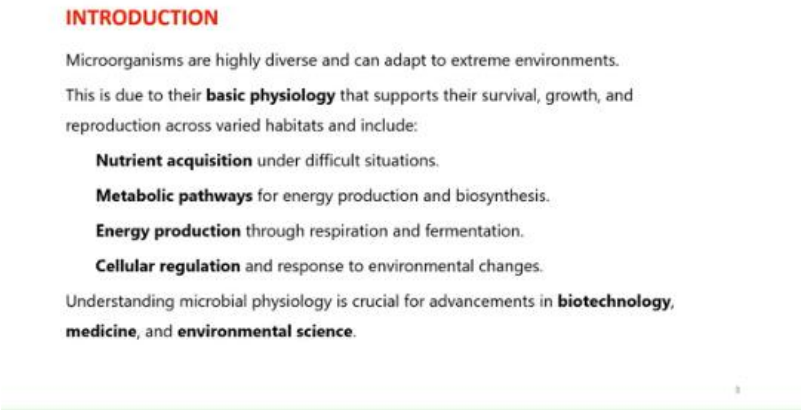
MICROBIAL BIOTECHNOLOGY

Prof. Utpal Bora
Department of Biosciences and Bioengineering
Indian Institute of Technology Guwahati

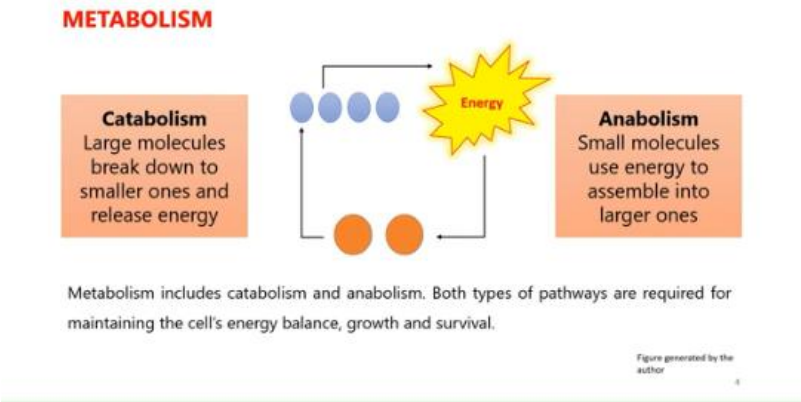
Lecture-10
Lec 10: Basic Physiology of Microorganisms

Hello friends, welcome to my course on microbial biotechnology. Today we are starting module number three, and in this first lecture, we will discuss the basic physiology of microorganisms. This lecture is divided into various sections—four sections. We will start with section number one, where we will give an introduction, then move on to metabolism. Nutrient acquisition in prokaryotes, then microbial nutrition—particularly the nutritional types—and then the nutrient uptake mechanisms through passive and active transport modes.

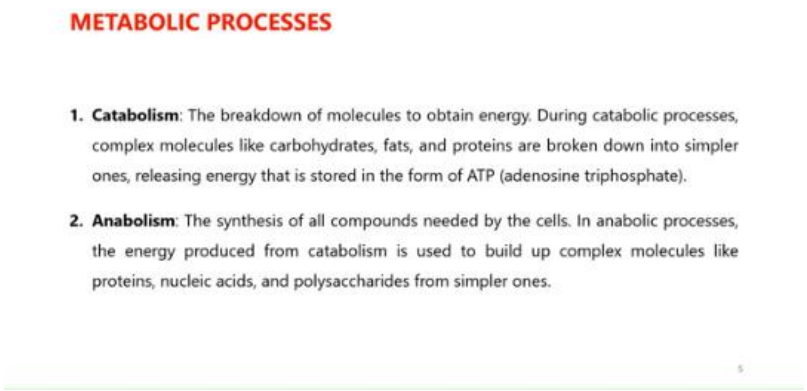
Microorganisms are highly diverse and can adapt to extreme environments: hot, cold, dry, and so on. This is due to their basic physiology, which supports their survival, growth, and reproduction across varied habitats. Some of these mechanisms include nutrient acquisition under difficult conditions, inherent metabolic pathways for energy production and biosynthesis, energy production through respiration and fermentation, and cellular regulation and responses to environmental changes. Therefore, understanding microbial physiology is crucial for advancements in biotechnology, medicine, and environmental sciences. Briefly, we can describe metabolism as the summation of two processes: catabolism and anabolism.



Both types of pathways are required for maintaining the cell's energy balance, growth, and survival. Now, what is catabolism? In catabolism, large molecules are broken down into smaller ones, releasing energy. In anabolism, small molecules use energy to assemble into larger ones. They are just the opposite of one another.



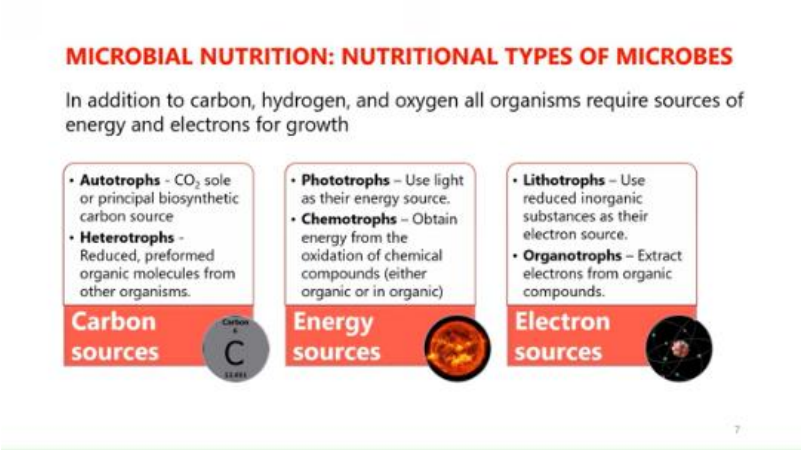
So the breakdown of molecules to obtain energy is catabolism. During the catabolic process, complex molecules like carbohydrates, fats, and proteins are broken down into simpler ones, releasing energy that is stored in the form of adenosine triphosphate. In anabolism, the synthesis of all compounds needed for the cell is completed. In anabolic processes, the energy produced from catabolism is used to build complex molecules like proteins, nucleic acids, and polysaccharides from simpler ones. So, catabolism and anabolism are tightly linked to one another and dependent on each other.



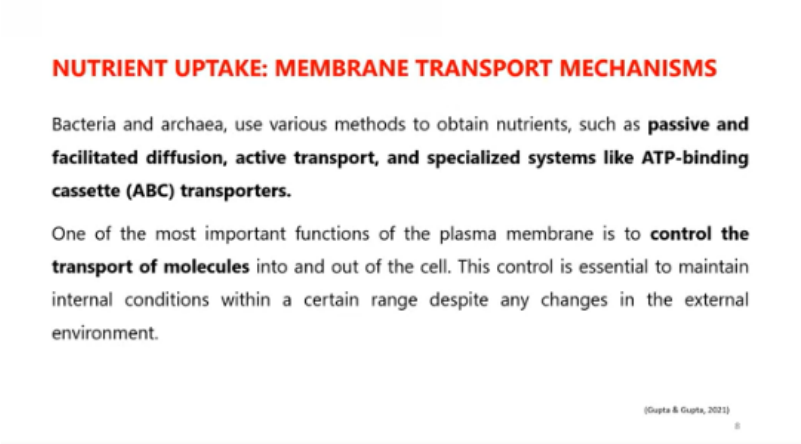
So let's try to understand how prokaryotes consume food or how they acquire nutrients. Unlike us, they do not have highly developed organs. In fact, they are unicellular. So microorganisms have evolved diverse mechanisms of nutrient uptake to efficiently capture and utilize nutrients from various sources. In addition to carbon, hydrogen, and oxygen, all organisms require sources of energy and electrons for growth.

So based on these, we can divide microbes into different types, which is basically the nutritional type. of the microbes. So they may be autotrophs or heterotrophs from the point of view of carbon sources. For autotrophs, carbon dioxide is the sole or principal biosynthetic carbon source. For heterotrophs, they use reduced preformed organic molecules from other organisms.

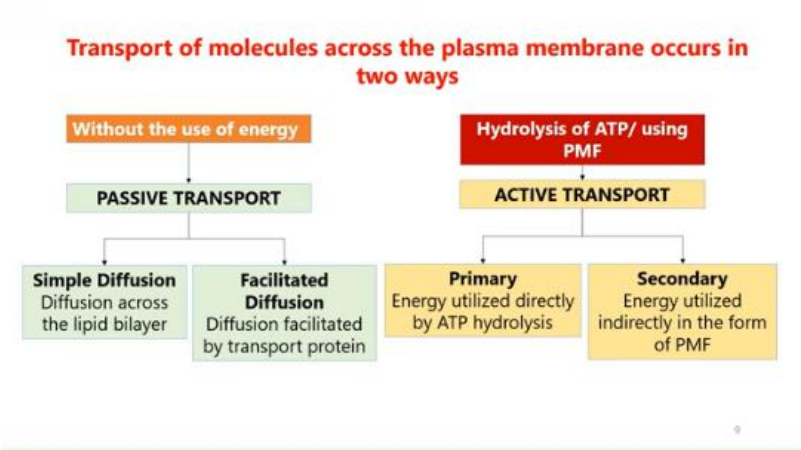
So when you look into the nutritional types from the point of view of energy, we can divide them into phototrophs that use light as their energy source or into chemotrophs, which obtain energy from the oxidation of chemical compounds, either organic or inorganic. From the electron source point of view, we can classify them as lithotrophs, which use reduced inorganic substances as their electron sources, or they may be organotrophs, those that extract electrons from organic compounds. So let us now look into the membrane transport mechanisms. Bacteria and archaea use various methods to obtain nutrients, such as passive and facilitated diffusion, active transport, and specialized systems like ATP-binding cassette transporters.



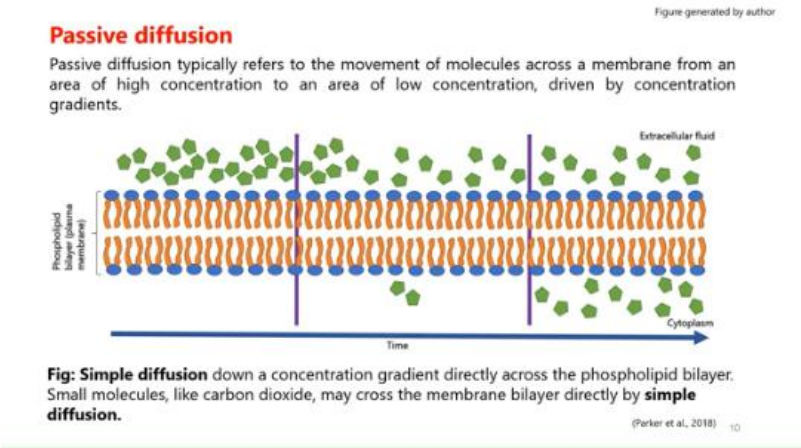
One of the most important functions of the plasma membrane is to control the transport of molecules into and out of the cell. This control is essential to maintain internal conditions within a certain range, despite any changes in the external environment; otherwise, the organism will perish. Transport of molecules across the plasma membrane occurs in two ways. The first way uses energy, and the other is without the use of energy. Let us focus on the pathway which does not use energy, known as passive transport, which may be further divided into simple diffusion, where diffusion across the lipid bilayer happens due to a gradient formation of nutrient concentration.



And there is facilitated diffusion, where diffusion is facilitated by the presence of certain proteins or certain structures. Then we have active transport, where we require the hydrolysis of ATP, and it uses the proton motive force. And this can be primary active transport or secondary active transport. In the primary one, the energy is utilized directly by ATP hydrolysis. In the secondary, energy is utilized indirectly in the form of proton motive force.



So, let's look into them one by one. For example, let's start with passive diffusion, which typically refers to the movement of molecules across a membrane from an area of high concentration to an area of low concentration, driven by the concentration gradient. So here, we can see the phospholipid bilayer plasma membrane, and then we have the cytoplasm, which is inside the cell, and this is the external area outside the cell. So here, in simple diffusion, there is movement down a concentration gradient directly across the phospholipid bilayer. Small molecules like carbon dioxide may cross the membrane bilayer directly by simple diffusion.



In facilitated diffusion, charged molecules like magnesium and large molecules like sugar cannot easily diffuse across the lipid membrane due to their size and charge. Instead, they rely on membrane proteins to help them transport across. We have ion channels and carriers, which facilitate the transport of ions like magnesium, by allowing them to move through the membrane down their concentration gradients. Then there are carrier proteins or transporters, which assist large polar molecules like glucose in moving across the membrane.

This process does not require energy and relies on the concentration gradient to accelerate the movement of molecules that cannot diffuse through the membrane directly. So, let us examine what a channel is and how it looks. So, you can see the protein channel over here, and this is the plasma membrane. Here, there are certain molecules in the exterior of the cell, and they are moving inside the cell into the cytoplasm through this protein channel. So, this is called facilitated transport through channels across the membrane. So, from this figure, you can see channels are basically membrane proteins with hydrophilic regions that create a hydrated opening, allowing rapid diffusion of specific substances across the membrane.

Facilitated Diffusion:

Charged molecules (like Mg^{2+}) and large molecules (like sugars) cannot easily diffuse across the lipid membrane due to their size and charge. Instead, they rely on **membrane proteins** to help them transport across:

- **Ion Channels** and **Carriers** facilitate the transport of ions (e.g., Mg^{2+}) by allowing them to move through the membrane down their concentration gradients.
- **Carrier Proteins** or **Transporters** assist large, polar molecules (e.g., glucose) in moving across the membrane.
- This process **does not require energy (ATP)** and relies on the concentration gradient to accelerate the movement of molecules that cannot diffuse through the membrane directly.

(Parker et al., 2018) 11

They are having different functions. For example, they enable high-speed transport, I mean millions of molecules per second. And they may be gated or open. The open ones allow transport all the time while the gated ones are regulating the movement of material inside. They may be voltage gated or they may be ligand gated.

Types of transport proteins: channels

Channels are membrane proteins with hydrophilic regions that create a hydrated opening, allowing rapid diffusion of specific substances across the membrane.

- **Function**: They enable high-speed transport (millions of molecules per second).
- **Gated vs. Open**: Channels can be open (always allowing transport) or gated (regulated by signals like voltage or ligands).
- **Specificity**: Channels are selective, transporting only specific ions or molecules.
- **Example**: **Aquaporins** are specialized channels that allow fast water movement, essential for maintaining cellular water balance.

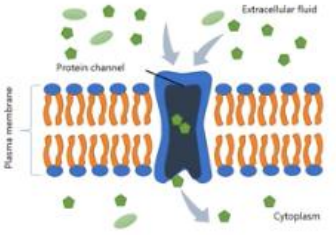


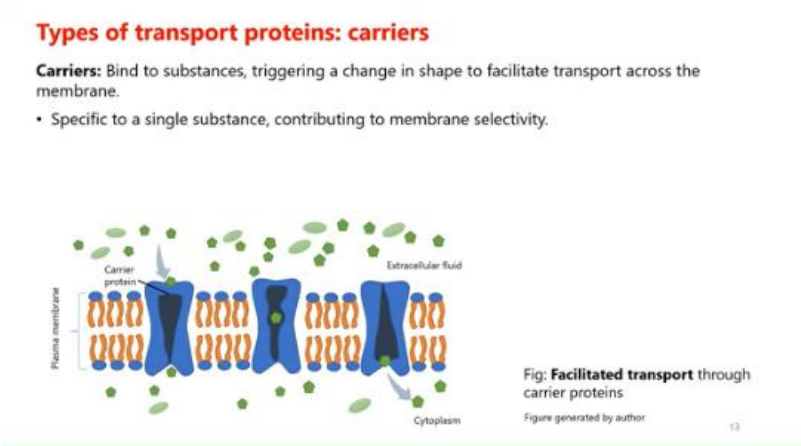
Fig: **Facilitated transport** through channels across the membrane

Figure generated by author

12

These channels are having specificity. They are selective, transporting only specific ions or molecules. Some examples are aquaporins that are specialized to allow the fast movement of water essential for maintaining cellular water balance. Then we have the carriers. They basically bind to substances triggering a change in shape to facilitate transport across the membrane specific to a single substance contributing to membrane selectivity.

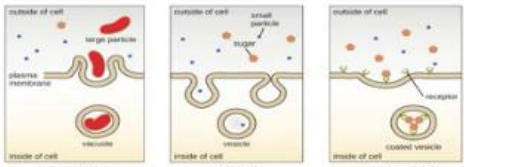
So you can see here this carrier protein which is in the plasma membrane and a particular molecule is binding to it due to which there is a safe change and this particular carrier finally delivers the molecule into the cytoplasm or inside of the cell. They have limitation in the number of carrier proteins and can affect the transport of necessary material for cell function. Carrier proteins work at a slower rate, a thousand to million molecules per second. Both eukaryotic and prokaryotic cells use simple diffusion, facilitated diffusion, and active transport for substance movement across the membrane. However, eukaryotic cells have the unique ability to perform endocytosis, where the



plasma membrane invaginates to form vesicles or vacuoles allowing the uptake of large particles or molecules. This process is not found in prokaryotic cells. So here in phagocytosis, you can see this is the plasma membrane and this plasma membrane is forming a vacuole. So, that is from the outside of the cell, it can bring in the material by the process of phagocytosis. The cell membrane surrounds the particle and pinches off to form the vacuole, which is internalized along with the material.

Both **eukaryotic** and **prokaryotic cells** use **simple diffusion**, **facilitated diffusion**, and **active transport** for substance movement across membranes.

However, **eukaryotic cells** have the unique ability to perform **endocytosis**, where the plasma membrane invaginates to form vesicles or vacuoles, allowing the uptake of large particles or molecules. This process is not found in **prokaryotic cells**.



Cell membrane surrounds the particle and pinches off to form an intracellular vacuole	Cell membrane surrounds a small volume and pinches off to form a vesicle	Uptake is targeted to a specific substance that binds at the receptor on the external cell membrane

(Parker et al., 2018)

Then we have the penocytosis. Here the cell membrane surrounds a small volume and pinches off to form a vesicle. So there are similar processes but phagocytosis happens for large particles and penocytosis happens for small particles. Then we have receptor-mediated endocytosis where uptake is targeted to a specific substance that binds to these receptors on the external cell membrane. And then again, the similar process like phagocytosis and penocytosis happens and the material is internalized.

But in all these processes, we can see that there is a kind of a pinching effect due to which the vacuole is formed. But in the case of receptor-mediated endocytosis, the transport is very, very specific because if there is no any receptor present for a particular molecule, it will not bind and this process will, this coated vesicle will be not formed. Now let us go to the next important mechanism which is the active transport. Here we have the primary active transport that uses ATP as the energy source to move substances against their concentration gradient from lower to higher concentration. This requires energy because the movement of substances is not passive and goes against the natural tendency of molecules to move from higher to lower concentration.

Active transport

Primary Active Transport:

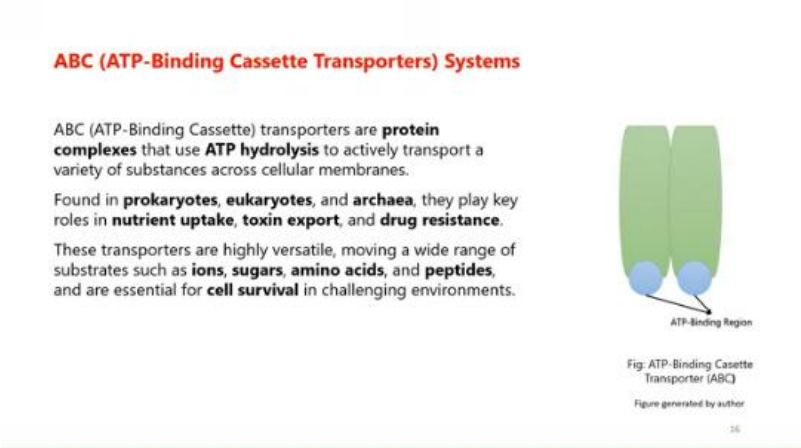
- **Primary active transport** uses **ATP** as the energy source to move substances **against their concentration gradient** (from lower to higher concentration).
- This requires energy because the movement of substances is not passive and goes against the natural tendency of molecules to move from high to low concentration.

ABC (ATP-Binding Cassette) Transporters:

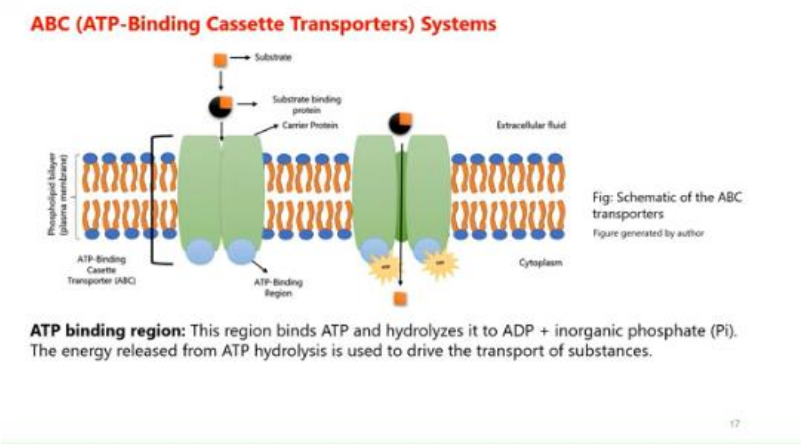
- **ABC transporters** are a large family of membrane proteins that use the energy from **ATP hydrolysis** to transport a wide range of substrates across cellular membranes.
- These transporters are found in **all three domains of life**: **Bacteria**, **Archaea**, and **Eukarya**, highlighting their evolutionary conservation and essential role in cellular function.

Then we have the ABC or ATP-binding cassette transporters. These are a large family of membrane proteins that use the energy from ATP hydrolysis to transport a wide range of substrates across cellular membranes. These transporters are found in all three domains of life: bacteria, archaea, and eukarya, highlighting their evolutionary conservation and essential role in cellular function. So here we can see an ABC system or ATP-binding cassette transporter system. These are protein complexes that use ATP hydrolysis to actively transport a variety of substances across cellular membranes.

They are found in prokaryotes, eukaryotes, and archaea. They play a key role in nutrient uptake, toxin export, as well as drug resistance. These transporters are highly versatile, moving a wide range of substrates such as ions, sugars, amino acids, and peptides, and are essential for cell survival in challenging environments. So you can see here in this ATP-binding cassette the ATP-binding region. So, here we can see a plasma membrane, and then this is the ATP-binding cassette transporter, and this is the ATP-binding region.

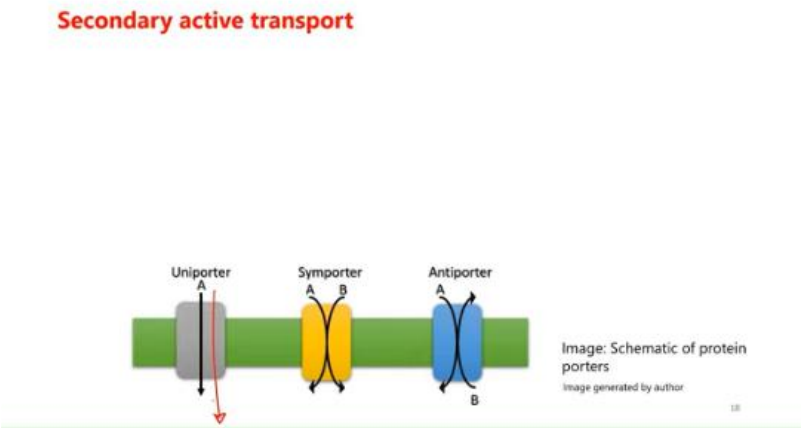


And this is a substrate; this is a substrate-binding protein. The substrate-binding protein binds to the protein and then interacts with the carrier protein, and you can see the substrate is transported inside. And because of the ATP-binding over here, there is a conformational change which facilitates the transport of the substrate from the external environment of the cell to the internal cytoplasm. So, basically, the ATP-binding region binds ATP and hydrolyzes it to ADP plus inorganic phosphate. The energy released from ATP hydrolysis is used to drive the transport of the substances.



The substrate binding protein binds to the specific substances that are being transported and ferries them to the membrane-spanning proteins. Let us now discuss the secondary active transport. Before that, let us understand some figures, as you can see here. Here, basically, we have a protein. We call it a uniporter.

This allows the movement of a substance or molecule only in one direction. It allows only the movement of a single species at a particular point in time. Then we have the symporters, where two unrelated molecules move together, and their movement may often be coupled. And then we have the antiporter, where the movement of two molecules is opposite to one another. So, when A moves inside, B will be moving outside.

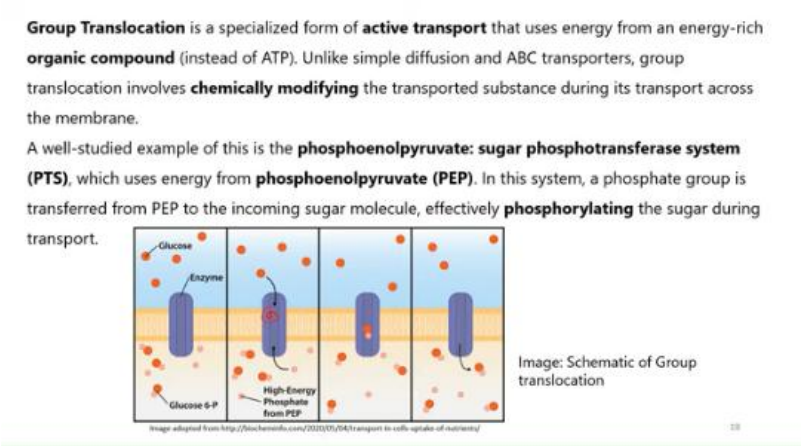


Now, the secondary active transport relies on energy from a proton motive force, which is created by electron transport processes. The protons accumulate outside the cell, creating a gradient. Simple transport involves uniport, where single substances are allowed to move in one direction. Then we have symport, where two substances move in the same direction,

and antiport, where two substances move in the opposite direction. This is facilitated by the specific protein porters, which we discussed just a few seconds ago.

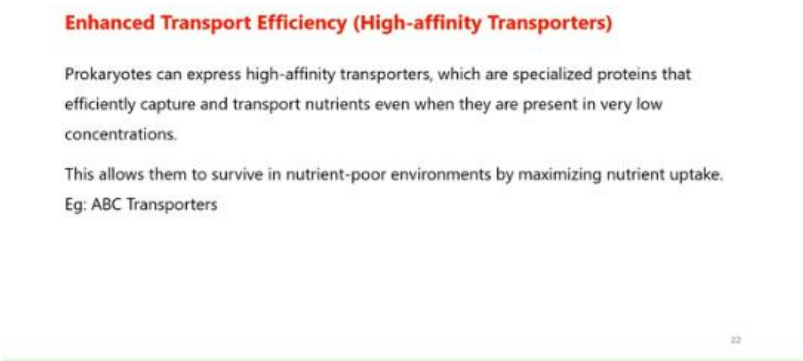
Then there we have the group translocation, which is a specialized form of active transport that uses energy from an energy-rich organic compound instead of ATP. Unlike simple diffusion in ABC transporters, group translocation involves chemically modifying the transported substance during its transport across the membrane. So, here we have this glucose, and you can see here an enzyme and then the high-energy phosphate from PEP, which you can see over here. So, this diagram basically shows the schematic of group translocation. So, one of the best-studied examples is the phosphoenolpyruvate sugar phosphotransferase system,

which uses energy from PEP. In this system, a phosphate group is transferred from PEP to the incoming sugar molecule. Here you can see this is a high-energy phosphate from PEP, and this is the incoming sugar molecule. And here, the phosphate group is transferred to the incoming sugar molecule, effectively phosphorylating the sugar during the transport. So, this glucose, which was outside, when it is internalized through this mechanism, will actually be phosphorylated.



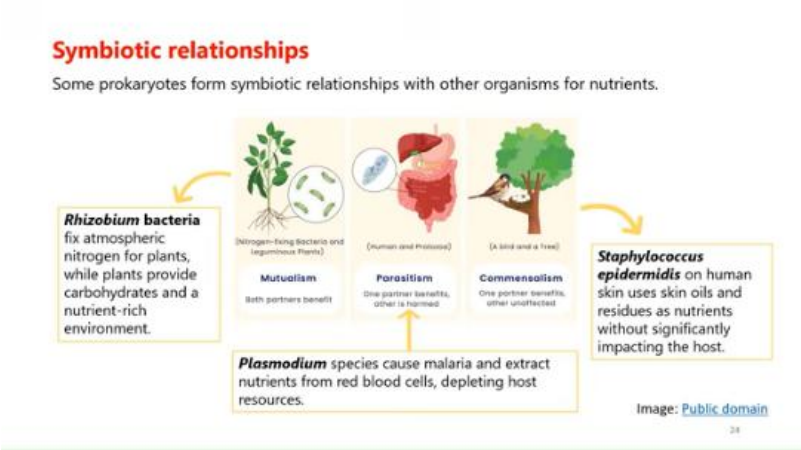
So, this is the glucose-6-phosphate. Let us now move to Section 2, where we will discuss the strategies for microbial survival and efficiency. Basically, this is done by enhanced transport efficiency or alternative metabolic pathways. Then certain special types of relationships, which we call symbiotic relationships, or biofilm formation, endospores, and cyst formations, which we have studied earlier. Let's start with the enhanced transport efficiency.

Procrease can express high affinity transporters which are specialized proteins that effectively capture and transport nutrients even when they are present in very low concentrations. This allows them to survive in nutrient-poor environments by maximizing the nutrient uptake. Some of the examples are the ABC transporters. Then there are alternative metabolic pathways in response to nutrient scarcity. Procreates can activate alternative metabolic pathways that allow them to utilize different substrates for energy and growth.



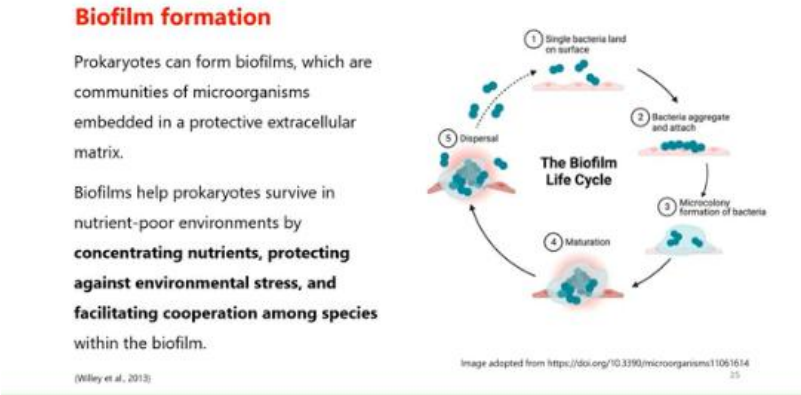
For instance, when glucose is limited, some bacteria can switch to using other sugars or even lipids and proteins as energy sources. This we will be discussing in detail in later part of this lecture. Some prokaryotes form symbiotic relationship with other organisms for nutrients and that may be mutualistic or parasitic or it may be a commensalism. In mutualism, both partners are benefited. In parasitism, one partner is benefited, the other is harmed.

In commensalism, one partner benefits, the other is unaffected. So some of the best example of mutualism is rhizobium bacteria, which fixes nitrogen, atmospheric nitrogen for plants, while plants provide carbohydrates and a nutrient-rich environment to the bacteria. Then we have parasites like Plasmodium species which causes malaria and extract nutrients from red blood cells depleting host resources and also causing damage or disease to the host. In conventionalism, we have the examples of Staphylococcus epidermidis, which is found on human skin. It uses the skin oils and residues as nutrients without significantly impacting the host.



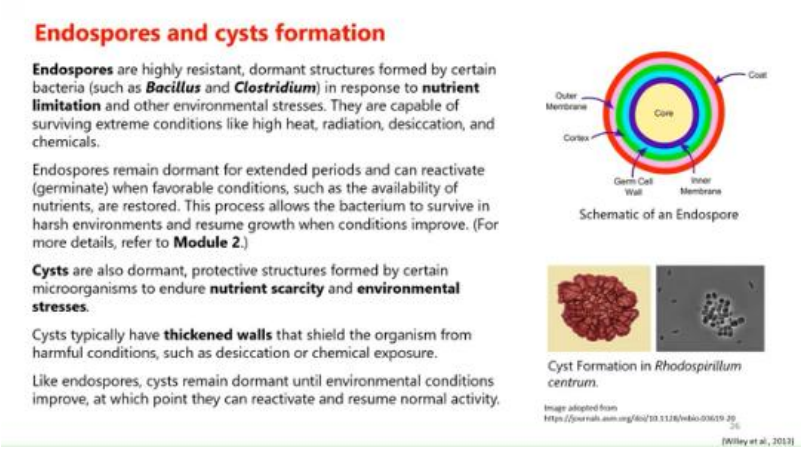
Biofilm formation is another survival strategy. Procreates can form biofilms which are communities of microorganisms embedded in a protective extracellular matrix. So this is a single bacteria which land on surface then the bacteria will aggregate and attach and then there is a micro colony formation of the bacteria and there is a maturation followed by the dispersal and then this cycle can go on. So we call it the biofilm life cycle.

These biofilms help prokaryotes survive in nutrient-poor environments by concentrating nutrients, protecting against environmental stress, and facilitating cooperation among species within the biofilm. Endospores and cyst formation are also one of the important survival strategies and here endospores are highly resistant dominant structures formed by certain bacteria such as Bacillus and Clostridium in response to nutrient limitation and other environmental stresses. They are capable of surviving extreme conditions like high heat, radiation, desiccation and chemicals. So this is a schematic of an endospore where we can see a core and then there is a inner membrane and surrounded by a gram cell wall and a cortex and then outer membrane and finally a coat. These endospores can remain dormant for extended periods and can reactivate, that is germinate, when favorable conditions such as the availability of nutrients are restored.

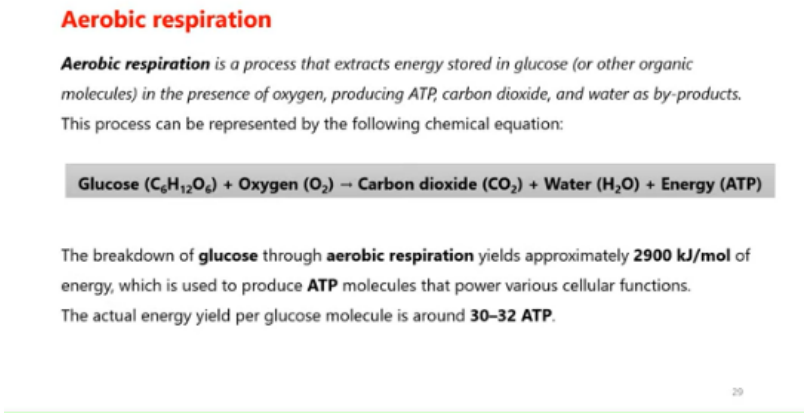


This process allows the bacterium to survive in harsh environments and resume growth when conditions improve. We have already discussed about these in details in module number 2. Then there are cysts which are also dormant protective structures formed by certain microorganisms to endure nutrient scarcity and environmental stresses. Cysts typically have thickened walls that seal the organism from harmful conditions such as desiccation or chemical exposure. Like endospore cysts remain dominant until environmental conditions turn favorable or improve, at which point they can reactivate and resume normal activity.

So you can see here the cyst formation in Rhodospirillum centrum. Let us now move on to section 3, where we will discuss the metabolic processes, mainly the various aerobic and anaerobic respiration processes. So, what is aerobic respiration? Aerobic respiration is a process that extracts energy stored in glucose or other organic molecules in the presence of oxygen, producing ATP, carbon dioxide, and water as byproducts. This process can be represented with the following chemical equation.

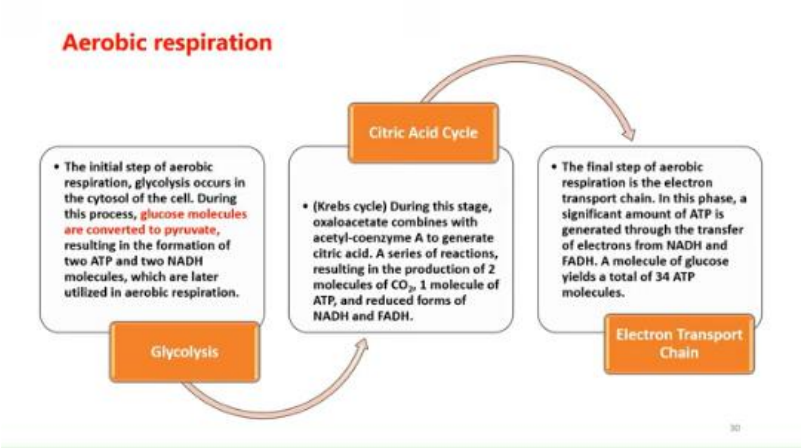


The equation shows glucose utilizing oxygen to form carbon dioxide, water, and energy in the form of ATP. The breakdown of glucose for aerobic respiration yields approximately 2900 kilojoules per mole of energy. This energy is used to produce ATP molecules that power various cellular functions. The actual energy yield per glucose molecule is around 32 ATP, and this is one of the most important sources of energy for the cell. So, let us look into the various steps involved in aerobic respiration.



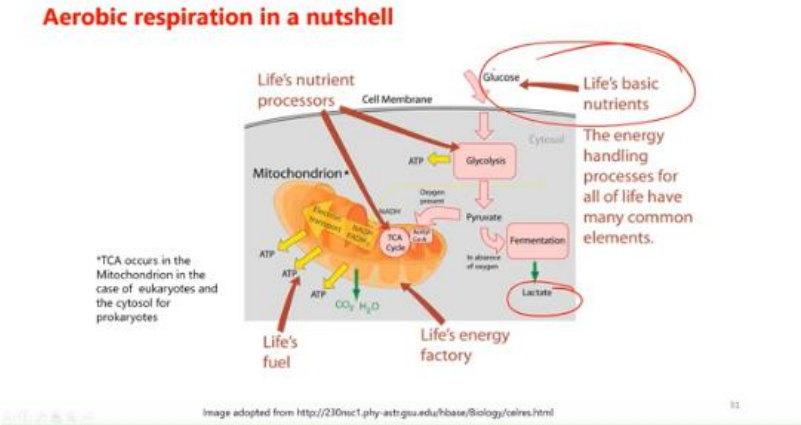
The first and foremost is glycolysis, which is the initial step of aerobic respiration. Glycolysis occurs in the cytosol of the cell. During this process, glucose molecules are converted to pyruvate, resulting in the formation of two ATP and two NADH molecules, which are later utilized in aerobic respiration. Then we have the Krebs cycle, during which oxaloacetate combines with acetyl coenzyme A to generate citric acid. A series of reactions results in the production of two molecules of carbon dioxide, one molecule of ATP, and a reduced form of NADH and FADH2.

Then, in the final step of aerobic respiration, is the electron transport chain in this phase. A significant amount of ATP is generated through the transfer of electrons from NADH and FADH2. A molecule of glucose yields a total of 34 ATP molecules. So, if we look into aerobic respiration in a NAD cell. So, this is the cell membrane, which delineates the external and internal environment of a

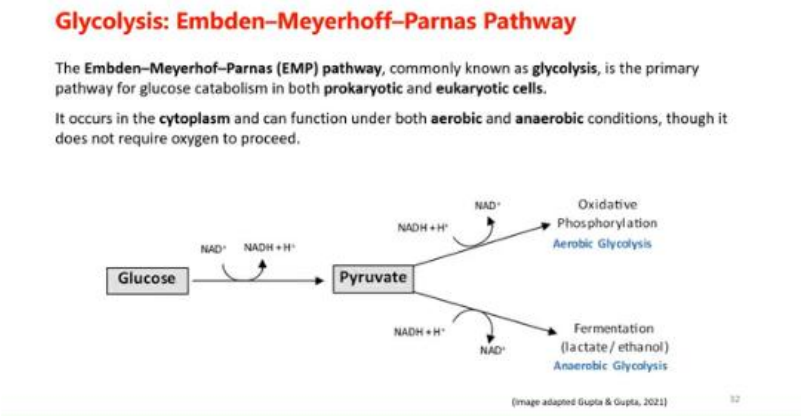


cell, and then glucose, which is life's basic nutrient, enters the cell by various transport mechanisms and undergoes glycolysis. The energy-handling process for all life forms has many common elements. So, this glucose undergoes glycolysis and produces some ATP molecules—two ATP molecules—and is also converted to pyruvate, which, in the absence of oxygen, will undergo fermentation and produce lactate. And in the presence of oxygen, it will be converted to acetyl-CoA, transported into the mitochondria, where it enters the TCA cycle. And from here, these NADH and FADH2 enter the electron transport chain, which produces many ATP molecules down the line.

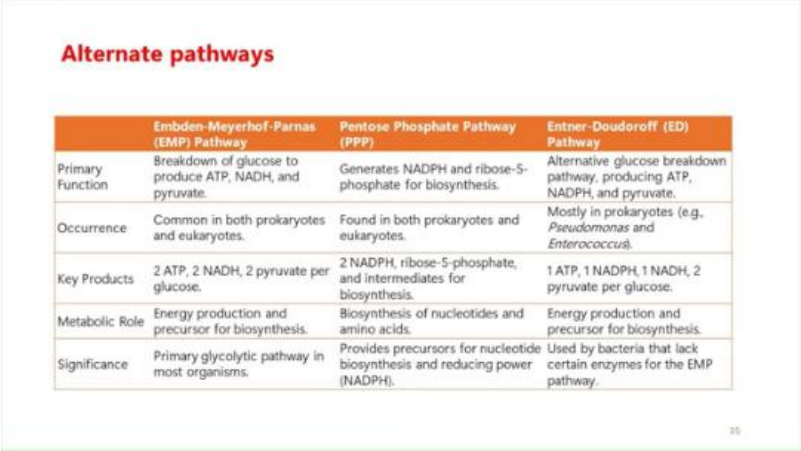
So, this is life's basic nutrient. And this is life's energy factory, which is the mitochondria, producing ATP, which is life's fuel. So, we'll be discussing many of these processes, as well as the fermentation process, towards the end of this lecture. So, let's start with glycolysis, which is one of the most important metabolic pathways. This is also called the Embden-Meyerhof-Parnas pathway or, commonly, glycolysis—a primary pathway for glucose catabolism in both prokaryotic and eukaryotic cells.



It occurs in the cytoplasm and can function under both aerobic and anaerobic conditions. It does not require oxygen to proceed. So basically, glucose is converted to pyruvate, where it may undergo oxidative phosphorylation or it may undergo the anaerobic pathway, which is the fermentation pathway, as we have shown in the earlier picture. Then there are alternate pathways like the pentose phosphate pathway and the Entner-Doudoroff pathway, which are alternative pathways to glycolysis. They play crucial roles in cellular metabolism, especially in prokaryotes.



The pentose phosphate pathway is involved in generating NADPH and ribose sugars for biosynthesis, while the Entner-Doudoroff pathway is important for energy production in some bacteria. Both pathways intersect with glycolysis at key intermediates. Here we can see a comparison between the EMP pathway, the PPP pathway, and the ED pathway from the point of view of primary function. The EMP pathway breaks down glucose to produce ATP, NADH, and pyruvate; in PPP, it generates NADPH and ribose 5-phosphate for biosynthesis. In ED, this is an alternative glucose breakdown pathway which produces ATP, NADPH, and pyruvate.



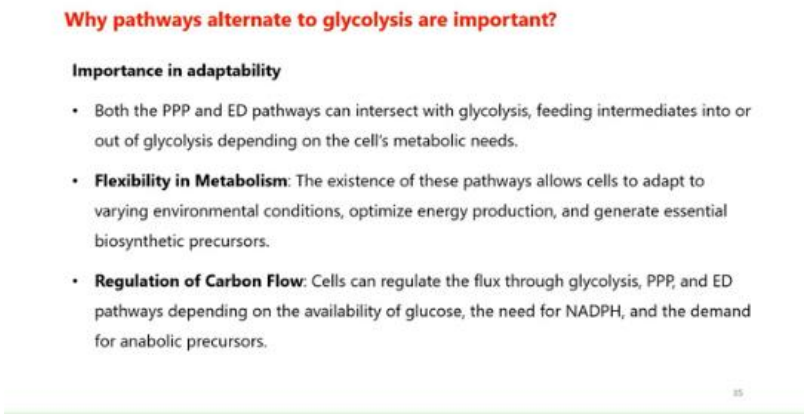
	Embden-Meyerhof-Parnas (EMP) Pathway	Pentose Phosphate Pathway (PPP)	Entner-Doudoroff (ED) Pathway
Primary Function	Breakdown of glucose to produce ATP, NADH, and pyruvate.	Generates NADPH and ribose-5-phosphate for biosynthesis.	Alternative glucose breakdown pathway, producing ATP, NADPH, and pyruvate.
Occurrence	Common in both prokaryotes and eukaryotes.	Found in both prokaryotes and eukaryotes.	Mostly in prokaryotes (e.g., *Pseudomonas* and *Enterococcus*).
Key Products	2 ATP, 2 NADH, 2 pyruvate per glucose.	2 NADPH, ribose-5-phosphate, and intermediates for biosynthesis.	1 ATP, 1 NADPH, 1 NADH, 2 pyruvate per glucose.
Metabolic Role	Energy production and precursor for biosynthesis.	Biosynthesis of nucleotides and amino acids.	Energy production and precursor for biosynthesis.
Significance	Primary glycolytic pathway in most organisms.	Provides precursors for nucleotide biosynthesis and reducing power (NADPH).	Used by bacteria that lack certain enzymes for the EMP pathway.

The EMP pathway is common in both prokaryotes and eukaryotes. The PPP pathway is also found in both prokaryotes and eukaryotes. The ED pathway is found mostly in prokaryotes like Pseudomonas and Enterococcus. Then, the key products of the EMP pathway are 2 ATP, 2 NADH, and 2 pyruvate per glucose. The key products of PPP are 2 NADPH, ribose 5-phosphate, and intermediates for biosynthesis.

Then we have the products of the ED pathway: 1 ATP, 1 NADPH, 1 NADH, and 2 pyruvate per glucose. The metabolic role played by the EMP pathway is energy production and precursor for biosynthesis. In PPP, it is the biosynthesis of nucleotides and amino acids. In the ED pathway, it is energy production and precursor for biosynthesis. The significance of the EMP pathway is that it is the primary glycolytic pathway in most organisms.

PPP provides precursors for nucleotides in biosynthesis and reducing power. And ED here is used by bacteria that lack certain enzymes for the EMP pathway. Why are the alternative pathways important? It is important from the point of view of adaptability. Both the PPP and ED pathways can intersect with glycolysis, feeding intermediates into or out of glycolysis depending on the cell's metabolic needs.

Then it offers flexibility in metabolism. The existence of these pathways allows cells to adapt to varying environmental conditions, optimize energy production, and generate essential biosynthetic precursors. They also help in the regulation of carbon flow. Cells can regulate the flux through glycolysis, PPP, and ED pathways, depending on the availability of glucose, the need for NADPH, and the demand for biosynthetic precursors. Let us now move on to the next important cycle, which is the TCA cycle or tricarboxylic acid cycle.



This is also known as the Krebs cycle. Here, it oxidizes acetyl coenzyme A from amino acids, fatty acids, and carbohydrates, producing NADH, FADH2, and ATP in prokaryotes.

It occurs in the cytoplasm, while in eukaryotes, it takes place in the mitochondria. This is the schematic of the TCA cycle. So, this pyruvate enters and is then converted into acetyl coenzyme A, which enters the TCA cycle and produces NADH, FADH2, and ATP.

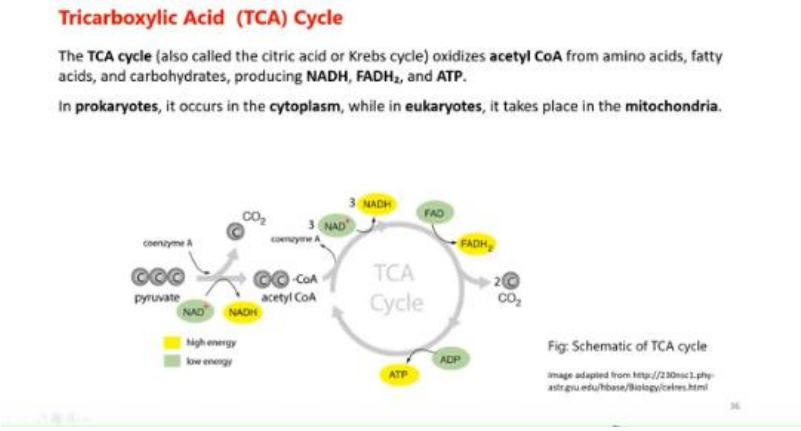
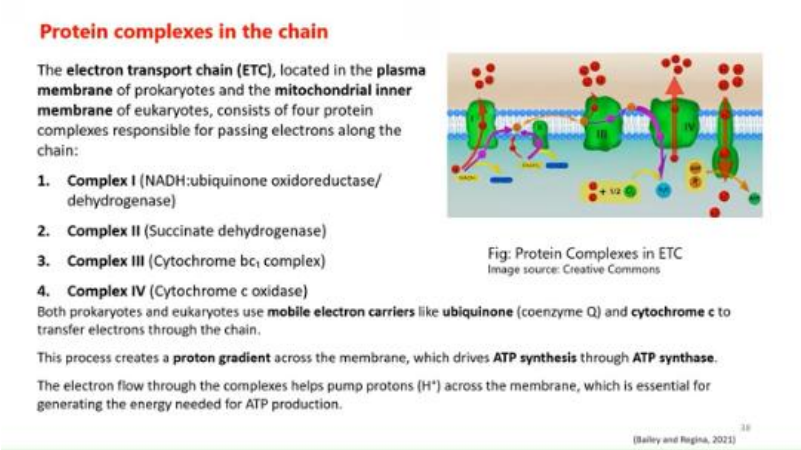


Fig: Schematic of TCA cycle

Then comes the electron transport and energy generation. The ETC is responsible for the majority of ATP production in the cell. The electron flow through the chain transfers electrons to oxygen and pumps protons across the membrane. This creates an electrochemical gradient. The return of protons through ATP synthase drives ATP synthesis, generating cellular energy.

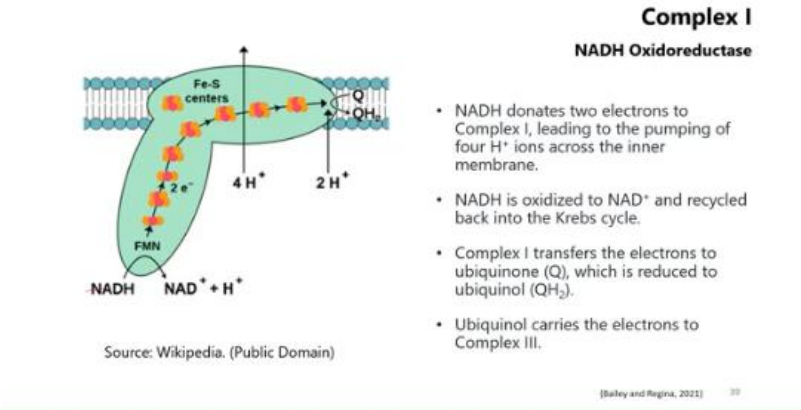
So, this ETC has various protein complexes. It is located in the plasma membrane of prokaryotes and the mitochondrial inner membrane of eukaryotes. It consists of four proteins: 1, 2, 3, and 4, which are named complex 1, 2, 3, and 4. Complex 1 is NADH ubiquinone oxidoreductase.

An alternate name is dehydrogenase. Complex 2 is succinate dehydrogenase. Complex 3 is cytochrome. BC1 complex and complex 4 comprise cytochrome C oxidase. Both prokaryotes and eukaryotes use mobile electron carriers like ubiquinone and cytochrome C to transfer electrons through the chain.



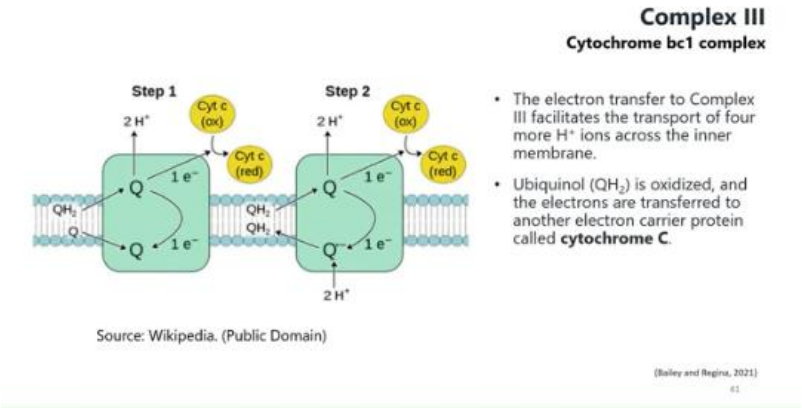
This process creates a proton gradient across the membrane, which drives ATP synthesis through ATP synthase. We will try to look into the structure and function of ATP synthesis at a later point in time. The electrons flow through the complexes and help pump protons across the membrane, which is essential for generating the energy needed for ATP production. So, let us see the role of complex one or NADH oxidoreductase. NADH donates two electrons.

to complex I, leading to the pumping of four hydrogen ions across the inner membrane. NADH is oxidized to NAD+ and recycled back into the Krebs cycle. Complex I transfers the electrons to ubiquinone Q, which is reduced to ubiquinol or QH2. Ubiquinol carries the electrons to complex III. In complex II, we have succinate dehydrogenase.



FADH transfers electrons to complex II, which are then passed to ubiquinone. Ubiquinone coenzyme transfers electrons from complex I and II to complex III. It is a lipid-soluble carrier that diffuses within the plasma membrane. Ubiquinol Q is reduced to ubiquinol QH2, which carries the electrons to complex III.

So complex III is basically the cytochrome bc1 complex. The electron transfer to complex III facilitates the transfer of four more hydrogen ions across the inner membrane. Ubiquinol is oxidized, and the electrons are transferred to another electron carrier protein called cytochrome C. This is basically the part of complex IV where cytochrome C is oxidized and is the main player. Cytochrome C transfers electrons to complex IV, which pumps protons across the inner mitochondrial membrane. The electrons are then passed to oxygen, which splits and combines with hydrogen to form water, as you can see here.



This process is crucial for the continuation of the electron transport chain and for cellular energy production. Cytochrome C is a mobile electron carrier. It transfers electrons from complex III to complex IV. In prokaryotes, it is often membrane-bound or loosely associated with the membrane. Let us now study a little bit about ATP synthase.

This is the structure of ATP synthase, which is basically a membrane-bound protein. So, this is the membrane, and this is the cytoplasmic side, while this is the external side, okay. Now, ATP synthase is a membrane-bound enzyme complex that synthesizes ATP from ADP and inorganic phosphate using energy from a proton gradient. In eukaryotes, it is located in the inner mitochondrial membrane. While in prokaryotes, it is found in the plasma membrane.

ATP synthase

ATP synthase is a membrane-bound enzyme complex that synthesizes ATP from ADP and inorganic phosphate (Pi) using energy from a proton gradient.

In eukaryotes, it is located in the inner mitochondrial membrane, while in prokaryotes, it is found in the plasma membrane.

ATP synthase functions as a molecular motor, playing a crucial role in cellular energy production.

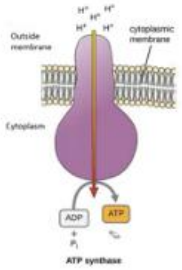


Fig: Schematic of ATP Synthase

ATP synthase functions as a molecular motor, playing a crucial role in cellular energy production. Let us now briefly discuss anaerobic respiration. Anaerobic respiration occurs in the absence of oxygen, where microorganisms are forced to use alternative terminal electron acceptors, such as nitrate, sulfur, sulfate, or carbonate, and through this, they generate energy. While anaerobic respiration is less efficient than aerobic respiration, it allows organisms to survive and produce energy in oxygen-depleted environments. So, based on the terminal electron acceptors, anaerobic respiration can be named as nitrate respiration, where nitrate is used as the terminal electron acceptor.

ANAEROBIC RESPIRATION

Anaerobic respiration occurs in the absence of oxygen, where microorganisms use alternative **terminal electron acceptors** (such as **nitrate**, **sulfate**, **sulfur**, or **carbonate**) to generate energy.

While **anaerobic respiration** is less efficient than **aerobic respiration**, it allows organisms to survive and produce energy in **oxygen-depleted environments**.

Examples of Anaerobic Respiration:

- **Nitrate respiration**: Nitrate (NO_3^-) is used as the terminal electron acceptor.
- **Sulfate respiration**: Sulfate (SO_4^{2-}) serves as the electron acceptor.
- **Sulfur respiration**: Sulfur (S) is reduced to hydrogen sulfide (H_2S).
- **Carbonate respiration**: Carbonate (CO_3^{2-}) is used as the electron acceptor.

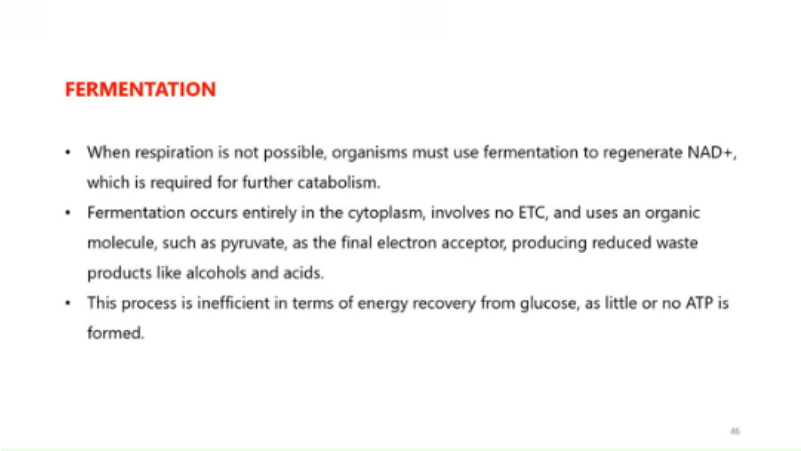
Other Terminal Electron Acceptors:

Some bacteria can also use **ferric iron (Fe^{3+})**, **manganese (Mn^{4+})**, and various **organic compounds** (e.g., humic substances) as terminal electron acceptors in anaerobic respiration.

Or it may be sulfate respiration, where sulfate serves as the electron acceptor. Or sulfur respiration, where sulfur is reduced to hydrogen sulfide. Then, it may be carbonate respiration, where carbonate is used as the electron acceptor. Then, we have other terminal electron acceptors. In the case of certain bacteria, they can also use ferric iron, manganese, or various other organic compounds, such as humic substances, as terminal electron acceptors in anaerobic respiration.

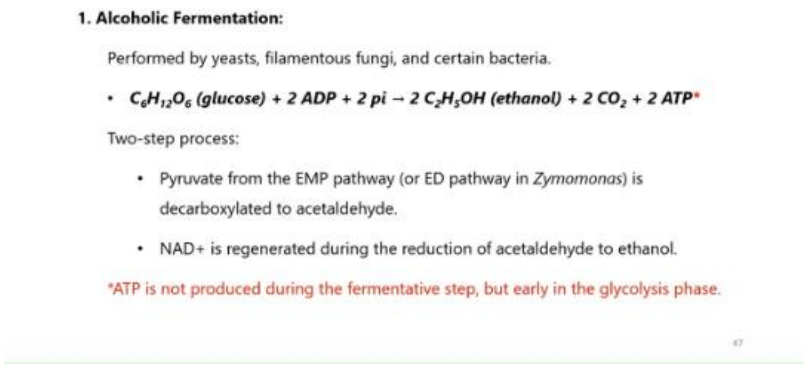
Now let us move to the last section of this lecture, which is fermentation. Briefly, we have various kinds of fermentation: alcoholic, lactic acid, mixed acid, 2,3-butanediol, propionic acid, or butyric acid fermentation. Let us try to understand what fermentation is. Basically, when respiration is not possible, organisms must use fermentation to regenerate NAD+, which is required for further catabolism. Fermentation occurs entirely in the cytoplasm, involves no ETC,

and uses an organic molecule such as pyruvate as the final electron acceptor, except for producing reduced waste products like alcohols and acids. And these are actually commercially available products. The process is inefficient in terms of energy recovery from glucose. As little or no ATP is formed, but from the product point of view, products like alcohols and acids are actually very commercially important. So in alcoholic fermentation, we have organisms like yeast, filamentous fungi, and certain bacteria which carry out alcoholic fermentation.

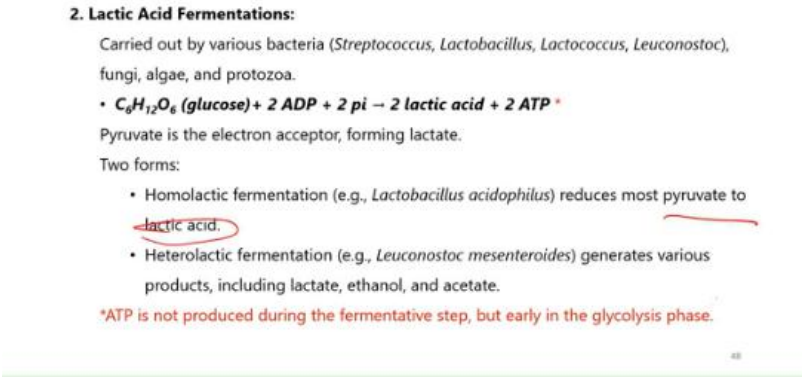


So here you can see glucose is getting converted to ethanol in the presence of 2 ADP and 2 inorganic phosphate, also producing carbon dioxide in the process. ATP is also produced here overall, but this ATP is not produced during the fermentative step. It was actually produced earlier in the glycolysis phase where glucose was broken down. This is basically a two-step process. Pyruvate from the EMP pathway or ED pathway in Xyminomonas is decarboxylated to acetaldehyde.

NAD+ is regenerated during the reduction of acetaldehyde to ethanol. Then we have the lactic acid fermentation, where various bacteria like Streptococcus, Lactobacillus, Lactococcus, Leuconostoc, fungi, algae, and protozoa convert glucose in the presence of ADP and inorganic phosphate to lactic acid. Here also, ATP is produced, but this ATP is not produced in the fermentative step. It was produced earlier in the glycolysis phase. Here again, pyruvate is the electron acceptor, forming lactate.



There are two forms. Homolactic fermentation, for example, Lactobacillus acidophilus reduces most pyruvate to lactic acid. Then there is heterolactic fermentation, as carried out by Leuconostoc mesenteroides, which generates various products including lactate, ethanol, and acetate. Now we have mixed acid fermentation. These are carried out by E. coli and related facultative anaerobes.



2. Lactic Acid Fermentations:

Carried out by various bacteria (*Streptococcus, Lactobacillus, Lactococcus, Leuconostoc*), fungi, algae, and protozoa.

- $C_6H_{12}O_6$ *(glucose)* + 2 ADP + 2 pi → 2 lactic acid + 2 ATP *

Pyruvate is the electron acceptor, forming lactate.

Two forms:

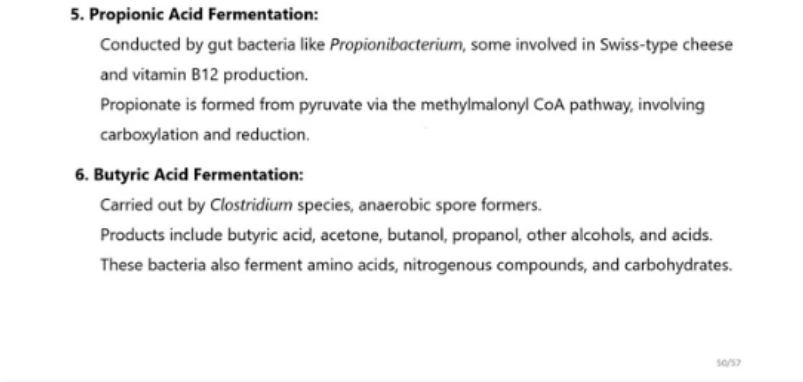
- Homolactic fermentation (e.g., *Lactobacillus acidophilus*) reduces most pyruvate to lactic acid.
- Heterolactic fermentation (e.g., *Leuconostoc mesenteroides*) generates various products, including lactate, ethanol, and acetate.

*ATP is not produced during the fermentative step, but early in the glycolysis phase.

Products include lactate, acetate, small quantities of ethanol, and formate. Some organisms can further reduce formate to hydrogen and carbon dioxide. Then we have the 2,3-butanediol fermentation performed by Enterobacter, Ureaplasma, Capsula, and Sericea. This is similar to mixed acid fermentation but generates butanediol along with ethanol and acids. Then we have the propionic acid fermentation.

This is mostly carried out by gut bacteria like Propionibacterium. Some are involved in sweet type C's and vitamin B12 production. Propionate is formed from pyruvate via the methylmalonyl-coenzyme pathway involving carboxylation and reduction. Then we have the butyric acid fermentation, which is carried out by Clostridium species, which are anaerobic spore formers. Products include butyric acid, acetone, butanol, propanol, other alcohols, and acids.

These bacteria also ferment amino acids, nitrogenous compounds, and carbohydrates. So with this, we come to the end of this lecture number one of module three. Thank you for your patient hearing. Amen.



5. Propionic Acid Fermentation:

Conducted by gut bacteria like *Propionibacterium*, some involved in Swiss-type cheese and vitamin B12 production.

Propionate is formed from pyruvate via the methylmalonyl CoA pathway, involving carboxylation and reduction.

6. Butyric Acid Fermentation:

Carried out by *Clostridium* species, anaerobic spore formers.

Products include butyric acid, acetone, butanol, propanol, other alcohols, and acids.

These bacteria also ferment amino acids, nitrogenous compounds, and carbohydrates.