Introduction to Cell Biology
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Lecture 52
Endomembrane System of Cells: Part 3

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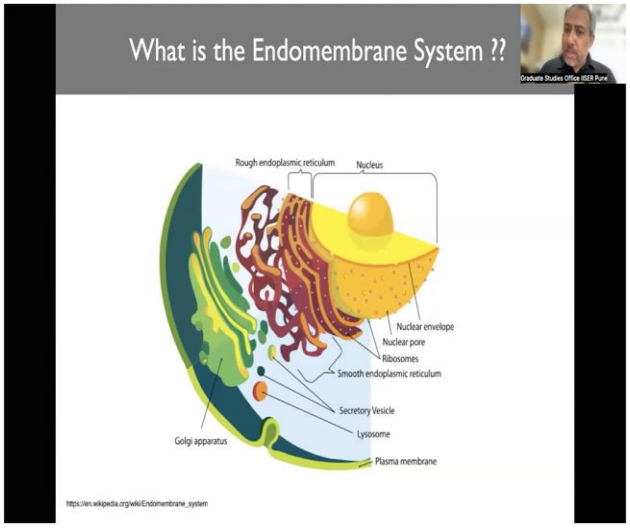

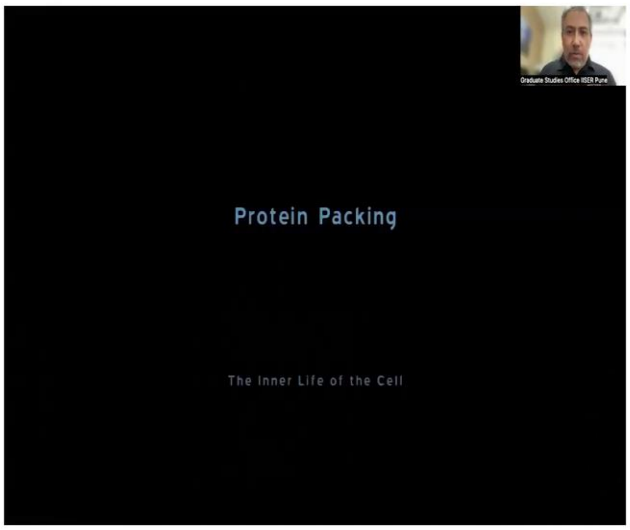

So we are going to try and continue where we left off with the endomembrane system and look at two additional players that we think could be of relevance. And both are interesting in what they are trying to do. And, both of them essentially originate from the fact that these are vesicles that

are coming together to make larger, and more complex vesicles, which have very unique properties or functions.

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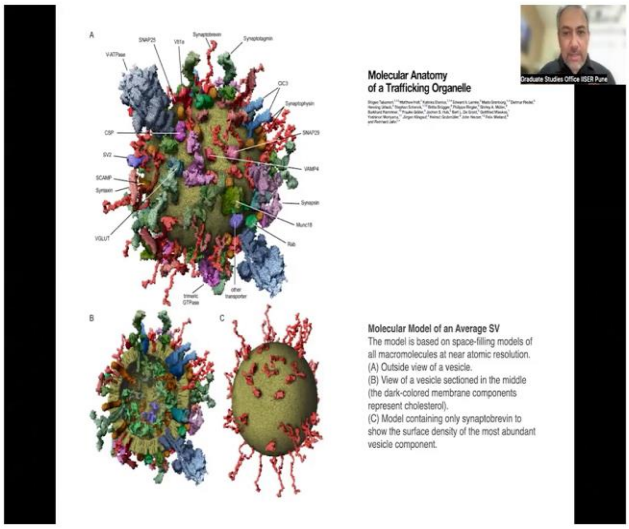

So, if you remember last time, we looked about looked at a vesicle that is used for in neurons, a synaptic vesicle. And, this kind of the idea of a vesicle, and how it could do very diverse things, is what we are continuing forward as well.

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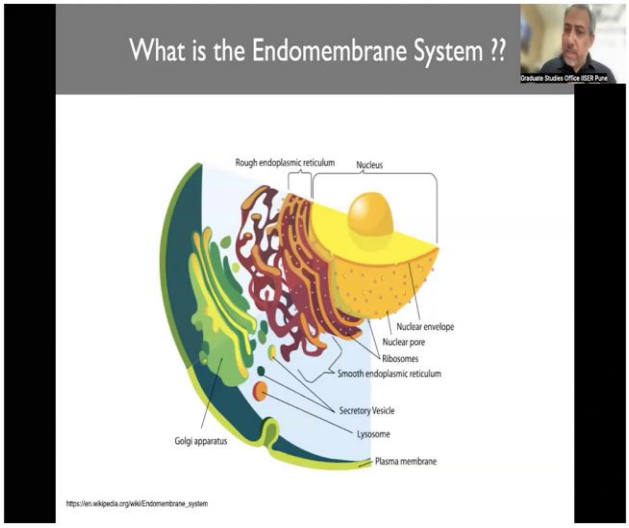

The organelle that we are going to talk a little bit about is particularly relevant, in context of an entire area of research that has developed in the last maybe 20 years. And we will try and

connect it to that, and this area has been well recognized now with a Nobel prize in 2016, as well. And I will tell you what, that area, per se is.

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The organelle that we are going to talk about, kind of reminds me of this. I do not know how many of you have been fans at some point of time of George Lucas's Star Wars. During the pandemic I have a 9 year old son and we see watched among other things, the entire series. And I do not know how many of you recognize what this is,

Student 1: Death star.

Student 2: Death star

Professor Nagaraj Balasubramanian: Death star that is right. So, there, so I think anybody who is seen this knows this thing. And the, the organelle that we are going to talk about today kind of reminds me of the death star. It is a very complex structure that is capable of destroying entire planets.

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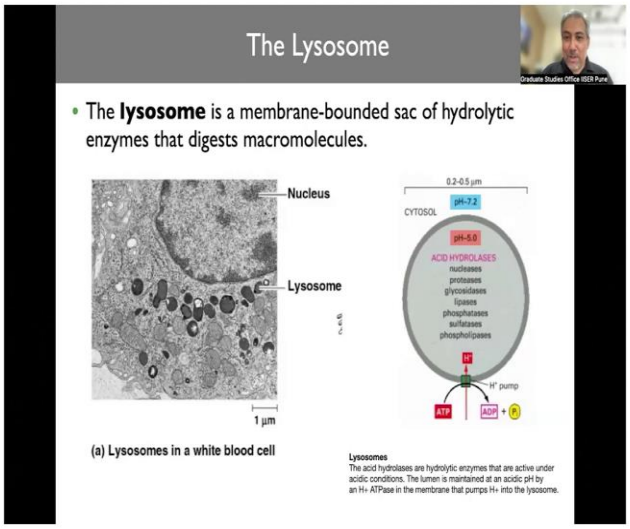

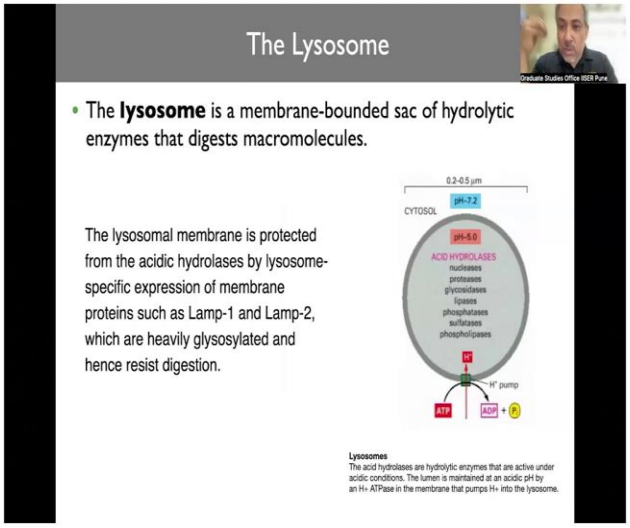

And, that is pretty much what lysosome does too. And it is a very potent organelle. Because it is essentially very dangerous, it is, it has the capability, as I said, of destroying much bigger complex structures. And in the cell, the lysosome is essentially a sack of hydrolytic enzymes that digest many different macromolecules.

The, pH inside the lysosome is maintained by a proton pump in them, and that pH is around 5 though the cytosol is 7.2. And now imagine this is like a bag of acid that is floating around. And it has a number of acid hydrolysis, nucleases, proteases, glycosylases, lipases, phosphatases, sulfatases, phospholipases, everything that can chew everything else up.

So it is a very dangerous bag that is floating around. And it is a bag of lipids and it is very interesting how the lipid membrane here is protected from everything that is inside. So, the lysosomal membrane, ,, expresses proteins such as ,, these proteins, lamp 1 and lamp 2, which are heavily glycosylated.

You remember when we talked about the Golgi, we spoke about the fact that the proteins that make it through the Golgi undergo among other changes, ,, this change in addition of sugar residues, which is called glycosylation. And so, they have a number of glycosylated proteins on the surface, which kind of protect them from being attacked by these hydrolysis. So, it is it is a mechanism which ensures that the sack is available is floating around and is able to chew on things, when required. But is not going around harming itself in the process.

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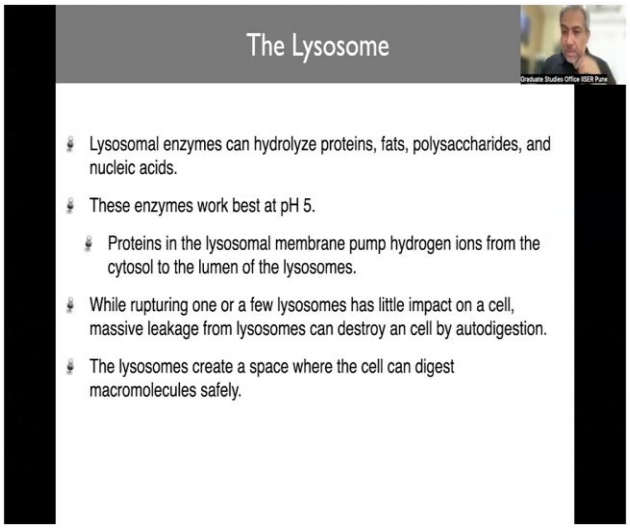

As we just mentioned, lysosomal, enzymes can hydrolyze a whole bunch of different things, they work around this pH 5, which is why the pH is maintained the way it is. And while rupturing one of few lysosomes has little impact on the cell, things will get diluted, and we will be fine. But a big leakage in the lysosome content can kind of damage the cell very badly.

So, they effectively provide a mechanism by which things can get digested, chewed up within the cell, and can be reprocessed, into their primary units, and sent back into the cell to build new things. So, it is a mechanism of self renewal of sustenance that the cells achieved through the lyso lysosome.

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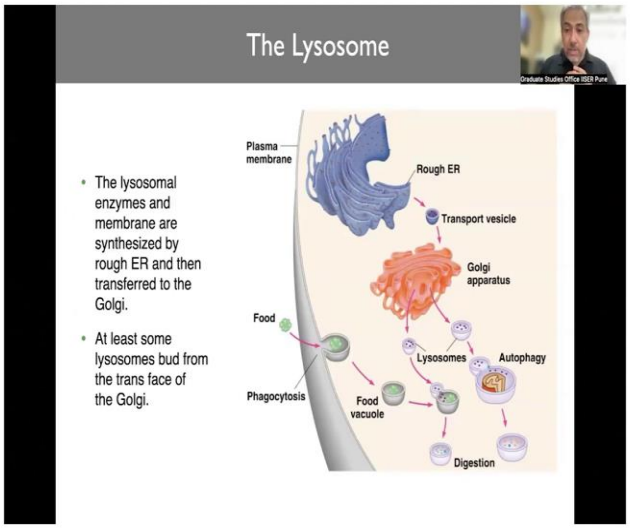

And, just to kind of paraphrase where things are, as far as the lysosome is concerned, these are membrane synthesized by the rough ER and then transferred through the Golgi, many of these modifications, as we talked about the glycosylation changes likely happened in the Golgi. And then the lysosomes are budded off and now available to act on different things.

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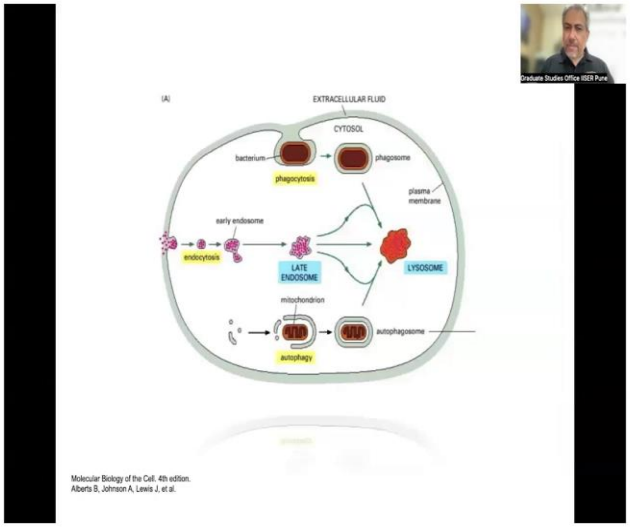

And they talk to different other vesicular structures that are present in the cell. So, they, for example, talk to late endosomes, late endosomes, are arising from early endosomes, which arise from things that are being endocytose or pinched from the plasma membrane, they talk to or

work with phagosomes, which are essentially again endocytosed compartments. But which carry among other things, it could be a way of taking in bacteria harmful things that need to be digested. They also work with this very interesting mechanism, which is called the autophagy, mechanism that is operational in sense, where the cells themselves make an auto phagosome, which captures old dead things that are need to be recycled in the cell.

An example of that is the mitochondria that is shown here. So, depending upon changes that the mitochondria have undergone, it is possible to identify mitochondria to send them into this into the autophagy pathway. And this is a mechanism by which you can break these things down and break them down into primary components, like amino acids, and, and then put them back into the system in the cell. Now making them available to synthesize new things.

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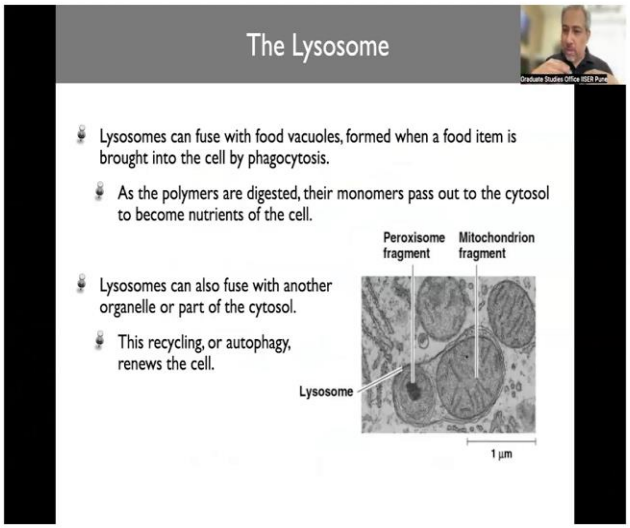

And the autophagy pathway is something that we will talk a little bit about. I mean, just to kind of expand on what we spoke there, along with autophagy, we have these food vacuoles, that can come in along with food particles that need to be processed, which also is something that the lysosome can fuse with, anything this that needs digestion and being broken down something that is harmful, some something that is that needs to be processed as food can be handled by the lysosome. So it is a remarkable machinery that the cell has.

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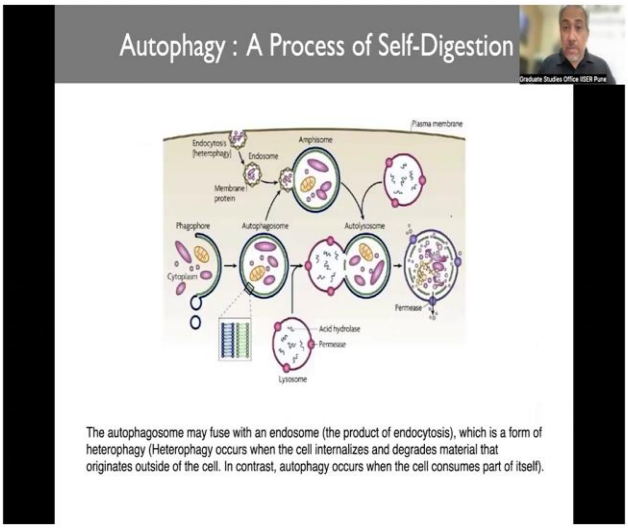

And, and it does really amazing things. Now, the autophagy pathway, we will talk a little bit about because it is rather interesting, and I will share some content that you guys can go read up a little bit about as well. Because there are many interesting implications of autophagy and what began as the identification or analysis of a cellular organelle now has become a pathway or process that is involved in so many diseases.

And the fact that autophagy happens or does not happen has serious consequences for cells. So, they can fuse with the auto phagosome that essentially is a bilayer vesicle that a multi layered vesicle not just bilayer which collects components that needs to be digested and broken down. They can, the auto phagosome can fuse with the lysosome it can fuse with things that are brought in from endocytosis and then fuse with the lysosome.

Essentially, eventually what happens is the components of the lysosome, enter the auto phagosome and it becomes an auto lysosome. So, essentially, it has components that needs to be broken down, it has the hydrolytic enzymes that are like, now available and flushing through this system, and they break everything down. And then things are carried through permeases out to the cell, and used by other mechanisms, machineries that are operational in the cell, as building blocks for building new things.

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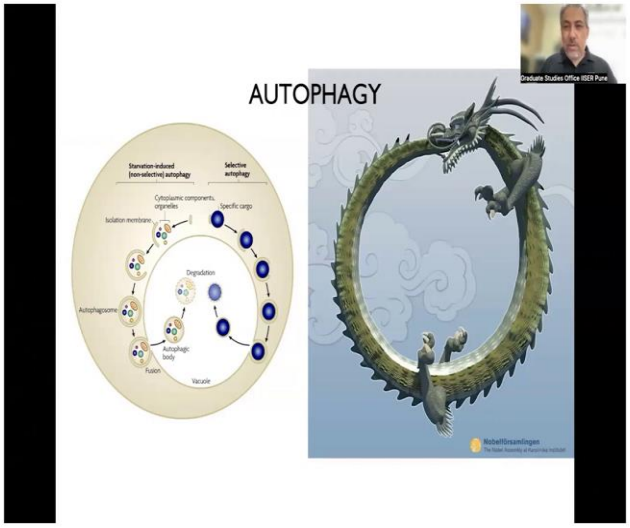

In 2016, the physiology and medicine, Nobel Prize went to Yoshinori Ohsumi, who pioneered the understanding of autophagy. And interestingly, the idea of autophagy, this kind of self renewal, if you want to call it is very beautifully captured in this image that was put up by the Nobel Committee when he was awarded this awarded this prize in 2016. So it is a kind of self-eating, something that cells can use to renew themselves and get rid of things that they do not need.

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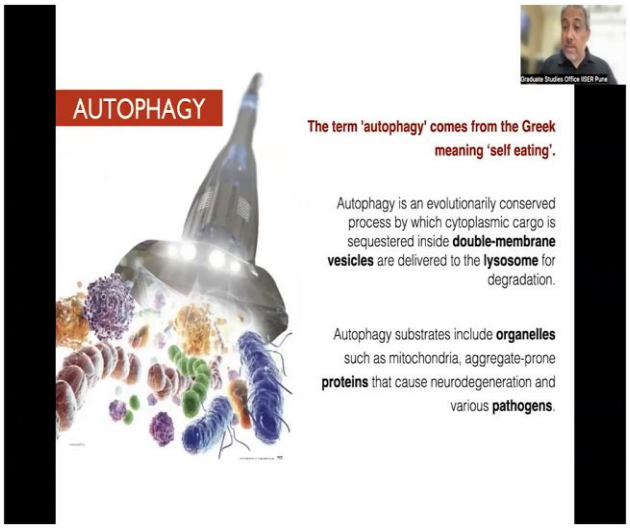

And autophagy comes from the Greek meaning self-eating, and it is an evolutionarily conserved a process by which a cytoplasmic cargo is sequestered in double membrane vesicles. And delivered to lysosomes for the degradation. Autophagy substrates include organelles, such as mitochondria, aggregate proteins that got that cause neuro degeneration, and various pathogens as well.

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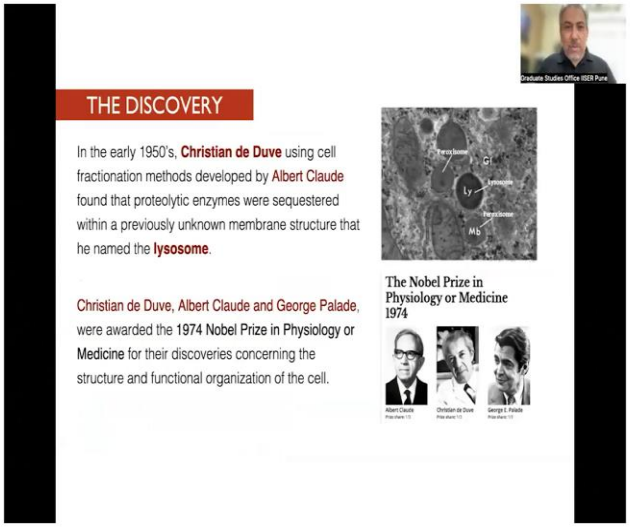

So, the a lot of this began, obviously, with the discovery of lysosomes. So, there is no autophagy. Till he we know about lysosomes. And lysosomes, interestingly were discovered by Cristhian de

Duve and Christian, Albert Claude and George Palade were all awarded Nobel Prize for Physiology and Medicine in 1974.

And it is 2016 almost 30 plus years, between the discovery of lysosomes and the Nobel for lysosomes, and the Nobel for autophagy. And it is very interesting, how our understanding has evolved over these many years. And how something that was identified as a cellular component, so many years ago, has now come on to come on board as a player in a major cellular pathway that is autophagy.

So, Cristhian de Duve when he identified, they did this by cell fractionation methods, you break the cell up and you separate components that were developed by Albert Claude and found proteolytic enzymes were sequestered within a previously unknown membrane structure that he named the lysosome. So, the identification of these enzymes is what defined this these particular structures.

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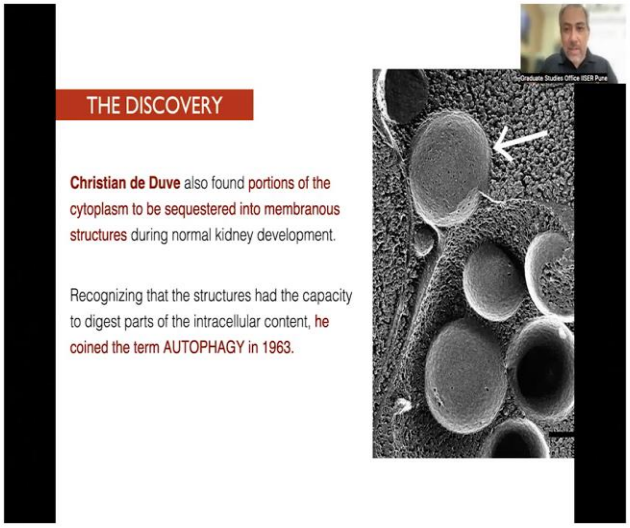

The along with this discovery Cristhian de Duve also found that portions of the cytoplasm are sequestered into membranous structures during kidney development, which they were studying and recognized, recognizing that these structures had the capacity to digest parts of intracellular content. He coined the term autophagy in 1963. So, it is very interesting. The idea of autophagy had existed a while. And it took a lot of time to discover pathways and processes.

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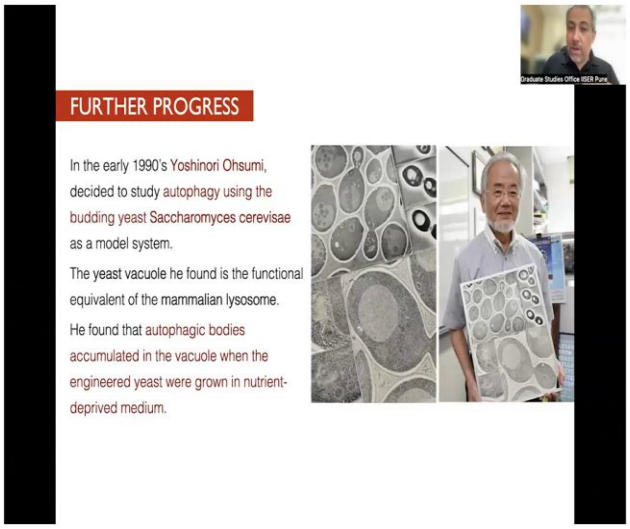

And a lot of that was made possible by Yoshinori's work, where they essentially decided to study autophagy using the budding yeast saccharomyces cerevisiae and with as a model system. And the yeast vacuole, he found is the functional equivalent of the mammalian lysosome. And he found that autophagy bodies accumulated in the vacuole, when the engineered yeast were grown in nutrient deprived medium.

So when the cell is starving, one of the things it does is it kind of gets rid of things that it does not need to kind of operate with minimum capacity. And that is what they used to track down, a whole bunch of different players. So, they delineated all the pathways, all the proteins that are involved in making this machinery work. And, figuring out how cells actually initiate the process and carry out the processes as well.

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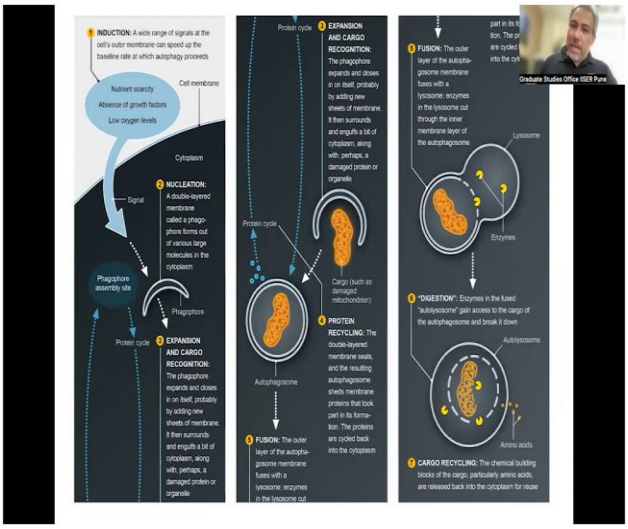

So there is this I mean this beautifully captured. It is a schematic from a scientific American article, which captures what might be happening, that there is nutrients, scarcity, absence of growth factors or low oxygen levels, which could all trigger this. There is nucleation, a double layered membrane called the Phagophore, which forms so this is two bilayers.

So it is that are coming together, expansion and cargo recognition, the Phagophore, expands and closes in on itself, probably by adding new sheets of membrane, with then surrounds engulfs a bit of the cytoplasm along with perhaps a damaged protein or an organelle. And so it kind of wraps around this cytosol with a with proteins or with cellular organelles.

And then things are, you have the auto phagosome a phagosome that is created the double layered membrane seals and the resulting auto phagosome sheds membrane proteins that took part in the in its formation, and the proteins are cycled back. So, some of the proteins that are required for making them are lost. And now this essentially goes and fuses with the lysosome. And the lysosome delivers all the proteolytic enzymes, which go in and digest, and then the cargo, the broken down cargo is now recycled back into the cell.

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So, that is the really interesting role that the lysosome particularly has in cells. And it is very interesting that and I have not discussed this in detail. But you know a couple of years ago, in 2016, when this Nobel Prize was awarded, there is a talk I gave that is on autophagy. And the discovery of autophagy itself, it is available on the science Media Centre's website, YouTube site, and you can go check it out.

And it talks a little more in detail about autophagy, what kind of disease conditions it is involved in, and I am not going into all those details you know not everybody is going to be interested in

that. But if you are interested, please go look this up. So the point that we wanted to make about the lysosome here was that there is no autophagy unless there is lysosome.

And these organelles, therefore, have had a significant impact on a mechanism or a process that now has wide ranging implications for physiology and disease per se. The other interesting organelle that, we wanted to kind of touch upon at the end is our vacuoles. And vacuoles interestingly, are vesicles they are like, there are some studies that suggest that these are essentially vesicles from the ER and Golgi that come together and fuse to make bigger vesicular structures. And there are different kinds of vacuoles, and vacuoles interestingly can have slightly different functions.

So, their size is an important factor, the fact that they are bigger than most other vesicles that are moving around inside the cell. So, for example, the synaptic vesicle that we saw, these vacuoles are going to be considerably bigger, they could be a way for the cell to pick up food. So, there are food vacuoles, that, that are initiated or that are created by phagocytosis. And these, again, then fuse with the lysosomes to kind of digest the food per se. There are contractile vacuoles found in fresh water protests, which pump out excess water from the cell.

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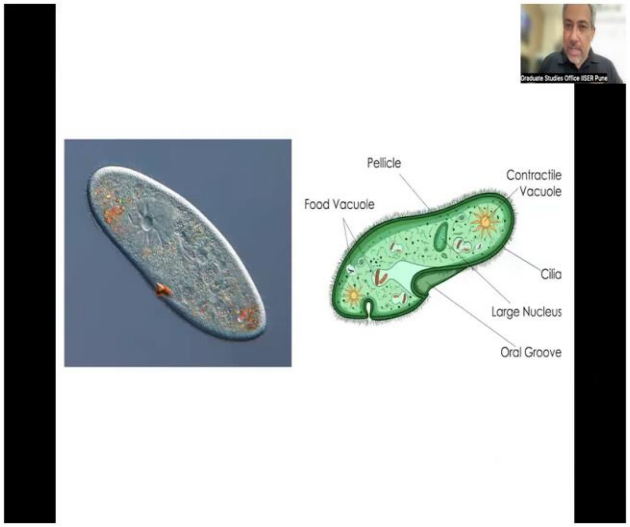

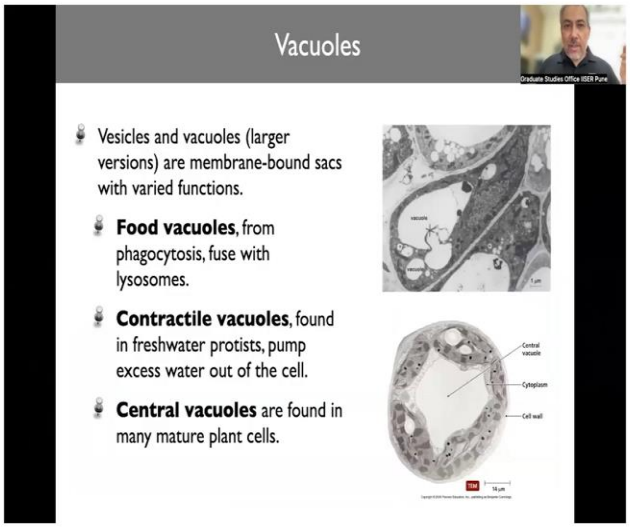

And we have structures like this, for example, in Paramecium. And on the left is an image of Paramecium and you can see the contractile vacuole. And it is indicated in the schematic on the right as well. So, these are considerably bigger than other small vesicles that is the first thing that you notice.

They are again made up of membranes and they have very distinct functions is being able to kind of pump out fluid from the organism itself. And then we have the central vacuole, which is essential integral part of the plant cell and plays a number of very important roles, particularly in the plant cell.

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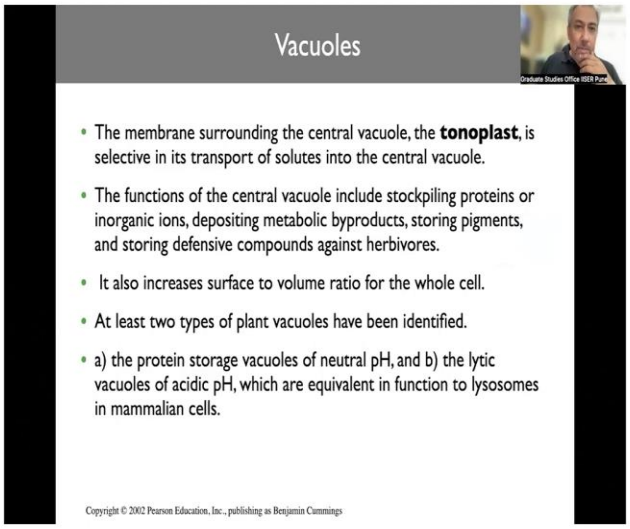

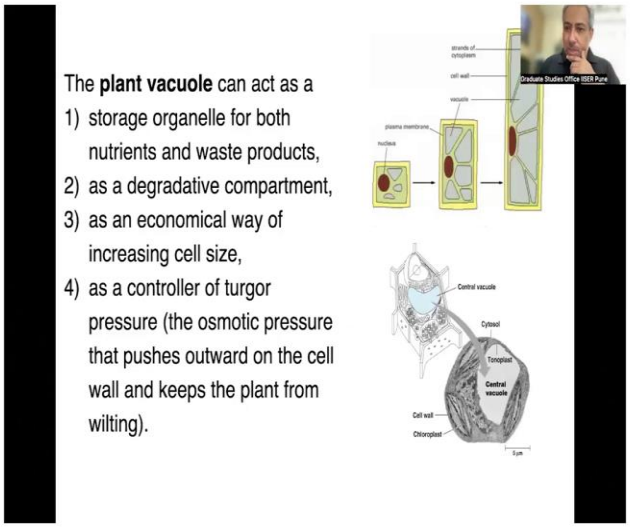

So, the vacuoles, the membrane surrounding the central vacuole, which is vacuole that is present in plant cells, and I am going to show you a schematic of what we are talking about here, this big central vacuole that is present there. It is made up of a membrane that is called the tonoplast, and it is selective in its transport of solutes into the central vacuole.

So, it can regulate what comes in what goes out. And in a cell along with processes like diffusion that actually move things around the cell, there are active mechanisms of transport of movement that can happen across the membrane as well. And vacuoles are a good example of how that can control not only the size of the vacuole, but it can also affect the content of the vacuole. What is stored inside and what is kept out, so to speak.

So, the function of the central vacuole includes stockpiling of proteins or inorganic ions, depositing metabolic by-products, storing pigments, storing defensive compounds against herbivores sometimes. It increases the surface to volume ratio of the whole cell. So the vacuole can grow in size and essentially grow the size of the cell.

And at least in plants, there are two types of plant vacuoles, the protein storage vacuoles of neutral pH, and the lytic vacuoles of acidic pH which are equivalent in function to the lysosomes as well. So it can act as a storage organelle as a degradative compartment, as an economical way of increasing size. So when, for example, water is added to plants, there is an increase in the amount of content that is present fluid that is present in the vacuoles, allowing them to grow in size that also makes a significant impact on the size of the cells.

And remarkably, in plants, these same vacuoles are also used as a controller of turgor pressure, which is the osmotic pressure that pushes outward on the cell wall, and keeps the plant from wilting, So it also ensures that when you add water and the leaves perk up, remember, that is turgor pressure, and that is that turgor pressure is need is mediated by this very vacuole.

So it is really interesting how these, when you think about the endomembrane system and you think about the fact that they are all membranous bags. How differently these bags of membrane have been adapted. We saw the ER, where the smooth and rough endoplasmic reticulum is present and protein synthesis happens, we saw this very nice arrangement, which is the parking lot arrangement for the ER, same kind of membrane put together assembled in a very distinct way along with ribosomes to do something.

We had the Golgi where, everything is kind of nice and stacked and things move from one compartment to the other and processing takes place, enzymes are kept apart from each other in the way the Golgi is built. And effectively things are processed and from the trans Golgi network budding of vesicles is taking place, we saw this movie about how clathrin which is a very beautiful mechanism for endocytosis allows us supports the budding of vesicles from the Golgi. So, and then things are carried.

And among the things that are produced from the Golgi are again these bags of lysosomes, which we now know are involved in binding to food vacuole and binding to the autophagosome in binding to the mechanisms that drive autophagy components that drive autophagy all this is and then you have vacuoles. Like this plant vacuole that essentially can change so many things about how plants work including something as fundamental as turgor pressure.

So, same idea, same components, a bag of lipids containing something inside, keeping some things outside. Having a certain composition and the membrane of that of that organelle affecting, what is retained and what is not present in that particular organelle. This is the basis of all these components. And they are all talking to each other, they work with each other, things get delivered to very specific points, these organelles can fuse with each other. All made possible because of the fact that there is this lipid bilayer around them.

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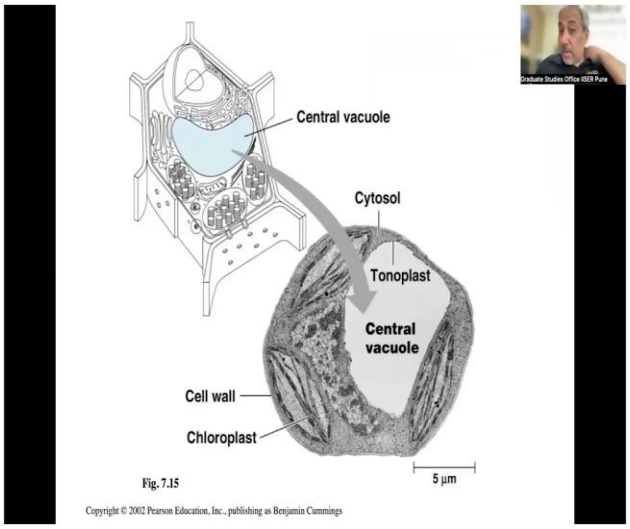

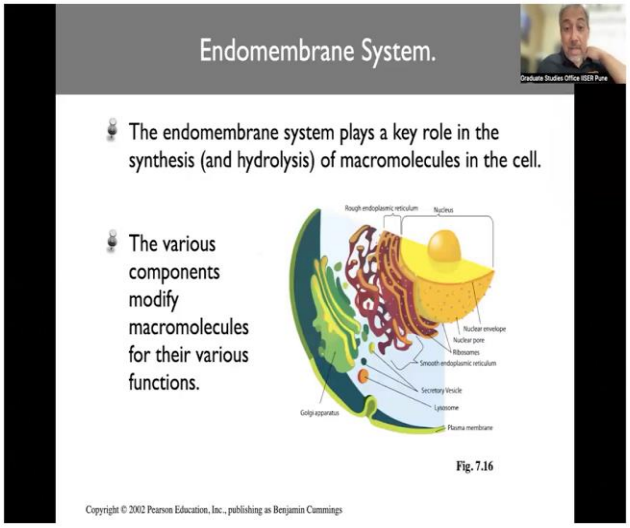

And that is the really remarkable thing about these structures about the endomembrane system at large, that they come together they work together in this very elaborating intricate manner. And in doing so they are able to drive so many complex pathways that work in the cell. In the next class, what we are going to focus on is something where a lot of this begins for the cell.

And we are going to focus on the nucleus, which is this big yellow dot at centre of the cell. where, now that, we began from outside, we have come all the way in and we have looked at all these components, we have seen the complexity with which they operate, we have seen the kind of molecular crowding, that exists in cells, cytoskeleton, cytosolic proteins, membrane vesicles,

Golgi, ER, all this in a very tight compact structure, and doing their own thing, talking to each other, recognizing what is happening to the other.

And then, allowing for the cell to function on minute to minute basis. And if you stop and think about this, it is really remarkable and staggering, that this network is doing this every single minute that you and I are talking to each other. And it is what is allowing for everything that we experience on a day to day basis to actually be possible.

So, that complexity is what I hope, we will also take away from this, that we know as we break this down into simple pathways and processes, we know what the microtubule looks like, we know what the lysosome looks like. We also step back and think about how all of this works in a very intricate, elaborate architecture, that is the cell.

And that is what we are, at least this set of lectures is aiming to do is to kind of introduce you to all these players. Let you see what each one of them does in their unique way. And also lets you appreciate the complexity that is in operation at any given point of time. So we will pause here with the endomembrane system.