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**Unit - III**

# RESPIRATION

Respiration is a chain of chemical reactions that enables all living entities to synthesize energy required to sustain. It is a biochemical process wherein air moves between the external environment and the tissues and cells of the species. As an entity acquires energy through oxidising nutrients and hence liberating wastes, it is referred to as a metabolic process.

## The Process of Respiration in Plants

During respiration, in different plant parts, significantly less exchange of gas takes place. They possess stomata and lenticels actively involved in the gaseous exchange. Leaves, stems and plant roots respire at a low pace

### Respiratory quotient (RQ)

- The respiratory quotient (**RQ**) is the ratio of CO<sub>2</sub> produced to O<sub>2</sub> consumed while food is being metabolized:
- $RQ = \frac{CO_2 \text{ eliminated}}{O_2 \text{ consumed}}$

## Respiration types

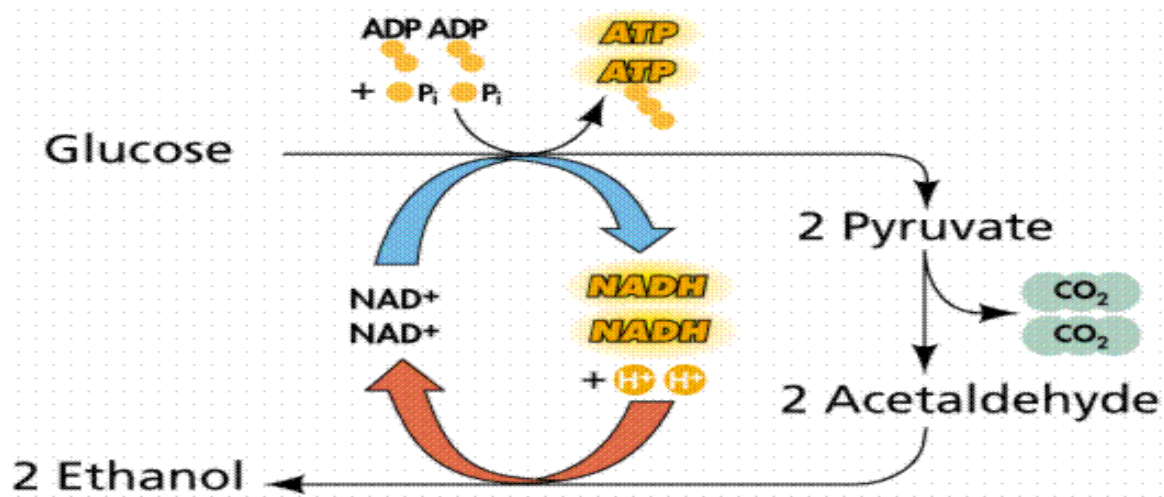
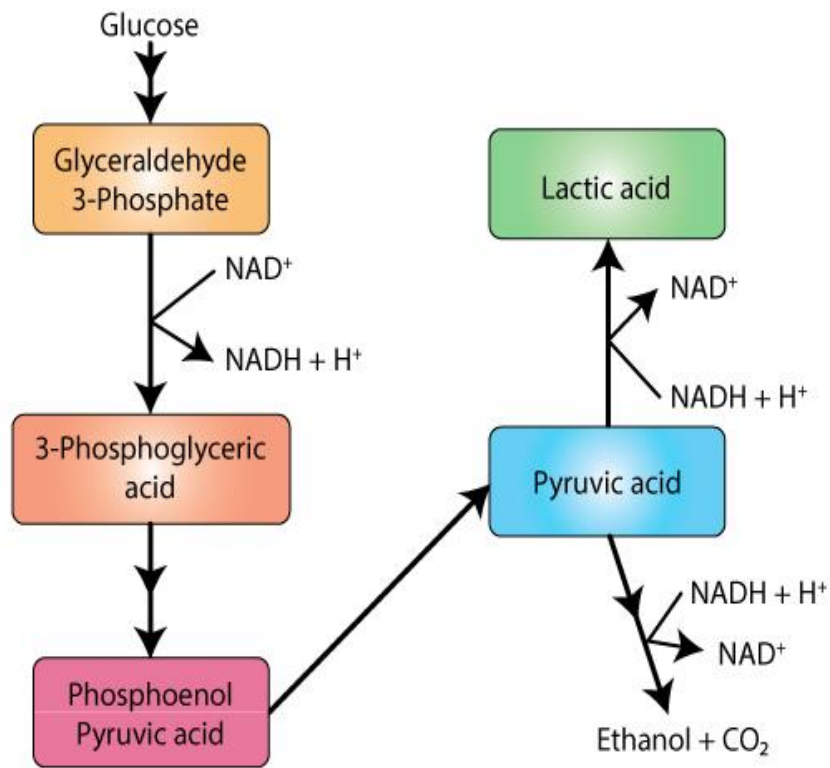
1. **Aerobic respiration, and**
2. **Anaerobic respiration.**

**Aerobic Respiration:** It is the process of **cellular respiration** that takes place in the presence of oxygen gas to produce energy from food. Cellular respiration is a biochemical process by which nutrients are broken down to release energy, which gets stored in the form of ATP and waste products are released. Cellular respiration is a four-stage process. In the process, glucose is oxidised to carbon dioxide and oxygen is reduced to water. The energy released in the process is stored in the form of ATPs. 36 to 38 ATPs are formed from each glucose molecule.

### Anaerobic Respiration

One of the best examples of anaerobic respiration is the process of fermentation in yeast.

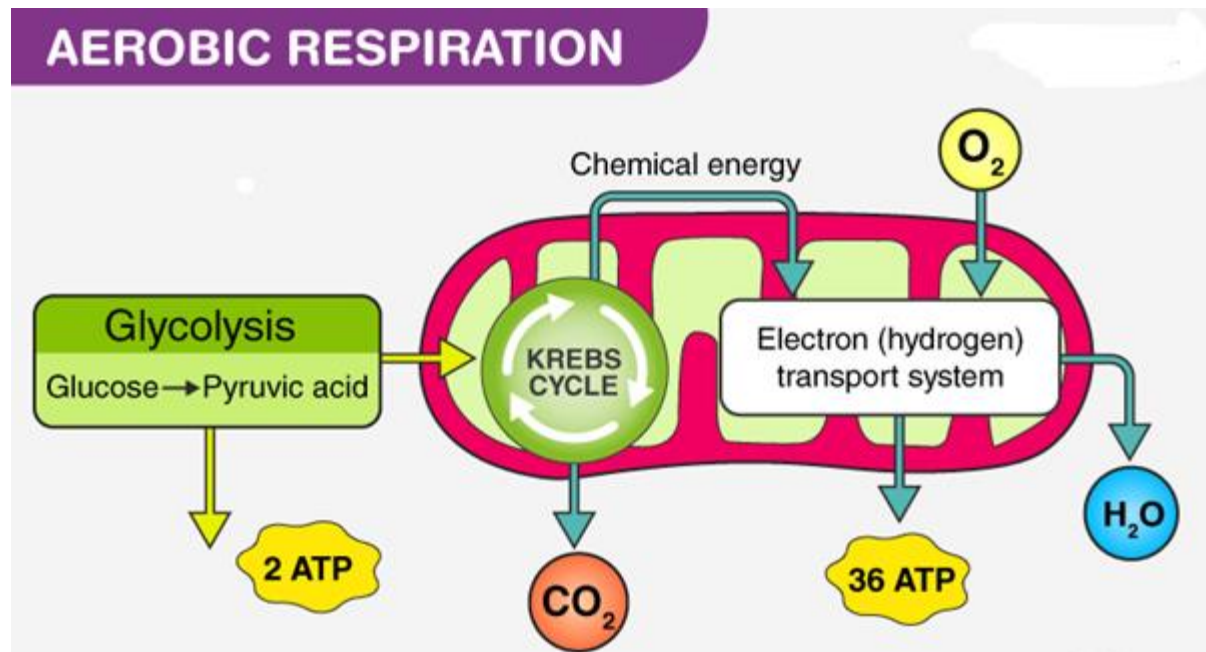
In this process, incomplete oxidation of glucose is obtained under anaerobic conditions by a set of reactions resulting in the conversion of carbon dioxide to ethanol, reactions catalyzed by enzymes – alcohol dehydrogenase, pyruvic acid decarboxylase. The various steps involved in fermentation are as follows:



In alcohol fermentation not much energy is released; less than seven per cent of the energy in glucose is released and not all of it is trapped as high energy bonds of ATP.

## Aerobic Respiration

Aerobic respiration is a biological process in which food glucose is converted into energy in the presence of oxygen. The chemical equation of aerobic respiration is as given below-



### Steps of Aerobic Respiration

The complete process of aerobic respiration occurs in four different stages:

#### Glycolysis

#### Formation of Acetyl Coenzyme A

#### Citric Acid Cycle

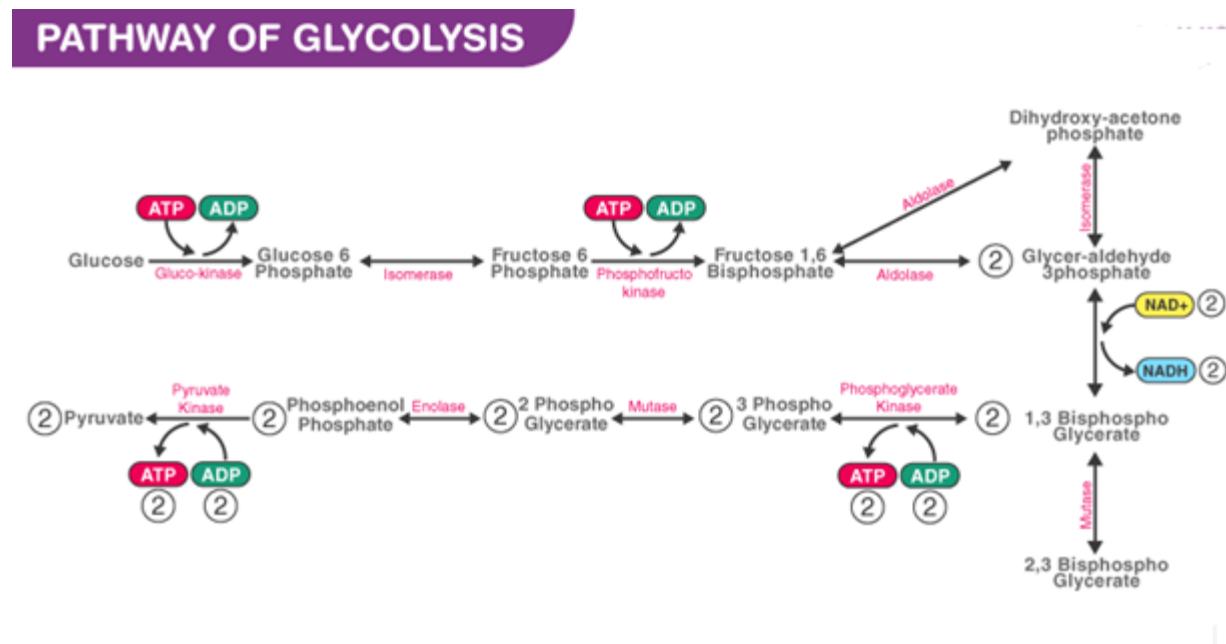
#### Electron Transport Chain

### Glycolysis

Glycolysis is the process in which glucose is broken down to produce energy. It produces two molecules of pyruvate, ATP, NADH and water. The process takes place in the cytosol of the cell cytoplasm, in the presence or absence of oxygen. Glycolysis is the primary step of cellular

respiration. This metabolic pathway was discovered by three German biochemists- Gustav Embden, Otto Meyerhof, and Jakub Karol Parnas in the early 19th century and is known as the EMP pathway (Embden–Meyerhof–Parnas).

The glycolysis pathway occurs in the following stages:



### Stage 1

- A phosphate group is added to glucose in the cell cytoplasm, by the action of enzyme hexokinase.
- In this, a phosphate group is transferred from ATP to glucose forming glucose,6-phosphate.

### Stage 2

Glucose-6-phosphate is isomerized into fructose,6-phosphate by the enzyme phosphoglucose mutase.

### Stage 3

The other ATP molecule transfers a phosphate group to fructose 6-phosphate and converts it into fructose 1,6-bisphosphate by the action of enzyme phosphofructokinase.

#### **Stage 4**

The enzyme aldolase converts fructose 1,6-bisphosphate into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, which are isomers of each other.

#### **Step 5**

Triose-phosphate isomerase converts dihydroxyacetone phosphate into glyceraldehyde 3-phosphate which is the substrate in the successive step of glycolysis.

#### **Step 6**

This step undergoes two reactions:

- The enzyme glyceraldehyde 3-phosphate dehydrogenase transfers 1 hydrogen molecule from glyceraldehyde phosphate to nicotinamide adenine dinucleotide to form NADH + H<sup>+</sup>.
- Glyceraldehyde 3-phosphate dehydrogenase adds a phosphate to the oxidized glyceraldehyde phosphate to form 1,3-bisphosphoglycerate.

#### **Step 7**

Phosphate is transferred from 1,3-bisphosphoglycerate to ADP to form ATP with the help of phosphoglycerokinase. Thus two molecules of phosphoglycerate and ATP are obtained at the end of this reaction.

#### **Step 8**

The phosphate of both the phosphoglycerate molecules is relocated from the third to the second carbon to yield two molecules of 2-phosphoglycerate by the enzyme phosphoglyceromutase.

#### **Step 9**

The enzyme enolase removes a water molecule from 2-phosphoglycerate to form phosphoenolpyruvate.

#### **Step 10**

A phosphate from phosphoenolpyruvate is transferred to ADP to form pyruvate and ATP by the action of pyruvate kinase. Two molecules of pyruvate and ATP are obtained as the end products.

## Formation of Acetyl-CoA

Formation of Acetyl-CoA through the transition reaction. The transition reaction connects **glycolysis to the citric acid (Krebs) cycle**. Through a process called oxidative decarboxylation, the transition reaction converts the two molecules of the 3-carbon **pyruvate** from glycolysis (and other pathways) into two molecules of the 2-carbon molecule **acetyl Coenzyme A** (acetyl-CoA) and 2 molecules of **carbon dioxide**. First, a carboxyl group of each pyruvate is removed as carbon dioxide and then the remaining acetyl group combines with coenzyme A (CoA) to form acetyl-CoA. As the two pyruvates undergo oxidative decarboxylation, two molecules of  $\text{NAD}^+$  become reduced to **2NADH + 2H<sup>+</sup>**. The 2NADH + 2H<sup>+</sup> carry protons and electrons to the electron transport chain to generate additional ATP by oxidative phosphorylation.

- The overall reaction for the transition reaction is:
- **2 pyruvate + 2 NAD<sup>+</sup> + 2 coenzyme A**
- **yields 2 acetyl-CoA + 2 NADH + 2 H<sup>+</sup> + 2 CO<sub>2</sub>**
- In **prokaryotic cells**, the transition step occurs in the **cytoplasm**; in **eukaryotic cells** the pyruvates must first enter the mitochondria because the transition reaction and the citric acid cycle take place in the **matrix of the mitochondria**.
- The two molecules of acetyl-CoA can now enter the citric acid cycle. Acetyl-CoA is also a **precursor metabolite** for fatty acid synthesis.

## Krebs Cycle

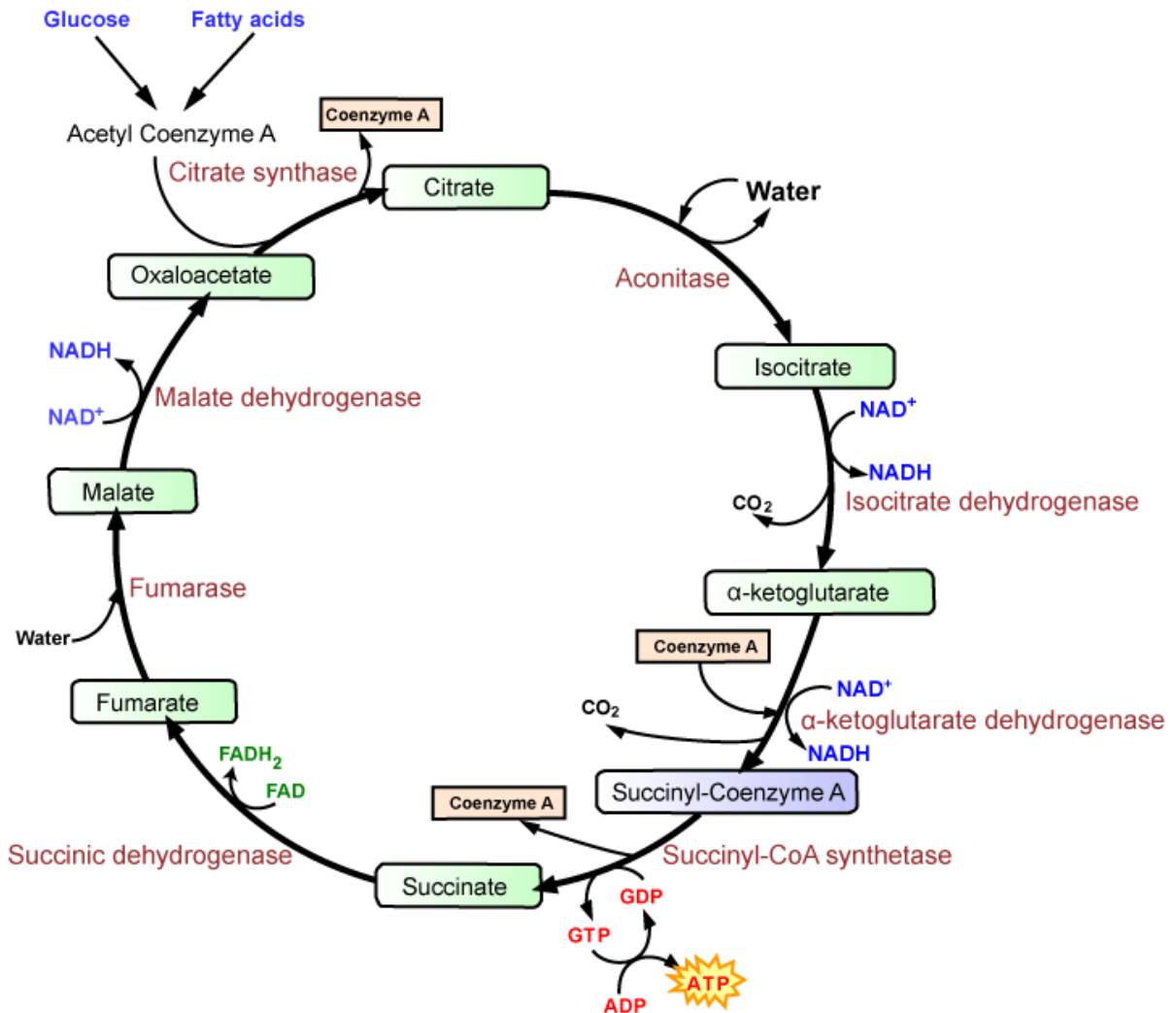
The Krebs cycle or Citric acid cycle is a series of enzyme catalysed reactions occurring in the mitochondrial matrix, where acetyl-CoA is oxidised to form carbon dioxide and coenzymes are reduced, which generate ATP in the electron transport chain.

Krebs cycle was named after Hans Krebs, who postulated the detailed cycle. He was awarded the Nobel prize in 1953 for his contribution.

It is a series of eight-step processes, where acetyl group of acetyl-CoA is oxidised to form two molecules of CO<sub>2</sub> and in the process, one ATP is produced. Reduced high energy compounds, NADH and FADH<sub>2</sub> are also produced.

Two molecules of acetyl-CoA are produced from each glucose molecule so two turns of the Krebs cycle are required which yields four CO<sub>2</sub>, six NADH, two FADH<sub>2</sub> and two ATPs.

# KREBS CYCLE (CITRIC ACID CYCLE )



## Krebs Cycle Steps

It is an eight-step process. Krebs cycle takes place in the matrix of mitochondria under aerobic condition.

**Step 1:** The first step is the condensation of acetyl CoA with 4-carbon compound oxaloacetate to form 6C citrate, coenzyme A is released. The reaction is catalysed by *citrate synthase*.

**Step 2:** Citrate is converted to its isomer, isocitrate. The enzyme *aconitase* catalyses this reaction.



**Step 3:** Isocitrate undergoes dehydrogenation and decarboxylation to form 5C  $\alpha$ -ketoglutarate. A molecular form of  $\text{CO}_2$  is released. *Isocitrate dehydrogenase* catalyses the reaction. It is an  $\text{NAD}^+$  dependent enzyme.  $\text{NAD}^+$  is converted to NADH.

**Step 4:**  $\alpha$ -ketoglutarate undergoes oxidative decarboxylation to form succinyl CoA, a 4C compound. The reaction is catalyzed by  *$\alpha$ -ketoglutarate dehydrogenase* enzyme complex. One molecule of  $\text{CO}_2$  is released and  $\text{NAD}^+$  is converted to NADH.

**Step 5:** Succinyl CoA forms succinate. The enzyme *succinyl CoA synthetase* catalyses the reaction. This is coupled with substrate-level phosphorylation of GDP to get GTP. GTP transfers its phosphate to ADP forming ATP.

**Step 6:** Succinate is oxidised by the enzyme *succinate dehydrogenase* to fumarate. In the process, FAD is converted to  $\text{FADH}_2$ .

**Step 7:** Fumarate gets converted to malate by addition of one  $\text{H}_2\text{O}$ . The enzyme catalysing this reaction is *fumarase*.

**Step 8:** Malate is dehydrogenated to form oxaloacetate, which combines with another molecule of acetyl CoA and starts the new cycle. Hydrogens removed, get transferred to  $\text{NAD}^+$  forming NADH. *Malate dehydrogenase* catalyses the reaction.

### **Products of the Citric Acid Cycle**

Two carbon atoms come into the citric acid cycle from each acetyl group, representing four out of the six carbons of one glucose molecule.

Two carbon dioxide molecules are released on each turn of the cycle; however, these do not necessarily contain the most recently-added carbon atoms.

The two acetyl carbon atoms will eventually be released on later turns of the cycle; thus, all six carbon atoms from the original glucose molecule are eventually incorporated into carbon dioxide.

Each turn of the cycle forms three NADH molecules and one  $\text{FADH}_2$  molecule. These carriers will connect with the last portion of aerobic respiration to produce ATP molecules.

One GTP or ATP is also made in each cycle. Several of the intermediate compounds in the citric acid cycle can be used in synthesizing non-essential amino acids; therefore, the cycle is amphibolic (both catabolic and anabolic).

# Electron Transport Chain

## Electron Transport Chain in Mitochondria

The final stage of aerobic respiration is the **electron transport chain**, which is located on the inner mitochondrial membrane

- The inner membrane is arranged into folds (cristae), which increases the surface area available for the transport chain

A complex could be defined as a structure that comprises a weak protein, molecule or atom that is weakly connected to a protein.

**Complex 1- NADH-Q oxidoreductase:** It comprises enzymes consisting of iron-sulfur and FMN. Here two electrons are carried out to the first complex aboard NADH. FMN is derived from vitamin B2.

**Q and Complex 2- Succinate-Q reductase:** FADH<sub>2</sub> that is not passed through complex 1 is received directly from complex 2. The first and the second complexes are connected to a third complex through compound ubiquinone (Q). The Q molecule is soluble in water and moves freely in the hydrophobic core of the membrane. In this phase, an electron is delivered directly to the electron protein chain. The number of ATP obtained at this stage is directly proportional to the number of protons that are pumped across the inner membrane of the mitochondria.

**Complex 3- Cytochrome c reductase:** The third complex is comprised of Fe-S protein, Cytochrome b, and Cytochrome c proteins. Cytochrome proteins consist of the heme group. Complex 3 is responsible for pumping protons across the membrane. It also passes electrons to the cytochrome c where it is transported to the 4th complex of enzymes and proteins. Here, Q is the electron donor and Cytochrome C is the electron acceptor.

**Complex 4- Cytochrome c oxidase:** The 4th complex is comprised of cytochrome c, a and a<sub>3</sub>. There are two heme groups where each of them is present in cytochromes c and a<sub>3</sub>. The cytochromes are responsible for holding oxygen molecule between copper and iron until the oxygen content is reduced completely. In this phase, the reduced oxygen picks two hydrogen ions from the surrounding environment to make water.

The electron transport chain releases the energy stored within the reduced hydrogen carriers in order to synthesis ATP

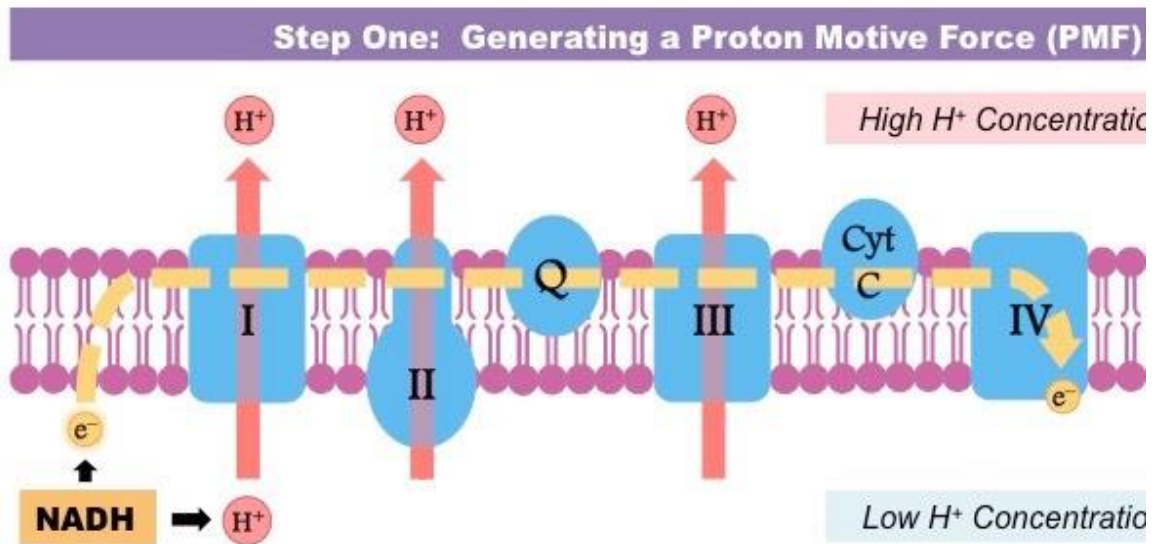
- This is called *oxidative phosphorylation*, as the energy to synthesis ATP is derived from the oxidation of hydrogen carriers

Oxidative phosphorylation occurs over a number of distinct steps:

- Proton pumps create an electrochemical gradient (proton motive force)
- ATP synthase uses the subsequent diffusion of protons (chemiosmosis) to synthesise ATP
- Oxygen accepts electrons and protons to form water

### Step 1: Generating a Proton Motive Force

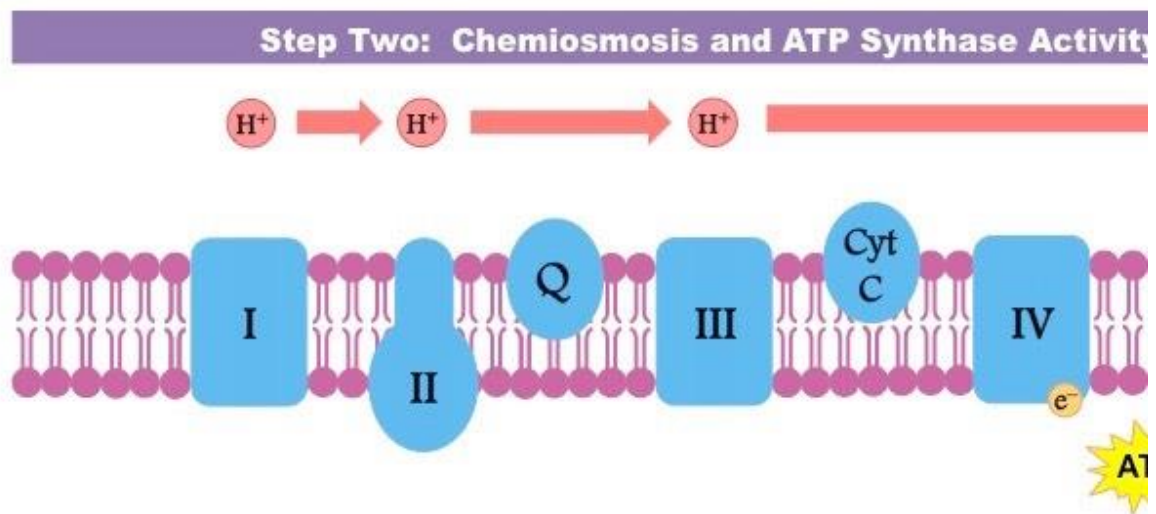
- The hydrogen carriers (NADH and FADH<sub>2</sub>) are oxidised and release high energy electrons and protons
- The electrons are transferred to the electron transport chain, which consists of several transmembrane carrier proteins
- As electrons pass through the chain, they lose energy – which is used by the chain to pump protons (H<sup>+</sup> ions) from the matrix
- The accumulation of H<sup>+</sup> ions within the intermembrane space creates an electrochemical gradient (or a proton motive force)



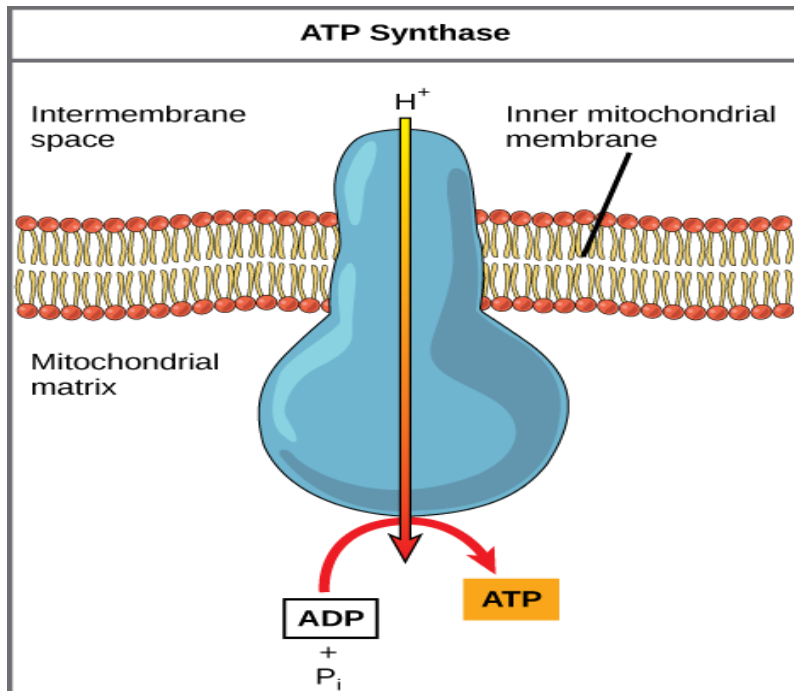
High energy electrons released by hydrogen carriers are shuttled through the electron transport chain. The released energy is used to translocate H<sup>+</sup> ions from the matrix, creating an electrochemical gradient.

## Step Two: ATP Synthesis via Chemiosmosis

- The proton motive force will cause  $H^+$  ions to move down their electrochemical gradient and diffuse back into matrix
- This diffusion of protons is called *chemiosmosis* and is facilitated by the transmembrane enzyme ATP synthase
- As the  $H^+$  ions move through ATP synthase they trigger the molecular rotation of the enzyme, synthesising ATP



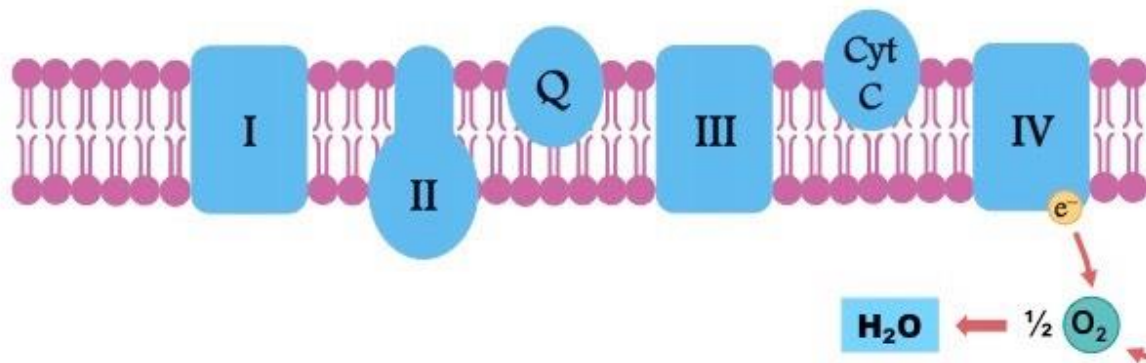
$H^+$  ions are transported down their electrochemical gradient by ATP synthase (ATP synthase uses this flow of protons back into the matrix to catalyse the synthesis of ATP).



### Step Three: Reduction of Oxygen

- In order for the electron transport chain to continue functioning, the de-energised electrons must be removed
- Oxygen acts as the final electron acceptor, removing the de-energised electrons to prevent the chain from becoming blocked
- Oxygen also binds with free protons in the matrix to form water – removing matrix protons maintains the hydrogen gradient
- In the absence of oxygen, hydrogen carriers cannot transfer energised electrons to the chain and ATP production is halted

### Step Three: Oxygen Acts as the Final Electron Acceptor



Oxygen acts as the final electron acceptor, removing the de-energised electrons. Oxygen also maintains the electrochemical gradient by binding to H<sup>+</sup> ions in the m

### Oxidative Phosphorylation

**Oxidative phosphorylation** or **electron transport-linked phosphorylation** or **terminal oxidation**) is the metabolic pathway in which cells use enzymes to oxidize nutrients, thereby releasing the chemical energy stored within in order to produce adenosine triphosphate (ATP). In most eukaryotes, this takes place inside mitochondria. Almost all aerobic organisms carry out oxidative phosphorylation. This pathway is so pervasive because it releases more energy than alternative fermentation processes such as anaerobic glycolysis.

During oxidative phosphorylation, electrons are transferred from electron donors to electron acceptors such as oxygen in redox reactions. These redox reactions release the energy stored in the relatively weak double bond of O<sub>2</sub>, which is used to form ATP. In eukaryotes, these redox reactions are catalyzed by a series of protein complexes within the inner membrane of the cell's mitochondria, whereas, in prokaryotes, these proteins are located in the cell's outer membrane. These linked sets of proteins are called electron transport chains. In eukaryotes, five main protein complexes are involved, whereas in prokaryotes many different enzymes are present, using a variety of electron donors and acceptors.

The energy transferred by electrons flowing through this electron transport chain is used to transport protons across the inner mitochondrial membrane, in a process called *electron transport*. This generates potential energy in the form of a pH gradient and an electrical potential across this membrane. This store of energy is tapped when protons flow back across the

membrane and down the potential energy gradient, through a large enzyme called ATP synthase; this process is known as chemiosmosis. The ATP synthase uses the energy to transform adenosine diphosphate (ADP) into adenosine triphosphate, in a phosphorylation reaction. The reaction is driven by the proton flow, which forces the rotation of a part of the enzyme; the ATP synthase is a rotary mechanical motor.

## **ATP synthesis**

The amount of energy (as ATP) gained from glucose catabolism varies across species and depends on other related cellular processes.

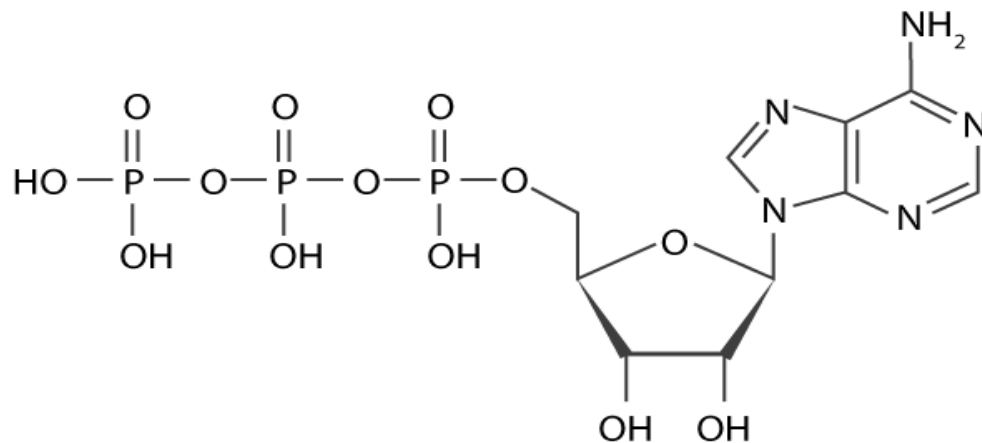
In a eukaryotic cell, the process of cellular respiration can metabolize one molecule of glucose into 30 to 32 ATP. The process of glycolysis only produces two ATP, while all the rest are produced during the electron transport chain. The number of ATP molecules generated via the catabolism of glucose can vary substantially. For example, the number of hydrogen ions the electron transport chain complexes can pump through the membrane varies between species. Another source of variance occurs during the shuttle of electrons across the membranes of the mitochondria. The NADH generated from glycolysis cannot easily enter mitochondria. Thus, electrons are picked up on the inside of mitochondria by either  $\text{NAD}^+$  or  $\text{FAD}^+$ . These  $\text{FAD}^+$  molecules can transport fewer ions; consequently, fewer ATP molecules are generated when  $\text{FAD}^+$  acts as a carrier.

## **ATP – the biological energy currency**

The ATP molecule was discovered in the year 1929 by German chemist Karl Lohmann. Later in the year 1948, Scottish biochemist Alexander Todd was the first person to synthesize the ATP molecule.

ATP is known as adenosine triphosphate, and it is a molecule containing carbon, hydrogen, nitrogen, oxygen and phosphorus. These molecules provide energy for various biochemical processes in the body. Therefore, it is called “Energy Currency of the Cell”. These ATP molecules are synthesized by Mitochondria, therefore it is called powerhouse of the cell.

## Structure of ATP Molecule



ATP molecules are largely composed of three essential components.

- The pentose sugar molecule i.e. ribose sugar.
- Nitrogen base- Adenine, attached to the first carbon of this sugar molecule.
- The three phosphate groups which are attached in a chain to the 5th carbon of the pentose sugar. The phosphoryl groups, starting with the group closest to the ribose sugar, are referred to as the alpha, beta, and gamma phosphates. These phosphates play an important role in the activity of ATP.

The three phosphate groups present in this ATP molecule are called high energy bonds as they are involved in the liberation of a huge amount of energy when they are broken. This molecule provides energy for various life processes without which life cannot exist.

Once after the energy is produced by the ATP molecules, they are stored in its bonds which are later utilized by the cells by breaking the bonds whenever required ATP is the most preferred energy molecule in the cell. Its preference is due to the following factors:

1. It donates its phosphoryl groups to release energy.
2. On hydrolysis, it releases a high negative Gibbs free energy which can be used to drive many important biosynthetic reactions in metabolic pathways.
3. The presence of adenine and ribosyl groups provide additional features for attachment to enzymes so it is able to regulate enzymatic activities.



## **Functions of ATP**

The ATP is used for various cellular functions, including transportation of different molecules across cell membranes. A significant role of ATP apart from energy production includes synthesizing the multi-thousand types of macromolecules that the cell requires for their survival. ATP molecule is also used as a switch to control chemical reactions and to send messages.

## **Importance of ATP Molecule in Metabolism**

1. These ATP molecules can be recycled after every reaction.
2. ATP molecule provides energy for both the exergonic and endergonic processes.
3. It is the only energy, which can be directly used for different metabolic process. Other forms of chemical energy need to be converted into ATP before they can be used.
4. It plays an important role in the Metabolism – A life-sustaining chemical reactions including cellular division, fermentation, photosynthesis, photophosphorylation, aerobic respiration, protein synthesis, exocytosis, endocytosis and motility.

### **Pentose Phosphate pathway**

The pentose phosphate pathway (PPP; also called the phosphogluconate pathway and the hexose monophosphate shunt) is a process that breaks down glucose-6-phosphate into NADPH and pentoses (5-carbon sugars) for use in downstream biological processes. There are two distinct phases in the pathway: the oxidative phase and the non-oxidative phase. The overall reaction for this process is:

**Pentose Phosphate pathway**

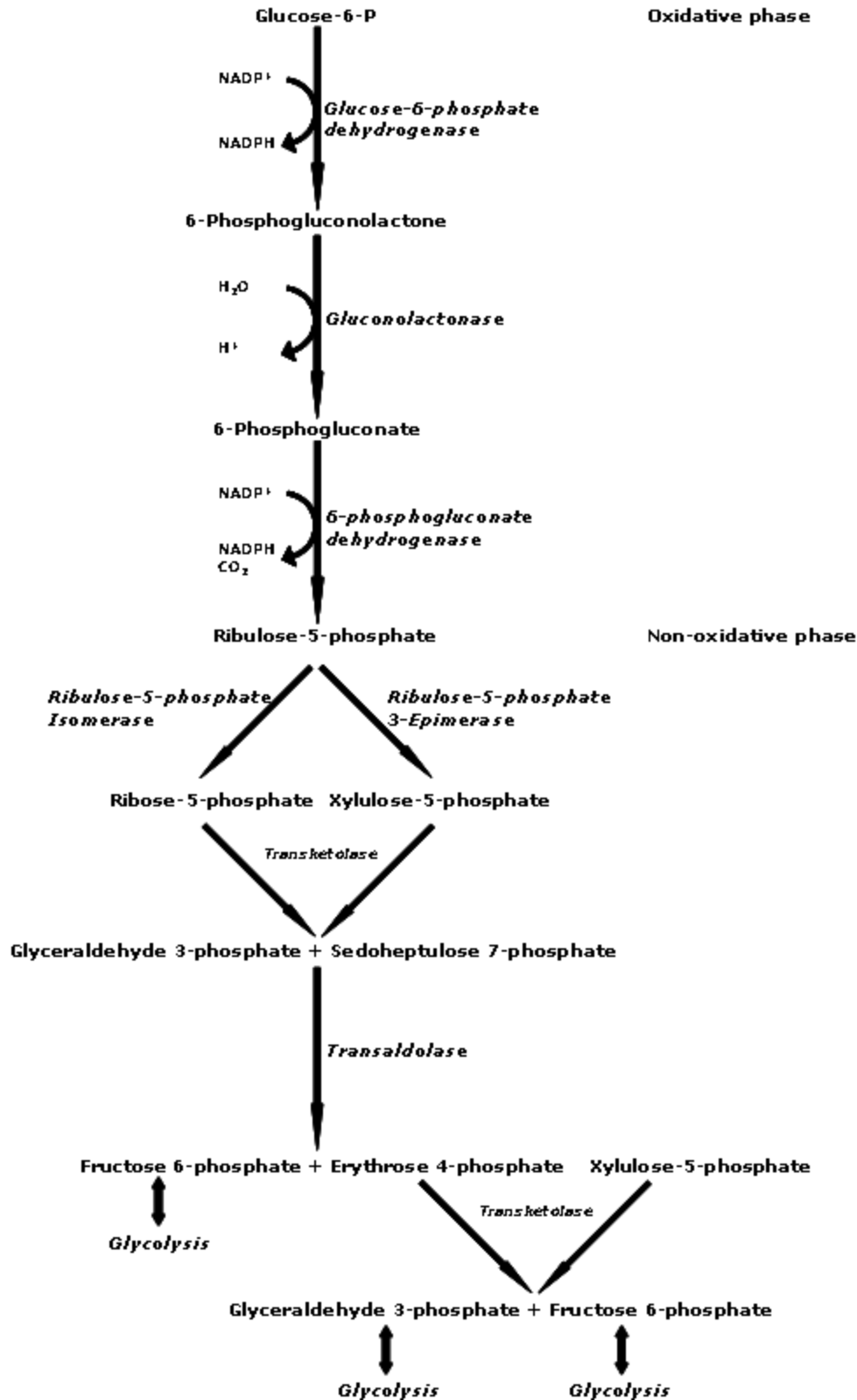
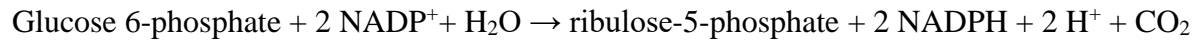


Figure: **Figure 1 The Pentose Phosphate Pathway:** The first is the oxidative phase in which glucose-6-phosphate is converted to ribulose-5-phosphate. During this process two molecules of  $\text{NADP}^+$  are reduced to NADPH. It produces ribulose-5-phosphate, used in the synthesis of nucleotides. It also produces nucleic acids and erythrose-4-phosphate, used in the synthesis of aromatic amino acids.



The second phase of this pathway is the non-oxidative synthesis of 5-carbon sugars. Ribulose-5-phosphate can reversibly isomerize to ribose-5-phosphate. Ribulose-5-phosphate can alternatively undergo a series of isomerizations as well as transaldolations and transketolations that result in the production of other pentose phosphates including fructose-6-phosphate, erythrose-4-phosphate, and glyceraldehyde-3-phosphate (both intermediates in glycolysis). These compounds are used in a variety of different biological processes including production of nucleotides and nucleic acids (ribose-5-phosphate), as well as synthesis of aromatic amino acids (erythrose-4-phosphate).

Glucose-6-phosphate dehydrogenase is the rate-controlling enzyme in this pathway. It is allosterically stimulated by  $\text{NADP}^+$ . NADPH-utilizing pathways, such as fatty acid synthesis, generate  $\text{NADP}^+$ , which stimulates glucose-6-phosphate dehydrogenase to produce more NADPH. While the PPP does involve oxidation of glucose, its primary role is anabolic rather than catabolic, using the energy stored in NADPH to synthesize large, complex molecules from small precursors. Additionally, NADPH can be used by cells to prevent oxidative stress. NADPH reduces glutathione via glutathione reductase, which converts reactive  $\text{H}_2\text{O}_2$  into  $\text{H}_2\text{O}$  by glutathione peroxidase.