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T.Y.B.SC.(MICROBIOLOGY)(CBCS)

NEW PROPOSED SYLLABUS -JUNE 2021

MB-502 BACTERIAL METABOLISM (THEORY)

UNIT :3 BIOENERGETICS

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CONTEN

- Different mode of ATP generation
- Electron transport chain
- Components of ETC
- Energy yield
- Anaerobic respiration
- Peptidoglycans biosynthesis
- Bacterial photosynthesise

Introduction of bioenergetics

- bioenergetics is the part of biochemistry concerned with the energy involved making and breaking of chemical bonds in the molecules found in biological organism.
- Growth, development and metabolism are some of the central phenomena in the study of biological organism.
- In a living organism chemical bond are broken and as part of the exchange and transformation of energy.
- Living organism obtain energy from organic and inorganic materials .for example lithotrophs can oxidize minerals such as nitrates or forms of sulfure such as element sulfure ,sulphites' and hydrogen sulfide to produce ATP.

Different mode of ATP generation

- ATPase (adenosine triphosphate)are a class of enzyme that catalyse decomposition of ATP to ADP and free phosphate ion.
- This dephosphorylation reaction releasee energy this process is widely used in all known forms of life .
- some such enzyme are integral membrane proteins and move solute across the membrane typically against the concentration gradients.
- there are called transmembrane ATPases.
- Transmembrane ATPases import many of the metabolites necessary for metabolism and export toxins ,wastes and solutes that can hinder cellular processes.

Type of ATPase system

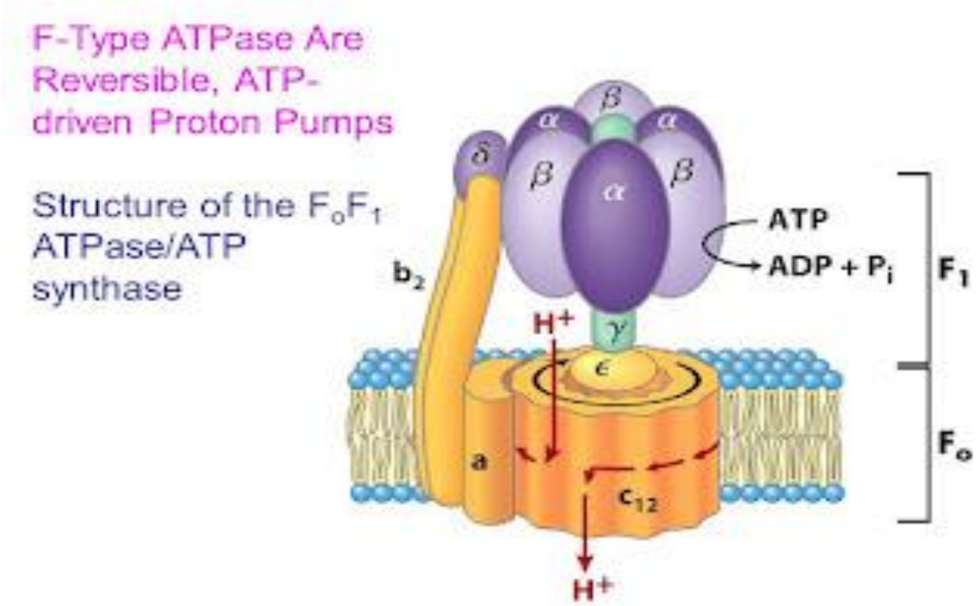
- The three major classes of the ATPase system are involved in transport ions and generation of energy.

1. F-type (F1F0)
2. P-type (E1E2)
3. V-type

1)F-type ATPase (structure of F1F0 ATPASE

- F-type ATPASE are similar structure and mechanism to the ATPsynthases of chloroplasts and eubacteria.
- This large enzyme complex of the inner mitochondrial membrane catalyses the formation of ATP from ADP and pi, accompanied by the flow of protons.
- ATP synthase also called complex V has two distinct components F1 a peripheral membrane protein and F0 which is integral to the membrane.
- F1 the first factor recognized as essential for oxidative phosphorylation.
- As a noted above the ATP synthase consist of two functionally distinct entities f1 and f0 ,which function in rotary –like manner.
- The f1 moiety retains ATPase function when stripped from the membrane.
- It consists of five subunits in unusual stoichiometric ratio of a3b3y
- The F0 pore consists of three subunits also in an unusual stoichiometry of a1b2c10
- F1 is built as a hexamer of alternating a and b subunits that surround an asymmetric core consisting of the y subunit.

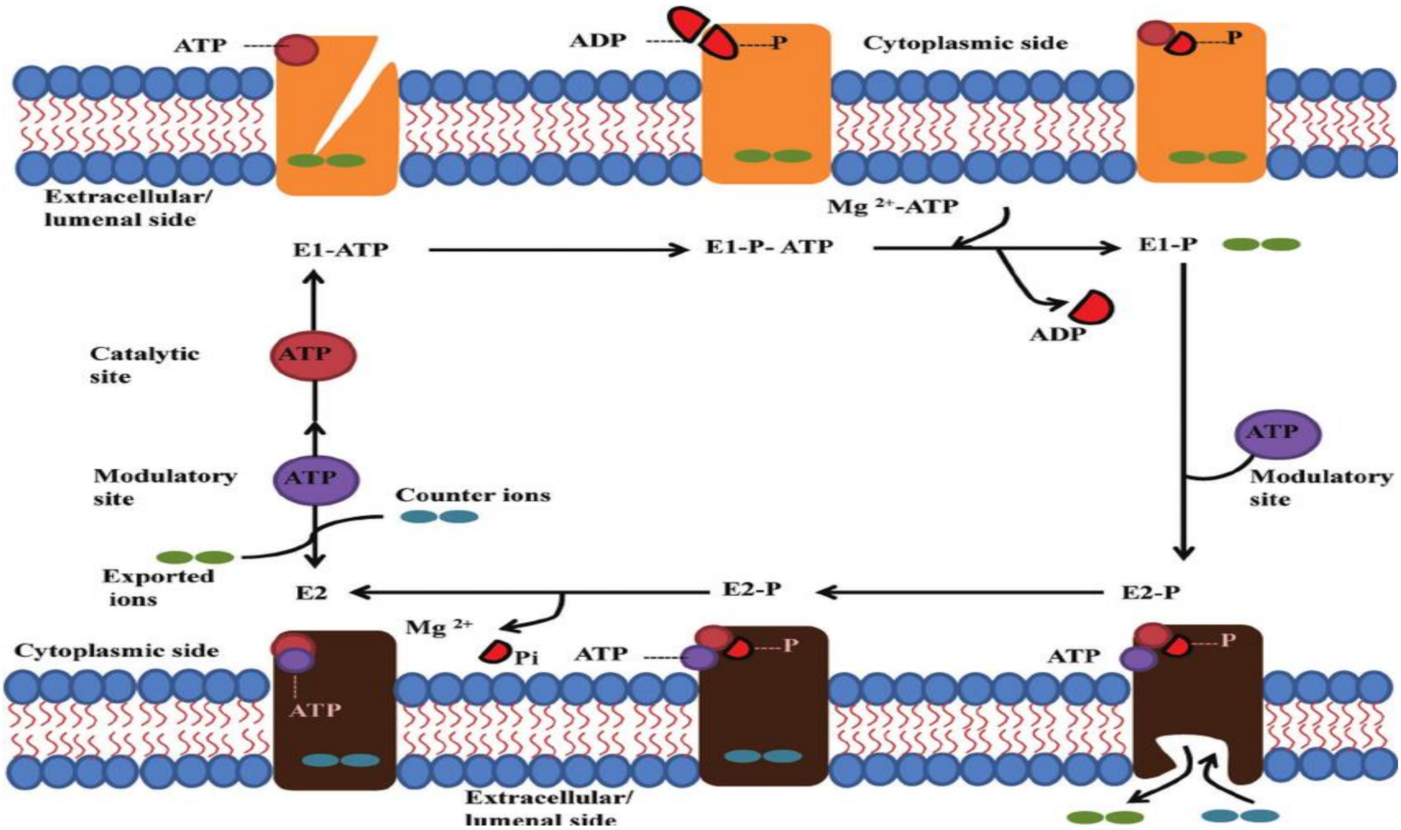
- The interfaces between a and b subunits from three nucleotide –binding catalytic sites where ADP is converted to ATP.



2) P-type ATPases (**structure of E1, E2 ATPases)**

- The p-type ATPases also known as E1,E2 ATPases related ion and lipid pumps that are found in bacteria archaea.
- They are a-helical bundle primary transport referred to as p-type ATPases because they catalyse auto phosphorylation of a key conserved asparted residue within pump.
- They all appear to interconvert between at least two different conformation denoted by E1,E2.
- most of members of this transporter family are specific for the pumping of large array of cations,

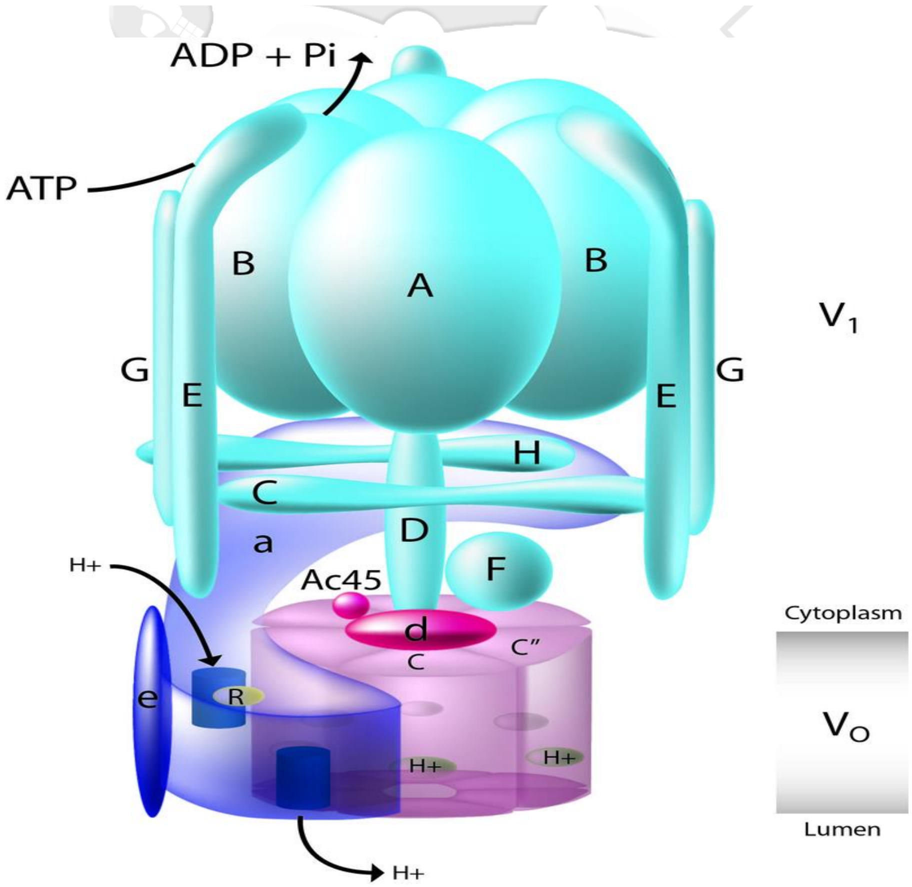
- Example
- p-type ATPases are the sodium –potassium pump , the plasma membrane proton pump , the proton –potassium pump and the calcium pump.
- A cytosolic ligand transported to extracytosolic ,space where an extracytosolic ligand is imported in to cytosol.
- In short ,p-type ATPase switch between two major conformation E1, with ligand binding sites facing the cytosol and E2 ,with ligand binding sites facing the extracytosolic side of membrane .
- The induced fit of ligand 1 binding in E1 promotes phosphorylation Mg+ ATP .
- in this E1-p state the ligand 1 becomes occluded .
- The rate-limiting E1-E2 transition is accompanied by major conformational changes reorienting the ligand –binding sites toward the extracytosolic space.
- This decrease the affinity of the binding sites ligand 1 whereas the affinity for in increased.
- As a result ligand is released in to extracytosolic space via an open exit pathway for ligand a and the counter transported can enterer the binding cavity .
- The result conformational change lead to dephosphorylation of E2p and the released inorganic phosphate expelled .
- The ligand become occluded where the pump is reset to the E1 state reducing the affinity for ligand 2 .
- The pump can now start a new cycle



3)V type ATPases (structure of vo, v1)

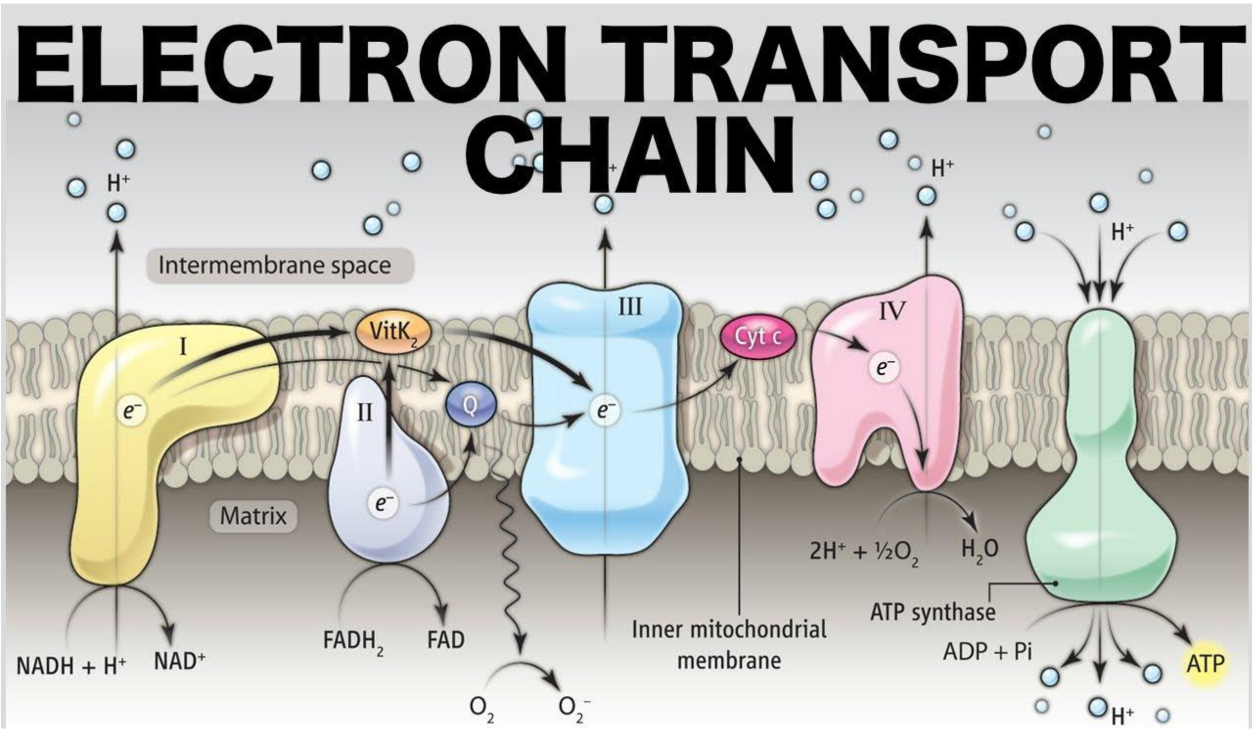
- Vacuolar –type H+ ATPase is highly conserved evolutionarily ancient enzyme .
- V type ATPase have diverse function in eukaryotic organism .
- V type ATPase a wide array of intracellular organelles and pump protons across the plasma membrane of numerous cell type.
- V-ATPases couple the energy of ATP hydrolysis to proton transport across intracellular and plasma membrane eukaryotic cells.
- V-ATPase are found in vacuoles and different from the other two types of ATP synthases due to the fact that they only hydrolyze ,not synthesize ,ATP.

- That is they ,break down ATP to ADP and pi
- The v-ATP ases hydrolyzing ATP releases the energy necessary for the tasks that vacuoles perform including endocytosis bone reabsorption and acid hydrolase activation.
- V-ATP ases like the other two types of ATP synthases have two type of ATP synthase have two domains called v1 and vo.
- They have 13 subunit in the stoichiometry.



Electron transport chain

- Electron Transport Chain is a series of compounds where it makes use of electrons from electron carrier to develop a chemical gradient.
- It could be used to power oxidative phosphorylation.
- The molecules present in the chain comprises enzymes that are protein complex or proteins, peptides and much more.
- Large amounts of ATP could be produced through a highly efficient method termed oxidative phosphorylation .
- ATP is a fundamental unit of metabolic process.



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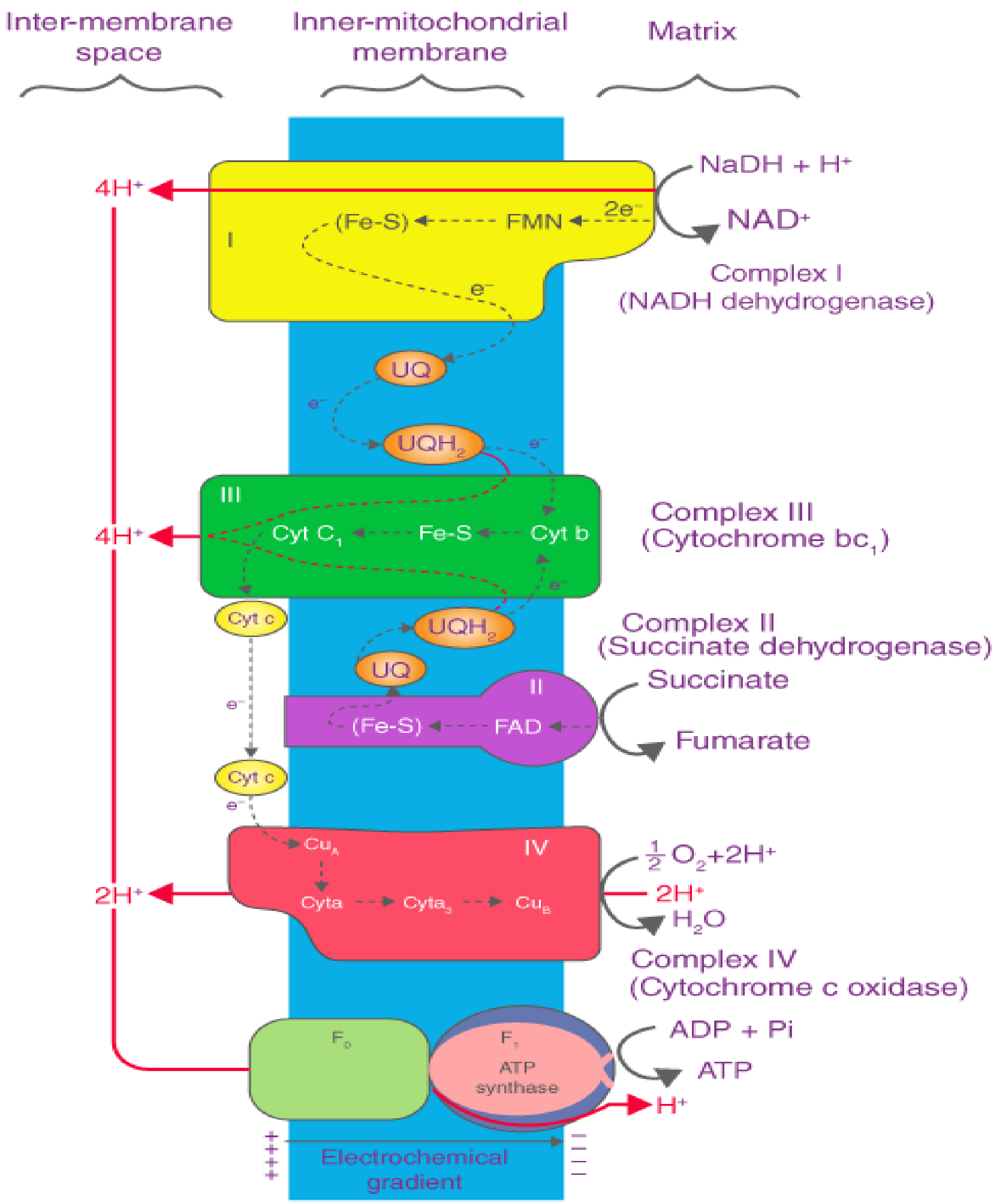
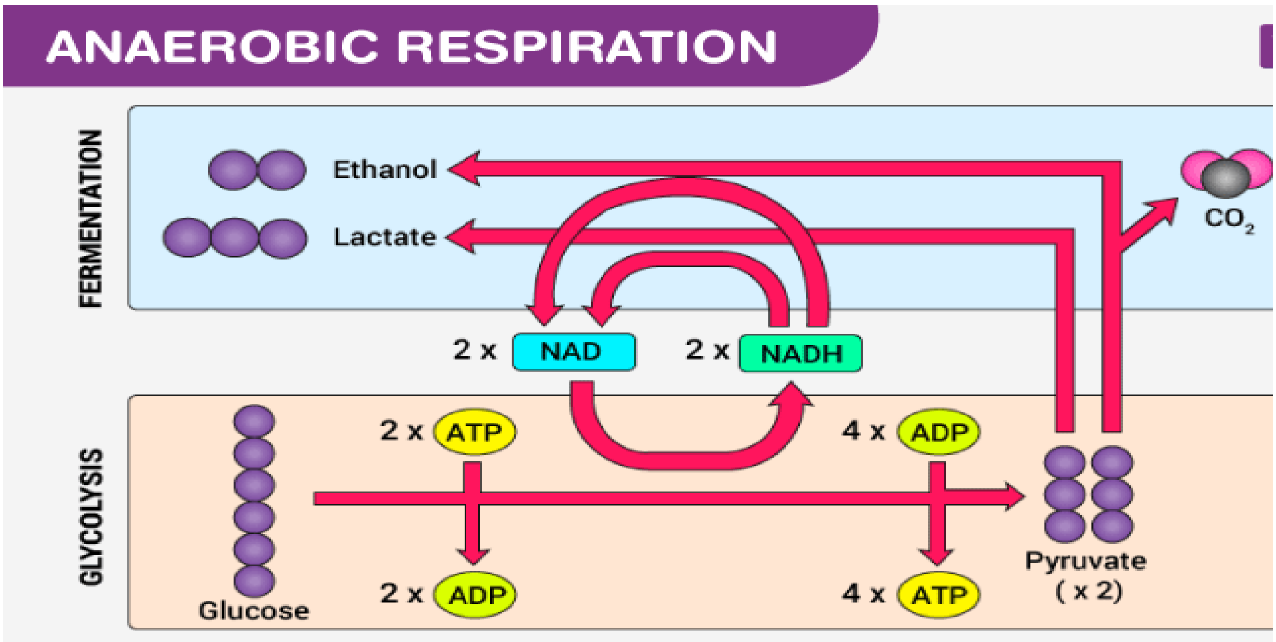
ELECTRON TRANSPORT SYSTEM



Electron Transport Chain in Mitochondria

- The requirement of oxygen in the final phase could be witnessed in the chemical reaction that involves the requirement of both oxygen and glucose.
- A complex could be defined as a structure that comprises a weak protein, molecule or atom that is weakly connected to a protein. The plasma membrane of prokaryotes comprises multi copies of the electron transport chain.
- **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:**
- FADH2 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 a3.
- There are two heme groups where each of them is present in cytochromes c and a3.
- 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.



Anaerobic respiration

- Anaerobic respiration is also used by multi-cellular organisms, like us, as a temporary response to oxygen-less conditions.
- During heavy or intensive exercise such as running, cycling or weight lifting, our body demands high energy.
- As the supply of oxygen is limited, the muscle cells inside our body resort to anaerobic respiration to fulfil the energy demand.
- Anaerobic respiration produces a relatively lesser amount of energy as compared to aerobic respiration, as glucose is not completely broken down in the absence of oxygen.

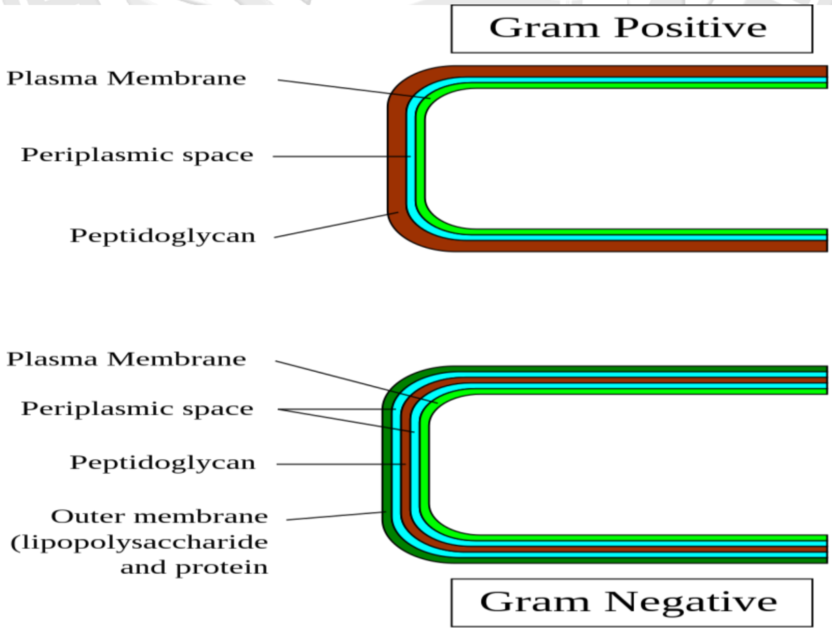
Glucose → Alcohol + Carbon dioxide + Energy

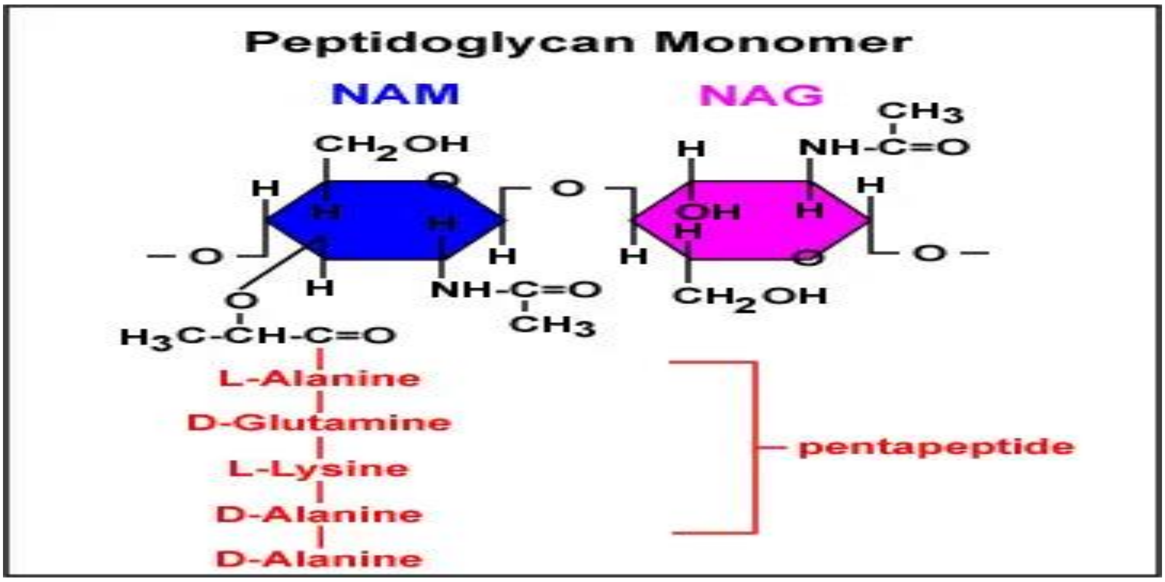
- The fundamental difference between aerobic and anaerobic respiration is the usage of oxygen in the process of cellular respiration.
- Aerobic respiration, as the name suggests, is the process of producing the energy required by cells using oxygen.
- The by-product of this process produces carbon dioxide along with ATP – the energy currency of the cells.
- It is carried out by many bacteria ,archaea ,and some eukaryotic microbes .
- The most common terminal electron acceptor used during anaerobic respiration are nitrate ,sulfate ,and co2.
- The anaerobic respiratory chain consists of dehydrogenase ,reductase and diffusible quinine to mediate the transfer of electron between dehydrogenase and reductase.
- In absence of oxygen E.coli can use either nitrate or fumarate and electron acceptor.

- The nitrate is reduced to nitrite and further reduced to ammonia.
- Many bacteria especially members of the family Enterobacteriaceae, can metabolize pyruvate to numerous products using serval pathways simultaneously
- One such complex fermentation is the mixed acid fermentation which result in the excretion of ethanol and a mixture of acids particularly acetic, lactic ,succinic and formic acids

- **Peptidoglycan biosynthesis**

- Peptidoglycan is a defining feature of the bacterial cell wall.
- Peptidoglycan is a rigid envelope surrounding the cytoplasmic membrane of most bacterial species.
- It helps protect bacterial cells from environmental stress and helps preserve cell morphology throughout their life cycle.
- Peptidoglycan biosynthesis is also an important regulator of bacterial cell division.



- The peptidoglycan layer provides structural support and maintains the characteristics shape of the cell.
- The structural, chemical composition, and thickness of the cell wall differ in gram –positive and gram-negative bacteria .
- most obvious difference is the dramatically thicker peptidoglycan layer of gram-positive bacteria.
- Peptidoglycan is a complex net work of sugars and proteins that surround the entire cell wall.
- The term “peptidoglycan “ is derived from peptides and the sugar (glycan) that make up the molecule.
- The peptidoglycan molecule has a carbohydrates backbone composed of alternating N-acetylmuramic acid (NAM) and N- acetylglucosamine (NAG) molecules.
- Attached to each a muramic acid molecules is a tetra-peptide consisting of both D and L amino acid the precise composition of which differ from one bacterium to another .
- Pentapeptide chains are attached to the NAM group and the polysaccharide chains are connected through their pentapeptides or by inter bridges .
- 

- The biosynthesis of bacterial cell wall peptidoglycan is a complex process that involves enzyme reactions that take place in the cytoplasm (synthesis of the nucleotide precursors) and on the inner side (synthesis of lipid-linked intermediates) and outer side (polymerization reactions) of the cytoplasmic membrane
- This review deals with the cytoplasmic steps of peptidoglycan biosynthesis,
- which can be divided into four sets of reactions that lead to the syntheses of

(1) UDP-*N*-acetylglucosamine from fructose 6-phosphate,

(2) UDP-*N*-acetylmuramic acid from UDP-*N*-acetylglucosamine,

(3) UDP-*N*-acetylmuramyl-pentapeptide from UDP-*N*-acetylmuramic acid and

(4) d-glutamic acid and dipeptide d-alanyl-d-alanine.

Bacterial photosynthesis

- *Photosynthesis is the process used by green plants and a few organisms that use sunlight, carbon dioxide and water to prepare their food."*
- The process of photosynthesis is used by plants, algae and certain bacteria that convert light energy into chemical energy.
- The glucose formed during the process of photosynthesis provides two important resources to organisms: energy and fixed carbon.

Site of Photosynthesis

- Photosynthesis takes place in special organelles known as chloroplast.
- This organelle has its own DNA, genes and hence can synthesize its own proteins.
- Chloroplasts consist of stroma, fluid, and stack of thylakoids known as grana.
- There are three important pigments present in the chloroplast that absorb light energy, chlorophyll a, chlorophyll b, and carotenoids.

Types of Photosynthesis

- There are two different types of photosynthesis:
- Oxygenic photosynthesis
- Anoxygenic photosynthesis

Oxygenic Photosynthesis

- Oxygenic photosynthesis is more common in plants, algae and cyanobacteria.
- During this process, electrons are transferred from water to carbon dioxide by light energy, to produce energy.
- During this transfer of electrons, carbon dioxide is reduced while water is oxidized, and oxygen is produced along with carbohydrates.
- During this process, plants take in carbon dioxide and oxygen into the atmosphere.
- This process can be represented by the equation:
- $6CO_2 + 12H_2O$ + LIGHT ENERGY → $C_6H_{12}O_6 + 6O_2 + 6H_2O$

Anoxygenic Photosynthesis

- This type of photosynthesis is usually seen in certain bacteria, such as green Sulphur bacteria and purple bacteria which well in various aquatic habitats.
- Oxygen is not produced during the process.
- The anoxygenic photosynthesis can be represented by the equation

Photosynthesis Apparatus

- The photosynthesis apparatus includes the following essential components
- **Pigments**
- Pigments not only provide color to the photosynthetic organisms, but are also responsible for trapping sunlight.
- The important pigments associated with photosynthesis include:
- **Chlorophyll:** It is a green-coloured pigment that traps blue and red light. Chlorophyll is subdivided into, "chlorophyll a", "chlorophyll b", and "chlorophyll c".
- "Chlorophyll a" is widely present in all the photosynthetic cells.
- A bacterial variant of chlorophyll known as bacteriochlorophyll can absorb infrared rays**.**
- **Carotenoids:** These are yellow, orange or red- coloured pigments that absorb bluish-green light. Xanthophyll and carotenes are examples of carotenoids.
- **Phycobilin's:** These are present in bacteria and red algae. These are red and blue pigments that absorb wavelength of light that are not properly absorbed by carotenoids and chlorophyll.
- **Plastids**
- Plastids are organelles found in the cytoplasm of eukaryotic photosynthetic organisms. They contain pigments and can also store nutrients. Plastids are of three types:
- Leucoplast: These are colourless, non-pigmented and can store fats and starch.
- Chromoplasts: They contain carotenoids.
- Chloroplasts: These contain chlorophyll and are the site of photosynthesis.

Antennae

- Antennae is the collection of 100 to 5000 pigment molecules that capture light energy from the sun in the form of photons.
- The light energy is transferred to a pigment-protein complex that converts light energy to chemical energy.
- **Reaction Centers**
- The pigment-protein complex responsible for the conversion of light energy to chemical energy forms the reaction center.
- Oxygenic phototrophs
- **What is a Phototroph?**
- **Phototrophs** are organisms that capture sunlight to carry out processes inside the cell.
- Although people commonly associate photosynthesis, the process that plants use to make their own food from sunlight with phototrophs, not all phototrophs are photosynthetic.
- All phototrophs, however, use light energy from the sun to power different processes within the cell.
- Some examples of phototrophs include bacteria, plants and algae.
- **Types of Photoautotrophs**
- There are two main types of photoautotrophs, oxygenic phototrophs and anoxygenic phototrophs. These two types are explained in more detail in the sections below.
- **Oxygenic phototrophs** perform the process of photosynthesis to create food and oxygen using light energy.
- The oxygenic prokaryotic prototroph use HO2 as the electron donor photosynthetic reduction of NADP.

- Most of the species belong cyanobacteria which are widely distributed in nature, occurring in fresh marine waters and terrestrial habitats.
- They have only one type chlorophyll (chlorophyll a) and light harvesting pigments called phycobilin's.
- Another kind of oxygenic microbial phototrophs is the prochlorophytes
- which are a diverse group of photosynthetic prokaryotes .

The complete process of photosynthesis is carried out through two process.

1. **Light reaction**
2. **Dark reaction**

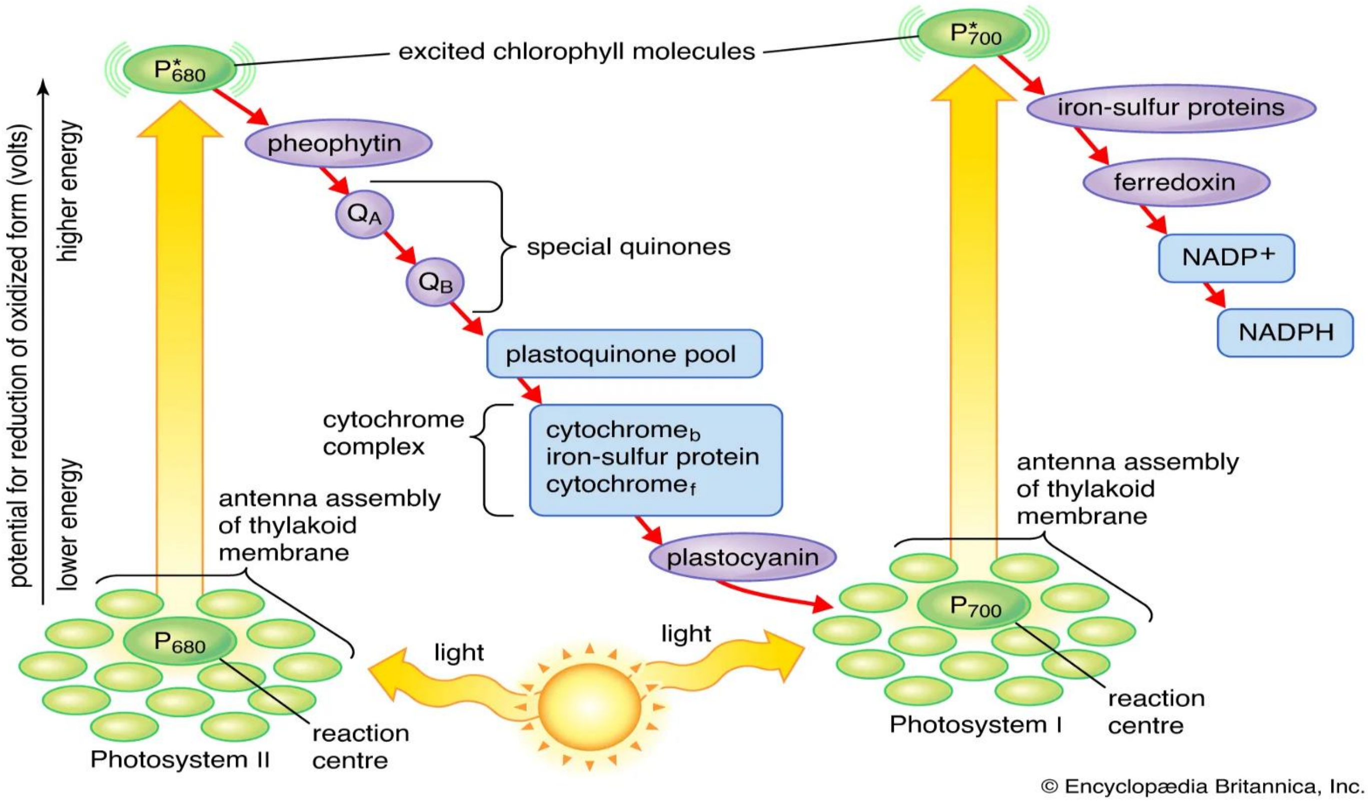
1) Light reaction

- The light reaction takes place in the grana of the chloroplast. Here, light energy gets converted to chemical energy as ATP and NADPH.
- In this very light reaction, the addition of phosphate in the presence of light or the synthesizing of ATP by cells is known as photophosphorylation.

2) Dark reaction

- While in the dark reaction, the energy produced previously in the light reaction is utilized to fix carbon dioxide into carbohydrates.
- The location where this happens is the stroma of the chloroplasts.
- Photophosphorylation
- Photophosphorylation is the process of utilizing light energy from photosynthesis to convert ADP to ATP.
- It is the process of synthesizing energy-rich ATP molecules by transferring the phosphate group into ADP molecule in the presence of light.

- **Photophosphorylation is of two types:**
- **Cyclic Photophosphorylation**
- **Non-cyclic Photophosphorylation**



Cyclic Photophosphorylation

- The photophosphorylation process which results in the movement of the electrons in a cyclic manner for synthesizing ATP molecules is called cyclic photophosphorylation.
- In this process, plant cells just accomplish the ADP to ATP for immediate energy for the cells.
- This process usually takes place in the thylakoid membrane and uses Photosystem I and the chlorophyll P700.

- During cyclic photophosphorylation, the electrons are transferred back to P700 instead of moving into the NADP from the electron acceptor.
- This downward movement of electrons from an acceptor to P700 results in the formation of ATP molecules.

Non-Cyclic Photophosphorylation

- The photophosphorylation process which results in the movement of the electrons in a non-cyclic manner for synthesizing ATP molecules using the energy from excited electrons provided by photosystem II is called non-cyclic photophosphorylation.
- This process is referred to as non- cyclic photophosphorylation because the lost electrons by P680 of Photosystem II are occupied by P700 of Photosystem I and are not reverted to P680.
- Here the complete movement of the electrons is in a unidirectional or in a non- cyclic manner. During non-cyclic photophosphorylation, the electrons released by P700 are carried by primary acceptor and are finally passed on to NADP.
- Here, the electrons combine with the protons – H+ which is produced by splitting up of the water molecule and reduces NADP to NADPH2.

Anoxygenic phototrophs

- The other phototrophic prokaryotes do not produce oxygen (e.. H_2O is not source of electrons).
- Instead they use organic compounds, inorganic compounds, or hydrogen gas as a source of electrons.
- These organisms will grow photographically only an aerobically or when oxygen tensions are low .
- There are three major groups of anoxygenic prototroph.

(1) Purple photosynthetic bacteria, which include the both Purple sulfur and purple non-sulfur bacteria.

(2) Green sulfur photosynthetic bacteria, Green non-sulfur photosynthetic bacteria, also called green gliding bacteria.

(3) Heliobacteria

1) Purple Sulfur Phototrophs

The purple sulfur phototrophic bacteria grow photo autographically anaerobic environments using hydrogen sulfide as the electron donor and CO_2, as the carbon source .

- Their natural habitats are freshwater lakes and ponds, or the marine water where the sulfide content is high due to sulfate-reducing bacteria .
- They oxidize sulfide to elemental sulfur, which accumulates as granule intracellular in all known genera.
- Which deposits sulfur extracellular site However, some can grow photoheterotrophically, and several have been grown chemo autographically under low partial pressure of oxygen wit reduced inorganic sulfur as an electron donor and energy source.

2) Purple non-sulfur Phototrophs

- Purple non-sulfur Phototrophs It was thought that these bacteria were not able to utilize sulfide as a source of electrons: hence the name "non-sulfur".
- However, it turns out that the concentration of sulfide used by the purple sulfur bacteria are toxic to the non-sulfur purples, and that, provided the concentrations are sufficiently low,
- some purple non-sulfur bacteria (Rhodopseudomonas and Rhodobacter) can use sulfide or thiosulfate as a source of electrons during photoautotrophic growth.

3) Green sulfur phototrophs

- Green sulfur bacteria oxidize inorganic sulfur compounds to use as electron donors for anaerobic photosynthesis, specifically in carbon dioxide fixation.
- They usually prefer to utilize sulfide over other sulfur compounds as an electron donor, however they can utilize thiosulfate or H_2.
- It is a group of photoautotrophic bacteria that can perform anoxygenic photosynthesis.
- Most of them are nonmotile and obligate anaerobes.
- They have bacteriochlorophyll pigments c, d, a or e. Also, they use sulfate as their ultimate electron donor for photosynthesis.
- Thus, they can thrive well in sulfur-rich environments with low light intensities.
- Most of these bacteria can reduce nitrogen to ammonia. This ammonia is later used to synthesis amino acids.

4) Green non-sulfur photosynthetic bacteria

A. Form flexible filaments; also called the green flexibacteria
B. Gliding mobility
C. Most do not have gas vesicles
D. Form chlorosomes

May have an Intramembrane system reminiscent of that in proteobacteria (e.g., *Oscillochloris*)