Introduction to Mechanobiology
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Week - 03
Lecture - 13
Focal adhesions: focal adhesion proteins

Hello and welcome to our lecture on introduction to mechanobiology. So, in the last class we started discussing about focal adhesions right. So, focal adhesions are those structures which stabilize cell shape. And they are those dynamic wells which connect the inside of the cell with the outside of the cell.

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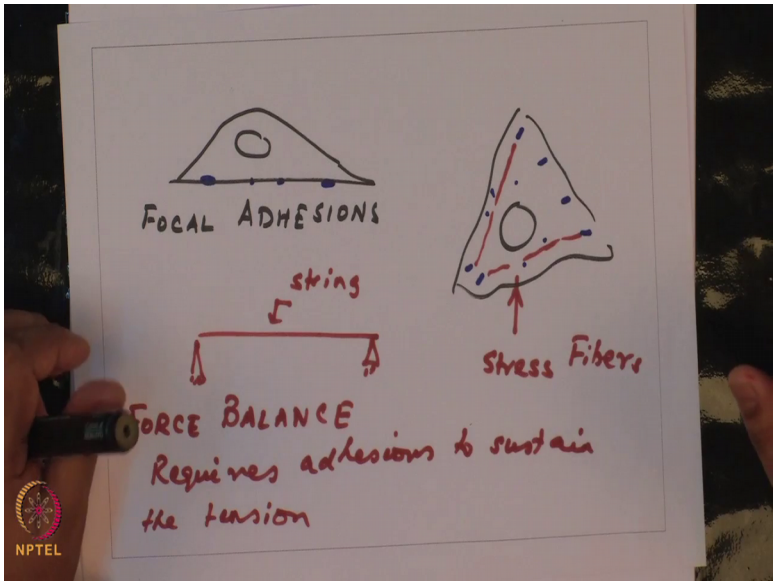

So, if you draw of cell with the focal adhesion for an idea and cell, you would draw it typically by depicting these adhesions like these dots. So, these are adhesions of the cell focal adhesions ok.

So, what are So these So if you draw if you take an image of cells with focal adhesions in top view, you would get an image. Let us say if this is a cell you would have these adhesion straight across. So, what you see is here I have drawn the focal adhesions as some of them are in the periphery of the cell which are relatively bigger in size, and towards the center of the cell I have just drawn them as dots ok.

So, focal adhesions are composed of more than hundred proteins, these are dynamic structures that is why it enables cells to migrate.

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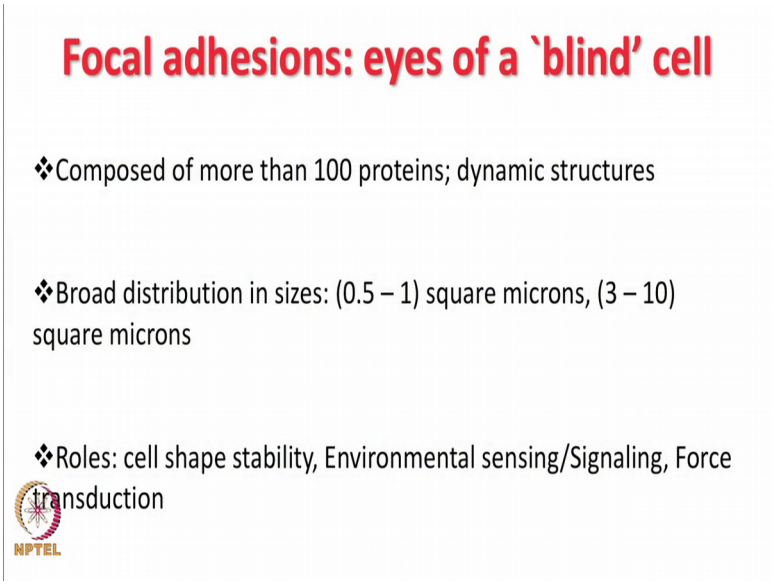

So, just like you walk you put your foot forward and then you put your foot on the ground. And that is equivalent to a forming a focal adhesion. So, if your substrate is very slippery or without does not provide any friction link class, then your additions are weak it is difficult to walk on glass ok.

So, focal adhesions are composed of more than hundred proteins actually close to 200 or even more. So, the list keeps on growing, you have a broad distribution in strizes. So, you might have adhesions which are 0.5 to 1 micron square versus 3 to 10 micron square or even larger. So, they shows you that there are various sizes possible for focal adhesions. So, what are the roles of focal adhesions of course, it provides stability to cell shape it stabilizes the cell. Just like it is not possible to stand in water why because the substrate does not resist ok.

So, your substrate has to resist the contractile forces that cells exert in order to stabilize cell shape. And these adhesions are the mechanisms which may which enable cells to actually sense or see the surroundings, and how does the cell see via exerting forces. So, these adhesions also develop as points through which forces can be excited by cells, in terms of pulling forces or contractile forces, and those who are the forces responsible for

inducing wrinkles on the substrate when play when cells are plated on a soft compliant substrate ok.

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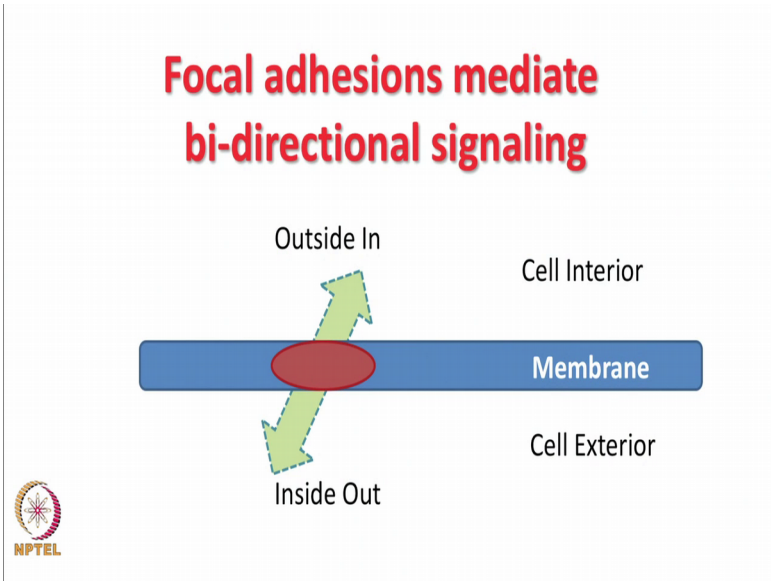

So, focal adhesions they would mediate bi directional signaling, in other words you can have outside in or when the cell is pulling it is transmitting information about the surroundings from the outside to the inside, or you can have inside out in which the cells probably pushing outside. Both these cases you have focal adhesions So, it is perhaps not surprising that given the gamut of functions that these focal adhesions perform, that there are. So, many different proteins within present within a focal adhesion. So of course, the most important among them is integrins.

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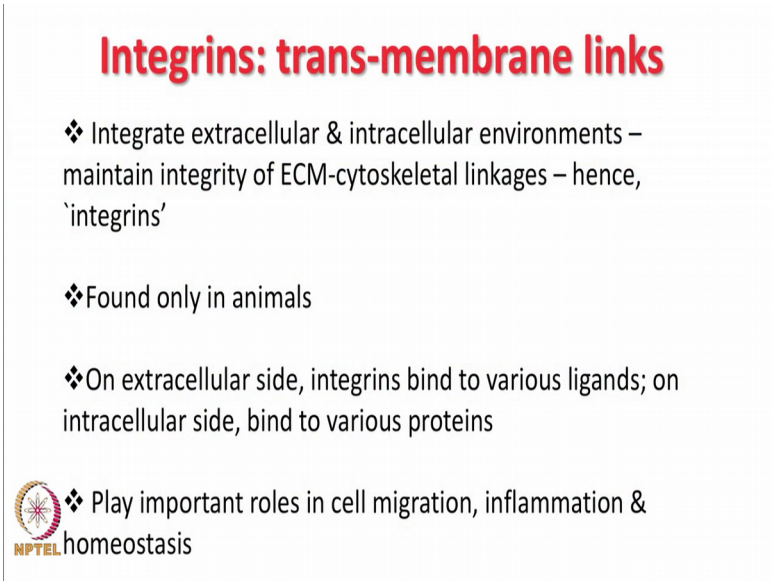

They So integrins serve as trans membrane links which integrate the inside to the outside. And that is the reason why they are called integrins. So, so the they maintain the integrity of ecm cytoskeletal linkages ok

Now, integrins are only present in animal cells. So, on the extracellular side these integrins would bind to various ligands and on the intracellular side to various proteins first of all within the focal adhesion itself, you have multiple proteins which interact with integrins. And these integrins play important roles in migration information homeostasis so on and so forth. So, if you look at the structure of integrins So they are non covalently associated heterodimers. So, they have 2 unit is alpha and beta then hence called the heterodimer, and these unit is associate in a non covalent manner depending on the outside signal ok.

So, only So these subunit is come together when in when they are active, and this conformational changes in the subunit actually lead to signaling through binding of cytoplasm proteins.

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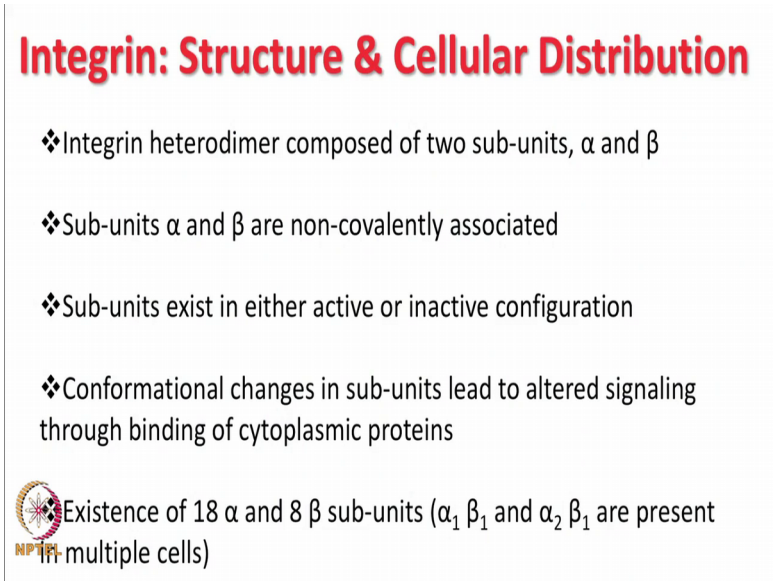

So, you have various combinations of integrins possible actually 18 alpha and 8 beta subunit is have been determined ok.

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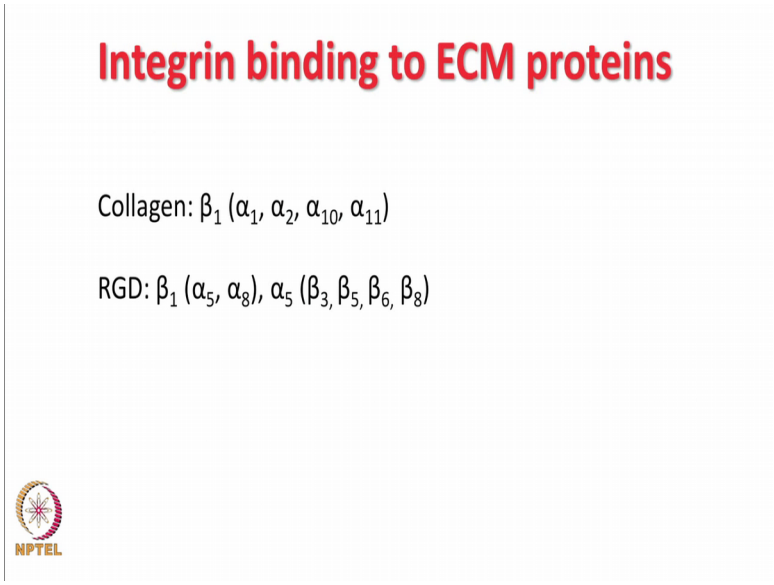

So, if you look at the kind of binding you can you can obtain. So, for example, for binding to collagen you can have various combinations. So, what I have written is beta 1 and then within bracket alpha 1 alpha 2 alpha 10 and alpha 11. This would mean that cells might have alpha 1 beta 1 alpha, 2 beta 1 alpha 10 beta 1 alpha 11 beta 1. Similarly for binding to RGD you can have various combinations in one in which you can have

alpha 5 beta 1 alpha 8 and beta 1, or you can have alpha 5 beta 3 alpha 5 beta 5 so on and so forth ok.

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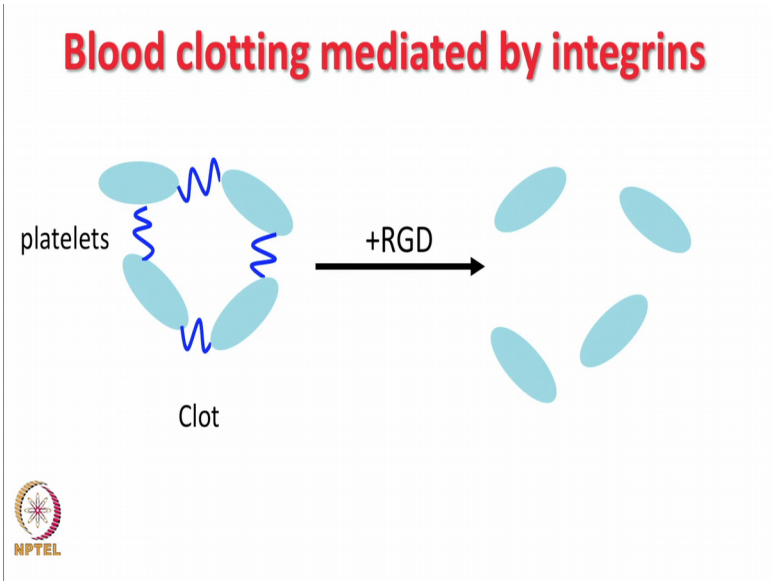

So, integrins one of the most important function of integrins was found in the case of mediating blood clotting. So, what I have depicted here is platelet us or white blood cells, which are connected by these blue spring like structures. So, these are fibrinogen which is engaged by 2 platelet us that 2 opposite ends. So, at this point of contact between a platelet and the fibrinogen you actually have an integrin connection, alpha 2 b beta 3 integrin is what binds to fibrinogen; however, when you add this peptide RGD you see that the clot falls apart. So, this is the clot blood clot and when you add a RGD it falls apart.

Why is that? Because RGD the affinity for RGD for alpha to be beta 3 is much higher than the affinity for alpha 2 b beta 3 towards (Refer Time: 06:43) as a consequence of which because of competitive on unbinding. So, the clot will fall apart. So, in a sense blood clotting is mediated by integrins. And integrins are central to So many different functions.

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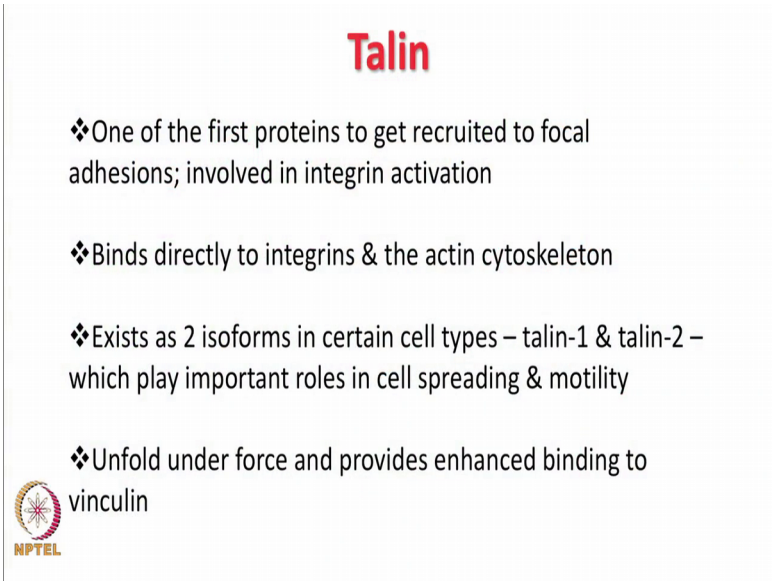

Let us look at another focal adhesion protein this is talin. So, this is one of the first proteins to get recruited 2 focal adhesions. And they are actually involved in activating integrins. So, this perhaps not surprising that talin directly binds to integrins as well as to the actin cytoskeleton, later on in today we will see how these molecules are located. So, you might have you have you will have 2 different isoforms of talin talin 1 and talin 2. And these have differential functions which regards to cell spreading or cell mortality.

Now one interesting aspect of the talin molecule is, it binds to another focal adhesion protein structural protein called vinculin, and what has been shown is talin domains unfold under force and expose binding sites for vinculin. And this recruitment of vinculin binding to talin reinforces focal adhesions.

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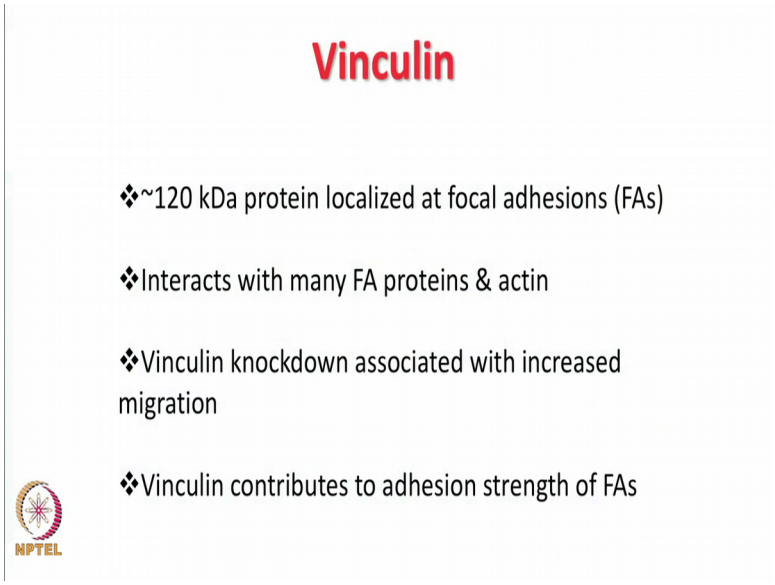

So, this brings us to the to vinculin. So, vinculin is a 120 kilo delta protein again localizing at focal adhesions, again it interacts with many focal adhesions proteins actin. Now if you knock out vin knockdown vinculin what you see the cells become faster migration.

So, suggesting that vinculin actually regulates the adhesiveness of cells to their substrate. So, if you increase vinculin that should lead to suppression of cell motility versus cells will become steady and they will form bigger adhesions with their substrate ok.

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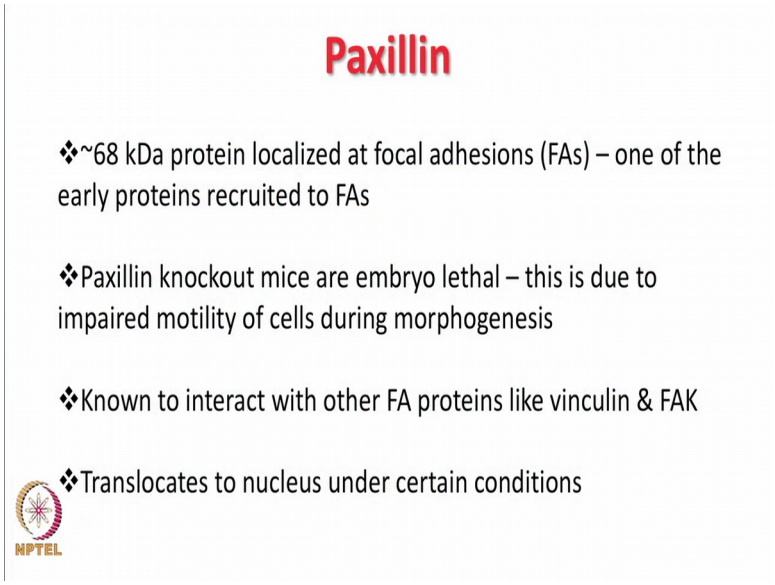

So, apart from vinculin and talin. So, is another very critical focal adhesion protein called paxillin. So, again it is localized at focal adhesions one of the earlier proteins to be recruited to focal adhesion. And what has been observed is when you knock out paxillin in mice the mice died. So, basically paxillin knockout mice maxon knockout is embryo lethal. And one of the reasons being that paxillin knock out paxillin then morphogenesis are development is stopped. It is known to interact with other focal adhesions proteins and interestingly under certain conditions paxillin in spite of being a focal adhesion protein, it can translocate to the nucleus and regulate other signaling boxes ok.

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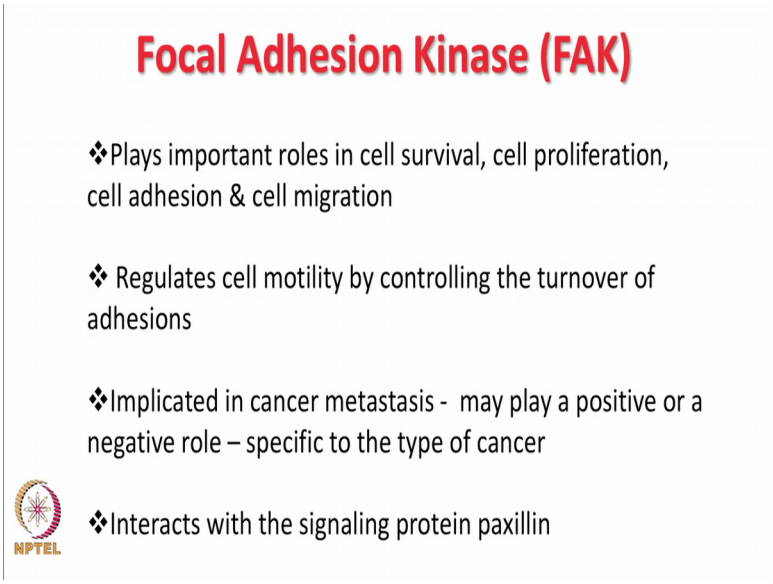

So, what you see here this molecule called FAK. FAK is nothing but focal adhesion kinase again it has important roles in cell cyber cell survival proliferation addition and migration, and what focal adhesion kinase has been shown to do is it regulates cell movement by controlling the turnover of focal adhesions. That is your additions once formed if they do not break then the cell cannot properly itself forward. So, you have to have breakage of additions and that is mediated by focal adhesion kinase.

So, in other words if you knock down the level of focal adhesion kinase in a cell then the cell will be less motile even though it has all the other machinery in places. And interestingly in the case of diseases like cancer FAK can play both a positive role; that means, it leads to potentiation of the disease or a negative role which will inhibit cancer. So, again it interacts with the signaling protein of paxillin ok.

So, you have these proteins which kind of bind interact mind and interact with each other one another. So, these are also focal adhesion kinase and paxillin are broadly called sing integrins signaling molecules. So, you have integrated your integrin signaling molecules. You have structural proteins like vinculin and you have So these focal adhesions. So, when you see an image of a cell what you will always find is there are at the end of each of the focal adhesions they are connected by these big stress fibers. So, these are nothing but stress fibers. So, it is equivalent to our anchoring a rubber band to the substrate via 2 adhesions. So, these are your focal adhesions and this is your string or stress fiber ok.

So, amount of tension you can put in the string is dictated by how much these adhesions can support. So, for a cell which exert a lot of force if here. So, force balance requires adhesions to sustain the tension. So, if you increase the tension too much then beyond a certain point focal adhesions will fall apart ok.

So, apart from these proteins you have some cytoskeletal proteins, which you see is these stress fibers what I have drawn are structures, where actinin myosin come together and these stress fibers are also put together in like they stay in place through actin associated proteins. And one of the most important actin associated proteins is this protein called alpha actinin. Alpha actinin is an actin cross linking protein. So, in cells apart from alpha actinin So, you have various actin cross linking proteins ok.

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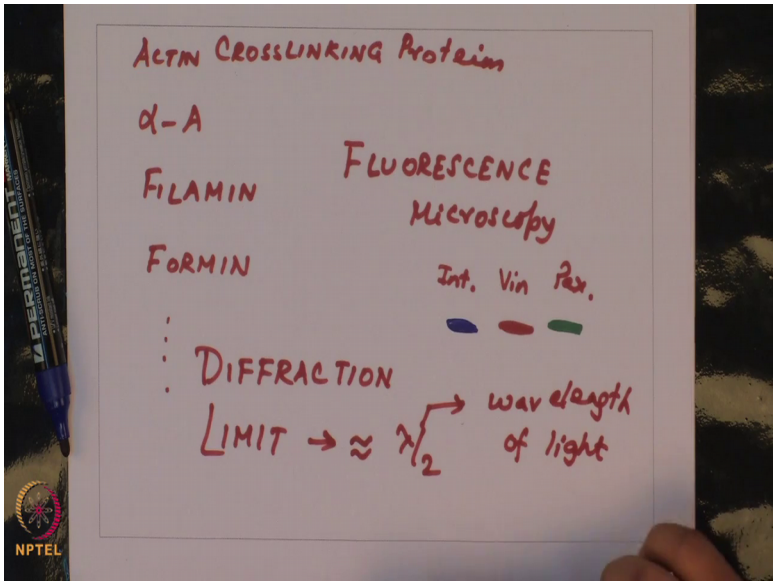

So, you have alpha actinin, I will write alpha A you can have filamin, you can have formin and various other protein. So, you have several different actin cross linking proteins. So, they differ in their sizes in how they cross link the actin network so on and so forth.

So, as the consequence the localization within different actin structures varies notably. So, for actin you have 4 different isoforms of actin 2 of them alpha actinin 2 and alpha actinin 3 are specific to muscle cells, the other 2 of them alpha actinin 1 and alpha actinin 4 are presented all non muscle cells.

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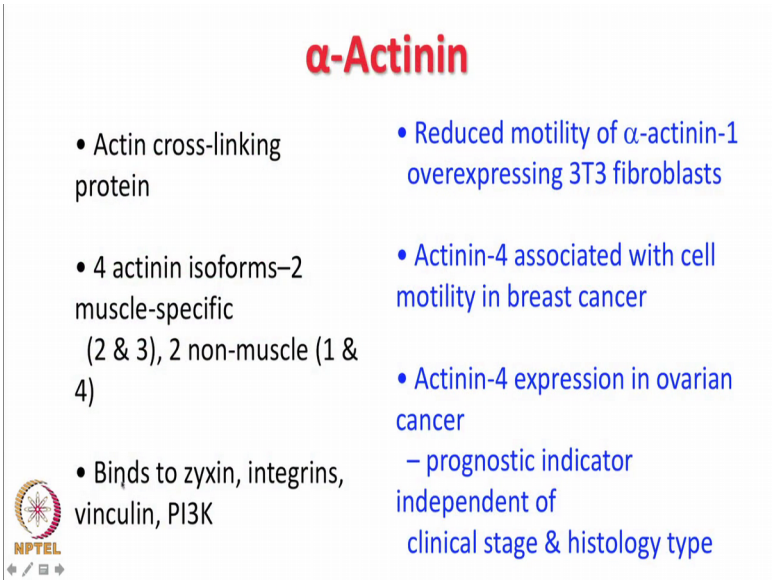

So, alpha actinin has been shown to bind to various integrins proteins including integrins vinculin. So, signaling, protein called pi 3 kinase and zyxin. What has been observed is alpha actinin 1 and alpha actinin 4 have been found to play opposite roles with regards to motility.

So, if you overexpress alpha actinin 1 in 5 blast cells become less motile, suggesting that alpha actinin one regulates cell motility or negatively regulate cell motility. In contrast alpha actinin 4 is associated with enhanced motility of breast cancer cells. And it is not just true for breast cancer cells across multiple different cancer cells, including brain cancer or tumor cells, ovarian cancer cells, pancreatic cancer cells so on and so forth. What is also been recently shown is actinin for expression in ovarian cancers has been

can be used as a prognostic indicator of independent of clinical stage and histology type ok.

So, these results suggest that alpha actinin which is an actin proxillin protein is a critical regulator of motility or invasion. So, this brings us to the question you have So many different focal adhesion proteins hundreds of them. If you were to put together at a single focal adhesion and we draw the very simple we schematic of a focal adhesion like this, just a dot or an or an ellipse, but it brings to question that they must be spatially organized. It is not that you just put hundred different proteins in a box and the entity will operate as a focal adhesion. So, because they have specific binding to several other proteins it is important that they are specially local localized. So, how would you address this question of localization at focal adhesion. So, if I were to imagine the focal adhesion as a 3 d entity, where are each of these proteins present. So, to answer this question if you rely on conventional optical microscopy you would not be able to answer that question ok.

So, if you were to stain if you were to use fluorescence microscopy. So, I am just drawing several proteins all of these in one channel you might see this, in another channel you might see this, in another channel you might see this, suggesting that they span the entire focal adhesion. So, this is 3 different channels you have labeled 3 different proteins let us say let us say this is vinculin, this is integrin and this is paxillin. All these proteins will show you this kind of localization. So, you it is impossible to know how they are specially localized within this validation. And the reason for this is because the resolution, the resolution of normal conventional microscopes is limited by diffraction ok.

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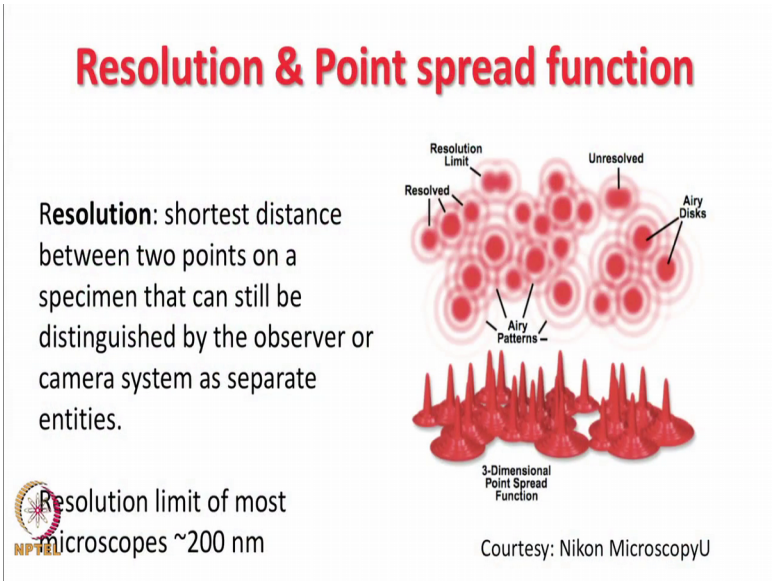

So, what we saw? Which will call is ah diffraction limit. So, how do we define resolution? So, the resolution is defined as the shortest distance between 2 points on a specimen that can still be distinguished by the observer or camera as separate entities. So, if you look at these pictures. So, what this is a representative picture of multiple dots which are positioned close to each other. And what it appears of a dot will appear as this particular structure of concentric structures if you replot in 3 d it looks as a point spread function. It is like a 3 d histogram which has a given peak, but it also has a spread as you go far out. So, if these So, these are also called airy disk. So, if 2 points are relatively spaced apart. So, even when you look took at these images there are 2 den spots representing the actual point center point of this dot. So, in this case the dots will be positioned here and here, but the dots will appear like these concentric circles.

So, in some cases if these points are far from each other you can resolve these points. This is called the resolution limit you can you can still make out. So, even though the histograms are coalescing or fusing, you can still make out where lies the center point of these dots; however, if you look at this particular point you cannot distinguish between the 2 points, because they have completely converged on each other. So, the resolution limit of most microscopes So, you call it the diffraction limit. So, diffraction limit is you can approximately say lambda by 2 where lambda is the wavelength of light. So, this is not the exact expression, but this is roughly gives you an idea of how much would be the resolution limit.

So, what is resolution limit is of most microscopes are ordered 200 nanometers, which is to say that an object which is less than 200 nanometers would look like 200 nanometers. So, even if you take a particle which is 10 nanometers in size, when you image in the microscope image it will appear that the that the that that particle has a width of order 200 nanometers. So, which would also mean that if there were 2 particles which were closely spaced this impossible to resolve them. So, then it is impossible to given that proteins have sizes order of nanometers and lesser. You cannot resolve 2 proteins within a single focal adhesion, you will just get is a block ok.

So, to circumvent this problem, scientists have come up with this alternate technique called super resolution microscopy.

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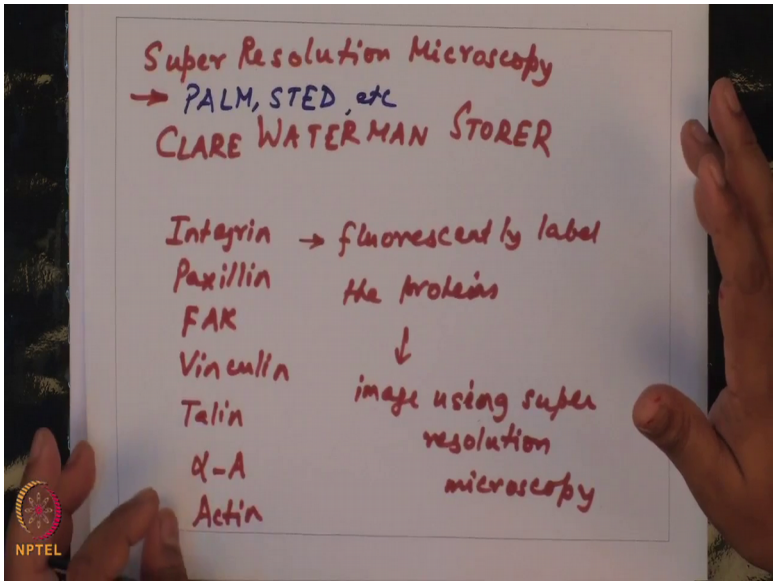

An in the super resolution microscopy. So, you can actually detect the 3 d position of proteins.

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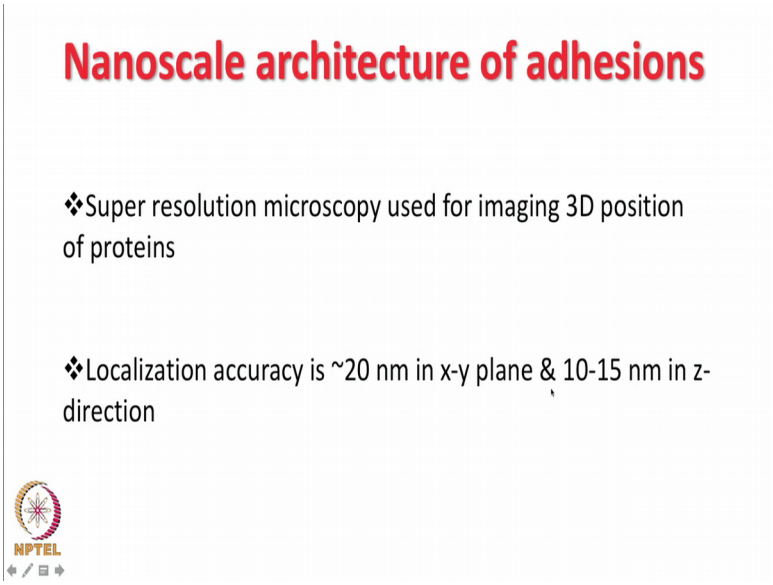

And why is that? Because if you look at the localization accuracy this within 20 nanometers in xy plane and 10 to fifty nanometers in z direction. So, this will really help you to identify how these proteins are located along the x y z plane. So, using this technique, using this technique clare waterman storer. So, what she did? She took some of the most important focal adhesion proteins in terms of integrin, paxillin, focal adhesion kinase, vinculin talin as well as alpha actinin and actin.

So, what she did was she labeled these proteins. So, you fluorescently label this proteins and then image using super resolution microscopy. So, there can be separate different types of super resolution microscopy. So, solve them are for example, palm sted etcetera. So, these are various techniques where in you can image super resolution you can image cells in super resolution ok.

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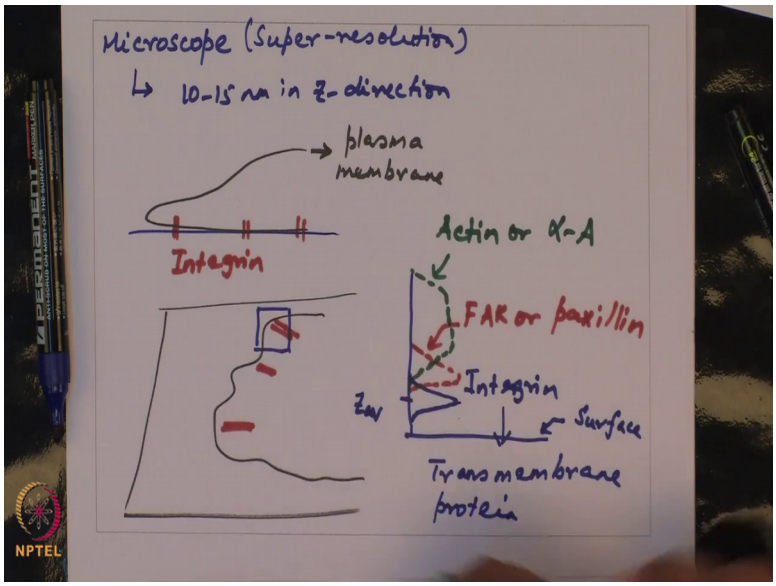

So, now let us say. So, you have a microscope super resolution. So, which means that it can detect 10 to 15 nanometers in z differences is in z direction. So, when you take a cell that this is the substrate and this is your cell. And let me draw the integrins let us say. So, this is your plasma membrane. So, inside view the same picture might look something like this. So, you have a 2 d plane and these focal adhesions would look like this. So, if we have fluorescently labeled integrin, let us say and we take a box here a small box here, and we ask the question that in this box how is the integral signaling distributed along the z direction. So, what you can get is So, if I were to draw this as a z direction you would get a distribution. And you can find out what is the z average of integrin.

So, similarly you can label one by one. And you can see So, for example, compared to integrin one would expect if you are staining with, let us say paxillin or ziksi or FAK you have some signaling. So, this would be FAK or paxillin. So, why is this expected? For the simple reason the So, your integrin is a trans membrane protein ok.

So, integrin being a trans membrane protein from the surface. So, let us say this is my surface, of all the focal adhesion proteins integrin should be the closest one from the surface. Similarly FAK or paxillin both these proteins are known to bind to integrin, and at the same time they do not have trans membrane domains. So, they are still cytoskeletal proteins. So, you must have them slightly above the integrins. In contrast actin or alpha

actinin should have a broad localization deep inside. So, this would be actin or alpha actinin ok.

So, based on this based on this what clare waterman storer showed was you have integrin layer on top of which you had ok.

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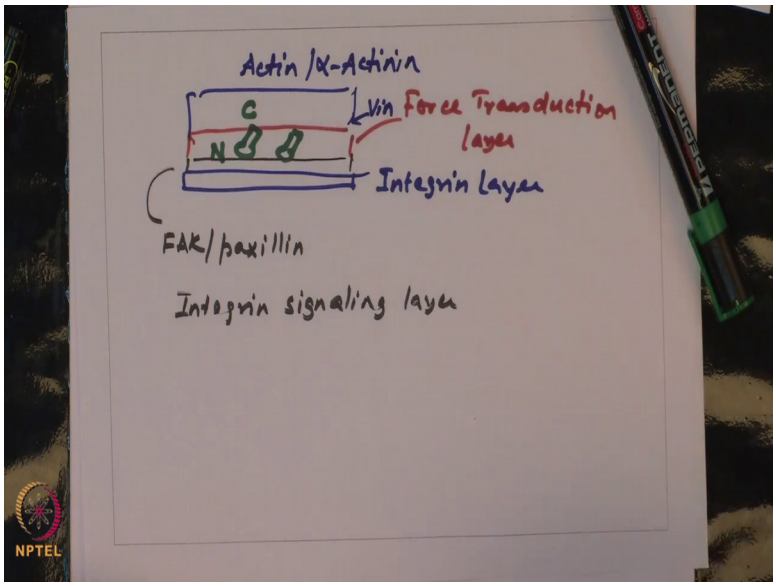

So, here you had proteins like FAK or paxillin in which localized and this is called the integrin signaling layer. On top of which, this layer was more thicker you had a force transduction layer. Now within the force transduction layer what was what she showed was the molecule called talin is actually localized something like this. With the n terminal protein closer to the integrin signal layer and the c terminal of the protein being away from it is closer to the actin there.

And on top of this, so here is where vinculin was also there, and on top you have actin and alpha actin. So, you this shows that there is a gradation or special localization of proteins within the focal adhesion it is not like a block. I would like to point you to read the following 2 papers this kanchanawong et al this is the clare waterman paper published in nature 2010 and the other paper patla et al nature cell biology 2010.

Thank you for your attention.