

# DP IB Biology: HL



Your notes

## 8.2 Cell Respiration

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## 8.2.1 Oxidation, Reduction & Phosphorylation

### Oxidation & Reduction

- Oxidation and reduction are commonly known as **redox reactions**
- These reactions occur at the same time and involve the transfer of electrons between molecules
  - **Oxidation** is the **loss of electrons**
  - **Reduction** is the **gain of electrons**
- Redox reactions also involve hydrogen, oxygen and energy transfer
  - Oxidation is also the **loss of hydrogen, gain of oxygen** and **releases energy** to the surroundings (exergonic)
  - Reduction is also the **gain of hydrogen, loss of oxygen** and **absorbs energy** from the surroundings (endergonic)
- Molecules that have a strong tendency to lose/donate their electrons, are known as **reducing agents**
- Molecules that that have a strong tendency to gain electrons, are known as oxidising agents
- Oxidation and reduction reactions feature in **cellular respiration** and **photosynthesis**

Table comparing oxidation and reduction

Oxidation	Reduction
Loss of electrons	Gain of electrons
Loss of hydrogen	Gain of hydrogen
Gain of oxygen	Loss of oxygen
Exergonic (releases energy)	Endergonic (absorbs energy)

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### Oxidation and reduction in cell respiration

- Respiration involves a group of molecules called **electron carriers** which accept or donate their electrons
  - **NAD<sup>+</sup>** is the primary electron carrier involved in respiration
  - **FAD** is another electron carrier used in respiration
- Both NAD and FAD serve as **oxidising agents**:
  - NAD<sup>+</sup> and FAD gain electrons and also gain one or more hydrogen ions (from molecules involved in respiration), switching to a slightly different form called **reduced NAD** and **reduced FAD**
  - $\text{NAD}^+ + 2\text{e}^- + 2\text{H}^+ \rightarrow \text{NADH} + \text{H}^+$
  - $\text{FAD} + 2\text{e}^- + 2\text{H}^+ \rightarrow \text{FADH}_2$

- These electron carriers are used to transport the electrons they have gained to other reactions in respiration
- When they lose these electrons they return to their original form releasing their electrons in the process
  - $\text{NADH} \rightarrow \text{NAD}^+ + 2\text{e}^- + 2\text{H}^+$
  - $\text{FADH}_2 \rightarrow \text{FAD} + 2\text{e}^- + 2\text{H}^+$
- This is an example of a **redox reaction**



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### Examiner Tip

To help you remember which way around loss and gain of electrons is from redox reactions, think OILRIG:

- **O**xidation Is **L**oss
- **R**eduction Is **G**ain

NAD is a collective term for the different forms NAD takes; NAD exists in an oxidised and a reduced form:

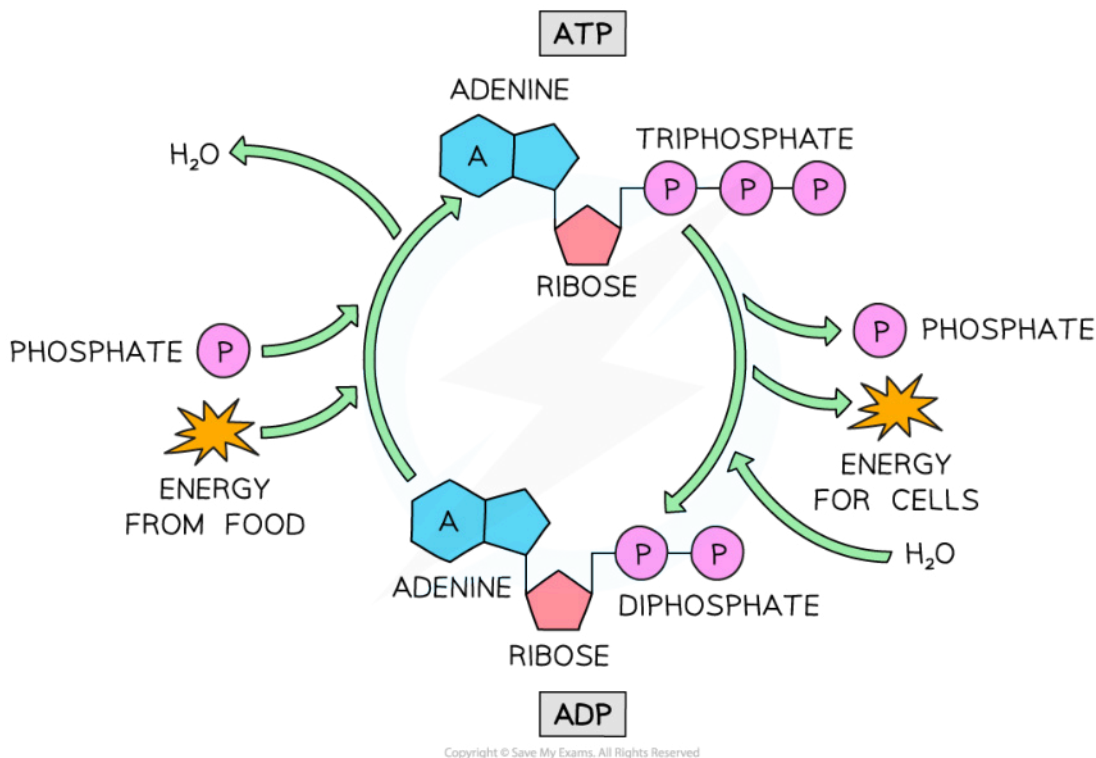
- $\text{NAD}^+$  is the oxidised form and acts as an oxidising agent
- $\text{NADH}$  is the reduced form and acts a reducing agent



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## Phosphorylation

- Phosphorylation occurs when a **phosphate ion is added** to a molecule
  - E.g. the phosphorylation of ADP to make ATP
- This makes the molecule **less stable** and therefore **more likely to react**
  - We can say that phosphorylation **activates** a molecule because it makes it more reactive
- There are two main types of phosphorylation:
  - Substrate level phosphorylation** where the phosphate ion is transferred from a donor compound
    - This takes place in glycolysis and the Krebs cycle
  - Oxidative phosphorylation** where phosphorylation is coupled with oxidation
    - This takes place in the the electron transport chain
- Phosphorylation is an **endergonic** reaction whereas the removal of the phosphate ion by hydrolysis in dephosphorylation is an exergonic reaction
  - Remember that the hydrolysis of ATP to ADP releases energy, therefore dephosphorylation of ADP is exergonic
- The addition of a phosphate ion to one molecule occurs at the same time as the removal of a phosphate from another; this is known as the **coupling** of reactions
  - E.g. the exergonic dephosphorylation of ATP is coupled with the endergonic phosphorylation of glucose at the start of glycolysis



**The cyclic formation of ATP from ADP by phosphorylation**



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## 8.2.2 Overview of the Stages of Respiration

### Stages of Respiration

#### An overview of respiration

- Respiration involves the transfer of **chemical potential energy** from nutrient molecules (such as carbohydrates, fats and proteins) into a usable energy form (through the synthesis of ATP) that can be used for work within an organism
- It is a vital process that takes place in the cells of all living organisms
- There are two forms of respiration depending on the oxygen availability of the cell:
  - **Aerobic** respiration
  - **Anaerobic** respiration
- **Aerobic** respiration is the process of breaking down a **respiratory substrate** in order to produce ATP **using oxygen**
- **Anaerobic** respiration takes place **in the absence of oxygen** and also breaks down a respiratory substrate but produces **less ATP** for the cell
- The main **respiratory substrate** involved in respiration is **glucose**

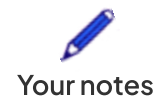
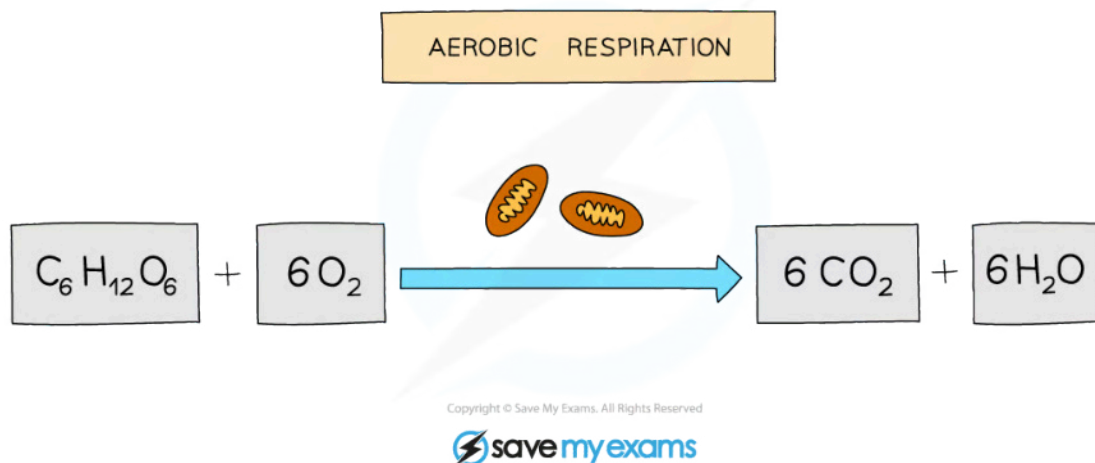
Comparison of Aerobic & Anaerobic Respiration Table

	Aerobic respiration	Anaerobic respiration
Stages	Glycolysis Link reaction The Krebs cycle Oxidative phosphorylation	Glycolysis Fermentation
Oxidation of glucose	Complete	Incomplete
Total ATP produced	High (~36)	Low (2)
Location	Cytoplasm and mitochondria	Cytoplasm
Products	CO <sub>2</sub> , H <sub>2</sub> O	Yeast: CO <sub>2</sub> , ethanol Mammals: Lactate

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#### Aerobic respiration

- Aerobic respiration can be summarised by the following equation
  - Glucose + oxygen → carbon dioxide + water + energy

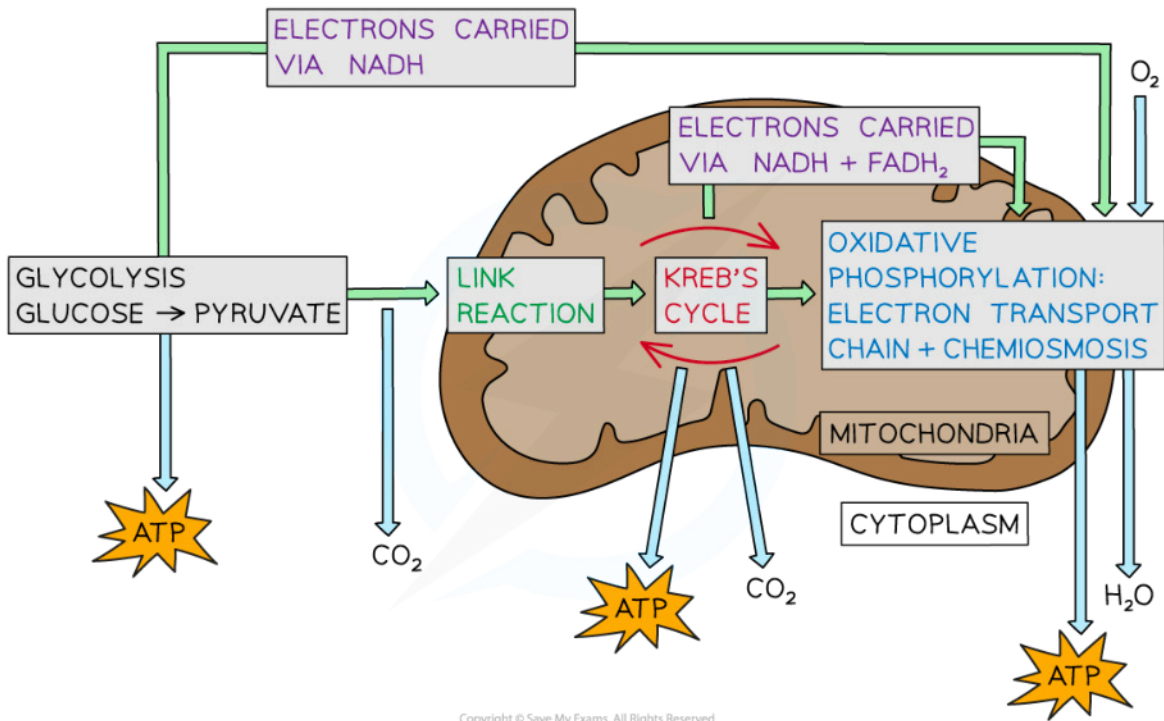


### The stages of aerobic respiration

- The process of aerobic respiration using glucose can be split into four stages
- Each stage occurs at a particular location in a eukaryotic cell:
  - **Glycolysis** takes place in the cytoplasm
  - The **link reaction** takes place in the matrix of the mitochondria
  - The **Krebs Cycle** takes place in the matrix of the mitochondria
  - **Oxidative phosphorylation** which involves the **electron transport chain** and **chemiosmosis** and occurs at the inner membrane of the mitochondria



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**Overview of the four stages of aerobic respiration**

**Four Stages of Respiration Table**



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Stage	Description	Location
1. Glycolysis	Phosphorylation and splitting of glucose	Cell cytoplasm
2. Link reaction	Decarboxylation and dehydrogenation of pyruvate	Matrix of mitochondria
3. Krebs cycle	Cyclical pathway with enzyme-controlled reactions	Matrix of mitochondria
4. Oxidative phosphorylation	Production of ATP through oxidation of hydrogen atoms	Inner membrane of mitochondria

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### Examiner Tip

It's important to know the exact locations of each stage. It is not enough to say the Krebs cycle takes place in the mitochondria, you need to say it takes place in the **matrix** of the mitochondria.





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## 8.2.3 Glycolysis

### Glycolysis

- **Glycolysis** is the first stage of respiration
- It takes place in the **cytoplasm** of the cell and involves:
  - **Trapping glucose** in the cell by phosphorylating the molecule
  - **Splitting the glucose molecule in two**
- It results in the production of
  - Two **pyruvate** (3 carbon/3C) molecules
  - Net gain two **ATP** (Four ATP are produced in total but two are used during the reactions of glycolysis)
  - Two reduced NAD

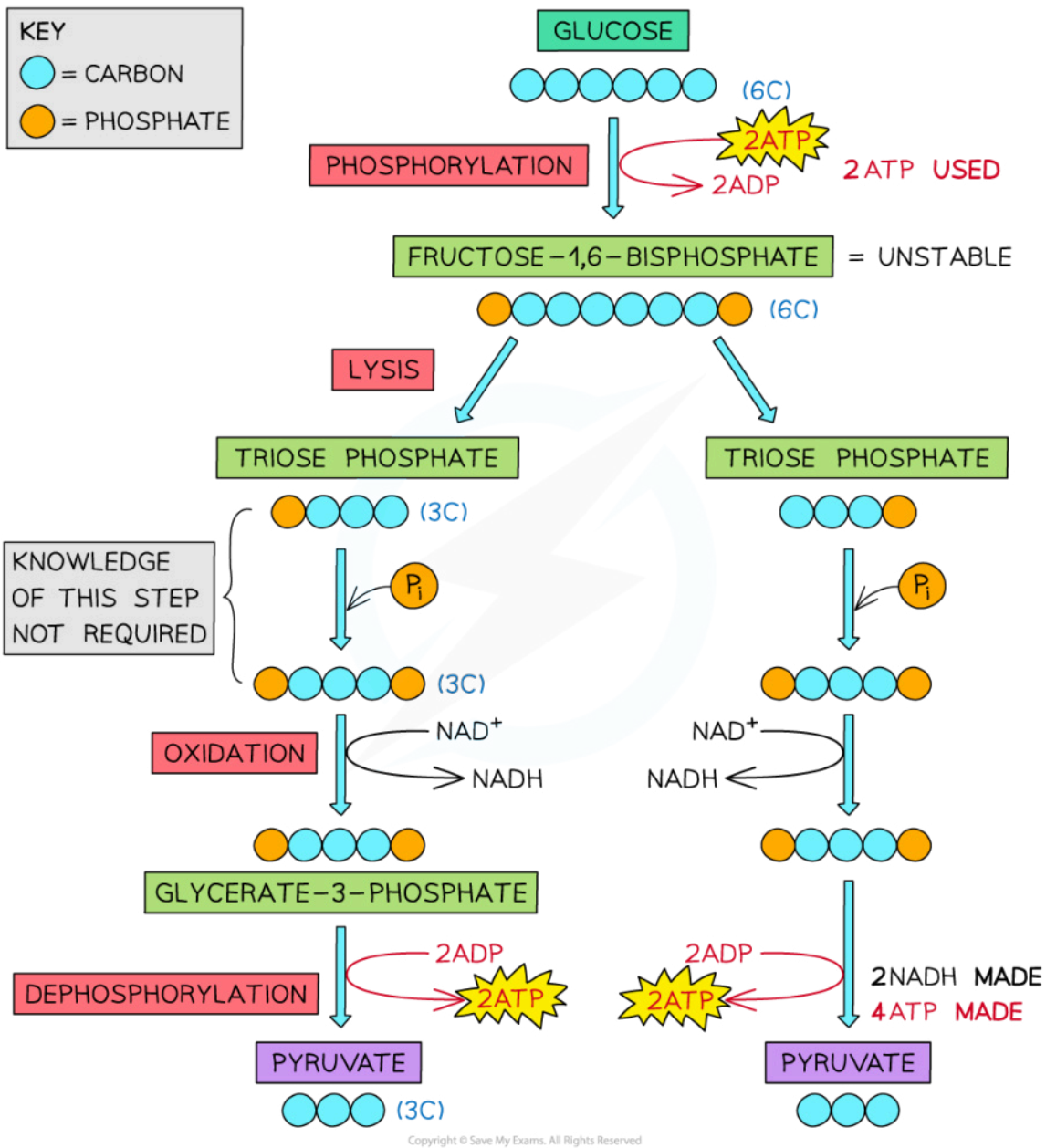
### Steps of glycolysis

- **Phosphorylation:** glucose (6C) is activated by phosphorylation from two ATP to form fructose-1,6-bisphosphate (6C)
  - This makes the 6C molecule less stable and therefore more **reactive**
$$\text{Glucose} + 2\text{ATP} \rightarrow \text{Fructose-1,6-bisphosphate}$$
- **Lysis**
  - Fructose-1,6-bisphosphate (6C) splits into two molecules of triose phosphate (3C)
$$\text{Fructose-1,6-bisphosphate} \rightarrow 2 \text{ Triose phosphate}$$
- **Oxidation:**
  - Hydrogen is removed from each molecule of triose phosphate by dehydrogenase enzyme and transferred to coenzyme NAD to form two reduced NAD
  - Triose phosphate is oxidised to form another 3C molecule glycerate-3-phosphate
$$2 \text{ Triose phosphate} \rightarrow 2 \text{ Glycerate-3-phosphate}$$

$$4\text{H} + 2\text{NAD} \rightarrow 2\text{NADH} + 2\text{H}^+$$
- **Dephosphorylation**
  - Phosphates are transferred from the intermediate substrate molecules to form four ATP through **substrate-linked phosphorylation**
$$4\text{P}_i + 4\text{ADP} \rightarrow 4\text{ATP}$$
- **Pyruvate is produced**
  - The end product of glycolysis which can be used in the next stage of respiration
$$2 \text{ Glycerate-3-phosphate} \rightarrow 2 \text{ Pyruvate}$$



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**Glycolysis, the formation of two pyruvate molecules from one glucose sugar molecule**

### Examiner Tip

It may seem strange that ATP is used and also produced during glycolysis. At the start ATP is used to **make glucose more reactive** (it is usually very stable) and to **lower the activation energy** of the reaction.

You may see 4H (four hydrogens) also written as  $2\text{H}^+ + 2\text{e}^-$

You do not need to know all the **intermediate** compounds of glycolysis apart from TP and G3P. The starting reactant, fructose-1,6-bisphosphate, and the product, pyruvate, in this chain reaction you do need to be able to name also.



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## 8.2.4 The Link Reaction & The Krebs Cycle

### Link Reaction

#### Entering the link reaction

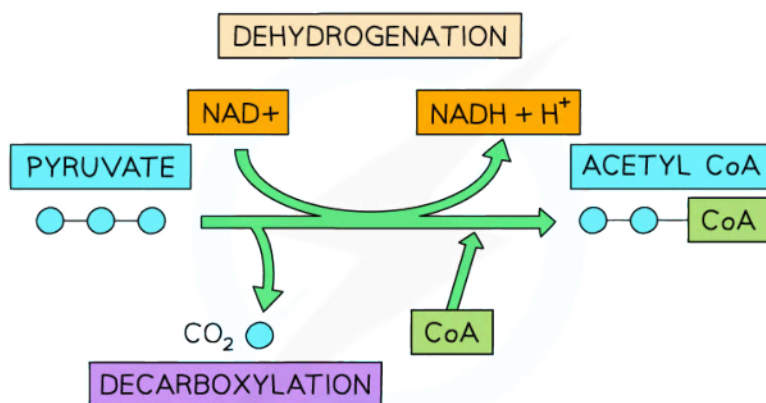
- The end product of glycolysis is **pyruvate** (3C)
- Pyruvate contains a substantial amount of chemical energy that can be further utilised in respiration to produce more ATP
- When **oxygen is available** pyruvate will **enter the mitochondrial matrix** and **aerobic** respiration will continue
- Once in the matrix pyruvate takes part in the link reaction

#### The link reaction

- The link reaction takes place in the matrix of the mitochondria
- It is referred to as the link reaction because it **links glycolysis** to the **Krebs cycle**
- The steps are:
  - **Oxidative decarboxylation** reaction in which:
    - **Carbon dioxide is removed** to produce a 2C molecule
    - **This 2C molecule is then oxidised** (loss of hydrogen and 2 high energy electrons) to produce an **acetyl compound** and thereby reducing NAD to NADH
  - **Combination of the acetyl compound with coenzyme A** to form acetyl coenzyme A (acetyl CoA)
- It produces:
  - Acetyl CoA
  - Carbon dioxide (CO<sub>2</sub>)
  - Reduced NAD (NADH)



- **Acetyl coenzyme A is supplied to the Krebs cycle** where aerobic respiration continues



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*The link reaction occurs in the mitochondrial matrix. It dehydrogenates and decarboxylates the three-carbon pyruvate to produce the two-carbon acetyl CoA that can enter the Krebs Cycle.*



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### Examiner Tip

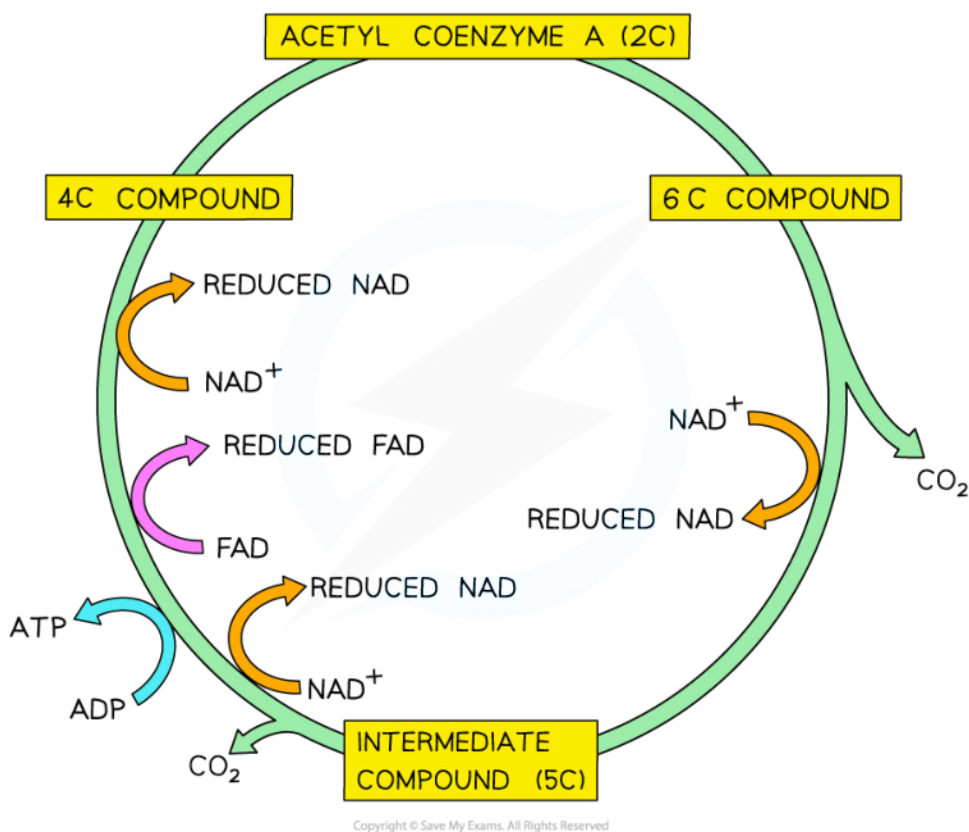
Remember that there are two pyruvate molecules produced per glucose molecule so you need to **multiply everything by 2** when thinking about what happens to a single glucose molecule in aerobic respiration.



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## Krebs Cycle

- The Krebs cycle (sometimes called the citric acid cycle) consists of a **series of enzyme-controlled reactions**
- The **Krebs cycle** takes place in the matrix of the mitochondria
- **Two carbon (2C) Acetyl CoA** enters the circular pathway from the **link reaction**
- A four carbon compound (4C) accepts the 2C acetyl fragment from acetyl CoA to form a six carbon compound (6C)
  - Coenzyme A is released in this reaction to be reused in the link reaction
- The **6C compound is then converted back to the 4C compound** through a series of **oxidation-reduction (redox) reactions**



*The Krebs Cycle uses acetyl CoA from the link reaction to produce reduced carbon dioxide, reduced NAD, reduced FAD and ATP*

### The reactions involved in the Krebs cycle

- The 4C compound is regenerated in the Krebs cycle through a series of **redox reactions**
- **Decarboxylation** of the 6C compound
  - Releasing **two  $\text{CO}_2$**  as waste gas
- **Oxidation** (dehydrogenation) of the 6C compound releases hydrogen atoms

- **Reduction** of coenzymes NAD and FAD (by the released H atoms)
  - $3 \text{NAD}^+ + 1 \text{FAD} \rightarrow 3\text{NADH} + \text{H}^+ + 1 \text{FADH}_2$
- **Substrate-level phosphorylation**
  - A phosphate is transferred from one of the intermediates to ADP, forming one **ATP**
- As the link reaction produces two molecules of acetyl CoA (one per each pyruvate), the **Krebs cycle occurs twice**
- **Per glucose molecule**, the Krebs cycle produces:
  - 4  $\text{CO}_2$
  - 2 ATP
  - 6 NADH +  $\text{H}^+$  (reduced NAD)
  - 2  $\text{FADH}_2$  (reduced FAD)

### **Examiner Tip**

The Krebs cycle is often referred to as cyclical or circular. This is because the 4C acceptor molecule is **regenerated** throughout the reaction so that it can start all over again by adding another acetyl CoA.

The names of the intermediate molecules in the Krebs cycle and the link reaction are not required.



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## 8.2.5 Oxidative Phosphorylation

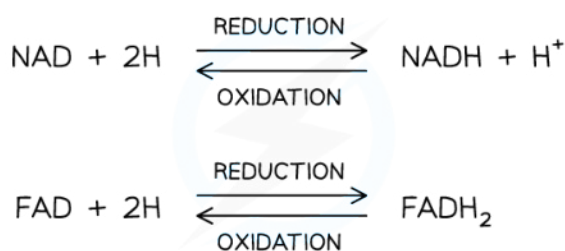
### NAD & FAD

#### Summary of oxidative phosphorylation

- Oxidative phosphorylation is the **last stage of aerobic respiration**
- It takes place at the **inner mitochondrial membrane**
- This is the most efficient producer of ATP in the process of aerobic respiration
- It also is the stage that produces water from oxygen
- Oxidative phosphorylation is comprised of the **electron transport chain** and **chemiosmosis**
  - During the electron transport chain, electrons are passed along carrier molecules forming an electrochemical gradient
  - Chemiosmosis describes the formation of ATP using this gradient
- **Coenzymes NAD<sup>+</sup> and FAD** play a critical role in oxidative phosphorylation by **transferring electrons** (from hydrogen) from the previous stages of aerobic respiration through a series of carrier molecules

#### NAD and FAD

- Coenzymes NAD and FAD play a critical role in aerobic respiration by transferring hydrogen through different stages of respiration
- When hydrogen atoms become available at different points during respiration NAD and FAD accept these hydrogen atoms
  - A hydrogen atom consists of a **proton** (hydrogen ion/H<sup>+</sup>) and an **electron** (e<sup>-</sup>)
- When the coenzymes gain a hydrogen they are '**reduced**'
- They **transfer the hydrogen atoms (protons and electrons)** from the different stages of respiration to the **electron transport chain** on the inner mitochondrial membrane, called the cristae (the site where hydrogens are removed from the coenzymes)
- When the hydrogen atoms are removed the coenzymes are '**oxidised**'



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#### *The reduction and oxidation of NAD and FAD*

#### Sources of reduced NAD & FAD

- A certain amount of reduced NAD and FAD is produced during the aerobic respiration of a single glucose molecule
- **Reduced NAD:**



- $2 \times 1 = 2$  from Glycolysis
- $2 \times 1 = 2$  from the Link Reaction
- $2 \times 3 = 6$  from the Krebs cycle
- **Reduced FAD:**
  - $2 \times 1 = 2$  from the Krebs cycle

### Examiner Tip

Note at all stages there is a doubling ( $2\times$ ) of reduced NAD and FAD. This is because one glucose molecule is split in two in glycolysis and so these **reactions occur twice per single molecule of glucose**.

Remember NAD can also be written as **NAD<sup>+</sup>**



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## The Electron Transport Chain in Respiration

### The electron transport chain

- The electron transport chain is made up of a **series of redox reactions** that occur via membrane proteins (also known as electron carriers) embedded into the inner mitochondrial membrane
- The chain is used to transport electrons and move protons across the membrane
  - Electron carriers are positioned close together which allows the **electrons to pass from carrier to carrier**
  - The cristae of the mitochondria are **impermeable to protons** so the electron carriers are needed to pump them across the membrane to establish a **proton (or electrochemical) concentration gradient** that can be used to power oxidative phosphorylation
- All of the electrons that enter the transport chain come from **reduced NAD** and **reduced FAD** molecules produced during the earlier stages of cellular respiration

### The importance of protons and electrons

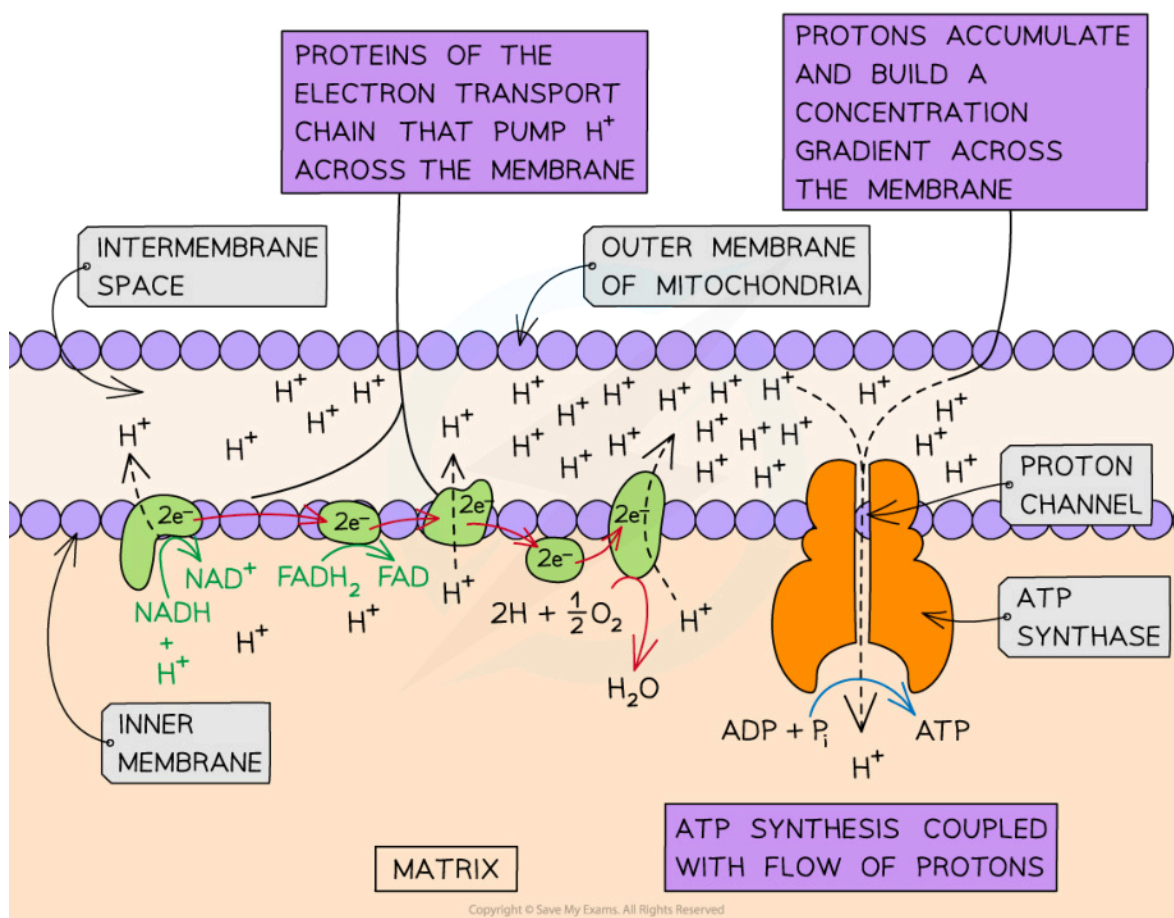
- **Protons** and **electrons** are important in the electron transport chain as they play a role in the synthesis of ATP
  - **Electrons** are **given to the electron transport chain** (from reduced NAD and reduced FAD)
  - **Protons** (from reduced NAD and reduced FAD) are **released when the electrons are lost**
  - The electron transport chain drives the movement of these protons across the cristae into the intermembrane space, **creating a proton gradient** (more hydrogen ions in the matrix)
  - Returning the protons down the gradient, back into the mitochondrial matrix, **gives the energy required for ATP synthesis**



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## Chemiosmosis in Respiration

- Movement of electrons through the electron transport chain causes a **proton or electrochemical gradient**
  - Positively charged protons accumulate in the **intermembrane space**
  - The movement of protons back into the matrix is then used to power ATP synthesis
- Protons that have built up in the intermembrane space can only pass through the phospholipid bilayer by facilitated diffusion through a membrane-embedded protein called **ATP synthase**
- ATP synthase acts a lot like a water wheel; it is turned by the flow of the protons moving through it, down their electrochemical gradient.
- As ATP synthase turns, it catalyses phosphorylation of ADP, generating ATP
- This process, in which energy from a proton gradient is used to make ATP, is called **chemiosmosis**.



*Oxidative Phosphorylation, involving the electron transport chain and chemiosmosis, generates a large amount of ATP*

**NOS: Paradigm shift; the chemiosmotic theory led to a paradigm shift in the field of bioenergetics**

- A **paradigm shift** is a fundamental change in an approach or already existing assumption
- Some examples of paradigm shifts that have happened in the course of scientific history are:
  - **CO<sub>2</sub> emissions** - Emissions of CO<sub>2</sub> did not register as a contributing factor to climate change until late into the 20th century
  - **Evolution** - Charles Darwin's theory of natural selection was a paradigm shift away from the traditional and religious views people held about the development of life on Earth
  - **Parasites** - Scientists once believed that illnesses were caused by "bad air" called miasma; it wasn't until the 19th century that a paradigm shift in this thinking happened
- The **chemiosmotic theory** was proposed by Nobel Prize winner Peter Mitchell in 1961
- His idea was a paradigm shift away from the **existing theory** that the energy for electron transfer was stored as a stable chemical intermediate
- Mitchell's **chemiosmotic hypothesis** started a revolution which has led to this paradigm shift in the field of bioenergetics
- The idea was rejected at the time for being too novel and radical
- His theory has shaped understanding of the fundamental mechanisms of:
  - Biological energy conservation
  - Ion and metabolite transport
  - Bacterial motility
  - Organelle structure and biosynthesis
  - Membrane structure and function
  - Homeostasis
  - The evolution of the eukaryote cell



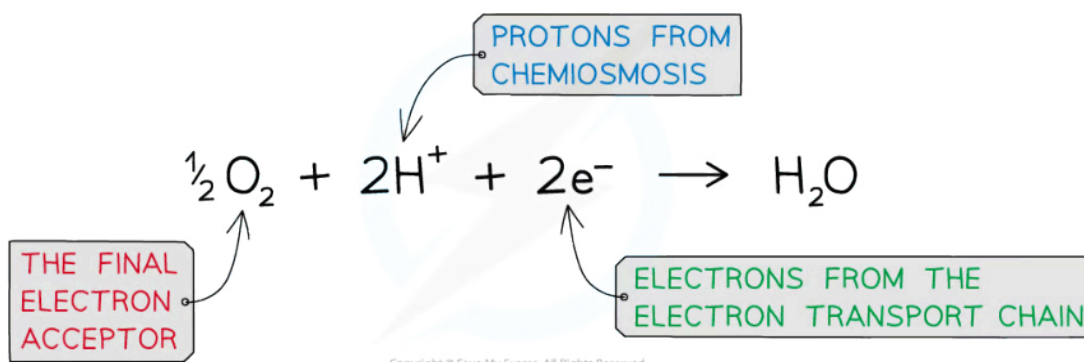
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## Oxygen as the Final Electron Acceptor

- The final link in the electron transport chain is **oxygen** and is referred to as the final or **terminal electron acceptor**
  - This is the **last acceptor of the electrons**
  - Oxygen is reduced by the **electrons**, forming **water**
- If oxygen is **not present** to accept electrons:
  - Reduced NAD** and **reduced FAD** will not be oxidised to regenerate NAD<sup>+</sup> and FAD, so there will be no further hydrogen transport
  - The **electron transport chain will stop**, and ATP will no longer be produced by chemiosmosis
  - Without enough **ATP**, cells can't **carry out the reactions** they need to function
- The electron transport chain is hugely efficient at generating energy in the cell but relies on an abundance of oxygen



### Examiner Tip

Examiners often ask why oxygen is so important for aerobic respiration, so remember the following:

- Oxygen acts as the final electron acceptor.
- Without oxygen, the electron transport chain cannot continue as the electrons have nowhere to go.
- Without oxygen accepting the electrons (and hydrogens) the reduced coenzymes NADH and FADH<sub>2</sub> cannot be oxidised to regenerate NAD and FAD, so they can't be used in further hydrogen transport.

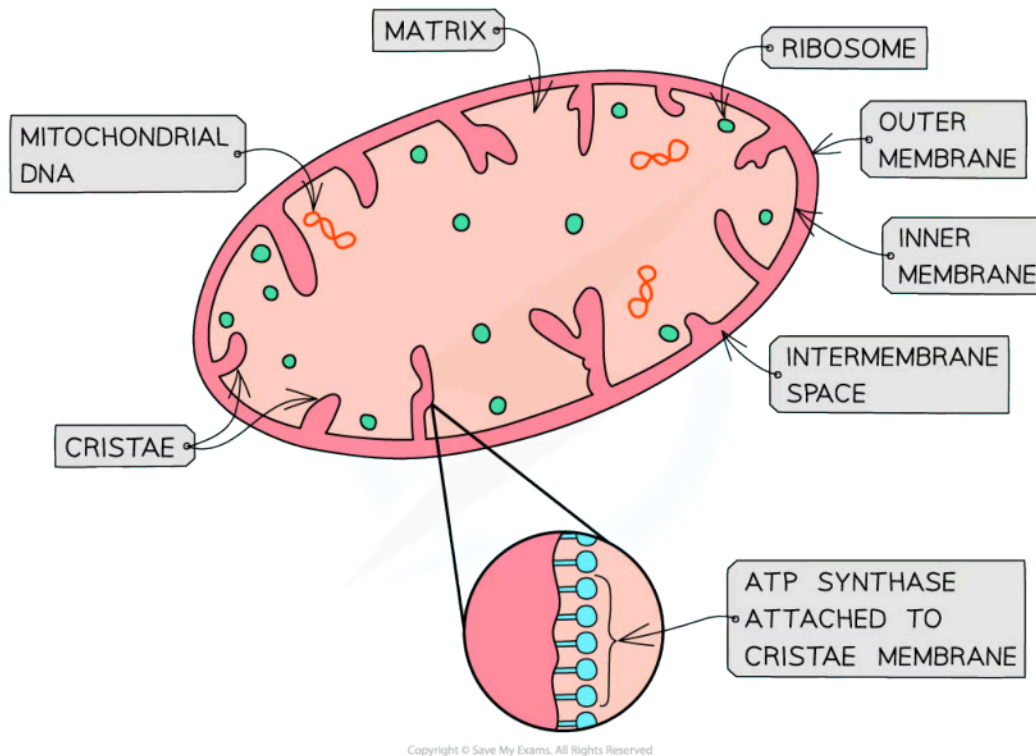


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## 8.2.6 Mitochondria

### Structure & Function of the Mitochondrion

- Mitochondria are rod-shaped organelles 0.5 – 1.0  $\mu\text{m}$  in diameter
- They are the site of **aerobic respiration** in eukaryotic cells
- The function of mitochondria is to **synthesize ATP**
- Synthesis of ATP in the mitochondria occurs during the last stage of respiration called oxidative phosphorylation
  - This relies on membrane proteins that make up the 'electron transport chain' and the ATP synthase enzyme – the details of this are covered later in the notes



*The structure of a mitochondrion*

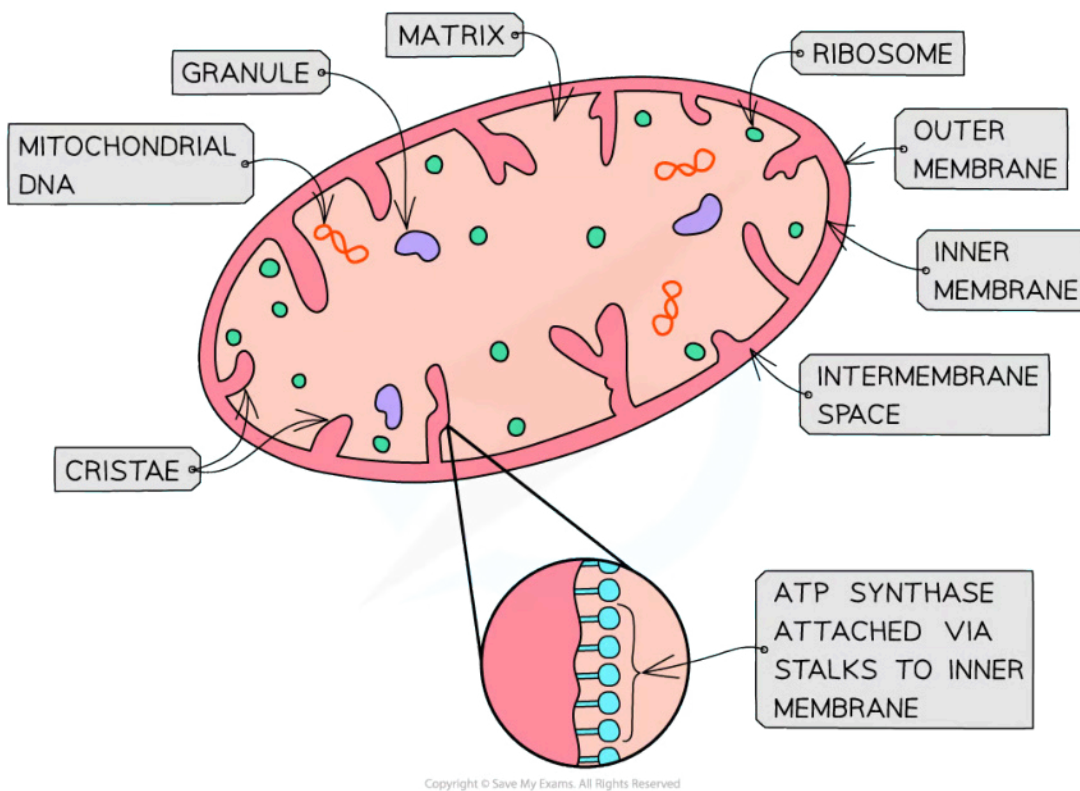
#### Structure

- Mitochondria have **two phospholipid membranes**
- The outer membrane is:
  - Smooth
  - **Permeable** to several small molecules
- The inner membrane is:
  - **Folded** (cristae)
  - **Less permeable**



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- The site of the **electron transport chain** (used in oxidative phosphorylation)
- Location of **ATP synthase** (used in oxidative phosphorylation)
- The intermembrane space:
  - Has a low pH due to the **high concentration of protons**
  - The concentration gradient across the inner membrane is formed during **oxidative phosphorylation** and is **essential for ATP synthesis**
- The matrix:
  - Is an aqueous solution **within the inner membranes** of the mitochondrion
  - Contains ribosomes, enzymes and circular mitochondrial DNA necessary for mitochondria to function



*The structure of a mitochondrion*

### Relationship between structure & function

- The structure of mitochondria makes them well adapted to their function
  - They have a **large surface area** due to the presence of **cristae** (inner folds) which enables the membrane to hold many electron transport chain proteins and ATP synthase enzymes
  - More active cell types can have larger mitochondria with longer and more tightly packed cristae to enable the **synthesis of more ATP** because they have a **larger surface area**
  - The **number** of mitochondria in each cell can vary depending on cell activity
    - Muscle cells are more active and have more mitochondria per cell than fat cells

### Examiner Tip

Exam questions can sometimes ask you to explain how the structure of a mitochondrion helps it carry out its function effectively. Make sure to follow through with your answer.

It is not enough to say that cristae increase the surface area of the inner membrane. You need to explain that an **increased surface area** of the inner membrane means there are **more electron transport chain** carriers and ATP synthase enzymes which results in **more ATP** being produced.

Be prepared to identify the different structures and locations in a mitochondrion from an electron micrograph.



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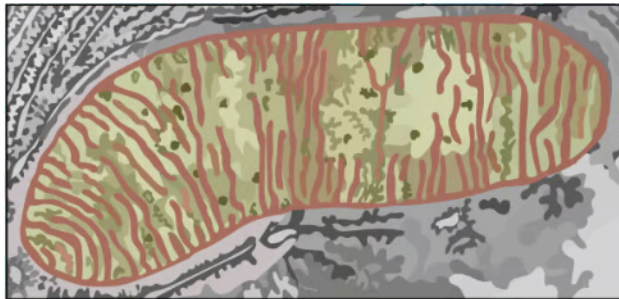


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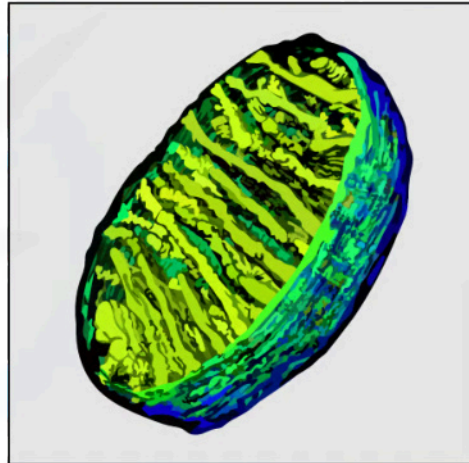
## Electron Micrographs of Mitochondria

- **Electron tomography (ET)** is a novel approach able to provide **three-dimensional (3D)** information on cells and tissues at molecular level
- The technique is an **extension of transmission electron microscopy**

ELECTRON MICROGRAPH



ELECTRON TOMOGRAPHY



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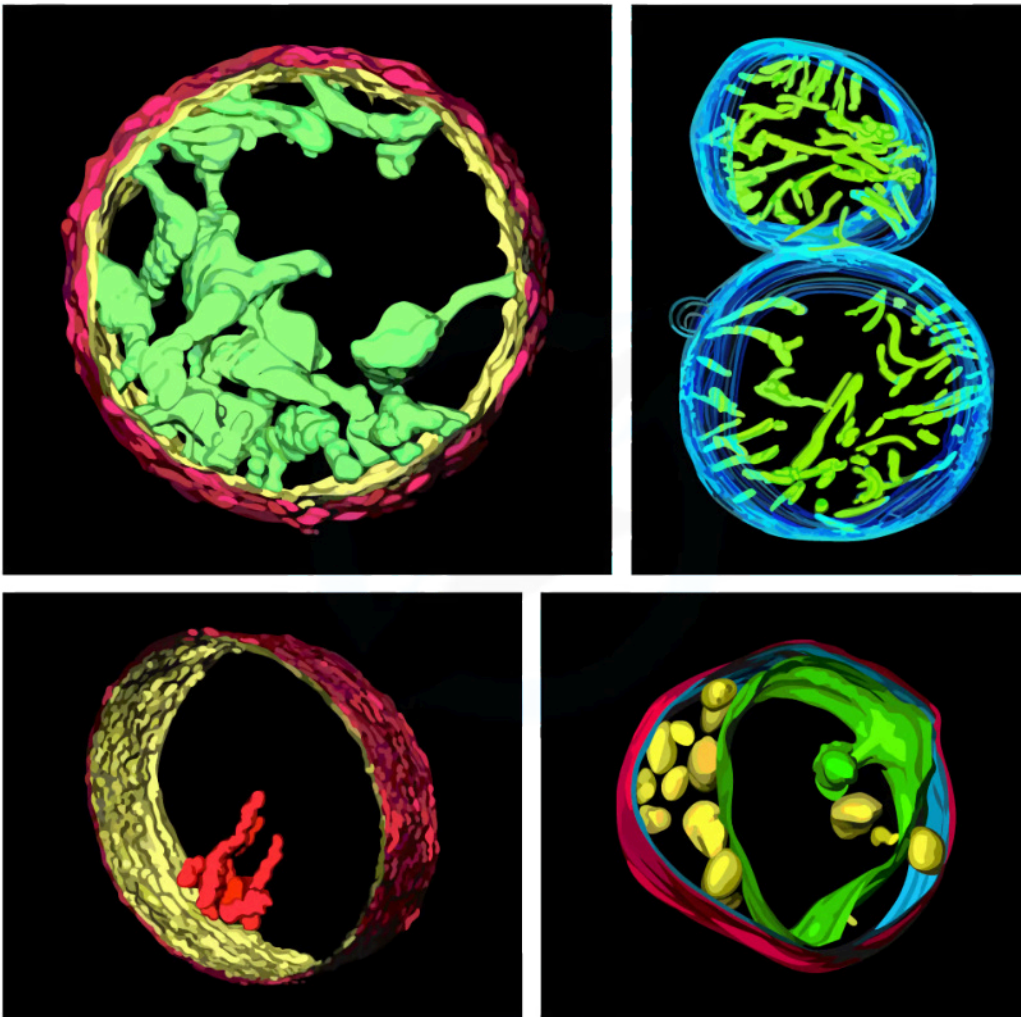
### *Transmission electron micrograph compared with electron tomography*

- **ET** has led to new discoveries about the mitochondria, including
  - The cristae are now known to be **dynamic in nature**, rather than static
  - The cristae have been seen to respond **physically and biochemically** to a changing environment with the mitochondria





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**Representation of EM images showing the dynamic nature of mitochondrial cristae**

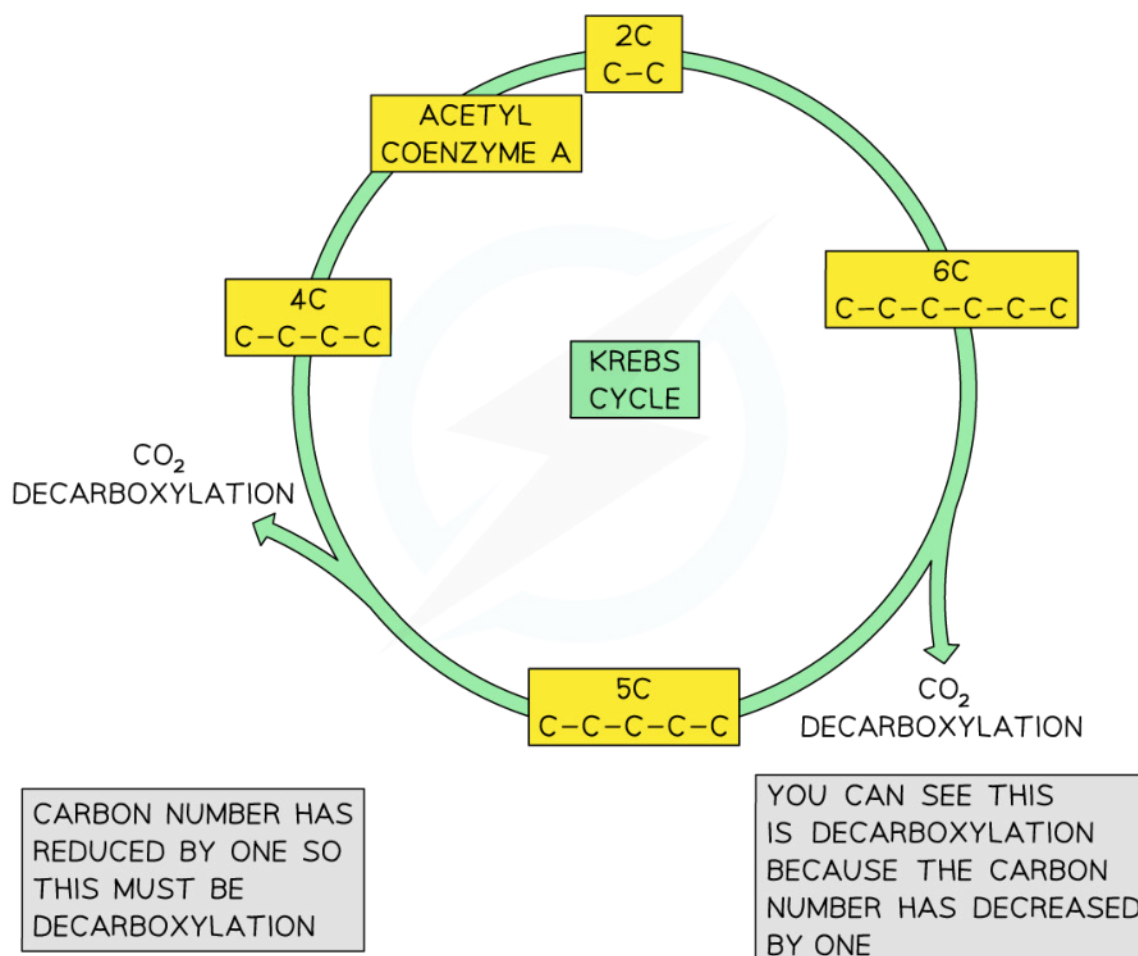


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## 8.2.7 Skills: Cell Respiration

### Identifying Decarboxylation & Oxidation in Diagrams

- The stages of respiration can be shown and summarised by different reactions which are shown in diagrams involving chemical molecules
- The diagrams can be used to deduce where oxidation and decarboxylation occur
  - Decarboxylation** is the removal of a carbon from a reaction, that releases carbon dioxide
  - Oxidation** is either the gain of oxygen, or the loss of electrons or hydrogens

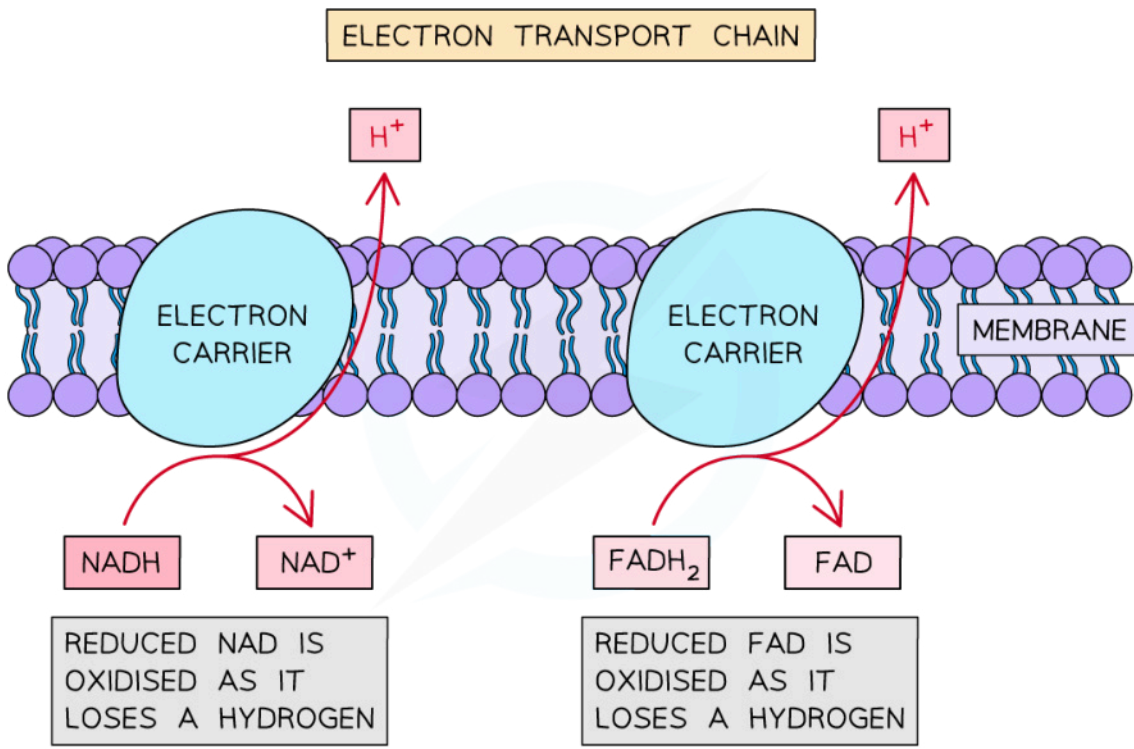


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#### Identifying decarboxylation in aerobic respiration



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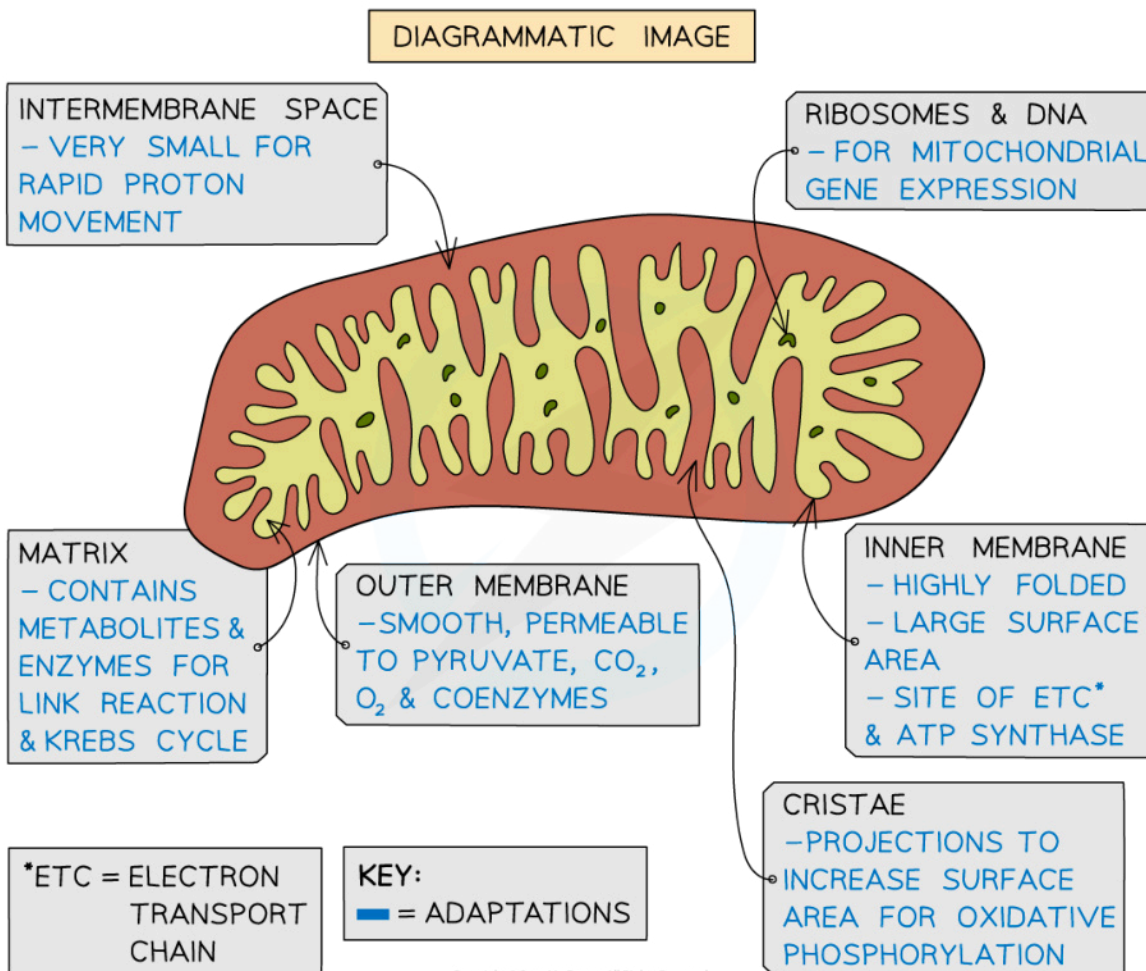
**Identifying oxidation in the electron transport chain**



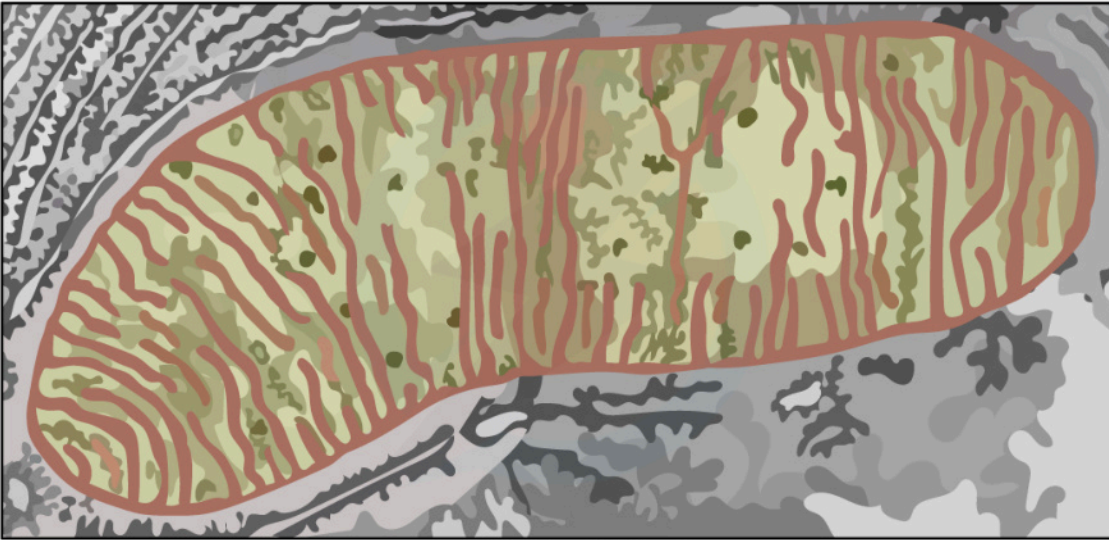
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## Annotating the Adaptations of Mitochondria

- The structure of a mitochondrion can be investigated by **transmission electron microscopy**
- Electron micrographs of a mitochondrion may differ in appearance depending on where the cross-section occurs
- The clarity of electron micrographs make it possible to produce a line drawing to show the mitochondrion's double membrane around the matrix, and the way the inner membrane folds to form cristae
- Typically, mitochondrial drawings should show the following features:
  - Usually **sausage-shaped** in appearance
  - **Inner membrane** contains many internal protrusions (**cristae**)
  - **Intermembrane space** is **very small**
  - **Ribosomes and mitochondrial DNA** are usually **not visible** at standard resolutions and magnifications



ELECTRON MICROGRAPH



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Your notes

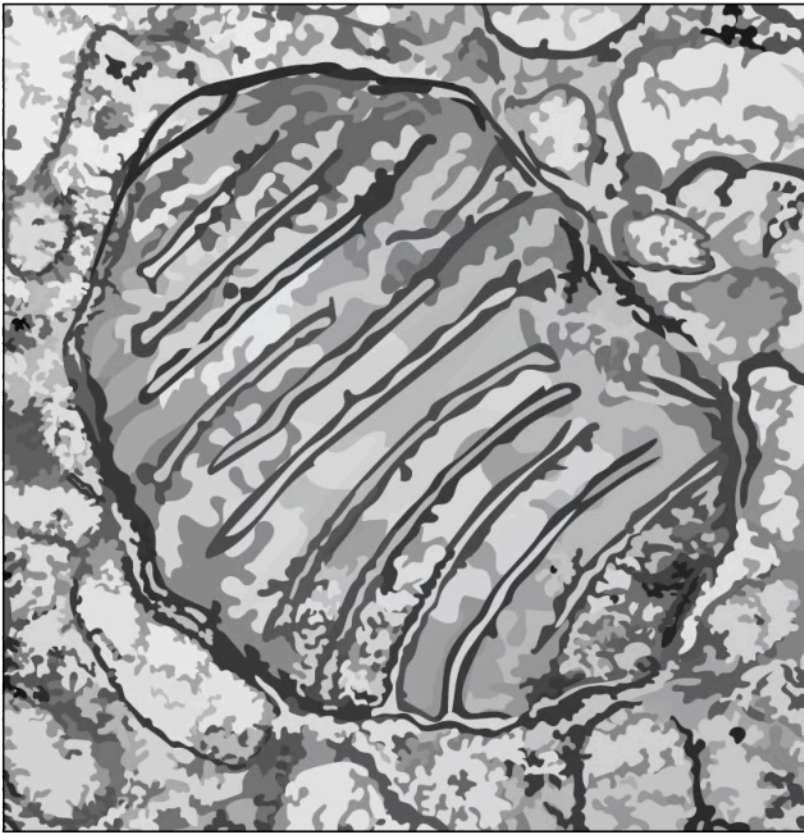
***Electron micrograph of a mitochondrion, with an interpretive drawing of the adaptations annotated***



 **Worked example**

Label the following structures on the electron micrograph of the mitochondria:

- Outer membrane
- Inner membrane
- Intermembrane space
- Cristae
- Matrix
- Ribosome



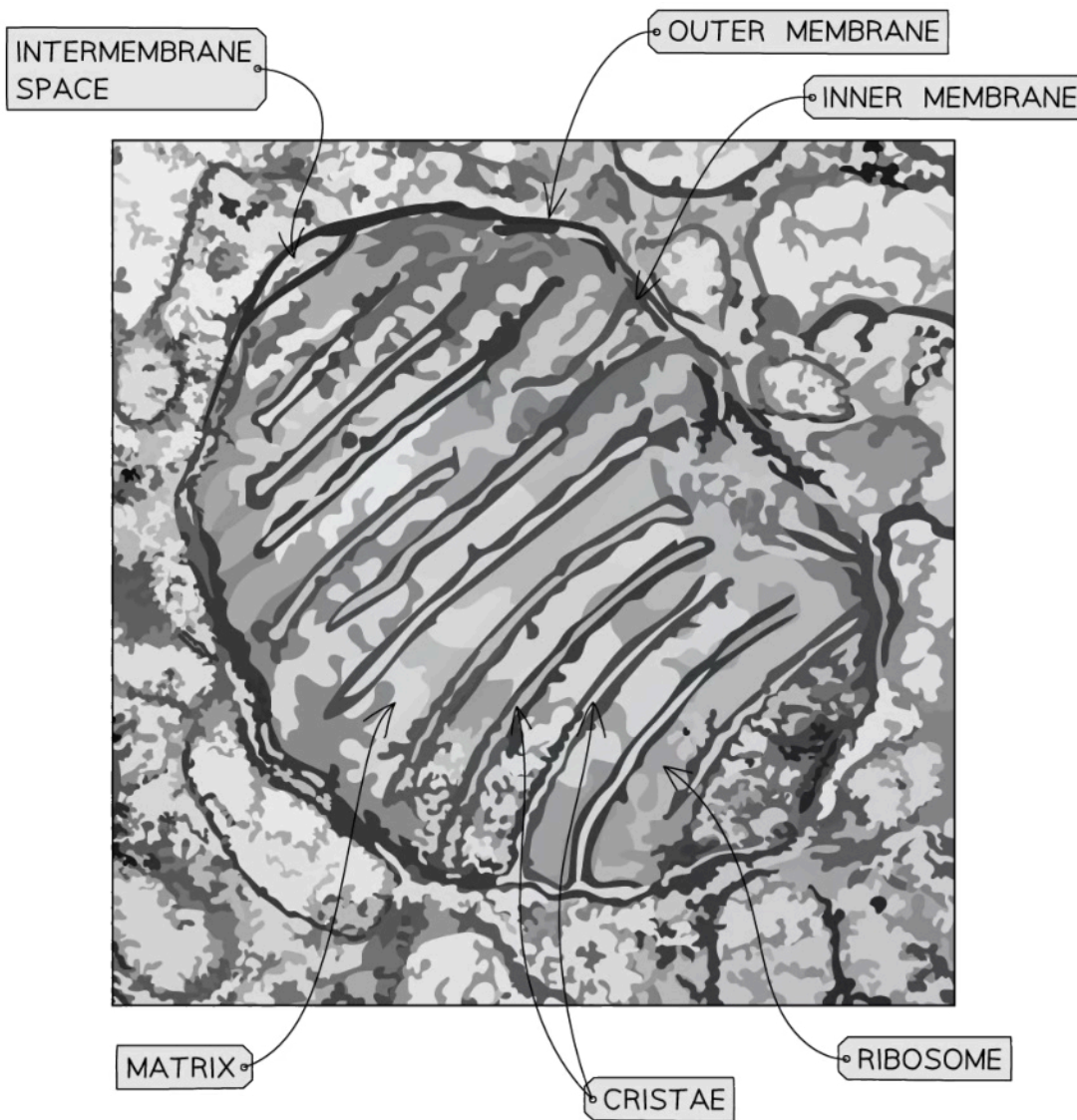
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