Essentials of Biomolecules: Nucleic Acids, Peptides and Carbohydrates

Prof. Dr. Lal Mohan Kundu
Department of Chemistry
Indian Institute of Technology-Guwahati

Lecture No. 20
Transcription - The Transfer of Genetic Information from DNA to mRNA II

Hello everybody and welcome to biomolecules again. So, we are discussing module 5 on the biosynthesis of proteins how proteins are synthesized in our biological cells. And we have seen that it goes through two distinct processes or two distinct steps.

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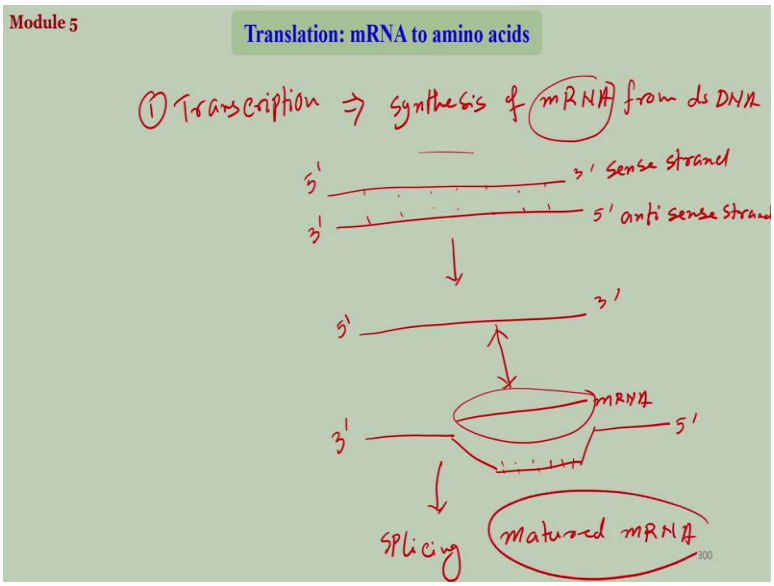

The first one being the transcription where we have seen how the information present in the nucleus comes out of the nucleus with the help of mRNA so this is the process of transcription was about synthesis of mRNA messenger RNA from the double-stranded DNA that was present in the nucleus of the cell. And here we have seen that the sequence of the mRNA is exactly the same as that of the antisense strand of the double-stranded DNA. So, very quickly if you have the 5 prime to 3 prime DNA and the 3 prime to 5 prime this is your DNA that is present in the nucleus and then so this is this was called the sense strand and this one was anti-sense friend.

And then we have seen that the anti-sense strand was taken as the template to synthesize the new mRNA with the help of the mRNA polymerase. So, therefore that's how the sequence of the

mRNA would be complementary to the antisense strand and therefore the sequence of the newly synthesized mRNA would be exactly the same as that of the sense strand. So, that is how all the genetic information that was present in the same strand has been transcribed or transcripted into the newly synthesized mRNA.

And then finally of course the mRNA after the process of splicing it under was maturation to the matured mRNA by removing all the unnecessary parts of the mRNA taking only the important part of the mRNA for the synthesis of protein. So, basically which means in the coded part of the mRNA would be kept and the rest of the parts which do not code for proteins or which do not code for amino acids would be removed. So, that is how you get a matured and functional mRNA that is fit to be used for the synthesis of the proteins and this is outside the nucleus of the cell in the cytoplasm. So, that was the first step about transcription.

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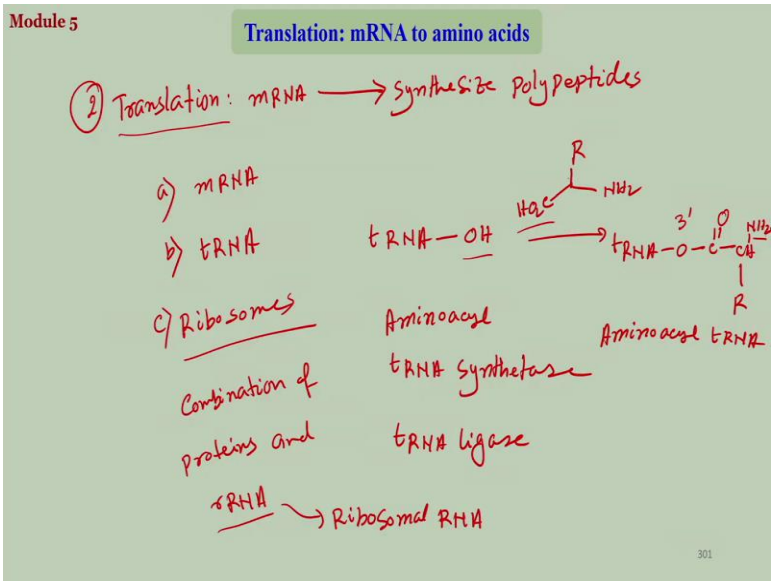

And then we have talked about the second step that was translation. So, in translation basically the imagine that you have synthesized from the previous tape would be used all the information present in the mRNA node be used to synthesize proteins or I will write polypeptides. So, mRNA to polypeptides is the process of translation and we have also discussed why this is called translation because it changes from one language to the other. The language of nucleic acids is been changed to the language of the amino acids that is why this is called the translation process.

And in the last lecture we have discussed that a fair number of biomolecules are involved in this process of translation. We have talked about of course this present mRNA that is one of the key ingredient that would be used we have also seen how tRNA was involved and we have discussed a detailed about the tRNA's.

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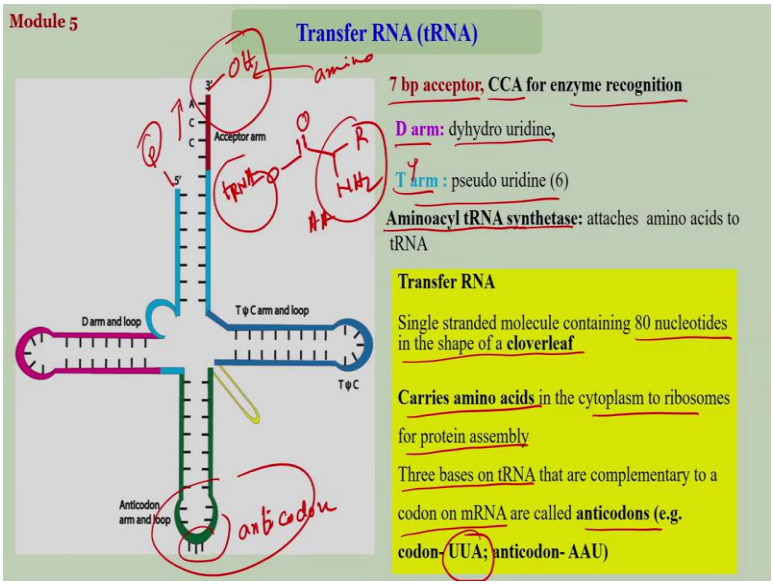

So, this is the structure of a typical structure of a tRNA which is basically a single-stranded RNA molecule starting with the 5 prime end and completed at the 3 prime end with a Hydra free hydroxyl group. So, it is a single-stranded RNA which has self hybridization in some regions of it this is one this is one this is one this is one self complimentary sequences present here. And we have seen that there are many some modifications here and they are present those modifications were important to give the flexible structure wherever necessary in the tRNA.

2 important parts in the tRNA which would be absolutely essential in determining the amino acid sequences or in synthesizing the polypeptide chains are one is this that every tRNA has a 3 letter code nucleic acid code this is RNA code 3 nuclear bases long which is known as anticodon this is very essential for us and the end of a three prime end of it has a free hydroxyl group that is also very essential for us and always the tail or known as the acceptor arm has a sequence of CCA this is basically a 7 mod sequence and the last 3 are CCA.

Adenine is the last nuclear base present in every tRNA with a free hydroxyl group at the 3 prime end and that 3 prime end actually reacts with amino acid to produce your monomer. So, tRNA here and we have seen in the last lecture how ATP was involved in this case. So, I am writing tRNA and OH which is the free hydroxyl group on the adenine and here there is the anticodon of it, anticodon is there. Now if you have an amino acid let us say R NH2 and the carboxylic acid with the help of ATP this forms R this is your NH 2. So, there is a amine group there is an acyl group so this is called amino acyl tRNA this is amino acid which is conjugated to tRNA via acyl bond so this is called amino acyl tRNA.

And this reaction is catalyzed by amino acyl tRNA synthetase this is the enzyme that catalyzes this reaction. This enzyme is also known as tRNA ligase it basically like it is the carboxylic end with the 3 Prime free hydroxyl end of the tRNA. So, ideally every tRNA carries a specific amino acid into its 3 prime end. So, this is the 3 prime end and every tRNA has an anticodon associated with it. So, every amino acid actually now has earned code attached to it which we call as anticodon.

So, these two are the ingredients we have talked last lecture and today we will talk about the other part of it another very important ingredient or another very important class of biomolecules is a ribosome or ribosomes. Ribosomes are absolutely essential for the process of translation. It is basically a combination of proteins and RNA known as rRNA, rRNA means ribosomal RNA. So, ribosomes is basically an assembly of proteins multiple number of proteins or many units of proteins are there plus some RNAs called rRNA ribosomal RNA here.

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So, ribosomes or ribosomal RNA has or are of globular safe it triggers the synthesis of the ribosomes. So, ribosomes are originally synthesized from our RNA and in the assembly both are present ribosome subunits are present at the same time our RNA also are present and this is very important what does it do for the protein synthesis it actually provides a workbench or a vessel or a container it acts as a container on which the synthesis of protein occurs. It acts as a workbench for protein synthesis rRNA and proteins make up the large and small subunits of ribosomes.

I will show you the structure of ribosomes. Ribosomes are the site of translation where the polypeptides are actually synthesized. So, these are the key people or the most important people who have worked pretty much on the ribosomes or to find out the structures of ribosomes plus to find out how the mechanism works how the polypeptides are synthesized on the ribosomes. Of course ribosomes were known much beforehand and there are many other properties and ribosomes were that I mean regarded as that it is very important for the synthesis of proteins, tRNA was also regarded as very important for the synthesis of proteins.

But actually how the mechanism works how the amino acids are coming close to each other what is the proper function of ribosomes all these things were developed by these 3 people who have received Nobel Prize in Chemistry in 2009 very recently actually. She is professor Ada Yonath from Israel he is professor of course I think you can recognize him Venkatramanan

Ramakrishnan he is also popularly known as Venki Ramakrishnan he is from the Cambridge University and professor Thomas A Steitz from USA.

So, these 3 people have worked to find out exactly the mechanism of the functions of the ribosomes and also they are the pioneers of showing how exactly antibiotics works on the ribosomes. So, what is the function of ribosome what does it do?

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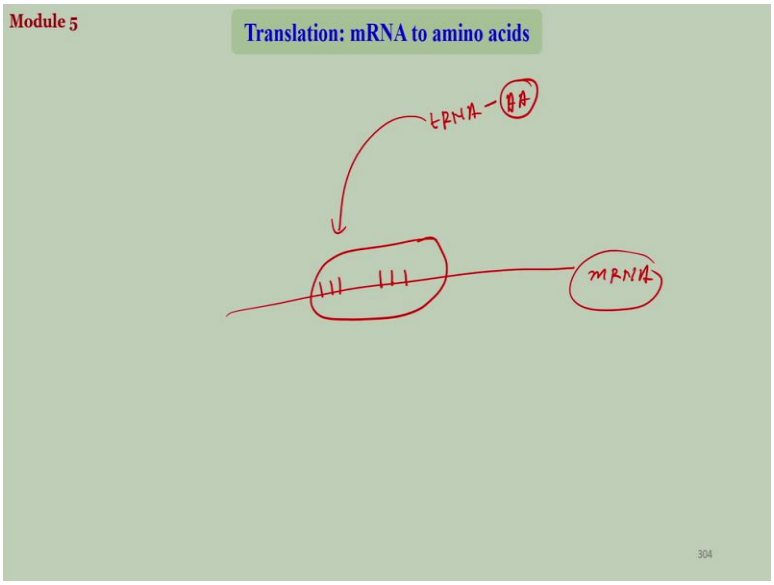

It actually works as as I have mentioned it works as a workbench. So. workplace or in other words it works as a container where all the other ingredients comes and will be accumulated in this place which means once you have a ribosome on the ribosome mRNA will come and bind. And then tRNA will come and bind so that is one tRNA there are many tRNA's that are present in the cells each carrying a specific amino acid and each carrying a specific codon anticodon.

If that anticodon is complementary to the codon of the mRNA then that specific tRNA I write A A for amino acid or amino acyl that particular tRNA which has a matching or complimentary anticodon to this will come and bind here. So, naturally that means that specific amino acid will also come and bind here. Similarly the other tRNA also has to come because you have to synthesize a peptide bond. So, you need two amino acids. So, ribosome provides that space on which you can have at least 2 numbers.

Actually three close to three number of tRNAs to come and sit down here side-by-side so that they can undergo reactions. And of course mrna is providing as the template where the tRNA will come or which tRNA will come that specificity comes from the mRNA. The workbench or the space is given by the ribosome.

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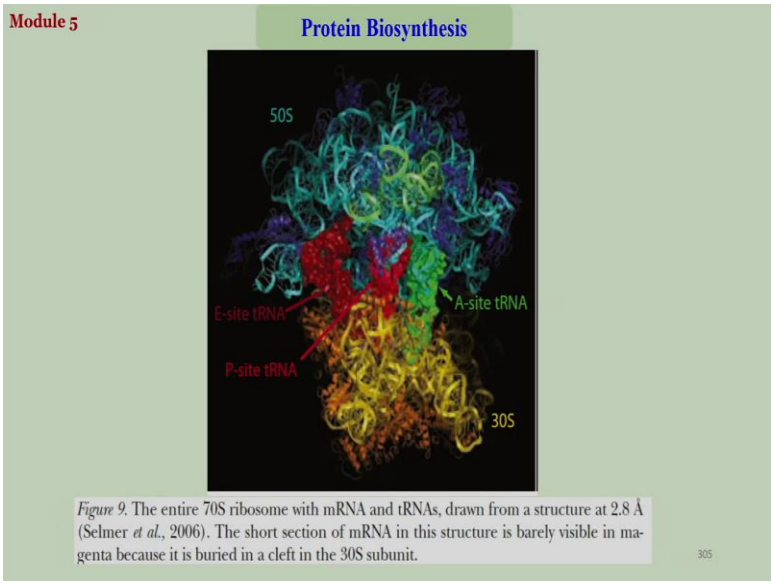

So, this is a structure typical structure of one ribosome this has two units called 50S subunit and another one is 30S subunit. 50S ribosomal subunit and 30S ribosomal subunit and each ribosome has as I was talking about space for one tRNA for 2 tRNA for 3rd tRNA. So, they are called sites in the ribosome so there are specific parts or spaces inside the ribosomes where the tRNAs can come and bind. So, one side one of these is called the ace site. So, this is a site where our particular tRNA will come and bind.

There is P site here this P site is also a space where another tRNA will come and bind and then there is a third one all the east side where there is a little bit of space for another tRNA. So, each ribosome contains 3 sites A site P side and E site. A site is actually known as amino acyl site, P site is where the peptide synthesis occurs so this is called peptidyl sites peptidyl site. And E site is where it knocks out or exits at it after the synthesis it goes off the tRNA goes of actually. I will see E site is called exit site and I have given our different names for this just to make it easier to remember.

A site I call them approach site because here the first tRNA is coming or the new tRNA is coming not the first the new tRNA is coming approach site continuing the amino acid. P site is where the reaction is actually taking place and I call it present site which is always there at the middle of it present site. And E site is exit site where it is going out. So, this picture I have taken from the Nobel lecture of I think professor Ada Yonath or professor Venki Ramakrishnan.

So the entire 70S ribosome with mRNA and tRNA so this is the picture drawn from a structure at 2.5 angstrom this is the resolution the short section of mRNA in this structure is barely visible because it is hidden somewhere here in magenta because it is buried in a clipped in the 30S that I was only trouble unit it is burried here actually.

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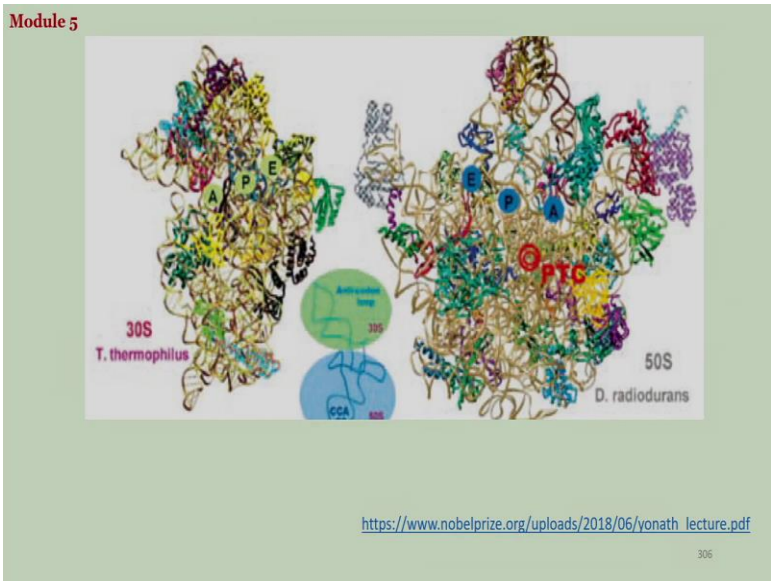

So, the reason professor Ada Yonath professor Venki Ramakrishnan and process states have received so much fame because that they have done something really beautiful, what they have done? They have crystallizes they have obtained the crystal structures of each step when the protein synthesis was occurring ribosome with tRNA's. How do they look like at each step so it is a huge work of course and really a new kind of idea of crystallization because they had to do they had to improve the method of crystallization they had to invent new equipment new instrument to crystallize them in the frozen form and in real time.

So, they basically have found out its real structure you can really see the structure in each step how they are working.

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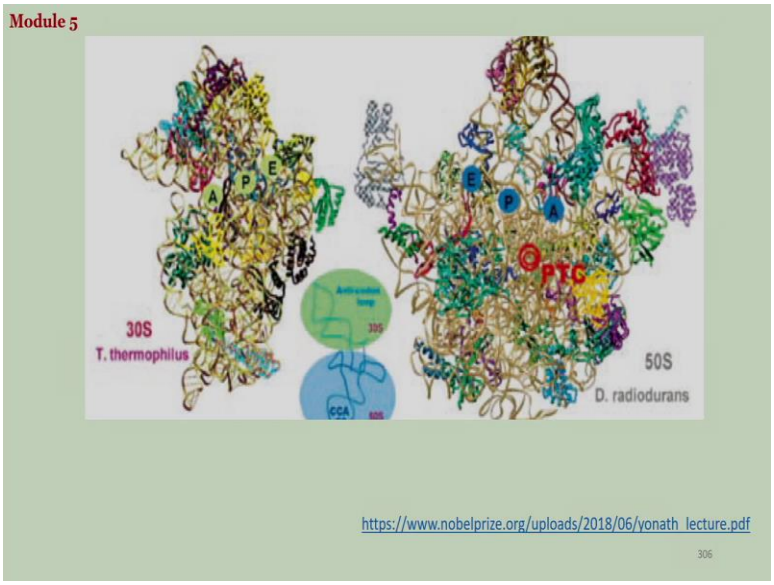

So, this is another picture of the ribosomal subunits 30s and 50s here this has been taken from the Nobel lecture of professor Ada Yonath and you can see this is the E site this is the P site and this is the A site same is here, the A site P site and the E site.

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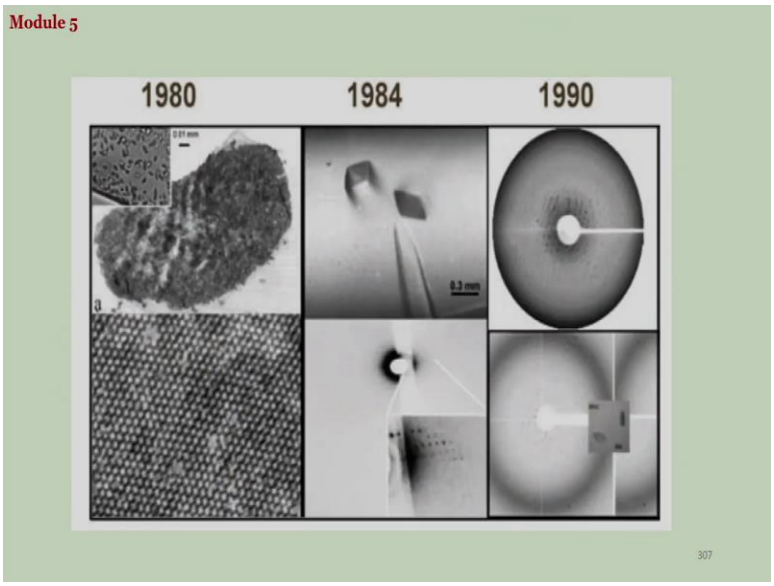

So, this source the crystal structures how the crystal property have been improved over time. So, 1980s you can see there is; these are the data that were available that term barely readable 1984

you can see there is fairly nice crystals single crystals that they could achieve and then 1990 of course and they have this hi very high-resolution crystal structures that have been obtained.

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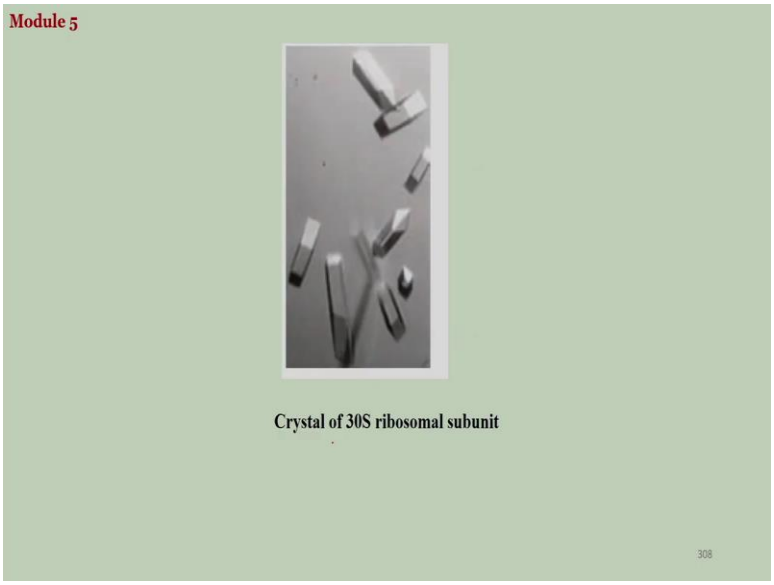

This is a pure crystal structure of a 30S ribosomal subunit, so in this really in the single crystal forms. All these crystals have been obtained in cryo form cryo crystals they call it. So, now comes how the synthesis works how the mRNA is used how the ribosome is used how the tRNAs are are used to synthesize the peptide bonds and this occurs in a sequential steps.

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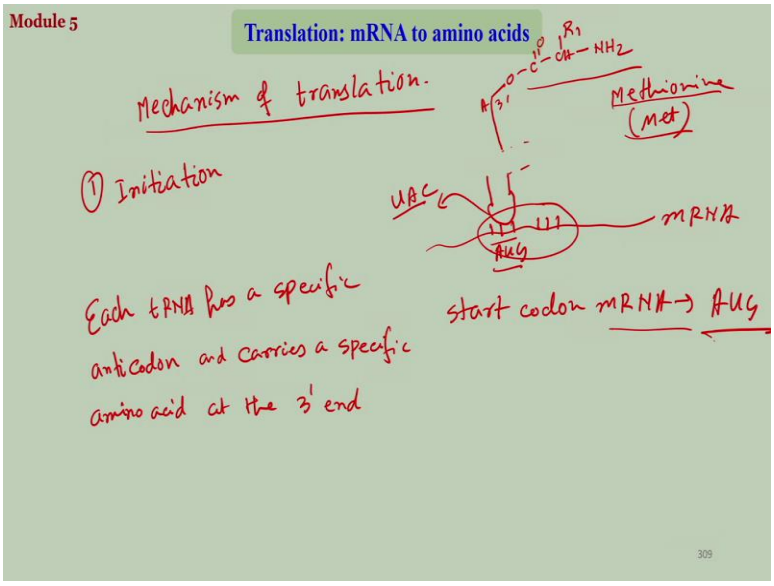

The first step so we are now discussing about the mechanism of translation. The first step is called the initiation. Initiation is the step where the first tRNA comes and assembles over the

ribosome and also the mRNA. So, if you have the ribosome here the mRNA comes and binds all the ribosomal subunits comes and binds here. So, it is basically an aggregation of the proteins as well as the mRNA that makes a ribosome. Once mRNA comes and gets into the; it docks into the protein structure or the aggregation structures and get stabilized there containing the codons the genetic information's.

Initiation is where the first tRNA would come and bind so I am not drawing the whole thing I will only draw the last one here this is the codon anticodon part which is important and this is what is important O CO CH let us call it R1 and amine NH2 the first time amino acid attached to the tRNA and here is of course your adenine. So, this is all happening at the 3 prime end of adenine. So, the first the RNA comes and bind to the mRNA sequence and of course when the tRNA comes it has to have the anticodon which matches or which is complementary to this sequence.

It happens that the first amino acid is always a methionine or we call it met and it has to start somewhere the synthesis process of initiation the binding of the first tRNA on the mRNA always happens with a start codon. So, mRNA wherever it has a sequence of AUG this sequence is called the start codon. So, mRNA has to have this sequence somewhere where the ribosome has bound or all the other ingredients have been bound and this sequence will be the starting point AUG will be the starting point.

So, the tRNA should have the anticodon which is complementary to Aug which basically is TAC not till U uracil I am sorry UAC would be the anticodon of the tRNA and this anticodon is always attached to this amino acid methionine. So, as I have mentioned that each tRNA this is again and again I am repeating and I am writing now because this is absolutely important. Each tRNA has a specific anticodon and carries specific amino acid at the 3 prime end so that is the identity of a tRNA it should have our specific anticodon and it should be our distinct amino acid that is all.

So, if the anticodon is complementary to the codon of the mRNA then they will hybridize. And this hybridization would be further stabilized by this assembly. So, that is the process of

initiation first tRNA carrying the first amino acid which is methionine comes and binds at the start codon of the mRNA which is AUG.

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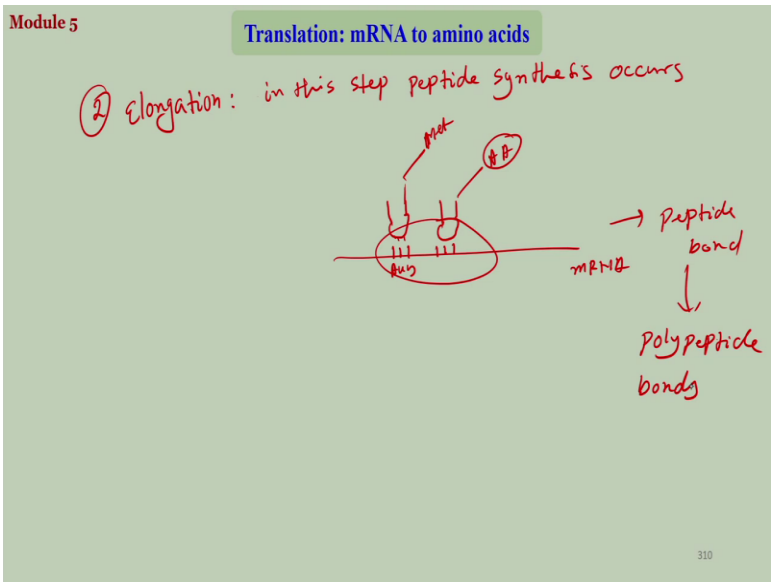

Now what next you need you need to bring another tRNA so that there will be 2 amino acids which may react. So, the second step is known as elongation. Elongation is the step where the peptide synthesis occurs in this step peptide synthesis occurs. So, basically here you need one tRNA to tRNA this is AUG, so that is the starting point and this, this tRNA carries 1 amino acid methionine and this tRNA carries is 1 amino acid I am writing AA. So, these two now are coming close in proximity and now they can react to produce a peptide bond.

And then it will go on and on subsequently so you get that is why it is called elongation the peptide number of peptide bonds is increased. So, you get a polypeptide. Polypeptide or I call poly peptide bonds that is the step of elongation.

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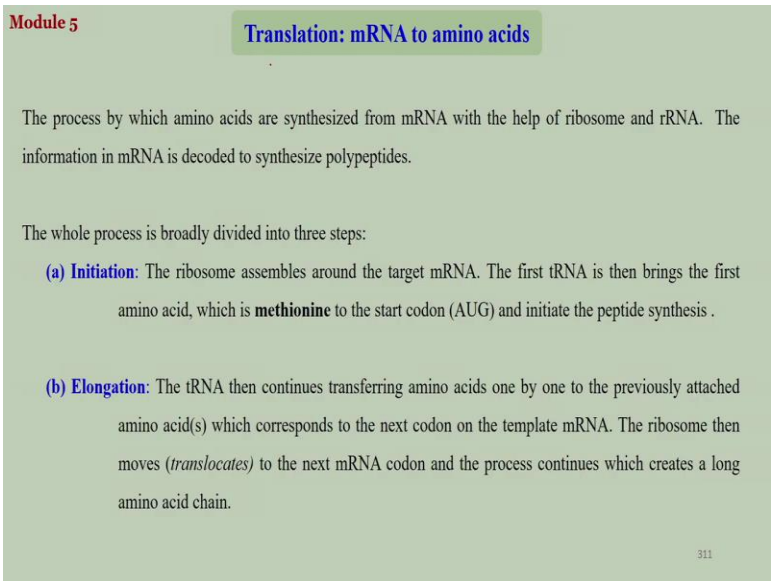

Here so now the translation overall then I will show you the chemical mechanism. The process by which amino acids are synthesized from mRNA with the help of ribosome and our RNA that is called the translation then information in mRNA is decoded to synthesize polypeptides that is what we have been talking about. So, the whole process is broadly divided into 3 steps 4th step is initiation. The ribosome assemblies or assembles around the target mRNA as I have shown here this is your mRNA ribosome would come or in other word the mRNA goes in find out the ribosome and stacks there.

So one important thing should be very well clarified that ribosome works as the as I have said as the workbench or the vessel which means ribosome is static this is very important. Ribosome is static it does not move ribosome stays in one place all the other ingredients they move if need be mRNA should would move tRNAs would move but ribosome would stay intact because it is a workbench so bench does not move, so initiation is where the ribosome assemblies around the target mRNA.

The first tRNA then brings the first amino acid which is always a methionine to the start codon and initiate the peptide synthesis. Then comes the elongation process elongation is the tRNA then continues transferring amino acids one by one to the previously attached amino acids which corresponds to the next codon of the template mRNA I will explain. The ribosome then moves to

the next mRNA this is mistake the ribosome does not move sorry ribosome does not move mRNA moves to bring the new tRNA or to accommodate the new tRNA.

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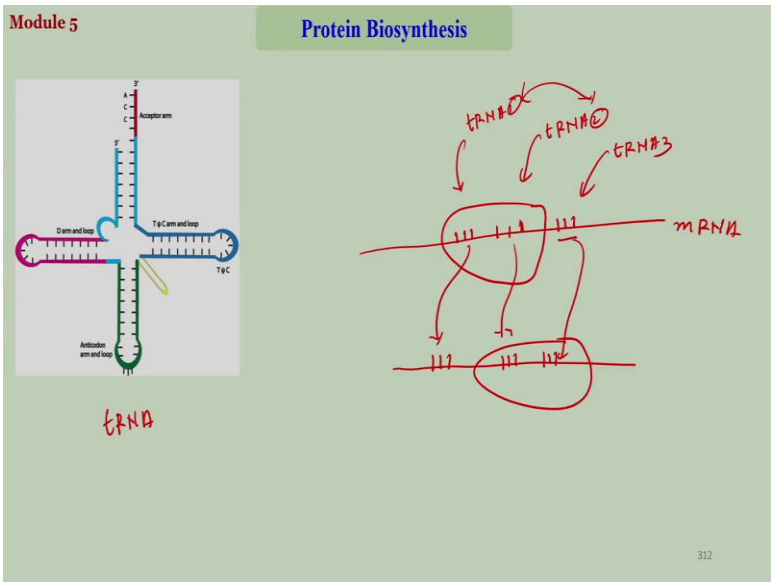

So, here so this is your tRNA you have a ribosome you have them RNA and then so this is a site this is if P site, so first tRNA will come here tRNA1 that is the first tRNA here comes the tRNA 2 now these 2 will react produce a peptide bond and then once this peptide bond is synthesized one of them would be knocked out because one of them will lose the amino acid I will show you and then the third tRNA which would be complementary to this codon has to come tRNA 3 but this is outside the ribosome.

So it has to get into the ribosome then only you can have the good hybridization. So, what is done is mRNA after this is the work of this first year and is finished it pulls the mRNA here, so that in the next phase this codon is out of the ribosome this is still there at this end and this codon comes very much into the workbench or into the vessel and that is how that new tRNA would be accommodated there and that is how the process would go on. Once a peptide is seen is synthesized mRNA node be pulled will bring the new codon into the ribosome subunit part or into the assembly and then it will go on and on.

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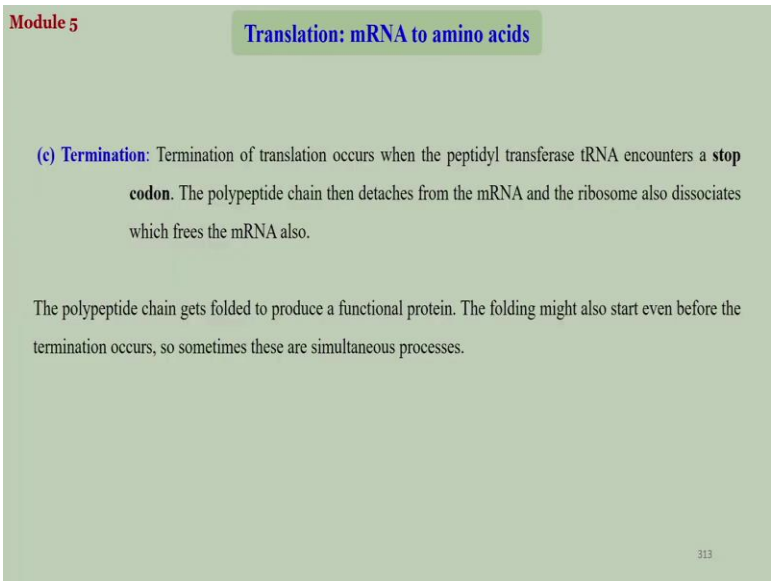

The third one is the last one the termination. Termination is the process is the termination of translation actually when the polypeptide chain is synthesized peptidyl transferase tRNA so that the enzyme that catalyzes the synthesis of the peptide bond is called the peptidyl transferase tRNA that encounters a stop codon. So, when it reaches the stop codon of the mRNA then the protein synthesis is stopped.

Because the stop codon of the mRNA that is complementary to an anticodon of a tRNA and that tRNA does not carry any amino acid is devoid of amino acid. So, the complementary sequence of the stop codon does not carry any amino acid so no one further protein synthesis can occur that is where the synthesis will stop. The polypeptide chain then detaches from the mRNA and the ribosome also dissociates which frees the mRNA also.

So the polypeptide chain gets folded so you have synthesized a long chain of a peptide with many amino acids and of course you have seen before that how internal interactions are the present in within the amino acids that will be responsible or that forces the structure to fold itself right. So, immediately or whenever the polypeptide chains are synthesized it will be folded to produce a functional protein.

Sometimes what happens the folding also starts during the synthesis itself so, even before the termination occurs so sometimes the folding as well as the translation occurs at the same time.

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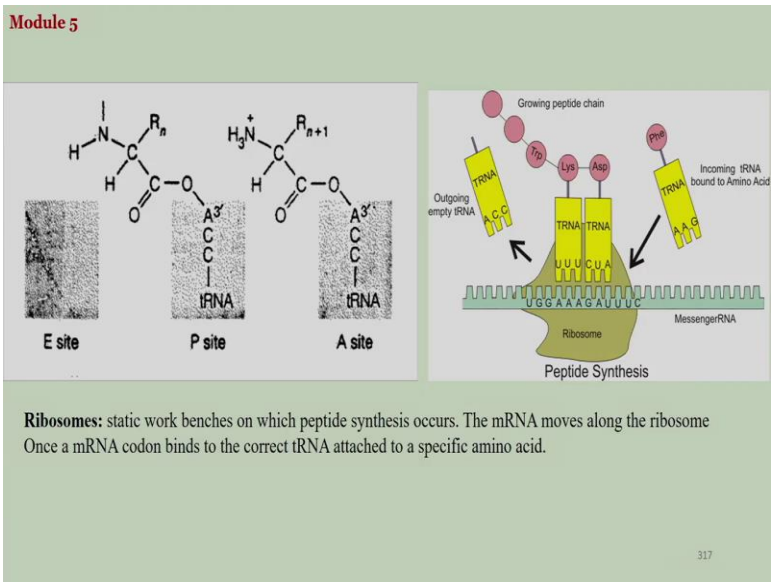

So, now I will show you the chemical reaction but before that here this will clarify better this is called E site or exit site this is P site peptidyl site and this is A site amino acyl site I call it approach this is where the new tRNA is coming in. So, it starts with this here the last this is the end of it and this is the start of it we have to decide or you can call it present site where things are happening and this is exit site.

So, here in this example it has been considered that you have already synthesized a peptide bond and you are trying to synthesize n + 1 n number of peptide bonds have been synthesized and you are trying to bring in the n + 1 amino acid so somewhere in between we are discussing. So, tRNA CCA 3 prime that is connected to our new amino acid and this has been a peptide. So, this is the nth number of amino acid which means you already have a polypeptide chain there.

This is the E site which is existing exit site empty for the time being and this is the new tRNA or the new amino acid attached to the new tRNA that is present in the A site. What happens it is these two will react I will show you the reaction these two will react and once it reacts after that this bond this whole thing would be transferred here as a result this chain this RNA this tRNA here would be devoid of the amino acid this does not have any amino acid now because that amino acid has been reacted here.

And it has become a free tRNA without the amino acid with the free hydroxyl group. Now since this does not carry and amino acid anymore it has to go out because that is how the new tRNA again will come so the new tRNA comes knocks it punches it that eliminates the tRNA which I was there to the E site. So P site after the synthesis become goes to the E approach side goes to the P so that is how it happens.

Every time a new tRNA comes and every time a polypeptide is synthesized this will be knocked this mRNA would be pulled to accommodate the new tRNA which has the codon here. So, this would be pulled this part will get into the ribosome this new tRNA will come in and the old tRNA would exit and always we have to remember that ribosomes act as the static workbenches on which peptide synthesis occurs.

The mRNA moves along the ribosome as I was saying that mRNA moves once the synthesis is done in a pulse itself. Once a mRNA codon binds to the correct tRNA attached to a specific amino acid. Now let us look at the reaction how it happens and then I will show you a real video where you can see how this is happening really.

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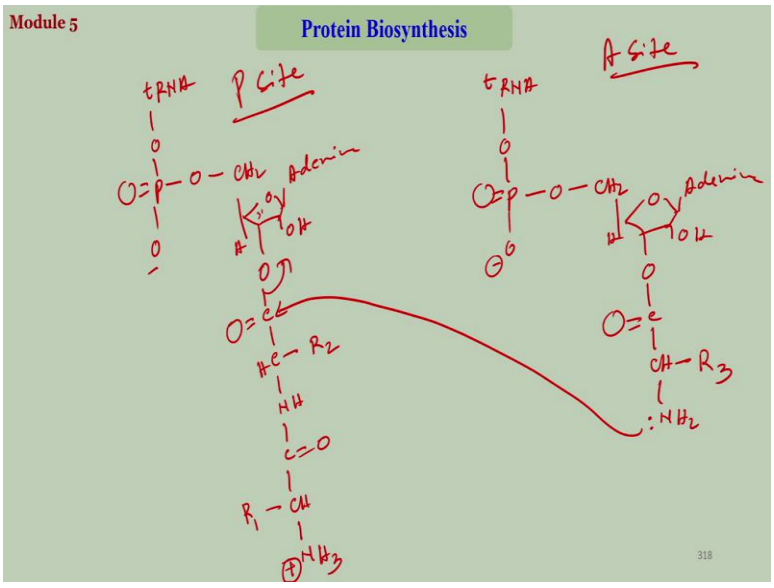

So, we have tRNA its phosphate here is your adenine, so here is your adenine this is the 3 prime end where the amino acid is bound and we will take that already one peptide bond has been synthesized. Let us say this is R1 NH CO R2 I will do it other way around this would be R2 this

should be R1 and here you have the terminal 1. So, we have taken the example that already one peptide one has been synthesized and a third one third amino acid is coming into the picture.

So this is the approach site added in OH this is HO and here the new amino acid is present let us say this is R3 and with NH2 or NH3 plus whatever it is. The reaction is the free amine group present in the A site this is A site this is P site that will react to the acyl bond eliminating this, so, cleavage of the ester bond and formation of an amide bond. So, if this happens then what do you get you see you get a cleavage of the P site.

In the P site what you will have you will have a free hydroxyl group on that adenine without any amino acid. And this whole of this peptide chain would be transferred or would be conjugated to the new site.

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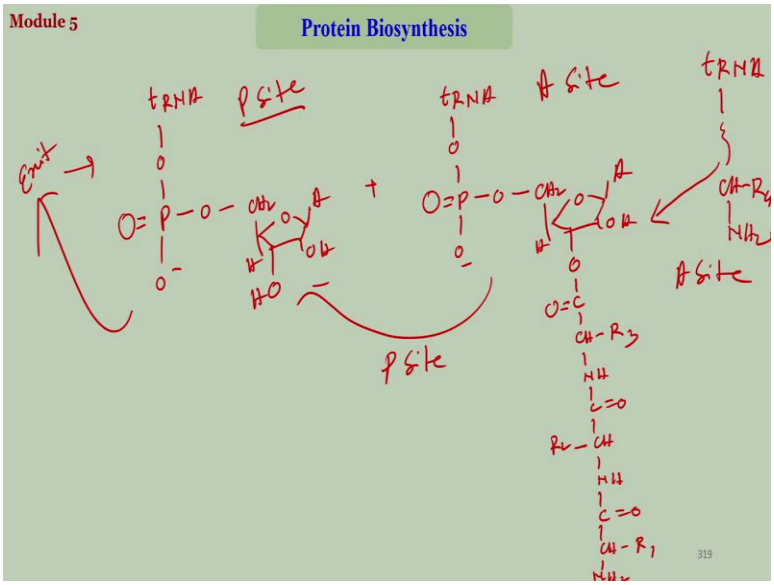

So, what you see what you get actually is tRNA O if adenine here also the free hydroxyl group plus the actual one O - O CH2 adenine OH O there would be three amino acids here. The new one and the new one would be their first because yes so the other one has been attached after the 3. So, this would be 3 NH CO CH R2 NH CO CH R1 and the last one is amine. So, all 3 amino acids would be attached here a site this is your P site.

So you see the job of P site is done the above this tRNA is done. It does not contain any longer any amino acid so it has to move it has to be relieved. On the other hand A site become your major one so what will happen tRNA that contains I am not writing the all the other part of it let us say CH R4 amine this is the new tRNA. So, this will come and this of course the anticodon has to get hybridized to the mRNA. So, the mRNA would be pulled this would come this would push you have limited space there this would push and this would knock this.

So the tRNA will exit which was originally present in this site P site would exist this comes will take the place of site and this will be your new a site so that is how the reaction goes.

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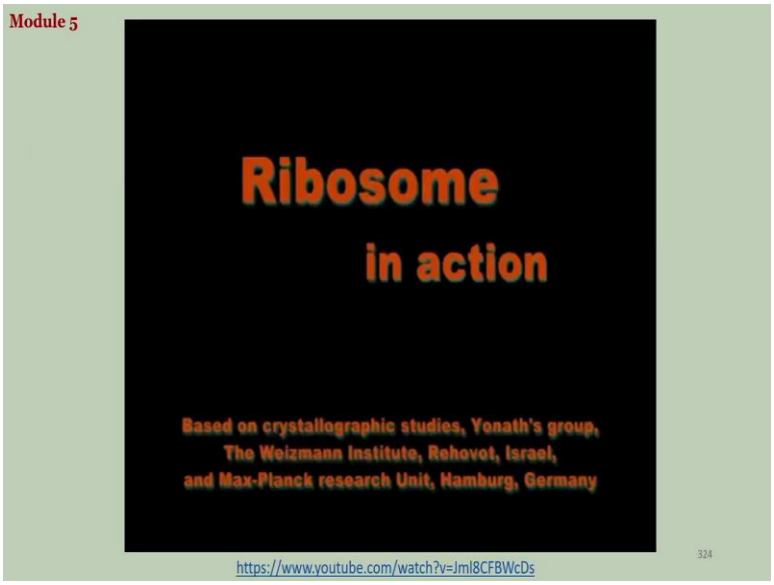

Now let us look I have a video here which actually models this video has been created by Professor Ada Yonath and she has actually visited our IIT few years before and gave a lecture. So, she has shown this video I have copied this video from YouTube from her site when this is the link you can find the video in this link which actually shows from their crystal structures what they have obtained. Hopefully it will be visible I have to show it couple of times I guess.

So this is called ribosomes in action so this video has been done based on the crystallographic studies and of course it is already mentioned here units group the Weizmann Institute, Israel and Max Planck Research Institute Hamburg Germany. So, here you go I have to pause a time to time to explain it.

(Video Start Time: 47:27)

So, this is the mRNA sequence that is getting and this is your ribosome. So, mRNA is going to get into the ribosome mRNA carrying the genetic code approaches the small ribosomal subunit and it is getting into the interior initiator tRNA charged, tRNA charged with an amino acid is brought IF2 to the P side inter subunit bridge of formed a little bit back I think I have to show so amino acid and tRNA approaches from the left assisted by L7 and stock it reaches the A site here.

So this is the exit site this is the structure of the tRNA and you will see if you look here as it will proceed there will be a small fluorescent blue bright fluorescent color on the tip that means that is the amino acid that is attached to the tRNA. This is tRNA here on the tip you can see something is glowing as it will move that is the amino acid. So, here it is not present because the amino acid has been taken away here and this is only the 3 prime hydroxyl and so it will it will age it knock out.

Here again it this is another tRNA that is getting out, so all tRNAs are getting out here you can see this is amino acid. This is the new amino new tRNA which is approaching so this is approach site when the new tRNA is getting into the ribosome this is the amino acid here you can see 3 of them so this is exit this is P site this is approach site. So, forget about this now this is exit site where the job of tRNA has been done free hydroxyl group so this will live.

This is the P site where you have already synthesized a good number of peptides the present site and this is the A site approach site which is carrying the new amino acid. So, these two will react whole thing would be transferred here this should become free and it will eliminate. So, It is knocking here this everything is transferred and it becomes like this and all the time the mRNA is moving itself in this direction.

Now the nascent protein which means the just right after the synthesis emerged from the exit tunnel opening from the exit site this is the protein. And it reaches the stop codon, stop codon reached the decoding site. So, again stop codon means it is the code the complementary of which

is the anticodon of the tRNA and that particular tRNA does not contain any amino acid. So, it will be stopped there, ready for next. So, these are catalysts basically.

(Video End Time: 52:11)

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So, end of the process the two subunits of the ribosome they dissociate themselves are ready for the next the next act. A new set of adenine will come or mRNA is already present a new set of tRNA will come and the new protein should be synthesized with the same ribosome. So, I will play this video again here you can see. You can find out by yourself I will not talk now.

(Video Start Time: 52:49)

(Video End Time: 54:31)

So, this is how the process the mechanism works and of course I have one second if I go back to the reaction you can understand how it was being transferred to the new one. And the new was working here this A site was taking the all other peptide that was already synthesized in the P site that was converted here and we were finally getting everything. And this A site was moving to the P site, P site was moving to the exit site and the new tRNA was taking the place of A site and the elongation goes on.

So that is the chemistry behind it and of course this is catalyzed by the protein which is as I mentioned is a transferase all right. So, with this I will conclude today still a little bit of content

is left on how the codons are present or how the codons came into the effect and some more calculations we can do and that we will do in the next lecture, thank you.