Introduction to Mechanobiology
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Week – 07
Lecture – 34
Nuclear Mechanotransduction: Gene regulation

Hello, and welcome to today's class of Introduction to Mechanobiology. So, in the last class we had started discussing about the nucleus and its structure and majorly I introduced, this complex are called LINC complex which stands for linker of nucleus skeleton and cytoskeleton ok.

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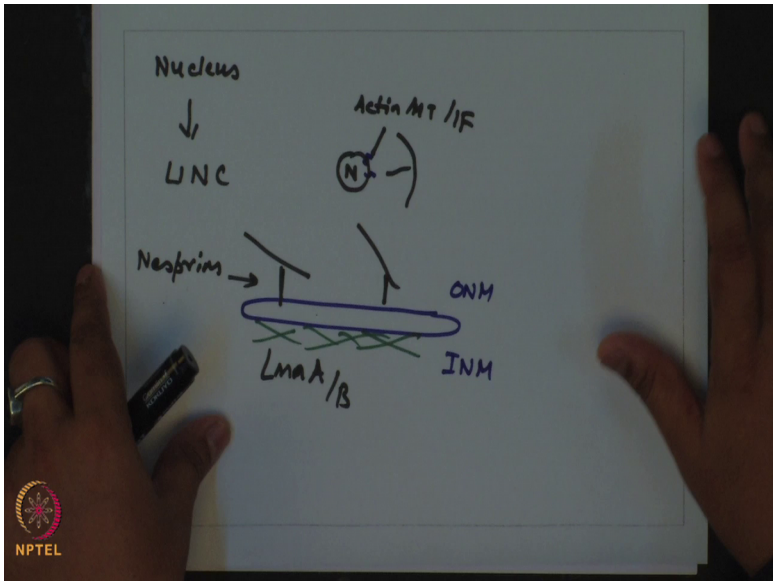

So, these LINC complexes just make sure, that if this is my nucleus you have a physical connection from actin or micro tubular or intermediate filament cytoskeletons to the inside of the nucleus through various connections. So, if I zoom in so the nucleus actually has is a double membrane system you have outer nuclear membrane and inner nuclear membrane.

So, inside the inner nuclear membrane you have the network of lamin proteins these include lamin A and B and outside. So, to the cytoskeleton you have these lincs which are mediated by nesprins. So, this establishes a direct physical connection from outside

the nucleus to the inside of the nucleus we also spend considerable amount of time in discussing how expression of lamins.

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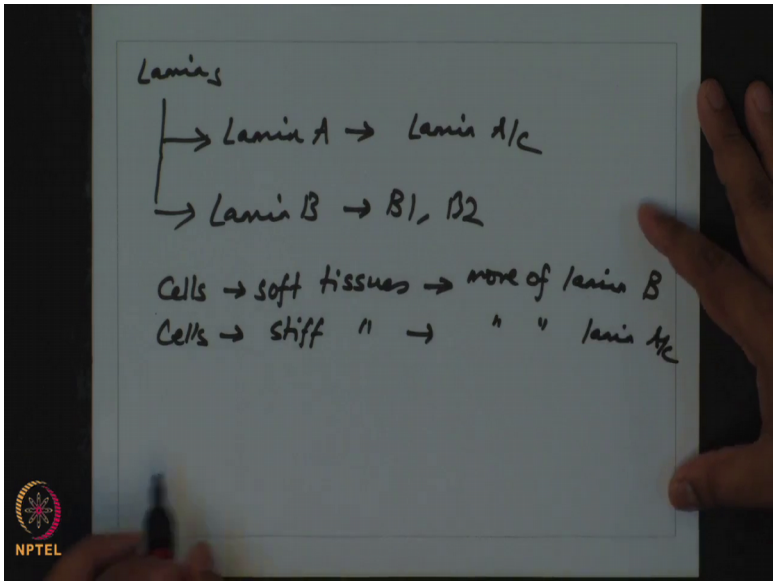

So, when we say lamins you have 2 major classes of lamins lamin as, but is encoded by the same gene, but because of splicing you have 2 forms lamin A and lamin C this is collectively written as lamin A C and you have the lamin B form found as B 1 and B 2. So, what has been recently demonstrated is that cells which reside in soft tissues, contain more of lamin B while cells which reside in stiff tissues they contain more of lamin A C. So, if you plot the stoichiometry of lamin A to B. So, you get this relationship where if x axis is tissue electricity and y axis is A to B stoichiometry.

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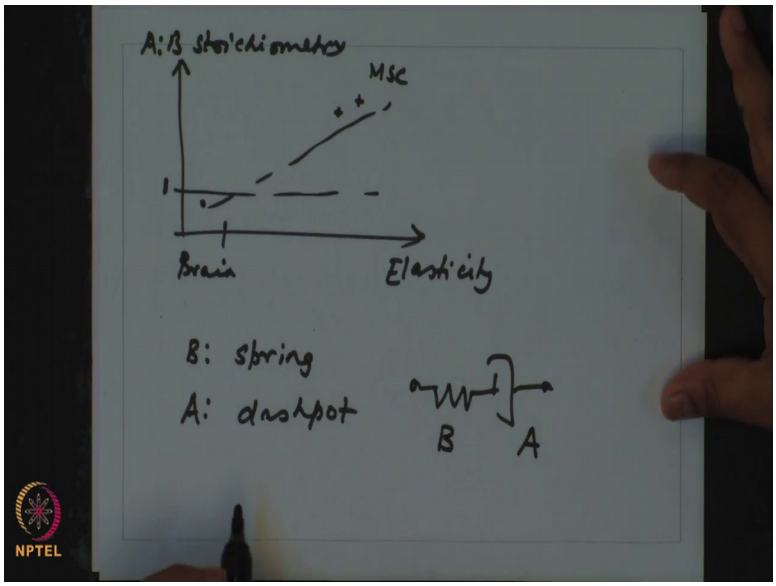

So, you have this scaling relationship, where below 1 here in soft tissues like brain soft tissues like brain you have cell types like deo muscles they would reside here where lamin A to be ratio is less than 1, while stiff tissues like cartilage, bone, muscle cells and even mesenchymal stem cells contain high ratios of A to B. Now mechanically A and B operated different entities lamin B operates like a spring and lamin A operates like a dashpot or of viscous object. So, collectively it is expressed as this where this is the your B responsive this is the a response.

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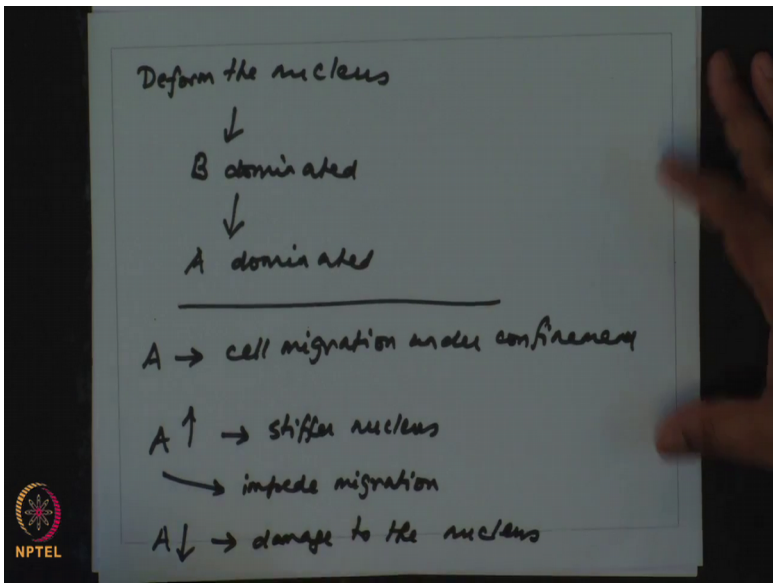

So, your short term whenever you deform the nucleus when you deform the nucleus you have a short term response which is lamin B dominated, and a long term response which is lamin A dominated.

Today so this was the first part of what I discussed in a nucleus, the second part was the importance of A in dictating cell motility or cell migration under confinement. So, since high levels of A lead to stiffer nucleus so high levels of A actually impede migration by migration I mean confined migration, but if you have A to low then you might have damage to the nucleus so in the next two lectures including today.

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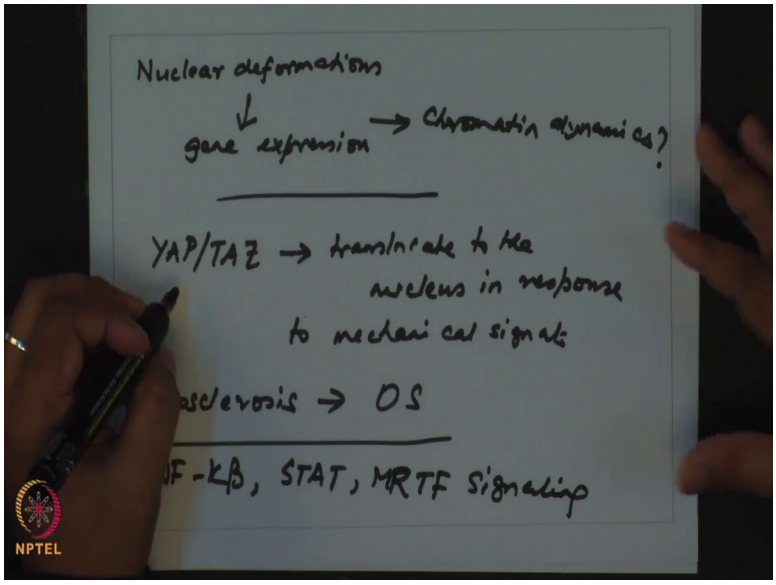

I will discuss two things that how the nuclear deformations, eventually influence gene expression patterns and this will be mediated by chromatin dynamics. So, I have already taken an example of the YAP TAS transcription factor, which translocate to the nucleus in response to mechanical signals; so in the context of atherosclerosis so this is oscillatory shear stress in response to oscillatory shear stress, YAP TAS as were shown to translocate to the nucleus.

There are other such transcription factors notable among them ins nf kappa B signaling, stat signaling and mr tf signaling pathways this also exhibit this fate where they do translocate to the nucleus and what do these translocation induce ok.

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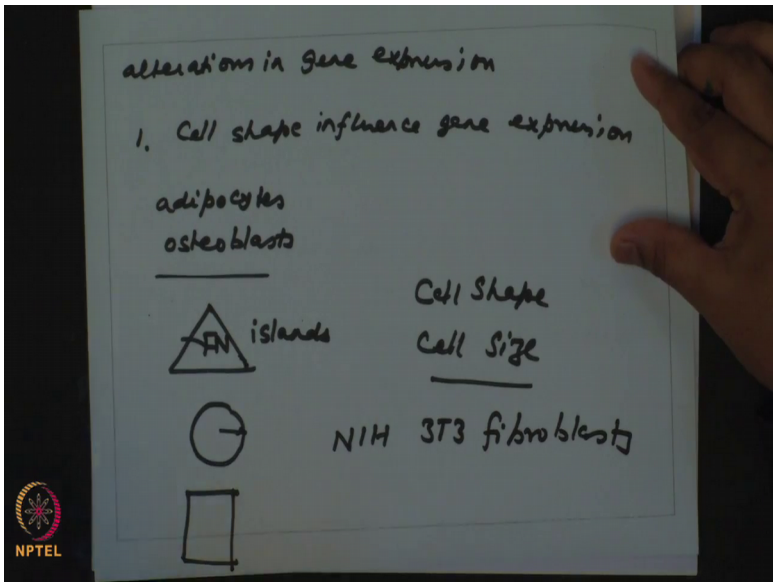

So, these actually lead to alterations in gene expression, so I would like to discuss two papers today briefly, one paper discussed how does cell shape influence gene expression. So, you are both aware of the earlier paper when we were discussing about mesenchymal stem cell differentiation, we showed that when cells are plated on small patterns then they tend to differentiate into adipocytes and then once on big islands they tend to become osteoblasts ok, so this is cell shape mediated.

So, what the author is did in this particular study was they took a variety of shapes of microfabricated islands coated with fibronectin. So, these are fibronectin islands and you they generated various geometries different shapes triangles, rectangles and circles, and they also altered the sizes of these. So, there are two things that they varied the cell shape and cell size. So, cell shape was changed modified by changing the shape of the islands that they used and cell size was controlled by the dimensions of the islands and then they asked that what happens when we will play. So, they used NIH 3T3 fibroblasts. So, they asked how do signaling altered when cells are cultured on these different patterns ok.

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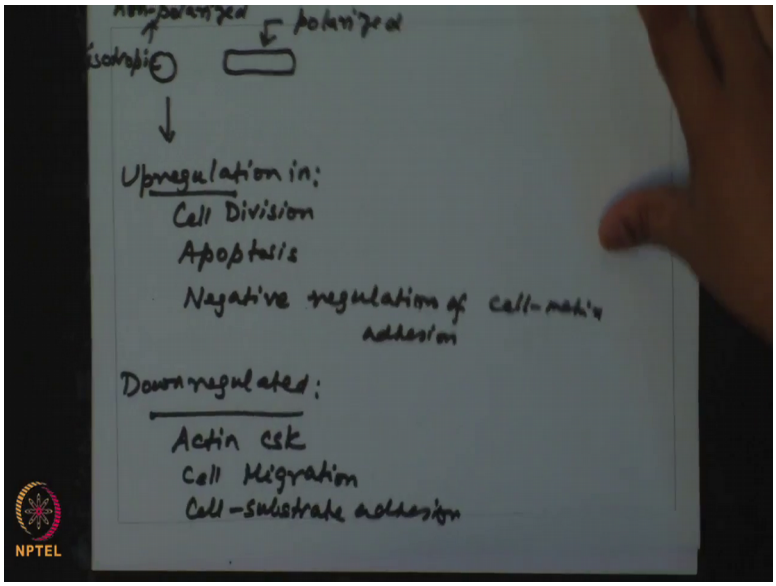

So, first they did a comparison between circular and elongated morphologies, and what they found was in this case of circular patterns. So, circular is a more isotropic geometry versus this is a more polarized geometry this is non-polarized or isotropic. So, what the form that on these circular patterns they had up regulation in cell division, proteins, apoptosis genes and regulators and negative regulation of cell matrix adhesion again, so there are some other signals which are down regulated on the circles.

So, when I say up regulated this is up regulated which respect to the elongated geometry similarly there were several things which were dying regulated you had actin cytoskeleton machinery all the actin cross linking proteins were down regulated proteins involved in cell migration their expression was down regulated cell substrate adhesion was perturbed so on and so forth.

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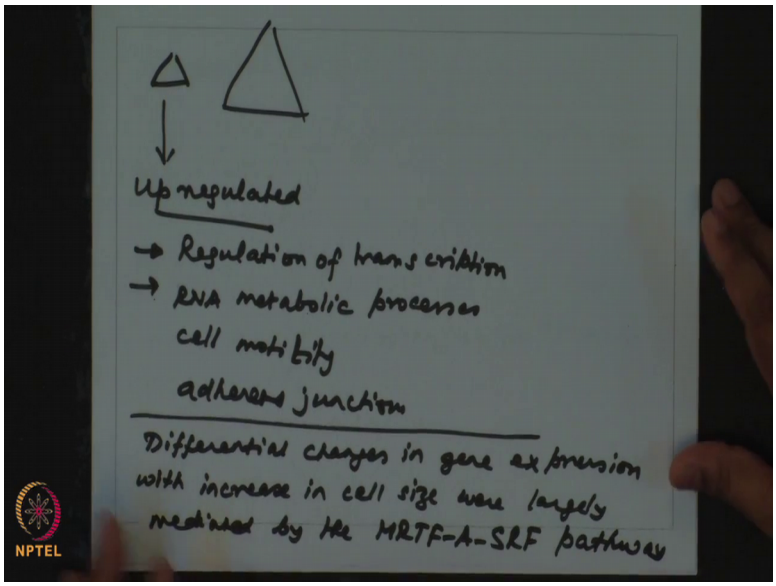

Similarly, they observed differences, so they used the triangular geometry they compared a small triangle with a big triangle and what they found was in the smaller triangles there are several signaling pathways which were up regulated, these include regulation of transcription RNA metabolic processes cell motility and adherence junctions . So, now all these differential changes in gene expression that were observed with increase in sizes. So, there was a correlation so the differential changes in gene expression, with increase in cell size were largely mediated by the MRTFA SRF pathway.

So, SRF is serums serum response factor. So, when you know that when we culture cells we always supplement with serum the serum has multiple factors including growth factors as well as ECM proteins which benefit cell you know cell growth ok.

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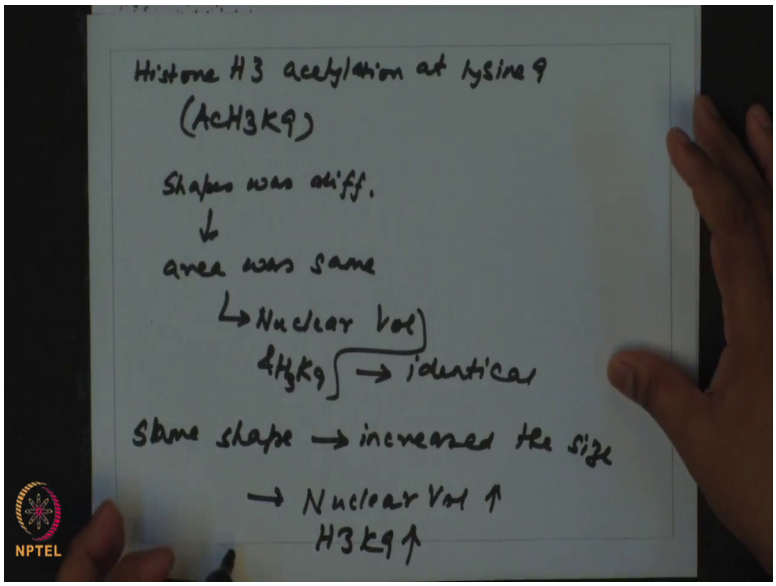

So, SRF pathway is involved in all of these processes, what they then checked was whether there was any alteration in histone acetylation, H 3 acetylation levels at lysine 9 this is typically written as ACH 3 K 9 H 3 K 9 is a histone acetylation. So, what they observed was when the shape was different. So, long as the area was same, so the nuclear volume and H 3 K 9 acetylation was identical. So, even though the shapes were different nuclear volume and acetylation levels were identical; however, for the same shape when the increased the size. So, they found increase in nuclear volume increased and H 3 K 9 acetylation also increased ok.

So, these experiments actually revealed that across the conditions there is a very linear relationship between H 3 K 9 acetylation levels and nuclear volume.

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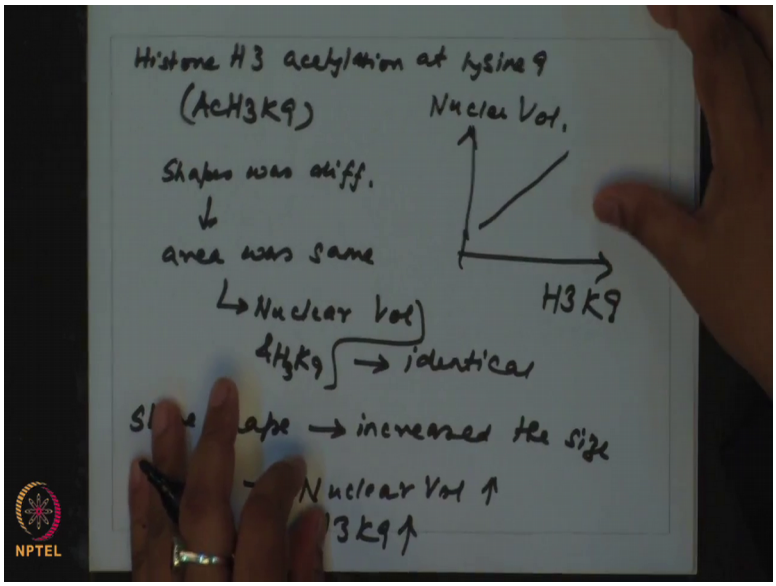

So, based on this they suggested a pathway in which any cell geometry that you regulate will influence the actin cytoskeleton ok.

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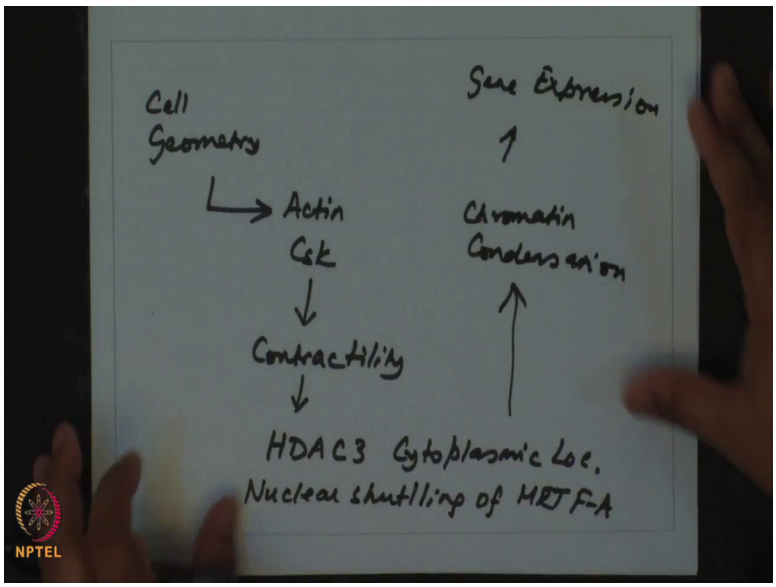

And by actin cytoskeleton when it is perturbed what it will change its contractility, and this contractility will eventually act upon HDAC 3 localization, cytoplasmic localization and nuclear shuttling, MRTFE all of these eventually perturbed chromatin condensation levels which dictates the gene expression patterns. So, this paper was published in pnas 2013, they have a more recent they have followed up on those experiments ok.

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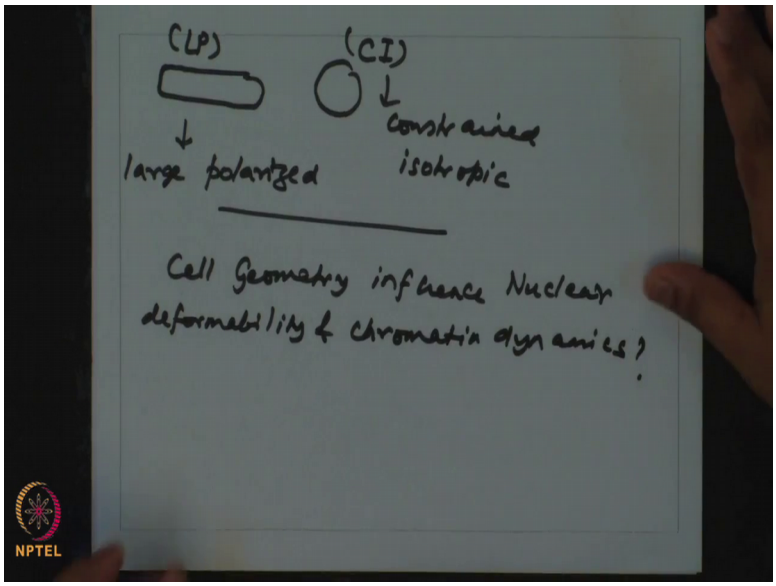

And then they chose these two geometries again a polarized geometry and a circular geometry ok.

So, this steroid has large polarized geometry or constrained isotropic. So, this is constrained isotropic and this is large polarized. So, through this they wanted to ask that how does cell geometry influence nuclear deformability and chromatin dynamics. So, this was a question, so what they did once again was the plated cells on these islands they plated 3T3 cells fireblast on these islands.

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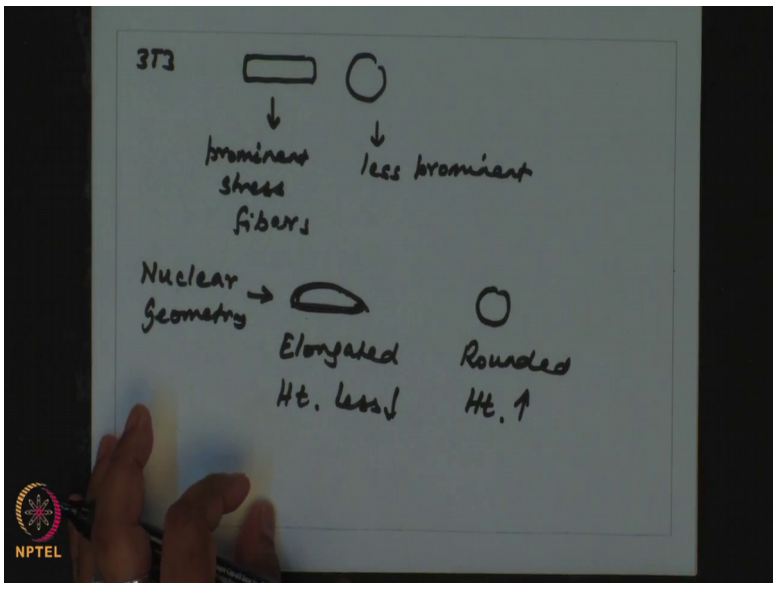

So, you have two geometries elongated and circular on work. So, what they observed was on this geometry they show up prominent, stress fibers, indicative of increased contractility while this was much less, less prominent stress fibers in this case and what they observed was on these two, if you were to compare the nuclear geometry ok.

Here if I look inside view the nucleus will have a geometry like this. So, in other words it is elongated and its height is less here for the rounded geometry you would assume that the nucleus would also be rounded and its height will be more. So, this is less this is more so given this they make fast that focus at this is static sheet how does the nuclear dynamics change ok.

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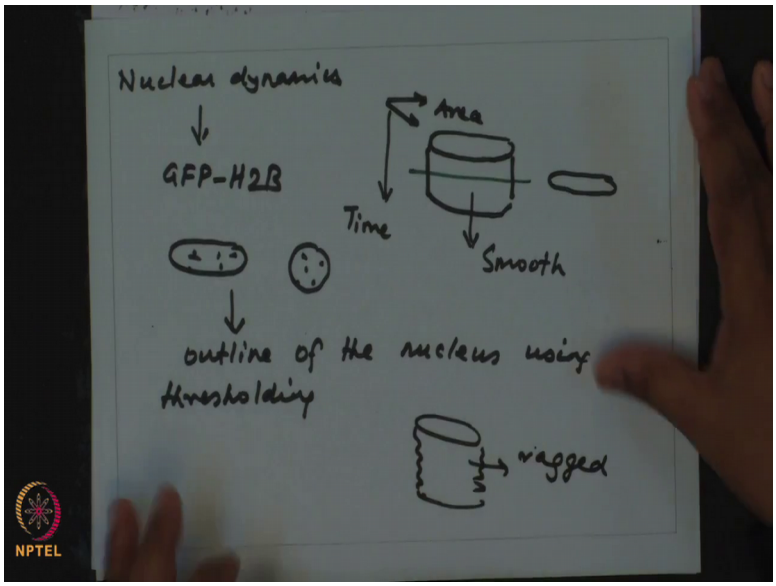

So, when what they did why for understanding nuclear dynamics, what they did was they transfected these cells with GFP H 2 B. So, H 2 B is a histone protein which is bind, which is not bind to the DNA. So, as a result, so when you transfect cells with H 2 B you can detect the entire nucleus round or elongated it will give you signal throughout the nucleus, and then once they transfected these cells they can actually track the outline of the nucleus using thresholding, and they asked that how do these shapes change as a function of time ok.

So, as a consequence you can have if this was a geometry at 1 given edge, at 1 given time instant you can track. So, let us say this top surface is the area and this vertical axis is my time you would get a 3D, surface where every section that you take every section

that you take will give you a given geometry. So, what they found was on the LP islands large polarized islands the nuclei this surface that they generated this was very smooth, the surface was very smooth in contrast on the circular islands the nucleus, if I were to draw the surface if you do the kymograph you get a surface which is much more ragged suggesting that nuclear area change nuclear area change is much more pronounced on the constraint IC dropping islands ok.

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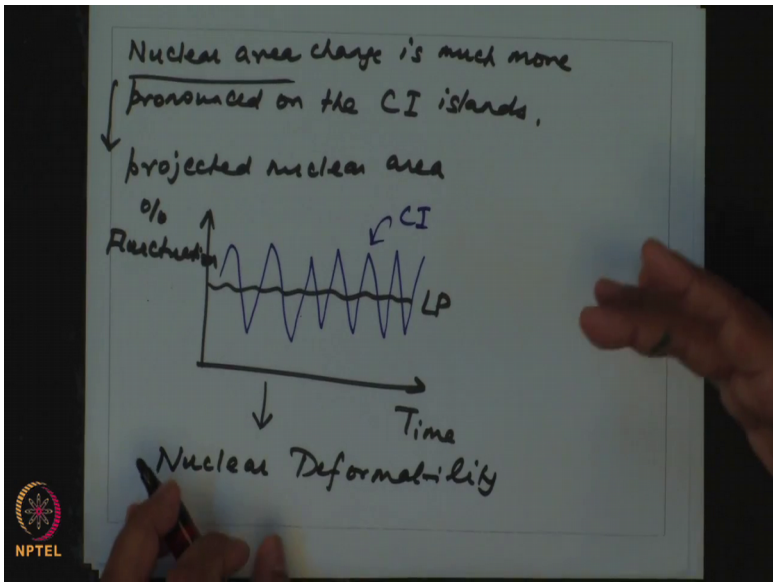

So, when I talk about nuclear area it is actually the projected nuclear area. So, to quantitatively detect how much does the nuclear area change what they did was they plotted the percentage fluctuation in the area the projected area and so this is your time axis. So, compared to on these islands that they on the LP islands if this was the fluctuation on the circular islands the fluctuation was much more. So, this is on the constraint CI islands and this was on the LP islands. So, this confirms that the area fluctuation is significantly higher on the rounded islands.

So, this shows so the higher amount of fluctuation. So, the higher amount of fluctuation is linked they linked it with nuclear deformability, the idea being if something is rigid then it will retain its shape for extended periods of time while if something is floppy particularly within the cell there is always, so much of activity going on the nuclear will be subjected to lot more changes in area as a function of time.

What did then ask was what is driving these fluctuations. So, they did experiments where they perturbed actin in two ways.

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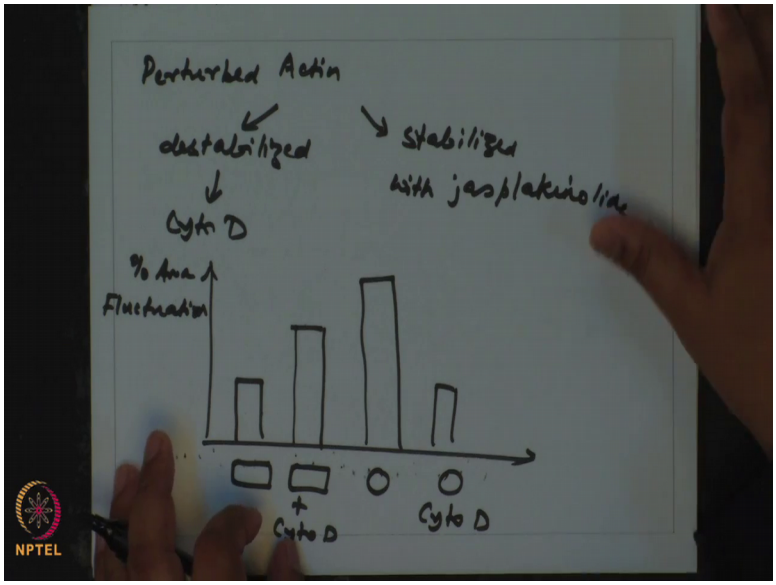

Either, they destabilized the affect in network which cytoskeleton D or they stabilized with jasplakinolide. So, what they found, so what they found is this, so my y axis is percentage area fluctuation. So, compared to the rectangle, so if rectangle this is your area fluctuation on the rectangle when they added with cytoskeleton d when they added cytoskeleton D plus cyto D this fluctuation increased, but on the circular pattern. So, where fluctuation is was significantly higher when they added cytoskeleton D this is decreased ok.

So, this suggests that these effects of polymerizing drugs are highly non monotonic, they also did experiment ok.

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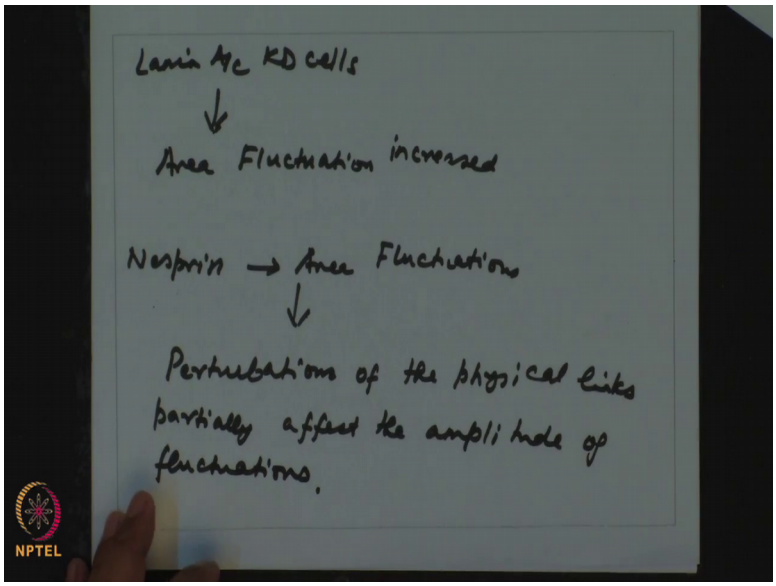

So, they didn't experiments with lamin A C knock down cells also found that with this on the circular patterns the extent of area fluctuation increased, increased then did experiments by perturbing being nesprin and still they observed area fluctuations. So, based on this they concluded that perturbations of the physical links partially affect the amplitude of fluctuations, because even when the perturbed with actin it dropped, but it did not go or it increase, but didn't go. So, as the last step for they did was they tracked chromatin dynamics.

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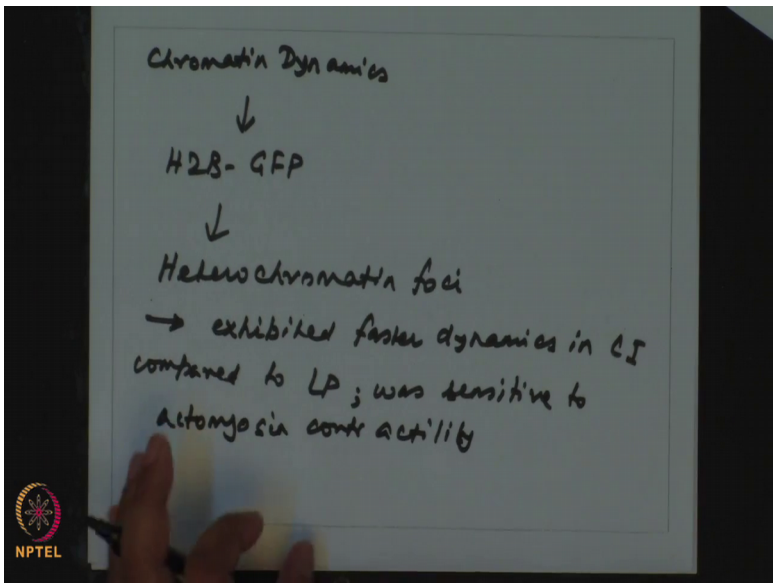

By labeling with H to B GFP, and tracking foci they tracked the heterochromatic foci within the nuclei and what they found was the this skymalglass.

So, this heterochromatin foci exhibited faster dynamics in CI compared to LP and this was sensitive to actomyosin contractility.

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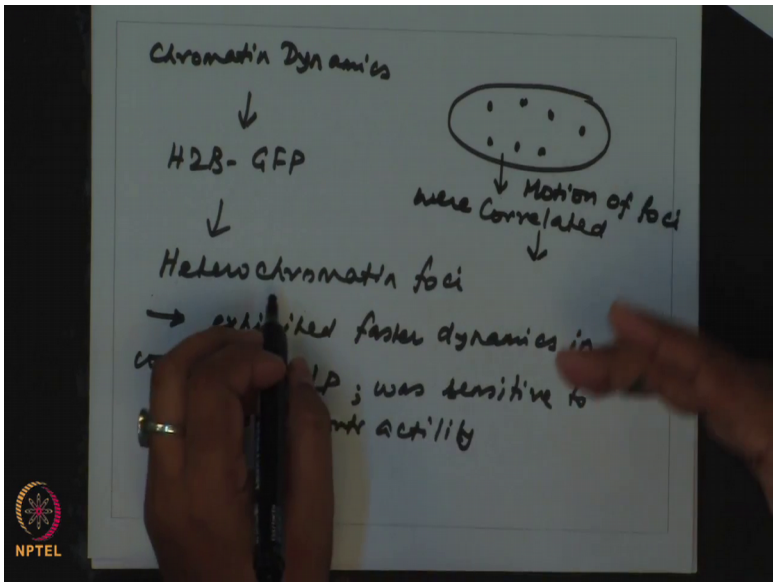

One more thing that this found was within the same nucleus, if you have these multiple foci they found that the motion of these foci were actually correlated the motion of the foci were correlated and these foci though they you know the extent of correlation was perturbed, when you treated them with drugs like actin or (Refer Time: 26:21) but when you remove then again when you remove these actions were removed.

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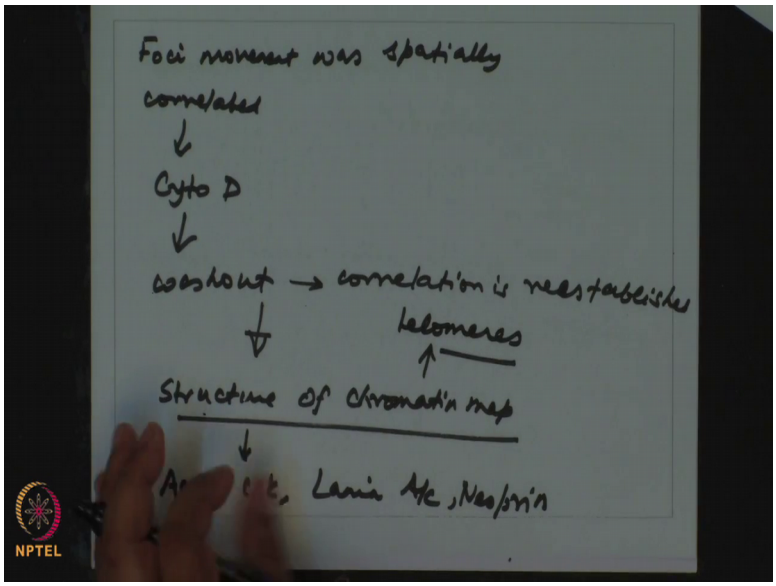

So, the foci trajectories, the foci movement was specially correlated and sensitive to cyto D, but after wash out the positions and the correlation map is reestablished established.

So, what all these experiments suggest is there is an imprint. So, there is a structure of chromatin map, and what they find that these structures, is controlled by multiple forces like contractility. So, you have actin cytoskeleton lamina A C nesprins or link domain chromatin and so on and so forth. So, these collectively regulate the telomeres which serve as the end points of the chromatin with that I stop here I would suggest to read two papers as reading assignment 13 ok.

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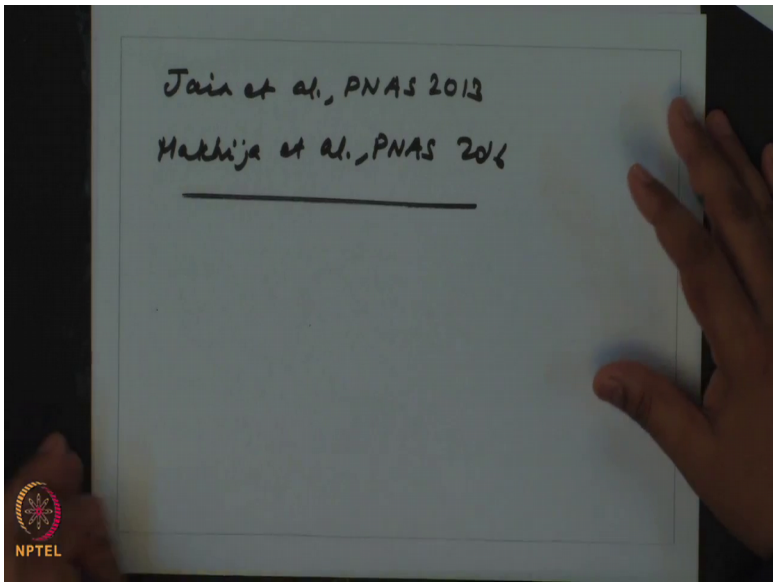

So, both these papers make use of a geometrical pattern and then study how you have these epigenetic modifications happening as a function of shape and size, and how telomere dynamics and within the nucleus how dynamics change when you perturb this cytoskeleton elements.

Thank you for your attention.