Introduction to Cell Biology
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Lecture 54
Endomembrane system of Cell: Part 4

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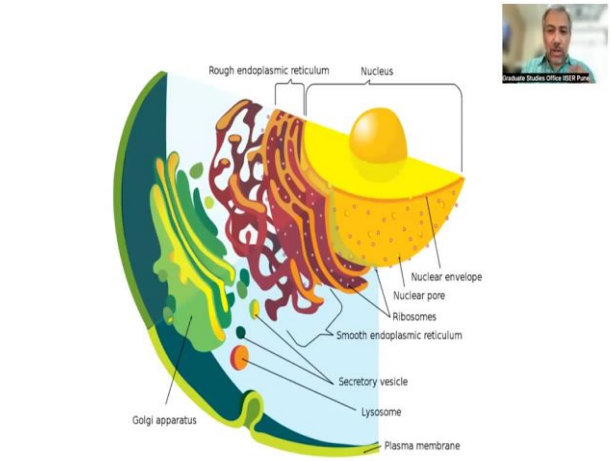

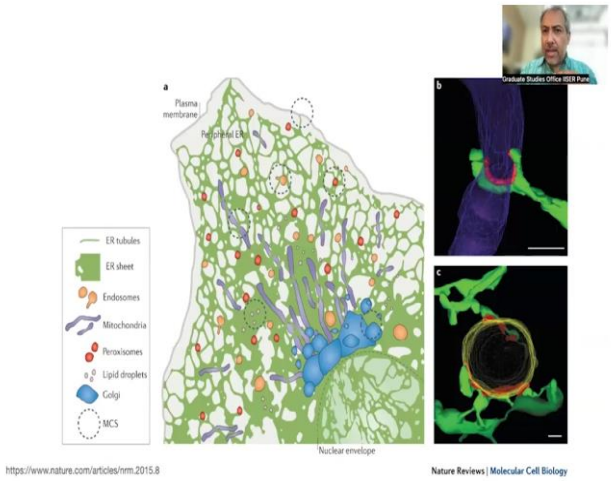

Welcome back, everybody. We will continue where we left off last time. And today we are going to talk about this rather important cellular organelle, called the nucleus. And we have been, as I mentioned right at the beginning, we have started from the cell membrane, and we have kind of slowly moved in. But before we talked about the nucleus, there was 1 or 2 things about the endoplasmic reticulum, and the related organelles that we had kind of raised last

time. And I kind of wanted to show you some stuff that is happening in the field in how these contents, these organelles actually talk to each other. And these are very recent studies.

The first point I wanted to make is I remember I mentioned telling you, I told you about the fact that the endoplasmic reticulum covers the entire cell. And in green is the endoplasmic reticulum. And there is a network of the endoplasmic reticulum. And now there are, there is thought to suggest that there are sheets of endoplasmic reticulum.

And as you can see, the nucleus is in the middle of the cell, the Golgi is in blue and the endoplasmic reticulum pretty much fills up the cell. And this is a fairly important thought to have when you are imagining the cell that just as the cytoskeleton network, the actin and the microtubules fill up.

There is a membrane, membranous organelle, which is the endoplasmic reticulum, that also fills up the entire cell. And increasingly, we are beginning to ask what that means. We are beginning to ask how the communication between different places in the cells, between different organelles is mediated by this network of membrane. So, not only do you have a membrane that is covering the entire cell, but you have this membranous structure that goes all the way.

There are some interesting circular markings which are points of contact that the endoplasmic reticulum has. The endoplasmic reticulum now is thought to talk to the plasma membrane, it is thought to talk to other cellular organelles. The figure B is the purple is the mitochondria and you can see how the endoplasmic reticulum actually wraps itself around the mitochondria holding it.

These kinds of contacts, and there are very specific proteins that mediate these contacts. We also have an endosome that they show at the bottom that is bound to the endoplasmic reticulum. The point being that many of these organelles, the endoplasmic reticulum now actually touches holds on to, and probably talks to and regulates. we do not fully understand how that regulation works. We are only beginning to understand that such a network exists and this kind of contacts also exists.

So, we really do not know much about that regulation. But that is where the field is going towards. It is trying to understand how this network of membrane that is present pretty much throughout the cell, that we are now able to see, using microscopy tools in a way that we could not earlier, could influence the behavior of the cells.

So, it is possible that 5 10 years from now, when we talk about the microtubule network and, and it going to different parts of the cell, we will also be thinking about the endoplasmic reticulum, and how its network regulates cellular function beyond just the rough and smooth endoplasmic reticulum which could be the sites where protein synthesis happens and where stuff gets delivered from the endoplasmic reticulum to the Golgi. So, initially, that was the understanding the fact that this network exists the way it does, challenges that understanding in more than one way. And as I said, this is a very recent review.

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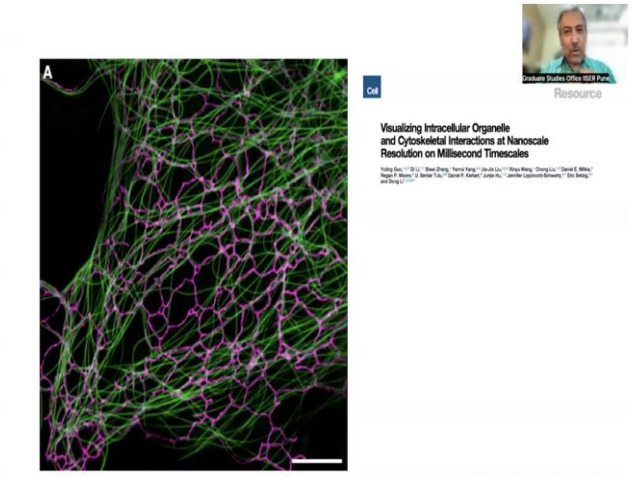

And there is now increasing evidence that this network exists. And I show this image also from a very recent paper in cell, which shows you the microtubule network and the endoplasmic reticulum network. And just as I was mentioning about the possibility that we will be looking at both of these networks, there are now actual evaluations of what that could mean. And as you can see, just as the microtubule network is spread as a mesh, the ER spread as the network too.

This could be a game changer in terms of how we look at organelles that regulate cellular function, particularly, and membranous organelle, like this which is spread as a network throughout the cell. So, this image of the microtubule network and the ER, sitting juxtaposed with each other in a cell is an image that is very powerful and I wanted you to have that with you. As you think about the microtubule network, and the endoplasmic reticulum, do not think of them as in the classical sense, the microtubule network being spread, and the endoplasmic reticulum sitting close to the nucleus, so to speak.

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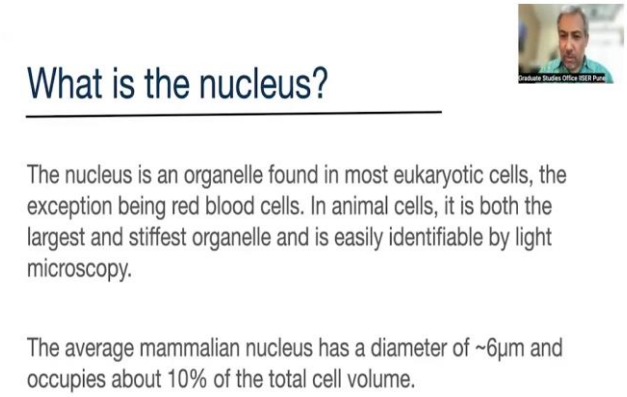

Which brings us to the organ and that we want to look at now which is the nucleus and the nucleus is an organelle that is found in most eukaryotic cells, the exception being red, red blood cells. And if you want to know more about what that means for the red blood cells and why. Please go read but it is one of the cells that actually does not have a nucleus in our body. In animal cells, it is both the largest and the stiffest organelle and is easily identifiable light by light microscopy.

So, when you put a cell under a light microscope you it is very difficult to see the Golgi, ER, the cytoskeleton network but the nucleus we will all be able to see and that in part has to do with not just the size of the nucleus but also the fact that it has a certain tensile strength, and it is one of the stiffer organelles. The stiffest organelles that is present in cells. It has a very robust architecture, which allows for that organelle to stay the way it is and be built the way it is. The average mammalian nucleus has a diameter of about 6 micrometers and occupies about 10 percent of the cell volume.

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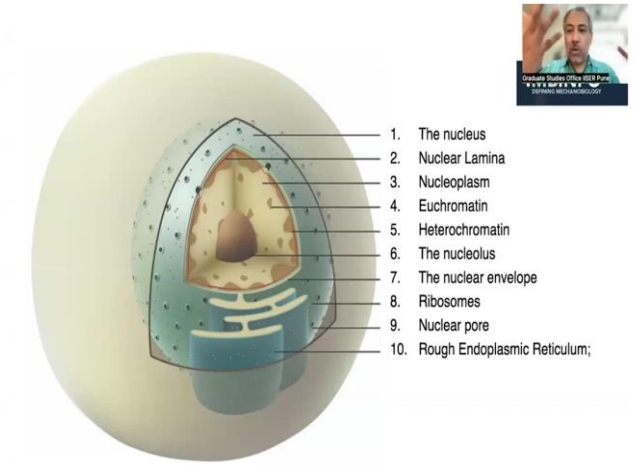

The if you look at the cell and the nucleus in it there are very specific components that make up the nucleus. And some of you are already familiar with we have the nucleus there is this nuclear lamina that provides architectural support to the nucleus, there is a nucleoplasm that exists between the 2 nuclear membranes. And I will show you what that means as we go forward. There is euchromatin that is heterochromatin that again, we will look at. It has a nucleolus and it is only now that we are slowly beginning to understand what the nucleolus does in cells.

There is obviously a nuclear envelope, there are ribosomes that are part of the smooth the rough endoplasmic reticulum that is actually directly connected to the nuclear membrane. And then there is the nuclear pore complex. And the nuclear pore complex or the NPC is a very vital segway or communication link between the nucleus and the cytoplasm and it is very elaborately constructed. And that is another interesting point to consider when we think about the nucleus is the way it is built.

And the way the kind of connections it has, the kind of regulatory components that interface of the nucleus with the cytoplasm has, because it obviously does very important and in some ways unique things. And the cytoplasm clearly has a lot of content that do not want necessarily all of that content to also be in the nucleus. So, there is a very clear boundary that defines what stays outside that what stays inside. And the nuclear pore complex is an important kind of segway between the 2 compartments that allows the content of the nucleus to talk to the external environment.

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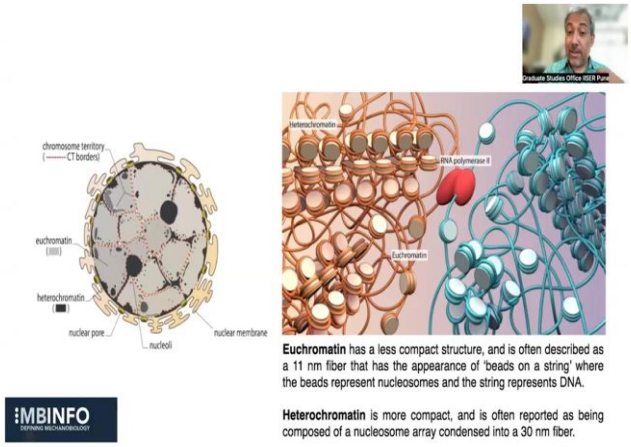

So, the architecture of the nucleus, if you see, you have the nuclear membrane that is connected to the endoplasmic reticulum, the rough endoplasmic reticulum. The nucleus also has something called chromosome territories and these are essentially this is a nucleus that is a where the DNA is still fairly opened up, they are not condensed into chromosomes. And there are very specific regions in the nuclei that genes and DNA per se is held in such a way that these regions contribute to very unique regulatory controls for the expression of these genes.

So, these chromosome territories essentially are so, you will think that all the DNA that is inside the cell is free floating around and is moving around the way it wants to and that was the understanding for a long time till it was discovered that no chromosome regions are held in very specific places inside the cell. Some for example, are closer to the center of the nucleus, some are closer to the periphery or the nuclear membrane. The expression of genes depending upon where these chromosome territories are, is carefully regulated.

Kundan Sengupta's lab studies this. They actually study chromosome territories, they study nuclear organization, they study proteins that keep the architecture of the nucleus. And the nuclear lamina proteins like lamins, for example, that we will quickly look at as well. They study these as well, and that nuclear lamina they now think is very vital for maintaining these chromosome territories. So, when you imagine the nucleus also imagine it with this regions of definition for where genes and DNA is sitting.

As I mentioned earlier, among the things that are present in the nucleus includes euchromatin and heterochromatic. Euchromatin has a has a less compact structure. So, remember, all DNA is not loosely lying around, it is wrapped around these proteins, which are called chromatin. And my favorite example is I do not know whether you have ever seen a yoyo. And if you have seen a yoyo there is a string that goes around that can be wrapped around the yoyo.

That is kind of the thought that you have when you look at chromatin and you look at DNA that is wrapped around it. And the extent to which they are packed, is variable. And that also affects the expression of genes. So, the way the DNA is read will depend upon how things are packed. You guys may have already read about DNA stretches or genes having exons and introns things that are read and things that are not read at least not in the conventional sense. And the chromatin bundling has a vital role in the availability of the exons for reading and introns being kept away from being read.

The euchromatin has a less compact structure and is often described as a 11-nanometer fiber that has the appearance of beads on a string where the beads represent the nucleosomes and the string represents the DNA. So, these are loosely tied to the nucleosomes and the heterochromatin is more compact. And because it is more compact it is more condensed and that not only affects the way the DNA is wrapped around and kept, but it also eventually affects gene expression.

Remember, the intent of the nucleus here as an organelle is to do a couple of things, one is carrying this information in the form of DNA, that is very vital to the entire machinery of the cell being replicated or being created again. So, so many things are dependent on the expression of genes. And so, these the expression of these genes, and the way these genes are held and protected becomes very vital to that information being passed on from one generation to another.

And so, that seems to be the primary objective of the nucleus to be able to protect all this genetic material that is held inside to find a way for this genetic material to be interpreted correctly and read out and for proteins to be made when required. And eventually to find a way to distribute this genetic material to the 2 daughter cells that this cell will generate. So, a nucleus will give rise to 2 nucleus which both which each of which will become part of the daughter cell.

And the way this content is now wrap is divided and distributed is hugely vital to how, this what the role of the nucleus is. Now, the other cellular organelles obviously have to do the same to and when we come to cell division, we will actually look at how that happens. So, the nucleus, at least we know, does could do this, but what about the rest is an interesting question.

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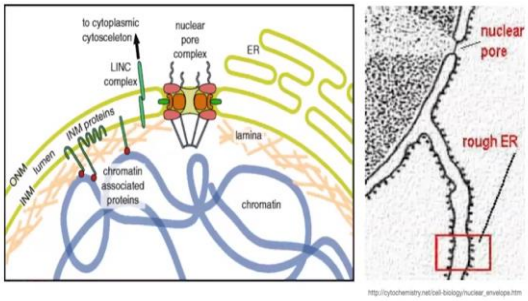

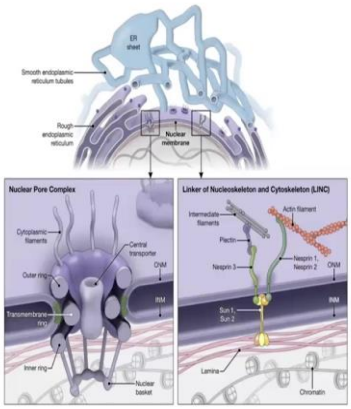

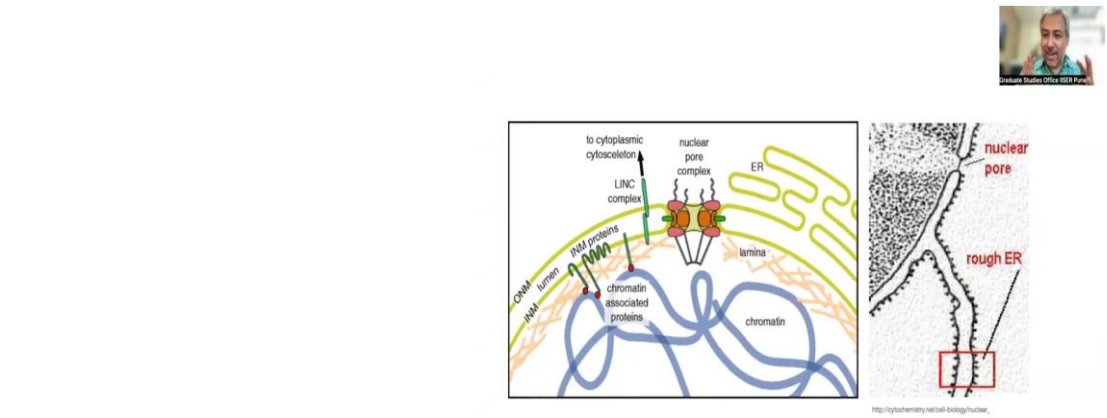

The way this architecture is put together you can see, the ONM, which is the outer nuclear membrane, there is an inner nuclear membrane, which is the INM and then there is a lumen there is a region that exists between the outer and the inner nuclear membranes and that is filled with the nucleoplasm. It also has inner nuclear membrane proteins that are attached to the inner membrane. And many of those inner membrane proteins actually talked to the chromatin.

So, when we when I showed you these chromosome territories that exists, where these DNA that is bound to nucleosomes is kept its location is actually mediated by the binding to many of these inner membrane proteins. The other interesting thing that you notice is the lamina which is essentially in the cell membrane on the plasma membrane. You remember the plasma membrane and the actin cortex that is lying right next to like underneath it.

The nuclear lamina is a similar structure that lies just below the nuclear membrane and has a very vital role in not only keeping the architecture but also talking to different components that are present in the nucleus. So, it is very interesting the architecture, the parallels in architecture that exist between what the cell membrane looks like, and what the nuclear membrane looks like. They may not exactly be made up of the same lipids, they may not have the exact same architecture, but there are some common themes that seem to be retained.

The fact that this is a membrane, the fact that it needs something to support the membrane. And so, there is a cortex, a lamina here and acting cortex there, that is mediating this and that plays a very vital role in not just maintaining the architecture, but how things are

communicated through. And then there is the nuclear pore complex, which already looks like a very complicated structure. And I will show you more recent studies that have very beautifully elaborated the kind of proteins that come together that to make that nuclear pore complex.

It is such an intricate structure and so much thought has gone into making it the way it is. The image on the right I kept here, because it actually shows you where the ribosomes attach. And it is interesting they are attached to the outer nuclear membrane the ONM. And which then becomes the rough endoplasmic reticulum. So, that connectivity is very beautifully illustrated here. And so, that is how the rough endoplasmic reticulum and the nuclear membrane are connected. They are effectively connected through the outer nuclear membrane. And then of course, there is the nuclear pore complex.

So, this essentially shows you a little more elaborately that architecture a bit more information now here. And again, the nuclear pore complex is present, the nuclear cytoskeleton and the cytoskeleton of the cell also talk to each other. So, what we saw earlier, as the lamina and the chromatin that is present there.

Now, the lamina and the chromatin are present here too. There are obviously inner membrane proteins, the Sun 1 and 2 that you are looking at here. But now look at the fact that there are proteins in the outer nuclear membrane like Nesprin, which actually bind to intermediate filaments and actin filaments which is now in the cytoplasm. So, the nuclear membrane is interesting in the sense that it is a double membrane. It has a nuclear lamina on the inside and on the outside, there is the actin cytoskeleton that can talk to it as well.

So, there is connectivity from both sides to cytoskeleton components. And then obviously, this connects the outer nuclear membrane transitions into the rough endoplasmic reticulum and then the smooth endoplasmic reticulum. The nuclear pore complex as it is shown yet is already has a very interesting and exciting architecture.

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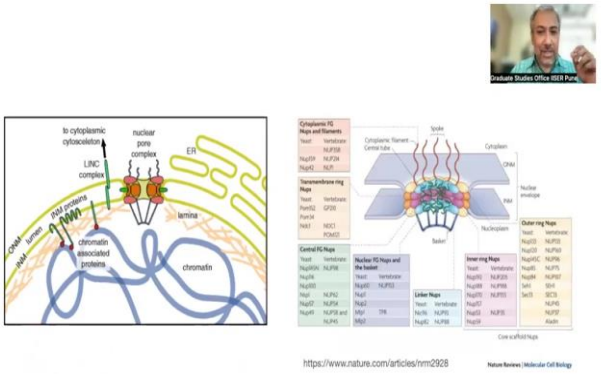

It is put together by a whole bunch of proteins. And it is interesting how there are so many proteins that have been come together to assemble this architecture. And this architecture is really remarkable in the sense of what it is able to do and how carefully it is able to regulate things.

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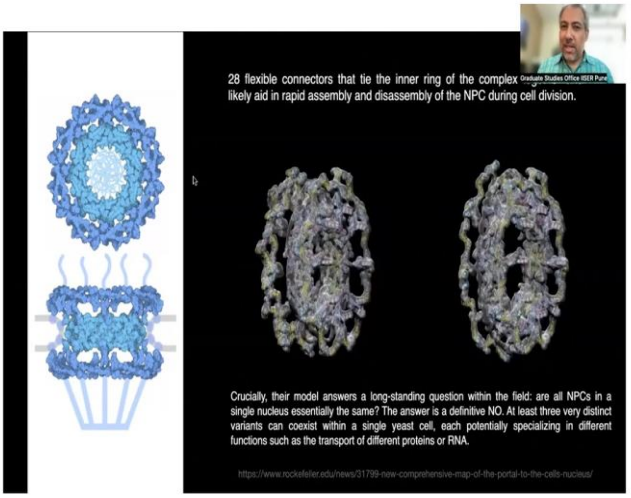

Actual X-ray crystallography structures of the nuclear pore complex have been made and these really show us what that architecture looks like. About 28 flexible connectors tie the inner ring of the complex together and likely add in the assembly and disassembly of the nuclear pore complex during cell division.

Remember, the nucleus and this architecture and we will see this at the end has to very rapidly break up and just as we, when we talked about the cytoskeleton, we talked about the fact that you need to put these together, and you also need to have a way to break them up such that it can be moved to new places.

The nucleus at very specific times in the cell cycle has to undergo that break up and, and a lot of this machinery has to be broken down and brought back together. And so, it is kind of programmed to be able to do that. And among the important things about the nuclear pore complex is this machinery is largely built to allow for it to assemble, disassemble very quickly. If you remember, the clathrin coated pit that actually buds of membrane from the Golgi. It has this very interesting triskelion like structure, which has tripartite structure that assembles very quickly to create a bud and then the structure completely disassembles.

And so, there are many examples of these number of subunits that are coming together to create a complex structure and then go apart. And the advantage of having subunits as you have the ability to do this, bring it together and take it apart as quickly as required. The other interesting thing about the nuclear pore complex that these recent studies and this is a very recent study about a year or 2 ago, have shown is, that the all-nuclear pore complexes are not the same. And there might be differences in the different nuclear pore complexes that exist on the on the cell membrane.

So, this is the architecture of the nuclear complex. And you can see, as it is moved around how intricate these proteins are in the way they have been put together to create this architecture. It is truly a remarkable thing. And I am not getting into too much detail. If you are interested, obviously, you should go read and I will share content that will allow you to go look at some of these in greater detail. if you are interested.

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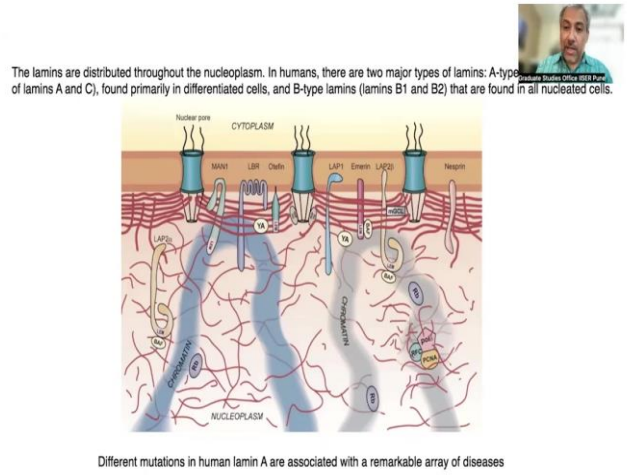

That now is our understanding of what that nuclear membrane looks like the red here at the bottom you are looking at the cortex which is where proteins like lamins, for example, play an important role in creating that cortex. Remember, these are intermediate filament proteins. The lamins are distributed throughout the nucleoplasm so, like just like there is a cytoplasm, there is a nuclear nucleoplasm as well.

And there are a bunch of you are looking at a bunch of inner membrane proteins, you are looking at the nuclear pore complex that goes through. You have chromatin that is now anchored to the cortex, the that is like below the nuclear membrane and that is responsible for these chromosomes' territory. So, now you understand what that distribution looks like. lamins are distributed throughout the nucleoplasm.

In humans, there are 2 major types of lamins A type lamins consisting of lamin A and C found primarily in differentiated cells, and B type lamins, lamin B1 and B2 that are found in all nucleated cells. Different mutations in human lamins, a lamins A, for example, are particularly associated with remarkable array of disease.

And there are many interesting outcomes of just targeting this protein. And this also tells you how important one single proteins that a protein that regulates the architecture of the lamins cortex in the nucleus could be that it changes in a whole lot as far as cells are concerned and this reflects in all these diseases that we can see.

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One of the interesting diseases is progeria which is a premature aging disease, which is called the Hutchinson Gilford Progeria Syndrome. And it comes because of lamins being mutated. So, there is a there is a movie recently, maybe 10 years ago that was made which looks at somebody who has this disease, and it is very interesting that the entire phenotype of this disease is governed by a mutation in one single protein and that protein is a lamin.

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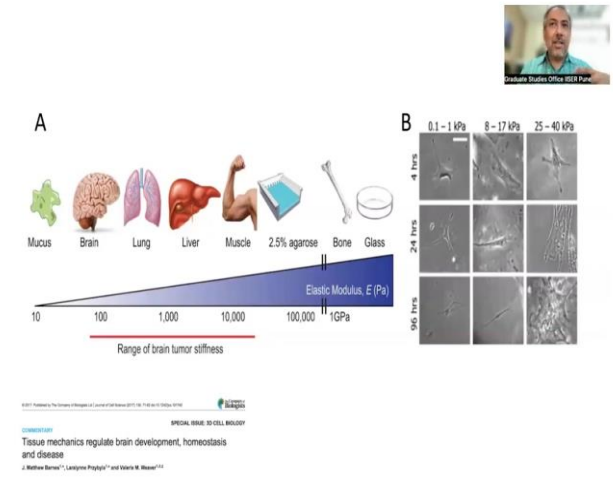

The other interesting idea that I want to bring in here and the nucleus is a great place to introduce this is that these cells, which are all part of different cellular organelles also have different are part of organs that have varying stiffnesses. So, everything from the brain to the

muscle, to the bone, have very diverse stiffnesses and the cells obviously on these stiffnesses behave very differently.

So, here you are seeing an example of cells that are held for plated for 2 hours, 4 hours, 96 hours on varying increasing stiffnesses. And when you put them on increasing stiffnesses the morphology of the cell changes, the behavior of the cell changes so much is altered in how cells feel and behave. And this is an important thought to also carry with you that when you are thinking about a cell along with all these characteristics that go to build the cell. What is also really important and interesting to keep in mind is cells are also mechanosensitive, and in many cases are mechanoresponsive that means, they respond to changes in the stiffness in their environment.

And this is mediated by many proteins that are present in the cells being responsive to mechanical stimuli. So, there are proteins that are receptors on the plasma membrane, that depending upon whether they bind to a stiff matrix or a soft matrix will have very different functionality.

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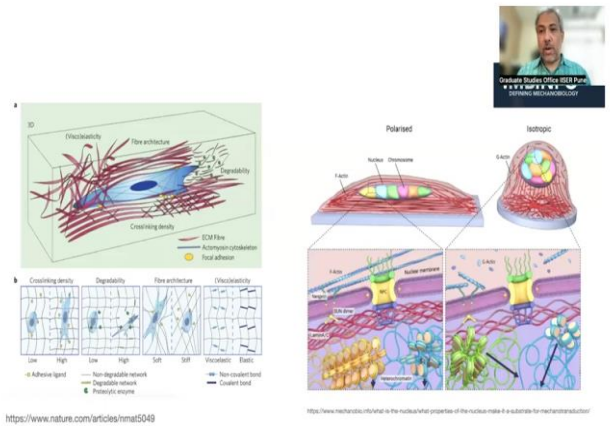

And that mechanosensitivity is also visible at the level of the nucleus. So, cells for example, and the image on the left is essentially showing you all the different properties of the environment that influence cellular behavior the extent of crosslinking that happens, the extent of degradation or degradability that happens, the fiber architecture that is present. The viscoelasticity of this environment, all our biophysical properties that affect how the cell feels and the cell responds to this.

And the nucleus in particular is very sensitive to mechanical stimuli largely also, because the good amount of the cell is response is governed by the gene expression that the nucleus has. So, depending upon how the nucleus is pressed or pushed, and what kind of tension it is experiencing, the response of the nucleus will change. So, a cell that is flat, versus a cell that is more or less around, the nucleus is under different tension.

Remember a nucleus that is pulled this way, versus a nucleus that is now experiencing less tension and is more or less around, the way these chromosome territories are distributed, the way the lamin architecture is, in response to this changing stiffness of the that the nucleus is experiencing affects the expression of genes.

So, remember this, the cell in itself is sensitive to mechanical stimuli, and among the many organelles that are responding to mechanical stimuli. The nucleus in particular, is a very important mechano-responsive organelles, something that changes its behavior and changes gene expression, if required, in response to mechanical cues.

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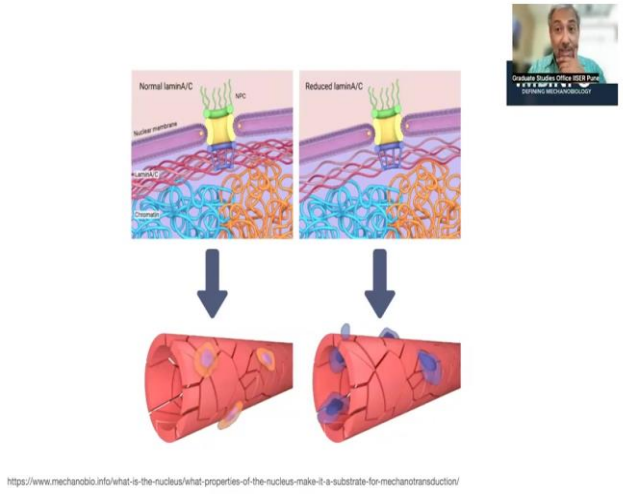

So, for example, one good example of where this kind of tension could be this mechanical cue could be experienced, is a very simple example is if you put your thumb on your pulse, and you can feel your pulse, what you are feeling is essentially flow of blood. It pumps every time your heart pumps, there is a flow of blood going through.

And if you put your finger on your pulse and you can feel the pulse it essentially says that there is in the blood does not flow continuously. It flows in waves and that force when blood

is moving through one small part, which you are touching, is what gives you the sense of the pulse.

It is strong enough for you to feel it over your skin. Imagine what the blood vessel is experiencing. Every time that blood vessel, there is a gush of blood going through this, it does this. And that is what you feel, and that happens to blood vessels in different parts of the cell. And that kind of rhythmic movement in mechanical cues is something that the cells experience and they react to this.

So, there are different kinds of mechanical stimuli that the cells will experience. Obviously, the stiffness of their environment is one, they could experience flow, like in blood vessels in the inner ear. There are cells that are floating in a kind of a viscous liquid. And the movement of these hair cells in the inner ear is vital to not just us being able to hear the communication that we are happening here. But also, the balance of the inner ear that the inner ear contributes to, is mediated by those hair cells. And they are extremely mechano-responsive and small changes, therefore, in that responsiveness can affect your balance, and also affect your hearing in that sense.

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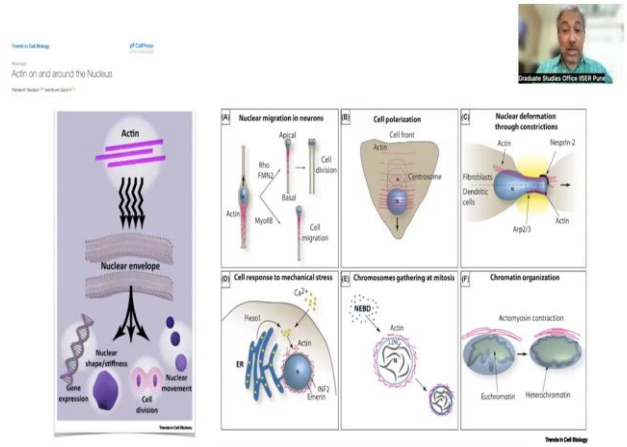

So, there are many cells in the body that actually respond to this. This is just to kind of capture the idea that we spoke about earlier that there is an actin cytoskeleton on the outside that also talks to the cell. And this actin cytoskeleton, talking to the nucleus does very interesting cells things for the nucleus. It plays a vital role in kind of polarized movement of the nucleus in specific directions, whether it be in migrating neurons, or in cells that are

getting polarized the nucleus moves to the back and that is mediated by the actin cytoskeleton.

Nuclear deformations, as the cell moves through small spaces. Remember, the nucleus is getting squished through and the nucleus is not as soft as the rest of the cell. The rest of the cell may actually make it through more easily, the nucleus actually has to be pushed through. And if that is the case, the cytoskeleton plays a very vital role in creating that response, the cell response to mechanical stress, we just talked about cells. Chromosomes coming together or gathering at the nucleus, during mitosis is, again driven by the cytoskeleton.

And the chrome, the chromatin organization just as I told you, the lamins on the inside of the nucleus, decide where the chromosome territories should be, the actomyosin on the outside can also affect by putting pressure on the nucleus where these chromosome territories are going to be. So, many interesting things that this cytoskeleton network on the outside does, to what is happening on the inside of the nucleus.

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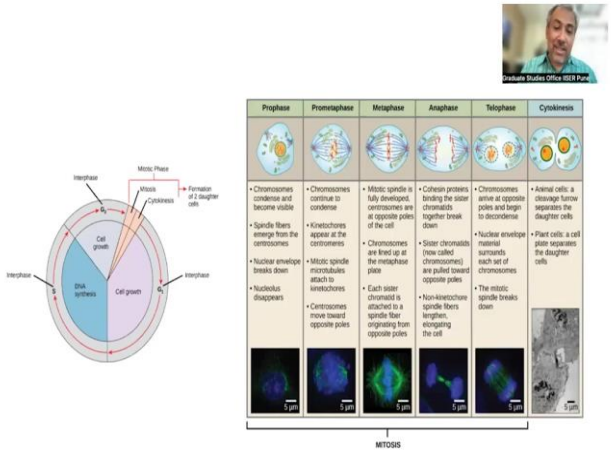

And as I said, a lot of this changes as the cell divides and during cell division, in particular, the nucleus, which is this yellow dot here has to kind of open up throw all its content out into the cell. Allow the chromosomes to form pull everything apart create 2 nuclei, which then reform and become this very nice compact nucleus like the parent's cell had.

And if you think about it, and if you think about all the things that need to happen in the nucleus, this opening up and breaking of the nucleus and coming back together is a remarkable event, because it has to happen at a very specific time in a very specific way. And

it has to maintain everything that the nucleus is required to do exactly, the same way as it reassembles.

And knowing how many moving parts there are in the nucleus, how many different players pieces that are coming together, the nuclear pore complex with all its different components, they all have to come back together to create the nucleus again, and have it functional perfectly. When you think about it, it just, it has to blow your mind, saying, and cells do this all the time, they do this with a certain degree of accuracy and efficiency that is staggering.

And that is why I pick cell division, because it is a great place to illustrate not just the complexity, but the effectiveness with which that complexity is handled by cells. All these proteins, all these pieces that you now know, make up the cell have to get separated, and then have to work into cells as perfectly as they did to begin with. And that is just remarkable every time I teach the course, and I talk about this, it still just amazes me, and we are still discovering things about how that process works.

When we talk about in the coming lectures, I will show you some data that that was discovered last year, on how, for example, the mitochondria get distributed in dividing cells, like, we did not know this till last year, that this is what happens and it is a remarkable thing to see. And cells have been doing this all the time and that is just amazing when you think about it.

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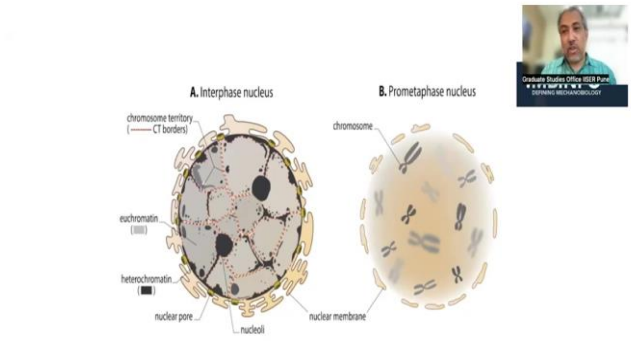

A series of processes must therefore take place that enable the cell to package DNA within the confines of the nucleus whilst retaining its ability to transcribe and duplicate the entire DNA sequence and maintain its integrity. This is achieved through an elaborate process of DNA condensation that sees DNA packaged into 46 chromosomes (or 23 chromosome pairs) in humans. The number of chromosomes varies from species to species; for example, there are 40 chromosomes (20 pairs) in mice, 8 chromosomes (4 pairs) in the common fruit fly and 10 chromosomes (5 pairs) in the Arabidopsis thaliana plant.

So, this is essentially, what I was just telling you about the kind of changes the nucleus undergoes, and how among the things that have to be that have to happen is not just the

nuclear membrane going apart, but also all the DNA that is present in this form as euchromatin and heterochromatic have to be put together in chromosomes. And then the chromosomes have to be pulled apart to create 2 daughter cells.

So, I think, any development that happens where a single cell or 2 cells coming together from the parents now fuse and the cell undergoes division and development and there is an embryo, I mean, it well and truly a miracle that this all happens and it happens repeatedly. And it happens with the degree of accuracy. That is just beyond belief.

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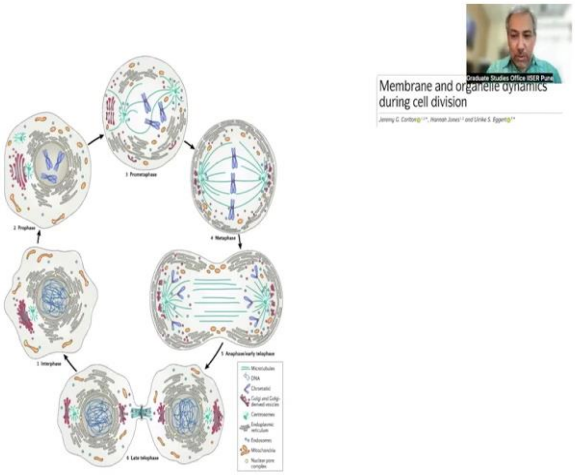

So, going forward we are going to talk about the membrane and organelle dynamics during cell division. There is an absolutely magical review article that I am going to borrow extensively from. It may be a little elaborate in some places, read the parts that you think are easy to understand. But it essentially tells you all the amazing things that the cell has to go through as it divides and as it goes through this process. And that is where I will stop today.