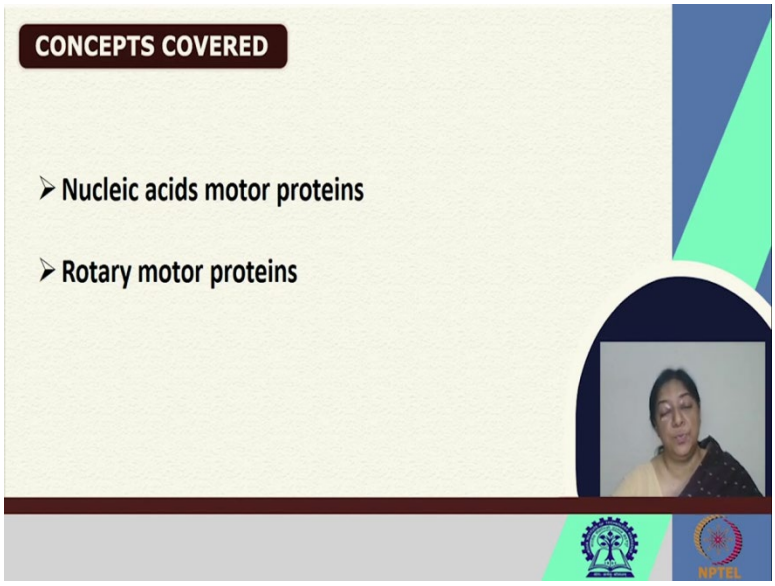
Fundamentals of Protein Chemistry
Prof. Swagata Dasgupta
Department of Chemistry
Indian Institute of Technology, Kharagpur

Module - 08
Motor Proteins and Metalloproteins
Lecture - 37
Motor Proteins - II

In our second lecture on motor proteins, we will be looking at the nucleic acids-based motor proteins.

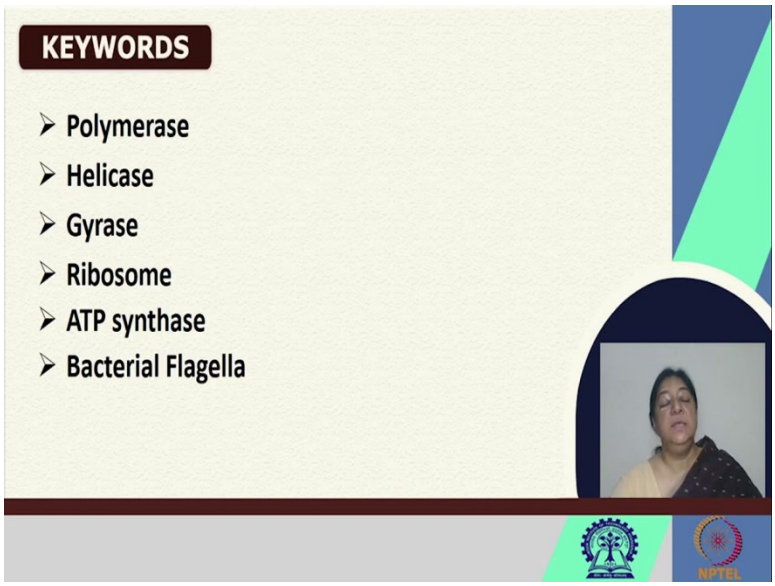
(Refer Slide Time: 00:22)



In the previous lecture we looked at the cytoskeleton filaments like motor proteins, where we saw that the motility was based on a specific type of track; whether it was the microtubule for the

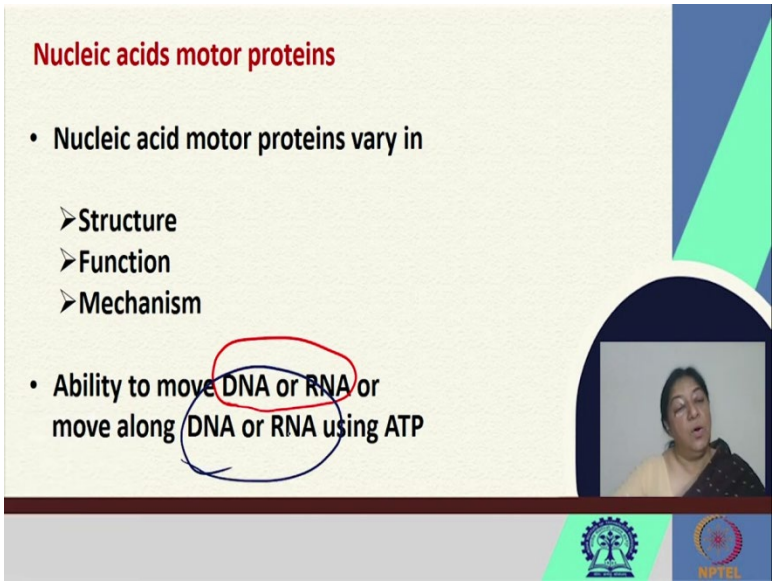
dyneins and the kinases or the actin-based filament for the myosins. In this lecture we will be looking at nucleic acid motor proteins and an example of a rotary motor protein.

(Refer Slide Time: 00:53)



The different types of proteins are the polymerase, the helicase, the gyrase, the ribosome, ATP synthase and bacterial flagella. This is what we will cover in this lecture.

(Refer Slide Time: 01:05)

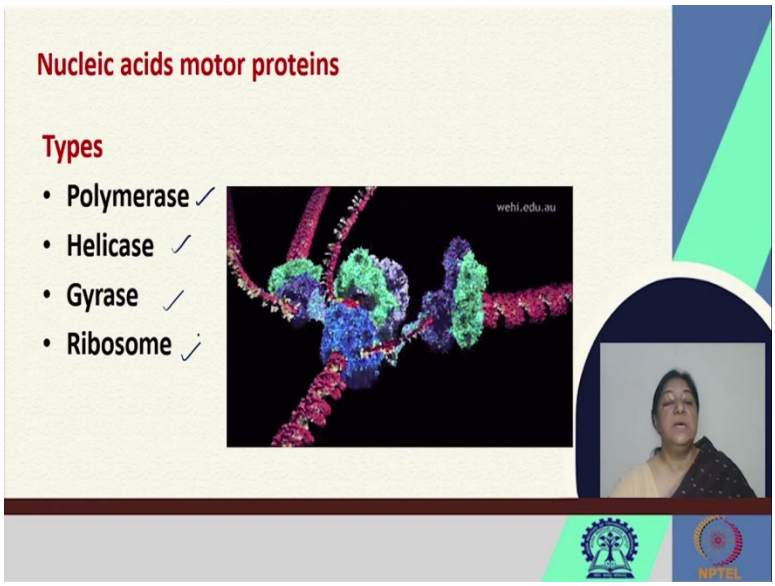


When we look at nucleic acids motor proteins, the name implies that they are motor proteins that are involved in the presence or the utility or the movement of DNA and RNA. Now when we look at nucleic acid motor proteins, we know that they vary in their structure, they vary in their

function and they vary in their mode of action or their mechanism. They have the ability to move DNA or RNA or they could also move along DNA or RNA.

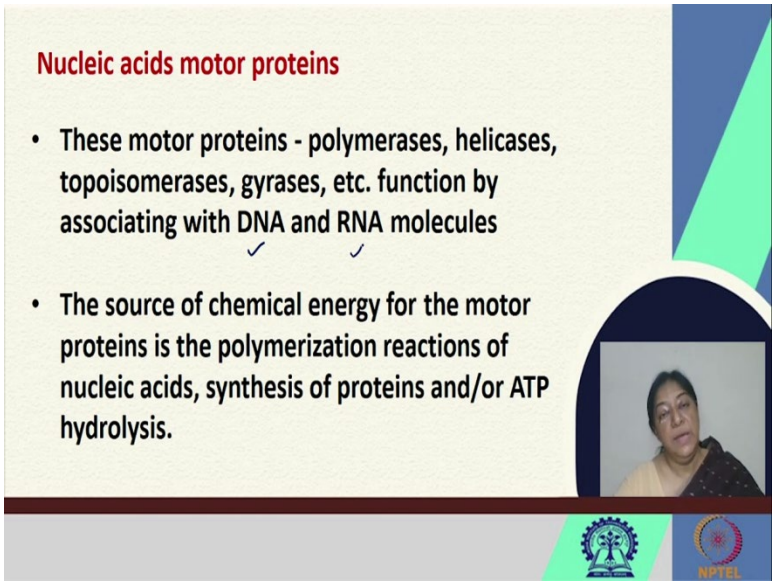
So either they will be moving DNA or RNA; or they themselves will move along DNA or RNA.

(Refer Slide Time: 01:59)



The nucleic acids motor proteins are of different types; they are polymerase, helicase, gyrase and ribosomes. We will be looking at each of these types and see what they can actually do and how they function.

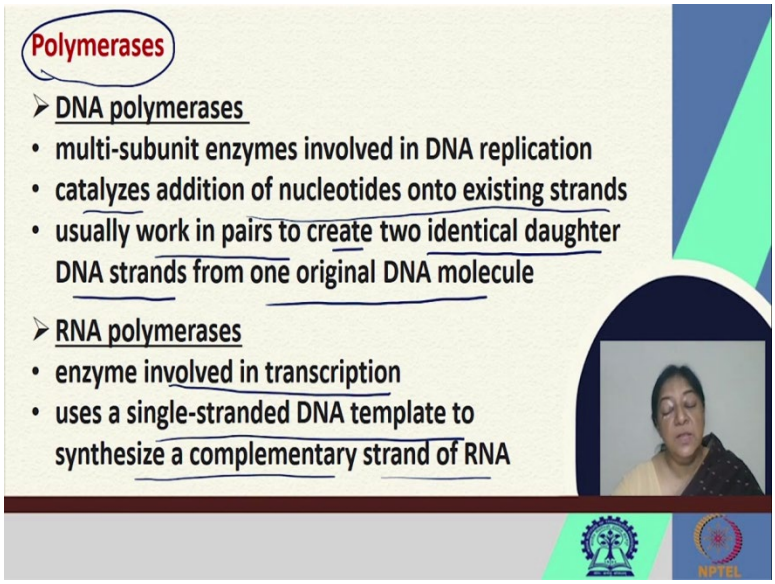
(Refer Slide Time: 02:17)



These motor proteins the polymerases, the helicases, the topoisomerases and the gyrase; they function as their name implies or the type of motor proteins that they are, they function by associating with the nucleic acids. The DNA and the RNA association; we will be looking at the types of association and how they interact in our discussions of protein nucleic acid interactions in a later lecture.

But to understand how the motor proteins work we need or we understand that they have to associate themselves with the DNA and the RNA molecules. The source of chemical energy for the motor proteins is the polymerization reactions of the nucleic acids, the synthesis of the proteins and/or ATP hydrolysis.

(Refer Slide Time: 03:18)

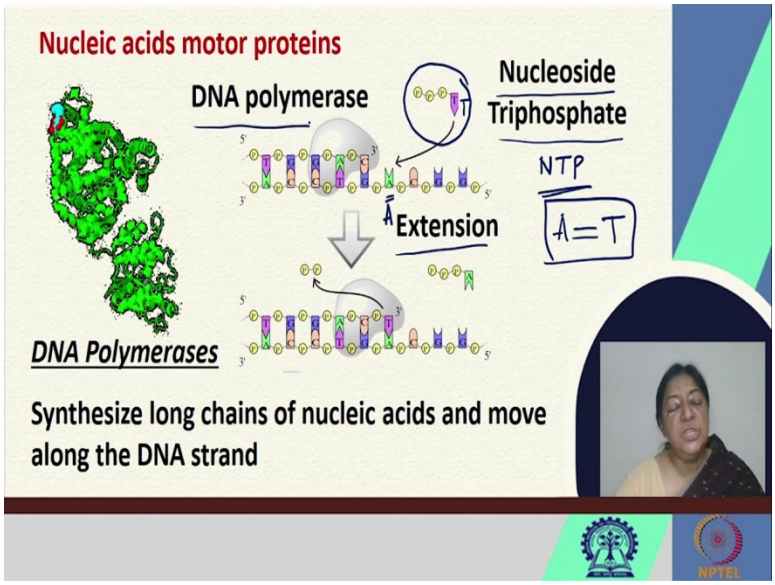


The polymerases as the name implies, will be in the polymerization of the specific nucleic acid that we are interested in. So if you look at DNA polymerases, they are multi subunit enzymes, that are involved in DNA replication. What they do, is they catalyze the addition of nucleotides onto existing strands. Which means that they increase the strands and it is a polymerization activity.

It usually works in pairs and it creates two identical daughter DNA strands from one original DNA molecule. So this is the function of the DNA polymerase and we realize its importance in DNA replication, where we have two daughter strands built from one original DNA molecule. RNA polymerases are enzymes that are involved in the transcription process. This uses a single strand DNA template to synthesize a complementary strand of RNA.

So, we realize the very important activity of these specific proteins involved in the way they function and what they can do.

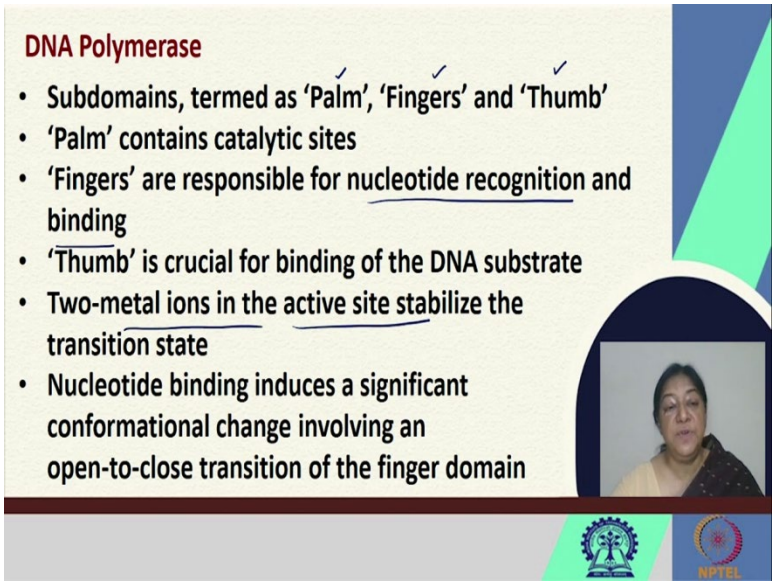
(Refer Slide Time: 04:53)



If we look at the nucleic acids motor proteins we have DNA polymerase for example and we have the nucleoside triphosphate; this is commonly referred to as NTP. This [refer to slide] is the Nucleoside Triphosphate and the DNA polymerase synthesizes long chains of nucleic acid and moves along the DNA strand. So we have our nucleoside triphosphate that comes and sits at its associated site. Here we have A that is the adenine base, here we have thymine and we know that we have the A=T.

So this is the connection with the hydrogen bonds between the two nitrogenous bases. Here we have our DNA stretch, the extension therefore, occurs where we have now the hydrolysis of the ATP and then we have the extension occurred due to the presence of the DNA polymerase protein.

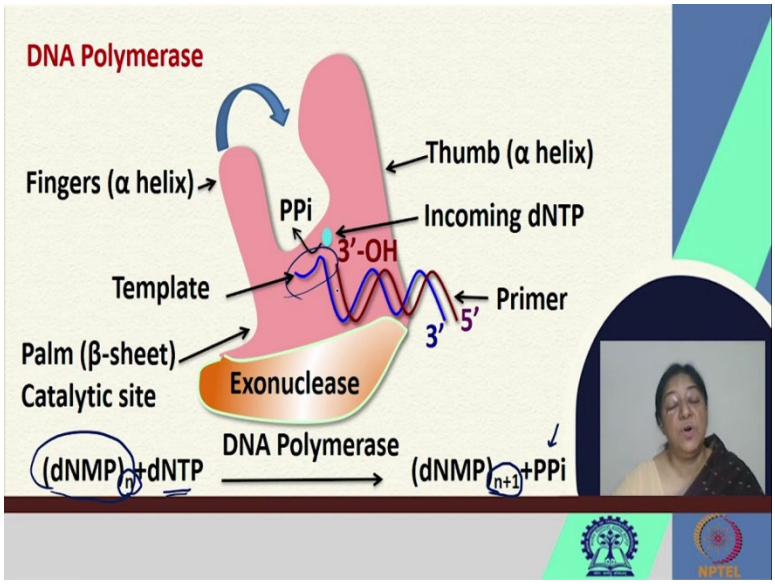
(Refer Slide Time: 06:15)



The structure of the DNA polymerase is such that it has sub domains. These sub domains are termed as the palm, the finger and the thumb. The palm contains the catalytic sides, the fingers are responsible for the nucleotide recognition and binding, and the thumb is crucial for binding of the DNA substrate.

We have learnt about protein ligand binding. In this case protein nucleic acid binding is a method of molecular recognition. And in addition to this there are two metal ions in the active site that stabilize the transition state, which we learned about in enzyme catalysis. This nucleotide binding induces a significant conformational change, that involves in an open-to-close transition of the finger domain.

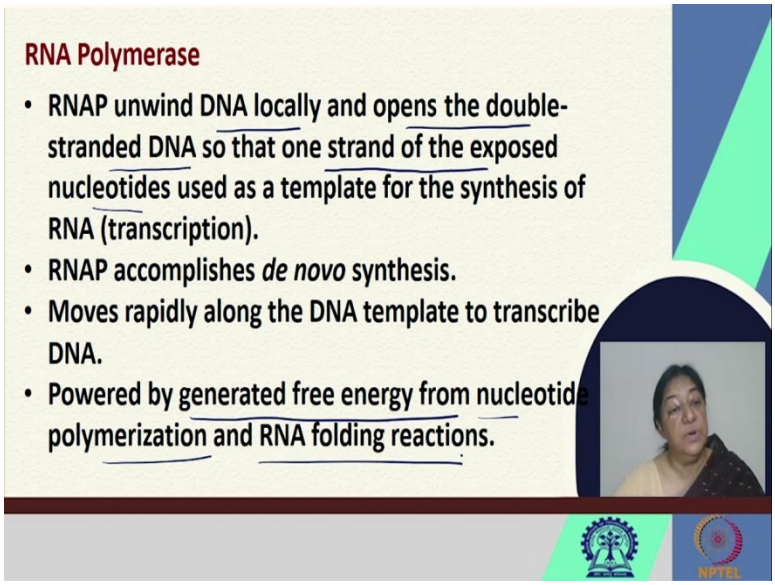
(Refer Slide Time: 07:16)



So this [refer to slide] is the palm, the β-sheet that is the catalytic site; the fingers are associated with the alpha helical conformation and so is the thumb. Then the exonuclease and the DNA polymerase works in a fashion that would form from our nucleotide triphosphate with n number, with the addition of another nucleotide triphosphate go to n+1, in the process releasing the PPi and the enzyme that is bringing about this reaction, is the DNA polymerase.

The incoming dNTP, that is the nucleoside triphosphate and we have the formation of the PPi, the removal of the PPi and the extension.

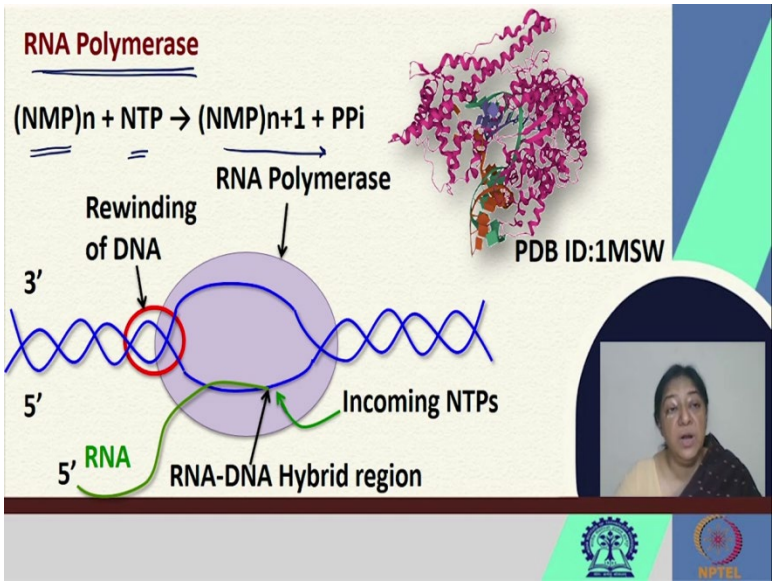
(Refer Slide Time: 08:12)



In the case of RNA polymerase, they unwind DNA locally and they open the double stranded DNA, so that one strand of the exposed nucleotides can be used as a template for the synthesis of RNA that is required in the process of the transcription.

We all know we will have DNA to RNA to protein. This RNA polymerase comes into the picture. It accomplishes the de novo synthesis; it moves rapidly across the DNA template to transcribe the DNA. And this is powered by the generated free energy from the nucleotide polymerization and RNA folding reactions.

(Refer Slide Time: 09:01)

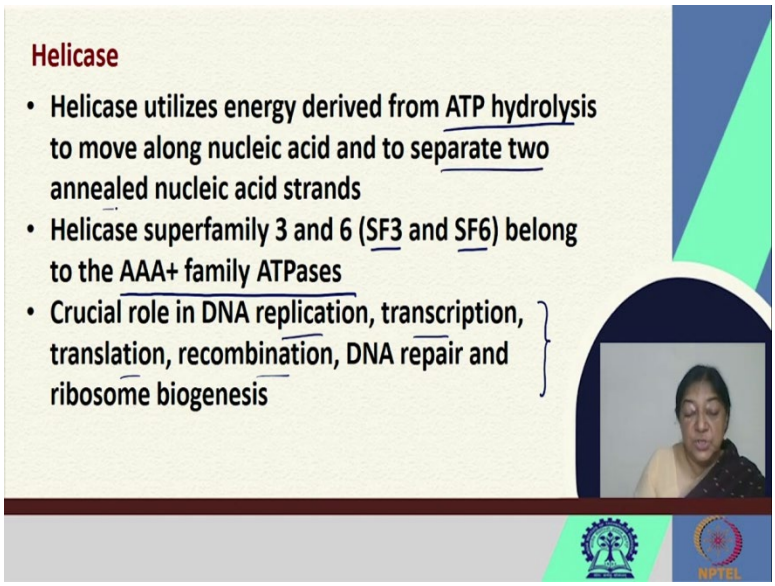


So we have our specific structure, this [refer to slide] is our RNA polymerase and this is where the incoming NTPs come into the picture. We have our nucleotide monophosphate, the nucleotide triphosphate and this is where we would have the formation of the specific set of

DNA. So the polymerization that occurs here, where we would have the template created; like we saw in what the role of the RNA polymerase is.

We have a region here where we are going to have the transcription process. The transcribing that is going to occur for the DNA, which we just saw in the previous slide, where we have the movement along the DNA template to transcribe the DNA and we would have this [refer to slide] form. The RNA polymerase, we would have the incoming NTP, the RNA itself, the rewinding of the DNA that would occur after the template was created. So there would be a region that would have RNA and DNA, known as the hybrid region.

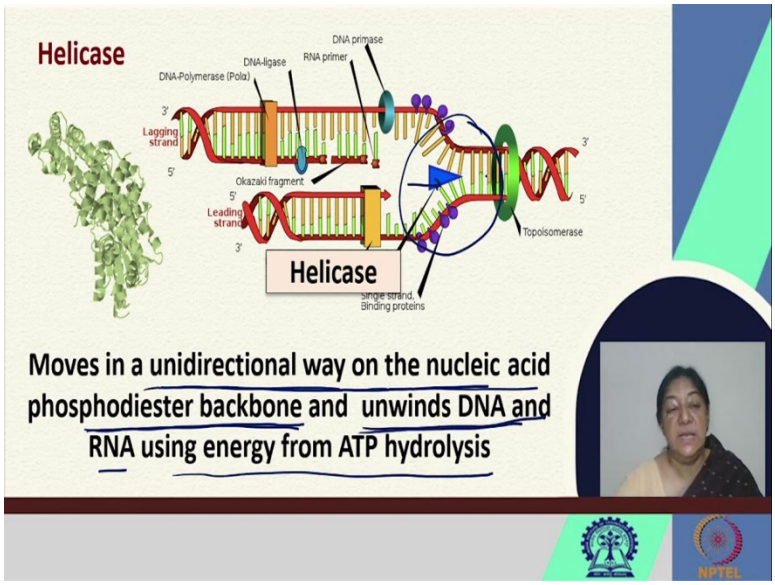
(Refer Slide Time: 10:16)



In the next set of examples of motor proteins, we have the helicase. The helicase proteins utilize the energy derived from ATP hydrolysis and they move along the nucleic acid to separate the two nucleic acid strands. So, again this super family called SF3 and SF6, belong to the AAA+ family of ATPases that we discussed in the previous lecture.

This plays a crucial role in DNA replication, transcription, translation, recombination, DNA repair and ribosome biogenesis. So, we understand the importance of this specific protein in the process of DNA replication, transcription, translation, recombination. This is where we have a separation of the two nucleic acid strands of the DNA.

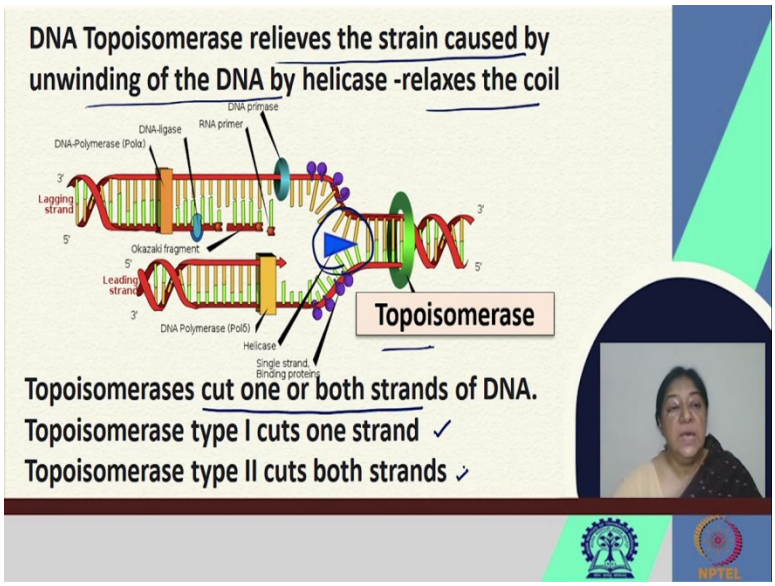
(Refer Slide Time: 11:18)



This is where helicase comes into the picture. This [refer to slide] is where we have the two strands being separated. This moves in a unidirectional way on the nucleic acid phosphodiester backbone. And it unwinds DNA and RNA using energy from ATP hydrolysis. The helicase is moving in a specific direction, where we have what is called a leading strand and a lagging strand.

A discussion of that is beyond the scope of this course, but what we do understand is there is a movement of the helicase in a unidirectional manner and as it does that, it unwinds the DNA as it goes along.

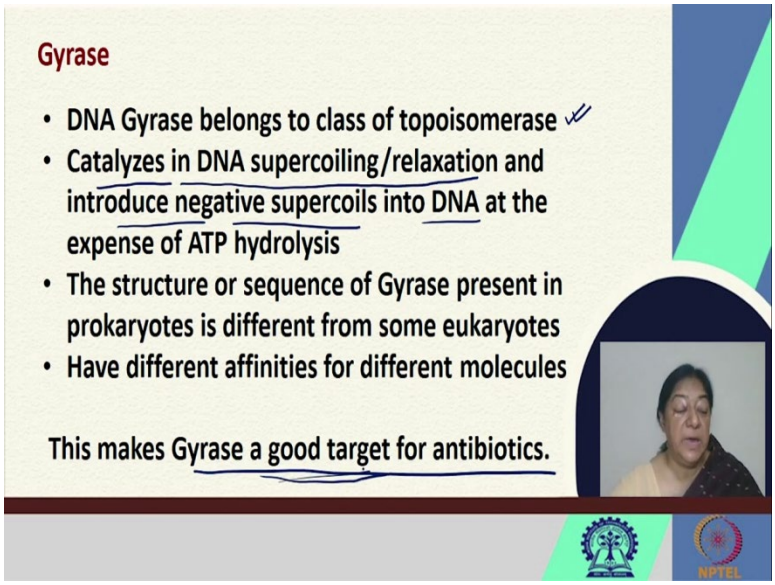
(Refer Slide Time: 12:16)



There is another protein called the topoisomerases and what the topoisomerase does, it relieves the strain that is caused by the unwinding of the DNA. So, helicase results in the unwinding of

the DNA and the topoisomerase helps to relax the coil as it is getting unwound by the helicase molecule. The topoisomerase therefore is an enzyme that cuts either one strand of DNA or both the strands of DNA. There are several types the topoisomerase type I for example, cuts only one strand and the topoisomerase II cuts both these strands.

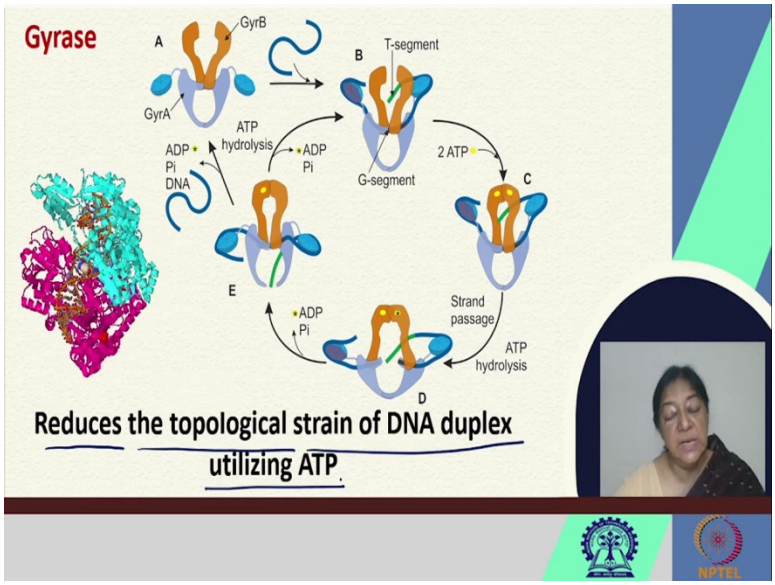
(Refer Slide Time: 13:02)



An example of one such topoisomerase is gyrase. So DNA gyrase belongs to the class of topoisomerases. It catalyzes in the DNA supercoiling relaxation and it introduces negative supercoils into the DNA, again at the expense of ATP hydrolysis. The structure of the sequences of the gyrase proteins present in the prokaryotes are different than those in the eukaryotes. Because they have different affinities for different molecules, gyrase are often times used as good targets for antibiotics.

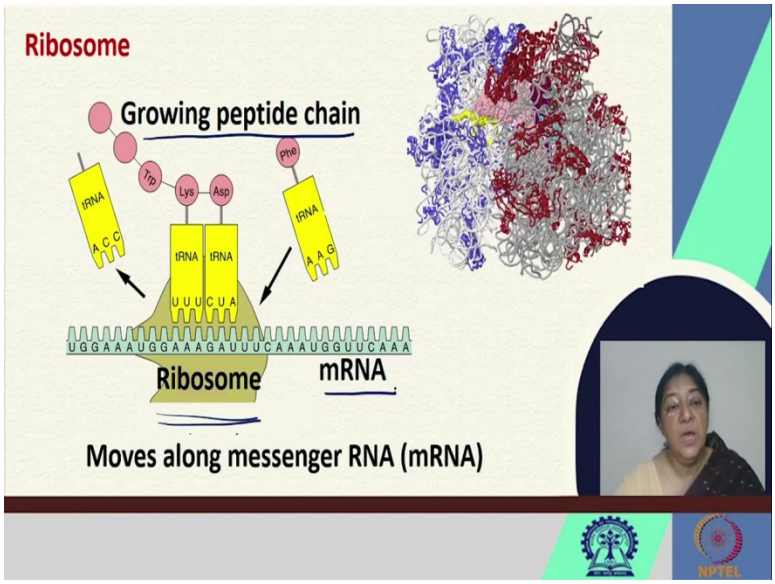
So this is a molecule that catalyzes DNA super coiling relaxation and it belongs to the class of topoisomerases and because it has different affinities for different molecules, it is often a very good target for the development of antibiotics.

(Refer Slide Time: 14:06)



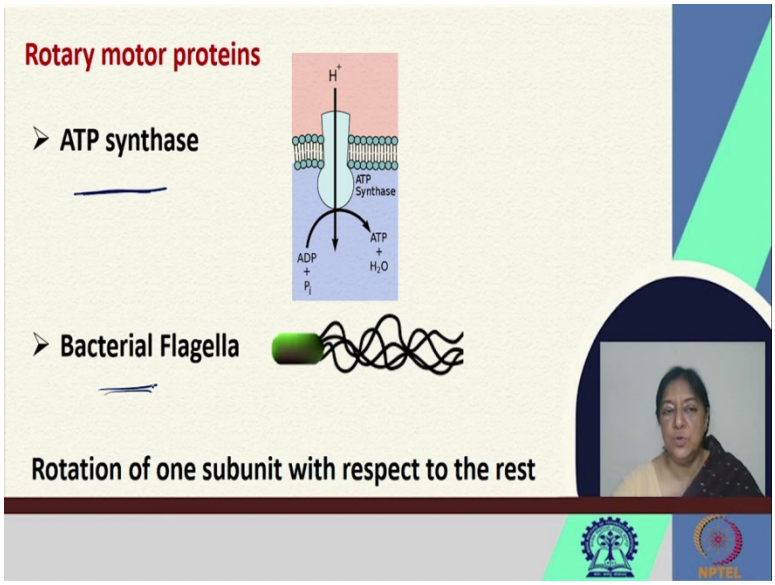
This [refer to slide] is our gyrase molecule and this is its mode of action, as it is one such topoisomerase. It reduces the topological strain of the DNA duplex using the hydrolysis of ATP, to release the strain due to the unwinding of the fragments, unwinding of the double helix. This unwinding is brought about by helicase.

(Refer Slide Time: 14:41)



In the ribosome protein, there is the development of the growing peptide chain. We have the ribosome protein that is the whole machinery for the formation of the peptide chain and we have the mRNA template. Now, in the process which we will visit in a later class as well, we will be looking at these specific types of proteins in the rotary motor protein, that is another type of protein.

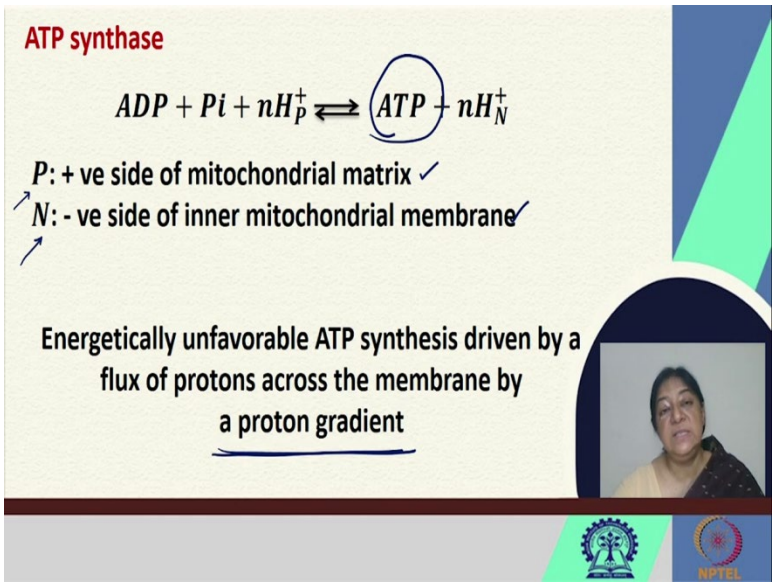
(Refer Slide Time: 15:09)



So we have the nucleic acids type protein. In the nucleic acids types proteins, we looked at the different polymerases, the helicase, the gyrase, the topoisomerase and the ribosome. The rotary motor proteins; an extremely important one is ATP synthase, as in all the discussion that we have based on water proteins, ATP hydrolysis is what is driving the reaction or the enzymatic function to occur.

The ATP synthase is one very important molecule that uses a specific process to generate ATP. The rotary motor proteins also contain bacterial flagella that are used for their movement and there is rotation of one subunit of these specific molecules with respect to the other ones.

(Refer Slide Time: 16:22)

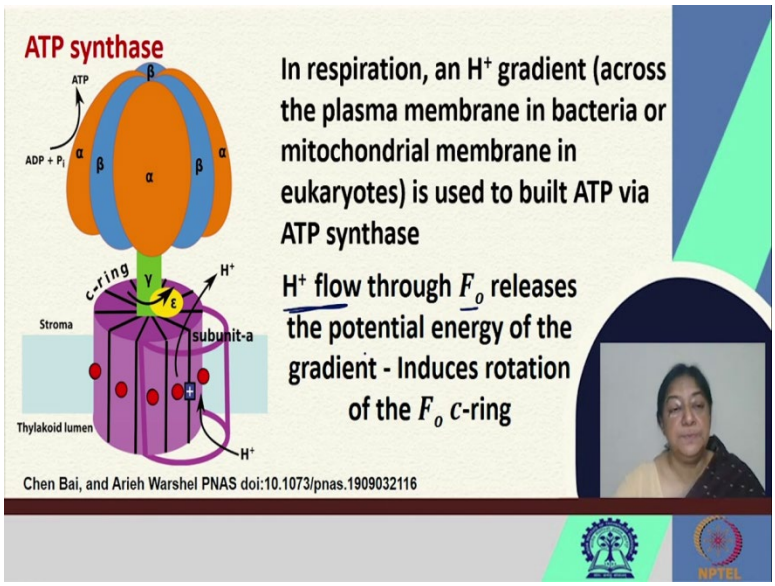


The ATP synthase is the formation of ATP. We were looking at ATP hydrolysis. ATP synthesis, is of course one of the most important reactions that would occur and this occurs in the

mitochondrial matrix, where there is a positive side and there is a negative side. We will be looking at this when we study membranes; membrane proteins, membrane potential and the transport across the membrane. In this case we have the proton transport.

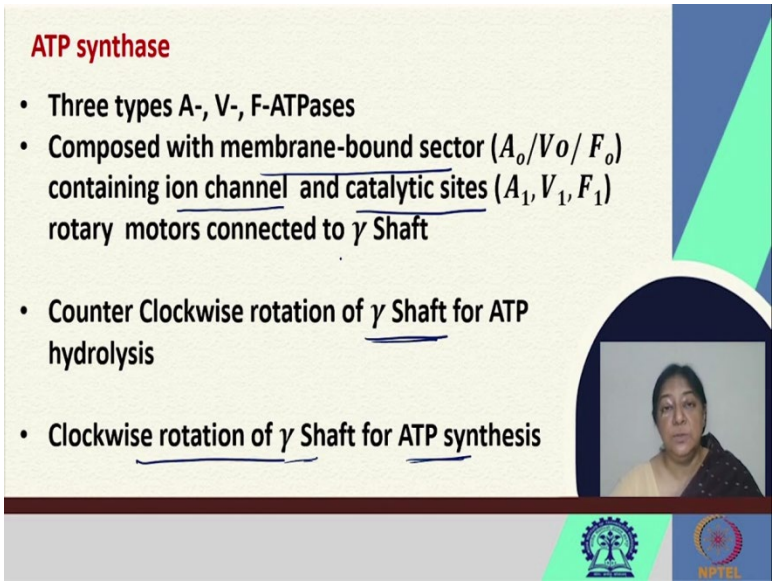
So P indicates a positive side of the mitochondrial matrix and N indicates a negative side of the inner mitochondrial membrane. So, energetically unfavorable ATP synthesis is driven by this flux of protons across a membrane, by what is called a proton gradient. What we are now concerned with, is the rotary motor movement of the specific protein that results in the synthesis of ATP.

(Refer Slide Time: 17:37)



This [refer to slide] is ATP synthase and in respiration what happens, there is a proton gradient across the plasma membrane in bacteria or mitochondrial membranes, that is used to build ATP via ATP synthase. What happens is there is proton flow through a subunit of this specific protein called the F_0. We will see what this means in a moment.

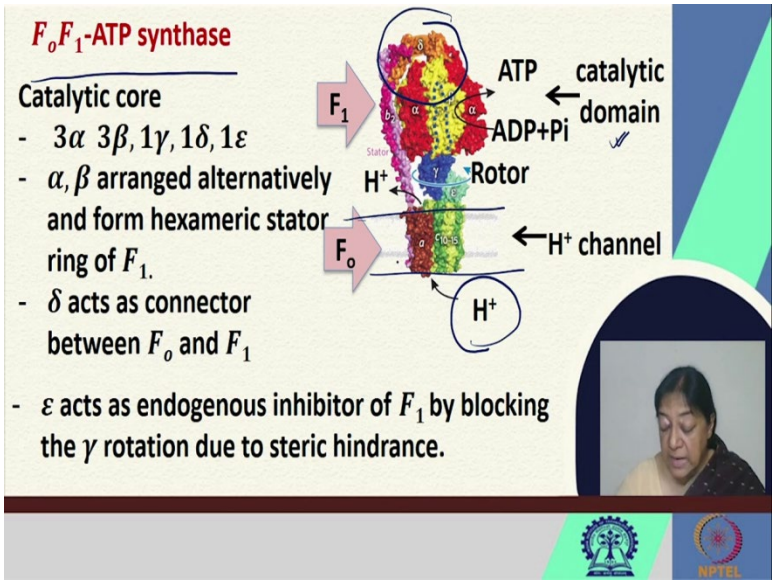
(Refer Slide Time: 18:04)



If we look at the protein there are three types A-, V-, F- ATPases. They are composed with membrane bound sectors that contain an ion channel because we know there has to be the flux of protons and specific catalytic sites and there are rotary motors that are connected to a gamma shaft. If we look at the structure, we will see what it means. The counterclockwise rotation of this particular shaft occurs for ATP hydrolysis and the clockwise rotation occurs of the gamma shaft for ATP synthesis.

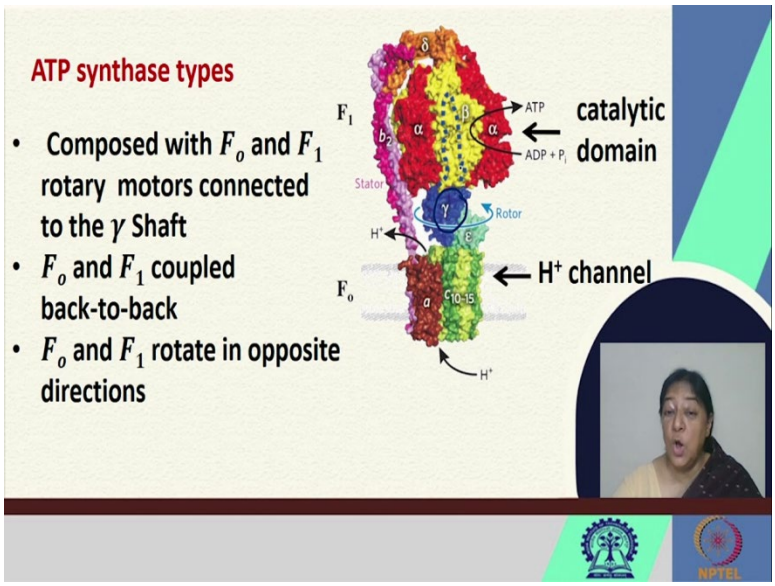
So there is a shaft movement due to the rotary motors. If there is a clockwise rotation there is a synthesis, if there is a counterclockwise motion there is hydrolysis. It is a wonderful molecular machine.

(Refer Slide Time: 18:59)



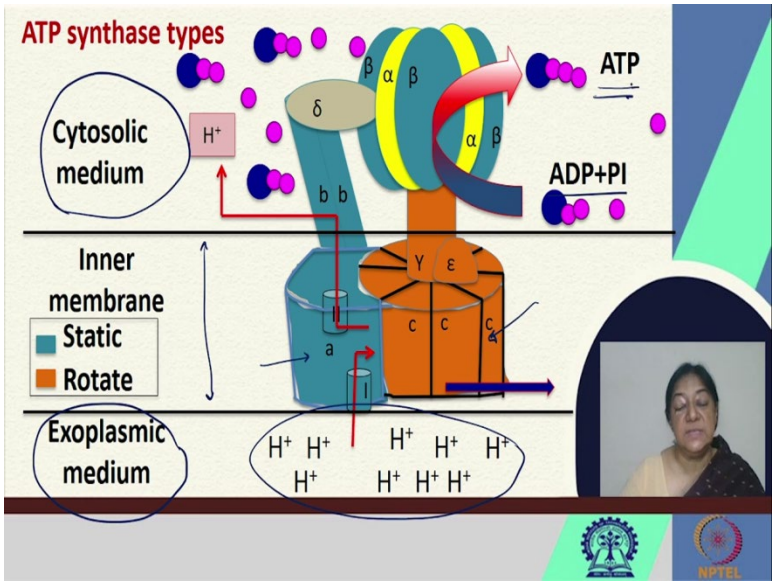
So we have in this protein an F_0 and F_1 domain. This is F_0 F_1 -ATP synthase. There is a catalytic domain that is comprised of 3α and 3β subunits and this is where we have our membrane channel, where we will have the proton flux movement. So we have the catalytic core which has 3α, 3β, 1γ, 1δ and 1ε unit. And the α and β are arranged alternatively to form this [refer to slide] specific hexametric sector of ring of the F_1 com subunit and the δ acts as this connector. This is the connector that is connecting the F_0 and the F_1.

(Refer Slide Time: 19:52)



The ATP synthase types, they are composed of the F_0 and F_1 rotary motors; they are connected through the γ shaft. So this [refer to slide] is the γ shaft and there is a counter counterclockwise movement or a clockwise movement depending upon whether they are going to be ATP hydrolysis or synthase. Now the F_0 and F_1 are coupled back to back and they rotate in opposite directions.

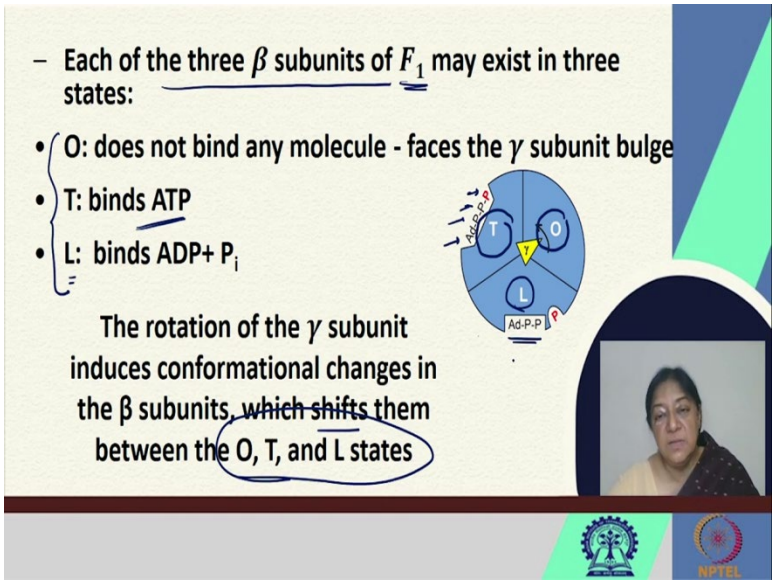
(Refer Slide Time: 20:17)



What happens is, there [refer to slide] is the cytosolic medium, there is the exoplasmic medium and this is the inner membrane. This remains static and this is where there is rotation.

This is where we have a proton flux and this rotation is going to bring us the formation of ATP. Then once we have the movement of this proton, we will have the ADP+Pi to ATP synthesis.

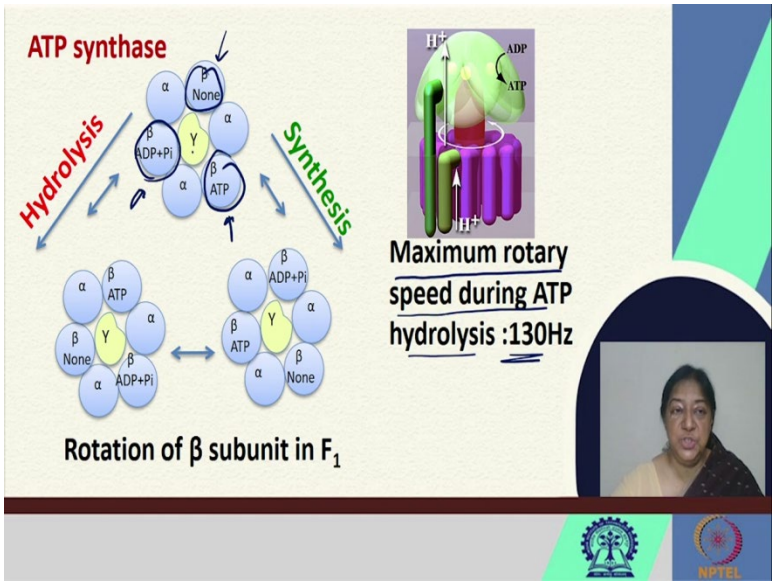
(Refer Slide Time: 20:54)



Now, this is important because there are three β subunits of the F_1 that actually exists in three states. There is an O state, kind of an open state that does not bind any molecule. The T state binds ATP, so we see [refer to slide] the adenosine and there are three phosphates associated here. So T binds ATP. L binds ADP+Pi. So there is an open state, kind of a tight state and a loose state.

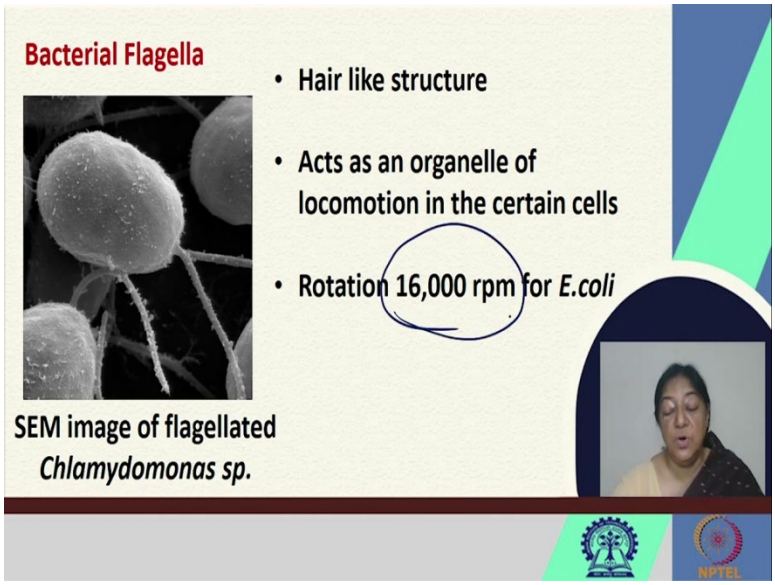
Now what happens is there is the rotation of the γ subunit. As this rotates there is conformational change that is induced in the β subunit. As a result of which, it shifts between the three states. So at one state there is O state and there is nothing bound to it, at one state there is ATP bond and at the other state there is ADP and Pi bond. So based on this rotation there will be the synthesis or the break.

(Refer Slide Time: 22:03)



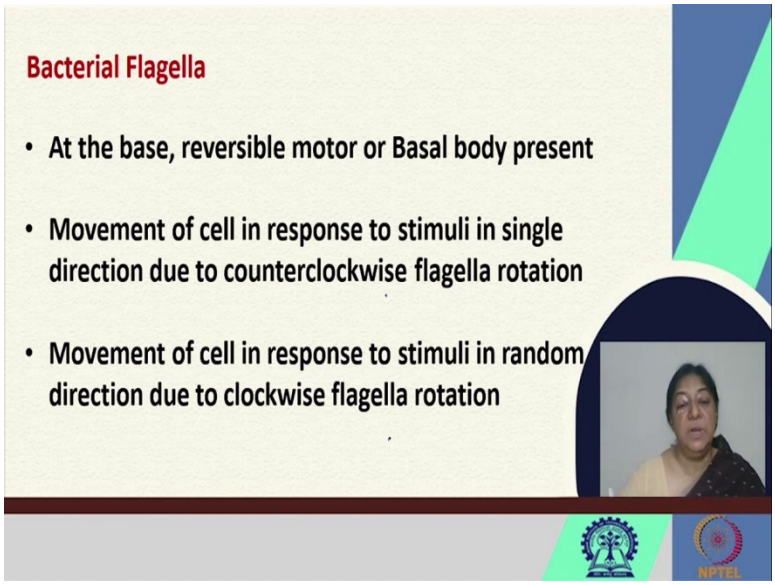
[Refer to slide] here is our B subunit which is either the open state, the tight state or the loose state and we have the γ shaft. Now the rotation of this γ shaft can lead to hydrolysis or synthesis and this is very important for the rotation of the β subunit in F_1, in bringing about the specific synthesis or hydrolysis of ATP. The interesting thing is that there is a maximum rotary speed during ATP hydrolysis of 130 Hz, of this specific machine.

(Refer Slide Time: 22:47)



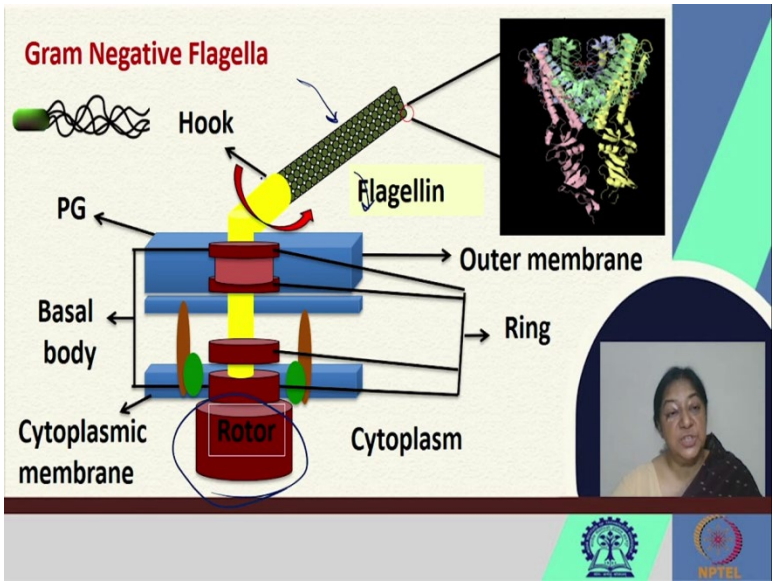
The other rotary protein that is important is the bacteria flagella. It is a hair like structure, it acts as an organelle of locomotion in several cells and here also there is a very high rotation for the E.coli.

(Refer Slide Time: 23:01)



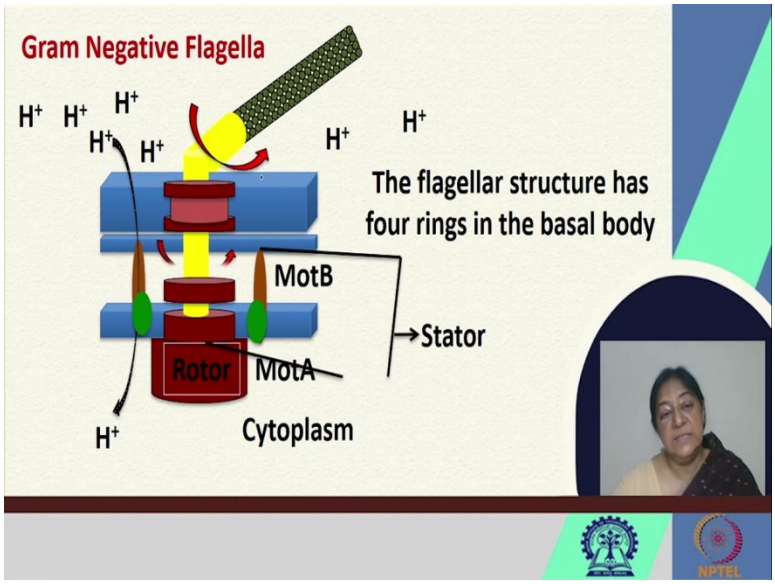
The flagella are at the base, it has a reversible motor and the movement of the cell is in response to stimuli in the single direction due to the counterclockwise of the flagella rotation. This movement of the cell in response to the stimuli occurs due to clockwise rotation. So, there are two types of motion. One movement that occurs due to the counterclockwise, this is called the run and one movement that occurs due to the clockwise, called the tumble.

(Refer Slide Time: 23:33)



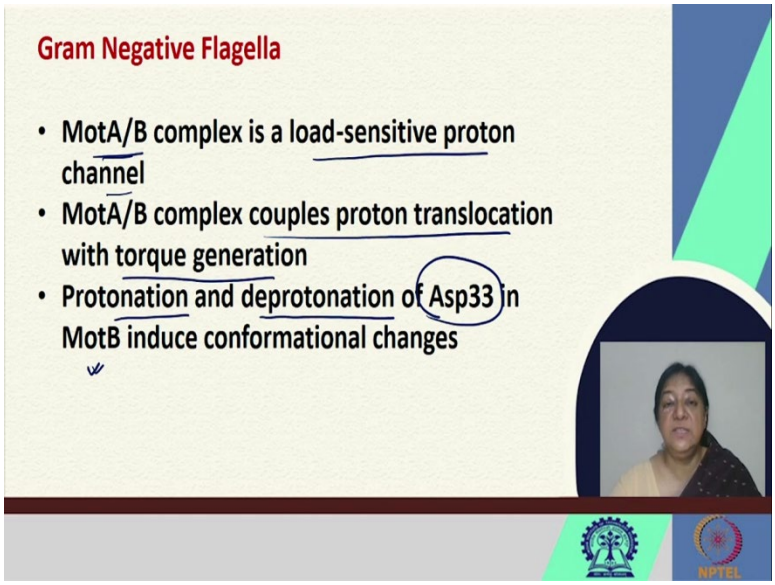
If we look [refer to slide] at the structural aspects of it, there is the outer membrane, the cytoplasmic membrane and there is a specific hook of attachment. There are rings and there is this motor that actually has the movement of the flagella. This movement causes the flagella to move.

(Refer Slide Time: 24:02)



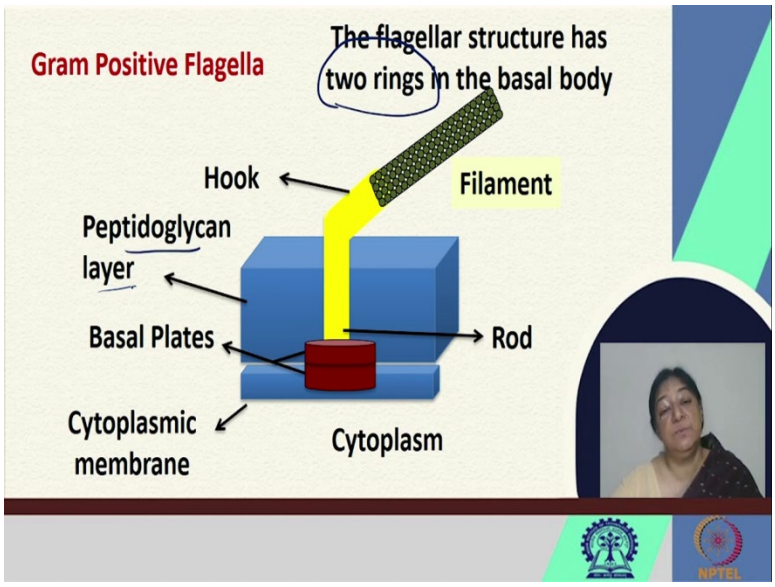
The flagella structure, in this case for gram negative flagella, has has four rings in the basal body.

(Refer Slide Time: 24:14)



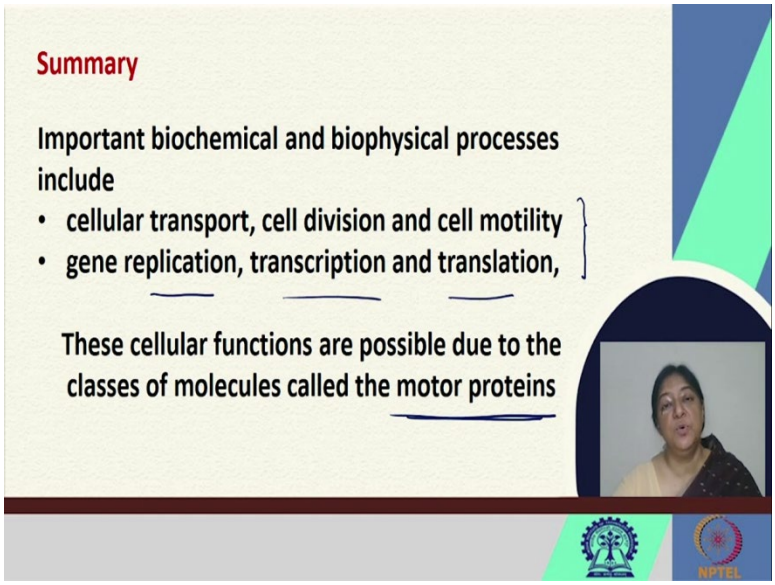
There is specific movement and there is a motor A and motor B complex, that is a load sensitive proton channel. What happens here, it couples proton translocation with a torque generation and this torque generation is due to the protonation and deprotonation of Asp33 in the motor B and this induces the conformational change. So, the proton translocation or the proton movement in this case results in a torque generation, that results in the movement.

(Refer Slide Time: 24:47)



The gram positive flagella on the other hand has two rings in the basal body, where there is a specific peptidoglycan layer in the gram positive bacteria, but the method of motion or its mechanism of motion is similar to that of the gram negative ones.

(Refer Slide Time: 25:03)

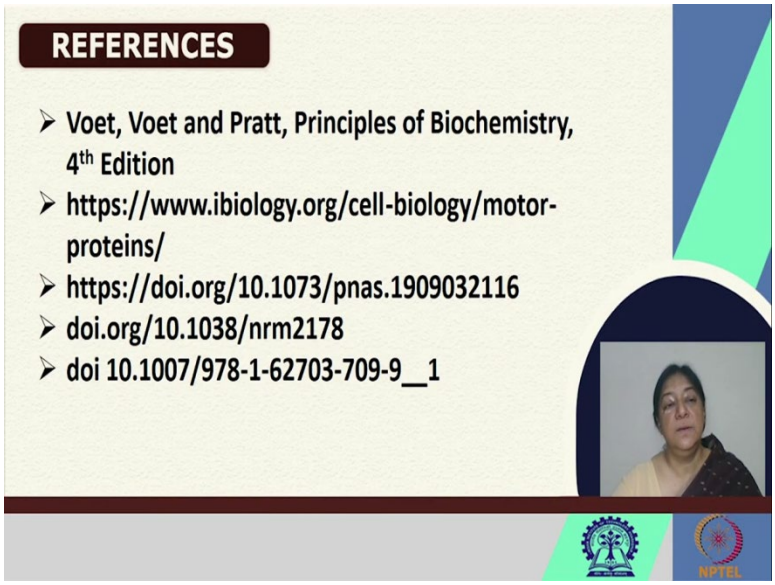


In summary; we have looked at the important biochemical and biophysical processes in this case, that include cellular transport, cell division and cell motility and we see the importance of the motor proteins in bringing about these specific processes, even gene replication transcription and translation.

So when we looked at cellular transport, we looked at a specific type of protein, the cytoplasmic types of motor proteins that use the actin filament or the microtubules for their motion, for their cargo transport. In this lecture we looked at nucleic acids and their motor proteins and how they are involved in DNA replication, transcription and translation.

All these cellular functions that are extremely important for the life processes, are possible due to these classes of molecules that are called the motor proteins. There is still a lot of research going on in these motor proteins, to understand them further. And there are several sites, several links, several books that are available that can provide you with more information based on the mechanism and workings of the motor proteins.

(Refer Slide Time: 26:31)



These [refer to slide] are the references.

Thank you.