Bioengineering: An Interface with Biology and Medicine
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Lecture – 06
Nucleic Acid & Central Dogma

A welcome to MOOC- NPTEL course on bioengineering an interface with biology and medicine, in last week, we started discussing about why biology is so important for engineering discipline, I try to give you various examples in which we; we can see that you know the bioengineering has started making huge impact in many applications, then we kind of; we talked about basics especially, the live properties, the cell; difference cell organelles and their function.

I also try to provide you a clinician's perspective by interaction with Dr. (()) (00:55) to give that you know in which way clinicians also looking at various engineering solution that is logical solutions for various medical problems.

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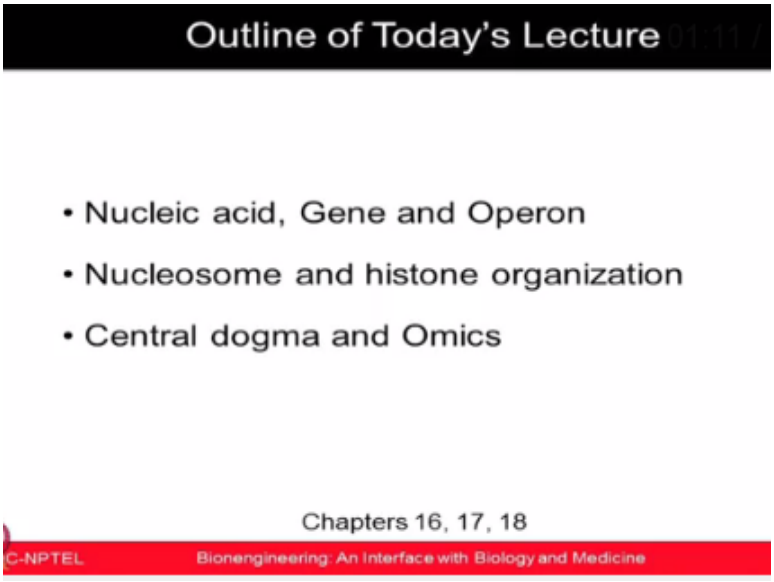

So, this week we are going to talk mainly about the DNA and DNA tools and how biotechnology has started making impact by knowing the DNA technologies, so we are going to talk about you know the various technologies involved in doing the molecular biology research especially, polymerase chain reaction, different gene cloning processes, those of all, we are going to cover in this week.

But I thought it will be important before we start going into detail of those technologies to first introduce you again with the nucleic acids and central dogma that is the theme for today's lecture although, I realised that you know you might have already studied this in your earlier courses, in other classes but just kind of refreshing you about some of the concept for the nucleic acids, gene and operon.

And then, I will talk to you about central dogma and in which where it is important in the Omics era and then these concepts, how we can you know, try to utilise these understanding, then in the next; next set of lectures in this whole week is going to utilise a DNA technologies and going to illustrate you in which we way can do research in these areas.

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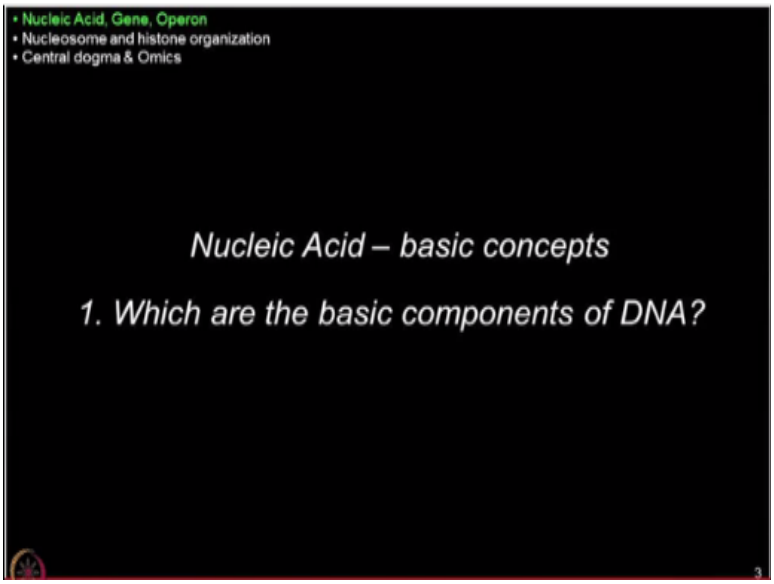

Let us first start with the basic concept of nucleic acid, which are the basic components of DNA?

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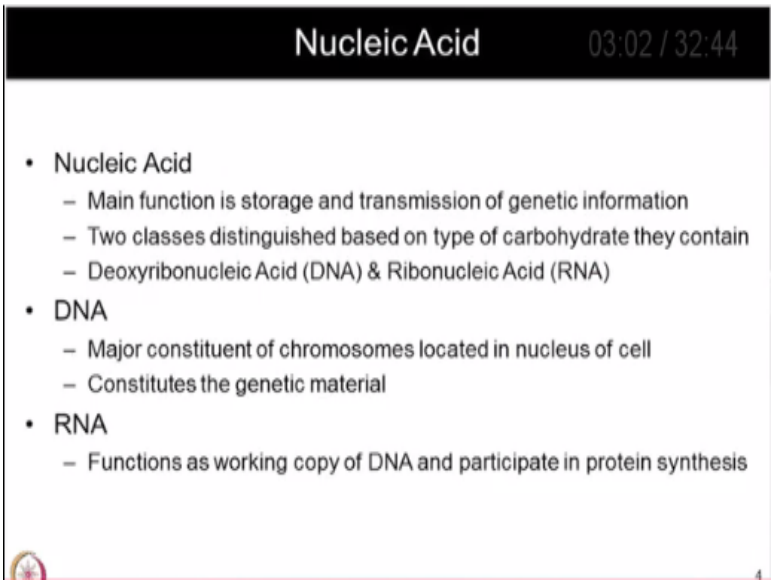

So, the main function of the nucleic acid is to store and transmit the inter genetic information and there are you know, 2 specific classes of sugars based on which the entire, the classification happens for nucleic acid, which is DNA or RNA, right, deoxyribonucleic acid; DNA and ribonucleic acid or RNA. So, the major constituents of the chromosomes which are located in the nucleus of a cell is the DNA, which constitutes the genetic material.

Whereas, the ribonucleic acid or RNA is the functional molecule or the working copy of DNA which then participate in the process of protein synthesis, so from DNA to RNA the process of transcription happens and then from RNA to protein, process of translation happens.

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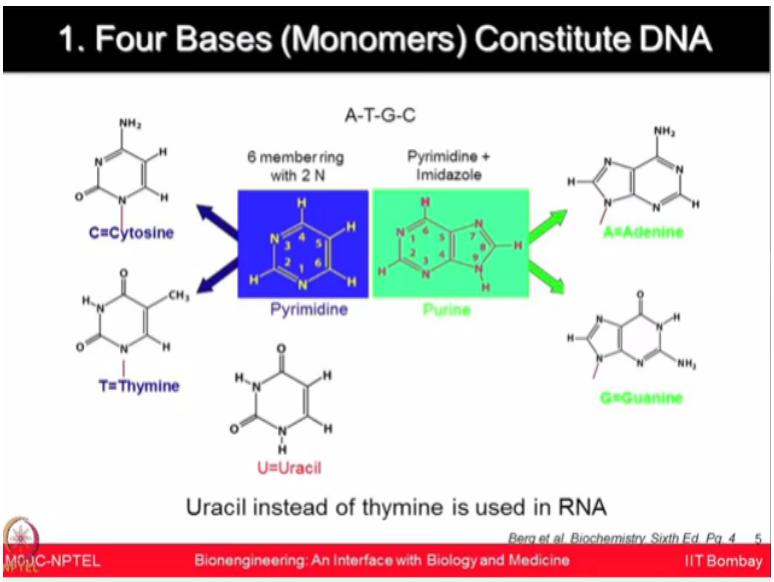

Let me now, show you again and more so refresh you about the DNA structure and the component and the Niels Bohr's involved in making DNA structure, so for example there are 4 bases; the monomers, which constitutes DNA and these are the structure shown on the screen which is cytosine, thymine, adenine and guanine; ATGC, you know cytosine and thymine, these are 6 membered ring with 2 nitrogen which is having the pyrimidine ring structure.

Or the adenine and guanine, they shed the structure with a purine ring and which is pyrimidine plus addition often, Imidazole ring. Now, Uracil is a; is unique because that is you know found in replacing thymine in case of RNA, so you can look at the structure of Uracil here.

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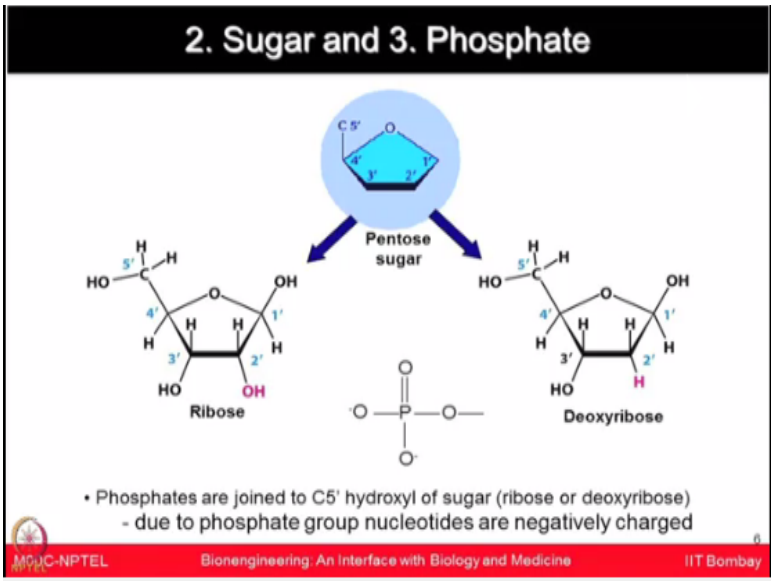

Now, let us talk about briefly these sugars; there are 2 sugars involved, one is the ribose sugar and another is a deoxyribose sugar, both of them have the pentose sugar backbone and now, on the second carbon you can see there is hydroxyl OH group there in the ribose sugar, whereas in the deoxyribose, it is hydrogen, so that is the different between the ribose and deoxyribose and then third important component is a phosphate chain which is joined with the; on the you know C5 carbon with hydroxyl of the sugar whether in case of ribose or deoxyribose.

And then because of this phosphate group, these nucleotides they are negatively charged and that property is heavily used in the DNA electrophoresis.

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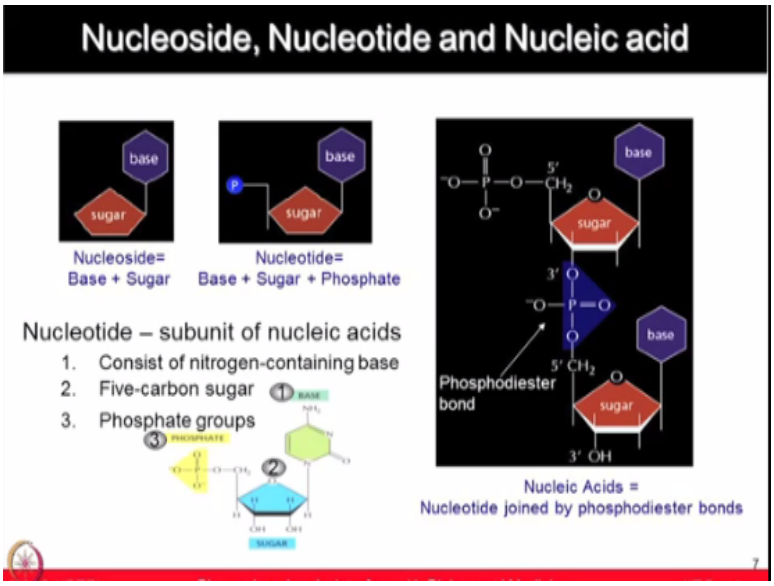

So, now let us kind of again look into these individual components, we talked about the bases, we talked about the sugar and we talked about the phosphate, right, these 3 components together form the nucleic acids and let us look at some terminology for example, nucleoside, when you are combining a base and the sugar form that together gives rise to nucleoside or when we add a base, sugar and phosphate chain that is nucleotide.

And then, when you are combining many nucleotides which are actually join with the phosphodiester bonds that is known as nucleic acids, so a nucleotide is a subunit of nucleic acid which consist of the nitrogen containing bases, which is having a 5 carbon sugar and the phosphate group, so again the structure is shown you here, which is for a nucleotide. So, now in this way now, you can easily decode the entire DNA structure which is you know straight forward know, we have good understanding that you know in which the nitrogen bases, the sugar phosphate backbone is constituting the DNA structure.

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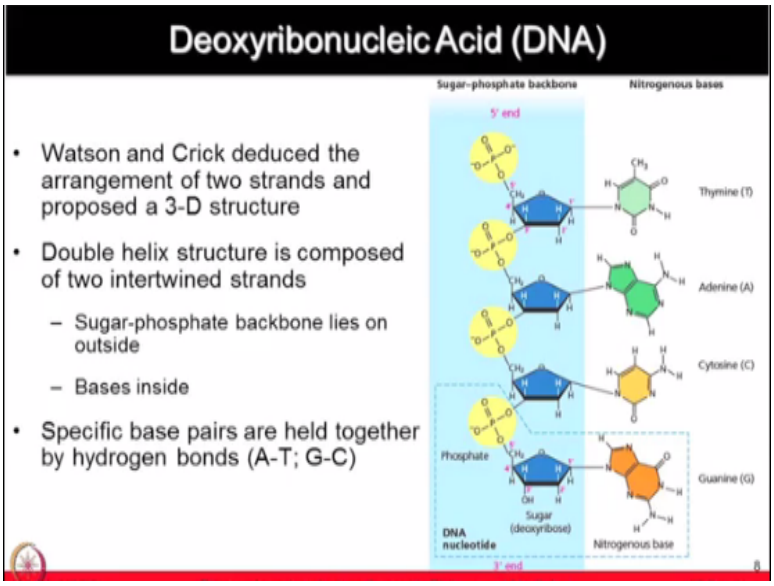

But of course, it was not known earlier and the scientist Watson and Crick, they get the credit for the deducing the structure of the DNA and they are you know, how these are the 2 strands are arranged in a helical form, how that are intertwined and the sugar phosphate backbone lies on the outside whereas, the bases are inside and then the base pairs are specifically forming the bonds, which is hydrogen bonds between A and T and G and C bases, right.

So, there was always that you know quest to elucidate the structure of DNA and many scientists you know started working in that area and try to find out in which way the DNA structure is made.

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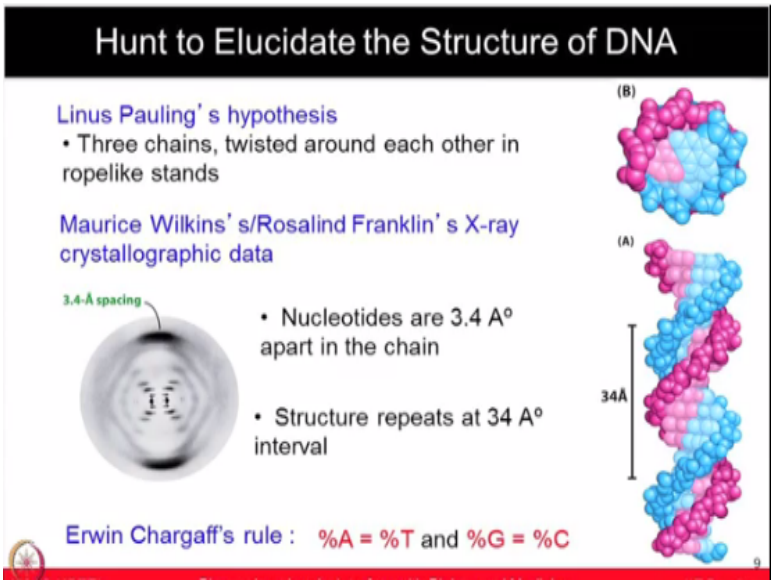

So, the Linus Pauling; he made a hypothesis that there are 3 chains, which are twisted around each other and they form you know, some sort of rope like a strands and that could be you know, how the DNA structure is made, so then Wilkinson and Franklin, these scientist; they provided x-ray crystallography data and then they found that you know, nucleotides are 3.4 angstrom apart in the chain and the structure repeats at found that the 34 angstrom interval.

So, there that gave much more clarity for the you know, the structure of DNA and then a scientist Chargaff, he provided the some basic rule that you know the if components are going to be equal to the T components of percentage of A = percentage of T and percentage of G = percentage of C base pairs.

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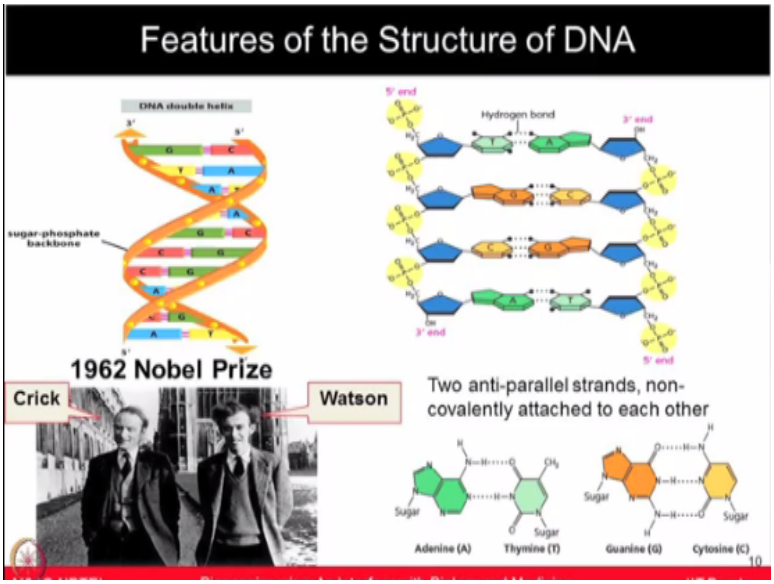

So, this summarises the kind of you know what we have discussed the structure of DNA in which way, adenine and thymine are they form the 2 hydrogen bonds and guanine and cytosine, they form 3 hydrogen bonds and the structure of DNA decoded by a scientist, Watson and Crick for which they were awarded the Nobel Prize in 1962. Now, then you know, how DNA makes its multiple copies, so the DNA duplication or replication of DNA that is another interesting concept.

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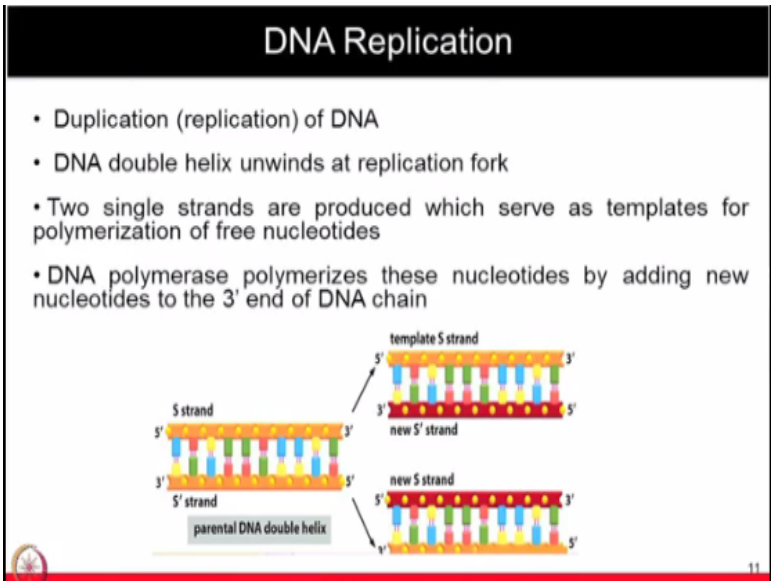

So, the DNA double helix is actually you know, starts unwinding at the replication for, so now 2 single strands are produced from this double helix DNA, which serves as templates for polymerisation of free nucleotides. Now, DNA polymer is; it starts polymerising these nucleotides by addition of some new nucleotides to the 3 prime end of the DNA chain and now, from the same DNA, now the 2 copies of DNA is made.

And as you can see that you know from the template S strand, now we have new strand being synthesised and now, we got 2 DNA molecules in the process of replication. So, the different theories which are involved in the DNA replication, I will talk to you about you know, the DNA replication and some of the classical experiments done in some other context that are on but just for the timing, I thought to you know, just give you the feel of that you know, how DNA copies are being made.

And why it is so important for us to understand DNA structure because you know, the DNA so much fundamental to our life, to our hereditary information that you know how DNA structure is, is found and how you know it is compatible with all you know, any possible sequence of bases that is very important, I think for us to appreciate.

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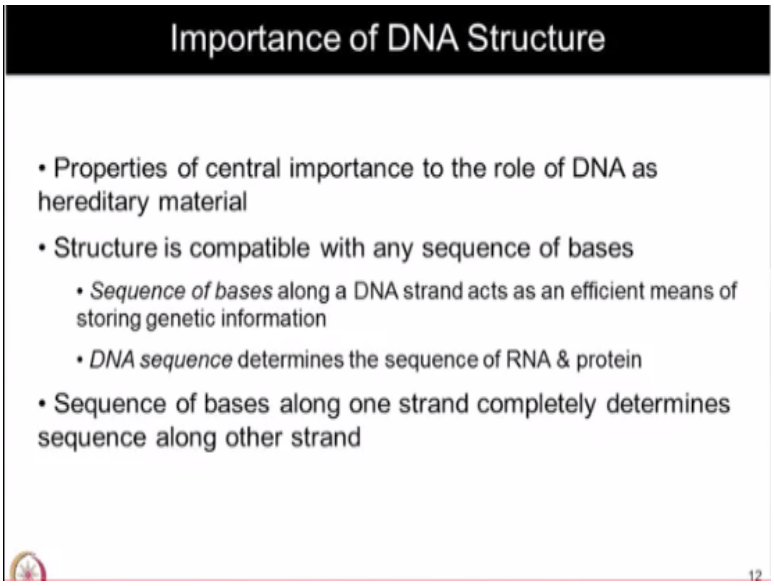

And understand that the sequences of bases along any DNA strand, they act as a very efficient means to store the genetic information, so knowing the DNA structure becomes very crucial and the DNA sequence actually ultimately determines the sequence of the you know, ribonucleic acid and eventually, the proteins are formed from that so, these sequences of bases along with one strand they are you know, completely determines the sequence of other strand.

And then, they are going to dictate the RNA formation and protein formation, so after you know revealing some basic concepts of DNA and DNA structure, let us now think about a question about what is the gene, right.

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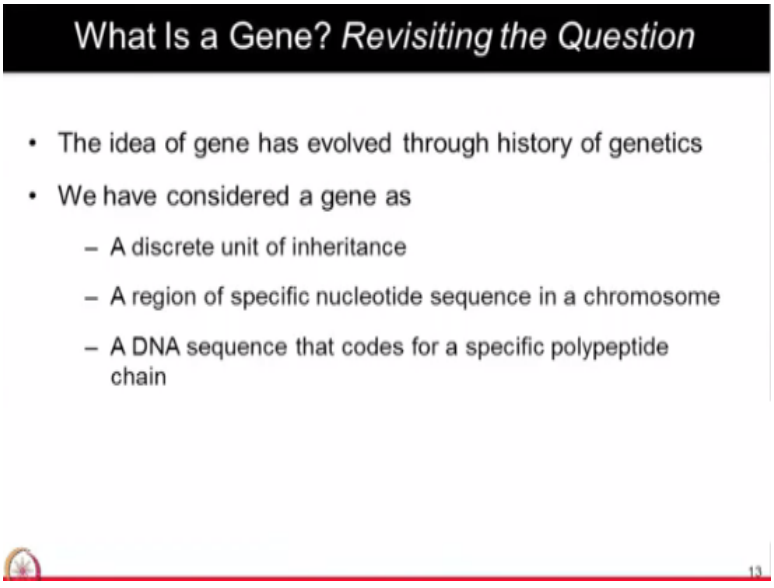

The concept of gene has actually evolved through the history of genetics starting from you know the scientists like Mendel who was thinking about you know, there is some hereditary factors which are; he did not know about gene that these formations are passing from one to next generation, probably there are some factors which are involved, which are having these information is stored.

And then, Morgan; kind of provided some further experimental evidences that you know these hereditary units are actually located on the chromosomes, so many scientists have contributed in the journey and now, finally we know that you know what we consider a gene is actually a discrete unit of inheritance or you can also defined that it is a region of a specific nucleotide sequences in a chromosome.

Or you can say, it is the DNA sequence that codes for a specific polypeptide chain, so if you just want to you know, get a broad overview of gene, I think we can summarise, it is a region of DNA that can be expressed to produce a final functional product, it can be a polypeptide or it can be an RNA molecule so, this is how you can think about a gene, one of the discrete unit of inheritance, which is providing these kind of function and formation.

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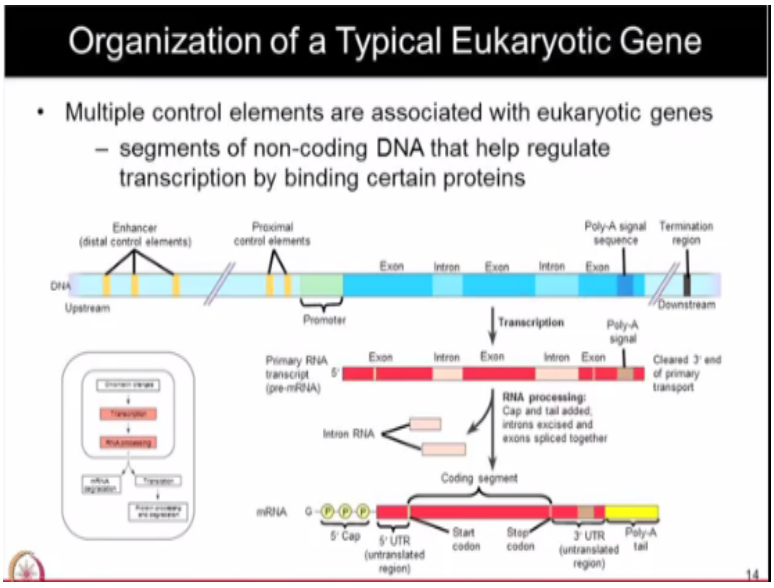

Now, the organisation of a typical eukaryotic gene is you know is really complex and having you know, many processes which are involving to; to shuffle from the DNA to make the RNA but

kind of this try to illustrate you are that we have you know various exons regions and we have a various introns, now in the; in the process of alternative splicing in which way now these introns are removed and the coded form the exons are coming together to give rise to the functional RNA molecule.

So, there are multiple control elements, which are actually associated with the eukaryotic genes and the segments of non-coding DNA which help to regulate the transcription by binding to the certain proteins. The concept of Lac operon becomes very crucial.

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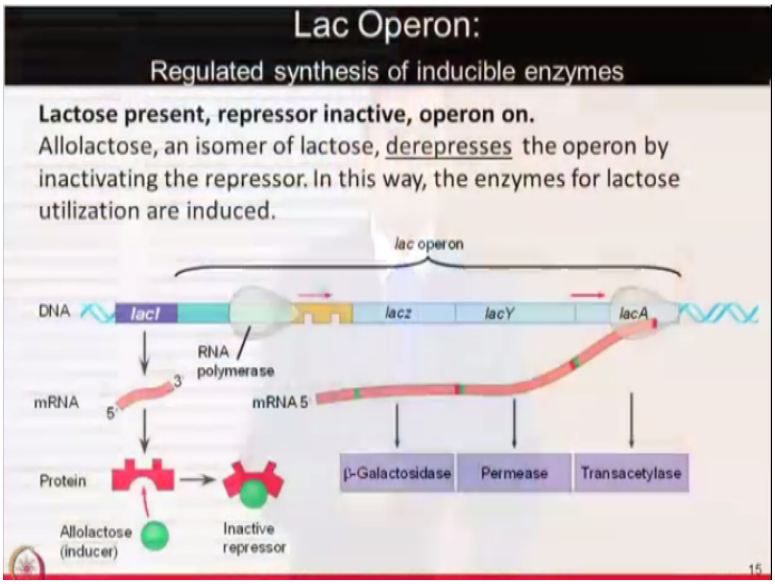

In this model, you can see that you know in the; if you have Allolactose, which is an isomer of lactose sugar that you know, D represents the operon by inactivating the repressor and in this manner, and this enzyme for the lactose could be utilised and then it can be further induced, so I am going to show you this in one of the animation and to explain you in much more detail.

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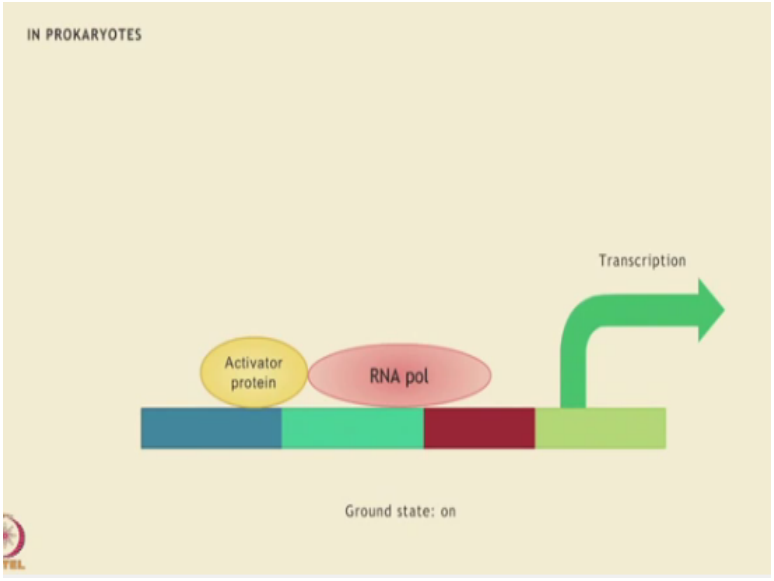

In prokaryotes, transcription by RNA polymerase can take place with the help of an activator protein.

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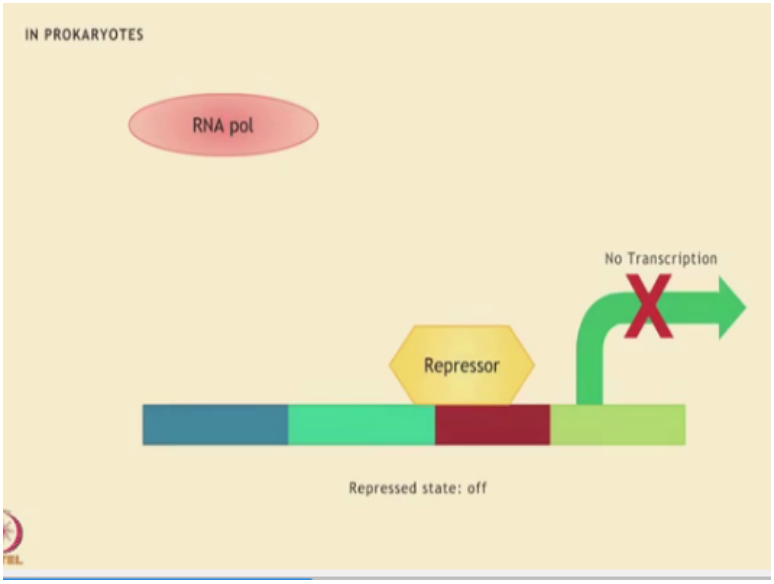

However, in the presence of a repressor molecule, the binding site for RNA polymerase is inaccessible due to which transcription does not occur. In the ground state, the repressor does not remain bound because of which the gene is turned own.

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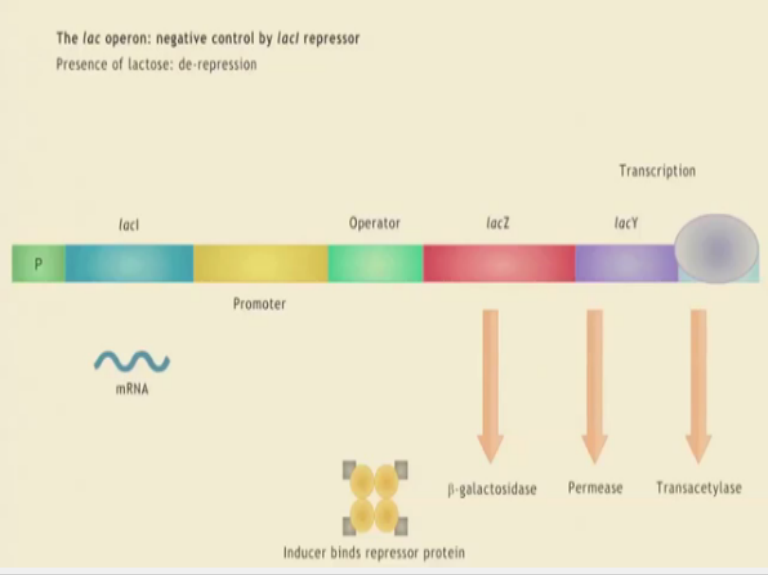

The lac operon consists of a group of genes that are responsible for transport and metabolism of lactose sugar in certain bacteria like E. coli, this operon is under negative regulation by the lack I; repressor protein. In absence of inducer, the tetrameric repressor binds to the operator region thereby, preventing transcription by RNA polymerase. In presence of inducer, the inducer binds to the repressor protein, which then prevents it from binding to the operator and therefore, allows gene expression.

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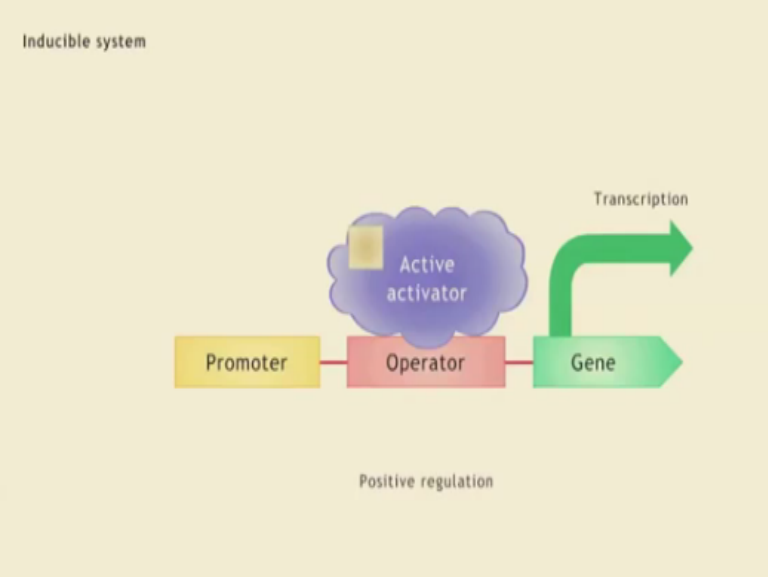

An inducible system is off state in its ground state and must be turned on by an effective molecule, which is known as inducer. In positive regulation mechanism however, the inducer

binds to the inactive activator to produce the active activator molecule which in turn facilitates binding of RNA polymerase to the promoter to turn on expression.

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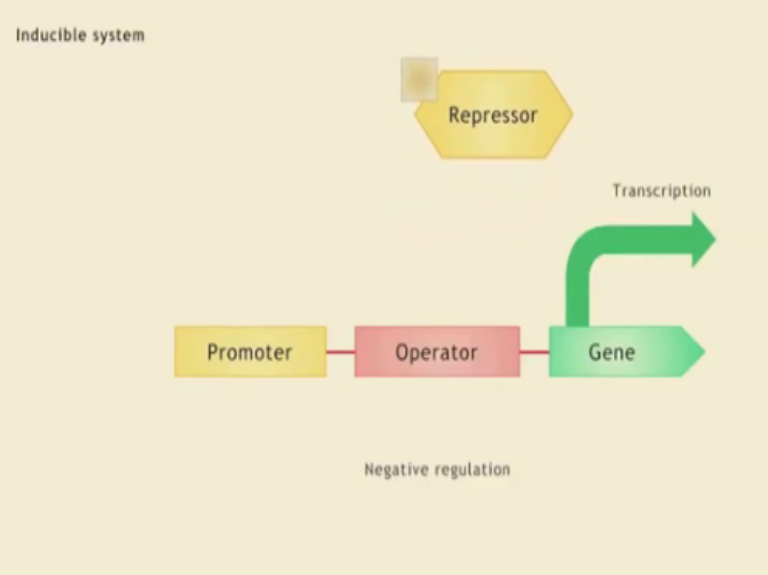

In the negative regulation mechanism, the inducer binds to repressor and prevents it from binding to the operator region; this allows RNA polymerase to proceed with transcription by binding to the promoter.

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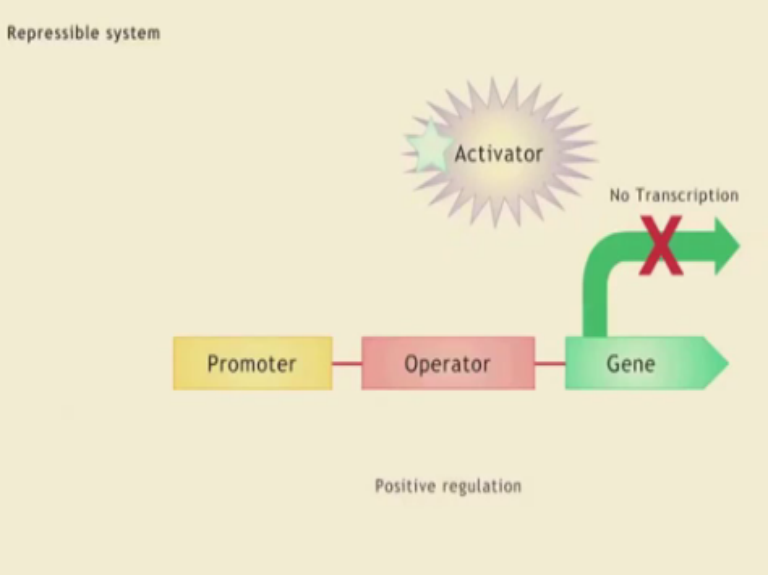

The ground state in case of repressible system is own, it has to be turned off by an effector molecule which is known as a co-repressor. In positive regulation, the co- repressor binds to the

activator molecule and prevents its binding to the promoter region thereby, turning of gene expression.

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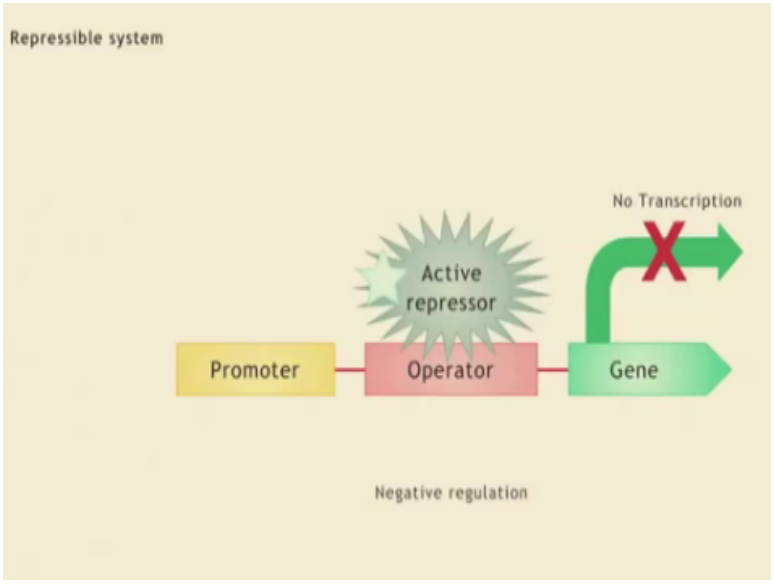

In case of negative regulation mechanism, the co-repressor binds to the inactive repressor molecule and activates it thereby, preventing gene expression.

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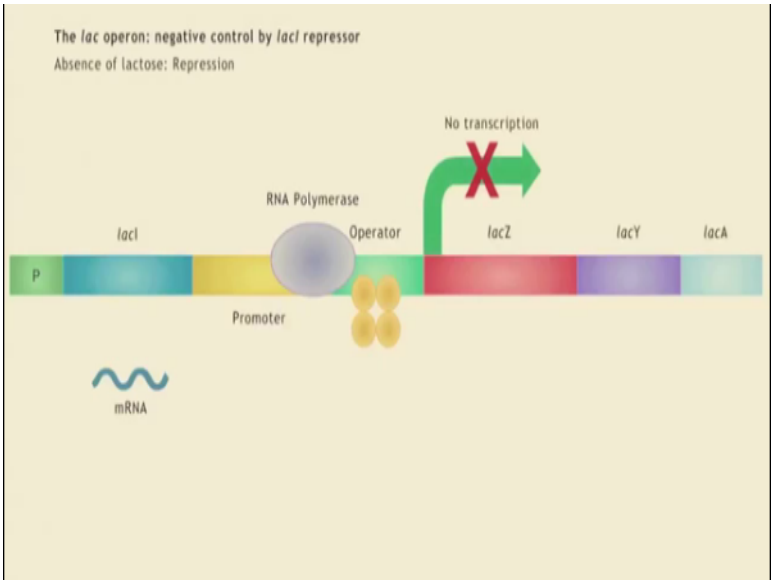

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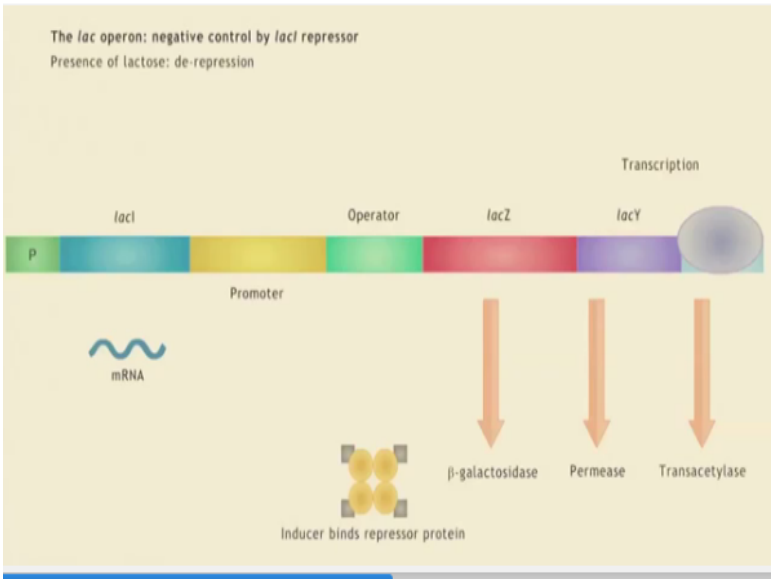

In presence of the inducer, the inducer binds to the repressor protein, which then prevents it from binding to the operator and therefore, allows gene expression.

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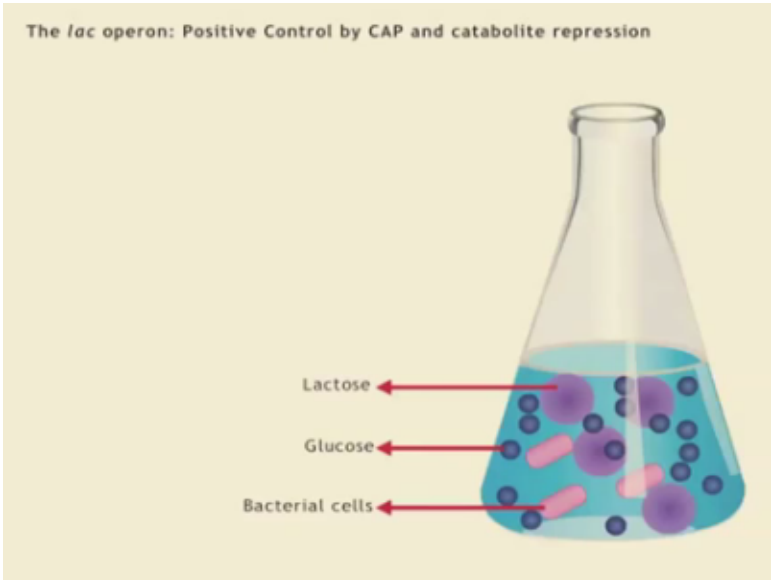

Lac operon also undergoes positive regulation by means of the cyclic AMP Cap system.

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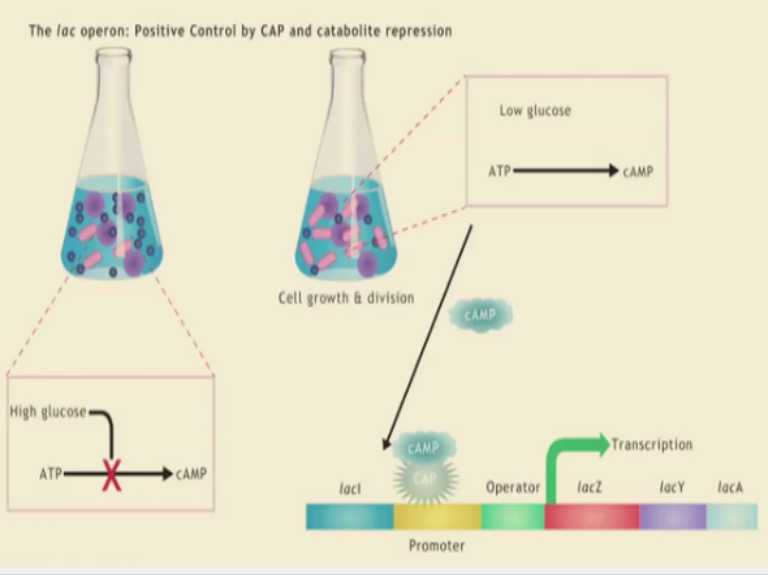

Glucose is a preferred energy source for bacteria and if both glucose and lactose are present, beta galactosidase enzyme, which metabolises lactose is not synthesise, high glucose levels prevent synthesis of the cyclic AMP which is essential for binding to the catabolite activator protein, this protein facilitates transcription of the lac operon. When glucose levels are low, cyclic AMP is produced, which binds to this gap which in turn binds to a distal part of the promoter region and facilitates transcription.

But this kind of you know, the slide illustrates you the broad model of lac operon and in which way it regulates the synthesis of inducible enzyme, okay. Now, let us move on to you know, thinking about from the cell, where we can find the DNA, right, so let us say the human body is made of billions and trillions of cells, we have discussed in the last class as well.

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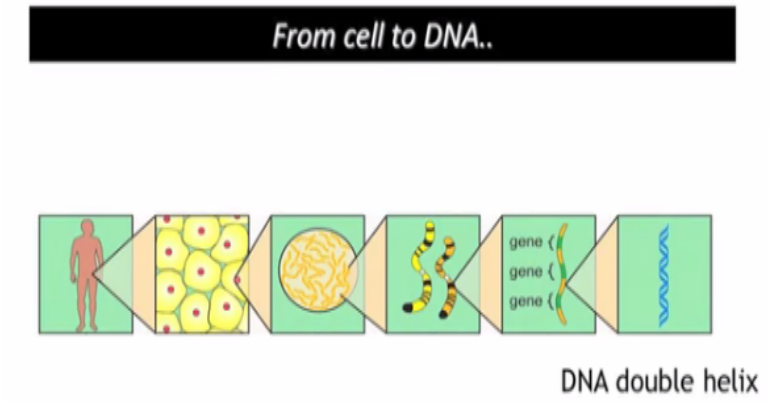

And then each of those cell is having these you know, the nucleus which contains the genetic material, now each cell contains 2 copies of these chromosomes and now, these chromosomes if you expand further, you can see the long DNA molecules, even the genes and the function region of DNA, we can; we can see over there and then, now you can see the tiny picture of you know how these DNA molecules you know, if you think about the cells, each cell having the nucleus, having the chromosomes, having the gene and their; the DNA part.

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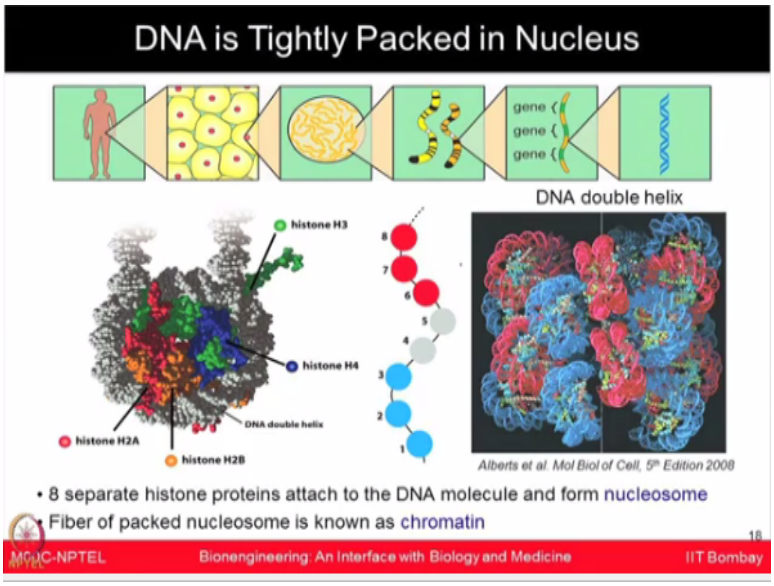

So, this is you know, really tiny bit of the molecule present in the cell but that it is so crucial which dictates all the hereditary information and it is you know, kind of packaging in this cell becomes very crucial as well and again, to refresh you from the previous lectures in the cell

context if you think you know how the nucleic acid contents are so tightly packed inside the nucleus in a small area with you know very intricate you know binding with histone proteins.

So, these histone proteins and these DNA molecules they formed these nucleosomes and these nucleosomes together are packed to form the chromatins, this how you know, these particular packaging happens inside the nucleus and let thinking about eukaryotic genomes, how they are organising the chromosomes, I think you know, knowing about the histone proteins becomes very crucial.

So, these histone proteins as I mention H2A, H2B, H3 and H4, they are positively charged proteins which could interact with the DNA molecule which is negatively charged and that actually helps to compact the DNA and these nucleosomes could be seen like the beads on a string, so this is how you can; you can think about from DNA to chromosomes.

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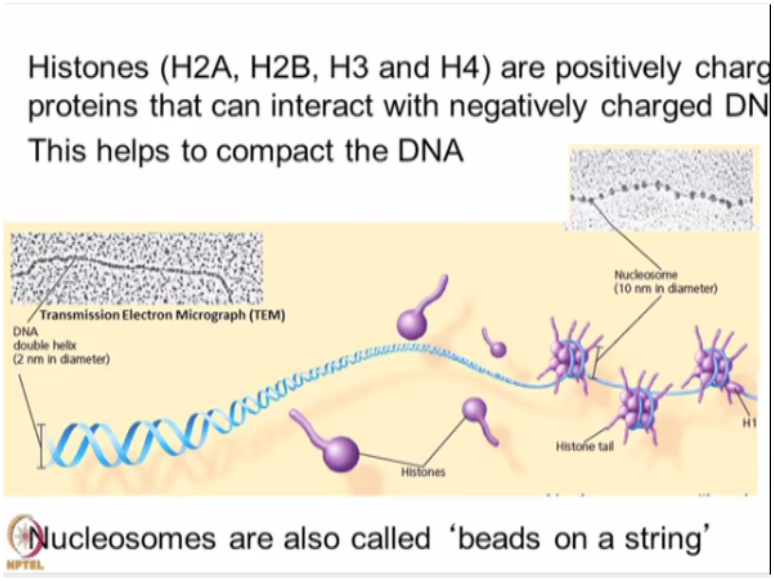

A chromosomes consist of a DNA molecule which is packed together with the proteins and now, these chromosomes could be seen which are having these you know, bead kind of a structures, right, alright, so human having 23 pair of chromosomes, 22 autosomes + one pair of sex chromosome; X or Y chromosome.

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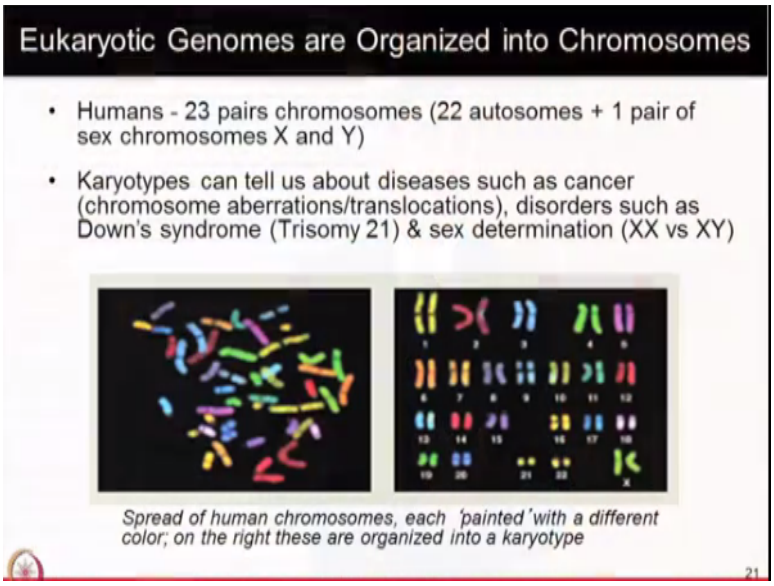

And a process known as karyotyping, where you want to look at the pattern of each of the chromosomes tells us about the you know, is the pattern of these chromosomes are normal or is there any abnormality can be seen and for many disorders like especially you know, chromosomal aberrations can be found and that actually, helps us to; to deduce is there is some sort of syndrome is present like down syndrome or some sort of you know the issues with any other chromosomes, abnormalities are there.

And even for sex determination, people look at the X and the Y chromosomes and their patterns, so this image just shows you know the colour painting of these various chromosomes but ideally, it shows us the organisation for the whole genome, alright, so there have been many discoveries which have contributed immensely to the field of you know, overall DNA related discovery which have contributed entirely from genetics to genomics areas.

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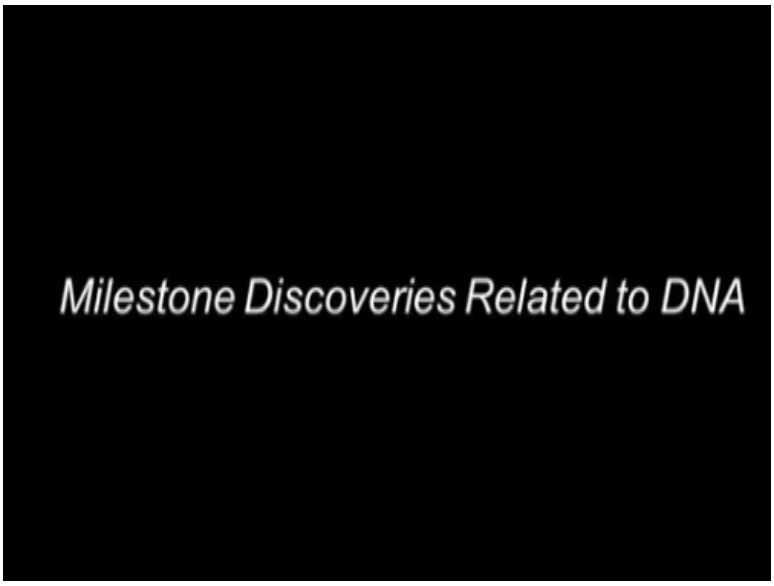

I am going to show you couple of mile stones discoveries although, you know we are going to talk in much more detail about you know many of these fundamentals and their applications in subsequent lectures.

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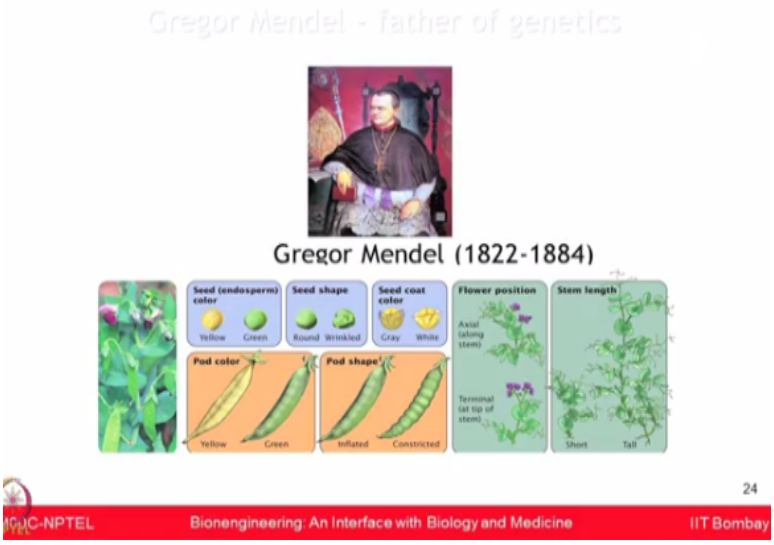

But just to kind of you know, refresh you and bring to the scale, starting from Gregor Mendel, the father of genetics, 1822 to 1844, lot of elegant experiment being done on the pea plant, which gives us the; the basic idea for you know the; how various laws of hereditary are governed and then with the ideas for discrete factors which Mendel mention, the genes are actually going to transmit characteristics from one generation to the other generation.

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Over the period, then we had you know the discoveries by Watson and Crick, which illustrated the structure of DNA.

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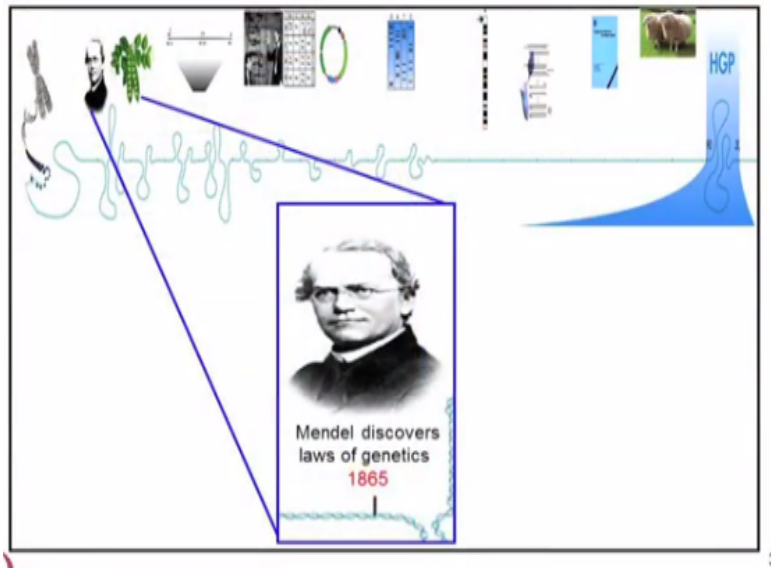

And but you know the initial part from 1865 for Mendel is you know gets credit and Mendel is known as the father of genetics for his contribution.

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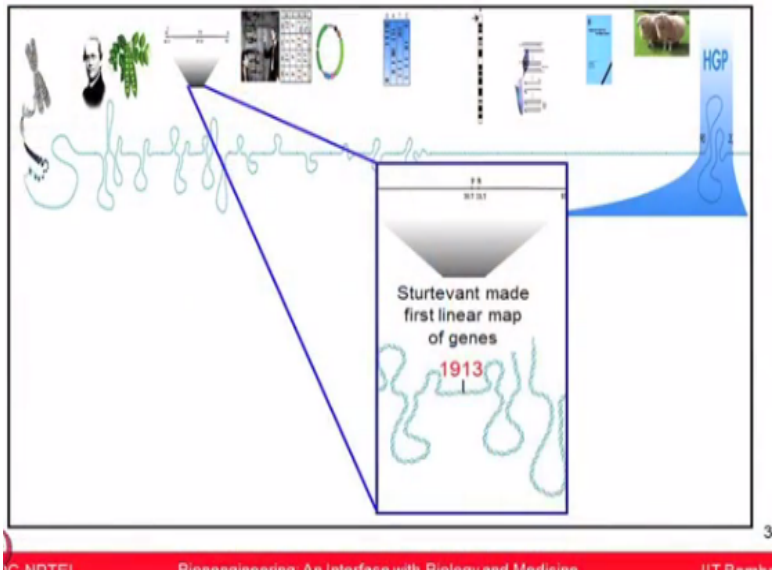

Then, as you go on the time a scale, a Sturtevant, he made the first linear map of the genes in 1913.

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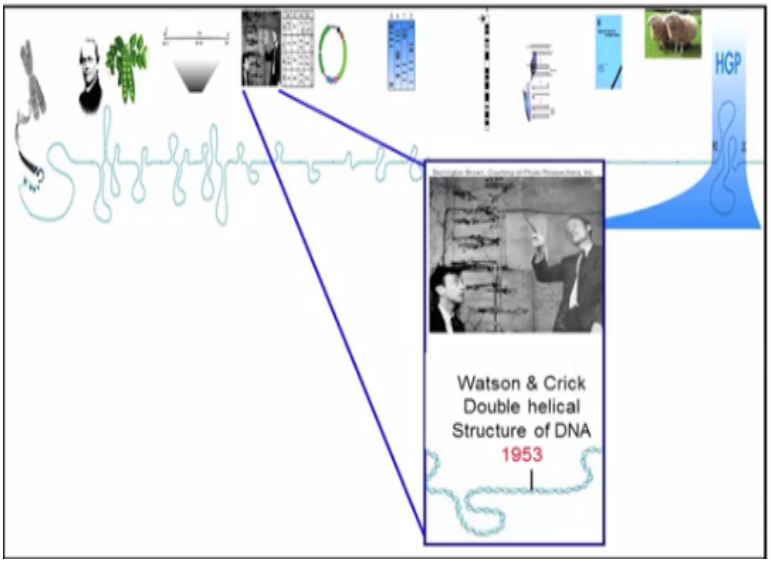

And then, came the Watson and Crick contribution for double helical structure of DNA in 1953.

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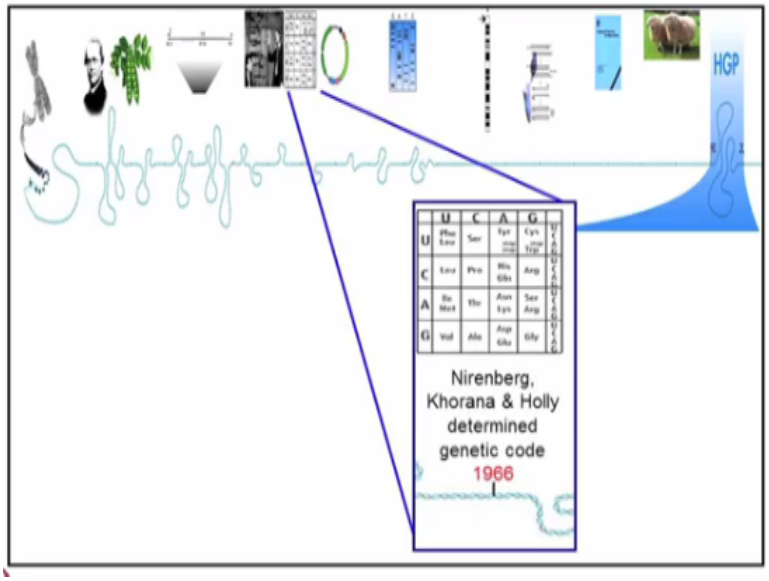

Then scientist Nirenberg, Khorana and Holly, they first mention the genetic code in 1966.

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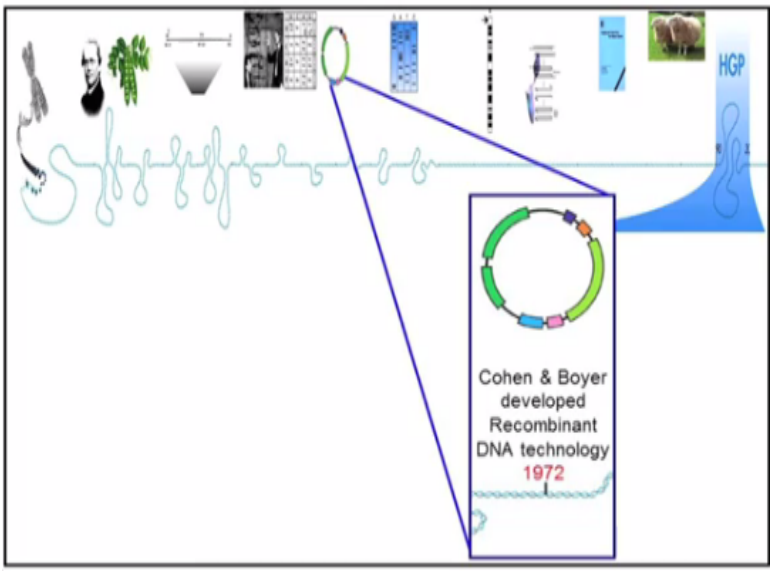

Scientist Cohen and Boyer, they developed recombinant DNA technology in 1972.

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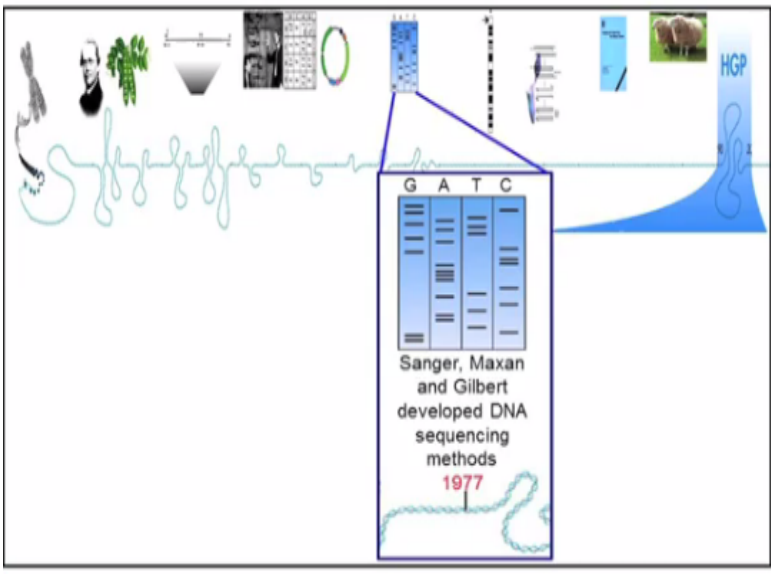

And then, Sangar, Maxan and Gilbert, they developed DNA sequencing methods in 1977.

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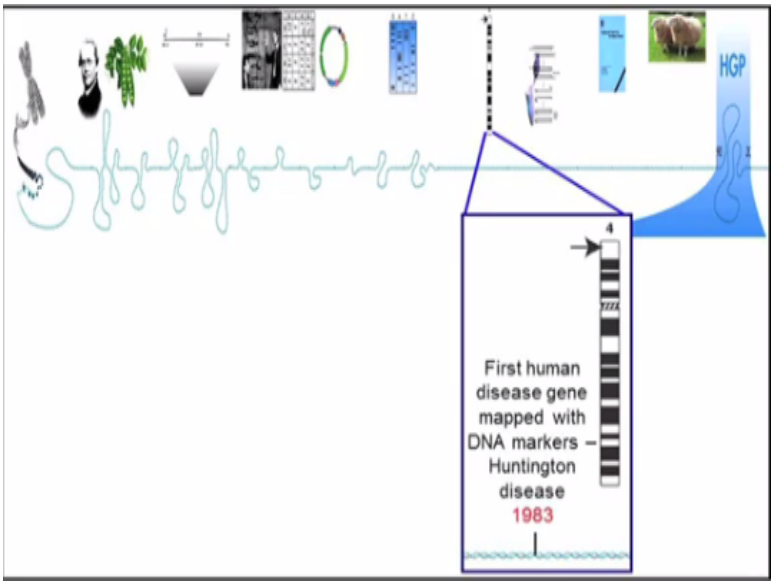

In 1983, the first human disease gene was mapped with the DNA markers especially in the disease of Huntington disease are shown.

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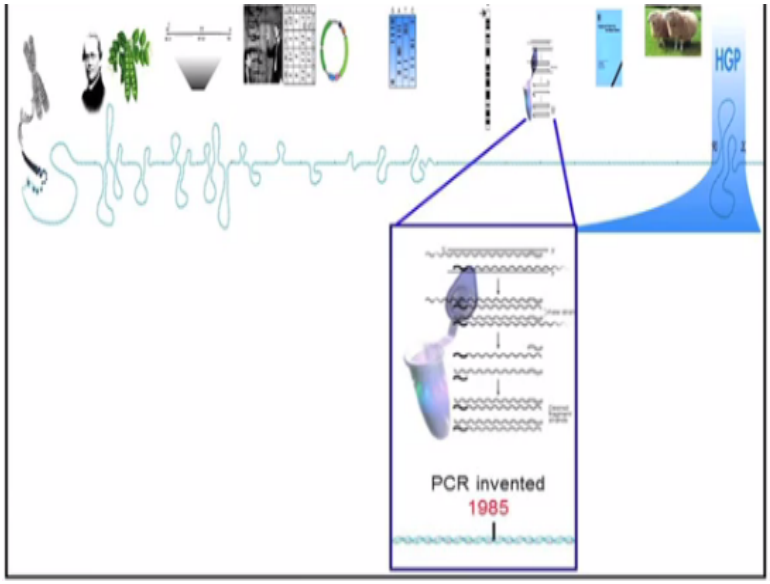

And you know, one of the milestone technologies polymer chain reaction was invented in 1985.

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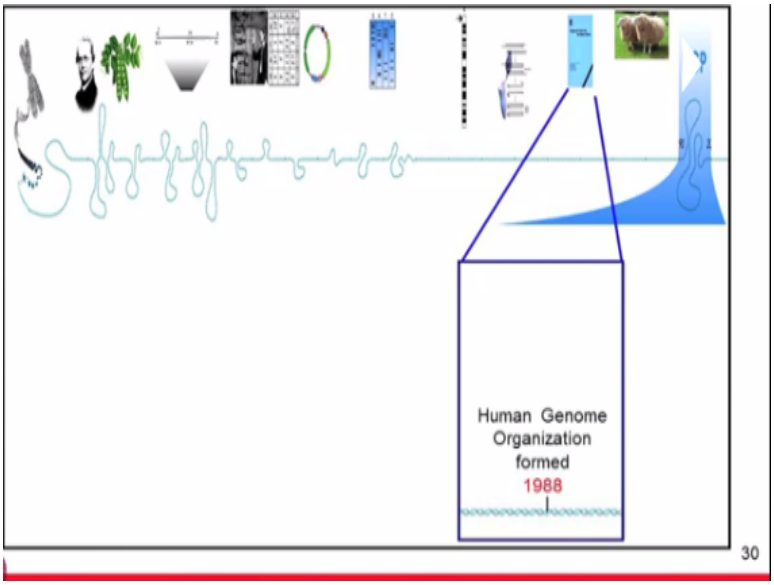

And then, the human genome organisation started you know, an ambitious project of knowing about all the human genes in 1988 and then while those things were happening, we started knowing more about the cell, about cloning, about you know development process and reprogramming.

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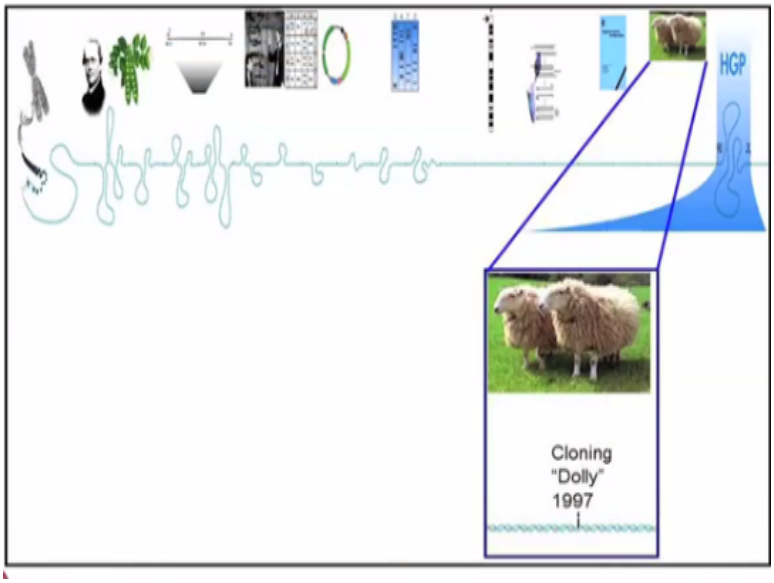

And that eventually, culminated into the cloning of an animal, Dolly by Ian Wilmut in 1997 and that is another you know one of the scientific fiction and an story in which one could produce a cell or organism with the same nuclear genome as another cellular organism.

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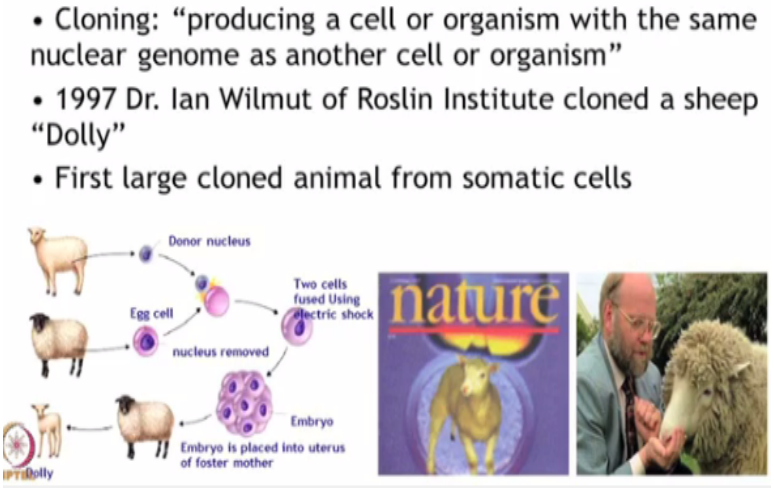

And Dr. Ian Wilmut of Roslin institute, they cloned this sheep Dolly which was major accomplishment at that time.

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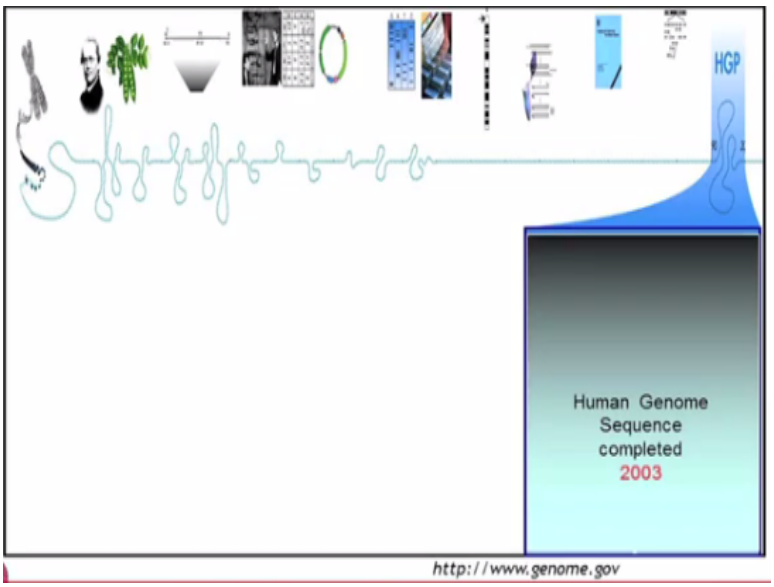

Then human genome sequence projects are getting competed in the years 2001 to 2003.

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And then, first draft of the human genome map was presented in cover page of nature and science and you know, those project actually, help us to, to really try to get a bigger picture of what is happening inside you know, the entire human genome, what are all genes present there and you know it is the first most ambitious project to really understand you know beyond moving on to the single gene and looking at you know just the characteristics governed from a gene that what is happening in the entire genome.

And all the gene, how they are governing the function, so that kind of you know was the; as the big accomplishment not only to understand the gene but also in the scientific community in which way we are able to now of work you know for the understanding the all the molecules of life. For example, Omics molecules, so this brings to the second part which is thinking about central dogma.

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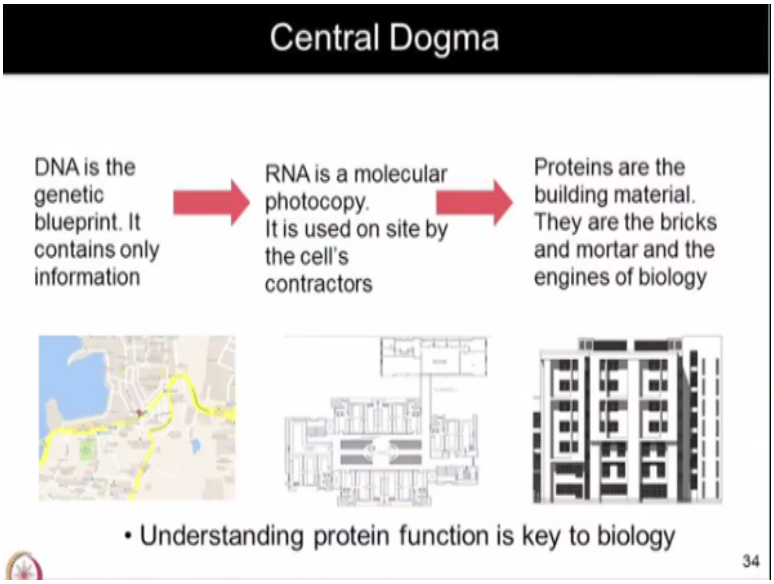

So, now we have studied about DNA, which is the genetic blueprint and just you know imagine that in a cell, now you want to; first of all, you want to know that you know where the DNA is and that DNA is going to make the molecular photocopy which is RNA and that is you know, like the functional molecule has to be initiated and from those RNA molecules, the proteins has to be made, so now use the same analogy for let us say making a building.

So, let us say you know we are in Mumbai in poor area in IIT Bombay and we want to make a building campus here, so from the map now you know that you know where the DNA, the genetic blueprint and in that area then, some contractors will come and then they will try to you know make a map that where the building has to be made and then the proteins are the building material will come which is going to be like you know, the mortars and bricks, which is going to create that building which is you know like the engines of biology.

So this something you know which helps you to have wide good technology in which way DNA to RNA to proteins, everything is crucial but in which way the proteins are much more you know direct in function they are providing much more functional molecules while the genetics blueprint come from the DNA.

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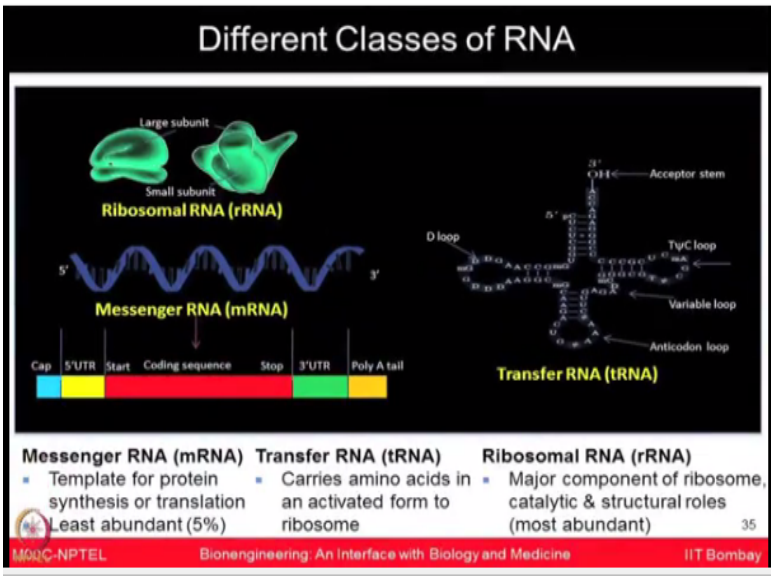

Now, let us see you know kind of briefly refresh the class of RNA's, we have the messenger RNAs, we have transfer RNAs and ribosomal RNAs. Messenger RNAs; they provide a template for protein synthesis for or the transition process to happen but they are very less abundant only you know 5% population is there for the mRNA, where the transfer RNA's, they are the carriers of amino acids and activate to form ribosomes.

Then the ribosomal RNA's, they are the major component of ribosomes, they provide catalytic and the structural roles and there actually, the most abundant among the RNA population, so RNA's, they are involved in the protein synthesis process, again which is say, you know an interesting but the complex subject which needs much more you know full lecture but just to brief you, the mRNA provides a long sequence of nucleotides, which serves as a template for the protein synthesis to happen.

Now, the tRNA's they are involved in the protein synthesis but that binds an amino acid at one end and the base pairs with a mRNA codon on the other hand and then that serves as an adapter that translates mRNA code into a sequence of amino acids.

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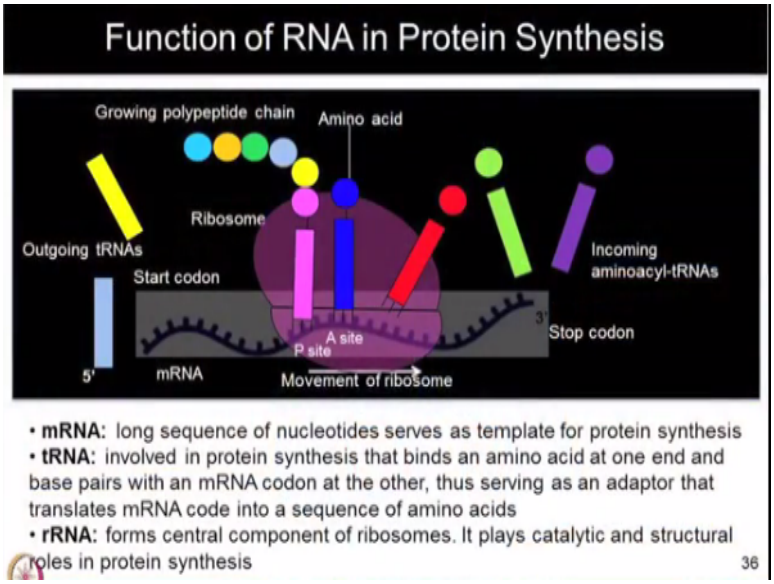

Then rRNA, it forms the central components of ribosomes, it also plays catalytic and the structural rules in the protein synthesis process.

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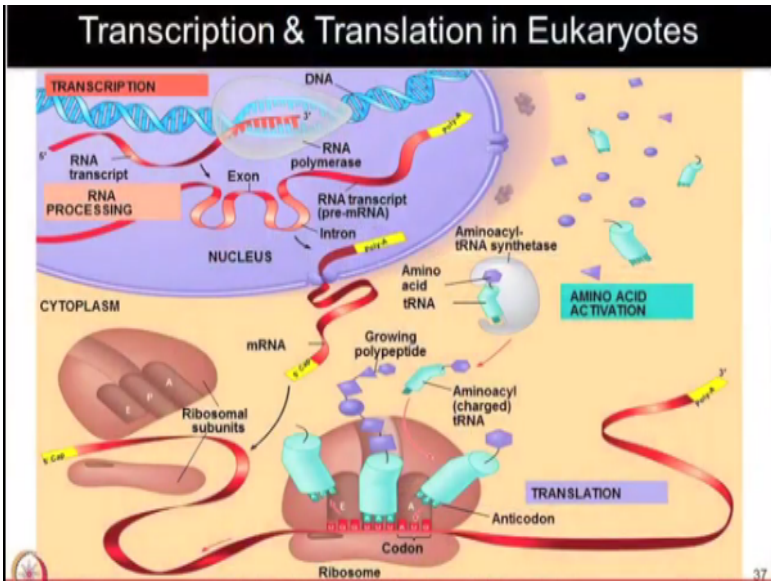

So, transcription and translation again you know, there is lot of fundamentals involved in understanding these processes, this slide just kind of gives you the illustration that in which way from DNA the RNAs are being formed in the process of transcription and then from there in

which way whereas, amino acids are formed in the translation process happens to generate the proteins.

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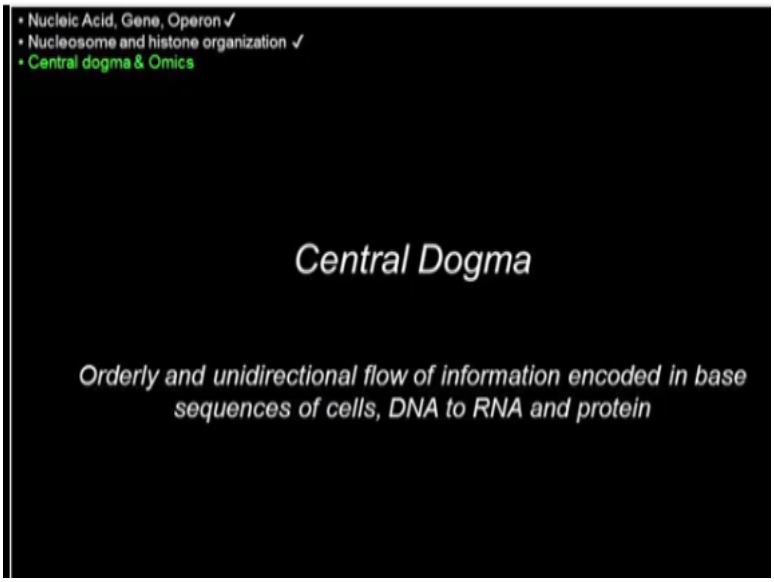

So, this is you know, we will talk as you go along but I just want to convey you that you know these orderly and unidirectional flow of information happens in the cell as a part of central dogma and that actually that information is in the base sequence of the DNA, which flows from DNA to RNA to the protein and this is what we say central dogma which involves 2 important steps of transcription and translation.

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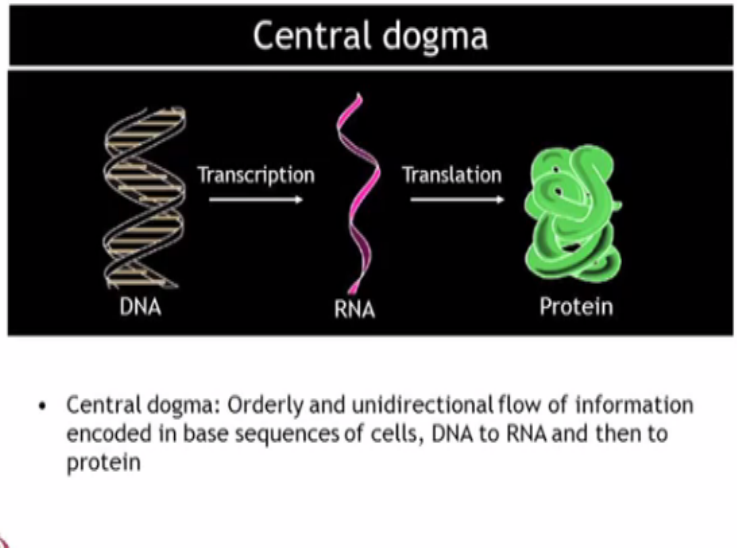

And of course, you know you are also aware that there could be reverse transcription as well but the information could also flow from RNA to DNA and that is also you know very crucial for many biological phenomenon to happen. An important thing you know, why from the same gene, we still see different type of RNA forms.

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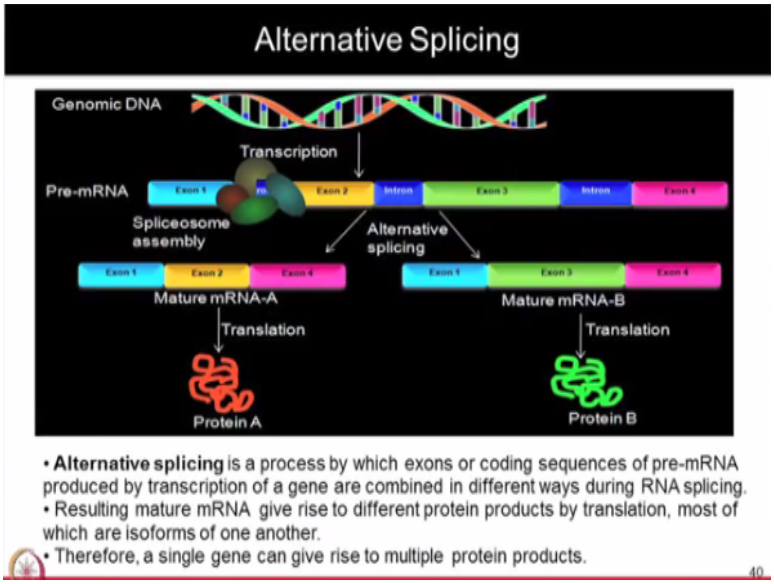

And by multiple protein forms you know becomes very crucial to understand and that is actually being dictated by 2 important phenomenon; one is alternative splicing, so in alternative splicing that is a process in which the exons or the coding sequence of pre-mRNA, they are produced by the transcription of a gene and then there are combined in different ways during RNA splicing. So, what happens then, the resulting mature mRNA, it gives rise to different protein products as the part of translation.

And then, they are the isoforms of one another, so now you have the single gene but actually that can give rise to multiple you know RNA forms in different protein products, so as you can see in the picture from the one of the pre-mRNA, we have mature mRNA-A and mature mRNA-B, they are being formed and they give rise to the red colour protein, protein A or the green colour protein, protein B.

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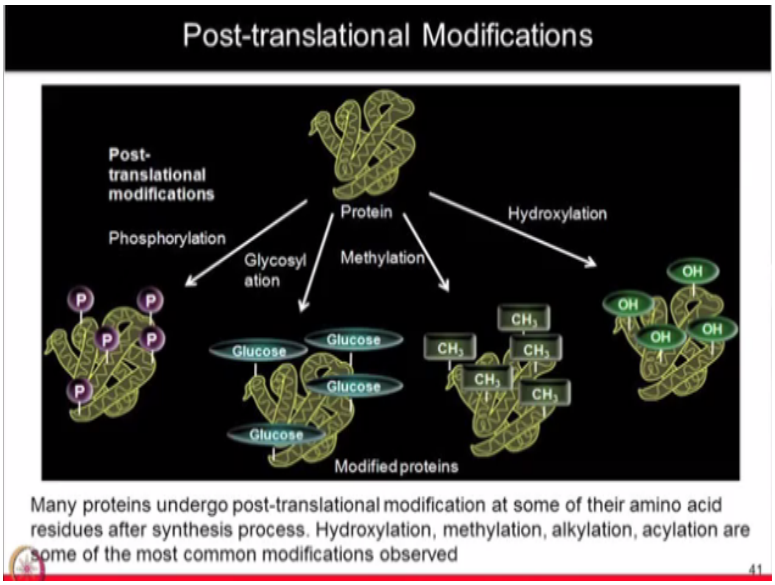

Then, after the proteins have been synthesised then further modification may happen at the protein level and that is known as the post-translational modifications, so many proteins undergo post-translation modification at some of their amino acid residues and some you know, molecules could be added for the sugar moieties as the part of glucosylation or phosphate moiety as the part of the phosphorylation or it can be hydroxylation, methylation, alkylation, acetylation.

And many kinds of modifications may happen which makes the protein very different functionally and that is where you know studying the RNA molecules or studying protein molecules provide much more function and formation because many of these modification are actually quite relevant thinking about the actual physiological question, right, these information what I just conveyed you are looking at the central dogma, all the genes, then the transcripts and the proteins.

Now, scientists are trying to study in much more totality for example, can we study all the genes of a given organism of a given system, let us say for human, we have no idea for the entire genome and that we say the human genome project or human genome sequences are available now and similarly, do we have idea for the entire human transcriptome or human proteome and that can help us to really understand the system much better as compared to thinking about just 1 or 2 protein at that time.

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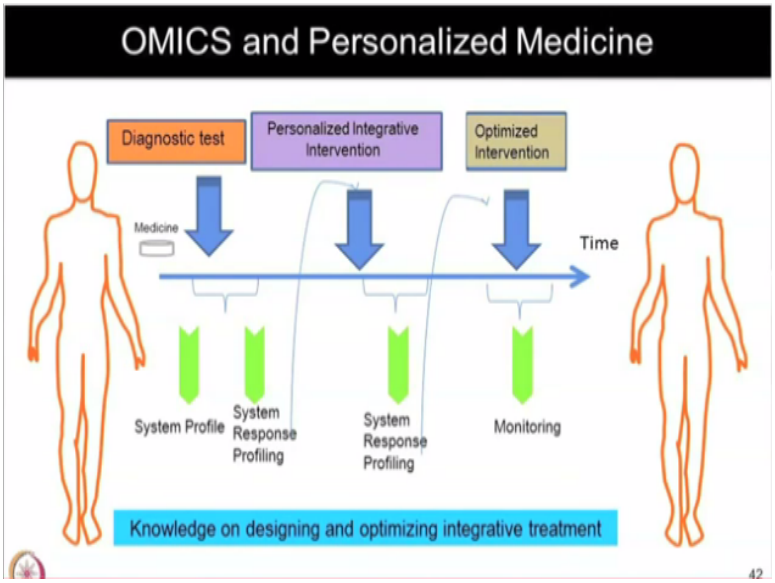

So, then the Omics understanding that so this whole field is known as Omics field, which aims to look at all the molecules present you know in a given system and then this information could be very valuable and useful for the patient's treatment and patient therapy, think about the personalised medicine which is an area upcoming right now, where intention is to look at these biomolecules you know from a given individual and use that entire information for their treatment.

So, whether you think about you know having the diagnostic test or think about you know the integrating these personalised interventions or optimising them over the period for the treatment modalities, all of them requires good understanding of you know these basic biomolecules and if possible, the technology to understand them at the OMICS level.

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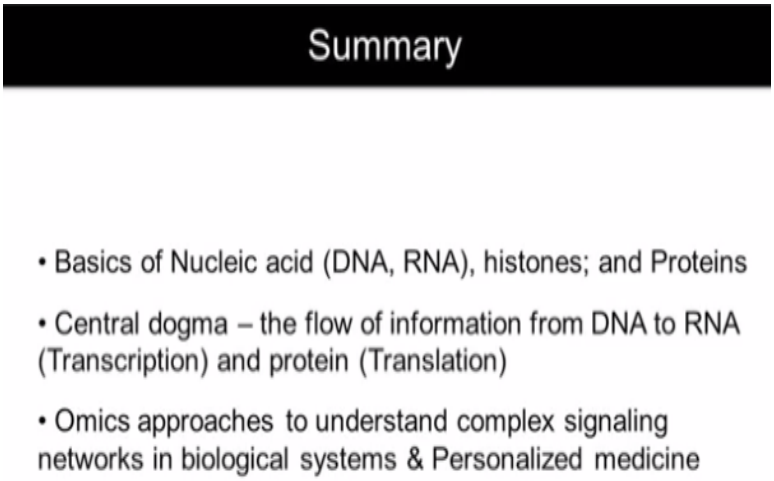

So, in summary today we just talked about the basics of nucleic acid especially, the DNA and RNA, we also try to cover briefly about histones and in which way the DNA are being packed, then I try to convey you there that in which were central dogma, flows information from DNA to RNA in the process of transcription and then protein in the form of translation and then in which way the new emerging field of Omics technology is able to you know is actually aiming to understand the complex signalling pathways, which could be involved in the biological system.

And that may lead us to do better cure at the personalised medicine level, so these are all some of the basic fundamental of course, you know to know more in detail, you have to either take some more advanced classes or you have to you know take more specialised biological classes but our intention was to give you some overview to refresh about these biomolecules.

And now, we are set to talk to you about various advances which are happening in the DNA tools and biotechnology area and that will be the main focus for the next of the lectures for this week. Thank you very much.

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References

- In house animations- OSCAR project IIT Bombay
- Laboratory demonstrations: Proteomics Lab students