Essentials of Biomolecules: Nucleic Acids, Peptides and Carbohydrates

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Lecture-23
Role of Ribosome in Protein Synthesis and The Concept of Codon

Hello, everybody, and welcome back to biomolecules, so we are discussing module 5 on the biosynthesis of proteins, how proteins are synthesized in living organisms. And in the last lecture we have been talking about the step called translation through which the coded information in mRNA is converted into the peptides or into the setup or sequence of amino acids. And we have seen that a number of biomolecules were involved in the steps, primarily the mRNA, the tRNA transfer RNA, which carries the amino acids in its 3 prime end.

And of course the ribosomes, and then we have seen how the ribosome actually works, how ribosome helps in bringing in all the ingredients to synthesize our new peptide bond. And of course, that process is catalyzed by a couple of enzymes that also we have seen.

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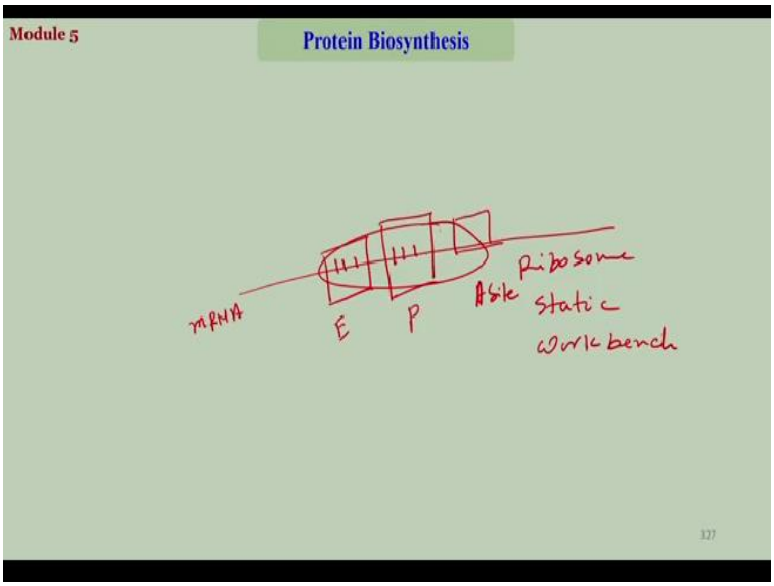

So the ribosome we have said that it is a group of protein plus the ribosomal RNA. So it is a constitution of the proteins as well as RNAs and we have seen the structures of how the ribosomes look like as well as the crystal structures. So, the function of ribosome as we have

mentioned is static and it works as a workbench or a platform on which the synthesis occurs. So, we have seen how the mRNA.

If you have the mRNA, how the mRNA goes back and forth into the ribosomal subunit into its binding pocket and then makes a certain part of it, where the synthesis works, synthesis occurs. And we have seen there are 3 sites called in 1 codon can be termed as E site and then there was another called the P site. And then there was another not the full from part maybe inside, but the rest of the part would outside of the ribosome space.

This is called A site and all are occupied by the tRNAs. And we have seen how the new tRNA comes in knocks this thing and the reaction proceeds in this direction, how that reaction goes on we have also shown the reaction actually how the peptide bond was forming by the reaction of the free amino acid with the bonded tRNA sequence. Now, it is something like this that we have already talked about, I will just briefly show you.

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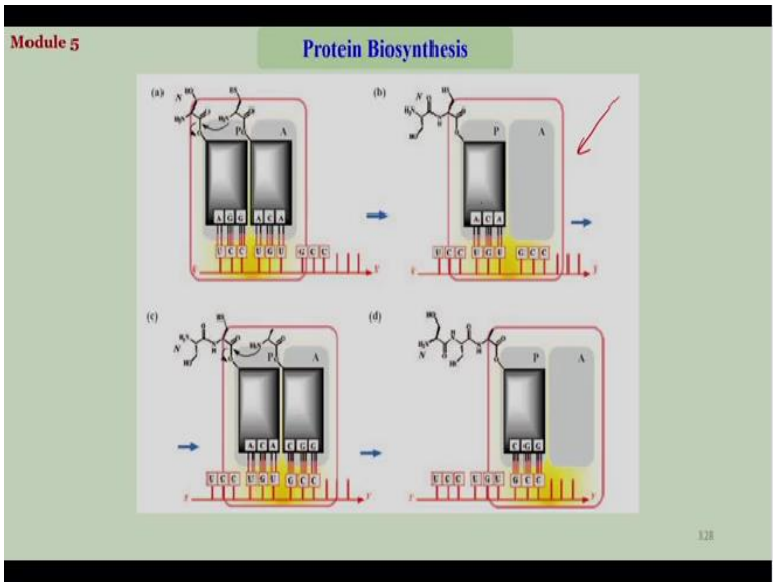

That you have one tRNA that carries from the terminal, this carries the amino acid, it can be last lecture we have started with the half made peptide here it can be the same thing. This is the anticodon of the tRNA. This is the codon of the mRNA. So, they will hybridize and they will stay into the ribosomal part, whether the ribosomes binds with the mRNA. This is called our P site and then comes the A site with the name tRNA with the different amino acid attached to it.

And a different anticodon which will hybridize to the codon and then you will have the reaction done. Now, once the reaction happens, it is transferred to this and then again the new tRNA has to come. So, the new tRNA will come here take the space of the A site that will push the A site to P and P site to the exit site and that is how the sequence of events will take place. And during the same time, the mRNA has to pull itself to bring the next codon into the cavity or into the platform of the ribosome.

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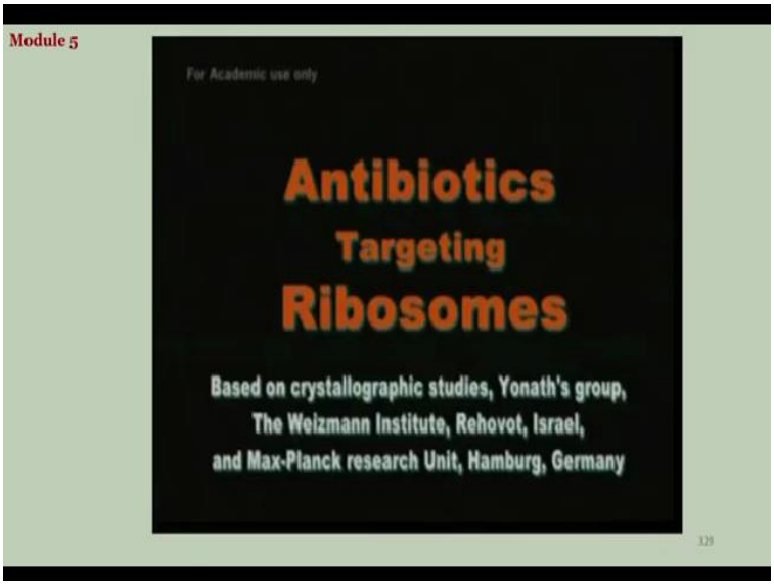

So, in the last lecture, I have also shown one video and that explains or that actually was a simulated model based on the experimental data crystallographic data that the professor Ada Yonath's group had obtained. They have made a video which shows how exactly the mRNA goes into the platform of the ribosomal subunit and how in real time, how the protein synthesis is occurring, how the peptide synthesis were occurring.

And then the protein final after the synthesis, the nascent protein was coming out of the ribosomal subunit. And that process is really, really fast per second, I forgot the exact number per second many numbers, I think 100 close to 100 amino acids are coupled actually. It is that fast. So today I will show you another video that from the same group professor Yonath's group they have made that shows how the antibiotics work.

So, most of these mechanism mechanistic studies on the function of ribosomes or action of mRNA, or use of mRNA in transferring the information to synthesize the amino acids were actually done based on the studies of many antibiotics. So now I will show how the antibiotics work in inhibiting the protein synthesis. So, there are several class of antibiotics that actually inhibit the synthesis of protein or that inhibits the function of mRNA in synthesizing the protein sequences and thereby if the proteins cannot be synthesized the organisms will die.

So, that is the mechanism of action of several class of antibiotics. And they have studied in a real vividness that shows how the antibiotic actually works in inhibiting the protein synthesis. And they have found out that, of course depending on the type of antibiotics depending upon their structure and properties, their mode of function or mode of actions are quite different. So, here it is. **(Video Starts: 08:01)**

So, let us say this is an antibiotic. So, this is edeine a universal antibiotic that hinders mRNA progression. Here you see the mRNA is coming in, mRNA is getting into the ribosomal subunit and on the other side of the cavity the antibiotic goes and binds. So, the mRNA will have a hindrance in it is path. So, roadblock, the mRNA cannot move further. Therefore, the process would be stopped. You see, the road is blocked for mRNA and it cannot go on.

Therefore, that protein synthesis cannot start. That is one way. That is the one way of blocking the synthesis of proteins or blocking the progression of mRNA. This is another antibiotic, very well known tetracycline that prevents A site tRNA binding the approach site. So you have already your mRNA in the ribosome, you have the tRNA that is conjugated to the amino acid sequence, which is in the blue fluorescent color.

This is your antibiotic. So this goes there, binds here, and let us see where it goes here. Yes. So what happens. This antibiotic goes and binds to the A site of the tRNA. So, A site tRNA and therefore, the P site or the new tRNA site, it can be that new tRNA site, it can be the old the P site that was existing when this comes together, they cannot come in close proximity because there is this antibiotic.

So, therefore, this amino acid which was already a peptide in the P site and the newly approaching amino acid cannot come in very close proximity to do the reaction. And therefore, the protein synthesis or the peptide synthesis will be stopped right there. See, therefore, apart from each other. So, they cannot come in the bonding distance. So that is the second way of function of antibiotics.

Third one is erythromycin, another very popular antibiotic that you know of what it does, it interferes with nascent protein, nascent protein progression by blocking the protein exit tunnel. So, in this case, it allows the full protein to be synthesized. But after the protein synthesis happens, the protein has to be detached out of the ribosome and comes out of the tunnel or comes out of the cavity.

So, antibiotic this particular antibiotic, it blocks that channel or the; it blocks the exit channel through which the protein could come. So, therefore, the protein cannot come out and it will eventually it will be destroyed. Here this is your antibiotic, clindamycin another class of antibiotic, what it does is it obstructs peptide bond formation. So, it will not allow to form the peptide bond. How it let us see, yeah, here.

So, this is your antibiotic and this is your amino acid. So, this particular class of antibiotic, they will bind to the amino acid sequence. So amino acid and the antibiotic will be bound together. If that is the done, then it is kind of the amino acid is kept by the antibiotic and cannot react further. So, this is the peptide that was synthesized and more to be synthesized new amino acid is coming in, but it is kept with the antibiotic.

And therefore, this reaction cannot happen, the reaction of the free amine of this newly approached amino acid to this peptide chain here cannot occur. So, it integrates the peptide bond synthesis. This cannot happen if it stopped then the other amino acids or other tRNA cannot come. Troleandomycin is another class of peptide that barricades tunnel passage. So, it will again block the tunnel.

Therefore, the protein up, the protein is getting synthesized, if it blocks the protein has to move this way as it is getting longer it has to move. Now it blocks that road. So, after some time when the length is enough it cannot move any further and then that will stop the further synthesis. **(Video Ends: 14:46)** Yeah, so, these are some of the ways through which the antibiotics work and therefore, once you know their mechanism of action then you can design new antibiotic to make more changes, or you can find out your own kind of pathways, which you want to block.

So, in order to do that, you have to know exactly how the ribosome and how the mRNA and how the protein synthesis is working. So, that was the previous video. So, once that picture is very, very clear, then only you can design the new kinds of antibiotics and that is what these 3 people have done that they have studied each and every stages of the protein synthesis process by crystallization and by other means also.

So, that has improved the understanding on the protein synthesis and understanding on the drug design to a very, very high extent. So, I also show you this video once again without any hindrance you can go through it. **(Video Starts: 16:05)** It is a first way of blocking. This is a second approach, third approach, the other one, this can be another way. Okay, there you go. **(Video Ends: 17:58)** So these are the ways through which antibiotics can function and you can program or design different antibiotics to act differently and of course given the time.

So in her lecture Professor Ada Yonath was actually explaining that although they have developed to understand the process of understanding how they work. How this antibiotic work, but over time the virus and bacteria they are smarter than us. So they will out cast our ideas. So over time these antibiotics will stop working and the microorganisms will find out ways how to bypass these things. And therefore constantly our job is to synthesize or design new antibiotics to find out new ways how the mechanism can work.

So that has to be a constant process to develop new antibiotics and to develop new mechanism of action apart from these which are explained alright. So, this is about the ribosomal part and how the protein synthesis works and now coming to the genetic code, which is known as codon.

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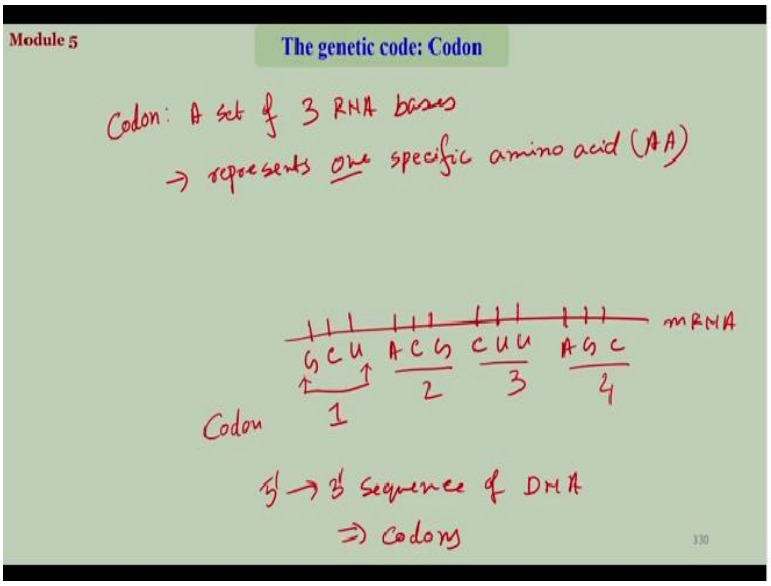

So, we have talked several times that tRNA contains the anticodon and the mRNA contains a codon and they hybridize is the 3 letter nuclear base sequence. So, these are called codons. So, today, little bit we will talk about which codons, how the codons came into effect, and how the codons actually represent the amino acids. So, what are codon, the definite is a set of 3 RNA bases, that is known as codon and what does it do.

A codon expresses or a codon represents one particular amino acid, it represents one specific amino acid now writing AA for amino acid. For example, if you draw a sequence maybe GCU. So, of course, I am writing an mRNA sequence here ACG. So, any arbitrary sequence actually CUU AGC. So, if this is your mRNA or any RNA, they will represent a codon. So, this constitute one codon, so you can call it codon 1. This is your codon 2, that is your codon 3 and this is your codon 4 and so on.

So, an mRNA sequence represents a set of codons. Similarly, you can also write down the codons using not using the mRNA what the original DNA, original 5 prime to 3 prime sequence of DNA to represent codons the 3 base pair sequence you can pick up that will also represent a codon.

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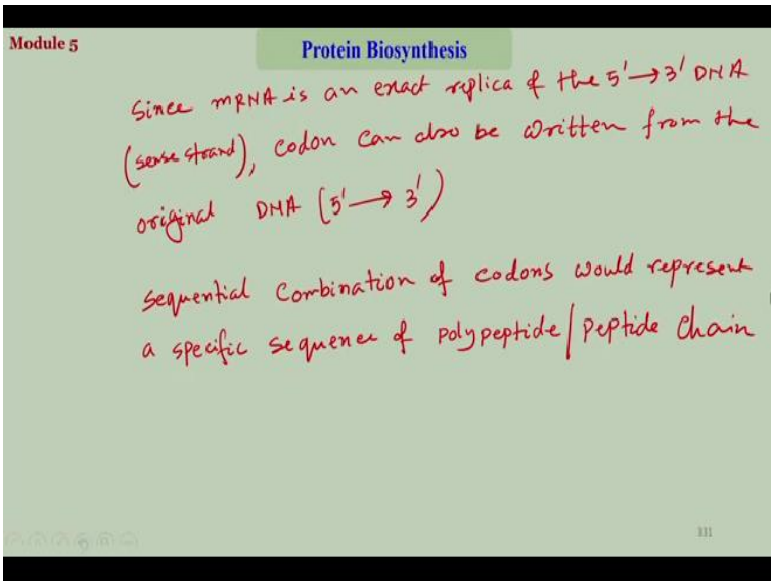

So, since mRNA is an exact replica of the 5 prime to 3 prime DNA, or we call it sense strand, codon can also be written from the original DNA of course, the 5 prime to 3 prime sequence of it. So what is the utility of codon. It is a combination of codon will generate a sequence of peptides, that we call proteins. So sequential combination of codons would represent a specific sequence of polypeptide or you can also call it a peptide chain okay.

So that is the definition of codons. What is the utility or use of codons. Now the question is how do you know that this particular codons represents this particular amino acid, of course, it is a real tedious job to find out, because direct there is no direct relation between the amino acid and the codons right, because amino acid is connected at the 3 prime end of the tRNA and anticodon is present at the bottom of the tRNA.

So, there is no as such direct relation between the amino acid and the anticodon. But still, they are specific to each other, they are complimentary to each other. A specific anticodon would represent a certain amino acid. So we have to find that out, what is the combination. A lot of people, a lot of experiments, many different kinds, of course, the first experiment that found out about the codon itself, what is codon.

And that a 3 letter base pair sequence is indeed responsible to express one particular amino acid was done by 3 or 4 people actually, in a particular experiment, it is known as a Crick and others experiment.

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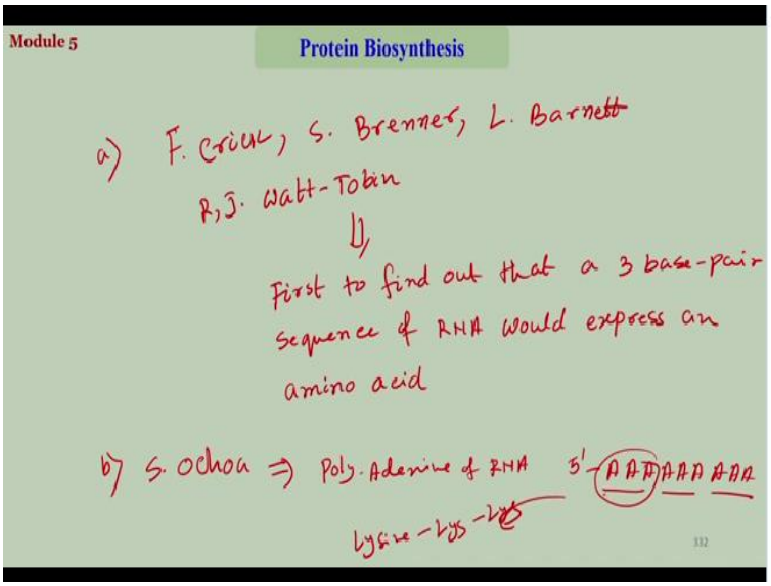

The same Francis Crick who has actually discovered the structure of the DNA Watson Crick. So, Francis Crick I will write the names of these people S. Brenner, Barnett and R.J Watt-Tobin. These are the people who in an experiment first found out that a 3 nuclear base sequence in RNA would represent or would express a specific amino acid. So, they are the first to find out that a 3 base pair sequence of RNA would express an amino acid.

How they had found out, what they have done is they have taken subsequently another experiment was done. So, that was the first experiment that talked about the codon, the second was S. Ochoa has found out, that if you have this kind of similar experiment, if you have poly adenine sequence of RNA which means basically 5 prime to 3 prime AAA AAA AAA and so on. So, 3 3 3, if you take this particular RNA with a poly adenine chain.

Then the protein or the peptide you synthesize out of this would be lysine or poly lysine. So, a poly lysine was synthesized out of poly adenine, RNA. And of course, the numbers was having the parity that 3 codons or 3 base pairs versus 1 amino acid. So, it was something like that. So,

from that the idea came in that a specific codon exists for a specific amino acid. So, in this case our AAA this codon would represent a lysine.

And since all are the same it represents or it synthesizes the same amino acids. So then came our own professional Hari Gobind Khorana.

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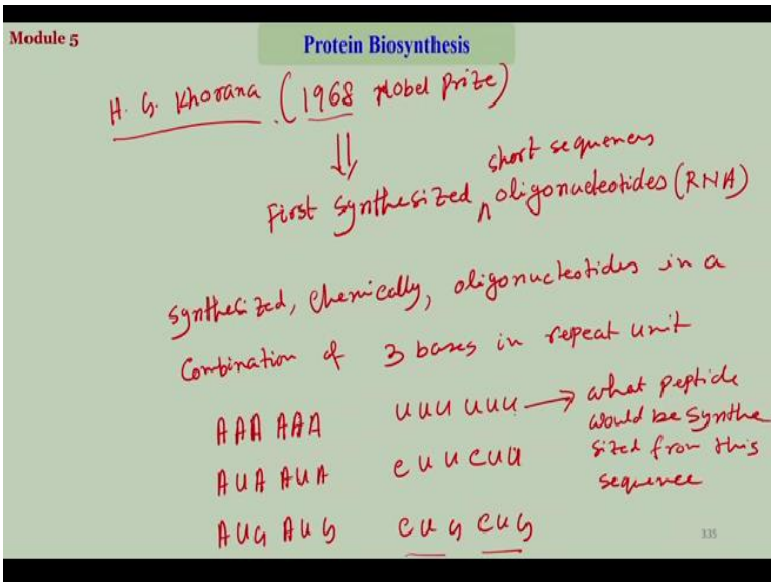

Professor Khorana was the person who has actually discovered the rest of the amino acid table, the rest of the codons versus the amino acid and for which he has received Nobel Prize very long back actually, all these things happened actually much, much before 1968, Nobel Prize in physiology and medicine. So what he has found out, he has done something really, really unique, which nobody has done until that time.

So, he has used the chemistry techniques, the organic chemistry tools to find out the amino acids. So, what he has done, he has actually synthesized all combination of the codons, chemically synthesized. So, that was the first synthesis of oligonucleotides. Of course, he has done the RNA, he was the first to synthesize oligonucleotides, RNA oligonucleotides that he has synthesized short sequences.

That we have seen in other modules when we have talked about solid phase DNA synthesis you remembered solid phase oligonucleotide synthesis. Nowadays we can synthesize quite a long

chain of them using the solid phase chemistry. But that was back he has done it really back in 1968 he received the Nobel Prize. So, things were done back in 1950s. So, that was one of the first times when that chemistry tools have been used to synthesize biomolecules.

So, RNA was synthesis, what he has done, he has synthesized a repeat units of all possible combination, for example so he has synthesized chemically oligonucleotides in a combination of 3 base pairs, or 3 bases rather to say because it is not double stranded DNA, usually we call base pairs for even for a single strand, so, 3 bases in repeat unit. So which means for example, he has synthesized adenine, adenine, adenine in the laboratory using the phosphor laminate kind of the phosphate chemistry.

So, AAA and then in a repeat of AAA AAA let us just take 2, I think he has taken 3 combinations at the time and then you ready you make all possible combinations. For example this U A U A and then A U A so, this is one RNA, this is another RNA and maybe A U G and similarly, A U G, so, we already have 9 combinations U U U U U U I am just writing to repeat units C U U it can be arbitrary any sequence you can fabricate.

But only thing is that it has to be the repeated units C U U C U U and then C U G C U G. So, likewise all very essence, all possible combinations he has actually synthesized and then he has studied what would be the proteins or what would be the polypeptides for the peptide that would be synthesized from these units, from this RNA. For example, what peptide would be synthesized from this sequence.

And of course, it is a double check or triple check, because you have the repeated unit. So, you expect the same amino acid to be appearing again and again. So, it should be a homo polypeptide. And that is what he has actually found out that using the repeat units the same amino acid was appearing. So, for this case, whatever the amino acid is here, that will be here also.

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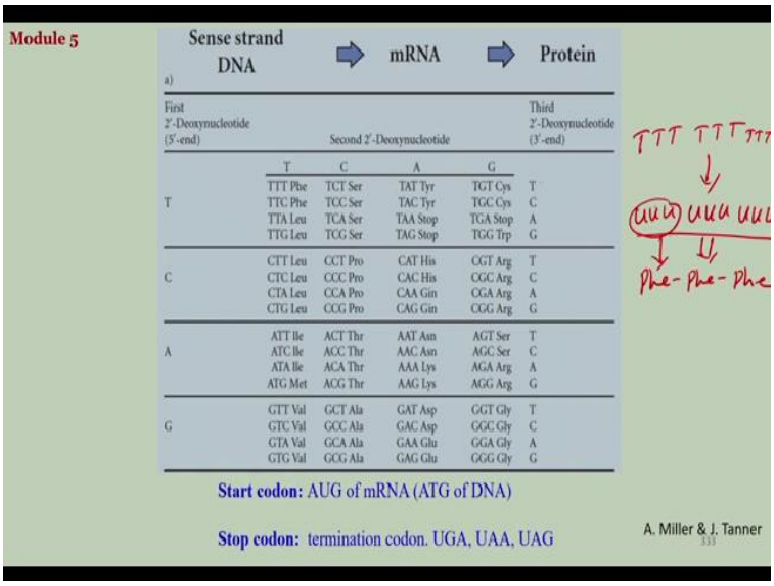

And that is how he has found out the whole lot of the table, which is known as the codon table basically. So, the first one is you can write it in 2 ways. One is you can directly take the sense strand sequence; of course, it is going through the mRNA and then protein. So you can take the sequence of the sense strand and find out the codons or you can take the sequence of the mRNA strand and find out the codon.

So, the table looks like this. So, 4 all the 4 nucleic acids, here also there will be all the 4 nucleic acids and in each group, in each column or each row, there will be again the 4 nucleic acids. So, that makes it 3, this, this, this, makes that one combination, because 3 nuclear base pairs makes 1 codon. So, for example, if this is a T, if this is T C A and G and then T C A and G then what are the combinations you can make, T T, the first one is T.

So, it has to be T T T, T T, second one is C. So, it has to be T T C. Similarly, T T A, T T G, so, this is for the T T column, T and T would be the first 2 nuclear base and the third one would vary according to this. Similarly, when you take C, then TC would be common for all and then it will vary here. The third one it will vary in the third nuclear base, same is this TA would be a TAT TAC, TAA TAG, G here, TGT TGC, TGA, TGG.

So that makes 1 2 3 4, 4 into 4 combination. So total 16 combination out of here, so each of them will have a 16 combination. So in total, you are getting about how many 64 combinations. So

Professor Khorana has synthesized all 64 combinations in the laboratory. And then he has started investigating what would be the amino acids that would be synthesized out of this sequence and this is what he has found out that our TTT sequence in repeat unit of course, would represent a phenylalanine.

So, when you have a TTT and another TTT and another TTT, I am writing the parent DNA strand. Of course, this would be going to the mRNA which would be UUU, he has actually synthesize the mRNA, he never, he has not synthesized that the DNA UUU UUU UUU and he has found out from here when you allow it to synthesize the peptides, it synthesizes phenylalanine, phenylalanine, phenylalanine which means this codon represents a phenylalanine.

So which is here. So that is how he has figured out all the individual codons and which amino acids they would express and there is a catch here. The same amino acid can be expressed by different codes. As you can see here TTT will represent phenylalanine, TTC would also represent a phenylalanine, TCT TC common with all 4 variations, all of them actually represent serine same amino acids.

So, that is also possible, different code on same amino acids possible. So, I will go to the next table where the mRNA was actually the real one actually, with the RNA sequence and amino acids.

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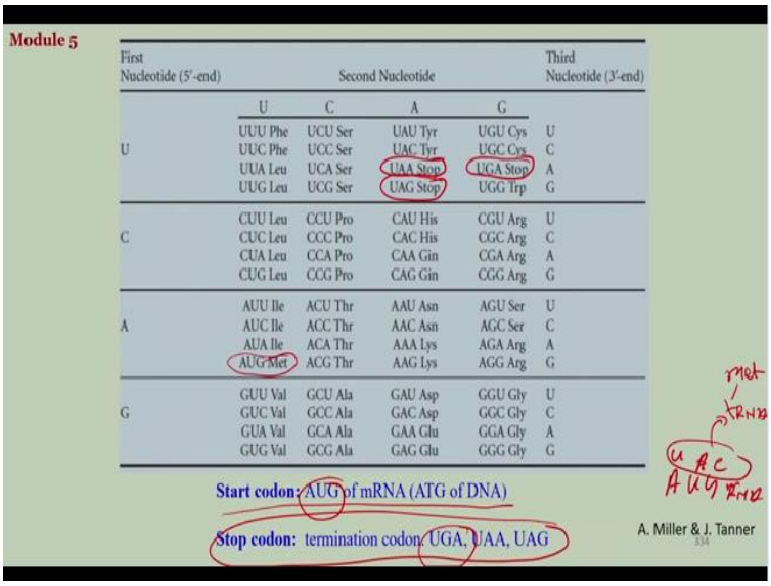

So, as I was showing, 4 columns here, 4 there and 4 here, that makes all these combinations. It is the same combination, but only thing is that this is now RNA. So, UUU is phenylalanine and so on. Now, there are a few things that we have to remember it is very, very important. Of course, I have talked about it, when you synthesize the peptide sequence or when you start synthesizing your protein at the stage of initiation; we have seen when the first amino acid is connected to the tRNA that is always a methionine.

And the codon is AUG, codon of the mRNA. So, the mRNA sequence will have the sequence AUG and that is the start codon. So, the anticodon tRNA, which will come and hybridize here will always carry on methionine there. So, AUG, this is the RNA, this is mRNA. Therefore, the tRNA will have the sequence of UAC; this would be the anticodon of the tRNA. And this tRNA would always carry methionine.

And this is called the start codon, that is where the your reaction starts, your protein synthesis starts. So, this is called the start codon, the codon AUG would represent a start codon, and therefore, I guess it should represent a methionine. Now, let us see, this is the A column. This is the U column and here is G. So, AUG it actually represents methionine, that is our start codon, this is where the synthesis starts.

And where does the synthesis stop here, when it reaches a stop codon, when a stop codon is reached in the mRNA as I have said that the stop codon does not carry amino acids, so, therefore, no further synthesis. So, these are the codes that are stop codons, if any one of them are present in the mRNA when that sequence is reached, then there will be no further reaction UGA is a stop codon, UAA. So it is all U sequence UAA is a stop codon.

UAG is also a stop codon. So, these 3 are the stop codons. So, you have to remember, whenever you stumble upon these sequences, then your amino acid synthesis will stop. So, if you are given the table, then you can pretty well find out what will be. And if you are given a sequence of your mRNA or even the sequence of the parent DNA, then you will be able to find out the sequence of the peptides. So, I will give you one question which can be regarded as an example.

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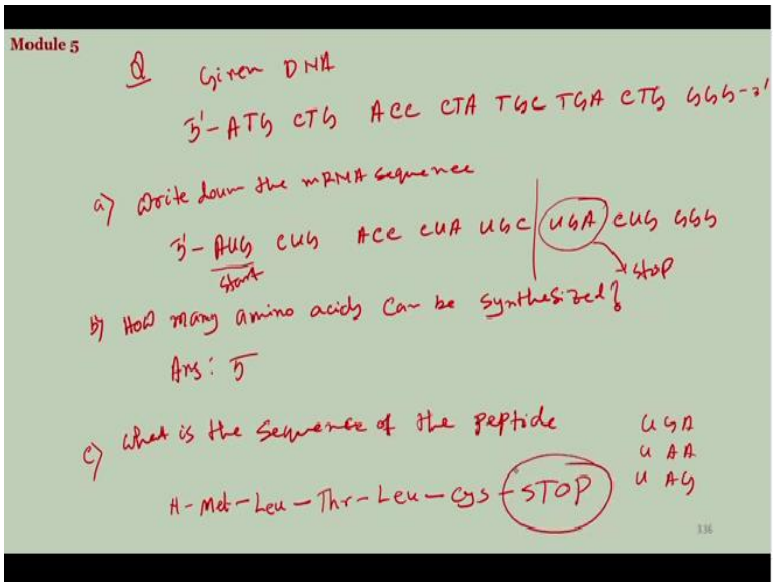

So, question is I will write a DNA sequence original DNA sequence given DNA that has a sequence of 5 prime ATG CTG ACC CTA TGC TGA CTA CTG GGG 3 prime. Now the question is question a, is write down the mRNA sequence. So, if this is the given DNA sequence what will be the mRNA sequence. This is very easy actually, it will be the same. So, your answer is here, it would be the exactly same only thing is that DNA bases would be converted into the RNA bases.

So your sequence would be AUG CUG ACC CUA UGC UGA CUG and GGG. That would be your RNA sequence. Second question is how many amino acids can be synthesized. How many amino acids synthesize from this mRNA sequence. So how many codons you have, you have 1 2 3 4 5 6 7 8, you have 8 codons. Would it synthesize 8 amino acids. It is actually if you look carefully, no, because I have included stop codon here.

So what is your stop codon. Let us see, what are the stop quadrants; UGA UAA UAG UGA UAA UAG. These are the stop codons. You have to remember the stop codons and the start codon. So AUG, see this is the start codon UGA, do you have any UGA. There is a stop codon right here. So, that is stop, this is start. So, once a stop codon is raised others one cannot come in because here itself your synthesis stopped.

So, therefore you can synthesize 1 2 3 4 5 amino acids. So, answer is 5, you can synthesize a sequence of 5 amino acids for this peptide out of this sequence out of this mRNA sequence. So, you always have to be careful that your stop codon would be hidden somewhere. And then the third question and the last question is what is the sequence. Sequence of the peptide of course, in this case, I will provide you with the table, with this table.

From here you can find out. So I will just write down because I have calculated it from the N terminal end. The first one is methionine because it is a start CUG. Let us find out very quickly. CUG it is a Leucine. I will do another one ACC, A column, C column ACC is threonine, THR. Similarly, you will have another Leucine here. You will have Cystine here. And then you have our stop signal. So this is what is going to be your peptide sequence.

So you can practice using different kinds of sequences permutation and combinations and try to read the table, try to use this table, use this table to figure out the peptide chain that could be synthesized from a given DNA or the mRNA. Now, with this, I will come to an end of this module 5, that what we have discussed in this module. So, this is fully about the biosynthesis of the proteins.

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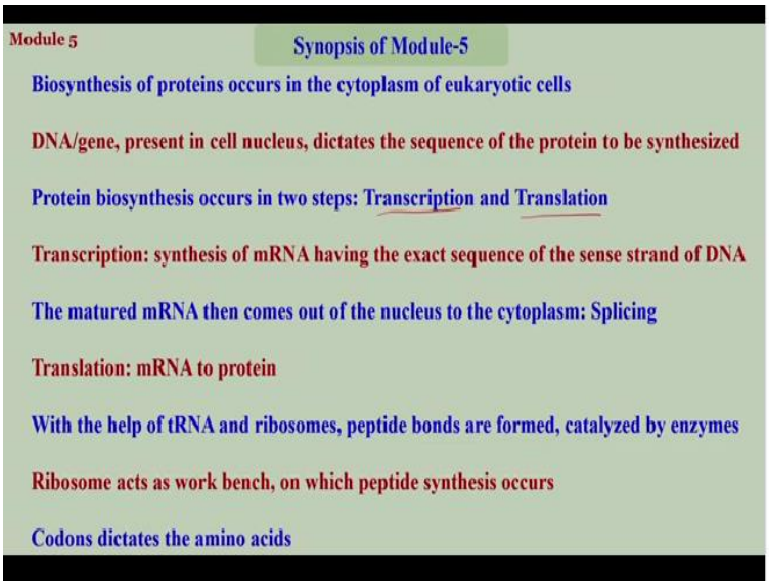

So, biosynthesis of protein that occurs in the cytoplasm of eukaryotic cells. How does it happen. It takes the information of the original DNA or the DNA gene that stays in the nucleus of the cell, DNA or gene present in the cell nucleus that would dictate the sequence of the protein that would be synthesized. The whole of this process protein biosynthesis occurs in 2 steps as we have seen, one is called the transcription other one is called the translation.

And we have discussed in detail what is transcription and what is translation and what are their mechanism. What is transcription that is synthesis of the mRNA having the exact sequence of the same strand of DNA that is all about transcription. And once the mRNA is matured, then it comes out of the nucleus to the cytoplasm through this process called splicing and that is how the information coded in the gene in the nucleus comes out to the cytoplasm and synthesizes the protein there.

Next step is the translation is the conversion of language from the language of nucleic acids to the protein from mRNA to the protein. How does it happen. We have seen that it occurs with the help of tRNA and ribosomes, new peptide bonds are formed. Of course, we have seen a couple of enzymes are involved. Ribosome becomes very, very important for us. It acts as a workbench, which provides a platform on which the peptide synthesis can take place.

So, this is mostly about this module 5 and in the next lecture we will talk about another module on the synthesis of the peptides. How can you develop the peptides in laboratory and also how to sequence a protein. Thank you.