Introduction to Mechanobiology
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Week – 03
Lecture – 15
Focal adhesions: role of forces

Hello and welcome to today's lecture of Introduction to Mechanobiology. In the last class we are started discussing about differences in the type of focal adhesions that you might observed limitation cells and what drives their assembly.

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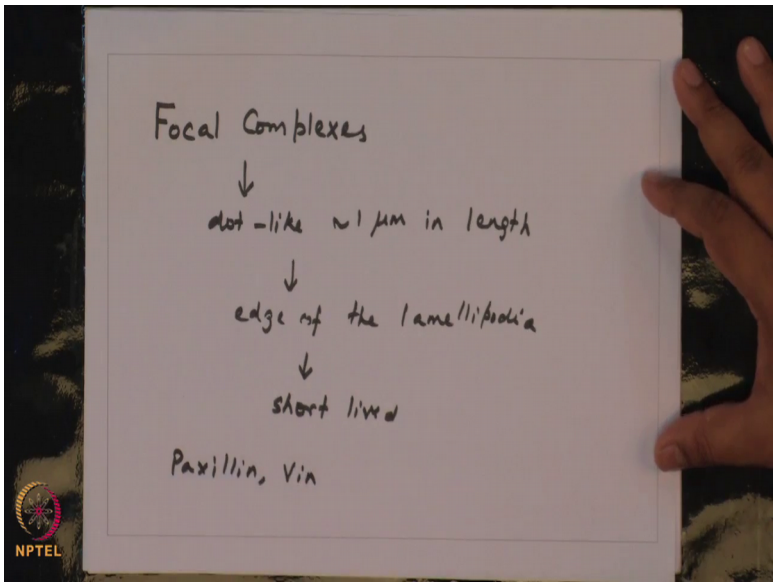

In that context we had said I had said that there are 3 different types of Focal Adhesions the smallest being Focal Complexes. These are more dot-like order 1 micron in length they are present at the edge of the lamellipodia, and these are typically short lived in terms of composition they have focal adhesion proteins like Paxillin and Vinculin present in them.

So, from focal complexes these focal complexes mature into larger Focal Adhesions.

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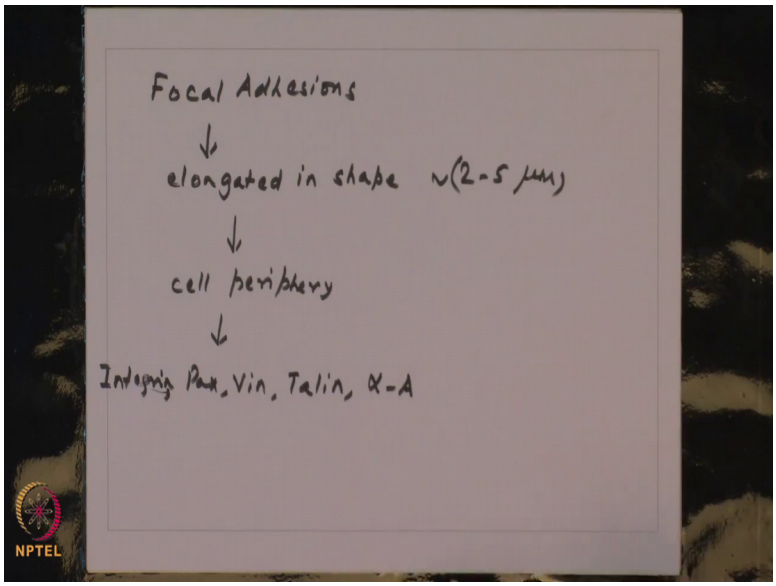

Focal Adhesions are more elongated in shape these are roughly 2 - 5 microns in length. And they are present at the cell periphery and in adhesion to Paxillin and Vinculin, of course, Integrins are always there you have many more proteins including Talin and alpha actinin. So, this (Refer Time: 02:07). These focal adhesions are present at the cell periphery.

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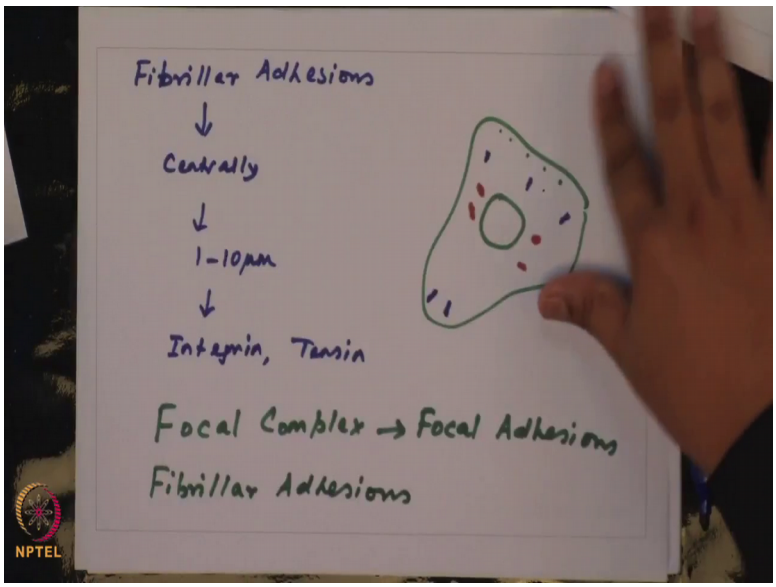

So, from Focal Adhesions in adhesion you can have Fibrillar Adhesions. As the name suggests their fibrillar in nature, but they are present central centrally towards the center

of the cell they can we 1 to 10 microns in length and in adhesion to Integrin they have this molecule called Tensin.

If I would to draw a schematic of the cell your focal adhesions are present here, and the fibrillar adhesions are present here, focal adhesions are also present at the real of the cell and you have focal complexes these appear like dots yeah really small in size. So, you have Focal Complexes mature into Focal Adhesions at the cell periphery and these become Fibrillar Adhesions at the cell center.

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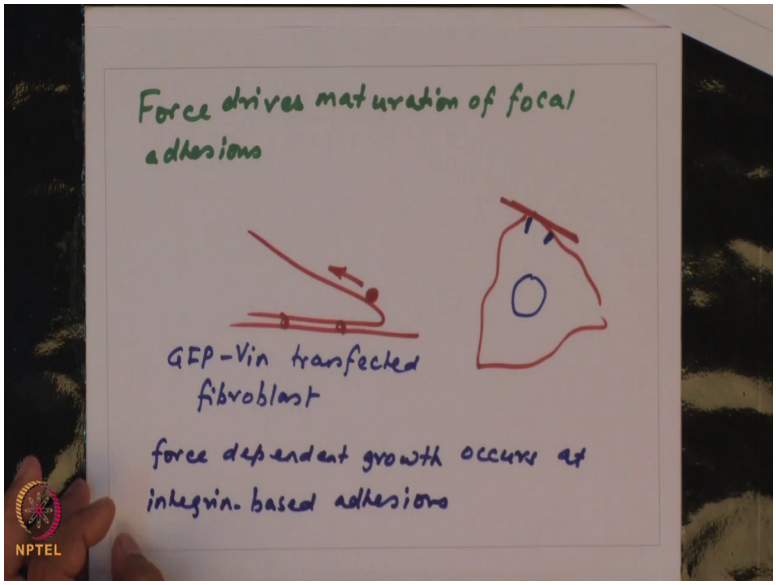

What drives this transition it is force. Force drives maturation of focal adhesions. So, this requires force and one of the first studies a disk which demonstrated that was by the group of Sascha Burchert Sket wise man.

So, what he showed was by exerting force. So, you have a cell here sitting on a substrate, connected by adhesions, and he took a pipette rolled on the cell edge. And he showed that, in tub you can see that wherever you put the pipette next to this you see growth of focal adhesions. So, this was done with GFP-Vinculin transfected fibroblast.

This paper I have also recommended you to read as a reading assignment you can download it through pubmed. However though this experiment demonstrated that forces are required for driving the growth of focal adhesions it is difficult to quantify the forces.

One more thing that this study demonstrated was this force dependent growth occurs at integrin adhesions at integrin based adhesions. If you were to do the same experiment on a polylysine coated surface you would not see it. So, the problem with this particular experiment not the problem the caveat is that you cannot measure the forces directly. So, this called for an alternate approach a technique to so, that you can quantify this forces. So, one of the approaches which was developed by Benny Geiger and Sascha Burchert Sket wise man was what they did was they took a substrate a patterned elastomer where, they coated fibronectin spots on top of this elastomer it subsets. So, this elastomer was PDMS.

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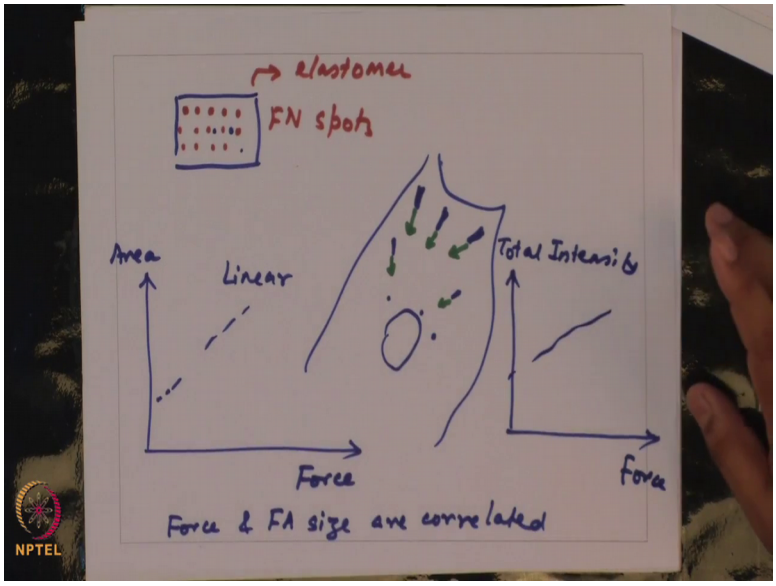

The grid like location spacing of these dots will allow you to quantify, how much one of these dots get displaced when cells are sitting on that. And by this by multiplying this by the local force you know what is the force exerted and at that particular point.

So, through this experiment what they found was if you take a cell you have these adhesions. These focal adhesions and at each focal adhesion they measured they were able to measure the forces, and what they found was that there was a linear correlation. So, there was a linear correlation between the force that they measured at a given adhesion and the area of this adhesion or the size of this adhesion, this was roughly linear. So, this was a linear dependence suggesting that there is a correlation between force and area force and focal adhesion size.

So, not only the correlation holds between force and focal adhesion size if you were to track the intensities because these experiment was done with gfp tranfected cells gfp vinculin transfected cells, you can track the total intensity of a given focal adhesion and the force measured at that point once again you have this linear correlation size and force and intensity and force which means that given if a focal adhesion is bigger there is a increased amount of local localization of that protein at this adhesion. Moreover the way I drew this picture I roughly aligned the direction of the force and the major axis of the focal adhesion.

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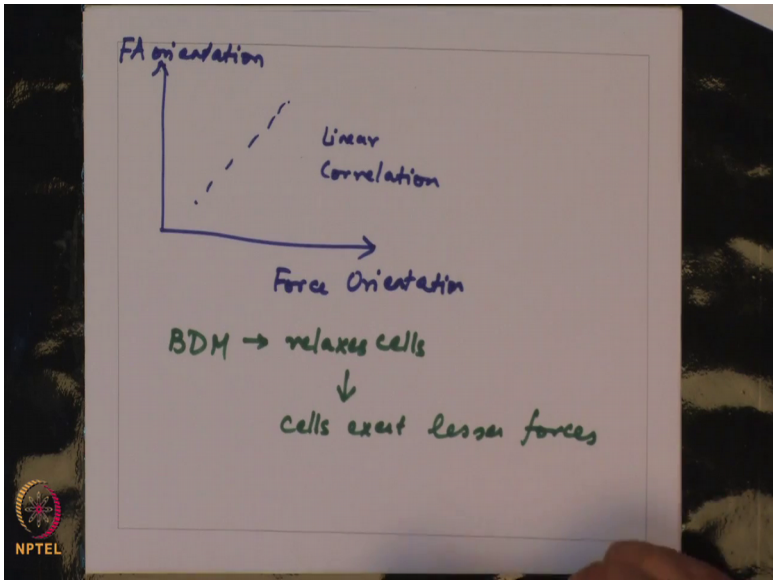

If you were to look at the force orientation and the focal adhesion orientation so, by fitting an ellipse to each of the focal adhesions you can find out the angle of the mac the major axis and they found was even there was some correlation.There was kind of a linear correlation.

So, still this establishes an association between force and focal adhesion size, but it does not say how what is the directionality of this association? So, to demonstrate that or to test that what they did was they did experiments in the presence of this drug called BDM. What does this drug BDM do it relaxes cells or in other words cells exert lesser forces. So, you are perturbing the amount of magnitude of the forces which the cells were exerting.

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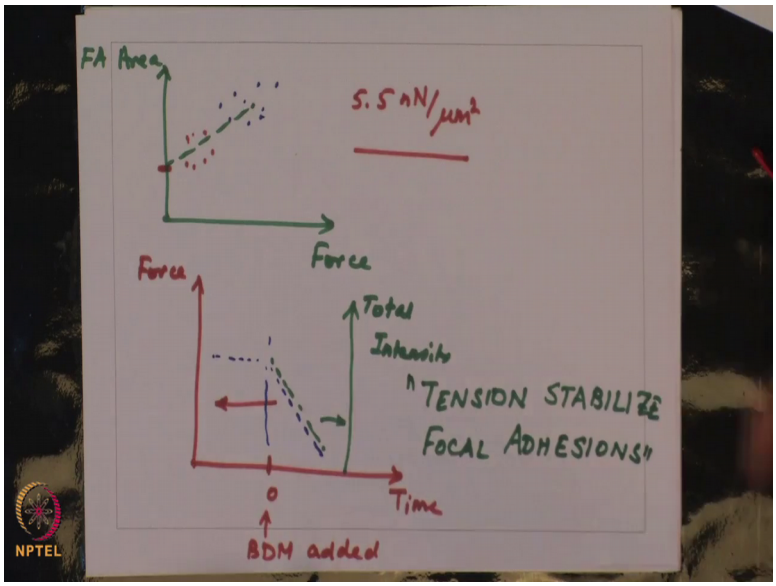

So, what they observed by doing this experiment. So, if I were to draw this curve again between force and area FA area the nature of the curves remains the same.

If earlier points were up here in the presence of drug these points shifted to the left, but they fell on the same line and on top of that you could find out a force. So, this line has a nonzero intercept. So, has a nonzero intercept and you can also find out the slope of this line the slope of this line was roughly 5.5 nano Newton per micron square. So, this was the average force sustained by a focal adhesion of size one micron squares. So, one micron square focal adhesion sustains a force of 5.5 nano Newton.

If when they did the experiment of trying to see as a function of time how the force dropped. So, when you administer the drug let us say this is time 0 when the BDM drug has been added.

So, before that point before that point you have some constant value and then these values after beyond this transition point they start to drop. So, correlated with this particular drop in focal adhesion force when they also plotted the focal adhesion the total intensity this also exhibited a drop. This is for this axis and the blue is for this red axis. So, once again concomitant with drop in force there was a linear drop in the total intensity of the focal adhesion and also, these were correlated perfectly correlated. So, this experiment suggests that tension or force exerted by cells at focal adhesions actually stabilize the focal adhesion.

So, force is required to stabilize focal adhesions. So, here you see, again if I were to go back to my analogy of cell as a tensed robbed or tensed rubber band right.

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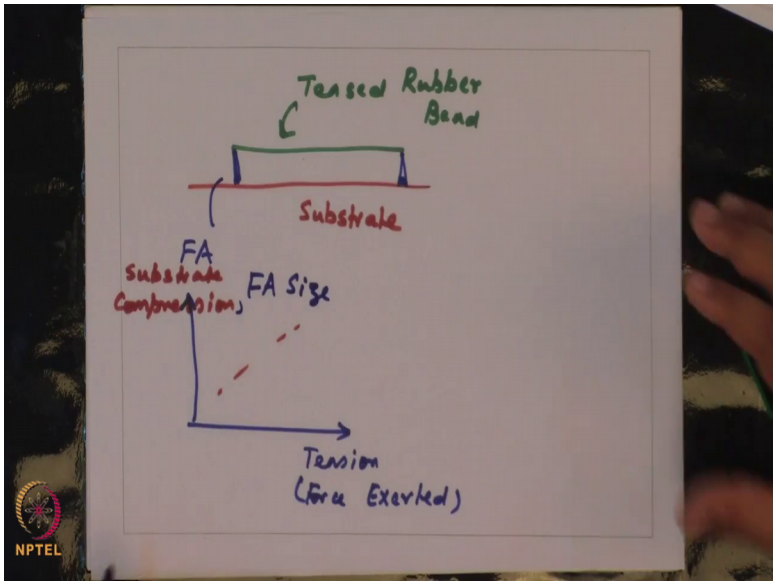

So, imagine a simplified picture where, this is my substrate, this is my theoretical cell which is like a, tensed rubber band and this blue represent my focal adhesion. The more amount of increase, if you increase the tension I can plot. So, if I is a tension of force exerted at the you know tension of force exerted at the adhesion versus the substrate compression.

So, you should see a linear relationship. The more the tension and if the focal adhesions are stabilized then the substrate will get compressed and not just substrate compression you can say along with this the FA size will also increase. So, that is how this entire system is stabilized. So, increasing tension allows stabilize stabilization of a focal adhesion the entire structure gets stabilized and that is how we are and the substrate the forces get transmitted from the cell to the substrate. So, that is why you get increase substrate compression.

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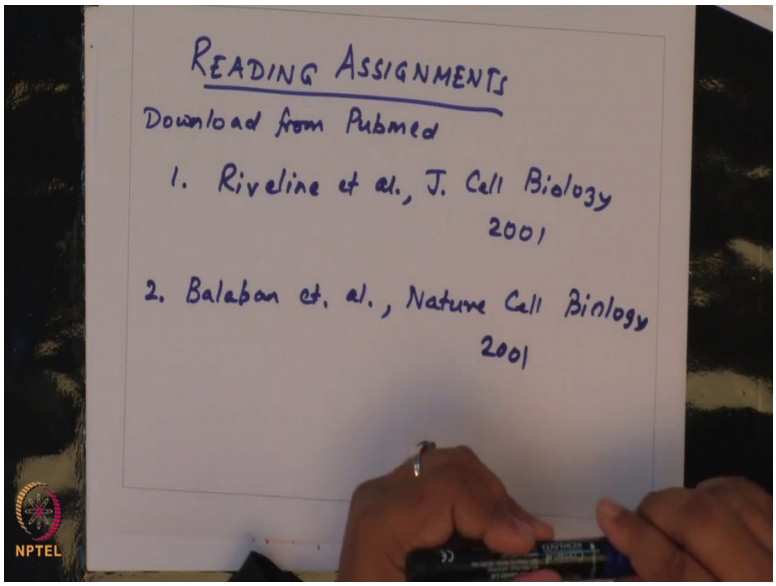

So, this brings to an end our discussion on focal adhesions I would once again give reading assignment of the 2 papers that I discussed over the course of the last today's lecture and last lecture. So, these are the 2 papers you must read you can download it. So, you can download from Pubmed, first paper is Riveline et al Journal of Cell Biology 2001. The second paper is Balaban et al, Nature Cell Biology again 2001.

These were 2 landmark papers which demonstrated the influence of forces in regulating focal adhesion size.

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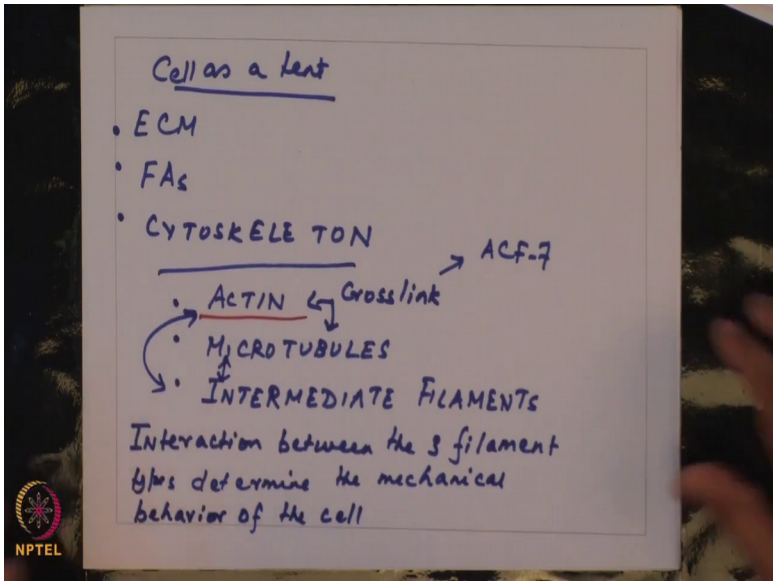

So, coming back to our analogy of cell as a tenth we have discussed the ECM, we have discussed focal adhesions, now we will come to the Cytoskeleton right. So, the cytoskeleton is the machinery which is required for cells stability, but also this is the one which regulates the forces which are acting at focal adhesions. So, for the cyto you most of you know what is cytoskeleton is, but I will still thought of giving a brief overview of the cytoskeleton. So, there are 3 different filament types one made of Actin, one made of Microtubules, one made of Intermediate Filament.

Today the overall properties of the cytoskeleton, though there are 3 different networks, but there are proteins which cross link or link actin and the microtubule cytoskeleton one example is this protein call ACF-7 or actin cross linking factor 7 similarly you have proteins which link the actin to the intermediate filaments and the microtubules to the intermediate filaments. So, interactions between the 3 filament types determine the mechanical behavior of the cell. So, today we will get started with discussing actin.

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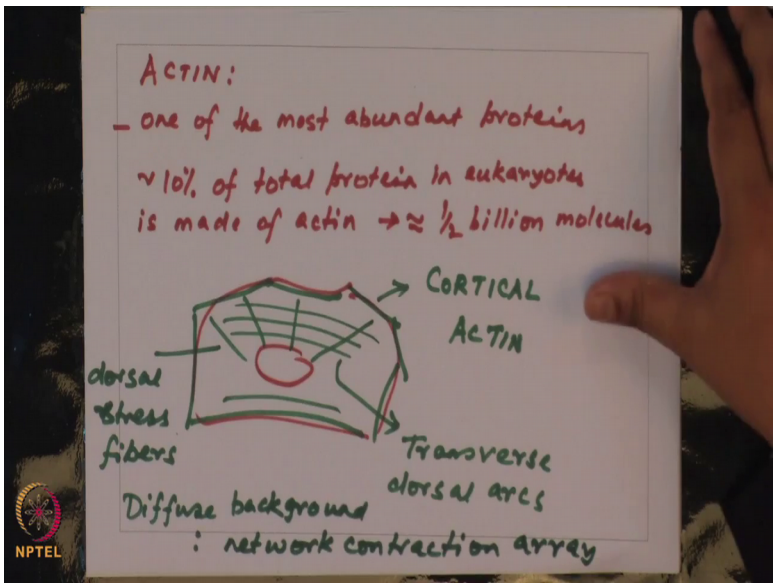

Actin actually one of the most abundant proteins, one of the most abundant proteins, so roughly so, 10 percent of the total protein in eukaryotes is made of actin. So, this amounts to roughly half billion molecules. So, if you look at an adherent cell see if I would to draw an adherent cell let us say if I draw a cell like this and I look at the actin cytoskeleton you might have as well as, I have drawn these peripheral stress fibers. So,

this is called the cortical actin network. So, these ones are called transverse dot cell arcs these ones are called dorsal stress fibers.

In adhesion to forming these fibers you will have a diffuse background. So, these are referred to as a network contraction array. You have various different actin structures if I would to draw the cell of a migrating cell if I would to draw a migrating cell.

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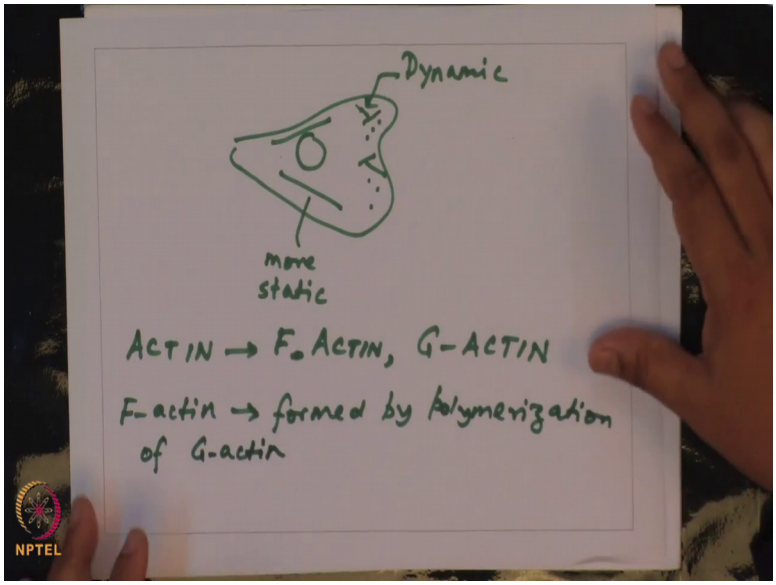

So, you can see branches which form, you will have monomers which will polymerize against the membrane and push it you will have these stress fibers as well. This some of these so, at the leading edge of the cell these are more dynamic and these are more static. These are the ones which actually transfer forces to the substrate. So, there are 2 pools of actin or actin exists in 2 forms have F-ACTIN and G-ACTIN. So, G-ACTIN stands for globular actin and F-ACTIN stands for polymerization if f filament is actin.

So, you are G-actin monomers, come together to form a filament. So, F-actin is just a formed by polymerization of G-actin. What has been demonstrated by a range of studies a lot of lot of researches that if you take monomers and these apply for multiple different type of processes. So, what you first need is the formation of a nucleus for some of these monomers and then.

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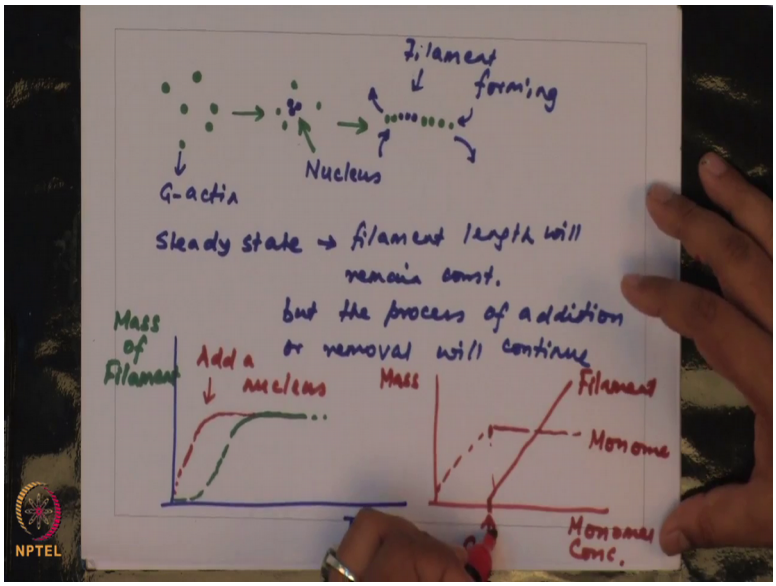

And then spontaneously these nuclei assemble in straight line and in then you have these monomers getting added and so these monomers then come. So, this entity is called a nucleus. So, these green dots are my G-actin and this is my filament form.

This filament in this filament you can have a new monomer getting added or coming out of it similarly you can have adhesion or removal from one end. So, in steady state in steady state say your filament length will roughly remain constant, but dynamics, but it is dynamic right the process of addition. So, addition process of addition is polymerization or removal which is depolarization will continue will continue. So, if I were to plot it in terms of time. So, it takes quite some time for these monomers to come together form a nucleus and then once the nucleus is formed you have quick formation of a filament. So, my y axis is the mass of filament of filament content, but if I provide a nucleus then this process is much hastened. So, this is when you add a nucleus.

Now this filament formation does not happen at all times, but will require a critical concentration. So, if I plot the monomer concentration. So, below a certain threshold below a certain threshold you will have if you keep on adding monomers your mass of the monomers will keep increasing, but once this threshold is cost your monomer concentration will remain constant, but your filament concentration will increase. So, this is a filament and this is a monomer. So, this is the critical concentration C c.

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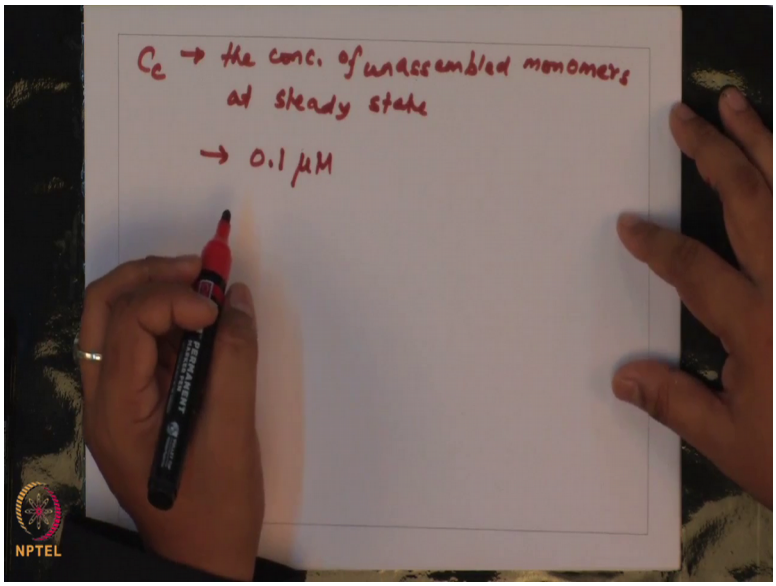

So, this represents the concentration of unassembled monomers at steady state and this has been found to be point one micromolar. So, in next class we will continue from here when discuss how actin filaments grow what kind of network you can form and how can you measure the forces exerted by these actin networks.

Thank you for your attention.