Physics of Biological Systems
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Lecture - 44
Structure and Treadmilling of actins and microtubules

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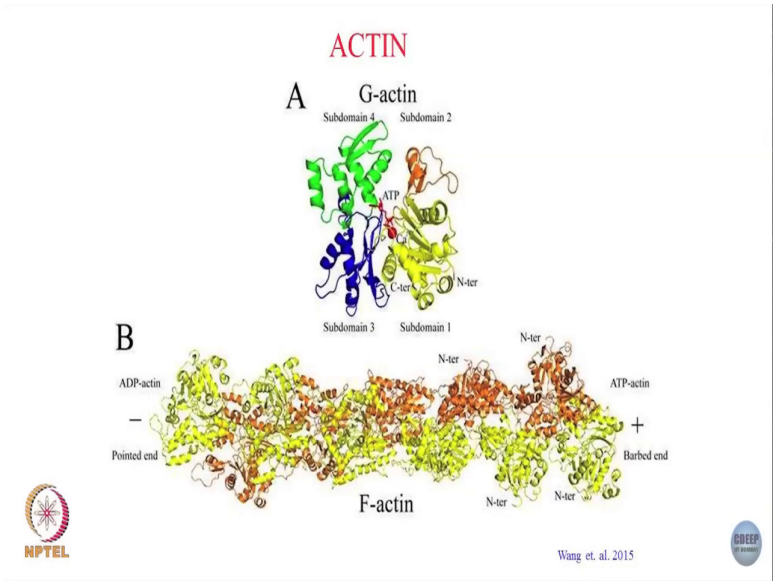

So, what we look at today is sort of how to write down dynamics of cytoskeletal polymerization.

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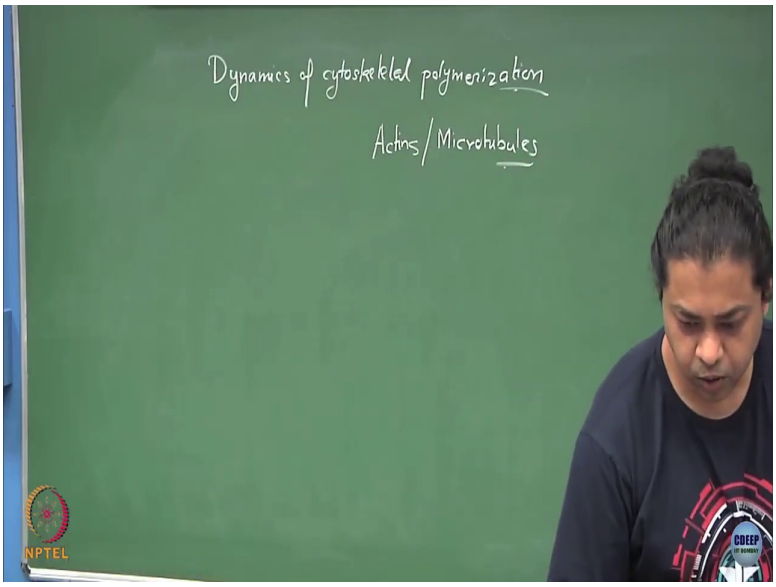

So, dynamics right see yesterday or rather last class I was discussing a little bit about the cytoskeleton, actins and microtubules. So, we will sort of look at different levels of models actins or microtubules and we look at models of different levels of complexity to sort of see what we can understand about polymerization and de polymerization of such (Refer Time: 01:09).

So, before I stop I thought, I will just give a brief introduction once more. So, we have all seen actins and microtubules, but maybe in a just a little bit of more detail what these polymers are ok. So, here is actin. So, this over here is what is called G actin or globular actin, the globular monomeric form of actin. This is a single monomer of this G actin proteins its, a protein consisting of these 4 sub domains 1, 2, 3 and 4 and if you see over here in the middle there is an ATP molecule sitting over there right.

So, this is the molecule that can hydrolyze. So, it can hydrolyze and form an ADP binding over there and that releases some energy that goes into stabilizing the structure of this filament. So, these monomers assemble together to form this actin filament, this is called F actin or filamentous actin. So, G actin is the monomeric form F actin is the filament is form. And, the filament is form consists of 2 sort of filaments wrapping coiling around each other in a helical form to give what is called this actin polymer or this actin filament.

Because, these the way this subunits assemble is such that there is a structural asymmetry and one end of these actins will look slightly different from the other end if you were to look at it in an electron microscope for example, and that leads to a sort of identification of two different ends. One end is called the plus end, another end is called the minus end and often in literature the plus end is called the barbed end and the pointed the minus end is called the pointed end.

So, when these actins are in solution the globular actins the monomeric forms of actin are in solution they generally. So, here is my actin monomer, here is my G actin G actin when they are in solution it comes associated with an ATP molecule then when it polymerizes. So, when many of these G actins polymerizes to form your actin polymer as it were these ATPs will hydrolyze to form ADPs.

So, the more you go towards the indeed let us say this is the plus end of the microtubule of the actin over here is the minus end on this side. So, the more you go away from the plus end the more likely are your that the ATP has hydrolyzed to become ADP. So, these are the 2 states that we were talking about the previous class. What does this do? This ATP to ADP conversion it introduces a structural a conformational change in the protein state.

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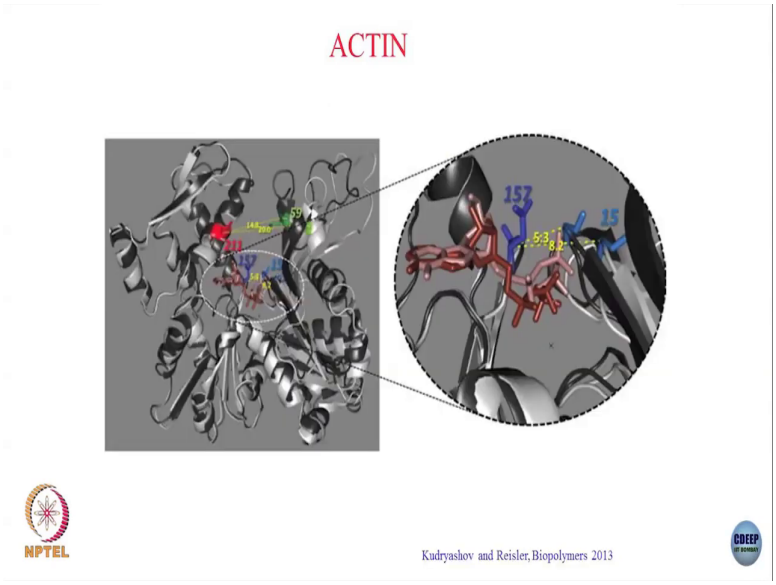

It introduces a conformational change in the protein state. I do not know if you can see. So, over here in the middle is this ATP binding domain of this globular actin, the black versus the white shaded paths show the change in the positions of the different amino acids of this G actin; when ATP is bound versus when ADP is bound.

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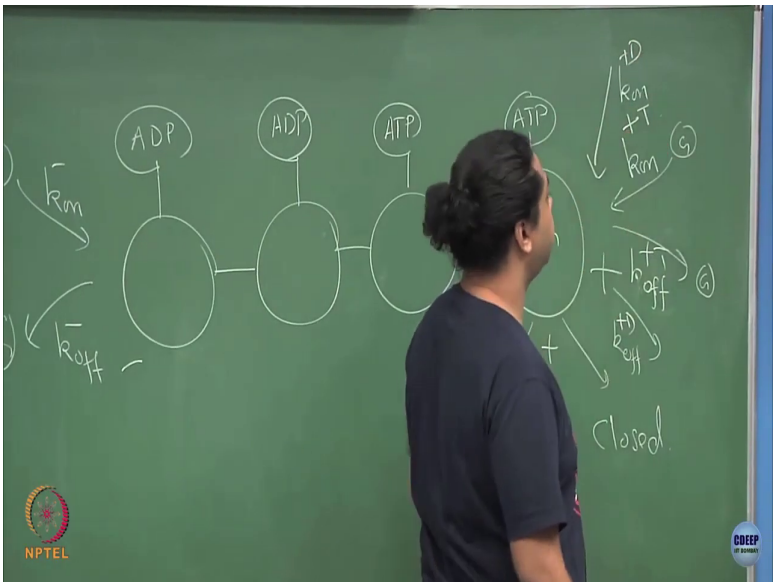

So, this G actin one says it can exist in 2 states open or closed; open or closed these are 2 conformational states depending on whether ATP is bound or ADP is bound. So, if you go back and think in terms of these multi state models that we did for example, this MWC model for hemoglobin and so on. Those often existed in multiple states. So, this is another example of a protein that exists in 2 such states open or closed and these 2 states have slightly different structures that leads to different stabilities ok.

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Similarly, if you look at this G actin in solution versus a G actin on the filament again you will see that there are certain structural differences. I think, I may be wrong, but I think the yellow one is the positions of the amino acids in the globular form and the white 1 is the position of the amino acids, when this actin is polymerized to form the F actin. So, again this polymerization leads to certain structural changes and that again contributes to the stability of this fall of this filamentous assembly

So, you have this G actin which can exist in 2 states ATP or ADP consistent with 2 sort of conformational states of this actin open or closed, it then assembles to form a filament. This filament is the structural polarity with a plus end and a minus end.

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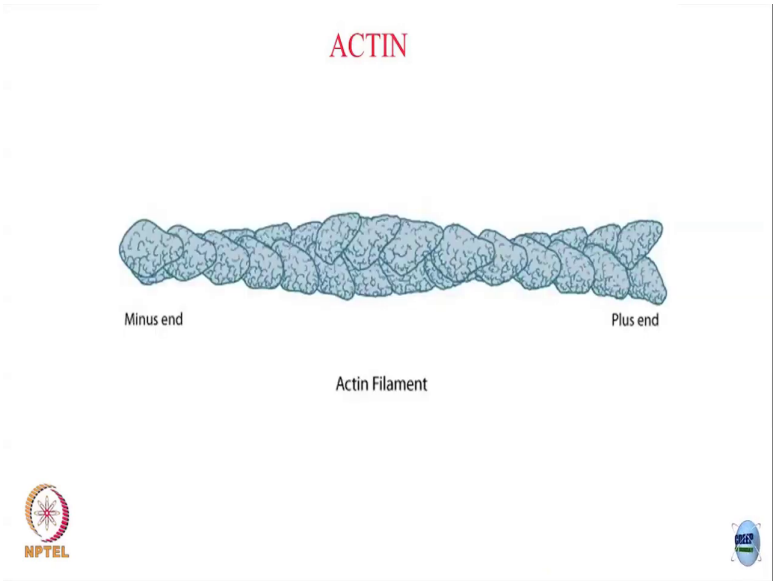

And thats how this is sort of a cartoon of this actin filament, these are two sort of strands intertwined with each other giving rise to a helical pattern. The two's ends look structurally different, and this one end is called the plus the other end is called the minus. As far as actin goes you can have monomers being added, you can have monomers being added or taken out. So, these are G actins from both ends, both the plus end and the minus end.

So, you can have monomers being added both to the plus end or monomers being taken off both from the plus end and the minus end is that the rates of these processes are different. Let us say it comes, it attaches with some rate k on plus and dissociates with some rate k on minus, where sorry k on k off plus let us say. And, in the minus end it is on and off rates which you call k on minus and k off minus ok.

So, generically these are different and for actins generally the growth is faster at the plus end. So, on an average you will have things that are being added in the plus end and on an average will of things that are being removed from the minus end ok. So, that is this phenomenon of tread milling.

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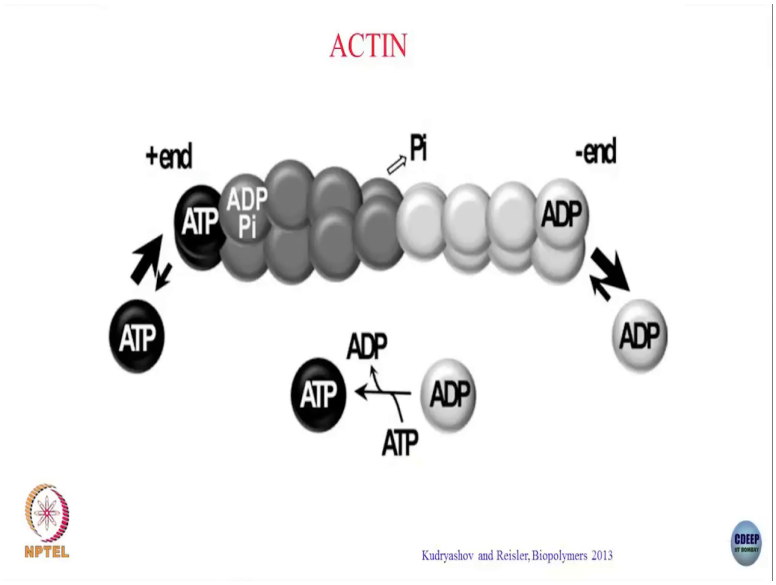

So, here is sort of the picture that these globular actins. So, this is my plus end that is my minus end, I have now forgotten about the structure of just everything is shown as balls. This globular actin comes with ATP this binds, the ATP then hydrolyzes to form ADP and this phosphate is released. So, in the interior of this filament most of these actins will have an ADP bound to them.

So, when stuff dissociates from the minus end what you are getting is this globular actin with an ADP bound to it. And, then in solution this ADP will again convert this ADP actin will

again convert to ATP actin and it will again come and bind it ok. So, it is a cyclic process right.

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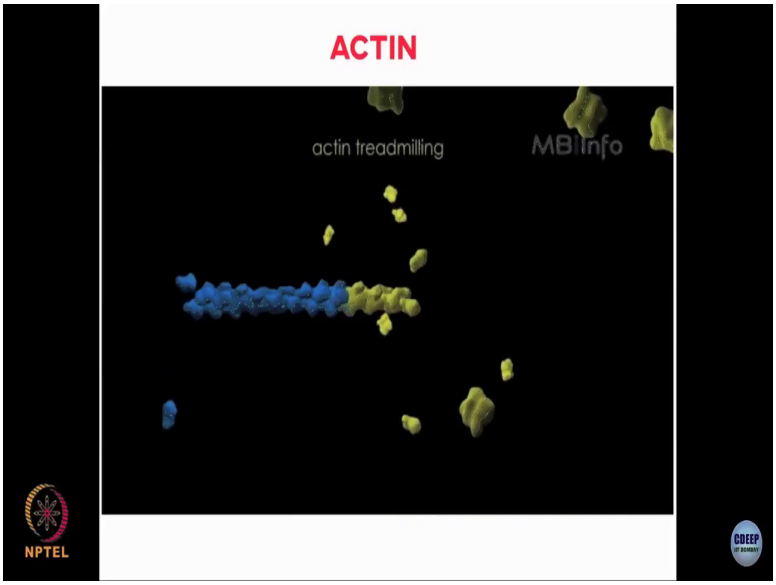

And, that will give rise to this cartoon that we saw the other day, this actin tread milling where things on an average will add on at the plus end. They will dissociate from the minus end and you get this phenomenon called treadmill.

So, it is not simply it is a complicated process, it is not simply monomers being added and taken off. There are these various internal states that you need to take care of if you were to do a full modeling. The picture is sort of similar for microtubules.

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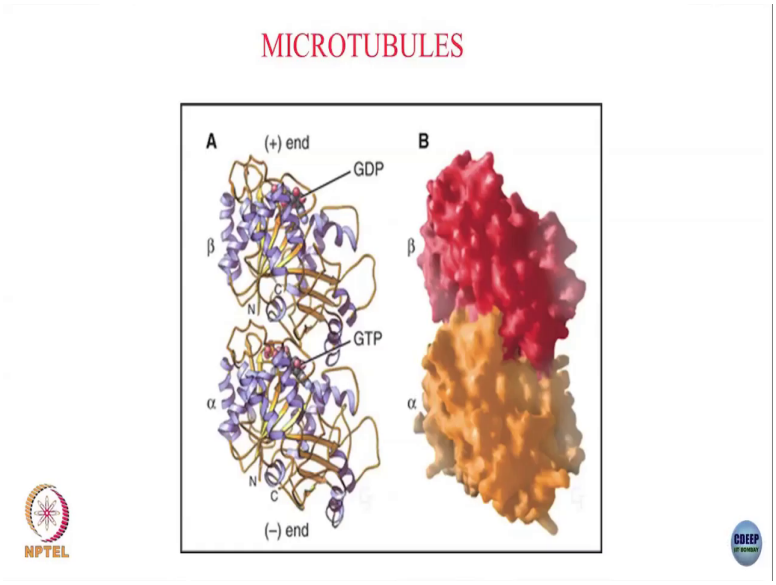

So, the situation is somewhat similar for microtubules except here the constituent monomer that makes up this microtubule polymer is actually a dimer. So, it consists of 2 proteins alpha tubulin and beta tubulin that form a dimer like this and then this dimer is the basic building block that forms your microtubules, again here both of these alpha and beta subunits. The alpha tubulin and the beta tubulin has a domain that binds GTP in this case instead of ATP both over here and here.

And, this GTP at this beta sub even that can again hydrolyze to form GDP and release energy exactly like ATP hydrolyzes and releases energy for actin.

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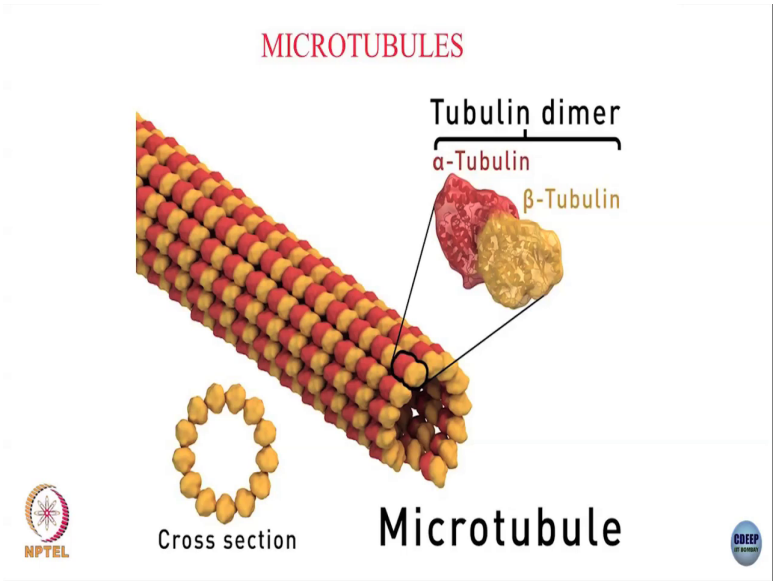

So, this dimer is then the basic building block of this microtubule. The micro tube so, it forms filaments like this which are called protofilaments and then 13 of these protofilaments from this hollow tube which is my microtubule ok. So, the structure is little more complicated than actin its not just 2 filaments winding in the helix, it is like this hollow tube made up of these alpha, beta tubulin heterodyne.

And, again there is a structural asymmetry because, in one end you will always see a beta tubulin exposed, at the other end you will always see an alpha tublin expose. And, that is why we again say that for microtubules is a structurally polar object, it again has a plus end and it has a minus end.

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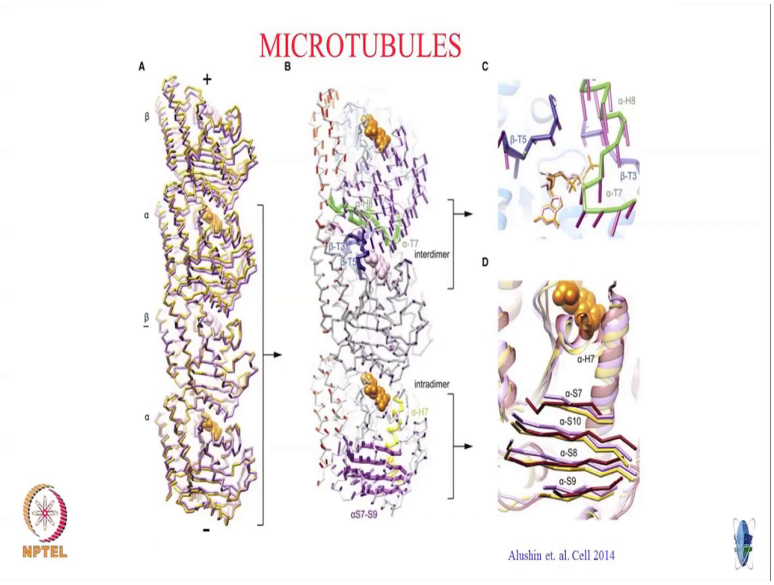

And again if you look at studies when you have this hydrolyzed hydrolysis of GTP to GDP, there is consequently a change in structure, this picture has not come very well. But if you see so, this is unhydrolyzed sort of alpha, beta; alpha, beta chain. This is a hydrolyzed alpha, beta chain. This one you see is slightly more compressed. So, there are changes in the conformation again of this tubulin heterodimer as a result of this hydrolysis ok.

So, again there are distinct conformational states that occur as a result of this GTP to GDP hydrolysis. Yes.

Student: (Refer Time: 11:01).

2. So, in a given heterodimer; in a given heterodimer the GTP on the beta subunit that hydrolyzes to give GDP. This one stays as GDP, why? I have no clue why. But it is just that

the as far as I remember I think, it is the beta 1 that hydrolyzes to GDP. So, the same sort of unit of currency that you can gain on polymerization, it can so, in principle you could say that I have a k on rate which has ATP for ATP bound subunits.

You could also say that, I have a k on plus d for GDP bound G actin and similarly to dissociation rates as well. It is just that this rate is known experimentally to be much smaller than this rate ok. So, for most modeling purposes we do not really take this into account and we say that only the ATP or the GTP form attaches and only the ADP or the GDP form detaches from the other, but in principle you should consider all 4 rates right.

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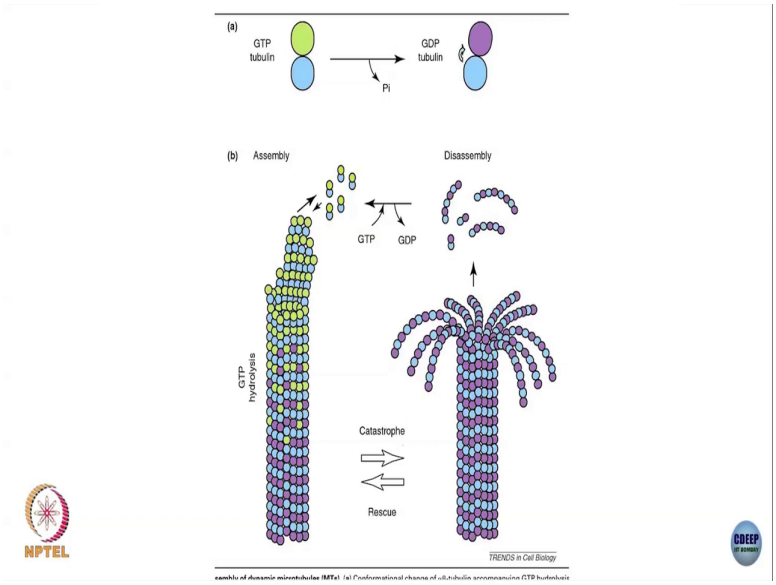

So, you can also see that this sort of structural, this conformational of the change is present which is there in the monomers for this alpha, beta tubulin; that is also sort of manifested in this macroscopic structure of the microtubule polymer itself. During hydrolysis when things

are being added, things add more or less as a straight this microtubule looks like sort of a straight cylinder, during disassembly the different protofilaments sort of peel off like flowers. So, there is sort of some intrinsic curvature through these protofilaments.

And, they peel off like this sort of curved sort of segments and the one of the major or one of the leading hypothesis is that at this leading edge you have the subunits that are attaching of these GTP bound subunits which are shown in the c l o and sign. And, in the interior they are all this GDP bound, and this GTP bound sought tubulin acts as a stabilizing cap which sort of prevents this disassembly.

So, if ever all of these were to be hydrolyzed into the GDP form you would switch from this growth stage to this shrinkage stage through a catastrophe and then microtubule would start to de polymerize ok. And, here the sort of instability manifests not as tread milling, but as this dynamic instability where.

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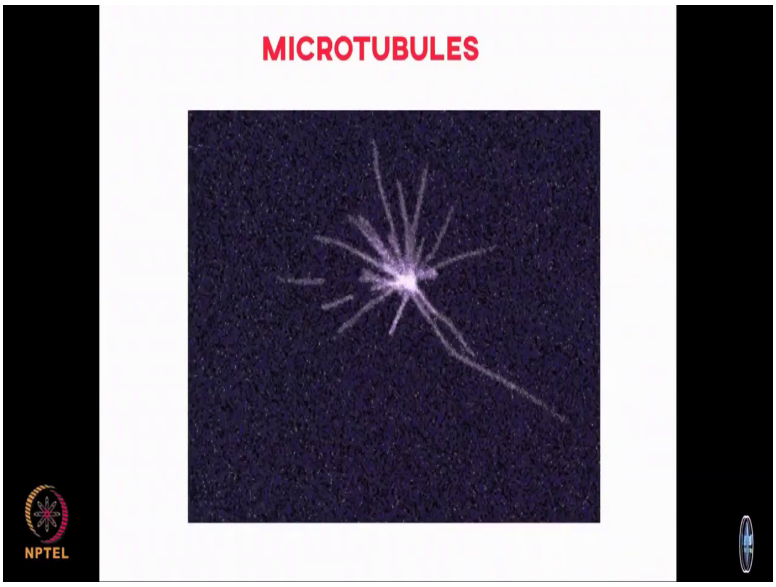

So, here generally for microtubules you could of course, polymerized them in vitro; if you left solution of free turbulence and you waited long enough you would see that after some time, it would start to grow. But, inside the cells there is the structure called the microtubule organizing center in the interior of the cell.

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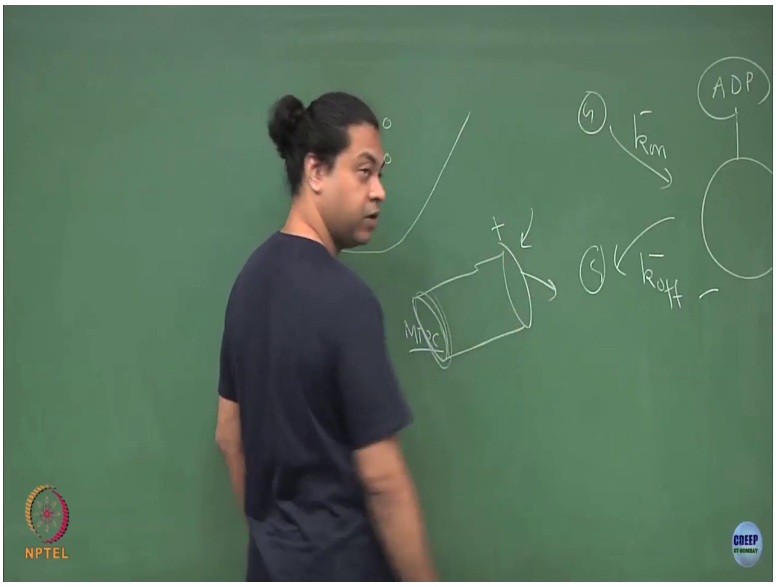

And, things microtubules nucleate from there. So, the minus ends are sort of bound to this and they do not have much binding, unbinding; most of the mining unbinding happens at the plus end. So, things attached and fall off at the plus end for microtubules, unlike for actin where it actually happens from both the ends.