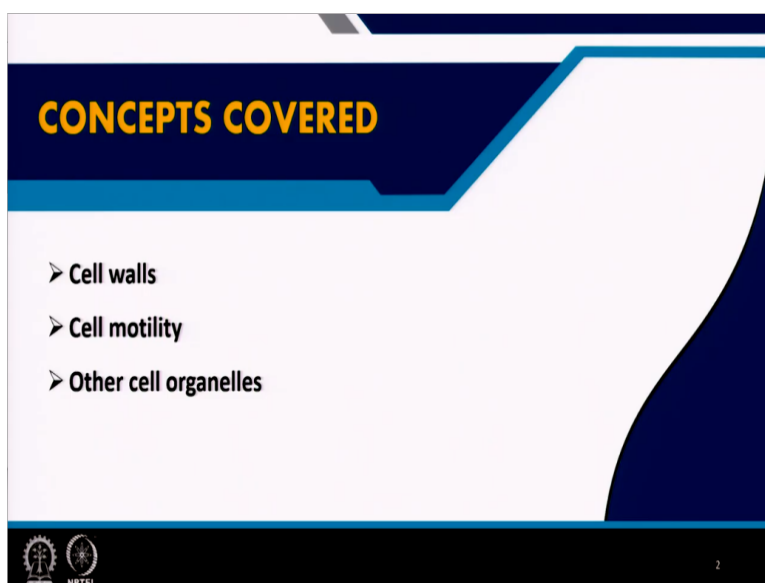


Environmental Chemistry and Microbiology
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Module - 8
Lecture - 41
Cell Biology - IV

Welcome everyone to the last part of Cell Biology. This is lecture number 41.

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So, we have already looked at cell walls and cell motility. We will now cover the remaining cell organelles.

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Capsule and slime layers

Capsule	Slime Layer	Biofilm

- A bacterial capsule excludes India Ink when dyed while the cells are stained black.
- The slime layer is not able to exclude India Ink while the cells are darker than the organic matter of the slime layer.
- Biofilms are composed of many cells and their outer barriers and stick to surfaces because of the slime layers
- The primary functions of both capsules and slime layers are protection and adhesion.

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So, when we think about cells, we should also be thinking about some of the other parts of the cell which are not always present, but are very important. So, here we have two parts; one is the capsule and the second is the slime layer. Often, cells in the environment are found in two forms. They can be encapsulated or they can be without a capsule. So, in the first case, you can see a diagram that shows you a cell with a capsule around it.

Now, this capsule can be seen in a very interesting way. So, if you take an encapsulated bacteria and dye it with India ink, it gets dyed. The cell itself will be dyed, but the capsule is not dyed. So, against a dark background, you can see the cells as very dark; the capsule is lighter in colour; and the other part of the background is very dark compared to the capsule part. So, that is how you can visualise the fact that there is a capsule around certain types of cells. And in chlorination, what we have found is that; it is there in the literature, that the capsule provides protection for these bacteria against disinfection. So, these encapsulated bacteria are more likely to survive disinfection compared to the non-encapsulated cells.

Then, cells are often found with what is called a slime layer. This slime layer, when it is dyed, is not going to be excluding India ink entirely. So, you can see that the cells will pick up most of the ink or the dye; and the organic matrix which is the slime layer will be dark in comparison to the background, but it has less of the dye, compared to the cells. So, you can see it as shown over here in this schematic. Now, why are these two important? Like I already said about the capsule, the slime layer is what allows the biofilm to attach itself to surfaces. I have already mentioned in the previous topics that the slime layer is when you know that there is bacterial growth on what seems to be a dry surface. So, the best examples are your bathroom fixtures, your buckets, your mugs, your sinks and basins. These are areas which are seemingly dry but they are transiently in contact with water. And there is sufficient moisture and nutrients in the water to allow for the growth of bacteria. So, very often, when I say, on rocks and on several other dry places, you will find a sliminess to the surface. And that is because of biofilm growing on these surfaces. And if they were to be examined, then this would be the result. So, you have this slime layer which can be seen on these surfaces which are basically providing a layer for the cells to grow; so, it is a layer for adhesion to the surface and it gets protection. This organic matrix provides protection against disinfection as well. So, in water supply systems, if you have biofilms growing on the inner side of the pipe surfaces and so on, they will be protected from being destroyed by chlorine or any other

disinfectant because of this slime layer. This slime layer acts as a protection for the bacterial cells.

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Structures internal to the cell wall

- ✓ Cytoplasmic or plasma membrane
- ✓ Nuclear Area or Nucleus
- Inclusions (prokaryotes only)
- Gas vesicles (prokaryotes only)
- Endospores (prokaryotes only)
- Mitochondria or Chloroplasts (eucaryotes only)
- ✗ Cytoplasm
- ✗ Ribosomes

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Then we come to structures that are internal to the cell wall. So, we have already taken a look at the cytoplasmic or plasma membrane and we have looked at the nuclear area. So, now we are going to look at the nucleus or the nuclear area. And now we are going to look at the remaining organelles shown over here. So, we are going to look at inclusions and gas vesicles as well as endospores. These are present in prokaryotes. And mitochondria, chloroplasts are present in eukaryotes only.

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Inclusions – sulfur globules

Sulfur deposits created when S^{2-} is available (See Fig. 4.53, Brock, 2003)
When S^{2-} is not available, deposits are used and oxidized to SO_4^{2-}

Inclusions – carbon and energy reserves

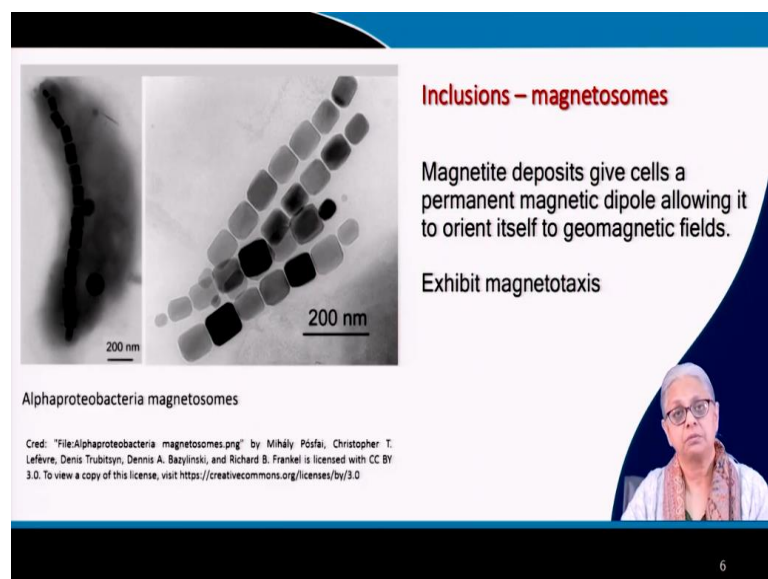
- Inclusions of poly- β -hydroxybutyrate are found in some bacteria (see Fig. 4.52, Brock, 2003).
- Monomeric unit shown in top right of slide.
- Globules of this polymer are formed when there are high substrate concentrations and serve as carbon or energy sources when they are lacking in the environment.

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Now, these inclusions are what the bacteria produce when there are conditions where these nutrients, a very important nutrients, essential nutrients are present in the environment. So,

they create these inclusions or storage granules. So, inclusion means, a storage granule that is inside the cell. So, sulfur deposits are created when sulfide is available. So, you can refer to the figure in the textbook. And we also have another one which I will come to. When sulfide is not available in the environment, you have certain bacteria, the sulfur bacteria, where they can use these deposits and oxidize them to sulfate. So, sulfur oxidizing bacteria will be able to create these sulfur globules. You also have another compound for carbon storage. Now, poly-beta-hydroxybutyrate, which is called biodegradable plastic; at this time, it is very popular these days. This is the form of biodegradable plastic. It serves as a carbon as well as energy source for certain bacteria. I have shown you the monomeric unit over here. Globules of this polymer are formed when the substrate concentration in the environment is high. And this will help the bacteria to survive famine. So, famine in the sense of nutrient availability. So, when there are insufficient nutrients in the environment of the particular bacterial cell, it can utilize these storage granules for both mass as well as energy. So, these are survival mechanisms that have been developed by bacteria to survive when the environmental conditions are hostile.

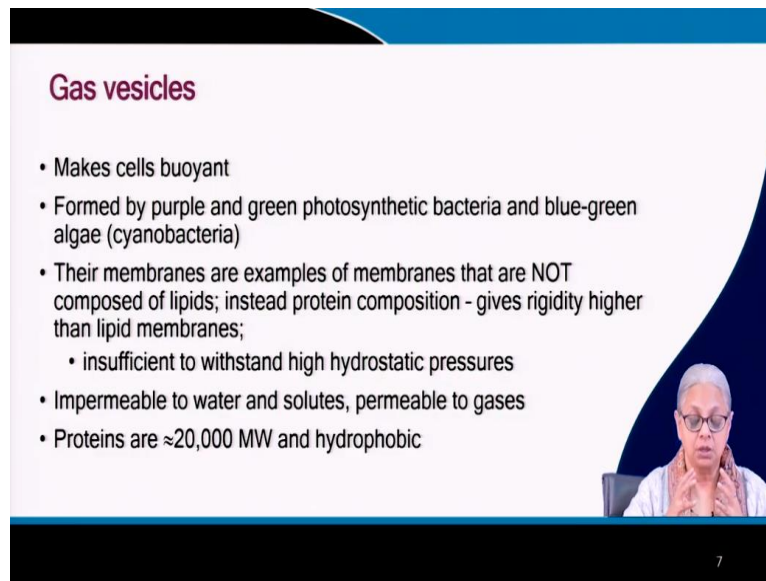
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Then, we come to other inclusions like magnetosome. So, there are certain types of bacteria. So, this is an alphaproteobacteria which has magnetosomes. And you can see that it has created these deposits of magnetite. Magnetite is a ferric mineral; and it gives the cells a permanent magnetic dipole. And you know that the earth has geomagnetic fields. So, the bacteria has been able to orient itself. So, entire colonies of bacteria orient themselves to the geomagnetic field. This is also considered an example of magnetotaxis. So, these bacterial

colonies will move in particular directions in response to the geomagnetic fields. So, fossil evidence of these kinds of bacteria have been found.

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Gas vesicles

- Makes cells buoyant
- Formed by purple and green photosynthetic bacteria and blue-green algae (cyanobacteria)
- Their membranes are examples of membranes that are NOT composed of lipids; instead protein composition - gives rigidity higher than lipid membranes;
 - insufficient to withstand high hydrostatic pressures
- Impermeable to water and solutes, permeable to gases
- Proteins are $\approx 20,000$ MW and hydrophobic

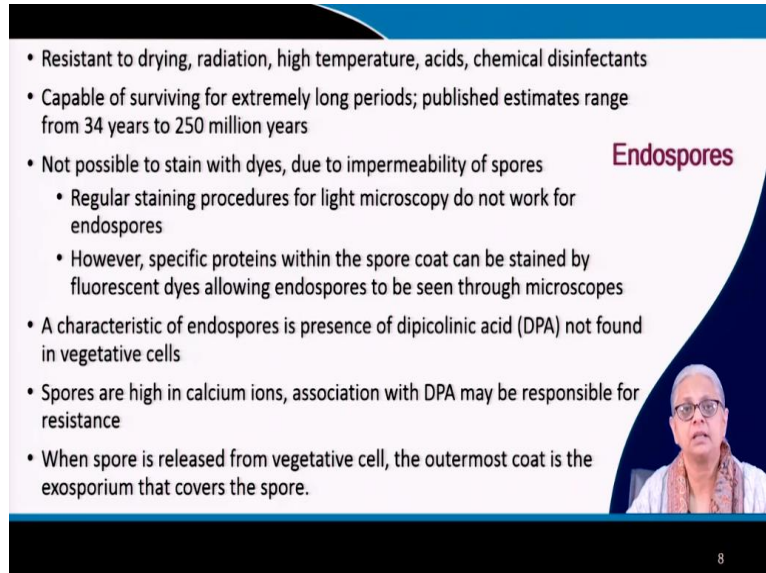
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Let us look at the next organelle and that is gas vesicles. Now, these gas vesicles are kind of like bubbles inside the bacterial cell. And that helps the bacterial cell or the algal cell to rise to the surface. It gives them buoyancy. This buoyancy is very important for photosynthetic bacteria and cyanobacteria or blue-green algae. So, these photosynthetic bacteria need to harvest solar energy. So, they can maximize their harvesting of solar energy by remaining at the top of the water column. So, these membranes are not made out of lipids; instead they are made out of proteins. And these proteins give them a rigidity. So, like I said, they are bubble like structures, because they are able to create; they are able to entrap gases; and those gases will give them buoyancy and allow them survival advantage by bringing them to the top of the water column.

However, they are not able to withstand high hydrostatic pressures. There is a very nice figure in the textbook that gives you a demonstration, experimental demonstration of the fact that they cannot withstand high hydrostatic pressures. So, let us say you take pond water in 2 different bottles, seal it without headspace and then hit one of the bottles with a hammer. Now, that hammer causes very high hydrostatic pressure. And you will find that the cells which were floating at the top will crash, and they will settle to the bottom as sediment. So, this is the proof that these membranes are rigid but not very rigid. They are not extremely rigid, they are somewhat rigid. These membranes are impermeable to water and solutes; and

permeable only to gases. They are made out of proteins that are approximately 20,000 in terms of molecular weight and these are hydrophobic proteins. That allows these proteins to create a bubble-like structure.

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Endospores

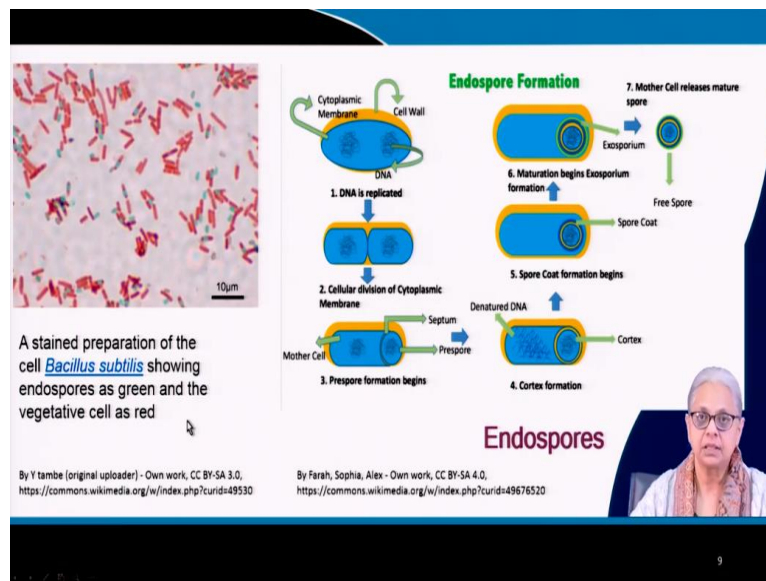
- Resistant to drying, radiation, high temperature, acids, chemical disinfectants
- Capable of surviving for extremely long periods; published estimates range from 34 years to 250 million years
- Not possible to stain with dyes, due to impermeability of spores
 - Regular staining procedures for light microscopy do not work for endospores
 - However, specific proteins within the spore coat can be stained by fluorescent dyes allowing endospores to be seen through microscopes
- A characteristic of endospores is presence of dipicolinic acid (DPA) not found in vegetative cells
- Spores are high in calcium ions, association with DPA may be responsible for resistance
- When spore is released from vegetative cell, the outermost coat is the exosporium that covers the spore.

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Then we come to endospores. Endospores are what the bacteria create when they are in an extremely hostile environment. So, let us say there is less moisture; or there is radiation, harmful radiation; or the temperature is too high; or the acid concentration in the environment is too high, meaning the pH is too low; and the chemical disinfectants are present. So, under any of these conditions, the bacteria is unable to survive in what we call a vegetative state. It cannot survive, it cannot reproduce. So, what does it do? It creates a different organelle called the endospore, just to survive in a hostile environment. In the published literature, there are claims literally by researchers saying that they have been able to isolate endospores from anywhere from 34 years to 250 million years. These are published reports in the literature.

It is not possible to stain these endospores with dyes. They are relatively impermeable to dyes. You need; the regular staining procedures that we use in light microscopy, they do not work for endospores. And there are specific proteins within the spore core that can be stained by fluorescent dyes. And that allows us to see endospores under the optical microscopes.

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Here is an example of endospores of *Bacillus subtilis*. So, you have this stained preparation. You can see, the green structures are the endospores and the red cells are the vegetative cells. These spores have very high calcium concentrations in combination with dipicolinic acid (DPA). Now, this dipicolinic acid is produced by the vegetative cells to create these endospores. So, it is present only in the endospores, not in the vegetative cell.

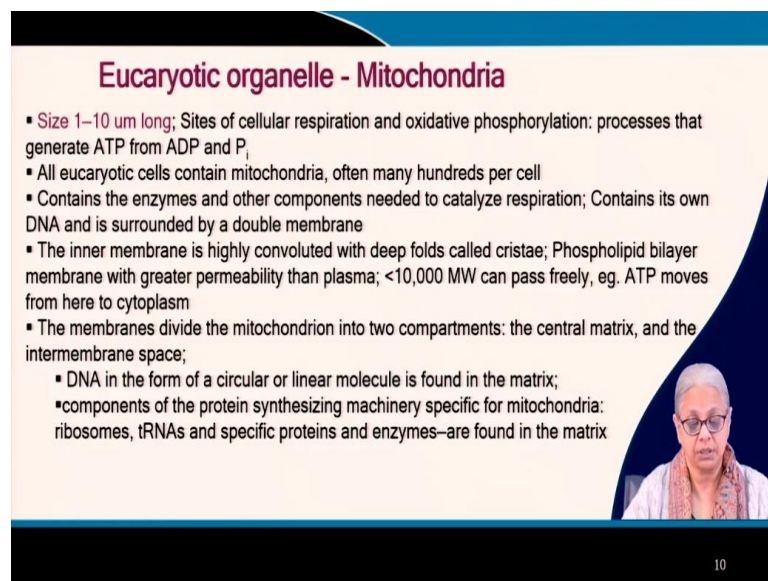
The calcium is assumed to be associated with DPA, and that may be providing resistance of the endospore to all these hostile conditions that are mentioned at the top. When the spore is released from the vegetative cell, the outermost coat is called the exosporium that provides protection to the spores.

So, let us just go to how endospores are formed. The first step is the vegetative cell. The vegetative cell is unable to reproduce. That is the reason for producing the endospore. So, this vegetative cell is going to replicate its DNA. So, the DNA has been replicated. Everything, the first part is the same as binary fission. So, you have replication of the DNA; cellular division of the cytoplasmic membrane; but the difference is that the daughter cells which should be equal and capable of living freely will not happen. So, here you have the mother cell. And you have the spore. The pre-spore is formed. So, a septum is formed, that separates the spore from the mother cell. In the next step, a cortex is formed around the spore. So, this DNA has been protected by the formation of the cortex. The DNA in the mother cell has been destroyed, because the mother cell is no longer producing. The spore coat is formed and this

endospore is now mature enough to survive in the environment, provided the exosporium or the final coat is there.

So, you have layers. You have the cortex, the spore coat, the exosporium. And when it is released, it is your independent endospore. Now, this endospore is not going to reproduce. It will remain in dormant form until the environmental conditions become conducive to reproduction. So, reproduction can take 30 years or millions of years. So, that is the nature of endospores. That is how the bacterial cell as a species is capable of surviving even millions of years.

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Eucaryotic organelle - Mitochondria

- Size 1–10 μm long; Sites of cellular respiration and oxidative phosphorylation: processes that generate ATP from ADP and P_i
- All eucaryotic cells contain mitochondria, often many hundreds per cell
- Contains the enzymes and other components needed to catalyze respiration; Contains its own DNA and is surrounded by a double membrane
- The inner membrane is highly convoluted with deep folds called cristae; Phospholipid bilayer membrane with greater permeability than plasma; $<10,000$ MW can pass freely, eg. ATP moves from here to cytoplasm
- The membranes divide the mitochondrion into two compartments: the central matrix, and the intermembrane space;
 - DNA in the form of a circular or linear molecule is found in the matrix;
 - components of the protein synthesizing machinery specific for mitochondria: ribosomes, tRNAs and specific proteins and enzymes—are found in the matrix

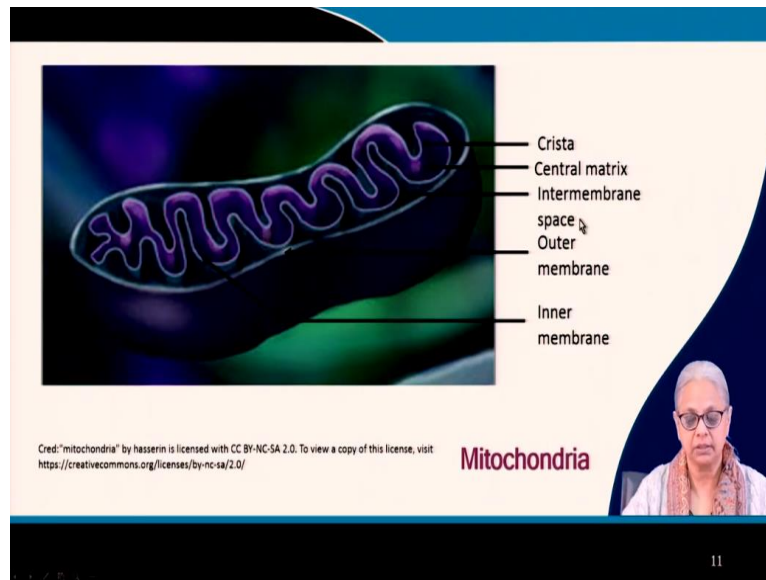
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So, eukaryotic organelles; we have mitochondria and chloroplasts. These are the organelles that are involved in ATP synthesis. So, generation of ATP is done by the mitochondria as well as the chloroplasts. Chloroplasts exist in photosynthetic organisms; and mitochondria in non-photosynthetic organisms or the eukaryotes. The size of mitochondria is about 1 to 10 microns long. These are the sites of both cellular respiration and oxidative phosphorylation.

And these are the processes. These are long words which you do not really understand at this point, unless you have studied some of this. But we will be covering this in detail in module 9. So, cellular respiration and oxidative phosphorylation is what helps any of these eukaryotes to generate ATP from ADP and phosphate. So, adenosine triphosphate (ATP) is generated when adenosine diphosphate (ADP) combines with another phosphate to form ATP.

All eukaryotic cells contain mitochondria; and they may be having hundreds of mitochondria. Mitochondria includes enzymes and other proteins needed to catalyze respiration. So, proteins; when I say proteins, I mean enzymes that are needed to catalyze respiration. It contains its own DNA and it is surrounded by a double membrane.

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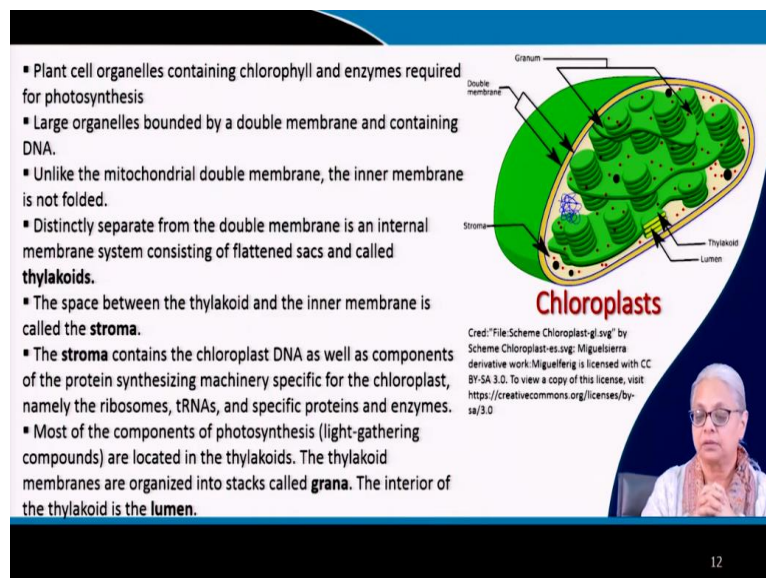
So, let me show you a graphic that shows you the mitochondria that exist within a eukaryotic cell. It does not exist in prokaryotes. And in fact, it is the lack of existence in prokaryotes and the size that has given rise to what is called the endosymbiotic theory. The endosymbiotic theory is that the prokaryotic cells have been engulfed by larger prokaryotic cells. And that is how the eukaryotic cell was born.

So, the mitochondria resembles a modern prokaryotic cell in terms of size, in terms of its double membrane. So, you have the outer membrane; you have the inner membrane; and you can see that the inner membrane is heavily folded. So, this is a convoluted inner membrane. There is a space; there is a lot of space between these folds and that is called the intermembrane space. And then you have the central matrix within the inner membrane. The folds are called crista; crista or cristae. So, this inner membrane is folded for a reason. Remember that ATP generation; I have already given you some idea that ATP generation happens at the site of the plasma membrane. So, the greater the length of the plasma membrane, the greater the amount of ATP that can be generated.

That is why the inner membrane is highly folded. So, it has deep folds called cristae that allow more ATP to be generated. Then you have phospholipid bilayer membrane, which has higher permeability than the plasma membrane. So, that is another advantage. Molecules less than 10,000 molecular weight can pass in and out freely. ATP has to move in and out of this cytoplasm of the mitochondria.

So, we are dealing with something that is very different from the prokaryote. The membranes can divide the mitochondrion into two compartments, the central matrix and the intermembrane space. So, the central matrix has its own DNA. And this is another argument in favour of the endosymbiotic theory. Then we have components of the protein synthesizing machinery that is specific for the mitochondria. So, the mitochondria has its own ribosomes; it has its own transfer RNAs; it has specific proteins and enzymes; all of them are found within the central matrix. And these are all the arguments that are provided in favour of the endosymbiotic theory. We will come to that much later.

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- Plant cell organelles containing chlorophyll and enzymes required for photosynthesis
- Large organelles bounded by a double membrane and containing DNA.
- Unlike the mitochondrial double membrane, the inner membrane is not folded.
- Distinctly separate from the double membrane is an internal membrane system consisting of flattened sacs and called **thylakoids**.
- The space between the thylakoid and the inner membrane is called the **stroma**.
- The **stroma** contains the chloroplast DNA as well as components of the protein synthesizing machinery specific for the chloroplast, namely the ribosomes, tRNAs, and specific proteins and enzymes.
- Most of the components of photosynthesis (light-gathering compounds) are located in the thylakoids. The thylakoid membranes are organized into stacks called **grana**. The interior of the thylakoid is the **lumen**.

Chloroplasts

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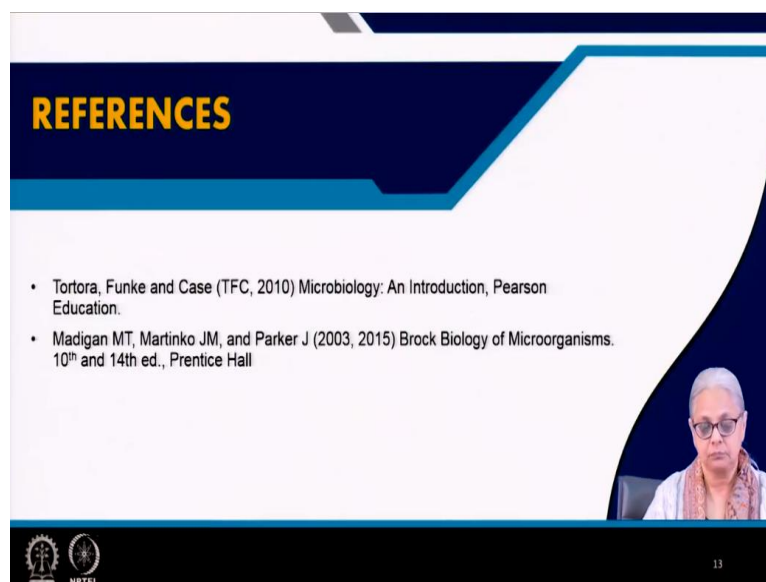
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Then we come to plant cells. Plant cells contain chlorophyll and enzymes that are required for photosynthesis. These are large organelles. The chloroplasts are where the chlorophyll is. These chloroplasts have double membranes and their own DNA. So, this is what a chloroplast looks like. It has a double layer membrane. Unlike the mitochondrial double membrane, the inner membrane of the plant chloroplast does not have any folds.

So, there is an outer and an inner membrane. And there are several other sub-organelles that are there inside the chloroplast. So, let us take a look at each one of them. So, distinctly separate from the double membrane is an internal membrane which contains flattened sacs. So, you can see these flattened sacs; and these are called thylakoids. So, these are the thylakoid, literally flattened sacs. The space between the thylakoid and the inner membrane is called the stroma. So, this empty space in light yellow colour is the stroma. Now, the stroma contains the chloroplast DNA. It also has the protein synthesising machinery that is required for the chloroplast. So, you can see the ribosomes. You can see the transfer-RNAs and the specific proteins and enzymes freely floating in the stroma.

Most of the components that are required for photosynthesis, basically the light gathering compounds are located in the thylakoid. The space inside the thylakoid, the interior of the thylakoid is the lumen. So, we will see it in subsequent modules. We will see how there are reaction centers and light harvesting compounds that are present in the lumen of these thylakoids. So, the thylakoid membranes are themselves stacked together. They are organised into stacks. And these stacks are called granum. So, one stack is a granum and multiple stacks are grana.

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NPTEL 13

We come to the end of this particular lecture. Thank you.