Introduction to Cell Biology
Professor Girish Ratnaparkhi
Professor Nagraj Balasubramanian
Department of Biology
Indian Institute of Science Education and Research, Pune
Cytoskeleton: Discussion Session 2

Student: Good morning, sir. So, I looked a little into the inner and outer mitochondrial membranes.

Professor: Okay, the question again could you repeat just for everybody's?

Student: So, the question was whether inner and outer mitochondrial membrane. Whether the inner and outer mitochondrial membranes are different or not? Whether they have different compositions and the quick short answer to that is yes; they have different compositions. More specifically, the inner membrane has much more protein content than lipid than the outer membrane. And I suppose the primary reason for this is that the inner mitochondrial membrane has cristae foldings.

Professor: Okay, sorry.

Student: And the foldings house is the electron transport chain? Sorry, where did I go.

Professor: No, no, you are fine now, go ahead, go ahead. So, you are talking about the cristae. What happens to the lipids?

Student: One minute, I could not hear you.

Professor: I was asking what happens to the lipid composition in the inner and the outer membrane? Is that.

Student: Yes, it does. Primarily, there are more lipids in the outer membrane. When there are.

Professor: More lipids, what does that mean if you have a sheet of lipid?

Student: The more lipids relative to the protein.

Professor: Now that is okay. So, the question here is the composition, protein content is another thing as far as the lipid composition goes. Is there a difference between the outer and the inner membrane? Dhairya can you hear me?

Student: Lipids is also different.

Professor: Okay, and are there lipids that are present in both? Some lipids that are different; is that how it is?

Student: So, there are lipids that are present in the outer (memb), in the inner membrane; but not too very concentrated in the outer membrane. One example is cardiolipidin.

Professor: Okay, okay.

Student: So, that is much more common in the inner membrane than it is in the outer membrane.

Professor: So, is there a lipid in the inner membrane? Can I ask you something? Is there a lipid in the inner membrane that is just not there in the outer membrane?

Student: Yeah. Wait, I did not catch you after inner membrane and all.

Professor: So, I was asking whether is there a lipid in the inner membrane that is completely missing in the outer membrane?

Student: I do not recall reading about such a lipid.

Professor: So, so it is very likely that there is a difference in composition and that there are certain lipids that are enriched in one membrane as compared to the other.

Student: Yes.

Professor: And it is possible that there is some kind of exchange that is happening, which means over a period of time, there is not really a lipid that is uniquely present only in the inner membrane, but not in the outer membrane. With proteins also, it is possible again that there are proteins that are enriched in one membrane versus the other. And over time, there is possibility that there is some exchange that has taken place; and you will see some of it in the outer membrane. But, you know that there is so much in the inner membrane that probably this all, at some point of time could have been exclusively in the inner membrane as well. So, that is probably how we want to think about this.

Student: On that note, I read about there being contact sites, where the inner and outer membrane come into contact.

Professor: Sure, sure.

Student: And there is a bit of diffusion of the lipids at those sites; around those sites, we find equal compositions in the outer.

Professor: So, this is interesting also for other organelles when we think about it, that the membranes can actually be in contact with each other. In the sense that for example, the ER and the Golgi will have contact sites. The ER and the mitochondria now are thought to have contact sites; and there are proteins that are actually present at these contact sites. So, a lot of these organelles that we talk about now, that initially were thought to be very distinctly different structures.

Increasingly, there is evidence to suggest that they all communicate with each other. So, there is a possibility that things can go back and forth between them as well in a way that we did not originally think of. So, thanks for that the idea.

Student: thank you.

Professor: Let us let us go to those who had questions last time. And let me see if I can find you here. Ajinkya had his hand up last time as well; Ajinkya can you go first?

Student: Yes sir, so just one.

Professor: Sure. I can not hear you Ajinkya. You are on mute; now I can hear you.

Student: Sir, I am audible now?

Professor: Yes.

Student: So, I wanted to ask that yesterday you said cytoskeleton showed rapid reorganization. So, can you specify in time how quick they do it?

Professor: So, this will vary, and these could be the depolymerization can happen in a matter of seconds, milliseconds. And it also depends on many of these regulatory proteins that are present. So, the presence of a regulatory protein can dramatically change the rate at which depolymerization or polymerization happens; it is a very rapid process. A lot of the cytoskeleton has been studied in vitro. So, you can actually put the components together and get them to

polymerize in petri dishes in slides, on in coverslips. And, and the rate is very rapid; so it is seconds to milliseconds for, for like one subunit to be added.

So, if we take a couple of seconds for that strand to grow, and it can grow very rapidly; it can depolymerize very rapidly as well.

Professor: Goraksh had put his hand up last time around.

Student: Yes sir, am I audible?

Professor: Yes, I can hear you, go for it.

Student: Is actin always a double helix or it can be triple or multiples as well?

Professor: No, no, no. So, so it almost always is a double standard structure. So, you will not get a single strand of actin. And it is a good question to think about because this may have something to do with how it has stabilized, how these strands are assembled. At some point of time, did a single strand exist before two were put together? We do not know, we do not know. And it is interesting that even in the early prokaryotes, the MreB that we just saw is is two strands wrapped on each other; which says that right at the beginning this may have, this may be vital for how these work. Yes, Chintamani Dhumal, Chintamani. Can you?

Student: Yes sir.

Professor: Yes, go for it.

Student: Am I audible?

Professor: Yes, yes. yes.

Student: Sir, yesterday you said that about the introduction to the cytoskeleton, that cytoskeleton provides and anchorages, anchorage for an organisms. Could you explain in short about that?

Professor: So, so they provide anchorage to organelles inside the cell. See now, now that you know how the cytoskeleton looks. Imagine the cell filled with this cytoskeleton; the actin is running everywhere. The microtubules are running as long strands across the cell. A lot of the (cyto) the intracellular organelles actually are needed to be kept in very specific places; like the nucleus, for example, stays more or less in the center of the cell. In a migrating cell, the nucleus

actually moves to the front of the cell. The Golgi, for example, needs to be right around where the MTOC is, the microtubule organizing center or the centrosome.

So, these organelles being kept in their place; they are for the most part bags of lipid that are floating around. But, they have things on the surface, they have proteins on the surface, they have motor proteins that could bind them as well. And so, that allows them to be anchored and tied with the actin or microtubule strands; and as a result they are kept in place inside the cell. So, the relative positioning of these organelles could be something that is governed or controlled by the cytoskeleton; that is what I meant to say.

Student: Yes, yes sir. And one or one small doubt, yesterday you talked about some concept of redundancy. Is it okay to say as a supplementary similar to redundancy?

Professor: So, supplementary is not redundancy, because supplementary could essentially say that what this does, this could come and help with, it could supplement that. That is not the case; in redundancy, this could do something; this could also do the same thing.

Student: Yes.

Professor: And that is the difference in redundancy.

Student: Okay sir, understood sir. Thank you.

Professor: Medha, you had a query?

Student: Yes, sir. I wanted to know, like your slides are mentioned that microtubules help to determine the position of organelles in the cells. So, I wanted to know how exactly do they do that? Do they have like, is there a fixed like template for the layout of the cell that they work off or is it more of continuous?

Professor: Good question, good question, good question. See, there is a kind of a loose template; it is not a fixed template. The organelles are also moving around inside the cell; and in response to certain stimuli, there might be very distinct changes in localization that happens. So, remember in a cell, the functionality of the cell is governed by these two parameters; it is governed by space and time. When I say space, it is what is happening where inside the cell? And time is what rate is it happening?

When we talk about dynamics of actin cytoskeleton, the kinetics here, the rate is the important determining factor; where the cytoskeleton polymerization is happening is another important factor. So, every functionality in the cell is governed by this space and time; so the same is true for organelles. They are being kept in places is by the ability of the organelles to bind to many of the cytoskeleton components. Because the cytoskeleton is present everywhere, it can hold on to the cytoskeleton, and try and keep its place. And that location may be determined by different cellular stimuli.

So, it is possible that, for example, when the cell has to move in a certain direction, the fact that the nucleus is repositioned to the front of the cell is a very elaborate mechanism; that allows the cell to do it. And that has benefits for the cell in terms of where the Golgi and the nucleus is relative to the front of the cell. So, so this is a dynamic thing. It is not like there is a direct template to say that if you look at it as a cell, and you you look at 37 degrees; you will see this organelle, no. But, ballpark, you will know for example, the nucleus is largely towards the center; the Golgi will be around the nucleus somewhere; so that relative positioning is maintained.

And that positioning again contributes to this idea of space and time, where both these parameters are vital for any functionality to be to be regulated in cells.

Student: I had a question regarding the microtubules that we talked about today, the polymerization and the depolymerization thing. So, in case of like when the cell is dividing, which side is the positive end and the negative end? Like if we does not it attaches on both the sides? One is.

Professor: So, the microtubules around the MTOC, the centrosome; is it a positive end or minus end? Do you know?

Student: I am guessing it is the minus end.

Professor: Look this up, that architecture is defined. See, remember plus minus is also our terminology. What essentially, you could call it x and y if you decide to? All it says is that the polarity is such that going this way, there is one one kind of polarity; going that way, there is a different kind of polarity. And if you are walking on the microtubules strand, you will feel it. You will feel what going this direction is like you will feel what going in the other direction feels

like. The centrosome and the polarity of the microtubules that is attached to the centrosome is the same.

So, this strand, if this is minus and it is plus at the other end, that then which is free is the one that is attached to chromosomes as it is being pulled up towards the centrosome. So, so that architecture remains the same; it just uses that architecture differently.

Student: No, my question was like, at the minus end it starts depolymerizing.

Professor: Good question, where does it depolymerizes is the query?

Student: No, no, no like, if it is attached to both the ends, then how does it depolymerize? The depolymerization molecule should be in the centrosome or somewhere.

Professor: Right, right, right. So, so I am going to send you something. As I said, the dynamic instability of microtubules is a very fascinating aspect of how polymerization happens, where it happens, where depolymerization happens. See, the critical thing is this being a brief introduction to cells; I do not want to overload everybody with too much content.

Student: So, so my doubt was regarding that early prokaryotes did not have any actin or motor proteins, then how did they acquire the food?

Professor: So they, they see there is a lot of it was diffusion. They were, an actin and microtubules are not the determining factor for acquiring food; they are doing many more complex things. So obviously, cells survived in very primitive forms as well. It is this complexity that is allowing them now to do many more intricate things. So, acquisition of food may not be stopped because they did not have motor proteins; diffusion and other methods were available. It was not very efficient; it may not have been very controlled, all that is fine.

But just if there was enough to kind of to survive, and that is what they were trying to do.

Student: Sir, I had a doubt like, in the last class, we studied that actin filaments and that microfilaments are same things.

Professor: So, microfilaments can be actin, but there are many specific proteins that also make microfilaments. Lamins for example, keratin for example are very unique cytoskeleton components that make the intermediate filaments. In some cases, it is possible for actin to make

thicker strands that resemble or make them resemble intermediate filaments. But, actin is not intermediate filaments. Okay?

Professor: Okay, okay. Yes, okay Satvik.

Student: My question is the same that I had a question last time. It was about like what maintains the structure in the mitochondria? I looked some stuff up and I found things about mitochondrial structure proteins; and the answer I got was that it is still being studied. So like is there, are there microtubules inside the mitochondria, like are there?

Professor: Not in the form that that we know in this in the in the eukaryotic cell; now, it is a good question. I do not know whether there is an FtsZ like molecule in mitochondria? I actually do not know whether they have actually been something?

Student: I read something about an experiment where they like modifications to the cytoskeleton affected the mitochondria structure.

Professor: That is possible. The modification of the cytoskeleton will affect the mitochondria; that does not mean the mitochondria itself has cytoskeleton. So, this is, that is a different question altogether. If the question is, what maintains the architecture of the mitochondria? There are enough proteins that are playing an active role to do that. Does the mitochondria have its own cytoskeleton? I actually do not know whether that is indeed the case. The mitochondria talk to the cytoskeleton of the eukaryotic cell; yes, it does. So, the location and even functionality seems to be affected by both actin and microtubules.

But that is not because they are inside the mitochondria.

Student: My doubt was just is there a cytoskeleton like structure?

Professor: I do not know, I do not know actually; I actually do not know. Quick, Elanshi.

Student: Yes sir, my question was regarding the regulator proteins that regulate the actin polymerization and depolymerization. The two that you mentioned right now, they were both either inhibiting the process of formation of actin filaments, or they were breaking up the already formed filaments? So, are there proteins that support or promote this process of polymerization?

Professor: Yes, the short answer is yes. And there are proteins that regulate the polymerization as well as the depolymerization. As I said, we just showed you a movie that kind of illustrates what is possible. And there is a lot of information about regulatory proteins, entire reviews that have been written on just the proteins that regulate actin. And then similarly, an entire set of proteins that regulate microtubules. So, it is actually remarkable that along with these structures existing in cells, the cells has have invested a lot of resources in fine tuning, how these structures are assembled, disassembled, put together; because that is vital to cellular function.

Student: Sir, my doubt was similar to microtubules have an anchorage at centromere from where they arise. Do actin also something like that?

Professor: No, they do not actually, that is why this, the idea of them being self nucleating is very very important for actin; because they have that ability that microtubules do not. Microtubules actually need a place from which to nucleate. So, they can not just randomly start nucleating somewhere, actin can; and this is a big distinction in how they assemble.

Student: I mean it needs a support, right Sir?

Professor: It support is itself, two such molecules can come together and two can become four; and before you know it, they are growing up strand.

Student: So, self-nucleating.

Professor: It is self nucleating, that is a big distinction. Good question, good question. Arnav, final query.

Student: Yes sir. So, you mentioned about how the microtubules present in the flagella, help in movement. So, my query was what what exactly controls the bending of the flagella? Is it the central microtubules or the peripheral?

Professor: So, I think it is a combination; it is not just the center. Actually, the control happens because of the motor proteins walking. And the motor proteins actually bind the strands like this; so these are the two motor proteins. This one is actually walking on this, this one is walking on this; and and their moment is actually able to do this. And allow the strand, the microtubule strands to move in this direction, which then when all of those strands do this effectively

translates into the movement of the cilia. So, so that is that is how this process works out. Kedar last question; your hand up late, very late, very quick.

Student: Sir, how the microtubules got direction to centriole to chromosomes? How they got attached to centromere and not other else?

Professor: So, so that has to do with where they originate; so, they originate from the centromere. So, it is not like microtubules were formed somewhere else and then found the centromere; they began at the centromere.

Student: I am asking that how they, they are arising from (cent) centriole, and they are attached to centromere no.

Professor: So they, you mean the kinetochore.

Student: Yes, sir.

Professor: Centromere is there, so kinetochore is at the chromosome.

Student: Yes Sir, attached to.

Professor: So, there the same tip complex. I told you remember that along with the originating end of the microtubule, the tip of the microtubule is very vital for functionality. And that tip is actually what goes and anchors. So, the tip has a tip complex, a whole bunch of proteins that are present there. Many of whom will actually bind to proteins that are present here and on the chromosome; and then we will be able to separate the chromosomes during cell division.