Introduction to Cell Biology
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Lecture 58
Discussion Session on Organization and Function of a Cell

Professor: So, this is, as you are aware, it is an open session. And, the idea is for you to be able to raise questions, anything that you wanted clarification on. And I can try my best to see if I can explain some of the stuff that. Sarthak has a query, how is filopodium different from a lamellipodium? A filopodium is this kind of very pointed membrane extension, it is almost like a finger that is getting out that the cell uses to probe its environment, the purpose of the filopodium and lamellipodium is very different.

The lamellipodium is actually kind of a front it is like a wave of membrane. So, this is when the cell keeps doing this in the front of the cell, this membrane that is being held up by the actin cytoskeleton underneath and is being pushed, that entire membrane is called the lamellipodium. This pointed projection is however, called the filopodium and the filopodium has a different architecture, because of the way actin bundles are put together to create that protrusion. filopodium can be withdrawn can be put out withdrawn. Lamellipodium can also be put out withdrawn.

The lamellipodium actually has a very distinct wavy pattern; where there are certain parts of the lamellipodium that are pushed forward, some are withdrawn, they both can happen at in a migrating cell. And the filopodium tends to happen early on when the cell is trying to probe its environment. So, the way they are assembled, the architecture of these structures are all different. The purpose they serve is also different, Sarthak. We will have queries, Sneha, you have a query? Go ahead.

Student Sneha: Sir, yesterday, I was reading the notes. So, sir, I was, I did not got one of the point. It was in the Golgi pinches of transport vesicles, and other vesicles that gives rise to lysosomes and vacuoles.

Professor: So, see vesicular structures, the Golgi, you remember, we saw a movie of membrane from the Golgi budding out, which is mediated by this clathrin that sits on this membrane, it

pulls it out, pinches it, and then the clathrin disassembles. And now vesicle floats away. So, there are a couple of different ways by which vesicles can be pinched off from the Golgi.

A lot of these vesicles make up that come out from the Golgi are the ones that depending upon the composition that they carry can become different vesicular components, including lysosomes and so, even vacuoles are formed largely from the coming together of vesicles to create bigger vacuole like structures, which they then eventually become vacuoles. So, a lot of the origin of these structures is from the fact that from the Golgi they can bud off. That is what that statement is meant to say.

RK Rishab Kulkarni, Rishab, you have a query about clathrin in endocytosis? See, I have not covered endocytosis and there is an intent here and not covering endocytosis. Because it if you cover one, you will have to cover a lot of other things. And, we are also doing things in a form that, everybody gets most of what is done in class, and it is all not too overwhelming. So, clathrin mediated endocytosis is not part of what we are covering here.

But, in the interest of the query, what I will tell you is clatherin is a very distinct kind of protein, it is a triskelion it has three kinds of structures if you want to call it three, the protein has three arms to it, if you want to call it that, and these structures fit into each other and they create what looks like a football, a structure that looks like the skeleton of a football and that triskelion attaches obviously to the membrane and plays a vital role in bending the membrane in such a way that now the membrane also acquires the shape of the football. And then of course there is a mechanism by which the final pinching of the membrane can take place.

And once pinching happens, then the triskelion, again, this is regulated by phosphorylation and other changes to this protein it can disassemble. So, it will assemble form the structure allow the membrane to acquire this shape of the structure once the membrane pinches off, this structure disassembles, and now you have a floating membrane vesicle that has effectively been pinched off. And that is one of the mechanisms of endocytosis, which is clathrin mediated endocytosis. There are obviously, other mechanisms of endocytosis, like caveolar endocytosis there is a CLIC/GEEC pathway.

And I will not get into what each of these means, but there are many methods by which endocytosis can happen. Not everything has an elaborate triskelion-like mechanism in that sense

clathrin is very unique, as far as endocytosis is concerned. Anand, has a query, does the viral DNA in case of infection enter the nucleus via the NPC? Can the NPC stop this viral DNA from entering sometimes? I actually do not know whether that is the only way for entry or whether there are other ways for entries? If you find out let me know as well I am not aware, If all viral DNA enters only exclusively through the NPC.

Abhishek has another query, how is motor protein traffic maintained without collisions happening between them? Or are there separate microtubules for anterograde? No, so, the clear no here is that there is not a microtubule that carries motor proteins in only in one direction, and one that carries in the other direction that does not happen. It is very clear that there is movement happening in both directions on a microtubule strand, and there is documented evidence for this as well. So, it is unlikely that there is a selected microtubule going in this direction, and one in the other direction.

Now, how do they avoid collisions? It is not entirely clear whether collisions are actually avoided, so, it is highly possible that collisions are part of what the motor protein has to encounter, and it just has to navigate through all the crowding that exists, and this obviously influences the rate at which motor proteins move, it will, it will kind of move it stops, and things like that, but that probably does happen in many of these circumstances.

So, I do not know how many of you ever traveled in trains in Bombay, I grew up in Bombay, and any train you take in the morning rush hour, if you want to get from one side of the train to the other side, you have to kind of, I do not know, how you manage it, but you somehow wriggle your way through, and so that if that crowd exists on a regular basis, obviously, your movement from one end to the other is affected by that crowding. And that is probably true with motor proteins as well.

There might be certain cells, there might be certain responses, where the crowding is more than the others, and those differences may exist, which will influence the rate at which things are moving around, but for a lot of practical purposes, the motor proteins and the vesicles that they carry, deal with the crowding and the net outcome, which means the rate at which something is being trafficked from one point to another is effectively a combination or an outcome of how

many motor proteins are bound which motor proteins are associated and then the crowding as well.

So, there will be crowding, there will be collisions, it will have to be, kind of work its way around that to get from point A to point B.

Sneha has a query how organelles hold each other? Is it by cytoplasm do they collide? Can you explain more about this? So, Sneha your query is how do they hold each other? Essentially means what is the kind of contact that happens between organelles, there is another holding which is their relative position in the cells and the relative position in the cells could be influenced by cytoskeletal components particularly, because many of these proteins do bind cytoskeletal components, proteins, sorry, many of these organelles. Many of these organelles, like the Golgi actually bind motor proteins.

So, the relative position of these organelles or these vesicular structures along the cytoskeleton component can be influenced by the motor proteins they carry, if you remove the motor proteins, their positions will change. So, that can happen for many of them. In some cases, this is well established, in some cases, it is thought to happen, but which motor protein? How does this take place? We do not fully know.

Organelles also now it is increasingly becoming, research is telling us this, that there are points of contact between organelles, and that there are proteins that are mediating this contact between the ER and the mitochondria, Golgi and the mitochondria, there are direct points of contact. And, these are mediated by special proteins that actually localized to those sites of contact.

So, there could be events like that, that also facilitate this, what exactly that contact means? An ER binding to the mitochondria, what does it actually do? We are just trying to understand this; I do not think there is a lot of information that clearly says what it does? So, we are still in the process of asking some of these questions.

And we do not know, answers to how that regulation could work. What we do know is those contacts now in the last five or seven years do exist, that there could be proteins that facilitate or exist at the site of that contract, which means the coming together is helped by those proteins.

And the cytoskeleton is what is keeping a lot of that relative position of organelles within the cell. Any?

Student: Sir, I have one more doubt. Sir, I did not got the difference between the lysosome and autosome.

Professor: So.

Student: Is adjusted digest itself and alliances digest others is it the thing?

Professor: So, autosome is something that forms later no as a result of lysosome fusing something.

Student: Okay, Sir.

Professor: You should just look this up my understanding is that is how they are created lysosome is what originates from the Golgi and can go fuse with anything and it can fuse with things that have to digest themselves it can fuse with stuff that is brought in from outside that has to be digested as well.

There is like phagosomes exists which come from the outside and when lysosomes fuse with the phagosomes, there is something an intermediate vesicle that is called the phagolysosome. And so, there are structures like this, where lysosomes talk to different other vesicles or fuse with them, and now facilitate breakdown or whatever that it is that they are carrying.

Student: Okay Sir.

Professor: Sure, (Abhijeet) Abhishek, has a query can tread milling of cytoskeleton cause occasional puncturing of the cell membrane? So this is your thinking, does it actually tear the membrane? Am I thinking that correctly, Abishek?

Student Abhishek: Yes, sir,

Professor: See the membrane is extremely flexible and there is an excess of membrane on the plasma membrane. So, remember, there are studies, and Thomas's lab here does these to where they take the cell, and attach a bead that binds to something on the surface, and then they hold it and they pull the membrane, they pull something called a membrane tether. And, what you

realize is you just can keep pulling, and pulling and pulling, and the cell is just still okay. Which means that all that excess lipid that exists is what is now going into the tether.

So, the lipid membrane, along with the flexibility also has a significant amount of lipid in it, which means any kind of so, if you can pull a tether that way and not break the membrane, then a simple poking of the membrane is not going to break it, so, the membrane has enough flexibility to accommodate that kind of event as a matter of fact, if you hold it and you keep pulling it, you can pull really long tethers from the plasma membrane.

And which is what really taught us that there is an excess amount of lipid and you can actually do that without harming this, you put this back, it gets integrated back into the membrane. So, it is a really remarkable structure and we are only beginning to understand, some of how that lipid architecture is put together and used by cells. So, it is unlikely that there will be a real puncturing of the membrane per se.

Close to the end of this session. There is one query in the chat box that I am going to take that has come from Padma Priya, where the query is that during cell division, how do lysosomes divide? Do they need to break open? And are they getting divided or are new lysosomes form formed from the Golgi, before the breaking of the Golgi during cell division?

So, a little of both happens, I do not think lysosomes divide this way, if that is what you are thinking, whatever lysosomes are present, may get distributed evenly. This again, I am not sure there are studies to look for how the lysosomes distribute. I think when it comes to structures, like the mitochondria, we are just beginning to understand how they are getting distributed. And so, lysosome, still much remains to be understood on how the distribution happens; it is very likely that new lysosomes are formed after the Golgi reorganizes in the two daughter cells.

And during cell division, remember there are many events that the cell actually pauses, and says okay, we are going through this, let us get this done, and then get back to doing, what we were doing earlier. So, among the things that could pause, could be the making of new lysosomes. Still, the Golgi reassembles. And now, the machinery gets activated again and a lot of the trafficking that needs to happen, including the making of vesicles, from the Golgi is restored.

So, that is probably the best way to think about this. Repeat myself, the intent here is to is for you to be able to raise any questions that you had that you wanted some clarity on. And I will see how best I can answer those. Kishen, you have a query go ahead, please.

Student Kishen: Sir, in the cell cycle, when we were discussing about mitochondria, you said that the, during mitotic exit the mitochondria fuses.

Professor: Yes.

Student Kishen: What happens?

Professor: No, during mitosis, it fragments and then during mitotic, exit (the) these fragments, then again, fused together to create more elongated structures.

Student Kishen: Is it just so that it distributes equally?

Professor: Yes, absolutely.

Student Kishen: within daughter cells

Professor: Yeah, the fragments, and the movement that we talked about, this stirring of the proverbial cell cup by the actin ensures that these fragments now get distributed everywhere. And then the cell divides, and then they form they reassemble into the network that allows them to function better. So, the point to remember in here in other situations is also that during cell cycle, there are certain processes events that the cell would normally do, that are paused, allowing the cell to divide and create this even distribution as well as possible. And then, many of these functions are restored.

Student Kishen: Sir, and also the slide on endoplasmic reticulum was a little bit confusing, like it had terms like GRASP65 when many small names which I couldn't understand?

Professor: So, I am not sure whether you mean Golgi.

Student Kishen: Golgi

Professor: Because GRASP65 doesn’t come at endoplasmic reticulum. So, these are structural proteins. So, what I would suggest is if there is a word there that you do not know what it is? Just

look this up. If you put GRASP65 in any of the textbooks and search for it you will be able to find what GRASP65 is? But it is not important to remember GRASP65 per se, it is a structural protein. And there are many other structural proteins that allow the Golgi to maintain its architecture.

I think your understanding at this point of time, we want you to get a sense of what that architecture means the fact that that architecture is regulated, those kinds of concepts are what you want to carry forward, you will get a chance to kind of understand read and read more in a more detailed way. How many of these structures are put together assembled? What kind of proteins are there? What is the kind of regulation all that we have not brought in here because that would just complicate things at this point of time. So, the idea of the concept is what you want to walk away with.

Student Kishen: Thank you

Professor: Deep, your query

Student Deep: As you said that it is mitochondria fragments like Golgi and other organelles also that fragment right we will do it for the same reason. So, where is the information stored to get them back quickly?

Student Deep: As you said that it is mitochondria fragments like Golgi and other organelles also that fragment right will do it for the same reason. So, where is the information stored to get them back quickly?

Professor: So, everything is regulated in a very protein or mechanism centric manner. So, there are proteins which when phosphorylated will either allow for fission of the mitochondria or fusion of the mitochondria, they are not thinking at that point of time, this fission fusion, what the implications are? Whether this is happening during cell cycle, all that this protein knows is that, if it is phosphorylated, and I am just giving you kind of a loose example, if it is phosphorylated, it has to do this, which will cause fission. And if it does say, dephosphorylated, it has to allow for fusion to take place.

It is not of course, as simple as that it is not like one protein doing just this. There may be a series of proteins that need to be involved in this manner. But, when that stimulus comes to them, they

essentially do their job. The fact that stimulus comes at the time of the cell cycle is probably governed by other factors in the cell, that are now again connected to events like it could be anything from centrosome division to the alignment of the chromosomes. That is why it cell cycle per se, the way we discussed, it, is extremely superficial.

I mean, we are just looking at the basic ideas of changes that are happening; we have not even scratched the surface of regulatory pathways that exists. Everything from what happens on the plasma membrane to the chromosomes being aligned, every little step allows for that pathway to talk to other pathways in the cell, which all kind of network in such a way that when this particular protein that regulates mitochondrial fusion, or fission or regulates Golgi organization, its phosphorylation has to be controlled. It is an amalgamation of all these different networks.

So, I think at some point of time, in the coming years, you are also going to look at and study about systems biology approaches, which essentially asks this kind of question, it asks if you have a protein and its activation, or phosphorylation has to be regulated in the cell, what is the kind of network that contributes to finally determining what that phosphorylation of this protein is? If you are thinking this is one pathway talking to this it isn't, and that network is very intricate, and all these players talking to each other, regulating each other is eventually going to reflect in what the phosphorylation of this particular protein is?

And that, in turn is going to drive a series of events that could either cause fusion or fission as the case may be. So, the same is true for the Golgi. GRASP65, for example is phosphorylated grasp talks to mitochondria, sorry!! talks to microtubules, microtubules have changes such as acetylation in microtubules that could differentially bind to grasp that is phosphorylated versus not a lot of such steps that eventually determine what happens to the Golgi. As I said, it is very easy to get carried away to talk about all the little, little intricacies.

We have consciously tried to avoid that in this particular course, the idea being to introduce you to the bigger concepts of how? Why is Golgi breakup important? Why that architecture being taken apart and being put together is irrelevant? Why is that happening in so many different processes in the cell? How this can be controlled? And so, all of these are broad concepts that you want to take away from here,

But if you are interested, for example, in one particular protein, how grasp regulates Golgi architecture, there are entire reviews that are written, describing that one protein and its associated proteins, and how they influence Golgi architecture, that is the level of detail that is available for some of these pathways and processes. I do not know if that answered the question Deep, it is a long-winding.

Student: No, sir, but I also have another question.

Professor: Sure.

Student: Like in the in the case of plant cells, we know that after cell division occur two cells are separated by the formation of that middle lamella so like there is the furrow formation like in animals cell, but certain bacterial cells in the video that I saw, seen, there is some sort of furrow formation that is they do go apart from each other and the cell membranes do collapse, but they also have a cell wall to take care of.

Professor: Plants cell?

Student: How does? No bacterial cell

Professor: Bacterial cell

Student: So, the peptidoglycan cell wall does exist. So, how do they manage that?

Professor: So, I am not sure whether there is a direct mechanism that is distinctly different in the peptidoglycan layer or whether once the cut happens essentially you have the peptidoglycan layer going above the cell membrane that allows for it to kind of seal up the bacteria. And I now, have the cell membrane and the peptidoglycan layer running all along.

Student: Sir, I also have a doubt. I was reading a review by the citable journal education for initial education and in that it was written endocytosis is a kind of reverse vesicle trafficking, how endocytosis is the reverse vesicle trafficking?

Professor: Yeah, so so it is this is assuming what they mean by vesicle trafficking is a vesicle pinching off from the Golgi, or a structure like that, and then making it to the plasma membrane, so, if that is vesicular trafficking, then endocytosis is essentially pinching off the plasma

membrane, and stuff being delivered into the cell, it could go to the Golgi, it could go to other compartments as well. So, I suspect that what is they are thinking of when they say it is reverse, because it is essentially a similar process, but working in opposite directions, this one coming in, and this one going out, they actually use the cytoskeletal machinery more or less the same way.

Microtubules for example, are required for delivery of vesicle in, they are required for delivery of vesicles out. And a lot of the endocytic machinery sometimes can be different like clathrin, for example, can work at the Golgi as well as the plasma membrane. But, things like caveolae happen work largely at the plasma membrane and but, when caveolae had to be made and delivered to the plasma membrane, they obviously pinch off from the Golgi similarly as well. So I think that is what they are referring to here.

Student: Thank you, sir.

Professor: Okay.

Student: Sir, we know that microtubules and the centrioles are extremely important for cell growth, cell division and so on. But, I just came across an article regarding mutations in flies that lacked the particular gene needed for centrioles replication. And so, eventually, what happens is that the adult flies they do not the cells do not have any centrioles, the cells are able to develop, I mean, they do die, these flies are not that smart once they grow up, but the development does happen, so, the abstract.

Professor: I also do not know why that would be the case. It is actually very interesting. I would have thought that. Do they provide an explanation for why?

Student: No, I was not able to find an explanation.

Professor: Please, is there something else that takes over the role of the centrosomes? I am not sure whether there are studies that have been done in mammalian systems that look at this very similarly. So, for example, the Golgi in flies is very differently organized as compared to the Golgi in mammalian cells.

So, I was, I mean, tempted to say that the Golgi could come in and do what the centrosome does. But I also know that the Golgi is organized very differently there. So, does that act as a

nucleating center like centrosome does here in mammalian cells? Does that do a similar role in flies? I actually do not.

Student: Does not because eventually the end result would happen so that the cells do not have any cilia flagella and so on.

Professor: Does it? Is this targeted in one specific cell?

Student: No, it does not seem like it we might just let the abstract and part of the results, but it does not seem like it.

Professor: Look this up and if required speak to Girish. I also I mean, I like to go read enough to know how this is happening. It is an interesting result, if that is the case, and they must have an at least a suggestion on what could be happening. As to why the cells are able to live through this? And what exactly compensates for what the role of the centrosome? Because obviously, there is a role for the centrosome and something has to compensate for it. And I do not know whether in flies, there is a system that allows for that.

Student: My doubt is like it is not exactly related to the course material. But in all the animations in the videos we saw, how exactly are the simulations being done, because I would guess that these are all like so biophysics would be based on statistical mechanics, but how are they also sure, like all the time?

Professor: So, my short answer here would be that this is this will be evolving this is based on current information that we have many of these is coming in small parts and pieces from different studies, which are looking at very specific aspects. Obviously, there is not one study that looks at all of this everything from the size of a vesicle, to say how many motor proteins bind cannot come from one particular study. So, the hope, I mean, the intent here was to kind of assimilate the current information, and see if that can be used to generate a model that looks like the best model right now.

So, it is not the perfect one, it may not also be how entirely accurate we may revise this? And, I think that is one of the important requirements, I think in any scientific inquiry that I think you have to be open to this kind of revision. And the hope is five years from now, there will be an

updated version, and a bunch of things will be different, 5 years from then there will be another updated version, and we will add new things to it.

And as I said, something as simple as how the mitochondria are distributed inside the cells during cell division? We just discovered it four months ago, and we have been looking at cells forever, at least for a few 50-60 years with some level of intent. So, yeah, so there is not it, it is the best that we have based on information that is available right now. And this will obviously keep evolving. I think some of that information is also coming from different cell types. So that is another point to consider, that (we do not think) I do not think we have all information from one type of cells.

So, you are putting all of this together and trying to see if you can generate a model that reflects what is going on, I think a lot of systems biology is essentially trying to do this in more than one ways is to essentially tie information together. That is coming from very diverse sources in a way that allows us to see a big picture, which is closer to the real thing. I think it will be a while before we can say this is the real thing.

Student: And I, read about this a while back. Proving proving mathematically that the DNA is coiled and packaged to fit within the nucleus is an unsolved problem. So how would like? How would you test the validity of DNA coiling models? If we do not have any…

Professor: I am the wrong person to answer this. So, no, I my short answer will be I really do not know. I think you have to ask people who you know who study this to kind of figure out what the understanding of the field is in terms of how that architecture looks? And how it is put together? Sneha you had a query?

Student Sneha: Oh, yes sir. Sir, in bidirectionality.

Professor: In bidirectionality of motor proteins?

Student Sneha: Yeah, the motor protein. So, in bidirectional because they move in both direction, so, if it gets cut at us at a point, so will it once, will it change it form?

Professor: Not understood the question in bidirectionality, what gets cut?

Student Sneha: No, sir, in bidirectionality, if the protein get cuts at a particular time, then Will it keep it state? Like will it be? Will it go in bidirectionality…

Professor: Motor protein gets cut? You are saying?

Student Sneha: Yes.

Professor: What do you mean by cut?

Student Sneha: If it breaks in between,

Professor: Like the motor protein snaps in between?

Student Sneha: Yes.

Professor: And it is a bidirectional motor protein goes in both directions.

Student Sneha: Yes, sir.

Professor: And your query is will it stay bi-directional?

Student Sneha: Yes.

Professor: It depends on where it breaks, no.

Student Sneha: So, if it breaks in the middle?

Professor: Middle means like this? Like there are two arms to walking of the motor protein. If the both arms do not are not attached to the same structure, it will not walk.

Student Sneha: Yes, sir, right.

Professor: So, it is, I mean, I understand your question, but maybe you are not framing it correctly. Because a protein breaking is a very loose term, I mean, if you are saying that it loses one of its legs, will it continue to walk? Chances are it will not? If protein breaks along its shaft such that the vesicle falls off, the base of the protein, which is actually of the motor protein that is walking will continue to. It may not be able to bind something, but it will probably walk.

Student Sneha: Okay, sir.

Professor: Vignesh in the chat has a query are not these animations more of a movie than true scientific simulation? Simulation, I think with something like the inner life of the cell, there is a fair amount of accuracy to what they have built. And those are fairly accurate. But there are other movies, which are largely meant to explain how a phenomena looks, which are more a schematic, or a cartoon rather than true scientific simulation. But in the life of the cell was built with the understanding that it is going to be, it is built with a lot of information that has come from scientific work. And so is meant to be fairly accurate for that particular time, off course.

Student: I have a question?

Professor: Yeah, go ahead.

Student: Yeah. I remember, this was a while back, I remember talking to someone who was working on using quantum computing techniques, like microscopy of cells, like obtaining image of living cells using quantum mechanics, so, there they used, like, you use the fact that these energy gaps are quantized to use light that does not stop disturb the cell. But like, similar to how there is a for even if you consider classical optics, there is a limit to the resolution, which you can get right. So is there some fundamental like the mitten microscopy of biology that should be the…

Professor: Yeah, right now, there is no, in terms of for the same reasons that you are pointing out. I think, see, electron microscopy is probably what gives you the kind of resolution in terms of cells, that is the best we have at this point of time. Now, the challenge with electron microscopy, obviously, is that, you know, we have not really been able to look at live cells, at least not yet. And there is not a method that allows us to, to do that, being able to look at just cross-sectional images in EM and put a 3D reconstituted image has probably happened in the last 10-15 years.

And, you know, that is obviously added a lot. Yeah, so there is a limit to what we can use, some of that limit is being stretched by the fact that you are, using algorithms that fill information in ways that is very intuitive. And it does not exist actually in the image. So, you can clean up the image in a way that allows you to see something that actually is not there in the parent image. And that has improved resolution a bit. But that is, yeah, that is cheating. I mean, effect, it is not really the action but the way being processed, by the by the way it is being processed.

So, yeah, so there is a limitation there. And there might be things that will come up in the future that allow us to achieve a greater resolution, without actually damaging the cell in such a way that we are able to see things with greater clarity. Right now, what we are doing is we are combining the kind of image and information that still images produced in EM with the kind of dynamics that we can achieve in live cells and trying to assimilate the two to kind of make sense of how this is all possibly working. To get that kind of resolution in this kind of a setting will be quite something. Yeah, it will ask allow us to ask things that we have not been able to ask so far. Yeah, still a lot remains to be done.