

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.

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CONCEPTS COVERED

- Nucleic acids motor proteins
- Rotary motor proteins

The slide features a video inset of Prof. Swagata Dasgupta in the bottom right corner. At the bottom, there are logos for IIT Kharagpur and NPTEL.

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.

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KEYWORDS

- Polymerase
- Helicase
- Gyrase
- Ribosome
- ATP synthase
- Bacterial Flagella

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.

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Nucleic acids motor proteins

- Nucleic acid motor proteins vary in
 - Structure
 - Function
 - Mechanism
- Ability to move DNA or RNA or move along DNA or RNA using ATP

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.



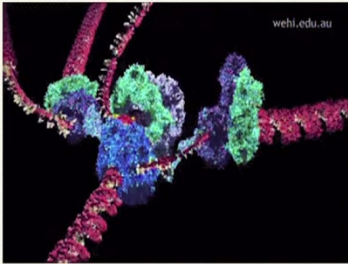
So either they will be moving DNA or RNA; or they themselves will move along DNA or RNA.

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Nucleic acids motor proteins

Types

- Polymerase ✓
- Helicase ✓
- Gyrase ✓
- Ribosome ✓


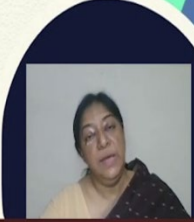


The nucleic acids motor proteins are of different types; they are polymerase, helicase, gyrase and ribosomes. We will be looking at each of these types and see what they can actually do and how they function.

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Nucleic acids motor proteins

- These motor proteins - polymerases, helicases, topoisomerases, gyrases, etc. function by associating with DNA and RNA molecules ✓ ✓
- The source of chemical energy for the motor proteins is the polymerization reactions of nucleic acids, synthesis of proteins and/or ATP hydrolysis.



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.

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Polymerases

- DNA polymerases
 - multi-subunit enzymes involved in DNA replication
 - catalyzes addition of nucleotides onto existing strands
 - usually work in pairs to create two identical daughter DNA strands from one original DNA molecule
- RNA polymerases
 - enzyme involved in transcription
 - uses a single-stranded DNA template to synthesize a complementary strand of RNA

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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.

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Nucleic acids motor proteins

DNA polymerase

Nucleoside Triphosphate



NTP

A=T

Extension

DNA Polymerases

Synthesize long chains of nucleic acids and move along the DNA strand



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.

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DNA Polymerase

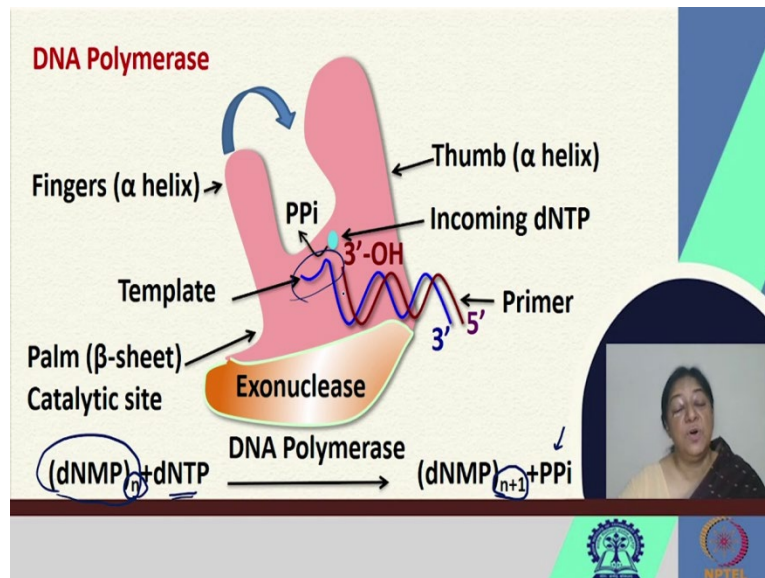
- Subdomains, termed as 'Palm', 'Fingers' and 'Thumb'
- 'Palm' contains catalytic sites
- 'Fingers' are responsible for nucleotide recognition and binding
- 'Thumb' is crucial for binding of the DNA substrate
- Two-metal ions in the active site stabilize the transition state
- Nucleotide binding induces a significant conformational change involving an open-to-close transition of the finger domain

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 sites, 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.

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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 P_i 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 P_i, the removal of the P_i and the extension.

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RNA Polymerase

- RNAP unwind DNA locally and opens the double-stranded DNA so that one strand of the exposed nucleotides used as a template for the synthesis of RNA (transcription).
- RNAP accomplishes *de novo* synthesis.
- Moves rapidly along the DNA template to transcribe DNA.
- Powered by generated free energy from nucleotide polymerization and RNA folding reactions.

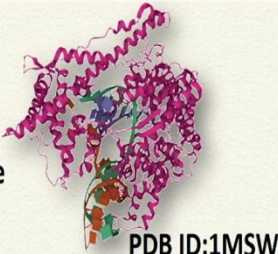



In the case of RNA polymerase, they unwind DNA locally and they open the double stranded DNA, so that one strand of the exposed nucleotides can be used as a template for the synthesis of RNA that is required in the process of the transcription.

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.

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RNA Polymerase

$$(NMP)_n + NTP \rightarrow (NMP)_{n+1} + PP_i$$


RNA Polymerase

Rewinding of DNA



3'

5'

5' RNA

RNA-DNA Hybrid region

Incoming NTPs

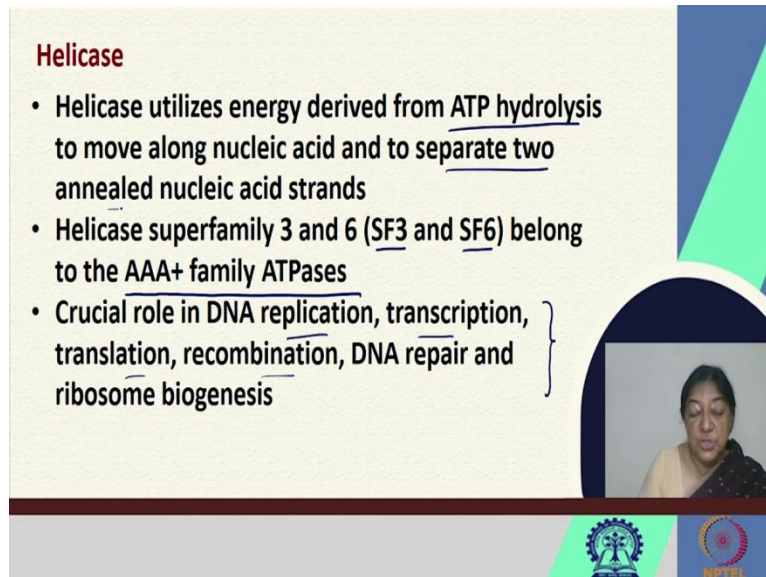



So we have our specific structure, this [refer to slide] is our RNA polymerase and this is where the incoming NTPs come into the picture. We have our nucleotide monophosphate, the nucleotide triphosphate and this is where we would have the formation of the specific set of

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.

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Helicase

- Helicase utilizes energy derived from ATP hydrolysis to move along nucleic acid and to separate two annealed nucleic acid strands
- Helicase superfamily 3 and 6 (SF3 and SF6) belong to the AAA+ family ATPases
- Crucial role in DNA replication, transcription, translation, recombination, DNA repair and ribosome biogenesis

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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.

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Helicase

Moves in a unidirectional way on the nucleic acid phosphodiester backbone and unwinds DNA and RNA using energy from ATP hydrolysis

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.

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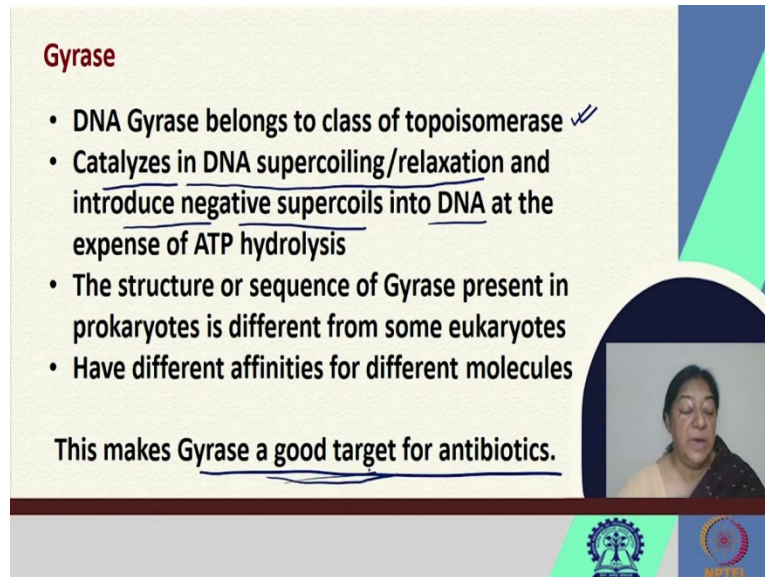
DNA Topoisomerase relieves the strain caused by unwinding of the DNA by helicase -relaxes the coil

Topoisomerases cut one or both strands of DNA.
 Topoisomerase type I cuts one strand ✓
 Topoisomerase type II cuts both strands ✓

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.

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Gyrase

- DNA Gyrase belongs to class of topoisomerase ✓✓
- Catalyzes in DNA supercoiling/relaxation and introduce negative supercoils into DNA at the expense of ATP hydrolysis
- The structure or sequence of Gyrase present in prokaryotes is different from some eukaryotes
- Have different affinities for different molecules

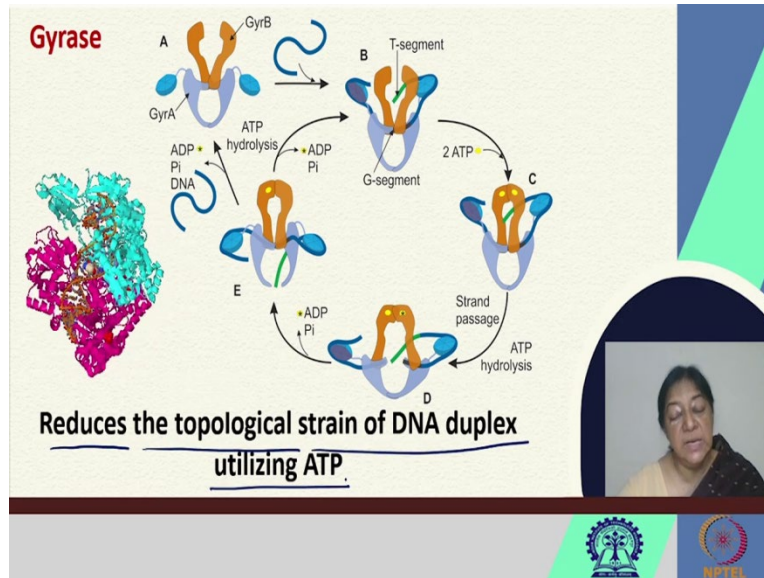
This makes Gyrase a good target for antibiotics.

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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.

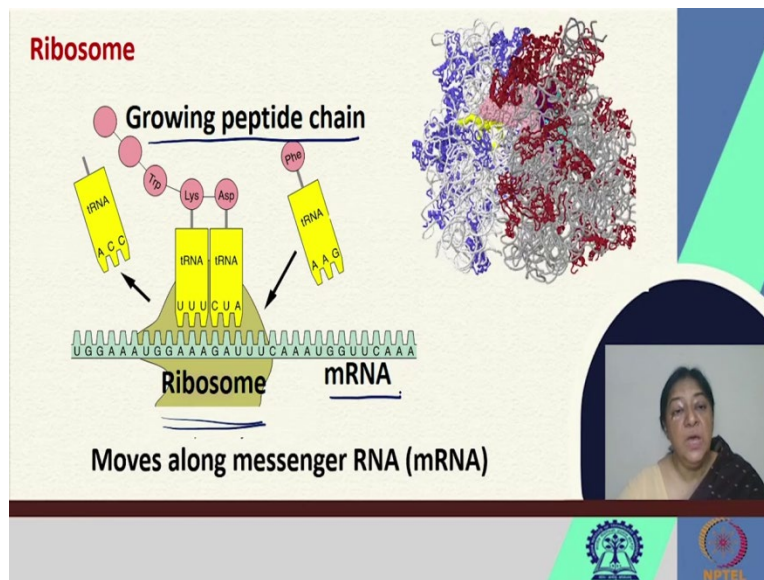
So this is a molecule that catalyzes DNA super coiling relaxation and it belongs to the class of topoisomerases and because it has different affinities for different molecules, it is often a very good target for the development of antibiotics.

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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.

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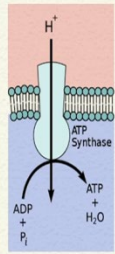


In the ribosome protein, there is the development of the growing peptide chain. We have the ribosome protein that is the whole machinery for the formation of the peptide chain and we have the mRNA template. Now, in the process which we will visit in a later class as well, we will be looking at these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

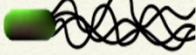
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Rotary motor proteins



- **ATP synthase**



- **Bacterial Flagella**



Rotation of one subunit with respect to the rest

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.



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ATP synthase

$$ADP + Pi + nH_P^+ \rightleftharpoons ATP + nH_N^+$$

P: + ve side of mitochondrial matrix ✓
 N: - ve side of inner mitochondrial membrane ✓

Energetically unfavorable ATP synthesis driven by a flux of protons across the membrane by a proton gradient

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.

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ATP synthase

In respiration, an H^+ gradient (across the plasma membrane in bacteria or mitochondrial membrane in eukaryotes) is used to build ATP via ATP synthase

H^+ flow through F_0 releases the potential energy of the gradient - Induces rotation of the F_0 c-ring

Chen Bai, and Arieh Warshel PNAS doi:10.1073/pnas.1909032116

The diagram illustrates the structure of ATP synthase. The F1 portion is embedded in the membrane, consisting of two α subunits (orange) and two β subunits (blue). The F0 portion is embedded in the membrane, consisting of a central ϵ subunit (yellow), a γ subunit (green), and a c-ring (purple) with H^+ ions (red dots) passing through it. The c-ring is connected to a subunit-a (purple) and a subunit-b (purple). The diagram shows H^+ ions flowing from the stroma (top) through the c-ring into the thylakoid lumen (bottom). This flow induces the rotation of the c-ring, which in turn drives the synthesis of ATP from ADP and P_i in the F1 portion.

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.

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ATP synthase

- Three types A-, V-, F-ATPases
- Composed with membrane-bound sector ($A_o/V_o/F_o$) containing ion channel and catalytic sites (A_1, V_1, F_1) rotary motors connected to γ Shaft
- Counter Clockwise rotation of γ Shaft for ATP hydrolysis
- Clockwise rotation of γ Shaft for ATP synthesis



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.

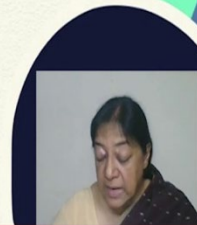
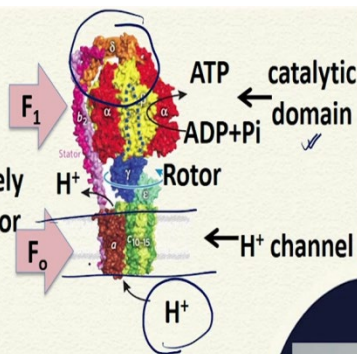
So there is a shaft movement due to the rotary motors. If there is a clockwise rotation there is a synthesis, if there is a counterclockwise motion there is hydrolysis. It is a wonderful molecular machine.

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F_oF_1 -ATP synthase

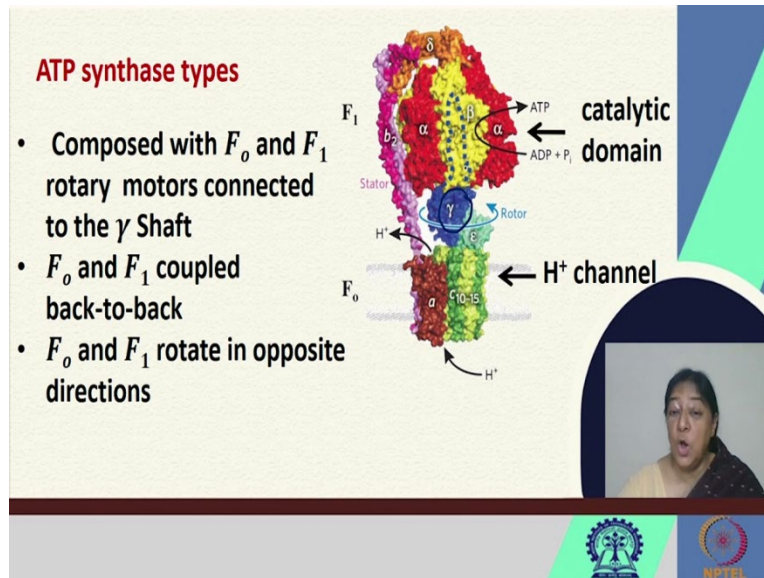
Catalytic core

- $3\alpha, 3\beta, 1\gamma, 1\delta, 1\epsilon$
- α, β arranged alternatively and form hexameric stator ring of F_1 .
- δ acts as connector between F_o and F_1
- ϵ acts as endogenous inhibitor of F_1 by blocking the γ rotation due to steric hindrance.



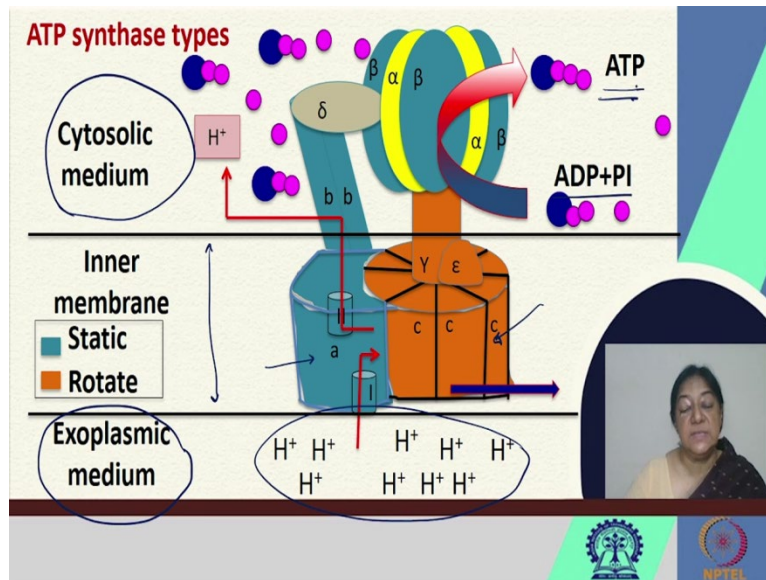
So we have in this protein an F_0 and F_1 domain. This is $F_0 F_1$ -ATP synthase. There is a catalytic domain that is comprised of 3α and 3β subunits and this is where we have our membrane channel, where we will have the proton flux movement. So we have the catalytic core which has 3α , 3β , 1γ , 1δ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

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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 clockwise 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.

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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.

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– Each of the three β subunits of F_1 may exist in three states:

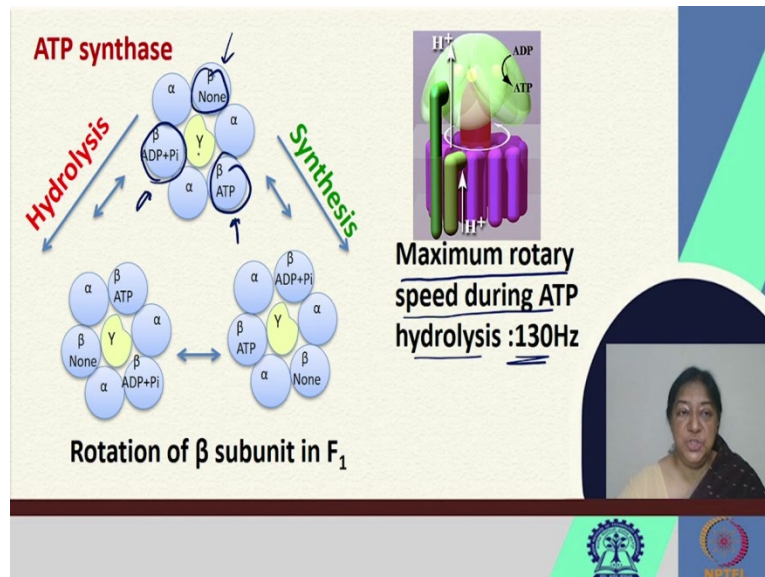
- O: does not bind any molecule - faces the γ subunit bulge
- T: binds ATP
- L: binds ADP+ P_i

The rotation of the γ subunit induces conformational changes in the β subunits, which shifts them between the O, T, and L states

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 bound and at the other state there is ADP and P_i bound. So based on this rotation there will be the synthesis or the break.

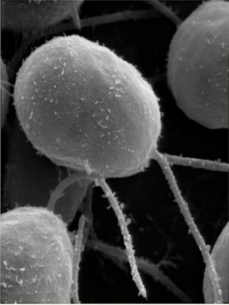
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[Refer to slide] here is our B subunit which is either the open state, the tight state or the loose state and we have the γ shaft. Now the rotation of this γ shaft can lead to hydrolysis or synthesis and this is very important for the rotation of the β subunit in F₁, 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.




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Bacterial Flagella



- Hair like structure
- Acts as an organelle of locomotion in the certain cells
- Rotation 16,000 rpm for *E.coli*

SEM image of flagellated *Chlamydomonas sp.*

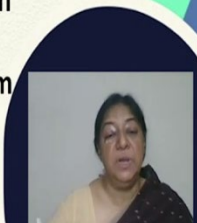






The other rotary protein that is important is the bacteria flagella. It is a hair like structure, it acts as an organelle of locomotion in several cells and here also there is a very high rotation for the E.coli.

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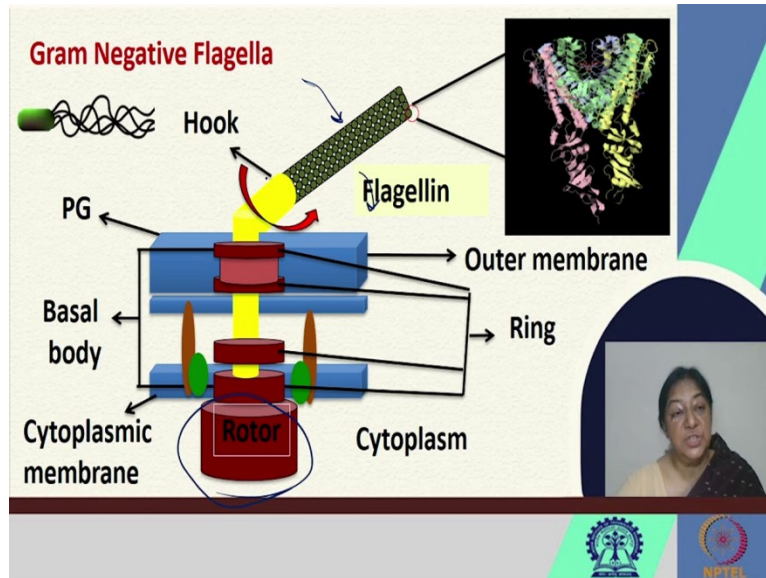
Bacterial Flagella

- At the base, reversible motor or Basal body present
- Movement of cell in response to stimuli in single direction due to counterclockwise flagella rotation
- Movement of cell in response to stimuli in random direction due to clockwise flagella rotation

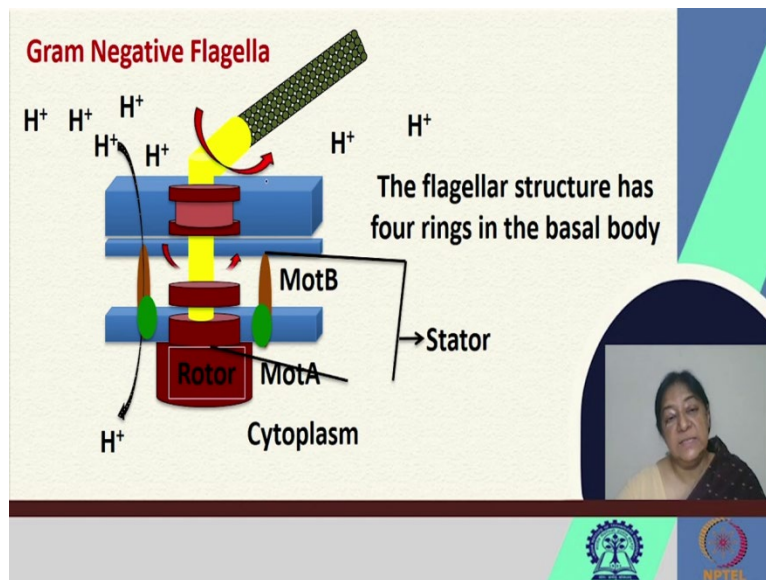
The flagella are at the base, it has a reversible motor and the movement of the cell is in response to stimuli in the single direction due to the counterclockwise of the flagella rotation. This movement of the cell in response to the stimuli occurs due to clockwise rotation. So, there are two types of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

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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.

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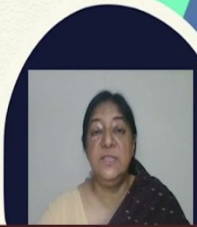



The flagella structure, in this case for gram negative flagella, has has four rings in the basal body.

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Gram Negative Flagella

- MotA/B complex is a load-sensitive proton channel
- MotA/B complex couples proton translocation with torque generation
- Protonation and deprotonation of Asp33 in MotB induce conformational changes

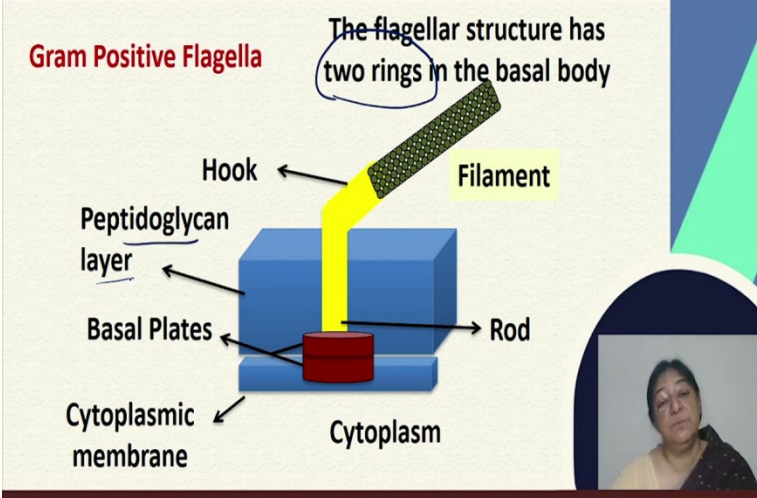
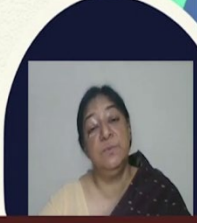




There is specific movement and there is a motor A and motor B complex, that is a load sensitive proton channel. What happens here, it couples proton translocation with a torque generation and this torque generation is due to the protonation and deprotonation of Asp33 in the motor B and this induces the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

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Gram Positive Flagella

The flagellar structure has two rings in the basal body

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.


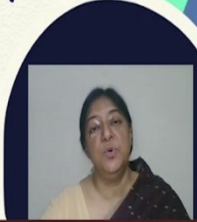
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Summary

Important biochemical and biophysical processes include

- cellular transport, cell division and cell motility
- gene replication, transcription and translation,

These cellular functions are possible due to the classes of molecules called the motor proteins



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.

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These [refer to slide] are the references.

Thank you.