Introduction to Mechanobiology
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Week – 03
Lecture – 12
Protein unfolding using AFM

Hello and welcome to today's lecture. So, in the last class we had discussed about unfolding of fibronectin and how you can go about designing experiments to determine the unfolding signature of individual domains within fibronectin stream module.

So, if I would to write down the steps you have to engineer multiple protein constructs. So, I wrote it as AB of n where n is a variable. So, n is chosen is n is experimentally determined so as to ensure the engineered protein folds properly.

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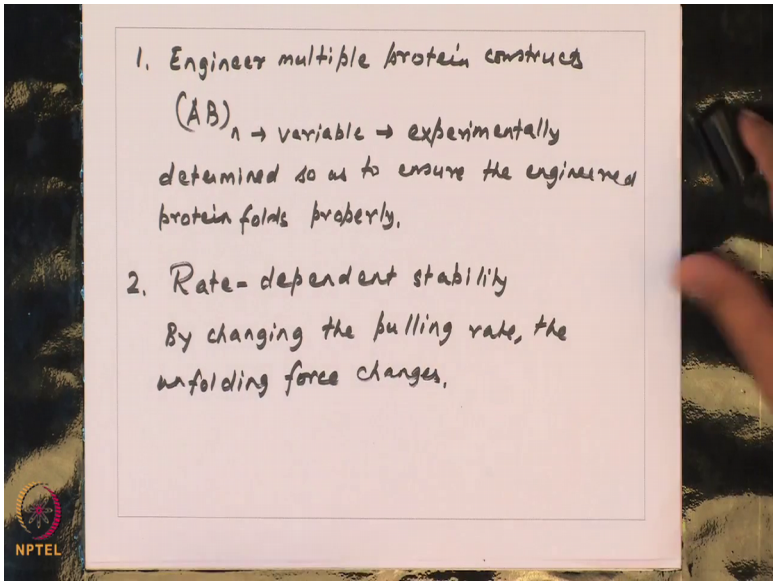

So, by doing this and choosing multiple different constructs you can determine the folding signature of multiple domains one thing I introduced in the last class briefly spoke about is rate dependent stability. So, what do I mean by rate dependent stability? By rate dependent stability I mean that by changing the pulling rate the unfolding force changes and how does it change.

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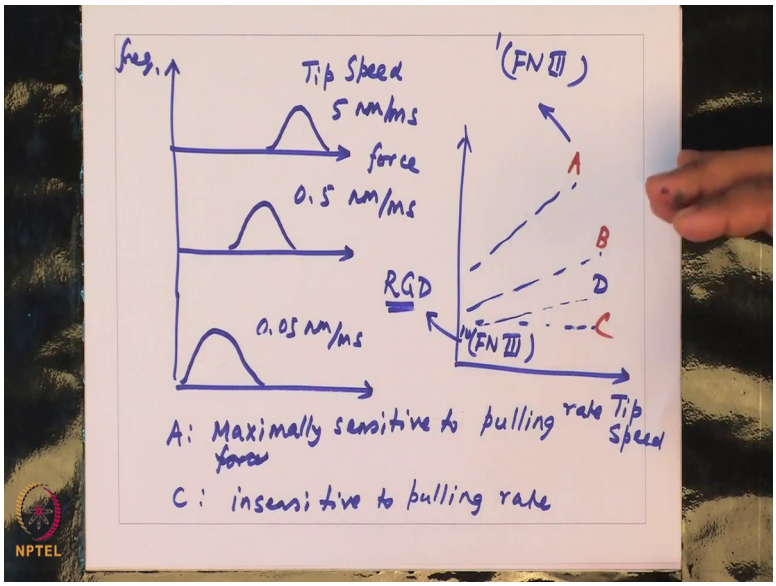

So, more often than not what you will see, this is let us say at a given tip speed. So, you have what you are changing is this tip speed. So, if this is let us say 5 nanometer per millisecond this is 0.5 nanometer per millisecond and this is 0.05 nanometer per millisecond ok.

So, this axis is frequency this axis is force. So, if you plot it for multiple different domains, this is tip speed or pulling rate. So, you will get. So, I have drawn representative rate dependent forces for three different domains in this case A is maximally sensitive to pulling forces pulling rate and C is insensitive to pulling rate.

So, for the case of fibronectin for example, it has been observed that if FN 3 first domain of FN 3 is maximally sensitive for forces and the tenth domain let us say D D is not, but FN 3 tenth domain. So, tenth domain of FN 3 contains the RGD sequence. So, this is on an average less sensitive to pulling forces.

So, what if a cell is exerting so much force that the entire protein folds will that be the end a function of that protein or not, what has been observed is there is another aspect and you can do that what it says that if you release the tip or hold it in one position.

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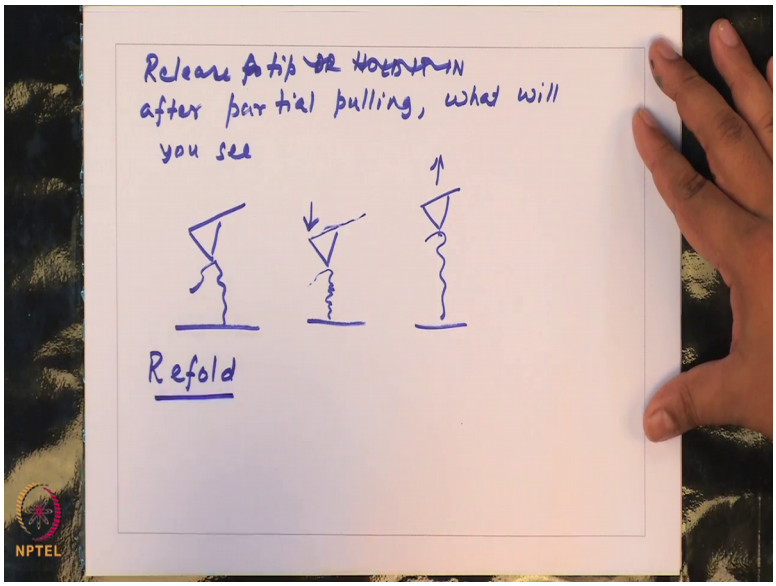

If release the tip after partial pulling what will you see. In other words, let us say this is a pulled configuration from this point the tip goes down and then you again pull you again pull. So, what you are introducing it is you would see that this time allows when you relax the tip release the protein it allows the protein to refold and how do you know what is the signature of refolding. So, let us say.

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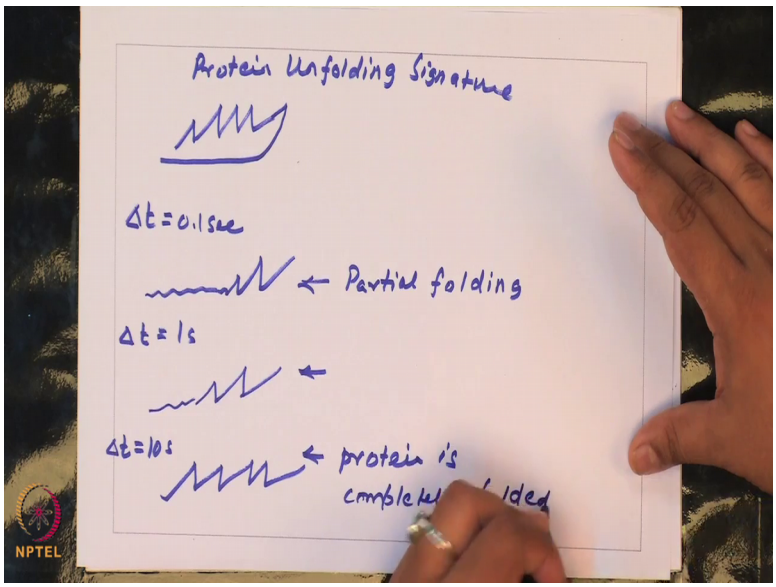

So, this is the protein signature right protein unfolding signature. Let us assume if delta t which is 0.1 second after that if you pull again what you will see is long no folding

signatures then something like this, if you put pull the protein after one second your signature might be and 10 seconds later you might have.

So, here you see that this is indicative of partial folding and here the protein is completely folded.

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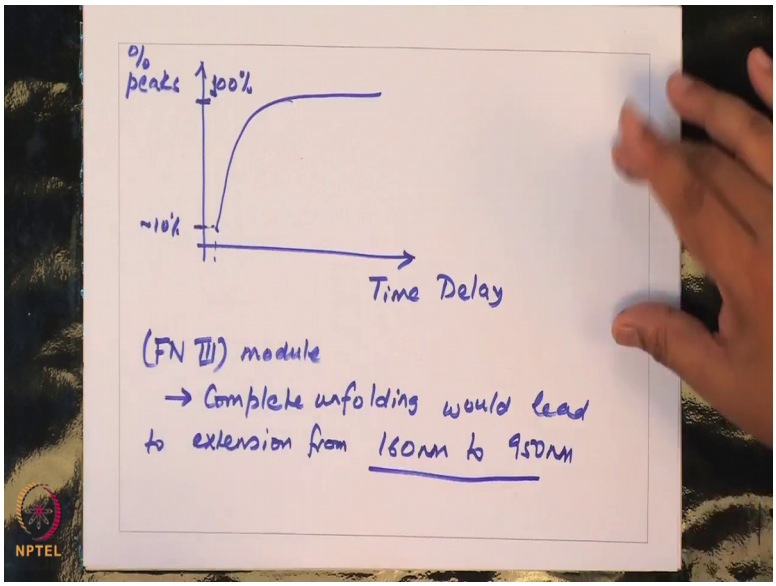

So, you can plot time delay percentage of peaks. So, what is this percentage of peaks? So, what you see is that how many peaks do you get. So, here at this value beyond a certain time delay this is going to be 100 percent ok.

So, if your time delay is negligible you would see a low value let us say 10 percent. So, that that would still indicate that one of the peaks has completely folded completely refolded. So, what you see over all is that the complete unfolding, so for FN 3 domain module, so complete unfolding would lead to extension from 160 nanometers to 950 nanometers. That is the increasing length that you observed even for unfolding of one FN 3 module one FN 3 module will lead to extension from 160 nanometers to 950 nanometers what are the other things. So, how do the unfolding forces look?

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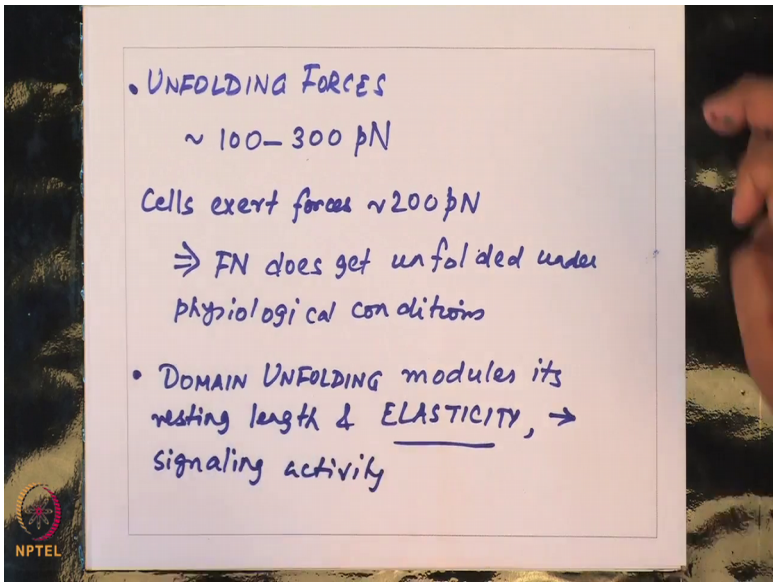

So, you have seen that unfolding forces unfolding forces have magnitude order let us say 100 to 300 pico Newtons and it is known that cells can exert forces order 200 pico Newtons.

So, this would be imply that fibronectin does get unfolded under physiological conditions. The last point, so what does unfolding achieve. So, domain unfolding, in a sense what it is modulates and elasticity and of course, the signaling.

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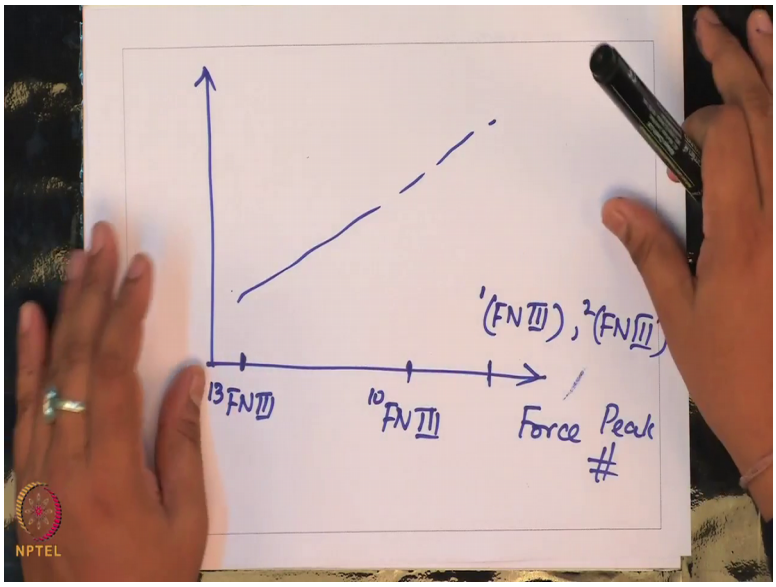

If I want to draw force peak number so at the initial ones, you will have you will have a linear force curve increase in forces and among the very first ones which unfold is thirteen FN 3 your FN 10 which contains the RGD sequence has an intermediate force and among the highest forces domain 1 and domain 2. So, these are among the highest forces. So, what you see is a great increase the amount of unfolding force and forces regulate the downstream signaling cascade as well as the effective elasticity of the protein ok.

So, with that I conclude our discussion on ECM proteins and how non-linear elasticity is engrained either at the level of a network ECM network or at the level of single proteins. So, as part of reading assignment I would like to assign the following two case.

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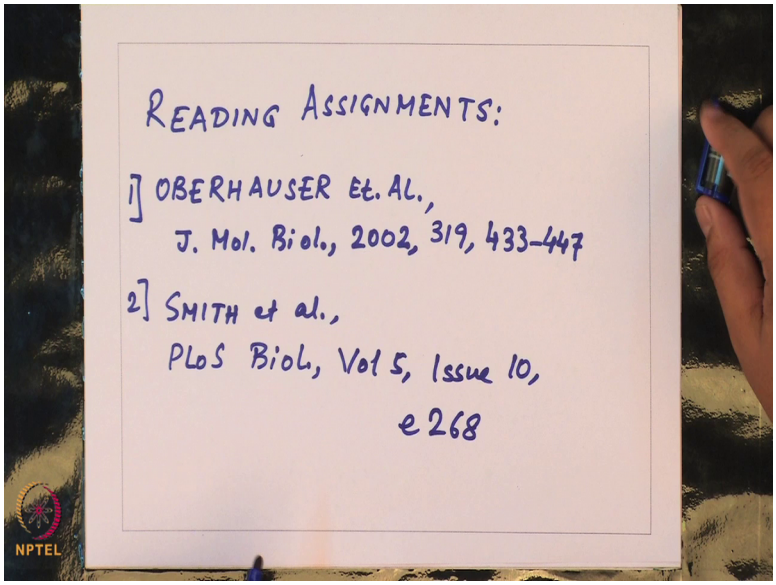

So these please read to these two papers you will get good understanding about fibronectin unfolding both these papers focus on fibronectin unfolding and most of what I discussed just now over the last two lectures two three lectures comes from the first GME paper. So, that concludes our discussion of ECM proteins.

Now, I will again come back to our analogy of cell as a tent.

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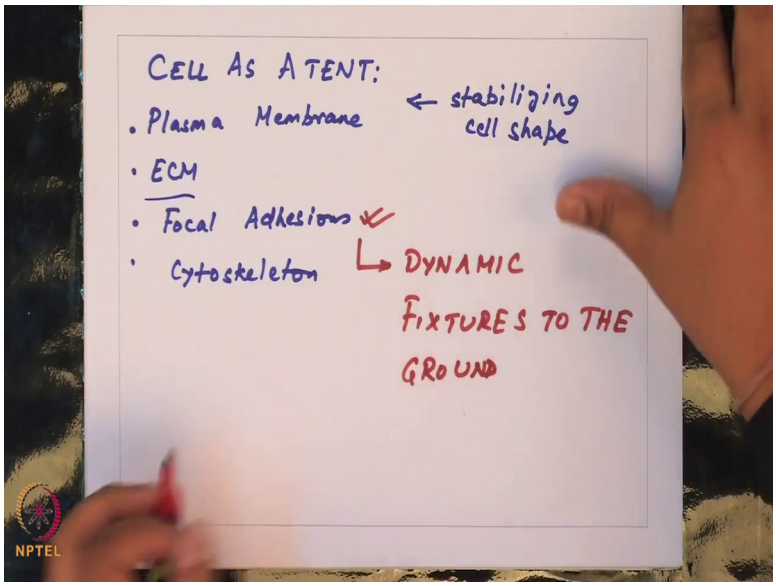

You have cell as a tent. So, we discussed, there are four things right plasma membrane ECM and cytoskeleton. So, there are these four things, so plasma membrane ECM focal adhesion and cytoskeleton ok.

So, these are the four things which are required for stabilizing cell shape. So, of these we discussed ECM and now we are going to start discussing focal adhesions what do focal adhesions do. So, this focal adhesions are nothing, but dynamic fixtures to the ground. So, now, we know that for adherent cells.

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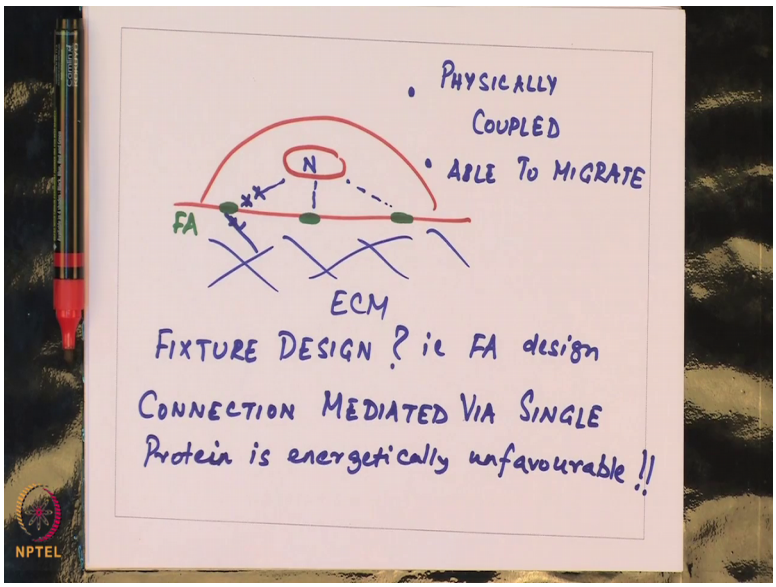

So, if I draw the cell and you have these ok. So, this is your nucleus, this is your focal adhesion, this is FA and outside you have the extra cellular matrix. So, if we ask that what kind of function these focal adhesions must serve. So, we know that for adherence cells the cell must remains stuck to the substrate, but at the same time it should be in a position to migrate. So, the cells should be physically coupled and be able to migrate.

So, what does what kind of requirement does it put on the design of the fixtures what can I say about the fixture design that is FA design. So, since the focal adhesion acts as the real link from the inside and the outside, so if you had it as a single protein. So, you would need for the cell to mood you would need to disengage the cytoskeletal as well as the ECM network it is connected to at all types, but that would be energetically very unfavorable.

So, connection mediated via single protein is energetically unfavorable. So, there must be some other signal the way that focal adhesions must be designed must be such must be such that so the design; must be such that cytoskeletal does not need to be fully dismantled as cells move ok.

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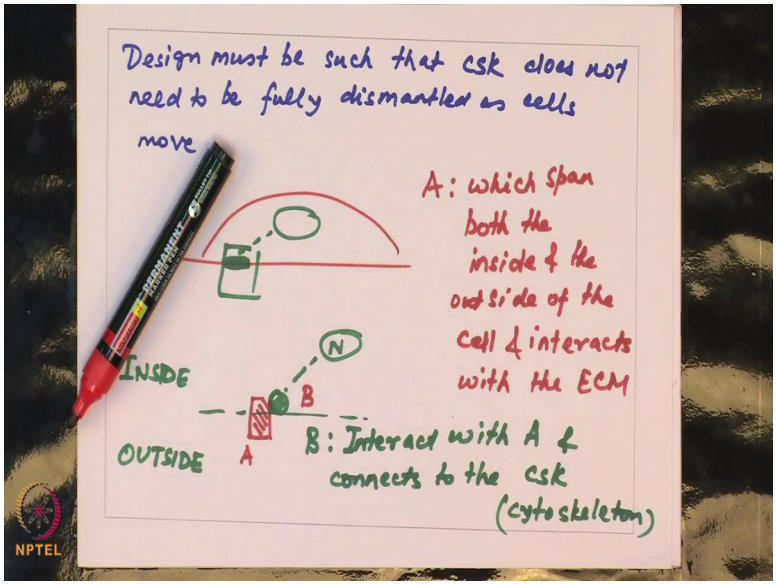

So, if I want to zoom in to the focal adhesion. So, this for your focal adhesions, if I zoom in to this structure what I propose, this is inside the cell and this is outside so the design of the focal adhesion probably might have some transmembrane protein linked some cytoskeletal protein and then you have the connection to the nucleus.

So, you have two entities - entity A which span both the inside and the outside of the cell and interacts with the ECM and you have another entity B which interact with A and connects to the CSK is short for cytoskeleton. So, in this way when the cell migrates just decoupling of B from A can allow A to again depolymerize or come off and re engage the outside and then as B connects you can have this dynamic turnover ok.

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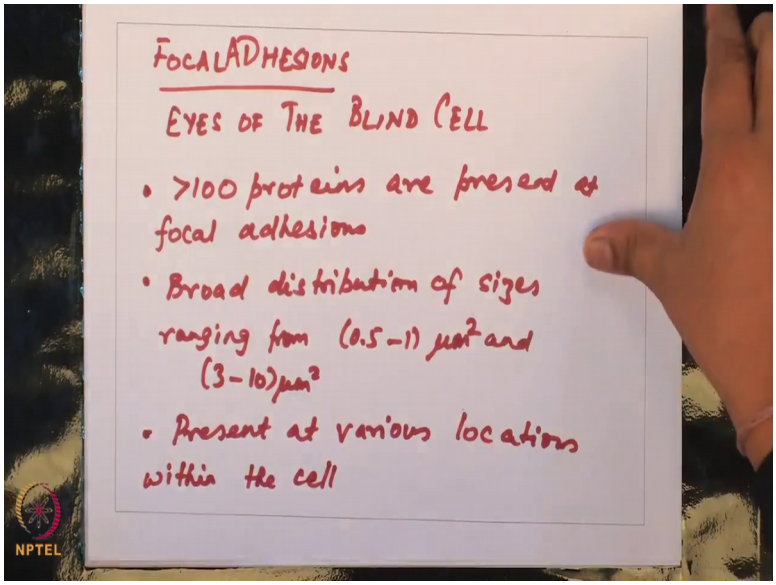

So, these fixtures or focal adhesions adhesions serve as the eyes of the blind cell and unlike the simply the way I simplified its design the focal adhesion does not comprise of one cell it has greater than 100 proteins. So, you can have more than 100 proteins localized at focal adhesion and the focal adhesions serve various functions more than 100 proteins. So, you will also have a broad distribution of sizes ranging from 0.5 to 1 micron square and 3 to 10 micron square. So, clearly you see that there is two different size distribution of these adhesions. And they are present at various locations within the cell.

So, what are the functions of this focal adhesions?

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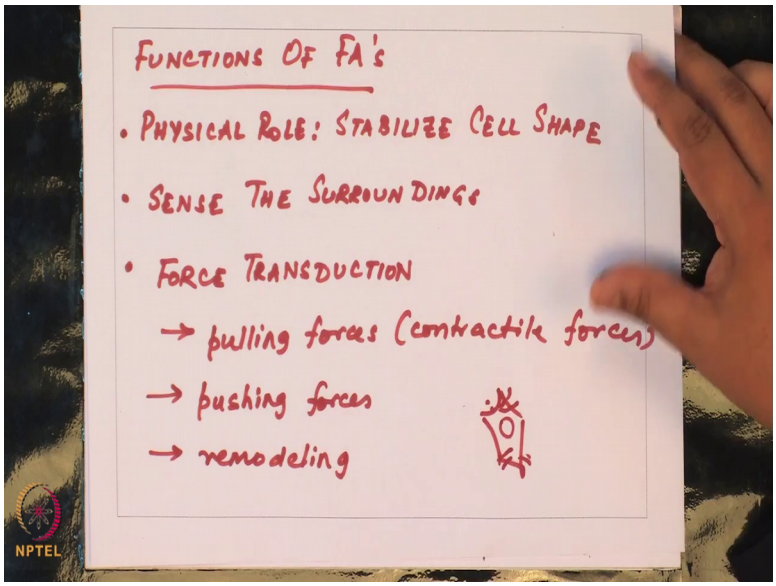

So, FA apostrophe s is short from powerful adhesions. So, they form the physical role that is stabilize the cell or stabilize cell shape and actually they are the ones which actually sense the surroundings and how do the cells sense the surroundings through focal adhesions by means of force transduction.

So, you can have pulling forces that is contractile forces you can have pushing forces pushing forces and you can have remodeling. So, pulling forces are contractile forces are what induced these wrinkles in cells right, I found seen this presence of these wrinkles and that is what pulling forces induce wrinkles in the matrix where would be an example of pushing forces ok.

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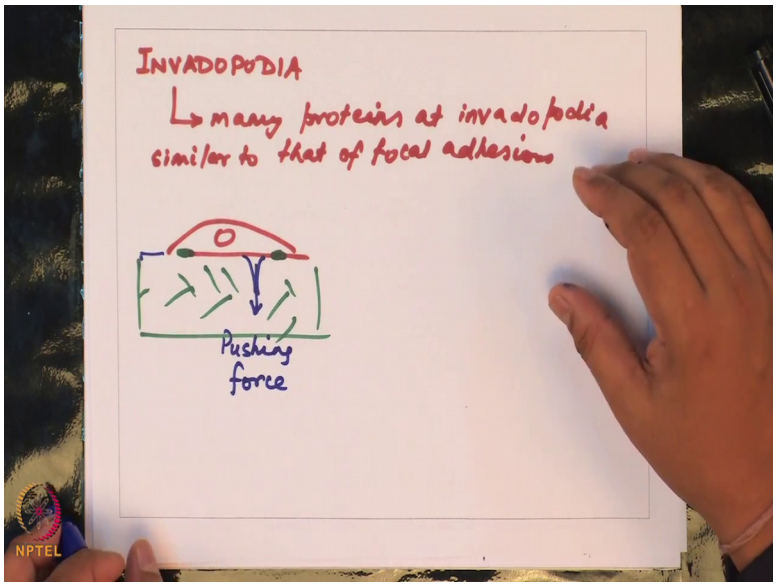

So, one case of pushing forces might be in the structures of invadopodia, it is not necessarily a focal adhesion structure, but invadopodia contains many proteins at invadopodia similar to that of focal adhesions. So, in contrast to focal adhesions which are formed on the plane and invadopodia actually protrudes into its matrix. So, this is the matrix. So, these are in basic structures where cells actually protrude into the matrix. So, you can imagine a pushing force being exerted. So, that completes our initial understanding of what focal adhesions are. So, I said that there are lot of proteins. So, there are lot of proteins which are present in focal adhesions and so one of the most important proteins is integrin.

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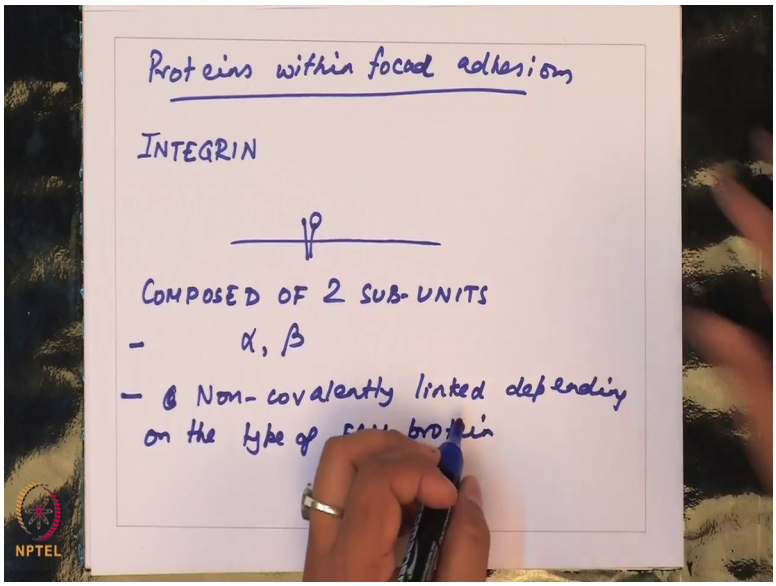

So, integrin is the one which is present transmembrane which is present across the membrane. So, you have an integrin, integrin is composed of two sub units. So, these are alpha and beta and these sub units are non covalently linked depending on the type of ECM protein. So, if I want to look at some of the combinations of integrins, so here I have drawn what I have drawn in the center is beta 1 and what you see is beta 1 is common to many of the integrin combinations. So, you can have for all these collagen receptors you can have alpha 1 beta 1.

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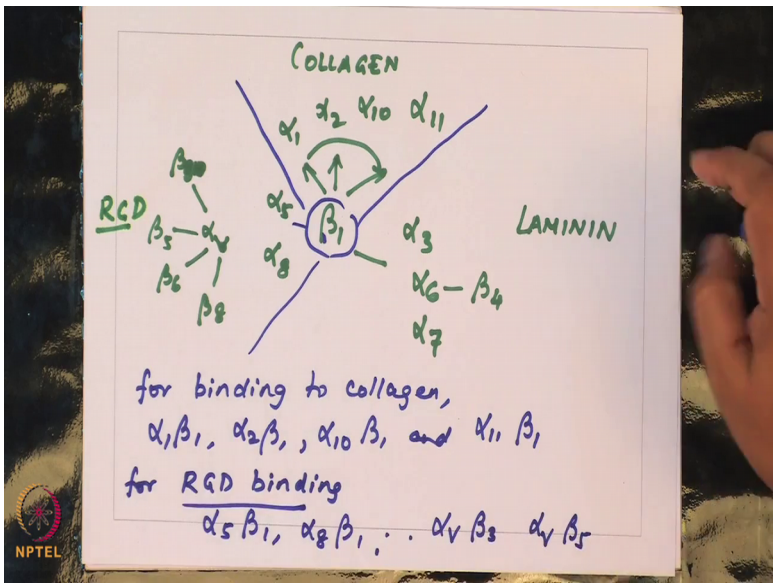

So, for binding to collagen you can have alpha 1 beta 1 alpha 2 beta 1 alpha 10 beta 1 and alpha 11 beta 1 ok.

Similarly, for binding to RGD you can have. So, for RGD binding you can have alpha 5 beta 1, alpha 8 beta 1 and also you have alpha v beta 3 alpha b beta 5 so on and so forth. Similarly you can have various other combinations for binding to laminin. So, in total there are 18 alpha and 8 beta sub units.

So, you have multiple different things for example, in fibroblast in most cancer cells alpha 2 beta 1, alpha 1 beta 1 are very common. With that I stop here for today and I will continue in next class discussing some more of the focal adhesion proteins and then discussing about their structural position or location within the focal adhesion.

Thank you for your attention.