Introduction to Biomaterials
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Lecture No. # 08

Cell Differentiation and Cell Death

Ok, in today's lecture we will be discussing the cell differentiation process.

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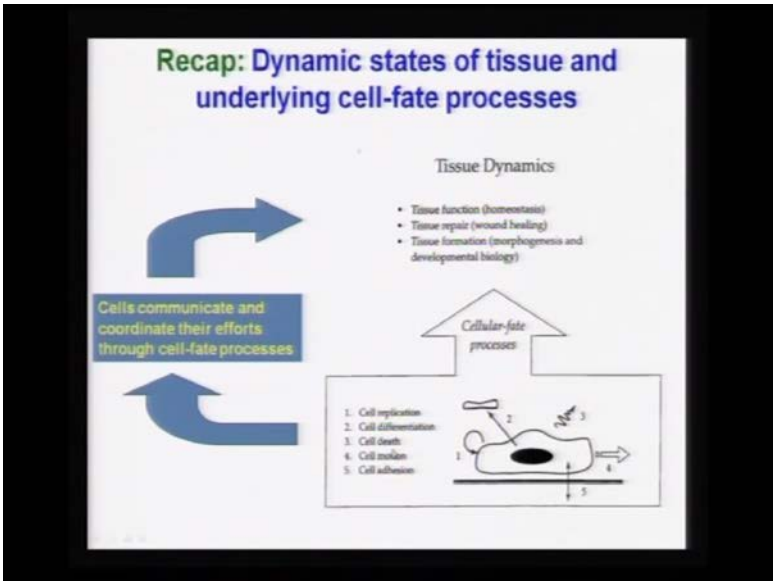

So, before we move on to the cell differentiation, let us recapitulate what I have shown in last one or two lectures. So, essentially, cell differentiation is one of the cell-fate processes and other cell-fate processes include, cell replication, cell death, cell motility or cell migration and cell adhesion. So, and all these cell-fate processes, they have direct relevance for the tissue function.

So, if a, if a tissue functions its normal functionality, then it is called homeostasis. Homeostasis means, homeo means same and homeostasis means, for the self-survival of the tissue, the functionality of a given tissue is known as a homeostasis. Tissue repair in biological term is known as wound healing and tissue formation, it is known as morphogenesis and it is part of developmental biology.

Now, as I have explained to you earlier that cell communication via different cell signaling processes are important in all these cell-fate processes, like replication, differentiation, death, migration and adhesion.

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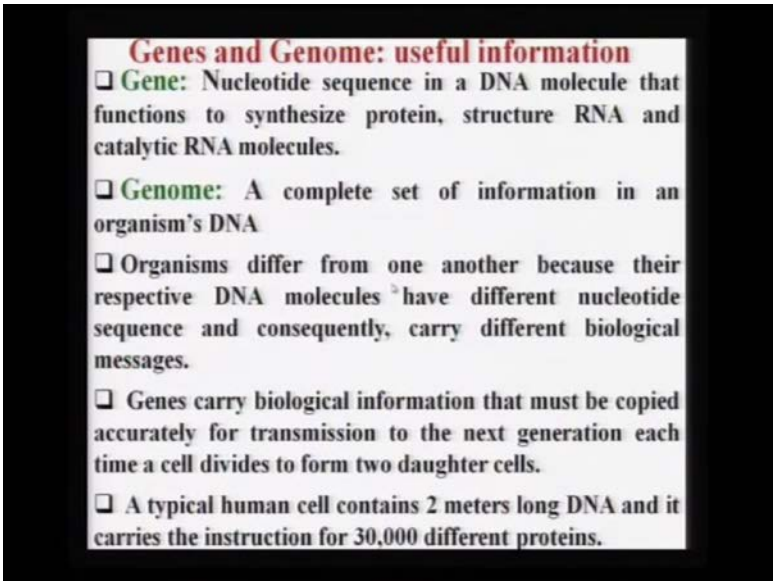

Now, as differentiation is known as the differential gene expression or differential cell functionality, we should first know that what is gene and genome? So, therefore, this slide provides you some important or useful information.

So, gene is actually nucleotide sequence in a DNA molecule; DNA stands for deoxyribonucleic acid. So, nucleotide sequence in a DNA molecule that functions to synthesize protein, structure RNA and catalytic RNA molecules. So, what is the function of DNA? DNA's function is to synthesize protein and what is, how this protein synthesized, that we will come to little later, that is, via formation of RNA and then, from RNA, the protein is synthesized. Now, structure RNA, catalytic RNA molecules, these are like different types of RNA molecules.

And genome, what is genome? A complete set of information in an organism's DNA is known as genome. So, gene is the nucleotide sequence in DNA and genome is the complete set of information, that are contained, in a gene, in an organism's DNA. So, organisms differ from one another because their respective DNA molecules are different

nucleotide sequence. So, two organisms, they are different because their respective DNA has two different nucleotide sequences, and what is this nucleotide sequence?

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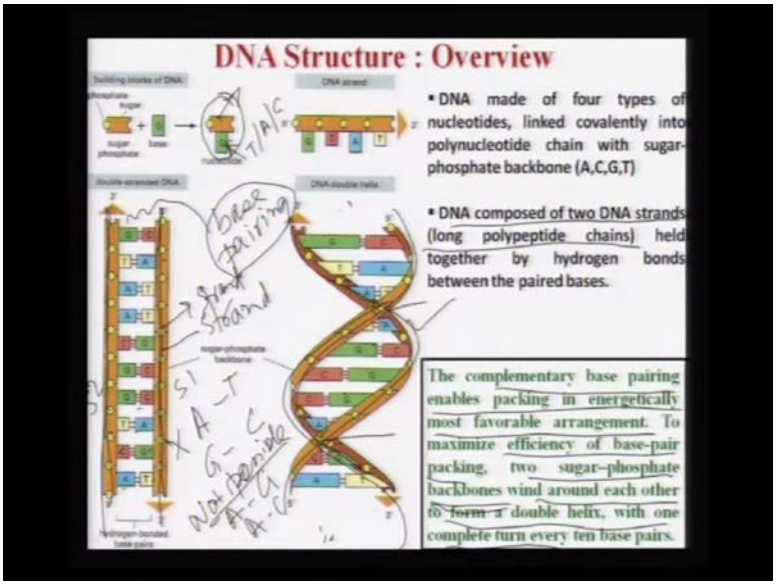

Typically, this is that important slide; so, this is DNA structure. Now, if you look at the, from the top of the slide's here, top left, so what is the building blocks of DNA? Building blocks of DNA are phosphate, sugar phosphate and there is one base, so this is called base paring. So, this for sugar phosphate plus base that is what is known as the nucleotide.

So, when I say, the nucleotide sequence, nucleotide sequence means, that this phosphate sugar or sugar phosphate, that remains the same, only your base, that is, base that is different, so this base is, here it is mentioned as G. G stands for, so there are four types of bases, A, T, G, C: A for adenine, T for thiamine, G for guanine and C for cytosine; so, these are like four bases. Now, A, T, G, C and the way this base pairing takes place, it is like A pairs with T, that means, adenine pairs with thiamine; C pairs with G, that means cytosine pairs with guanine, that is a typical base pairing that is observed in a typical DNA sequence.

Now, I repeat, now if you remember, that what is the protein structure, what is the building blocks of a protein structure, that is amino acids, right. Now, two amino acids, they come together, they join by the peptide bonds and then, they form, what is known as polypeptide and this polypeptides, they can interact, they can fold and all those things

and they can give different shapes of a protein structure, be it primary structure be it secondary structure, be it tertiary structure, be it quaternary structure.

So similarly, DNA molecules, the base structure or base is known as the nucleotide base structure, means that is, the building blocks of DNA, is known as the nucleotide. Now what is this nucleotide? Nucleotide contains one phosphate sugar molecule, so phosphate molecule means PO 4 3 minus and there is a sugar molecule and this phosphate sugar, that forms the backbone kind of a structure just like any polymeric molecule, CH-CH bonds, they form a backbone kind of structure. Similarly, for DNA, it is the sugar and phosphate, that forms the backbone structure and in this backbone structure you have four different bases, I repeat, adenine, thymine, guanine and cytosine.

Now, these are known as ATGC and so, this is your one backbone here, this is your another backbone here, in between this is known as the base paring. Now, this base paring, as you notice, that there is very specific base paring, A always pairs with T and G always pairs with C, that, that means, A would never pair with G or A would never pair with C. So, A-T is possible, G-C is possible, what is not possible is that, what is not possible is that A-G or A-C. So, and another thing is that, that so, these are four different.

Now, why four different nucleotides? The moment you replace G with T or A or C, then this nucleotide also would be different. So, similarly, you know, in a protein molecule when you have this polypeptide chains, now the moment you change one (( )) radical in one of the amino acids, then entire protein molecule composition and configuration also will change.

Similarly, here you have a, this one is the backbone and one is the base pairing, one is the G here, so the moment G is replaced by C, that is complete different nucleotide. The second (( )), now this is known as the individual strand. So, this is one strand and, and another one is the, this is second strand, so this is strand number one, s 1 and this is strand number two, s 2. So this backbone, in biological terminology, it is known as the strand. So, that is why, it has been written, the DNA composed of two DNA strands, long polypeptide chains and these two strands in between, these two strands, you have the base pairing A-T and G-C; A-T and G-C. The 3rd one is more important.

So, so, now you know, that DNA composed of four nucleotides, it has a strand. The strand is made of a sugar and phosphate molecule and this, there is base pairing, there is specific combination of base pairing, A-T and G-C, that is good enough.

Now, 2nd thing, that is important here is, that this complementary base pairing enables packing in energetically most favorable arrangement; energetically most favorable means, for any stable structure it has to minimize the total energy of the system, that is the law of nature.

So, here, these DNA molecules, if they have this double helix kind of a structure, then that has been biologically shown as the energetically most favorable structure. So, DNA molecules stable structure is not this one, but DNA molecule stable structure is this one. Now, how this structure, they just wind around against each other, this DNA structure and what you see here, that to maximize efficiency of base pair packing, two sugar phosphate backbones, two sugar phosphate backbones means, two strands wind around each other to form a double helix with one complimentary turn. With one complete turn every 10 base pairs, with one complete turn means, suppose from here to here, how many are there, 1, 2, 3, 4, 5, then similarly, in next one is that, so the one turn means, the moment it comes and makes the complete turn, so this, this complete turn, in this complete turn you have 5 here again, there will be 5 down, so 10 or 5 up would be there, so that would be 10, so total 10 base pairing makes 1 DNA strand, make complete turn. So, that is the way these structures are determined, is it clear?

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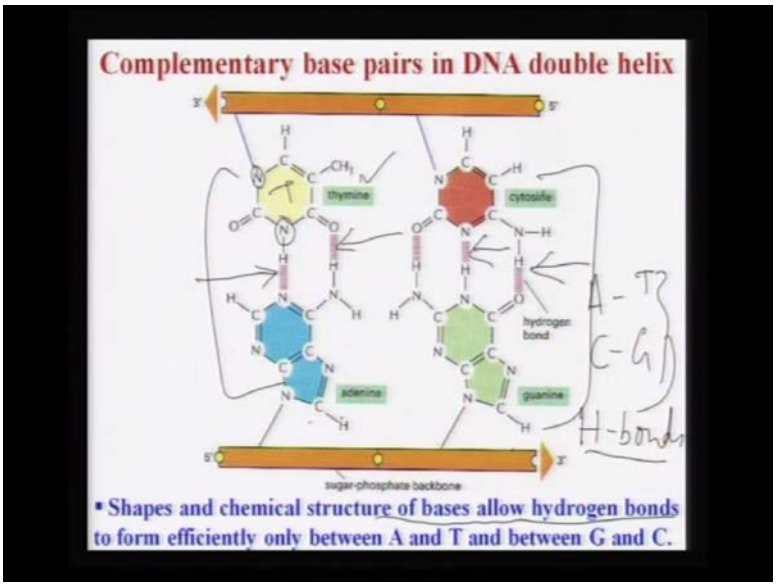

So, here, what is that? Now, again, all these nucleotides sequence, it would be difficult for to remember, but to show you, that what is the typical organic structure of this A, T, G, C, that is the base pairs here, so T is here thymine. What you see in this base paring is, that in case of the T, you have a typical benzene like ring here and, but here, it is not all carbons, some, some carbons are replaced by nitrogen and there, and there is some double bonds, and then some hydrogen and oxygen and hydrogen are attached to each of the carbon atoms.

Now, another thing, that you notice here in this base pairing between A and T there is hydrogen bonds are there, so this is like non-coherent bonds or weak bonds between C and G, that is also again hydrogen bonds.

So therefore, what you notice here, that this A-T and C-G, this base pairing by hydrogen bonds and hydrogen bonds, one of the uniqueness of the hydrogen bond is, that it can be broken if it is so desired. So, shapes and chemical structure of bases allow hydrogen bonds to form efficiently between A and T and between G and C, that is what is known as, that is what is uniqueness of the structure. So, A-T and C-G, this is known as the complementary base pairing in DNA double helix.

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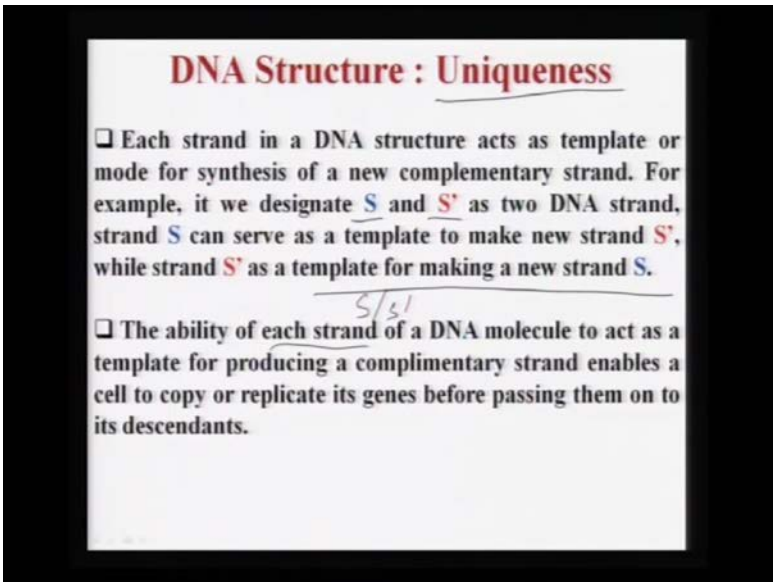

The other things in the DNA structure, that is, that has been, that I have mentioned, that uniqueness. Each strand in a DNA structure acts as template or mode template or mode for synthesis of a new complementary strand. For example, if we designate S and S prime as two stands, then S can serve as a template to make a new strand S prime and while S prime as a template for making a new strand S.

Now, let us go back and try to understand what it means. Suppose, this is strand S, this is strand S prime, so what it means is, that this strand S has the unique property, then it can make a complementary strand S prime. Similarly, S prime individually has the capability to produce a complementary strand of S.

So, this individual strand S and individual strand S prime can serve as a template to produce the opposite strand, either S prime or S, is it clear. So, this S and S prime can serve as a template, so that is uniqueness of the structure and then, ability of each strand of a DNA molecule two act as a template for producing complementary strand, enables a cell to copy or replicate its genes before passing them on to its descendants.

So, what it means is, that now look at this slide and try to understand. First of all, as I said, that between A and T and C and G, there is hydrogen bonds. Now, if required this here laterally, this hydrogen bonds can be broken. Now, 2 S, what are the bases that will be attached to nucleotides? One is T and one is C; 2 S prime, what are the bases that will be attached? One is A, another one is G, right, you understand.

So, now, S has the capability to produce a complimentary strand as S prime and S prime can produce another complimentary strand as S. Now, only thing, that there is required is that opposite bases. Now, T is there, C is there, the moment they find A and moment they find G, so simply, A and G can be fixed to the S prime and can form another DNA molecule. So, that is what has been mentioned here is, that the ability of each strand, each strand means that is, either S or S prime of a DNA molecule to act as a template, actually enables for producing a complimentary strand, enables a cell to copy or replicate its genes.

So, genes replication or copying is possible because simply, because S can produce S prime, S, S prime can produce S. And secondly, because between A and T and G and C there are weak hydrogen bonds, which can be broken if they, if it is desired.

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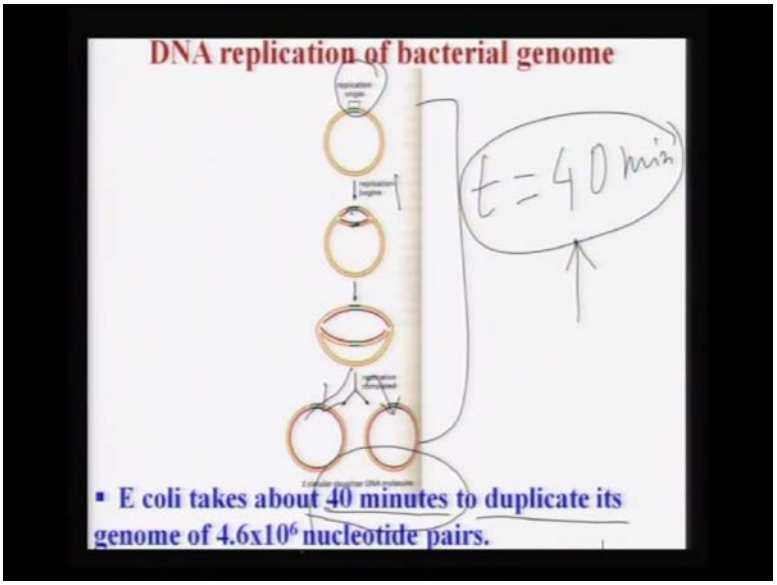

For example, DNA replication in a bacterial genome. Now, bacteria, you know, that DNA, RNA, all this genetic materials, they are not contained within a nucleus, but they are just dispersed in a cell. Now, this bacterial case, for example, this is a replication origin here.

Now, this replication origin here, you can see, that it will start this green one, it will start to grow and subsequently, this two daughter DNA molecules will be formed and for bacterial cells, typically takes around 40 minutes. So, this entire length time span is that t is equal to 40 minutes or less than an hour for, two dot, two daughter DNA to be

produced. And that is the reason, typically bacterial cells, for the proliferation and growth we check in the laboratory for 4 hours. 4 hours means, you give approximately 8 times, then one individual, two daughter DNA molecules to produce. And then, these 40 minutes, you, as you can see here, it duplicates genome of 4.6 into 10 to the power 6 nucleotide pairs. So, in these 40 minutes, huge number of nucleotides, there normally are produced and this is of the order of 10 to the power 6.

In the beginning what we have mentioned is that DNA molecule, that what is the role of the DNA molecule? It produces or synthesizes a protein structure RNA and catalytic RNA molecules and then, other thing is that genes carry biological information that must be copied accurately for transmission to the next generation, each time a cell divides to two daughter cells. What it means? From one cell if it goes to two daughter cells, that this each daughter cell must have a copy of its parent's gene very accurately or precisely. The second thing is, that a typical human cell contains 2 meter long DNA. So, 2 meter long DNA means, it is more than a person's height. So, if a guy is 6 foot tall, then it is how much? It is around 180, 180 centimeter or around close to 2 meter, so more than 6 foot height a person's length is there in the DNA in the humans, typically human's cell.

But how this is possible? The 2 meter long DNA can be squeezed because it has a double helix kind of structures, so that is, that is what is required to make this energetically most favorable structure. You can squeeze it, but you can, if you stretch it and put it all together, then you will find, that it is a kind of 2 meter long DNA structure and how many nucleotides, and, and how many proteins it has? It has almost, like thirty thousand different proteins, that it carries the instruction.

Now, let us see, that how this DNA can form the proteins?

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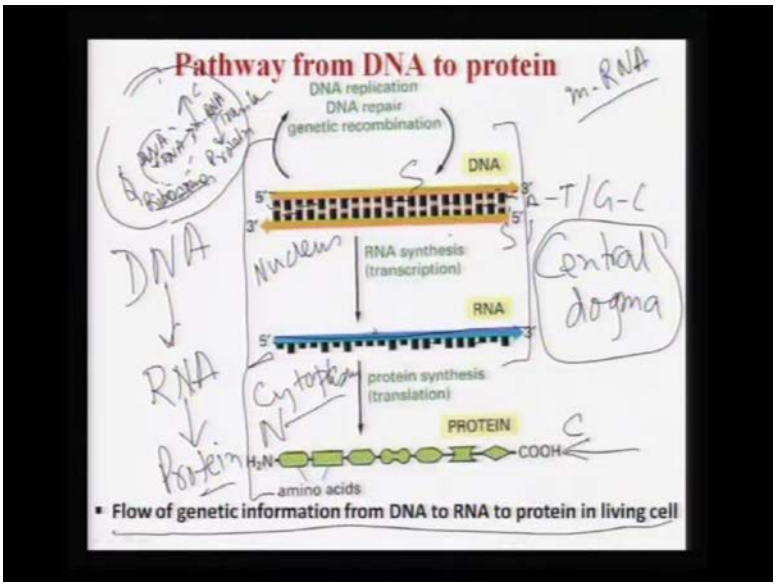

You have this S and S prime strand and here you have this A-T or G-C kind of base paring, this is the, and this red one is all the hydrogen bonding here. Now, what happens during that, from DNA to RNA, RNA is ribonucleic acid and DNA is deoxyribonucleic acid? Now, what happens? From DNA to RNA, just one strand and this, what is this strand, this 5 prime and 3 prime, that strand is getting detached from the DNA molecule, and if you see closely, that whatever, five point, three prime, that whatever this bases were attached to this and same bases were attached here also, it is just, that this entire DNA molecule has been transformed to one RNA molecule. So, this type of DNA to RNA, this is known as transcription, transcription means… So, this transcription means the nucleotide sequence in a chromosome is first copied into RNA and this process is known as the RNA synthesis or transcription.

Now, here the proteins are not formed, proteins are formed in the next stage when RNA goes to another process called translation. So, translation is the stage where the proteins are synthesized. And you can see here, that this is the long polypeptide chain, this is C terminal and this is N terminal. So, that means, this long polypeptide chain molecule has formed and this is that exactly the flow of, so you have to take a note of it, that irrespective of the cell dying in all living cells, this is the way proteins are synthesized. DNA goes to transcription to RNA; RNA goes to transcription to, sorry, translation to protein. So, this is that, and this is known as, in molecular biology language is known as the central dogma. Central dogma, what is central dogma? Central dogma in molecular

biology language is known as the process of DNA transcription to RNA and from RNA to protein.

Now, the question is that how this thing happens? This is, now, what are the different parts of the RNA? What are the different types of RNA? One, RNA, you must be knowing is that mRNA, what is the mRNA? That is messenger RNA; now, DNA you know, so suppose, this is an eukaryotic cell and you have a nucleus here; you know, this nucleus has a nuclear pore complex, now DNA you have, DNA you have in the nucleus.

Now, DNA to RNA synthesis, DNA to RNA synthesis, therefore must happen inside the nucleus. Now, this RNA, once it is synthesized, that is, once is transcripted, it goes through certain processing stages, which is known as RNA splicing; RNA splicing means, the simple RNA, then it goes to mRNA, but the way I am writing here it is not that simple. The biologically, the RNA being processed to a more refined step, called mRNA, before this mRNA goes outside the nucleus to the cytoplasm; you understand the whole process.

So DNA, so the up to this process, DNA to RNA, this is all takes place in the nucleus; up to this process, new RNA to protein synthesis, this all takes place in the cytoplasm. Now, how this thing happens? DNA to RNA transcription happens in the nucleus, then RNA goes through RNA splicing or some processing stages to transform to mRNA. Now, this mRNA, now goes to the cytoplasm, this mRNA now goes to the translation stage and then forms a protein on the cytoplasm or on the cytoplasm matrix. Remember, in the cytoplasm you also had certain organs, which is known as the ribosomes; ribosomes are another way, another place, where proteins are synthesized in large numbers.

So, now let me summarize what I have said on this slide, this slide is important. So, how, what is the role of DNA? DNA actually contains genetic information and DNA, also DNA's role is also to synthesize proteins and RNA. So, DNA has a double strand S and S prime and DNA has four different nucleotides sequence A, T, G, C. Now, in this base pairing, when DNA transforms to RNA, what happens? One of the strand, either S or S prime, along with the different nucleotides they get detached from the double strand and then, they form the RNA molecule. Now, this RNA molecule, DNA to RNA synthesis is known as the transcription process. In the subsequent stages, which take place in the cytoplasm outside the nucleus, these RNA molecules, they undergo translation process

and then, they make the proteins. And this is the typical protein structure, right hand side as per as that convention, it is c terminal; left hand side, it is, as per as the convention, it is n terminal. Next question, that what is the biological difference between DNA molecule and RNA molecule? Now let me try to answer this one.

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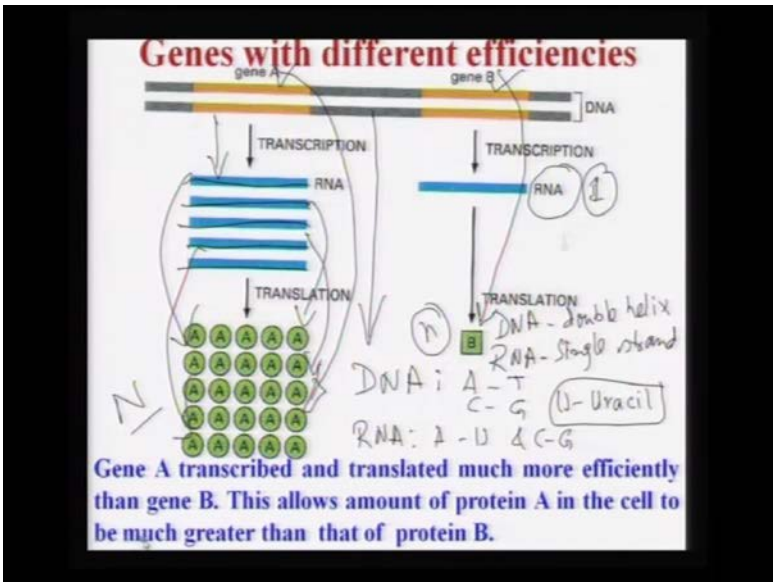

Biological difference is that DNA has a double helix kind of a structure, now RNA has a single strand, so RNA does not have a double strand or double helix kind of a structure. Now, DNA, it has a typical base pairing of A-T and C-G. RNA has also four nucleotides, but typical base pairing would be A-U and C-G. So, in case of RNA, T, thymine is replaced by U. What is U? U is known as uracil that is another different base, U means uracil. So, in RNA, the base paring would be A-U and C-G, so this is the 2nd difference. Here, 3rd difference is that nucleotides in RNA or ribo-nucleotides, they contain sugar ribose rather than deoxyribose that comes from the basic nomenclature itself.

So, since DNA is that deoxyribose, so they are the nucleotides at the, they contain the deoxyribose; in case of the RNA ribonucleic acid, so they contain the ribose, that is, the sugar molecule. So, this is the major difference between DNA and RNA.

Now, coming to the genes with different efficiencies, let me explain, that you know, that although the transcription and translation processes takes place on different genes, but two different genes, let us say A and B, they can have different efficiencies. Efficiencies

means, one gene can produce large number of protein molecules where other genes can produce very small number of protein molecules.

Now, how it happens? Now, in this particular case, gene A, when it goes to the transcription, the RNA molecules, if you see in number of RNA copies are made, now this individual RNA copies, then they can individually produce a large number of protein molecules and as a result, you end up of having capital N number of protein molecules here. Now, in case of gene B, although it produces the same similar RNA copies, but the number of RNA copies is just 1 and therefore, you have very small N number of protein molecules. Therefore, gene A is known as highly efficient, gene B is known as less efficient.

The 2nd thing is, that in biological language if you, if you consider, that the gene a can be expressed or expression of gene A would be much more than expression of gene B, what it means by expression? Expression means here, how much protein molecules a particular gene can produce by transcription, translation process. So, therefore, this entire biology of this transcription, translation leads to more gene protein molecules formation in gene A, so there gene A has the higher expression. So, here it is mentioned, that gene A is transcribed and translated much more efficiently than gene B and this allows amount of protein A in the cell to be much more greater than that of the protein B. So, essentially, what it means?

Here, gene A, ultimately will synthesize protein of type A, gene B would be ultimately produced in the protein of type B. However, quantity-wise, A protein is much, much larger in numbers than B protein. So, although the message, that I am trying to say, although gene A and gene B, they are contained within the same DNA, but individually, gene A and gene B has different efficiency, because of that any difference in the base paring or some other biological properties.

Now, from that, you can, now you can say, that you know, that now I can start explaining what is cell differentiation?

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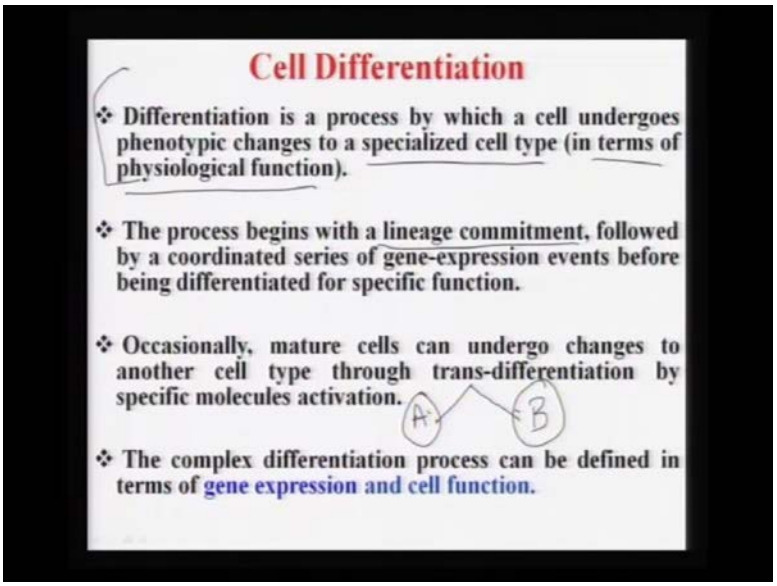

So, differentiation means, as I, I said earlier, that is the differential gene expression. Now, differential gene expression means, that means you know, that how two different genes they are expressed differently, you have the idea, like through transcription and translation process and because of two different efficiencies.

Now, therefore, suppose this is cell A and this is cell B. If I say, cell A has different gene expression than cell B that means, that in the A cell, in the nucleus DNA, if both are eukaryote cell in the nucleus DNA, they have different efficiency of protein synthesis compared to gene in cell B. So, ultimately, the function of these genes is related to the function of the protein synthesis.

So, their first thing is the definition, what is definition? Differentiation is a process by which a cell undergoes phenotypic changes to a specialized cell type in terms of physiological function. So, therefore, two cells, if they are differentiated, that means, A has a different physiological function than B. And why they have, they can perform two different physiological functions? Simply because their DNA structure, their gene structures are different and their efficiency is, the efficiency of both the genes are also different. The process begins with a lineage commitment and what is lineage commitment I will come back to later, followed by a coordinated series of gene expression events before being differentiated for specific function.

Now, let us see, that what is meant by lineage commitment.

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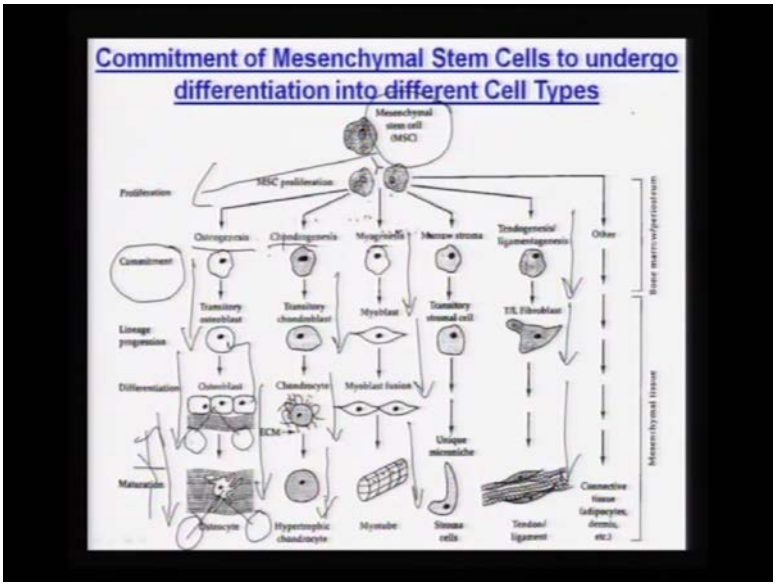

So, this is, you start with stem cells here, mesenchymal stem cells. Now, what is mesenchymal, what are stem cells? I will take a different; I will explain may be later if time permits. Now, this stem cells is the primary cells, you have to remember only the one thing, at this point is, that stem cells is the primary cells, all the other cells, they are known as the transformed cells. Transformed cell means, that cell must have transformed from some primary cells by differentiation process. Now, if you, if you see the stem cells here, they can have chondrogenesis. Osteogenesis means, it is forming the bone cells; chondrogenesis means, it forms chondro sites or cartilage tissue cells.

Now, commitment means, if you remember, that you know cell cycle, I have shown you, that when a cell goes from S 1 to G and G to S 2, that is checkpoint; checkpoint means, that the cells are now ready to enter from S 1 to G. So, now, once the cell is committed to enter to G, it will remain in G, it cannot come back to S 1, that is the simple logic. Now, similarly, here the commitment means, once the cell is committed to differentiation, that means, it will go to first transitory osteoblast, then it will be differentiated to osteoblast. Now, transitory osteoblast and osteoblast cells, they have different gene expression or they have different cellular functionality and then it will go to more matured, matured cells is, that called osteocytes. It is like, you can always consider or you can always compare this with the development of a child. As the age goes up, a child becomes more and more mature and that you can see in their expression, they can learn the things, they can understand things, they can respond to the different things.

Similarly, here the moment transitory osteoblast forms the osteoblast, they can form bone cells, but its more matured stage is the osteocytes. So, some of the physiological functions that osteocytes can perform osteoblast may not be able to perform, because osteocytes is the differentiated form of osteoblast. I repeat, osteoblast and osteocytes, both are bone cells, however osteocytes are more differentiated stage of the osteoblast, therefore they have different gene function.

Similarly, here in case of chondrogenesis, you have transitory chondroblast and then, you have chondrocytes, and then you have hypertrophic chondrocyte; hypertrophic means, atrophy, hypertrophy, hypertrophy means increase in the cell size. So, similarly here, so when you say, the hypertrophy, chondrocytes means, simply chondrocytes cells are increased in size.

Then, similarly, there is myogenesis; there are myoblast cells, then myoblast fusion. Myoblast fusion means, two myoblast cells coming together and then you have the myotube. So, similarly, you have these tendrogenesis or ligamentagensis. Ligamentagensis means, it is the formation of ligament; tendrogenesis means, it is the formation of tendal. Now, you have the fibroblast cells here, now this fibroblast, you know, it is cells of the connective tissue. Now, all these fibroblast cells together with the ligament and then it forms ligament cells. So, this is known as the lineage commitment; lineage commitment means, that a cell comes from origin.

It is just, because if you look at the family history of a person, any person is coming from his ancestor, right, so, and then it comes, like grandfather, great-great grandfather, so similarly, here in the cell case also, osteocytes' father is osteoblast, grandfather is transitory osteoblast and this all to comes from the stem cells. So, stem cell is the primary cell, where it can be differentiated into many cells and all the cells, once it is differentiated, their genes are also copied and there they have different genes.

So, other things in differentiation process is, that suppose, at any stage, osteoblast cells is divided into two daughter osteoblast cells, then that gene expression would be the, because there are two genes, that copied here from one single DNA, if osteocytes cells, if they are divided into two osteocytes, daughter osteocytes cells, again they will have same gene expression. So, differentiation, division, proliferation, adhesion has conceptually different meaning. That is what I am trying to repeat here, that occasionally, mature cells

can undergo changes to another cell type through trans-differentiation by specific molecular activation. Now, I will possibly give an example on that.

Then, 4th one, the complex differentiation process can be defined in terms of gene expression and cell function that is what has been already realized by you. So, different gene expression and different cell function, that is, what is known as the cell differentiation. Differentiation involves expression of unique genes that are specific according to a cell type with an irreversible change towards a particular cellular phenotype process. What I mean is, that is, that osteoblast cannot be, can be differentiated, osteocytes and it is a re-reversible process. So, if you go back to that, so this thing cannot take place, osteocytes to osteoblast; do you understand what I am saying? So, what I am trying to say, that osteoblast can only be differentiated, you know, irreversible manner; it is not a reversible phenomenon, this differentiation process.

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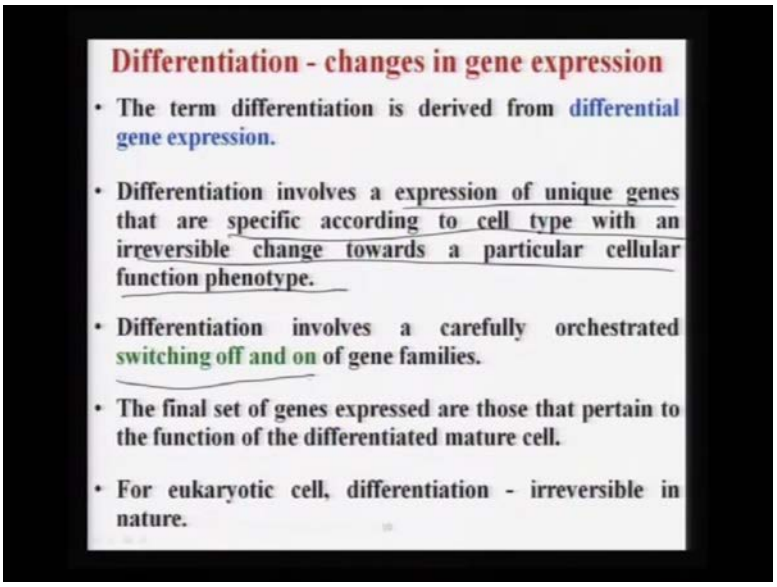

This is like very, kind of, bookish type of definition, that is involves a expression of unique genes specific to the specific cell type, that means, for osteoblast it is a different genes, for chondrocytes it is a different genes, for fibroblast it is a different genes, which are responsible for the differentiation process.

Differentiation also involves, yeah, carefully orchestrated switching off and on of gene families. What it means is that many genes are inactive, but the moment it gets certain

signal for cell differentiation to take place, those inactive genes are switched on. So, they are become active. Now, once the differentiation process is over, then again they become inactive. So, it, it goes through switching off and switching on type of process. The final sets of genes expressed are those that pertain to the function of the differentiated matured cell. And for eukaryotic cell, differentiation is irreversible in nature that I have already mentioned to you. So, always you remember, the differentiation is irreversible in nature.

We are discussing the differential of the cells, some of the key points to remember is, that one, there is a, once there is a commitment for a given cell to differentiate, to different cell types, then it follows the lineage. That means that osteoblast cells should be differentiated to osteocytes in a very irreversible manner. So, these goes from top to down, not from down to top, that is not possible.

So, similarly, chondrocytes, that is the cartilage tissue cells, they will go to the hypertrophic chondrocyte cells is myoblast cells, will go to myotube and so on, so forth.

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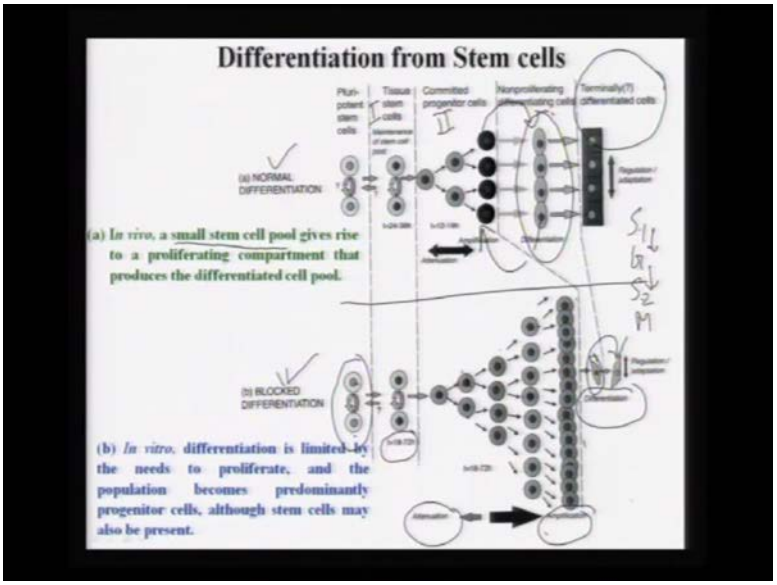

There is another type of differentiation that is called… There is two type of differentiation, one is called normal differentiation, another one is block differentiation. So, block differentiation is, what happens in case of the in-vitro study, in-vitro means, that is, the culture media in a laboratory, so here differentiation is limited by the needs to proliferate. So, there are two things here, one is the differentiation and one is

proliferation. This differentiation is limited by the needs to proliferate and the population becomes predominately progenitor cells, all those stem cells may also be present. Now, what is show in the right hand side, this bottom, bottom here, so there are two cells, which goes from one cell to one type, to another type. Now, in 18-72 hours, then these two cells will start getting differentiated to different types of cells. As you can see, that 1 becomes 2, 2 become 4, 4 becomes 8, 8 becomes 16, and so on.

And then, subsequently, that after this amplification, so this is attenuation and this is amplification, that one particular cell type goes to another particular cell type via differentiation process. So, this is, this is one type of differentiation, what is known as block differentiation. In the normal differentiation case, it is small stem cells, as I said, the stem cells is the primary cells.

Now, this stem, a small stem cell gives rise to a proliferating compartment that produces a differentiated cell pool, so this is the case for the non-proliferating differential cells. So, non-proliferating means, they are not proliferating, but they are just differentiating and here, this one is called terminally differentiated cells where it is almost on the terminal stage and it cannot be differentiated any further.

In the 2nd stage, here you see, that if I, if, if call it stage 2 and this is called as stage 1, this is called committed progenitor cells; committed cells means, this cells will now be bound to undergo differentiation process, is just like, as I said in the cell cycle, it was going from S 1, G, S 2, M. Once it comes from the check point between S 1 and G, the cell is committed to enter into G and once it goes to check point from G to S 2, cell is committed to enter into S 2 phase. Similarly, this commitment means, now this progenitor cell must differentiate, it cannot go back to the earlier position. So, this is like, takes place in a re-reversible manner.

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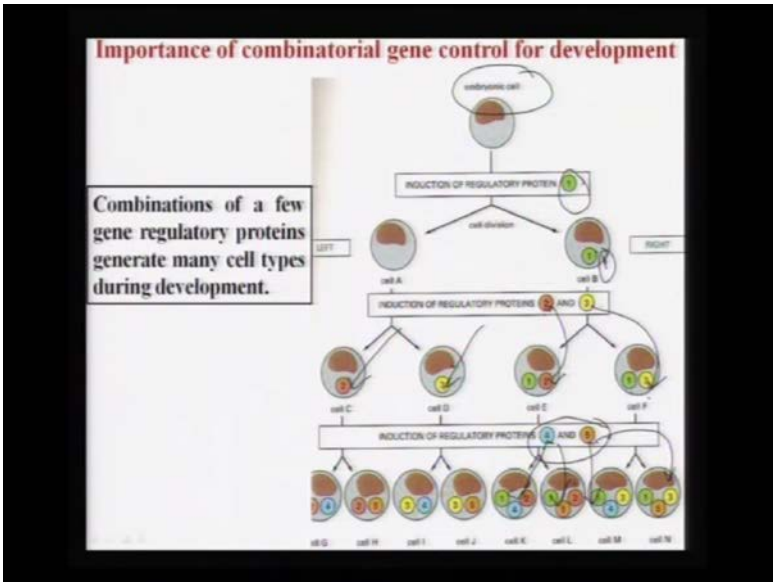

This is, that combinations of few regulatory gene proteins, as you can see here, that they can actually induce differentiation and form a large number of cells. So, this is the embryonic cells, it can be stem cells. Now, induction of the gene regulatory protein here, one, so here this protein is already inside the cell, this gene regulatory protein.

So, these cells are divided into two cells, daughter cells. Now, this is that induction of gene regulatory protein two and three, now this one goes already inside, two is also coming, so three is coming here and two is coming here. Now, here, this gene regulatory proteins are also coming in individual two daughter cells, cell C and cell D, then again they will induce, that other gene regulatory protein is that four and five. Then, similarly, this gene regulatory proteins also will enter each of the daughter cell, so each time the cell divides into two daughter cells, the gene regulatory proteins are internalized in the cytoplasm of the cells and this internalization process actually helps cells to further differentiate to many different other cell types.

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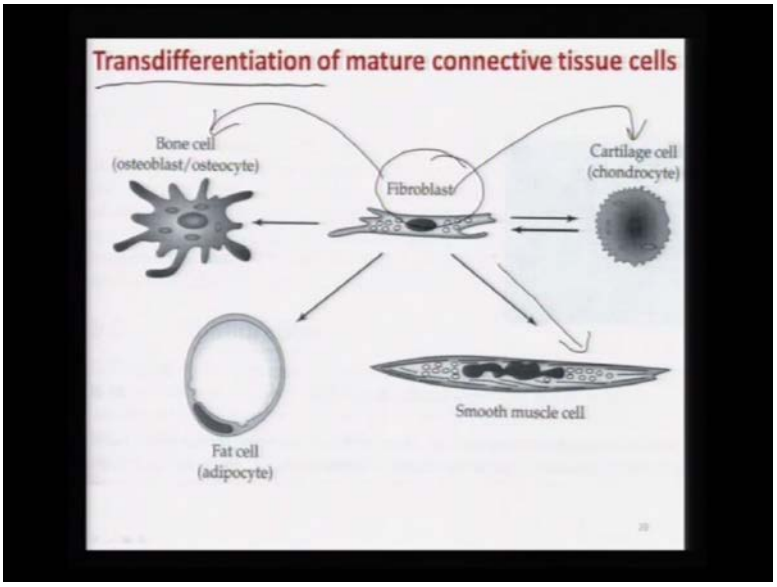

Now, this is a unique example, what is known as transdifferentiation, I have mentioned to earlier, that in the, in the, in the initial discussion of the differentiation. Now, what is transdifferentiation?. Suppose, you have that one type of cell fibroblast, which is a connective tissue cells, now fibroblast cells can be transdifferentiated to smooth muscles cells. Now, what is the performance of the muscle cells? These actually help in the contraction muscles, means that can contract and expand, right, so this kind of contraction. Fibroblast cells can be transdifferentiated into cartilage cells, chondrocyte cells; fibroblast cells can transform to bone cell, osteoblast and osteocyte, osteocyte is the more matured differentiated cells formed from the osteoblastcytes, osteoblast cells.

So, osteoblast and osteocytes are both the same cell type, the osteocyte is the most matured and differentiated cell type. So, what you can see here, that this is a unique example where fibroblast cells can be differentiated into two or three different type of cells. In one case it forms a cartilage cell, chondrocyte cells; another type, another, another case, it forms the smooth muscle cells; another case, it forms the bone cell, that is the osteoblast cell. So, this is called the transdifferentiation case.

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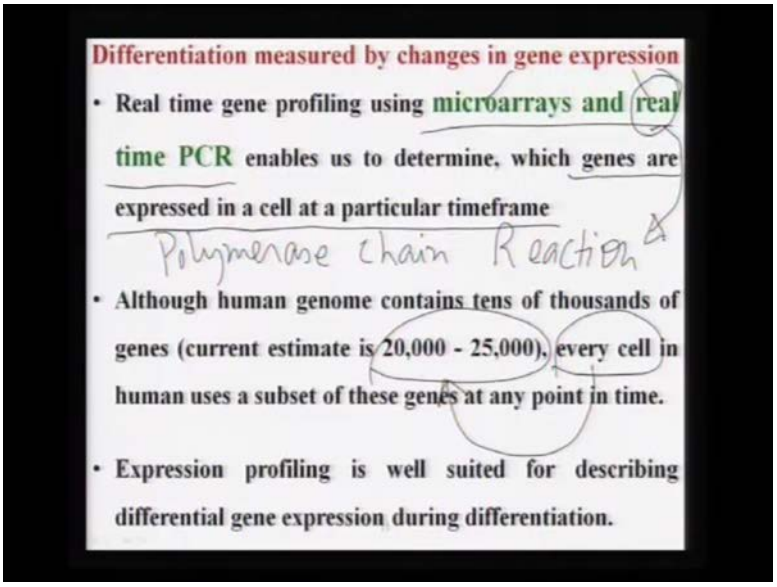

Now, real time gene profiling. So, now, as I said, that you know, that there can be, yeah… So, as I said earlier, that although gene A and gene B, they are contained within the same DNA, but gene A and gene B can have different efficiency. Efficiency is measured here in the context of the gene is, that in terms of how much or how many protein molecules, that it can synthesize finally. So, here you can see from gene A, that is, RNAs are formed by the transcription, from translation from RNA to protein molecules, it forms a huge number of a protein molecules, but gene B, only few B protein molecules are formed.

So, what it means? That means, gene A is highly efficient compared to gene B or in other words, gene A can be expressed more than gene B. So, expression, expression, in the biological terminology means, that a particular molecule, with how much concentration they can be measured. Suppose, if I say, that gene A has higher expression that means, that gene A can be measured with much more higher concentration by a certain analytical equipment, yeah.

So, real time gene profiling means, that means, that when the actual differentiation process occurs, so this differentiation process means, that your gene expression changes, that how this gene expression can be changed. That, changes can be monitored by two techniques, one is the microarrays and another one is the real time PCR; PCR stands for

polymerase chain reaction. So, PCR stands for polymerase chain reaction and these, these two techniques.

Now, what is the principle of operation, I, I may be able to explain this it to PCR, but not the other one, so that, what is that principle of operation of PCR? Then, when I will explain, then you will be able to realize, that PCR can actually be very useful to quantify that, which are the genes, which are expressed during the cell differentiation process. So, human genomes typically contain tens of thousands, typically estimate is 20 to 25000 different genes. And every cell in human uses the subset of this genes at any point in time, that means, that you take any cell, what is present in a, in living conditions, they just uses, that out of 20, 25000, a few genes for its survival or for its functioning.

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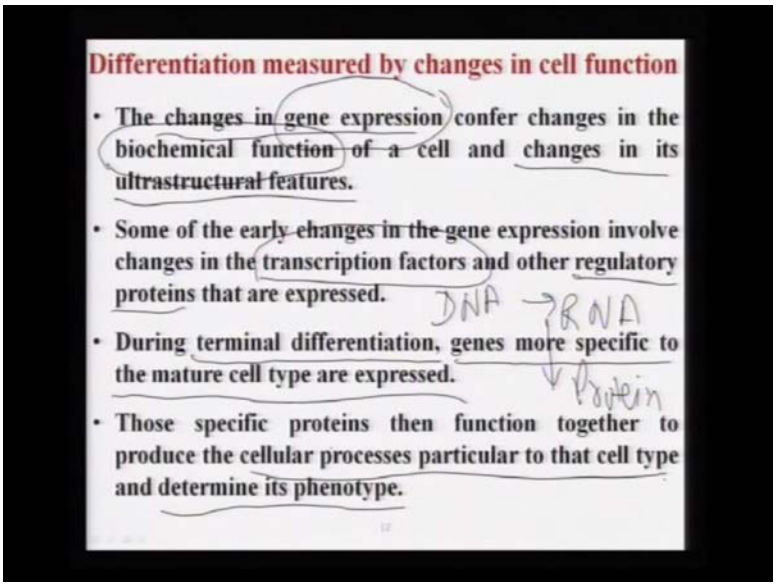

Now, what is that gene expression changes? Changes in gene expression actually confer changes in the biochemical function; biochemical function means, that is cell, its always supposed to do certain functions, like connective tissues or endothelial cells, they are supposed to provide support. Connected tissue cells, they can also form, they can, they can support mechanically to the ligamented tendon. So, all these changes, all these functions are possible because they have a particular gene expression and also many times changes in gene expression means, changes in these ultra structural features. Ultra structural features means, the way the cellular organelles are organized inside the cell.

Some of the early changes in the gene expression involve changes in the transcription factors. What is transcription? Transcription is a DNA to RNA synthesis, and regulatory, and regulatory proteins, that are expressed. What is regulatory protein? That, can be metabolic regulatory proteins; that, can be gene regulatory proteins, different type of proteins are expressed. Terminal differentiation means, as I said earlier, once the cells reaches terminal, anything terminal, like a human being, it goes to a terminal stage means, it cannot come back, right, so it is almost towards the end of the life.

So, similarly, here terminal differentiation means, genes more specific to the matured cell type. That means, once the cells actually achieved most matured stage, cells cannot be differentiated any further. So, if you see, that osteoblast lineage, it is the osteocyte cells, which are the most matured cells. Osteocyte cannot be differentiated to any other bone cell type; that is the last stage of the differentiation. So, therefore, that is, that is what is mentioned here, that during terminal differentiation genes, more specific to the mature cell type are expressed.

Fourth point, that mentioned is, that those specific proteins, that function together to produce the cellular processes particular to that cell type and determines its phenotype. So, that means, that from DNA to RNA to proteins by translation process, whatever set, particular set of proteins, that are generated, this proteins, they function together to produce the cellular processes. That means, whatever the cellular functions that they are supposed to carry out.

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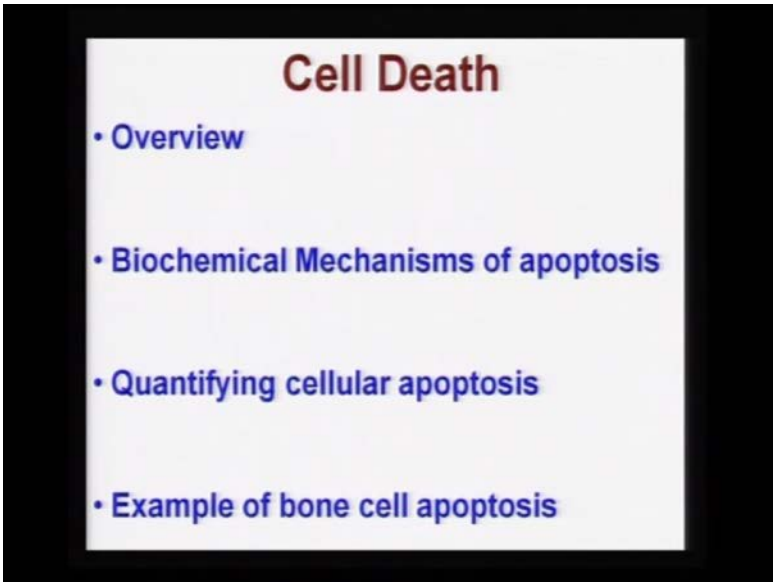

The next topic is the cell death process, so in the cell death or apoptosis process, I will first give you an overview of the cell death processes followed by the biochemical mechanisms of apoptosis. Third one is that how to quantify cellular apoptosis process; fourth one is that example of the bone cell apoptosis.

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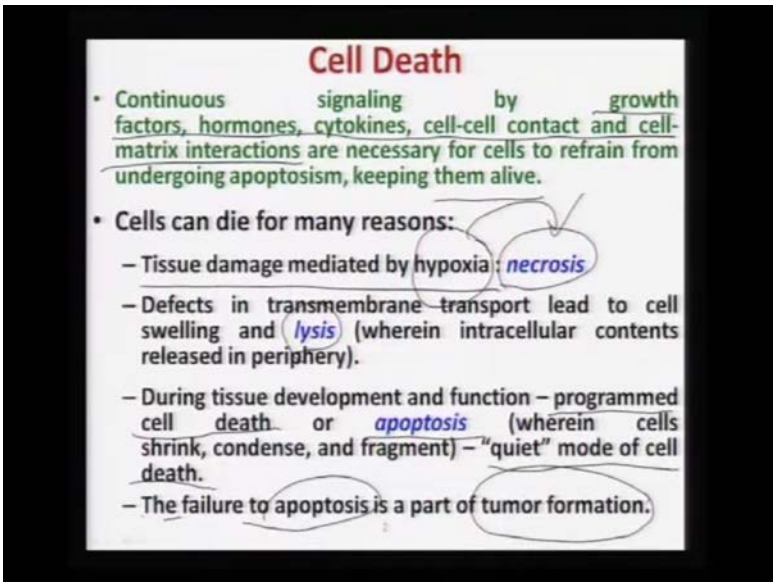

Now, cell death is that continuous signaling by growth factors, hormones, so these are like different signaling process growth factors hormones and cytokines and cell-cell

contact and cell-matrix interactions are necessary for cells to refrain from undergoing apoptosism, keeping them alive.

So, what it means is that as long as the cells get or receive all the signals necessary for its survival, cells will survive. The moment the cells are deprived of this signals, cells will undergo apoptosis or death, it is as simple as that. If you, if a human being is deprived of taking food every day, that automatically, that human being cannot survive. So, similarly here, in this particular context, the cells, if they are not getting survival factors or survival signals, then it will undergo apoptosis.

Then, what is necrosis? Necrosis, essentially mediated by the tissue damage, mediated by hypoxia; what is hypoxia? I have, I told you earlier, hypoxia is the lack of oxygen supply to a particular cells or that particular tissue has inability to use the supplied oxygen for its own survival. So, either of these cases, this hypoxia actually leads to necrosis stage.

Now, defects in the transmembrane transport lead to cells swelling and lysis. So, that is also another case of the cell death. During tissue development and function, program cell death or apoptosis, that take place, now this apoptosis is the quiet mode of cell, quiet mode of cell death. Many times you might have seen or you might have heard from people, that a particular person undergoes silent heart attack. Silent heart attack means, that there is not much enough symptoms earlier that the person will get a heart attack and suddenly, he, he sleeps and the next morning you will find the person is dead; that means, the person had a very silent heart attack. So, you can consider, that apoptosis is also very quiet mode or very silent mode of the cell death and this apoptosis takes place in a very programmed manner, it is not a very irrational or illogical manner, this apoptosis takes place. So, many times, if a cell does not undergo apoptosis, then what will happen? This (( )) cells, a number of cells, they can cause a tumor locally at that particularly infected place.

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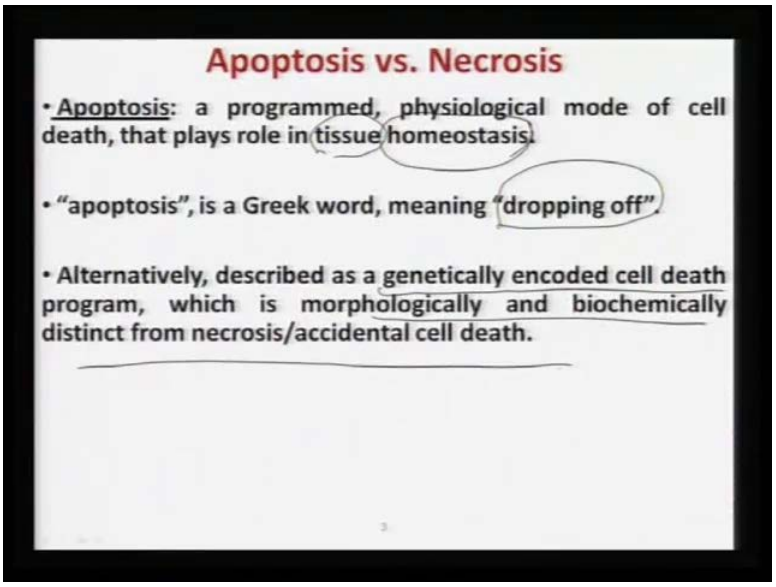

Now, apoptosis, like many of the scientific words, it is a, it is also a, origin of a Greek word. Now, this Greek word, the apoptosis means dropping off; dropping off means, in a particular season you might have seen, that all the trees there leave, there leaves are dropped off, right, so they just, they just, this leaves are dropping off from the trees. So, similarly, apoptosis means, in the context of cell biology means, the cells are also kind of dropped off, like they are not any more dead, so, so, they are, they are not any more alive or they are now dead.

Now, what is that kind of text book type of definition of apoptosis? It is a programmed physiological mode of cell death that plays role in the tissue homeostasis. Tissue homeostasis means, that a particular tissue, as long as it is able to perform its own function, that is, what it is destined for, that is called tissue homeostasis. Alternatively, apoptosis is also described as a genetically encoded cell death program, which is morphologically and biochemically distinct from necrosis or accidental cell death.

In the case of necrosis, what happens? The cell membrane is ruptured immediate, some instantaneously and then the cell undergoes necrosis process. This is more like a accidental death process. So, again, coming back to the, that thing, that I have mentioned, so necrosis can be death due to road accident, apoptosis can be described as death due to the silent heart attack. So, silent heart attack means, it is a very quite mode of cell death, that is the apoptosis and necrosis means, like road accident, that it is,

immediately a person die and that is what is meant by necrosis process. So, this is, that you know, general language it is. If we mention, then it is for you, to very easy to remember.

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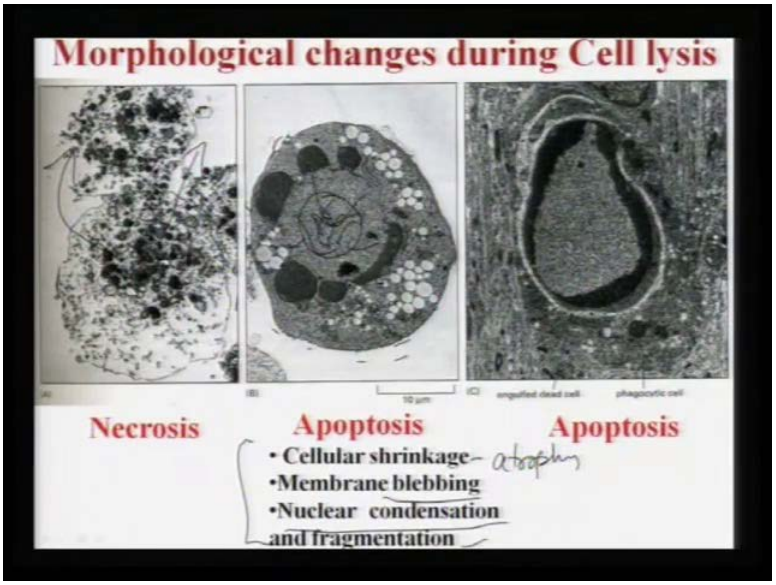

Now, if you look at this slide and you will be able to realize, that what are the morphological changes, that takes place during cell lysis process? So, left one is that necrosis. As you can see, that cell membranes are ruptured here at different places and all the intracellular organelles, they are like coming out of the cell. Now, apoptosis is, actually you do not see much changes in the cell membrane structure, so that is cell membrane is more or less intact here.

But cells, they shrink in size. So, cells shrink in size means, shrinkage of the cells in the adaptation process; the biological term is atrophy, right. Atrophy is the biological, biological term, then there is membrane blebbing, that is also occurred and 3rd one is that many times a nuclear condensation or fragmentation. (( )) means, that whatever, nuclear, nucleus was here in a stable vital cells or live cells, the nucleus, they become condensed or many times they fragment or there is fragmentation of the nuclear membrane. So, these are the kind of science, morphological science of the apoptosis, but in the necrosis, one can immediately understand, that cell membrane itself, they are ruptured and all the intracellular organelles, they can, they come out and then they form the necrotic cells. Sometimes in our lab we have seen, that once you do the electric field

experiment, if you apply too much electric field or electric voltage, the cells undergo necrosis, not the apoptosis process, because we can see, that you know, cell membranes are ruptured and all the intracellular elements are coming out.

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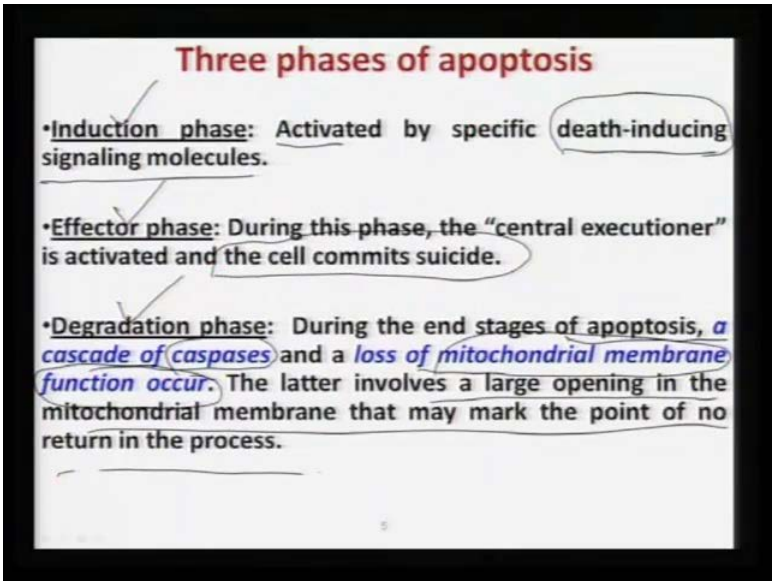

Now, there are three phases of the apoptosis: one is the induction phase, one is the effector phase and 3rd one is the degradation phase. Induction phase is, that this is activated by certain death inducing signaling molecules. So, as I said earlier, that for every cell-fate processes you require some set of intracellular signaling processes. So, some death inducing signaling molecules are there, we are not getting into details of these molecules, but we are discussing the mechanisms here.

So, in the 1st stage it sees, this 1st stage is activated when the cells receives some death inducing signals. 2nd one is the effector phase; effector phase means, that this is the phase when cell commits suicide. So, as I said, that it is also a kind of more like a suicidal mode, but in a very silent manner. Third one is the degradation phase. During the end stages of apoptosis, a cascade of caspases, caspases is a particular signaling pathway, that are activated and loss of mitochondrial membrane function occur.

Now, why mitochondria are important? Mitochondria is a place where ATP to ADP, ADP to ATP, all these transformation takes place and mitochondria is known as the power house of the cell, like it produces energy. Now, at any certain instance, if the

mitochondrial membrane does not function, then what will happen? These mitochondria will, will not be able to produce the energy required for that particular cell and therefore, it can lead to the cell death process, clear. So, that is, that is what is mentioned here. This mitochondrial membrane functions stops or that is, there is a loss mitochondrial membrane function that involves a large opening in the mitochondrial membrane that may mark the point of no return. In the process the point of no return means, now cells will definitely undergo death, it cannot come back any more. So, it is from that point onwards, it is more like an irreversible process of going to death.

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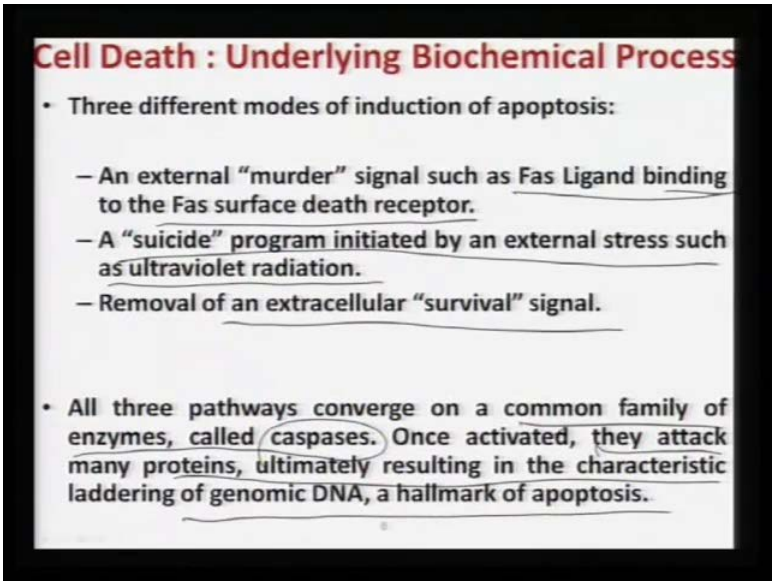

So, the other things, that I have mentioned now here, it is more clearly written, that an external murder signal, that is, the FAS ligand binding to the FAS surface death receptor, that is, that induces apoptosis. And the suicide program that is initiated by an external stress, such as ultraviolet radiation for example, if you expose some cells to a very ultraviolet radiation, UV radiation, the cells can die, that means, the cell apoptosis here is instigated or is triggered by the ultraviolet radiation. Removal of an extracellular survival signal; so, either you induce the death signal or you simply remove whatever signals that were necessary for the survival of that particular cell. So, then also you can do, so then also you can induce the apoptosis.

So, all three pathways typically converge to a common family of enzymes that is called caspases, as I mentioned earlier. Once activated, they attack many proteins and ultimately result in the characteristic laddering of the DNA, leading to the apoptosis.

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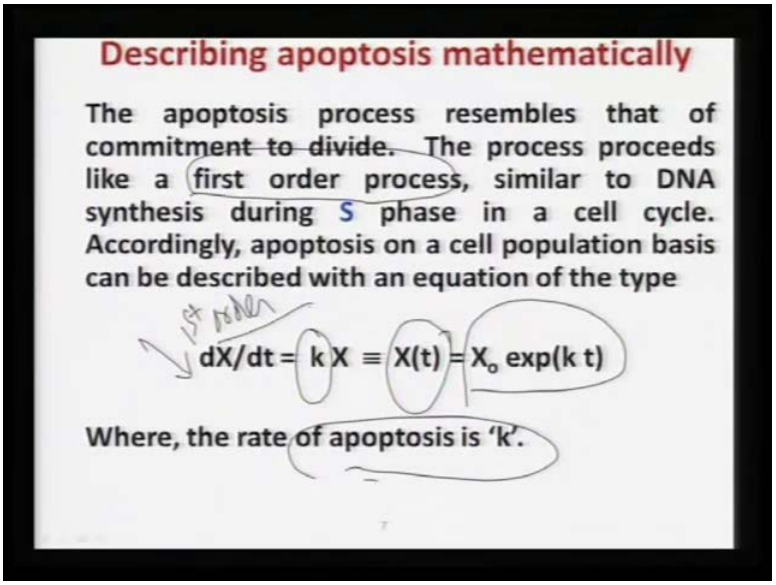

Little bit of mathematics here, but very simple, the apoptosis process resembles that of commitment to divide. If you remember, the similar expression we have also seen in case of the cell division process. So, the rate of apoptosis, that means, the number of cells, that is undergoing death, dX by dT is proportional to the population at any particular instance X and the proportionality constant here is known as K. So, dX by dT is proportional to X, that means, dX by dT is equal to X and therefore, at any given instance XT, you can written as, that x naught exponential K, t and what is K? K is the rate of apoptosis, that is, process proceeds by a 1st order kinetic process. So, these apoptosis is a first order kinetic process, that means, that dX by dt is proportional to the X to the power 1.

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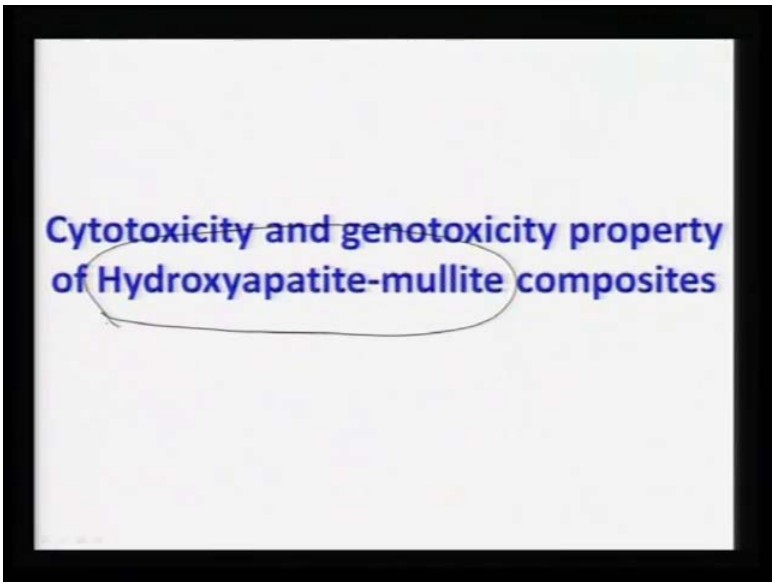

Coming to the some examples of that cellular apoptosis, that how it happens, I will give an example, that when a cell is treated with a particular particle, evaluates like hydroxyapatite-mullite; how this apoptosis take place?

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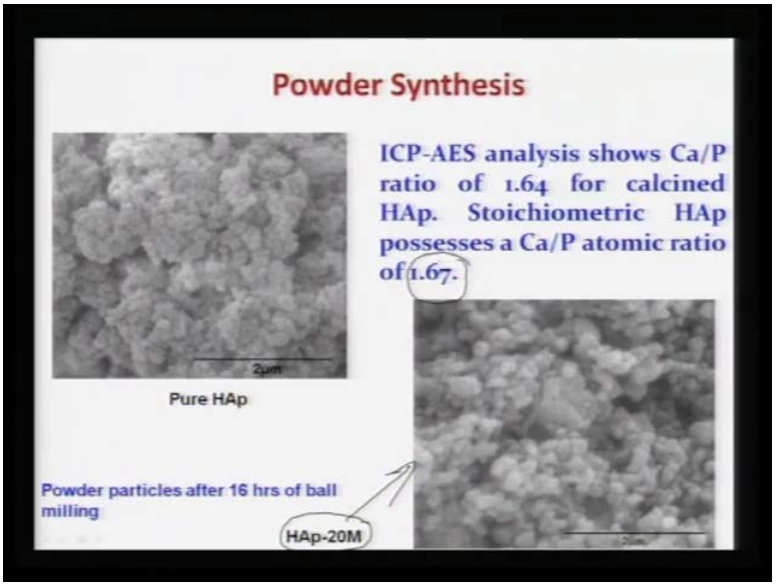

So, this is the hydroxyapatite particles here. So, these particles are typically in the nano size, 100 nanometer or less and there are different other particles, that we have taken is the hydroxyapatite, 20 percent mullite. So, there also the particle size is very small,

hydroxyapatite, that what we have used in our experiments. It has a Ca by p ratio 1.67, that means, it is more or less stochiometric hydroxyapatite.

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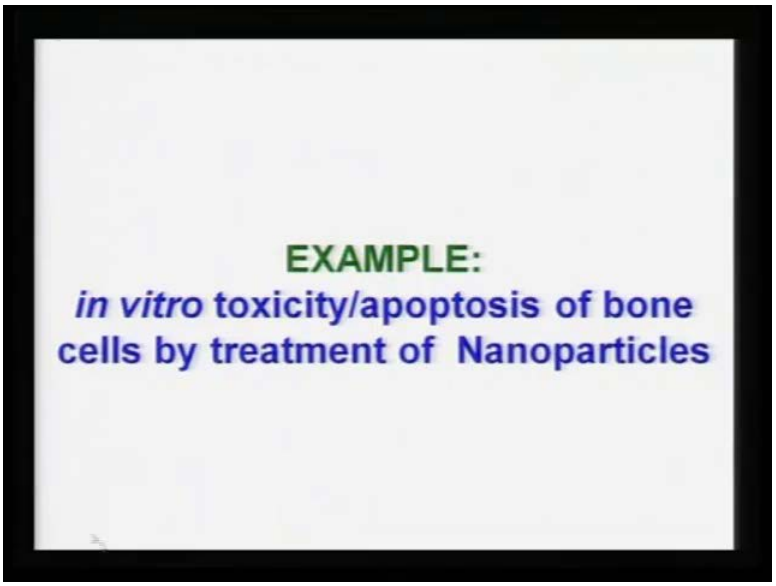

Now, with some experiments, particular experiment, we, I will show you, that these particles, when these particles, they are in the culture medium where you were culturing the fibroblast cells or osteoblast cells, how the DNA is damaged?

So, this is a typical double helix pattern of the DNA. Now, when it is treated with biomaterial helix what happens, that DNA in the double helix pattern, there are some breakage will happen. So, that means, in the strand, like DNA, there is a stand S and there is a strand S prime. So, S and S prime will not be intact, they will be broken in the double helix pattern and therefore, it is causing the damage to the DNA. And these, once it is damaged, this DNA to RNA, RNA to protein synthesis cannot take place. So, this is known as the damaged DNA or fragmented DNA.

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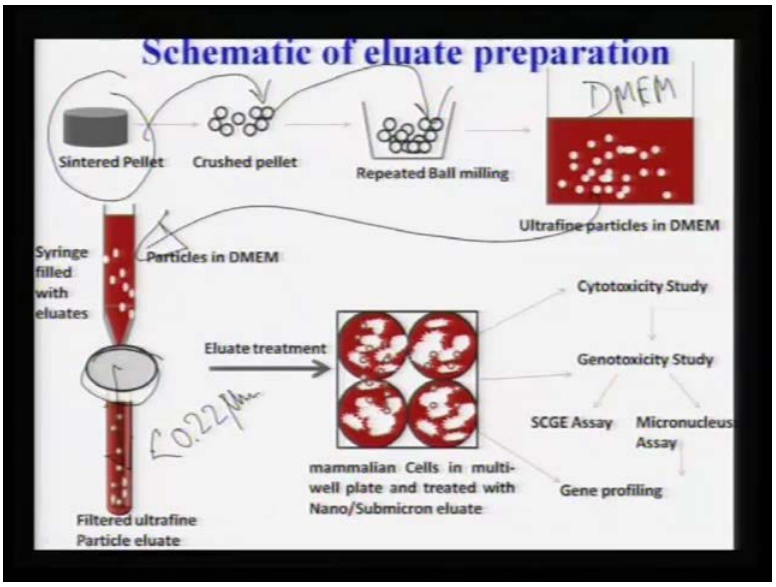

Now, how these particles were generated? So, after sintering, we get a sintered pallet. Now, this pellet can be crossed into small particles, then you can to ball milling and in this ball milling process you can make fine particles. Now, this fine particles you can put it in the DMEM, that is, the typical culture medium, you can disperse them, then you can simple sinter them with a filter. After the filtration you get a particle size, which is less than 0.22 micron, very fine particles and these medium, you can directly treat the different cells, like L9 to 9 cells and other type of cells.

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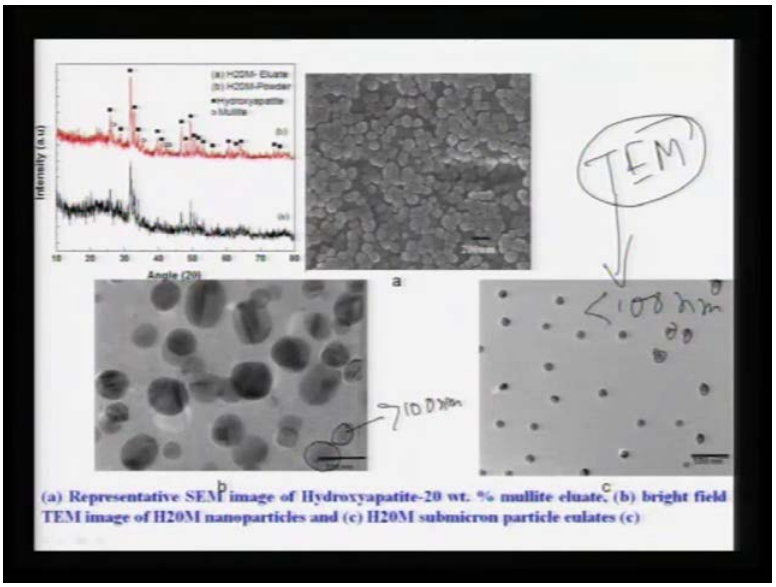

So, this is the transmission electron image, a transmitter microscope image, TEM image, brightfield TEM image, and you can see, this is 500 nanometer, means, individual particles are somewhere between 70 to 80 or 80 to 90 nanometers, which is less than 100 nanometer. Here, this is 500 nanometer means, individual particles here is greater than 100 nanometers, so this is less than 100 nanometers, other one is greater than 100 nanometers. So, that we can see, that what is the effect it can have as far as the toxicity is concerned.

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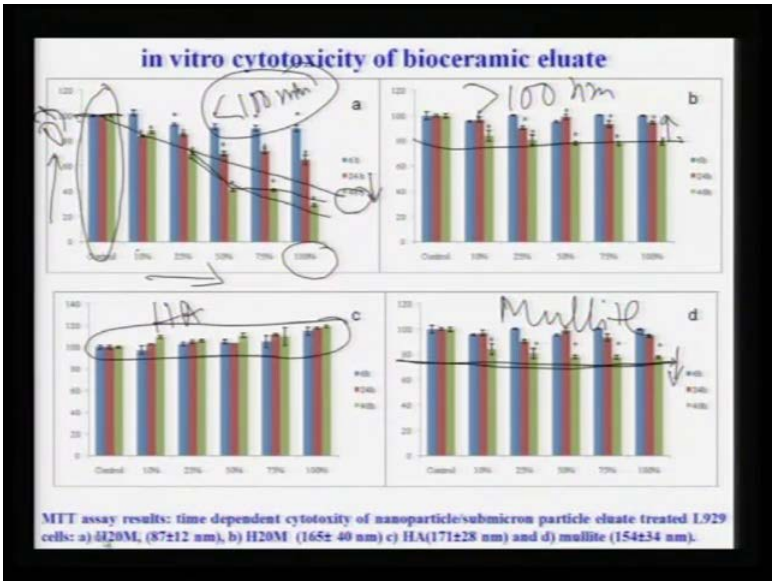

So, on that x-axis it is the optical density is plotted and along the y-axis, along the y-axis, it is that different concentration of the hydroxyapatite-mullite that is plotted here.

Now, control means, there is no particle treatment. So, in the control case, all the cells are surviving. The moment you are adding the 10 percent to 100 percent concentration of the hydroxyapatite-mullite particulates, then what will happen? You see, after 24 or 48 hours, the optical density decreases steadily, that means, the cells are, less cells are becoming less and less survivor. So, lower the optical density means, lower number of cells are surviving in the culture medium. So, therefore, after long time, like after 2 days, that all these less and 100 nanometer particle size, that cells are, cells are dead and then it shows some cytotoxicity.

However, when the particles size is greater than 100 nanometers, what you see? That, you do not see that much drastic optical density deduction, the way you have seen in the case of 100 nanometer, because here OD value is more than 80 and the above 80. So, you cannot call it as an indication of the cytotoxicity, this is the case for the pure hydroxyapatite. As expected, you do not see anything cytotoxicity, this is the case for pure mullite and you normally see, that is, 80 percent or less and above that, 80 percent or 75 percent or 100 percent mullite concentration.

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This is the typical cell morphology of the L929 fibroblast cell. What you see here, that cells are proliferated in some cases, but there is limitation of the number of cell proliferated when you were treating them with the mullite particles and some small, small particles. What we have seen, they are becoming internalized in the inside the cytoplasm of the cell.

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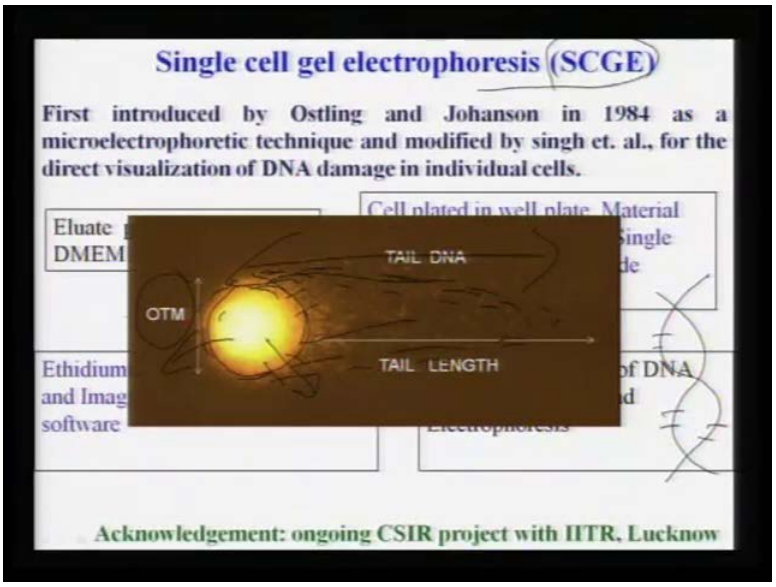

This is what you can see when you do the some experiments, what is known as single cell electrophoresis. This is the nucleus of the cell and on this nucleus, outside the nucleus you can see, the DNA is fragmented. If you remember, that earlier one of the slides, that I have shown you that in the double helix pattern of the DNA, so this is the double helix pattern. Now, if that some part have broken, that means, DNA is becoming fragmented and once the DNA is fragmented, they also spread out both, inside and outside the cells because why spreading out is possible? Because cell nucleus has also nuclear pore complex, that means, there are certain pores at there and through this pores, this fragmented DNA can be outside the nucleus.

Now, how to quantify DNA damage? Now, you have a certain tail here, you can measure this tail length, that is called DNA tail length or you can measure the OTM. OTM stands for olive tail movement; Olive is the name of the scientist who defined that, what is the tail movement Olive had? Thereby, this DNA this comet has and then you can find out that what is the tail DNA length also.

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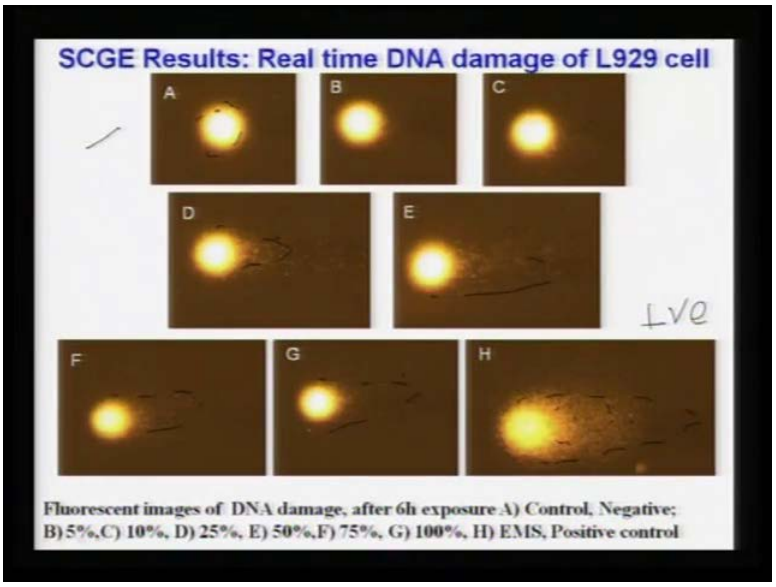

Now, if you measure this, you will see that this is the fluorescence image. This is, A stands for control, B stands for 5 percent, 10 percent, 25, 50, 70, 100 and this is the positive control. This positive control means, you can see extensive DNA damage here, lot of fragmentation; negative control means, no DNA damage.

In between positive and negative, these two extremes, you see, the moment you are adding 10 percent, 25 percent, 50 percent, 75 percent, that means, they are progressively more and more DNA being damaged here and that is what is being shown here in this particular plot.

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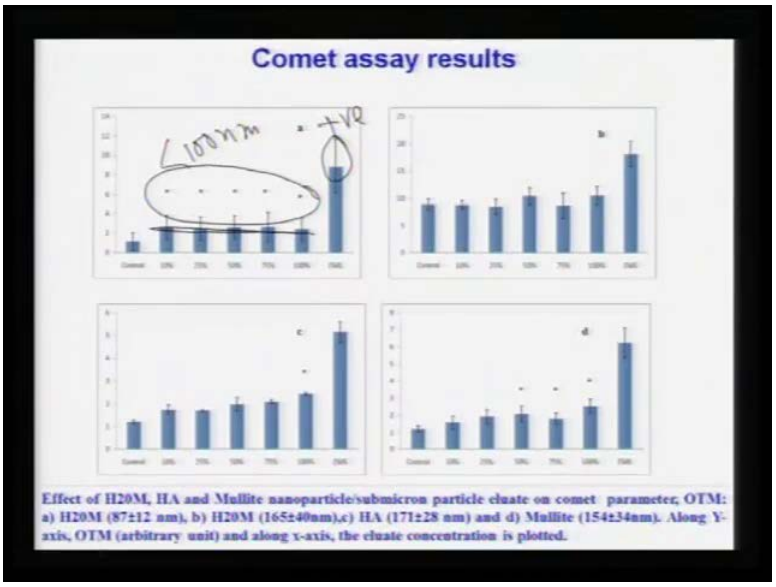

You can quantify it and then, once you quantified you can see, this is the less than 100 nanometer H 20 m particle size and you can see, that statistically significant DNA damage because there is a much higher tail DNA length compared to the control, which is negative control. But certainly, this tail DNA is much less than, which is known as the positive control. Positive control means this one, so compared to this one, you do see, that is a reduced, reduced value of the DNA fragmentation in this materials.

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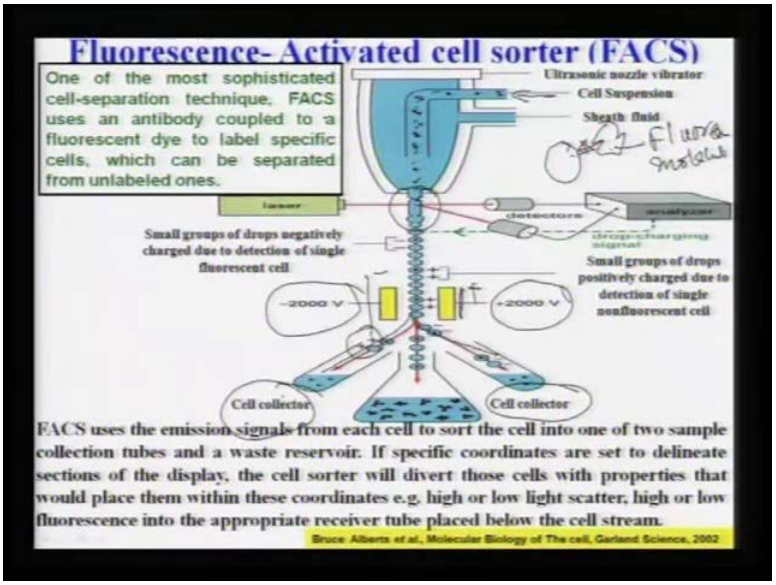

This is the last thing that I want to discuss in the cell apoptosis. This is kind of very unique equipment, which is known as fluorescence-activated cell sorter or a FACS machine. What, what is the basic principle of this machine is that all the cells, they are tagged with certain fluorescence molecule.

So, suppose, it is a cell here, now there is certain fluorescence molecule here and this fluorescence molecule are being attached to a particular cell here. Now, every molecule performs different function, I will come back to that. Now, this fluorescence molecule, the tag fluorescence molecule, they come with a cell stream. Now at certain point they will experience certain electric field and typically, this electric field is that 2 kilovolt. So, there is a voltage difference, one side is positive, one side is negative, so this is the two kilovolt voltage difference. So, depending on the molecules, which are being tagged to the cell surface, certain cells will be separated from this time onwards. This way certain other cells will be separated from other test tube and the cells, which are unaffected by the electric field, they will go straight and then they will be accumulated at the bottom.

Now, what are type cells they are being accumulated? This will be the two as the positive electrode, that depends on what kind of, what kind of property this fluorescence molecule has, and how this fluorescence molecule are being attached to the individual cell.

So, this, uniqueness of this process, this is, that one of the unique process, which is also known as the, which is also used in the cell sorting process. So, this is known as the cell collectors, these are like different cell collectors and then, before it is being, before it is entered in this electric field, there is a laser. There, this laser is focused on the cell stream and once this laser is focused to the cell stream, this laser, depending on what is the population of the total cells, which are still alive, that population can be detected.

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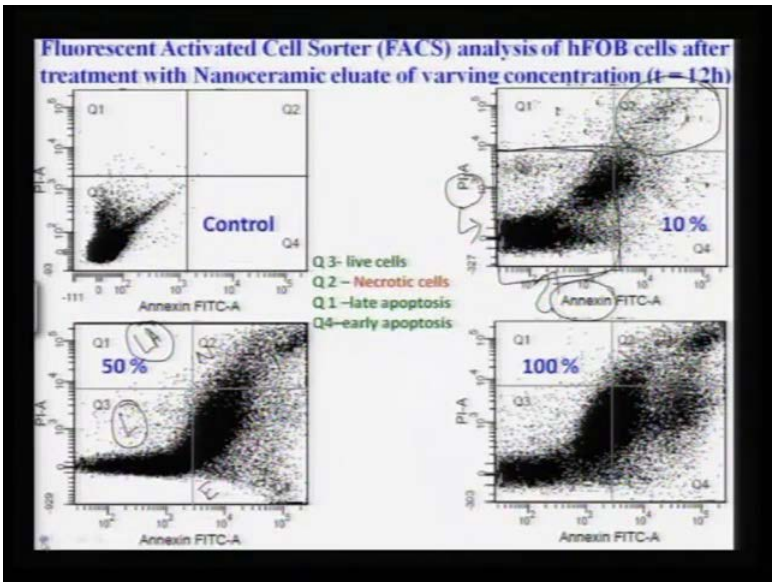

This is the typical results. Now, this Annexin is one of the fluoromolecule; propidium iodide is another kind of fluoromolecule. Now, what is the uniqueness of this FACS analysis? This will tell in a given cell population what are fractions of the cells, which are alive. This is the, L stands for alive. Now, what is the cells, which are in the necrotic stage? That, is n. What are the cells, that are in the early apoptosis stage? That, is E and what is the stage when, what is the, how many cells are that is in the late apoptosis process?

Let me summarize. You see here four quadrants: one, two, three, four and the individual data points here, that individual data points are collected from one cell. So, each data point means one cell information, the second information, that second thing that you must remember is there, that these particular coordinates, the way it is mentioned, that is coming to this one.

This particular coordinate stands for one of the collector tube, so that how many cells, that are being deflected in a particular direction, that individual coordinate data points is actually deflecting the cells in that particular collector tube. Now, if you go to the different coordinates here, if you go to different coordinates here, that means, these data points means, this many cells which are, which are collected in a different collector tube. Similarly, this coordinates means, this information being collected from some other collector tube, like that there are different collector tubes are there. Now, each coordinate

has a particular meaning and where, what is that meaning? This meaning is that in one of the coordinates if it is the bottom one, then PI is negative, propidium iodide is negative and Annexin also is negative.

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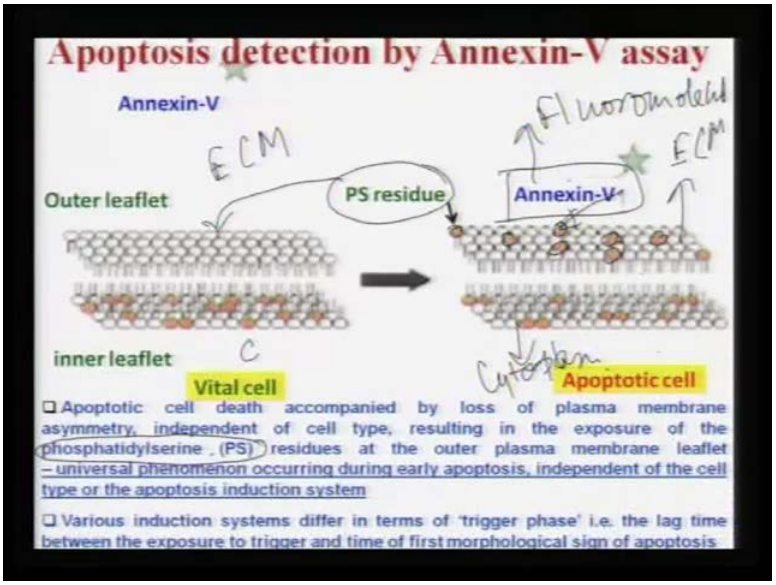

Now, what is this negative and positive? They two, in this FACS process, they are two cells, that has been shown here, left one is the vital alive cells, right one is the apoptotic cells. Now, in these two cells, this, this is called plasma membrane. This plasma membrane has a particular structure, as I mentioned while discussing the cell structure, plasma membrane has a typical double layered type of structure, means, certain molecules are exposed to the extracellular space, certain molecules are exposed to the cytoplasmic space.

So, this is your cytoplasm, this is your ECM. Now, what happens when a cell undergoes apoptosis? Some molecules, which is known as, that phosphatidylserine, that is, the phosphatidylserine molecules, PS, this is the red one here or different colored ones, this colored molecules, there be in, being increasingly more exposed towards the ECM, compared to the cytoplasm.

But when a cell is alive you do not see any of the phosphatidylserine residues here in the outer leaflet, which is exposed to the extracellular matrix. Now, Annexin-5 is a particular fluoromolecule. Now, what it does? This fluoromolecule has the specific binding

capability to the phosphatidylserine. So, this, what, when a cell is undergoing apoptosis, this Annexin-5 comes, they are being attached to the PS molecules and then, more the number of data points you have, that means, more number of Annexin-5 molecules are being attached to the more number of cells during the cell apoptosis process. That means, when the Annexin-5 is negative, what it means? It means that the cells are live cells.

When Annexin-5 is positive and more concentration, that means, more number of apoptotic cells is present, is it clear?

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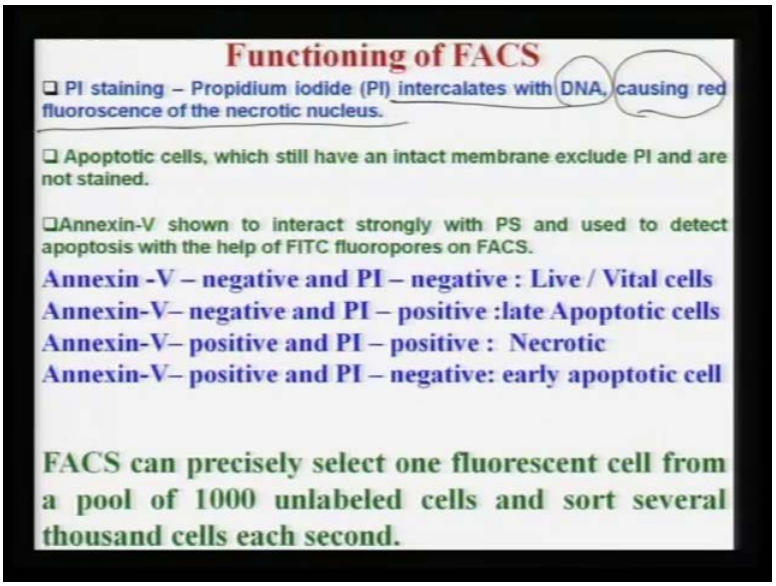

So similarly, there are different, there, there is another type of staining agent is, that propidium iodide. What is propidium iodide? Propidium iodide directly intercalates with DNA and causing red fluorescence of the necrotic nucleus.

Now, if propidium iodide is negative, what it means? It is live cells. If propidium iodide is positive, that means, it is necrotic cells. So, similarly, if Annexin-5 is positive, it is apoptotic cells; if Annexin-5 is negative, it is simply apoptotic; Annexin-5 negative means live cells, Annexin-5 positive means, it is apoptotic cells. So, both the case, Annexin-5 positive and PI positive, that means, cells are not anymore alive.