Cell Biology: Cellular Organization, Division and Processes

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Lecture 15

Cytokinesis

Hi, everyone my name is Saravanan Palani. I am a faculty at the Department of Biochemistry, Indian Institute of Science, Bangalore. Today I am going to talk about cytokinesis, which is one of the cell division processes that happens at the telophase. And the previous lectures would have covered the different cell cycle stages from G1 to metaphase and what happens in metaphase, which is where the cells exit mitosis and then go on to a physical separation happens in the telophase.

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So, before going into the topic I would like to give a overall view about what is cell division is all about? Cell division is a process consists of many phases as I said earlier G1, S phase, G2M and then the metaphase where you have an anaphase and then the telophase where the physical separation happens. And cells ensure all of the genetic material has been duplicated, segregated to the daughter cell, and then this has been monitored throughout the cell cycle by different checkpoints and if there is any problem, until the error is rectified the cell cycle will not move on to the next phase. And then such as like a DNA damage checkpoint, spindle assembly checkpoint and then they moved into the mitosis where cells exit mitosis and then give the license to the next phase called telophase where the cells can go for a physical separation.

If there is a problem it cannot be rectified and that is where the cell division fail and that leads to an aneuploidy of cells and that can happen to move into a cancer cell or tumour formation and then leads to metastasis and so on where the cell division fails. And a cell division happens in a proper sequential manner will lead to healthy progeny cells and that will go on and then propagate and then we will get more healthy cells.

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So, before we move on to the topic I would like to give a brief history about how do you know about cell division in general. In early 1970s and 80s, three great scientists across the world, they identified

a major discovery in the field of cell cycle. They identified the cell division cycle genes and that leads to understand what we know about cell cycle today it is coming from their understanding from different model systems.

And they were identified a mother kinase which regulates most of the cell cycle stage called cyclin dependent kinase and its regulatory subunit cyclins. And their discovery led us to come to a conclusion about cell cycle and what are all the different stages and we are still trying to explore is there a more signalling molecules and how they are regulated all the cycle cycles or the cell cycle stages in a variety of model systems. And most of the work was being done by Professor Paul Nurse, Tim Hunt and Lee Hartwell.

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I am giving a simple example about the cell cycle for us to easily understand the *Saccharomyces cerevisiae* it is one of the well studied model systems, nothing but budding yeast. And as you can see in budding yeast is the bud comes from late G1 and then the bud site has been determined in in early G1 and then the bud start growing from that site and then you have a spindle pole body duplication which is nothing but mammalian equivalent centrosomes and then you have a chromosome segregation happens. And then the cells have to exit mitosis in order to give the license in telophase so the cell can have a physical separation from the mother cell and that leads to a cell separation.

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And in general mitosis consists of four different phases; we call it a PMAT which is a prophase, metaphase, anaphase and telophase where you have chromatids attached to spindles and then it can be segregated to moves in the opposite pole direction and then you have a new nuclear envelope forms of the new chromatin chromosomes are kept separately from the mother and daughter cells. And then all the organelle separation is finished then the cells will exit mitosis. How they exit mitosis is that the way they all the cell cycle have been done using a cyclin dependent kinase activity and that kinase activity has to go down in order cells to exit mitosis. As I was as I mentioned earlier that cyclin-dependent kinase is a mother kinase which regulates all throughout all the cell cycle phases and also that it inhibits the cytokinesis. So if you wanted to proceed for cytokinesis so that cyclin-dependent kinase activity has to go down and cell uses many ways to inhibit the cyclin-dependent kinase activity such as degrading this mitotic cyclin, which is a regulatory subunit. As well as activating the cyclin dependent kinase inhibitor which is Sic1 in budding yeast Kip1 in higher eukaryotes. And they use multiple ways in order to regulate the cyclin dependent kinase activity to level zero so that the cells can easily exit mitosis and then proceed on to a cytokinesis followed by the physical separation of cells.

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And using the budding yeast as a model system the major discovery has been done in early 1990s they have identified a pathway called mitotic exit network signalling cascade, which is called MEN that already clearly tells what this pathway does. So this pathway happens at the spindle pole body, it is an equivalent to mammalian centrosomes and there is a protein called Nud1 which act as a scaffold and holds a couple of signalling molecules starts from Tem1 which is a GTPase and which has got a GEF which is Lte1. And then there is a GAP Bub2-Bfa1 complex and this activates a series of kinase called Cdc15 and Cdc15 kinase activates Ndr kinase family called Dbf2-Mob 1, Mob1 is a regulatory subunit. Very recently in early 2010 that have been the function of this particular kinase playing a major role directly on cytokinesis and what was known in early 1990s and 2000 that this cascade activates a conserved phosphatase called Cdc14 which is nothing but a dual specificity phosphatase which dephosphorylates Serine/Threonine phosphorylation as well as tyrosine phosphorylation.

Cdc14 is a dual specificity phosphatase tend to counteract always cyclin dependent kinase substrate because they both share the similar motif in order to a Cdk uses the same motif and Cdc14 to phosphorylate and then Cdc14 you also tend to dephosphorylate that similar kind of motif. And this particular phosphatase is kept inactive in most of the cell cycle stages in the nucleolus by its competitive inhibitor called Net 1 and then when cell reaches an anaphase and this cascade MEN cascade gets activated and via Cdc5 polo like kinase.

And then Dbf2 Mob 1 phosphorylates Net 1 as well as Cdc14 nls at the c-terminus and that allows Cdc-14 gets released from its inhibitor called Net 1 from nucleolus and then it reached nucleus cytoplasm and eventually to the cytokinetic site nothing but a budneck. As you can see on the left-hand side I put an time-lapse series image which comes from my own paper was published in 2012 that where I showed the first evidence that this particular phosphatase has kept most of the time in the nucleolus. And then gets released and the beginning of the time phase where it gets released to the nucleus and also it localized to the daughter , predominantly in daughter spindle pole body. And later you can see in the zero minute where it localizes to the cell division site and that is the time that where the actomyosin ring started contracting. The moment the Cdc14 the phosphatase localizes to the cytoplasm and that is where the exit of mitosis is initiated by activating Cdh1 which is an APC proteosome component and Cdh1 specifically recognized the mitotic cyclin to send out for degradation.

At the same time Cdc14 also activates Sic1 which is a Cdk inhibitor and it is also downregulate Cdk activity. While doing Cdc 14 all this job at the same time Cdc14 also goes to the division site where the physical separation is actually happening and then to activate the cytokinesis by dephosphorylating certain critical substrate by removing them which are the inhibitors and activating them some of them are going to initiate the actomyosin ring contraction followed by septum formation and so on.

And in general the signalling cascade we know and what are all structural molecules are playing a role in in order to execute this job. Signalling molecules of course control the structural components but what are all the structural components are involved in this process.

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And before going into it the cytokinesis or the actomyosin ring dependent cytokinesis and I would like to give a brief introduction or history behind it. So in late 1970s and 80s the three great scientists Albert and Ray Rapaport and Andrew Huxley has identified a type 2 myosin non-muscle type 2 myosin it is a ATP dependent motor along with F-actin which is like a track a globular actin gets polymerized by in the presence of formin.

And they have identified this particular motor protein which is a myosin and then sits on actin and moves actin filaments. And later in early 1980's and early 1990's the two great scientists Tom Pollard and Jim Spudich have done an enormous work on the biochemical characterization of all these components. And over the last 40 years we have identified many more components of this particular machinery.

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So as I said in early 60's and 70's uh Rapaport, Huxley what they found is that they have identified a myosin which is a ATP dependent motor and it moves the F-actin filament and that leads to contractility. And over the last 50 years what we identified many more components almost more than 100 proteins are involved in order to make us such a unique cytokinetic apparatus with nothing but an actomyosin ring.

And the ring consists of more than 100 to 150 proteins and as I said, you have a globular actin which is a monomeric actin gets polymerized by the presence of formin and profilin and then followed by protect, this F-actin has to be protected from the severing factors. So that in order to maintain certain integrity and stability of the ring by tropomyosin which wraps around this F-actin complex.

And all of once it is everything is organized there is a signalling molecules comes in when the myosin can sit on this actin filaments and then pull the actin filaments together and then that leads to a contractility. And this all this work was done in the last couple of years and we are trying we are making a better understanding of all how this machinery is working.

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And as I said this the entire process of the cytokinesis happened which is an acto-myosin ring dependent it is not just an actin and myosin there are more than 100 proteins are involved in it. And such a mechanistic apparatus are regulated by multiple signalling cascades. So I if I can simplify split into two, one is the preparatory period because the actomyosin ring assembly happens and then execution period once the assembly is done and all the organelles all the chromosomes has been you know segregated to the daughter cell and the cells will make a decision. Now it is a time to initiate the constriction of the ring and that allows in the case of yeast that allows the membrane invagination followed by the septum synthesis based on the chitin which is a primary septum and then the glucan which is the secondary septum, the physical border has been closed between the mother and daughter cells and then there will be no more cytoplasmic exchange happens between the mother and daughter.

In the case of mammalian cells there is no cell wall so in this case that what happened at the same process but in the cleavage furrow formation happens it is just in a membrane invagination and then it closes the border. And finally the cleavage happens via escort protein and why people have used yeast as a model system to study this unique process because most of the proteins are involved in this process are highly conserved. Especially the structural molecules such as actin myosin tropomyosin formin profilin and signalling molecules GTPases kinases and phosphatases and that gives them a very unique strength to studying such a dynamic and a conservative process using a simple yeast model system.

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And here I am giving an example of *Saccharomyces cerevisiae*, how it puts the actomyosin ring and how it divides the physical separation of the daughter cell happens from the mother cell. The *Saccharomyces cerevisiae* budding yeast it is a unique feature that it determines the cell division site that means where the division is going to happen in the late G1 and then the bud started growing after the site has been marked by septin and septin associated proteins called Bud3, Bud4, there are annilin proteins and then the bud grows. And once the bud started growing in and then you have all other cell cycle stages process like duplication, chromosome segregation, all happens and the ring gets mature and the septin here function as a kind of a collar like a sandwich and the actomyosin is nicely fit in the middle of the septins. And one cell reaches the phase in the anaphase and then where the cells are ready to exit mitosis and then septin split into two rings and that allows many other proteins to localize to the actomyosin ring and that is the time the activators come in the inhibitors has been removed and then the cells go on to constrict the ring and the constriction of the ring allows the primary secondary septums to follow the membrane invagination and then the physical closure of the mother and the daughter cell.

As you can see here the time-lapse series image shows that where the previous cell is kind of going out of the mother and then the new buds started coming and you can see the acto myosin ring and septins are very nicely merged here. And the when cells ready to exit mitosis and then you can see a split then you can see a light magenta colour in the middle of the blue where the septins are marked here as a blue colour. And you can see the magenta colour is visible where the septin splits and then after actomycin constrict at the last frame of the timelapse move you can see you can see only the splitted septin rings. So, you do not see any more actomyosin ring because the border has been closed and the septum has been formed. So you can take it as a very simple thing here take home message is that actomycin ring organization is one most of the part of the cell cycle in the case of budding yeast from G1 to metaphase until early anaphase. And then the late anaphase where the acomyosin ring initiate the constriction it is kind of a sequential steps first the septin splits. That septin split happens only when the mitotic side Cdk level goes down and that allows the septin splitting and then recruitment of new cytokinetic components which activates a couple of factors such as activating chitin synthase 2 which produced the chitin for the primary septum formation. And then also a couple of components which are ready to activate the cytokinetic ring constriction.

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So to make it very simple here what I have said earlier and here with the EM picture I am showing here is that as you can see in the red colour schematic shows that is an actomyosin ring you can believe that and then the ring constrict and where you can see the primary septum kind of follows the ring. And then closing the border okay and once the primary septum comes close the border.

And then you have a secondary septum made up of glucan and then they will also come and close and that makes a very thick gate between the mother and daughter cell. And once all this process has been done the same phosphatase Cdc14 does a unique job is that activating one of the transcription factor at the daughter nucleus and that is a key factor to activate the production of the production of chitinases and glucanases and they will get secreted outside and then they will come and then chew the chitin and glucan and then that leads to physical separation.

And this are all happens in a very sequentially the MEN activates in the early anaphase in order the cells to release the Cdc14 phosphatase in late anaphase and where the cells exit mitosis at the same time they release Cdc14 in cytoplasm activates and transcription factor and that goes wait until the ring constriction happens and that the closure of the mother and daughter cells by the chitin of primary septum and then the glucan the secondary septum and then the chitinases and glucanase are secreted outside and then they will come and chew this off and then you have a physical separation.

And this is a complete sequential order of events happening at the late anaphase and then telophase.

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And as you can see here the comparative analysis of a budding yeast as well as the fission yeast as you can see in budding yeast that once the old cell goes off it means the previous cell division is finished and then you can have a new bud comes in where the septum mark. And then the new bud started growing from where the septin has been marked where the bud that means the bud site has been selected by septins and then the new bud comes out of it. And then the septin splits and then the separation happens and this is what the natural process happening all the time. In the case of fission yeast as you can see on the far right that the ring formation happens based on the cytokinetic nodes in the G2M phase and then the ring formed and then get matured and ring constrict and then your split happens. So this is the difference but the components are involving in this process are highly conserved and this is a similar case also happens in a mammalian cells.

So with this I covered mostly about what happens in the late anaphase events after cell exit mitosis and then the unique phosphatase called Cdc14 which is highly conserved and that not only down regulating Cdk activity and also that dephosphorylating the substrate which are crucial for executing cytokinesis and cell separation at different level. And that leads to cell separation and then you can get leads to a healthy progeny and then that the cells can propagate further. With that I would like to thank everyone for your attention.