Introduction to Cell Biology
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Lecture 56
Cell Division

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So, welcome back what we have done up to this point is we have looked at the organization of cells, we have gone from the cell membrane, all the way in towards the nucleus, we have looked at, many of the players that are involved or are part of the cell organization, how they possibly could have originated? What kind of functional roles they play? How they are put together.

And we also looked at certain common ideas or themes, one among them being that a lot of the cellular machinery is put together in a form that allows it to assemble and disassemble, we have cytoskeleton subunits, for example, that come together to make complex cytoskeleton structures, and can break dissipate and then come back together again.

So, there is a lot of that happening in cells, there is a lot of redundancy that happens in cells, the same process being regulated by different players in slightly different ways sometimes. We also have seen how, within a cell, the same kind of machinery is used in slightly different ways in different places.

And all of this is happening, to allow the cell to thrive, to function, to talk to its neighbors, its environment, respond to it accordingly and react in that way. And we have also familiar with the

kind of molecular crowding that exist inside cells. This is an image of a cell that obviously does not have the crowding here; it is a (simple) simplified image. But you can imagine now, when you look at a cell like this, and if I say molecular crowding, what that crowding would look like? So, all of this is happening in the cell. And one of the places where a lot of this comes together to regulate a cellular event is the process of cell division.

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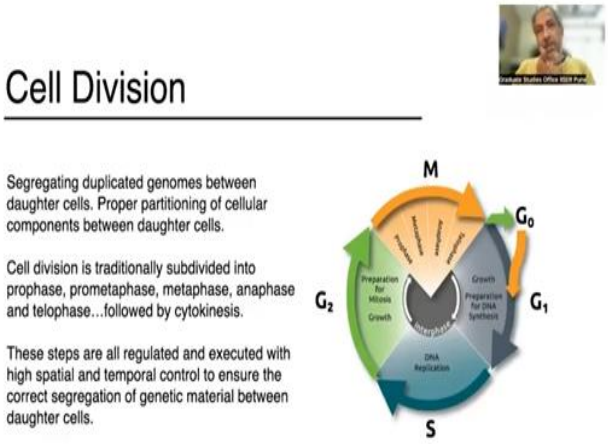

And we are going to try and look at cell division in this particular lecture and particularly focus on the process of mitosis. So, there are obviously different steps to the process of the cell cycle, (we) you have growth and preparation of DNA synthesis that happens in the G1 phase, you have DNA replication that happens in the S phase, you have preparation for mitosis and growth that happens in the G2 phase and then mitosis itself which has many steps with prophase, metaphase, anaphase, telophase, cytokinesis, and then the formation of two daughter cells.

In the interest of time, and also in the interest of not throwing too much information at you, we are going to focus on the mitotic phase largely because a lot of the cellular components that we have talked about how they are being distributed among the daughter cells is the point that we are trying to kind of look at, and a lot of that happens in the M phase in the mitosis phase. So, that is the step that we are going to focus on and look at and see how it behaves.

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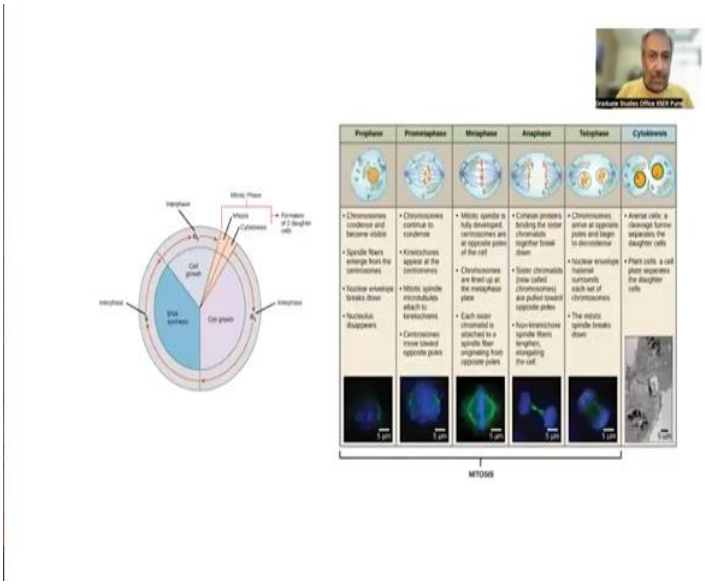

As I mentioned, this has many steps in it, you have prophase, pro metaphase, metaphase, anaphase, telophase, and cytokinesis. And, some of you may have read about this already, and have some sense of what kind of changes that are taking place. What is interesting at this point, when you think about the changes, is many of the players that we are going to talk about you now have a fair sense of how they are put together, whether it be the nucleus that opens up, whether it be the DNA that is condensed into chromosomes, whether it be the centrioles that have to go apart and now microtubule strands have to attach to the chromosomes and kind of pull them apart, whether it be the breakup of the ER, and the nuclear membrane right at the beginning, whether it be the breakup of the Golgi, and how that gets dispersed? Whether it is the mitochondria? And how the mitochondria needs to be distributed among daughter cells?

What we do know, is that, since we have looked at a cell, and we have looked at all the major machineries that are operating in a cell, we have a sense that this machinery, there has to be a mechanism to give to the two daughter cells as well, and the hope in looking at these next slides is we will get a glimpse of how that is achieved. And you now have a sense that along with all the complexity that exists in a living cell, being able to deliver this complexity to the next generation, the daughter cells, becomes that much more important as well.

And the cell has developed very ingenious ways of doing it; it is using many of the same properties of each of these players to do this. Properties that we have talked about in a different

context, and we will try and see how that ties in, so, I am not going into too much detail on each aspect and this is just to give you a flavor of what that diverse set of mechanisms could be that allows for all these players that we talked about to be given two daughter cells.

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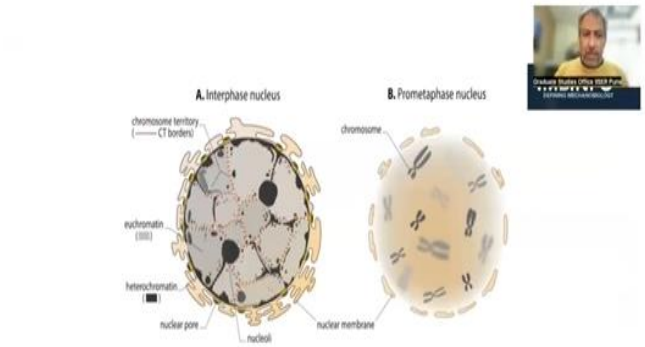

A series of processes must therefore take place that enable the cell to package DNA within the confines of the nucleus whilst retaining its ability to transcribe and duplicate the entire DNA sequence and maintain its integrity. This is achieved through an elaborate process of DNA condensation that sees 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.

We looked at this earlier, where the cell has these distinct chromosome territories and we know how these chromosome territories were or how these, this DNA is then, during the pro metaphase stage of the cell cycle wound up into very compact structures which are chromosomes, there is obviously division of the DNA that has taken place in the S phase. And, when it reaches the pro metaphase nucleus, the DNA is now packed into two sister chromatids as they are called, which are now ready for separation as well.

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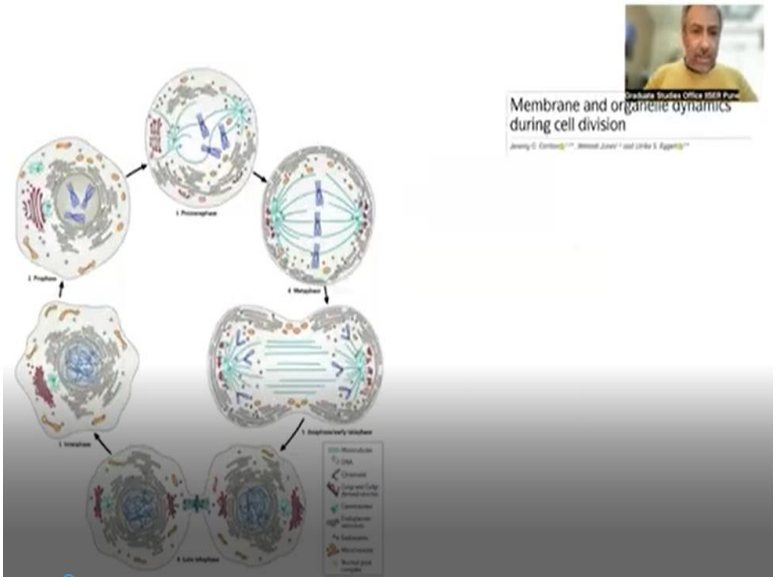

So, the packing of the DNA is one of the first steps that happens, and then there is a plethora of events that are taking place, that segregate this and we will look at all the different players, there are many players that are now listed, for example, in this schematic, and thanks to all the lectures that we have done up to this point, we know most if not all of these players.

We know microtubules we know DNA, we know chromatids, we know Golgi and Golgi derived vesicles, we definitely know centrosomes, we know the endoplasmic reticulum, we know the endosomes that are pinched from the plasma membrane, we know the mitochondria, we know the nuclear pore complex as well. And all of these players are now affected or regulated in some way during cell division.

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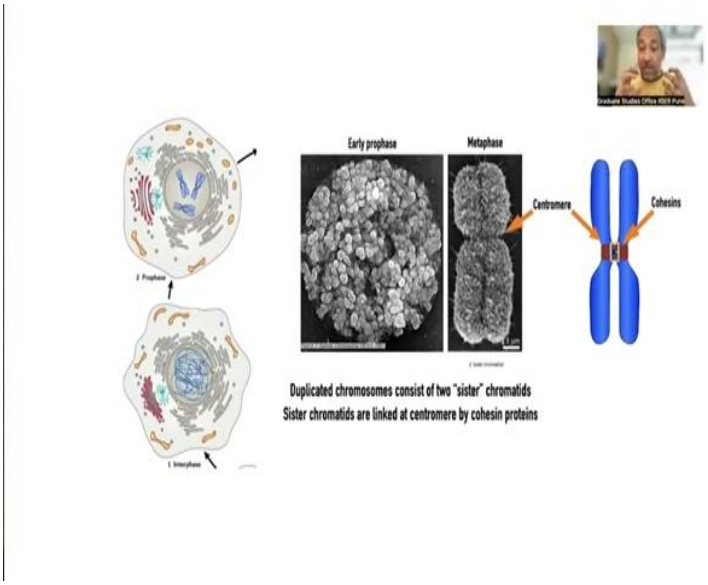

As I mentioned, during the early prophase the duplicate the chromosomes, the DNA material that is duplicated is packed into these chromosomal structures. And they have something known as the centromere that, as shown here, kind of holds the two sister chromatids together, and there are these molecules called cohesins that are essentially what allow the microtubules to come and bind the sister chromatids and pull them apart.

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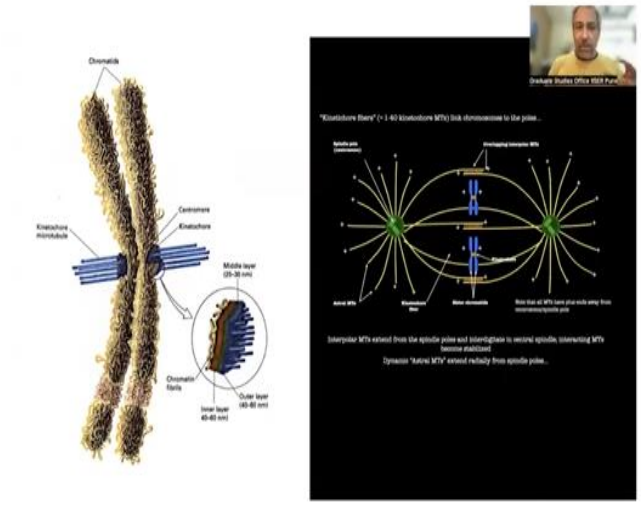

And that is kind of the schematic that shows you in great detail, what that architecture of the Kinetochore could be like around centromere? And you have cohesins as being among the

molecules that are present there at the kinetochore and microtubule strands that now come and attach microtubules we are familiar have a very distinct polarity. On the right you can see where the sister chromatids are aligned, where the two centrosomes are present? What the orientation of the microtubules strands is like? So, the plus end of the microtubules is what comes to bind the centrosomes per se.

Now, the microtubules being distributed in this kind of a manner is one of the early steps of the creation of the identity of the two daughter cells. And that separation of microtubules is initiated by the fact that the centrosomes divide. So, before the entire cell divides among the early steps is where the centrosome divides, and the centrosome which has two pieces as part of the centrosome becomes four and they then distribute to either side of the of the cell that is still beginning to divide.

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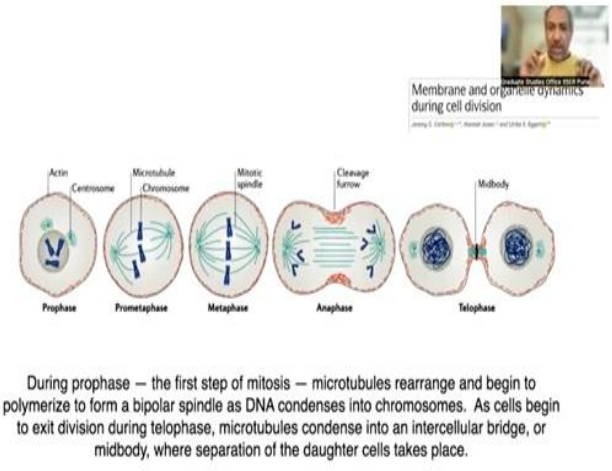

During prophase — the first step of mitosis — microtubules rearrange and begin to polymerize to form a bipolar spindle as DNA condenses into chromosomes. As cells begin to exit division during telophase, microtubules condense into an intercellular bridge, or midbody, where separation of the daughter cells takes place.

So, that opening of the centrosome or breaking up of the centrosome is what, among the early steps that happens in prophase. And then, they move to either end of the cell, and some of that, we will look at is achieved by the lipid composition of the plasma membrane, so, the fact that the centrosomes actually go to the ends of either side of the cell is mediated by lipids that could be present on the plasma membrane, none of the players that we looked at, is inert during this process, that is the other point I wanted to make that each one is doing its bit, and they are all working in tandem to achieve, this outcome of the cells actually dividing.

So, we have during the pro metaphase the chromosomes being aligned During metaphase, the alignment of the chromosomes to the center of the cell happens and then there is the pulling apart of the chromosomes, we have the creation of the cleavage furrow, you can see the actin cytoskeleton is continuing to play a role here and just as the microtubules are involved, so, is the actin and then, there is this formation of an intercellular bridge around the mid body, which is mediated by microtubules.

So, during prophase which is the first step microtubules rearrange and begin to polymerize to form a bipolar spindle as the DNA condenses into chromosomes, as cells begin to exit division during telophase microtubules also condense into an intracellular bridge or mid body when separation of the daughter cells takes place, this is one of the major changes that are taking place with microtubules.

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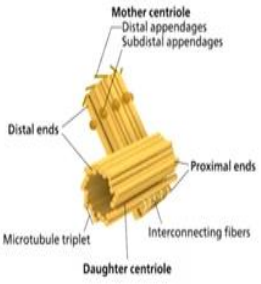

https://sciencetrends.com/centrosome-function-plant-vs-animal-cell/

A lot of this as I mentioned is initiated by the division of the centrioles and the centriole the daughter centriole actually branches out from the parents centriole, and then makes a daughter centriole, and then the parents centriole separate.

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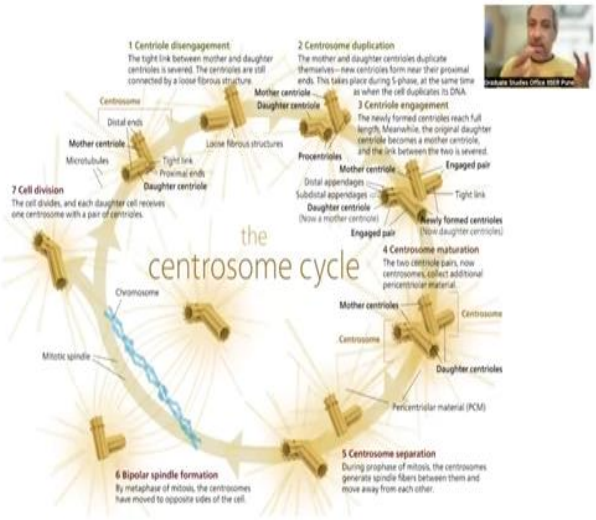

So, this is the there is a centrosome cycle as well in cells, where there is, if you look at step one that is (centry) centriole disengagement, there is the duplication of the centrosomes, you have the centriole engagement, the newly formed centrioles reach their full length and they become a pair now, and then there is maturation where the two centriole pairs now, centrosomes collect additional pericentriolar material, and then they separate, and that separation is also helped by the fact that microtubules are growing the way they are, and they push the two centrioles apart.

And the fact that there are microtubules that are anchoring to the plasma membrane on either ends, also facilitates the coming apart of the centrioles. And then you have these mitotic spindles coming to bind the chromosomes that are arranged in a way that allow them to now be pulled apart. And, now two daughter cells being generated. So, the centrosomes are vital to triggering a lot of these events that we are looking at.

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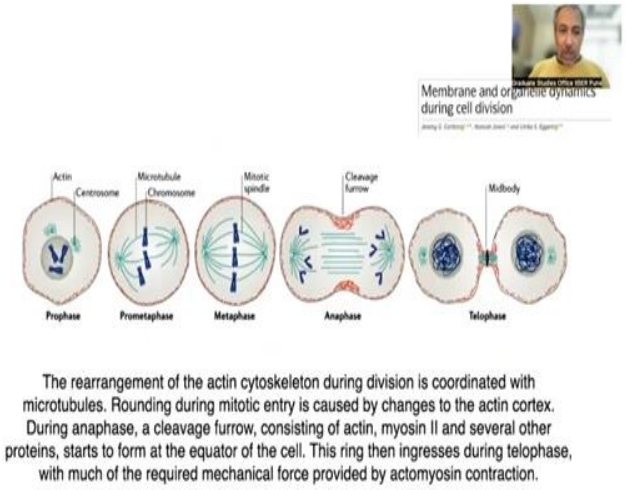

The rearrangement of the actin cytoskeleton during division is coordinated with microtubules rounding during mitotic entry is caused by changes to the actin cortex. I will talk a little bit later about how this process of mitotic cell rounding happens, cells that are normally flat and spread out during mitosis at some point become round, before the separation of the two daughter nuclei takes daughter cells takes place, and then they attach and spread again, and that mitotic rounding may have implications for what the cell for many of the events that happen during the last stages of cell division.

During anaphase a cleavage furrow consisting of actin myosin, and several other proteins starts to form at the equator of the cell, this ring then increases, which with and along with the actomyosin, contractility provides many of much of the mechanical force that is required for pinching the two cells apart effectively.

As I mentioned, during the process of endocytosis there is pinching of the membrane, and whenever you want two membranes to fuse together, one easy way to do this is to bring them very close to each other, and that allows for the fusion to take place, but obviously, bringing them together requires force.

And that is mediated by very specialized proteins sometimes like, proteins like dynamin, that we have not really discussed about in endosomes do this, that they create enough pressure and force that allows for the coming together of the two membranes and then (fuse) fusion of the

membrane causing it to pinch off, the same happens for during cell division as well. And there again, there is a ring of actin and so, the actin along with the myosin you remember, we talked about the fact that the actin strands can slide one above the other because of myosins that are attached to them.

And that kind of movement, which is very similar to, there are many purses for example, that have a string that you can pull and constrict the mouth, that is kind of the action that the actin cytoskeleton is trying to achieve and squeeze the membrane and then create this mid body that allows the cells to separate from each other.

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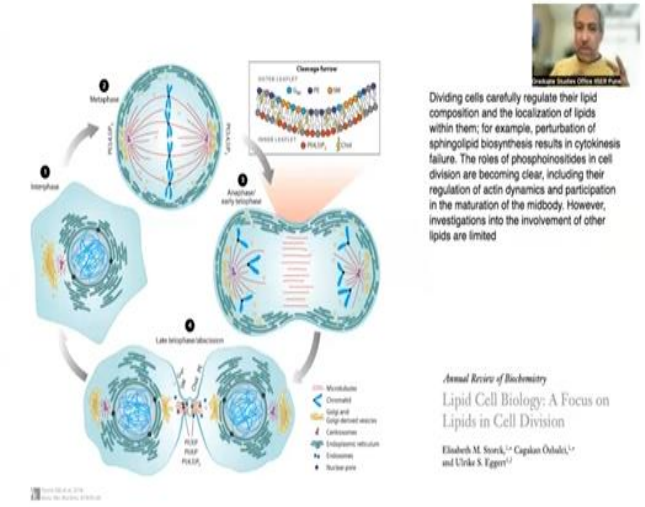

As I said, a lot of this is also governed by the fact that lipids are there everywhere and the composition of the lipids is very vital to how, many of these steps are taking place. There are three lipids which are present at the two ends of the cell, which is where the centrosomes are pulled towards, and some of that is mediated by the binding of microtubules to those two lipids and proteins that are present bound to the membrane at that point, another place where the role of lipids is very vital is lipids are also very integral to creating curvature. So, depending upon composition of lipids, some lipids will facilitate the creation of a curvature.

And so, those kinds of lipids are also enriched in the cleavage furrow. So, when you want the membrane to bend this way, it makes sense to have certain kinds of lipids here, which then can be pinched. So, it allows for the bend and once the bend happens, the actin cytoskeleton, the

actomyosin (contact deliv) contractility will come and pinch that particular region. So, you have very interesting lipids that are accumulated in that mid body where the coming together of the two cells is taking place. So, lipids are integral as part of the membrane, plasma membrane and other membranes to in regulating the cell cycle as well.

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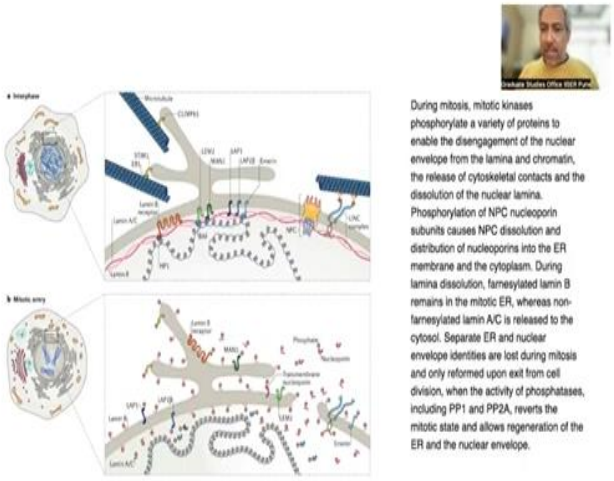

A lot of the internal components now, during various stages of the cell cycle have to be broken apart, and that includes the nuclear membrane, that includes the endoplasmic reticulum. And, you remember the nuclear pore complex, which is part of the nuclear membrane, which then connects to the endoplasmic reticulum. That is what we are looking at here, in interphase, and during mitotic entry, these now undergo breakup as the chromosomes condense, and you essentially lose this, lose this defined architecture, the pieces of the nuclear membrane are retained and distribute among the two daughter cells.

One of the questions that we do not completely know is, is there an active mechanism that allows for the distribution of structures? A lot of the mechanisms that have been thought to be operational in cells that allow for separation of membrane structure like the endoplasmic reticulum, or the Golgi are that these things break up and then the general movement of the cytosol inside the cell distributes things around. And, I will show you at the end, a very recent paper that was discovered late last year, which is a very remarkable study that shows that there could be active mechanisms that do this as well.

So, we are looking at the ER and do not worry about all the individual players, you just need to know, the major components that are here you can see microtubules you can see the ER membrane, you can see the nuclear membrane, you can see where lamins are present in the inside, the chromosome territories and how they are attached to the lamins, where the nuclear pore complex is, where the link complexes which attaches to the microtubule as well. So, the architecture now, and, you are looking at the change that happens as this gets broken down.

So, during mitosis, various mitotic kinases phosphorylate, a variety of proteins to enable the disengagement of the nuclear envelope from the lamina. So, the lamina that is lying underneath, which is kind of holding this entire architecture disengages from the nuclear envelope, and the chromatin, the release of cytoskeleton contacts and the dissolution of the nuclear lamina takes place, which then allows the nuclear membrane and the endoplasmic reticulum to now break up.

And, allow the nucleus as we know it to effectively disintegrate, phosphorylation of the NPC and a nuclear pore complex and nucleoporin subunits causes the NPC dissolution and distribution of nucleoporins into the ER membrane and the cytoplasm. During the lamina dissolution farnesylated lamin B remains in the mitotic ER, whereas non-farnesylated lamin A and C is released to the cytosol. Do not worry about what farnesylation is? It is essentially a lipid modification of these proteins but, what is important to know is that some of them make it to the ER, some are in the cytosol.

Separate ER and nuclear envelope identities are lost during mitosis and only reformed upon exit from the cell division when the activity of phosphatases including PP1 and PP2A, reverts the mitotic stage and allows regeneration of the ER and the nuclear envelope, so, there is phosphorylation of a bunch of proteins that initiates or begins a lot of this change, causes the breakup and then eventually there are phosphatases that come and remove the phosphorylations that have been added that now bring back this machinery to what it was, at the beginning. So again, this theme of things being able to be taken apart and put back together is very vital to how cellular machinery works.

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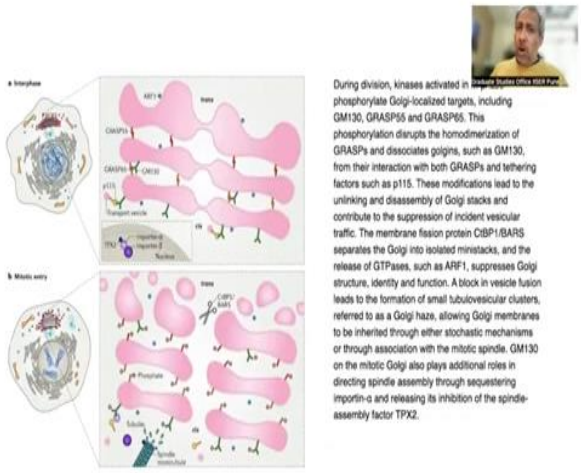

Along with the ER and the nucleus, we also see that the Golgi undergoes very rapid change in its architecture. During division kinases is activated in the M phase phosphorylate again Golgi localized targets, including GM130, GRASP55, GRASP65. These are all proteins that are vital to keeping the architecture of the Golgi, these are structural proteins. The phosphorylation disrupts the homodimerization of GRASPs and dissociates golgins such as GM130 from their interaction with both GRASPs tethering factors such as p115. What the cell is essentially trying to do like with the ER and the nucleus, is there are proteins that are keeping this architecture.

These are getting modified in such a way that they lose their functionality, they lose their ability to keep the structures together. And so, things now start breaking apart. These modifications lead to unlinking and disassembly of Golgi stacks and contribute to the suppression of vesicular traffic. Once the Golgi breaks up the delivery of things from the Golgi is also now getting affected the membrane fission proteins CtBP1/BARS separates the Golgi into isolated mini stacks and the release of GTPases such as ARF1, suppresses Golgi structure, identity and function.

So, proteins like ARF1, which are required for keeping that architecture together are lost and you can see that Golgi now breaks into smaller pieces, and these pieces now then get moved around by the cytosol and distributed throughout the cell. And then when division happens you pretty much get an even distribution between the two daughter cells. There is a block in vesicle fusion

leads to the formation of small (tubule) tubulovesicular clusters referred to as Golgi haze, allowing Golgi membranes to be inherited through either stochastic mechanisms or through association with mitotic spindles.

So, it is possible that some of the Golgi structures are also bound to the mitotic spindles and that could allow for them to be separated between the two daughter cells and some break up into this haze which is like, which pretty much fills up the whole cell. And then once the cell divides, there is a machinery that initiates, the re-putting together of the Golgi, so, a lot of these phosphorylation changes that triggered the breakup, get lost, and proteins like ARF1 whose activation may have changed is now restored. And, now the Golgi comes back very quickly.

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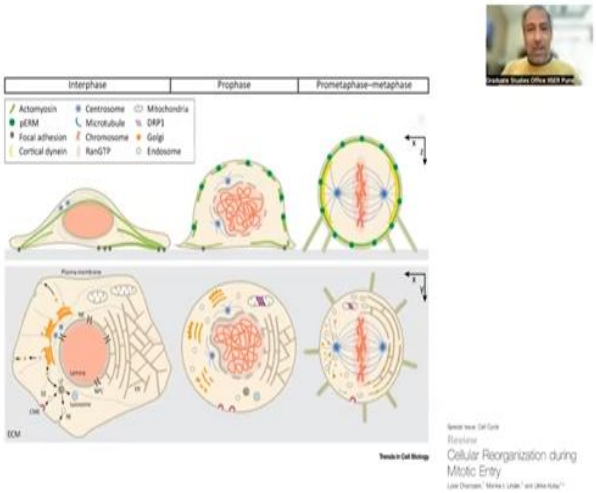

And one of the things that can drive this is actually mitotic cell rounding. So, I told you we in the lab, study cell adhesion, and how adhesion regulates the Golgi organization. And, I showed you how if you detach cells the Golgi just goes poof inside the cell. As the cell divides, one of the things you have to see and this again is interphase, prophase, pro metaphase, that the (gol) the cell actually starts rounding up and this rounding up of the cell means that there is change in the way this cell is adherent to its matrix.

So, normally, it would be nice and spread and now it becomes a round structure where adhesion is dramatically reduced. And that could also in part contribute to the breaking up of the Golgi. So, there could be many different mechanisms that are contributing to making this happen. So,

the ER, the nuclear membrane, the Golgi are all finding ways to break up and go to the two daughter cells.

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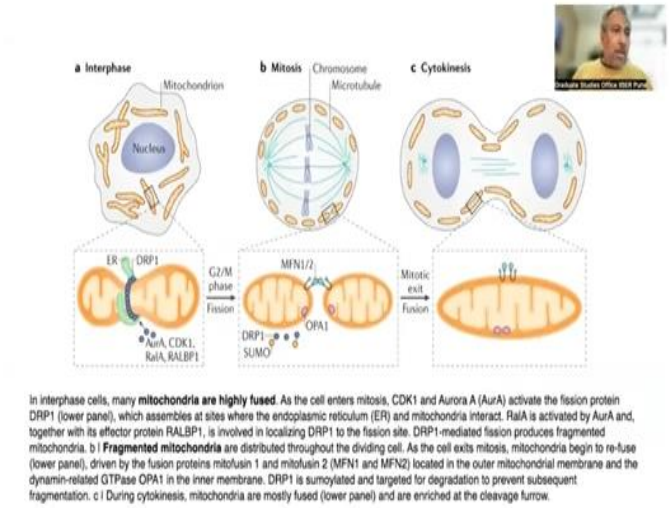

In interphase cells, many **mitochondria are highly fused**. As the cell enters mitosis, CDK1 and Aurora A (AurA) activate the fission protein DRP1 (lower panel), which assembles at sites where the endoplasmic reticulum (ER) and mitochondria interact. RalA is activated by AurA and, together with its effector protein RALBP1, is involved in localizing DRP1 to the fission site. DRP1-mediated fission produces fragmented mitochondria. b | **Fragmented mitochondria** are distributed throughout the dividing cell. As the cell exits mitosis, mitochondria begin to re-fuse (lower panel), driven by the fusion proteins mitofusin 1 and mitofusin 2 (MFN1 and MFN2) located in the outer mitochondrial membrane and the dynamin-related GTPase OPA1 in the inner membrane. DRP1 is sumoylated and targeted for degradation to prevent subsequent fragmentation. c | During cytokinesis, mitochondria are mostly fused (lower panel) and are enriched at the cleavage furrow.

The mitochondria is not going to be far behind, the mitochondria are in interphase cells are highly fused elongated structures. And they use mechanisms of fusion and fission; just as all other membranes just as there is pinching up to cause endocytosis there is pinching to separate the two daughter cells that same kind of pinching mechanism can work to break up mitochondria as well.

So, as the cell enters mitosis, there are very elaborate set of proteins CDK1, Aurora kinase A which activate the fission protein DRP1. Richard's lab, in biology here, works on DRP1, they actually look at how mitochondrial organization changes during development in drosophila. Thomas's lab, who study fusion and fission also work on DRP1 and they are interested in actually how DRP1 acts on the membrane? What does it bind? how does it actually cause vision to take place? But what effectively happens is that the DRP1 activation assembles at sites where the endoplasmic reticulum and mitochondria interact.

RalA is activated by Aurora kinase A and together with its effector RALBP1 is involved in localizing DRP1 to fission sites and DRP1 mediated fission produces fragmented mitochondria. So, essentially big long mitochondria now cut into pieces. And now, these pieces are distributed

throughout the cell. And then when the cell divides, you have a more or less even distribution of the fragment and mitochondria between the two daughter cells.

Like with the machinery that was involved in Golgi breakup, the mitochondrial distribution also was thought to be a stochastic mechanism more than anything else, essentially, this breaks up and because there are no pieces, the cytosolic movements are distributing things, the mitochondria throughout the cell, and at some point the cell divides, and both cells get more or less even amount of microtubules.

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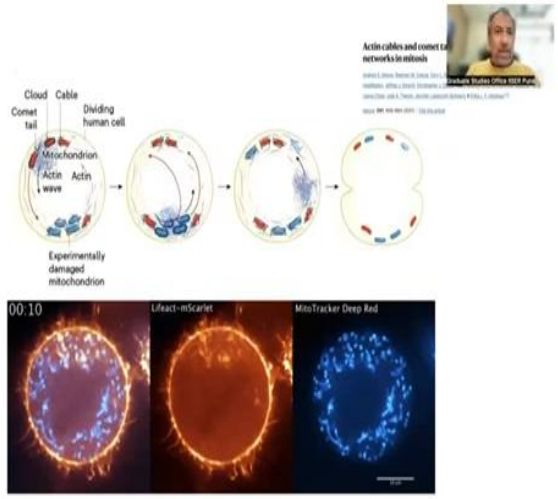

This was still last year, that there was a study that remarkably showed that there is an active mechanism that is mediating this. And this is very interesting because the mitochondria, in some cases actually have tails of actin that move it around. And along with this, the actin also undergoes very distinct movement in the cell, that effectively shakes up the mitochondria, this actin architecture and movement could also contribute to the distribution of other membrane organelles. Because, imagine, these are all pieces that are lying around. And now you want somebody to kind of move them around.

So, what you would do is you would shake this cell around a bit and make sure everything gets distributed. And then say, now go divide. And, if you want to mix something in a bowl, you will put a spoon and essentially stir it that is what the actin cytoskeleton is doing. And I am going to show you this movie, which I think the first time I saw just blew me away, knowing what we

know and about these mechanisms, and it is very interesting. This is the first time in an introductory biology course; I am actually showing this movie. And, till last year, this was not part of our understanding.

And this year, now, we have this very beautiful mechanism that was just discovered, a couple of months ago, that allows you to see how the actin cytoskeleton could act as a spoon to stir the entire cell up. Look at what the acting does, so, the Middle images of actin of the mitochondria. And you can see that the actin creates a wave that runs through the cell. In many cases, this actin is attached, or in close proximity to the mitochondria and actively moving the mitochondria.

Look at the blue on the right, which is the mitochondria only, you can see the movement of the actin wave., if you have been to a cricket or a football stadium, there is this Mexican wave that runs through the stadium at times, and it is quite a remarkable thing, because it kind of has a life of its own. And, this actin ring does that, it kind of runs around the cell obviously (shakes her up) shakes up the microtubules. But it probably does this, this kind of movement also does, redistribution of many of the membranes that are all been kind of broken around. And so, whether it be the ER, the Golgi, such a wave could make a difference to all of them.

So, the cell essentially has, broken up all of these things and now you put a spoon in there, and then you stir. And that stirring is mediated by the actin cytoskeleton. It is truly a remarkable observation and the fact that, it happened a few months ago, says that there is so much that we still do not know about these processes, even though cell cycle has been studied for such a long time. There is a lot that still remains to be discovered, so, a lot of those structures that we now know, break up in the cell, or run like this could distribute them in a way that ensures that when the cell divides, there is indeed equal distribution, and what a remarkable way to do this as well.

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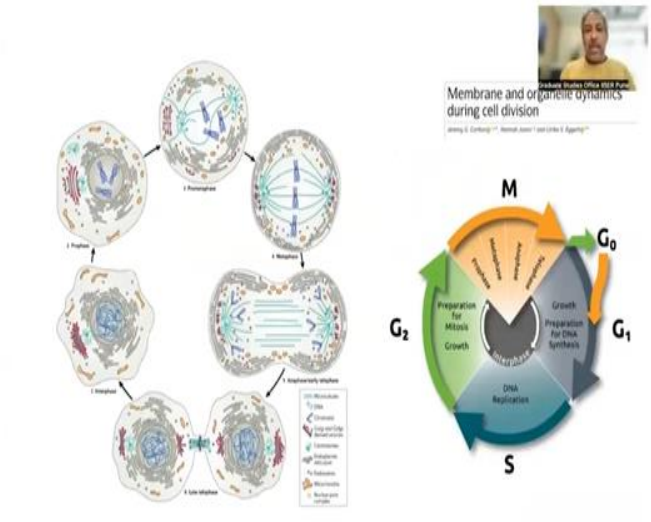

So, that is kind of in a nutshell looking at all the players that we that we spoke about and how their functionality is regulated in the process of the cell cycle. Obviously, there is a lot more to this than what I have just told you just now. And there are very intricate mechanisms for how they are regulated? I will share some of these reviews that you should go look at, there are two very wonderful reviews; one is on the membrane and organelle dynamics during cell division and another is this particular review, which is the lipid cell biology of focus on lipids and cell division, both are very wonderful reviews. And when you get a chance, go read it out of curiosity to see what you make of it. So, we will stop here.