Fundamentals of Protein Chemistry
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Module - 08
Motor Proteins and Metalloproteins
Lecture - 36
Motor Proteins - I

We begin our discussion in module 8 on motor proteins and metalloproteins. In the first two lectures we will be looking at motor proteins the way they work, the way they function and what we mean by these motor proteins.

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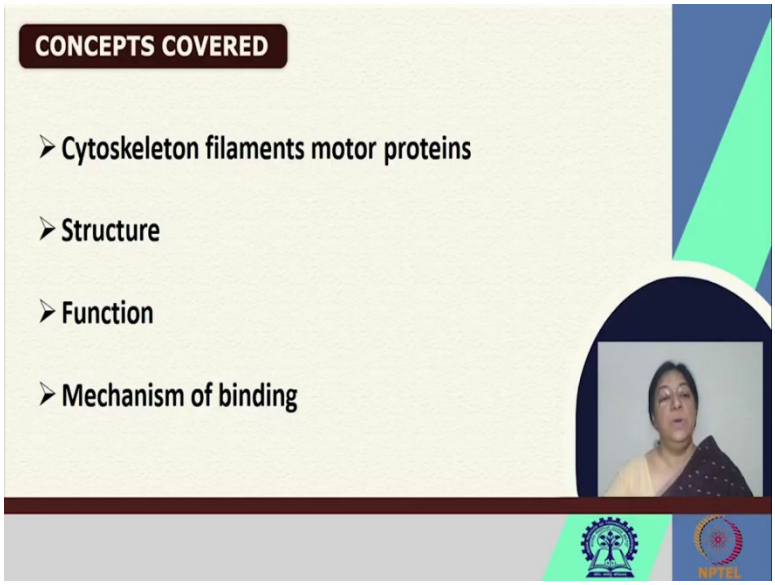

In our discussion we will be looking at cytoskeleton filaments motor proteins, their structure, their function, their mechanism of binding and exactly what they do in a set of biological processes that go on in the body.

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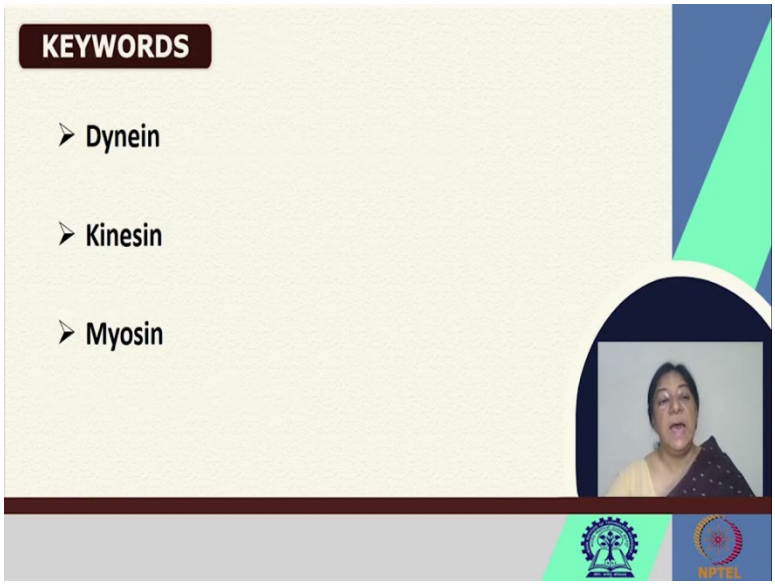

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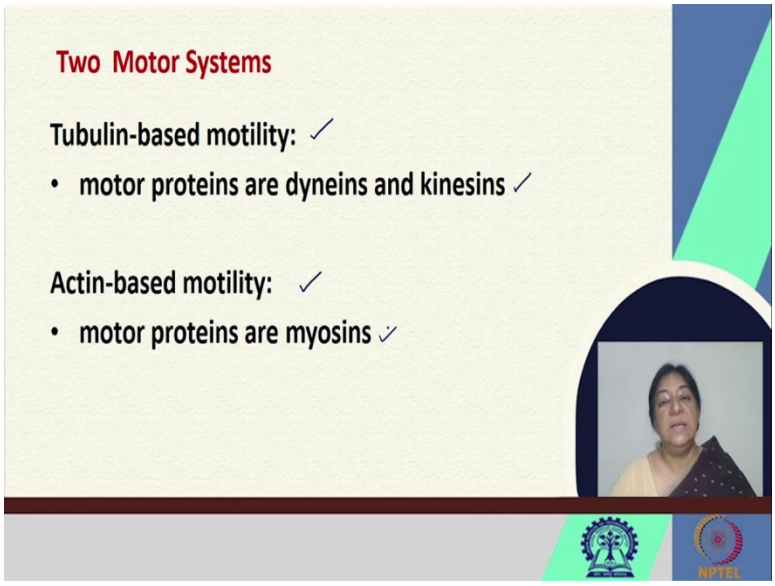

There are two motor systems. The motor systems that we are going to talk about in their basic motility is the tubulin-based motility; the motor proteins there are the dyneins and the kinesins and we have an actin-based motility, where the motor proteins are the myosins. Now, what do we mean by the tubulin-based motility and the actin-based motility.

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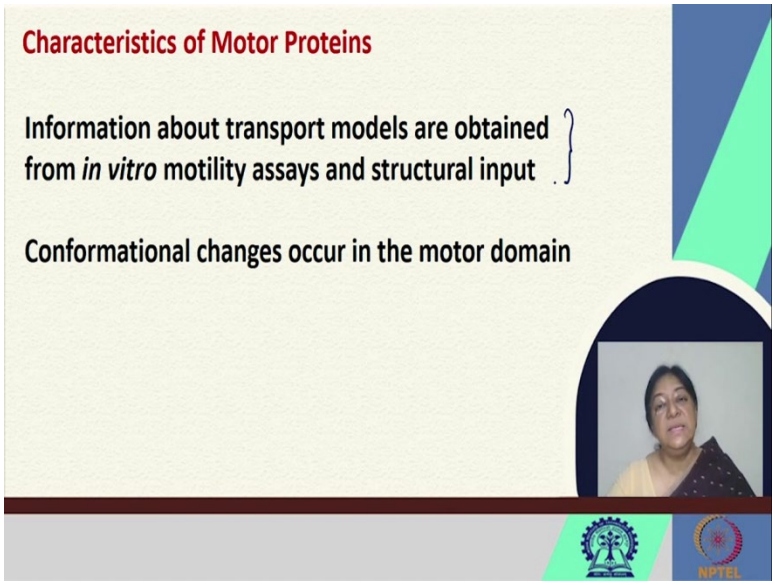

To begin with, we need to know what the characteristics of these motor proteins actually are. What they do is they transport information, they transport material from the inside to the outside of the cell and have various important roles. So, the information about the transport models are actually obtained from a set of in vitro motility assays and structural input available from different experimental methods.

There is a lot more to be known about the structure, the function of these motor proteins. And there are conformational changes that occur in the motor domain of the proteins and this is what brings about a motion.

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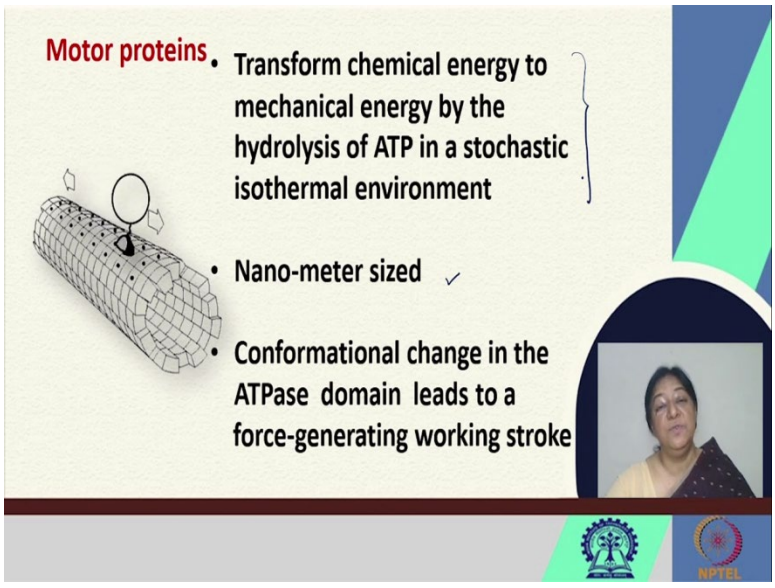

If we look at the motor proteins that work in our bodies, they are nanometer size. Their importance is that they transform chemical energy to mechanical energy and this chemical

energy is obtained by the hydrolysis of ATP, in this isothermal environment, in our biological cells. The conformational change for example, in the ATPase domain, that we will look at in the next lecture, leads to what is called a force generated working stroke and we will see what that is important for and why it is necessary for this to occur.

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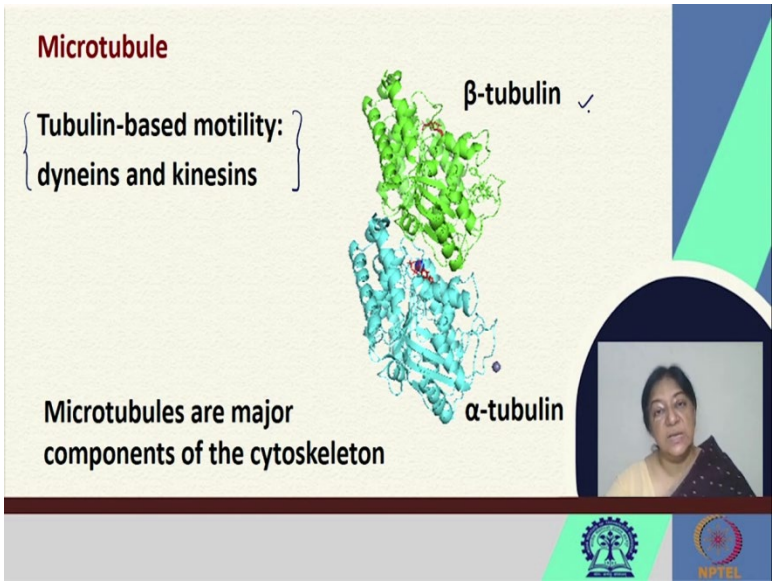

So when we look at the microtubule motility ones, where we have the two motor proteins dynein and kinesin, we need to know what the structure of a microtubule is.

Now when we consider the motion of the motor proteins in this case, we need a track for the proteins to travel on because the motor proteins have to bring the material in and out of the cell; the vesicular material the protons and the proteins and so on and so forth. The microtubule is composed of two proteins the α-tubulin and the β-tubulin. These are major components of the cytoskeleton and have structural aspects to them.

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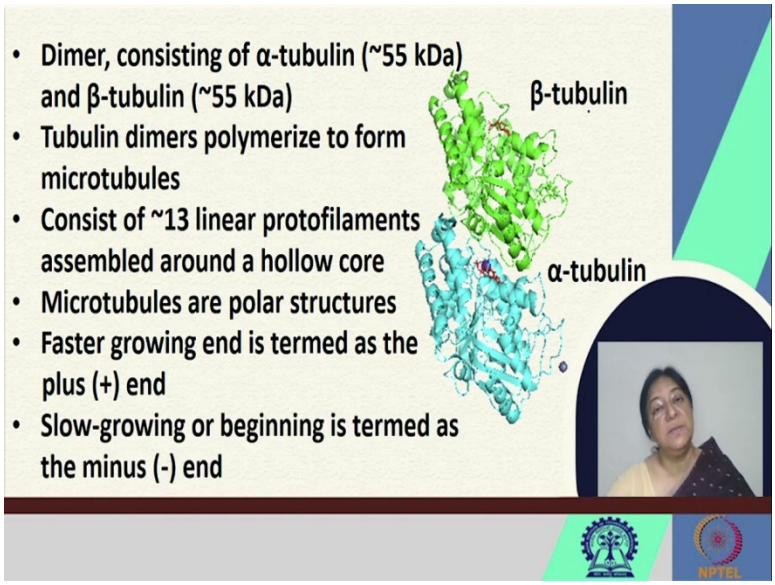

This [refer to slide] is a dimer that consists of the α-tubulin and the β-tubulin and they polymerize to form what are called microtubules that are tracks for the dynein and the kinesin to work on. They consist of about 13 linear protofilaments, that are assembled around a hollow core. This forms a tube like structure. They are polar structures and the faster growing end is known as the plus end and the slower growing end or the beginning is termed as the minus end.

We can have proteins that go from the minus to the plus end or those that go from the plus to the minus end and we will be discussing those in a moment.

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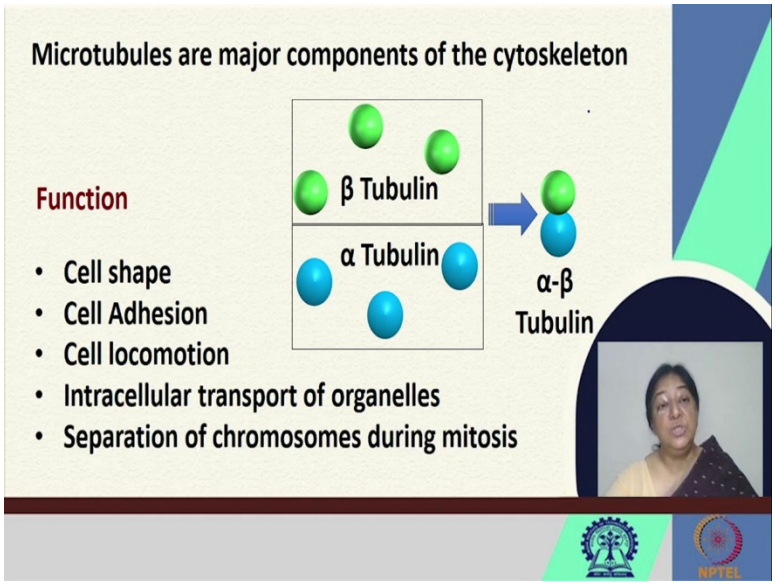

When we have the microtubules that are these major components of the cytoskeleton, their function is to maintain the cell shape, for cell adhesion, for cell locomotion and the intracellular

transport of organelles. It also has a major role to play in the separation of the chromosomes during mitosis.

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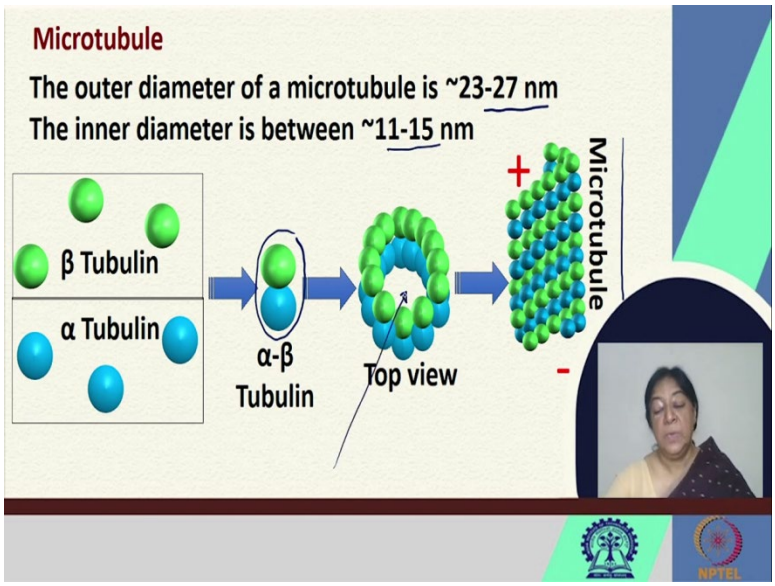

The outer diameter of this microtubule is around 23 to 27 nanometers and the inner diameter is 11 to 15 nanometers. We have the formation of this α-β tubulin that then forms a tube like structure like this [refer to slide], that is then formed into this microtubule like structure, that has this hollow core.

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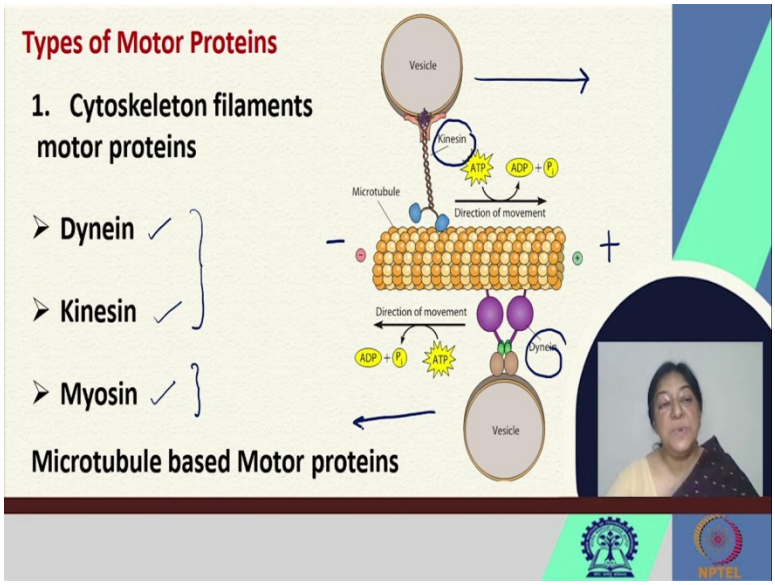

This tubular structure now will then be used as a track for the motion of dynein and kinesin, that work on the tubulin track system. So these cytoskeleton filaments motor proteins are the dynein,

the kinesin and the myosin. Dynein and kinesin are the ones that use this tubulin and myosin use the actin filament as its track for motion.

So if we look at the microtubule based motor proteins, we have the kinesin and we have the dynein. Now the kinesin moves from the negative end to the positive end and the dynein moves in the direction from positive to negative. We will see a bit more about their motion and what their specific roles are.

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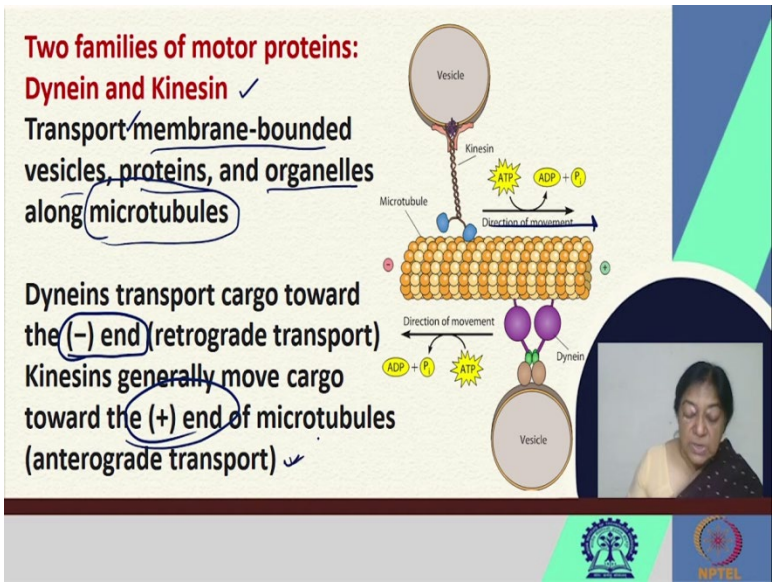

The two families of motor proteins that we are looking at here that are microtubule based, are dynein and kinesin. These are transport membrane bounded vesicles. They transport membrane bound vesicles, proteins and organelles along the microtubules that are the tracks for the dynein and the kinesin.

The dyneins transport cargo towards the minus end, known as retrograde transport end and kinesins go towards the plus end and this is known as anterograde transport.

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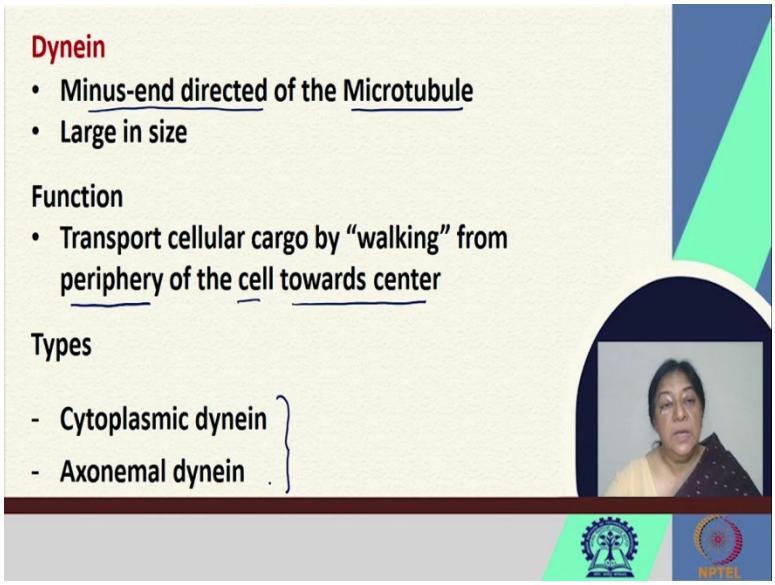

The dynein molecule goes towards the minus end, direction of the microtubule. It is relatively large in size and we look at the specific functions now. It transports cellular cargo by "walking" from the periphery of the cell towards the center. This is going from the plus direction to the minus direction. So, from the periphery of the cell it walks towards the center and the road or the track on which it walks is the microtubule.

The two types of dyneins that are known are the cytoplasmic dynein and the axonemal dynein.

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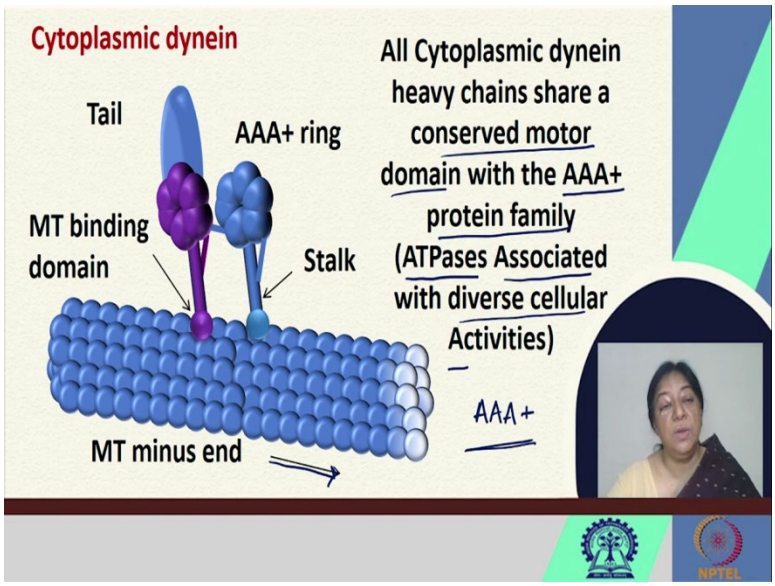

Let us look at the cytoplasmic dynein. Now all cytoplasmic dyneins heavy chain share a conserved motor domain as it is called, with the AAA+ protein family. This is a family of proteins that are marked as ATPases, but these are associated with diverse cellular activities. This is a very large group of proteins which we will discuss in brief; the AAA+ types of proteins.

Now, this [refer to slide] is our microtubule, this is the MT minus ends. So we will have the motion in this direction and this is our dynein, the cytoplasmic dynein that has this specific mode of motion and specific conserved motor domain, that is similar to the ones with the AAA proteins.

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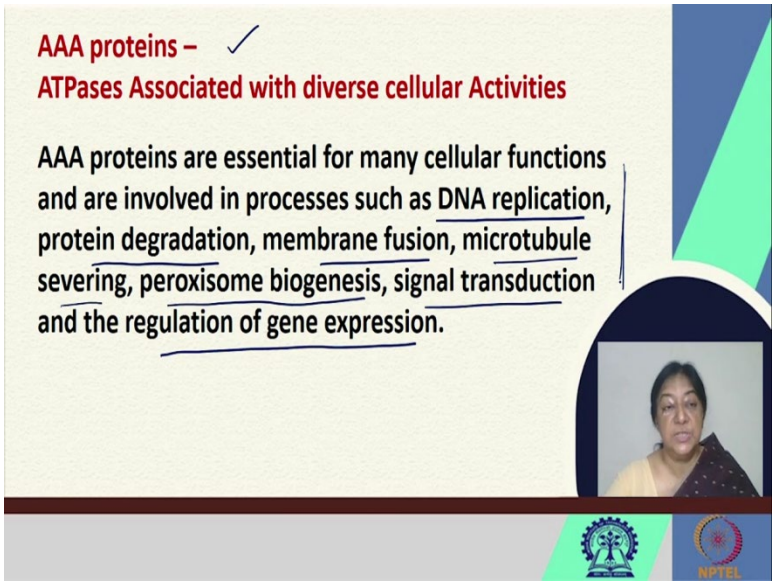

Now the AAA proteins, are the ATPases that are associated with several other diverse cellular activities. And these AAA proteins are essential for several cellular functions that are involved in the processes such as DNA replication, protein degradation, membrane fusion, microtubule severing, peroxisome biogenesis, signal transduction and the regulation of gene expression.

We realize the importance of these AAA proteins in bringing about several biochemical, biological processes that are important for life functions.

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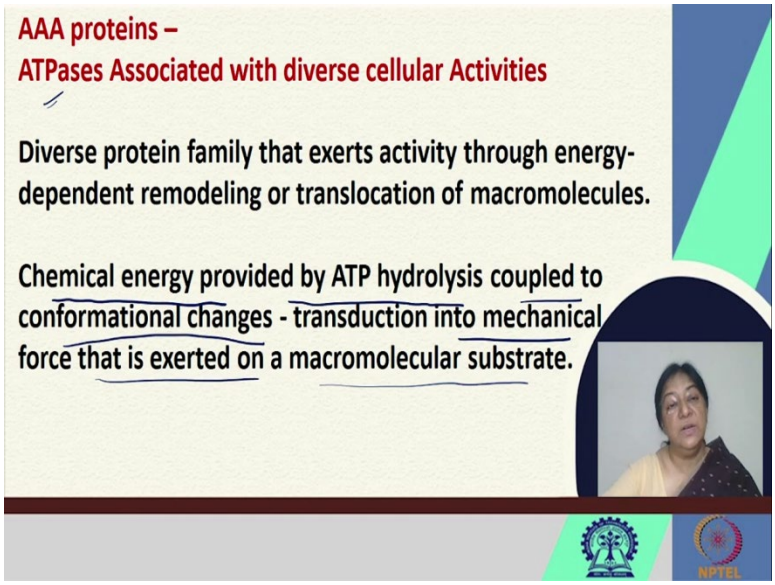

These proteins are a diverse protein family and they exert their activity through energy dependent remodeling or the translocation of macromolecules. The chemical energy that is provided by the ATP hydrolysis, is coupled to conformational changes. And these conformational changes are transduced in a manner that brings about a mechanical force that is exerted on a macromolecular substrate.

We have a mechanical force generated due to the ATP hydrolysis. So there is a conversion of chemical energy to mechanical force, that is required for the specific motion.

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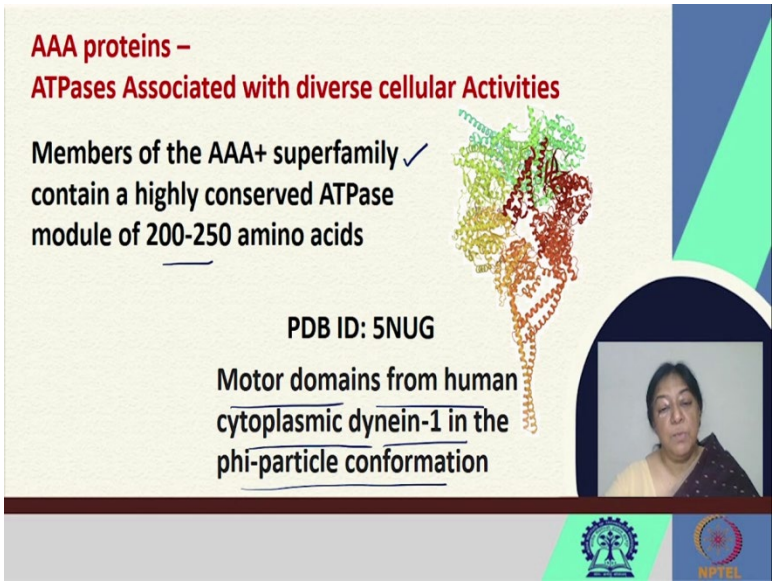

The members of this AAA+ super family contain a highly conserved ATPase module, that comprises 200 to 250 amino acids. This [refer to slide] is a motor domain from the human

cytoplasmic dynein-1 in the phi-particle conformation. This is a specific type of conformation which this molecule or this macromolecule adopts.

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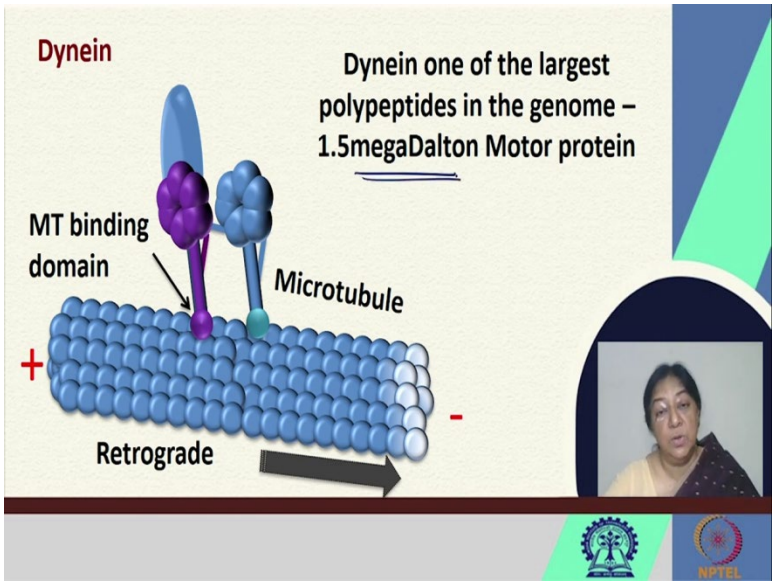

When we look at our dynein molecule, we know that it is a microtubule based motor protein and it has a retrograde motion, meaning it goes from the positive to the negative direction. It is one of the largest polypeptides in the genome and has a 1.5 mega Dalton molecular weight.

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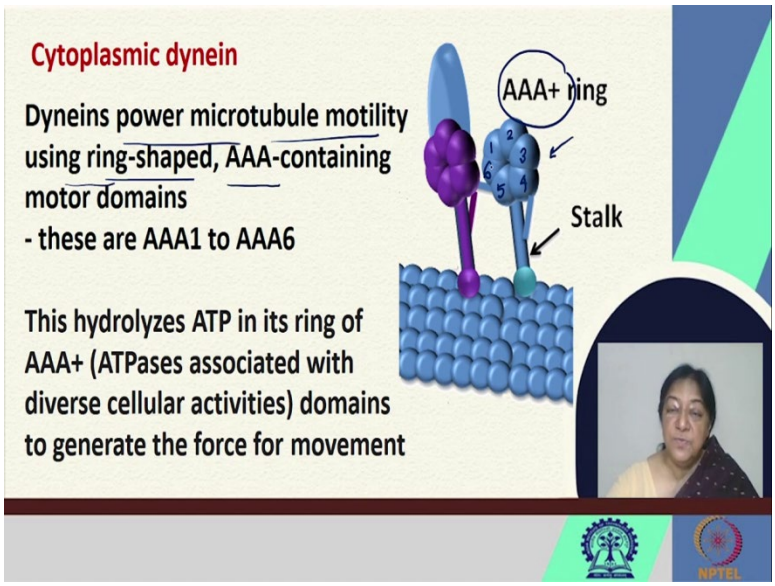

When we look at this AAA+ ring now, it has six of these domains. So they power the microtubule motility using ring shaped, AAA containing motor domains. These [refer to slide]

are the motor domains. We have 6 of these. And these are AAA1 to AAA6. And this hydrolyzes ATP in its ring and generates the force for the movement required.

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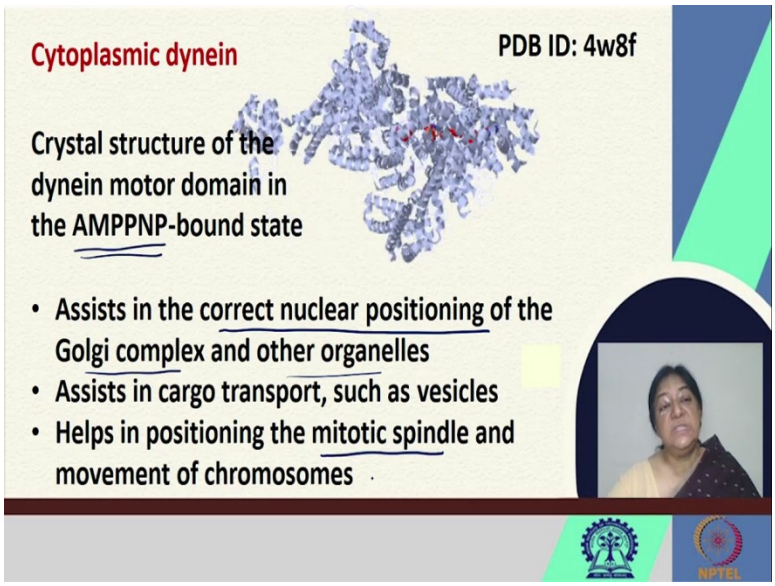

The crystal structure of the dynein motor domain in a specific bound state, is shown [refer to slide]. But to understand that the importance of the cytoplasmic dynein is in the fact that it has this microtubular assistance and it helps in the correct nuclear positioning of the Golgi complex and other organelles. It assists in cargo transport and it helps in the position of the mitotic spindle and the movement of chromosomes.

So, we understand the importance of this cytoplasmic dynein, the motor protein that we are talking about.

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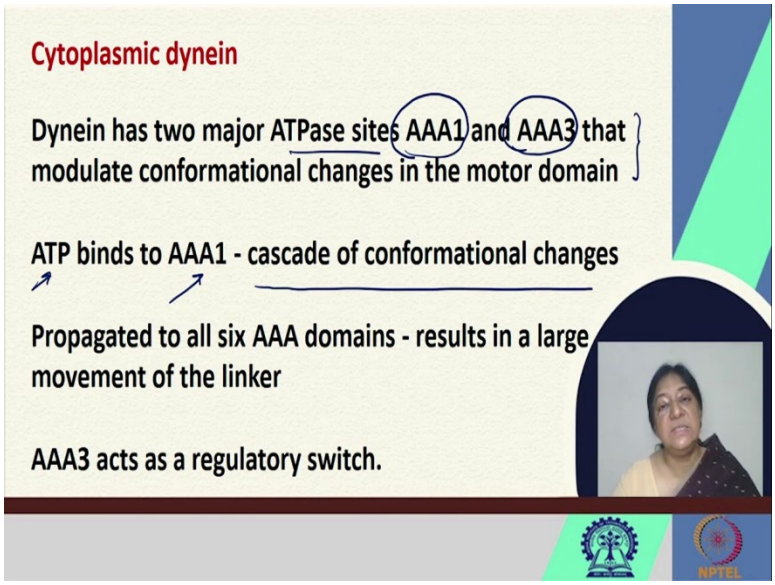

So the dynein has these two major ATPase sites, the AAA1 and the AAA3. What they do is, they modulate the conformational changes in the motor domain. And this ATP will bind to AAA1, result in a cascade of conformational changes that are propagated to the six AAA domains that results in a large movement of the linker. AAA on the other hand acts as a regulatory switch.

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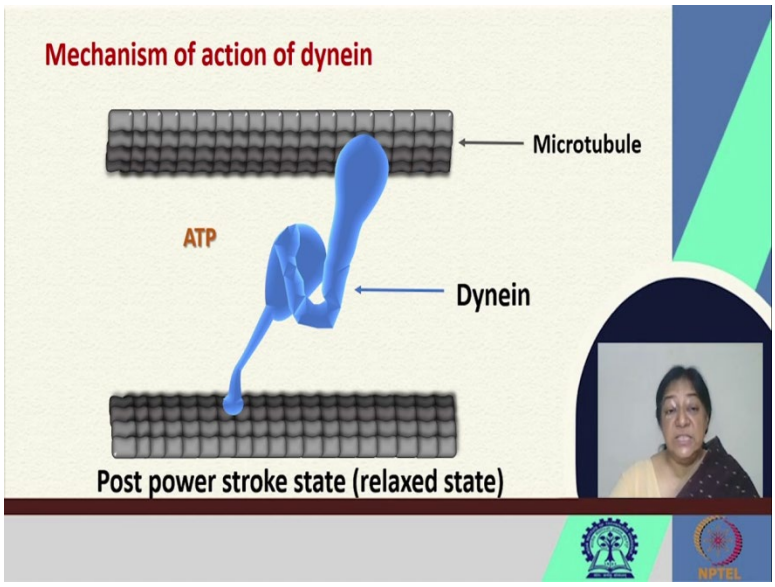

If we look at the mechanism of action of the dynein, we have the microtubular structure and we have a linker, the dynein and this [refer to slide] is the post power stroke state that is the relaxed state.

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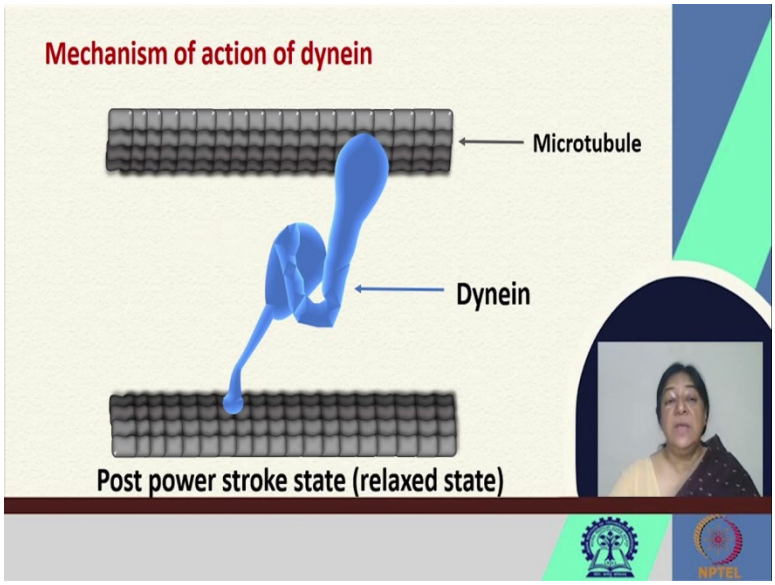

We have ATP coming to bind to it. The hydrolysis now is going to take place, resulting in what is called the binding of the ATP, that drives this motion.

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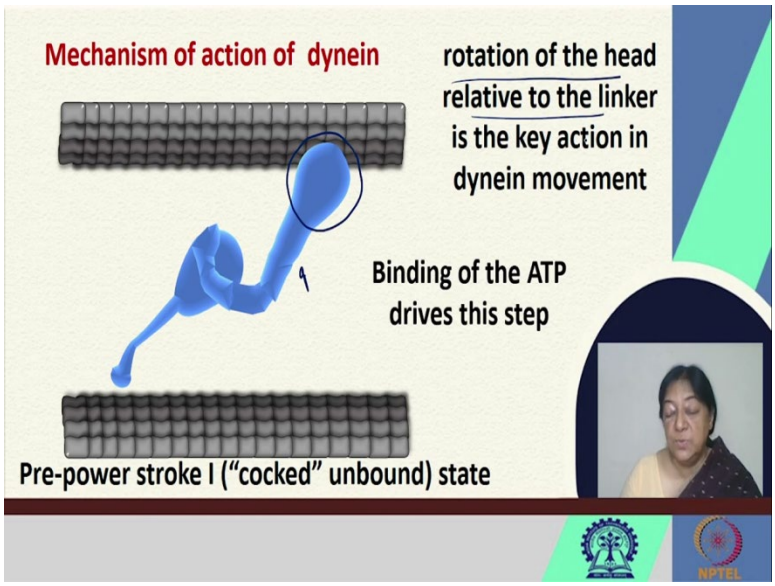

We have the relaxed state; the post power stroke state and we will have a motion based on the binding of the ATP that is going to drive this step. So the rotation of the head, relative to the linker results in the dynein movement.

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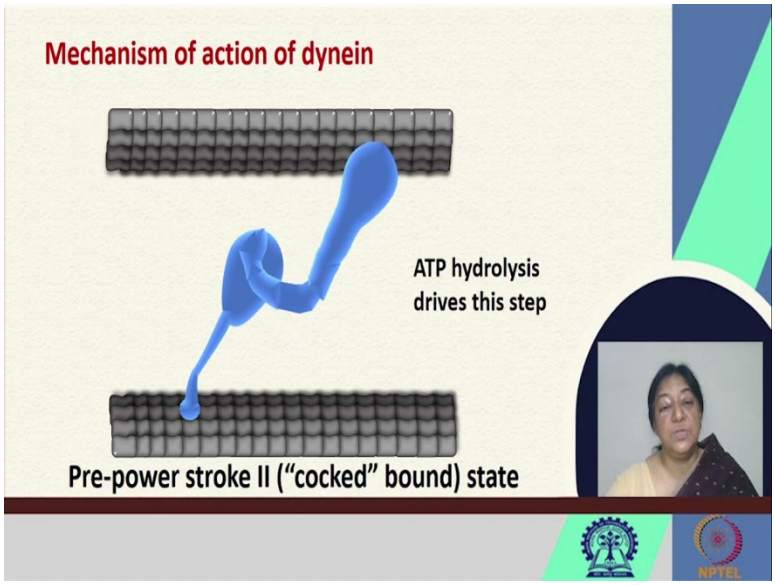

So this movement is extremely important because it is ATP hydrolysis that is driving this step.

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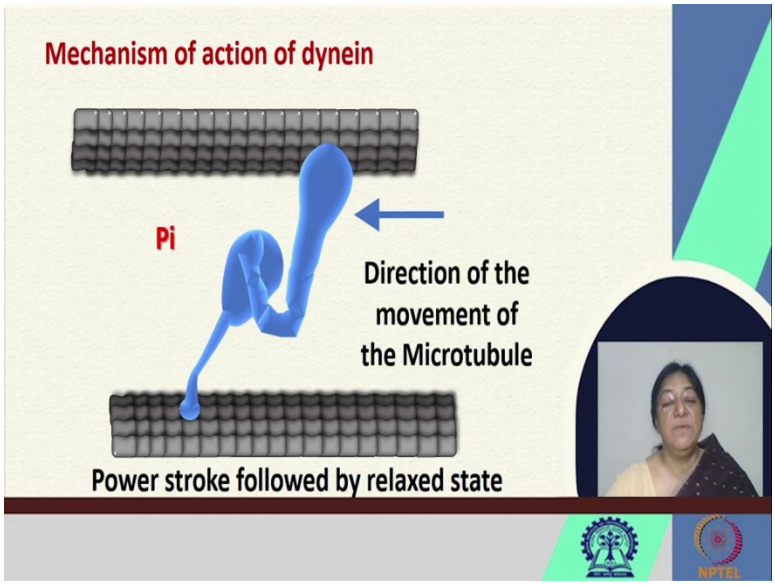

There are conformational changes associated with this and this [refer to slide] is the direction of the movement of the microtubule, which we can see. So when we have the ATP hydrolysis, we will have the direction of the movement of the microtubule.

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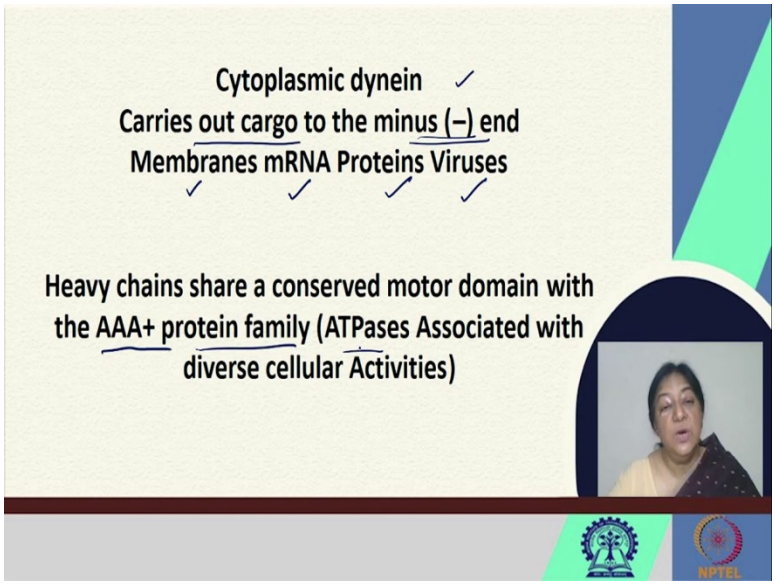

So the cytoplasmic dynein carries its cargo to the minus end and this cargo can comprise of membranes, mRNA, proteins, viruses. And the heavy chains share a conserved motor domain with the AAA+ protein family which are ATPases that are associated with diverse cellular activities.

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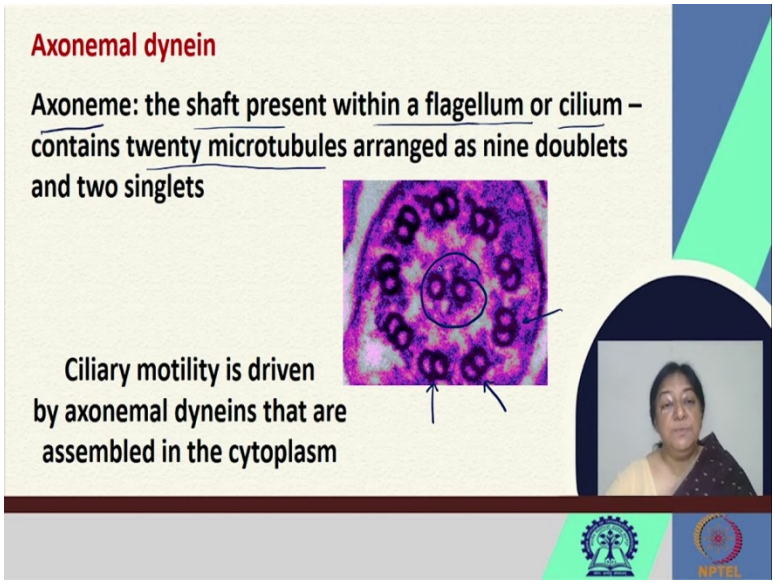

The axonemal dynein is another type of dynein involved in ciliary motility. What do we mean by this? The axoneme is the shaft that is present within a flagellum or a cilium that contains twenty microtubules arranged as nine doublets and two singlets. These [refer to slide] are the nine doublets that are on the periphery and there are two singlets here. So there are twenty microtubules.

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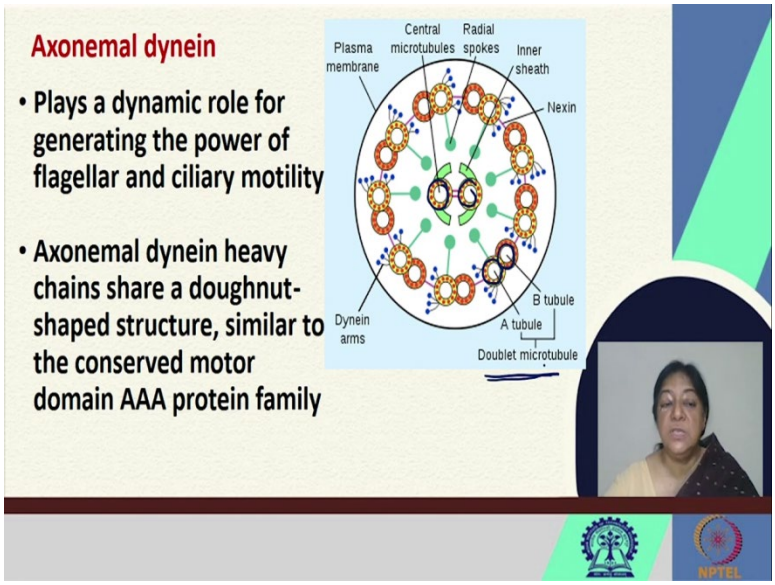

These microtubules have a specific motion in the way that they move. The central tubules are located in this [refer to slide] fashion here and we have the A tubule and the B tubule that are slightly overlapped, forming what is called a doublet microtubule. Now, this plays a dynamic role for the generating of the power of the flagellar or ciliary motility. The axonemal dynein heavy chains this doughnut shaped structure, it is similar again to the conserved motor domain of the AAA protein family.

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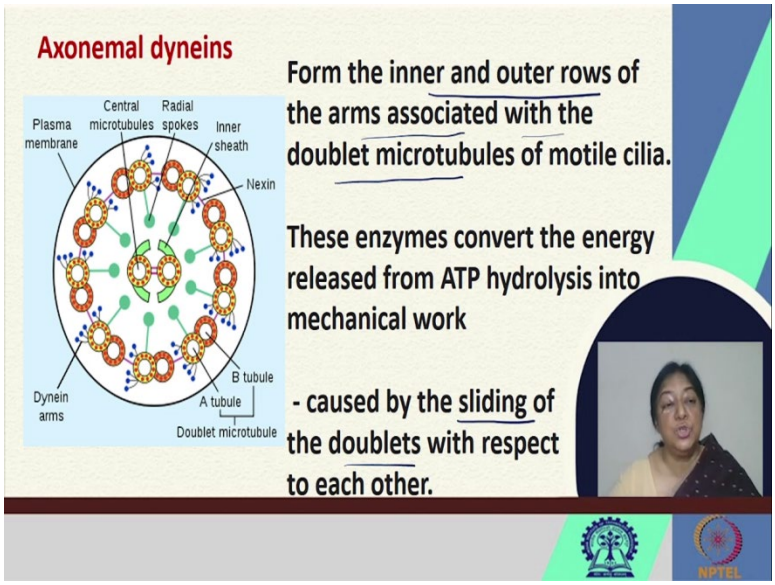

So there are inner and outer rows of the arms, that are associated with the doublet microtubules of the motile cilia and these enzymes convert the energy again released from the ATP hydrolysis into mechanical work and this is caused by the sliding of the doublets with respect to each other. This sliding motion brings about the movement of the flagella or cilia.

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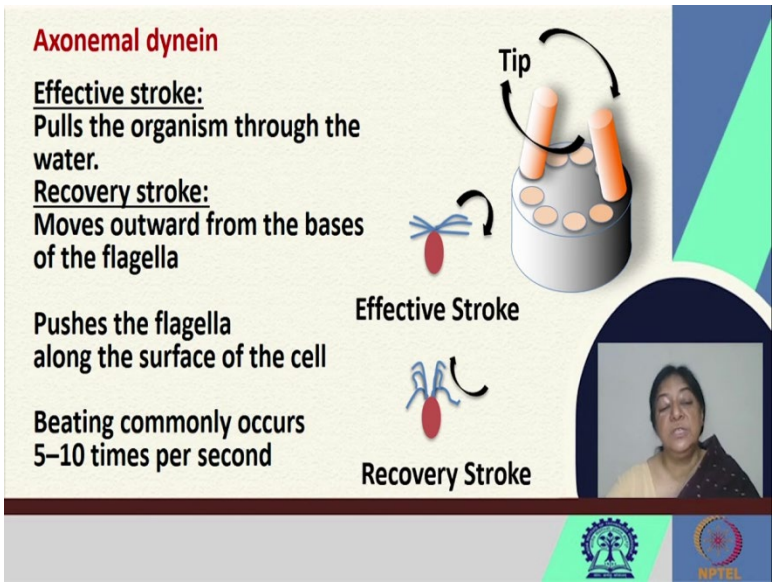

So these [refer to slide] are the nine sets of doublets that we have here and we have a motion associated with it. If we look at an effective stroke, there is going to be a stroke like this and this pulls the organism through the water. There is a recovery stroke that would take it back and this moves outwards from the bases.

This motion pushes the flagella along the surface of the cell, in the effective stroke and the recovery stroke and this is how the motion can take place. The beating commonly occurs about 5 to 10 times per second.

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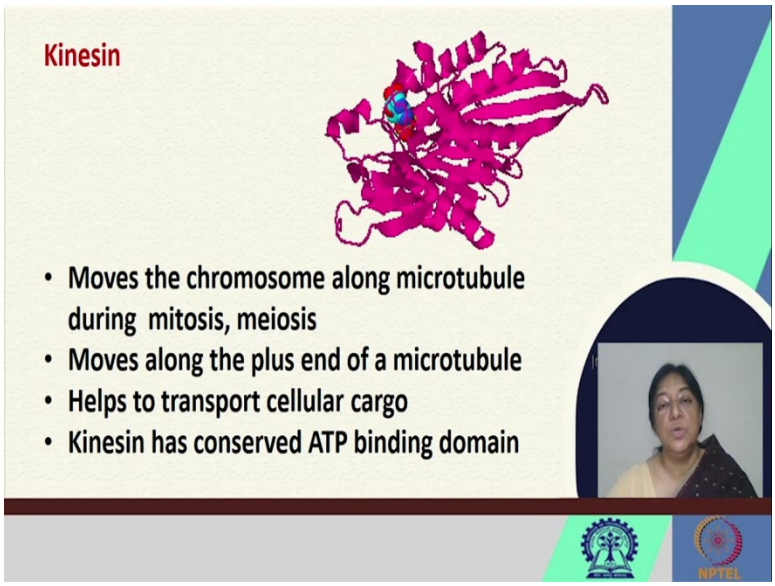

The other protein that is associated with the microtubules, is the kinesin. This moves the chromosome along the microtubule during the process of mitosis and meiosis. And it moves along the plus end of a microtubule and again helps in the transport of cellular cargo. This also has a conserved ATP domain. We have to remember that this ATP hydrolysis is what drives or brings us the chemical energy that is going to be transformed to the mechanical energy.

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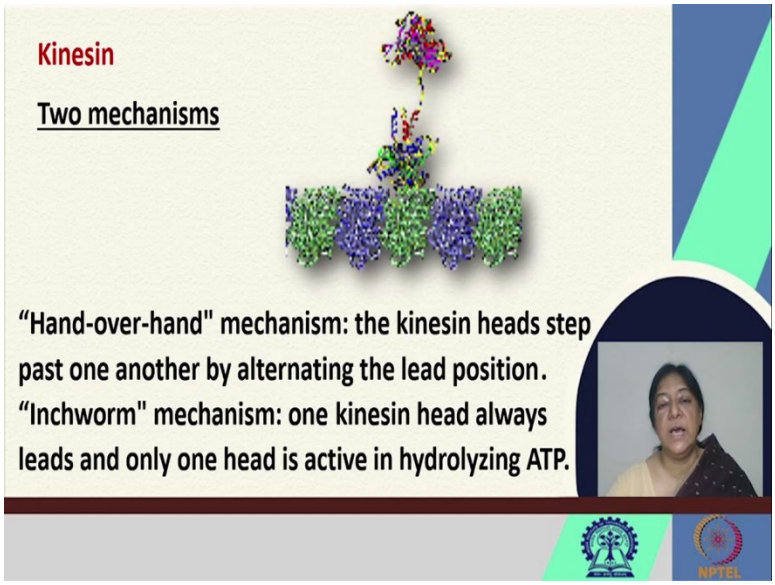

So this kinesin has two mechanisms. One is called a "hand-over-hand" mechanism, where the kinesin head steps past one another by alternating the lead position. Another is an "inchworm" where one kinesin head always leads and the other is active in hydrolyzing the ATP. This is like a typical motion for the kinesin molecule, where there will be a hand-over-hand mechanism, where it will pass through over the microtubule.

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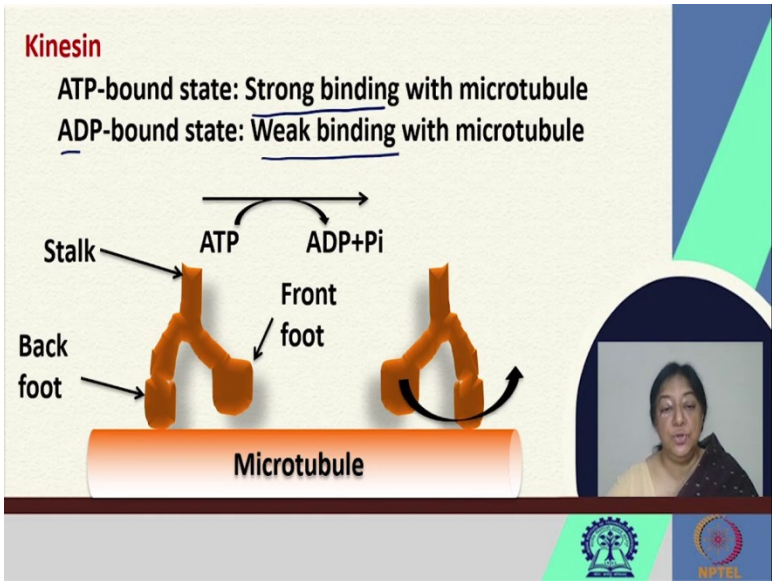

The ATP bound state, brings about the strong binding and ADP is the weak binding. So what happens is, when we have the microtubule and we have the stalk and we have the specific back foot, each of these feet can be bound to ATP. If it is bound to ATP, it will have a strong affinity with the microtubule, but if there is hydrolysis of the ATP to ADP and Pi, then there is weak binding with the microtubule and it will release its foot from the microtubule.

So the ATP to ADP+Pi transformation will give us the release and connection to the microtubule. This [refer to slide] is the way it would go.

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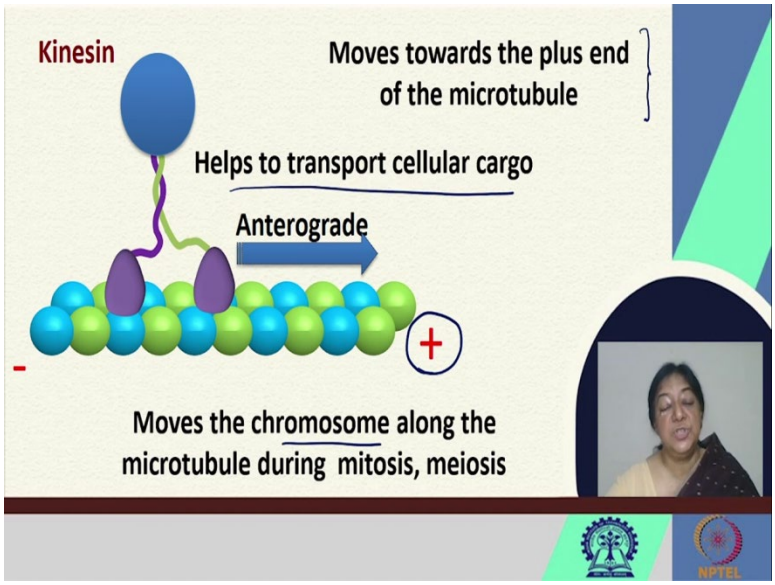

It has an anterograde type of transport, where it moves towards the positive end of the microtubule. It helps to transport the cellular cargo and moves towards the plus end. And the

importance is the movement of the chromosome along the microtubule during mitosis and meiosis.

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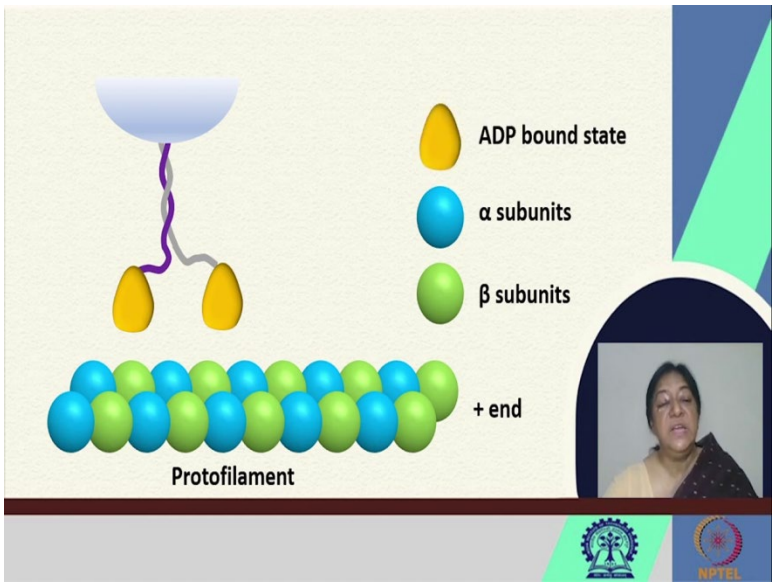

So if we look at the way this works, we have the protofilament that is the microtubule structure of the α-tubulin and the β-tubulin.

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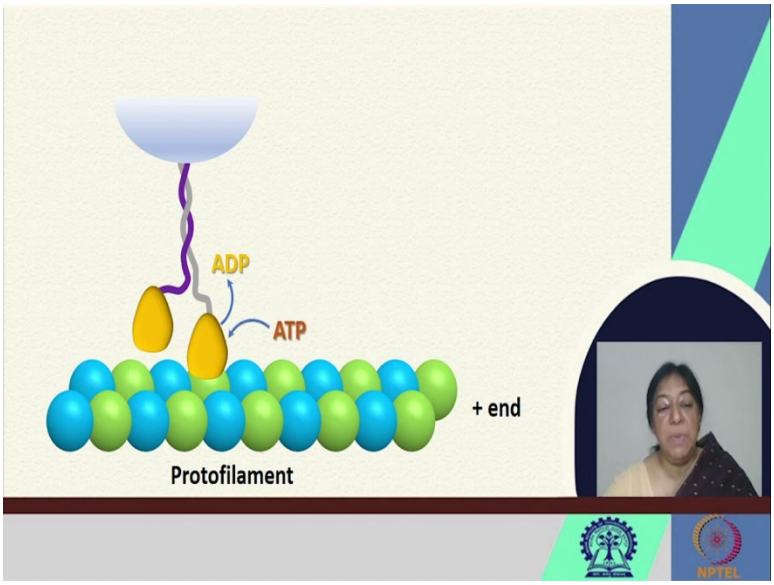

And we have our feet that are going to connect to the microtubule, depending upon whether we have ATP bound or not bound or hydrolyzed.

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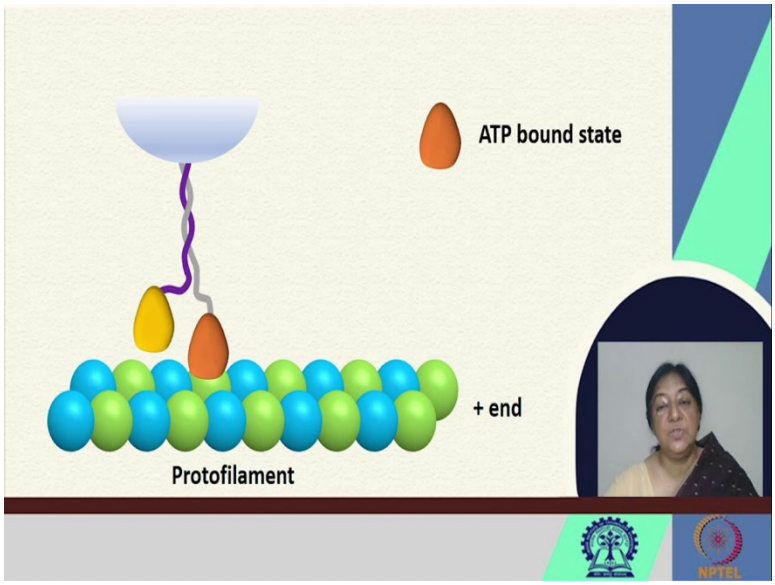

So once this [refer to slide] ATP binds then what happens is, it has a strong affinity.

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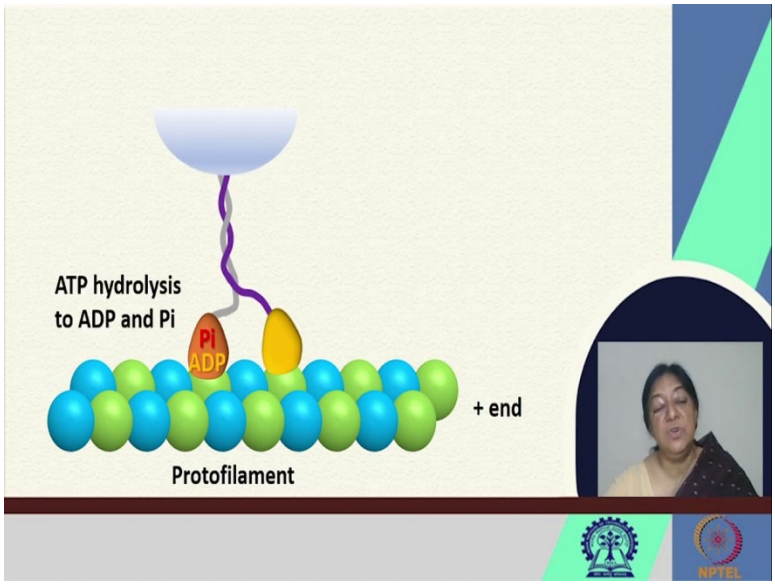

When the ADP comes into the picture then it will release this foot. So when the ATP hydrolysis occurs to ADP and Pi, it will release the foot.

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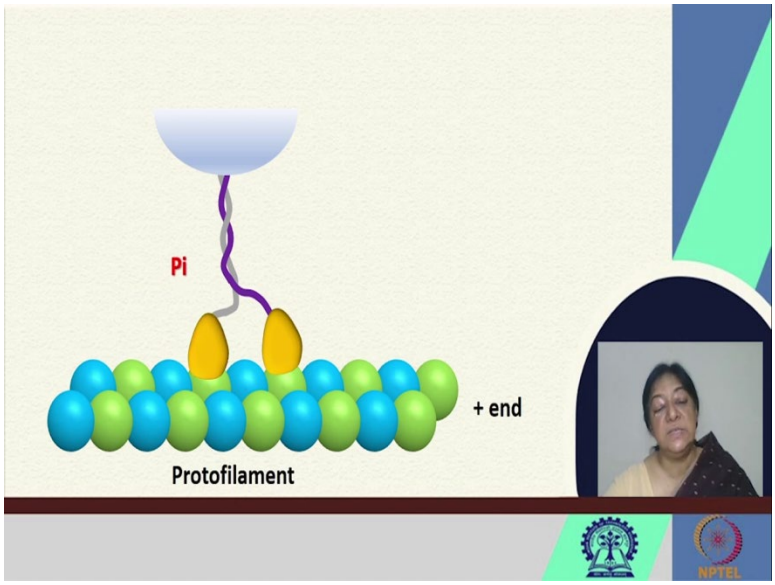

And then we move along.

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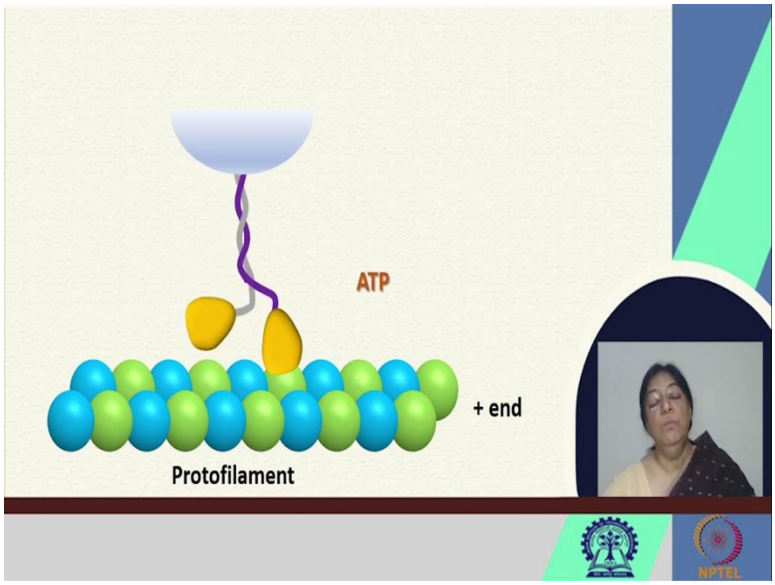

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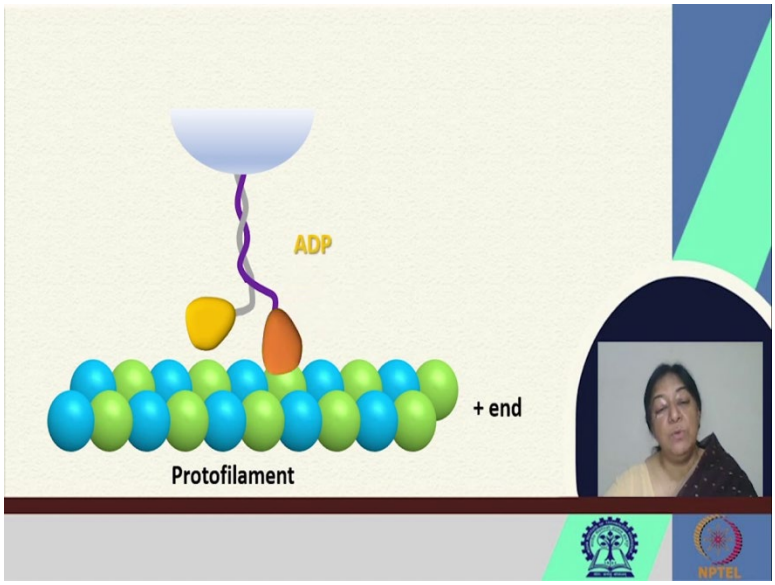

We have another ATP now bound to this foot, which will have a strong binding and so on and so forth.

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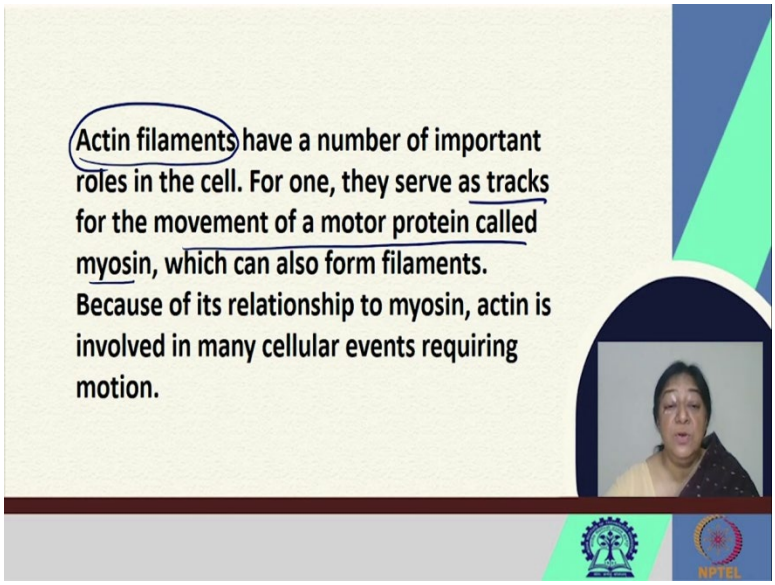

Now actin filaments have a number of important roles in the cell. They serve as these tracks for the movement of the motor protein called the myosin. These are the other types that we are going to look at; the actin filaments.

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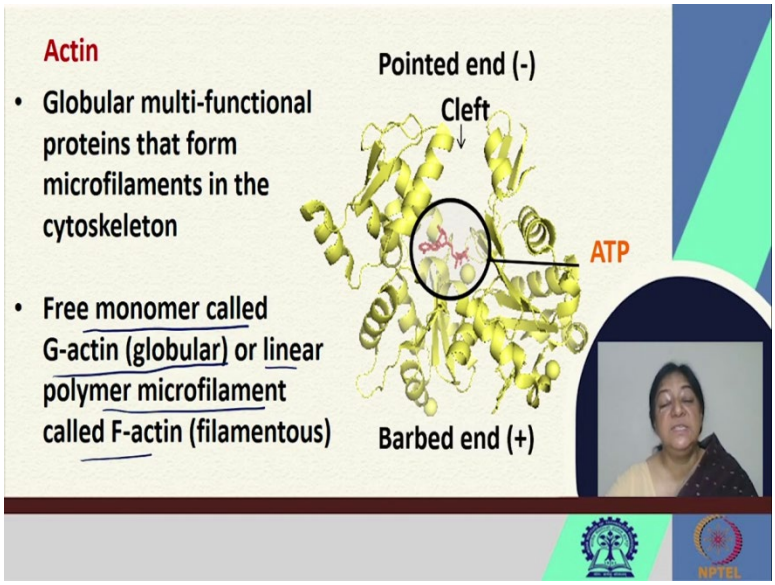

This actin again is involved in many cellular events that require motion. Actin itself is a globular multifunctional protein that forms the microfilaments in the cytoskeleton. If there is a free monomer called the G-actin, that is the globular or the linear polymer microfilament, called the F-actin.

When we look at this [refer to slide] actin molecule we again have the ATP bound and there is a cleft to which this ATP can bind this is the pointed end and a barbed end.

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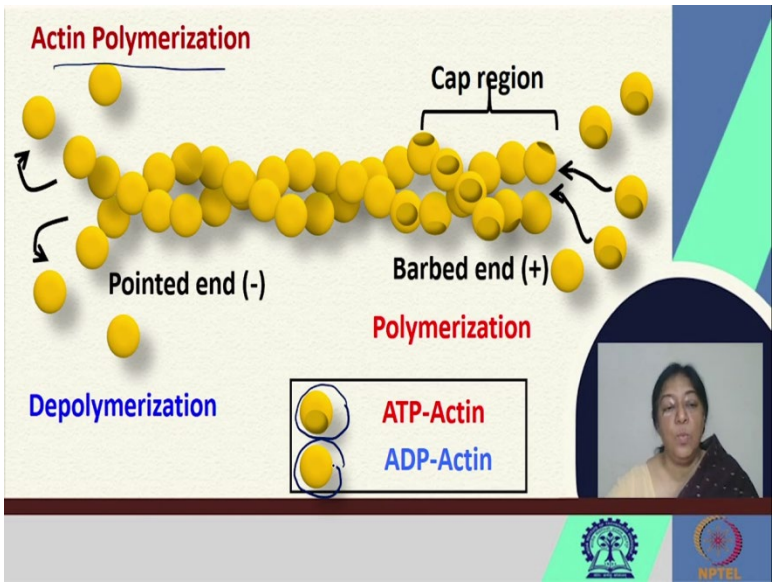

What we have is, we have an actin polymerization where we have the ATP bound actin and the ADP bound actin. And as was mentioned, we have a polymerization in a specific direction, where we have the barbed end which has the ATP bound to it and we have the ADP bound to it. One is the pointed end, one is the barbed end that results in depolymerization.

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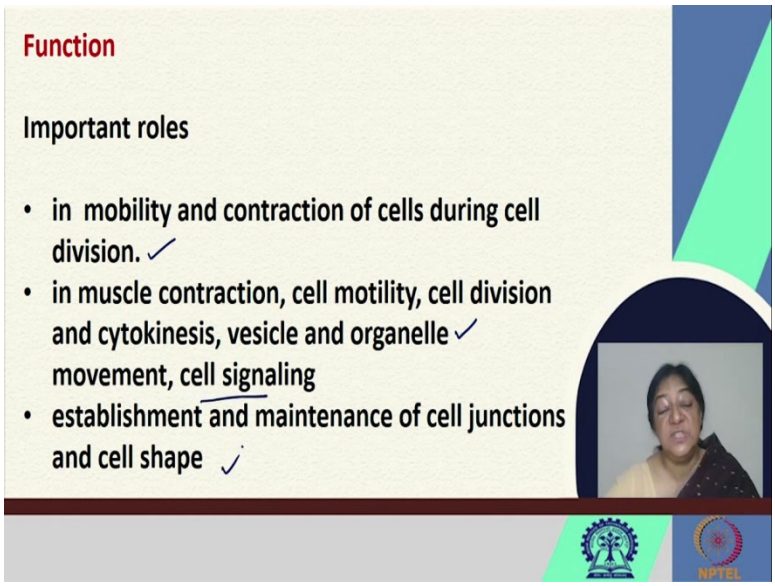

The important roles of this are in mobility and contraction of cells during cell division, in muscle contraction, cell motility, cell division, cytokinesis and movement of vesicle and organelle as well as cell signaling and the establishment and maintenance of cell junctions and cell shape.

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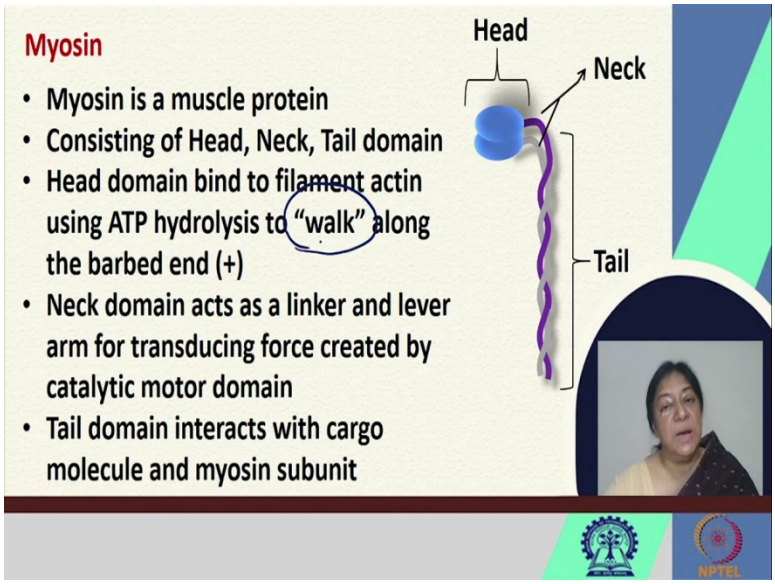

This myosin now, is a muscle protein that consists of the head, neck and tail domain. The movings or the workings of the myosin motor protein require the actin filament for its motion. The head domain binds to the filament actin, using ATP hydrolysis and during that again we see that it will "walk" along the barbed end because that is where the ATP is bound.

The neck domain acts as a linker and a lever arm, that transduces forces that are created by what is called a catalytic motor domain. Then the tail domain will interact with the cargo molecule and the myosin subunit. We will see what this looks like.

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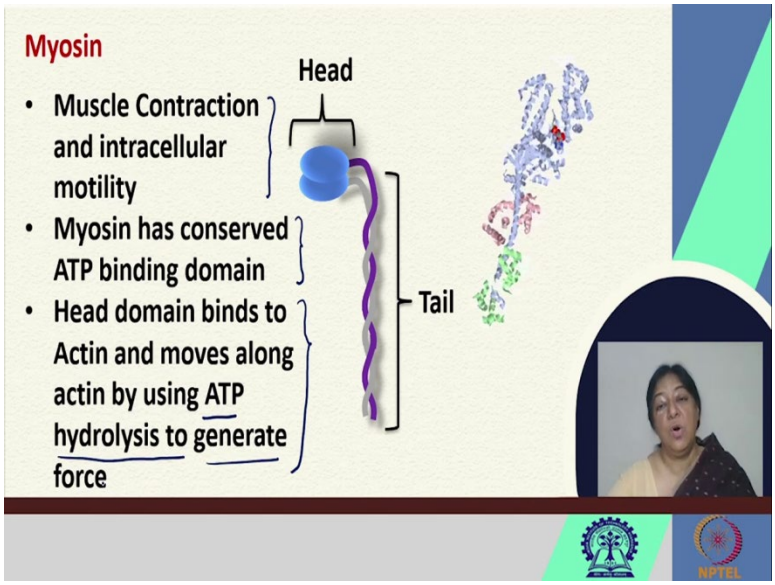

So here [refer to slide] we have the head, then we have the tail and there is muscle contraction and intracellular motility that occurs because of this myosin movement. The myosin also has an ATP binding domain and the head domain binds to actin and moves along actin again, by using ATP hydrolysis to generate the force. So the head will bind the ATP and it will move along its track, that is the actin and cause the motion.

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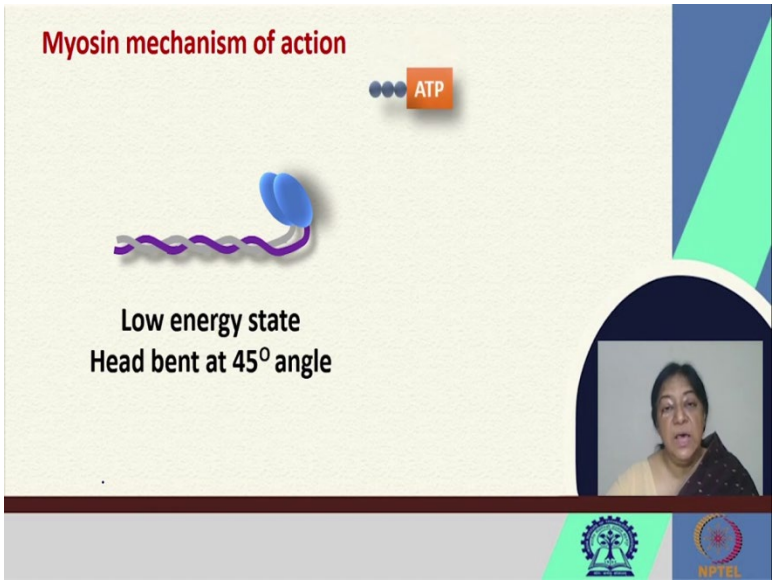

The myosin mechanism of action has a low energy state, where the head is bent at a 45° angle.

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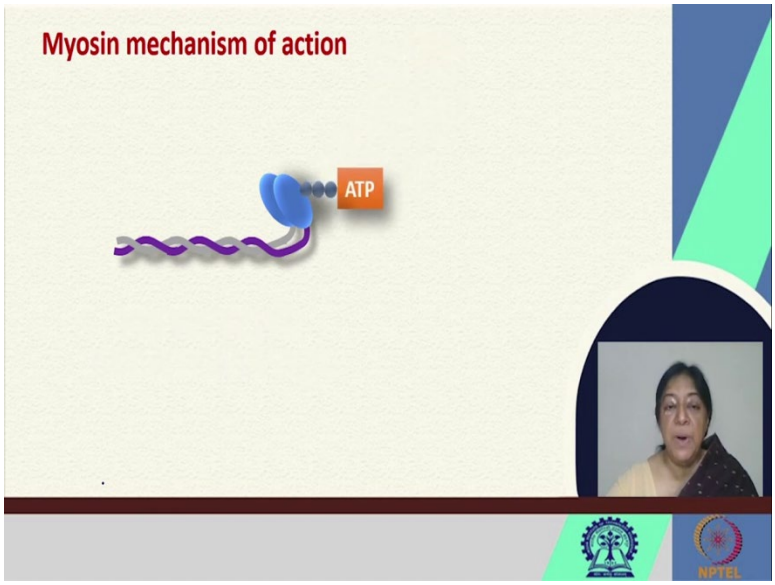

The ATP molecule comes into the picture and binds to the head and forms ADP and Pi.

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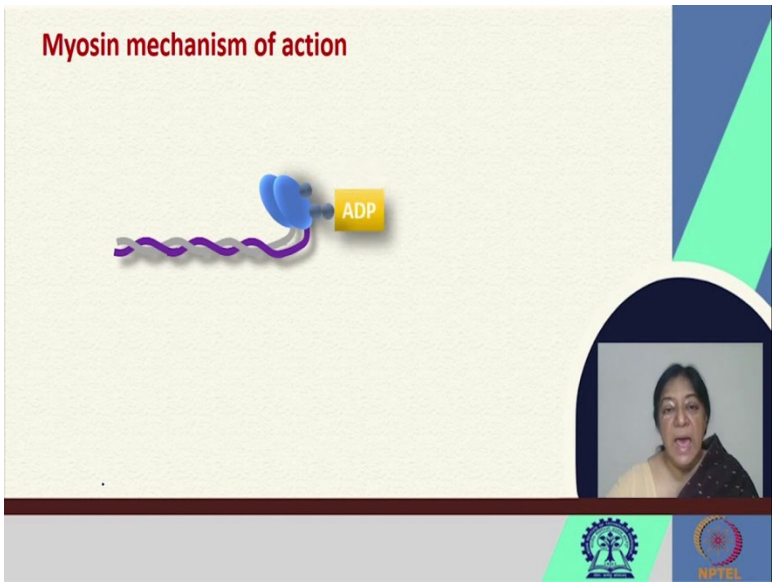

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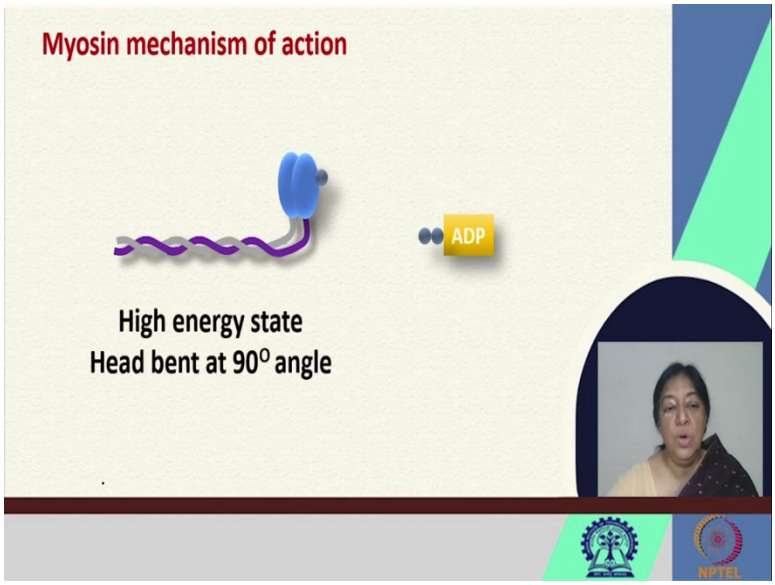

As it does so, the high energy state makes the head bend at an angle of 90^{o} now.

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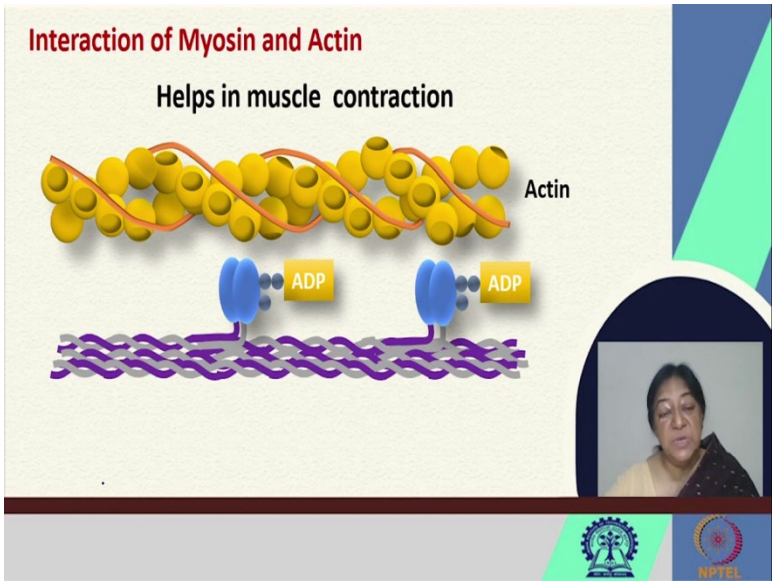

The interaction of this myosin and actin coupled with the ATP hydrolysis, is going to allow in the movement. So we have our actin profilament, we have the myosin and now the ADP and the ATP has bound. So it is now in it's head strong state, in the 90^{o} angle state.

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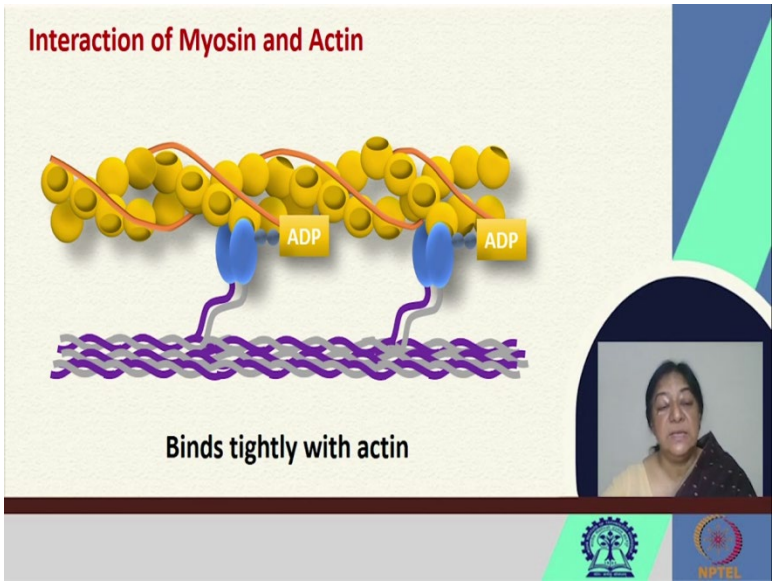

And then what happens, it binds tightly with actin.

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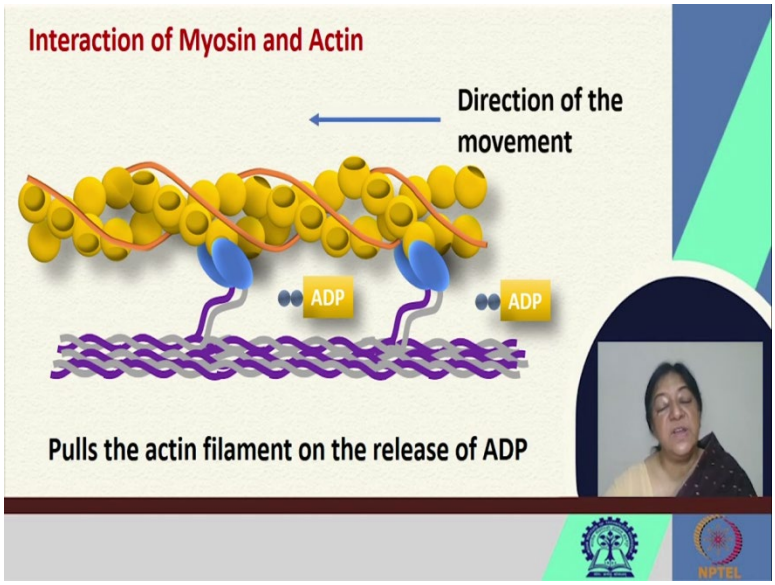

As it binds tightly with actin then once the ADP is released, it pulls the actin filament.

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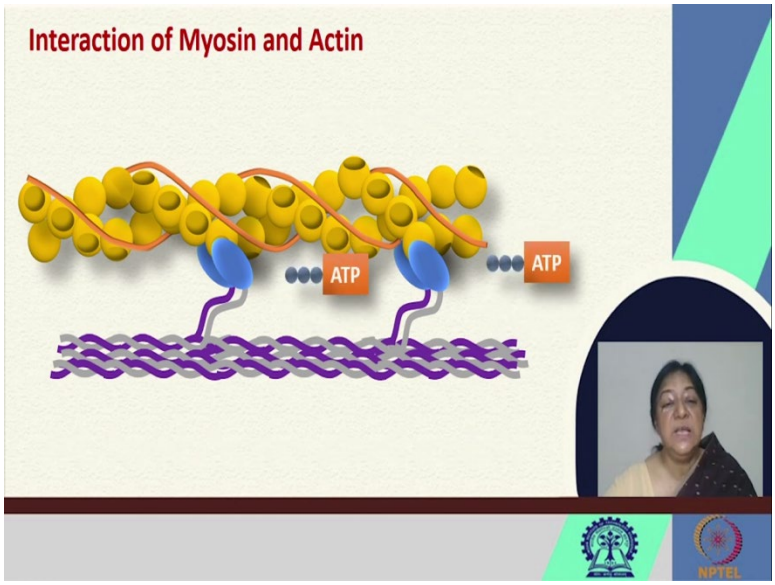

There is a specific direction of the movement and this helps in the movement of the muscle. So what we have is we have the interaction of the myosin and the actin, in this specific motor protein.

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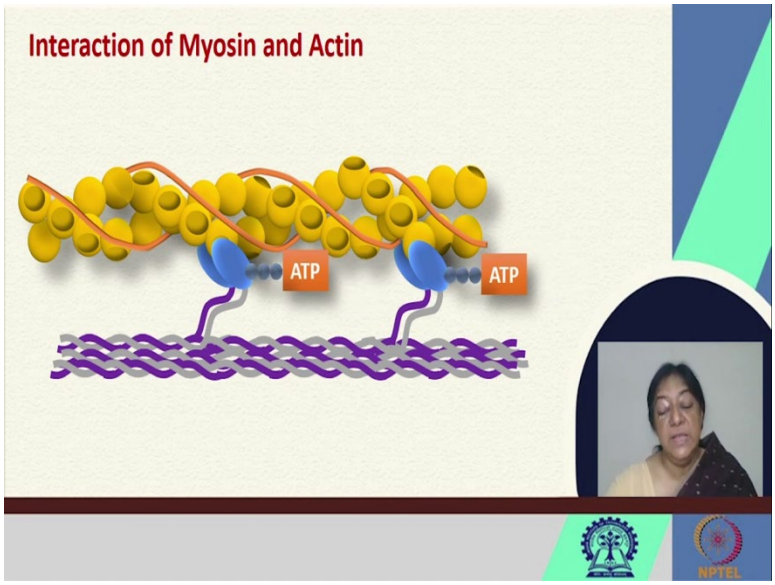

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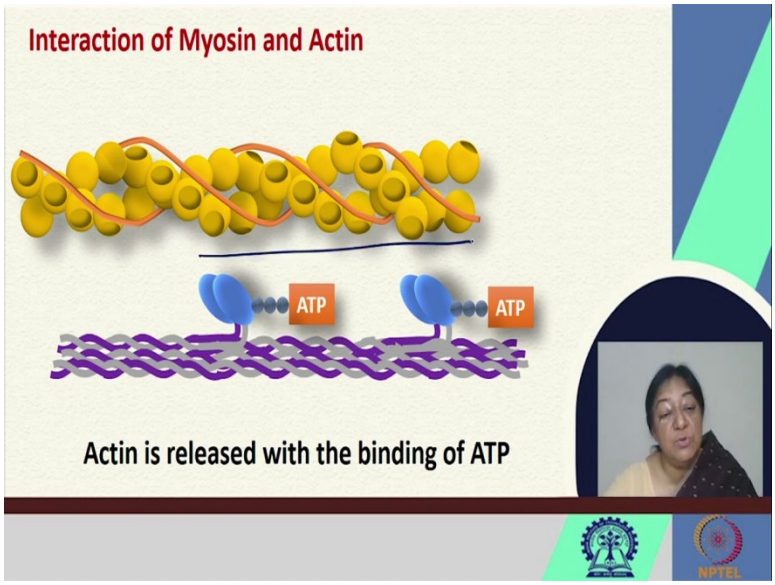

Where we have the ATP bound and once we have this hydrolysis, it does not interact anymore because the head position has now changed and the actin is released.

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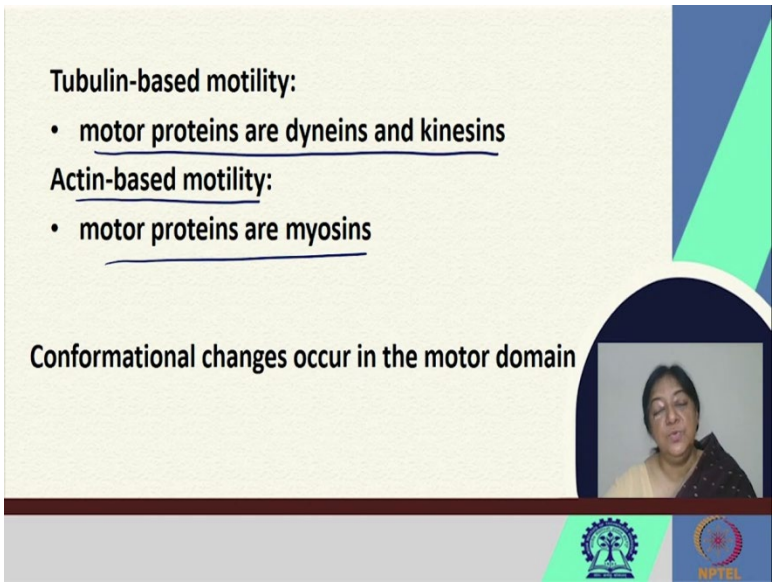

In the tubulin based motility, we looked at the motor based proteins dyneins and kinesins and in the actin based motility we looked at the motor proteins that are the myosins. We found out that there are conformational changes that occur in the motor domain and these conformational changes occur due to ATP hydrolysis, where this chemical energy is transformed into mechanical force and there is movement due to the presence of these motor proteins.

This is the transformation of the chemical energy to the mechanical energy by the hydrolysis of ATP. We have these nanometer sized microtubules or active filaments and we have this

conformational change in the ATPase domain, which we will see in the force generating working stroke in these cases.

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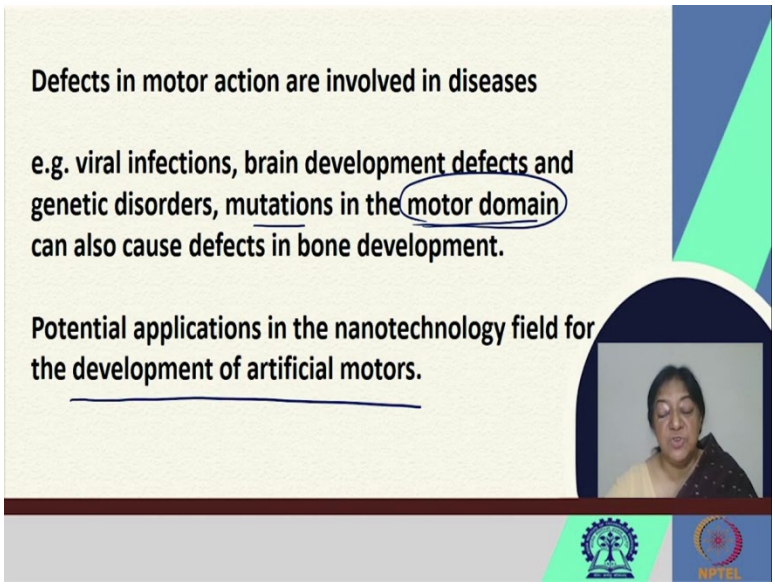

The defects in motor action as we can see, can also be involved in diseases. We could have viral infections, brain development defects and genetic disorders because there may be even mutations in the motor domain cases and this would cause several deformations. There are potential applications in the field of nanotechnology, a lot of research is going on in the development of artificial motors, to look at how cargo may be transported.

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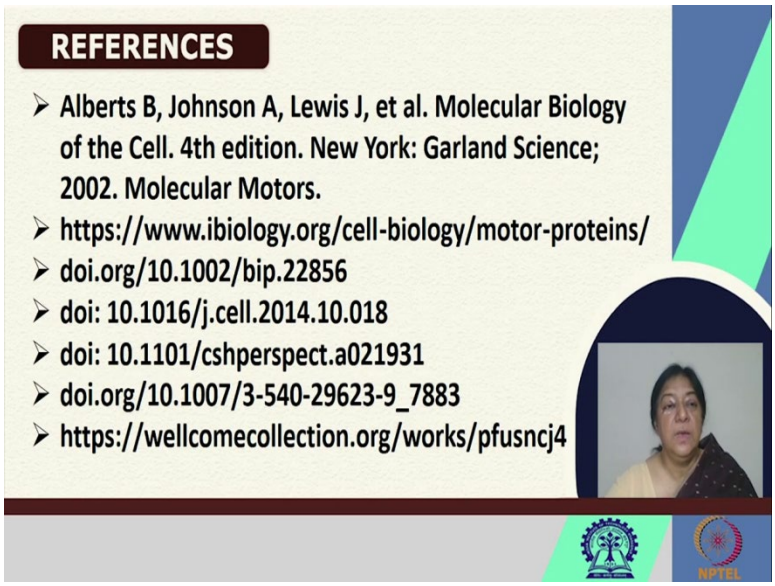

These [refer to slide] are the references.

Thank you.