Molecular Biology
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Module - 02
Basics of Biological system (Part 2)
Lecture-09 Cell Cycle and Control (Part 2)

Hello everyone, this is Dr. Vishal Trivedi from department of biosciences and bioengineering IIT Guwahati. And what we were discussing, we were discussing about the basics of the biological system and in this context so far what we have discussed, we have discussed about the cellular structures. So, initially we discuss about the prokaryotic structures followed by the eukaryotic structures and when we were discussing about the eukaryotic structures, we have also discussed about the different organelles what are present in the eukaryotic cells and how you can be able to separate them with the help of different types of fractionation techniques. We have discussed about the density gradient identification and as well as the differential certifications and in the previous lectures, we have also discussed about the cellular metabolism. So, we have discussed about the anabolic reactions and we also discuss about the catabolic reactions.

So, within the catabolic reaction, we discuss about the carbohydrate metabolisms and lipid metabolisms whereas within the anabolic reactions, we discuss about the synthesis of the different types of amino acids and so on. So, in today's lecture, what we are going to do is we are going to discuss about the how the cell is utilizing this energy for different types of activities and one of such different activity which is very crucial for the cell is the cell division and the cell cycle. So, I hope you have understood the how the flow cytometry is actually analyzing the different types of cells and then you can actually be able to exploit this particular technique not only for studying the cell cycle, but also for the other kinds of applications. So, these are the some of the classical flows and dye what you can actually use into the flow cytometry.

You can actually use the FITC, PE, per CP, APC, Texas red, you can actually use the PE Texas red and PE silane and these are the emission wavelength. So, all most of these emission wavelengths are available in the and they will actually go into the very clear cut you know separation from each other so that you can be able to use them either singly or in a combinations. So, now come back to the our original question how you can be able to use the flow cytometry to study the cell cycle analysis. So, for the cell cycle analysis what we have is we require to stain the cells for the DNA binding dye. So, one of such DNA binding dye is the propodium iodide.

So, if you stain the cells with propodium iodide, so this is the propodium iodide structure and it is actually going to stain the DNA by the you know by using this positively charged residues. So, the propidium iodide is a flow-set dye frequently employed in the analysis of cell cycle to label the DNA. PI has a unique property of being unable to enter the life cell but capable of permeating the cell membrane of the seeds or the diet cells through its ability to DNA and subsequent examination of red flow set upon excitation with a specific wavelength. PI enables the evaluation of individual cells DNA content and facilitate the examination of their distribution within the cell cycle. Remember that what we are supposed to study we are supposed to study how which cell has the 2x amount of DNA and which cell has the 1x amount of DNA.

Apart from that it is also going to combine this information with the size and they using these two property you can be able to study the whole cell cycle. Remember that G1 phase the amount of DNA is going to be 1x and size is also going to be small whereas S phase you are going to have the 2x whereas G2 phase you are going to have this bigger size and as well as the 2x amount of DNA. So, that is a way in which you can be able to use the combination and you can be able to study the cell cycle using that. So, if you want to do the cell cycle analysis these are the material what you require. So, I am not going to go through with the content right.

So, you require the well plate, propionate iodide, RNAs and all that and this is the protocol what you are going to do. So, you take the cell in a 6 well plate you on the day of experiment wash these cells and then treat the cell with desired concentration and incubate. So, mostly people do the cell cycle analysis when they are doing the treatment right. For example, the cancer cell was if they are under the treatment their cell cycle maybe get disabulated and that is how they will actually going to undergo the apoptosis or cell death. So, for those kind of experiment what you are going to do is you are going to treat the cells with a particular anti-cancer agents or compound and then you are going to study whether it is affecting the cell cycle or not.

So, for example, after the 24 of treatment remove the media and scrap the cell with a 16 or 0.6 percent ETTA, centrifuge at low rpm and discard supernatant using the 16 and retain the cells as a pellet. Then you add the 250 microlitre of EBS to resuspend the pellet cell and keep the suspension on ice for a couple of minute, add 1 ml of 70 percent ethanol and under work test condition for the fixation of the cell transfer the sample from ice to minus 20 for 3 to 4 hours. And then you are going to have the incubation with the 70 percent ethanol centrifuge at 5000 rpm discard supernatant and save the pellet then you add 1 ml of ice cold PBS and gently resuspend the cell centrifuge the sample at 5000 rpm at 4 degree for 10 minutes to remove any residual media and debris remaining from the cell. So, you take the washing step with the PBS two more times after washing the

cells with PBS add the RNAs so that you are actually going to remove all the samples all the RNA set signal actually because RNA signal is going to be very high because it is present in the cytosol.

So, you do not want that kind of disturbance. So, with RNAs one where the final condition would be 20 to 30 microgram and incubate at 37 degree Celsius for in water bath for 2 hours. After the RNA degradation by the RNAs a stain the cells with propodium iodide final consideration is 50 to 70 microgram for 30 minutes before acquiring the data flow cytometry. And when you are doing the data acquisitions, analyze the stain cell using the flow cytometer equipped with the appropriate filters for the propidium iodide. Usually there is an excitation at 488 and the emission at the 585 nanometer.

So, you should use slice the standard flow cytometer where you can have the different these type of lasers in a you know the detectors. Adjust the flow cytometer setting for appropriate sources and forward and side scattered. Run the stain cells on a flow cytometer collect the data for at least 10,000 events. So, every event means the cell actually. So, you have to collect 10,000 cells data.

Analyze the acquired data using the flow cytometry software, plot the DNA content and onto the x-axis and the cell count or the event onto the y-axis. The resulting histogram will typically show the distinct peak in the different phases of the cell cycle such as G1, S and the G2. Data analysis, the data analysis can be done with the FCS-5 XPREN software. These are the some of the precautions what you are supposed to take and to avoid any disruption in the distribution of cell population is recommended not to seed or treat the cells when they reach the confluency between 90 and 100. So, you should not take a very old cell.

So, it should be cells which are under the actively dividing cells should not be like confluentish because that the cells are going to be old and they will not be dividing. To avoid obtaining unwanted background signal, it is important to run the cells labeled with the propidium iodide in a PBS when using a flow cytometry instead of using the complete or the incomplete media. Ensure to avoid excessive pipetting when the scraping the cells from the plant because that is actually going to damage the cells. So to explain you how to perform these and how you can be able to study the cell cycle using the flow cytometry, we have prepared a small demo where I will take you to my lab and where the students are actually going to explain you how you can be able to stain the cells, how you are going to acquire the sample and so on. Hello everyone, in this video we will be discussing about how to perform cell cycle analysis on a FACS equipment.

So basically the cell cycle analysis gives us the details about how much population of a given number of cells is present in which phase of the cell cycle. For example, the G0 or the G1 phase, the synthesis phase or the G2 or M phase. So we can design the experiment according to our needs. For example, if we want to find out how a chemical compound inhibits a certain population of cells, we can do a time dependent experiment or also we can do a concentration dependent experiment. So in both the ways we can find out which particular phase of the cycle is being inhibited.

The procedure for cell cycle analysis is very simple. First we trypsinize the cells from 100 mm cell culture drish and seed approximately 1 million cells in a 6-well plate. After the cells are seeded, we incubate it for 12 to 14 hours so that the cells get adhered completely to the dish. After the cells are adhered, we give the appropriate treatment but after washing it twice with PBS. After the appropriate amount of treatment, let's say 12 hours or 24 hours or 48 hours, we trypsinize the cells and collect the pellet.

We wash the pellet two times with PBS and then centrifuge again and then resuspend the pellet in 1 ml of 70% ethanol. And then this mixture is kept in minus 20 degrees for 12 hours for fixating the cells. After the cells are fixed with 70% ethanol, we wash the cells with 5 ml of PBS and then collect the pellet and resuspend the pellet in 1 ml of PBS. Then we provide the appropriate RNA treatment to remove all the RNA from the mixture because RNA, if present in the mixture, might interfere with our cell cycle analysis. After incubating the sample with RNAs for 2 hours, we give the appropriate Propeidium iodide treatment.

The working concentration for Propeidium iodide in cell cycle analysis is 50 microgram per ml. After giving the treatment with Propeidium iodide for around 1 hour, we analyze the cells in fax equipment. After the samples are prepared, we need to analyze the data in the fax equipment. The first thing we do is open the cell Quest Pro software. After that, we connect it to the cytometer.

After connecting to the cytometer, we need shooting such as counters, detector and amps, status, and also we need the dot blot, acquisition and analysis. We need another dot blot for FL-2A and SSC because in the FL-2A channel, the Propeidium iodide emits red fluorescence. We also need a histogram to see the cell cycle phases. For that purpose, we need the FL-2A channel. We need another dot blot in order to see whether there is a presence of any doublets.

There is a chance that there might be a presence of the doublets in the data like for example the clumps of cells, so we have to exclude that from the data. For that purpose, we take the FL-2A and FL-2W channel. Now that everything is ready, now we can

change the directory for acquiring the data. We can also choose the file name. So we will name it as unwitted1 and we will keep the file count to 1.

For checking the data, we have kept it on the setup so that we can see whether the data is coming properly or not and then we can remove it from the setup and then acquire the data. So now we are going to load the sample onto the sample injection port and then press acquire. As we can see that the number of events have started recording. In the FSC and the SSC plot, we can see that most of the population is seen between the 200 and the 200 mark. The population which is coming away from the doublets.

In FL-2A and SSC, we can see that there are three different populations, G1, S and G2. And in the FL-2A and the counts, we can see that the longest peak is the G1, the between one is the S and the smallest peak is the G2. So before acquiring the data, we have to set the number of events. So we go to acquisition and storage and press record 10,000 events. So in order to record the data, we have to remove it from the setup and then setup and then press acquire.

In the FL-2 and the FL-2W channel, we can see that we can see there are two different populations. One in line and another is the presence of some doublet or clumps of cells. This dating population might be presence of the doublet cells or clumps of cells. So while acquiring the data, we can see this but in the FCS Express software, we can remove that. So now that we have acquired the data, now we can take the data from the untreated sample.

We have to remember that we don't have to change the parameter conditions in order to compare the untreated and the treated samples. Now we have to change the sample name from untreated to treated and also change the file count to 1. Press ok and then acquire. We can see that there is a little shift in the S and the G2 phase but we can't say for sure that there is a change in the data. So for that, we have to acquire, we have to process the data on the FCS Express software in order to see what is the difference between the untreated and the treated sample.

So now we will remove it from the setup and then acquire the data for the treated sample. Sometimes we can see that the events per second will be low. So in order to increase the events per second, we can pause the recording and then tap the sample once or twice in a while to shape the sample and then the flow will be continuous again from the sample injection port and the events per second will increase. Now we can see that the events per second has increased because we have tapped the sample and the data acquisition will be little bit faster. So now we have acquired the data for the untreated and the treated samples.

In order to see in which phase of the cell cycle the arrest has taken place, we have to process the data in the FCS Express 5 software. So now that we have acquired the data on the FACS equipment, we have to now process the data in order to see the difference between the untreated and the treated sample. For that purpose, we use the FCS Express 5 flow software in order to process the data. So for that purpose, we use the new layout and then change the mode to landscape because it is easier to work in the landscape. And then after that, we go to the data in the toolbar and press open and then go to the folder where our data is saved and press open the untreated file.

As we can see that there are multiple options available, dot density, color, dot contour, surface histogram and multi-cycle DNA and kinetics. For this cell cycle DNA, we only need multi-cycle DNA plot and the dot plot. So we are going to open these two. Because we have recorded our data on the FL2A channel and also because the propidium iodide is only shown in the FL2A channel, we are going to open the FL2A channel. So now after we have opened the dot plot and the cell cycle DNA plot, we can see the, means how much population of cells is represented by the G1s and G2 by right clicking on the plot and then selecting statistics and then show DNA cycle statistics.

So a small window will appear in which it will show that the %G1, %G2 and %s. So this is for the untreated sample. Let's see for the treated sample. As we can see that there is some change in the untreated and the treated sample both in the both plots, the dot plot and as well as the cell cycle plot. So in order to see how much % of the cell cycle phases have changed, we right click on the plot, then show DNA cycle statistics and then place it right beside the untreated one in order to compare.

So we can see that there has been a reduction in the G1 phase from 61 to 49 and from G2 also from 15 to 12. But there has been an increase in the %s phase which has increased from 23 to 38. So we can say that there is a significant change in the phases of the cell cycle. But we can only be sure after doing the experiment in the triplicate so that we get the proper standard deviation and also the standard error.

So this is one way of processing the data. Another way of processing data is by gating. As we can see in the FSC and the SSC channel that there are some debris near the 00 point and also there are some population of cells which are very far from the 00 representing clumps of cell or may be doublets which we have to take care using gating technique. So in order to explain the gating, I will use another page and then open the data and then press OK. So for gating we need multiple plots. The first one is FSC and SSC and the second one is between FL2A and SSC and the third one is between FL2A and FL2W.

The fourth one is between FL2A and FL2H and the last one is between FL2H and FL2W. So we have to start the gating from the first plot, the FSC and the SSC. So in order to do the gating, we have to go to the gating option in the toolbar and then we can choose any one like ellipse or the rectangle, polygon, freeform. These are the shapes of the gating. So we will go with the polygon gating because it is easier to handle and then we can get our required population using polygon gating tool and then just select the required population.

So here we are excluding the debris and the clumps of cells which might be interfering with our actual data and then we can select what gate colour we can give and also rename the gate 1 to our particular type but we will go with the standard one and then press OK. And then we have to apply this gating to the second plot. We can just drag and drop it on the second plot. So we can see here that we have excluded some population. So but still there is some population which can still interfere with our data.

So we will do the gating again and then we will exclude some more population which might not be helpful to us. And then we will name this as gate 2. So in order to apply this one, this gate to the third plot, just drag it and drop and then we can see that another number of population have reduced and in the third plot as well we can reduce this population which is a little bit distinct from the singlet cells. So we have taken another gating using polygon and then we are only now going to select the singlet cells and then we will name this as gate 3. From gate 3 onwards we just have to apply the gates to the next plot and then we can apply the plot, we can apply the gate to the plots using just by just selecting the plot and then going to the top left corner and then selecting the gate.

So as we have seen that we have reduced significant number of population and we have only selected the population which might be helpful to process the data. And then finally we apply the gate 3 which is the final gate and then we will see the change in the cell cycle statistic plot. So this is for the untreated one. In order to show the cell cycle statistics just press statistics and then show DNA cycle statistics. As we can see in the ungated one we have seen that the percentage of G1 was 61 whereas in the gated one it is 67.

1 and the percentage G2 is 8.9 and the percentage S is 23. Similarly we can do for the rated one but we don't have to follow the whole procedure. We can just copy all the plots to a new page and then press paste and then go to data. And then select the treated one. It will just replace all the plots with the treated data. So but the treated data and the untreated data is little bit different so we just have to move the gate.

So in order to see the cell cycle statistics for the treated one we can select the statistics DNA cycle statistics and then we get the DNA cycle statistics. So in this way we can get the process the data for the untreated and the treated sample in the cell cycle DNA statistics using gating as well as non-gating technique. So hopefully this video was helpful for everyone. So at the end of this experiment what we are going to do is what you are going to see is you are going to see all the cell cycle stages.

You are going to see the G1. You are going to see the G2. You are going to see M. You are going to see the S phases. And all these are going to be analyzed by a flow cytometry analysis software. You can use any standard software what is available into your laboratory.

You are not bound to use only the FCS-5 receptor. And what will happen is that when you analyze that it is actually going to give you the different phases. So when you plot the FL2 versus the number of cells this is the unfiltered sample and this is the sample which has been treated with the anti-cancer compound. So what you see here is that this is the G1 phase right. This is the G2 phase and this is the other phases right.

So what you see here is that this is going to be the G1 phase. This is going to be the G2 by M phase and this is going to be the S phase right. So what you see here is this. This is the S phase okay. These are the cells what are present in the S phase.

And these are the cells that are present in the G1 phase. And these are the cells what are present you see this red color right. These are the cells which are present in the G2 by M phase. So although the separation of the M phase from the G2 is very very different because they both have the same amount of DNA and they also have the same size. Because at the end of the M phase you are going to have the 1X of DNA right.

But before that it is actually going to have the 2X of DNA. So that is why it is very difficult to separate out the G2 by G2 phases G2 phase from the M phase but it will actually going to give you an idea right. So this is the what it says is that out of the total number of cells the 54% cells are under the G1 phase, 6.72% cells are into the G2 phase and only the 39% cells are under the G2 by G1 phase. What you see here also that S phase is actually the phase which is responsible for the DNA synthesis right.

And what you see here is that the S phase is now 20%. This means there is a disturbance within the S phase and that is how it is actually going to suppress the growth of the cell right. And these are the some of the information what you are going to get when you are going to do the analysis of the facts analysis. Apart from the flow cytometry you can also be able to do the traditional methods where you can actually be

able to you know prepare you can take the plant tissue and you can actually be able to extract the chromosomes and you can actually be able to visualize the cells and you can be able to study the different types of phases using the other methods. And in this the advantage is that you can not only be able to study the G1, G2 and S phase you can also be able to study different phases within the mitosis such as interphase, you know interphase, metaphase, telophase and anaphase.

So this is what we are going to discuss now. So if you want to study the M phase the flow cytometry is not good for studying the M phases right or different phases within the M phase okay. So for that we are actually going to prepare the chromosome samples and it is actually going to give you the different sample. So mitosis and meiosis you are actually going to prepare the chromosome preparations okay and that chromosome preparation can give you the clear idea about what are the different sets of cells present in the mitosis and within the mitosis how many cells are present in the interface, how many cells are present in the metaphase, anaphase and telophase. And for doing this what you require is you require a plant sample so you can actually be able to do this in a root tip right and you require a plant cell samples, you require the if you want to study the same thing in the meiosis then you require the flower beds, a flower bud right because flower bud is actually going to be actively divided by the mniotic phases and then you require all these material. Apart from that you require a compound microscope and you also require a testimulator for storing the prepared reagents.

You also have to prepare the different types of dyes such as the acetone or the cetin dye, you also require the coronary solutions and so on. You require so how you are going to prepare all these solutions this is all the recipe is already been given and then you require the two different types of methods. So you can actually be able to prepare the mitotic chromosome preparations. So mitotic chromosome preparation this is the lengthy procedure and we are actually going to you know so these are the methods what you are actually going to follow. So this is the protocol if you follow this step by step it is actually going to give you the chromosome preparations okay.

And that and then apart from that if you want to study the meiosis you can actually be able to prepare the chromosome preparation in a different way. You are actually going to use the flower buds if you want to study the meiosis whereas if you want to study the mitosis you are actually going to use the root tips or the somatic tissues. And at the end of this what you are going to do is you are going to prepare you are going to take some precautions such as you are going to use the, collect the plant material which is you know which should be performed in a bright sunny day to ensure the proper mitotic stage of the development. All the collection timing may vary depend in between the plants. Cloudy rainy should be avoided for the material collection and use of the freshly prepared

solution of the acetooxygen solution should be used during the preparation of the slides.

So I have prepared a small demo clip into one of my colleagues lab and there the students are actually going to show you how you can be able to prepare the chromosomal preps for studying the mitosis and meiosis. Hello everyone I am Rajendra PhD student from IIT Guwahati. In this tutorial I am going to demonstrate you the practical aspects of mitosis cell division. In this method we will explain you each and every details of mitosis starting from sample preparations to the microscopic observation.

We will explain you each and every details. 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, carnois solution, Glacial acetic acid 45%, 1 normal hydrochloric acid, 2% acetoarsin, wash glass, cover slip and cover glass, burner, glass beaker, pipettes, various size of the forceps, blades, filter papers or the blotting sheets and ependop. Root tips from healthy onion plants were collected at 9 am in morning.

Initially epical region of the root tips were cut by using surf blade. 2-3 pieces of root tips 1 cm in length were selected. 8-hydroxyquinoline 0.02% were previously prepared and kept in amber glass bottle. 1 ml of 8-HQ previously kept in ependop tubes were used and those cutted root tips were kept inside ependop tube. After that this ependop tube will be stored in refrigerator at 4 degree Celsius temperature for 4 hours.

This whole process is called pretreatment. After 4 hours of pretreatment at 4 degree Celsius temperature the 8-hydroxyquinoline has been discarded. Therefore, the pretreated root tips now will be fixed in carnois solution. Carnois solution containing absolute alcohol, chloroform and glacial acetic acid at 6 x 3 x 1 ratio. The ependop tube containing 1 ml of carnois solutions with pretreated root tips now will be stored at room temperature for 48 hours.

After fixation in carnois solution for 48 hours that solution has been removed. Therefore, that fixed root tips has been transferred in vase glass followed by addition of one normal hydrochloric acid. one drop and 2% aceto oresin. The mixture of aceto oresin and hydrochloric acid has been heated gently over the burner. This process is called staining.

Now the stained root tips has been placed over the glass slide. Lower portion of the root tips has been removed by sharp blade. Only the meristematic region has been taken for analysis. Root tips were then squashed and mounted by cover slip. This process is very careful to prevent entering of air bubbles. Now the sample was covered by the filter

paper and gradual pressure has been applied by fingers in order to spread out the cells.

Now the slide is ready for observation under microscope. Now the slide has been placed under microscope. Photomicrographs were taken with Carl Zayes microscope having 10x, 20x, 40x, 60x objective lenses. We can see here from the microscopic field that chromosomes of onion root tip cells is clearly visible along with cell divisional stages like anaphases and telophase also visible. In this method we have explained each and every steps of mitosis starting from sample preparation to microscopic observation. Hope this video will help you to prepare the slide of any plant sample for the study of mitosis.

The meiotic cell division process starting from sample preparation to microscopic observation. We will explain you each and every steps in detail. Flower buds of onions will be used for mitosis study. Initially flower buds were collected during the flowering season in morning between 11 to 11.30 am and has been fixed in carnoise solution containing absolute alcohol, chloroform and glacial acetic acid 6H to 3H to 1 ratio.

Those fixed sample has been placed over a watch glass and a single flower bud has been selected for smear process. Flower buds size 1mm in length has been taken and placed over a surface of glass slide. After that sepal and petals were removed initially from that selected flower buds. Therefore the anthers were removed.

Special isolated anthers are clearly visible on the surface of glass slide. One drop of 2% acetoarsin has been given over the anthers. With the help of iron needles the anthers were ruptured and pollen mother cell has been released. Very gentle pressures has been applied over the anthers. This process is called smear technique. Now the anthers walls has been removed so that we can observe the various stages of pollen mother cells that is undergoing meiosis.

After that one cover slip has been placed over the sample with the help of pointed iron needle. This process needs extra precautions to prevent the entry of any air bubbles in between slide and cover slips. We can use blotting sheets or filter paper to remove excess stains. Now the slide is ready for microscopic observation. After that the slide has been placed under microscopes to capture photomicrographs. Different objective lenses has 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 after second meiotic division two cell stages is formed but the four distinct nuclei has been reached in two different poles. In this process we have explained each and every

step of meiosis cell division process starting from sample preparations to the microscopic observations. Hope these videos will helps you to prepare the slides from any flower buds in your plant sample. Thank you for listening. So, this is all about the cell cycle and let us discuss about what is the role of cell cycle into the different properties or different section of the biological function of the cell.

So, the role of cell cycle is actually required for development of the growth. So, the development of cell cycle single cell into the multicellular system is possible due to the cell cycle and division. Then it also require for the cell replacement. So, eukaryotic cells have the predefined lifespan after that period it needs to be replaced with the new one. It is possible due to cell division and making more cellular properties.

For example, the human RBC has a lifespan of three months. New RBCs are formed from the bone marrow by the cell division. Then it also requires for the regeneration. So, cellular damages and injury is an integral part of the living system and the cell division is the primary event required for the synthesis of the lost or the damaged organ. Then it is a very very important method for the asexual reproduction. So asexual reproduction is common in the lower invertebrates such as and in these organisms the cell divide to form the new cells and these newly formed cells give rise to the new organism for example, hydra or planaria or amoeba.

Now the last part is that how you are actually going to control the cell cycle because if it is a cell division it actually increases the cell mass it has to be very precisely controlled. So, cell cycle is controlled at the multiple stages. It is actually going to be controlled at the interface of the G1 to S phase. It is going to be controlled as the S to G2 phase and also going to be controlled at the G2 to M phase and all that. So at this stage when the cell is entering into the G1 phase it is going to be checked for different types of parameters so that it should not happen spontaneously.

So cell cycle at different step is tightly controlled by the cell cycle check proteins. The cell cycle check proteins are used to ensure the completion of different steps and repair of the DNA damage. The main checkpoints are present at the G1 to S phase, G2 to M phase and the M phase. Each checkpoint is controlled by the mutual interaction between the cyclin protein and the cyclin dependent protein kinase or CDKs. P53 protein are known to control many events through the G1, S and G2M checkpoints.

So what will happen if there will be a dysregulation or there will be a control gone right. So what will happen if these events goes wrong right. Dysregulation of the cell cycle and control mechanism give rise to the enormous growth of that particular cell and that is nothing but the tuber actually. Under certain number of cell division every cell will enter

into the G0 phase and ceases its cell division. In that case of tumors cells lost the control mechanism and multiply indefinitely to give rise to the cell mass.

These cells are taking nutrition but not performing the functions. Retinoblastoma or the RB proteins, P53 are the crucial factors cellular factor responsible for the cell cycle control and play crucial role into the tumor deployment. If you want to study more or if you want to study more about the cell cycle control, I have given you this reference and you can be able to study or you can actually be able to go through with the content. So what we have discussed, we have discussed about the cell cycle and control. We have discussed about the different phases of the cell cycle whether it is G1 phase, G2 phase, M and S phase and we also discuss about the relevance of these phases.

Apart from that we have discussed about the two different methods. One is the traditional method where you are actually going to prepare the chromosomal preps to study the mitotic phases and the other is the more advanced technique of the flow cytometry where you can actually be able to separate the cells based on the DNA content at the size and both of these techniques are going to be very robust to study the cell cycle and the different phases of the cell cycle. So with this I would like to conclude my lecture here in our subsequent lecture we are going to study or we are going to discuss some more aspects related to the biological system. Thank you.