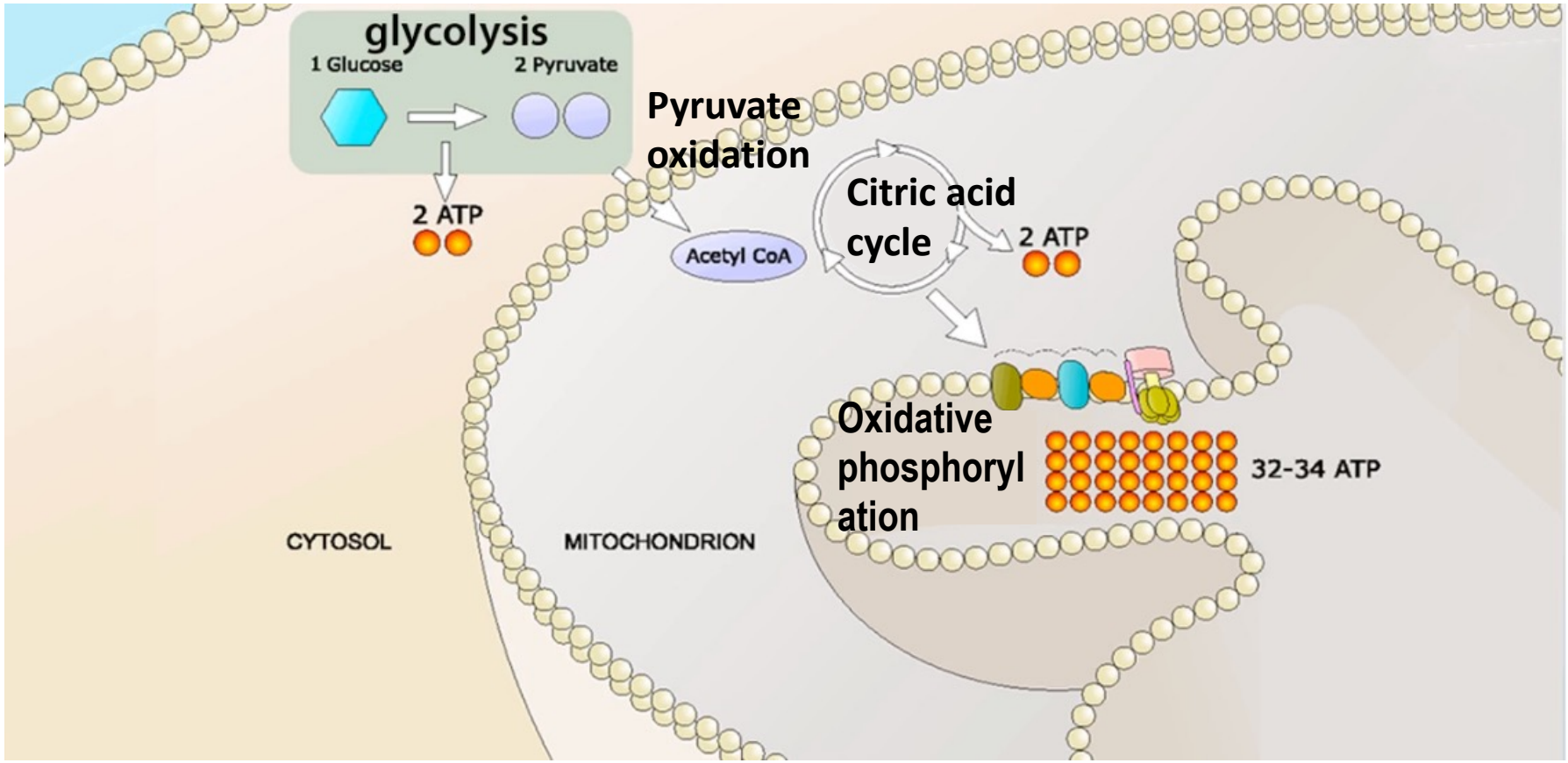


1. **Electrons** carried by molecules like NADH and $FADH_2$ are transferred through the **electron transport chain (ETC**, a series of proteins embedded in the **inner membrane** of the mitochondrion)..
2. As electrons move down the chain, energy is released and used to pump protons out of the matrix, forming a gradient.
3. Protons flow back into the mitochondria through an enzyme called **ATP synthase**, making ATP.
4. At the end of the electron transport chain, oxygen accepts electrons and takes up protons to form water.

Image modified from "Etc4(Opens in a new window)" by Fvasconcellos

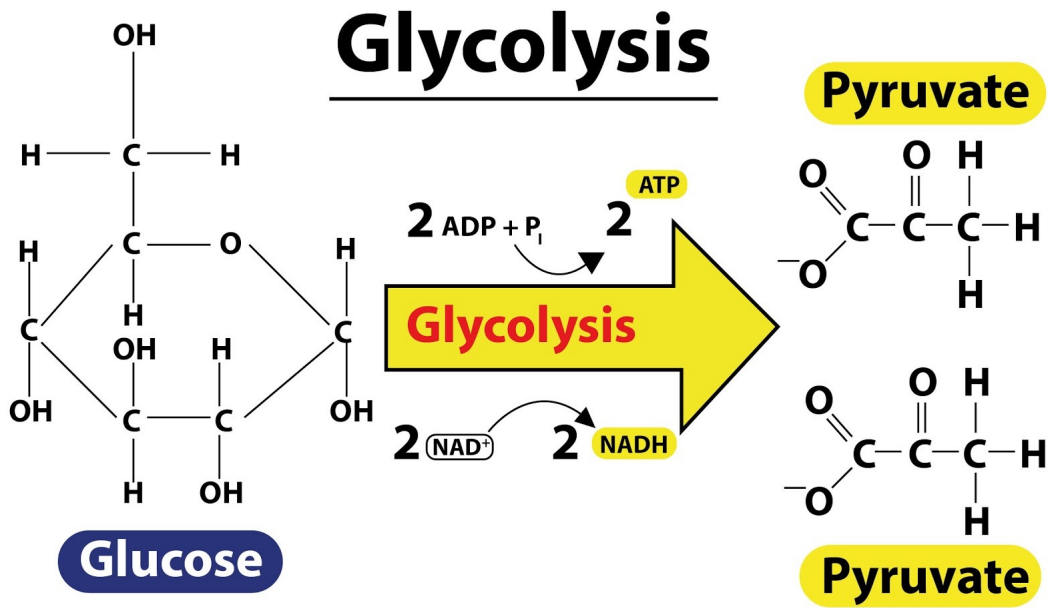
Cell respiration in mitochondria

- Cellular respiration is the process in which cells convert organic molecules, like glucose, into ATP energy. It occurs in the **mitochondria** of eukaryotic cells (or the cytoplasm of prokaryotic cells). This metabolic process fuels cellular activities and consists of **four stages - glycolysis, pyruvate oxidation, the citric acid cycle, and oxidative phosphorylation**, ultimately producing ATP, carbon dioxide, and water.



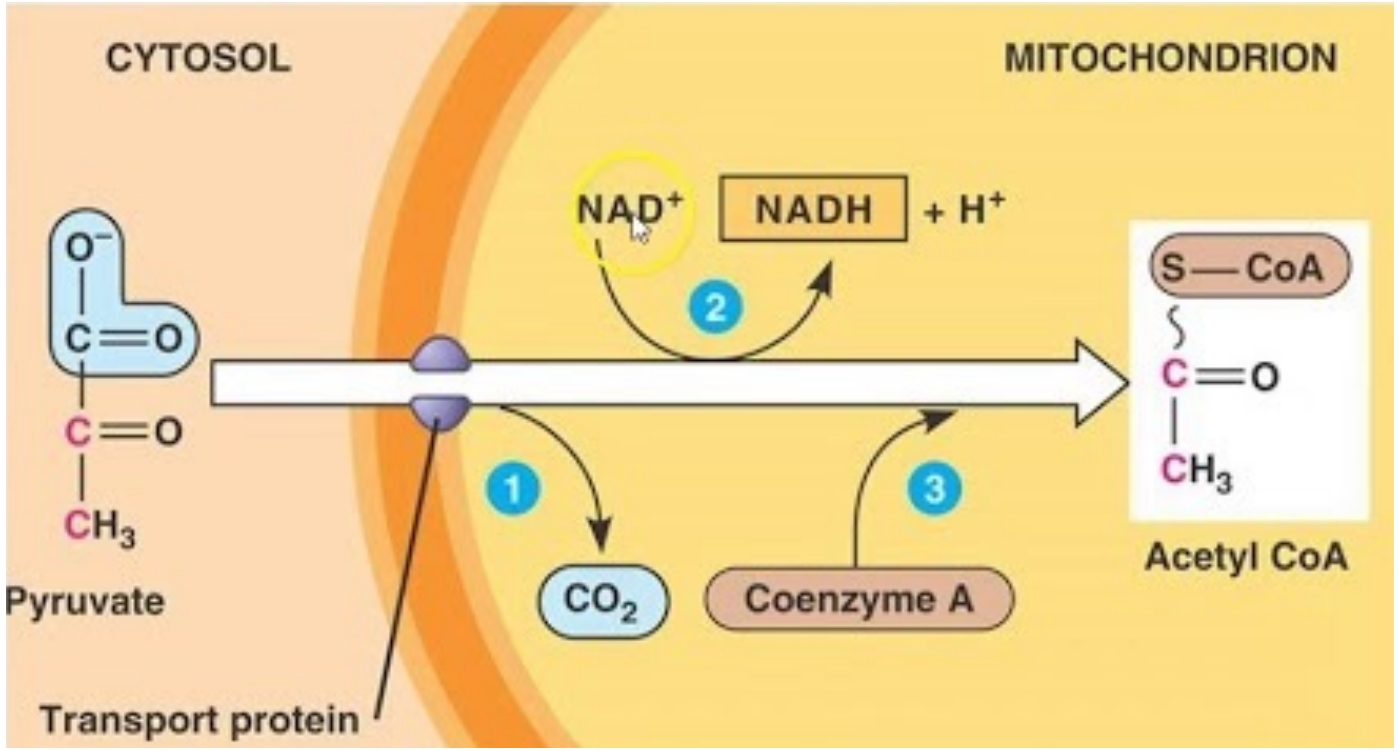
1st step - Glycolysis

- **Glycolysis** is a series of reactions that extract energy from glucose by splitting it into two three-carbon molecules called **pyruvates**. The net products of this process are two molecules of **ATP** produced and two molecules of **NADH**. Glycolysis takes place in the cytoplasm of the cell.



2nd step - Pyruvate oxidation

- **Pyruvate oxidation:** Each pyruvate from glycolysis the pyruvate is transported into the **mitochondrial matrix**—the innermost compartment of mitochondria. There, if the cell has oxygen available, it's converted into a two-carbon molecule bound to Coenzyme A, known as **acetyl CoA**. **Carbon dioxide** is released and **NADH** is generated (No ATP is generated in this step!).
- **Acetyl CoA** acts as fuel for the citric acid cycle (CAC) in the next stage of cellular respiration.



Oxidation of Pyruvate

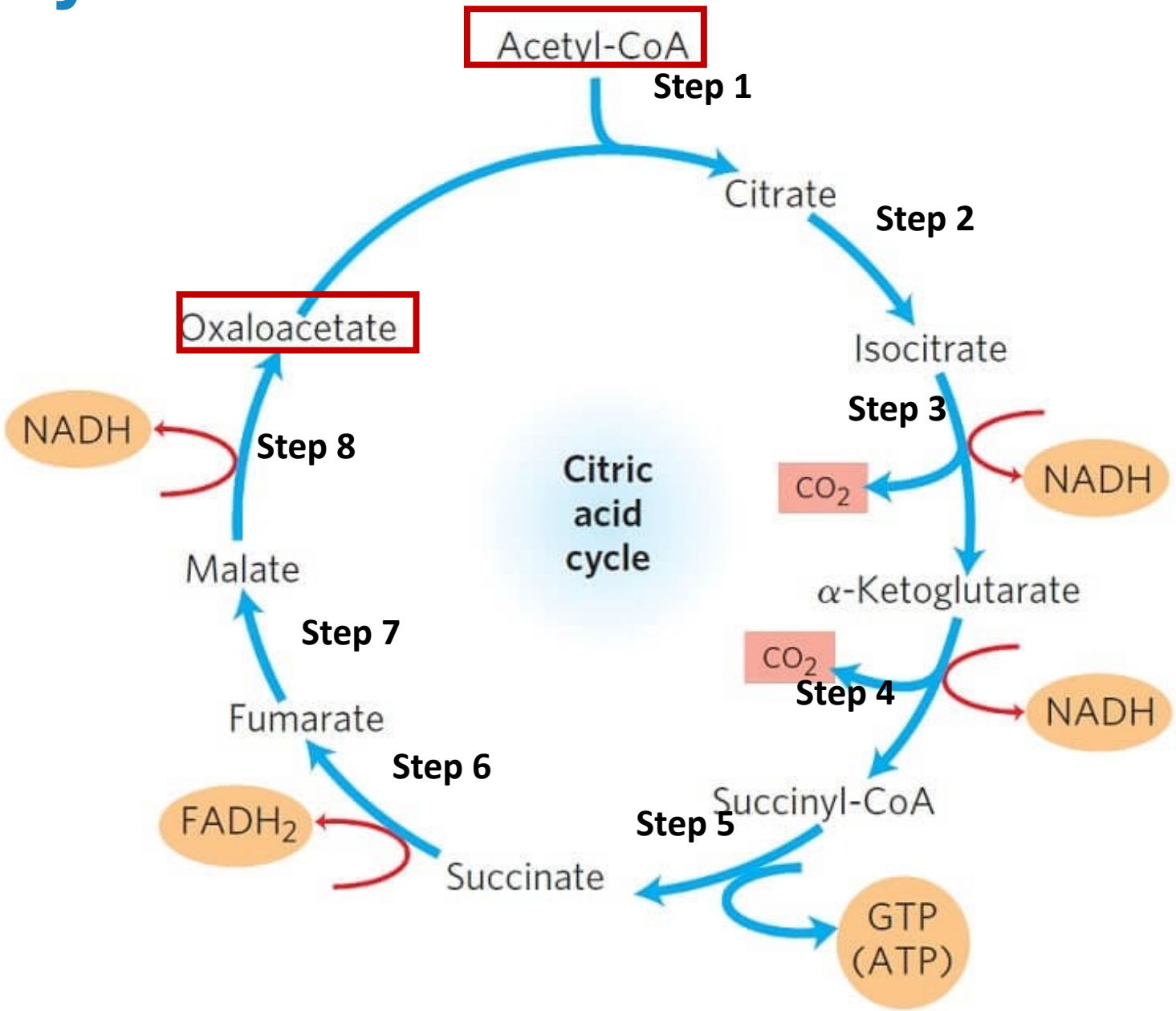
Pyruvate	Oxidation reaction	Acetyl CoA
1 A carboxyl group is removed from pyruvate, releasing carbon dioxide.	2 NAD^+ is reduced to NADH.	3 An acetyl group is transferred to coenzyme A, resulting in acetyl CoA.

OpenStax College, Biology

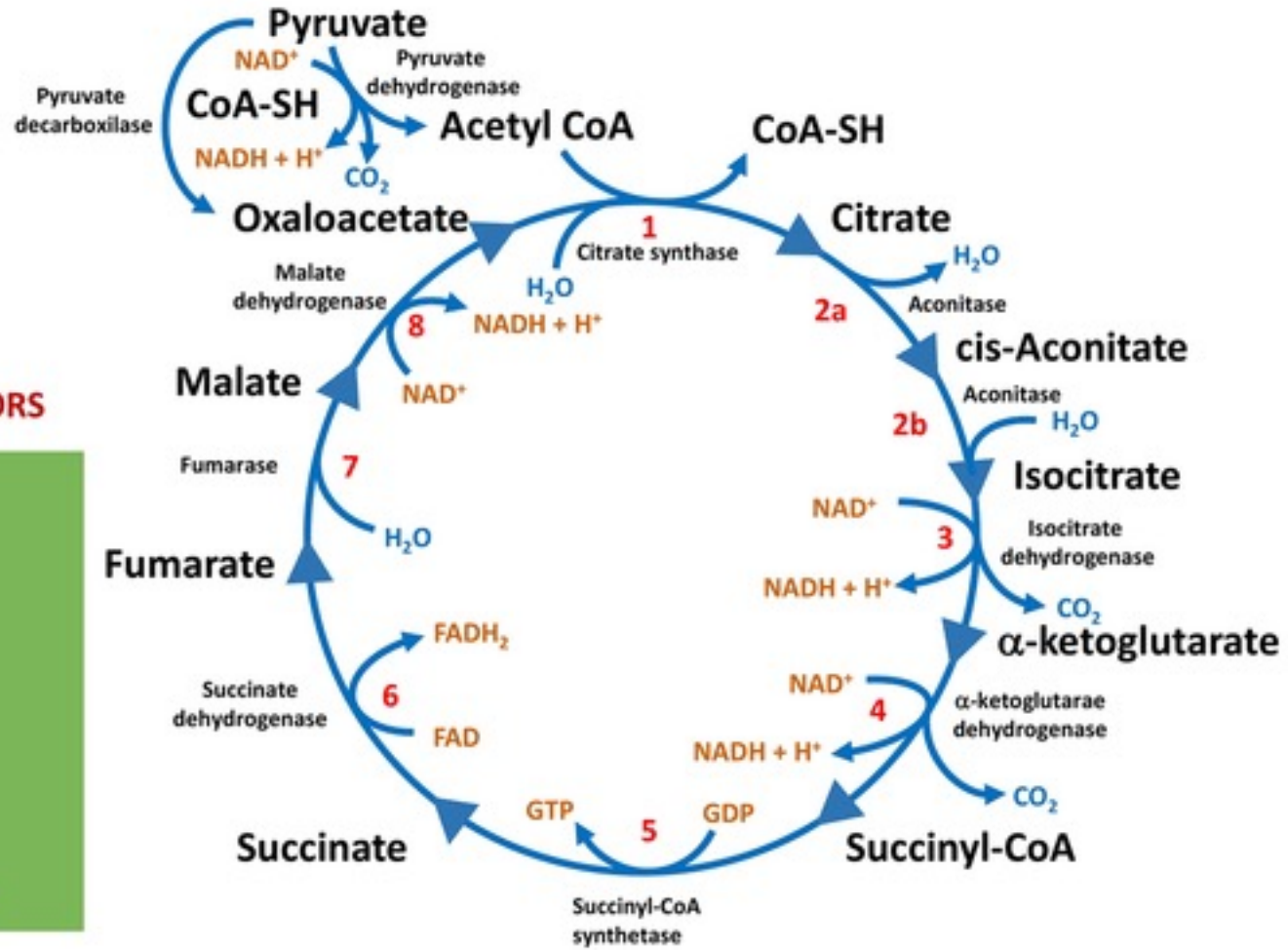
3rd step - Citric Acid Cycle (CAC)

- The **acetyl CoA** from the step of pyruvate oxidation combines with a four-carbon molecule called **oxaloacetate (OAA)** and goes through a cycle of reactions named the **Citric Acid Cycle** (CAC: also called the Kreb's cycle or the tricarboxylic acid (TCA) cycle) that occurs in the mitochondria, ultimately regenerating the OAA.

- **ATP, NADH**, and **FADH2** are produced, and carbon dioxide is released.



Inhibitors and activators of the CAC



CYCLE ACTIVATORS

- Glutamine
- Glutathione
- Fe
- NADH
- Carnosine
- Physical exercise

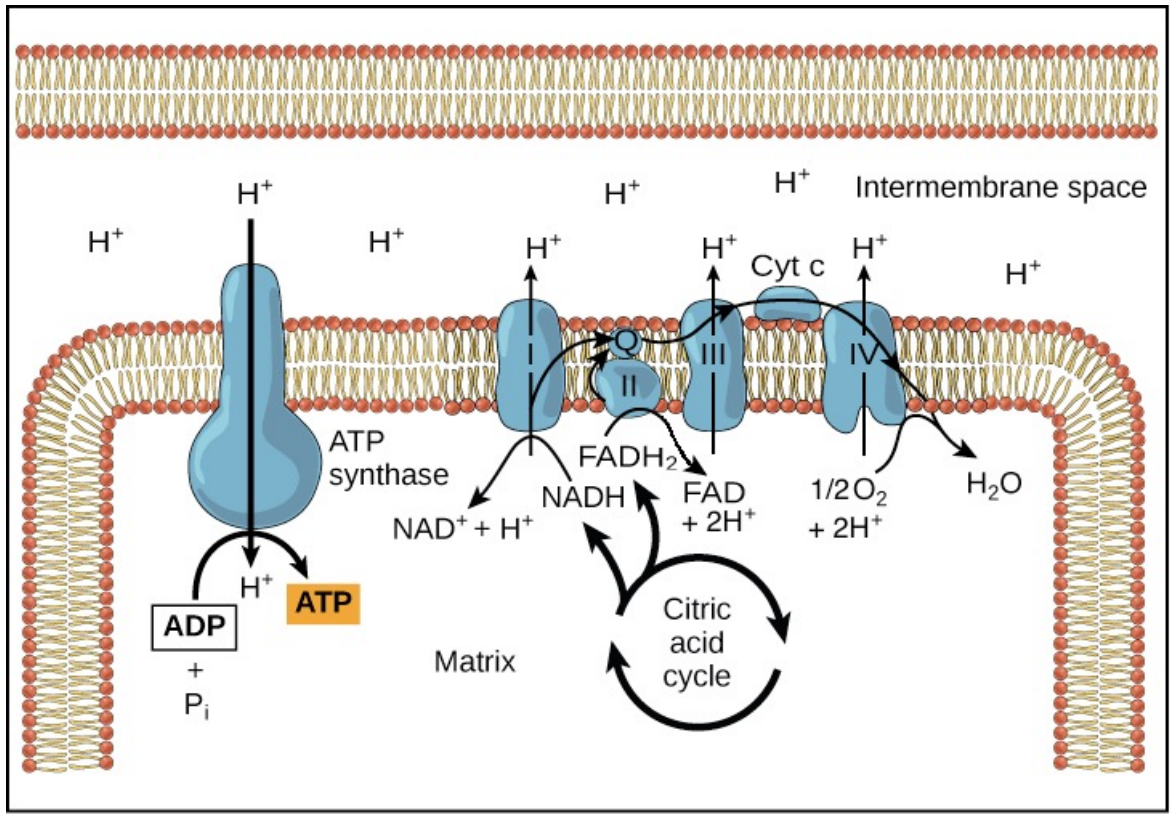
CYCLE INHIBITORS

- Aconitase inhibition: Zn excess and other inhibitors
- Metals: Pb, Cd, Cr, Mn, Al, Fe excess
- Metformin
- Vitamin B12 Deficiency

Gasmi, A.. Krebs cycle: activators, inhibitors and their roles in the modulation of carcinogenesis. Archives of Toxicology 95, 1161–1178 (2021).

4th step - Oxidative phosphorylation (revisit)

- The reduced electron carriers —NADH and $FADH_2$ —generated in the CAC will pass their electrons into the electron transport chain and, through oxidative phosphorylation, will generate most of the ATP produced in cellular respiration.

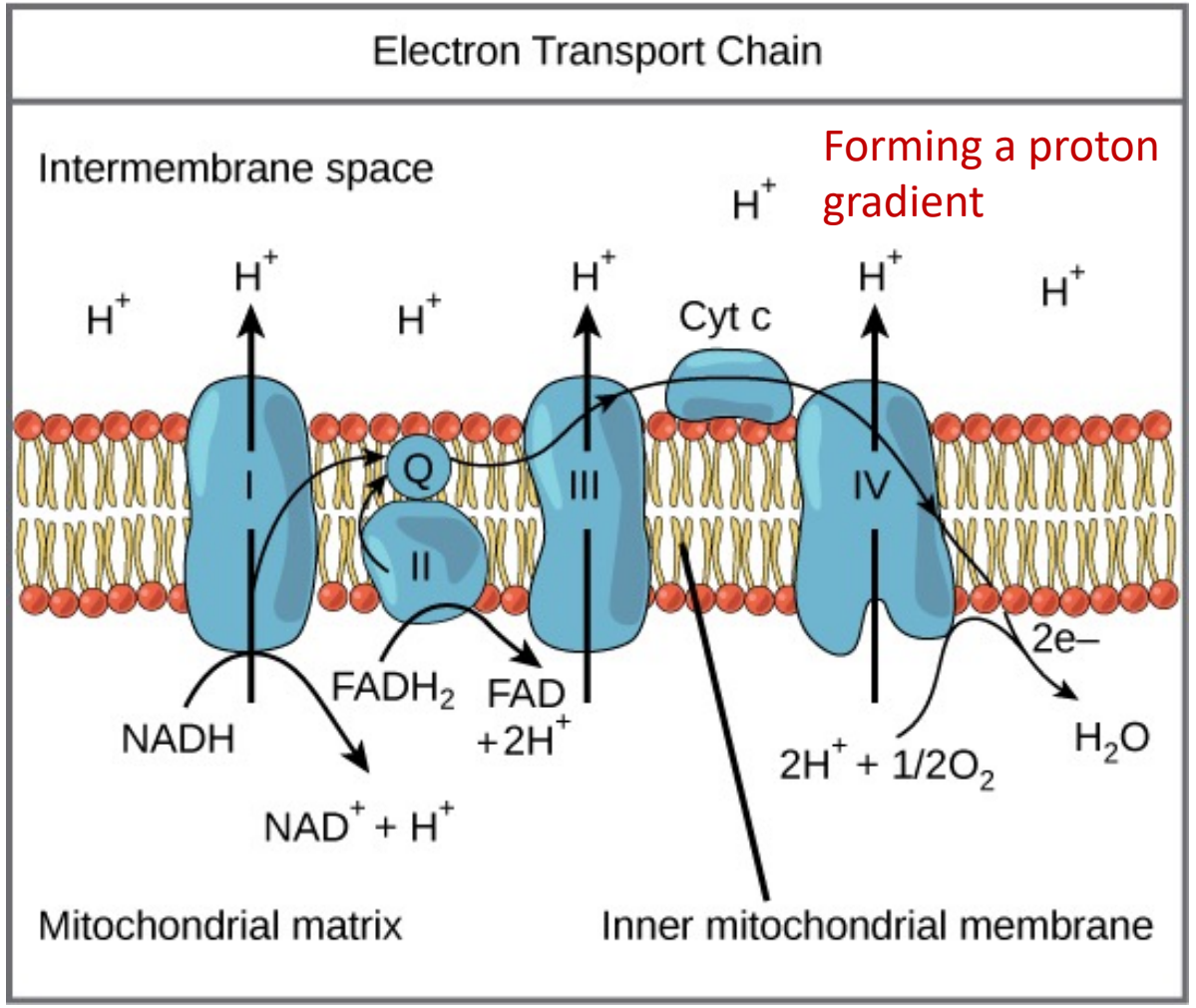


1. **NADH** and **$FADH_2$** made in other steps deposit their electrons in the electron transport chain (ETC, a series of proteins embedded in the inner membrane of the mitochondrion), turning back into their "empty" forms NAD+ and FAD.
2. As electrons move down the chain, energy is released and used to pump protons out of the matrix, forming a gradient.
3. Protons flow back into the matrix via **ATP synthase** to generate ATP.
4. At the end of the electron transport chain, oxygen accepts electrons and takes up protons to form water.

Correa, Jakob, Takala, 2015

The electron transport chain (ETC)

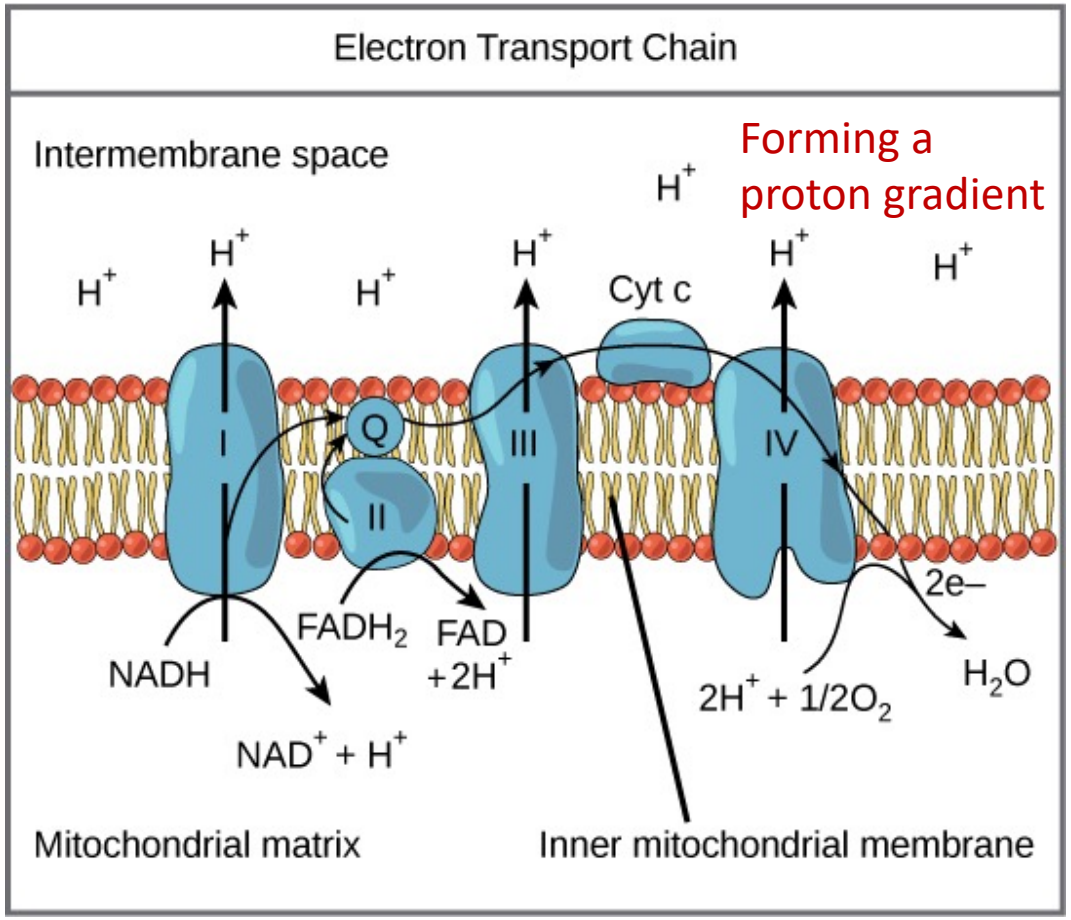
- **ETC** is a collection of membrane-embedded proteins and organic molecules, most of them organized into **four large complexes** labeled I to IV. In eukaryotes, many copies of these molecules are found in the **inner mitochondrial membrane**. In prokaryotes, the electron transport chain components are found in the plasma membrane.
- All of the electrons that enter the transport chain come from **NADH (via complex I)** and **$FADH_2$ (via complex II)** molecules produced during earlier stages of cellular respiration: glycolysis, pyruvate oxidation, and the citric acid cycle.



Correa, Jakob, Takala, 2015

The electron transport chain (ETC)

- Complex I and Complex II pass electrons to the mobile electron carrier, **ubiquinone (Q)**. It becomes reduced, forming **QH_2**, and traverses the membrane.
- QH_2 delivers electrons to **Complex III**, while also pumping H^+ ions across the membrane.
- Electrons continue their journey through Complex III, leading to more H^+ ion transport. The final destination is **Complex IV**, where electrons are transferred to another mobile carrier, **cytochrome C (cyt C)**.

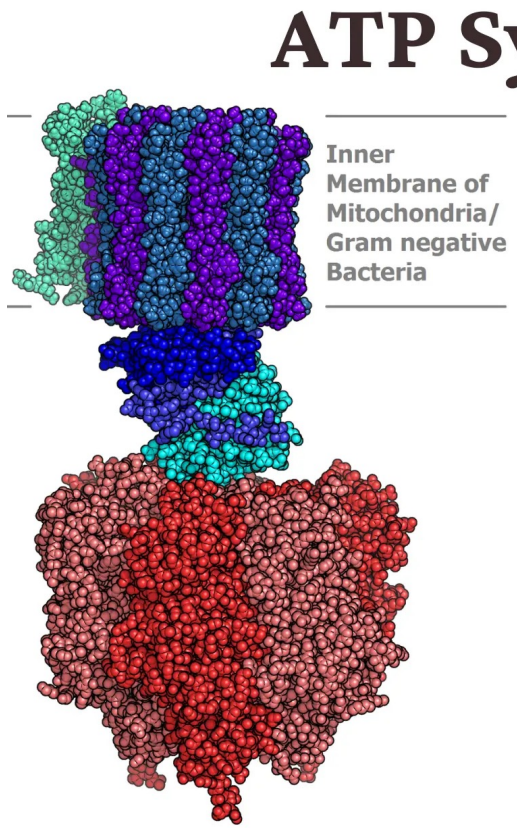


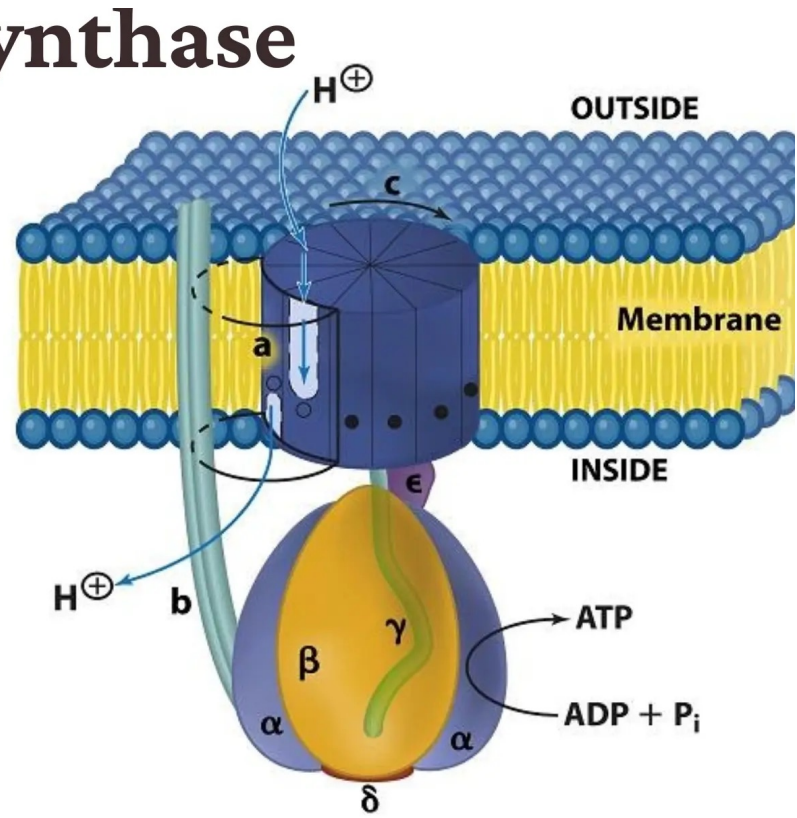
Two main functions of ETC

- **Regenerates electron carriers.** NADH and $FADH_2$ pass their electrons to the ETC, turning back into NAD+ and FAD. This is important because the oxidized forms of these electron carriers are used in glycolysis and the citric acid cycle and must be available to keep these processes running.
- **Makes a proton gradient.** **Complexes I, III, and IV** of the ETC are proton pumps. They pump H+ ions **from the matrix to the intermembrane space**. This pumping forms an electrochemical gradient across the inner mitochondrial membrane. The gradient is sometimes called the **proton-motive force**. This gradient represents a stored form of energy that can be used to make ATP.

Proton Movement and ATP Synthesis

- **Chemiosmosis:** In the inner mitochondrial membrane, H+ ions have just one channel available: **ATP synthase**. It's turned by the flow of H^+ ions moving down their electrochemical gradient. As ATP synthase turns, it catalyzes the addition of a phosphate to ADP, capturing energy from the proton gradient as ATP.
- Chemiosmosis isn't exclusive to cellular respiration; it's also involved in photosynthesis.
- It accounts for over 80% of ATP production during glucose breakdown.

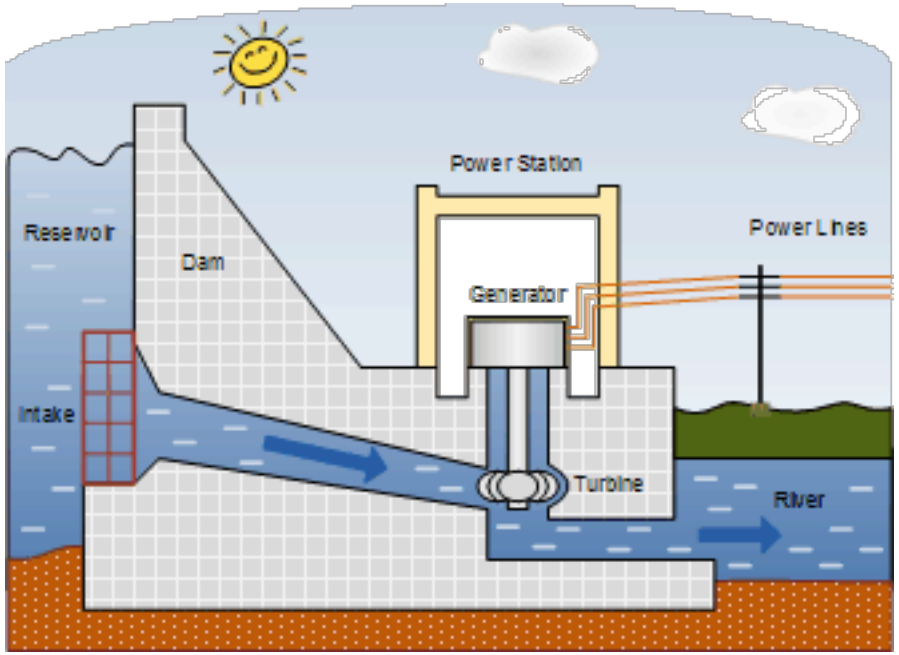




- If the proton gradient's energy isn't used for ATP or other cellular work, it's released as heat. Some cells in hibernating mammals (such as bears) like brown fat cells deliberately use proton gradients for heat generation.

The Analogy to a Hydro-electric power Dam

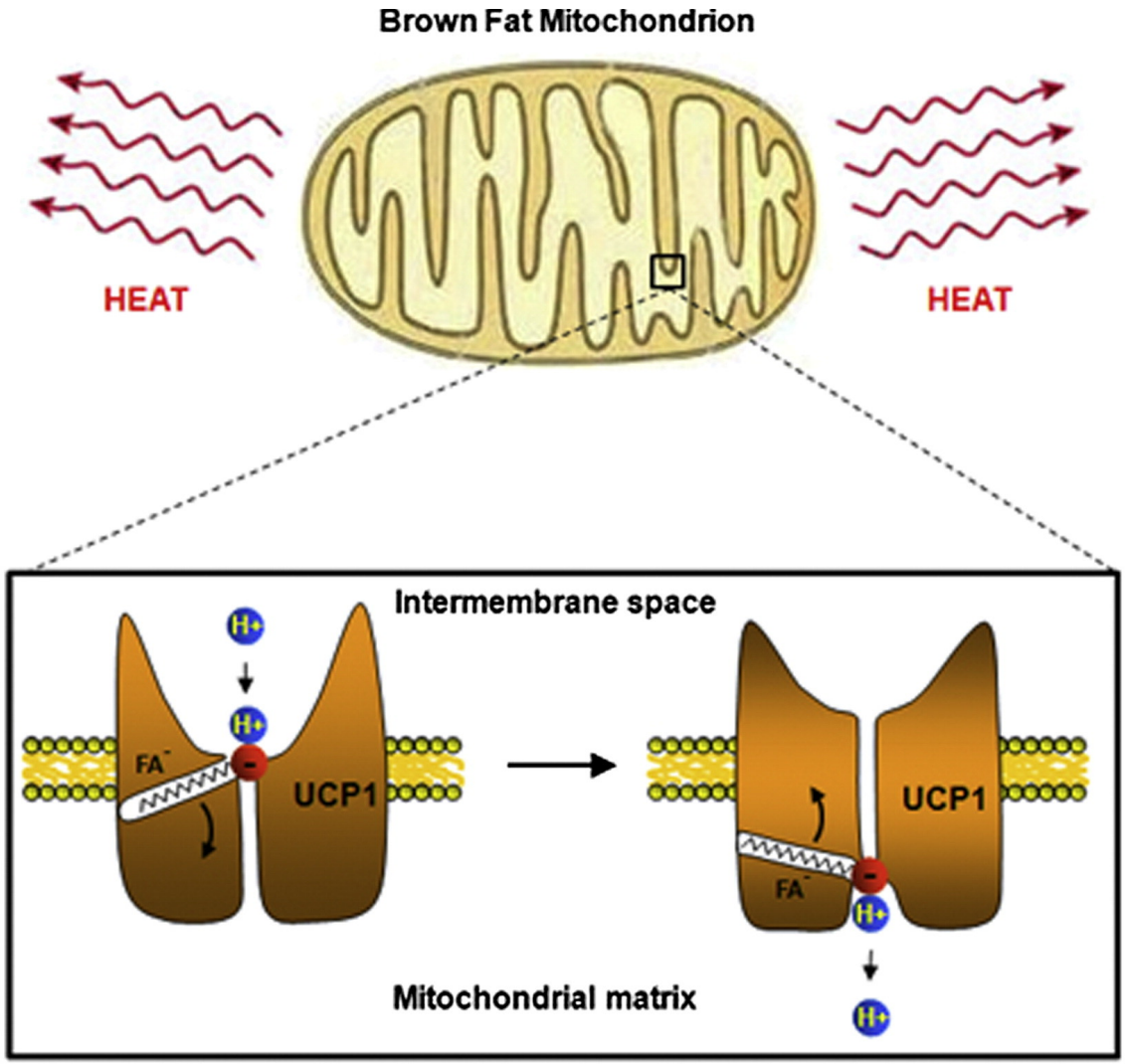
- The pumping of water (protons) into a reservoir (intermembrane space) contained by a dam (inner membrane).
- As water flows out through a channel in the dam, it is used to drive turbines (ATP synthase) to create hydroelectric electricity (ATP).



https://www.alternative-energy-tutorials.com/hydro-energy/hydroelectricity.html

Uncoupling

- Uncoupling disrupts the coupling of electron transport and ATP synthesis in mitochondria → Decreased ATP/Increased ADP + enhanced oxygen consumption/NADH oxidation → energy from ETC's proton gradient dissipates → released as heat
- Uncoupling naturally occurs in some organisms for heat generation and temperature regulation, involving tissues like brown adipose tissue and uncoupling proteins (**UCP**) like thermogenin.



DOI:https://doi.org/10.1016/j.cell.2012.09.010

Requirement for oxygen

- This requirement for oxygen makes the electron transport process the respiratory chain, which accounts for the greatest portion of the body's use of oxygen.
- When oxygen is absent in the mitochondrion, the electrons cannot be removed from the system, and the entire electron transport chain would back up and stop. The mitochondria would then be unable to generate new ATP, and the cell would ultimately die from lack of energy.
- This is the reason living creatures must breathe to draw in new oxygen.

The ATP Yield

- A high-end estimate that the maximum ATP yield for a molecule of glucose is around 30-32 ATP

Stage	Direct products (net)	Ultimate ATP yield (net)
Glycolysis	2 ATP	2 ATP
	2 NADH	3-5 ATP
Pyruvate oxidation	2 NADH	5 ATP
Citric acid cycle	2 ATP/GTP	2 ATP
	6 NADH	15 ATP
	2 $FADH_2$	3 ATP
Total		**30-32 ATP**

Mitochondrial Toxicity

The harmful effects of various compounds or agents on the structure and function of mitochondria, disrupt their vital role in cellular energy production and other essential processes. Mitochondrial toxicity can be caused by:

1. **Chemicals**
2. **Medications**
3. **Metabolic Disorders**

Mitochondrial Toxicity can result in a spectrum of consequences, including:

1. **Energy Depletion**: Impaired ATP production, leading to cellular fatigue.
2. **Oxidative Stress**: Increased production of damaging free radicals.
3. **Cellular Damage**: Disruption of vital cellular functions and even cell death.

Chemicals and Mitochondrial Toxicity

- **Organophosphates:** These are a group of insecticides and herbicides. They can inhibit mitochondrial complex I and IV, leading to a decreased production of ATP. Examples include chlorpyrifos and diazinon.
- **Carbon Monoxide (CO):** A silent killer, CO binds to cytochrome c oxidase (complex IV) with much higher affinity than oxygen, inhibiting the electron transport chain and disrupting ATP synthesis.
- **Heavy Metals:** Metals such as lead, mercury, and cadmium can accumulate in the body and cause mitochondrial dysfunction. They might induce oxidative stress, interfere with mitochondrial enzymes, or even cause mtDNA damage.

Medications and Mitochondrial Toxicity

- **Nucleoside Reverse Transcriptase Inhibitors (NRTIs):** This class of antiretroviral drugs, used to treat HIV infection, can inhibit **mitochondrial DNA polymerase**, leading to mitochondrial DNA depletion. Examples include zidovudine (AZT) and stavudine (d4T).
- **Statins:** While primarily known as cholesterol-lowering drugs, some statins can cause myopathy (muscle pain or weakness). This adverse effect is believed to be linked to the drug's interference with the synthesis of **coenzyme Q10**, an essential component for mitochondrial electron transport.
- **Non-Steroidal Anti-Inflammatory Drugs (NSAIDs):** Some NSAIDs may disrupt mitochondrial function, contributing to gastrointestinal toxicity. For instance, aspirin and salicylate can inhibit the **mitochondrial enzyme succinate dehydrogenase (SDH)**, which is a component of both the citric acid cycle and the electron transport chain.

Metabolic Disorders and Mitochondrial Toxicity

- **Mitochondrial DNA Mutations**
- **Defects in Nuclear DNA that Affect Mitochondria**
- **Peroxisomal Disorders:** Peroxisomes are organelles that share some functions with mitochondria. Diseases related to peroxisomal dysfunction can indirectly affect mitochondrial functions.
- **Pyruvate Dehydrogenase Complex Deficiency (PDCD):** PDCD affects the conversion of pyruvate to acetyl-CoA, a critical step linking glycolysis to the citric acid cycle in mitochondria. Symptoms include developmental delays, lactic acidosis, and neurological issues.
- **Lipid Storage Diseases:** Dysregulated lipid metabolism can impact mitochondrial function, especially in tissues heavily reliant on fatty acid oxidation, like the heart and skeletal muscles.
- **Carnitine Cycle Disorders:** Carnitine plays a vital role in shuttling fatty acids into mitochondria for oxidation. Disorders here lead to issues in energy production, especially during fasting.
- **Coenzyme Q10 Deficiency:** This is a condition where the body cannot produce enough coenzyme Q10, a vital component of the electron transport chain. It can lead to seizures, neurological issues, and muscle breakdown.

Thank you
Next lecture, we will discuss Mitochondrial Disorders