Biomaterials for Bone Tissue Engineering Applications
Professor Bikramjit Basu
Materials Research Centre
Indian Institute of Science Bangalore
Module 04
Lecture No 16

So after the discussion on this cell division let me give you some numbers for you to realize that how fast or how slow a typical eukaryotic cells, they grow. This slide actually shows you some of the numbers for some specific cells.

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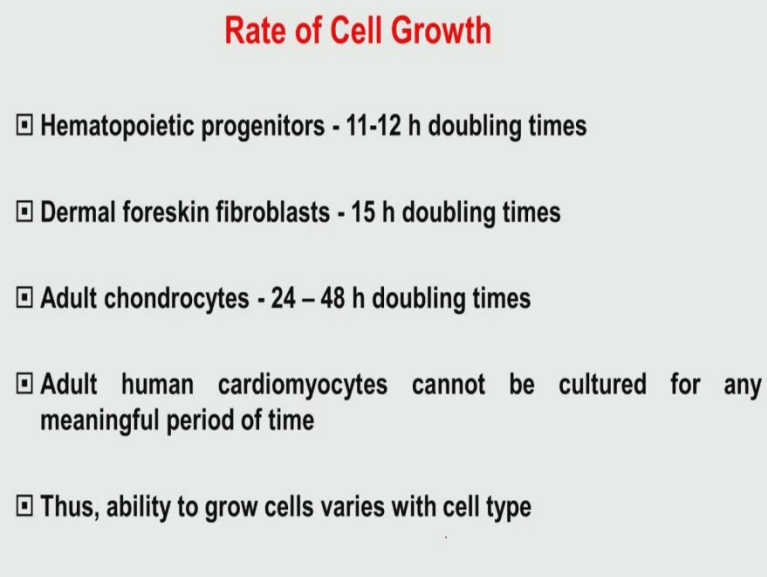

Adult chondrocytes the cell division doubling time is 24-48 hours so chondrocytes means this is a cartilage cells. Dermal Fibroblast the cell doubling time is 15 hours. Whereas hematopoietic progenitors they typically double in 11-12 hours. Cardiomyocytes which is a cardiac cell, this cardio myocytes cannot be cultured in any meaningful period of time because it is very difficult to culture.

So clearly the cell division or cell doubling time depends on the cell type. Human osteoblast their division time, cell doubling time is typically 48 hours. Whereas fibro blast they grow little fast 15 hours.

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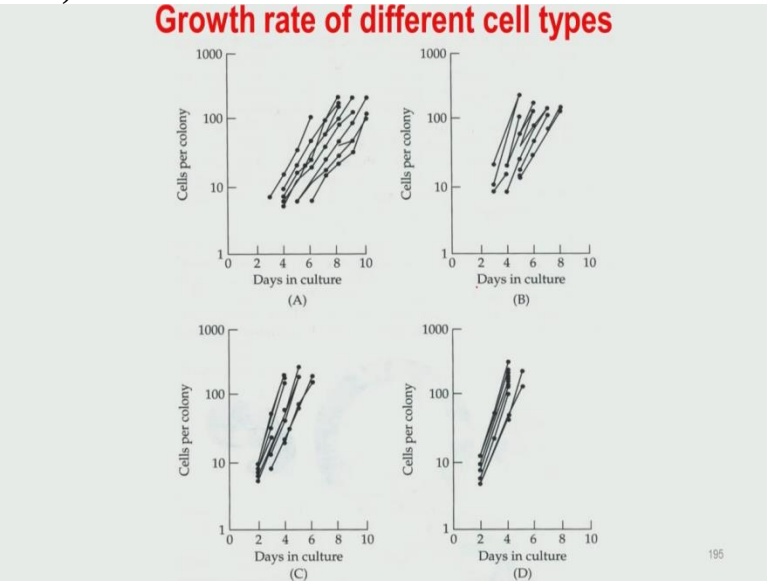

Now when you grow the cells in culture one of the immediate parameters that researchers they use just to see that whether this cells grow in a predictable manner or not, that is, they count the number of cells after growing them in different time scale like 4 days, 6 days, 8 days, and so on like over a week or more than a week. Now that depends on what is a typical cell doubling time.

Now this slide shows you some of this data taken from different cell types and mostly you can see that this cell growth is actually taking mostly the linear shape so essentially the number of cells that linearly grow with time and culture.

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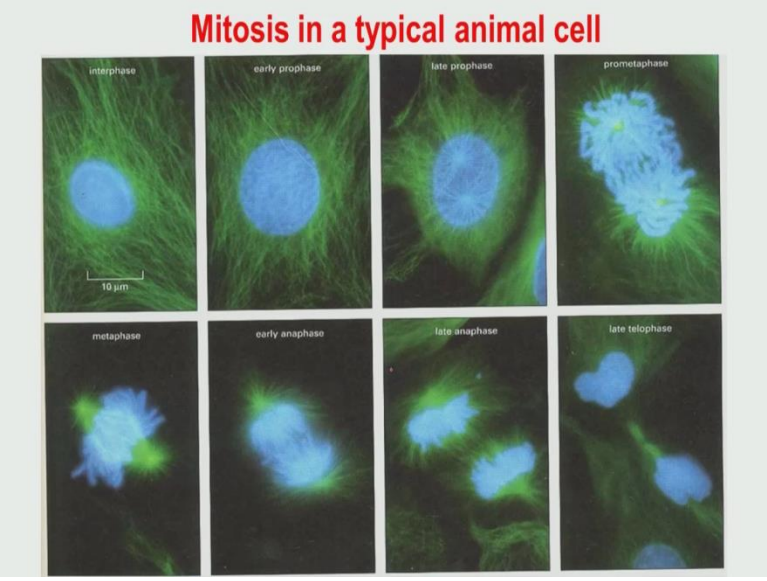

This is some of the (())(2:29) microscopic images just to show the progress of cell division of a typical animal cell and what you see that slowly cells increase in size. You see that nucleus that all that significantly increases in size during the early stage of the cell division. And then there is typical increase in this so increase in the cell nucleus is an indication that cell is preparing itself for the cell division.

And slowly that in during the different stages of the cell division, at the late stage you can see that this the cytoplasm of one daughter cell is almost physically separated from another daughter cell. And this cytokinesis is the last step where the formation of the two daughter cells from a mother cell is complete.

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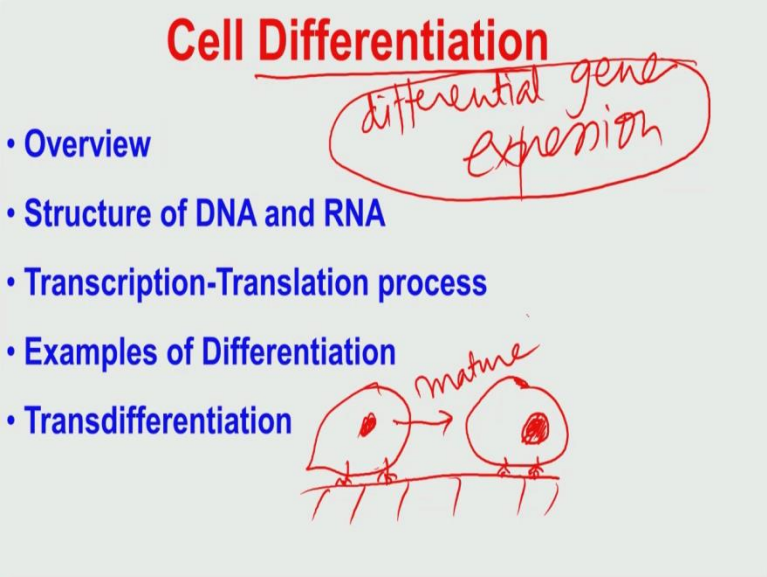

So next thing I would like to start with and I would like to complete in this particular module is the cell differentiation, and to this extent I will start with the typical description of the structure of DNA and RNA. Because as I said differentiation means this is the different Gene expression, so it is a kind of cell fate processes, it is one of the cell fate process which involves differential Gene expression.

Now to go back to my earlier description of a cell fate processes, when a cell adheres on a material substrate, the question that we need to address that whether the cell changes in cell fate processes, changes in cell fate. So that means that whether the cell functionality is changed and the cell functionality changes can be assessed by certain differentiation assess and see that whether cell goes to a more mature cell type.

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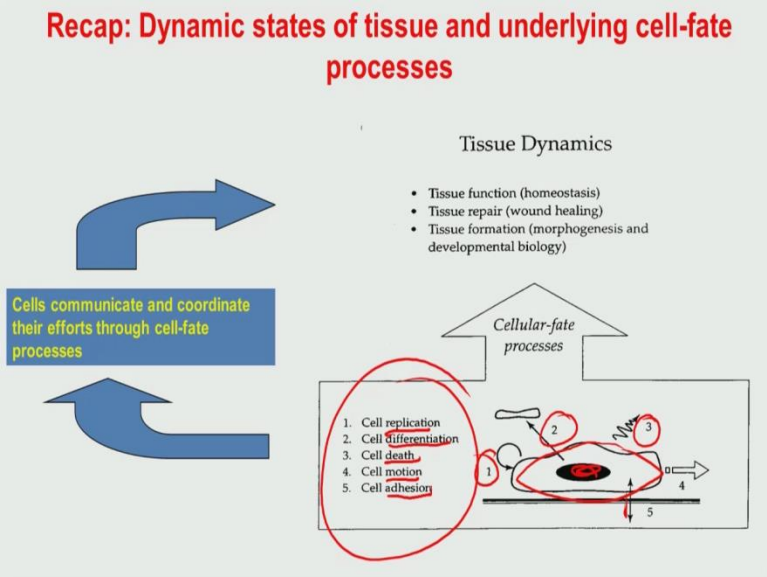

Now to understand the differentiation and to quantify the gene expression you need to first start, you need to first understand that what is the structure of two nucleic acids of importance that is the derybo nucleic acids and rybo nucleic acids, DNA and RNA. So these ones I have mentioned you before that once a cells adheres on a material substrate that what are the different fate processes that a cell can decide or cell can choose to take it up. Replication that is the division, differentiation, death, cell death, cell motion and cell adhesion.

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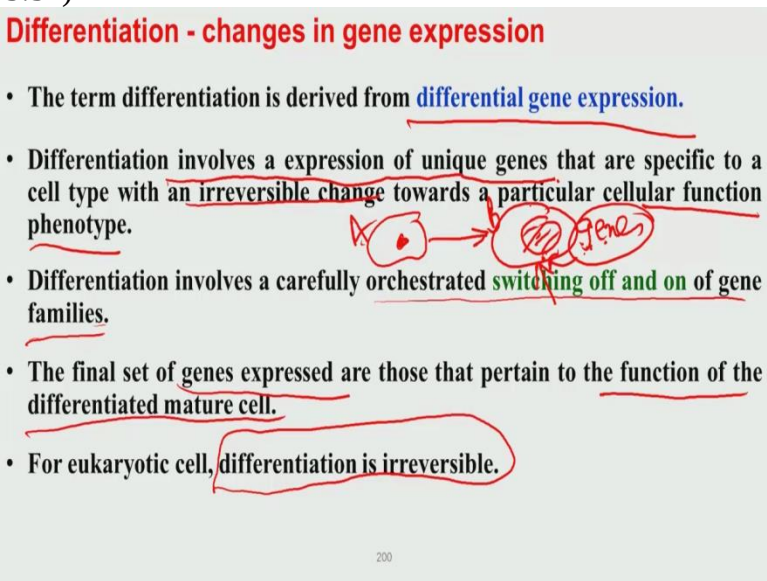

So I already mentioned the differentiation essentially means the differential gene expression. So therefore this involves, one of the things that you must remember in the differentiation process, that involves the expression of unique genes that are more specific to the mature cell type. So that means that you have one cell type which is little bit pre mature and one cell type which is much more mature cell type.

So genes which are specific to these particular mature cell type, that should be expressed and that are specific to, with an irreversible change towards a particular cellular function phenotype. So the gene specific to the mature cell type that should be expressed or that should be up regulated and then only you can confirm yes a cell A undergoes differentiation to cell B and certainly cell B will have different functionality compared to cell A.

Ok so the third point that has been mentioned here that is, it involves orchestrated switching off and on, of certain gene families. So these certain specific gene families which are switched on that means that will be expressed now or that will be up regulated now which will be related to the more mature cell type here. And final set of genes which are expressed or which are up regulated that will pertain to the function of the differentiated mature cell itself. And like cell division process, differentiation is irreversible process, that means that once a cell goes from A to B, that is this initially this is irreversible in nature.

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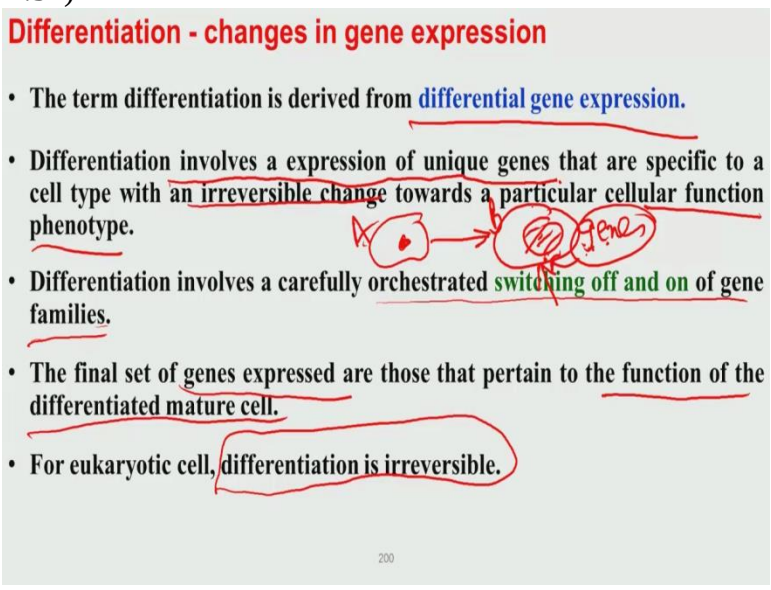

Now gene, what is gene? Gene is nucleotide sequence in a DNA molecule that functions to synthesize proteins, structure RNA and catalytic RNA molecules. So essentially gene is defined as a nucleotide sequence in a deoxyrybonucleic acid. And genome is a complete set of information in an organism is DNA. Now this information undergoes transition or change from DNA to RNA that means information is carried over form DNA or RNA.

Organisms differ from one another because their respective DNA molecules have different nuclei type sequence and therefore carry different biological messages. And genes which carry biological information that must be copied accurately for transmission to the next generation each time the cell divides to form two daughters cells.

That means once a cell goes to two daughter cells, so these genes are copied accurately. So these genes from that mother cell is to be copied accurately to two daughter cells each time the cell divides. A typical human cell contains 2 meter long DNA. 2 meter long DNA means, you can understand that it is much longer that the height of a even a tall person.

That this long DNA, this the DNA length that means height can be much larger longer than the height of a single person but it is essentially squeezed in a nucleus in an extremely coiled form and it also carries the in instructions for 30000 different proteins.

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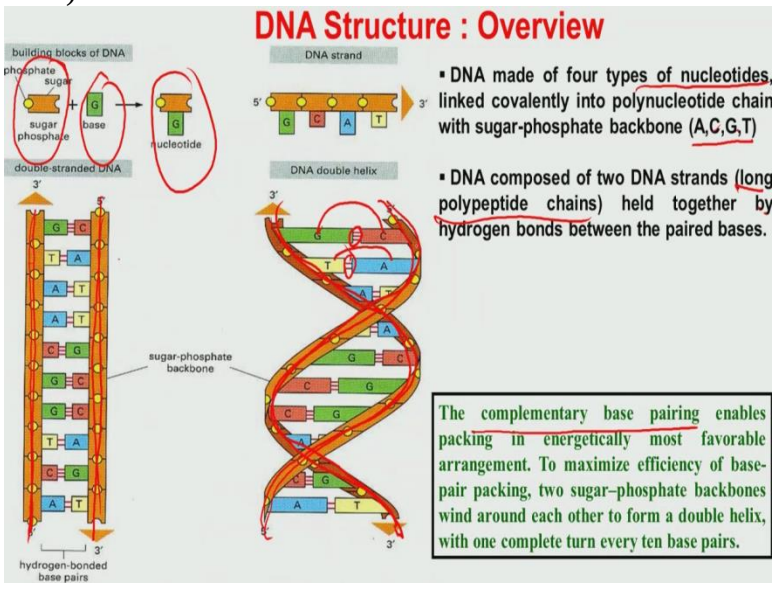

Now DNA structure, it appears often on the cover page of many standard biological text book and many of you have read the DNA structure even in your school biology as well. So this is a typical double helical structure. And DNA double helical structure was discovered quite some time back. Now you can describe this structure in a more simplistic or to start with you can describe this structure as two backbone chain so this is your two, so DNA is essentially made of four type of nucleotides.

A, C, G, T so adenine, cytosine, guanine and thymine. So this A is to always pair with T and C has to always pair with G. And this pairing is involved through some chemical bonding here. So you have two chemical strand, this is strand no 1, this is strand no 2, another thing you can see that this strand is essentially replica of one another. So these two long polypeptide chains are there and along this chain you have the sugar phosphate and base that is it forms a nucleotide here.

So the complementary base pairing that is a very important thing in DNA structure, enables backing in energetically most favorable arrangement. What it means that this double helical structure essentially represents the lowest energy configuration, because you know from basic thermodynamics any structure which in its equilibrium shape must represents the lowest energy configuration then only that structure would be more stable.

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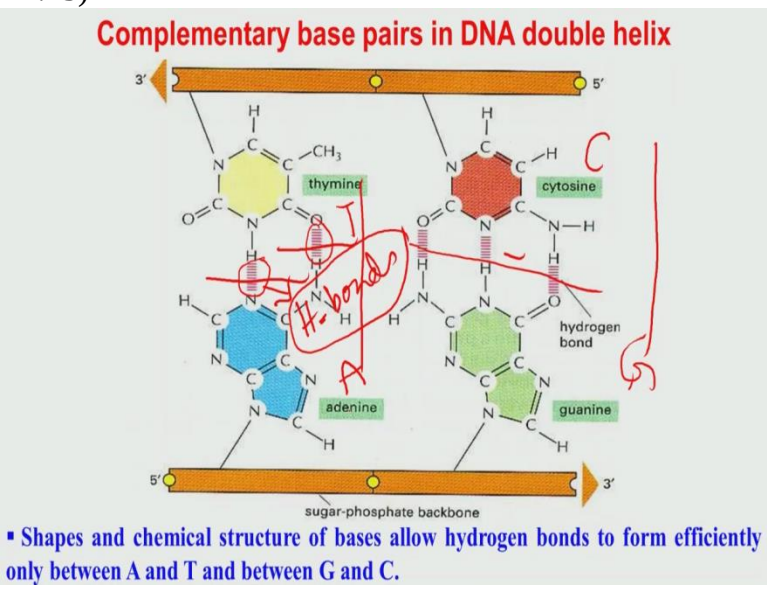

So this is more elaborate view of this A, T, C, G . So this your A adenine, this is your thymine, so you have this specific bonds here. And this is your C and this is your G, so these are the two base pairing. So this is one base pairing and this is another base pairing. And these bonds are essentially very weak bonds.

So these bonds are hydrogen bonds. So the advantages of having hydrogen bonds in this base pairing is that, that if required they can be simply removed and therefore these two strands can be separated easily, simply because these bonds which enable or which mediate the pairing between C and G or A and T are extremely weak that is the hydrogen bonds.

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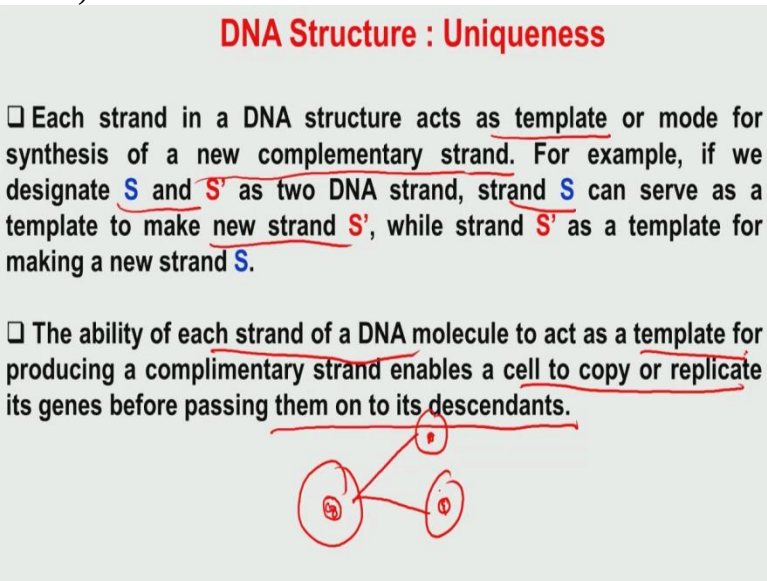

So there are certain uniqueness of the DNA structure which has been summarized here. So each strand in a DNA structure access a template as I told you that it forms, it helps to synthesize new complimentary strand and, so if you designate S and S prime as two DNA energy strands so strand S essentially serve us to template for the new strand that is S prime and the ability of each stand of a DNA molecule to act as a template enables a cell to copy or replicate its genes accurately down to two daughter cells. So you have one mother cell, it goes to two daughter cells, so essentially the DNA sequence here is to be exactly to be replicated in two daughter cells.

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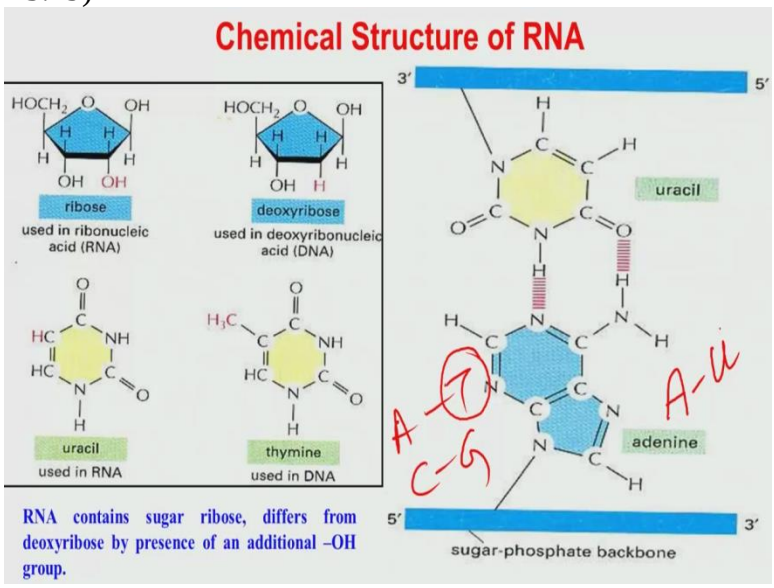

Ribonucleic acid, their point of dissimilarity with respect to DNA structure is that in the Ribonucleic acid in a DNA structure you have A, T and then C, G. In Ribonucleic acid you have uracil in place of thymine. So therefore you are A will base pair with U that is uracil.

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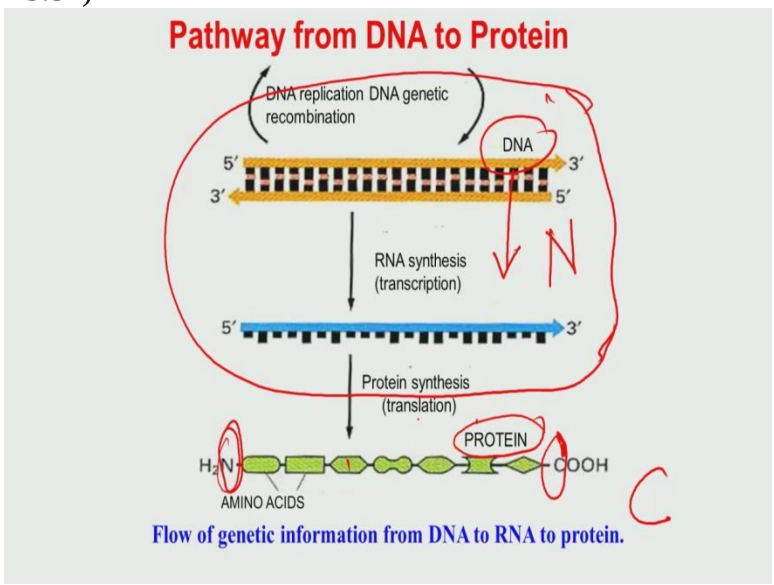

So one of the characteristics of a cell, eukaryotic cell is that the DNA replication or DNA genetic recombination. So from DNA you can, DNA can undergo transcription process to form RNA, so that takes place within the nucleus itself. And this is your cytoplasm so that is your nucleus, so in the cytoplasm this RNA can undergo translation to from a protein molecule. As I mentioned to

you before that protein in a given protein molecule, n terminal will always be kept at the left hand side. And c terminal will always be kept at the right hand side.

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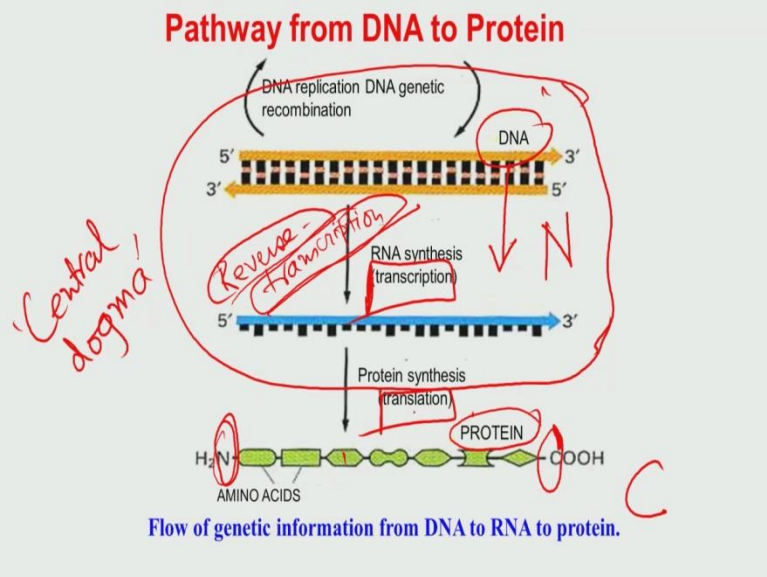

So this DNA to RNA and RNA to protein, so first one is your transcription process and second one is a translation process. The way this was discovered, early stage of this, at the early stage people used to think that this transcription and translation process is one way process. So once DNA comes to RNA, RNA cannot go back to DNA, but later on people have found out that reverse transcription that means that RNA also, the information contained in RNA can also go and change and form to DNA.

So this reverse transcription process is also possible. And therefore this entire theory of transcription, translation in the molecular biology its mentioned as the central dogma, in the molecular biology theory.

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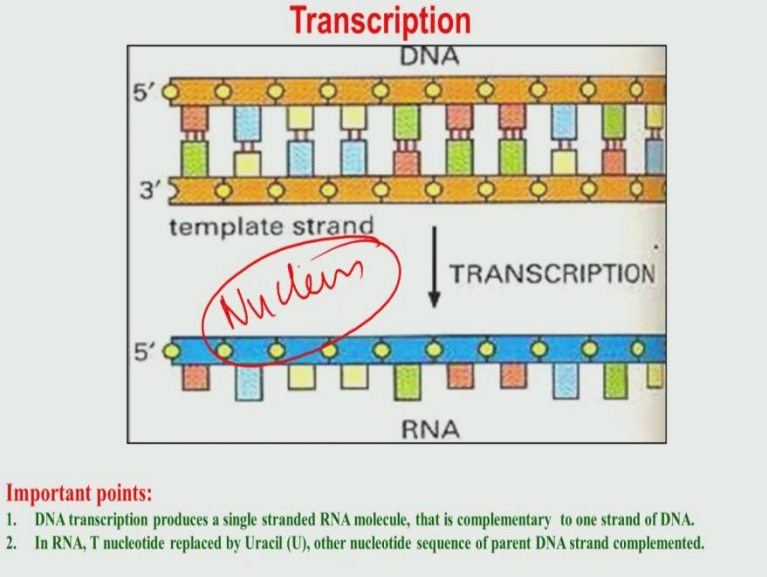

So this is more mentioned here. This DNA transcription produces a single stranded RNA molecule and that is complementary to one strand of DNA. What it means is that, so 5.3 prime. And then it is complimentary when it forms that RNA in the transcription process. And remember in a eukaryotic cell this transition process always takes well within the nucleus.

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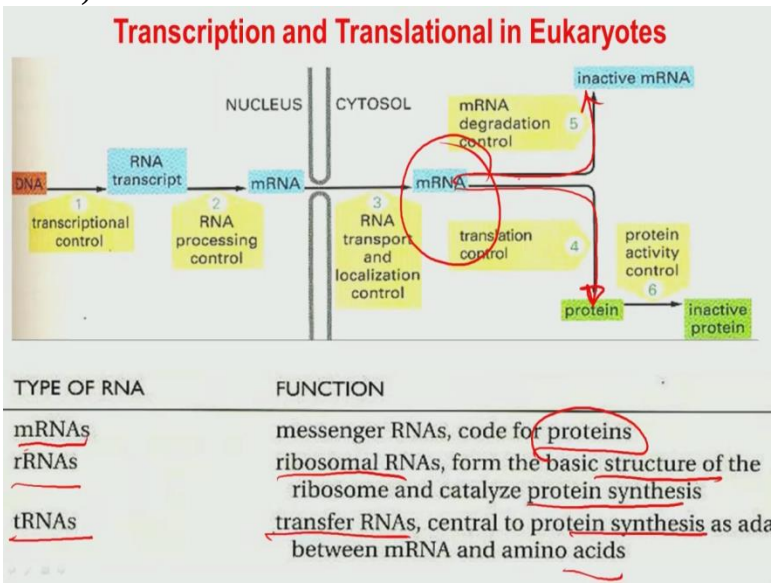

So this one has been shown here. So you have different types of RNA. M RNA stands for messenger RNA and it contains the codes for protein which will be ultimately synthesized in the

cytoplasm of the cells that is outside nucleus. Then you gave ribosomal RNA that is r RNA and this actually basic structure of ribosome and catalyzes the protein synthesis.

Then you have a transfer RNA that is t RNA, that is is central to protein synthesis and also this essentially, is also this is one of that another RNA type which also takes part in certain biological process. So DNA to RNA, mRNA so mRNA is now comes to the cytoplasm, through this nucleus core complex that mpc, which I have mentioned before. And then from mRNA it has two options either it can be undergo transcript or it can undergo translation to synthesize protein in the cytoplasm.

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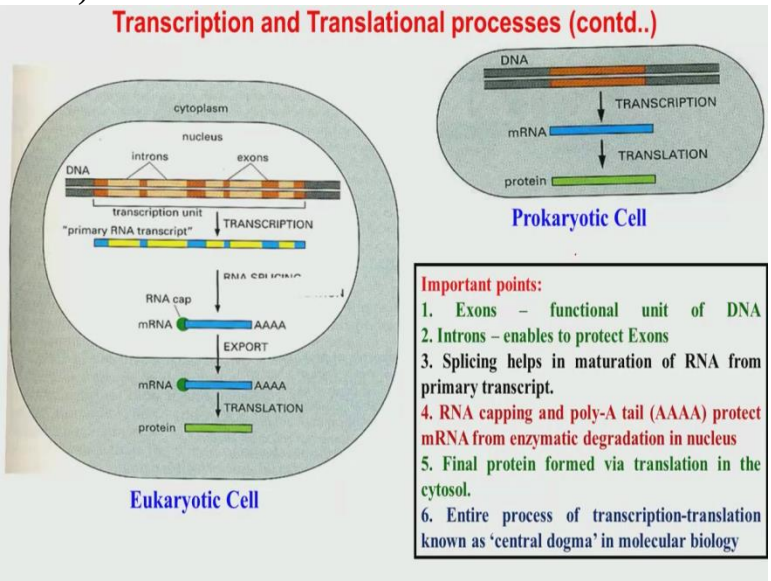

This entire thing has been shown here in the prokaryotic cells, that within the cytoplasm itself the transcription, translation process takes place. Simply because prokaryotes does not have very well defined nucleus within the bacterial, within the prokaryotes.

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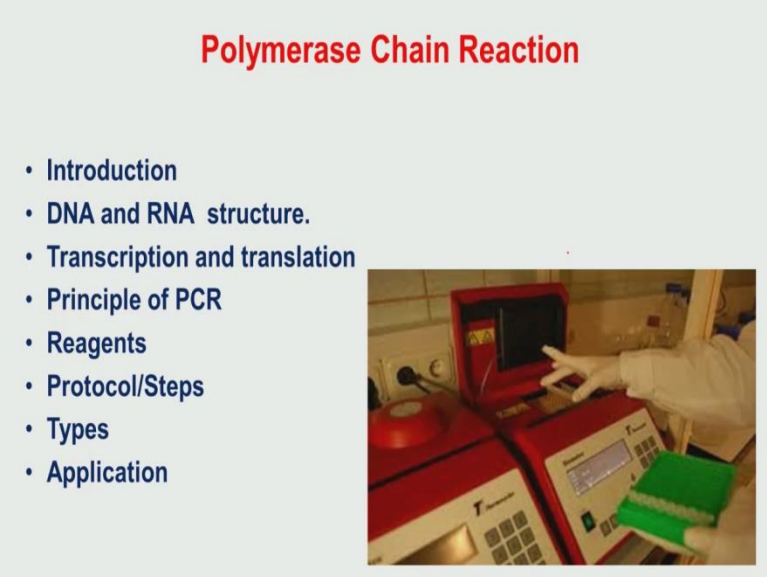

So this one of the techniques that is used in the cell biology. It is a polymerase chain reaction. Now what is this polymerase chain reaction? So once a cell differentiates to another cell, a cell is committed to undergo differentiation to another cell type, then you have to extract mRNA from the culture cells. Now the quantity of mRNA can be extremely small.

So you have to amplify the this mRNA expression and this amplification take place in using, this this amplification is possible if mRNA undergoes now polymerase chain reaction.

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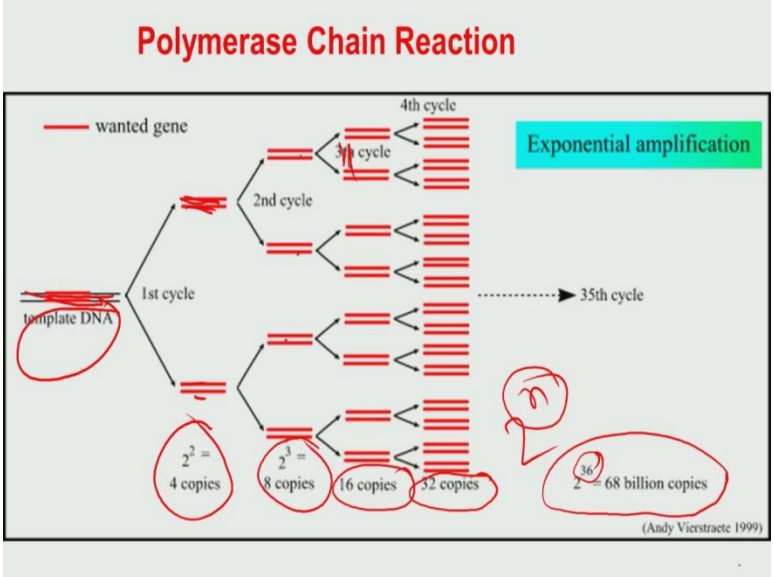

So it is essentially a technique which is used to amplify the number of copies of a specific region of DNA in order to produce enough DNA to be adequately tested. So there is a detailed biology involved in discussing that PCR, but what is more important and more simplistic for you to realize that you have the double standard DNA. So this is your template DNA, in the first cycle it forms two copies here. So this essentially this is the two pairs for copies so the total copies is four.

In the second cycle each of these copies will now produce another four copies so the second cycle essentially, 4, 4, 8 copies that will form. In the third cycle it again that each of this, each of this pair of copies will produce again four, so its 16 copies finally and likewise it will undergo exponential amplification and then it would be 2 to the power n stop so if n is the total number of cycles, and then let is say if it undergoes 36 cycle or at the end of 35th cycle you have such a large number of copies like 68 billion copies that will be produced which will be quite good enough to now quantify or to be quantified using this particular technique.

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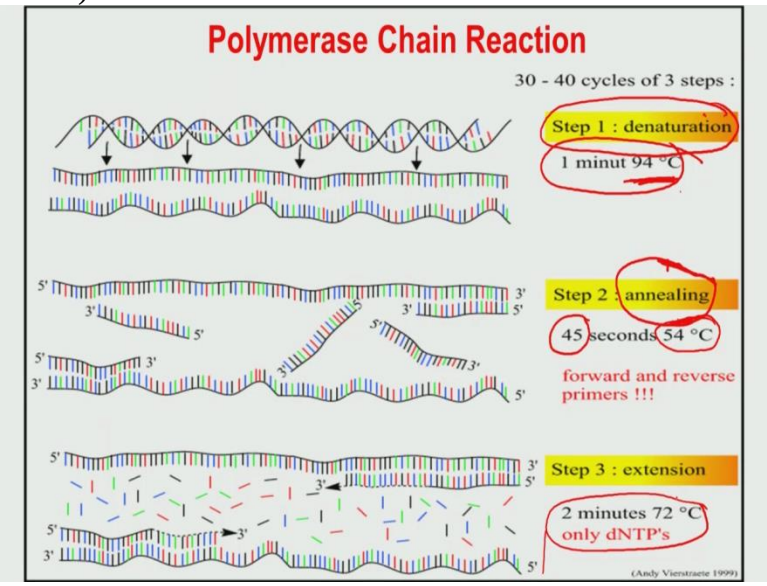

So this is that more PCR cycle what they call the polymerase chain reaction. So first step is that step one is the denaturation. Denaturation takes place and these number are very specific and this is this typically takes place at 94 degree Celsius, below 100 degree Celsius, then something called annealing. The term annealing means it is one of the term which is used in material science which is like heat treatment.

So essentially this heat treatment in the biology essentially takes place at a much slower time, much lower temperature like 54 degree Celsius and very short time period like 54,45 seconds, less than a minute and then you have a extension, and extension is 72 degree Celsius and that is for 2 minutes.

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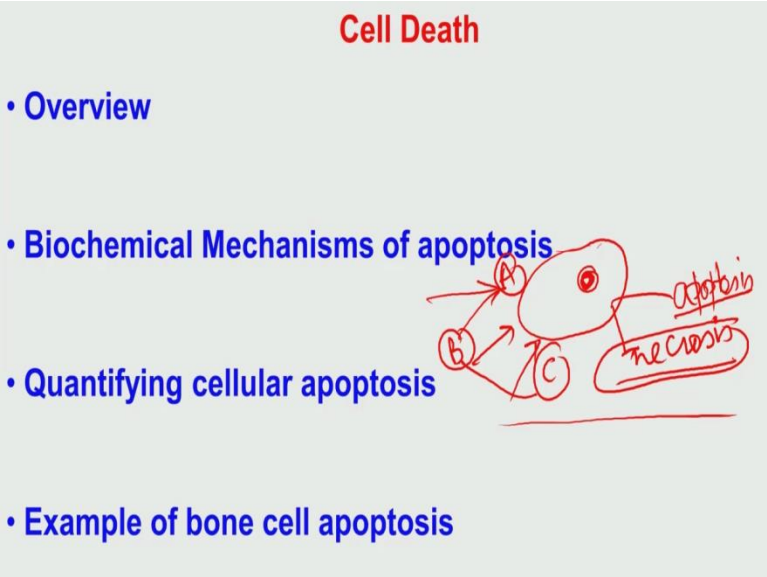

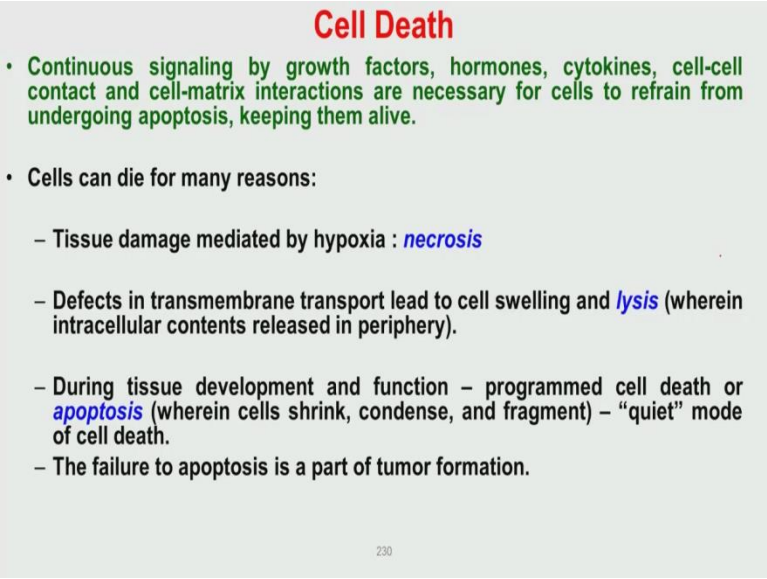

Ok so I think that, so from the PCR, expression then you can see that from the PCR experiments if you can quantify that whether this certain specific genes related to the mature cell type, is now up regulated or it is been quantified then only you can confirm the cell differentiation process.

Third one is the cell death so essentially if you go back to my earlier discussion or you can recall my earlier discussion that a cell to be viable or cell to survive cell has to receive different type of signal molecules A, B, C.

And then if each of these or some of these signaling signal molecules are removed or now absent in the cellular micro environment then cell has two options, then either cell will undergo apoptosis that is the programmed cell death or in case cell receives some physical injury or tissue receives some physical injury then it can undergo unprogrammed cell death and this is called necrosis. The way apoptosis and necrosis can be distinguished that is by morphological changes.

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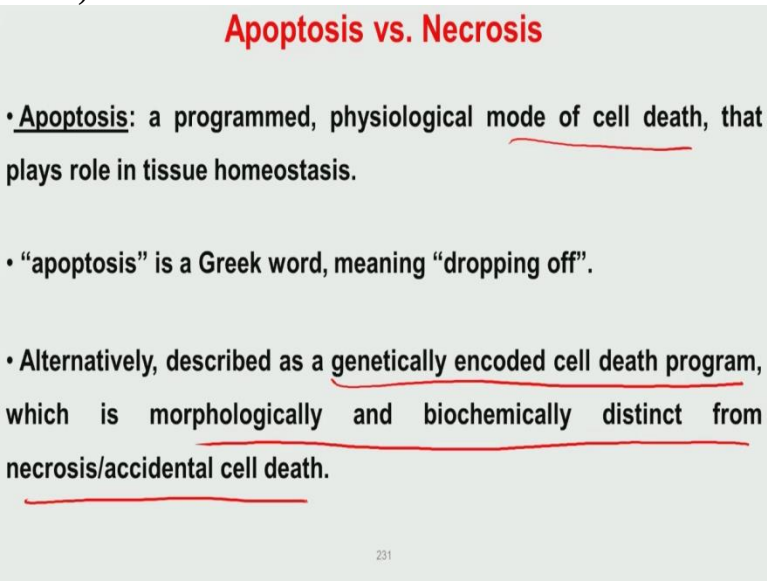

So apoptosis is a Greek word meaning dripping off. So dropping of leaves like the way leaves in certain specific season, they drops off from the plant, so similarly apoptosis is a programmed and physiological mode of cell death and that plays in tissue homeostasis and it is also described as a genetically coded cell death program which is morphologically and biologically different from necrosis or accidental cell death.

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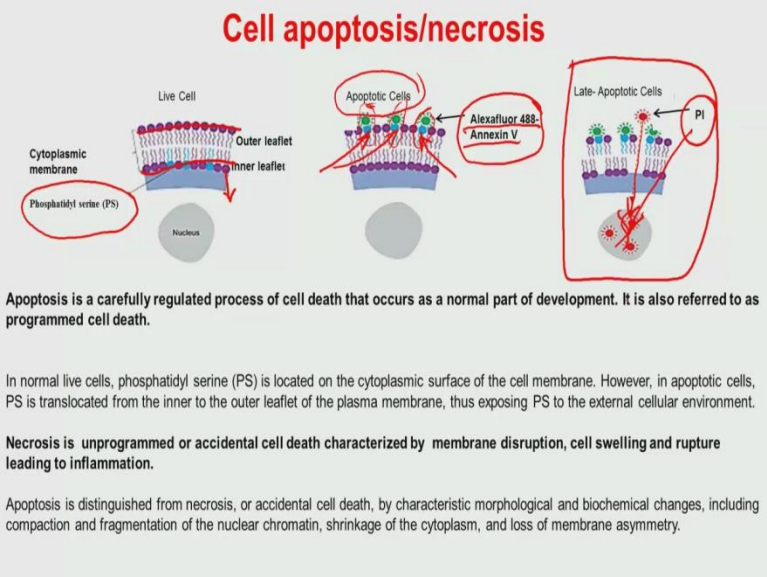

So how to quantify or how to identify this cell apoptosis process? In the cell membrane you have the double layer, double lipid bi layer structure. In a live cell, when the cell is live, then you have specific molecules, biology, protein molecules, phosphatidylserine, which is always located towards he cytoplasmic space of the cell membrane. That means it is attached to the inner leaflet of the cell.

When a cell undergoes apoptosis this phosphatidylserine which is shown here like a green sphere, this phosphatidylserine molecule will now be flipped towards the extra cellular side. That means this phosphatidylserine molecule will now be switched to the outside the, outer leaflet of the cell membrane. And this once it is located at the outer cell membrane then there is certain fluorescentdyes like Alexa 488 or Anacin, these can be tagged to the phosphatidylserine, so these local tagging can be quantified by technique called fluorescent activated cells analysis or flow cytometry technique.

Another thing that has been shown here, so there is another fluorescent dye is called propodeumiodate . Now this propodeum iodate it has unique property, like it can inter collect with the DNA of the cell. And once it inter collects with the DNA of cell then it will process in the blue stained region and then you can see that where the nucleus is located inside the cell.

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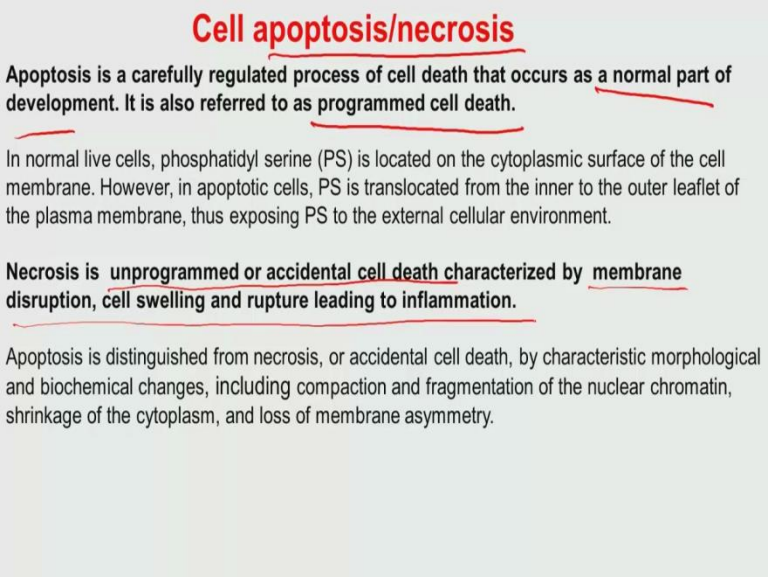

So these two mode of the death, one is the apoptosis and necrosis has been summarized here in this slide. So one is known as the very regulated process of cell death that occurs as a normal part of that cellular development and it is called phosphatidylserine its known as the programmed cell death. Another one is unprogrammed or accidental cell death, which is characteristically morphologically different by membrane disruption, cell, swelling and rupture often leading to inflammation.

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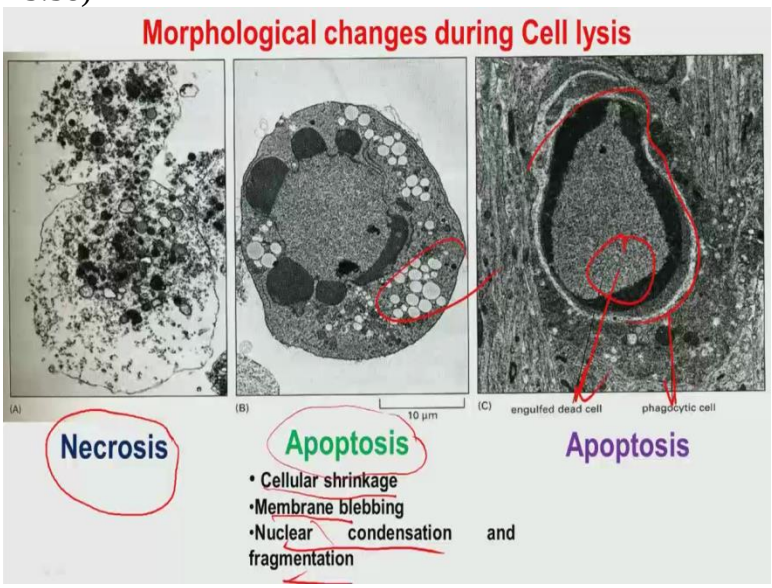

So this is the cell morphological feature during these two type, modes of death process one is the necrosis, you can see all the intra cellular substances are now coming out and the total destruction of the cell. And then apoptosis which is essentially characterized by cellular shrinkage, also membrane blabbing here you can see and nuclear condensation and fragmentation that you can see here, not that clearly but it is the end of dead cell. And this is a phagocytic cell.

So there is distinct morphological difference between necrosis and apoptosis by which you can identify that when you see that there is no morphological change in the cell when its trying to adhere on a material substrate it remains spherical, that means you can well realize that cell is not willing to grow on this material substrate, and probably cells are not alive and cells are dead.

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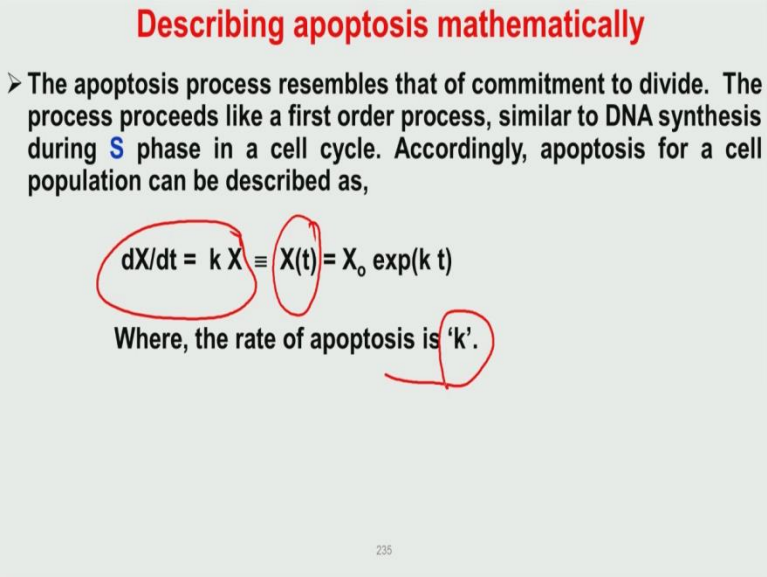

Like in the cell division process cell death processes also can be expressed by certain mathematical formulae and this is one of the things that has been shown very generally in the cell death process. Like rate of change of cell numbers with time dx by dt is equal to kx where k is the rate of apoptosis, so at any given point of time t, xt is equal to x not exponential kt, so this rate of apoptosis k essentially tells you that how fast the cell would undergo apoptosis.

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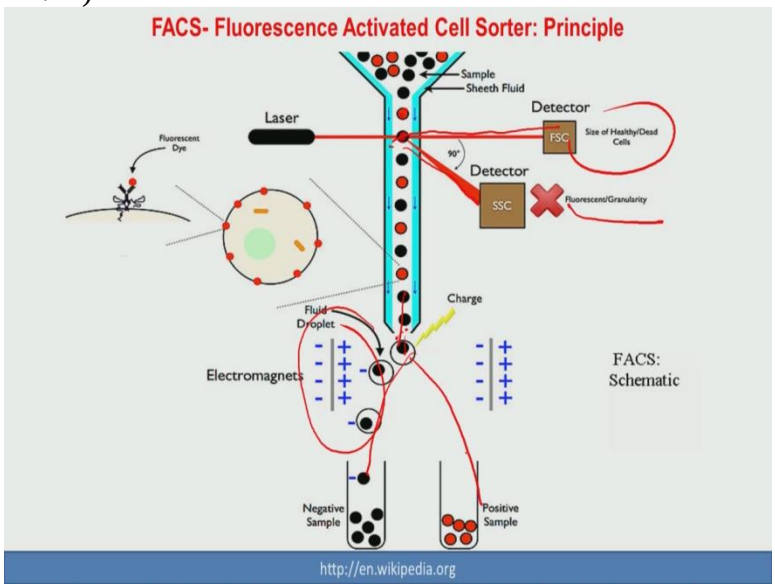

So one of the things that I have mentioned in the last two modules is that fluorescence activated cells sorter analysis, so this slide explains that what it means essentially. So fluorescence activated cells analysis is quite important because it allows single cell or one cell or single cell specific analysis like it can allow the identification of the cellular fate proceeded for a single cell. So you have a certain fluorescent stained cells which is made to flow through a column here and once it goes through this column then laser will be focusing on, this specific laser will be focusing on this individual cells.

Now these red stained cells and black stained cells are showing they have either in different sizes or they have a different cellular fate process. Now there is two different scatter, one is called side scatter and one is called forward scatter. So side scatter and forward scatter is shown here. So side scatter essentially tells you that a size of a healthy or dead cells, size of healthy or dead cells and forward scatter and side scatter essentially will tell you that what is the fluorescent and granularity changes.

Now once the cell goes through this fluorescent, this is the fax column, then if you induce certain or if you allow certain electromagnets to be placed there at the end of the column, then it will be, it will enable you to sort the cells depending on what kind of negatively stained samples and positively stained samples.

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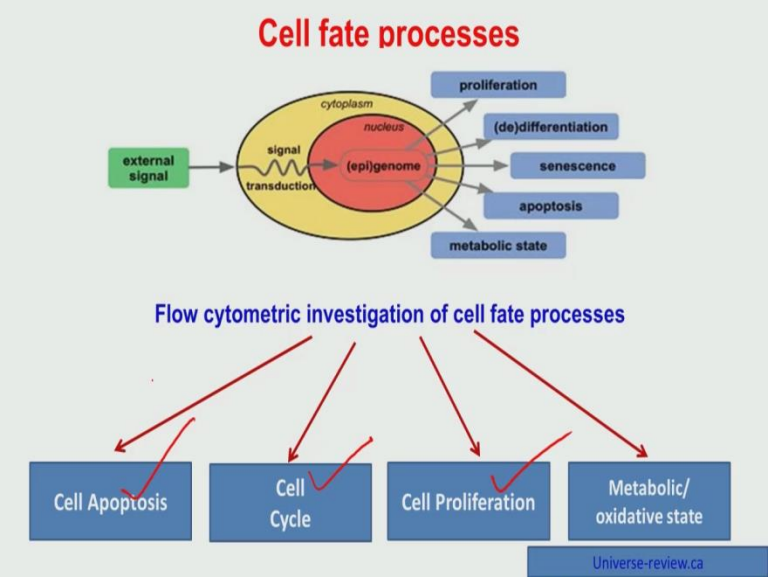

So this is this important process can be very useful to quantify cell proliferation or cell cycle analysis or cell apoptosis on a single cell specific basis. Now why this is unique in nature is because all other biological, all other bio chemical assess, they essentially deals a given cell population where a large number of cells are present. So one cannot therefore identify whether individual cell, what kind of cell fate processes they are actually at.

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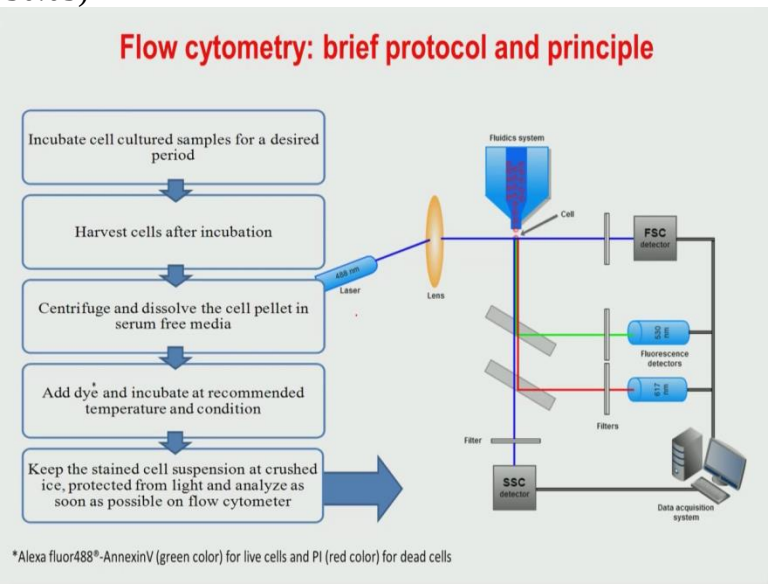

So this is again shown here in the side scatter and forward scatter and this is a typical protocol that that a researcher follows while analyzing, while using the cell date processes.

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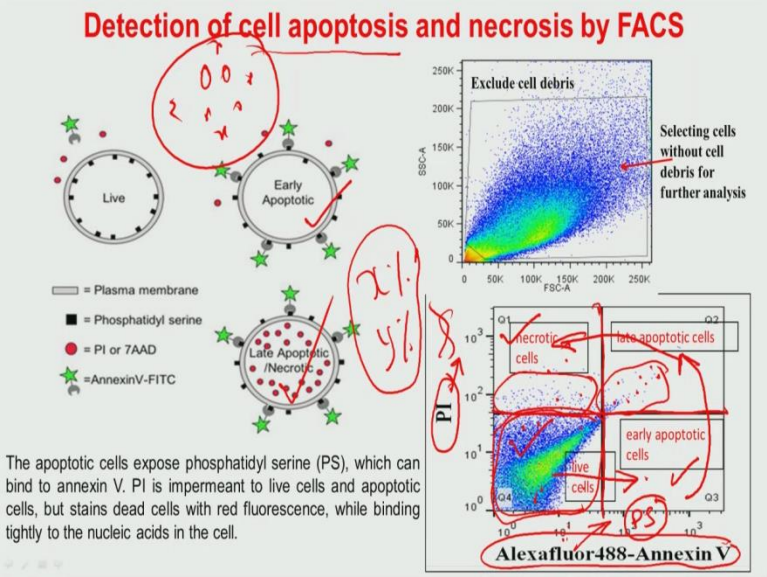

Now one of the things that can be very useful for in to analyze is that how to quantify in a given cell population, suppose you have a large cell population, let is say 10 to the power 3, 10 to the power 4 number of cells, we need to know whether that x percentage cells are apoptotic cells and then y percentage cells are necrotic cells. Now this distinction can be made by this fluorescent activated cells analysis, even you can also see that whether the cell is early stage of apoptotic or late stage of apoptotic.

So what are the stain fluorescent dye that you have to use. One is the Alexa Fluor 488. So this one will tag two phosphatidylserine molecule as I mentioned before. Propodea myodite will inter collect to DNA double helical structure as I mentioned before. Now what you can see here, this is the last slide before the end of this module. So this is the four quadrants stop so q1 that is quadrant no 1, here you have a very large expression of pi but very small expression of, lower expression of Alexa 488.

So this is the necrotic cells. The live cells means it is stained with the PI but the Alexa Fluor 488 also stained but it is in the lower most quadrant here. Each dot in the fluorescent activated cell sort essentially represent the information from individual cells. So the more number of dots in a particular given quadrant means more number of cells or larger cells fractions are present in that particular quadrant.

If you go from this quadrant to this quadrant that means cells are in the different stages of cell fate process. So this one is your live cells, this one is earlier apoptotic, then this goes to later apoptotic and finally necrotic. So from these simple things what you can see here, very few cells are in the later apoptotic and necrotic so more than 95 percent of cells are in the live cells so that means that this particular cell population is largely alive with negligible fractions of the cells are in the apoptotic or necrotic stage.