**Cell Culture Technologies**
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**Lecture - 05**
**Dividing Cells**

Welcome back to the lecture series in Cell Culture Technology.

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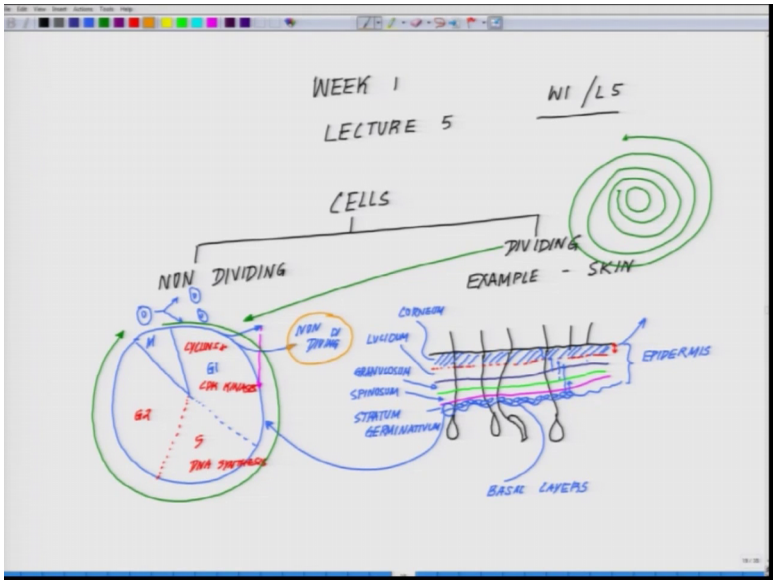


Today we are in the 5th lecture of the 1st week it is put that down otherwise I will miss it out it is Week 1 lecture 5 W 1 slash L 5.

We closed on in the previous lecture in the fourth lecture on cell cycle, where is the fag end of the lecture I introduce the 2 cell types broadly speaking; the Dividing and the Non dividing cell. And in the non dividing cell I give the example of a neuronal cell, cardiac cell and in the dividing type I talked about the skin cells I am just taking example there are so many of this. Whenever you talk about dividing cell think of it say if I take the let us start it. So, talking about the cells Non dividing and this side and Dividing on other side.

So, my example was skin now if you look at the skin itself skin consists of 2 layers epidermal layer and the dermal layer. If you look at your own skin to the something like this is. The skin the hairs are coming out like this and this here cell bodies are kind of

sitting like this and underneath it we will see first layer like this, second layer like this I am just broadening it up, but is not that thick of layer third layer like this, fourth layer like this and fifth layer which is something like this. So, I am just kind of you know this is the epidermis. And within an epidermis the layers are this is stratum Germinativum stratum this one is Stratum Granulosum in between is Stratum Spinosum, Stratum Lucidum, and Stratum Corneum.

The interesting part is this layer this layer contains some apart from other cells like morcal cells cells and melanocytes this layer contains something called basal cells. These cells you can considered them as the stem cells or the germ cells for you know the germ cells is wrong word sorry let me take back the skin stem cells which divide to form the skin layer. So, whatever divides here they move like this layer by layer them move and then they come to the uppermost layer. And this whole process this migration of the cell takes around 15 to 20 days and at the top layer these cells survive for 2 to 3 weeks before they are sluft off and the previous layer moves to the upper layer.

It is always like if the lowermost layer is 5 next is 4 then 3 2 1. So, from 5 something germ to germs to 4 from 4 that 1 germs to 3 from 3 to 2 2 to 1 and then form 1 0 it get you know sluft of from the skin similarly this whole process continuous now coming said this now start let us talk about this cell cycle process. This individual cell has can go through something called a process of mitosis M phase what we call as the mitosis is the M phase after the mitosis where the chromosome separates out and the 2 daughter cells are formed. This is the cell now and here the nucleus it is chromatic condensers and the 2 daughter cells are formed.

At this stage next stage pose mitosis this cell has a choice this is called the gas phase G 1 phase it has a choice and it is a decision to make whether it is going to terminally go out of dividing and it becomes non dividing or non dividing or it temporarily goes out of division and then in due course it again comes back to do the division cycle. There are several chip points here sa this several cyclin and cdk kinases which are involved I am not getting in into those. So, there are several such molecules which are dealing with it cyclins and cdk kinases and there are several several check points, which ensures whether they are going to go further or not. Then comes a phase called S phase if they decide to you know go further and this is the S phase where DNA synthesis happens.

And followed by a G 2 phase where the again have a choice again have a choice and there are lot of checkpoints out here.

So, cell essentially goes through this cycle. Now that brings us to a very interesting question that how many times a dividing cell will be going through this cycle is this number finite or is this number infinite say for example, a particular cell like this how many cycle 1 2 3 4 likewise one and so forth how many cycle it can do. Because with every cycle there is something in it is chromosome called telomere the telomere length reduces and as a matter of fact the length of the telemeter can tell you which is part of the chromosome can tell you that what is the edge of it? Because as more and more telomere lengths are lost eventually the cell loses it is viability loses it is original characteristics. If cell at this stage say for example, at G 1 phase out here decides to finally, land up that it will become non dividing it will permanently go to the non dividing phase, then it has to decide what final function it will attain what does that mean.

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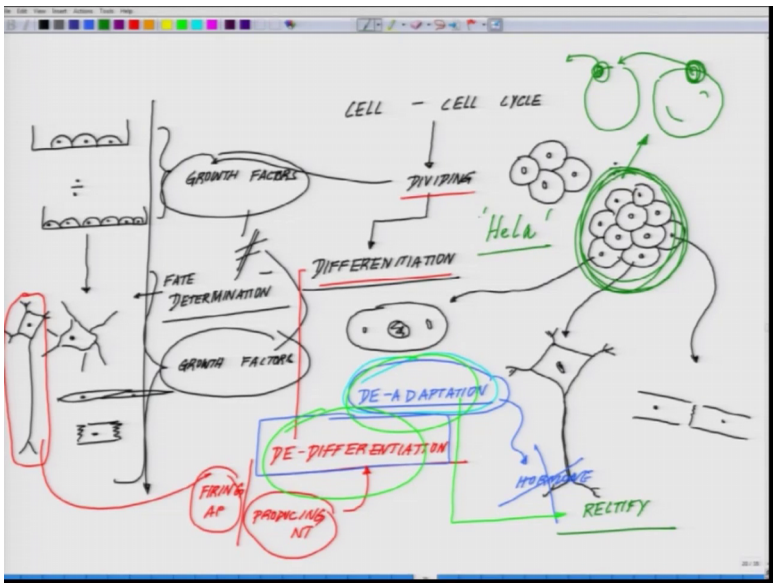


So, there are 2 3 phases apart from the cycle it goes through broadly speaking cell of course, it goes to cell cycle. Now while it is cell cycle it is doing 2 options some point or other it has it has been dividing maybe for few days or few hours whatever it has must have dividing, and after dividing if it decides at a certain point of it is division that it is not going to go any further division then it has to take it is final identity what I meant by it is final identity means whether it will be a cell which will be producing a particular

kind of hormone, or it will be a cell which will be producing a particular kind of hormone, or it will be a cell which will be present in the alveoli, or it will be a cell present in the heart as cardiomyocytes, or it will be a cell producing insulin, or it will be a cell produce saying a some other hormones like you know gonadotropins. So, that paid determination of a cell post is division is called differentiation.

So, this is a mass of cell sitting out here which is dividing now it divide form a bigger mass this. Now from this mass some of the cells decided that you know they will decide their own fate. Some of them say becomes like fully differentiate itself they will not divide any further they may secrete something some from specific population may decide that they become neuron and they want you know divide any further there is some from in population a specific population decided that they will become muscle cells or some other cell time. This fate determination process weight determination is called differentiation and the conditions for dividing cell is different from a differentiation.

So, since that brings us to a point while we are culturing cells in a dish we have to make a call are we holding the cell type on a dividing phase or they will be differentiated. So, say for example, I have a cultured dish here where I have these cells which are sitting and suppose they are dividing and spreading further. Now at this the conditions which will be there the dividing conditions the growth factors and the surrounding milieu will be entirely different for these cells when they decides they are fate determination whether they will be come from here they will be come and neuron or they become you know some real cell something like this astrocytes or they will become schwann cells or they become cardiomyocytes that is it. So, the conditions out here if you talk about the growth factors here they are totally different these 2 conditions are very very unequal.

That is brings us to another point that whenever we try to emulate a biological system outside, we have to emulator dynamic system and that was the reason why in the previous class before I teach this aspect or highlight this aspects to you. I talked about the concept of introducing micro fluidic systems, where you can change milieu and a very nano molar picomolar concentration by you know flowing certain specific growth factors certain point of time and then secret then sending another set of growth factors which you know will may promote differentiation or some other aspect. In that whole process there is another word which will be coming very handy and that I am just putting

in grade now this is the word called between division and differentiation there is another word called De-Differentiation, what is de differentiation?

De-differentiation is a situation, when a cell forgets or loses it is ability of it is differentiated behavior what does the mean say for example, this becomes a neuron. Now this loses it is characteristics of say Firing action potentials or Producing certain kind of neurotransmitters it loses it is ability; it means this cell has reached a de differentiation it has forgot10 or it has lost permanently well this part we will kind of keep we will take it with a bench of salt because we do not know the future of cell biology may tell a totally different story that we can again get back de differentiated cell back into all it is definition statistics. So, I will be I will be little cautious on it.

So, but for the time being a de-differentiated cell loses it is that unique capability of is differentiated state. And there is another one another word which comes pretty handy here called De Adaptation say for example, a cell is adapted to produce say a particular hormone. Now this particular hormone production for some reason or other it is adapted in that particular milieu you keep this cell out in this milieu it will behave differently say for example, let me pick up that previous class example previous say for example, here you have a milieu.

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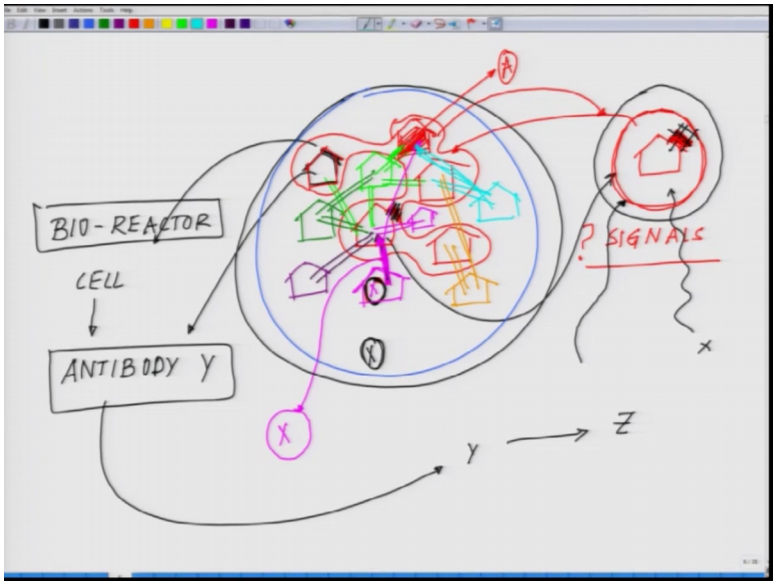


Now if you keep this red I consider each house as a cell in this milieu it will do certain XYZ stuff say for example, it produces compound A.

But if you remove it and keep grow it in isolation it may not be able to produce at compound a because in order for this red one to produce compound a may need influence from this pink one, from this green ones, or from this blue one. And if these influences are missing then what will happen is this cell will be de-adapting to that situation.

This process is called the de adaptation. These words are very very important that we understand that is it a de differentiated cell we will come later when all these things we will be dealing more and more or is it de adapted could this de adaptation be rectified could you rectify the de adaptation. Similarly those who are using cell lines. So, I am just using a word which I have been introduced. So, cell lines are cells which are continuously dividing and people isolate such colonies of cell and store it in liquid nitrogen and take them out and again culture them again subculture them we will come to these words do not worry about it what is culturing what is sub culturing and all those things.

But just for the time being think of it key I am a mass of cell, unique mass of cell, and I can grow them, I can divide them, I can take a part of it like this, and I can store it, and I go this once and do my experiment I pulled out this part and take again growth and, again store a part of it; likewise every time whenever I am dividing them I am taking a part and store it in liquid nitrogen when after that again I am using it and I can make it in number of aliquots of it and I take aliquot I grow it and I again a take small part of it. This processes called sub culturing your continuously growing them, but I told you something and one such say line which is very commonly used call ‘Hela’ cell. Again will after the name of henrietta lack we will come to this story of this hela cell, but here what I wish to highlight is something much more I should say interesting and precautionary.

Every time a cell is going through this cycle it loses I told you part of it is telomere because telemeter is activity changes. Now, there are several labs in the world who use hela cells somewhere in 19 50 this whole cells line has developed. We have no clue that how many cycles these cells have gone through and still we are using it, and there are several reports I will dug out I will try to dug out those reports some of them for you to read those cells behave now very deserved several places this is happening.

Cell lines over period of time growing them in an synthetic artificial conditions time and again time and again time and again time and again gives us results which are fairly (Refer Time: 22:12) and why is it I told you that every cell is this division process, what I tried to tell you is this division process what I am telling is it never ending you can divide it forever possibly no it has a threshold limit and many a times we do push it beyond that limit.

Whenever you are sub culturing or you know this process this process for passaging the cells over generations after generations one has to keep track that how many time this is divided at least because of course, you do not even know that when you receive the stock how many times it has already divided before this, but at least you can keep a tab or at an what you one can do is something called a carrier typing process, where you can analyze the chromosomes time to time if you are a person who are using those kind of you know cells cell lines you should carrier type it an see, how close or it is resembling to the original stock or the description the original stock some 40 50 years back how close you are too that or has it reached to a point that in a it has to be you know thrown away it is no more really worth using .

One has to always go through that exercise time and again time and again to figure out, that hope I am not using something which is which will give me ambiguous results. And in order to appreciate that one has to understand this basic fundamental the this process is a chemical process, it goes through that cycle and it has it is checkpoints check and balance in the form of cyclins and cdk kinases which are playing tremendous role what I am I interrupting what is the question I am having does this question matters about it is genetic integrity of that cell maybe it does not matter may be you are using it is a sensor it does not matter as long as that sensing a protein is being synthesized by it is perfectly fine.

But maybe it matters you were doing some other kind of experiments with it. These are the basic questions as a cell culturist one has to ask without asking this questions I mean it will be a blind walk in (Refer Time: 24:44) we will get some results and you will of course, in a paper also, but are you sure what you are talking, or what you are documenting.

That is why understanding of this fundamental concept is very very important we will come back. So, do not worry for those who are unable to track what is this cell line concept I am introducing we will come back to this, but for the time being understand that the cell either will divide an or some after division they will reach a point where they would not divide any further there will be differentiated. Yet there will be certain cells who will lose their permanently as of now what we know from the literature their differentiation ability and they are called de-differentiated cells. And yet there are certain cells which does not lose it permanently that differentiated potential their de adaptive cells. There will be several such things which will happen and then there are terms called transformations and all which will come which will be coming later once we will talk about the cell line cultures.

As of now what all things we have talked about is the Oxygen and Carbon dioxide milieu we have talked about the extracellular matrix, we have talked about the dynamic nature of in vivo system and how we can emulate that in vivo system using the modern technology is a micro fluidic systems and we will be talking later about all those end up detail and the most recent studies in that area till 20 20 20 20 17.

And now we are talking about the cell cycle very briefly to give you an feel about the challenges what you will be coming across while we will be talking about the cell lines and primary culture again this is another new term, we will come back later into it. I will close in here in the next class we will talk little bit more about the biology of the cultured cell before we move on to the other aspect of cell culture technology.

Thanks for your patience listening.