Introduction to Cell Biology
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Introduction to Cytoskeleton: Part 2

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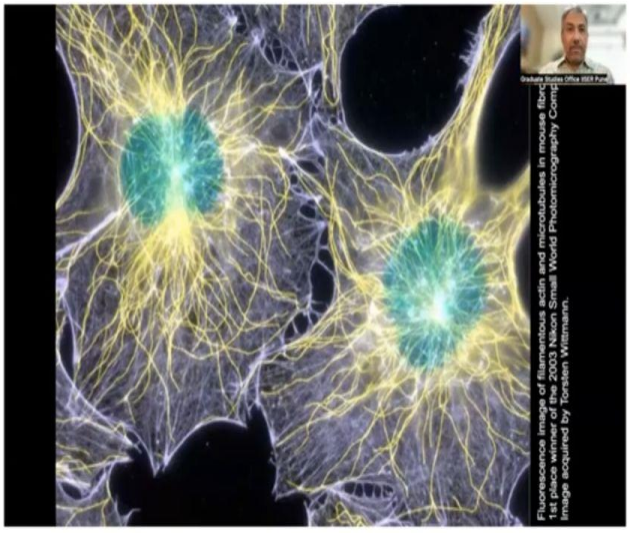

We have been discussing the cytoskeleton and then just as a refresher, we talked about the actin and microtubule network. We talked about the fact that how these two networks are similar and different. Similar in the way they are put together by sub-units, different because of the kind of sub-units that are coming together. Similar because they are both requiring energy; one requires GTP, one requires ATP. Different because of the way they are organized and this picture captures that. The fact that these are microtubules are radiating from a central point inside the cell and appear as big long strands.

These are kind of highways that are running from one end of the city to the other. And their intent is to kind of take you from point one point to the other. The microtubules along with the centrosome, also I mentioned has a tip; and a tip complex that plays a vital role in anchoring it to very specific sites inside the cell. The actin cytoskeleton, on the contrary is more branched. It has the capability of making these very well defined branches, which the microtubule network does not the actin cytoskeleton therefore plays an important role in holding up the membrane in talking to the plasma membrane.

So, there is something called a cortex that actually sits below the membrane and kind of holds it up. And any type of force generation in the cell, like pushing of the membrane, moving the cell forward, bending the membrane, will all require the actin cytoskeleton. The microtubules on the contrary, act as major trafficking or movement highways. It is not like actin cannot allow for movement to take place microtubules; because, they originate from the center of the cell and move all the way to the periphery; can be used to carry things from organelles like the Golgi that kick off at the center that are present around the center, and take things all the way to the periphery of the cell. So, so that is among the distinctions that exist.

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We spoke about the fact that this assembly happens through defined subunits that can be put together in various forms. And I gave you this example of Lego and for some of you who may not used or seen a Lego building block, they look like this; and they come in very specific defind shapes. And they can be assembled into various structures, depending upon what what you are trying to build. And and that is kind of what the cytoskeleton is also like. There are a couple of movies we'll look at and these are just refreshers on how, how these are put together, how the cytoskeleton is put together.

One is a very nice movie that is from the mechanobiology institute that shows you how actin is assembled. As I said, you do not have to remember all the players, you do not have to know what each component is. The critical thing is for you to conceptually understand how energy

requirement is there, and how the structures are assembled and disassembled; and that is the take home from the movie. So, the movie does not have a commentary; so we will watch the movie first.

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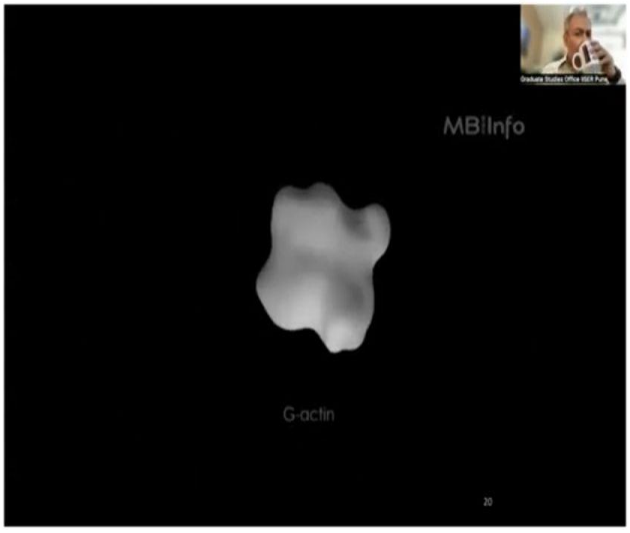

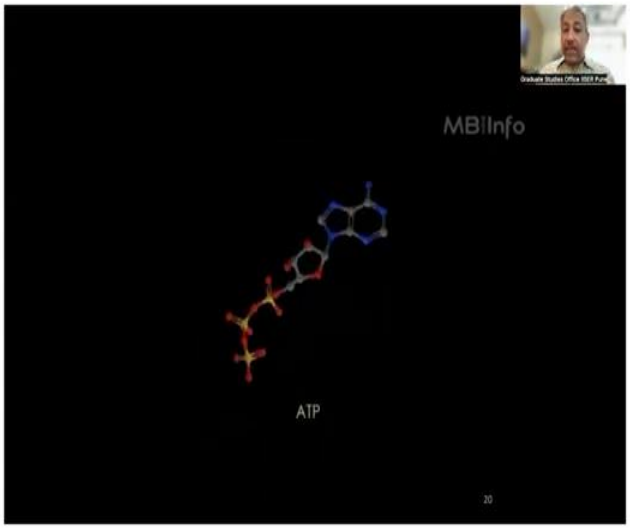

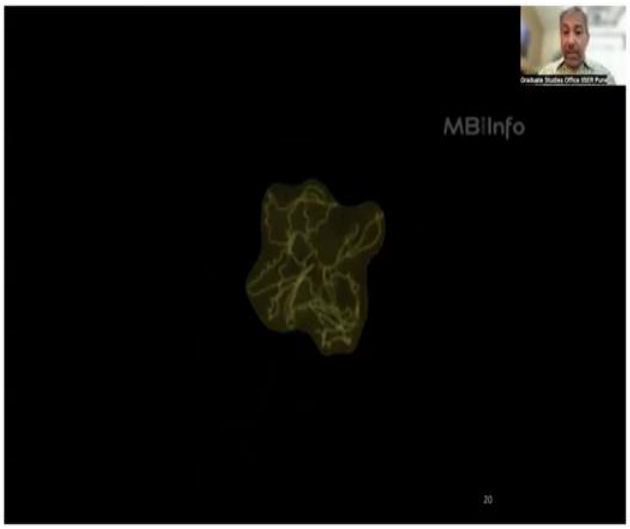
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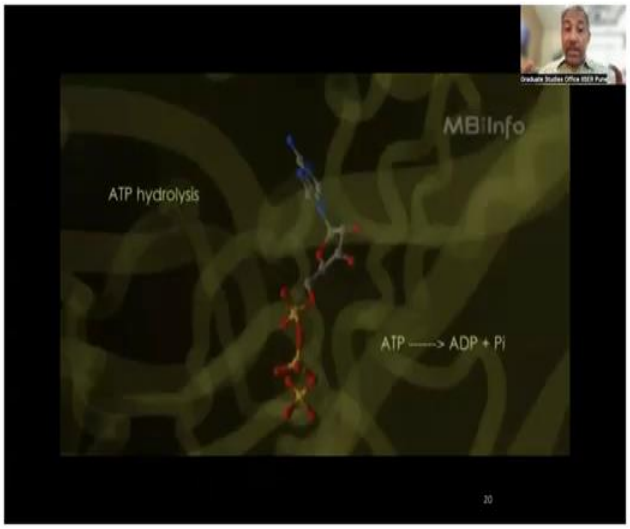
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ATP ------> ADP + Pi
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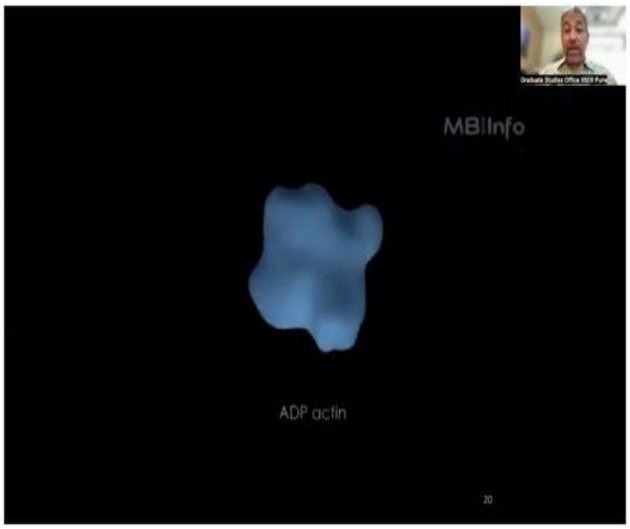
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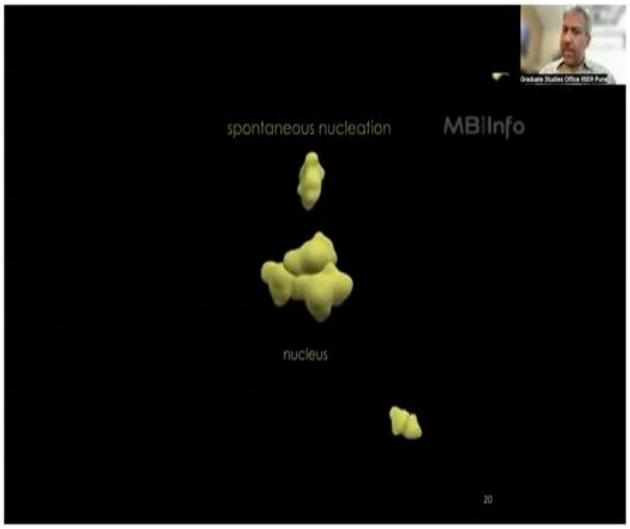
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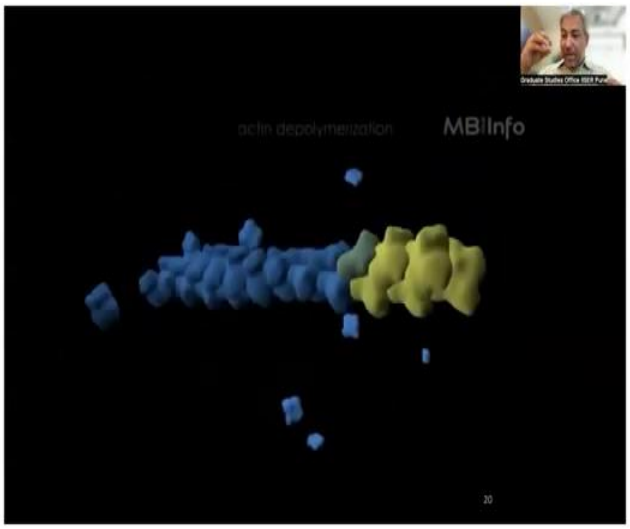
actin depolymerization
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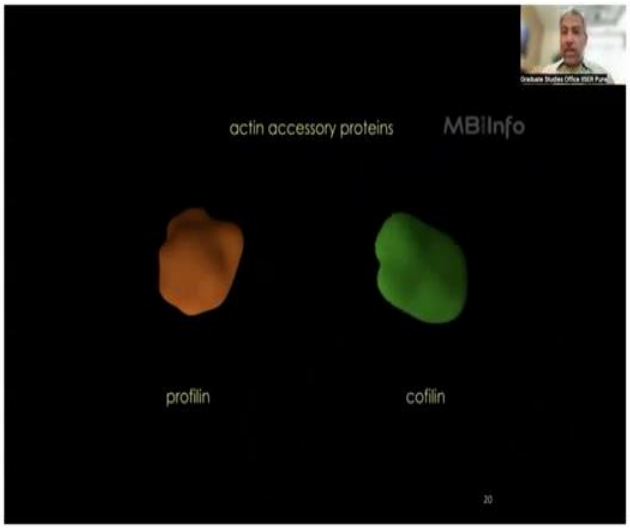
actin accessory proteins
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accessory proteins regulate
actin dynamics
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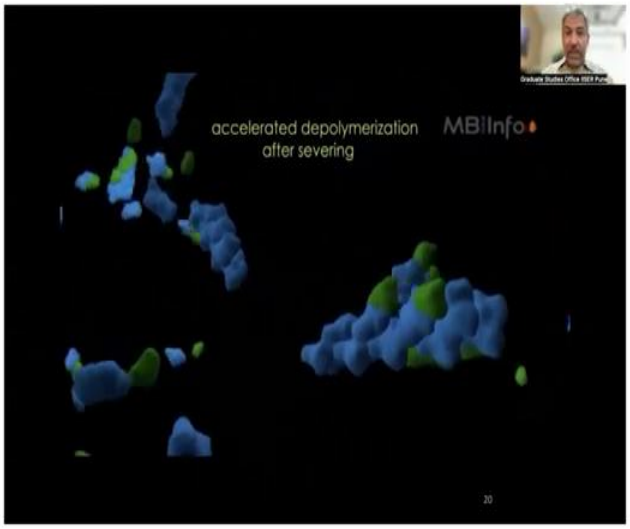
accelerated depolymerization
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That is the single actin subunit which is globular actin, which it is called G-actin; and that is the architecture of that. This is the ATP molecule that we spoke about and which can come in bind actin; and convert into it into ATP bound actin, which now then has the ability to assemble. So, then there is hydrolysis of ATP to make it ADP bound actin; and that cycle of ATP to ADP is what is vital. There is something called spontaneous nucleation for the yellow ATP bound actins, where they can come together and sit together. And when they sit together, they start building a strand and, and then there is hydrolysis. So, you can see the ATP bound actin getting converted to ADP; and then now the ADP strand starts disassembling.

And there are proteins that are required for both parts of the process. So, there is polymerization and depolymerization, both happening in the same strand. And the rate at which both happen is going to determine the length of the strand, for example. A number of proteins again, as I said, you do not have to worry about names and such. This is just to know that there are proteins that are regulating these processes; so, polymerization as well as depolymerization. Profilin for example, inhibits nucleation; that means it when the actin is bound with it, it will not do this spontaneous making of a or initiation of a strand, which will happen otherwise.

There are other proteins that similarly regulate actin as well. And profilin is one of them and will look at that as well. So, as I said, accessory proteins regulate the dynamics. So, not only is, because this is a dynamic process, you have to remember that things happen at a certain rate; and the rate at which assembly and disassembly is happening, is going to determine how stable the cytoskeleton strand is. And how long it stays the way it stays. And so there are proteins that are

mediating all aspects of this, proteins that chop off actin; so, there is an actin severing protein; proteins that regulate polymerization, the rate at which extension happens, proteins that regulate depolymerization.

And this happens also for tubulin by the way, not just actin. So, the take home from all of this for us is that along with the cytoskeleton components being structures that are assembled this way using Lego blocks; there are components that come to also regulate the rate at which the Lego blocks are being assembled. And the rate at which they are be they are being taken out.

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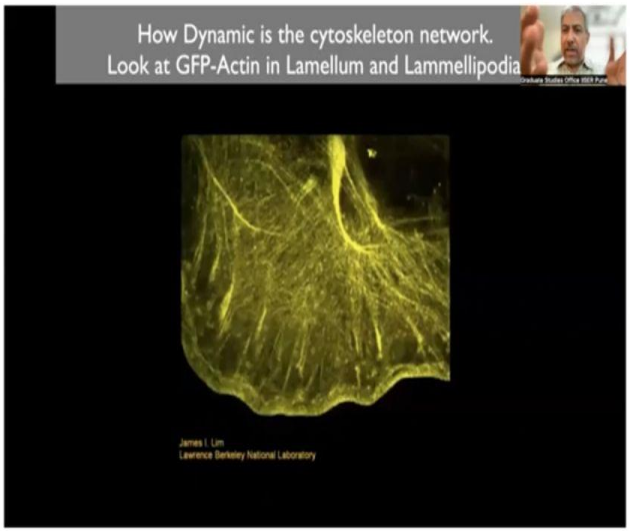

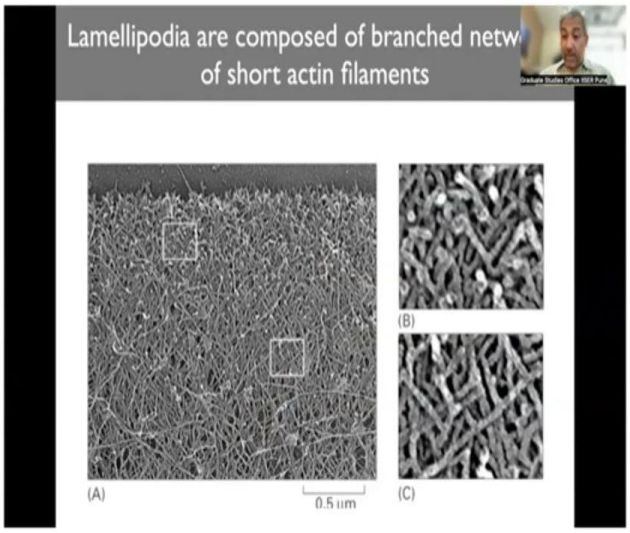

And so and that plays a vital role in determining what the final architecture looks like, how long a strand of actin or microtubule is stable, where it goes and where it is retracted? All of this is determined by this dynamics. Last time somebody asked about the importance of dynamics. That is how important dynamics here is that because everything in the cell is very dynamic. And I am going to show you a movie there the actin has been labeled by fluorescent actin. So, the actin strands are present in the cells and we put a very small amount of fluorescent actin, which when I say fluorescent will kind of emit light.

And this fluorescent actin will go and incorporate into the endogenous actin. So, you get to see what the actin that is present inside the cell is actively doing as the cell is, all it is, all the cell is doing is sitting and trying to move. And this is the front of the cell, something that is called the lamellipodium. And you can see these waves of actin as they go to the front and come back; it is literally like a wave at the ocean. And and this is a real cell and we are looking at actin inside the cell. So first, take a look at the movie and see what all you see. You can see how actin at the periphery at the edge of the cell; the content is very high.

You can see how it is it is being disassembled and is being carried back into the cell. And this actin at the front looks like that mesh I told you about; here, this mesh. And it is, there is a membrane that is in the front; and it is kind of actively pushing on the membrane.

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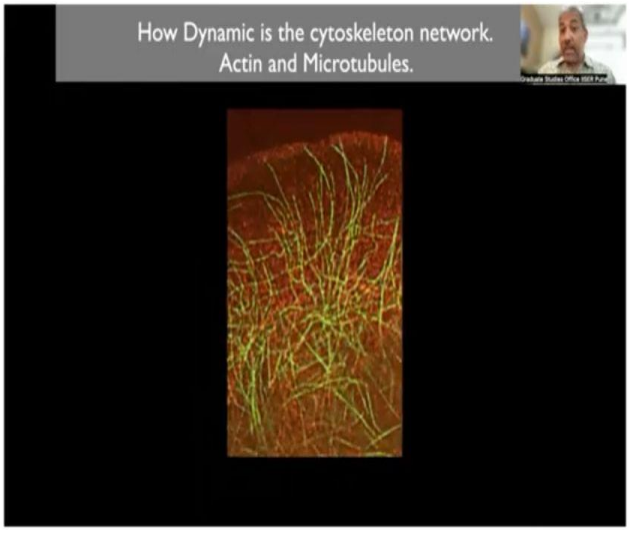

And that is what we saw here too, the front of the cell. The actin that you see in red is kind of actively pushing. Can you see that the front has moved forward in the time that the movie has been playing and microtubules are poking through. These are the highways that are coming right up to the front of the cell. They are carrying things that are required for the front of the cell to do what it is doing as well. So, this is the kind of combination of actin and microtubules that works together.

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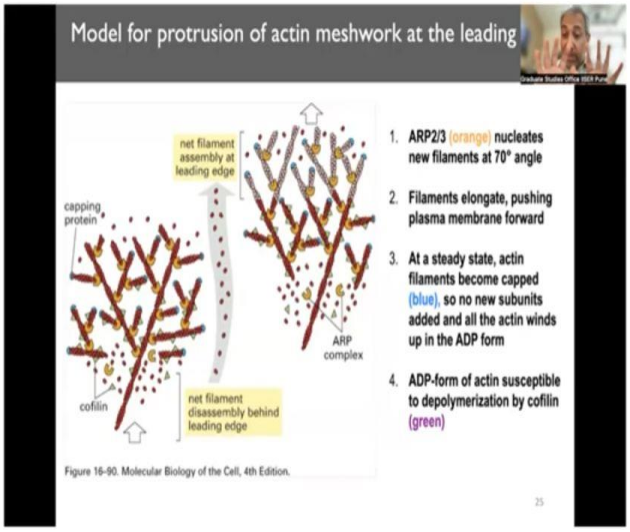

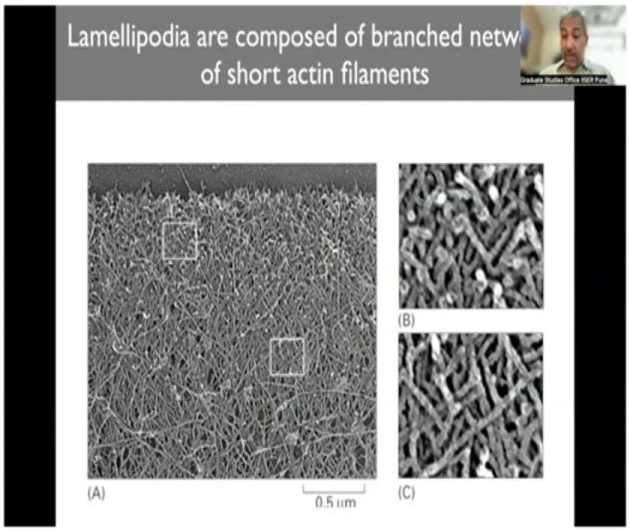

The branching of the microtubules that we just saw here of the actin that we saw here, is made by this protein called the Arp complex Arp 2/3 complex. And it binds actin and creates a starting

point for a new strand at a very specific 90 degree angle; so, all the branching that you see in actin will happen at a very specific angle. And the reason that it happens as a specific angle is there is a protein here, that sits and binds to this actin that will allow for a new strand to form only at a very specific angle. And this branching is a big distinction between actin and microtubules.

And this is something that only now we are starting to see one or two cases where there is a, there is some thought to suggest that maybe just maybe microtubules under certain circumstances may also branch. But, for the most part, actin branches extensively microtubules do not and actin, because of the branching is able to generate force in a way. It is like spreading your fingers and being able to do this versus using a single strand of microtubule and trying to push on anything. So, this is able to generate force in a way that this will not, and that is one major distinction.

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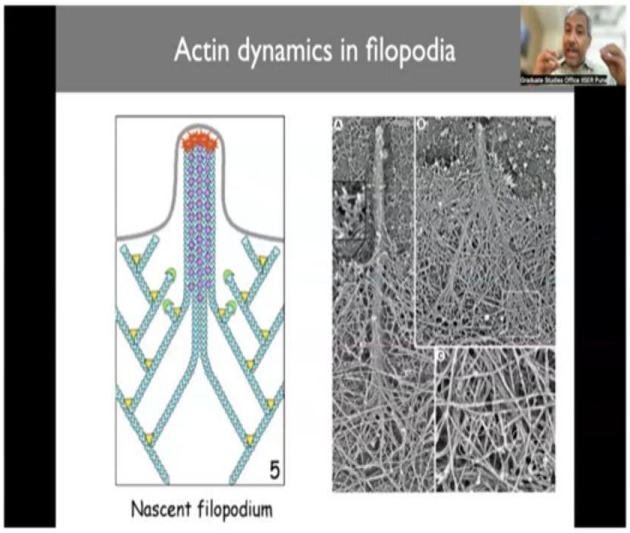

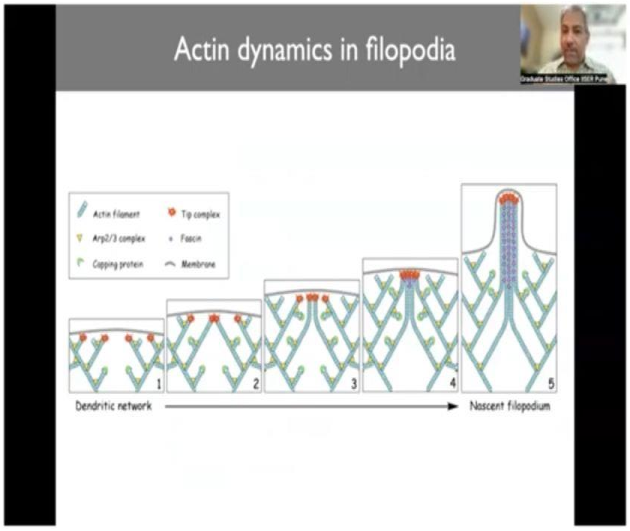

I spoke to you about the fact that actin can be assembled, just as microtubules can be into very different looking structures. And one of those structures is this filopodia, which is essentially the gray line that you see on the outside is the membrane. So, the membrane is covering the filopodia. And the filopodia is essentially like a like a bar of actin, that kind of pushes on the membrane and creates a very nice structure like this. So, you can see how the actin comes together to create that kind of filopodia like structure. And all of this is mediated by the proteins that we saw that regulate the assembly and disassembly of microtubules, so of actin.

So, these actin and microtubules can with the help of these accessory proteins be made to do very different things. And that is an example of how this structure is assembled. And you can see again, very distinct proteins a tip complex, fascin all coming together to meet that filopodia Like structure.

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It is also interesting that these actin structures are exploited by bacteria; and this is a good example of a bacterium called listeria. What you can see is listeria that has infected a mammalian cell; and it uses the actin that is present in the mammalian cell to make nice tails of actin. So, the bacteria has a mechanism has proteins that will allow you to take the actin that is present in the cell in which it is sitting. And now build these things which allow the bacteria to move. So, the bacteria by itself does not have this tail, till it enters the cell; and now it has the ability to move around.

So, if you see these black dots, the black dots are the bacteria; and the fuzzy tail that you see is the tail of actin that now allows the bacteria to move. So, there are very interesting examples of how the coming together the fact that this actin is present in eukaryotic cells. The prokaryotic cells that are entering the cell have have also found a way to exploit and use the cytoskeleton components.

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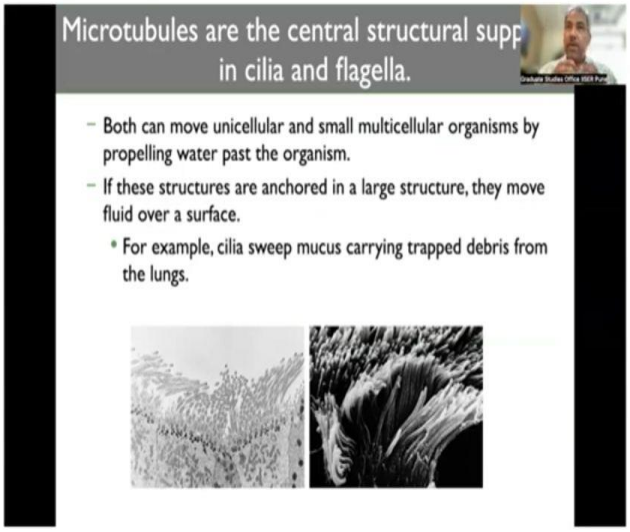

Another example of how the same cytoskeleton component can be repurposed or used very differently, is the example of microtubules being present in cilia and flagella, which are very thick structures that move around, and kind of allow cells in our body. For example, the esophageal lining cells; there are many places where microvilli like structure or cilia and flagella like structures are present.

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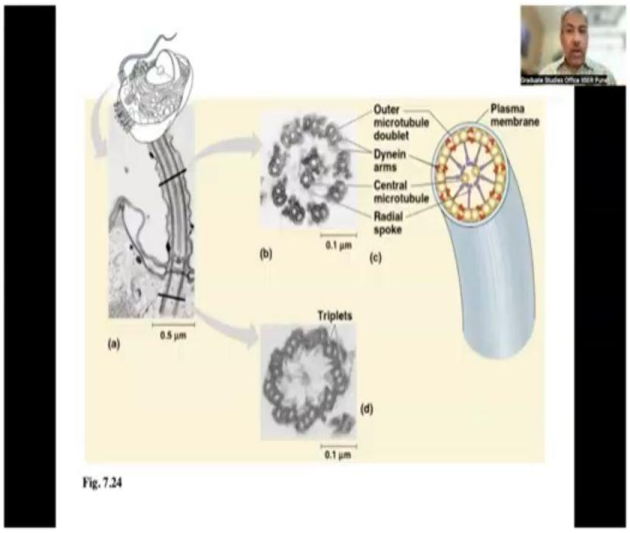

And so, this is largely put together by the same microtubules. But, in a cell you saw the microtubules starting from the center and radiating outwards; here they are assembled into a ring

like this. So, this is a pair of two microtubules strands, 1, 2, 3, 4, 5, 6, 7, 8, 9 strands; and two in the center. And they are all held together by motor proteins that bind to one strand of actin and bind to the other and vice versa. So, these two strands if my hands are the two strands, the fingers are the two motor proteins, and and they are linked to this way. And these motor proteins, all they do is because they have the ability to walk on these microtubules strands, they walk. So, they do this, this one does this.

And when they both do this in a very specific manner, the these strands are getting moved accordingly. And as a result, the entire structure moves, and you are able to create this movement of cilia and flagella that we all know of. The reason to highlight this is, it is interesting that the same cytoskeletal component is used in very different ways. Microtubules also play an important role when the cell divides. And when we look at cell division, we will look at this briefly to kind of pull the chromosomes apart. The chromosomes were arranged around the center of the cell, and then will get pulled towards the two corners.

And the two, the two corners are essentially the centrosome. So, the centrosome that is in the center of the cell will divide, make two centrosomes; and now you will have an array of microtubules from both of these. They are essentially doing what they do in the normal cell. But, now the tip of the microtubule instead of going and attaching to the plasma membrane and the places, attaches to the chromosome at a site or a place in the center of the chromosome. And that allows the two chromosomes to be pulled apart. And the way the pulling apart happens is because this is microtubule; and this microtubules have the ability to polymerize and depolymerize. You know when they depolymerize, they will shrink.

So, they just use the mechanism that they already have. That is they can grow or they can shrink and they decide to shrink. And when they shrink, they apply force because they start pulling these apart; and that is how the chromosomes gets separated. So, the microtubules that act as a highway in these cells that are normally growing, and the cell has to divide now act as a way to kind of pull apart chromosomes as well. And that is really remarkable. It is, it is essentially what we talked about last time, one player playing multiple roles and all-rounder; somebody who can be a batsman, if required a bowler, if required a wicket keeper if required.

And and that kind of diversity in function these cytoskeleton components have. So, depending upon where they are doing, what they are doing; their role and their architecture could change.

And this may have been a very strong reason for the cytoskeleton components to have to have survived through evolution and be part of cells; because they have this ability to repurpose to go from doing this to that to this whenever required.

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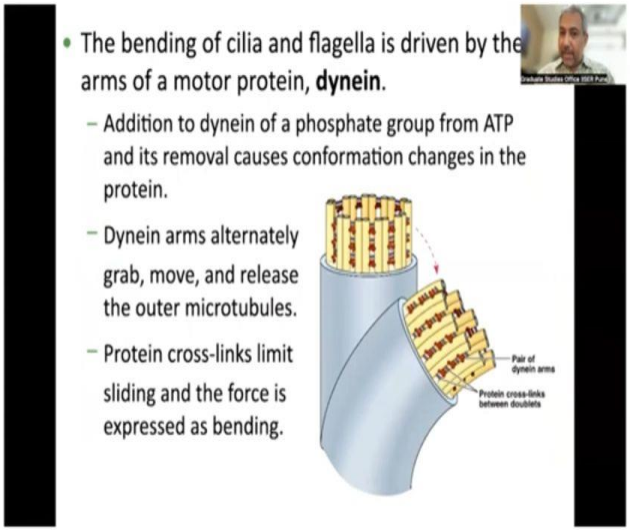

And and this is again, the example of the strands moving around with actin; and I just showed you what what that would look like earlier. Again, this is an ATP dependent process. And so we have microtubules actin, and we have the intermediate filaments that we wanted to quickly look at.

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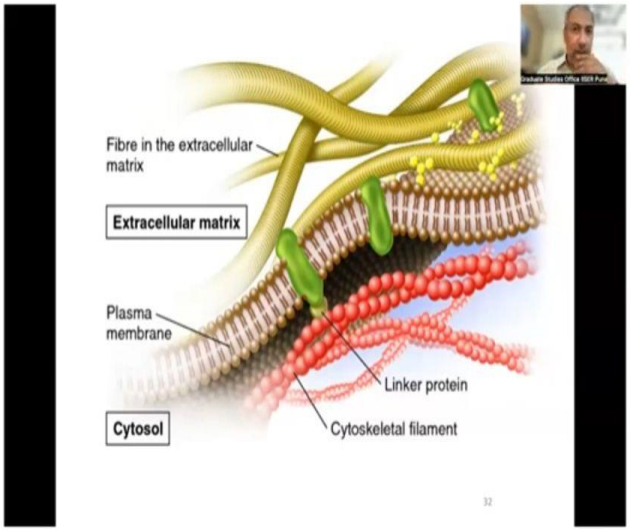

The other interesting point that I wanted to mention is that because we talked about membrane and the cytoskeleton being present underneath. Remember on the outside of the cell, there is something called the extracellular matrix; and this is again a mesh. So, that mesh is made up of proteins that are secreted by cells; we are not talking about the extracellular matrix in these set of lectures, but at a later point you will run into them. And so when you imagine the membrane and you imagine the mesh of actin that is right underneath the membrane, you also imagine a mesh that is on the outside.

So, there is a mesh on the outside, there is a mesh on the inside, and a membrane that is in between. And that is generally the architecture with which cells are sitting. There are places where cells are sitting next to other cells and the membranes are actually attached to each other. And that happens, in which case there will not be an extracellular matrix. But, for most other places, you have the matrix mesh on the outside, you have the membrane, and you have the actin mesh on the inside. And that is the image of the cell that I want you to carry with you.

We come to the microfilaments at the end, which are again uniquely different from actin and microtubules. And we talked about actin microtubules. You know, how they are similar, how they are different.

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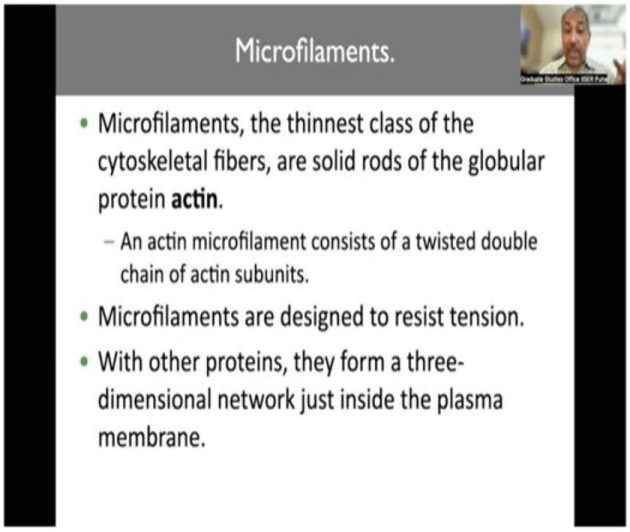

Let us look at the microfilaments and see how they are similar or different. So, first thing they are the thinnest class of cytoskeletal fibers fibers and are solid rods of globular protein actin. So, the subunit that is used to make microfilaments is effectively actin; but, it is assembled and put together in a very different way. Again, this idea of the same player being somebody else; and this might be one more reason why actin is still around. Because, not only can it do all those branches and other things, it also has the capability to form these very robust microfilament structures; they are designed to resist tension.

So, these are among the, those structures in the cellular cytoskeleton, that can take the most amount of pressure. So, they do not buckle very easily, they do not break or bend very easily. And because of that they provide a certain stability to the cell in places, where that kind of stability is very important. So, actin, microtubules very dynamic, and the dynamicity is very vital to their function; but not everything needs to be dynamic in a cell. And there are places where the cell wanted a cytoskeleton that is not dynamic. And you remarkably the same actin now assembles into a structure that is very different.

So, with other proteins, they form a three dimensional network just inside the plasma membrane as well.

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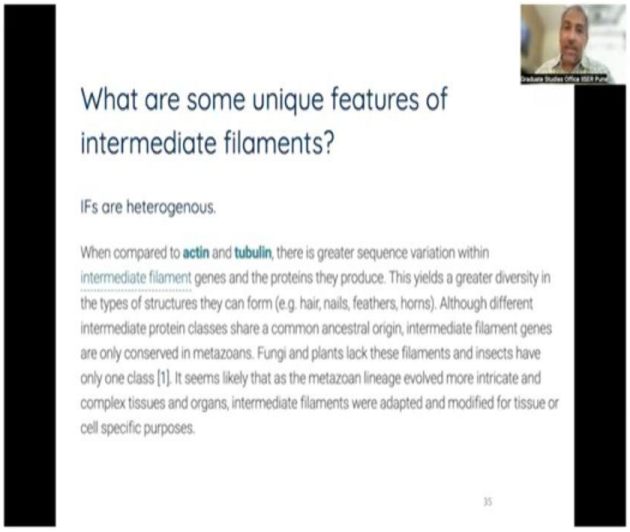

So among the unique features that when compared to actin and tubulin, there is a great sequence variation within the intermediate filament genes, and the proteins that they produce. This yields a great diversity in the type of structures they form; so they could form things like hair, nails, feathers, horns are all examples of these intermediate filament protein. Keratin is therefore a good example of an intermediate filament protein. Fungi and plants lack these filaments and insects have only one class. It seems like they are the that as the metazoan lineage evolved more intricate and complex tissues and organs evolved intermediate filaments were adapt and adapted, and modified for tissue or cell specific purposes.

So, when you are trying to build bigger and complex structures, this stability became more and more important. So to begin with, you may have had eukaryotic cells that live by themselves. And as they started making complex structures, you now have the ability; you needed these kinds of intermediate filaments.

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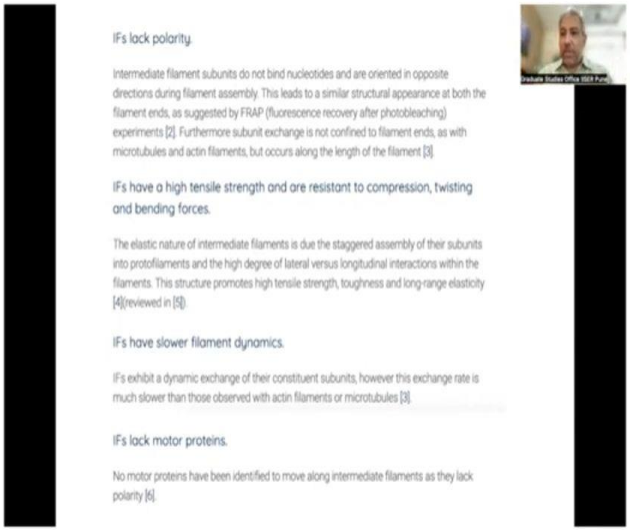

Among the properties of intermediate filaments that really sets them apart is one; these intermediate filaments lack polarity. So, unlike actin and microtubules that are very clearly defined as plus and minus ends these do not carry that kind of polarity. They do not have polymerization at one end, depolymerization at the other end. They have high tensile strength and are resistant to compression, twisting and bending forces. So, so they really have this ability to withstand pressure in a way that conventional cytoskeletal proteins do not; they have slower filament dynamics.

And as I said, unlike actin and microtubules, intermediate filaments are much more stable. Their intent is not to form and deform very quickly; their intent is to form, and stay formed for long periods of time; and so their filament dynamics are very different. They also interestingly lack motor proteins. And this is another important aspect of how these structures have evolved. So, you have actin, you have microtubules, you know all the things that they are capable of doing. What kind of dynamicity and the fact that they can have motor proteins that can move up and down, bind and bring these two structures together.

All of that happens there, intermediate filament is all about stability; I form, I sit, and I hold my place. And so, it is not something that if it is not dynamic, chances are motor proteins that go up and down very quickly. Do rapid changes, does not make sense. So, they formed intermediate filaments were formed with without the presence of any specific motor proteins.

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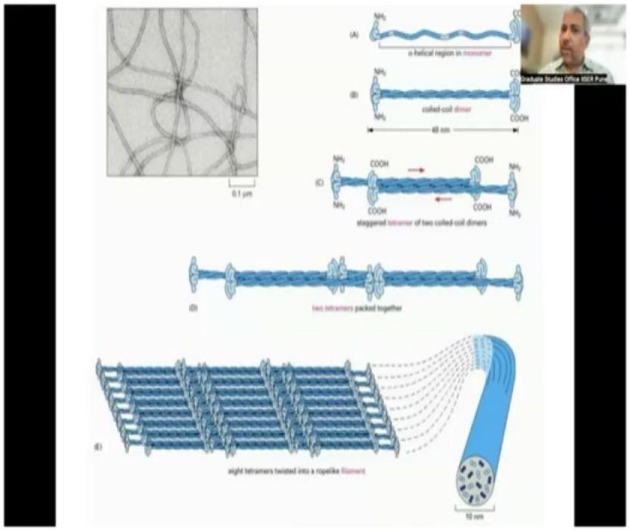

So see, this is an example of how they can be assembled into coiled-coil structures, that are the, my favorite example is of these big twines that are made out of choir. And these big threads, which are essentially individual coils that are kind of assembled into to create a big strength; and that is what the intermediate, what the intermediate filaments look like.

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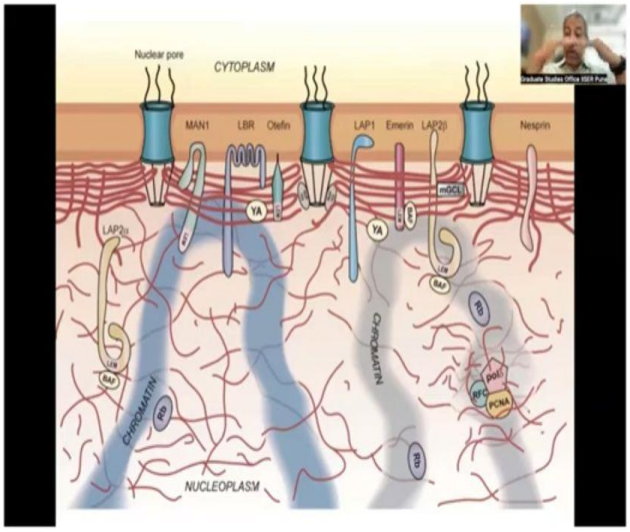

And there are many of them that are present in cells; and a lot of them, for example are present in the nucleus. This is at the at the nuclear membrane, and a bunch of intermediate filaments like lamins, for example, are present lining the nuclear membrane. So, they are just as actin is present

below the plasma membrane; these are present below the nuclear membrane in cells. There is one slide that I am missing, which is a listing; I will bring that up later. I will try and share that with you guys; because we talked about the evolution of the cytoskeleton. I normally do not share the slides here.

But, I am just going to quickly show you something here, which tells you how these have evolved.

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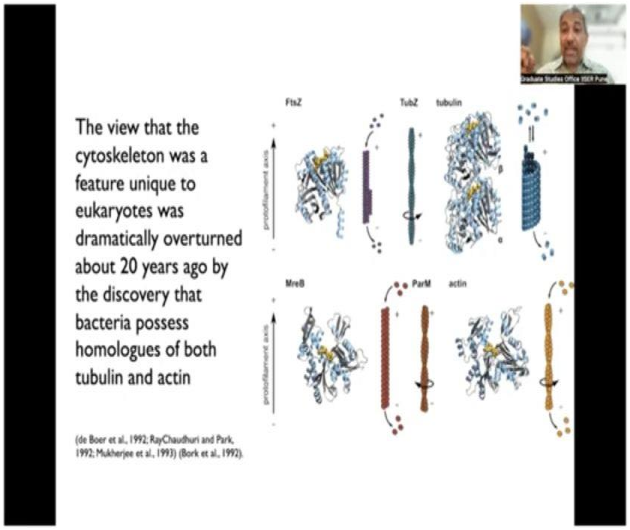

And you remember I mentioned how prokaryotes have something that is similar to tubulin. This is that molecule called FtsZ, which is what Gayatri's lab studies here. And it is it is kind of an ancestor of tubulin. Now, the strand that it makes has a bit of has polarity, but is like actin is a strand that is two strands that are wrapped around each other. Tubulin that you know of in the mammalian cells; it is like a hollow tube. So, there is a version of tubulin that existed in prokaryotes, which looks very different, has some similarities structurally to the tubulin that we see here.

And you can see the structure of the two proteins, and you can see how similar they are. But, the way they have assembled, how they are put together very different. Same for a protein called MreB, which is thought to be the kind of original actin. And MreB is again similar in structure to actin; it is again assembled as a strand that is two strands that are wrapped around each other. And that is very similar to the actin that we know of today. And it is interesting that these

molecules existed in some form then, and then somehow adapted evolved to create the complexity that exists in the tubulin strands that we know of today.

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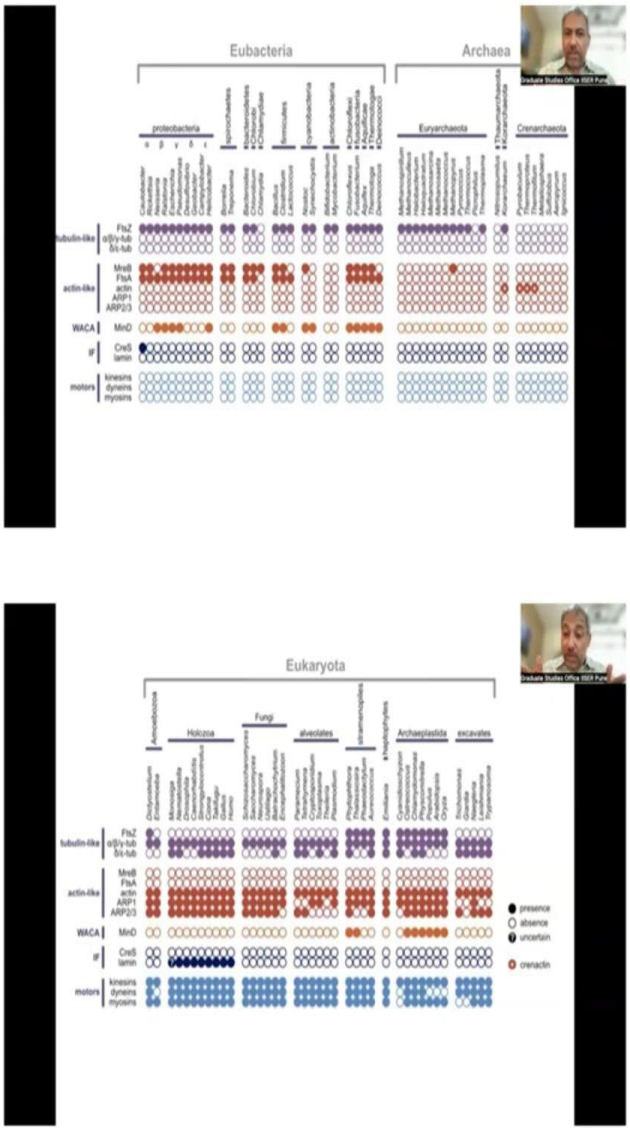

This is a very beautiful table and do not worry about all the complexity here. All I want you to see is that wherever the dots are red, it says that that particular molecule existed. And look at where the MreB, and FtsZ, sA-sB, FtsZ are present. And you can see all the early Eubacteria and to some extent, the Archaea have FtsZ. There are some Archaea which have an MreB version; they were all present in the early the early stages. The intermediate filaments like lamins, for

example completely missing in those early organisms; actin Arp, Arp2/3 all missing, tubulin all missing, motor proteins completely missing.

And then there is a point where all of this kicks in; you have now alpha and beta tubulin, your actin, you have the Arp2/3 complex. When actin is present in the form of actin in the early eukaryotes, the branching is already in place. It somehow existed with that branching as part of the of its architecture. The motor proteins are in, so you can see the bottom blues kinesins, dyneins, myosins, which will come to in the next set of lectures. All of them are now present in these eukaryotes; and this is happened at least with the actin and microtubules. You can see that there were versions of this with motor proteins, for example, boom, it is there.

Now, the it is possible that there is a version of motor proteins that is present in those prokaryotes that we know, do not know of. I mean till 20 years ago, we did not think that the there was an FtsZ in prokaryotes. So, it is possible that this is just waiting to be discovered; and this table will change. And we will start seeing things here as well, as we understand this better.

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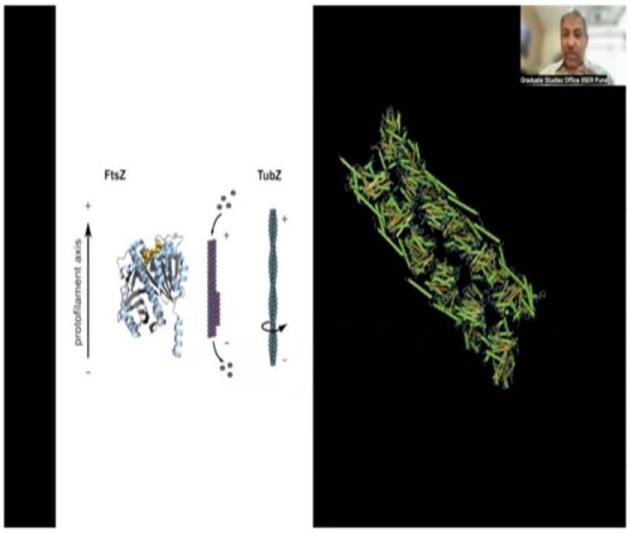

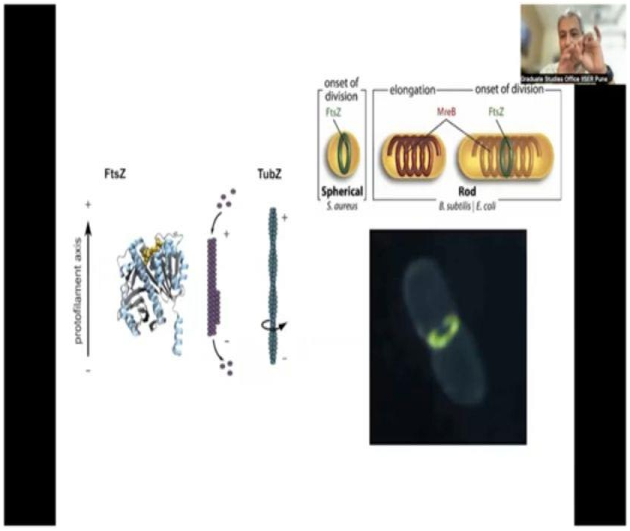

But, this is just to give you a flavor of what this would have looked like in prokaryotes. Again, the architecture of this tubulin strand, for example; and it is assembled in very distinct structures. So, it is not the same architecture that you know of in mammalian cells, but in in in kind of forced generation and making a ring to kind of divide bacteria. The FtsZ ring is thought to play a very important role. So I am going to stop here.