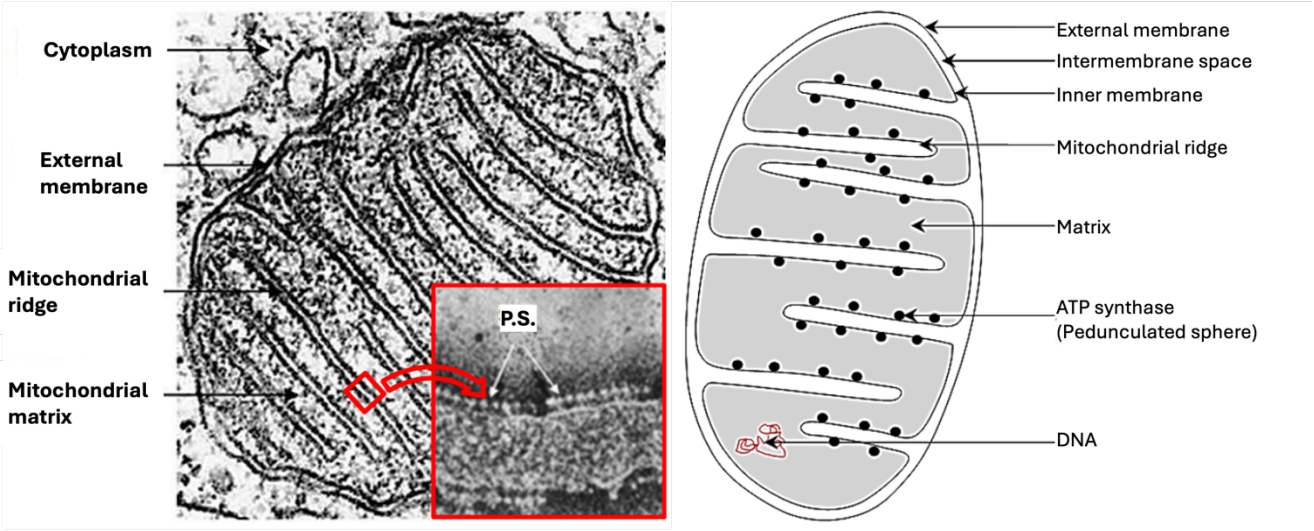
From Pyruvate to ATP: What happens in the mitochondrion?

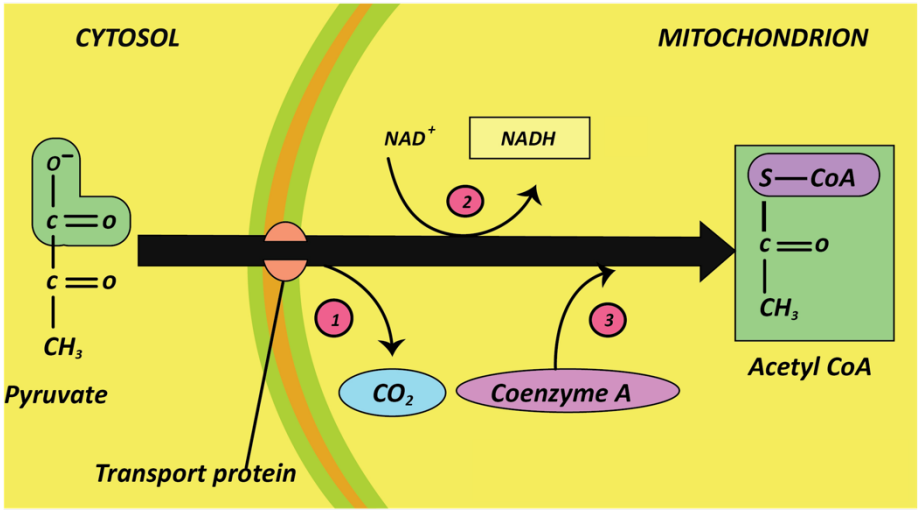
Document 1: Structure of a mitochondrion

Partially autonomous organelle (due to the presence of mitochondrial DNA), the mitochondrion measures approximately one micrometer. The mitochondrial matrix is surrounded by 2 membranes:

- The external membrane separates the mitochondrion from the cytoplasm of the cell.
- The inner membrane features invaginations called mitochondrial ridges. These are covered by granular, spherical structures known as pedunculated spheres (PS). Analysis of these spheres reveals that they are ATP synthase enzyme complexes.



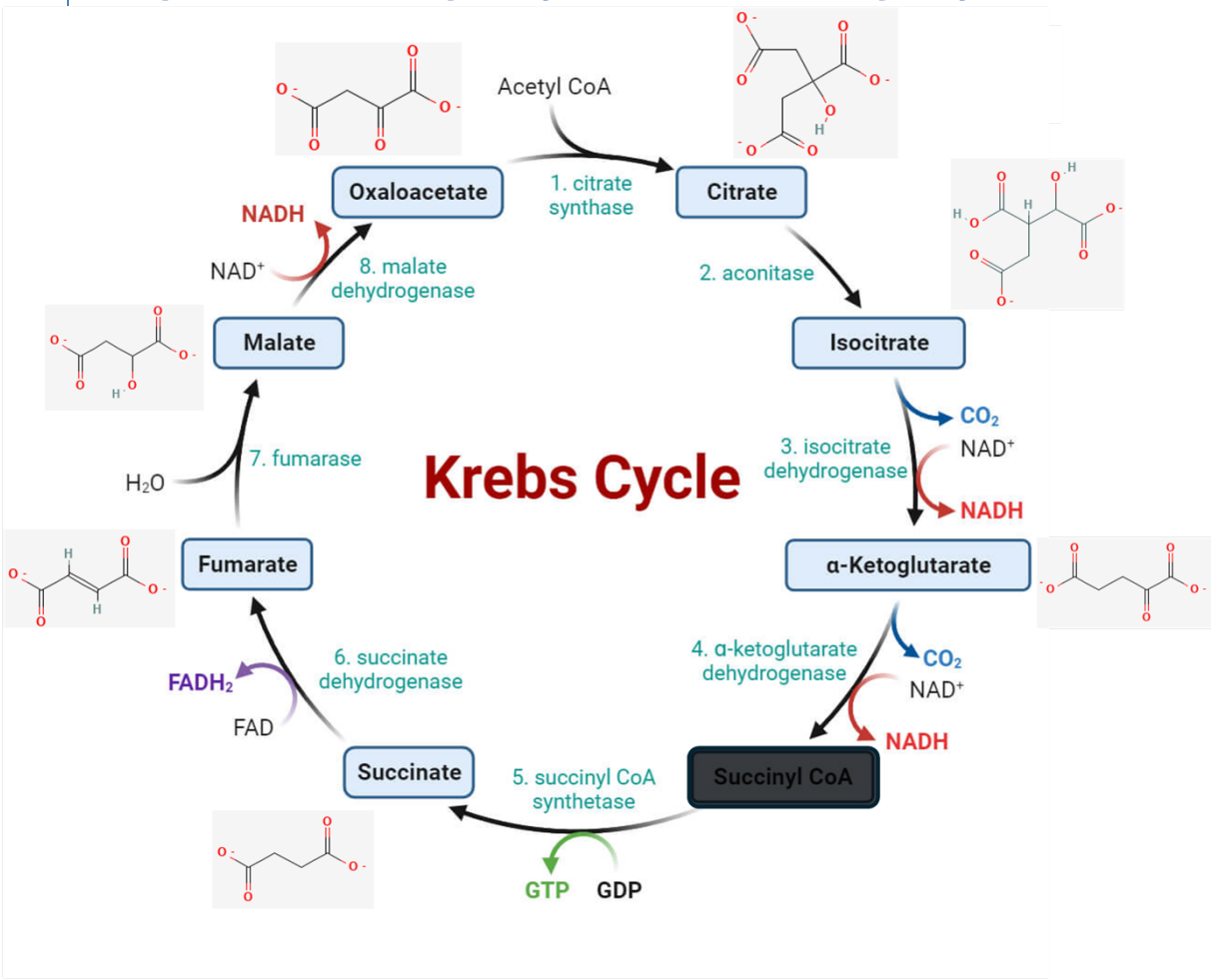
Step 1: Pyruvate oxidation



1. **Where does this step happen?**
2. **How do we know that it involves a redox reaction?**
3. **Suggest a method to show that the CO_2 molecule comes from the part of the pyruvate molecule highlighted in green (carboxyle group)**

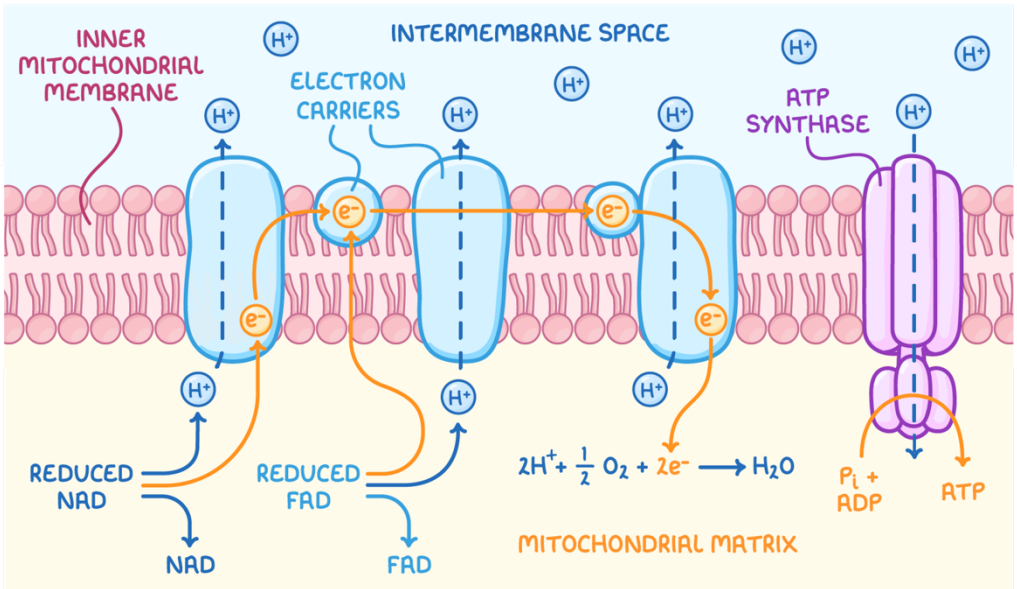
   Hint: This method has been succesfully used in the study of photosynthesis.

Step 2: Krebs cycle (AKA Citric Acid Cycle)



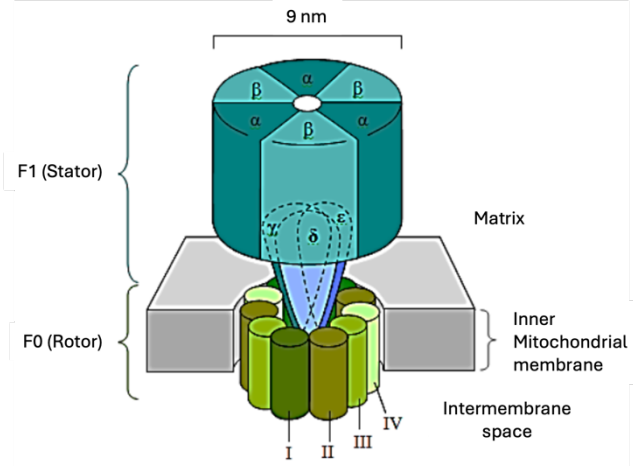
4. **Identify the steps in which an oxidation happens**
5. **How many CO_2 molecules are produced during the cycle?**
6. **By combining the pyruvate oxidation and the Krebs cycle, explain how 6 CO_2 molecules are produced from 1 glucose molecule.**

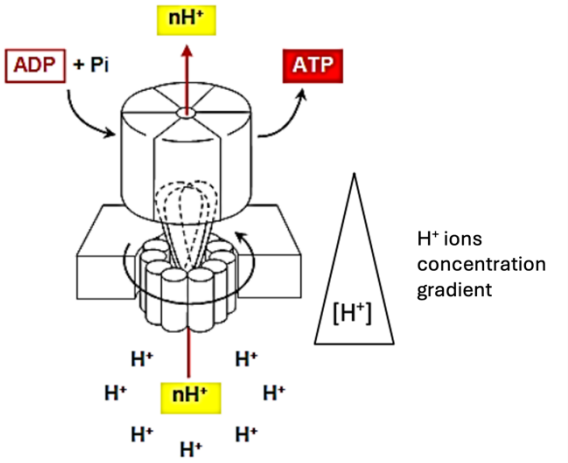
Step 3: Oxidative phosphorylation of ADP



Document 2: Structure of ATP-synthase

- ATP synthase is a bulky protein complex made up of 2 parts: the stator (blue), which is embedded in the mitochondrial crest membrane, and the rotor (green), which protrudes from the membrane and is exposed to the mitochondrial matrix.





- It is also a channel for H^+ ions. When these pass through the complex, the rotor starts to move (rotate). The gamma subunit then rotates within the stator and deforms the alpha and beta subunits, forming ATP from ADP + P_i (inorganic phosphate).

Note: The representation is only an example. The displacement of H^+ ions can be in both directions.

7. **Show that the transformation of O_2 into H_2O is susceptible of creating the H^+ ion gradient needed for the rotor to start moving.**

In 1955, biologists B.Chance and G.R.Williams showed that in absence of ADP, no oxygen was consumed.

8. **How is this experiment related to step 3?**

In 1978, P.Mitchell received the Nobel Prize in Chemistry for his chemiosmotic theory, first published in 1961. His theory stated that the electron transport chain and ATP synthesis are embedded in the same membrane, that the membrane is impermeable to protons, that compounds involved in the electron transport chain and ATP synthesis are in contact with one another or the other side of the membrane, that there is an exchange of protons across the membrane, and finally that the electron transport chain and ATP synthesis are separated.

9. **What can be deduced from Mitchell's theory about the role of the electron transport chain?**

In the late 1960s, E.Racker and P.Stoeckenius set up the following experiment:

- They built artificial lipid vesicles, in which they inserted:
  - Bacteriorhodopsin, a light-driven proton pump from halobacteria.
  - ATP synthase, purified from mitochondria.
- They then illuminated the vesicles with light.

They observed that ATP was produced.

10. **To what extent did this experiment confirm Mitchell's theory?**

Note: Uncouplers (like DNP) were later used to show that disrupting the proton gradient prevents ATP synthesis even though electrons still flow.

Estimating the energy production in the mitochondrion

In the late 1940s and early 1950s, scientist were able to use radioactive phosphate (^{32}P) to study the production of ATP. They observed that this happened primarly in mitochondria, and only when O_2 was present.

11. **Using this experiment, suggest in which step of cellular respiration the highest amount of ATP is produced. Explain your answer.**

The P/O ratio corresponds to how many ATP are made per atom of oxygen reduced. Studies have shown that the P/O ratio of cellular respiration is approximately 3.

12. **Estimate the amount of ATP produced, in mol, for 1 mole of glucose consumed.**