Genetic Engineering- Theory and Applications
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Module 1
Introduction and Basics of Biological System
Lecture 2
Cellular Structure (Part II)

Hello everybody, this is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering, IIT Guwahati. And in the previous lecture, what we have discussed? We have discussed about the definition of the biotechnology, its potential, the different millstones through which the different technique have been evolved and that is being used by the humans to exploit the microorganism as well as to improve the crop yields and in following our discussion, we have also discussed that what are the different types of host which are available for the biotechnology applications and in that context, we have also discussed about the differences between the prokaryotic cell versus the eukaryotic cell.

And then in detail, we have discussed about the structure of a typical prokaryotic cell such as the bacteria and then we have also… and in that contest, we have also discussed about the cell wall of gram positive as well as the gram negative bacteria and at the end, we have also discussed about the gram staining which is the staining which is been used to distinguish between the gram positive and gram negative bacteria. So let us continue our discussion about different types of hosts which are available for the biotechnology applications.

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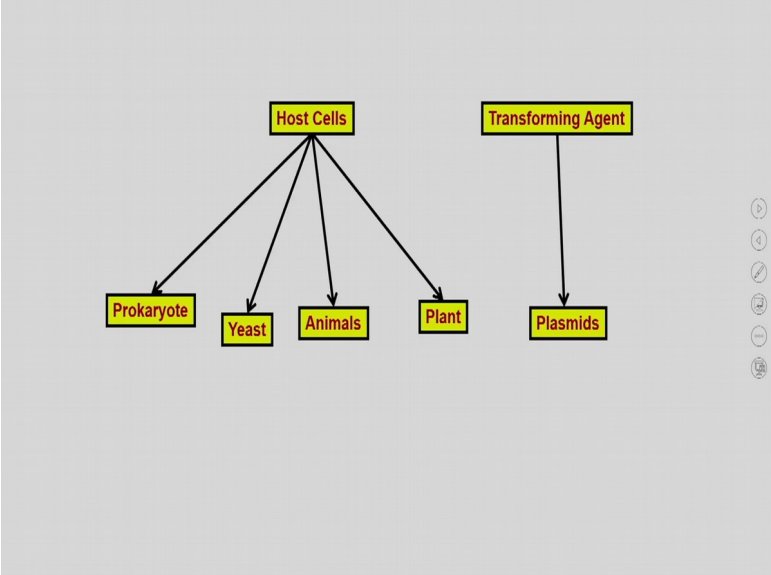

So what are these hosts? As we discussed in the previous lecture, we have the prokaryotic hosts or the yeast, animals, plants and we have already discussed about the minute details of the structure of a prokaryotic cell such as the bacteria. We have also discussed about the cell wall, cell wall of gram positive as well as the gram negative bacteria. So in the today's class, we are going to discuss about the typical eukaryotic cell and the different organisms which are present in the eukaryotic cell.

If you remember, in our previous lecture we have also discussed the differences between the prokaryotic and the eukaryotic cell and one of the major differences between the prokaryotic and eukaryotic cell is that the eukaryotic cell contains the membrane bound organelles. So in the eukaryotes what we have? We have two different types of cells. One is the animal cell, the other one is the plant cell and both of these, the animal or the plant cells are sharing the various similarities as well as the eukaryotic cell is concerned but they are also having the differences from each other.

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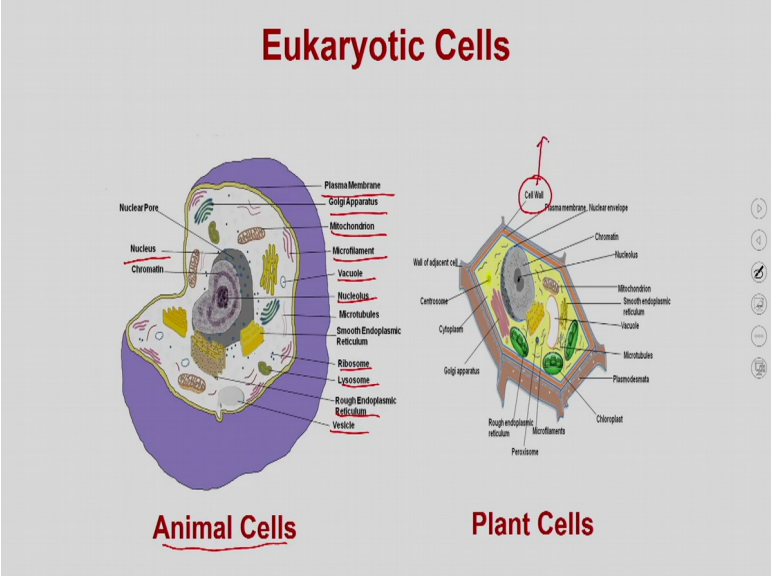

So in a typical animal cell, you have the plasma membrane, you have the golgi apparatus, you have mitochondria, you have Micra filaments, then you have the vacuoles, then you have the nucleolus, then you have the ribosome, lysosomes, rough endoplasmic reticulum and vesicles, you also have the well-developed nucleus which is actually the retistic feature of the eukaryotic cell compared to the prokaryotic cell and all these properties of an animal cell is also present in the plant cell except that the plant cell also contains the cell wall similar the bacteria.

And the cell wall of the plant is different from the bacterial cell wall. Apart from that, the plant cell also contains the chloroplast which is completely absent in the animal cell and apart from this, there are also some striking differences between a animal as well as the plant cell. Let us see what are these differences?

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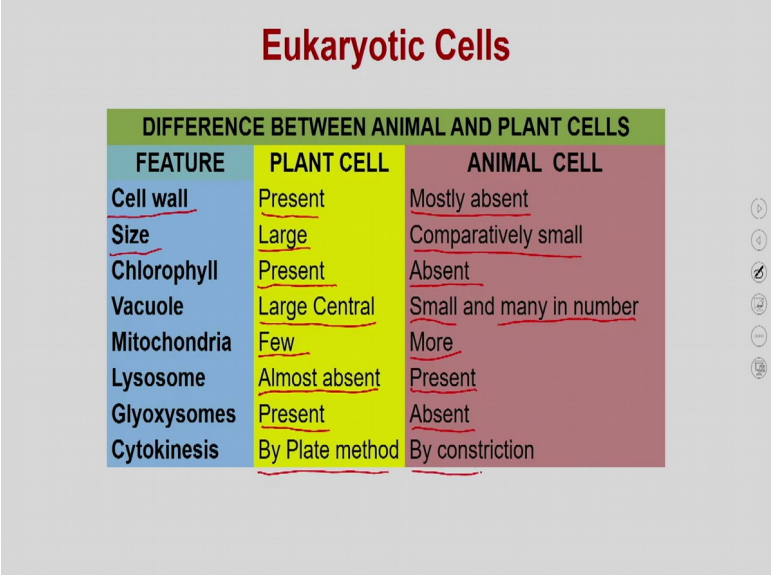

So these differences are, one of the major difference is that cell wall, the cell wall is present in the plant cell whereas it is completely absent in the animal cell, except there are a few exceptions where the animal cell also contains the cell wall. Mostly, the plant cells are very large. You might have seen the larger leaves and the cells which are present in the leaves. Similarly, compared to the plant cell, the animal cells are relatively smaller. As I discussed in the previous light, the chlorophyll which is completely present in the plant cells whereas it is absent in the animal cell.

The vacuoles are the spaces which are present in the eukaryotic cell and the vacuoles are very large in the case of plant cell where are they are very small as well as their number is very small. Mitochondria. Mitochondria is the energy producing organelle which is present in the eukaryotic cell and this number is very few compared to that the animal cell contains a very large number of mitochondria because animal cells are energetically more active and that is how they actually require more energy for their metabolic as well as the other kind of activity.

Lysosomes, Lysosomes are almost absent in the plant cell whereas the lysosomes are present in the animal cell and they have a very very unique as well as the typical function inside the eukaryotic animal cell. Glyoxysomes, Glyoxysomes are present in the plant cell but they are absent in the animal cell. Then the major difference is in the way the animal as well as the plant cells are dividing. The plant cells are dividing by plate method which is different from the animal

cell which is actually dividing by the constrictions and these constrictions are being mediated by the microtubule.

So let us continue our discussion about the different organelles which are present in the eukaryotic cell and you might have noticed that the bacteria does not contain any membrane-bound organelles. Whereas eukaryotic cell contains different types of organelles which are membrane-bound. So what is that major advantage of a eukaryotic cell to contain the membrane organelles, the organelles which contains the membrane around it? Membrane-bound organelles always give the advantage of developing a concentration gradient.

So there are multiple advantages of a eukaryotic cell to have the membrane-bound organelle. You can imagine a situation where the mitochondria is catalyzing a reaction and that reaction product is required in a very high quantity. If the mitochondria has to work without the membrane around it, then it has to, it requires a very high concentration of that particular substrate. Whereas if it is a membrane-bound organelle, a lower, a very low amount of that particular substrate will develop a high concentration and that high concentration can be crucial for driving a particular metabolic reaction.

The other point is that the reaction whatever you are doing in one organelle or the other organelle will not going to interfere. That 3rd is, you can actually make a very fine balance between the different organelle if they are membrane-bound so that you can exchange the material very very precisely and in a controlled fashion. And because of that, the eukaryotic cells are more advanced compared to the bacterial cells. So let us continue our discussion about different organelles present in the typical eukaryotic cell.

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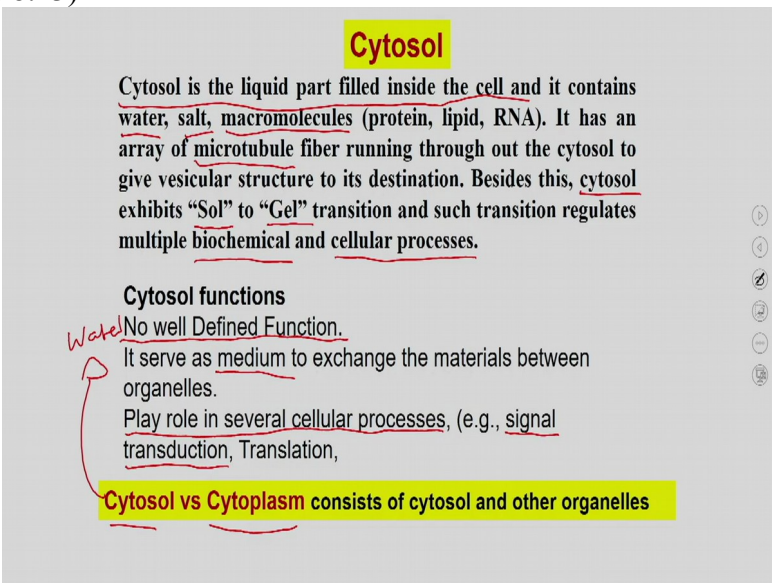

We will start with the cytosol. So cytosol is the liquid part which is present in the, inside the cell and it contains water, it contains salt, it contains the macromolecules such as protein, liquid and RNA. It also has an array of microtubules. So microtubules are the fibrous which are running throughout the cytosol to give vesicular structures and these microtubule mediated vesicular structures are having a role in providing the destination to the vesicles which are going from one organelle to another organelle.

This means this vesicular transport system is actually similar to just like a roads in our city. Suppose you want to go from one destination to another destination, you have a typical set of roads which you can use to go from one place to another place. Similarly, the microtubules are also running within the cell and having the similar function. Apart from that, the cytosol is going from one state to another state and one state is called Sol State and the other one is called as the gel state and these transitions are always happening inside a eukaryotic cell.

It depends on the weather as well that when it is winter, the cytosol is going from the sol to gel conditions and as well as, it has a similar, it has a implications in controlling the reaction of the bar chemical as well as the cellular processes because in one form or cytosol is more liquidified, in the other form, it is more jelly like. So because of this loss of water or the density of the cytosol, it actually can control the biochemical as well as the cellular processes. So what is the function of cytosol?

The function of cytosol is not a very very well-defined because it is a liquid which actually is present inside the cell but it also as I said, it serves as a medium to exchange the material between the different organelles. So if one organelle for example, if the mitochondria wants to send some molecules to the lysosomes or to endoplasmic reticulum, then it will be mediated through the cytosol. It plays various roles. It plays a role in various cellular processes, for example, signal transduction, translation. So the RNA which is present in the cytosol is being translated by the translational machinery which is in the cytosol.

Similarly, the signal transduction, as you know the cells contain different types of receptors, for example, the insulin receptor. So once the insulin binds to these receptors, the insulin receptor is driving a signalling cascades and that case, signalling cascades give the message to the different cellular organelles to change its metabolic reactions and as a result, we are actually regulating the glucose as well as the lipid metabolism.

One question which is very very interesting is that what is the difference between the cytosol versus the cytoplasm? So the cytosol is actually the water part. Cytosol is the water whereas the cytoplasm is the cytosol as well as the other organelle. So whatever you have inside the cell is called a cytoplasm if you remove the nucleus. So if you remove the nucleus, the remaining whatever is there inside the cell is called as cytoplasm.

Whereas the cytosol is the water part or the liquid part which is filled inside the cell. So cytosol does not contain the, is does not mean the organelles. Whereas the cytoplasm is the liquid part as well as the different organelles except the nucleus.

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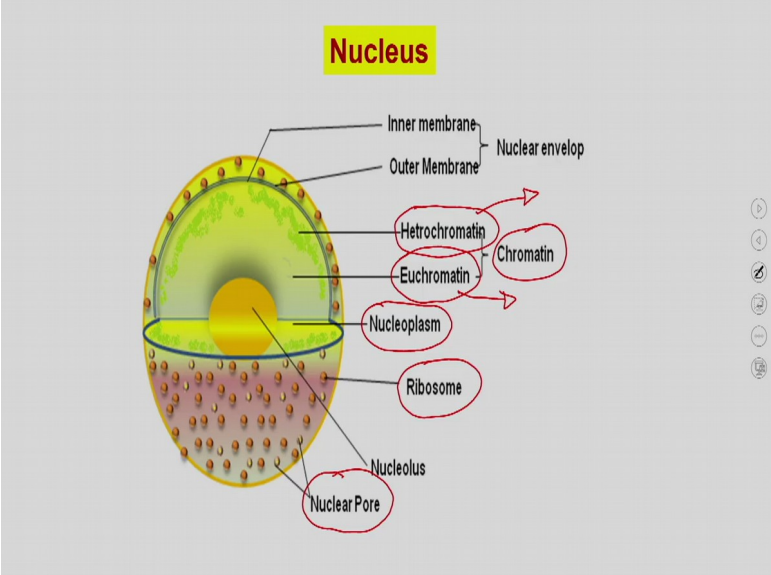

Now we will move on to the next organelle and that is the nucleus. So nucleus is considered to be the central processing unit. As you might know that in a computer, the central processing unit, what is the role of the central processing unit is to control the different types of metabolic as well as the biological processes within the cell. So the nucleus has the similar function and how it is happening? Because the nucleus contains our genetic material. The nucleus contain the genetic material which is called as chromatin.

This chromatin is present in the 2 form, one is called heterochromatin, the other one is called as the euchromatin. So heterochromatin is the condensed genome or the genome which is transcriptionally very inactive whereas the euchromatin is the loose genome or the genome where the genes and the other parts are accessible to the machinery and as a result, the euchromatin is the region which is transcriptionally very active. Apart from that, you have the nucleoplasm.

This nucleoplasm is the liquid part which is present inside the nucleus. The nucleoplasm contains the nucleotides, DNA polymerase and other enzymes as well as it contains the transcription factors and they all are going to work in modulating the transcriptional activity within the nucleus. Apart from that you have the ribosomes which are present on the outer surface of the nucleus and then the nucleus also contains a nuclear pore. The nuclear pore is very important in terms of regulating the material within the nucleus or outside the nucleus.

So the nuclear pore is a very very controlled pore which actually regulates the exchange of material between the nucleus as well as the cytosol. It has a very very complex structure. And apart from that, you have the nuclear envelope which contains the inner membrane. So its nuclear envelope is a double membrane structure which is a inner membrane as well as the outer membrane and a nuclear pore which actually regulates the exchange of material between the nucleus to the other part of the cell.

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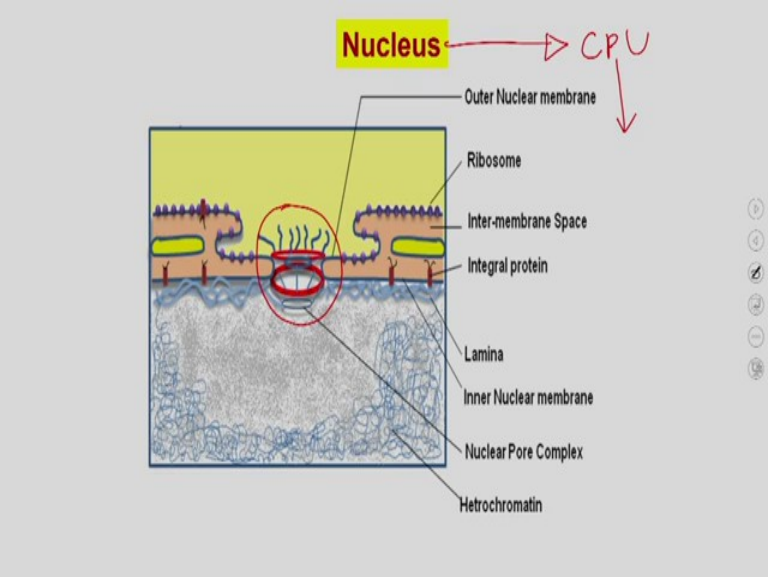

In a typical nuclear pore is having the outer membrane as well as the inner membrane. And then, inside that you have the genomic content and then you have a nuclear pore complex. This nuclear pore complex is actually regulating the exchange of materials from the nucleus to the external, to other part of the cell and it is very selective. It can allow even the entry or the exit of the RNA as well as the other bigger proteinicious molecules.

It can allow the entry and exit of different types of transcription factors and so on. But all these is very selective in the case of within the cell. What is the function of a nucleus? As I said, nucleus is the central processing unit of a cell and you know all that what is the role of a CPU or the central processing unit in a computer. So what is the role of a central processing unit of a computer is that it actually going to regulate and monitor all the metabolic as well as the cellular processes happening in the cell and it actually governs that simply by modulating the transcriptional as well as the translational activities within the cell.

And that is all done by the signal which the cell is receiving from the external stimuli. So when you have an external stimuli, for example, we have already taken an example of insulin. If the insulin is binding to the insulin receptor, that actually drives a signal transduction and that actually, eventually ends up into a set of transcription factors. This transcription factor goes and bind to their respective genes inside the nucleus and that is how they actually increases the transcription of those particular genes and that in turn changes the different types of proteins which are present in the cell and that in turns actually regulates the carbohydrate as well as the other metabolic reactions within the cell.

That is how the insulin is actually controlling the different metabolic reactions in the cell. But it is all been mediated through the nucleus or with the help of the nucleus. In the other word, you can also think about that if you go in sun and you are actually having very hot weather, then in that case, the other kind of stimuli or other kind of receptor is going to give the signal to the nucleus and accordingly, the nucleus is going to produce different types of protein which has been mediated through the transcriptionally modulating the different set of genes.

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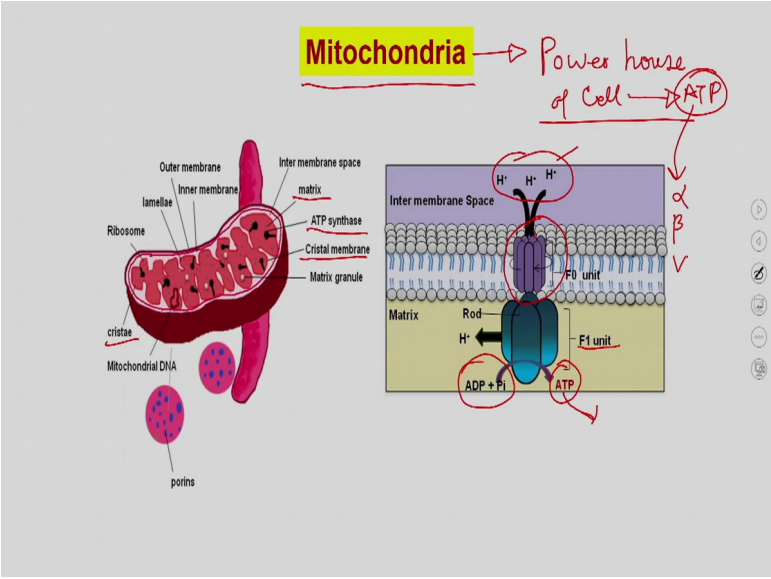

Now let us continue to the next organelle and the next organelle is the mitochondria. The mitochondria is considered to be the powerhouse of the cell and what is the function of a powerhouse of a cell? The powerhouse in our city is required to generate the electricity. Whereas similarly, the mitochondria is the powerhouse in the cell and its function is to generate the

electricity which is actually in the case of cell, it is ATP. ATP is actually the currency or the energy equivalence which are required for running the different biochemical as well as the other metabolic activities within the cell.

And mitochondria is a very very complex structure. It contains a outer membrane and a inner membranes, the inner membrane is folded in the form of a cristae. On this cristae, you have the, so you have the cristae and in this cristae you have the ATP synthase which is actually the enzyme which is required for ATP synthesis and the ultimate electron transport component is called as the ATP synthase and that ATP synthase is important for generating the ATP. You can see that this is a ATP synthase where you have the 2 subunit.

One is called FO one and the other one is called F1 subunit. These 2 subunits are coming together to make the ATP synthase and ATP synthase is utilising the hydrogen potential and that hydrogen potential when it is running through the ATP synthase molecule is generating the ATP so it is combining the ADP plus phosphorus to generate the ATP. This ATP is then available for the cell to utilise it for the different metabolic reactions. In a typical ATP synthase, you have the as I said, you have the 2 subunits. One is called FO, another one is called F1 unit.

And the FO unit is consists of 3 different proteins which is called A, B and C. And the FO subunit is a membrane-bound subunit whereas the F1 particle is the present inside the matrix and that also contains different types of proteins. So it contains the alpha, beta, gamma and all these, alpha, beta, gamma proteins are having its exclusive functions and with the help of the proton gradient, the ATP synthase is rotating. So with the help of this proton gradient potential, the ATP synthase is rotating and as a result, it is actually combining the ADP and PI to synthesise the ATP. Let us see how it happened.

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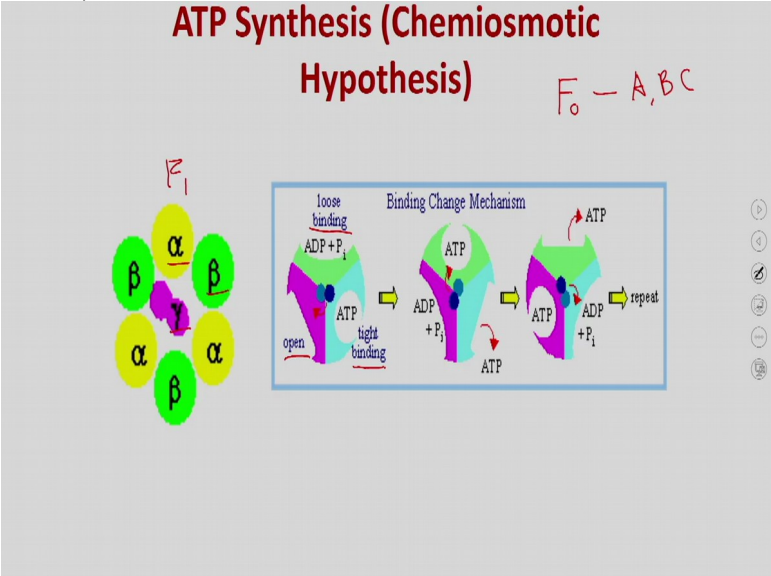

So ATP synthase is a membrane-bound enzyme or enzyme complex. It mostly has 2 subunits or 2 components. It is called, the component number 1 or A is called as FO and the component number B is called the F1. The FO is the membrane-bound component and F1 is the actual enzyme which is catalysing the combination of ADP plus PI to synthesize the ATP and that is not the membrane-bound. That is in the free into the lumen.

So in the FO one subunit, that is made up of 3 different proteins known as the A, B and C. Whereas the F1 subunit is made up of different types of proteins and these proteins are called as the alpha protein, beta protein. So alpha, beta, gamma, delta and epsilon. Okay. So there are 3 units of alpha, 3 units of beta, one unit of gamma and one unit of Delta and epsilon. And if you see the arrangement of the ATP synthase, it is like this.

So this portion is the FO subunit. Then after that it has a ball like structure and this is the F1 subunit okay and within this ball, what you have is, so this is the C protein okay, then you have B and this is called (())(23:26) of FO subunit okay. Whereas in the case of the F1 particle or F1 subunit, you have the alpha, you have the beta and then alpha and beta actually alternate with each other to make this ball like structure. Then they have a gamma in between.

So this is the gamma and apart from this, they also have the epsilon. So this is the structure which is responsible for converting the ADP plus PI to ATP. So let us see how this happens okay. So if I show you the structure, the working structure of the ATP synthase, so ATP synthase

as I said you know has the alpha, beta and gamma subunits and these are actually the subunits which are actively participating into the catalytic mechanisms.

So in the catalytic mechanism, what we have is, so we have alpha, beta, alpha, beta, alpha, beta. Okay, alpha, beta. So we have alternate arrangement of alpha, beta and in the middle, we have the gamma, okay. So this is the middle balloon like structure what you have seen earlier okay. And this, I showed you the beta subunit. So beta subunit has 3 catalytic site okay or 3, or its catalytic site can be modulated in 3 different ways.

So it can actually been modulated by the conformational changes induced by the gamma chain. So it has 3 different types of conformational changes. One is called open confirmation, the other one is called loose confirmation and type confirmation. So what happened is that the open confirmation or the open site is actually the active site when it can receive the substrates. So this open site actually gets the initially ADP and PI.

So they come and bind to this site and then there is a conformational changes in the beta subunit because of the movement of the gamma chain and as a result, the ADP and PI goes and bind to the loose binding site. Okay. As soon as they bind to the loose binding site, the gamma subunit also again induces the conformational changes and as a result, these 2 molecules comes and bind here in a tight confirmation. And once they bind in a tight confirmation, the coupling reaction takes place between the ADP and PI and as a result, they both get combined and it regenerates the ATP.

So in a tight confirmation, the ADB and PI actually get combined into each other and generates the ATP. As soon as the ATP is generated, again the gamma chain is inducing a conformational changes and because of that, the ATP which is bound very tightly in the tight confirmation, the active site changes its confirmation. As a result, the ATP, it comes out because the active site confirmation is now in the open confirmation and because of that, ATP is released and the new molecule or the new series of ADP and PI comes in.

So the cycle continues for several down and as a result, it actually assimilates the proton energy which has been used to couple the ADP and PI to generate the ATP. Now the question comes, how actually this is happening. So how it is happening is that as I said, you know you have the

membrane-bound complex okay and on this side, you have large quantity of H plus, okay. So because the H plus has a potential and this potential energy can be utilised by this enzymatic machinery to generate a mechanical force and because of that mechanical force, it actually can induce the confirmation changes within the beta subunit and that actually allows the enzyme to couple the ADP and PI to generate the ATP.

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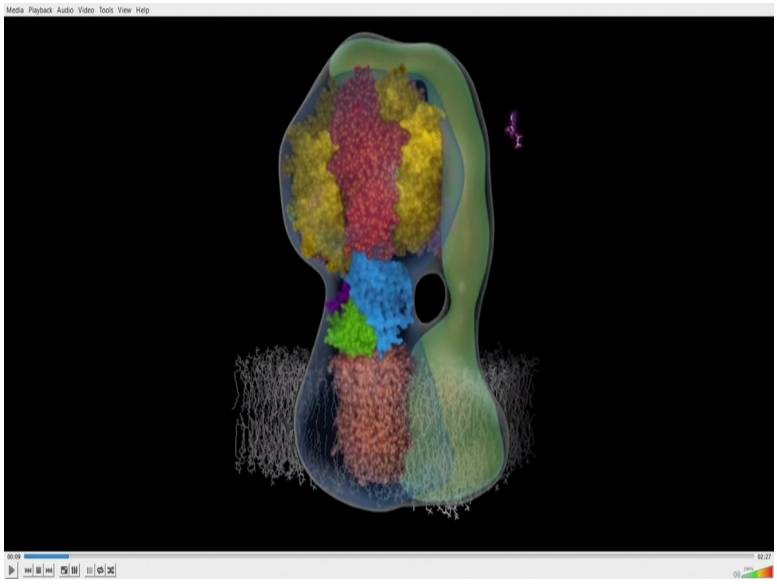

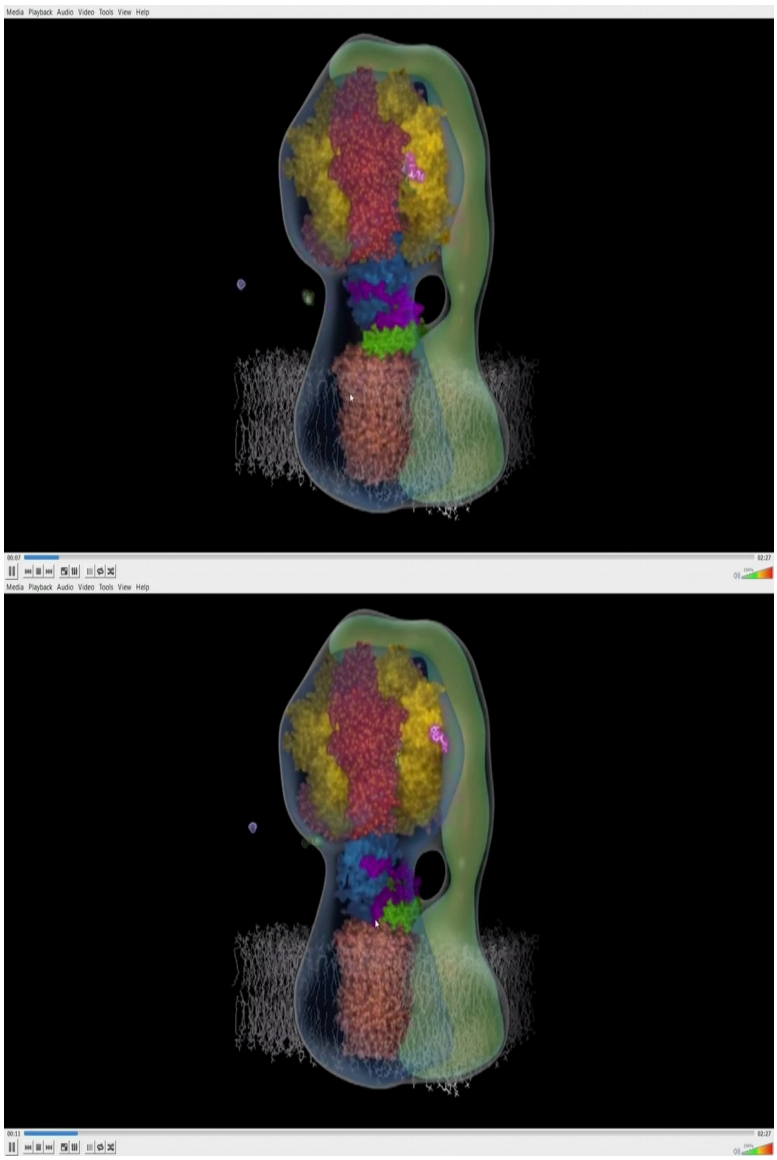

You can easily see this same phenomena in a more schematic animation. This animation has been provided to us by the Prof Volcker. Prof Volcker is a renowned professor in the MRC, UK. What you can see here is the complete the ATP synthase and what you can see is actually a rotating rod actually. So this rotating rod what you see the pink colour, is actually the sea chain of the FO particle.

So free chain is actually in the, which is actually integrated within the membrane and that rotates, keep rotating and because it rotates and the gamma chain is attached to or is in co-ordinance with this particular sea chain, so as the sea chain runs, it also induces the conformational changes in the gamma chain, that is what you see in here as a cyan coloured or a yellow, blue colour protein

and the top portion what you see is the yellow and the red colour proteins are actually the alternate arrangement of the alpha and beta subunit.

So in totality, in a ATP synthase, what you see is actually the sea chain, C protein of the FO one particle which is integrated within the plasma membrane and that is rotating. Because of its rotation, it induces the conformational changes in the gamma chain which is which you see in the blue colour and that conformational changes is also being directed towards the alpha and beta chain.

So what happened is, once the proton comes out from this ATP synthase, it because the proton has the potential energy, it once it runs through this particular protein complex, it gives a kind of the mechanical energy into the sea protein and that allows the sea protein to keep rotating in the membrane.

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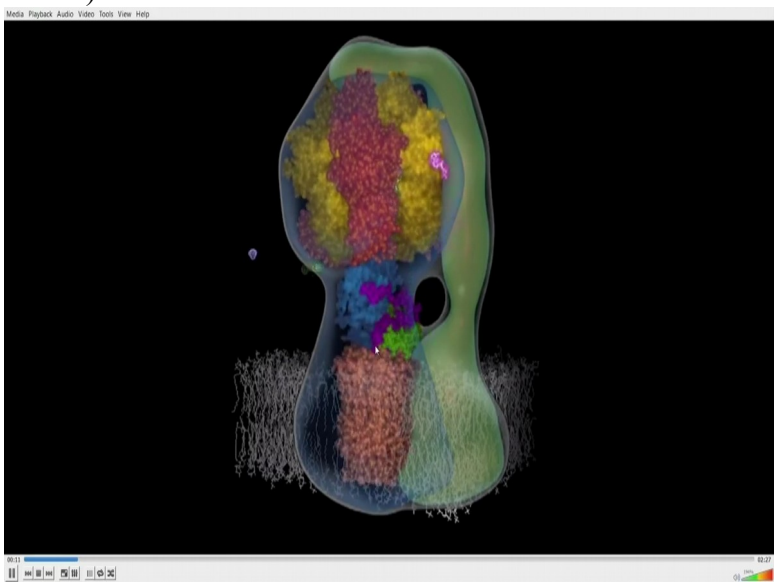

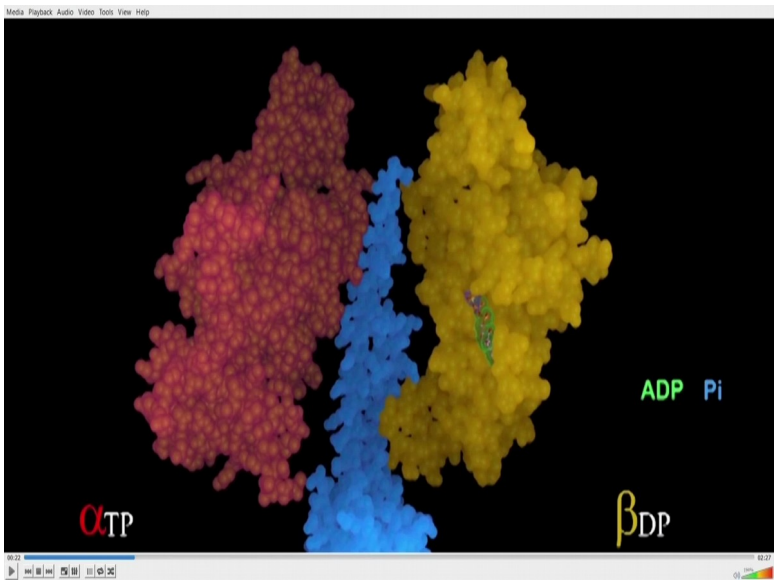

And as a result, it actually gives the conformational changes throughout the molecule. And these kind of conformational changes are being used. So now what you see is actually the 3 different confirmation of the beta chain. So what you see now is actually the so what you see now is the open confirmation and in the open confirmation, the molecules are not binding, okay. So the active site is relaxed and it can actually bind the ADP and PI.

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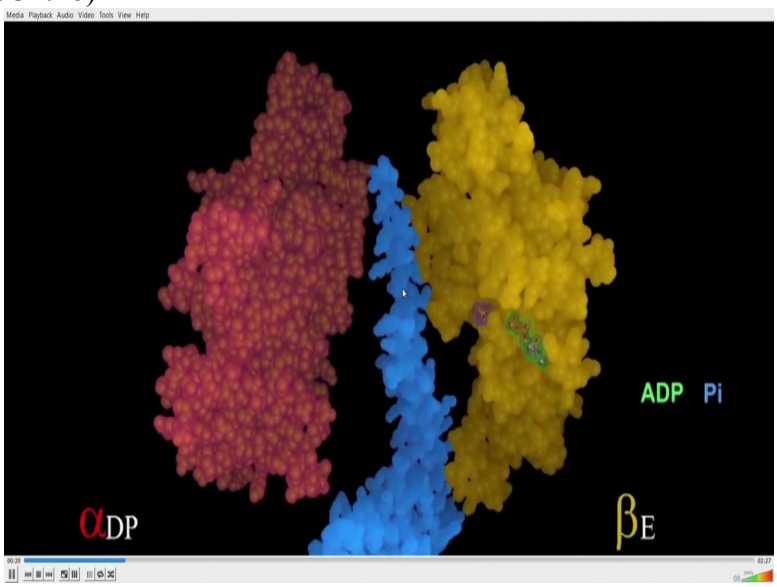

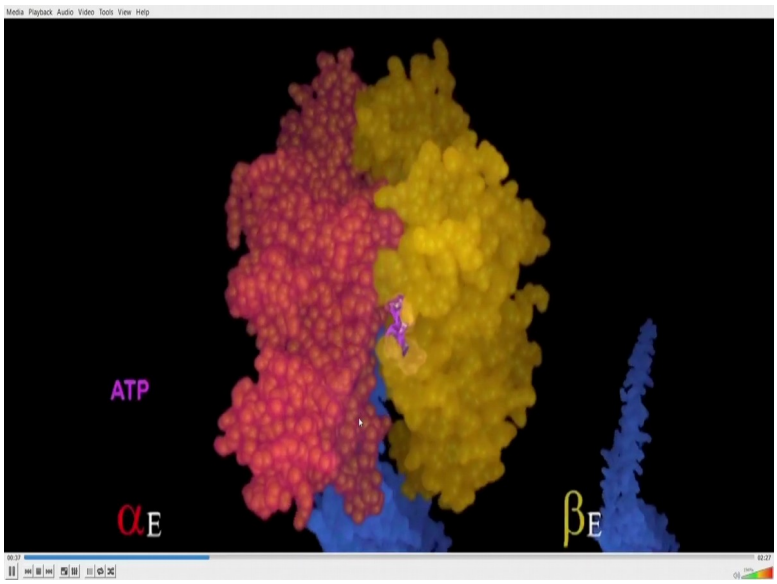

As soon as it happens, you can see that the blue is actually making a conformational changes and because of that the beta subunit is turning its active site into 1st into the loose binding site and then again into the tight binding confirmations and because of that, it actually coupling the ADP and PI and generating the ATP. As soon as the ATP is formed, the active site is getting converted into the loose, into the open confirmation.

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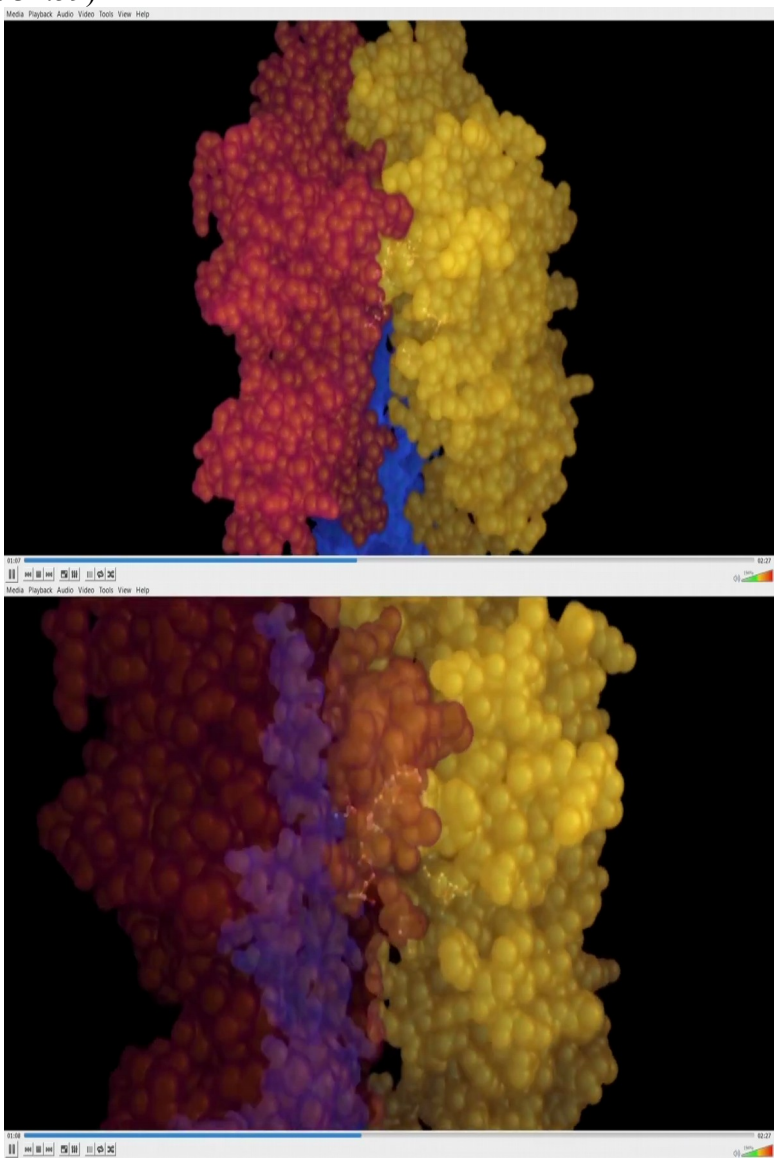

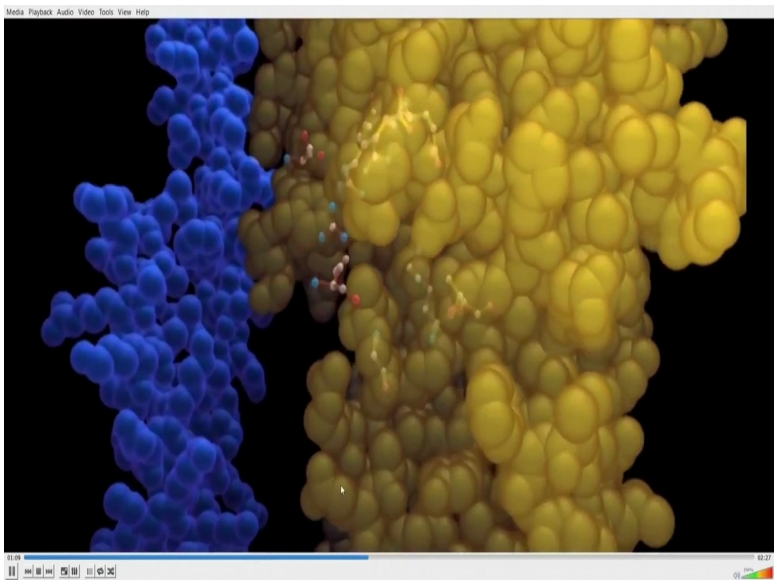

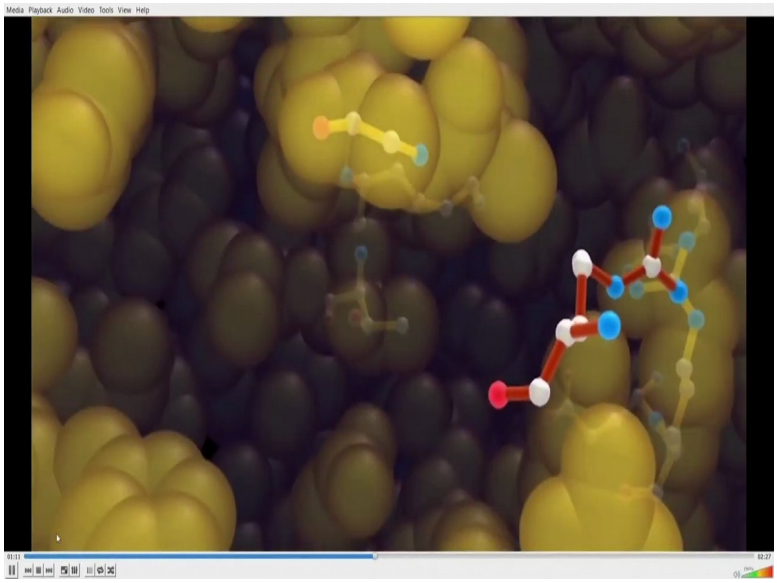

And as soon as it gets open into the open confirmation, it gets, it releases the ATP from the active site. Okay.

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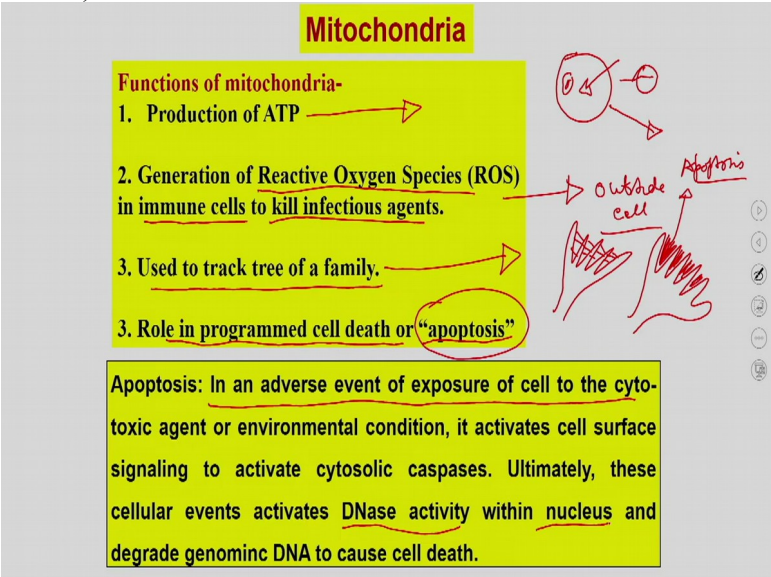

So let us continue. What are the functions of mitochondria? So function of mitochondria as we said, function of the foremost function of the mitochondria is to play the role in the production of ATP, then the mitochondria is a site which actually is important for generating the free radicals. And these free radicals or the reactive oxygen species are known in the immune cells to kill the infectious organisms.

So what happen is when somebody is getting the infection, it actually generates the signal transduction and that signal transductions within the immune cells ask the mitochondria to increase its free radical generation capacity and because of that, these free radicals are being released outside the cell and these radicals are very very toxic. So they will be going to kill all the microorganism. Similarly, the mitochondria is the only organelle which is being used to trap the family tree of any humans or any animal. Why it is so? Because the mitochondria is the only organelle which it remains within the ovum of a particular human being or…

So during the fertilisation what happen is that you have the ovum and then you have the sperm. Why? After the fertilisation the mitochondria which is present inside the sperm is been lost because the only the nucleus part is going inside the ovum whereas the ovum contains the mitochondria. And that mitochondria continues from one generation to many generation and because of that, if you follow the mitochondria, you can be able to trap the family tree.

The mitochondria is also having a role in program cell death or the apoptosis. So apoptosis is a program cell death it is actually having the different cascades in which the cell is undergoing the death and this is actually a controlled process. So it does not create any kind of damage to the organisms and one of the classical examples is the development of a hand or the pentaductily feature in a human being. You might have observed that in a frog, what you have is a hand like this where the fingers are connected to each other by a membrane.

Whereas in the case of humans, what you have is a typical pentaductily hand. So the region among these fingers are being dead by a process known as apoptosis or the program cell that. So the in the case of apoptosis the mitochondria is actually releasing the cytochrome C. So cytochrome C is inducing the different types of reactions within the cell and that actually is going to kill the cells. So mitochondria is controlling the apoptosis in the cell.

In an adverse event of exposure of the cell to the cytotoxic agent or an environmental condition, it activates cells are they signalling to activate cytosolic caspases. Ultimately, these cellular events activates a DNases. So these DNases are going to degrade the DNA which is present in the nucleus and once the genomic DNA is been degraded inside the nucleus, it eventually leads to the death of particular cell.

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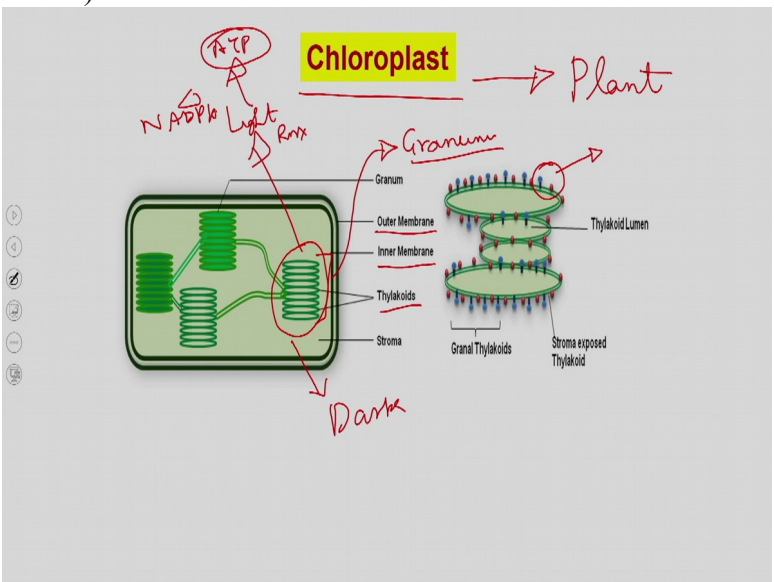

Now move onto the next organelle. And the next organelle is chloroplast. Chloroplast is only present in plant. So it is completely absent in animal cell. And the chloroplast is, the

anatomically or structure wise, the chloroplast is very closer or very similar to the mitochondria. It has the double membrane, one is the outer membrane, the other one is the inner membrane. And the inner membrane is folded in the form of thylakoids. These thylakoids are arranged to each other to form the granum. So you can imagine that this is the granum part and the thylakoid membranes are arranged in a coin like structure and that is called as the granum.

This granum contains the different types of photoreceptors such as the photoreceptor 1 and photoreceptor 2. As you know that the chloroplast is using the potential energy or it is using the ATP as well as the sunlight to synthesise the food and that is happening within this stroma part. So in this stroma, the chloroplast, it is running the dark reaction. Whereas the thylakoid membrane region is being used for the light reaction and light reaction is being used to produce the ATP.

Similar to mitochondria, the chloroplast also contains the ATP synthase complexes and that utilises the light reactions to generate the electromotive forces and then these light electromotive forces are again being used by the ATP synthase to generate the ATP as well as the NADPH and the both of this NADPH as well as the ATP is being used in the dark reaction to synthesise the sugar with the help of taking the sunlight as well as the carbon dioxide from the environment.

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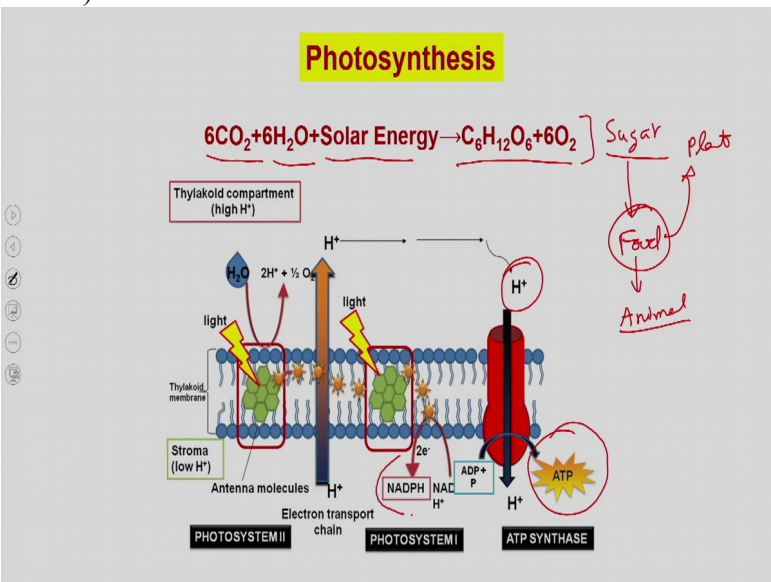

So in the typical photosynthesis, you have carbon dioxide, water, then the sunlight and what you produce is the sugar molecule or the glucose molecule. This sugar is eventually being a food for

the plant as well as the animal. So this food is being used by the plant for its own, running its own metabolic them as well as the same food can be used by the animal for their own nutrition.

So this is a typical the photosynthetic reaction which are happening inside the chloroplast and that actually is generating the proton gradient and that proton gradient is being utilised by the ATP synthase to generate the ATP as well as the NADPH. And that NADPH and ATP is being used in the dark reaction which is given on the top to generate the food.

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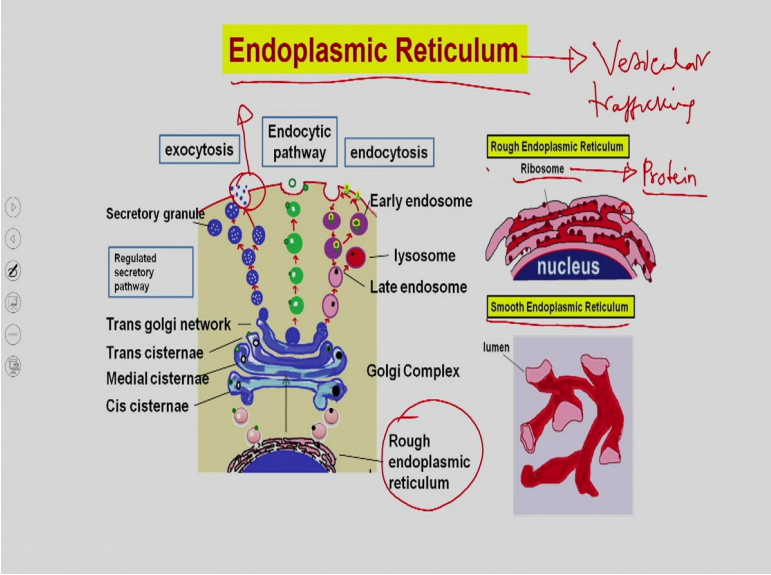

Now we move onto the next organelle that is called endoplasmic reticulum. Endoplasmic reticulum is also be a part of vesicular trafficking which means endoplasmic reticulum is working similar to the roads. So endoplasmic reticulum job is to distribute the substances or the material between the different organelles of the cell and it is done by simply by endoplasmic reticulum with the help of the golgi body as well as the lysosome.

So the endoplasmic reticulum, golgi bodies and the lysosomes are, both are coming together to form the vesicular trafficking system and how it is happening is that in the rough endoplasmic reticulum, so you have to different types of endoplasmic reticulum. One is called rough endoplasmic reticulum, the other one is called as smooth endoplasmic reticulum. In the rough endoplasmic reticulum, you have the ribosomes which are being attached onto the ribosomes, onto the endoplasmic reticulum.

Whereas the smooth endoplasmic reticulum does not contain any of the ribosomes. So these ribosomes are synthesising the protein by translation and once these proteins are being synthesised inside the endoplasmic reticulum, they are being processed and then they are being sent to the golgi bodies and then these golgi bodies are processing these protein containing vesicles and tagging them to their destinations.

For example, if our vesicles has to be exocytosed or it is a secretary substances, then the vesicle will not have any tag and as a result what will happen is the musical will fuse to the plasma membrane and it will release its content in the outside world. You can imagine that this is what happened when there is a, when we would like to secrete the antibodies in the external media by the immune cells. Similarly if there are substances which need to be go to the mitochondria, the golgi bodies will receive those proteins from the endoplasmic reticulum.

They will process it, tag it with the mitochondrial localising sequences and that is how those vesicles will go to the mitochondria.

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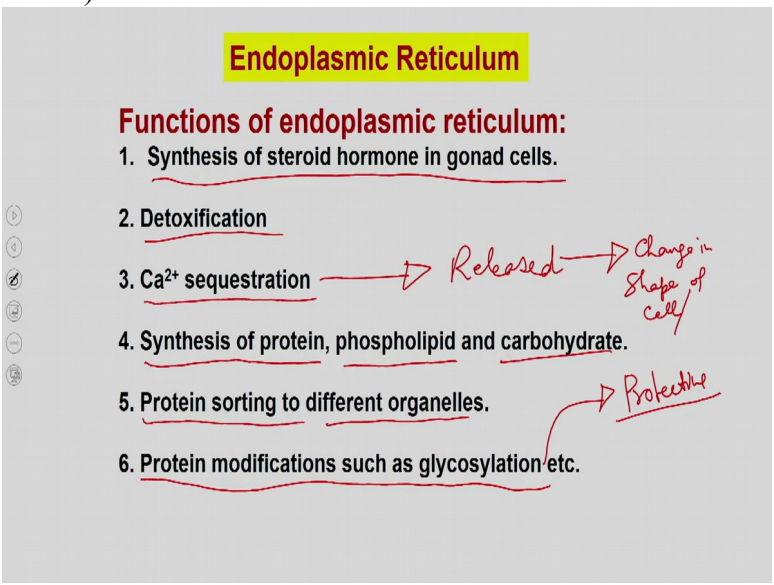

What is the function of endoplasmic reticulum? So function of endoplasmic reticulum is very simple. In the case of smooth endoplasmic reticulum that is required for the synthesis of steroid hormone in the gonad cells. Endoplasmic reticulum is also a site for detoxification of different metabolites which are being produced inside the cell. Endoplasmic reticulum is the major site for calcium sequestration. This calcium is being released in the event of any signal transduction and

that released calcium is causing the change in shape of the cell or it can also allow the cells to make the movement towards a particular chemo attractant.

Endoplasmic reticulum is also having a role in synthesis of protein, phospholipid as well as the carbohydrate and the major role what the endoplasmic reticulum is in the protein sorting to different organelle and that is being done with the help of the golgi body which actually is receiving these substances from the endoplasmic reticulum and then processing and tagging it with the different organelles to, for the different organelles. And then lastly, the endoplasmic reticulum is also been involved in glycosylation of different proteins.

So this glycosylation pattern which the endoplasmic reticulum is tagging to every protein is deciding their destinations and this glycosylation is also protecting the cell or protecting the protein for the proteolytic degradations and sometimes this glycosylation pattern is also deciding the fate of these proteins or the destination of these proteins within the cell. Let us move onto the next organelle.

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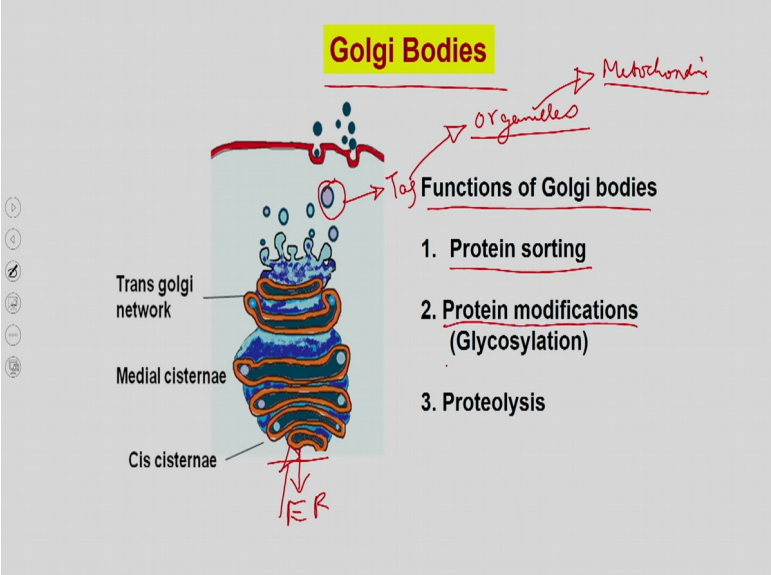

The next organelle is the golgi bodies. So golgi bodies is also a part of the vesicular trafficking system. The golgi bodies is a membranous organelle whereas you have the cist. You have 2 regions, one is called cis golgi as well as the other one is called as the trans Golgi. This cis golgi is facing towards the ER and it receives the substances from the ER. Then within these

membranous structures, the protein is being processed and then those processed protein is being released from the trans golgi part in the form of a vesicle.

These vesicles are containing the tag for the organelles which they want to be the destination, which is being the destination. For example, if these vesicles are being tagged for mitochondria, then they will be tagged with a mitochondrial localisation sequences and they will be, go to the mitochondria and supply that particular protein to the mitochondria. So what is the function of Golgi bodies? The function of Golgi bodies is in the pace of protein sorting which means, it receives the protein from the endoplasmic reticulum, then it sorts those proteins for its different organelles.

It may receiving a protein which is for the lysosomes, it may be receiving a protein which is for mitochondria, it may be receiving a protein which is for the nucleus and so on. So that is sorting part is being done by the Golgi bodies and then it actually tags those vesicles with a particular localising sequences for a destination and that is how it actually helps in vesicular trafficking. Golgi bodies are also playing a role in protein modification or the glycosylation and the ultimately, it is also playing a role in proteolysis or the degradation of the proteins.

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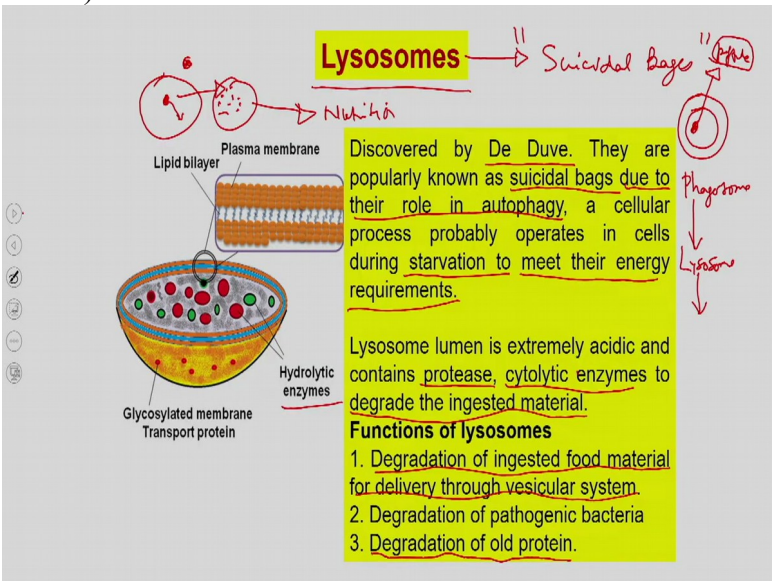

Let us move onto the next organelle and the next organelle is known as the lysosomes. Lysosomes are popularly known as suicidal bags which means the lysosomes are containing the substances which are very very harmful for the cell and these substances are if they will be

spilled over within the cell, it actually induces the suicide of that particular cell. Lysosome is been discovered by the scientist De Duve and they are popularly known as the suicidal bags due to their role in autophagy.

So you might have read about the autophagy. Autophagy means eating yourself which means in a particular condition, when the cell does not have the energy to run its metabolic reaction, it actually goes undergoes in the process of autophagy. And in the process of autophagy, what happen is the lysosomes are actually releasing its content within the cell and that actually started degrading the different types of organelles and that, by degradation of a different organelles, it produces some amount of biomolecule as well as some amount of energy and that actually allows the cell to sustain for a longer period of time.

And that happens if you keep a cell under starvation. And that is required for meeting the energy requirements. So as you can see, lysosomes are the double walled membranous structures. It is containing the glycosylated proteins on the surface and the membrane of or the lumen of the lysosomes contains hydrolytic enzymes, it contains, its pH is very low and it is actually a, it contains the cytolytic enzymes, proteases and the purpose of these substances is to degrade the ingested material which means what is the function of lysosomes?

The function of lysosome is the degradation of ingested food material for delivery through vesicular system which means if the organism is taking a food material, for example, if amoeba or if some organelle is taking a food material, once the food material enter inside the cell, it is actually of very large in size. So this food material has to be disintegrated into individual biomolecules. That is the function of the lysosomes which means the liposome will take up this food material and with the help of its proteases and cytolytic enzyme, it actually going to disintegrate or divide these material into the smaller particles and these smaller particles are good enough for, cell to use for nutrition.

On the other hand, it actually degrades the pathogenic bacterias. Similar to the food particles, if a cell has taken up the bacteria, so bacteria is also made up of biomolecules. So once the immune cells catches up any bacteria and they are been taken up all these bacteria, this bacteria is being encapsulated in a double membrane structure, these are called phagosomes. These phagosomes

then deliver the bacteria to the lysosomes and with the help of the proteolytic enzymes as well as the cytolytic enzymes, this bacteria is being digested and as a result, what will happen?

The bacteria will going to form the peptides. So bacteria will be digested to a level where the individual peptides chain of the bacteria is being developed and these peptides are being displayed along with the MSC molecules to keep the signal to the other immune cells about the infection and that is how it is actually going to help in generating the robust immune response within the host. The lysosome is also required to degrade the older proteins.

So if a protein is aged and it is required to be older, it is required to be degraded, then this protein is also being delivered to the lysosomes for its degradation because once you degrade this particular protein, and you generate the amino acids then these amino acids can be used by the cell for the synthesis of other biomolecules. What we were discussing? We were discussing about the lysosomes. So lysosomes as we discussed, they are called as the suicidal bags and their job is to provide the, to degrade or to destroy the ingested bacteria as well as the digest the proteinaceous substance or aged proteins.

So let us discuss further and now what we are going to discuss is the most important organelle which actually defines a particular cell. So this particular cellular organelle is not been considered as an organelle but it is the most important organelle present in the cell and this particular organelle is known as the plasma membrane.

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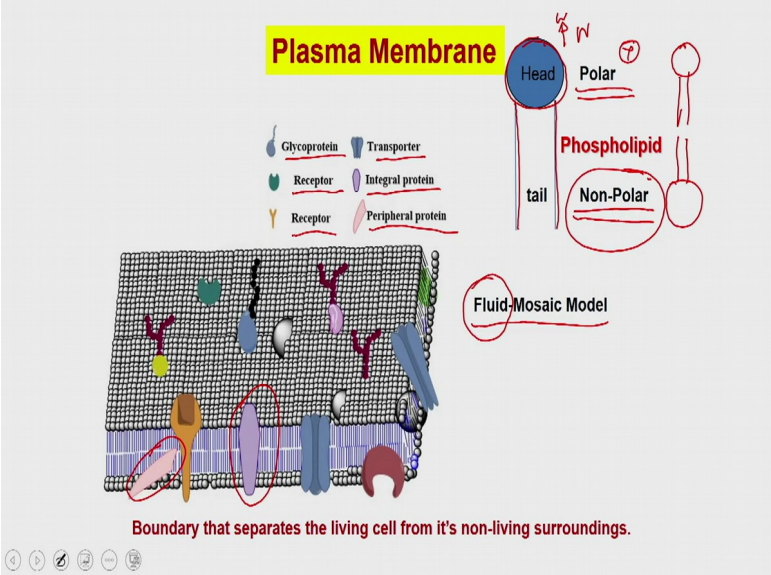

So the plasma membrane is actually the only boundary and plasma membrane actually defines the boundary which actually separates the living cell from the nonliving surroundings. So in plasma membrane, what you have is, you have the lipid molecules as well as the proteins and the lipids are arranged in 2 layers and that is called as the bilayer and why they are arranged in 2 layer? Because the phospholipids which are making the plasma membrane has a unique feature. What you have is in a typical phospholipids, what you have is a head on the top and then you have the tails.

This head is polar which means it is going to be a charged molecule. Whereas the tail is nonpolar which means this particular portion is not going to like the water which means the head is always directed towards the water or the aqueous environment. Whereas the nonpolar tail is going to be directed towards the nonpolar interaction. That is why, the 2 phospholipids are arranged from tale to tell and head to head. Because of that, you have the bilayer present in the plasma membrane and within this bilayer sandwich, you are going to have the different types of protein.

Some proteins are the integral proteins which are present throughout the depth of the plasma membrane whereas some proteins are the peripheral proteins such as peripheral protein like either they will be present on the inner leaflet or they will be present on the outer surface. So apart from these proteins, the plasma membrane also contains the receptors, these receptors is

also different types and then they also contain different types of glycoproteins, transporters, and the receptors.

And the classical model which explains the arrangement of liquid and protein in a plasma membrane is called as the fluid mosaic model. According to the fluid mosaic model, the lipid molecules are making a fluid like situation and because the lipid molecules can rotate or move within the plasma membrane, the plasma membrane is dynamic in nature. It can change the position of the proteins as well as the position of different lipids which are present in the plasma membrane.

So apart from the phospholipid the plasma membrane also contains the cholesterol, the spingocytes and as well as the other kinds of nonpolar lipids. And the composition of these lipids can be modulated under different environmental conditions in such a way so that it protects the cell from the different types of environmental as well as other kind of damages. So let us see what is the function of plasma membrane.

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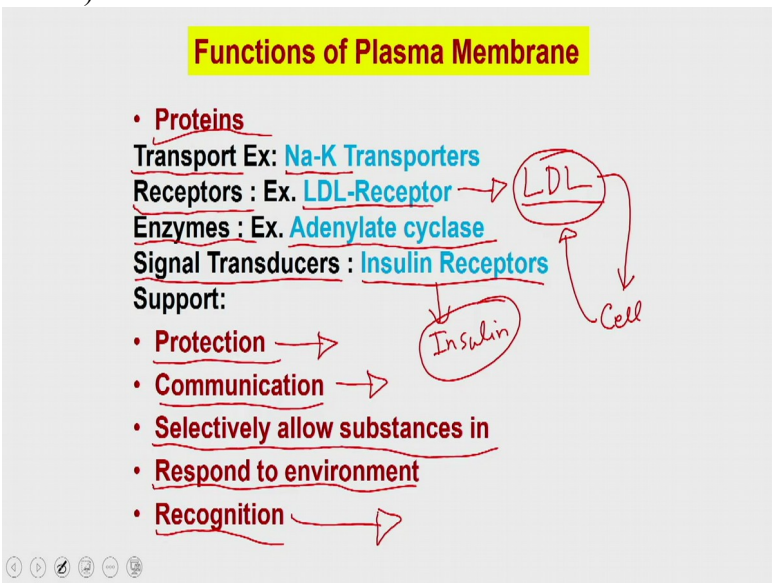

So the plasma membrane as we said, is containing different types of proteins. These proteins are falling in 5 different categories. One is called transporters. The transporter's job is to transport the specific analyte across the plasma membrane. It could be analyte, it could be for the sodium potassium or it could be for the different types of nutritious substance such as the glucose or other food particles. Then we have the receptors. These receptors are, there are 2 classes of

receptor, one receptor which is actually helping the cell to take up the food molecules from the extracellular media.

One of the classical example is the LDL receptor. The LDL receptor is responsible for the uptake of the lipid known as the LDL. And this receptor is present on the plasma membrane and it getting re-circularised. What is the LDL receptor is doing? It is catching the LDL which is present in the microenvironment and then putting this LDL into the cell and once this is done, then the LDL receptor returning back to the plasma membrane and the cycle continues for several round and that is how the cell will take up the LDL which is present in the extracellular media.

Apart from the receptors which are important for the uptake of the food particles, you also have the receptors which are doing the signal transductions or which are relaying the signal which is present on the, which has been exerted from the extracellular agonist molecules. One of the such classical example is the insulin receptor which is working when the insulin is present outside and when the insulin is present outside, it binds to the insulin receptor and that is how it actually drives the series of phosphorylation cascades and that actually changes the gene expression profiling from the nucleus and that in turn changes the cellular metabolic is for the carbohydrate as well as the filled lipids.

Apart from these 2 classes, you also have the different types of membrane-bound enzyme. One of the classical example is adenylate cyclase. And this also functions while it is on the, present on the plasma membrane. Apart from these enzymes, you also have the electron transport train which is also present on the plasma membrane of the mitochondria. And you also might have seen that the photo system 1 or photo system 2, those are also the membrane protein which are present on the thylakoid membrane.

The thylakoid membranes are also made up of plasma, is also of the same composition as the plasma membrane. And apart from these proteins which are present on the plasma membrane, the function of the plasma membrane is to provide the protection. So plasma membrane is actually making a boundary which actually separates the cells, cellular content from the extracellular media and because of this important role, the plasma membrane protects the internal cellular organelles from getting the damage by providing a support of cushion.

You can imagine a situation where the cell is eating up the food particles and expanding. In those cases, the plasma membrane will grow and that is how it will not allow the cells to burst actually. Vice versa, there is a, when there is a loss of water, then also the plasma membrane will try to overcome these losses and try to protect the cell from these kind of damages. Communications. The plasma membrane also contains different types of receptors or different types of ligand molecules.

One of the classical example is the catrin molecules or the integrin molecules and all these molecules are, they are the cell is using for communication purposes. In some cases, the cell is directly coming in contact with the other cell and relaying the signal. In other cases, the plasma membrane is secreting the molecules which are actually going to the other cell and giving the signal. Then the most important part if the plasma membrane is the selective uptake of substances in or out which means the plasma membrane is a selective semipermeable membrane which means it does not allow anything which is coming inside passively.

So you can imagine a situation if you have a bacteria or infectious organism or the toxic substance outside the cell, the plasma membrane may not allow these substances to come inside and because of that, the plasma membrane protects the inner machinery from getting the damages. Then the plasma membrane because it contains different types of receptors, it responds to the changes in the microenvironment, for example, if there is a change in the pH, if there is a change in food substance or presence of food, all these changes are going to be responded by the plasma membrane because it also contains receptors on the cell surface and this receptor will respond to the changed environment.

And then the lastly, the plasma membrane also allows the cell for making it recognised. For example, the different types of cells have the different types of ligands or the receptor present on their surface and these ligands are specific for that particular cell type and this is very common in terms of immune cells because the immune cells are containing different types of CD molecules and these CD molecules are characteristic to a particular cell type and because of presence of these particular molecules, these cells could be recognised by the system or by the body. So that is why the plasma membrane which is actually making the boundary of a cell is playing a very

very crucial role in maintaining the cellular integrity as well as in maintaining the, it is doing the different types of functions in (())(62:55).

So in totality and in summary what we have discussed so far? What we have discussed about the different organelles present in the eukaryotic cells and what we have discussed about the different, the function as well as the structure of these organelles and how these organelles, what is the advantage of eukaryotic cells to have the plasma membrane-bound organelles. And now in subsequent lecture what we are going to discuss is how to separate these organelles and how to isolate individual organelles so that you could be able to make the recovery of a product which is being produced inside a particular organelle.

So for the protein production point of view, it could be done in a different way as far as the prokaryotes or the eukaryotes cells are present. So there are different sites where of the protein can be produced inside a prokaryotic cell as well as in a eukaryotic cell. That is why it is important for us to isolate that particular cellular fraction and so that we could be able to make the purification easier as well as the downstream processing would be easier and at the end, that may help us to make the recovery, make the better recovery of the product from these host cells which are over expressing your protein or the product what you are trying to develop. Thank you.