Cell and Molecular Biology
Prof. Vishal Trivedi
Department of Biosciences and Bioengineering
Indian Institute of Technology, Guwahati
Week 03
Cellular Homeostasis
Lecture - 13
Mitosis and Meiosis

Hello, everyone. This is Dr. Vishal Trivedi from the Department of Biosciences and Bioengineering, IIT Guwahati. And in this particular module, we are discussing cell growth and cell division. So for what we have discussed, we have talked about the importance of growth and how growth is regulated within the prokaryotic system or the eukaryotic system. In this context, we have discussed the cell checkpoints and how cell growth is regulated by following the different types of stages.

And each stage of cell growth is going to be regulated by cellular machinery or a set of proteins. Subsequent to that, in the previous lecture, we also discussed the different types of events, what is happening within cell growth, and how you can provide nutrition to the cellular system. So we have discussed the preparation of the media for the prokaryotic system, and then we also discussed the media for the eukaryotic system. Subsequent to that, we have also discussed the different types of methods for monitoring the growth of prokaryotic cells as well as eukaryotic cells; within the eukaryotic cells, we have discussed the monitoring of growth for animal cells as well as for plant cells.

So now it is clear that growth is very important for the longevity and survival of living organisms. And the growth is being divided into different stages. Right. And all these stages are part of the cell cycle, right? So when a cell is growing through the different stages of the cell cycle, it actually follows a very regulated manner. And that's how it is actually going to be accomplished.

The purpose of cell division is to ensure that it does not give rise to abnormal cells or any kind of abnormality. So when you talk about the cell cycle in the eukaryotic system, the cells undergo different stages. And these different stages are being regulated by the cyclins, CDKs, and all those different molecules that we have discussed in this particular course. So in today's lecture, we are going to discuss the cell cycle and how it can be studied within the biological system, as well as how you can study the different stages of the cell cycle. Now, talking about the cell cycle, you can actually have, you know, within the M phase, the different distinct processes that are happening within the M phase.

So actually, the mitotic phase is using four different events or processes through which it divides the cells or the nuclear content between the different cells. So after the G2 phase, each cell enters into mitosis and divides the cell equally between the two daughter cells. Each mitosis has four distinct phases to precisely divide the DNA content of the cell. One is called prophase; the first phase is called prophase. During this phase, the nuclear membrane is dissolved and the chromatin condenses into chromosomes.

The nucleolus in the nucleus disappears. In the beginning, the yeast cell has one centromere which replicates along with the DNA to give rise to a pair of centromeres to coordinate the downstream events. Each centrosome has microtubules to form the spindle and assist in the distribution of nuclear content during mitosis. Centrioles are considered to organize microtubule assembly, but they are not essential. So this is what is different stages of mitosis sort of being shown.

So this is a very classical experiment. So what you can actually do with the onion leaves are those kinds of experiments. So once the interprophase is done, the cells will enter metaphase. And in this phase, the two centromeres start pulling the chromosome using the attached chromatids towards the end of the cell. As a result, the centromeres are aligned along the metaphase plate or equatorial plane.

Since the pulling power of both centromeres is almost equal, they eventually arranged the chromosome on the metaphase plate. The alignment of the chromosomes along with the metaphase plate is a crucial event in deciding the entry of cells into anaphase. The signal required for this control is created by the mitotic spindle checkpoint. So this is exactly what is going to happen. Cells are going to enter prophase, then they are actually going to enter metaphase.

After metaphase, it is going to enter anaphase. The protein attached to each chromatid is cleaved and the sister chromatids are segregated as the daughter chromosomes. The chromosomes lined up on the metaphase plate are pulled by the microtubules and move towards their respective centrosomes. Although the exact mechanism of generating forces required for centrosome movement is unknown, it is suggested that a reactive assembly and breakdown of microtubules may provide the force for this movement. At the end of this phase, the chromosomes are prepared for distribution among the data cells.

So this is exactly what is going to happen, and then the cell is going to enter telophase. In this phase, the daughter chromosomes move and attach to the opposite end of the cell. A nuclear membrane forms around each set of separated chromosomes, and the nuclei disappear. And in this event, several processes that occur during prophase are reversed to give the two daughter nuclei. So this is exactly what is going to happen.

You are going to have two daughter nuclei at the end of telophase. And then it is going to undergo cytokinesis. So at the end of telophase, mitosis is over, but cell division requires the distribution of cellular content equally between the daughter cells. In this animal cell, a cleavage furrow is formed along the metaphase plate. So it's actually going to form in the metaphase area, right? So that the cells divide and have an equal amount of cytosol, the individual nuclei are divided as separate cells; during this process, it is ensured that, besides the nuclei, all other cellular organelles are distributed equally between the daughter cells.

In plant cells, the cell plate is formed and divides the cellular content between the daughter cells, so this is the process that is happening within mitosis and what the

purpose of mitosis is. The purpose of mitosis is to increase the size of an organism, replace worn-out cells or repair damaged tissues, and reproduce identical organs or clones. So how are you going to study mitosis? So remember that when I was showing this site, we were actually going to do an experiment where you would be able to see all the phases of mitosis. Right. So, for that, we are going to take you to my lab where the students are actually going to show you the experiment.

So, to conduct these experiments, you actually require different types of materials, and you are also going to make different types of preparations. To conduct the experiments, what you require is the apical region of the root tip. Because this is, you know, fast-dividing plant material. You can take any other material where, you know, mitosis is happening. Then you can acquire the flower bud for the meiotic studies.

Then you require the blotting papers, glass slides, and hydroxyquinoline, which is actually going to be for staining. Then you require acetic acid, chloroform, methanol, HCl, orcein. Absolute alcohol, Carnoy's solution, and so on. And then you require the aceto-orcein stain, Eppendorf tubes, sharp blades, watch glasses, and a compound microscope so that you can observe the stained sample, and you also require refrigerators. First, you are going to prepare staining solutions so you can actually prepare the 2% aceto-orceine stain by adding the orceine powder to boiling 45% acetic acid, followed by storing it in an amber glass bottle.

It requires a Carnoy's solution, so you can mix absolute alcohol, chloroform, and glacial acetic acid in a 6:3:1 ratio and store it in the agent bottle for further use. How are you going to perform and study the mitosis? So you can prepare these staining solutions correctly, and then you'll have the methods to take the root tips from the healthy plants in the experimental garden that were collected in the morning. So it is important that you collect it in the morning so that all the cells will be under the active division stage; then you excise the root tips, wash them under tap water to remove the soil particles, take the apical region of the root tip, and cut it into the Eppendorf containing 0.02% of 8-hydroxyquinoline at 4ºC for 4 hours inside the refrigerator for pre-treatment. Thereafter, the pre-treated samples were fixed in a modified Carnoy's solution containing absolute alcohol, chloroform, glacial acetic acid, and methanol for 48 hours.

Thereafter, the fixed root tips were transferred to a mixture of 9 drops of 2% aceto-orcein and 1 drop of 1N HCl in a watch glass and heated gently. After the root tip was placed in a drop of fresh aceto-orcein on a glass slide, the root tips were then squashed and mounted by putting a cover slip on the sample and applying gradual pressure with the thumb in order to spread out the cells and observe them under the microscope. And then you can take the images under the different types of magnifications, like 10x, 30x, 40x, or 100x, and then you will be able to count the chromosomes. What precautions do you have to take? So the collection of the plant material should be performed on a bright sunny day to ensure the proper mitotic stages of development. Although collection time may vary between the plants, cloudy, rainy days should be avoided for material collection.

And then the freshly filtered solutions of aceto-orcein should be used during the preparation of the slide. So we have prepared a small demo clip so that you can understand each and every step. And for that, I am going to take you to my lab where the students are actually going to, you know, explain each and every step to you. And then they are also going to perform the study of mitosis in the plant material. In this tutorial, I am going to demonstrate the practical aspects of mitosis cell division to you.

In this method, we will explain to you each and every detail of mitosis. Starting from sample preparation to microscopic observation, we will explain each and every detail to you. In today's demonstration, somatic chromosomes will be studied from onion root tips for mitosis, and flower buds will be used for meiosis studies. Various chemicals and materials will be required, such as 0.02% 8-hydroxyquinoline, Carnoy's solution, glacial acetic acid 45%, 1 N HCl, 2% aceto-orcein, wash glass, cover slip, cover glass, burner, glass beaker, pipettes, various sizes of forceps, blades, filter papers, blotting sheets, and Eppendorf.

Root tips from healthy onion plants were collected at 9 am in the morning. Initially, the apical region of the root tips was cut using a sharp blade. Two to three pieces of root tips, each 1 cm in length, were selected. 8-hydroxyquinoline 0.02% was previously prepared and kept in an amber glass bottle.

1 ml of 8 HQ previously kept in Eppendorf tubes was used, and those cut root tips were kept inside that Eppendorf tube. After that, this Eppendorf tube will be stored in the refrigerator at 4ºC for 4 hours. This whole process is called pretreatment. After 4 hours of pretreatment at a temperature of 4ºC, the 8-hydroxyquinoline has been discarded. Therefore, the pre-treated root tips will now be fixed in Carnoy's solution.

Carnoy's solution contains absolute alcohol, chloroform, and glacial acetic acid in a 6:3:1 ratio. The Eppendorf tube containing 1 ml of Carnoy's solution with pre-treated root tips will now be stored at room temperature for 48 hours. After fixation in Carnoy's solution for 48 hours, the solution has been removed. Therefore, the fixed root tips have been transferred to a watch glass, followed by the addition of 1N HCl (1 drop) and 2% aceto-Orcein. The mixture of aceto-Orcein and hydrochloric acid has been heated gently over the burner.

This process is called staining. Now the stained root tips have been placed over the glass slide. The lower portion of the root tips has been removed by a sharp blade. Only the meristematic region has been taken for analysis. Root tips were then squashed and mounted with a cover slip. This process is very careful to prevent the entering of air bubbles.

Now the sample is covered by the filter paper, and gradual pressure has been applied with fingers in order to spread out the cells. Now the slide is ready for observation under the microscope. Now the slide has been placed under the microscope. They were taken with a Carl Zeiss microscope having 10x, 20x, 40x, and 60x objective lenses. We can see here from the microscopic field that the chromosomes of onion root tip cells are clearly

visible, along with cell divisional stages like anaphase and telophase.

In this method, we have explained each and every step of mitosis, starting from sample preparation to the microscopic observations. Hope this video will help you prepare the slide of any plant sample for the study of mitosis. Thank you for listening. So within the M phase, some cells are actually undergoing mitosis, while others are undergoing meiosis. And we know that meiosis is a reduction stage.

So it happens only in the cells that are actually undergoing sexual reproduction. Why it is important to do the meiosis that we are going to discuss in the next couple of slides. So in sexual reproduction, you know that each organism has one pair of chromosomes, right? So, one pair of chromosomes, right? So, each organism has one pair of chromosomes, right? Which means it actually has two copies of each chromosome, correct? And if it wants to, you know, divide with sexual reproduction, it actually has to divide these two copies into one plus one. Right. So that each copy, right, can be given to one of the offspring.

Right. Because in sexual reproduction, you are actually going to have two individuals. Right. So, for example, suppose this is a male. Right? You're also going to have a female. Right? And the female is also going to have the two copies.

Right. And she also has to divide that into two. Right. So one plus one. Now, when the male and female gametes are going to fuse, then they will again come back and give you a 2. If there are no reduction steps not performed, then the ploidy of the cells is going to be increased. So imagine that you are not undergoing meiotic division.

Then what will happen? The female is going to produce the gamete, which is also going to have the two pairs of chromosomes. Male is also going to do the same, and it is also going to produce the two numbers. Right. And when they fuse, the ploidy will go up and it will become 4n.

Right. And after the 4n, in the next generation, it is going to be 8n. And next to that, it is going to be 16n and so on. So that is actually going to destroy or disturb cellular physiology. Right. So what you want to do is change this 2n into 1 plus 1.

And that's how these organisms can actually return by mating, and that's how you are actually going to maintain the ploidy of those particular organisms. This is all achieved with the help of meiosis. So in sexual reproduction, each organism must inherit a single copy of every gene from each of its parents, right? And each of these organisms' gametes must contain one set of genes. So that is what we have explained here already. And when the gametes are formed, there will be a process that separates the two sets of genes.

So each gamete gets one set actually. And this is happening because you are actually going to have the homologous chromosomes. So, a chromosome that has a corresponding chromosome from the opposite sex is present in the parents. For example, the fruit fly has eight chromosomes: four from the female and four from the male, right? And that's why

this particular type of stage is called the diploid stage. So what is the diploid stage? The diploid stage is where you are actually going to have one pair of chromosomes, right? So diploid means two sets of chromosomes, right? And cells that contain both sets are homologous chromosomes, and cells contain two complete sets of chromosomes, right? Two complete sets of genes, and the number of chromosomes in a diploid cell is represented by 2N. And for example, in Drosophila, 2N is 8, which means it is actually receiving 4 from the female side and 4 from the male side.

And so, how are the haploid gametes made from the diploid cells? That is done by the process of meiosis. So, the process of reduction division is when the number of chromosomes per cell is cut in half due to the separation of the homologous chromosomes in a diploid cell. So you have the two distinct stages of meiosis. You have meiosis I, where you are actually going to do the reduction division, and then you are going to have meiosis II. So a diploid cell enters here, and then you are actually going to have meiosis II, where the diploid cell that entered through meiosis has become 4 haploid cells, right? So basically, in meiosis I, a diploid cell will enter, right? And there will be a reduction division, which will actually form haploid cells, right? And then two haploid cells are going to give you the two haploid cells, and then these two haploid cells will enter into meiosis II, and that's how they are actually going to go through with the further division, and that's why they are going to give you the four haploid cells.

So let's first discuss meiosis I. So, before meiosis I, each chromosome is going to replicate. Right, and then they divide like in mitosis. So what happens in mitosis? You are actually going to have all these events that we have already discussed.

Right. So you're going to have the PMAT. You are going to have tetrads. And then tetrads are the structure made when each chromosome pairs up with its homologous chromosome. So this has already been discussed when it is going to happen in the prophase. And then you're going to have the four chromatids, right? And so on. In prophase one, each chromosome pairs with the homologous chromosome, making a tetrad, right? Then, as they pair up in tetrads, the chromosomes exchange portions of their chromatids in a process called crossing over.

So in crossing over, what happened is that suppose these are a pair of homologous chromosomes. You see that they have different types of gene sites. You have A, B, and C. Here, you also have A, B, and C. And then, when they come for, you know, pairing, they are actually going to have the crossing over.

And because of the crossing over, some portion of one chromosome is going to be exchanged with the homologous chromosome, and that's how at the end of the crossing over you are actually going to have four different types of chromosomes; earlier you had pure chromosomes: A, B, C, and ABC. Now, the homologous chromosomes will come together and actually undergo crossing over. And because of that, you are going to have hybrid chromosomes where the material is being exchanged. So this is going to be the original chromosome, and this is going to be the crossing-over chromosomes.

And then it will enter metaphase one. So within metaphase one, you are actually going to have the spindle fibers, which are going to attach to the chromosomes. And then it is going to enter anaphase. And within anaphase, the spindle pulls the homologous chromosomes apart to opposite poles. And because of that, each chromosome is going to be divided into two, correct? And then it will enter telophase.

And within telophase, the nuclear membrane is going to be formed. And the cell is going to divide into two new cells. And these two new cells are going to be haploid cells. So this is exactly what is going to happen. In prophase 1, it is going to start using one pair of chromosomes. And within that, it is actually going to make pairs of homologous chromosomes.

Then in metaphase, it is going to be arranged on a metaphase plate. And then in anaphase, each chromosome is going to be separated. So homologous chromosomes are going to be pulled in opposite directions. And because of that, it is going to be divided into two halves. And then in anaphase, the nuclear membrane is going to be formed between the two genomic DNAs or the two individuals. And that's how it is; within the telophase, you are going to have the two nuclei containing half of the chromatin or half of the genomic DNA, and that's why you're going to have the two cells: one is at one end, and the other one is also at one end.

Right, you started with the two ends, and now it is actually going to be one end. Now these cells will enter into meiosis II. But before that, what will happen next? So you are actually going to have two new daughter cells. Are they identical to their parents? They are not identical to the parent.

They are due to the crossing over and the other kinds of parents. Because the parents actually have four chromosomes, whereas these cells are going to have two chromosomes, right? So each daughter cell only has two chromosomes because the parents are 2N, correct? Whereas these cells actually have the N, each daughter cell has a set of chromosomes that is at least different from each other and different from the parents because of crossing over as well. So now the cells will enter meiosis II. And within meiosis two, unlike meiosis one, it is not going to be a reduction division. It is actually going to just follow just like mitosis.

So neither cell goes through a round of chromosomal applications. And each cell's chromosome has two chromatids, right? So each chromatid and then the meiosis results in two haploid cells, each with half the number of chromosomes in the original cell. And then it is going to enter metaphase II, and within metaphase II, you are going to have the chromosomes lined up in the middle. Then, in anaphase, the cells are going to separate; the sister chromatids are going to separate and move to the opposite poles. In telophase, meiosis II results in four haploid daughter cells. And four haploid daughter cells containing the haploid number of chromosomes, just two each, right? So this is exactly what happens in the case of prophase, right? So at the end of prophase two, you are actually going to prepare both of these cells, right? So this is daughter cell number one, and this is daughter cell number two, right? They both will get into prophase II, and then

they will form metaphase.

Within metaphase, the chromosomes are going to be arranged on the metaphase plate, and then they will be distributed toward the poles during anaphase. At the end of telophase, both of these cells will actually form separate nuclei. After this, you will have four daughter cells. So, daughter cell number one, daughter cell number two, daughter cell number three, and daughter cell number four. And each of these daughter cells is actually going to have only one set of chromosomes, which means that their ploidy is going to be N instead of 2N.

So in meiosis II, you are not going to have the reduction division because it is just going to take the same cells and give you the duplications. So how are you going to study meiosis? So you can actually request the different types of materials. You're actually required to prepare this kind of dye, right? And all of these, I think we have already discussed when we were discussing meiosis and mitosis, right? A similar kind of material is required, except that you need the flower buds for the meiotic study, and for mitosis, you need the root tips. And you can actually prepare all these dyes.

So first, you are going to prepare the mitosis chromosome preparation. You can take the flower buds of different developmental stages, and you can collect them from the fields during the morning and during the flowering season. Thereafter, your sepal and petal will be removed from the selected brand, and anthers will be fixed in Carnoy's solution containing absolute alcohol. Then the anthers were directly smeared into a drop of 2% aceto-orcein stain; therefore, the slide was slightly warmed by quickly passing it through the flame. Finally, the prepared slide was wrapped in a fold of filter paper to remove the extra stain, and then you can observe these cells under the microscope and collect the images at 10x, 40x, and so on, and that's how you are going to obtain the meiosis cells. What precautions do you have to take? So you have to ensure that you collect fresh material and prepare all the reagents that are fresh.

So we have prepared a small demo clip on how you can study meiosis in the flower buds. And I hope the students will actually be able to explain all the processes to you in detail. We are going to explain the meiotic cell division process. Starting from sample preparation to microscopic observation. We will explain each and every step to you in detail.

Flower buds of onions will be used for a meiosis study. Initially, flower buds were collected during the flowering season in the morning between 11 and 11:30 am and have been fixed in Carnoy's solution containing absolute alcohol, chloroform, and glacial acetic acid in a 6:3:1 ratio. Those fixed samples have been placed over a watch glass, and a single flower bud has been selected for the smear process. Flower buds sized 1 mm in length have been taken and placed on the surface of a glass slide. After that, sepals and petals were initially removed from the selected flower buds.

Therefore, the anthers were removed. Single, isolated anthers are clearly visible on the surface of the glass slide. One drop of 2% acetorcein has been given over the anther.

With the help of an iron needle, the anthers were ruptured, and pollen mother cells were released as very gentle pressures were applied over the anthers. This process is called the smear technique. Now, the anther walls have been removed so that we can observe the various stages of pollen mother cells that are undergoing meiosis.

After that, one cover slip has been placed over the sample with the help of a pointed iron needle. This process needs extra precautions to prevent the entry of any air bubbles between the slide and cover slips. We can use blotting sheets or filter paper to remove excess stain. Now the slide is ready for microscopic observation.

After that, the slide was placed under microscopes to capture photomicrographs. Different objective lenses have been used, such as 10x, 20x, 40x, and 60x, for capturing the various stages of meiotic cell division. Here, we can see some earlier stages of pollen mother cells. In this image, we can see that after the second meiotic division, two cell stages are formed, but four distinct nuclei have been reached in two different poles. In this process, we have explained each and every step of the meiosis cell division process, starting from sample preparation to the microscopic observations.

Hope these videos will help you prepare the slides from any flower buds in your plant sample. Now, what is the difference between mitosis and meiosis? Right. So let's talk about mitosis. Mitosis is going to happen in the somatic cells. If you cannot recall the somatic cells, all the cells, you know, all the cells present in the body except the cells that are present within the gonads are called somatic cells.

So all other cells that are present in the body are going to be a part of somatic cells. Then the results in the production of two genetically identical diploid cells, which means it is actually going to produce the cells by conserving, you know, the diploidy levels. Then the daughter cells will have one set of chromosomes identical to each other and to the parent cells. So daughter cells and parent cells are not different. Then mitosis allows the body to grow and replace other cells, so mitosis is actually the cell division that is required when you sustain an injury or encounter any kind of tissue problem.

When cells age, they are replaced; mitosis is the event that will occur. And then it is actually a part of asexual reproduction. Now talking about meiosis, it is going to happen in the sex cells, which means it is going to happen in the gonads. And at the end of meiosis, you are actually going to produce the germ cells, which are called sperm and ovum. So sperm is produced in males and the ovum is produced in females.

Then it results in the generation of four genetically haploid cells. So it is actually going to change the polarity of the cells like the daughter cells. And because of that, the daughter cells are not going to be the same as the parents, right? The parents are diploid, whereas the daughter cells are actually haploid. Meiosis is where the sexually reproducing organism makes the gametes. So, with this, I would like to conclude my lecture here. Thank you.