Introduction to Mechanobiology
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Week - 04
Lecture – 18
Mesenchymal cell migration

Hello and welcome to today's lecture of NPTEL course introduction to mechanobiology. So, in the last class I had discussed about force ferocity relationships of actin networks.

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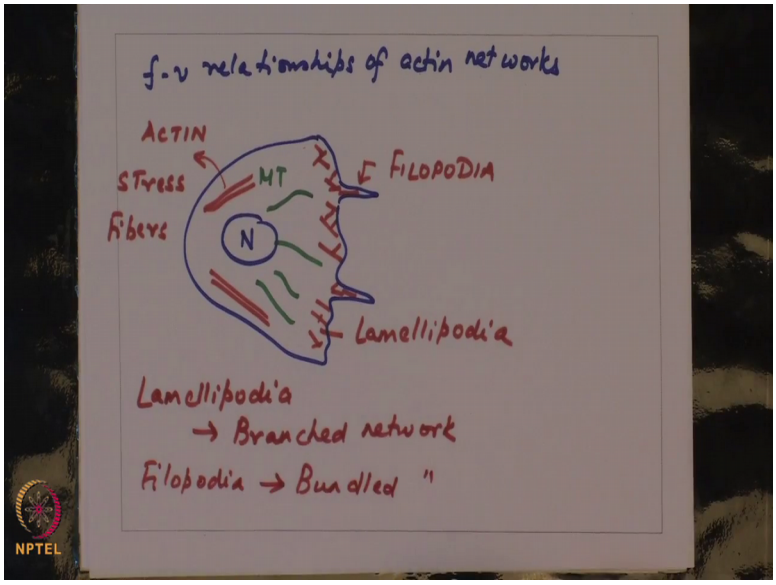

So, the advantage of having a branch, so if I draw cell, this is your nucleus the polarization of the cell is dictated by the microtubule cytoskelet uson, but you have your actin filaments which are forming various kind of structures. So, these structures your actin stress fibers this long pointed extensions that you see are called filopodia and this branched network this portion of the network is lamellipodia ok.

So, filopodia in the way the actin is cross linked by various proteins is different in case of filopodia versus in case of lamellipodia. In lamellipodia the actin is more branched. So, branched network versus in filopodia the actin is more bundled. So, actin plays a key role in cell migration. So, actin plays a key role in cell migration.

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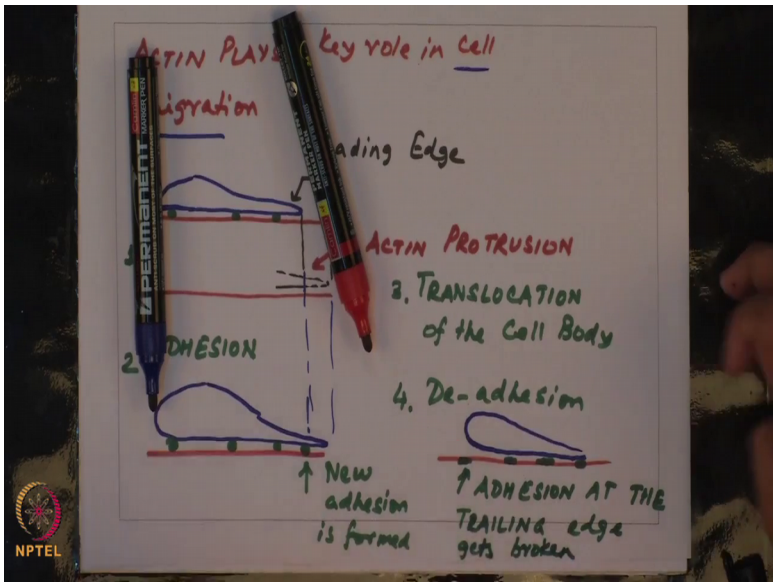

So, what we are going to discuss today is to study how actin participates in directing migration of cells. So, for that let us first look at the various steps which are include involved in cell migration. So, in cell migration, if I were to draw in side view let me draw the substrate these your substrate your cell is anchored by multiple adhesions as a first step what you have is actin protrusion.

So, at the leading edge of the cell, this is the leading edge first thing which happens is you have elongation of the cell. So, this remains the substrate I am not drawing the entire cell you have actin protrusion the second step what you see is the formation, let us for this for the second step I have to draw the entire cell once again this is a protrusion. So, earlier you had focal adhesions here here here what you also have is formation of an adhesion here new adhesion is formed. So, first you have protrusion then you have adhesion.

So, what adhesion does it stabilizes this protrusion and subsequently in steps 3 and fou4r. So, in step 3 you have translocation of the cell body and finally, in step 4 you have de adhesion. So, what is de adhesion I will draw de adhesions for you. So, this adhesion gets broken adhesion at the trailing edge. So, trailing is a this is the trailing edge of the cell gets broken and as a consequence a cell moves forward.

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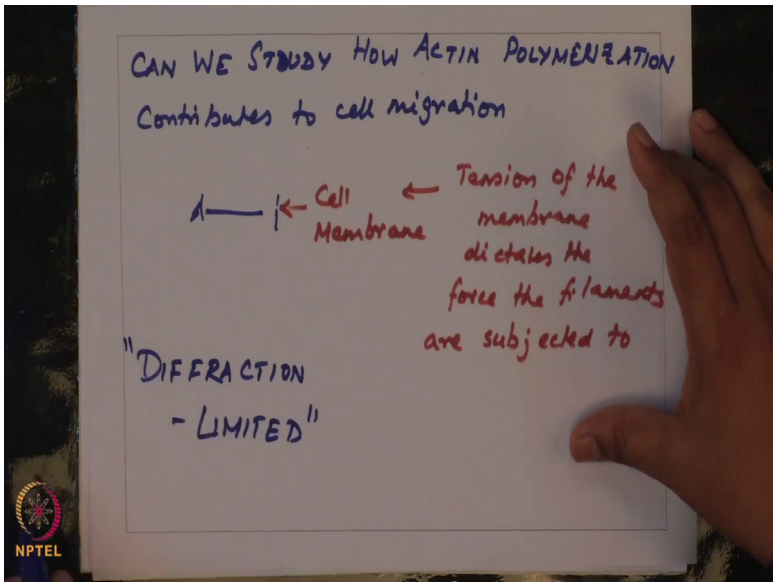

Now, ahead, the question for today is can we study how actin polymerization contributes to cell migration. So, it is obvious that if you have a filament against the wall so, what is the wall in case of a moving filament moving cell? The wall is nothing, but this is nothing, but the cell membrane itself, because of some tension in the cell membrane, tension of the membrane dictates the force the filaments are subjected to.

So, we know from in optical microscopy right, I had introduced this term called diffraction limited. So, it is impossible to see a single monomer under an optical microscope. So, you cannot see it objects which are less than 250 nanometers. So, if I were to draw filament, imagine I have drawn an actin filament.

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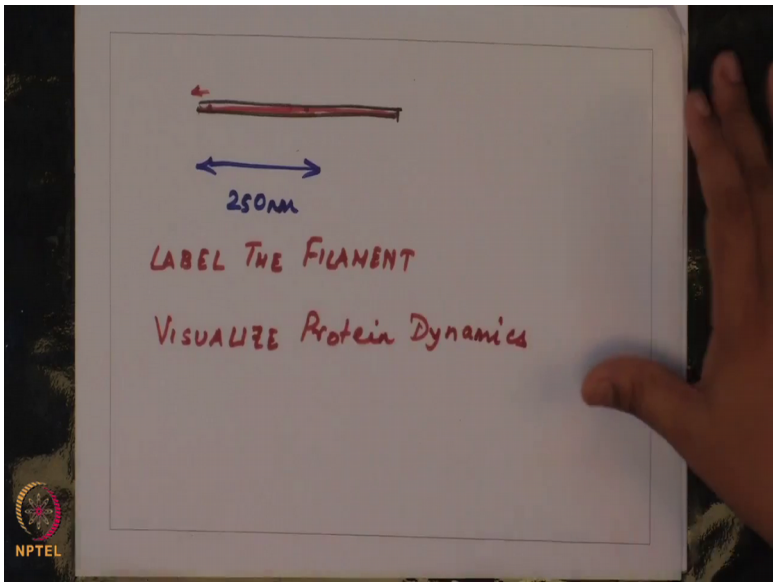

So, this is an actin filament and a monomer. So, let us let us say this distance is 250 nanometers. So, you cannot see any event let us say like a monomer going in this direction or a monomer being added here these events are impossible to see, but if you label the filament if you label the entire filament.

So, then this entire thing will turn red in this case also you cannot see individual monomer level polymerization or de polymerization events. So, how can we devise an approach such that we are able to visualize protein dynamics? So, you want to visualize, so the approach one of the approaches developed is called fluorescence speckle microscopy or FSM.

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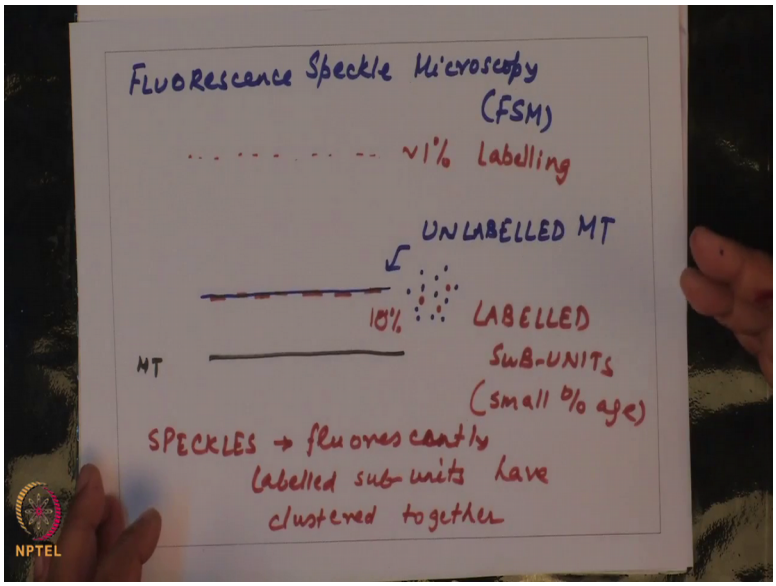

So, what this technique does. So, let us assume in the simple case you have a filament or even let us say let us say this is be a microtubule which is entirely labeled. So, in that case you are in no way to track the dynamics, but let us say you do the following experiment in which you take unlabelled filaments.

So, this is a unlabelled microtubule and what you add is among the; you have all these sub units which are unlabeled and within this mix of unlabelled sub units you add some labelled sub units. So, this is a small percentage. So, let us say if in this case if the percentage of labelling was 10 percent. So, what you will see you would see these roughly the labelled zones interspaced which black zones which are not visualized, but if you reduce the concentration of labelling significantly then what you will find. So, this is let us say order one percent labelling.

So, now you do not see the entire structure, but you can guess what that structure is. So, you can see multiple of these dots now what do these dots represent. So, these dots are called speckles where, so fluorescently labelled sub units have clustered together.

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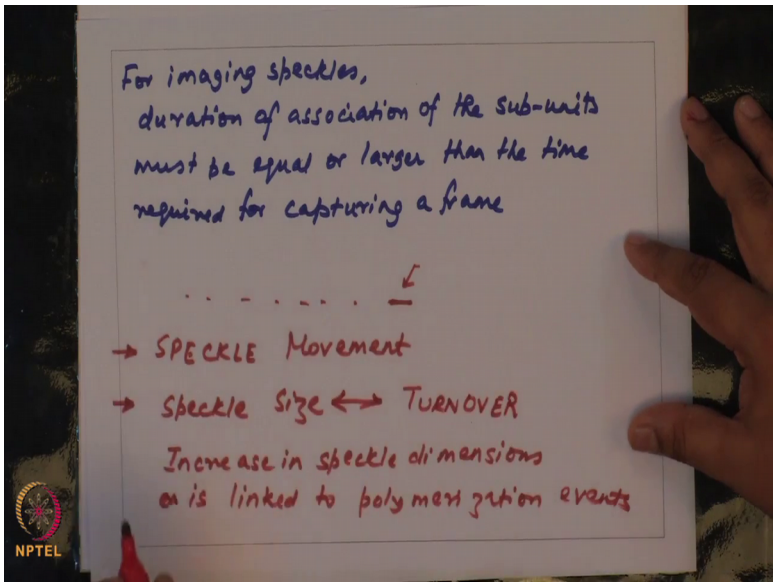

So, in order to image these speckles the duration of association, for imaging speckles, for imaging speckles your duration of association of the sub units must be equal or larger than the time required for capturing a frame.

So, what is the advantage of having of these fluorescent speckle microscopy. So, I have drawn this particular filament it might been actin filament or a microtubule filament which is, so I have intentionally drawn dots of various sizes because the chance or the size of these speckles will vary because this association is completely random, but once you see this speckle geometry what you can track there are two pieces of information that you can determine from this. So, you can track each speckle and you can track speckle movement you can track speckle movement and you can track speckle size.

So, if locally at let us say if this particular speckle is growing in size then I know other there is some polymerization event going on. So, increase in speckle dimensions is linked is linked to polymerization events. So, in a sense speckles size is correlated to turnover.

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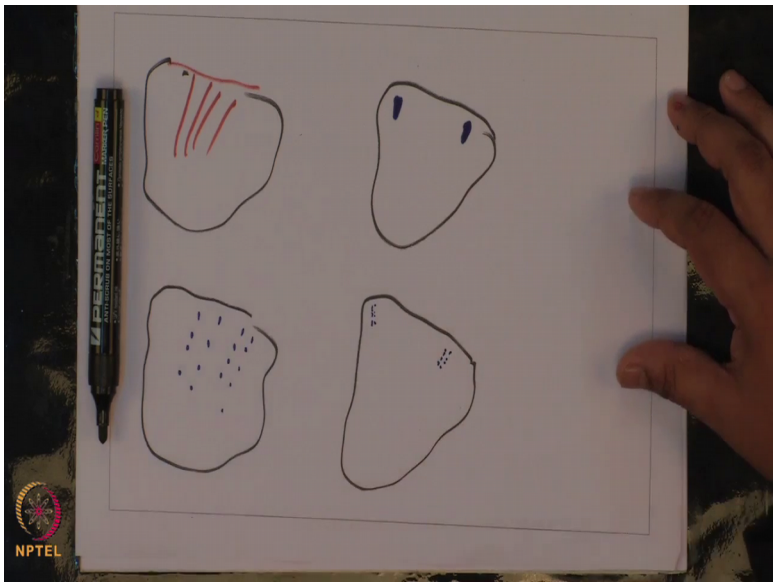

So, if we take let us say. So, if we take a cell let us say which is stained imagine this is one stress fiber network this is portion of a cell where you see stress fibers in this. If the same cell where imaged in speckle microscopy then what you would see you though you will not see the entire structure, but you can gauge the orientation of these filaments from the speckle microscopy.

So, similarly if your adhesions let us say for this cell you had these adhesions which were like in speckle microscopy. So, what you would have found was, you would still see get an idea of where the adhesions are, but not just that in adhesion to the static information now using speckle microscopy you have access to dynamic information. So, now let us say you use speckle microscopy to image actin dynamics in migrating cells you want to ask what is the nature of actin dynamics in a migrating cell.

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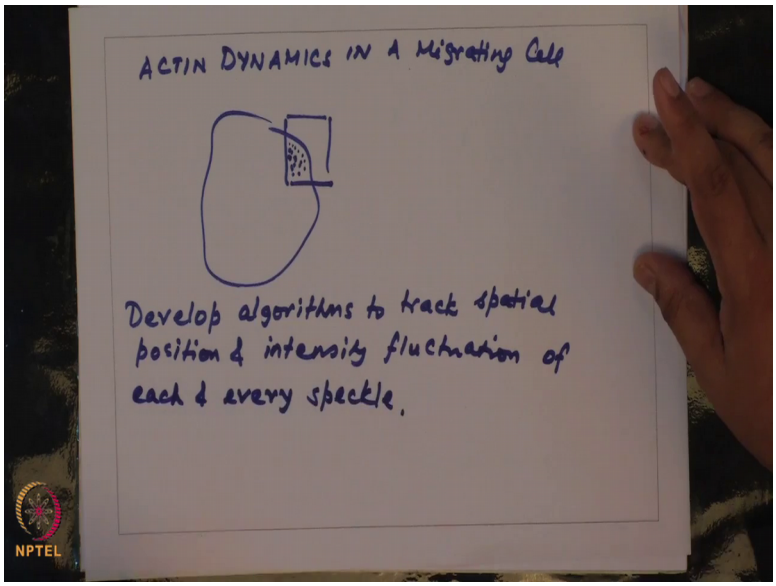

So, if you have the cell let us say a section you have this whole cell you can zoom into one corner you can zoom into one corner and if you look at the speckle images you would see tons of dots tons of these dotted particles your speckles. So, in order to gain any quantitative information you need to develop algorithms to track spatial position and intensity fluctuation of each and every speckle. So, this is no mean task. So, there are authors Goderns Danuser is a mathematician who has significantly contributed to our development of fluorescence speckle microscopy.

So, if you do now the question is if you do this speckle microscopy what is the nature of the movement of the speckles?

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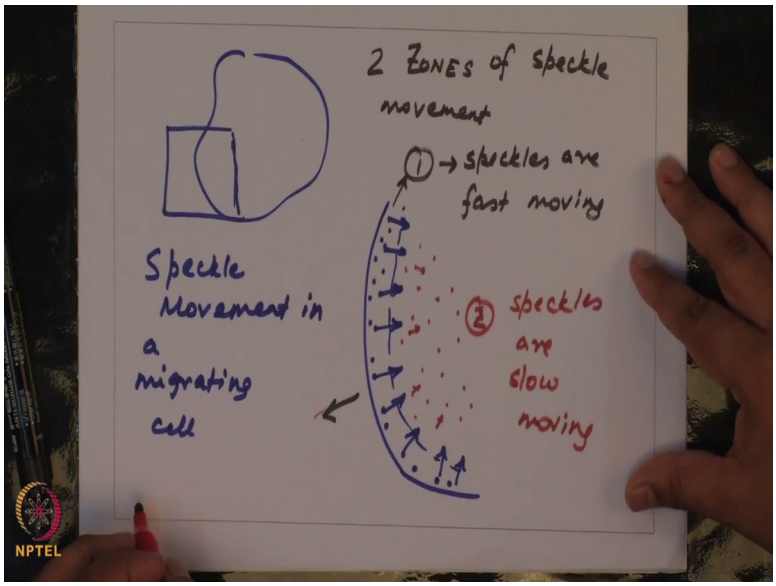

So, what has been found was, let us say that this cell you are looking at this edge of the cell. So, what I will do is instead of dotting plotting the speckles I will plot what is the overall motion of the speckles. So, what has been observed is. So, this edge let me draw it little bigger. So, there are actually, what I have drawn here is the distribution of speckle movement. So, what you are looking at is speckle movement in a migrating cell.

So, this is the direction this represents roughly this represents the direction the cell is moving at this point of time. So, what you see that there are two populations of speckles or two zones of speckle behavior or movement there is this zone at the periphery zone one speckles are fast moving and zone two speckles are slow moving, but consistency is all the speckles tend to moves inward. So, the cell is trying to move outward in this direction the cells speckles are moving inward.

So, this motion inward flow is referred to as retrograde actin flow. So, you have retrograde actin for all the speckles tend to move inward that is why retrograde or backward. So, backward compared to the direction in which the cells are moving and you have fast moving speckles right at the periphery of the cell0 so, this width of the region where the speckles are fast moving or is approximately one to two micron in width. So, one to two microns in width at the periphery and then there are slow moving speckles towards the center of the cell.

Second point what they found. So, you have two regions of retrograde flow what you can also do is for the cell you can track. So, you can track the intensity fluctuations to find out how does the intensity fluctuation change along the length.

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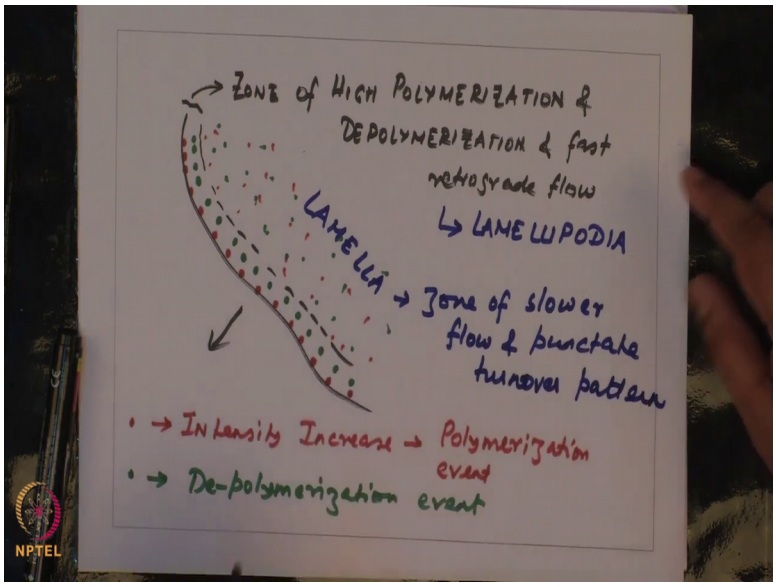

So, what was observed and then once again, what I have done red corresponds to intensity increase in other words it signifies the polymerization event and green corresponds to a de polymerization event.

So, once again this is the direction in which the cells are moving you have this. So, again based on this I can segregate the entire cell into two zones, the zone of the cell periphery where the speckles were exhibited fast retrograde flow had a layer of more of speckles where there was indicative of polymerization and right next to it a layer of de polymerization and then inside within the slow speckle zone you have mixture of both red and green dots. So, polymerization and de polymerization both.

So, what the authors did was called this zone of, zone of high polymerization and de polymerization and fast retrograde flow. So, this is the zone which they refer as lamellipodia and this zone internal zone is called the lamella. So, what is the lamella? It is a zone of slower flow and punctate or mixed turnover pattern.

So, you have two zones which seem to be physically distinct. The lamellipodia which has speckles fast, fast retrograde flow high turnover and second is zone of slow flow and

punctate turnover pattern. So, this suggests that you have these two zones which are right next to each other, but probably they are physically distinct zones. So, what they went on to see was to check that what is the spatial localization of some of the key proteins, actin associated proteins and what they found was Arp 2 3 which is associated with branching of actin it is present in the lamellipodia.

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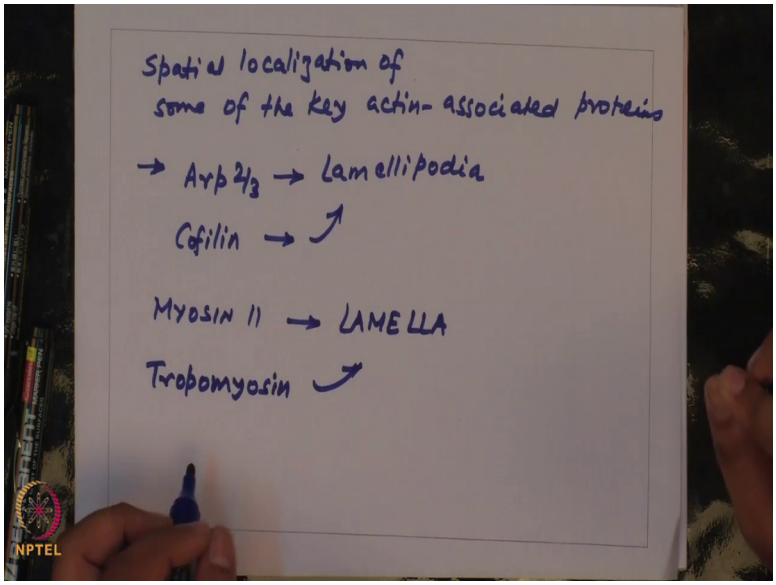

Similarly, cofilin it is actin de polymerizing. So, it is a capping protein contributes to actin de polymerization this is also present in the lamellipodia. In contrast myosin to which actually pulls on actin which pulls on actin fibers first enriched in lamella and same was tropomyosin this was also present in lamina. So, in short what you have is two zones which exhibit distinct retrograde flow distinct turnover and also are molecularly distinct. So, suggesting that there must be some dynamics which is different in these two zones.

With that I stop my lecture today and I will continue from here the next day.

Thank you.