Organic Chemistry In Biology And Drug Development
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Lecture - 31
Central Dogma: DNA Replication, Transcription and Translation (Contd.)

In the last session, we talked about translation process and we have discussed the differences between the prokaryotic translation versus the eukaryotic translation. In prokaryotic translation, whatever mRNA is obtained by transcription depends upon the position of stop and start codon. That entire portion between the start and the stop will be transcribed into a protein.

However, in case of eukaryotes, the mRNA obtained by transcription contains alternate segments. One segment codes for amino acids and the next segment does not have any function. It contains junk sequences that is not required to make the protein.

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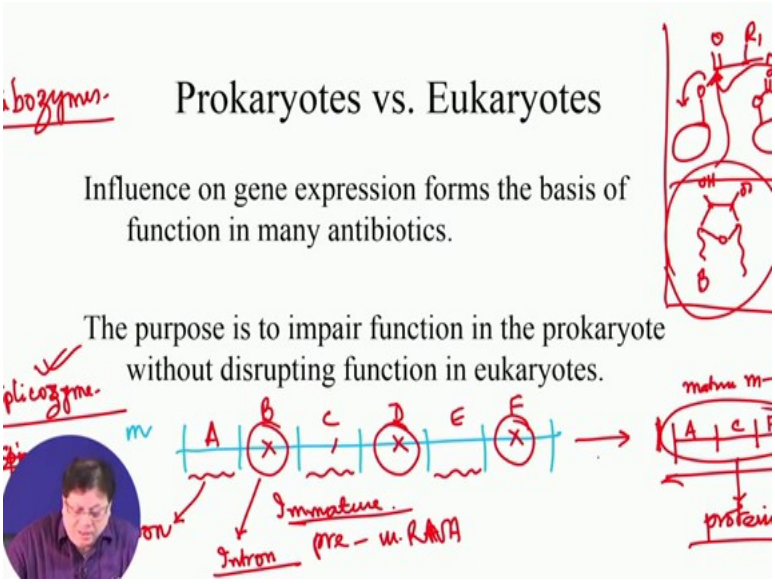

In eukaryotes, suppose this is m RNA. It has got different segments and some of the segments are functional segments. Some portion codes for a protein but some portions do not.

This has to be processed and this junk portion which is not coding for any amino acid that has to be removed. Then the pieces have to be again stitched together. Suppose it is

A B C D E F. So, if you have to remove the B D and F from it and you now join A with C and with F.

This is the one which will now uninterruptedly code for the different amino acids in a protein. Now this mRNA called i.e. mature mRNA can synthesis the protein. This is called immature or pre RNA and this can be called as fully functional mRNA.

This part is called exon and this part called intron. A pre mRNA contains intron and exon. So, this complication is present in the eukaryotes. The enzyme called splicozyme removes the introns and joins the exons together.

For a reaction to happen proximity is very important. Ribosome brings two tRNAs together so that they are very close to each other. Ribosome can do so because of the codon anticodon interaction.

Different tRNA attached to different amino acid will not react to form peptide in absence of catalyst. The RNA that is present in the ribosome that catalyzes this reaction.

RNA has a nucleophilic two prime hydroxyl group. It has been found that the two prime hydroxyl group first attacks this acylated tRNA and release this one. It is formed via tetrahedral complex.

Then the second NH_2 comes, attacks and releases the RNA. It is very similar to your protein enzyme chemistry. For example, just like the Chymotrypsin that hydrolyses the peptide bond. At first, the nucleophile attacks to form tetrahedral intermediate. Then water comes and attacks. RNA also follows same mechanism.

So, the 2 prime OH is attacking this acyl releasing this one and the next step is the NH_2 comes and releases the 2 prime OH. It is an example of nucleophilic catalysis. We are seeing that an RNA molecule also has catalytic activity.

It is basically nullifying o going against the central dogma of biology because in central dogma biology it is the proteins which are enzymes that carries out all the reactions. But this is an interesting fact where RNA can act as an enzyme.

So, when RNA act as an enzyme they are called ribozyme. One important reaction is the formation of the peptide bond by trans acylation pathway where ribosomal RNA acting

as a nucleophile ok. Now, let us come to the other one the splisozyme again they splisozyme again this as I said this splicing is done by the by RNA molecules the RNA molecules itself does the splicing and then join the exons together.

So, another example where RNA can act as an enzyme was Nobel Prize winning work by Thomas. There are the two examples. That I know that we wear the RNA can act as enzyme. So, one is this peptide bond formation. Another is this splicing of the introns and conversion of immature mRNA into the matured form.

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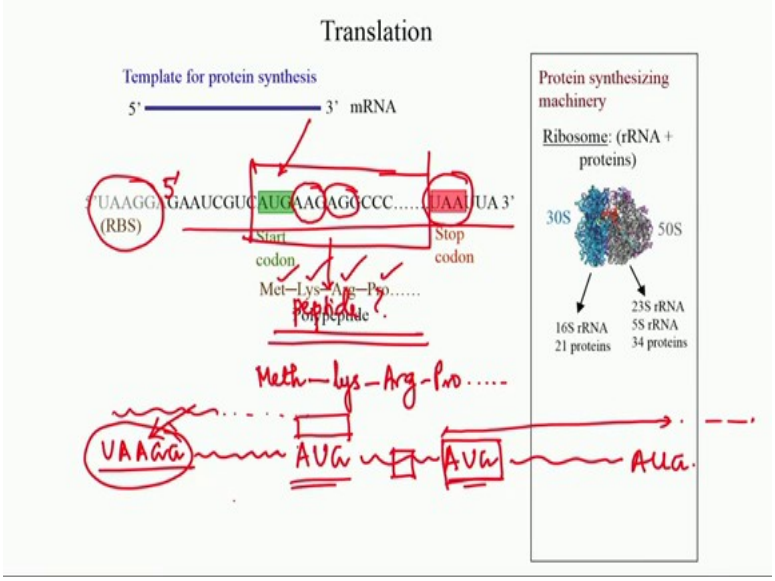

Suppose this is the sequence of the mRNA. mRNA provides the template for the protein synthesis depending on the sequence of the codons in the mRNA and accordingly the primary structure of the protein will be dictated. Suppose this is the sequence of the mRNA and the structure of the peptide that is going to be obtained from it by translation.

So, what is the sequence of the peptide? How can we solve this? First find out your start codon i.e 5'-AUG-3' and it codes for methionine. So your first codon or first amino acid should be methionine. What is the second amino acid? Here your next genetic code is AAG.

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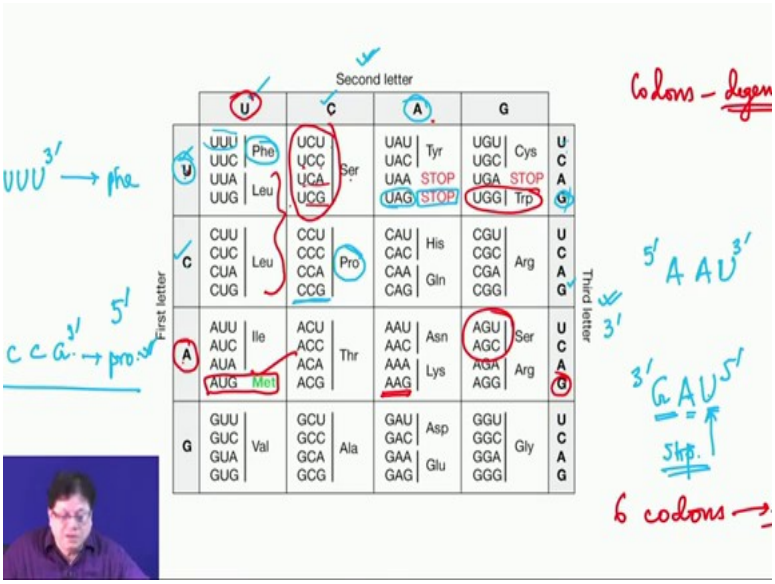

So, it will be lysine. Third one is AGG that codes for arginine. Fourth one is CCC that codes for proline.

Then suddenly you see that there is a UAA i.e. stop codon. So, your protein synthesis will stop here.

The mRNA has to bind to the small ribosomal unit. This is your RNA binding site present in the ribosome on which the mRNA is anchoring.

Suppose, I have a mRNA and its sequence is UAAGG. Suppose this is your anchoring sequence. It may happen that there may be several AUG. This anchoring sequence is important. Then protein synthesis will start from which AUG that depends on the position where the mRNA has anchored. If the anchoring sequence is in between two AUGs then the first 5'-AUG-3' from the right side will be your start codon.

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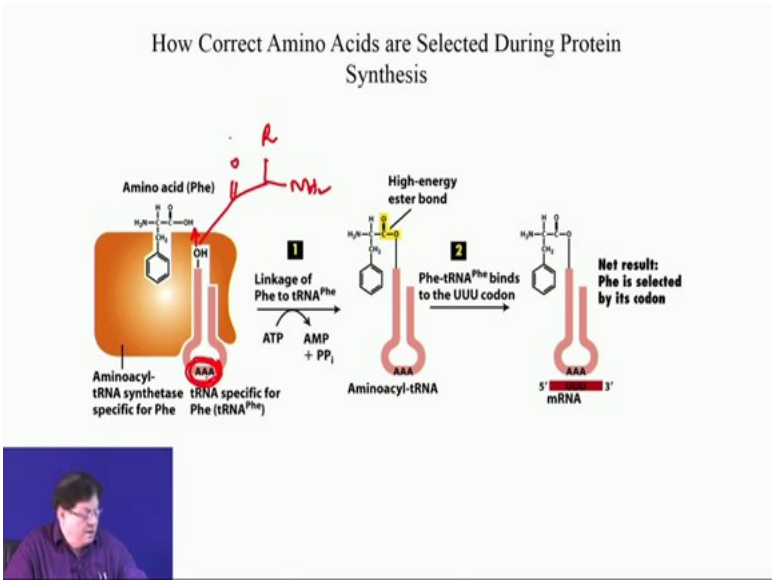

Amino acids that are selected during protein synthesis will depend on the sequence anti codon.

The RNA can recognize the anticodon. How it will know that the OH is attached to this acylated amino acid. It is like human body system i.e. if there is any small needle which pricks to my leg immediately I know because my head is the realizing point. This stop point knows the anticodon sequence. Then accordingly it puts the amino acid. There is actually an enzyme called tRNA amino acyl synthatase which does this job this acylation

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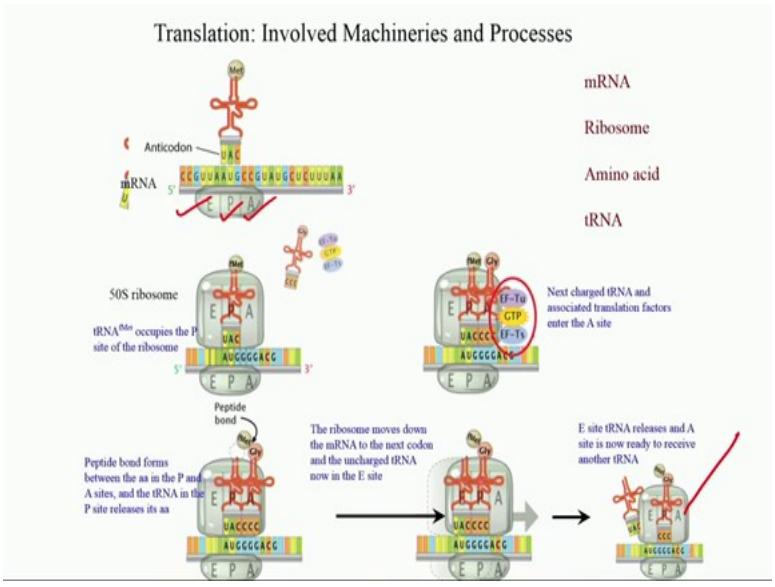

It is shown here schematically that this is the first amino acid methionine. Then this is GGG and according CCC will come.

That codes for glycine. So, methionine and glycine will combine. Methionine is transferred to the glycine and this becomes free. Next it goes to the exit site and ultimately that is released. That comes to the P- site.

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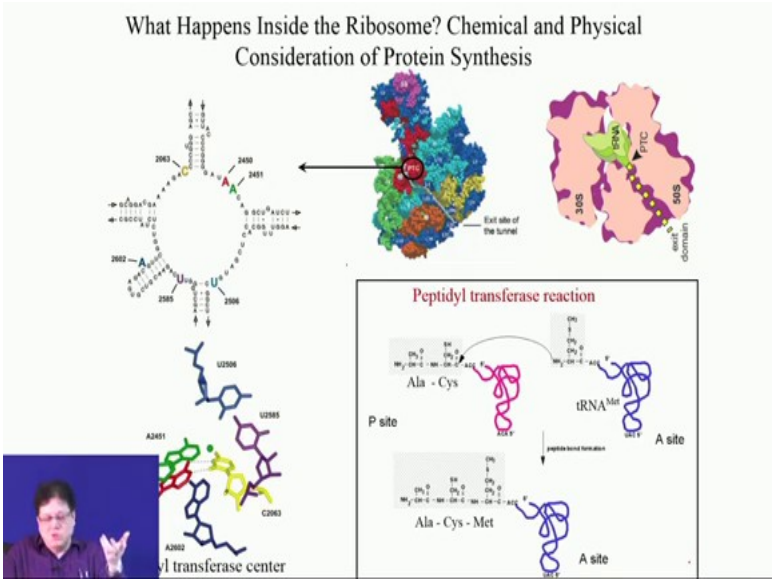

According to this sequence, next tRNA will come. If you stop the replication by a small molecule then cell division will not occur.

You can stop transcription also. Replication, transcription, translation are vital process. If a small molecule can stop these processes then it can act as drug.

I am giving you the simple examples of bacteria i.e prokaryote. Prokaryotes do not need any splicing. Their mRNA does not need any modification. So mRNA goes and binds to the ribosome. Now, if you have a small molecule which binds to the A site or P site or exit site this shifting will not take place because your sites are already occupied by small molecules. That can give rise to death of this prokaryotic organisms and antibiotics which work on this principle.

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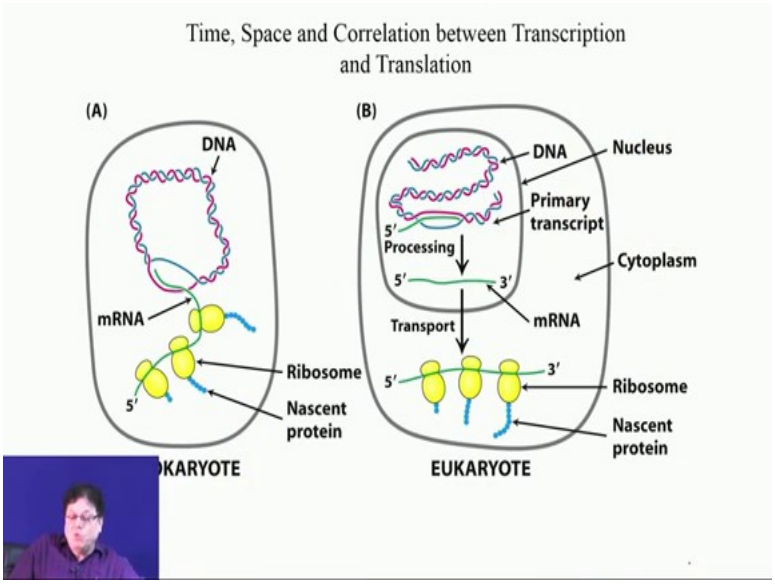

The genetic code is having 64 codons, but we have 20 amino acids. Many codons here are coding for the same amino acid. For example, 6 codons are coding for serine.

So, many of the codons are degenerate i.e. more than one codons are coding for one amino acid. The word ‘degenerate’ is used many times in describing the orbitals in connection with the MO theory. Only 2 amino acids has got a single codon and one is AUG. AUG codes for methionine. If the start codon is not a fixed one there is a lot of degeneracy. Then the whole machinery will become very complicated that where to start.

We have covered replication, transcription and translation process. We will do some problem solving once the biology part of it is done.

In the next video, I will go to the next topic. If you have double stranded DNA that will make the RNA and ultimately RNA makes proteins.

Suppose, I am sitting here in this room and nobody is here. But somebody has come earlier in this room. I want to know who has come before me in this classroom. I will search for any body material like piece of hair which is present in this room. One or two hairs usually fall. So, I can get a piece of hair from the person who was entered last. So, I take that piece of hair and I isolate DNA from hair. I will get a tiny amount of DNA.

Now, I have to analyze the DNA i.e. I have to get the DNA sequence. Then whoever is in my suspect list, I will check the DNA sequence there. Then I will match and then from that matching I can tell who has actually come.

We have to make multiple copies of the DNA that I isolate from a single hair. There is one technique that is called Polymerase Chain Reaction i.e. PCR. PCR is an essential tool in any biology laboratory and it is continuously being used. Basically PCR is a technique by which you can make copies.

Another way is that whatever DNA you isolate even if it is small you can insert this piece of DNA into a non-pathogenic bacterial DNA. As bacteria grow, multiple copies of that DNA is made in the bacteria. You have to isolate those pieces of DNA from the bacteria.

So, these are the two different ways by which you can take multiple copies of DNA. Because of the simplicity the PCR technique has attracted the most. However, the other technique where a foreign DNA inserted into a bacterial cell also has got one advantage because you can always grow the bacteria. In the next lecture, we will discuss amplification of DNA.

Thank you.