Cell and Molecular Biology
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Week 03
Cellular Homeostasis
Lecture - 09
Cell Growth and regulation

Hello everyone, this is Dr. Vishal Trivedi from the Department of Biosciences and Bioengineering, IIT Guwahati. And what we were discussing was the different properties in the course Cell and Molecular Biology. So far, what we have discussed includes the evolution, the origin of life, and subsequently, the different cellular structures of both prokaryotic and eukaryotic cells. In that context, we have discussed the prokaryotic cell, including the cell wall and other properties. Whereas in the case of eukaryotes, we have discussed the different cellular structures.

We have discussed the different types of organelles and we have also discussed the function of these organelles. Now, in today's lecture, we are going to discuss some more aspects related to cell and molecular biology. When we talk about living organisms, the basic feature of any living organism is that it actually has complex organization. It should be responsive to the different types of environmental stimuli, such as temperature, wind, starvation, and so on.

But apart from that, the fundamental property of any living organism is that it should be able to give rise to offspring. That means you should be able to reproduce it. On the other hand, it should be able to achieve endogenous growth. So these are the two properties that actually discriminate a living organism from a non-living organism. And if you recall, we have discussed the different types of cells.

We have discussed the prokaryotic cell. We have discussed the eukaryotic cell. Within the eukaryotic cell, we have discussed animal cells. We have discussed the plant cells. We have discussed the yeast as well as the fungi.

But the basic phenomenon of any living organism is that it wants to contribute to two different types of fundamental activities. One, it wants to increase its numbers, and it also wants to grow in size. I'm sure you might have seen that when you are born, you are born as a small baby, and in due course of time, you take nutrition from outside, and that's how you actually develop into adult individuals. The same is true for even the tiny cells. So if you see the cell, it is actually going to perform different types of activities, and these cells mostly would like to do all these activities so that they can grow in size.

They can make more of their type, or they will be able to divide and give you different types

of similar kinds of cells. So growth is the fundamental nature of an organism, right? Growth is the outcome of cell division, which means one cell divides into two, and that's how it actually increases in number. But all these tiny cells are also going to grow in size in due course. Cell division is a process in which each parental cell reproduces by dividing into two daughter cells under specific conditions. The resulting daughter cells can themselves grow and divide, giving rise to the new cell population formed by the growth and division of the single cell.

In this context, I am sure you might have studied when you were doing the microbiology experiments in your laboratory that when you inoculate a single tiny bacterium, it actually grows, divides, and gives rise to a complete cell culture. Similarly, if you plate these few bacteria, you will see that they are actually going to form colonies. These colonies actually contain thousands of different bacteria and so on. So in a simpler format, the continuous cycling of growth and cell division allows the single bacterium to form a colony consisting of millions of progeny bacteria following the overnight incubation on the nutrient agar media. And this is what you might have done in your microbiology lab.

In a more complex form, when you are going to work with eukaryotic cells, they are actually going to develop as a single cell. So, for example, in humans, when the sperm and ovum fuse, they actually give rise to a single cell individual that is called the egg or the zygote. And then these zygotes are going to undergo repeated divisions, and that's how these divisions give rise to the 10^{14} cells, which is how it is actually going to give rise to the small baby. and then that baby is also going to take up the nutrition from outside and that's how it is actually going to grow up into an adult individual So the division of the cells must be tightly regulated and coordinated with cell growth and DNA replication to ensure that the proper flow of genetic information is maintained. So this is the most important part of what we are going to discuss in this particular module: that the cell is going to divide.

So we are actually going to discuss the division mechanisms, how the different types of cells are utilizing the different types of division mechanisms. On the other hand, it is also very important that all these division mechanisms be tightly regulated by the cellular machinery and be under coordination so that they do not result in irregular cells, irregular multiplications, or irregular divisions. And we are also going to discuss what will happen if such a thing actually happens. Now the fundamental question is why we need to grow, right? Why is growth required? So for that, I will take you to one of the examples: suppose you are cutting the vegetables in your kitchen. Or suppose you are doing any kind of, you know, paper cutting and all that, and all of a sudden you cut your finger.

So what happens when you cut your finger? When you cut your fingers, the blood starts oozing out, and it oozes out because you are actually cutting the blood vessels, and blood vessels are made up of specialised cells. These are called the endothelial cells But it doesn't happen that you actually keep losing blood; what happens is that the body is getting the signal from the cutting site, and that's how you are going to start repairing the tissue. And

all those kinds of things, and the tissue repairing requires that the neighbouring tissue, which is still healthy and does not get any kind of injury, is going to grow, and that's how it is actually going to pile up or complete the tissue site, okay? So when you get a cut or when you get injured, the surrounding tissue of the injury undergoes inflammation. So inflammation is a protective immune response. This anyway, we are going to discuss when we are going to talk about basic immunology.

And it is actually going to release many types of growth factors; it is also going to release many types of chemotactic factors so that the neighboring tissue or neighboring cells should come and protect this particular cut site. And then it's also going to induce different types of signaling pathways, and that's how it is actually going to induce cell division, because if you are getting a cut here, the neighboring tissue is actually going to start developing. I'm sure you might have seen that when you get an injury in your hand or feet, it is actually forming a clot, and that's how all the cells are covering this wound site. And then, in due course of time, they get completely healed, and then this proportion is going to be peeled off, and that's how you are actually going to have the recovery of the injury. So your body is made up of billions of cells, and those cells make multiple copies of themselves to fix the injured tissue so that you can function properly.

This is very important because if you don't recover from your injury, it will actually damage the cell machinery. The average human body contains 10^{14} cells, and these cells contain more than 200 differentiated cell types. Cell division is crucial to replace the cells that have died due to necrosis for tissue maintenance. So this is also one of the portions that we are going to discuss at the end of this particular course, where we are actually going to talk about what happens when the cell is not getting enough nutrition and how the cell will go through the death pathways. Now, as I said, cell division is a controlled event, right? So, cell division is a controlled event because you have to divide the cells in such a way that it does not give you, you know, enough cells.

So, in high eukaryotes, the progression to the different stages of the cell cycle is controlled by a series of protein kinases that have been conserved from the simple yeast to the highly complex animal cells. And this happens in bacteria as well as in higher eukaryotes. The cellular machinery is itself tightly regulated by the growth factors that control proliferation, allowing cell division events to occur only when the organism requires it. So this is also very important. And the defects in the regulation of the cell cycle caused by genetic instability are a common cause of the abnormal proliferation of cancer cells.

And this is very, very important. What we should understand is that if there is dysregulated cell division, which may actually not be under tight control, then these cells are only going to differentiate in the course of time. And that's how they are actually going to develop the cancer cells. So cancer is one of the leading causes of death, claiming millions of lives every year across the world. So first, we have to understand how the cell goes through the different types and stages of cell division so that it can be understood that at every stage,

there is a way to control the cell cycle.

So, the cell cycle, which is the cell's reproduction, is the most common characteristic of the cell, right? Every cell wants to grow; everyone wants to make its own copy so that it can remain in the environment. All cells reproduce when the parental cell divides into two daughter cells and after every successful completion of the cell cycle. The cell cycle of most cells consists of four well-coordinated processes. One is cell growth; the second is division applications. Then we have the segregation of the replicated chromosomes.

And then we have cell division, which means there will be a division of the grown cells. So the cell growth is actually also called the G1 stage. DNA replication is going to occur in the S phase. Then, segregation of the applicator phase is going to happen in the M phase. So, after the M phase, the cell will enter cell division, and that's how one large cell is going to divide into two daughter cells.

So, in bacteria, cell growth and DNA replication occur throughout most of the cell cycle, and the duplicated chromosomes are distributed to cell division in association with the plasma membrane. In high eukaryotes, the cell cycle is more complex and consists of four discrete phases, where the chromosomes are duplicated in one phase and the duplicated chromosomes are distributed into the daughter nuclei before continuing the cell division. So, what we have said is actually the basic fundamental aspect of what is going to happen, whether it is a prokaryotic cell or a eukaryotic cell. So, you're going to have cell growth, which means the cell—suppose this is the normal cell. First, it is actually going to synthesize the cytosol so that it will have the large cell, right? There will be cell growth.

Then, suppose this contains one nucleus; then this cell is actually going to contain two nuclei, and that is what is going to happen in the DNA applications. Then, these two nuclei are actually going to be segregated in the third step, and then there will be cell division. So it is actually going to be divided in half, and that's how you are actually going to have the two daughter cells: one which contains this nucleus, the other one which contains this nucleus, and this is what the fundamental events are that are going to happen irrespective of whether it is a prokaryotic cell or a eukaryotic cell. In prokaryotic cells, since the machinery is small, the cellular machinery is also small, and the protein machinery is also small, the number of components required for controlling cell division or the cell cycle is small. But in the case of eukaryotes, all these are actually going to be under tight control, and that's how they are actually going to be forming these kinds of different stages where you have the G1, then you have S, and after the M phase, you are actually going to have the division of the cells.

Now this cell cycle and the discovery of the cell cycle don't stay in a single day, right? People have done a lot of experiments and made a lot of discoveries so that you can understand cell division. It started with the simple discovery of the compound microscope. Because when you discover the cells, you discover the cell because there is a compound

microscope. And that was done by Janssen and Janssen in the year 1590. So they have discovered the first compound microscope.

And because they have discovered a compound microscope, you will be able to see the cells. You will not be able to see the single cell before that. In the year 1661, the Italian microscopist Marcello Malpighi was among the first to use a microscope to describe thin slices of human tissue. So this is the scientist who first saw the animal's tissue. In the year 1665, Robert Hooke coined the term "cell" after observing a thin slice of dried cork under the compound microscope, and he noted that he saw dead cells, which appeared to form hexagonal compartments, and that is how these compartments came to be called cells.

What he observed was the dead and dried cork. Then, in the year 1675, Antonie van Leeuwenhoek was the first to observe free-living cells. Then in 1824, René Dutrochet correctly concluded that all animal and plant tissues are aggregates of globular cells. Then, in the year 1838, Schleiden and Schwann actually concluded the cell theory. And in the year 1852, Virchow published the classical textbook called Cellular Pathology, which correctly states that the functional unit of life is the cell, the primary site of disease and cancer.

In the year 1953, Watson and Crick actually discovered the structure of DNA, and that's how people began to understand the cell and molecular biology of different organisms and what different types of events are being regulated. So one of the major important things related to the context of this particular module is that we should understand what the cell theory is. Cell theory was proposed by the German scientists Theodor Schwann and Matthias Schleiden in the year 1838. And it explains the basic nature of all living organisms. What it says is, number one, living organisms are made up of cells.

Right. So, that is very, very fundamental information. What it means is that cells are the smallest units of life. Each cell contains cytosol with a phospholipid bilayer, and all the cells contain DNA, which holds the information necessary to produce the molecules for survival. So, Schleiden and Schwann postulated the cell theory, stating that all living organisms are made up of these cells. And these cells actually contain the cytosol.

Right. So these cells actually contain the cytosol, which is covered by a plasma membrane on the outside. So this is actually going to have a covering of lipids and proteins. And then it is also going to have the DNA so that the DNA actually contains all the information required for controlling all the activities of this particular cell. Then the cells are the basic structural and functional units of life. So an organism can have a single cell, or it can carry all the necessary molecules for metabolism, or there can be complex tissue that contains different cells to perform specific tasks collectively.

So, a group of tissues contains what can actually form the organs. So irrespective of whether the organism is unicellular, multicellular, or specific to organs or tissues, what it says is that the cell is going to be the basic structural and functional unit of life, which

means you can actually have different types of organization. You can have cellular level organizations, tissue level organizations, organ level organizations, or organ system organizations. Irrespective of the organizations, the basic function that is going to be performed is going to be performed by the single cell, and the cell is the structural and functional unit of life. All cells are derived from pre-existing cells, which means all the cells actually divide, and that's how they form their own copies, and these cells perform different types of functions.

So all cells are derived from pre-existing cells only. Only the cell grows in size and reaches a threshold; then it undergoes cell division, giving rise to two daughter cells. So, this is the photograph of Schleiden and Schwann, right? So, this cell theory was very, very important and it actually gives very important information about how living organisms are made up of these cells. Cells are the structural and functional units of life, and every cell comes from pre-existing cells, which actually gives the idea that there is cell division, which is very important. So now the first question is, is the cell division in prokaryotes and eukaryotes the same, right? So what you see here is the generation time of the different organisms.

So what you see here are E. coli, Bacillus subtilis, Mycobacterium tuberculosis, Euglena, Giardia, and Saccharomyces cerevisiae. So these are actually the eukaryotic organisms, and these are actually the prokaryotic organisms. And what you see here is that the doubling time for these organisms is much faster, or much smaller, actually, I will say, compared to the eukaryotic organisms. Eukaryotic organisms take 2 hours, 18 hours, and more than 10 hours, while prokaryotic organisms actually work in the frame of minutes. So basically, the cell division and the fundamental machinery, or the fundamental process, remain the same between prokaryotes and eukaryotes.

But the mechanisms, the players are different. So although the basic events that occur during cell division are similar in both prokaryotes and eukaryotes, cell division is more complex and highly regulated in eukaryotes. And because of that, only the prokaryotes actually have shorter lifespans or shorter generation times, whereas the eukaryotes actually have longer generation times. And that is understood because you have to perform a lot of functions, and you will see in due course of time why it is bigger in eukaryotes versus why it is smaller in prokaryotes. Eukaryotes have several checkpoints where cell regulation is involved in the progression of the cell cycle, and each checkpoint of the cell cycle must be cleared to progress further into the cell cycle. Otherwise, there will be a cell cycle arrest at that particular point.

The multiple checkpoints increase the time required to complete one cell division. You can observe this by using simple tiny cells to complete one cell division. A simple bacterium, E. coli, takes only 20 minutes to complete its cell division, whereas budding yeast only takes 90 minutes, and human cells actually take more than 24 hours. As the complexity of the cell increases, the time taken for the cell to divide also increases.

And this is what is very clear from this particular table: if you are a prokaryotic cell, you are going to take less time to divide. If you are the eukaryotic cell, then it is actually going to take more time. And why are the prokaryotes dividing so fast? So the cell cycle in prokaryotes actually takes fewer steps and divides. So why are the prokaryotes dividing fast? Because prokaryotes have a simpler cell cycle and most probably undergo binary fission. First, the cells grow in size by accumulating the nutrients under favorable environments and conditions, and once they reach the optimal size, they initiate chromosomal duplications.

Chromosomal replication is a very crucial process in cell division, and it must be tightly regulated. Once the chromosome replication is accomplished, the molecular mechanism must ensure the successful segregation of the daughter chromosomes into the daughter cells. Prokaryotes tightly regulate their chromosome replication, and this is what we are going to discuss. Prokaryotes are actually regulating the distribution of chromosomal replication. Remember that prokaryotes do not have any kind of partitions.

They don't have any kind of nuclear membranes, and so on. Right. So, within the single cell, you actually have the DNA. Right? Which is part of the genome.

Right. So this genome is going to be duplicated. And then this duplicated genome is actually going to be distributed within the cell so that when there is a cell division, it is going to be equally divided. So how is the regulation of the chromosomal replications? Remember that when we are going to discuss the replications, you will understand that there are different modes of replication, but the most acceptable mode of DNA replication is the rolling circle model, right? So in the rolling circle model, you are actually going to use one point that has been called the origin of replication. So, in the bacterial chromosome, the replication of the chromosome starts at a specific sequence that is called the origin of replication. So, the original replication is the site from where the DNA is going to start replicating, and then it is actually going to form the mechanism that is being described for the rolling circle model. So, when we discuss the replication in prokaryotes, you will understand more details about the rolling circle model.

A replication factor called DNaA binds to the specific sequence within the oriC and then initiates the unwinding of the DNA and the loading of the replisomes. DnaA is regulated by ATP binding, and it becomes active when ATP is linked to it. Other regulatory mechanisms that inactivate the DNaA include competitive binding at the OriC, repression of the DNaA, titration of DNa-ATP away from the chromosome, and inactivation of the DNaA-ATP. So all these mechanisms are very very important and you see all these are linked to the ATP, right? So the molecule that is central to this whole activity is ATP, and ATP is linked to energy.

Right. So if the bacteria do not have enough energy and energy is linked to nutrition, right? So if there is not enough nutrition, it will not be able to produce enough ATP. And if it

cannot have enough ATP, then this whole event is actually going to be slowed down. Right. Because the DnaA is not going to bind to the specific sequences on the OriC, it requires ATP and other kinds of molecules. And that is all linked to energy, and energy is linked with nutrition.

Before the initiation of replication, both strands of chromosomes are methylated at the adenine in the sequence GATC. Immediately after replication, only the parent strand has the methyl group and is hence called hemimethylated DNA, which facilitates competitive binding between the DnaA and SeqA. Hemimethylated DNA is strongly bound by the Seq-A. Binding with Seq-A at OriC blocks the DnaA binding and hence prevents reinitiation of the chromosomal applications. Then another regulatory mechanism, so you have what we have seen: one mechanism where the DNa-A and ATP interactions are very crucial, and the availability of ATP itself is very, very important.

The other is where you are actually going to have a decrease in the ratio of DNa-A ATP to DNa-A ADP. The conversion of DnaA ATP to DnaA ADP is catalyzed by the ATPase enzyme called HdaA. and which binds to the DNA close to the replisome and specifically targets the DnaA ATP. This is also related to energy. Remember that energy is very important when it comes to the prokaryotic system because it is linked to the nutritional status of the environment.

And if the bacteria are under less nutritional status or the environmental factors are not very conducive, Then all these events will lead to a state where replication will be slowed down, or the bacteria will increase their generation time, and so on. And that's how the bacteria actually have the immense power to withstand the different types of adverse conditions. But genome replication is fast in bacteria, such as Yersinia pestis or E. coli. Why is it fast? Because the genome size itself is very small compared to that of eukaryotic cells.

The circular nature of the chromosome facilitates faster replication as DNA replication occurs bidirectionally from the original replication and the formation of multiple forks further decreases the time requirement for DNA replication. So you know that if you have a circular object, right, then you are actually going to start with the original application. So one fork will go in this direction; the other fork will go in this direction. And that is what the mechanism is, what we are going to discuss, you know, when we discuss the replication in the subsequent modules.

And because they go in different directions, the replication is faster. And apart from that, the size itself is also very small. So once you have the two copies of the chromosome within the bacterial cell, it is actually going to segregate. Which means it is actually going to distribute it to this particular parent cell so that ultimately it will go through with the cell division, and that's how you are actually going to have the two copies of the bacteria, right? So how are we going to have the segregations? So during cell division, the segregation of the chromosomes to the poles of the cell ensures that each daughter cell gets a copy of the

genome, and it also facilitates septum formation. Most of the bacteria use a mechanism that is called the Par system to distribute the chromosome and its plasmid equally into the progeny cells. This system is composed of the protein called ParA ATPase, ParB chromosome-binding proteins, and the PopZ complex, along with the chromosome, like the ParS sequences located near the origin of replication.

The Par system does not segregate the fully replicated chromosome; instead, Pop Z localizes to the old pole of the cell and facilitates the anchoring of the chromosome to this location by interacting with ParB, which is itself bound to the parS sequences. Once the replication begins, the newly synthesized power sequences bind to another ParB. which then pulled to the pole of the new cell by the ATPase activity of ParA and PopS not only anchor the parental chromosome at the old pole but also recruit ParA on ParB bound to the newly synthesized power as sequences This means that what it says is that you are actually going to use a Par system where one Par protein is actually going to bind to the older chromosome and the other proteins are actually going to bind to the newly replicated chromosomes. And that's how, at one end, the parental DNA is going to remain, and on the other side, you are actually going to have the newly synthesized DNA. Then you are actually going to have cell division, right? And cell division is going to be done by binary fission, right? Several proteins play a role in cell division.

Collectively, they are known as the Fts proteins, and a key one of them is FtsZ, right? Similar to the tubulin of the eukaryotes, which plays an important role in binary fission. FtsZ proteins interact with each other to form a division apparatus called the divisome. So this is a segregation of the chromosomes. The chromosomes are segregated to the two poles of the cells. Then in the rod-shaped bacteria, the divisome formation begins with the arrangement of the FtsZ protein in a ring shape around the center of the cell, which then acts as a central division plane.

The FtsZ binds to the other divisome proteins, including FtsZ and ZipA. ZipA acts as an anchor that stabilizes the FtsZ ring onto the cytoplasmic membrane. FtsZ is also involved in recruiting FtsZ and other chromosomal proteins. Another group of proteins known as the Min proteins, including MinC, MinD, and MinE, interact to help position the FtsZ ring in the center of the cell. As cell elongation continues and receptor formation begins, the two copies of the chromosomes are pulled apart, each into the titer cells. This is exactly what is going to happen here: in the middle of the cell, you are actually going to have these cell division proteins, which are going to aggregate, right? Or we are going to localize.

And then they are actually going to have the other cell proteins, which are called Min C, Min D, and Min E, and then they are actually going to form the elongation of this particular cell. As the cell constricts, the FtsZ rings depolymerize, which triggers the inward growth of the cell wall material to form a septum and seal off the daughter cell from others. So this is all about the cell division within the prokaryotic system. What we have discussed is that whether it is a prokaryotic system or a eukaryotic system, you are actually going to have

four distinct phases.

One, the cell growth is where the cell is actually going to grow to size. The cell is actually going to grow in size. So if the cell is like this, it is actually going to start synthesizing cytosol. And so all the cytosolic proteins are actually going to be synthesized. And that's how it is actually going to increase in size. Right? And apart from that, the chromosome that is present here is also going to be duplicated.

So in the second step, then in the first step, there will be a synthesis of cytosol. In the second step, there will be a synthesis of DNA. So you have one copy of the chromosome, right? And that one copy is going to be duplicated. And that's how each portion of the cell is actually going to have two cells.

And then it will proceed with the division. So this is cell division. And that's how you are actually going to have the two cells that are going to contain their individual chromosomes. And it is under very tight control. You have control over the ATP level. You are actually going to have control with the help of the different types of protein that are participating in these events. But these events are, you know, the molecular players regulating the cell cycle within the prokaryotic systems.

And the events are also very fast. For example, the chromosome size is very small, and then you are actually going to have multiple original folks that are going to run within the genome, and that's how you are actually going to have faster replication. And that's how we have seen that generation time in prokaryotes is very, very fast. Now, let's move on to the eukaryotic system and see how the eukaryotic system performs similar kinds of events and cell divisions. So in the eukaryotes, the phases of the cell cycle have actually been broadly divided into two events.

One is called as the interface. The other one is called the metaphase or the M phase. Within the interface, you have the G1 or the G0 phase. Then you have the S phase, and then you have the G2 phase. And within the M phase, you are going to have mitosis or meiosis. And these are the specific types of cell division that we are going to discuss in due course.

And then you are also going to have cytokinesis so that it actually divides the cells. So the interface is actually the phase that is going to prepare the cells for the M phase, and the M phase is what is actually going to be used for dividing the cells, right? So let's first discuss the interface. The phases of the cell cycle. So a eukaryotic cell divides into two phases. One is called interface, the other is called the M phase. During the cell cycle, the cell spends approximately 95% of the time in interphase and only one hour in mitosis.

The interface can be divided into discrete phases like G1, S, G2, followed by the M phase. G0 phase, so within the G1 you also have the G0 phase. The cells are metabolically active but are not committed to the devices. One of the classical examples is the neurons or the RBC.

So these are the cells that are actually metabolically very active, but they were in the G0 phase. Then we have the G1 phase, during which proteins involved in DNA replication are expressed and the cell prepares for DNA synthesis.

So, within the G1 phase, you are actually going to have the synthesis of cytosol. And within the cytosol, you are actually going to acquire the machinery that is required for the DNA application. So, you are going to have the synthesis of the DNA polymerases, and you are going to have the synthesis of the nucleotides, and so on. And then this machinery is going to be utilized in the S phase, or the synthesis phase. So DNA replication commences and the chromosomal number is going to be doubled.

So, during the S phase, you are actually going to have the duplication of the DNA. Then you have the G2 phase, or I will say GAP2. The cell continues to grow and expresses the proteins that are involved in mitosis. So remember that the G1 phase is actually required for the synthesis and preparation of the S phase, whereas the G2 phase is required for the preparation of the M phase. Then mitosis is the most dramatic event of the cell cycle, where the separation of the daughter chromosomes takes place, and at the end of the M phase, you are going to have cytokinesis. So this is what it is showing, right? You are actually going to have the G0 phase, you are actually going to have the G1 phase, then the S phase, then the G2 phase, and then the M phase, and within the M phase, you are going to have cytokinesis.

How does the cell cycle begin? So let's understand this by using a model organism, such as Saccharomyces cerevisiae. So these are the simplest unicellular organisms or unicellular eukaryotes, where you can actually understand the different types of diseases and the different processes occurring within the cell cycle. So there are various regulatory points in the cell cycle driven by extracellular signals from the environment as well as internal signals that monitor and coordinate the functions.

In S. cerevisiae, the first regulatory point occurs in the late G1 phase, and it is known as the start. Passing through the start is highly regulated by the availability of nutrients in the environment, mating factors, and the size of the cells. Hence, the start acts as a checkpoint where the cell checks for the availability of the nutrients required to complete the set of stages of the cell cycle. Once it proceeds from the start and enters the S phase, it will become committed to undergo one of the seven cell divisions. So there is a checkpoint before the cell enters the cell cycle, and that is called the start site or start checkpoint. And at the start checkpoint, the cell is actually going to analyze whether there is proper nutrition available, whether there is an environment that is conducive to division or not.

And you remember that these things are very important for unicellular organisms, right? If the environment is not very conducive or is not good, for example, if the temperature is very high and there is a loss of water, then it will decide that, okay, let's conserve these nutrients, and let's conserve these...

And then it will go through those conservative modes. We are also going to discuss these conservative modes. When the cell goes through or experiences these kinds of harsh conditions at the end of this particular module. Budding in the yeast produces progeny cells of different sizes: a large mother cell and a small daughter cell. So what you see here is a large mother cell and small daughter cells. The smaller daughter cell must grow to the size of the greater and the mother cell before it divides.

The start mechanism allowed the cells to reach a minimum size before they can divide. Now, if you talk about the cell cycle regulatory points, you have the regulations by one of the protein complexes, which are called Cdks and cyclins. Then you have regulation by DNA damage, and you also have regulation by different types of growth factors. And within the DNA damage, you can have different types of proteins. So you have the ATM and ATRs, a p53, and the Rb proteins.

Let's first talk about the regulation by the CDKs and cyclins. Cdk and cyclins are a pair of proteins that participate in the regulation throughout the cell cycle. What are cyclin and the Cdk? So cyclins and Cdks are the two pairs of proteins that work together to regulate the cell cycle. So cyclin is a protein that binds the Cdks and causes major conformational changes that render it in an active form. So once the Cdk binds to the cyclin, it will turn into an active protein. A Cdk is known as the cyclin-dependent kinase, right? So Cdk's full form is cyclin-dependent protein kinase, right? So once the Cdk is active, it is actually going to start catalyzing protein phosphorylation.

And that's how it is actually going to, you know, initiate the different types of cascades of reactions. So Cdk is a protein kinase that, after getting activated, phosphorylates different target proteins. About the start protein in yeast. Cdk1, which regulates the progression to the start and also enters into mitosis, although the cyclins involved in both regulatory points are different. So if you see the production of the cyclins, what you see here is the level of cyclin with the different types of stages within the cell cycle. So what you see here is when the cell is under the interface and the mitosis, or from the inter; this is the complete cell cycle.

Right, this is the complete cell cycle. First cycle, this is the second cycle, right? So when it is in the interface and the mitosis, there is always an increase in the level of cyclins and the corresponding Cdks, and that's how it is actually going to regulate the different events of the cell cycle. So, you have the Cdk1, which is in association with the G1 cyclin, and that's what's actually going to bind cyclin 1, cyclin 2, cyclin 3, and that's how it's actually going to allow the cells to pass the start site. And once it passes the start site, it is actually going to be committed to cell division. So, Cdk1 then associates with the different B cyclins, namely Clb5 and Clb6, to regulate the progression to the S phase, and if the G2 to M transition is driven by the CDK1 complex with the mitotic cyclins. Such as Clb1, Clb2, Clb3, and Clb4, and that's how you see that the Cdk-cyclin complexes are actually regulating the different phases of the cell cycle.

Starting from the interface to the G1 phase, then the S phase, and then the G2 phase. In animal cells, the Cdks perform different types of functions, such as the Cdk4-cyclin D complex, which is responsible for the progression from the restriction site to G1. Then you have a Cdk2-cyclin E complex required for the transition from G1 to S phase. And then you have a Cdk2 cycle in A, which functions in the progression of the cycle to the S phase, and then you have a Cdk1 complex with the cycle in A to progress from the S to the G2 phase, and then a complex with the cycle in B to regulate from the G2 to M. So this is exactly what is showing here: you have the Cdk4 when it is making a complex with the cyclin D; then it is actually allowing the passage through the cells from the restriction point.

If Cdk2 is forming a complex with cyclin E, then it is actually allowing passage through the G1 to S checkpoints. So these are the checkpoints you actually have. And then it will enter the S phase. Then you have Cdk2 and cyclin A, which will allow the cell to proceed through the S phase. And then you have a Cdk1 and Cyclin A, which is actually going to allow the cell to progress to the G2 phase, and then it will actually enter into the G2 to M phase, right? And then you have a Cdk1 Cyclin B, which is actually going to help regulate the G2 to M phase. So this is actually the different types of Cdk and cyclins that are going to be essential for regulating the eukaryotic cell cycle, whether it is a single-celled organism such as yeast or multicellular higher eukaryotes.

Then Cdks actually have their own regulatory mechanism, right? So once the Cdks bind their cyclins, they will be activated, right? So they are actually going to start phosphorylating the different types of proteins, but they are also going to have the checkpoints, right? They're also going to have other points and other proteins, which are actually going to regulate their own activity. So Cdk one has three phosphorylation sites, which are threonine-161, tyrosine-15, and threonine-14. So phosphorylation at threonine 161 actually keeps the Cdk1 in the active form. Phosphorylation at Tyr15 and Thr14 by the protein kinase V1 inactivates Cdk1, leading to the accumulation of the cyclin-Cdk1 complex in the cell, and when the cell is ready for the transition from the G2 to M phase, the protein phosphatases such as Cdk50 remove the phosphate group from Tyr15 and Thr14, which then form the actin-cyclin Cdk1-cyclin complexes.

So basically, the Cdk, when it forms the complex with the cyclin, gets into the active form. And that's how it started to phosphorylate the downstream substrates. When cycling within the Cdk, you have three sites: one is T161, one is Y15, and one is T14.

Right. So this one is actually a site that is required for activation. Right. So if there will be a phosphorylation of this site, it is going to get activated. But if phosphorylation is occurring at this site, then it is actually going to have a deactivation effect.

Right? So this is exactly what is going to happen. Right. So Cdk has three sites. One is Thr161. The other two are Tyr14 and Thr14. Right. So when will there be a phosphorylation

of Thr161, right? And when there is phosphorylation of Tyr 15, then this phosphorylation of the cyclin, Cdk cyclin complex, right? So, for example, from here, it is actually going to be that there will be a dephosphorylation, and because of this dephosphorylation, only the CDK1 and cyclin complex, where the phosphorylation is at the Thr161, will be in its active form, and that's how the cell will enter the mitotic phase. And slowly, cyclin B is going to be degraded, and then Cdk1 is actually going to be available.

But this Cdk1 is still active because 161 is phosphorylated. So, what will happen then? There will be a dephosphorylation, and the phosphate group from the 161 is going to be removed. And then there will be a reassociation of the new molecule of cyclin, and that's how it is going to form this complex, and then there will be a phosphorylation; because of that, there will be a phosphorylation of these three molecules. Then we have the different modes of Cdk regulation.

So you can actually have Cdk regulation through three different modes. One is associated with the cyclins. Then you can also have the association with the Cdk inhibitors or CKIs. Then you can also have the inhibitory phosphorylation of Thr-14 and Tyr-15. And then also going to have the activation by the phosphorylation of threonine-161.

So you can basically have different modes of Cdk regulations. One is the protein interactions, the second is the phosphorylation. So if there is phosphorylation of 161, then it will actually activate the Cdks. If there is a phosphorylation of Thr 14 or Tyr 15, then there will be an inhibitory effect. So now what we have understood is that the cell divides when there is a need, like in the case of an injury or to replace old cells. Do you know how these processes are induced? So when there is a loss of cells, the body secretes some signaling factors such as growth factors, which induce cells to enter the cell cycle to replace the lost cells.

And these are some of the growth factors, such as EPDGF, FGF, PDGF, TGF beta, and NGF. So, growth factor, what are these growth factors doing? Both factors are stimulating the various signaling pathways, such as Ras/Raf and MEK/ERK, to induce the expression of cyclin D1. So they are actually inducing the expression of different types of cyclins, including cyclin D1, and then you have cyclin B and all that. And then CDK4 and CDK6 complex with cyclin D1, allowing the cells to pass through the quiescent stage, which is the G0 phase, and the restriction point to enter the G1 phase. Cyclin D1 is readily degraded in the absence of the growth factor to check cell division. Initially, we have seen that cell division may also cause cancer, but under normal conditions such a thing doesn't happen because there are some cellular proteins that play an important role in preventing the progression of controlled cell division into uncontrolled cell division.

So it is crucial that the cell does not enter into mitosis until the replication of the genome has been completed. So until the S phase has not been completed, the cell will not enter the M phase. Several cell cycle checkpoints and DNA damage checkpoints function to ensure

that the DNA damage is not replicated and will not be passed on to the daughter cells. This means it actually ensures that no mutated or damaged DNA is passed on to the daughter cell so that the daughter cell does not replicate or harbor these damaged nucleic acids from the parent. And that's why it is actually going to have a higher probability that they will develop into uncontrolled cell division, which will lead to the production of cancer cells. There is also some sense in the damage or incomplete replication of DNA to prevent the cell cycle progression before the DNA gets repaired or replication gets completed.

DNA damage checkpoints, such as the G1, S, and G2 phases of the cell cycle, are important. Failure of the DNA damage checkpoint will lead to the accumulation of mutations in the progeny of the cells and increase the probability of developing cancers. How does the checkpoint respond to DNA damage and unchecked unreplicated DNA? So, how are these kinds of methods ensured within the cell, or will they be present within the cell to keep the cells in an unproliferated state or actually keep the cell cycle in check? Cell cycle arrest occurs upon DNA damage. So if there is DNA damage, this DNA damage could happen because of many different types of events. All these we are going to discuss when we discuss the DNA damage and the repair mechanisms.

So you are actually going to have the two important proteins. One is called the ATM. The other one is called the ATR. And there are two crucial proteins that actually play an important role in the cell cycle arrest. Upon sensing DNA damage, it is very important to ensure the complete replication of DNA; if there is DNA damage, that portion will not be replicated. And that's how the daughter cell will not have the information of that damaged DNA. So if you have damaged DNA, and the parents are actually having the damaged DNA, it is better to first take care of that damaged DNA, replicate that damaged DNA, repair the damaged DNA, and then proceed with cell division. So, basically, these proteins, the ATM or the ATRs, are ensuring that the DNA damage is sensed, is repaired, and then you proceed to cell division.

So ATM and the ATRs are the protein kinases that then activate when there is unreplicated or damaged DNA. When there are any double standard breaks in DNA, ATM is activated and phosphorylates the CHK2 protein or the checkpoint kinase. Whereas upon unreplicated DNA, ATR gets activated and phosphorylates the CHK1 protein kinase. CHK2 and CHK1 phosphorylate Cdk25, Cdc25, and Cdc35. Upon phosphorylation, the phosphate activity of both proteins is inhibited. As we have seen before, both phosphatases are required for the activation of Cdk2 and Cdk1, and they are involved in removing the inhibitory phosphate group from the Cdk.

Its inhibition leads to cell cycle arrest at the G1 to S phase or the G2 phase. And because of that, it is actually going to help the cell get enough time to repair the damaged DNA, or it will give enough time for the cell to complete the DNA duplication. And that's how it is going to help the daughter cells have complete information about the genetic information present in their parents. So there are two different proteins that participate in this. So Rb protein is a

tumor suppressor that plays a crucial role in the negative feedback mechanism of cell division and is often mutated into different types of cancer.

Rb checks the DNA damage before the cell proceeds into the S phase to prevent cell division. In humans, the Rb is encoded by the RB1 gene on chromosome 13. Mutations in both alleles of the Rb gene in retinal cells lead to the uncontrolled division of the cells and the development of retinoblastoma. Hence, the name Rb is given. The activity of the Rb protein is regulated by changes in the phosphorylation state.

Rb binds to a member of the E2F family of transcription factors, which regulate the expression of several genes involved in cell cycle progression. In the unphosphorylated state, Rb suppresses the activity of the E2F transcription factor, and hence the gene expression mediated by E2F is going to be regulated. The phosphorylation of the Rb by the Cdk4, 6, and cyclin D complex results in the dissociation of the Rb from the E2F and hence the activation of the expression mediated by the E2B transcription factor. Rb, or the retinoblastoma protein, is very important because this is the protein that is being isolated from the eyes and is responsible for the development of cancer, and that's how the name Rb is given to this particular protein. And what the Rb protein is doing is actually ensuring that no damaged DNA is passed on to the daughter cells. And when there is a mutation in the Rb protein, they cannot actually function properly, and that's how they are responsible for allowing the uncontrolled division of cells, irrespective of whether the DNA is damaged or DNA replication is not complete.

And that's how it is actually going to allow the production and development of the different types of cancers. P53 is another mutated protein in cancer and is commonly known as the guardian of the genome. In mammalian cells, cell cycle arrest upon DNA damage is also mediated by the action of the protein P53. P53 is another tumor suppressor gene that gets phosphorylated by both ATM and checkpoint 2 in response to DNA damage. Upon phosphorylation, the P53 is stabilized, which would otherwise be rapidly degraded, resulting in an increased P53 level. The P53 is a transcription factor, and the increase of its level induces the expression of the Cdk2 inhibitor P52, which inhibits the Cdk-cyclin E complex or the cyclin A complex.

Inhibition of the Cdk cyclin complex arrests the cell cycle at the G1 phase, and the inhibition of the Cdk2 cyclin A complex arrests the cell cycle at the F phase. So basically, when there is a double-strand DNA break, the ATM will actually recognize these breaks, and that's how it will phosphorylate p53; when p53 is phosphorylated, it will bind to the damaged DNA. And that's how it is actually going to increase the level of the Cdk inhibitor P21, which inhibits the complex of Cdk2 with cyclin E or cyclin A. And that's how it is actually going to arrest the cell cycle at the G1 phase or the S phase. And that's how it is actually going to allow the cells to repair these double-stranded breaks, and that's how it's actually going to ensure that the daughter cells receive the complete genomic DNA from the parent cells.

So this is all about what we have discussed in terms of cell division and growth. What are the different types of events happening, and what are the different ways in which the prokaryotic system or the eukaryotic system is regulating the cell cycle? And what we have discussed is that the cell cycle is a very, very complex event.

Right. It requires cell growth. It requires the duplication of chromosomes. It requires the distribution of chromosomes. And lastly, it requires cell division. Right. And all these four fundamental events are occurring differently in the prokaryotic system and the eukaryotic system. Even the machinery required for regulating these events is very different. But the purpose of the checkpoints and the purpose of the regulatory protein is to ensure that DNA is replicated completely from the parents so that the complete genomic information is passed down to the daughter cells.

And in this context, we have discussed the Cdks and cyclins. We have discussed the DNA pair and the checkpoints. And we have discussed the role of the Rb protein as well as p53. So, with this, I would like to conclude my lecture here. In our subsequent lecture, we will discuss additional aspects related to cell division. Thank you.