Introduction to Cell Biology
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Introduction to Cytoskeleton: Part 1

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This next two lectures will focus on a component that is part of the cell, which is extremely vital for what the cell does, and is important in the cell being in how the cell is put together and is also important for the fact that this allows the cell to do everything from getting from point A to point B and being able to, for example, take things into the cell, push things out, talk to the external environment and remarkably also help generate cells of different sizes and shapes and this component is referred to as the cytoskeleton.

And the these two images that I have right at the beginning, are kind of meant to build this picture of what the cytoskeleton could mean for a cell, and how there are examples of such structure being vital for maintaining the shape of art or architecture of things that we probably know of. And one common example is a camping tent that some of you may have used.

And if you have used a camping tent you know there are these beams or bars that are kind of have to be connected to build the scaffold on which this tent is then mounted. The other interesting thing about a camping tent is the fact that this is kind of held down with different things, and we will come to that at a later point of what could be holding the cells down as well.

The other interesting structure is a structure like this geodesic dome, and this is used in many different places, greenhouses, for example, have structures like this, this is an example of a tent that can be used for many purposes. But you have this sheet that is lining the outside, and this network or mesh of metal here, which has clearly a different architecture, it also has the ability to provide some stability to this mesh or this net that is sitting on top.

Now imagine the net here in this image on the right hand side, but without this metal structure, underneath, and what you will have is this whole thing will kind of collapse on itself.

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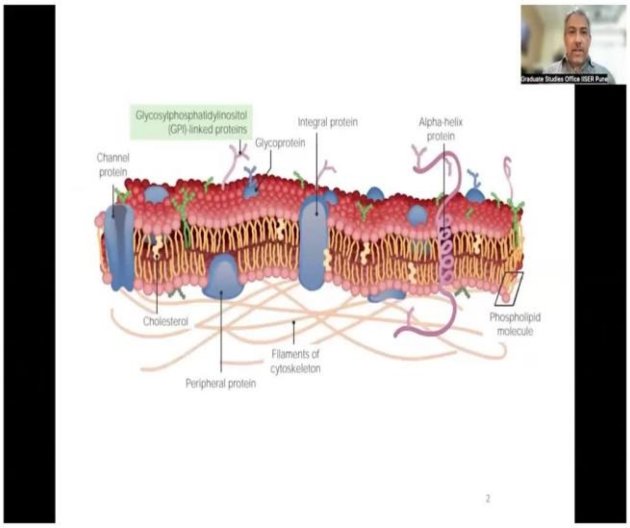

So it is very similar for cells as well, the cell membrane as we looked at earlier is a very fluid structure, it is made up of lipids, it is also a structure that is very floppy can be moved around easily, and began probably as a sphere, which then grew in size over a period of time. And when you think about larger cells, you also have a significant amount of membrane now.

And this membrane left to itself will largely collapse, on to the membrane itself and will not be held in the kind of shape or architecture that we wanted to, which is where the cytoskeleton comes into play and an image of the membrane, like this one seen in textbooks, you have the two bilayers, you have proteins going through it, and you have this layer of the cytoskeleton that is underneath the membrane.

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And this like the scaffold that we just saw earlier, is able to prop up the membrane hold the membrane and provided shape. So for example, if in such a dome that we are looking at the scaffold had a very different architecture like a long tube, and then you put the membrane on top of it, what you will see is a shape that has the shape of the cytoskeleton, even here, the shape of the dome is essentially the shape of the cytoskeleton.

So the cytoskeleton has the ability to provide this kind of architecture or shape for cells in a way that becomes very useful to define what cells look like and how they behave and how they talk to each other.

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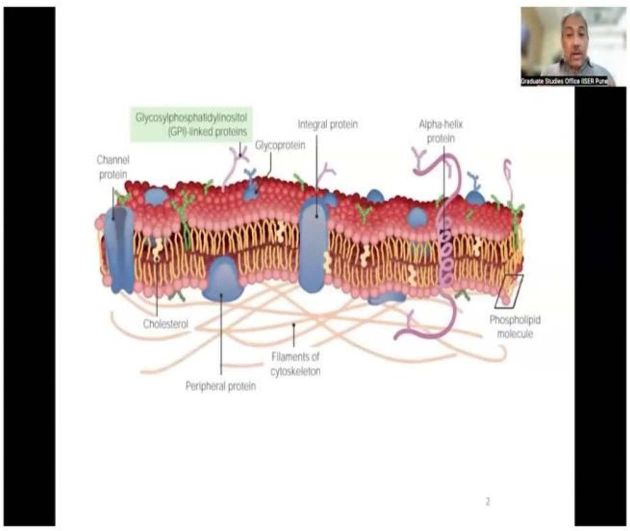

Along with being this support that exists on the outside the cytoskeleton interestingly also acts as important transport highways inside the cell and we will we will come to the role that they could have as transport highways. So you now see that the cytoskeleton not just has one role it may have more than one role, the cytoskeleton is involved in making structures like flagella or Pilli or in cells, there are structures called filopodia, which are essentially small pointy things that come out of the cell, which are all covered by membrane.

So here you have a cytoskeleton structure and a membrane and now that allows the cell to reach out with a structure like that. So there are many different roles that the cytoskeleton can play. It may have originally evolved as a structure that is vital for supporting the cell membrane, but with time, it has developed many other functions and now it has become a very integral part of the cell in more than one ways.

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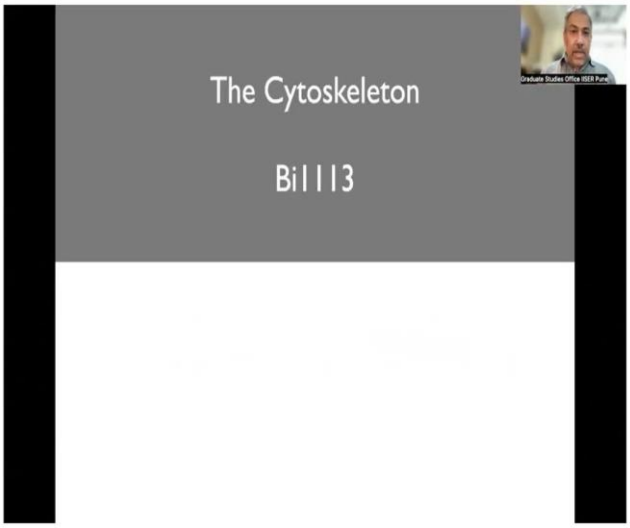

And we are going to try and look at the cytoskeleton and the components that are coming together. The other really interesting and important part of the cytoskeleton is that if you have a cytoskeleton or if you have a structure in the cell that needs to meet all these requirements, it should have, it should be able to provide a mesh or a network, it should be able to assemble in those structures.

It has to be acting as has to be actively remodeled, put together, taken apart, to create different kinds of structures as needed. It should act as a structure that can be assembled into things like filopodia, or cilia, it has to have the ability to transport things inside the cell, you are asking us for a structure that has the capability to adapt, and, make different kinds of things inside the cell.

The question that also then arises is whether there is, the cell can do with just one kind of cytoskeleton or does it lead multiple kinds of cytoskeleton? To begin with, it is highly possible that the cells evolved with a certain kind of cytoskeleton and over time different cytoskeleton components were brought together to now make a cell that has more than one cytoskeleton component that does very distinct things.

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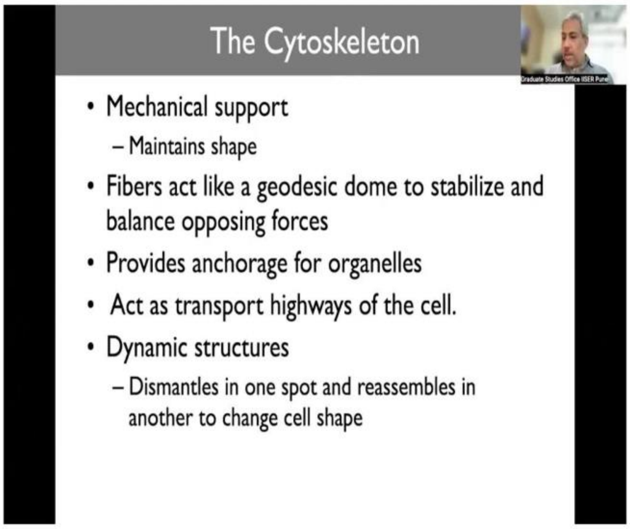

So the cytoskeleton as I just told you, it provides mechanical support, it has a vital role in maintaining shape, it is able to stabilize and balance opposing forces. So if there is a structure inside the cell like this and something is getting pushed from one side this is able to communicate it to the rest of the cell. So a way to stabilize forces that happen inside the cell is another important function.

The cytoskeleton also provides a way to anchor cellular organelles inside. So now you have a bag of lipid, and you have thrown a bunch of things inside. And each of those things is trying to do specific things for the cell has specific functions, and their functions could be dependent on where they are inside the cell and how they talk to each other.

So there is merit to keeping these organelles in very specific locations in the cell, even being able to move them around. Now if it was just a bag of fluid and things were sloshing around, there will be very little control on where something is kept and how exactly it is moved to another place. The cytoskeleton now could provide the architecture on which these things could be held and also be moved around.

As I mentioned, they also act as transport highways in the cell and most importantly, they have to be dynamic structures. So the dome that we saw at the beginning, imagine that is one structure, but suppose you want to change that dome from being semicircular to a square or you wanted it to be a long box.

Depending upon the requirement of the cell if there is a dome that can be put together taken apart put together again, you have the capability to create a structure like this. In tomorrow's class, I will show you something that we may all have used and played with that has this same idea or concept that is to create very complex elaborate structures with minimal parts. So you have one kind of part that you make many of and then you put them together in all kinds of shapes and forms.

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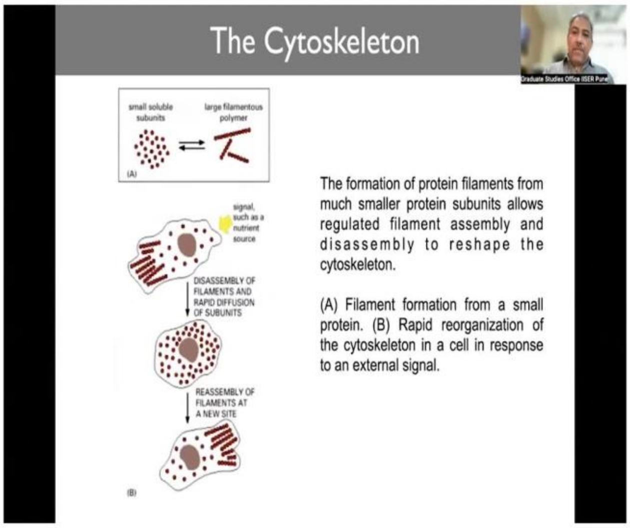

The cytoskeleton components, unlike the membrane, which is made up of lipids are made up of proteins. So one of the things is to remember is when it comes to making of the cytoskeleton using of the cytoskeleton the advent of proteins and the making of proteins, should have taken place.

So cytoskeletal components and I asked this question earlier, and we will take it at the end of the class as well as to whether prokaryotes and eukaryotes both have cytoskeleton. But what it suggests is that this kind of complexity that exists in eukaryotic cells, the architecture of the cytoskeleton that I am talking about, the complexity that I am talking about, is in context of eukaryotic cells.

So they have a protein making machinery and there are many ways proteins could be assembled or put together. The interesting thing about cytoskeleton is that their are coming together into complex structures is mediated by small subunits, which are identical almost, which can come together, assemble and disassemble as required, and create all kinds of architectures.

A good example in that I was going to refer to is Lego, I do not know how many of you played ever with these Lego building blocks, it is a remarkable thing. And these blocks are all identical, they come in different colors, but the original Lego block is a block that every block looks the same, but it has the ability to be assembled into all kinds of complex structures. So there is immense power in having one single subunit that can be assembled disassembled into various structures.

And the cytoskeleton is actually the original Lego block, if you ask me, because it has been put together in this manner.

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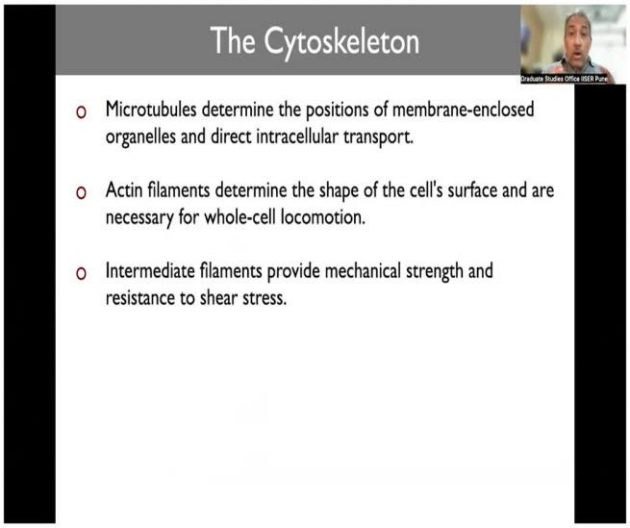

So along with this, the other interesting thing about the cytoskeleton in eukaryotic cells, is that there are 3 flavors of the cytoskeleton. So this again, took time to evolve and reach this point where all these 3 flavors are found in the same cell.

It is very possible though we do not have a remnant of this in eukaryotic cells, that at some point of time there were cells that contain one cytoskeleton component, there are some cells which contain the second one and there are cells that we have contained the third, and they may have come together in some kind of combination.

And the coming together of these two cells could have generated a cell that has now two components, which made this particular cell that much more efficient in doing many of the things. And this could have led to the selection of a such a cell, and it doing well and the same could have happened for all the 3 cytoskeleton components. But in eukaryotic cells, now, we have microtubules, we have actin, and we have intermediate filaments.

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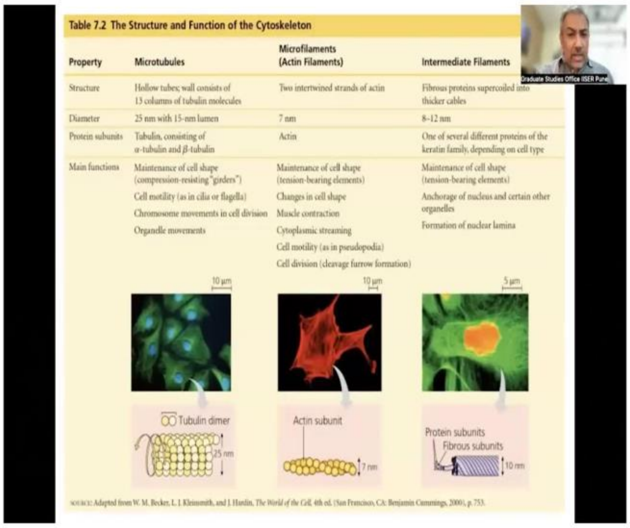

Table 7.2 The Structure and Function of the Cytoskeleton

Property	Microtubules	Microfilaments (Actin Filaments)	Intermediate Filaments
Structure	Hollow tubes; wall consists of 13 columns of tubulin molecules	Two intertwined strands of actin	Fibrous proteins supercoiled into thicker cables
Diameter	25 nm with 15-nm lumen	7 nm	8–12 nm
Protein subunits	Tubulin, consisting of α-tubulin and β-tubulin	Actin	One of several different proteins of the keratin family, depending on cell type
Main functions	Maintenance of cell shape (compression-resisting "girders") Cell motility (as in cilia or flagella) Chromosome movements in cell division Organelle movements	Maintenance of cell shape (tension-bearing elements) Changes in cell shape Muscle contraction Cytoplasmic streaming Cell motility (as in pseudopodia) Cell division (cleavage furrow formation)	Maintenance of cell shape (tension-bearing elements) Anchorage of nucleus and certain other organelles Formation of nuclear lamina

SOURCE: Adapted from W. M. Becker, L. J. Kleinsmith, and J. Hardin, *The World of the Cell*, 4th ed. (San Francisco, CA: Benjamin Cummings, 2000), p. 753.

And we will look at each one of them, and see how they are similar how they are different. And then think a bit about, why the cell would invest in having 3 different cytoskeleton components. And whether you think it is actually a sensible idea at all. The first thing to consider is that each of these cytoskeleton elements to be put together assemble, disassemble, requires energy.

So we are now entering this era or period and we are talking about all these components that, eukaryotic cells have, knowing that they do have access to a reasonable amount of energy, they have they can use ATP or other energy sources to build things inside the cell and so the structures that we now begin to encounter are things that are not about saving energy.

They are saying okay, we have the energy, we will be able to use it, let us find the best way of doing something that may require energy and you will allow the cell now to do well. So the interesting thing about the way microtubules and actin are put together is very visible in the architecture they have. So let us, first let us not look at intermediate filaments, let us focus on microtubules and actin and make a distinction between the two.

The first thing that you notice is the microtubules are essentially spiral. So they are actually a hollow tube, unlike actin, which is like a pleat, two strands that are woven on top of the other, which means the width of the actin cytoskeleton is about 7 nanometers. The tubulin filament however, is about 25 nanometers. So it is about almost 3 times the thickness of an actin cytoskeleton, this is actin, this is microtubule.

The things that are common here, obviously, is that they are both made up of individual subunits, actin is made up of actin monomers, that are assembled into the actin strand, microtubule is made up of alpha and beta tubulin, that are assembled in a very distinct architecture, there is a lot of information that we will not cover here that, looks at how the strands are put together, what the alpha and beta subunits mean, how they bind and assemble a lot has been done with the cytoskeleton components.

And in the advanced cell biology courses, you will be looking at more aspects of how the cytoskeleton is assembled and works. Here we are just introducing you to the idea of cytoskeleton. And also thinking a bit more about what having such structures in cells could mean. So you have microtubules, and you have actin as two major cytoskeleton components, they are made up of different subunits.

They use different energy sources and since we were talking about energy, I am going to flag that here, it is not listed here, but it will come up later, which is that the microtubules, use GTP as the energy source and actin use ATP as the energy source. And one important question, therefore that this raises is that one, did microtubules and actin actually evolved or come into existence as cytoskeleton components at different times, during the process of evolution? And what do you think, could have come first and later.

Does their energy source have something tells you something does it tell you something about, when this could have come into play? So think a little bit about this, this is an open question that I am going to kind of raise to the class, which is ATP versus GTP, 2 different energy sources. Did they have a timeline, did one come before the other?

They could have come at different times and existed together, and the cytoskeleton components could have evolved at around the same time also, we really do not know if that is the case. One way to evaluate the cytoskeleton and this is actually used as a very interesting tool to address the question of how evolution itself happened is to follow cytoskeleton components and their forms if any that exist in early prokaryotes, early eukaryotes and eukaryotes that we know now. When did they when did microtubules come when did actin come is something that a lot of people study. So we will come to what these structures are.

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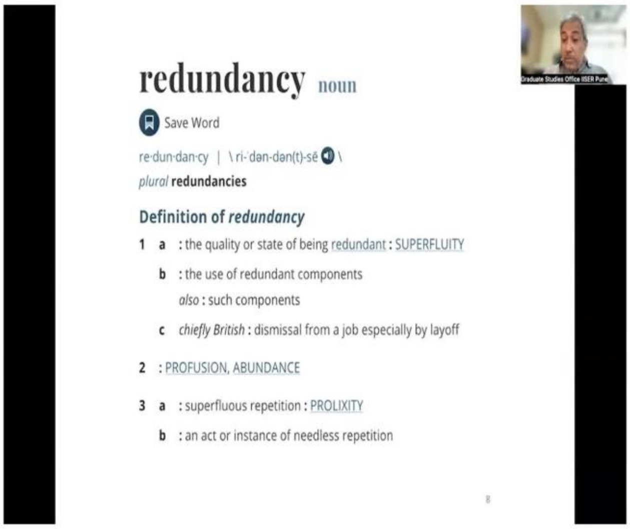

And before we do that, I want you to introduce you to this term that some of you may know and some may be hearing for the first time, which is this term called redundancy. And when it comes to cells, this is a term that you will run into repeatedly, which is one more reason to introduce it here. It essentially means having more than one way of doing something or having more than one mechanisms that are available to address a situation or a problem.

I think a good example for us, is having two wicket keepers in any cricket team that the Indian team sends out that the Indian cricket team. So whenever they go abroad to play inevitably you have seen that there are two wicket keepers that travel with the team and this is just in case somebody gets hurt and things like that you have another wicket keeper to fall back to that is redundancy.

So we have this tour of Sri Lanka that is coming up and Rishab Pant and there is a new wicket keeper batsman who is traveling with us with the team called Bharat and this is his first big tour, but that redundancy has existed all along. And there is a first wicket keeper and the second wicket keeper this person will play first and if there is something wrong with their health, then you go to the second one that means there is some hierarchy that may exist as well in redundancy.

In some redundant situations, there is no hierarchy that means both wicket keepers could play they are both equally good and it will depend on other things that will decide which wicket keeper to pick, it could be on the current form they are in, it could be about other requirements for the team, so there is no preference here to say one over the other in terms of them just being wicketkeepers.

So redundancy could have some level of hierarchy built in, redundancy could also lack hierarchy in sometimes. And in cells, this is a very common theme that the cells have had different ways of doing the same thing and they have used it in very interesting ways to kind of adapt and be able to accommodate situations that they have had to accommodate or withstand or put up with.

So something like that happens, so for example, in the Indian cricket team, if both the two established wicket keepers, if they have not been able to play we have gone to sometimes a batsman and said can you keep wickets. A good example is our current coach Rahul Dravid, who is kept wickets for India in certain situations. Sometimes it happens that you are in the middle of a match and we get keeper gets hurt, one of the other players only now has to chip in, so that is another form of redundancy that exists.

So what you see is that cells similarly have different ways or different mechanisms by which they use redundancy. And the cytoskeleton could actually be a good example to illustrate this redundancy, which is you seem to have very more than one way of essentially doing a certain kind of thing and we listed a whole bunch of functions that the cytoskeleton needs to do.

The question now is do each of these cytoskeleton components are all 3 of them capable of doing this, everything? Or have they evolved certain unique characteristics that make them partly redundant, which means there are certain things they can both do, but there are certain unique things also they can do. Like, I do not think that we will have a batsman, wicket keeper like MS Dhoni and he went on to be captain of the Indian team as well.

So as much as he had redundant functions as a wicket keeper, he was also captain of the team which means he had certain unique functions, which made him very difficult to replace, and you had to you cannot say that, the redundant wicket keeping function somebody else would come into, but hey then who will be the captain there. So there are some redundant functions, and there are some unique functions. And that is probably what happens with the cytoskeleton.

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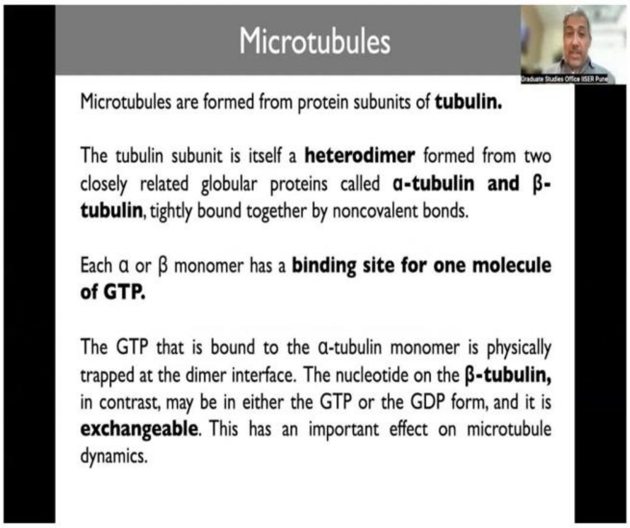

So we will look at this idea of redundancy as we go through the cytoskeleton and as we look at other components as well. So there will be terms that will there will be other places where this term will come up again. So as I told you, the microtubules are put together by alpha and beta subunits, they bind GTP as their energy source, and they cycle from a GTP to a GDP bound form, which allows for their coming together and disassembly.

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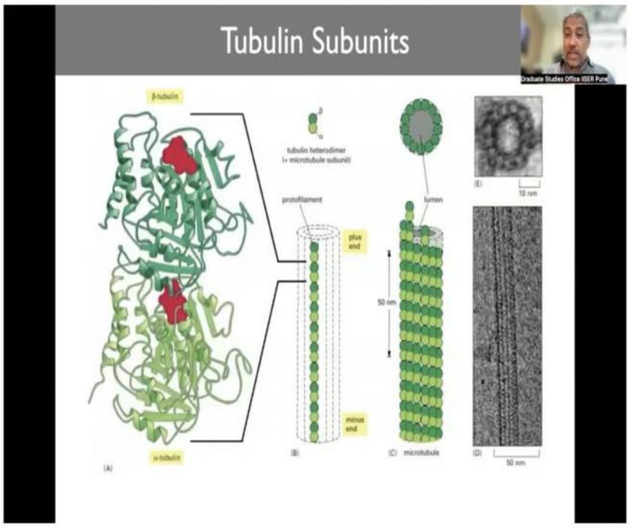

So the coming together of the cytoskeleton elements and of the subunits is an energy dependent process and that is what you need to remember. There is a process called dynamic instability that exists in microtubules, which allows them to disassemble very quickly as well.

Right now, I am not even going to get into dynamic instability again, as I said, when you do advanced cell biology, we will be looking at that, all you need to know is that there are methods by which these structures can be assembled quickly, can be taken apart quickly as well.

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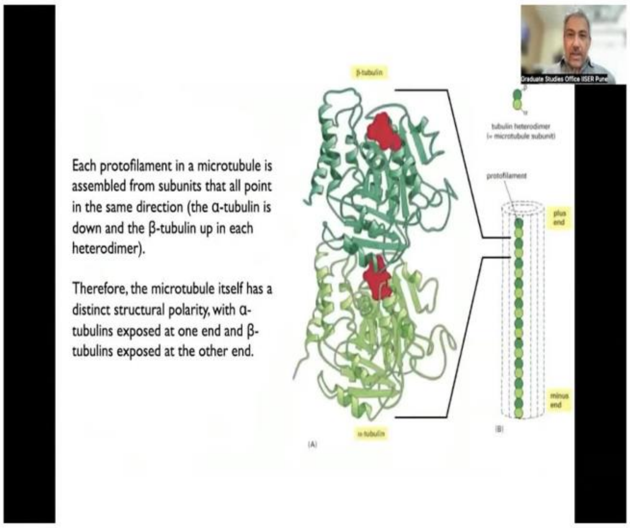

The and with tubulin, they are assembled as alpha and beta subunits repeating with each other and each ring of microtubule has 13 subunits that are assembled together, let me say that correctly and that kind of defines the thickness of the structures. One of the open questions that still remains to be fully addressed is a lot of what we know about what happens on the microtubules, either happens at the tip of the microtubules, where the microtubule goes and attaches to something or happens on the surface of the microtubules.

So if this is the tube like structure that we are talking about, we know about what happens on the surface, what binds and things like that. This is a hollow structure, if it is a hollow structure, there is space inside, is there stuff happening inside with the inside the microtubules, we still do not know at this point. And a lot of it has to do with how we are able to image and look at the structures.

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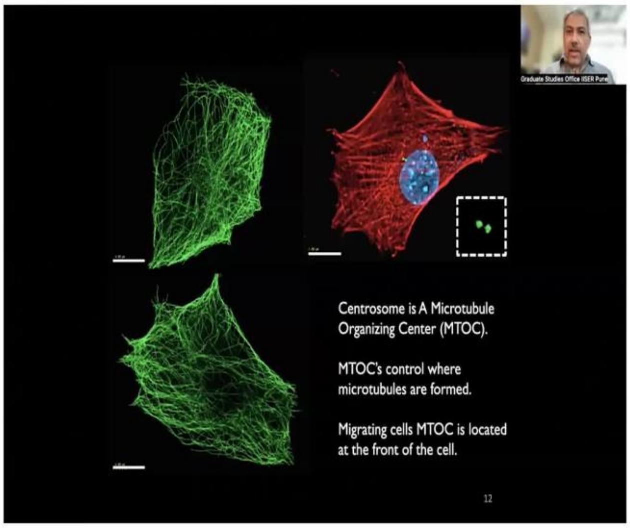

The microtubules, interestingly, originate from one particular point inside the cell, called the centrosome. So there is something called a microtubule organizing center, it is stained, these two green dots that you see is the centrosome and this images that are taken in my lab, where we have seen the microtubules and the actin.

The red is actin, the blue is the nucleus and can you see those two green dots that is the microtubule organizing center. The microtubules originate from there and so they start there, and they go in all directions inside the cell. The green you can see on the left hand side are two cells, which are where the microtubule network is stained for and you can see this very beautiful architecture that they have in cells.

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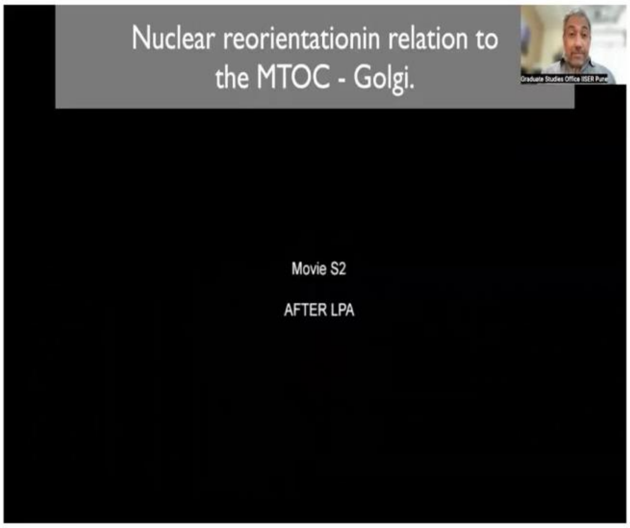

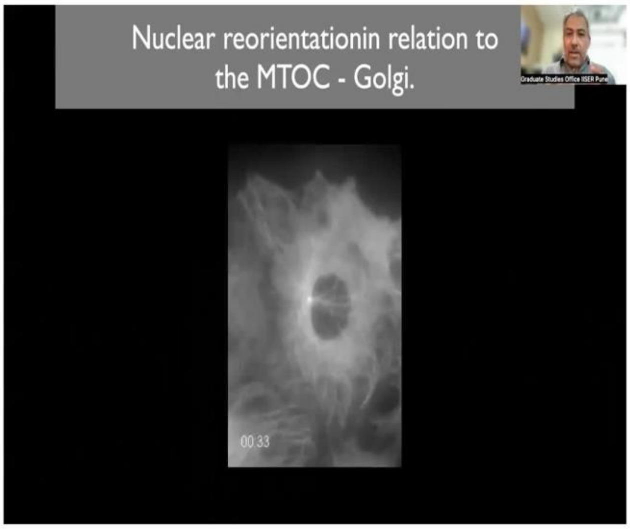

And so this is a good example of the microtubule organizing center and you can see the nucleus in a cell that is migrating, I am not getting into what this movie essentially means. This is a cell that is responding to a particular stimulus and is beginning to migrate. Here, what you need to look at is can you see that spot, that is where the microtubules are beginning from, and the nucleus as an organelle in the cell is kind of this microtubules are all wrapped around it, they are wrapped around the nucleus, they are going above and below.

So the nucleus is kind of trapped in this mesh of the microtubules. Because this is a movie, you can also see that the microtubules are very dynamic. Look at the top part particularly, you can see how they are forming, they are extending, they are pushing along, so a lot is happening with these strands. And this is happening because they have the ability to assemble and disassemble very quickly. So that is how the microtubule architecture is put together.

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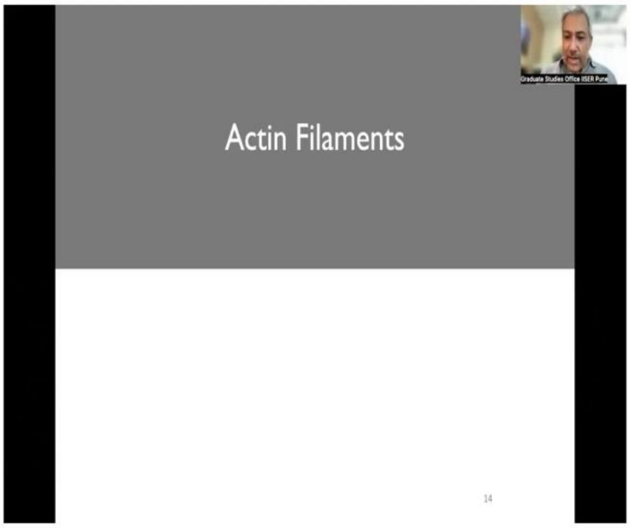

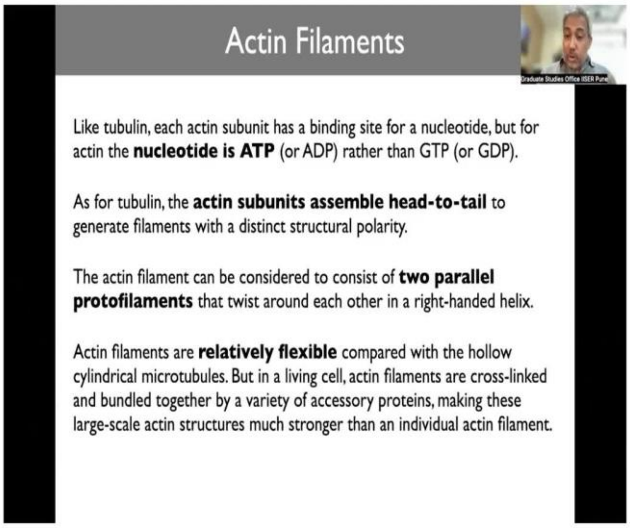

The actin on the contrary is again made up of subunits. And these two subunits, as I said mentioned, as I mentioned earlier, come together to create this kind of overlapping structure, which makes up the actin strand, they require ATP to be assembled unlike microtubules, that require GTP and unlike microtubules.

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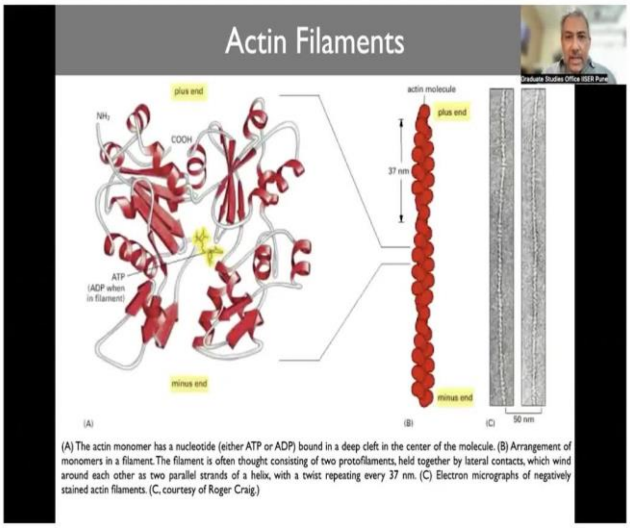

So this is how that strand is assembled and you can see that this also has a plus and minus end, which is again an important property or characteristic of the microtubules as well. So along with subunits along with energy requirements, along with dynamic, that is their assemble disassemble quickly.

Both actin and microtubules seems to have some polarity in them, which essentially means for a cell or anything in a cell that actually binds to the microtubules actin strand. It knows immediately, which is this direction, which is this direction, I mean, it knows this is plus this is minus, plus and minus are the terms that we have used.

But it essentially knows the difference between going right and going left and this is very intriguing, because this then allows the cell to fine tune a lot of the movement that happens on these strands. If they have to be acting as highways. Imagine, Bombay to Pune, we are traveling, there is this expressway here that we are all familiar with.

Imagine you are blindfolded and you are taken to a place on the Express Highway, and you do not know where you are, you do not know which direction you are in, there are no vehicles on the road and you are dropped off in the center of the highway, and your blindfold is now removed and you are asked to go towards Bombay.

Now you have all these external cues, you know what is outside and what kind of buildings may be present and so on and so forth that may give you a sense, you have the sun also, which we can use to kind of navigate. Now imagine we removed all that and you are just dropped off on the highway and you are told you walk towards Bombay, you really have no clue, till you meet somebody and ask them, maybe you really do not know whether you are going in the right direction or the wrong direction.

Now imagine, the highway has an inbuilt system that allows the moment you put your feet down that tells you this is Bombay, this is Pune and so you can decide which way to go without having any other cues, just because of the fact that the highway has this information, it could be a set of arrows on the highway, that are pointing in one direction, that is the Pune direction.

And you know if you have to if how to read the arrows you know that the arrow is always pointing towards point Pune, so if I have to go to Bombay, I should go the other way. This kind of information being built into the highway itself is the kind of analogy I can give for this polarity that exists in actin and microtubules and this becomes very useful when they have to act as parts on which things have to be carried or move in one direction or the other direction.

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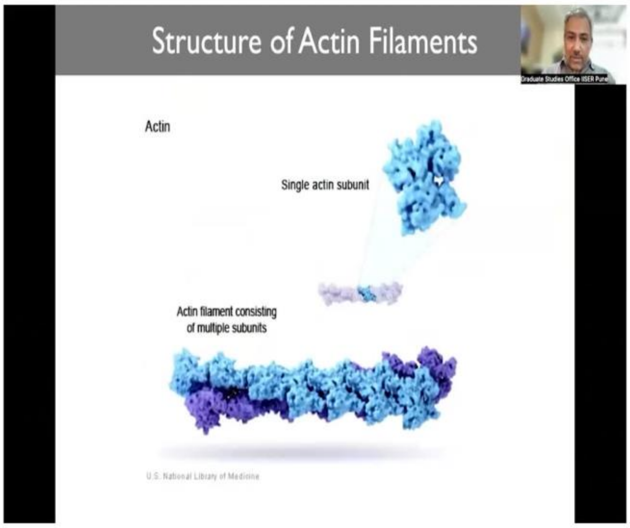

This is again, an example of how those subunits are put together, and how these strands are twisted around into each other.

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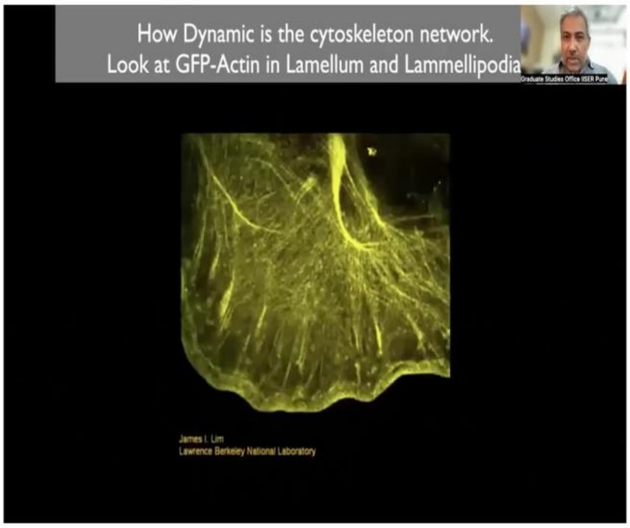

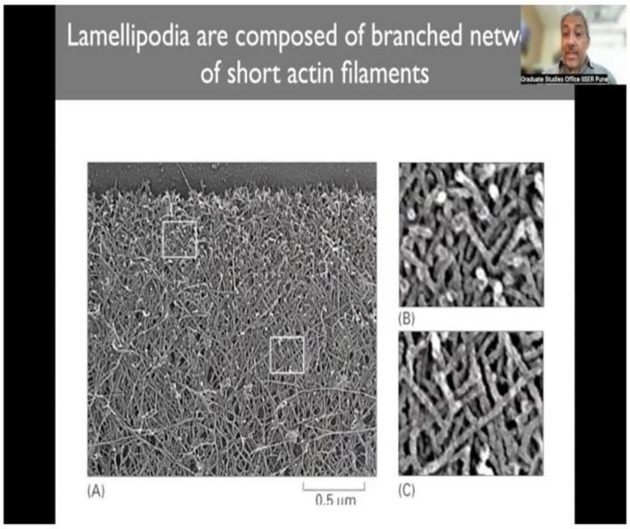

So when you look at a cell, and this is a very beautiful image, where the nucleus is in green, you can see the yellow which is microtubules and you can see how they are radiating from within the center of the cell out to the periphery, you know that center is the microtubule organizing center, and the purple is all the actin.

Now immediately, you can tell the difference in how the microtubules are organised in the cell versus the actin, you already know that microtubules are bigger structures, they are hollow tubes, actin cytoskeleton have the ability to, are much thinner structures, along with that actin also has this capability.

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And I will skip this movie very quickly to kind of show you that has the ability to branch. So unlike microtubules, that actually are long strands. Actin makes very interesting branches and these branches arise from the binding of very specific proteins here at the in the actin strand, which are called Arp 2/3 proteins.

And you do not have to worry about names and things like that, all you need to know right now is that they have the capability of branching, which means they can create a mesh that looks like this, which is very different from what the actin microtubules do.

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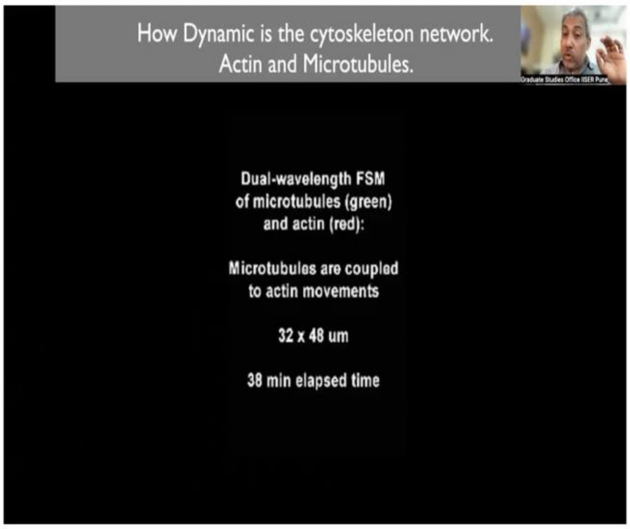

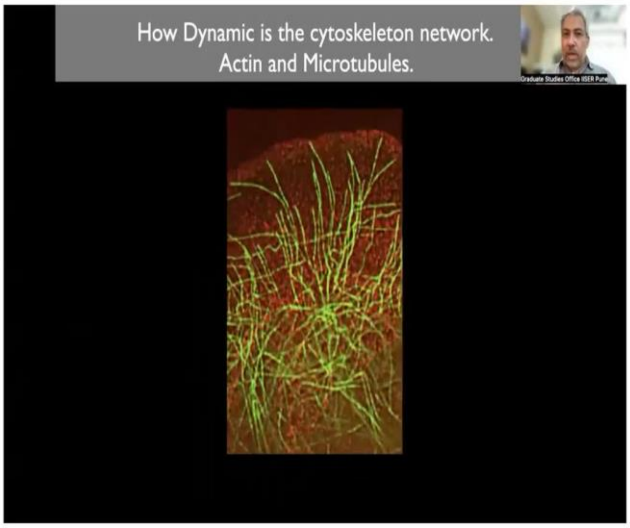

So if you look at microtubules and actin together in a cell and I am going to show you a movie of a live cell, where microtubules and actin are both labelled, and let us look at what this movie looks like. The red that you see is actin and it is labelled in a very special way for microscopy method called speckle microscopy, again something we are not discussing at this point of time.

The thing for you to know is look at how the mesh of actin looks and now look at what the microtubules look like at, microtubules are nice pointy structures that are like snakes moving around, the actin is more of a mesh and is arranged very differently. So we are going to stop here, we will pause here with this movie, because this is a good point to kind of break before we think further about what actin and microtubules do.

And as I said, this distinction in the way they are assembled distinction in their properties, and the way they are assembled are put together is both interesting and important for the cell. So think a little bit about and you into tomorrow's class, we will take this up again, as we also look at intermediate filaments and see how they are different.

Think of what the cell would have looked with just actin, think of what the cell would have looked with just microtubules, and then ask, what kind of benefit the cell that had both of them coming together may have had, that allows that to be the predominant form. We actually at this point, do not have a single cell type which is has only one kind of cytoskeleton eukaryotic cells I am talking about, which has only actin or only microtubules.

They may have existed at some point of time and this became their coming together could have created this, really spectacular cell that now had all these advantages over having either one or the other. So this is a good example of certain properties being redundant. So it is a wicket keeper batsman that is our wicketkeeper captain is how I would define this. Some roles that both these could do, which are overlapping, but they also have very unique capabilities and roles that allowed them to do something that the other cannot. So we will stop here.