Introduction to Cell Biology
Professor Girish Ratnaparkhi and
Professor Nagaraj Balasubramaniam
Department of Biology
Indian Institute of Science Education and Research, Pune
Motor Proteins in Cell

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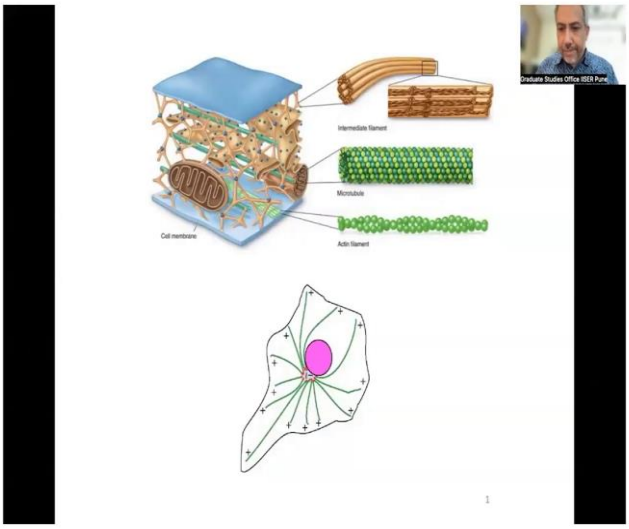

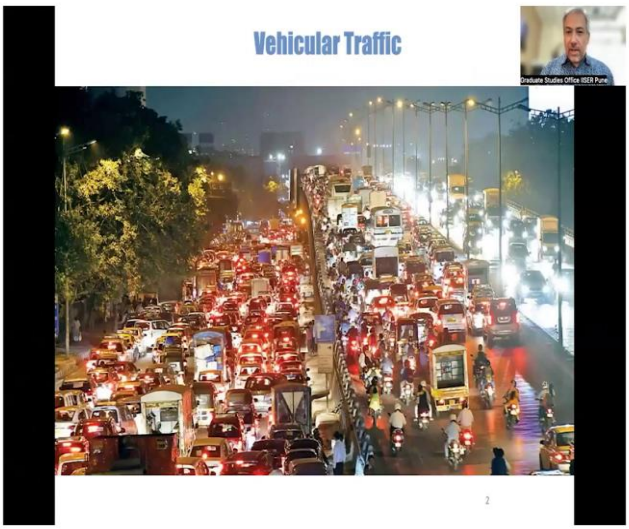

Okay welcome back, we are picking this up from where we left last time. And we are going to discuss this additional component that is an integral part of the cytoskeleton, but is also very distinct clear in how the cellular architecture is organized, how cellular function is mediated. And many of the things that the cytoskeleton does, that we spoke about, is driven by this particular component, which are the motor proteins that are present in cells.

This is just to kind of bring you back to this image of all the cytoskeleton components that we talked about and discussed, the intermediate filaments, the microtubules, the actin filaments. You are also familiar with the idea that these structures have an inherent polarity. And we talked about this idea that the polarity of having a plus end or minus end could play a major difference in how mobility happens on these structures. And that ensures that there are many functionalities that are taking place that use this mobility.

And the motor proteins are essentially the things that are actually mobile on these cytoskeleton components and we will be talking a little bit about the motor proteins that are present. And about their directionality, about where and how they could come and play a role. And, if possible, at the end, we will try and imagine a life without motor proteins. And as we add each of these components, our thought process has been like that. We have been thinking about the membrane and the cell boundary. And then we spoke about the cytoskeleton and how the cytoskeleton adds to what the membrane enclosure does.

And so, we are imagining constantly life for a cell with or without a certain component and we will try and do that for the motor proteins as well. We are all familiar with sites like this. And we have been thinking about the cytoskeleton components in many places, particularly the microtubule network, which starts from the center towards the periphery of the cell, as being major highways in the cell. And we are all familiar with this kind of crowding on highways. The interesting thing about the picture when I chose it was the fact that there is directionality here. There is stuff that is going in one direction versus the other. There is a highway that is a flyover that is moving up and one that is moving down, which means there are more than one track to get in a particular direction as well.

And so, like our the vehicular traffic that exists on our highways having these structures or having the highways built is only one aspect of how to regulate their functionality or how they are able to control contribute to the environment that they are in. The highways will be nothing without the vehicles that go that travel on them. And one of the things we notice here, when we look at these vehicles, there are different kinds of vehicles. We have two wheelers, we have auto rickshaws, we have cars, we have trucks, we have buses. They all inherently have the ability to move on these tracks, but they are also very distinctly different.

So, they are similar and they are different. They are also different in for example, how they are built. They are also different in what kind of cargo they can carry? A bike can carry one or two people. A bus can carry 30. A truck can carry a lot more things than a bike or a car

would. So, there are differences in what the capacity of each of these components is and that also contributes dramatically to how these pathways are being used. And the idea of having these vehicles with different capacities with different capabilities like in our lives, there are times where a bike can get into get through a traffic jam in a way that a truck cannot, so the truck can carry so many more things, but if you are trying to get from point A to point B quickly, you probably need a bike to be able to make it there quickly.

Each one therefore, has a slightly different functionality. And so, they may be that functionality may be exploited very differently by us and cells are essentially trying to do the same thing as well.

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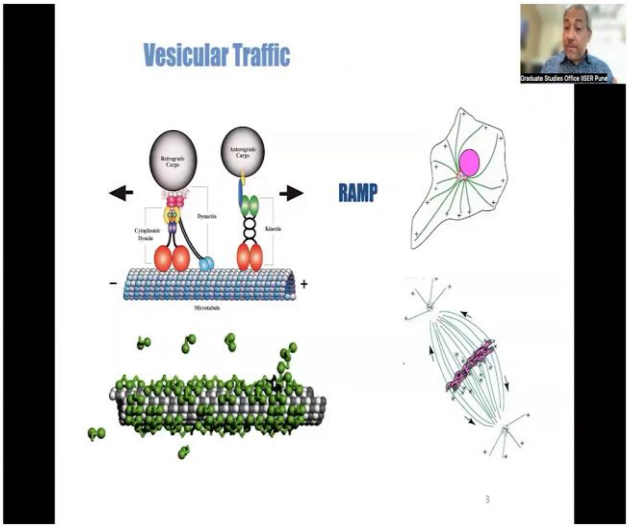

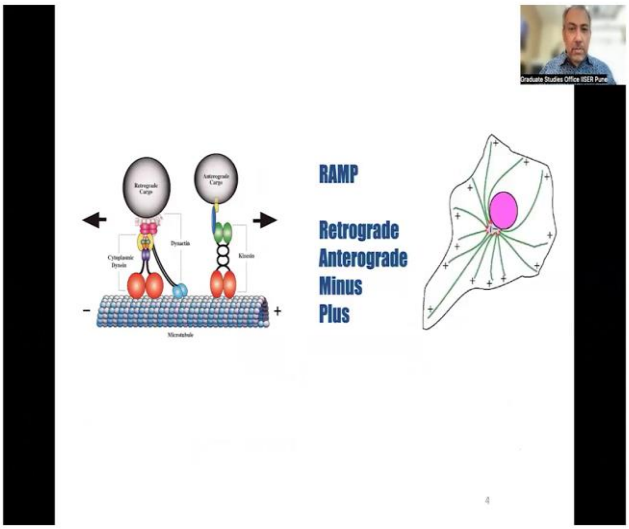

So, like with vehicular traffic that we see on the roads, we have vesicular traffic in cells and this is essentially vesicles with distinct cargo that are carried around the cell. The because these are vesicles. Remember they effectively are like one or more of these vehicles that carry things around vehicle, a vesicle can carry, just as a vehicle can carry varying number of components. Vesicles can carry varying number of components, that can be proteins, that can be lipids, remember, this is a bag of lipid. And will take a look at the snapshot from that movie inner life of the cell that we saw right at the beginning, where we saw this giant big vesicle that is floating around.

And that is carried by a motor protein that is working on microtubules. It is a very remarkable and striking image. And it kind of captures what, how to think about a vesicle in the context of a motor protein. So, these are big giant vesicles that are carried by one or more motor proteins and these also utilize the directionality that exists in the cytoskeletal components. So, there are motor proteins that go in one direction, kinesins that go towards the plus end, dynein that go to the towards the minus end and that directionality inherently plays a role in allowing these motor proteins or these vehicles to actually do their job.

So, if you imagine the vesicle as being the vehicle in which people or cargo is carried, then the motor proteins are effectively the wheels of these vehicles. And unlike our roads, where the vehicles where we are dependent on the driver to kind of make decisions about where to go. Motor proteins are like Elon Musk's driverless cars, they effectively have a plan, they know what they are going to do and all you need to do is hop on and they will do their bit. And we will talk a little bit about how that regulatory process works for motor proteins, what for example, directionality, unidirectionality or bidirectionality could mean to them and all of that will come in the next coming slides.

The other thing I wanted to mention is that most pictures for motor protein look like the one on top where you have one single motor protein very nicely walking around, but remember, just like there is crowding in traffic for vehicles on roads, there is crowding in vesicular traffic as well. There are a large number of motor proteins that can be accommodated on cytoskeleton strands and that means you can expect things to bump into each other and there is a fair bit of crowding that is happening there as well.

People have done studies where they have actually seen real motor proteins which are labeled, when they walk on microtubule strands that they kind of pause. They jiggle around each other and then move past or they get trapped sometimes because of the molecular

crowding. So, it is not like the motor proteins have open roads. The highways on in the cell are also likely as crowded as the highways out here in Pune. This idea of movement towards the plus and minus end is also referred to as the anterograde or retrograde transport. And this is a very useful term to remember. And just as these microtubules act as ramps to kind of carry things around.

We have a very simple way of remembering retrograde and anterograde that I was taught when I was an undergrad and it is something I still use. And the term to remember is ramp which is retrograde, anterograde. Retrograde is towards the minus end. Anterograde is towards the plus end. So, if you remember, ramp, you now will never forget, which direction towards the plus end is called anterograde, towards the minus end is called retrograde. And you know what the orientation of the plus end minus ends are. So, it is this movement that we are going to talk a bit about today.

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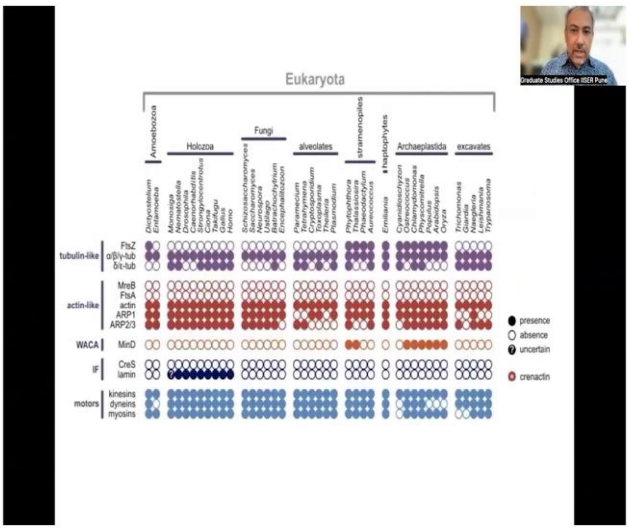

Just as a reminder, I wanted to bring in the fact that last time we spoke about the evolution of motor proteins and I am just reminding you, that these motor proteins that we are going to talk about kinesins, dyneins and myosins. All, if you remember, suddenly evolved in the eukaryotic systems and in the early prokaryotes, we do not see any of these motor proteins. We may have something that is the equivalent, because till 20 years ago, we did not think there was cytoskeleton components in those early organisms, but now we know. So, it is possible that there is an equivalent of a motor protein there. And it is just waiting to be found.

And we have a version, a more advanced version of this in the eukaryotes. But at this point of time, we do not have evidence for that. So, when you plot a map like this, you see motor proteins kicking in at a particular point of time.

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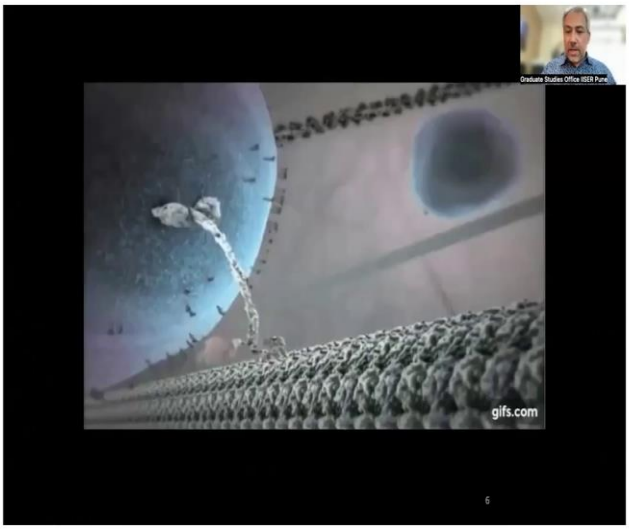

This is the movie I was telling you about. And this is just a refresher of what this would look like. You can see the cargo that is sitting things that are sitting on the vesicle membrane. Another point to make is, I do not have that image here. But there is a really, I will show it to you next time an image of a neuronal vesicle that is in the neurons, which has been completely mapped to show you all the proteins that are present on the vesicle. And just as the size of the vesicle you can see relative to the motor protein and how big the vesicle is.

The vesicle can also be very crowded. So, it can have a whole number of different proteins. The vesicle is made up of lipids. So, remember, the lipids are also contributing factors. So, when a vesicle gets delivered to a particular point, we are not just carrying its protein cargo, we are also carrying its lipid carbo there.

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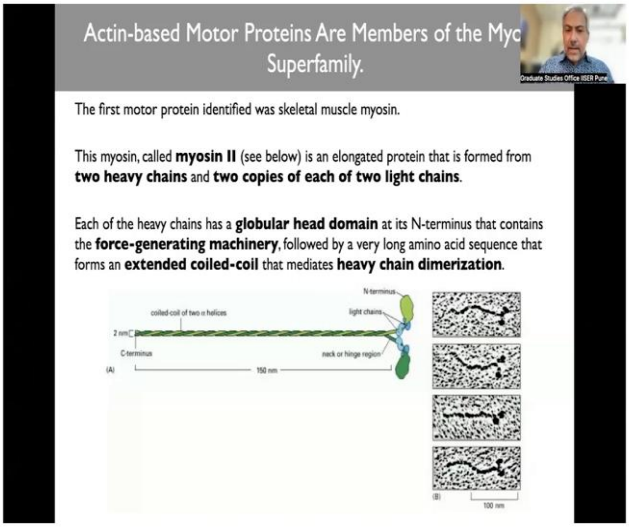

There are three major classes of motor proteins, so that is one class of motor proteins that works with actin called myosin. And we will try and focus on what the inherently similar or different characteristics are, of all these classes of motor proteins. The first thing you notice, that I should mention is that they all seem to have this kind of a structure where there is a head and a fairly long tail. And it is interesting the head is what actually sits on the microtubule strand is what generates the stroke that allows for the motor protein to move.

And that stroke is generally energy dependent in case of myosin, so it is ATP dependent, if I remember correctly. And you have this tail. And the idea of the tail here is essentially it is a way for cargo to be kept away from the base of the motor protein. So, the vesicle is attached here. This particular thing will have some amount of sway. It can move in directions. And remember, if they are walking along the same microtubule strand and we are thinking about crowding, it really helps if the motor protein can bend and as does the vesicle and two vesicles can actually pass by each other.

So, the height of the tail is actually interestingly variable across different motor proteins. So, we will take a quickly look at how this clocking action works. And again, the critical thing for you to remember is not to kind of completely understand how these motor proteins work. There is a there is a lot of work that has been done to characterize this particular aspect of how energy is utilized to create the turn in the arms or the base of the motor protein that allows for them to make this moment and walk along very specific cytoskeleton components. So, this is a quick movie, please listen in and then we will move forward.

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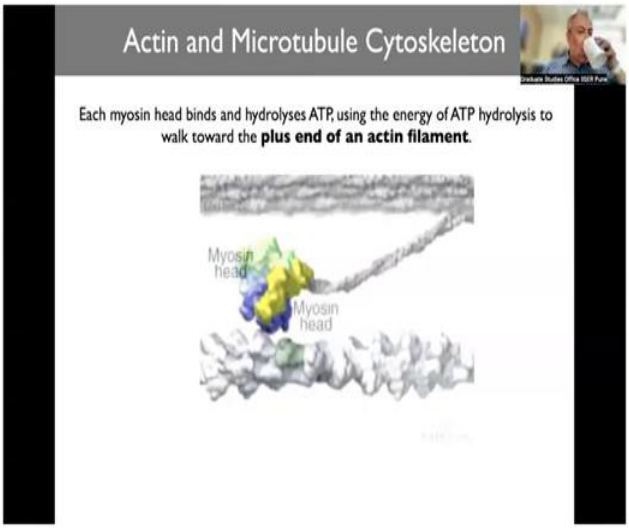
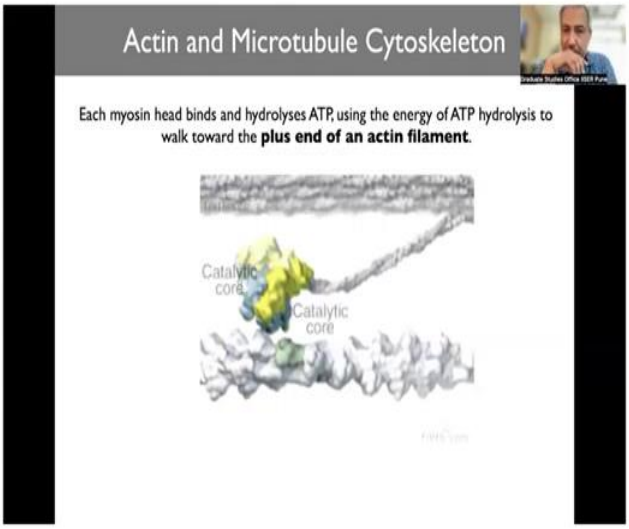
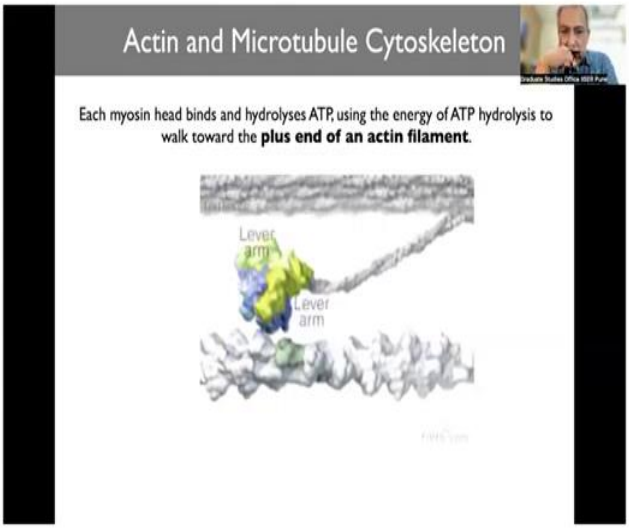
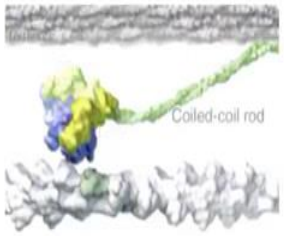






Actin and Microtubule Cytoskeleton

Each myosin head binds and hydrolyses ATP, using the energy of ATP hydrolysis to walk toward the **plus end of an actin filament**.



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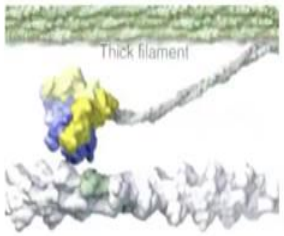

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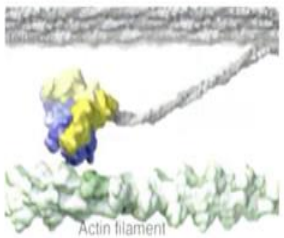

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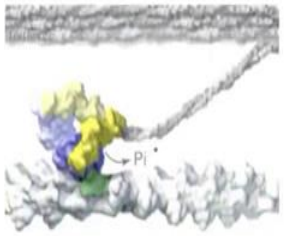

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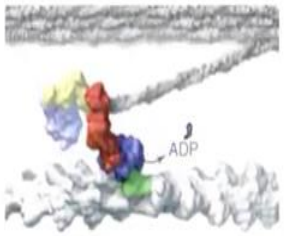

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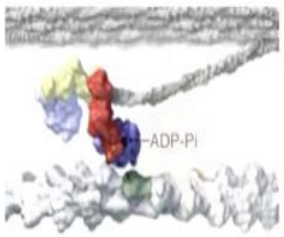

Narrator: Muscle myosin is a dimer with two identical motor heads that act independently. Each myosin head has a catalytic core and an attached lever arm. A coiled coil rod ties the two heads together and tethers them to the thick filaments seen on top. The helical actin filament is shown at the bottom.

In the beginning of the movie, the myosin heads contain bound ADP and phosphate and have weak affinity for actin. Once one of the heads docks properly onto an actin subunit, phosphate is released. Phosphate release strengthens the binding of the myosin head to actin and also triggers the force generating power stroke that moves the actin filament. ADP then dissociates and ATP binds to the MT nucleotide binding site, causing the myosin head to detach from the actin filaments.

On the detached head, ATP is hydrolyzed, which we cocked the lever arm back to its pre stroke state. Thus, like a stretch, the arm stores the energy released by ATP hydrolysis and the cycle can repeat. The actin filament does not slide back after being released by the motor head because there are many other myosin molecules also attached to it, holding it under tension.

Professor: So, it is a simple lever.

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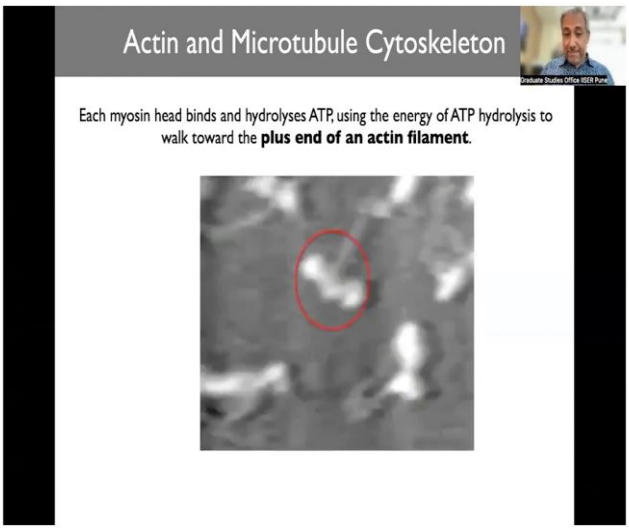

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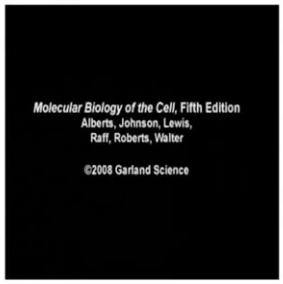

Swing of the lever arm can be directly observed on single myosin molecules. Here visualized by high speed atomic force microscopy.

Professor: So, there is this very crisp moment of it being turned on and off. A lot of the wonderful work on motor proteins is done by Ron Valle. And Ron is a leading contender for the Nobel in the coming years. And is a pioneer in helping us understand how these systems are put in place.

What is important and interesting to note is that having a single strand of a cytoskeleton and a motor protein walking on it is not the scenario that inevitably happens in cells. So, it is not like an open empty space with a strand. Strands can be lying right next to each other. Motor proteins can be anchored to other things that could be vesicles, that could be crowding, between all of that this kind of movement is taking place and is allowing for the motor proteins to do their job.

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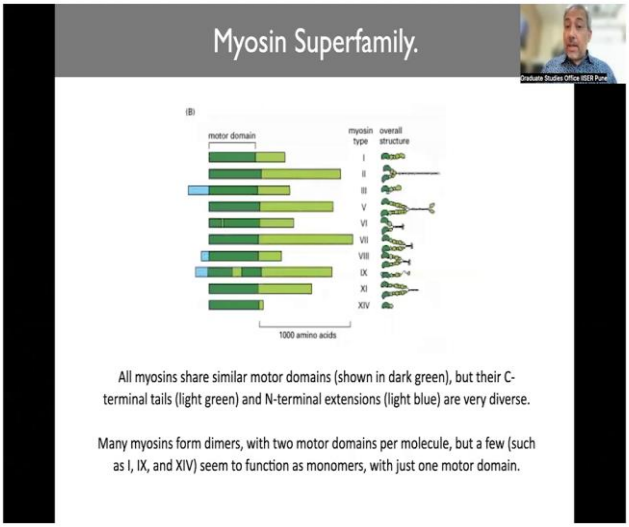

The myosin with all motor proteins, we also do not have one single kind of motor protein. So, this is again, a possible example of this term that we heard about, which is redundancy. In some cases, there are indeed, more than one kind of motor protein that is actually working to do similar things. But this class of motor proteins or this group superfamily of myosins. The thing that you notice is as I mentioned earlier, they all have a head, which is where the binding and the movement is regulated, but the tale of these myosins could be variable. The specificity of one or more of these myosins could also be variable. That means they could bind slightly different things. So, they could also be localized in different parts of the cell.

In some cases, certain myosins can be present in certain types of cells. And that all adds to the diversity that exists in the role of these proteins. So, along with the redundancy that can happen at times, there is also diversity in how and where myosin proteins, one or more of these myosin proteins could work.

Remember, in a cell, it is the I do not want you to go leave thinking that a cell will have one particular kind of myosin that is not true. They could have more than one kind. Different cells could have different flavors of my myosins. And that is contributing to how the role of myosins is controlled in those cells.

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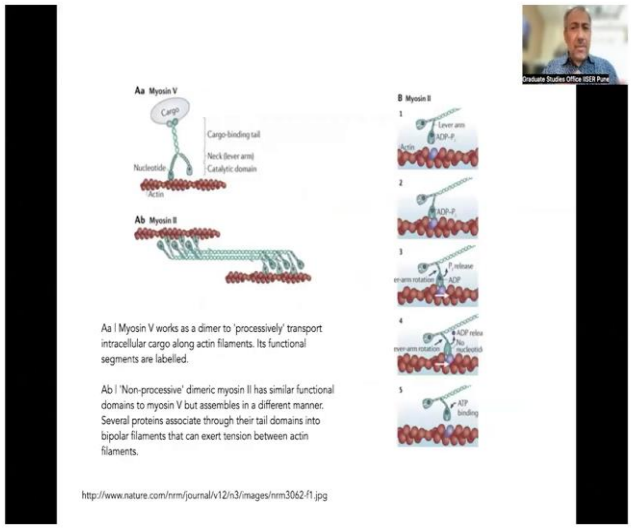

We looked at this just captures this arm rotation and release. And that video actually does a fairly good job of it. And as the video pointed out, remember that there is not, it is not that there is only a myosin with a vesicle that is walking on a track. You have this image of two actin cytoskeleton tracks and myosins that are connected to two. So, the moment of two actin tracks like this could also be mediated or driven by myosins that connect them. And a lot of the muscle myosin play a very vital role in this manner.

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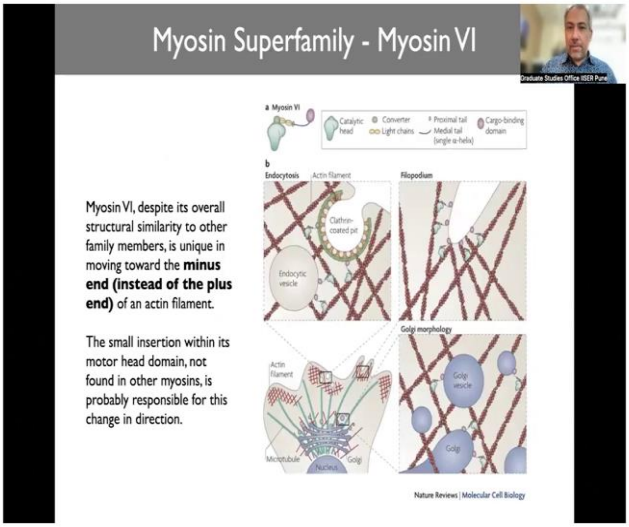

They also play an important role with actin in being able to generate the force that we now know, actin contributes in the cell, that means, we know that the actin can make a mesh, we know that it can push, it can generate forces, it can bend the membrane, if you remember structures like filopodia, these long tracks of the membrane are generated by actin. A lot of that bending endocytosis pulling something in is all governed by the force that actin cytoskeleton generates.

And a lot of that force generation by actin is made possible by the kind of crosslinking that the myosin subunits allow for. So, actin can bind to many of these components like membranes, like endocytic vesicular structures, as can myosins as well. And that allows for them to kind of pull and contract and generate forces when needed. So, it is important to remember that the ability of, the force generating ability that exists in myosin in actin is actually coming from the actomyosin component. So, you will hear this term actomyosin and it essentially is a combination of actin and myosin, which are coming together to kind of do things as far as force capabilities of the cells are concerned.

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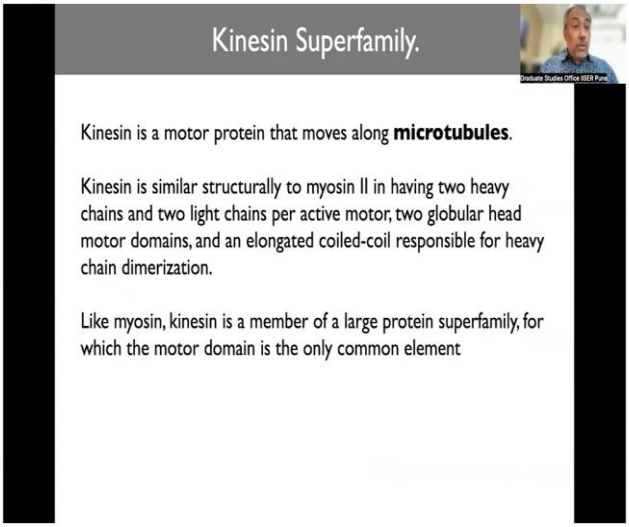

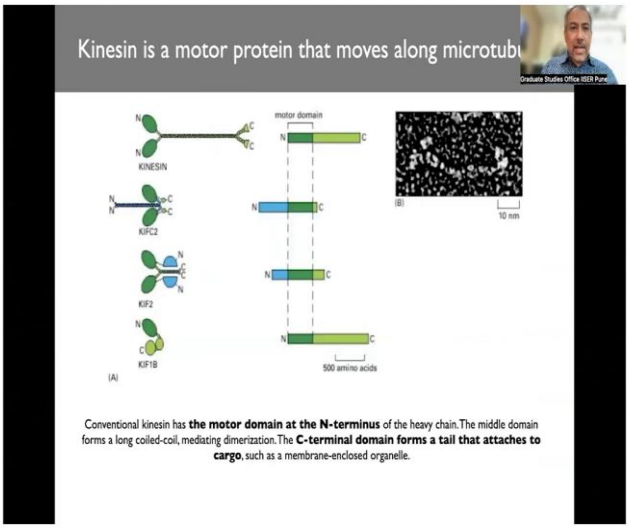

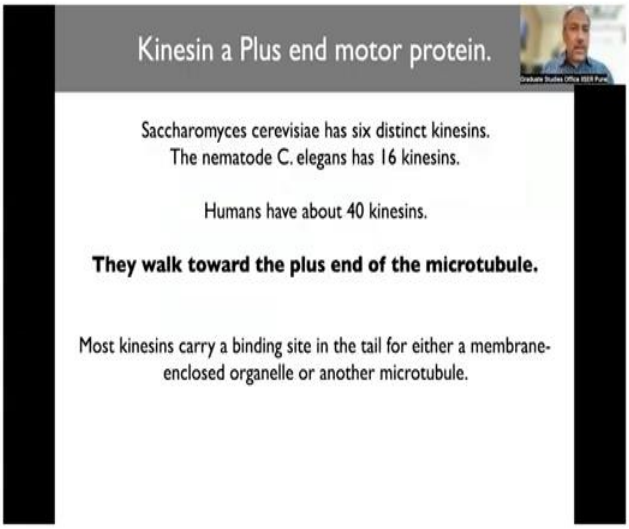

With microtubules, interestingly, we have two very distinct classes of motor proteins. We have one motor protein that walks to one end and the other motor protein that walks to the other end, like myosins, kinesins are similar in the sense that they have a globular head, they have a tail. So, this idea of the structure of motor proteins, irrespective of whether they are working on actin or they are working on microtubules seems to be fairly conserved. They all have a head, they all have a tail and the length of this tail could be variable. They all utilize energy to kind of drive this movement, the clocking of the motor head and that allows for them to move in very specific ways.

With myosin, with kinesin like proteins, again, there are many versions of them. So, that is another conserved point, like and that is allowing for very fine tuning or controlling of the behavior of these motor proteins. They can carry very specific cargo. You remember, I mentioned this idea of everything in cells being controlled in context of space and time. And a lot of that space and time control is mediated by these fine players. So, having so many versions of myosin or kinesins could go a long way in allowing for that to take place.

The kinesin is essentially a plus end motor protein. In humans, for example, there are 40 different variants of kinesins that can exist. As I said, this could exist in different cells or cell does not have just one kind of myosin, it could have a mixture of kinesins and this mixture of kinesins could vary depending upon the type of cell you are looking at. So, a cell A could have kinesin 1, 10 and 15. I am just giving random numbers here and cell B could have 1, 10, 15, 22, 33.

And that may have to do with what cargo they are carrying, that may have to do with what functionality they have or they are trying to achieve and together this comes to drive the movement of vesicular cargo and other components that are present along microtubules.

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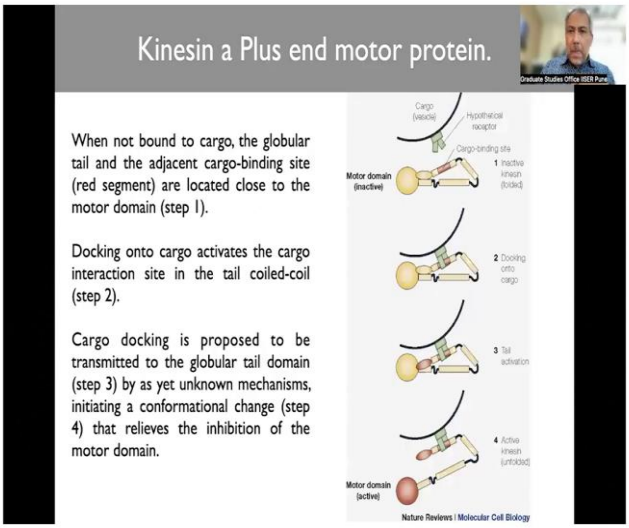

Like with myosin motor proteins they have very distinct binding capabilities and the motor promoter heads are able to move clock open, collapse back and then open again. And that allows for this movement to take place. The directionality of these motor proteins along microtubules, is also actually governed by their ability to bind to very specific sights on the motor protein on the cytoskeleton components.

So, along microtubules, the reason the kinesins are able to move in a certain direction. They really do not understand the fact that they are actually moving in a particular direction, they are essentially walking on the microtubules in a way that they can. And the fact that they are able to bind to very specific sites and make this clocking movement and bind again, over time, we realize allows them to move only in one particular direction.

And this in a very simplified way, I am trying to explain how this could govern the fact that there is movement in one direction for a particular motor protein and movement in the other direction for a motor protein. So, these motor proteins inherently are not thinking that I want to be a plus end or minus end motor protein. They bind to the microtubules. And they walk in the direction that their architecture and the architecture of the microtubules and the way they bind, this allows. So, this binding and this moment, is the only thing that is regulated and the fact that they bind in a certain way and can move in a certain way is what is determining which direction they move in.

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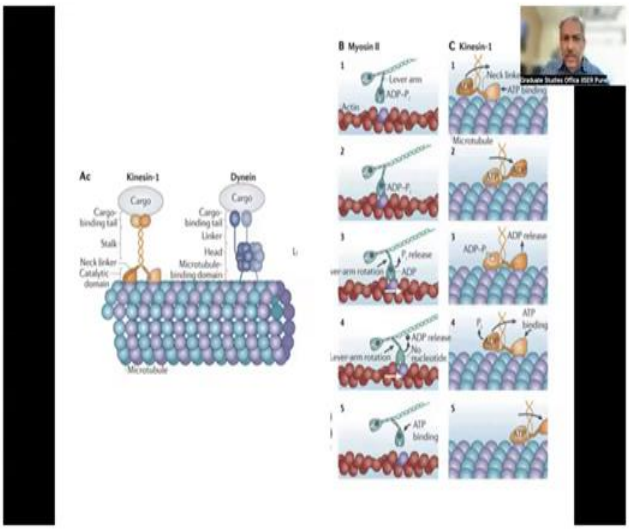

So, that is an important thing to keep in mind. And this is just to kind of give you a comparison between the motor proteins. On the right, you can see myosin and kinesin. Again, this is an ATP dependent process. So, as we talked about, actin and microtubules and they are coming together requiring energy, we now see that the motor proteins and their mobility along the cytoskeleton is also an energy dependent process. So, you can see how energy becomes an important rate limiting step. And the evolution a lot of these is likely to have happened because of the availability of an energy in these systems. Along with kinesin we have dynein, which actually walks in the other direction.

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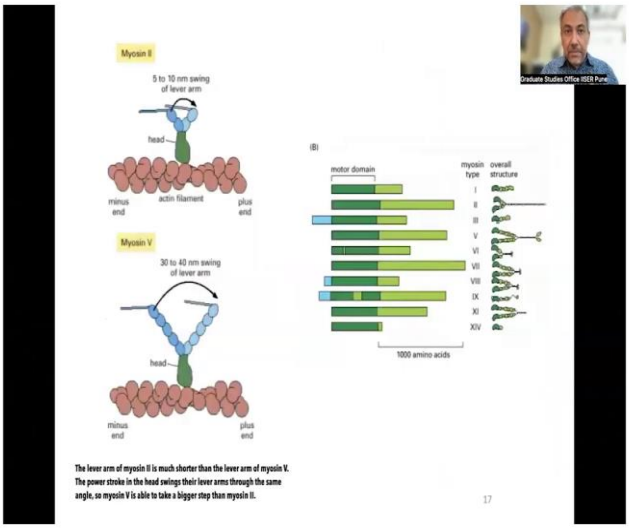

This is just to kind of, this is a okay, this is myosin. I think the slide got misplaced they should have come earlier. It essentially was to kind of make the point that they have arms of different lengths. And so the length of the stroke they make, could be variable and that is another important parameter, because if you have a shorter head and you essentially each step you make the number of steps that are needed to make travel a certain distance will also be variable.

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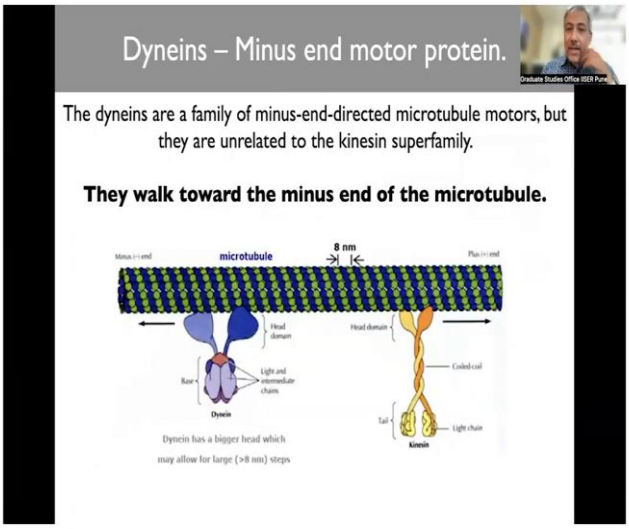

Dyneins like kinesins, as I mentioned earlier, which are plus end motor proteins. Dyneins are minus end motor proteins. Again they require energy.

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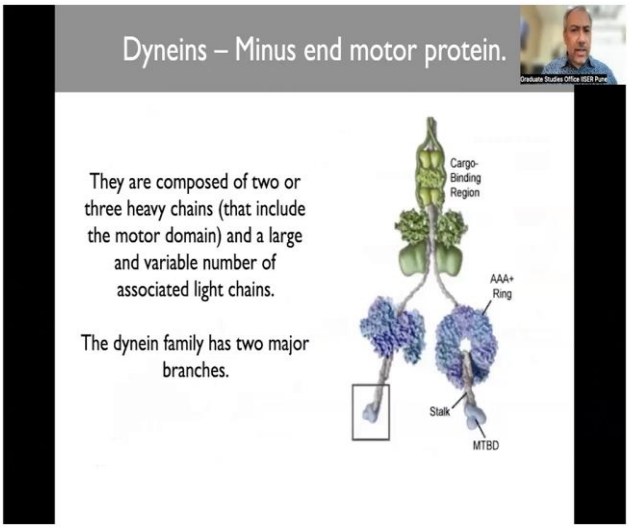

There are variations of it that exists. And all those aspects are fairly conserved, extensive evaluation of the architecture and of these motor proteins has been made, crystal structures for many of these are available. And we have a very, very detailed understanding of how they are assembled and how they are put together to do what they do. And it is quite remarkable.

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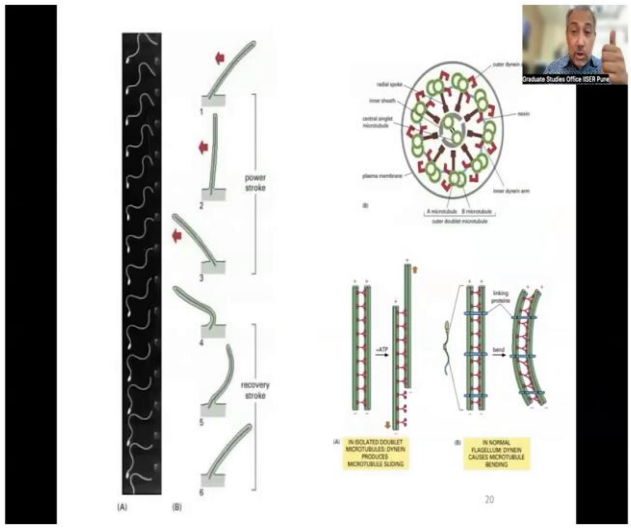

As we talked earlier, the ability of motor proteins to attach and bind two cytoskeleton components could also make an important role in the movement of the cilia, for example. And you remember, we saw this image earlier of microtubule components being arranged in such a way linked by dynein and now that you know what the dynein motor protein would look like and what it is capable of. You can now imagine what kind of movement it will generate, if it is actually binding to neighboring microtubule strand.

And the fact that this kind of movement is happening across the length of the cilia is what helps the cilia make this very elaborate power stroke, that is discussed here. So, this is again, a good example of how motor proteins could play a very vital role. We also saw right at the beginning of how motor proteins of cytoskeletal components plus and minus ends. Microtubules, particularly are arranged during cell division. And we talked about the chromosomes that are in the center that has been that are being pulled apart.

Some of that movement could also be facilitated by motor proteins along with the polymerization depolymerization that we talked about.

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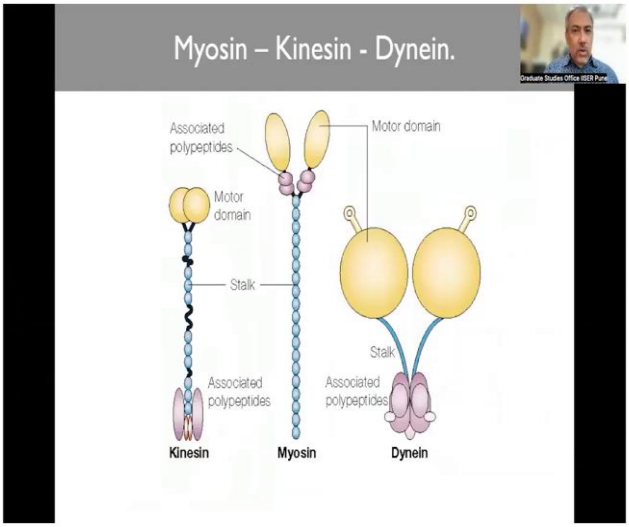

So, there are different kinds of roles these could play and among the things if you look at their architectures, you can see that there is a presence of a distinct motor domain. They all have tails of differing sizes. And together these come together to drive their unique capabilities. Their ability of the motor heads to bind, very specific kind of cytoskeleton components is also very vital to them being able to function with unique cytoskeleton components and also be able to have directionality within that when they do.

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Actin filaments
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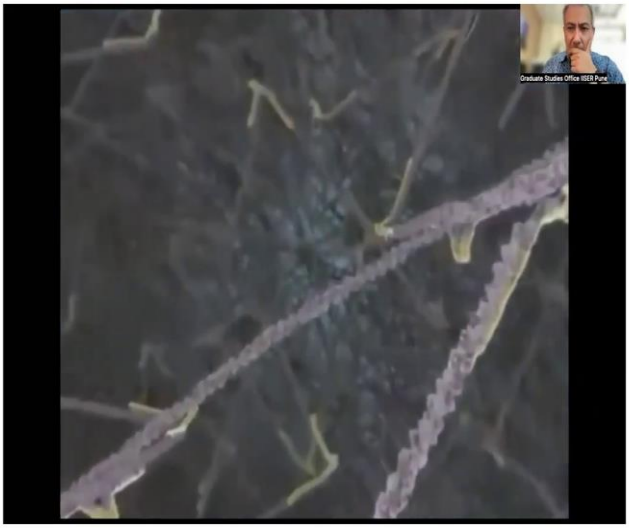
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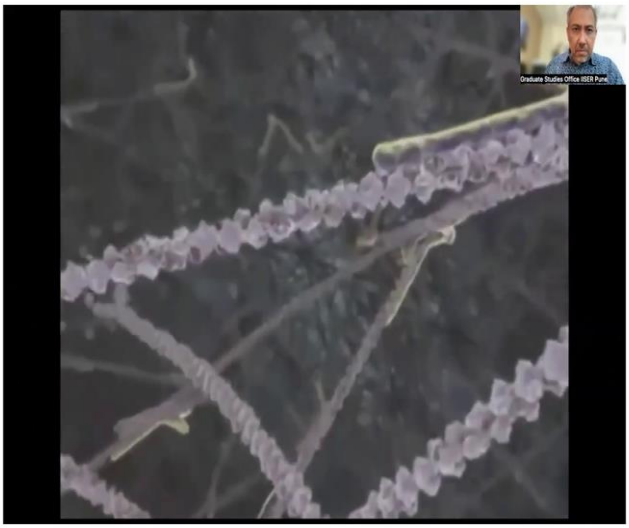
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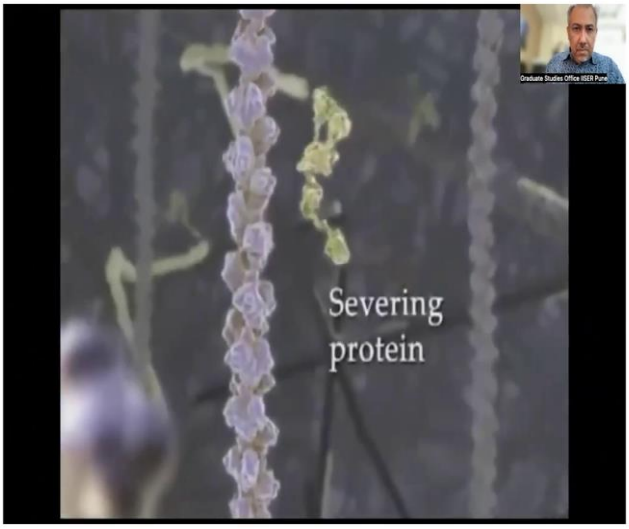
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Severing
protein


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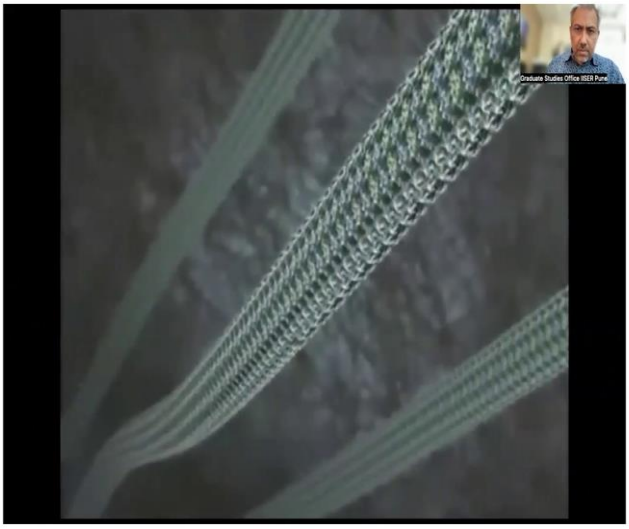
Microtubules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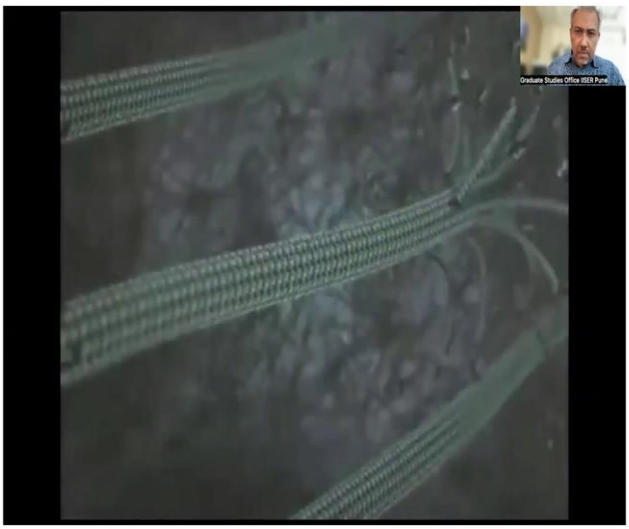
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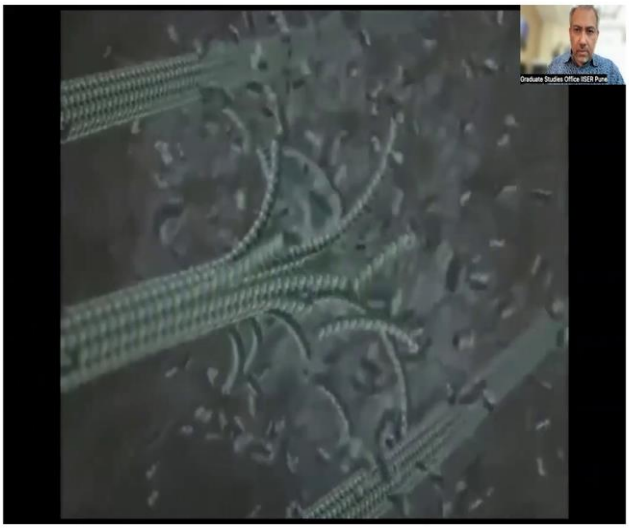
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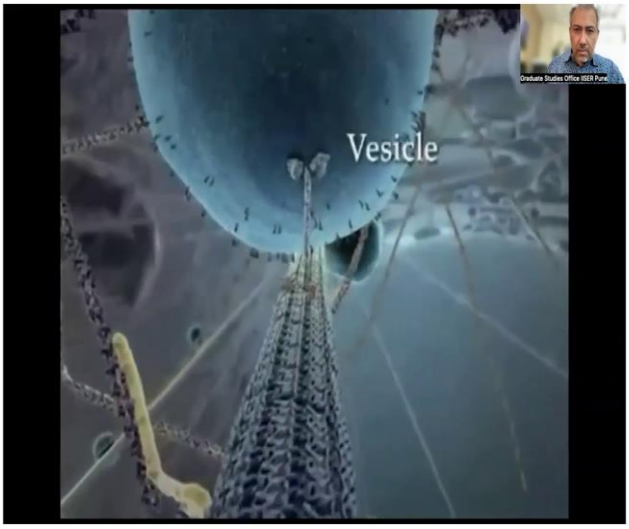
Vesicle
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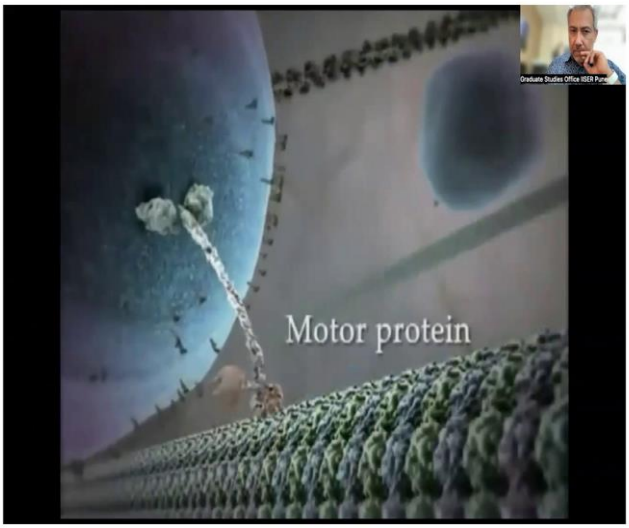
Motor protein
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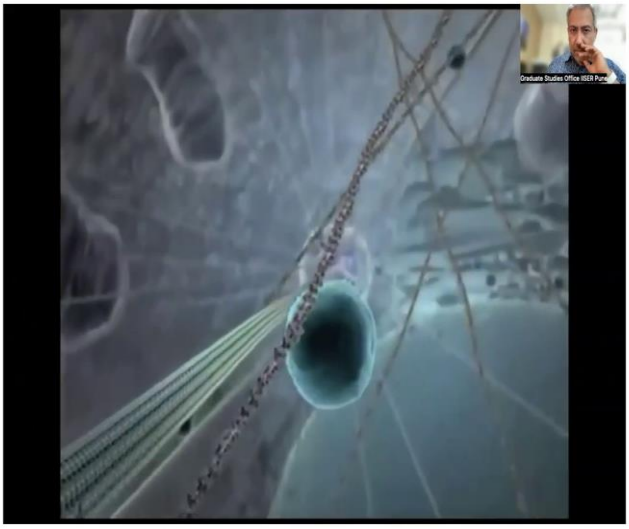
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Mitochondria
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We will look at this movie, we will just close with this today, where we are looking at the movie that is taken from the inner life of the cell, this is again a portion and as I said, we will keep coming back to these movies. And now you will recognize more things here. You will understand a bit more and they will also, we will also be looking at motor proteins and how they are involved in things within the cell.

Narrator: At works, filamentous proteins that are responsible for the spatial organization of cytosolic components. Inside micro villi actin filaments form tight parallel bundles, which are stabilized by crosslinking proteins. While deeper in the cytosol, the actin network adopts a gel like structure stabilized by a variety of actin binding proteins. Filaments kept at their minus ends by a protein complex grow away from the plasma membrane by the addition of actin monomers to their plus end.

The actin network is a very dynamic structure with continuous directional polymerization and disassembly. Severing proteins induce kinks in the filament and lead to the formation of short fragments that rapidly depolymerize or give rise to new filaments. The cytoskeleton includes a network of microtubules created by the lateral association of protofilaments formed by the polymerization of tubulin dimers. While the plus ends of some microtubules extend toward the plasma membrane. Proteins stabilize the curved conformation of protofilaments from other microtubules causing their rapid plus end depolymerization.

Microtubules provide tracks along which membrane bound vesicles travel to and from the plasma membrane. The directional movement of these cargo vesicles is due to a family of motor proteins linking vesicles and microtubules. Membrane bound organelles like mitochondria are loosely trapped by the cytoskeleton. Mitochondria change shape continuously. And their orientation is partly dictated by their interaction with microtubules.

All the microtubules originate from the centrosome. A discrete fiber structure containing two orthogonal centrioles and located near the cell nucleus.

Professor: So, a lot of these players now are familiar to you.

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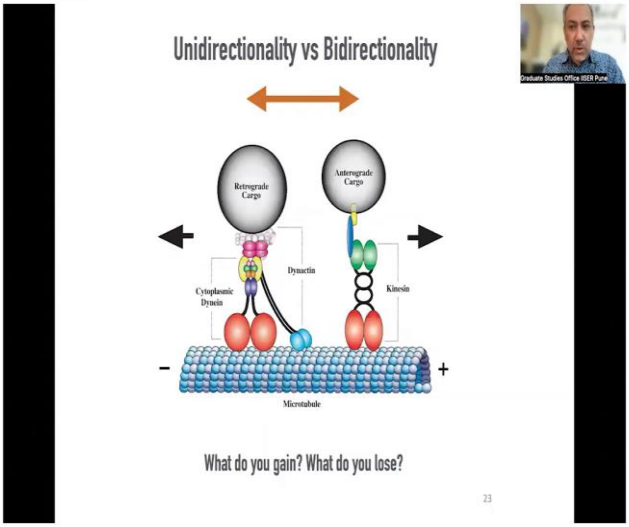

You have a sense of how they are organized. And now when you start imagining all of them inside the cell. You get a sense of what they are capable of doing. One important point to consider is that and I keep repeating this is the kind of crowding that exists within cells. And it is very easy to imagine open big spaces and those probably do not exist in most cellular systems.

The cell is a fairly crowded structure. The cell also has a cytoplasm. So, when you saw the vesicle being moved, remember, the vesicle is not heavy. And if you are wondering why this motor protein is able to just kind of hold it and walk with it, it is got to do with the fact that there is the cytosol in which this vesicle is floating.

So, it is almost like zero gravity where the vesicle is bobbing around. And now this allows for the motor protein to kind of bind and pull the vesicle. Is only one motor protein bound to a vesicle? No. There could be more than one motor proteins, that could be also motor proteins that go in opposite directions that can bind a vesicle. And the number of plus end, a number of minus end motor proteins that are pulling on a vesicle could eventually determine which direction is it goes.

So, if there are more plus end motor proteins that are pulling, then the vesicle eventually makes it in this direction. But remember, more than one kind of motor protein can bind and to a vesicle. And that binding can determine which direction the vesicle goes to.

One important question that I am raising here at the end and this is something that I want you to think a bit about and we will hear your thoughts right at the beginning next time is we talked about the directionality and the fact that these vehicles or these motor proteins are actually going in very uniquely plus or minus end directions. So, but the question here is unidirectionality, which is the ability to go only in one direction versus bidirectionality, which is the ability to go in this or in this direction as the need be.

There could be a very important choice here that has been made by cells, because most motor proteins are unidirectional. They are not bidirectional. There are some evidences of some motor proteins under very specific circumstances having bidirectionality, but it is rare. It is not the norm. And the question for you is what do you gain, what do you lose here? The fact that we, the cell has chosen unidirectionality, what do you think, is the biggest advantage it gives the cell and what exactly is the kind of disadvantage that the cell may have because it has chosen unidirectionality over bidirectionality.