Introduction to Mechanobiology
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Module - 05
Lecture - 21
Adhesion Independent Migration

Hello and welcome to our todays lecture of NPTEL course Introduction to Mechanobiology. In the last 2 lectures I had discussed Actin Dynamics in cell Migration.

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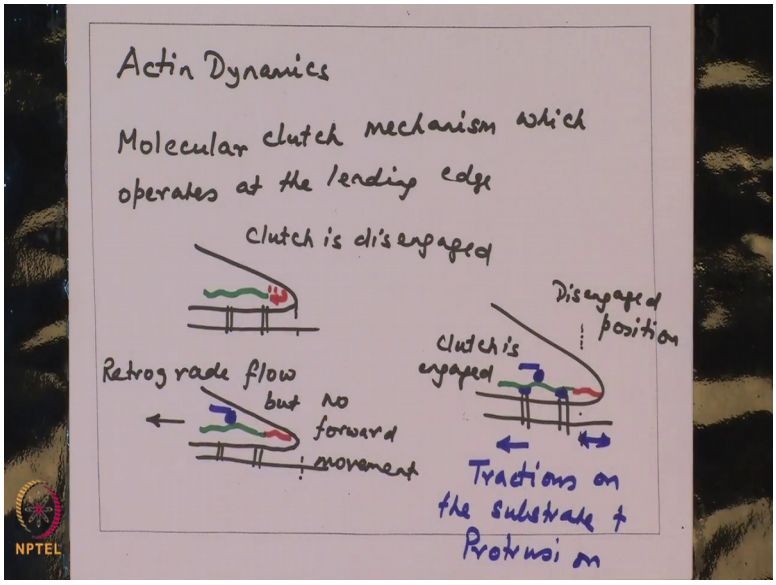

And while discussing how cells migrate we had found out that you have a Molecular clutch which molecular clutch mechanism which operates at the leading edge. What you have we have 3 situations. What I am drawing here these are your integrins and let us say this is your Actin filament and you have monomers which are getting added to the Actin filament.

So when the clutch is disengaged. This is a situation where the clutch is clutch is disengaged. When the clutch is disengaged the polymerization. Let us say this is my leading edge position no matter how much the filaments form they will just keep getting pushed backwards. This is your new filament which has got10 added to the existing filament and this is called your rit. This overall you have this back flow and partly this is mediated by your myosin molecule which pushes pulls on it.

So, you will have retrograde flow, but no forward movement. In the other case when you have clutch is engaged, you have various adapter proteins which linked with the filament. Let us say this was the earlier position in disengaged position this was the leading edge position. So, when your clutch is engaged, in this case your clutch is engaged. So, you are retrograde. Even when myosin motors are pulling on it, what you will have is tractions on the substrate and plus you will have protrusion.

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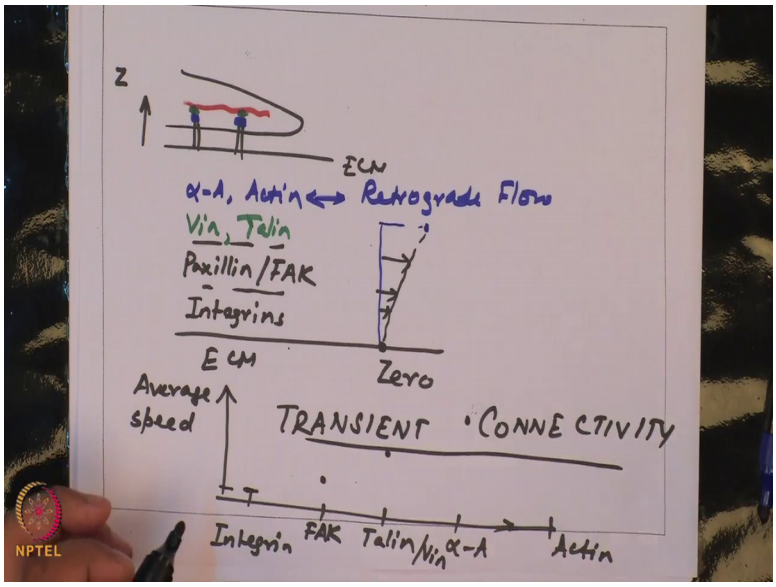

So, this is the distance by which the cell moves forward. This is the principle of a molecular clutch now. So, imagine a situation you have. This is a cell membrane this is your ECM you have your integrated molecules here integrins here and then various other proteins right. I am just drawing these layers and eventually you have your actin filament. So, you know that as a function of Z if this is my Z height you have your integrins then you have molecules like Paxillin and FAK on top of which you have Vinculin and Talin. So, talin exhibits and direct orientation and on top you have alpha actinin and actin.

If actin is exist, you have measured retrograde flow right. So, you have measured retrograde flow. So since at the bottom at the bottom you have your ECM. So, flow at this point has to be zero. So, if I were to characterize the speed what you should have here is Zero there is no slip condition at the base and at the maximum let us say at this blue point you have a certain speed here. Let us say this is my axis this would mean that

all these intermediate proteins will have some speed some sliding speed which scales with height and this is what has been found if you track the motion of different proteins at focal adhesions, you have Integrin, you have FAK slash paxillin, you have Talin ,you have alpha Actinin and Actin.

If you track the average speed of these molecules what you find is this guy has the high speed and then you have reducing speed. So, paxillin and vinculin are paxillin and FAK are way less than talin or vinculin and lowest very close to zero is integrins. So, this shows you that there is a hierarchy and this. So, in order to achieve this kind of speed you must have transient connectivity you have to have transient connectivity of focal adhesion proteins for this motion to be transmitted from the top to the base where speed is zero at the base and as you go up you have different speeds.

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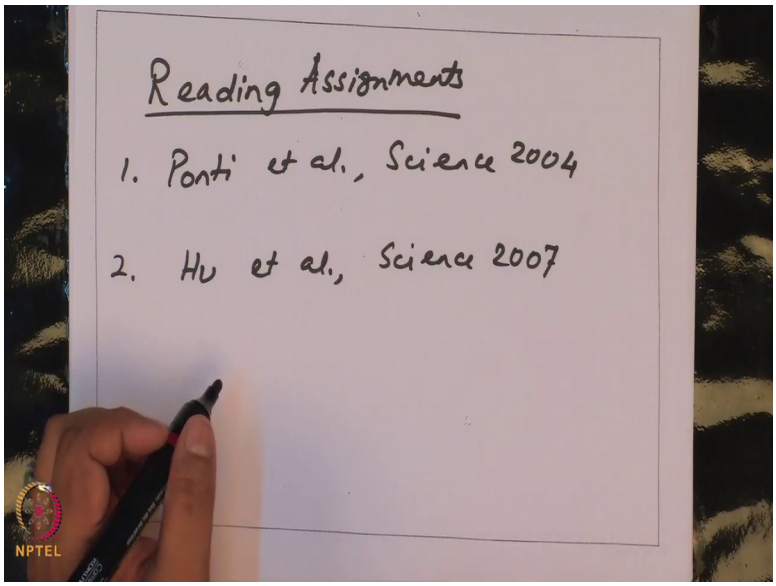

These 2 papers that we discussed would be our next reading assignments. First one you have Ponti et al science 2004 paper, so this is about the acting dynamics. And the second paper Hu et al also a science paper in 2007 this discusses about this relative sliding of different focal adhesion proteins. So, this concludes our discussion of mesenchymal migration.

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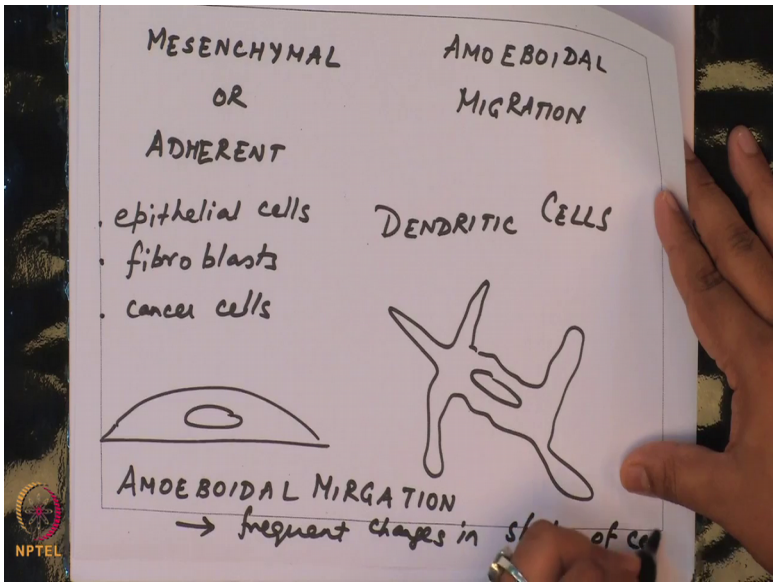

Now, compared to Mesenchymal Migration, you have mesenchymal or adhesion dependent or adherent. So, this kind of migration is exhibited by epithelial cells fibroblasts cancer cells.

There are various types of cells which exhibit this migration, but you also have another mode of migration which is called Amoeboidal Migration. So, today we will start discussing amoeboidal migration. What is amoeboidal motility? So, if you look at dendritic cells if I were to outline the shape of a dendritic cell it will be more like this. So, what you see is as suppose to the well defined shape of these adherent cells these dendritic cells they do not have a well defined shape you have multiple different protrusions which are getting you know which are evolving in space and time and amoeboidal motility or amoeboidal migration is characterized.

So, amoeboidal migration is characterized by frequent changes in shape of cell. So, amoeboidal migration is exhibited. If I were to measure the average speed of fibroblasts or epithelial cells it is order let us say 20-30 microns per hour.

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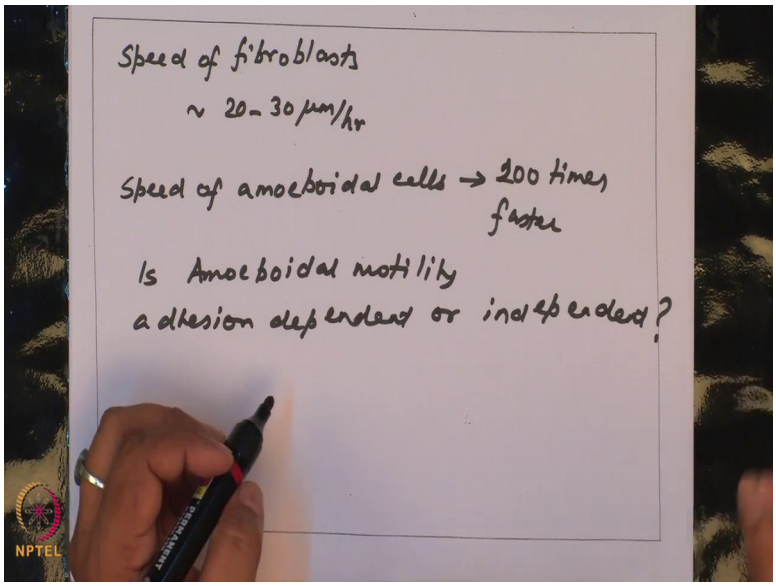

In comparison to this adhesion dependent type of migration the speed of amoeboidal cells can be 100 times faster. So, the question that I want to ask is motility or is amoeboidal motility adhesion dependent or independent. The idea being if something is adherent you are protruding your and then you are breaking your rear adhesions. So, that should limit how fast as I can move in contrast amoeboidal migration can happen very fast if there is no adhesion. So, is there any justification for saying that amoeboidal motility must exhibit adhesion independent migration?

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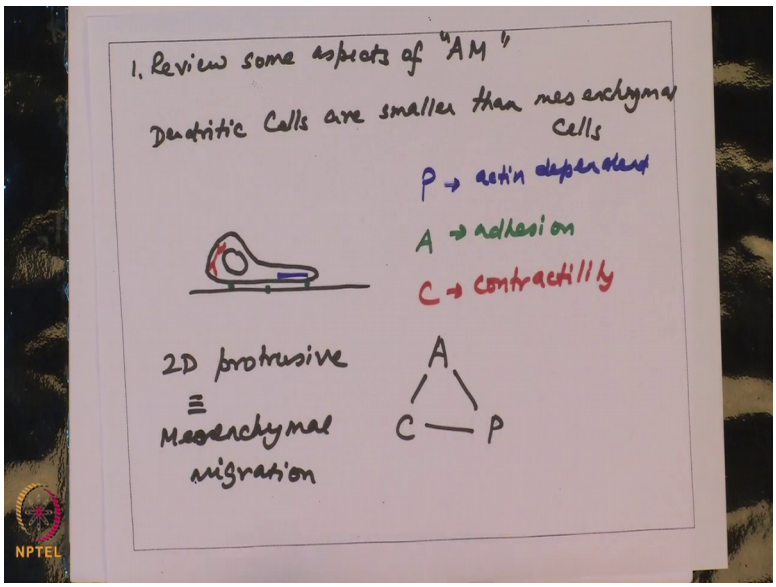

So, to answer that question what I will do is I will first review I will first review some aspects of amoeboidal migration, “AM” is shortfall for amoeboidal migration. So, generally dendritic cells are much smaller then cells like fiber than mesenchymal cells. There are in case of amoeboid cell motility there are even on a 2 D plane you can have 3 different types of motility. Let me draw again you have these adhesions and you have the nucleus what I will also draw these protrusions I can draw in blue. So, you have 3 things blue is protrusion actin dependent A is adhesion and what I have depicted here in red A C or contractility.

So, 2 D protrusive a 2 protrusive can we drawn in the following way you have 3 different aspects of it protrusion adhesion and contraction when all 3 are dominant this is nothing, but almost equivalent to mesenchymal migration. So, cell protrudes adhesion stabilizes the protrusion by adhering and then the cell contracts. So, you have all 3 of these parts playing major roles, but you can have. So, remember that I made this statement dendritic cells are much smaller than mesenchymal cells.

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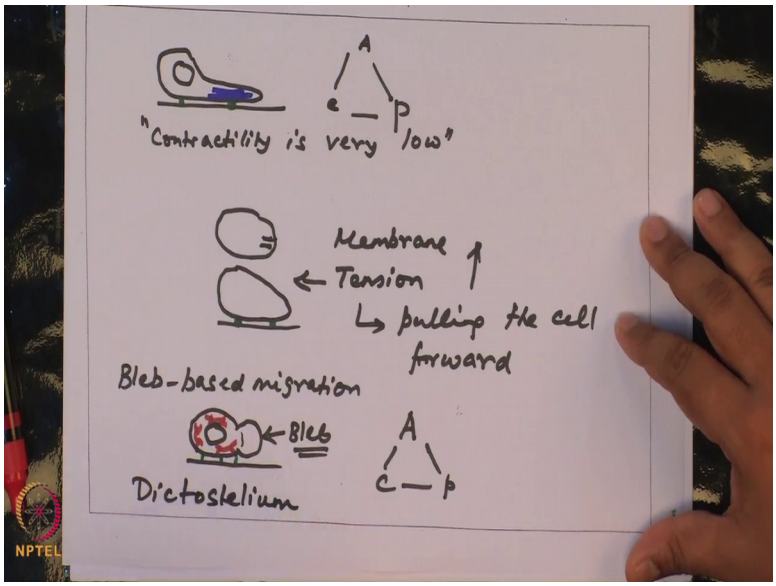

What has been observed is these cells they can migrate just as well if you have small. In this case I have drawn C to be very small suggesting your “Contractility is very low”. Even with less contraction if the combined protrusion and adhesion the cell is able to migrate and how is this possible.

So, it is possible only because the cell is very small imagine that you take a balloon a balloon and you poke from inside the balloon you poke with the pin then what you will have is the balloon will slowly take this kind of a shape. So, what you are doing is the balloon membrane you have a tension a membrane tension which goes up as a consequence it is this tension. If you are adherent then the membrane tension itself can lead to pulling the cell forward.

This is an alternate mode where protrusion and adhesion are dominant and contractility is very low increasingly for these cells there is exist a third mode of migration nucleus. So, what it does. So, this is a called a Bleb-based migration. So, this is called Bleb-based migration in which your protrusion is very small, adhesion is large and contractility is large. So, the cell actually it is like you are you are exerting this contractile forces from the back which leads to flow of the cytoplasm leading to rupture of the membrane and creation of a bleb, so this is called a bleb. And you can keep on having doing this repeatedly that you have a contraction cell moves forward adhesions are broken and so on and so forth. This kind of bleb-based migration has also been observed in dictostelium. So, you have these 3 cases.

Now, this is on 2 D surfaces in 3 D content in 3 D context you can have various different situation you can have a situation.

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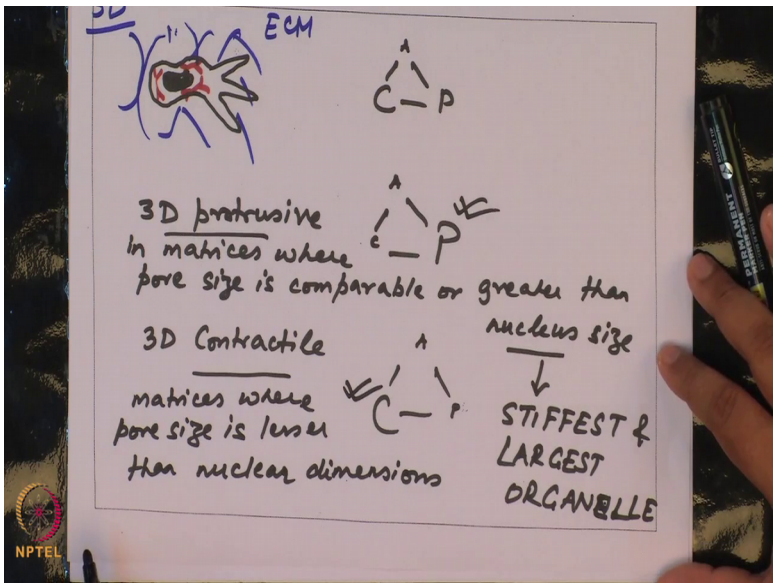

So, what I am drawing around the cell, this is 3 d; that means, within interstitial matrices you can have this kind of migration where adhesion is small, contractility and protrusion are large, this is one type of migration. You can have another piece of migration where you are adhesion and contractility is small and protrusion is large. So, this is 3 D protrusive and the third mode is 3 D contractile in 3 D contractile your adhesion and protrusion are small and contractility is large.

So, 3 D of 3 D protrusive can be sustained in matrices where pore size is comparable or greater than nucleus size this is because the nucleus. So, the nucleus is the stiffest compartment stiffest and largest organelle. So, the nucleus being the stiffest and the largest organelle for 3 D contractile this is where matrices where pore size is lesser than nuclear dimensions.

So, here you have protrusion picking a bigger roles here you have contractility playing a bigger role.

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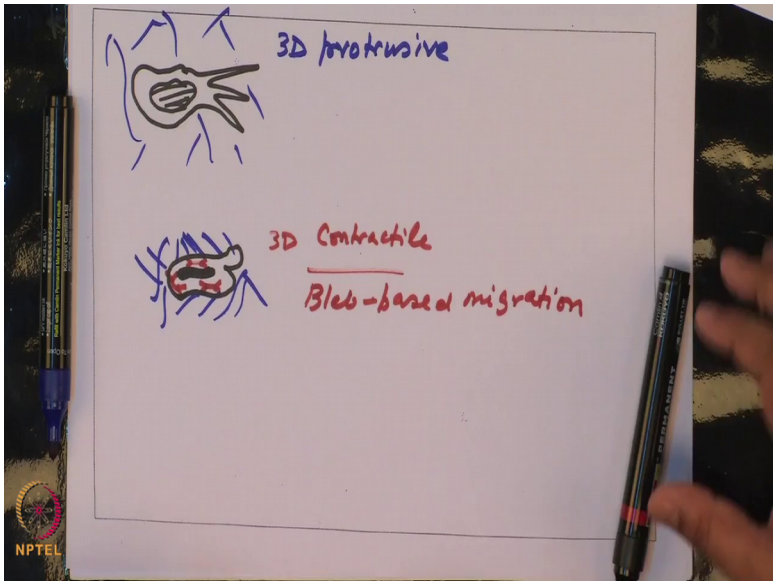

So, in 3 D contractile see if I were to draw a 3 D protrusive and you have, the way I have drawn it. So, this is 3 D protrusive and in 3 D contractile. In this case the nucleus the 3 D contractile. So, this is 3 D contractile. In the case of 3 D contractile the nucleus has to be deformed. Again in 3 D contractile you have a bleb based migration. So, this is just a brief overview of all the different types of amoeboidal motility you might have.

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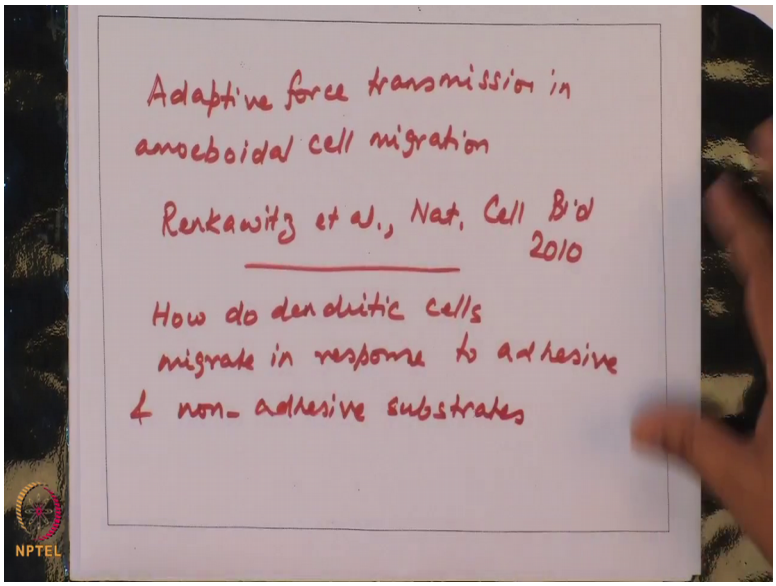

Now we will initiate discussion about one particular paper. So, the name of the paper is Adaptive force transmission in amoeboidal cell migration some nature cell biology paper 2010 paper. So, the broad goal here of this paper is to understand. How do dendritic cells migrate in response to adhesive and non-adhesive substrates?

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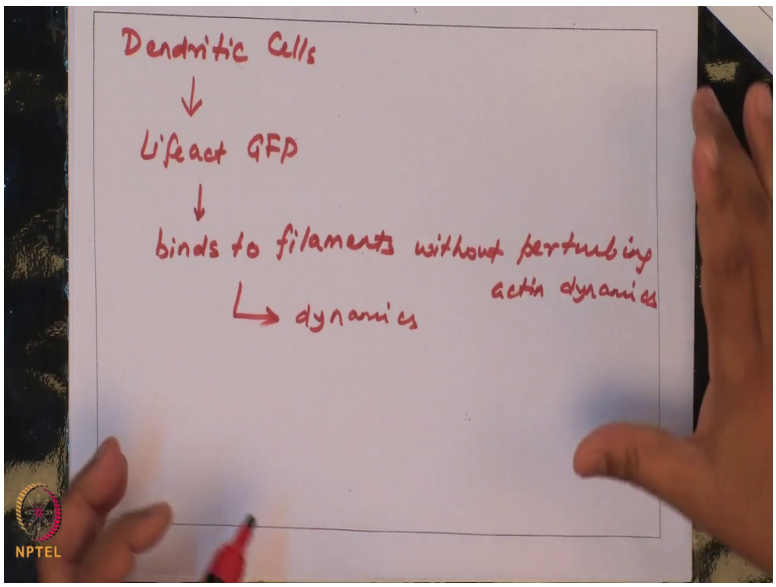

The authors work with dendritic cells you have dendritic cells these were transfected these were transfected with Life act GFP. So, life act is an entity which binds to filaments. So, as a consequence you can track the dynamics.

Now, binds to filaments, but without perturbing actin dynamics. Basically the first question the authors asked whether they would observe retrograde flow in these cells.

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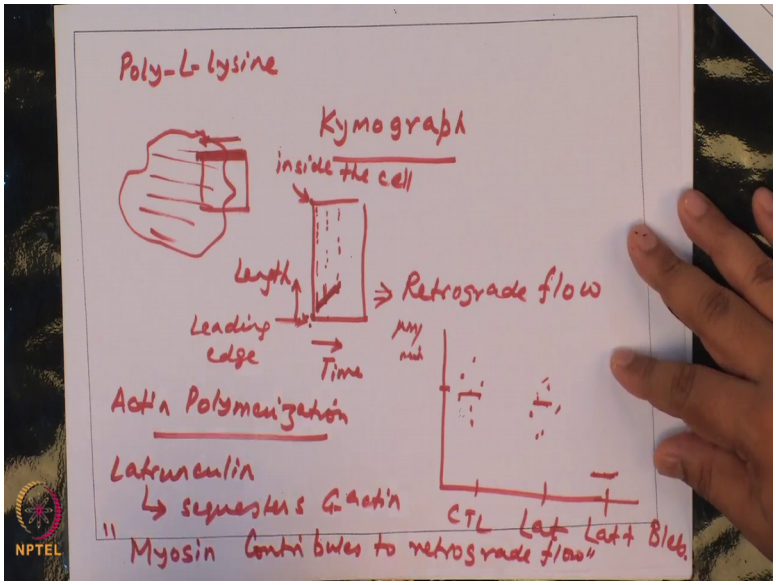

So, to answer this question the cells were plated on Poly-L-Lysine coated surfaces and what they observed. How do you quantify retrograde flow using life act GFP. So, this is what is done if you have a cell. So, when you transfect with the life act GFP you will see the entire thing fluoresce.

Let us take a portion a small window of the cell where the cell is actually protruding. So, what you can do you can generate what is called a Kymograph and what the kymograph does is along a very small length of it along a very small length of the membrane the cell tracks how the florescence and signal and the membrane front evolve. So, as a consequence if you look at the same line if you look at the same line and see how the fluorescence will evolve what you can get. So, you have the following thing you have a time axis here and you have a length axis here.

Let us say at time at time t equal to zero you had some let us a distribution you had some distribution of the fluorescent signal, let us say I draw it like this is this is one strip the next time in the next time instant I would plot it again similarly the next time instant I would float it again. So, what I will get is these treks I will get and if I connect these treks. So, this is how a given point in the membrane evolved as a function of time and

what you would see is a line. So, this is time and this is length, so this length is taken from this direction backward.

So, this point is the Leading edge and this is for the width it is some point which is inside the cell. So, by connecting these dots I will get these lines and what you find is. So, because this is the leading edge and this is the trailing this is some point inside the cell a slope this sort, but this would give us Retrograde flow. So, you have a given retrograde flow. You can measure you can do multiple measurements and you can get some value of retrograde flow, so in terms of microns per minute or microns per hour. So, under control treated conditions cells the author source of some retrograde flow, when they perturbed Actin Polymerization with this drug called latrunculin. What latrunculin does is it sequesters G-actin as a consequence your polymerization is stopped and what they found is latrunculin treated cells you have the same amount of retrograde flow. We have 2 conditions in which the retrograde flow does not change when you perturbed the polymerization.

What does it suggest it suggests that probably it is again the myosin motors behind the lamellipodia which is pulling the entire network backwards if? If this was true then if you do experiments with Latt plus blebbistatin blebbistatin is the myosin inhibitor what they observed was that in latt plus blebbistatin this was completely eradicated your retrograde flow is completely eradicated. This suggests that Myosin Contributes to retrograde flow. So I will stop here for today and we will continue the from here in the next class.

Thank you for your attention.