Cell and Molecular Biology
Prof. Vishal Trivedi
Department of Biosciences and Bioengineering
Indian Institute of Technology, Guwahati
Week 03
Cellular Homeostasis
Lecture - 11
Cell Growth Monitoring

Hello, everyone. This is Dr. Vishal Trivedi from the Department of Biosciences and Bioengineering, IIT Guwahati. And in the course Cell and Molecular Biology, we are discussing the different aspects of the molecules, how they interact with each other, and how these interactions result in the different types of processes. In this context, so far, we have discussed the origin of life, the evolution, and the cellular structures, whether they are prokaryotic or eukaryotic. And then in the previous lecture, we discussed why growth is very important for the different types of organisms.

So, we have discussed that growth and reproduction are the fundamental aspects of any living organism. So, there are distinct properties of a living organism. It should be responsive to the different types of stimuli. It should be, you know, very complex in its organization.

The most important property of a living organism is that it should be able to have growth potential, and this growth potential should be endogenous rather than exogenous; it should also be able to reproduce and give rise to offspring. Now, in the previous lecture, we discussed the growth, how the different phases of growth are present in both the prokaryotic and eukaryotic systems. What are the different places where it can be controlled so that you can have growth in very, very tightly controlled processes? We have discussed how the growth is being controlled at different stages in the prokaryotic system and what different cellular machinery is required for controlling cell division within the prokaryotic system. And then we also discuss the eukaryotic system, and we have discussed the different stages where growth can be controlled. Now, when you talk about growth, it is very, very important.

And if you recall from the previous lecture, we discussed that for growth, energy is very, very important. And because the energy is required in terms of, driving so many interactions, whether it is being done by the ATP and ATG complexes and all that. So when you talk about growth, the growth requirements are very different. We have discussed prokaryotic cells, eukaryotic cells, and within the eukaryotic cells, we have also discussed animal cells, plant cells, fungi, yeast, and so on. So when you talk about growth, every organism's requirement for growth is very, very different.

So once you are done with the media, you are actually going to use this media for growing the cells, right? These cells could be prokaryotic in nature, or they could be eukaryotic in nature. According to that, you are going to use the different growth monitoring methods, how you can use them for monitoring the growth of these cells, how they are actually increasing their numbers, and all that. So when we talk about bacteria, you can actually use different types of methods. So you can actually use the Petroff-Hausser counting chamber. And you can actually use the direct microscopy method where you are going to use the Petroff-Hausser counting chambers.

So the Petroff-Counting chamber is a thick glass microscopic slide with a ruled surface of 0.02 mm depth and 0.02 mm by square in volume. So to determine the number of cells, what you are going to do is put the cells here, and then you are going to cover them with the cover slips, right? So you are going to put the cover slips like this, and then you are going to count the chambers, right? And the number of cells per ml, you're going to use the number of cells counted in 16 squares. So you see here we have the 16 squares: one, two, three, four, and like this; and three, one, two, four, like this.

So if you count the number of bacteria in 16 squares, then you can actually multiply that by the dilution factor and then multiply by 50,000. And that will give you the number of bacteria present per mL. This method is rapid, simple, and easy, with minimal equipment requirements. What equipment do you require? You require a microscope, right? Whether it is an inverted microscope or an upright microscope, it doesn't matter. And you require this particular counting chamber? Then, these are error-prone but difficult to achieve precision, so basically this method requires a lot of, you know, a lot of experience, actually, to identify the bacteria and, you know, to count them, actually.

Then the second method is the turbidometric method, so the turbidometric method is actually going to use the spectrophotometer. So cells scatter light and therefore look cloudy and turbid, right? So that is the basic principle of turbidometric methods because they actually exhibit the Tyndall effect. So, a spectrophotometer is used to measure the turbidity, or I would say the optical density, of the suspended culture incubate, which gives an estimation of the cell mass. So, if you use the Beers-Lambert law, which states that A is equal to εcl, you can use that to calculate the concentrations. Where you can actually use the formula $a = \ln(Io/I)$, where Io is the incident light, I is the output light, c is the concentration of the solution, or I will say the concentration of the bacteria, and then l is the width of the cuvette.

So all these components are constant except for the concentration of the bacteria, so if you input that, you are actually going to get the concentration of bacteria. Then you could also use the serial dilution method and spread plating. So in this condition, what you are

going to do is actually perform the serial dilution. So, in this technique, the parent microbiology culture is first serially diluted. So 1:10, 1:100, 1:1000, 10,000, or 1:100000.

So what you're going to do is take the parent culture, the first parent culture; you take 1 ml and put it into 10 ml. So that has become a 1:10 dilution. And then you take the small amount and plate it onto a dish, right? And then it will give you the number of colonies. The number of colonies could be very large, couldn't it? So similarly, you do all this plating in different media, right? Different culture plates and in some of the plates, the number of counts or the number of colonies is going to be very, very low. For example, if you have 100 colonies here, if you have 1,000 colonies here, if you have 10,000 colonies here, if you have 1 lakh colonies here, and if you have 10 lakh colonies here.

So, ten lakh colonies are very difficult to count. One lakh colonies are also very difficult to count. 10,000 is also very difficult to count. But if you count 1,000 or 100, 100 is a very small number, and 1,000 is also a reasonable number. So if you precisely count the 1,000 cells, then you will be able to go back and calculate the number of bacteria in this 1 ml.

And that's how you can calculate the number of bacteria per ml. This is a lengthy process; it is a time-consuming process, but it is very precise and quantitatively very good, actually. So 0.1 ml from each dilution is spread onto neutral media and incubated overnight. Each bacterium gives rise to one colony, which can be seen on the agar plate the following day; hence, the colony has been counted from one bacterium.

The number of bacteria was then multiplied by the dilution factor to determine the bacterial count per ml of the original culture. You can also use the dry and wet weights, so this is actually a method where you are roughly going to calculate, right? This way, you can actually pellet down the bacteria, and then you can count them or dry them and count them, so the cell density can be quantified by the two methods: dry weight or weight per liter of sample for wet weight. The culture media is first centrifuged to pellet the cells. Carefully discard the supernatant and scrape the cell paste into a weigh pan and measure the weight. Similarly, for the dry weight, you dry the cell paste in an oven at 100 degrees and measure the weight periodically until there is no further decrease in the dry weight.

These two are actually rough estimates; they are being used for comparison. So when you are treating a particular cellular population for different types of observations, that is the only time you can use the wet weight or the dry weight. So this is all about how you can measure the growth or how you can monitor the growth of prokaryotic cells. Then we'll talk about mammalian cells. So within mammalian cells, we have two different

types of mammalian cells.

We have plant cells; we have animal cells. Let's first talk about monitoring the growth of animal cells. So the first method is that you are going to use the inverted microscopes. So in the animal cell culture lab, inverted microscopes are routinely used to monitor cell growth and health. The cell culture in T flasks is directly placed under the objective.

So remember that you are always using the inverted microscope to monitor the cell culture, right? And then you take the, you know, the culture flask and put it into the objective, and then you are actually going to observe. Inverted microscopes provide direct observation of living cells in culture without any use of stains. And in this same method, you can actually use the counting chamber. You can actually use the counting chamber for counting the cells. And since this is a very interesting aspect, how you can count the cells and calculate the concentration of the cells.

I will take you to my lab where the students are actually going to show you how to put the cells into the counting chamber, how to count the cells, and how to calculate the concentration of the cells per ml. Hello everyone, in this video we will show how to subculture the cells and count the cells. First, we have to remove the remaining media, then trypsinize the cells, and then we will count the cells and see. Now I will show how to perform trypsinization. Now I am going to add the drip seal to detach the cells.

After the cells are detached, we have taken them into clean Falcon tubes, then we have to centrifuge the cells. As the cells are very delicate, we have to centrifuge at 1500 rpm for 2 minutes. Now we have to remove the supernatant and resuspend the cells in fresh media. After resuspension, we have to count the cells. So, I am going to take 20 microliters of this cell suspension and mix it with 20 microliters of trypan blue and count under the Neubauer chamber.

Before counting, we have to see what a counting chamber or hemocytometer looks like. This is a typical hemocytometer, also called the Neubauer Chamber, which contains squares on the upper and lower sides, with each square having a depth of 0.1 mm and an area of 0.0025 millimeters squared. Now I am going to put a cover slip on this chamber, then I will slowly add the suspension through capillary action, and it will spread all over the squares.

So we check how many cells there are in all the squares. Now we have to count: how do we count the cells? So here is a typical Neubauer chamber that contains five squares. So we have to count the cells in these squares. So each square is 0.0025 mm^2 and there are a total of 16 small squares, so the total area of this whole square is 0.

04 mm^2. The depth of each well is 0.1 ml, so what is the volume? 0.04 multiplied by 0.1 is a total of 0.

004 $millimeter^3$ or 0.004 microliters. So say we have combined the cells in every way. Say this is A, B, C, D. Here we have 100, here we have 150, here we have 110, and here we have 100 again. So, to find the total cells, we have to take the average. That means 100 + 150 + 110 + 100 divided by 4.

We are counting a total of 4 squares. The average is 150. So, there are 115 cells in a 0.004 microliter volume. So, how many cells are there per 1 ml? So that we can simply calculate 115 / 0.

004 x 1000. That will give the volume of cells per 1 ml. Now, apart from this, we can also use flow cytometry. So, flow cytometry is a highly used analytical tool for quantitative analysis of cell number. This involves the hydro-focusing of the cell into a single chamber, which then passes through the laser beam. When the single cell passes through the laser beam, the laser gets distracted, and this distraction is measured by the photodetector, which then gives us the count of the cell.

So, flow cytometry is a very vast topic. So we are not going to cover flow cytometry in this particular course, but flow cytometry is going to use the hydro focusing technique. And because of that, it is actually going to pass the single cell in a single event. And when the cell is passing through the light path, it is actually diffracting the light beam. And therefore, that diffracted beam can be used for calculating the number of cells that have been passing through the light chamber.

And that can be used to count the cells. Then we can also use the different types of assays. So we can actually use the biochemical assays like the MTT assay or the Resazurin assay. The MTT assay is a colorimetric assay to estimate the number of cells present in a cultured media. You are going to use the MTT dye, which is called 3, 4, 5 dimethyl triazole, 2, 5 diphenyl tetrazolium bromide or tetrazolium salt. which can actually cross the plasma membrane, and since this dye gets reduced, MTT gets reduced into the insoluble formazan, a purple-colored compound, by the dehydrogenase enzyme present in the mitochondria of the viable cells.

So the cells actually have the viable dehydrogenases that are present inside the mitochondria. So when the MTT is getting into the cell because it can cross the plasma membrane, it can also cross the mitochondrial membrane, and then it actually goes and aggregates into insoluble aggregates like formazan, and these insoluble formazan can be

used for calculating. So these insoluble PharmaZone compounds can be dissolved in DMSO and then they can actually be measured at an absorbance of 570 nanometers. The intensity of this color gives rise to the estimate of the number of viable cells present in the culture.

So this is the structure of the MTT. And when it actually enters the mitochondria, it is processed by the mitochondrial dehydrogenases. All the viable cells actually have a different amount of mitochondrial enzymes, and then it gets converted into a formazone. This formazone is actually blue in color, right? And this is actually colorless. So the MTT is colorless, whereas this is going to be blue, and that blue color is actually going to have a very strong intense absorbance at 570 nanometers, which you can use. So the OD values at 570 nanometers are going to tell you about the number of live cells.

Now, similarly, we have the resazurin assays. So the Resazurin assay is another type of colorimetric assay to estimate the number of viable cells in the culture. Resusurin is a non-fluorescent blue color dye. When the resveratrol enters the cell, it is reduced by the dehydrogenase enzyme of the mitochondria into a red-colored resource of wine. So it is a non-fluorescent blue color dye. So this is a blue color dye, and then it gets converted into a fluorescent dye called resorciphene.

The amount of resorciphene found is proportional to the number of viable cells measured by taking an absorbance at 590 nanometers. Then we can also have the quantification or growth monitoring of the animal cell by quantifying the DNA and RNA. So a viable cell is metabolically active with active gene expression profiling. The more the number of cells, the higher the amount of cellular content will be. Hence, the quantification of cellular DNA and RNA will give a direct estimate of the cell number.

Extraction of the DNA or RNA can be performed using the available extraction kits and their respective protocols. The concentration of the DNA or RNA in the final extract can be determined using the NanoDrop. You can use the A260/A280 to determine the purity, and a ratio of 1.8 indicates pure DNA, while a ratio of 2 indicates pure RNA. You can use these absorbance values to calculate the amount of DNA present in a particular biological sample, and that's how you can estimate the number of cells present.

So this is all about cell growth monitoring in the prokaryotic system as well as in the eukaryotic system. Let's move on to the slightly bigger eukaryotic system, which is called plant cells. So the plant cells are actually being used because, you know, the plants are starting from unicellular organisms to multicellular trees. Right. So that's why the plant cell monitoring actually has different types of basic principles that you can use.

So the first one is the image-based technique. Right. So you can actually have either the 2D imaging or the 3D imaging. For example, you have a tree. Right? Now this tree, how this tree is growing, you cannot use any of those biochemical methods that we have just discussed for the animal cell or the proper prokaryotic system. What you're going to do is take a camera and take the image, right? You're going to take the image, either the 2D image or the 3D image, which means you can actually be able to. You know how to use the drone and all those kinds of apparatuses to calculate what the height is and use this angle? You can calculate what the increase in height will be.

So if you suppose this is going up, right? Suppose this is the height when you measured a month before, and when it goes to this height, right? The angle is going to change. For example, if you are changing it like this, it is going to change like this, right? So this change in angle can be measured and can be done using the camera to measure the phenotype of a plant, such as the broadness of the leaves, stems, height, and leaf counts. It makes use of a camera through which the observer tracks the growth of the plant from a distance. It also allows recording the growth over a period of time. It is a simple mode of visualization where the accuracy of the growth estimate largely depends on the observation.

This is actually an observational method. Where you are actually going to use the simple camera observation is whether the plant is growing, whether it is increasing the number of leaves, and whether the leaf size is increasing. In 3D imaging, you can actually use the three-dimensional analysis of the phenotype of the plant, which ultimately gives rise to the growth of the plant and is much more accurate than 2D imaging. It involves the construction of a 3D model of the plant using a camera that rotates around the plant to capture images and then reconstructs the 3D images from them to track the coordinates of the plant. So this 3D imaging is actually going to tell you whether the thickness of the plant is increasing or not, right? So for what we are measuring with the 2D, whether the height is increasing, whether the number of leaves is increasing or not, but whether it is increasing in width that you are going to get with the 3D imaging. The increase in stem height, leaf surface area, broadness of the stem, and the number of branches over a period of time is used to estimate the rate of growth as coordinates of the plant change.

Then, the second method is the biochemical method. So, in a biochemical method, what can you use? You can use the different types of biochemical markers. So, for the first marker, you can actually use the chlorophyll content. Right. So chlorophyll content is a basic life-trapping pigment that is present in the leaf and aids in the photosynthesis process. So the cellular content of chlorophyll in plant leaves increases with the growth and the age of the plant.

Hence, the estimation of the chlorophyll content can give a good estimate of the growth rate of the plant cells. Then we also have Arnon's method of spectrophotometry, which can estimate the chlorophyll content of plant cells. And the Arnon method involves the following steps: So in step number one, the leaves are collected and ground using a pestle and mortar with the addition of acetone. So you're actually going to extract the chlorophyll into 80% acetone. The homogenate is then filtered using the Whatman filter paper, and the filtrate is collected.

The homogenate on filter paper is washed 3 to 4 times with 80% acetone until the filtrate becomes colorless. The absorbance of the chlorophyll extract was measured using the spectrophotometer at 670 nm and 663 nm. The total amount is calculated using an equation.

Chlorophyll per tissue is 20.2 absorbance at 675 plus 8.02 absorbance at 663 multiplied by V/100 into W, where V is the final volume of the chlorophyll extract and W is the weight of the fresh tissue extracted. Then you can also use the DNA and RNA quantification. Similar to the animal cell, you can also use the OD at 260 nanometers to estimate whether there will be an increase in the DNA content or the nucleic acid content. So, with this, I would like to conclude my lecture here. Thank you.