Cell Biology: Cellular Organization, Division and Processes

Monica Bettencourt Dias

Instituto Gulbenkian de Cienca

Lisbon, Portugal

Lecture 12

Centrosomes in Development, Evolution and Disease

Hello everyone! I would like to thank this opportunity to speak to you about centrosomes in development and evolution and disease. My name is Monica Bettencourt-Dias and I am a group leader at the Instituto Gulbenkian de Cienca close to Lisbon in Portugal.

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So, in our group we are really fascinated by how cells are organized in space and time. So, how do they execute such diverse functions such as a nerve cell, a bone cell, skin or blood. And in particular we are very interested in a small structure in our cells called the centrosome, which is composed of centrioles and which is very important actually for many different functions in our cells.

And in particular to also organize a skeleton that then defines polarity and organization of structures in space and time.

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So, these are very tiny structures the centriole that I was just showing you and here in a scheme. They are made of nine triplets of microtubules and that are shown here and they have two very different personalities in our cells. So, they can form the centrosome where you have two of them which with material that surrounds the centrioles actually helps to anchor and nucleate microtubules. And these are important for cell division, cell polarity, cell migration and also cell signalling.

But this structure can also tether to the membrane where they nucleate the formation of cilia and flagella that are very important for signalling and also for motility such as in the sperm flagella. So, at this moment in time in your body these structures are having many different functions from sensing the light in your receptors in your eyes to expelling particles in your trachea.

To seal in many different parts of your body, the hypothalamus sensing whether you have eaten enough, your kidneys, but also the centrosomes in cells either in division or in interphase in organizing the skeleton. And because of these multiple functions they actually are involved in many different diseases.

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For example, if you have changes in the genes that codes for centrosome proteins then actually can have diseases of development such as microcephaly where people have a small brain as compared to the wild type. It is known also that the Zika virus can change centrosomes in cells. We also know that changes in centrosomes are very common in tumours and actually that if you alter centrosome numbers you can induce tumours in mice.

And it is also known that if you change cilia you can have many different diseases including cystic kidneys, retinal degeneration, obesity, multiple fingers. So, there are many different symptoms that are associated with different diseases depending on the molecules that are affected.

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So actually these structures were found really long ago by these famous biologists and already at that time they were asking some of the questions that we are still asking today such as what is the role of these structures in human disease? What is their role in cells but also how are they inherited? But it was only in the last 15 years or so, that we started to have the tools to address some of these questions because the structures are really small. And with the advent of genomics, comparative genomics, microscopy, sensitive proteomics, we started to have a list of the molecules and RNAi screens. A list of the molecules that are needed to form these structures centriole and cilia toolkit which actually it is very interesting because these are present in many different organisms in the eukaryotic tree of life that have these structures. So, which suggests that the last common ancestor of these eukaryotes already had these structures and they were coded by the same molecule. So, this would be a pathway that is two billion years old.

So, there are many different questions in particular in our lab we are very interested in several of them including principles of centriole and cilia biology. So how you define the number of the structures? How you define a structure such as length? How you define how are they are assembled in space and time but also how you break some of these rules to generate diversity within a single organism. So, that you if form different structures in different tissues, within evolution, but also in human disease. So, how do how, are they altered in human disease and contributes to human disease.

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And some of these questions as I said are old questions. So, already when Beneden and Boveri were already discussing how the centrosome is formed and suggesting that it is an inherited structure. And when you have fertilization the egg does not have centrosomes but it would be the sperm that would bring in its place the centrosome that would be needed. So, that the new organism is formed with centrosomes.

However, it was also known that actually some certain organisms can develop without fertilization and this is what is called virgin birth. So, you have no fertilization you have development of an organism which would suggest that centrosomes could form de novo without template and so, these were two opposing views of whether the centrosome was a continuous structure being transmitted from generation to generation or whether they would form de novo.

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This is a question that has remained for a long time but now we actually have studied many different organisms and we know that this might not be exclusive. So, you have certain cells like our cycling cells where you have you start the cell cycle with one cell with two centrioles in a centrosome and then they duplicate. So, close to the one that already existed almost like a template where you have new centrioles forming there that will elongate. So, that in mitosis you have two centrosomes each one with two centrioles in a highly coordinated fashion and this is a what you call canonical way. But at the same time certain organisms, the wasps for example can develop without fertilization and so, centrosomes form de novo. But there are also other organisms where cells normally do not have centrosomes they only form them to generate the sperm like the mosses. And then they form centrioles that will form the flagella that is needed for cell motility.

So, there are different things here that contrast. So, these guys are regulated by the cell cycle they form close to centriole it is one and only 1 centriole. Here the timing we do not really know it may depend on what type of cell we are talking about the location is not very clear where they form and the numbers it really depend on the species it can be very controlled. Like for example in mosses or it can be very diverse like for example in human cells when you remove centrioles and they form de novo.

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And this is actually something that you can see in many different organisms as I was telling you. So, you can have de novo for example in flatworms, you can have also de novo in different plants. So, and you can have de novo in different parts of the eukaryotic tree of life.

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But also you can have de novo in human cells. So, if in human cells you ablate centrioles either by using a laser and you kill the centrosome effectively or you prevent the centrosome from forming new ones by preventing or inhibiting the trigger of centriole biogenesis. Then you have a cell without

centrioles and if you now actually remove this inhibitor then you can allow centrioles to form. And actually in this case many of them will form the novo, So, there is no control over the formation. So, the question is, are they different processes the canonical and the de novo? But you have in the same cell you can form de novo but you can also form in a templated fashion. So, this would be the same cells and how is this process regulated in space and time?

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So, already a while ago when we were studying a trigger of centriole biogenesis called polo like kinase 4 which is a molecule of the polo like kinase family which are protein kinases important for cell cycle progression. So, this molecule we knew it is actually necessary for centriole formation. And if you have too much of it you form many centrioles and what we saw was very interesting. So, this is an egg of the fly it is unfertilized eggs. So, you only see the products of meiosis and you are seeing it because they are labelled with tubulin. So, tubulin is labelled. If you fertilize this embryo you have the first spindle in the first mitosis and you have the two centrioles at each pole forming the centrosome. If you now have too much PLK4 which is a trigger of mitosis what you can see that you start seeing a lot of centrioles forming de novo. And you see the centrioles here bilateral microscopy and but if you already are fertilized you see that the centrioles form close to the one that already exists.

So, this suggests that again the same cell can form them de novo or in this canonical fashion but also that if the centrioles are present new centriole will form close by suggesting that the centriole either recruits molecules, it works as a catalyzer so, that new centrioles form close by or it inhibits other structures from performing elsewhere.

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So, a while ago looking into the regulation of PLK4 we saw that actually PLK4 autoactivates in a trans fashion. So, PLK4 phosphorylates another PLK4 molecule in this T-loop, which is important for kinase activation. So, normally PLK4 has a low activity but when it encounters another PLK4 molecule they can phosphorylate itself and it can become active. So, normally you would have if you look at total levels of PLK4 at low levels the probability that these molecules encounter is low. And therefore, there is very little PLK4 which is active but if you give more PLK4 to the cell you will have an increase in PLK4 activity because these molecules start to encounter themselves. And this suggests that there would be a critical threshold that if when you overcome this threshold then centrioles could be formed. And perhaps this threshold would be lower if you have a centriole because molecules such as PLK4 would be recruited there and then you have this positive feedback loop where PLK4 activates PLK4. There are also some other positive and some negative feedback loops and centrioles will be formed there. And if there is no centriole you would expect that you need more PLK4 to accumulate. So, that you start having this positive feedback loops occurring in the cytoplasm even without centrioles there.

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So, is it the same molecular pathway and how is it controlled / encoded? What ensures the right structure? Already, a while ago using comparative genomics our group and other groups have looked into some of these molecules that are needed to form centrioles. So the trigger of centriole biogenesis, it actually only exists PLK4 only exists in Apistoconcha so, fungi and animals and it might be substituted by a different molecule in other organisms such as even the founder of that family PLK1.

But other molecules that are needed to form the structure of the centriole actually exist in many of the different organisms that have centrioles and they are only absent from genomes that do not have these structures. Suggesting that whether you form centrioles de novo or in a canonical fashion you would use the same molecules. We also know that when we allow cells to form centrioles de novo they need these molecules. And if you allow them to form them in canonical fashion they also need these molecules suggesting that the same pathway is involved. And we also know now that these similar molecules are involved even if you look in plants and if you look also in different animals which suggest again that similar pathways are involved.

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So, it would be not two different mechanisms but a similar mechanism should be involved in this canonical way of forming centrioles or de novo whereby you use this complex pathway with many different molecules and centrioles would be self-assembling whether here or here. And that if you have a centriole components will be recruited there you have a positive feedback loop that makes that centrioles are formed there.

If you do not have centrioles and if you have high levels of these molecules you could have positive feedback loops occurring in the cytoplasm and there are new centrioles forming. So, an interesting question is really how you regulate this process for example how you regulate numbers but also how you create different numbers and diversity.

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So, I told you that this process of centriole formation is highly coordinated with the cell cycle and in fact we know that the cyclin-dependent kinases that regulate the cell cycle also contribute to regulate this process. For example Cdk1 is very important here to prevent centrioles from forming here. So, they only form at this stage. But also this cycle is a cycle whereby you normally would produce two centrioles and cells would be born with two centrioles.

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Yet we see that there are certain cells in our body that show themselves without these structures. So, the question is what is happening to these structures?

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And this is also interesting because as in the case of the eggs this could have an important function the fact that these structures are not there or are eliminated because we know that in *Xenopus* or frog eggs. So, here they do not have centrioles but if you actually inject centrosome there, they can form develop into an embryo and a small frog, which suggests that centrosome is really important and it is important that you eliminate it otherwise there could be a risk of parthenogenesis.

On the other hand, this experiment done in *C. elegans* also shows that these structures are very stable. So, if they disappear it is strange because they are really stable. So, if you label a centrosome in the sperm of a worm *C. elegans* and then you fertilize, in this paper, it was seen that actually even when you have 550 cells in the embryo you see that you still see the labeled centriole that came from the sperm which suggests that these are very, very stable structures.

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So, looking into eggs what we saw is that these centrioles disappear with time during the egg. So, that you end up with an egg without centrioles and the reason why they disappear is because this matrix that I told you in the beginning and surround centrioles and it is important for microtubule nucleation is not only important for microtubule nucleation but also it is important to protect centrioles from actually being eliminated.

And therefore, what happens in the eggs actually these components disappear with time and then centrioles disappear. So, this is very important. So, it means that even though these structures supposedly are very stable this is not a process that once you make centrioles they are stable forever this is a process where centrioles need to be actively maintained. And they can be unstable in certain tissues if that is if that makes sense for that tissue.

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So, the other question that is very interesting is how you create the diversity that you observe here. And this is work performed in my lab by a variety of different people including Swadhin Jana and what you can see here is that if you look in a fly an olfactory neuron that has one cilia here another cilia here. And the sperm cell where it has cilia here and another cilia that is perpendicular to it what you see is that actually these cilia are very very different.

So, you have triplets here in the centrioles these guys have doublets, these are really long these are very short, these are a longitudinal fashion. These ones are perpendicular to each other. So, there Are multiple multiple differences these guys have a cartwheel at the base, these guys have no cartwheel; they are very, very different.

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And we have actually been able to observe that even though there are many different components that are common to the different centrioles even in different species what happens when these cells are developing is that they lose some of these components. So, that actually the centrioles at the base of the different cilia have different components or actually have some of the common components localizing to different structures suggesting that this process is highly regulated.

And this is actually very interesting and maybe relevant for human disease because we actually see that sometimes the same molecule that is important and is what we call a core structure a core molecule a component of the cilia, cilium. This molecule when it has mutations in different places it can generate different diseases. And this makes sense if these different tissues are regulated in the different ways the molecule is regulated different ways in different tissues therefore different mutations could give rise to different phenotypes.

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The other very interesting thing is not just when you look we did not want organism when you look at these structures. For example your transition zone but if you look at also different species of organisms you can actually see that these structures can also be very different. And this is very nice showing that there is also a lot of interspecies variation and not just intraspecies variation.

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So, finally I would like just to discuss a bit this role of centrosomes in human disease and this is something that was actually suggested long ago by Theodore Boveri that centrosomes could cause cancer. And in recent years people have seen that in many different cancers actually show deregulated centrosome numbers. And that changes in centrosomes can actually lead to tumor formation in mice.

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We have actually seen that if you have if you pick different cells from different tumour types and you look at centrioles, this is a wild type cell you can see that in different tumours in different cell lines you can see many different centrosomes for example or even really long centrosomes. And we have actually observed that this deregulation is quite common.

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As I will show you here in work that we have done looking at the NCI-60 panel of cancer cell lines whereby if you look at the percentage of cells that show centriole amplification. So, these are cells with more than say 8 centrioles per cell or cells that have 5 to 8 centrioles per cell which is also not normal the normal is up to four looking at mitosis. And you can see here that in many different cell lines that are cancer cell lines in a panel of cancer cell lines that is called the NCI-60 panel of cancer cell lines.

And if you compare this with non-cancerous cells we see that many of the cancer cell lines actually have centrosome deregulation in numbers and also in size. It is very interesting that if you look at breast cancer you see that the breast cancer that is less invasive luminal has less amplification as compared to the more invasive basal breast cancer. And we could actually look in tissue samples from breast cancer and we see the same profile where basal actually shows more deregulation as compared to luminal breast cancer.

So, to finalize I think these are very fascinating questions that already these guys were studying. We now have the tools to address these and we can ask questions about how are these structures formed in space and time, how are they maintained? But also how are they formed in different tissues? How do they form in different organisms and can even contribute to evolution and how do they participate in human disease.

So, many, many different questions to address and which I did not have time to address today but that you can look at many different papers.

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So, I would like to thank people in my group that contributed to the work that I have shown here today. I would also like to say that the work that I have shown in cancer has also had the participation of many different research groups including the group of David Pellman, Susana Godinho, Joana Paredes and Nuno Morais that helped with that particular study and I would like to thank these organizations for funding. The work on centrioles was done by Swadhin from the people that are here.

But also Susana Mendonca and the work on cancer has been done by Gaelle Marteil, Adan Guerrero, Carlos (())(23:09) also worked on cancer, the work on centriole disappearance in oogenesis has been done by Anna Marcus here but also Ines Bento ((23:19))(23:21) who is not here. So, variety of different people and thank you very much for listening.