Bio - Microelectromechanical Systems

Prof. Shantanu Bhattacharya

Department of Mechanical Engineering

Indian Institute of Technology, Kanpur

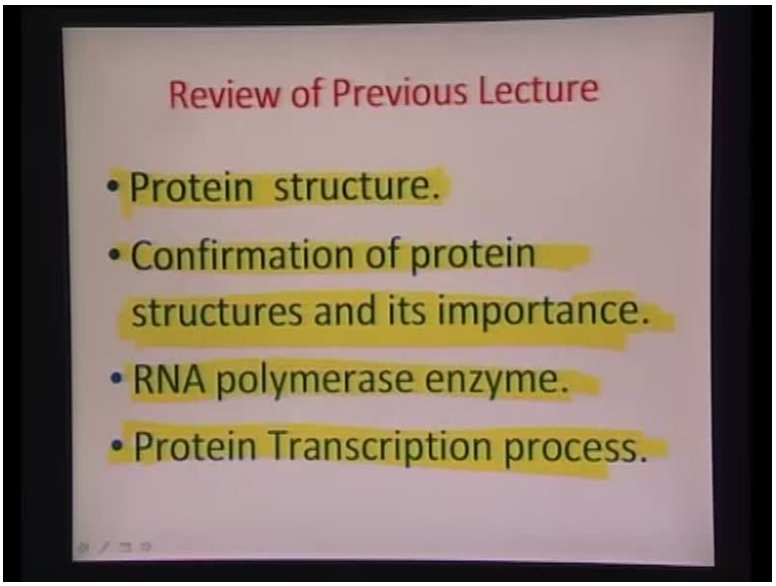
Module No. # 01

Lecture No. # 23

Hello and welcome to this 23rd lecture on bio-microelectromechanical systems. Today, I will be briefly reviewing what has been done earlier. We will delve into the process of translation and then we will also look into some other aspects related to this beautiful codification process, where there would be a change of language from nucleotides into amino acid sequences.

We will also talk about what would be useful for coding - what terms of amino acids, talk a little bit about the degeneracy component in encoding and then compare this whole system of compression of information and translating of information as if it were a floppy drive computer model. Then, we will go on to study some other binding mechanisms between different molecules used for immobilization on surfaces, following which we will talk a little bit about enzyme kinetics using Michaelis Menton equations.

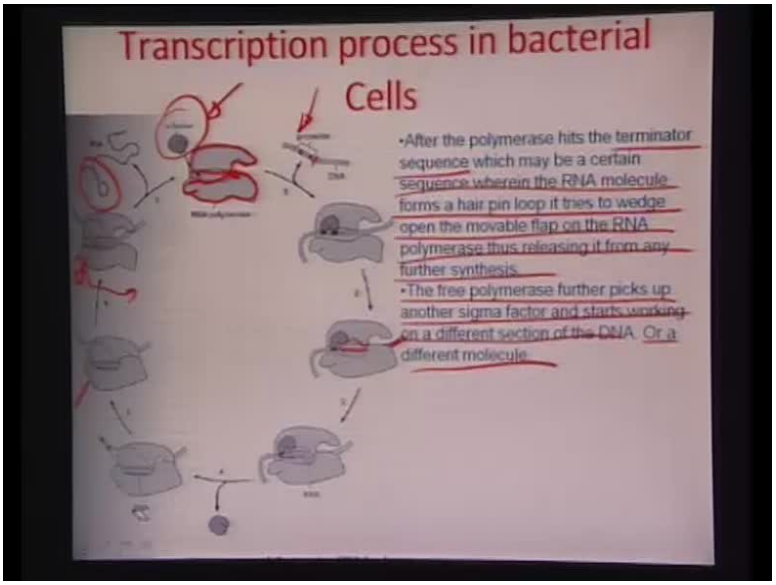
(Refer Slide Time: 01:28)



Let us talk a little bit of the review of previous lecture. Last time we discussed in detail about the primary, secondary, tertiary and quaternary structure of proteins, and also looked at how proteins can be formed as enzyme catalyzed processes of condensation reactions. We talked about the various confirmatory stages of structures that proteins have and its importance, and deciphered the importance of the r group and its categories into polar, non polar, positive and negative charges for the final structure formulation of the protein.

We then talked about the RNA polymerase enzyme and its whole business of how it is used for compression of information from the DNA or the Deoxidizing Ribonucleic Acid and convert that into a compressed mRNA. We looked into the details of how this transcription takes place.

(Refer Slide Time: 02:33)



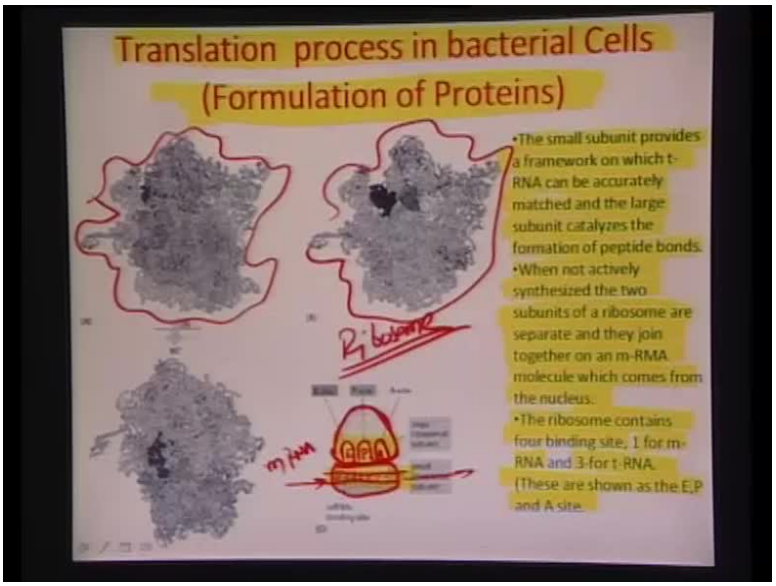
Let us me just quickly review the transcription process once more. If you look at the transcription, it starts with this RNA polymerase, we call it a magician's hat -the structure here and there is a sigma factor which comes as information to find out what would be a region from where the binding and the opening of the DNA chain would start. So, it is used to identify the promoter region on the DNA. This is the DNA, the double strand DNA right here. You have this promoter region here on which the sigma factor goes and bites and starts opening the chain of the DNA into two. The idea is - as the child chain passes to the other side and goes out of the RNA molecule, it again zips

back, it unzips in this region, and then because of the binding of the sigma factor and then as it binds and opens, there is an assembling of the nucleotides from the solution onto these structures here, and these nucleotides are sutured again by the RNA polymerase enzymes. So, what it does is, it sutures both the nucleotides of phosphate groups together by a sugar molecule in between.

After the 10 nucleotides are kind of operated, the sigma factor automatically loses its grip on the DNA and then it is of course, a process downhill where the unzipping action and the movement of RNA polymerase automatically occurs until it hits upon something which can give a jerk to the RNA polymerase and it can ask the RNA polymerase to decouple. Once it decouples by a change in confirmation- before that -there is an automatic stoppage of the synthesis of the mRNA which is also defined by something called a stop codon or a terminator sequence and because of this sudden change in confirmation of the RNA polymerase, it also disengages the RNA molecule and sends it out.

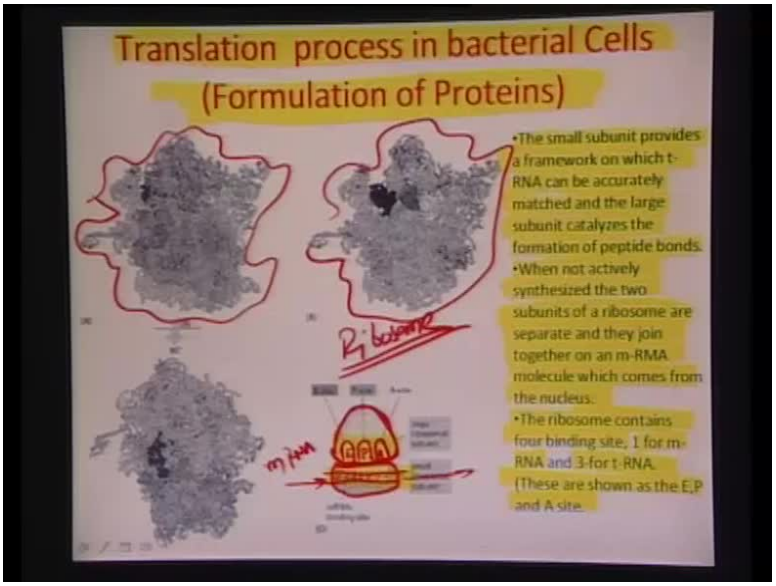
As you can see here, the RNA molecule is being continuously generated in this portion and whenever it hits up a sequence where it can formulate a hairpin loop or something, it changes confirmation and detaches itself from the RNA polymerase and the polymerase actually finds out another sigma factor to repeat this particular cycle. This whole beautiful process is about compressing the information on DNA into this small stand here which is the ribonucleic acid.

(Refer Slide Time: 05:19)



Let us now look into a very different aspect of this process of translation. As the prior process was discussed in sense of bacterial cells, this process also we will be considering what happens normally in the bacterial cells. The translation process as applied to the million cells also is similar to this translation process with a few minor variances here and there, but the basic mechanism is almost same as that is in DNA, it is in a bacterial cell.

(Refer Slide Time: 05:19)



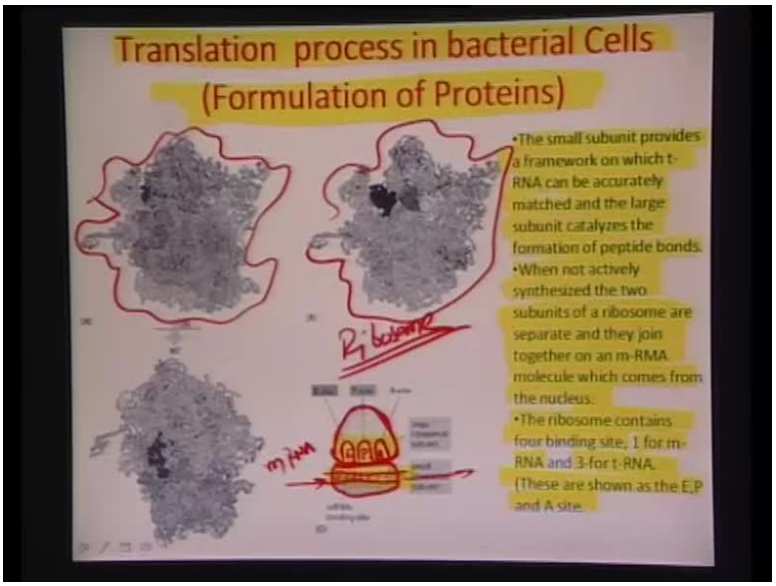
How does a translation process take place in a bacterial cell? You have this mRNA chain which has gotten out and from a certain region of bacterial cell, a unicellular organism which does not have a nucleus. So, DNA is present all through inside the membrane and there are organelles dipped within the cytoplasm and the cytoplasm also contains all DNA. All these mRNA synthesis work is inside there is no nucleus specifically for this purpose. It is just inside the solution and the mRNA then hits onto the endoplasmic reticulum where these ribosomes or protein assemblies on that RNA and try to sequence the amino acids based on the codons which are available.

Let us look at it how - a small subunit - this ribosome is one of the most intelligent nano machineries ever available in nature or given by Mother Nature. It is a small subunit which provides a framework on which tRNA can be accurately matched and the large subunit catalyzes the formation of the peptide bonds. If you look at the basic ribosome here, this is the ribosome - we can classify the ribosome is in upper half and lower half and it is like a jaw. You must have seen these Pacman videos where there is a jaw which comes and starts eating the balls or some kind of moving objects.

So, here you can consider that these ribosomes are like Pacmans which kind of move through the solution and they target the mRNA. the mRNA is slipped through in the framework on the lower portion of the ribosome and goes there and attaches and the upper portion is ready to suture the transfer RNA, which is there around the solution by matching the sequence of the transfer RNA onto the sequence of the mRNA. However, there is only a set of three sequence or three base pairs which are available onto transfer 1 RNA.

Therefore, the readouts of assembly of the tRNA on the mRNA is a 3 by 3 by 3 combination. You have three base pairs at a time which is to be a read and the corresponding tRNA has to be inducted which also has three active sites for binding onto these three codons and then the next three groups are taken up and the next three groups are taken on. Therefore, there is a set of the transfer RNAs which are sutured onto this chain. So, for the Pacman model, the lower portion engages the mRNA and upper portion is able to suture the tRNA on the mRNA, three at a time - three base pair at a time. That is how this kind of protocol works. I am going to illustrate this in detail a little more.

(Refer Slide Time: 05:19)



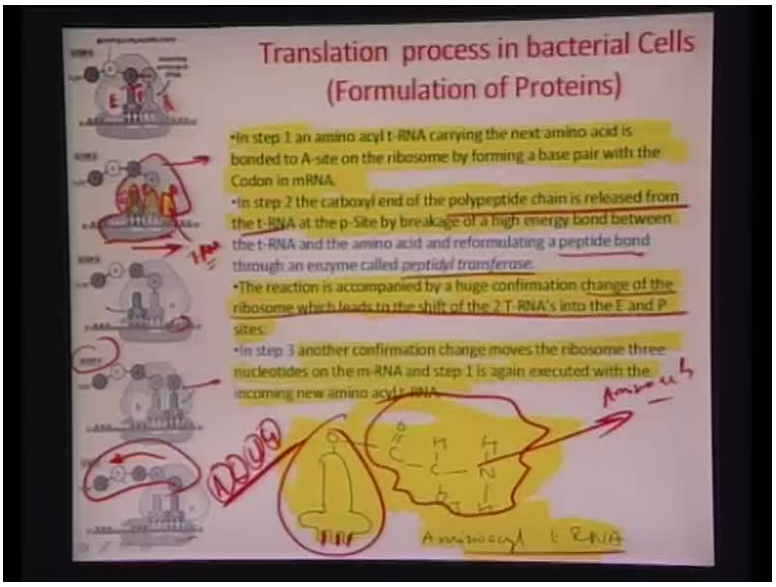
Let us look at what these are comprised of. The ribosomes contain about 50 different kinds of proteins and several different kinds of mRNA structures. It is made up of a jaw like arrangement with an upper and lower part. This is how it really looks like. There are so many different proteins folded in different confirmations and also many different RNAs within this particular network.

You can look at it as a set of jaws, this is the upper jaw, this is the lower jaw and these jaws have three different binding sites here, which is represented by E P and A. These essentially are pockets. It is not very clear in the diagram here. These E, P, and A sites, they are pockets on the upper portion of the ribosome. They are pockets in which as we will see later - the transfer RNA will go and fit really. The lower portion - this rail here- through which you can actually pass a small mRNA molecule. So, the mRNA can go through this rail and this is the mRNA. You have these different base pairs on the mRNA which can pass through this rail; it goes in here and out of this particular site.

When not actively synthesized, the two subunits of a ribosome are separate and they join together on an mRNA molecule which comes from the nucleus, in this case, because it is the bacterial mRNA, it is produced within the solution and it comes to the ribosome. The jaw only closes whenever there is an mRNA; if there is no mRNA, the rails are open and the jaw is also in the open state. The upper half is open like a mouth. Ribosome contains four binding sides, one for mRNA as I already illustrated here, this area which is like a

rail and three for the transfer RNAs as E, P, and A site as you can see here in this particular region on the upper flank of the ribosome.

(Refer Slide Time: 12:23)



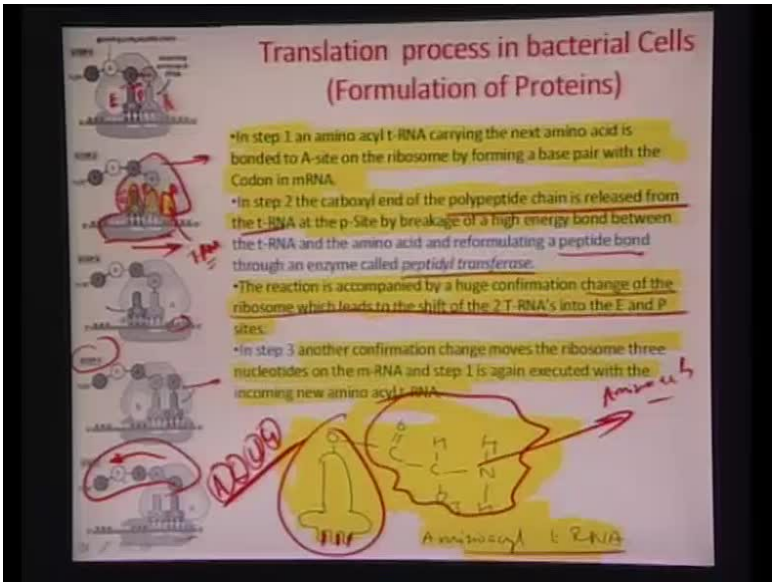
Now, very interestingly, there are bunches of amino acyl transfer RNAs which are available in plenty within the environment of the cells. This is actually a amino acyl tRNA. I will just like to illustrate here that there are about 20 different amino acids which have sequence some way or the other through this amino acyl tRNA. This is the protein part of it, amino acyl group.

Let me just illustrate this through little more appropriately. This part is the amino acyl group hooked onto this mighty here- which is a transfer RNA. The beauty about transfer RNA is - these three groups here for binding. They are essentially three among the four different groups that normally RNA would have, that is adenine, cytosine, uracil, and guanine. There is no thiamine on the RNA as we told before.

These three groups are bonding sides comprising of three of these four molecules. Based on the sequence here, you can find out a sequence on the mRNA and try to bind this particular amino acyl tRNA, but what is important is that this sequence matching essentially leads to development of another sequence here of all these different amino acids and that is what a protein are is all about.

In step 1, as you can see here in this particular figure, the amino acyl tRNA carrying the next amino acid is bonded to the A site first. This is the A site here, you can consider this as the A site. Let me just illustrate this a little more clearly. This is the A site. This particular transfer RNA is with the three groups here and it bonds over three different base pairs on the mRNA.

(Refer Slide Time: 12:23)



Incidentally these three base pairs on the mRNA is also known as the codon sequence. It is called a codon. Therefore, the synthesis of these amino acids one by one on the mRNA is codon by codon. So, you read three base pairs at a time and try to match it with the three base pairs complementary to the codon sequence present on the transfer RNA and it has an amino acyl group at the end of the transfer RNA, which gets synthesized because of the binding of the tRNA with the mRNA at a particular site of interest.

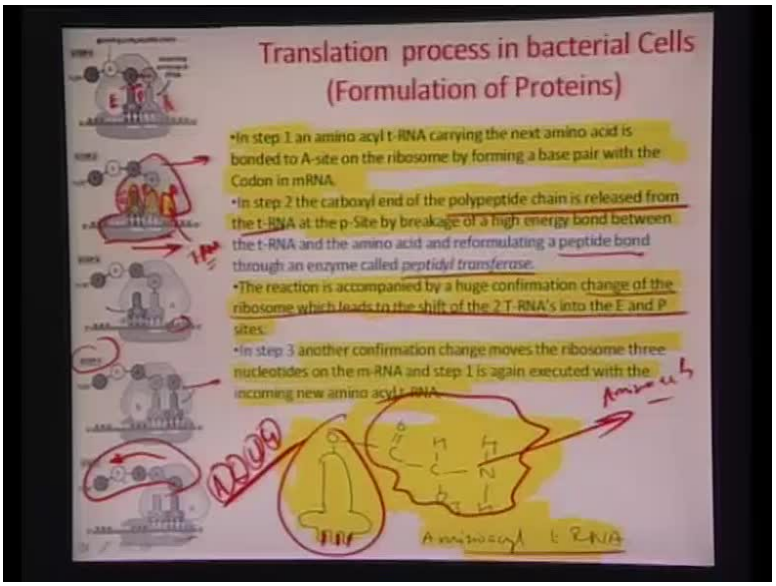
So, this is the site A in which the first transfer RNA is assembled. Now, note here that based on the sequence of three RNA, the ribosome would pick up a molecule, a transfer RNA molecule which has exactly in the complementary three base pairs. It will not do something where there is a mismatch. So, the very fact that a certain sequence is only determined by the sequence of the mRNA comes very clear. There is a matching between complementary for forming a basis of binding of the tRNA with the mRNA.

So on site A, they bind first and then leave an amino group here as you can see in this particular illustration. Amino group is the circle here which has been left. Let us say you

have an amino acid 4 which you were trying to add to this chain. In step two, the carboxylic end of the polypeptide chain is released from the transfer RNA and at the p site, the breaking of a high energy bond between the same.

There was a previous transfer RNA which was already immobilized as you can see here in this particular example to another - that is the p site here. This is the p site, this is the A site and this is the E site as I talked about in my last slide. There is already a transfer RNA which has been bonded to this amino acid here 3 which is already formed a bond with are their produced amino acid 2. So, this chain is kind of how exiting out of the ribosome in this particular manner.

(Refer Slide Time: 12:23)



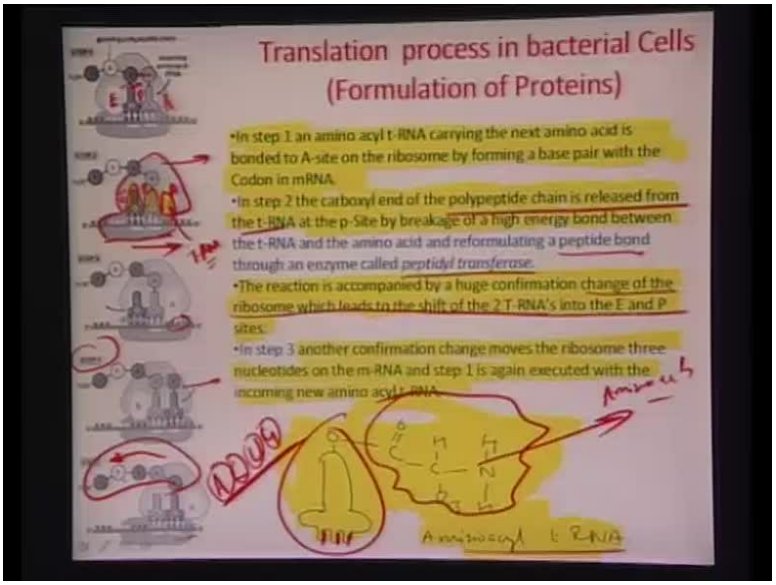
You have a amino acid 4 here. After the suturement of this or filling up of this active site A with the transfer RNA of a particular kind, as this brings up the particular amino acyl group or the amino group here close by. The polypeptide chain is released from the tRNA. We are talking about releasing this particular portion of the molecule.

Let us just close all this. We are talking about breaking this particular bond, here of the molecule. It is a polypeptide chain with the tRNA -disengaging this portion of the molecule by breakage of this high energy bond. This is a very high energy bond between the transfer RNA and the amino acyl group. Simultaneously what this ribosome does is - it creates another bond or another peptide linkage between the 3 and the 4. So, it is not only breaking this high energy bond but also developing a bond between the 3 and the 4.

This is again a poly condensation reaction; you need an enzyme catalyzed poly condensation reaction here. If you remember from your first slide how peptide linkages made between 2 groups of a chain which has OH on the carboxylic N group and NH 2 on the other hand. You have H 2 O coming out.

So, it is a condensation reaction in the presence of an enzyme. You are making this bond and you are breaking this particular bond so that you are disengaging the transfer RNA which there in the site p and engaging the amino acid on the bound transfer RNA on the site A. So, the enzyme that does this activity is also known as the peptidyl transferase. This is an enzyme which would be able to break the high energy polypeptide chain from the transfer RNA and at the same time suture the peptide linkage between the 2 amino acids.

(Refer Slide Time: 12:23)



Because there is a sudden liberation of the amino acid from the transfer RNA and sudden bonding of this particular amino acid with another amino acid group on the last transfer RNA, there is a huge changing confirmation or huge change in size of the particular ribosome. So, there is a confirmatory change in the ribosome suddenly due to which there is a change leading to shift of the 2 tRNAs into the E and P sites respectively.

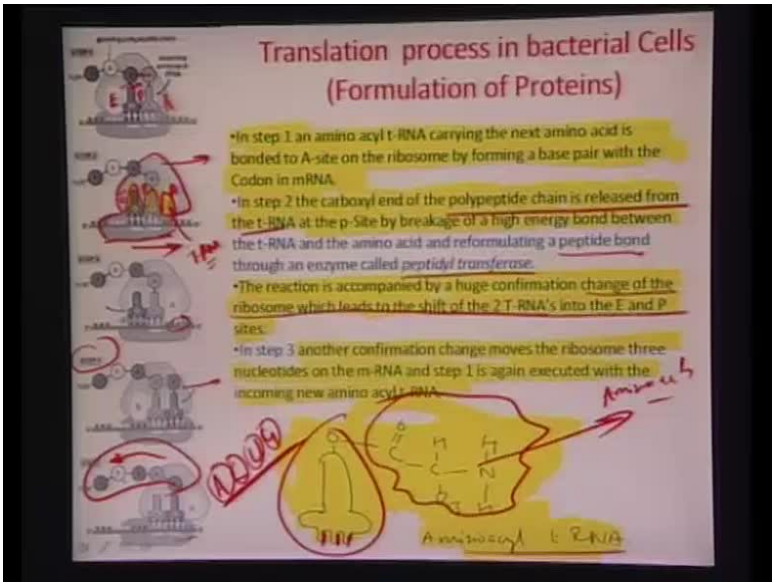
This is the E site, this is the P site, and this is the A site. There is a confirmatory change suddenly because of this additional bond breakage here and this bond formulation here there is sudden change which leads to this upper jaw as you see to move one unit on the

right. What happens is that if you look at these different pockets E, P, and A, the 2 transfer RNA shift from pocket P and A to site E and site P. A is left open and what A does? It takes in another tRNA, amino acyl tRNA from the solution.

Therefore, the reaction is accompanied by a huge confirmation change of the ribosome which leads to the shift of the 2 tRNAs into the E and P sites and that is step 2 here. There is a confirmatory change leading to the opening of this A site and closing respectively of the E and P site - transfer or exchange their position.

Now, in the following step, there is another confirmation change which moves the ribosome 3 nucleotides on the mRNA. What you are doing is - this lower group if you see here - the lower part of the jaw is shifting in this direction and it changes by three groups and the three next groups are inducted inside here. It is a biting and moving process. You have this chain of mRNA; it has bitten let us say on these my three fingers and so there are these three fingers on the upper jaw of the ribosome and this lower one has a track like this.

(Refer Slide Time: 12:23)

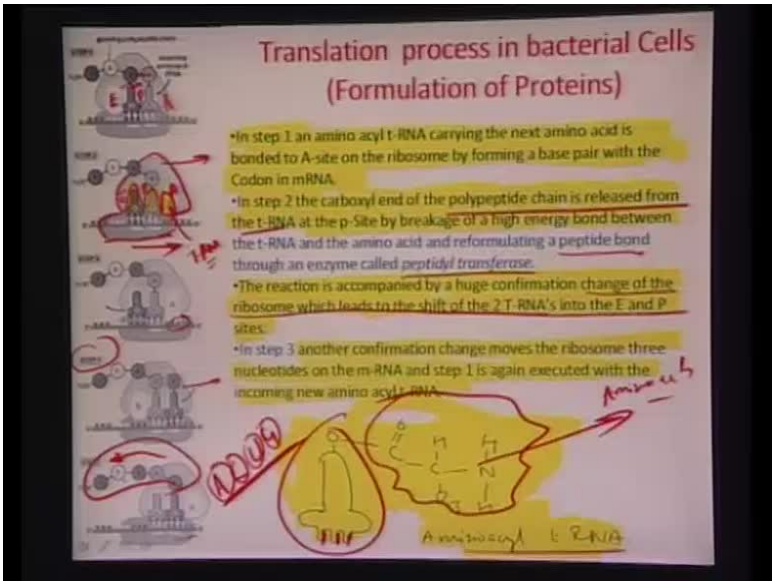


So, you have bitten here and then there is a sudden confirmation state change of this three and then following it - there is a confirmation change state of the lower jaw and it keeps on crawling like this over the whole mRNA. So, there is a continuous rail of this mRNA passing through the lower jaw as there is a slow pickup of the three sites to the next due to confirmation change in the upper half and then the lower half coming back

and then again the next three sites for the three new transfer RNAs and the lower half again coming back. So, it keeps on reading on the whole RNA chain like this.

The same process is again repeated after step 3 where a new transfer RNA is inducted and mind you - all these coding is happening because there is a match between this codon the 3 new induced groups on the mRNA and the 3 on the amino acyl tRNA. So, what the ribosome really does is the matching between the transfer RNA, 3 N groups and the mRNA. That means there is a complementary nest, which it defines for attaching the right transfer RNA which would also transport amino acyl group by amino acyl group.

(Refer Slide Time: 12:23)



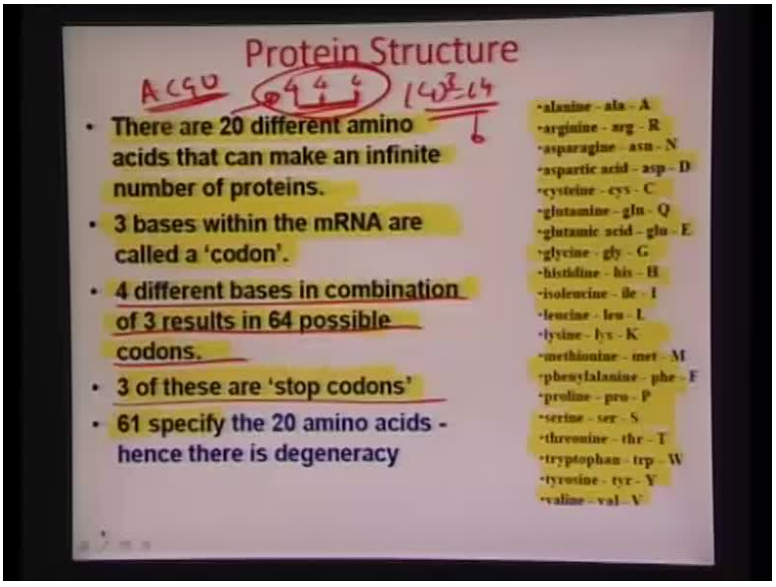
Then this enzymes pep till transfers etcetera they would just cleave off the polypeptide chain and make a peptide between the 2 amino acid sequences. So, that way as this thing is sliding, there is a continuous production of amino acids. As you can see here, this chain grows with time in this particular slide. You can see this chain here - keeping on adding these different groups 1, 2 then this is the third group, this is the fourth group, fifth group, and this chain is getting liberated as the RNA is slowly moving by upper half moving then lower half following again upper half moving to the right and then the lower half again following. So, the steps are repeated again and again. There is a continuous production of these protein chains and these are of primary interest because the sequence that you are bringing as an mRNA from all the way from the nucleus in a normal cell or in a bacterial cell from the solution general is really dependent on the

portion of the DNA that your binding - that is number 1 and the number 2 is that sequence is translated into another sequence of molecules, which are so called proteins of the particular reaction.

This brings us to a very interesting aspect of cell biology; in a sense this is probably one of the most fantastic nanomachinary that Mother Nature has provided us. The rate at which the synthesis can be done is tremendous. There are thousands of proteins which are generated in a single second by the several different ribosomes which are floating around in the cell and at the same time there are lot of these mRNAs which are being produced on the nucleus and they are based on the signaling which the sigma factor or some other chemical signals which the RNA polymerase gets an information where to start biting and start the production. So, there is a rapid production of all these different groups matching each other and thousands of mRNA is getting generated every second which leads to thousands of proteins.

These proteins sometimes get to the surface express on the surface and try to get back the physiological state of a cell back into equilibrium. Every time there is some kind of disequilibrium created by a process, it is essentially a chemical signal which would give you more protein so that the process can brought back into the equilibrium state of the body.

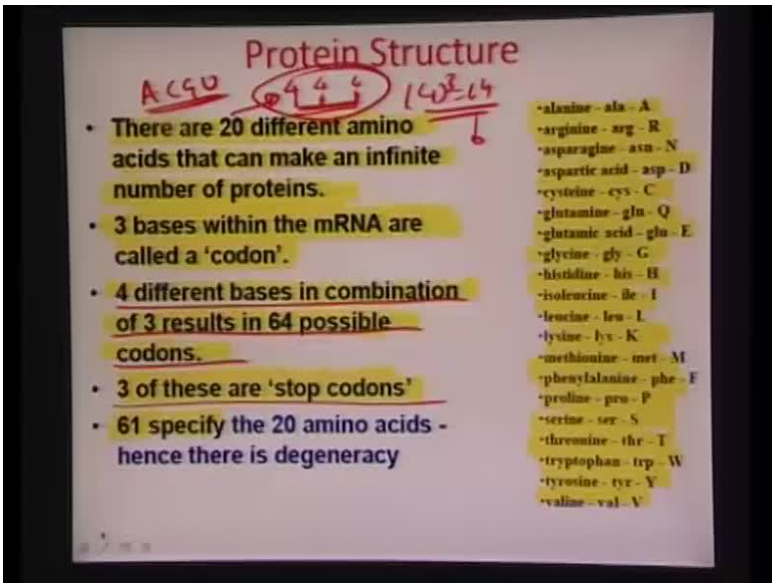
(Refer Slide Time: 26:17)



Looking into a summary of whatever we have done so far and then trying to do some facts and figures. We know there about 20 different amino acids that can make infinite number of proteins. You already know what happens if there is a 25 amino acid sequence - there about 20 to the power 25 different combinations of proteins which are available. This is a big a number only for about 25 amino acids, let alone the other proteins which are probably thousands of amino acids knit together. There, the commentarial is really very high and can produce many combinations of these different base pairs together.

3 bases within the mRNA which can coat an amino acid are called a codon. The word codon comes from the word coding. You are using three base pairs successively placed on an mRNA molecule to essentially code certain amino acid. Then there are 4 different bases in combination of 3, if you look at the 3 sites - let us say you have 3 sites here and you have 4 bases A C G and U. If you put the different combinations that may exist in the sequence, there are 4 that can come on 1 with another 4 on the another 4 - assuming that there is a repetition of the particular group.

(Refer Slide Time: 26:17)



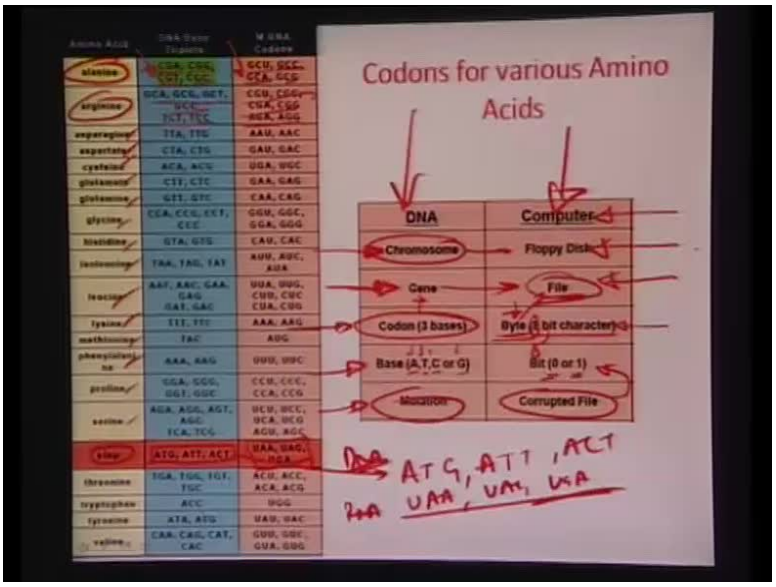
There are about 4 to the power 3 or 64 different such combinations of base pairs which can exist in 1 codon. There are different the about 64 different codons that are possible based on these arrangements. So, 4 different bases in combinations of 3 results in 64 possible codons.

Now, as we know there are some terminating sequences of stop codons on the RNA where there is a disjointing action because of the confirmation change of the RNA polymerase from sliding over the DNA. So, it stops the process of mRNA production and disengages the mRNA from the RNA polymerase and also folds back the DNA or zips back the DNA into a normal double standard structure.

These 3 codons are also known as stop codons or terminating sequences. Let us say there are 3 codons. How many are really left with? You have about 64 possible combinations out of which 3 are always used for termination sequences. Therefore, up there are about 61 different combinations that may be used for specifying amino acids but the unfortunate part is that there are only about 20 amino acids as is explained here in this particular illustration.

There about 20 different amino acids as you can see in this particular illustration and there are about close to 61 different combinations of these sequences or codons, which are possible to code these 20 amino acids. Definitely there is going to be some degeneracy.

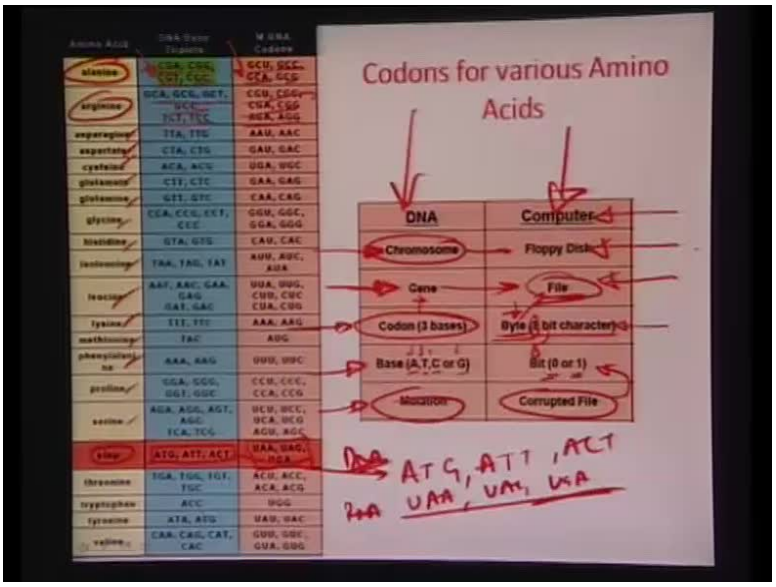
(Refer Slide Time: 29:27)



Let us look into some facts and figures - right here is an illustration of what happens. This is the particular amino acid and this is been coded by these DNA base triplets, C G A, C G G, C G T, and G C G C and correspondingly the mRNA codons that are produced out of wetter G C U. If you look at a little more closely, there is

complementariness in this. Therefore, if there is C, there is a G on the mRNA, there is a G on the DNA base triplet, there is a C on the mRNA, there is a adenine here in the DNA base triplet, there is a U or a uracil on mRNA. I would just like to remind you that the uracil is the substitution for thiamine group on the RNA molecule.

(Refer Slide Time: 29:27)



Therefore, if you see more there are these, C G G on the DNA base triplet corresponding to G C C and so on so forth. This alanine amino acid is being coated with these many base triplets and these many mRNA codons. So, definitely there is going to be degeneracy as I told before. There about 61 codons which can code only 20 different amino acids that means there more than one codons which could be responsibly coding one amino acid.

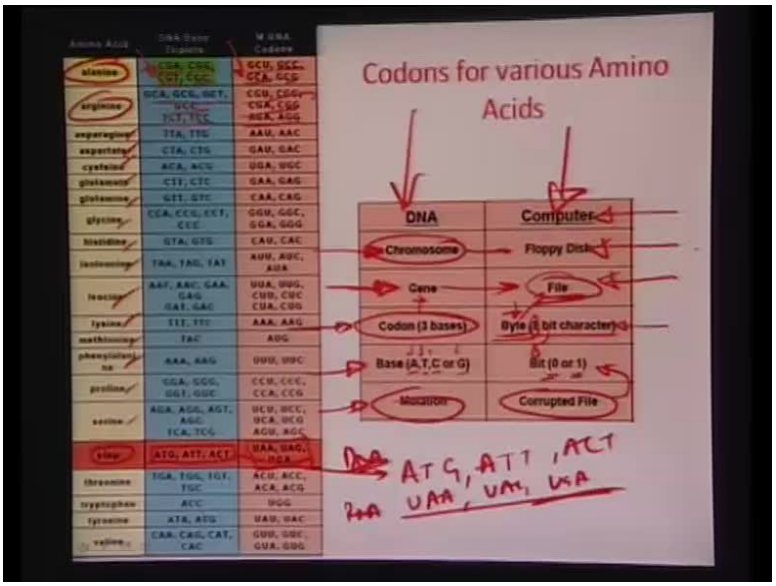
This is called the degeneracy component of the coding. There is arginine here, amino acid and you can see the number of base sequences on the DNA as well as on the RNA mRNA which would be used for coding this arginine. Similarly, these things go true for asparagine, aspartic cysteine, glutamate, glutamine, glycine, histidine, isoleucine, Lucien, lysine, methionine, phenylalanine, tralon, serine so on and so forth.

What I am also interested in looking at is- these top codons here. If you look at this particular portion of the molecule, it is actually a stop codon. This is a stop codon and there are these sequences here which I will just illustrate here in little bit. So, the base triplets for the DNA- A T T, A T G, A C T are essentially stop codons, this correspond to

mRNA sequence U A A, U A G, U G A. These are the RNA triplets; this is the DNA triplet and so wherever the RNA polymerase hits one or more of the sequences there is almost always a detachment and there is almost always a separation of the RNA and stopping of the process.

That is why it is a termination sequence of stop codons. These are the stop codons. The other 64 combinations which are there coding these different amino acids, these 20 different amino acids. So, it is like a mini computer system. I would like to draw a very strong analogy between what happens on a computer as to what happens on this DNA as you do the transcription and translation.

(Refer Slide Time: 29:27)



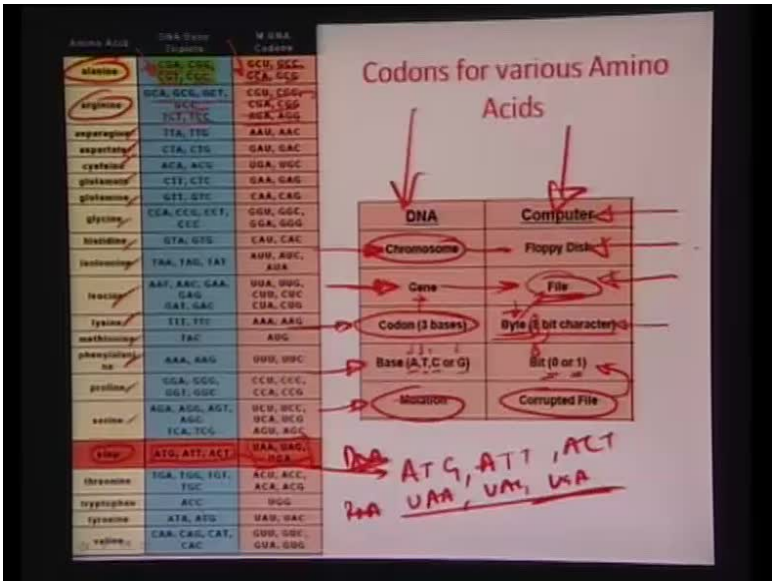
On a computer if you look at, you have something like a floppy disk and you can consider that in this DNA codification and translation process, the chromosomes are like floppy disks. They are readouts of data points where this compressed DNA is compresses all this information that is present.

So, on a floppy disk you have files, you have several files to store whereas, in the chromosomes you have these different genes or these different DNA which are super coiled and placed inside the chromosomes. Chromosome is equivalent to the floppy disk here. The gene is equivalent to the file on the floppy disk here. Therefore, if you consider a file, it is made up of electronic information called bytes and 1 byte is basically an eight bit character. A bit could be either 0 or a 1. You have like an 8 bit character which

comprises a byte and then there are several kilobytes, megabytes which can be the size or the total information on a single file as you can see here.

So, the bit here is either a 0 or 1 correspondingly if you look into a gene, it is the codon of these 3 base pairs on the mRNA which is comprising of the same information as is there are on a byte which can correlate to a file. In this case, the codon is the information a 3 base pair on the DNA or base triplet on the DNA which is the codon which actually comprises of the gene. Several of these codons altogether comprise of the gene just as several of these bytes, kilobytes, or megabytes comprise of the file here.

(Refer Slide Time: 29:27)

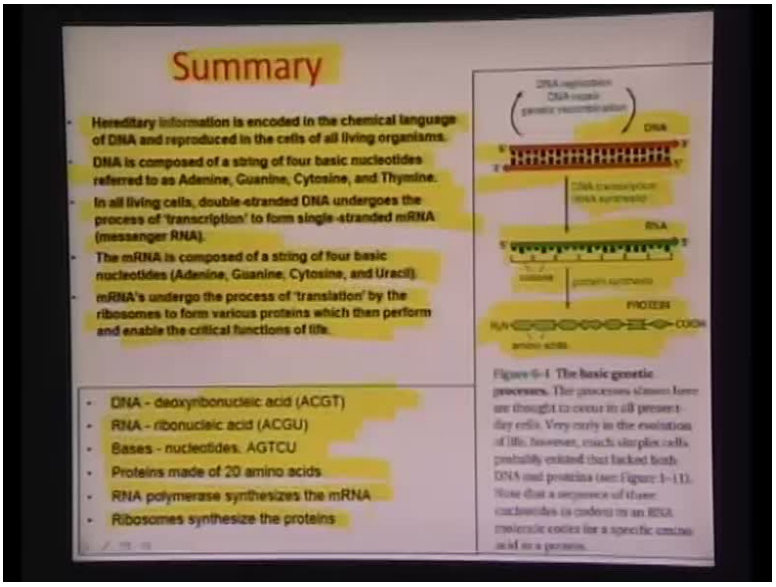


Just as in a byte you have 8 bit character, where 1 bit could be either 0 or a 1. You have 8 such numbers or 8 such combinations of zeros and ones for representing 1 byte. In this case, there are these 4 different combinations A, T, C, and G – adenine, thiamine, cytosine, and guanine which are the 4 base pairs of DNA which comprises of this codon.

So, these 3 bases are made up of 4 rather than 2; so, do you see the complication here? In a computer, what is done with two characters in the real life DNA, it is done with 4 different characters. The computation increases 2 to the power 2 times. You are considering here, instead of zeros and ones, 4 different characters to write a bit of information and then from that 8 bits are taken together to a byte and here probably several of these bases are taken together to make a codon which is 3 base pair long.

What is important here for me to tell you is that a corrupted file in this kind of a case may be something which has an information or a link missing in case of a DNA, this corrupted file is a mutation where there is a certain sequence mismatch or there is a certain sequence redefining which may modify altogether the way that a DNA performs in all its processes including codification including everything that it particularly does.

(Refer Slide Time: 37:07)



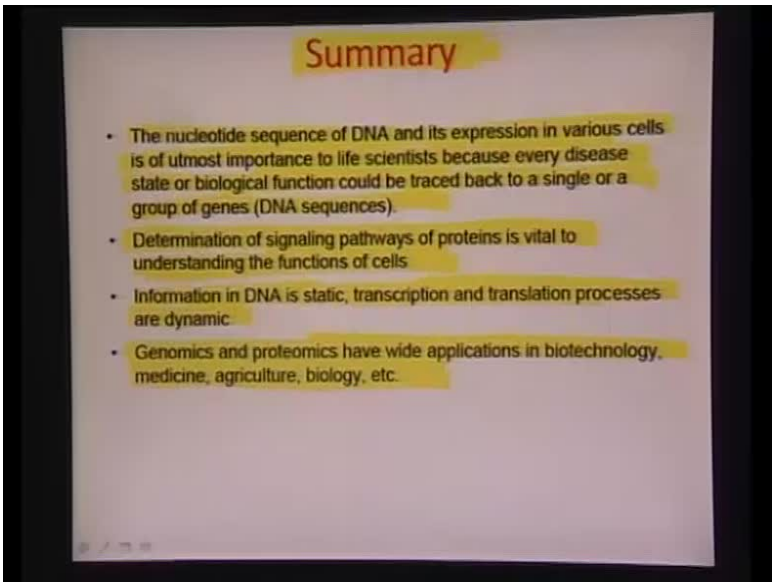
So, there is definitely a correlation between what the DNA does and what a computer does. This is a very interesting model which shows how the human machinery can behave in a preprogrammed manner and in fact the way that a cell dies also is preprogrammed or prior programmed inside the cell using this information. That is the most amazing part of what we are really and what probably Mother Nature has made us off so that we have predefined timelines of existence. Some philosophical thoughts on that and then let us look into another kind of summary of the whole transcription translation process.

In summary, what we would say here is that essentially the hereditary information is encoded in the chemical language of DNA. This is also a basis of production cells in all living organisms. The DNA, as we know is comprised of 4 basic groups: adenine, guanine, cytosine, and thiamine. In all living cells, the double stranded DNA undergoes the process of the transcription to form a single stranded messenger RNA and it is a much more compressed code of information that on the DNA itself.

The mRNA of course, is comprised of adenine, guanine, cytosine, and uracil. Uracil is a new group which is added onto essentially all RNAs and which replaces the thiamine on the DNA. That is how they are built. The mRNAs which are generated this way undergoes this translation mechanism in the ribosome wherein a group of amino acids just stich on the bases of 3 base pairs at a time or a codon in the mRNA and that way you can have a sequence of amino acid from a sequence of mRNA.

In a nutshell, what all these processes are comprising of - you can look at this is a nice cartoon on the right here, which talks about this DNA structure as you can see with the help of RNA polymerase you can generate this RNA and from the RNA again you can produce a protein using ribosome as the enzyme. Some facts and figures to take home- the DNA comprise of A C G T, RNA ribonucleic acids comprise of A C G U and nucleotides are bases essentially in A C G T U all of them, proteins are made up of 20 different amino acids in different combinations in a variety of combinations, the RNA polymerase synthesizes the mRNA from the DNA and the ribosome synthesizes the protein from the mRNA. These are in general take homes.

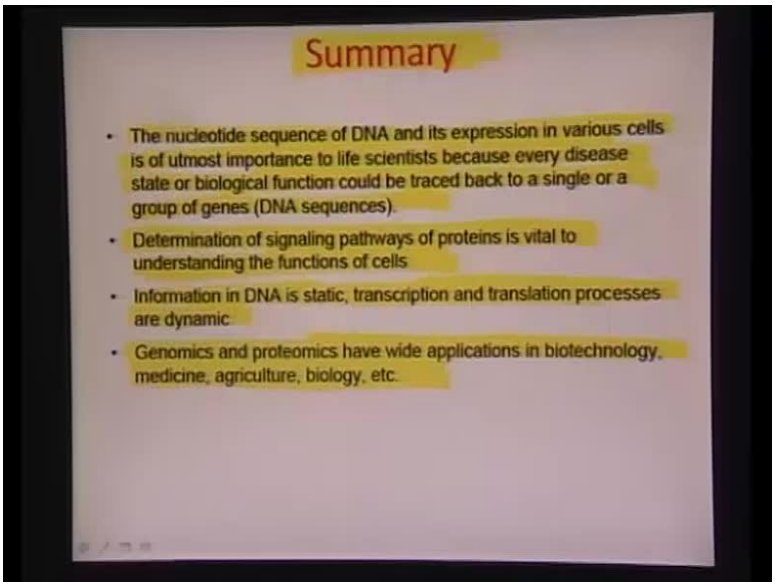
(Refer Slide Time: 39:27)



In summary, I would like to say the whole area that we have been discussing so far of this translation and transcription is based on or revolving around the fact that the nucleoside sequence of DNA and its expressions in various cells is of utmost importance because every deceased state or biological function of a cell could be traced back to a

single or a group of genes in the DNA sequences. It is very important to find out how a cell can be able to revive itself from a disease state into an otherwise equilibrating state where they do not perform any malfunction and this whole route of convergent from a DNA to RNA to a protein is essentially helping it to do that.

(Refer Slide Time: 39:27)

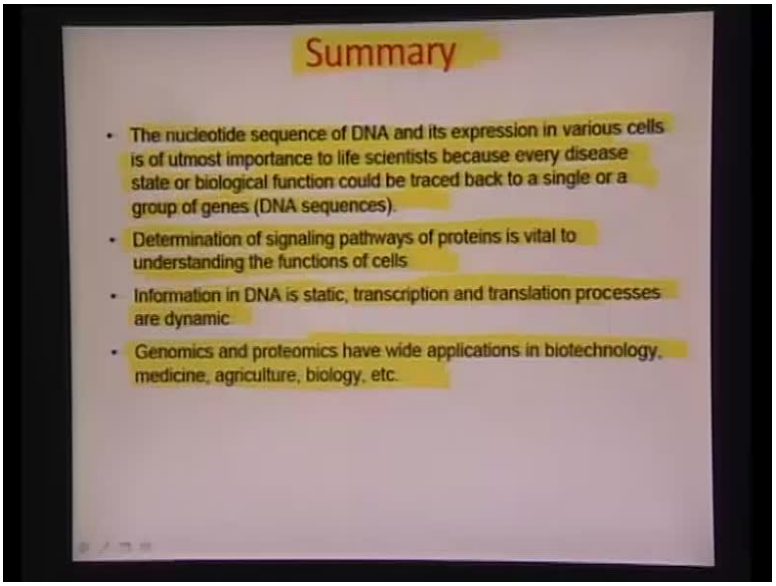


So, the determination of the signaling pathway of proteins is vital to the understanding the function of cell. This is essentially what the signaling pathway would mean. Information in DNA is pretty static and it does not change much with time except when there are mutations. However, the mRNA which is transcribed onto the DNA is a continuously generating a form of molecule. Several copies of these would be produced time and again and the translation processes are very dynamic along with a transcription processes. It is not that stable as the DNA is. It is very metastable molecule and therefore, sometimes in detection assays also it is a better idea to look at the messenger RNA instead of the DNA as a basis of detection of the particular entity, biological entity.

Of course there are two fields to study - what is on the gene, and trying to code a particular protein, trying to study what is on the amino acids in the protein- they do makeup this area of genomics and proteomics and these two standalone have very wide applications as on date in the field of biotechnology, medicine, agriculture, biology, etcetera. Therefore, in a nutshell, this is what the life process all revolves around for getting back into an otherwise healthy state from the deceased state.

If you could work out in a better way on this whole principle of transcription and translation and could identify the individual pathways which would cause these signaling to happen to produce proteins or not to produce proteins or produce at a certain rate, you could control diseases within the human body. Therefore, this whole area of systems biology now is actually more concerned with working and biology from the systems point of view wherein all these information pieces that you get that are stored up in a organized bases where you could have a proper control on the signaling processes on the way that the RNA would be essentially produced from the DNA, or the way that the protein would be produced from the RNA and any of these mechanisms if it is balanced or cut short or altered can produce altogether different set of processes for the cell.

(Refer Slide Time: 39:27)



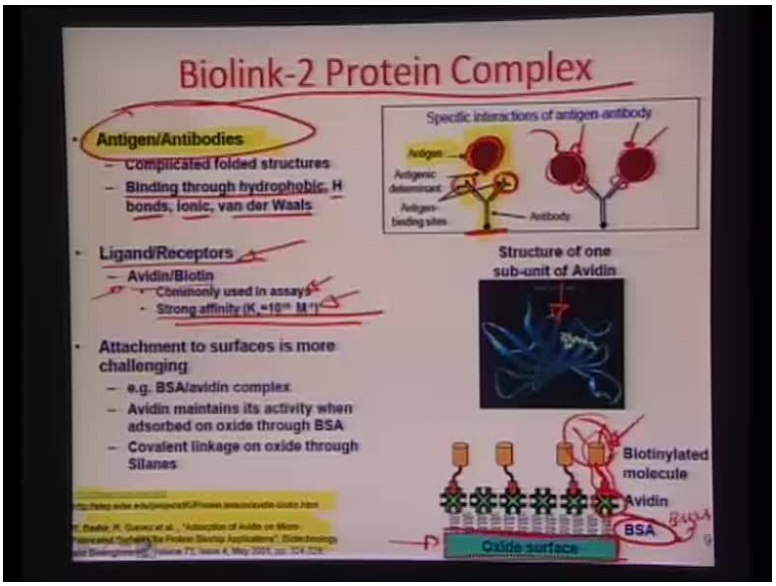
So, looking from that angle, the system biologist derives his theory and database accordingly. We are towards the end of this transcription translation process. Another very interesting factor which I would like to discuss before moving on to the next topic is that how proteins can be used as linker molecules and how they can be used to immobilize different moieties, biological entities on to the surface.

For that we need to study the protein complexes as bio links. We have just a few pieces of information put together here which talks about and this paper has been taken up from variety of literature which is available worldwide. There are certain references which are

also coded here and this paper on adsorption of Avidin and micro fabricated surface for protein biochip applications has been used for citing this particular slide.

There are several bio links that one uses for doing or capturing cells or different biological entities on the surface. Why is capture needed, of course, because we have been talking about the whole area of electrochemistry of sensing, you have to immobilize something in order to find out more details about the information contained in the acting. So, immobilization is a primary goal of any biosensor. How you can immobilize a recognition element, how you can mobilize a particular biological entity - that is the first primary goal of biosensor.

(Refer Slide Time: 44:46)



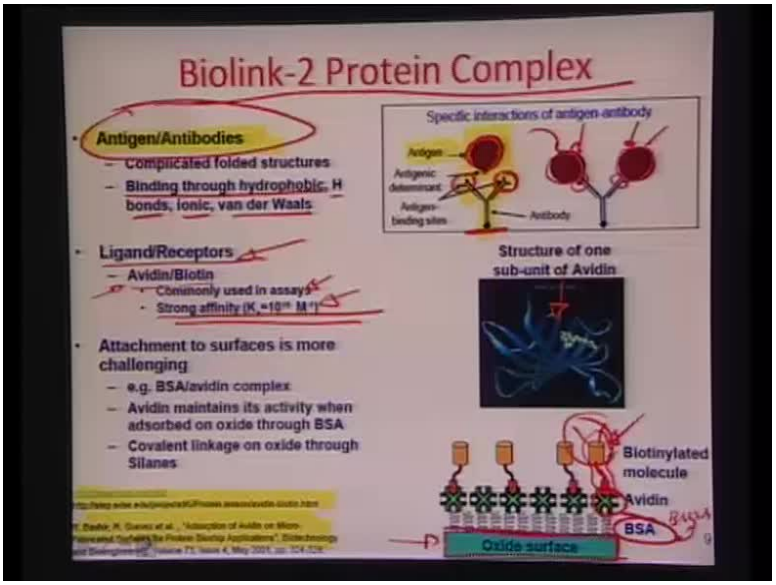
On the first such bio link of course, is this chemistry between antigens and antibodies. As we already know that antibodies are essentially proteins which has a complicated white shapes, there are 2 light chain sides of the chain which are arms of the y and there is a heavier chain which is at the bottom or stem of the y and this kind of linkage on the lighter chains you have these epitope sites or chemical sites which would be able to recognize sometimes selectively in case of monoclonal and sometimes non selectively as in case of polyclonal, the surface proteins of these antigens, antigens could be some infecting pathogen cells etcetera.

As you look at this balloon like feature here, it is the antigen and you have these 2 moieties which are the lighter chain moieties and this is the heavier chain of the y or

stem of the y, right along this particular region here on the top you have so called the epitope sites. Let me just quickly change the ink color here, it causes better visibility.

This is what is there on an epitope site. Similarly, you have another site here in this region which is also an epitope site. The way it works is that the proteins which are on the surface of this particular antigen here would be able to bind specifically to these epitope sites. Therefore, whenever there is an antibody which is somehow immobilized somewhere, you could bind two or more of those cells on the basis of their surface proteins using the epitopes which are there on the antibodies.

(Refer Slide Time: 44:46)

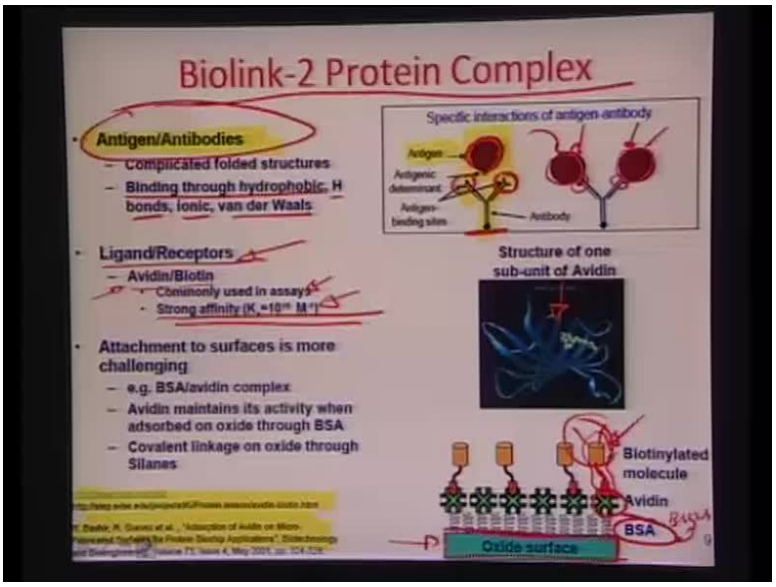


So, the binding could be through hydrogen bonds, it could be through ionic, or van der Waals or even covalent at times among the proteins on the surface and the epitope sites. That is the one mechanism where you could actually use this as a molecular hook for trapping a flow sample. Now, how you put the mobilize or immobilize the antibody on a surface is another kind of backward integrated processing which I am just going talk in the next few minutes. We have this ligand receptor binding. This is one of the very famous locks in biochemistry called the Avidin biotin lock.

You can see how this structure of 1 subunit of Avidin really looks like. It is a very complicated molecule as you can see here. Biotin is again an organic molecule; it is a vitamin. If you have a bonding between the Avidin and biotin where the affinity

constants are as high as about 10 to the power 15 per molar, they are commonly used assays molecular locks. This is something that biochemistry has to offer.

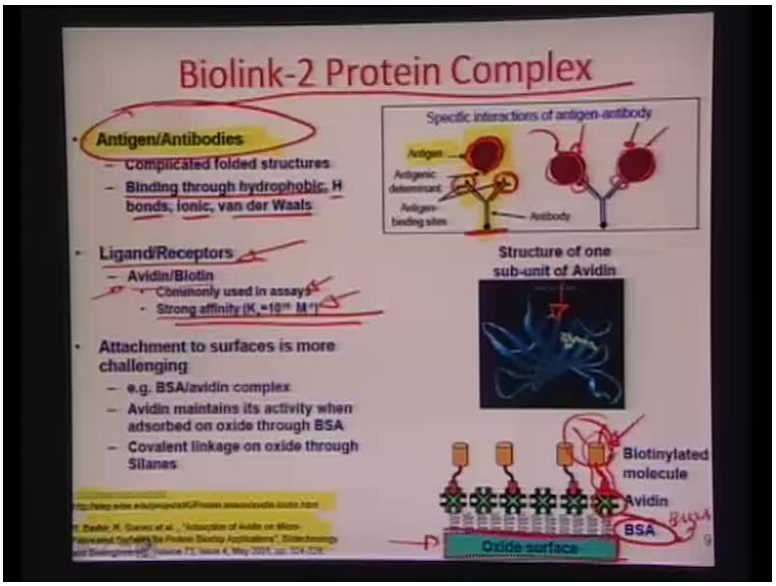
(Refer Slide Time: 44:46)



Another important point is the binding of antibody using this concept of biotin Avidin chemistry. Here what you do is - let us say you have a surface which you want to bind something on and you create a protein in its positive state at a certain pH and once the protein is protonated, you can actually create discharge surface of the oxide to have the exact opposite charge so that there is a binding of the protein, in this case, the protein BSA - bovine serum albumin onto the surface of the oxide as you can see here. What you also do is that this BSA is somehow biotinylated; you have a biotinylation done over the BSA. You put a biotin moiety on the BSA.

On the other hand, let us say this is some kind of a biotinylated molecule may be it is an antibody. You biotinylate intentionally the N group here, you have a biotin on both ends. One is in the BSA end and another in the molecule end that you are capturing. You are using Avidin linker between these 2 biotin groups, one on the BSA and one on the antibody; you are actually trapping the antibody in this manner onto the surface.

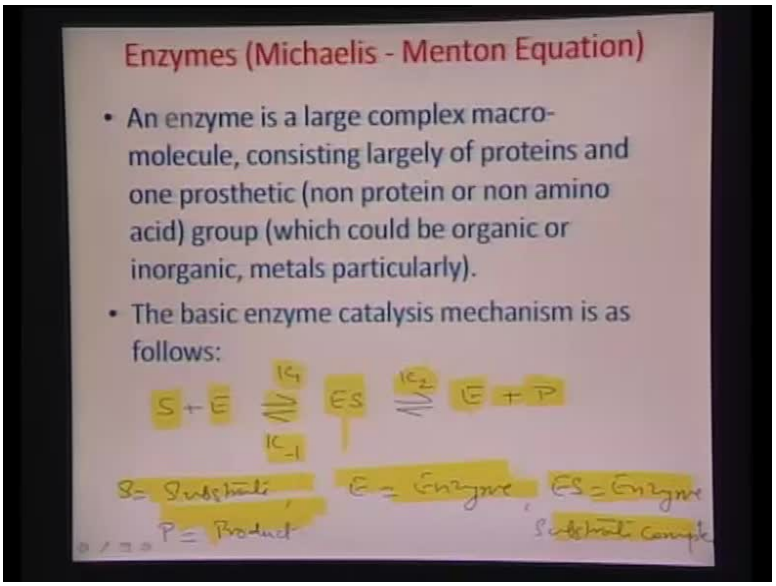
(Refer Slide Time: 44:46)



So, whatever you have done here which is concerned with probably the upper portion of this molecule in trapping the cells, the lower portion is fixed using the biotin Avidin chemistry using some kind of a species which can adsorb or freezes or organically bond electrostatically bond itself to the surface oppositely charged surface, any surface in fact silicon dioxide, any metal surface so on and so forth.

This a very interesting approach of how you could actually bind protein complexes to surface or immobilize protein complexes to a surface and further immobilize the different biological entities by using links like antibodies to identify or select. Common mechanisms like Eliza we have looked up before also considered this antibody antigen kinetics in order to determine changing coloration from where we can figure out what is the immune response of the patient or whether he has been afflicted by HIV or not.

(Refer Slide Time: 51:02)



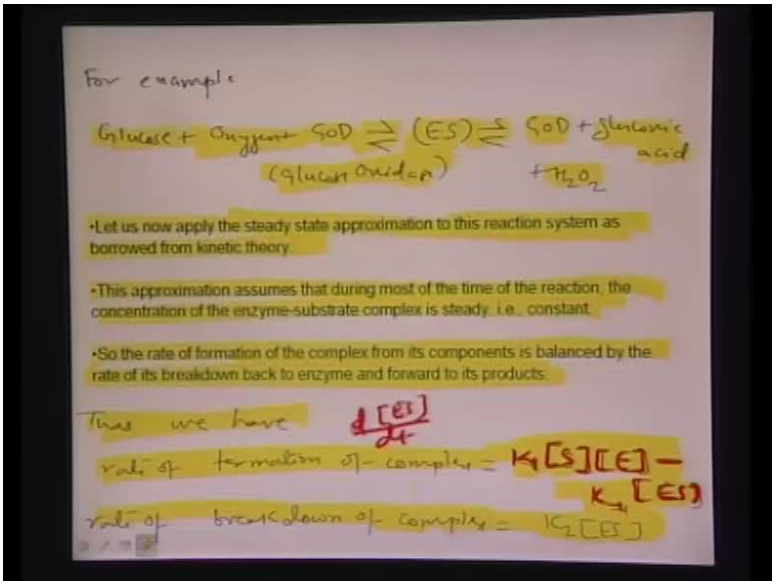
After doing this, we would like to do a little bit of more work in understanding how enzymes behave and for that we need to find out some mathematical formulations related to the rate constant of various reactions as the enzymes catalyze these reactions. As I have discussed many times earlier, the enzymes by themselves do not react permanently to substrates but they do participate in the reaction and create an intermediate complex with themselves as a part of that complex and then later on they dissociate the molecule making the product and dissociate themselves completely as a free enzyme which are available for the next cycle. If you would look at definition, the enzyme is a large complex macro molecule consisting largely of proteins and one kind of prosthetic group which could be either non protein or non-amino acid or even sometimes organic or inorganic group may be metals sometimes.

The other part by and large is made up of proteins except one which is a non-protein, non-amino acid kind of a moiety. That is was an enzyme is. If you look at the basic enzyme catalysis mechanism, let us suppose we start with a substrate here and by substrate what I mean again is some molecule which we need to modify into a product and you have an enzyme E and there is a binding kinetics going on between S and E at a certain rate K 1 and this is an equilibration with an intermediate enzyme substrate formulation which has been formulated here. There is the equilibrium set up so; there is a reverse rate constant which is also same of the forward rate constant and there is

equilibrium between the enzyme and substrate independently in the enzyme substrate complex.

Now, if you look at what happens to this complex, this complex is again converted into an enzyme in a product and that there is a rate constant K 2 in the forward direction in this case. We are not concerned K minus 2 here for reasons which I will disclose little bit later. So, we have from the substrate to enzyme, and from enzyme to product, the enzyme is retained as it is and the substrate is converted into product or catalyzed by this enzyme.

(Refer Slide Time: 53:28)



So, that is how this whole enzymatic complex formation process is defined. Let us look into some examples. For example, we have been talking about this enzyme GoD glucose oxidase. When you convert this or when you mix this with glucose and oxygen and you break it down there is an enzyme substrate complex which would be formulated and which it would generate back into the GoD, the gluconic acid in the H 2 O 2 which are products of this particular reaction. So, enzyme gets separated as it is.

Now, you want to apply the steady state approximation of this reaction system as borrowed from the kinetic theory. We want to find out what in terms of concentrations of various substrates, enzymes, or enzyme substrate complex can be really modeled the rate constant as. Some approximation we assume that during most of the time of the reaction the concentration of the complex enzymes substrate complex is very steady, that is

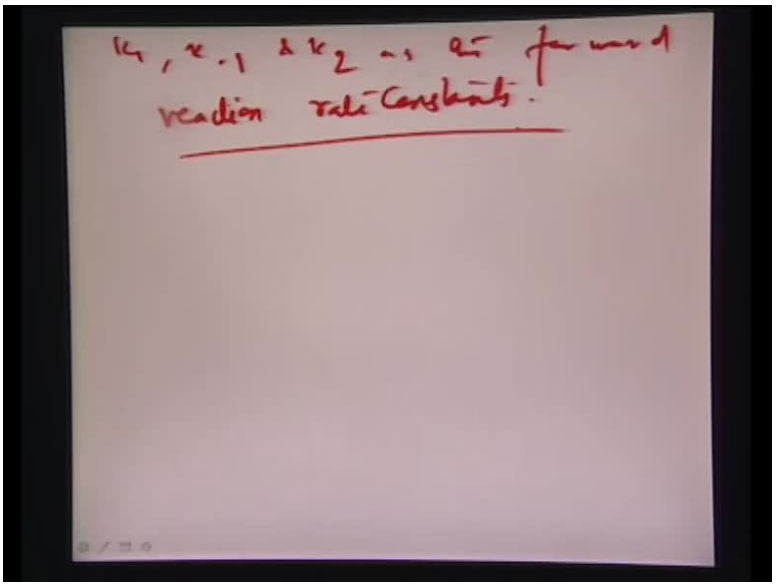
constant. So, there is one approximation that we make here and then also from this we can derive the fact that the rate of formation of the complex from its components is balanced by the rate of break down. So, there is equilibrium between formulation of enzyme substrate and formulation of the enzyme product and also there is an equilibration between the formation enzyme substrate and the kind of back formation of the enzymes and substrate individually.

So, there is equilibrium in the forward and reverse direction as you are forming the complex and then there is equilibrium in the forward direction as you are forming the enzyme product from the enzyme substrate. So, the rate of formation of the complex from its components is balanced by the rate of break down back to enzyme forward to its product.

Thus, we have the rate of formation of the complex as represented in this manner. K 1 is the equilibrium constant, so, you can have the rate of formation of the complex, d ES by d t, is a positive rate. You have formulating this K 1, which is the rate constant times of the concentration of the substrate times concentration of the enzyme minus the reverse rate which is K minus 1 times of concentration of ES which also shows the breakdown of ES into individual enzyme and substrates.

On the other hand, the rate of breakdown of the complex essentially into the enzyme and product, this is called the breakdown of the complex into the enzyme itself from the product - that is dependent on the forward rate at which ES complex is converted into the enzyme in the product that is K 2 times of ES this K 1 S E minus K minus 1 ES.

(Refer Slide Time: 56:51)



Let us look at some of these formulations here. Let me just write it down once again. We have K 1 K minus 1 and K 2 as the forward reaction rate constants. Because we are just more or less towards end of this lecture, what I am going to do is to pick this particular topic in the next lecture and try to derive what you call the Michaelis Menton equation. I would like to close the lecture with this. Thank you!