**Introduction to Mechanobiology**
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Week - 07
Lecture - 32
Nuclear Mechanotransduction: LINC Complex

Hello, and welcome to our todays lecture of Introduction to Mechanobiology. In the last few lectures we discussed about various diseases including cancer ok.

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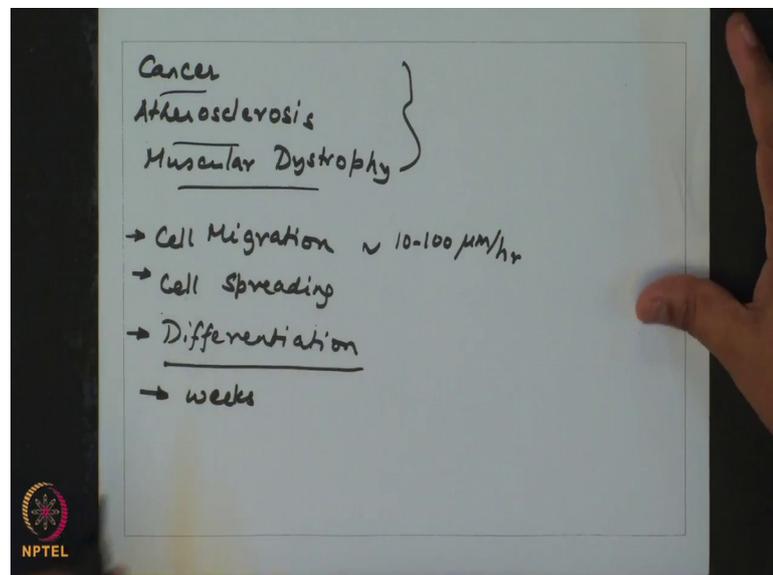
Atherosclerosis and muscular dystrophy, apart from these diseases we also discussed some basic cellular processes like cell migration, cell spreading and differentiation. So, processors like differentiation or any of these diseases are take much more longer time to manifest ok.

While cell migration and cell spreading requires the order of so cells migration might have a cell speed of ten to hundred microns per hour. So, for these processes you require that the molecules which participate are right there at the proper location. So, that they can enact what they supposed to do; however, processes like differentiation this is weeks in time. So, there are lot of changes which are associated for a stem cell to differentiate into a neuron or a muscle cell or a osteoblast ok.

So, what dictates it requires that these physical signals which are encoded by ecm in the form of topography or stiffness reaches the cell nucleus ok,

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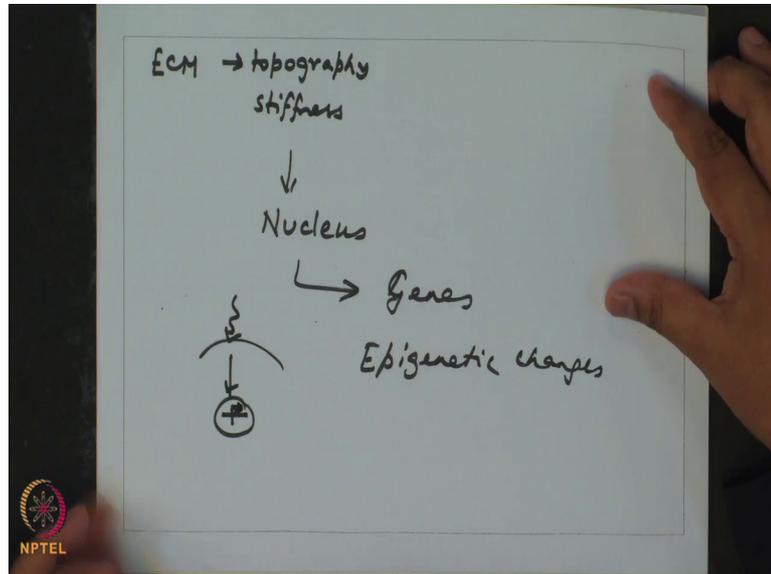
And activates various genes, and it is not just genes you also have epigenetic changes. So, it is important to understand how from a cell periphery to the cell nucleus you have a signal, which is transmitted and eventually activates a given gene ok.

So, in that regard the nucleus becomes an important command center. So, if you think of the nucleus. So, over the next 3, 4 lectures we will discuss about the nucleus.

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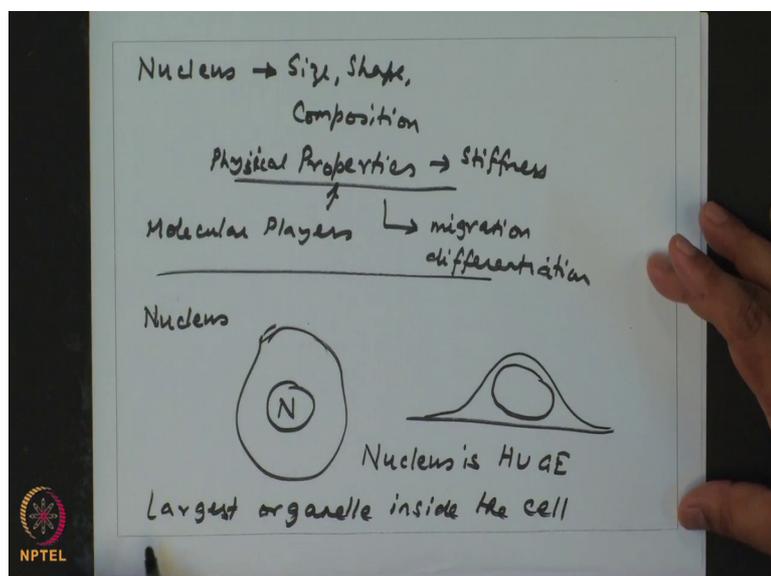
What are its size, shape, composition and properties physical properties like stiffness, and how do molecular players dictate these properties, and how are these properties relevant to processes like migration to differentiation ok.

So, we begin with discussing the nucleus in general. So, as we all know that in contrast to cells like bacteria, in a cell the genetic information is stored inside the nucleus ok.

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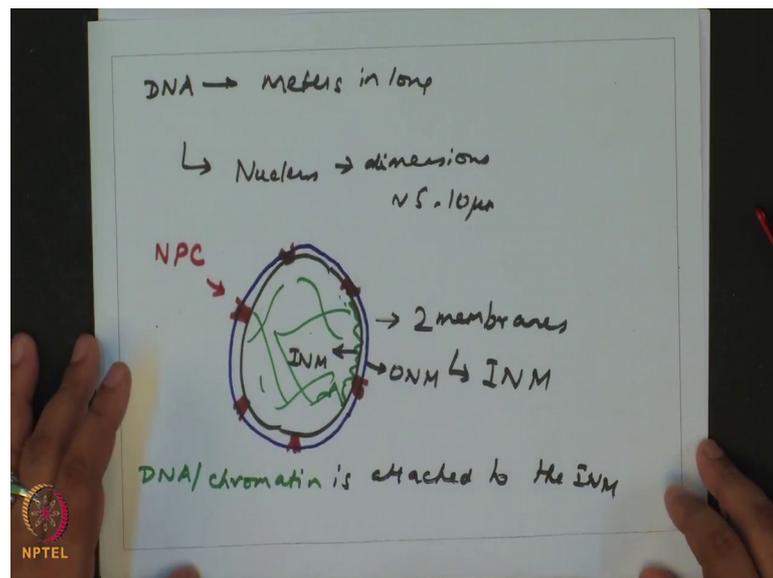
And though we like to draw a pictures our schematic of a cell with a nucleus might be like this, but if you look in side view. So, if I draw a cell with a nucleus in side view accounting for its proper shape and size the nucleus will look something like this.

So, clearly what we see is the nucleus is huge, and it is perhaps not surprised, so it is of course, the largest organelle inside the cell and it is also the stiffest component. So, why is the nucleus is a stiffest component? Thing is because you have the entire DNA if you stretch out the DNA, you would have meters long and this you put inside a nucleus, with dimensions ranging from order of 5 to 10 microns gain size.

So, when you compact such amount of DNA within the nucleus, the nucleus becomes very stiff, now what are the constituents of the nucleus? So, if I want to draw the nucleus, you have actually, so the nuclear membrane has two membranes the nucleus has two membranes the inner nuclear membrane or INM.

So, this is your INM, and this is your ONM or outer nuclear membrane and within this you have the entire DNA, it is present as chromosomes. So, some portion of the chromosome or the DNA is attached to the membrane in a nuclear membrane. So, you have some portions, where the DNA or chromatin is attached to the INM and what I have drawn in red, these are nuclear pore complex ok.

So, these are the holes through which proteins can enter and exit the nucleus and typically proteins, which entered the nucleus by themselves, have what is called as a nuclear localization signal and this entry is mediated by proteins like importance and similarly you have other proteins which mediate the exit ok
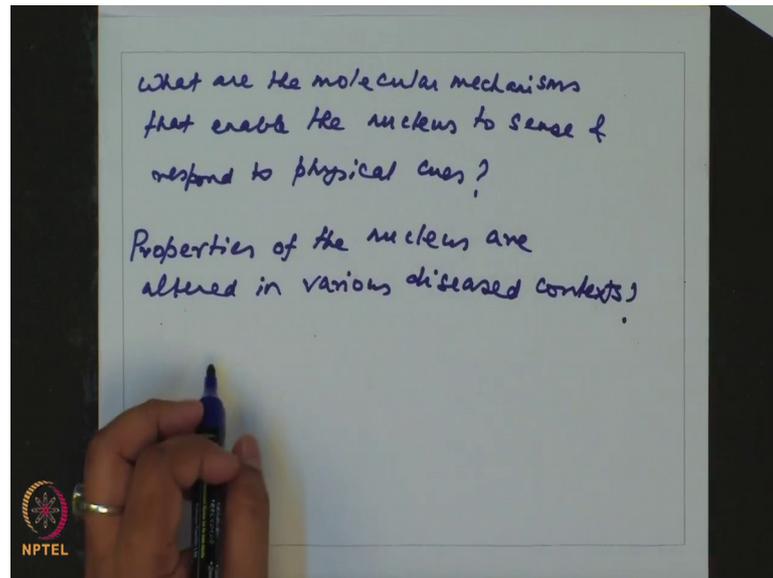
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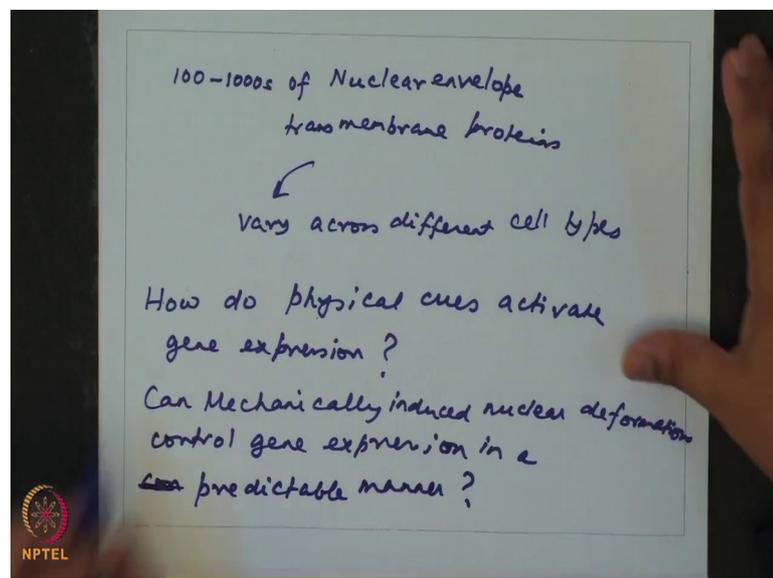
So, it is now emerging that there are 100 to 1000s of nuclear envelope proteins trans membrane proteins ok.

So, this expression can vary across cell types. So, the question to ask is, how do physical cues activate gene expression? And can mechanically induced nuclear deformations control gene expression in a control level in a predictable manner.

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So, what are the molecular mechanisms that enable the nucleus to sense and respond to physical cues? And how properties of the nucleus are altered in various disease contexts?

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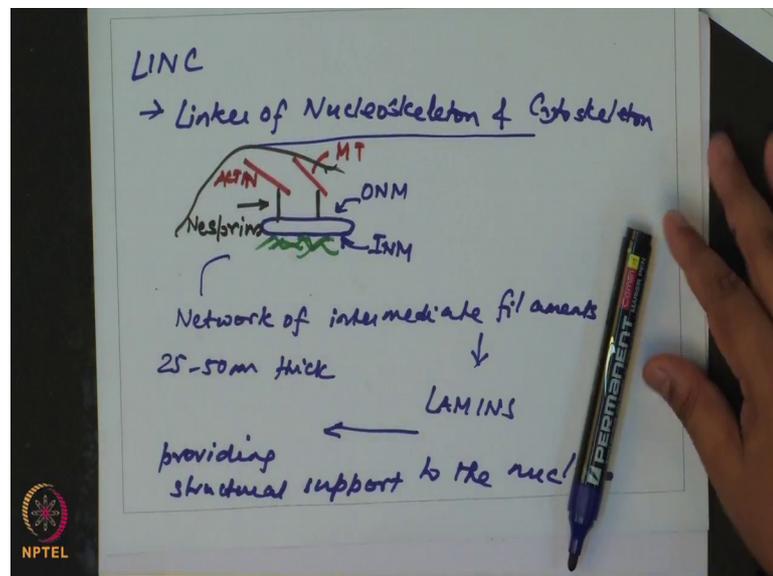
So, in this regard, I would like to introduce this complex called a LINC. LINC is the short form for Linker of Nucleus Skeleton and Cytoskeleton ok.

So, inside the inner nuclear membrane so if I draw a short segment of imagine that this is my outer nuclear membrane and this is my inner nuclear membrane. So, just underneath the inner nuclear membrane, you have a network of intermediate filaments. So, this is a

network of intermediate filaments. So, this is 25 to 50, nanometer thick and these intermediate filaments are called lamins ok.

So, laments are associated with providing structural support to the nucleus. So, while inside you have lamins. So, this is only one way connection, now as this term says linker of nuclear skeleton and cytoskeleton; that means, that there must be proteins. So, imagine you have this network of microtubules or actin. So, there must be some connections which exist which connect this. So, then you have a physical network from outside. So, this is your entire thing is your cell and this is just a short section of the nuclear membrane ok.

So, this might be your actin stress fiber, similarly this one this will be microtubule. So, these molecules ok.

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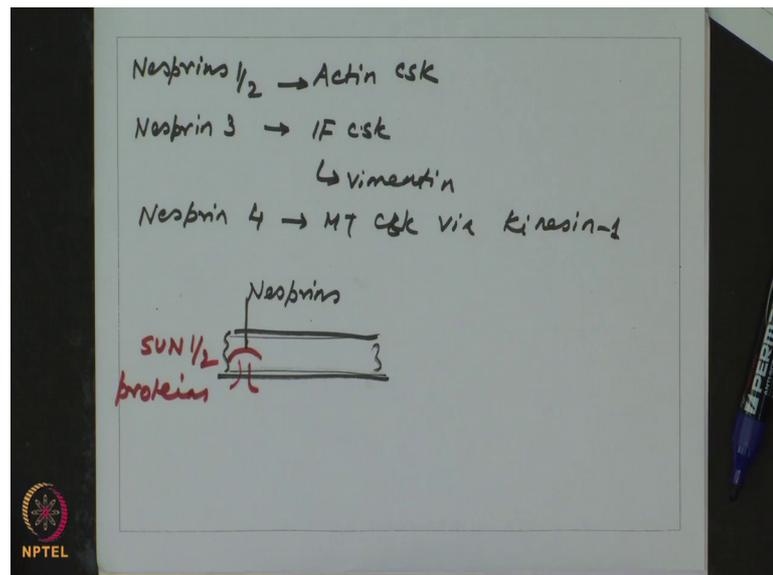
Which connect the nuclear membrane to either the actin or the microtubule cytoskeleton are called Nesprins. So, you have different type of nesprins. So, you might have nesprins 1, 2 which are known to connect to the actin cytoskeleton ok.

You can have nesprins 3, which connects to the intermediate cytoskeleton. So, by intermediate cytoskeleton I mean vimentin network, and you have nesprins 4 which connects to the microtubule cytoskeleton via kinasin 1. So, if I draw the membrane in a little more detail .

So, let us say this is a if you have these nesprin molecules, they are known to bind to some proteins called sun proteins, you have sun proteins which link the nesprins to the nuclear membrane and the sun in turn. So, you have sun 1 or sun 2 and these in turn associate with the inner nuclear membrane, through the cytoskeleton of the nuclear lamina ok.

So, lamins they bind to large other nuclear mineral proteins ok.

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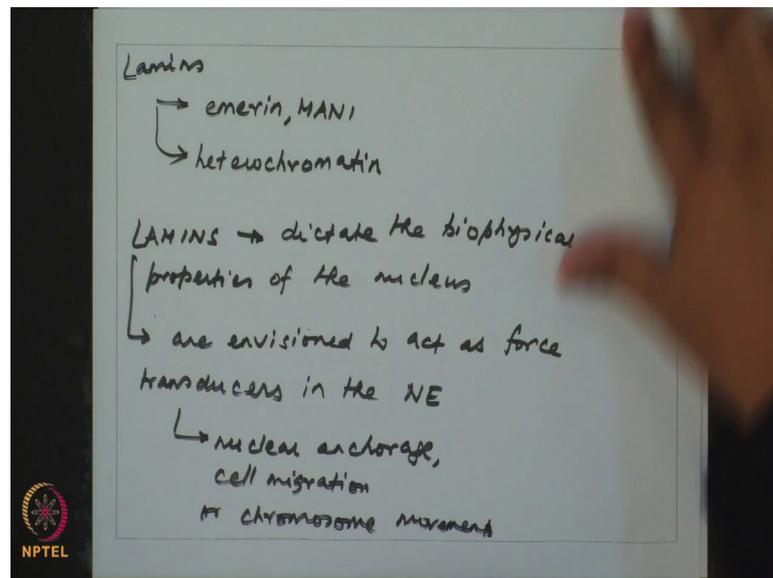
And that is where you have other proteins like emerin or MAN1 as well as heterochromatin. So, lamins are known to bind to all of these, now lamins given their structural role they are associated. So, they actually dictate the biophysical properties of the nucleus ok of the nucleus, and it is envisioned that these lamins are envisioned, to act as force transducers in the nuclear envelope and this is key to processes like, nuclear anchorage cell migration or chromosome movements. So, there are various mechanisms which have been proposed as to how forces transmitted to the nucleus, might lead to a signaling, cascade, controlling, gene, expression ok.

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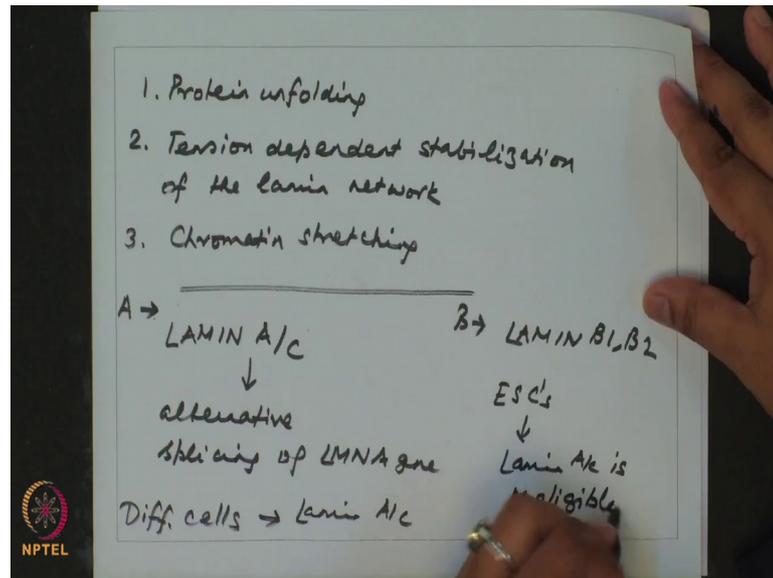
One is like we saw for ECM proteins like fibro protein you might have protein unfolding as one of the mechanisms, second mechanism you might have tension dependent stabilization of the lamina of the lamin network, and some cases you might have chromatin stretching thereby exposing spaces for binding. So, if you look at lamin expression so there are two types of lamins in you having lamin AC. So, this is because of alternative splicing of lamina gene, and you have lamin b1 and b2. So, you have a type lamins and b type lamins. So, most differentiated cells express lamin AC and in stem cells for example, in embryonic stem cells lamin AC is very low negligible.

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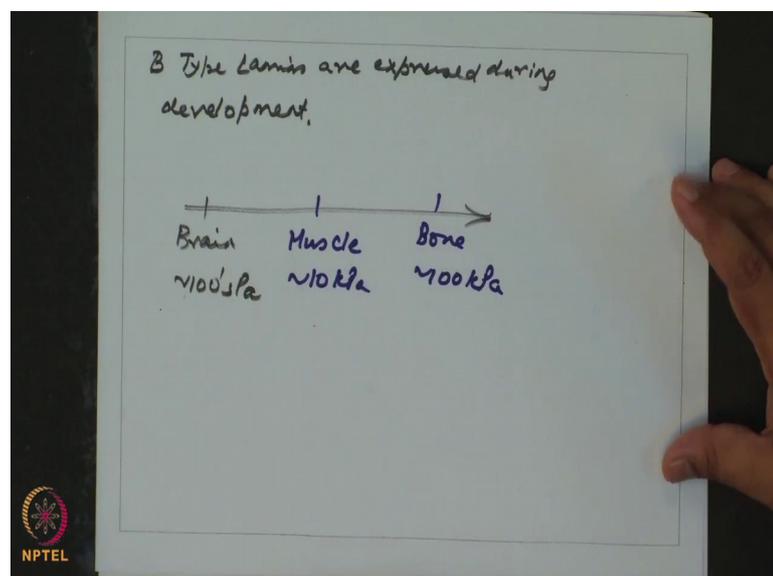
So, it has been demonstrated that lamin b type lamins, are expressed during development. Now you know that within the body you have this intact tissue stiffness scale right. So, brain being 100s of pascals to muscle order 10 kpa to bone collagen is bone order 100 kpa. So, in an seminal work discher, DENNIS discher and coolies showed that ok.

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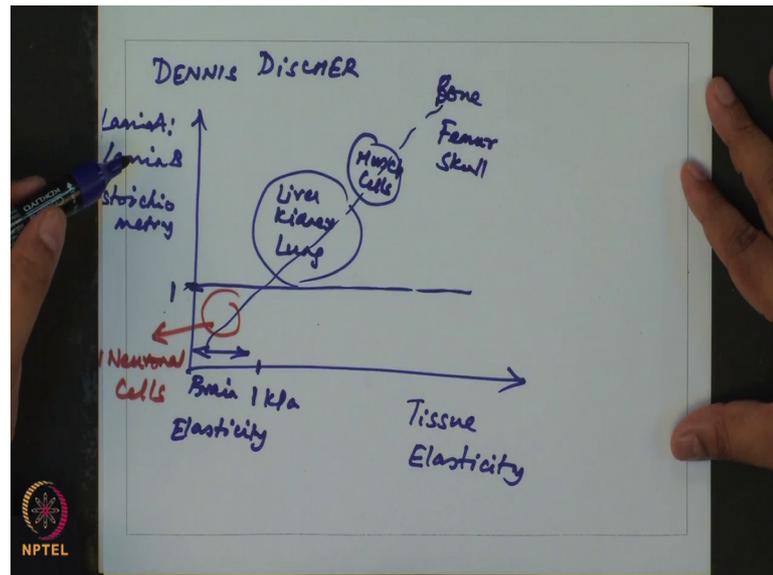
So, if you have an axis of tissue elasticity, versus lamin a is to lamin b stoichiometry. So, you would find a linear relationship. So, let us say this is 1, so below 1 you have in soft tissues, so let us say this is 1 kpa order brain. So, this is your brain elasticity, so you have cells.

So, in these zone you will have neuronal cells, in somewhere here you would have liver, kidney, lung and high here, then in this cluster you will have muscle cells and high up you will have bone tissue like femur or skull. So, in other words in soft tissues your lamin a to b ratio is less than one which means lamin b is higher lamin a ratio is very low ok.

Why in stiff tissues this keeps on increasing. So, there is a increase in lamina expression, now along this if you also plot how collagen levels scale with tissue stiffness. So, you would get a scaling relationship of collagen 1 scaling as bulk tissues stiffness to the power 1.5.

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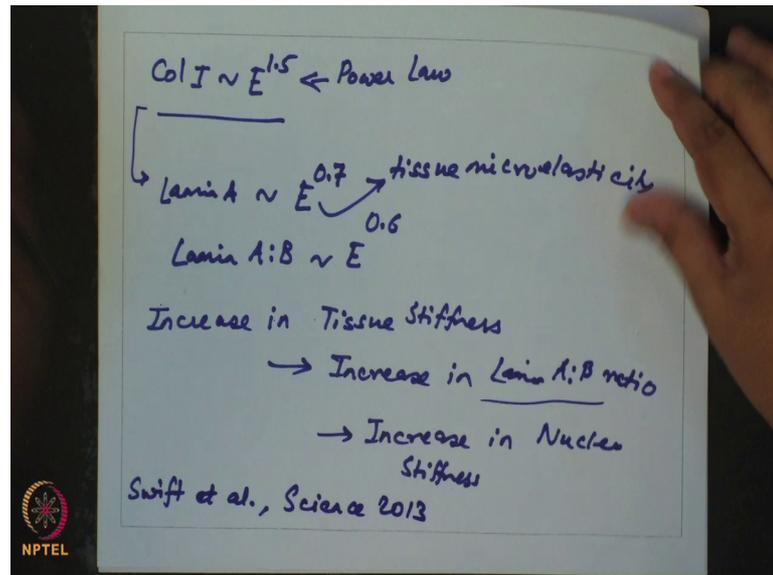
So, this is called the power law relationship similarly, so you have this lamin ratio lamin a ratio scales as e to the power 0.7, and lamin a to b ratio scales as e to the power 0.6.

So, here e corresponds to the tissue micro elasticity, so what it tells you is that as you increase in stiffness. So, increase in ecm stiffness or increase in tissue stiffness is linked to increase in lamin a to be ratio, an increased lamin a to be ratio is associated with increase in nucleus stiffness. So, this shows you that if a cell resides in an environment which is stiff.

So, it is likely that the forces on it is higher, it is subjected to more compressive forces or higher amount of tensile forces and under those conditions the nucleus responds, by increasing its expression of lamin a compared to lamin b and the nucleus stiffens. So, this work we will reading assignment, I recommend you to read this paper.

So, what in this study the authors also showed, so when they look at the nuclear membrane, if this lets say if this is my lamin a staining, AC staining I and if this is my lamin b staining ok.

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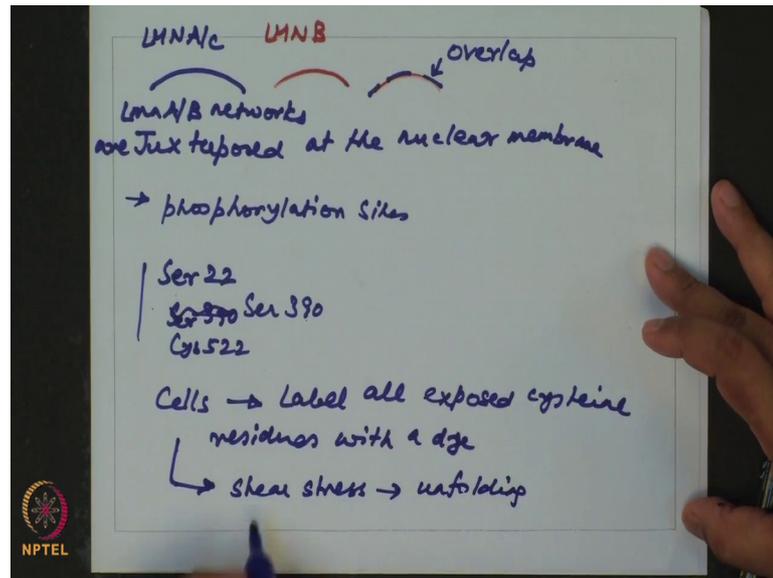
So, if you co localize, so there are segments, in which they overlap there is complete overlap. So, suggesting that these two networks are jux tuposed.

So, lamin AB networks are jux tuposed at the nuclear membrane. So, lamin a is known to have multiple phosphorylation sites. So, including one at serine 22, one at serine 390, one at this is a sorry, this is a cysteine 522, here serine 390 and many others ok.

So, what the authors showed, that if you do an experiment in which you take cells and you label all exposed cysteine, cysteine residues with one dye, with the dye and then you subject the cells to shear stress. So, what force is known is going to lead to unfolding and this unfolding, so you have unfolding which leads to phosphorylation ok.
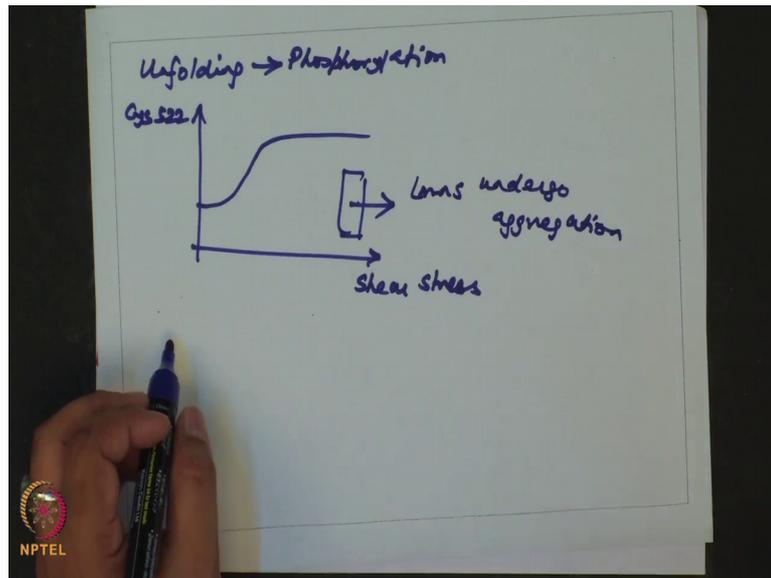
So, what they have shown is if you track the labeling of cysteine 522, the amount of labeling of cysteine 522 as a function of shear stress, you would see an increase in the amount of labeling, suggesting that as you expose forces on to the cell these get transmitted to the nucleus and as a consequence of this there is unfolding of this lamin network, and that is associated with increased amount of labeling of cysteine reactive probe ok.

Now, if you increase the shear stress at high stress zones, this actually the cells this lamin undergo aggregation. So, in this zone the cells actually undergo aggregation.

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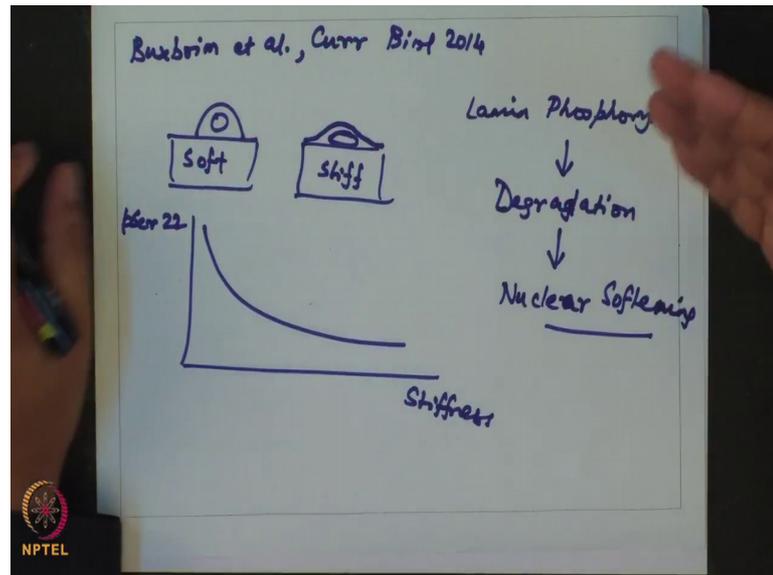
Now another interesting study was performed this study by bux. So, what they did they plated cells on soft gels, where nuclei where the cell is rounded and the nuclear is also rounded and on stiff checks, where the cell splits and the nuclei is expected to be under lock mode stress ok.

And what they found, that there was an inverse relationship between phosphorylation of serine 22 as a function of stiffness. So, as you increase stiffness phosphorylation decreases, on soft substrate which means that amount of lamin phosphorylation is lot high and increase in lamin phosphorylation is associated. So, when you when lamin gets phosphorylated, it leads to degradation, and degradation would mean that it would lead to effective nuclear softening ok.

So, this is one of the mechanisms, by which depending on a soft or stiff substrate the extent of phosphorylation changes and as a consequence the nucleus stiffness can also change, on a stiff surface when the cell is exposed to lock mode stresses internal stresses also you have stress fibers pushing on to the nucleus, in that case the phosphorylation decreases and there is higher levels of effective lamin, which contributes to the stiffness of the nucleus ok.

With that I stop here for today, in the next class I will connect these observations of lamin phosphorylation and lamin expression to how these effects cell migration.

Thank you for your attention.