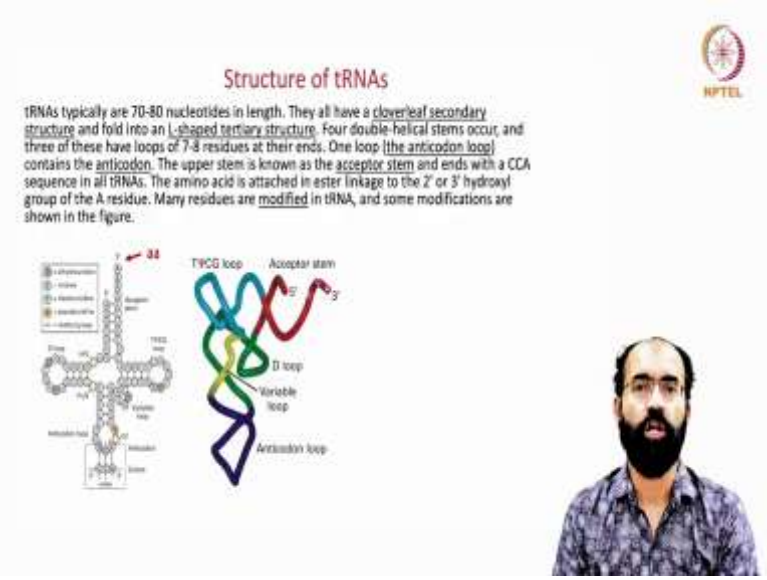
RNA Biology
Prof. Rajesh Ramachandran
Department of Biological Sciences
Indian Institute of Science Education and Research, Mohali

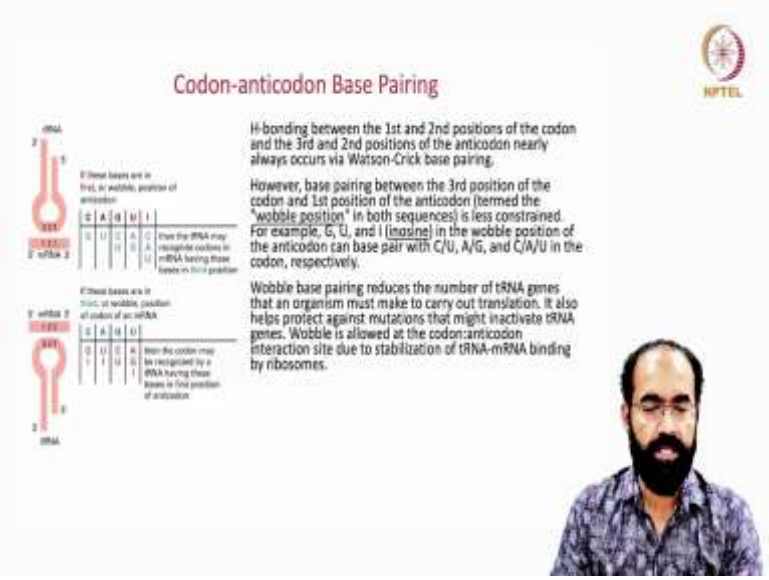
Lecture - 62
Epitranscriptome and Protein Synthesis: Mechanism of Translation

(Refer Slide Time: 00:22)



Hello everyone, welcome back to another session of RNA Biology. So, we were here in the previous class that we were looking into the structure of the transfer RNA with regards to its aminoacylation and the modification of the tRNA ends.

(Refer Slide Time: 00:42)



So, now let us move on to the codon-anticodon base pairing and we have discussed extensively about this codon and anticodon pairing. And it is quite simple to understand codons are there in the mRNA each codon is consisting of 3 bases and each codon corresponds to 1 amino acid. And same way the anticodon also is 3 bases and it is present in the tRNA and it base pairs with the codon as a process of delivering the amino acid during protein synthesis.

So, the hydrogen bonding H bonding means hydrogen bonding between first and second positions of the codon is strong and very precise. And the third and second positions of the anticodon corresponding to the anticodon are nearly always occur via Watson-crick base pairing. That means A pairing with U with a double bond and G pairing with the C with a triple bond. So, this is followed strictly.

However, the base pairing between the third position of the codon and the first position of the anticodon as a part of the complementary pairing is termed as the wobble position. Wobble position indicates it tolerates little flexible bonding. Tolerates non-strong bonding need not necessarily be very strong in both the sequences is less constrained. Less constraint mean it is not so stringent.

For example, GU and I, I stands for inosine which occurs in the tRNA as a post-transcriptional modification. In the wobble position of the anticodon can base pair with C U, A G or C A and U because I can pair with any of these bases in the codon

respectively. That means G can pair with C or it can imperfectly pair with U and same if U is there it can pair with A which is its normal pair pairing partner and also it can weakly pair with G also.

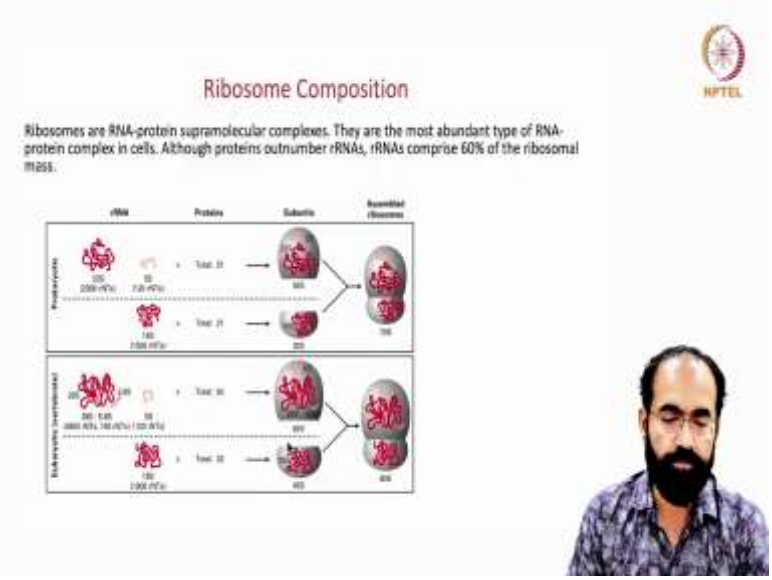
And if I is present in the tRNA it can allow pairing with C, A or U. So, we also have seen in earlier classes that I is replaced as the in the third position of the codon for facilitating the distribution of the amino acid arginine in various codon positions with minimal amount of tRNA genes, when it is present in an organism through rRNA editing tRNA editing.

So, the wobble base pairing reduces the number of tRNA genes that an organism must have to carry out the protein translation. It also helps protect against mutations that might inactivate tRNA genes. Some mutations can occur and which can make some tRNA debilitated.

So, you wanted some protection from that. Wobble is allowed at the codon-anticodon interaction site due to stabilization of tRNA mRNA binding by ribosomes. So, we should understand the specificity is maintained, but the stringency at the wobble position is moderately low compared to the 1st and 2nd base in the codon.

So, that allows the flexibility as you can see here. So, 1, 2, 3 is the codon base, 1st base, 2nd base and 3rd base of the codon and this is the tRNA anticodon, 1st base, 2nd base and 3rd base. So, remember the first base of the codon pairs with the 3rd base of the anticodon like so on and so forth. And the 3rd base of the codon pairs with the first base of the anticodon. So, you can see here that this pairing is always followed stringently at the 1st base and the 2nd base whereas, the third base is the wobble position.

(Refer Slide Time: 04:52)



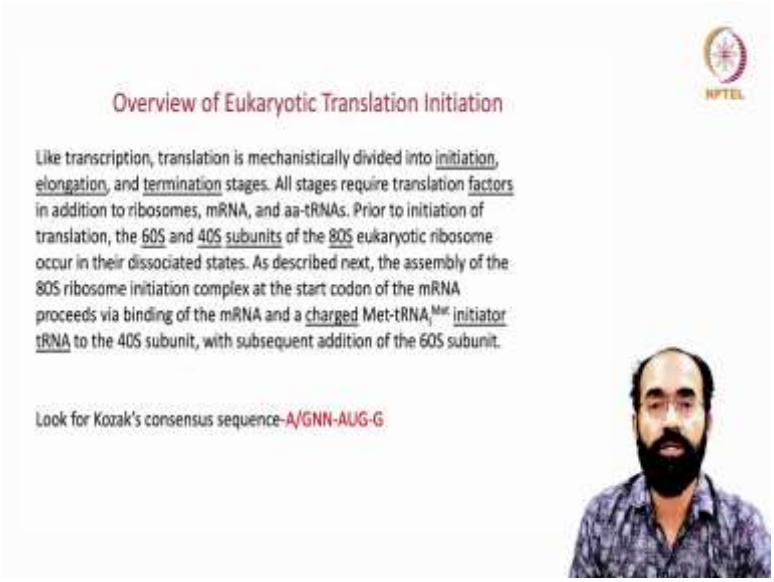
So, now let us see the ribosome composition. Ribosomes are RNA protein supramolecular complexes. We have seen ribosomes from the beginning of this RNA biology that it is a classic example of ribozyme.

They are most abundant type of RNA protein complex in the cells which accounts for a significant proportion of the total RNA. Although proteins outnumber the ribosomal RNAs, ribosomal RNA comprise 60 percent of the ribosomal mass. Because that constitutes the active component or the functional component of the ribosomes.

You can see here and in both in prokaryotes and eukaryotes the different types of ribosomal RNA 23S, 5S, 16S and also around 31 different proteins and it constitutes larger subunit and then smaller subunit, 16S and 21 16S ribosomal RNA and 21 different types of proteins constitute the smaller subunits and that will assemble together to become the 70S ribosomes.

And same way in eukaryotes you have got 28S, 5.8S and 5S ribosomal RNA and around 50 different proteins constitute the larger subunit which consists of 60S, Svedberg unit of molecular mass whereas, the smaller subunit is 18S RNA and around 23 different proteins constitute the smaller subunits. Both together assemble to become 80S subunits which actually participates in the protein translation.

(Refer Slide Time: 06:35)



So, if you look into the overview of the eukaryotic translation it has 3 phases initiation, elongation and termination. Similar to what we saw in the case of RNA transcription. So, like the transcription that is RNA production translation is mechanistically divided into initiation, elongation and termination stages. And all stages require translation factors in addition to ribosomes, mRNA and amino acylated tRNAs.

So, all these three are a must and also lot of associated factors also comes into picture for the initiation, elongation and termination step. Prior to the initiation of translation, the 60S subunit of the ribosomal structure and the 40S subunit which constitute together constitute the 80S ribosomes of the eukaryotic ribosome machinery and they are remaining in a dissociated state.

You will not find an 60S and 40S assembly in the absence of an mRNA, 60S will be separate, 40S will be separate and they remain in a dissociated state. They assemble because of an mRNA available for translation. As you can see, we describe the assembly of 80S ribosome initiation complex at the start codon of the mRNA proceeds via binding of the mRNA and a charged methionyl tRNA.

That means amino acid methionine is added that is why it is written as Met. Met is the triplet name of or the three letter name of an amino acid. Every amino acid have got a single letter unique code and also a three letter name code. For So, Met stands for methionine.

Met tRNA means this tRNA carries the methionine and this is called the initiator tRNA. So, methionine tRNA in this case is the initiator tRNA. But methionine can come elsewhere also in the proteins. Not that every protein will have only one methionine that is in the first methionine. Not like that, methionine can come later on also.

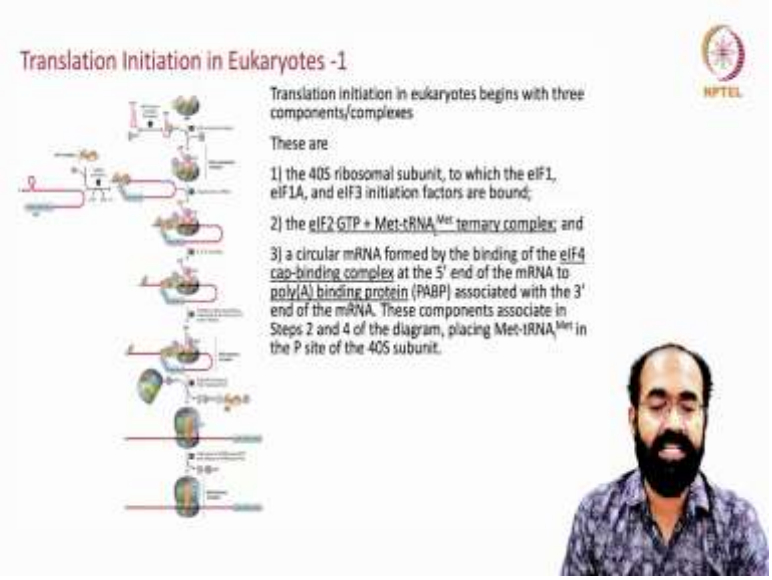
And this initiator tRNA to the 40S subunit. So, what we are seeing the assembly of the 80S ribosome initiation complex at the start codon of the mRNA proceeds the binding of the mRNA and the charged tRNA that is methionylated tRNA that is the initiator tRNA to the 40S subunit.

So, mRNA is bound with the 80S and the tRNA is bound with the 40S subunit with the subsequent addition of the 60S subunit on to this complex. So, like that this assembly will continue. And usually when an RNA has encountered the ribosome, ribosome will scan the RNA and look for not just an ATG or not an AUG. ATG will be in the DNA, AUG is in the RNA, but it will look for the Kozak's consensus sequence.

What is that sequence? It should be A or G at one place and any two nucleotides means it can be any of these four nucleotides and then you should have an AUG and then next one should be preferably G. It is not a compulsory G preferably G. So, AUG is must and left side of this AUG upstream of this AUG should be any two base, any base is allowed, but before that.

So, technically we can call this minus 1, minus 2 and minus 3, minus 3 position it must be A or G. 90 percent of the cases it is A, 10 percent of the cases it is G. And this N can be anything and this N can be anything and this plus 4, plus 1, plus 2, plus 3 and this plus 4 need not necessarily be G, but many a times it is seen as G. So, this is the Kozak consensus sequence. So, the ribosome look for the Kozak consensus sequence.

(Refer Slide Time: 10:51)



So, translation initiation in eukaryotes stage 1. So, translation initiation in eukaryotes begins with three components or complexes. These are first one the 40S ribosomal subunit to which the elF1, elF1A and elF3 initiation factors are bound. You may be remembering that as soon as A mRNA comes to the cytoplasm through the nuclear pore complex we have discussed that the cap binding protein is replaced by elF in the cytoplasm.

So, this is what elF we are talking about. So, the elF2 paired with GTP, GTP is an energy yielding molecule. elF2 paired with GTP and also methionylated tRNA and it forms a ternary complex. As step 1 the 40S subunit and elF1, elF1A and elF3 forms a complex. And at step 2 elF2, GTP and the methionyl tRNA pairs to form a ternary complex. And as a 3rd step a circular mRNA form by the binding of the elF4 cap binding complex at the 5 prime end of the mRNA to poly A binding protein.

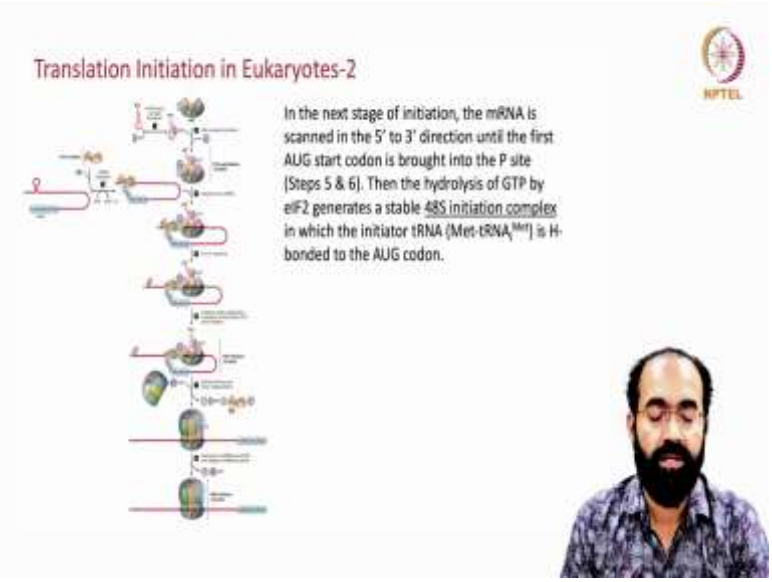
So, understand RNA forms like a bangle that is PABP that is the poly A binding protein and it forms a cap binding complex which is in the 5 prime end forms a circular assembly. And this PABP is in the poly a tail whereas; the cap binding complex is in the 5 prime cap. And these components associate in steps 2 and 4 which you can see in this diagram and placing the methionyl tRNA in the P site of the 40S subunit.

P site is the peptide bond forming site. Because usually RNA enters through an A site acceptor site which is as the protein synthesis takes place. But here it directly goes into

the P site because you do not need to start from the A site because the P site is lying empty which will not be the case in subsequent step onwards because the previously entered tRNA will be there in the P site.

But now P site is empty. So, directly it is entering into the P site as you can see here the RNA forms a circular shape as you can see this complex and the directly the tRNA methionyl tRNA enters into the P site.

(Refer Slide Time: 13:37)



What happens next? In the next stage of initiation the mRNA is scanned in the 5 prime to 3 prime direction. Just like the synthesis of mRNA the protein synthesis also takes place 5 prime to 3 prime direction. Just like if I give you a book to read you will read from left to right that means, you cannot read from right to left because although you can read it will not make any sense.

So, you have to read every sentence from left to right. Something like that they there may be other languages which are read from right to left I am just telling general say English something like that. So, mRNA scanned from 5 prime to 3 prime direction and for the detection of the AUG.

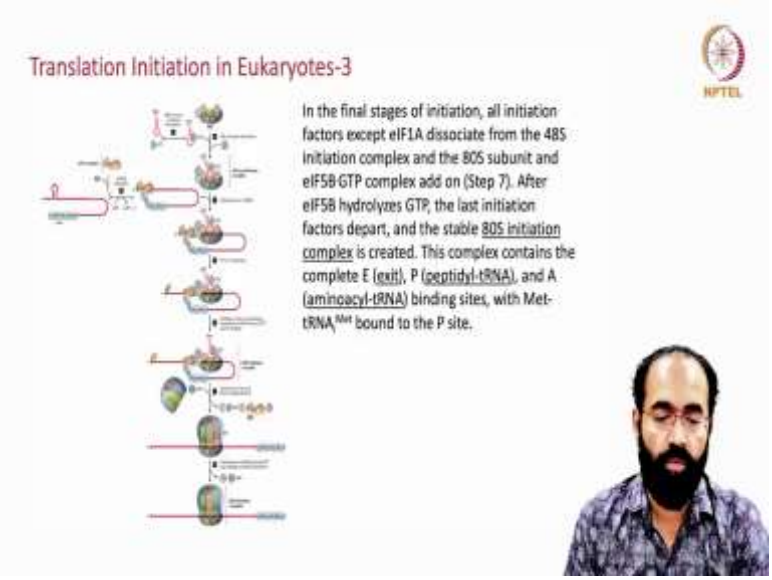
And keep in mind in Eukaryotes it is not the first AUG usually it is the first AUG, but if the first AUG is lacking the features of the Kozak consensus sequence that will be

skipped it will go to the next AUG or it will go to the next AUG until it satisfies the Kozak consensus sequence. Otherwise it will not be a preferred AUG.

So, that idea should be because some mRNA will have lot of untranslated region 5 prime untranslated region which may have an AUG, but it will not start unless it satisfies the Kozak consensus sequence. The hydrolysis of GTP attached on to the elF2 by the elF2 hydrolysis hydrolyzing the GTP generates a stable 48S initiation complex in which the initiated tRNA that is methionine tRNA is hydrogen bonded to the AUG codon.

Because the tRNA has the corresponding anticodon and it is now paired on to the first codon of the mRNA that is the step number 2.

(Refer Slide Time: 15:31)



What is step number 3? The final stages of initiation all initiation factors except elF1A dissociate that is the means this will continue the peptide bond formation will continue in the end. Because A site the tRNA enters and P site the peptide bond is being formed. So, next when a tRNA comes with say alanine, first one was methionine which occupied the P site and next one is say alanine and that tRNA will enter through the A site and now you have the methionine and its tRNA in the P site.

So, now what will happen? This methionine bearing tRNA will move on to the E site exiting site and the newly arrived alanine bearing tRNA will occupy the P site, but during this process the amino acid methionine that was present in the first tRNA will be

bonded to the second, it will be bonded to the second amino acid that is alanine bond which I gave an example, do not think that after methionine it has to be alanine, it can be any amino acid there is no bias it can be one more methionine also.

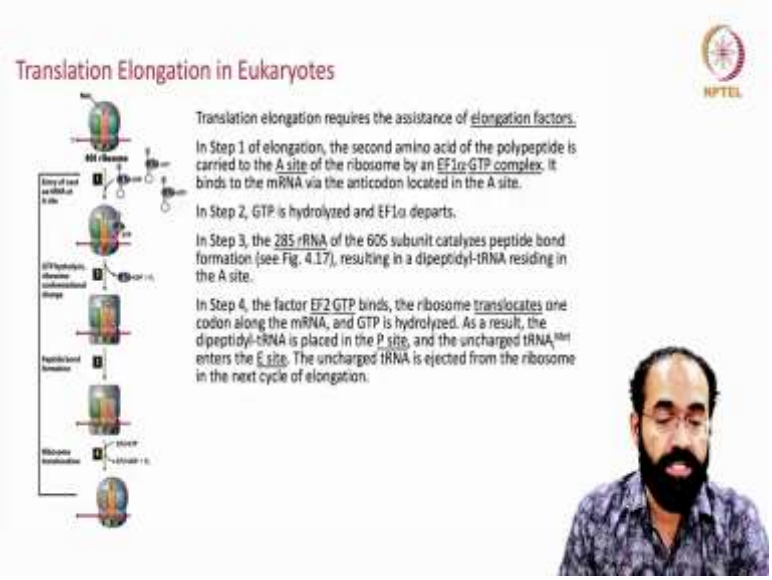
So, this amino acid first amino acid is now peptide bonded with the second amino acid and that bond is called a CO-NH bond it is an amide bond and that we call it as a peptide bond. So, now you have got two amino acid attached on to this tRNA staying in the P site that is methionine alanine and it will stay there. So, it is a dipeptide.

Now, you have a third amino acid that is coming in because A site is empty and A this tRNA it can may have it can have a glycine. So, the P site having tRNA that has got a methionine followed by alanine and attached onto the tRNA. When a third amino acid bearing tRNA comes in through the A site it will jump onto the P site while this P site occupied tRNA will move onto the exit site. And this methionine and alanine its now added onto the glycine bearing tRNA.

So, you have methionine, alanine and glycine onto. So, it is a tripeptide added onto the P site. So, like that this jumping will continue. So, once it is completed what happens in the final stage of initiation all initiation factors except elF1A dissociate from the 48S initiation complex and 80S subunit and elF5B GTP complex and as you can see here in the step number 7 after the elF5B hydrolysis of GTP.

And the last initiation factors depart and the stable 80S initiation complex is created. And this complex contain the complex that is complex E that is the exit complex and the peptidyl tRNA P and A aminoacyl tRNA. So, A site you have fresh entry of that is A site is called acceptor site and where the fresh aminoacyl tRNA comes, P site is the peptidyl bond forming site, E site is the exit site. And so, this will continue as you saw in a sequential manner which I described.

(Refer Slide Time: 19:13)

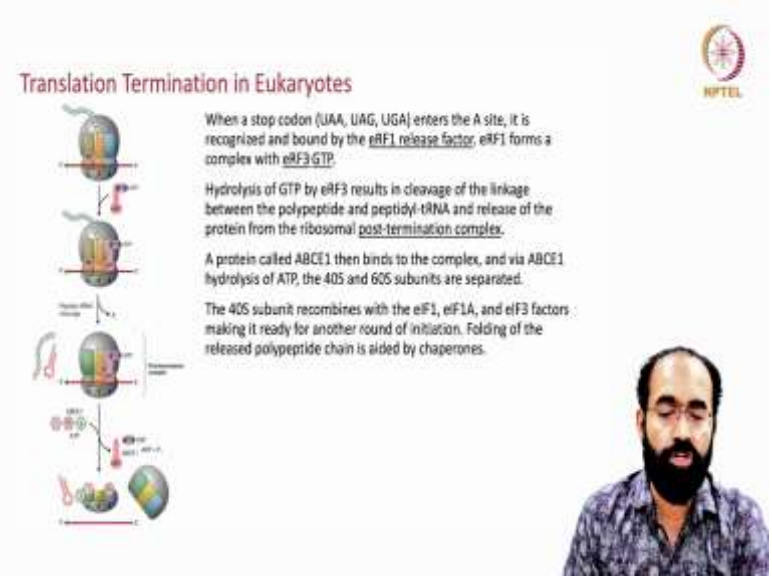


So, now in the next phase is the elongation. Translation elongation requires the assistance of various elongation factors. In step 1 of elongation the second amino acid of the peptide is carried to A site of the ribosome by EF1 alpha GTP complex, again GTP is utilized. It binds to the mRNA via the anticodon located in the A site which is the entry site.

In step 2 GTP is hydrolyzed and ElF1 alpha departs. It moves away. In step 3 the 28S ribosomal RNA of the 60S subunit catalyze the peptide bond formation the CO-NH bond formation and resulting in a peptidyl dipeptidyl tRNA. That means I gave an example of methionine and alanine I gave an example dipeptidyl tRNA residing at the A site. And in step 4 the factor EF2 GTP binds the ribosome translocates one codon along the mRNA and the GTP is hydrolyzed. So, as a result the dipeptidyl tRNA is placed in the P site.

So, first the dipeptide is transferred or the initial the methionine is transferred onto the incoming tRNA and then at the cost of energy it the dipeptide bearing tRNA moves onto the P site. The uncharged tRNA ejected from the ribosome in the next cycle of elongation through the E site or exit site.

(Refer Slide Time: 20:59)



So, when stop codons such as UAA, UAG, UGA enter the A site it is recognized and bound by a, no tRNA can come because there is no tRNA corresponding to the no tRNA has the anticodon corresponding to the stop codons. So, you end up getting a eRF1 release factor. And eRF from eRF1 forms a complex with the eRF3 GTP. This we kind of discussed when you are talking about nonsense mediated decay etcetera when the RNA stability was discussed.

So, the hydrolysis of GTP by eRF3 results in cleavage of the linkage between the polypeptide and peptidyl tRNA and release of the protein from the ribosomal post termination complex. So, the presence of eRF1 and eRF3 is essential for the termination purpose. Without which you know like we have saw seen many cases if an mRNA do not have the stop codon what it will do it will never see it will come all the way till the tip of the mRNA.

And that time you cannot accept eRF1 the complex cannot accept eRF1 because there is no stop codon. So, stop codon is must for the entry of eRF. So, the hydrolysis of GTP present on the eRF3 results in the cleavage of the linkage between the polypeptide and peptidyl tRNA and release the protein from the ribosomal complex and that we call it as post termination complex. So, it helps in the disassembly.

So, a protein called ABCE1 then binds to the complex and via this ABCE1 hydrolysis of ATP that also utilize the energy yielding molecule instead of GTP it is using ATP. The

40s and 60s subunits are separated. So, eRF1 and eRF3 occupy the stop codon and attracts the ABCE1 which are the cost of ATP disassemble the 40s and 60s subunit.

The 40s subunit recombines with the eLF1 and eLF1A which is required for the initiation purpose for a next round of translation. And eLF3 factors making it ready for another round of initiation. So, 40S is readily going and interacting with the elongation or the initial initiators of or the part of the initiation complex.

So, folding of the released polypeptide chain is aided by various chaperones. Chaperones are proteins that is present in a cell that will help in the proper folding of a protein. Every protein has a plenty of possibility of folding. So, if it is folded properly then only it can function, it is correct just like your shirt and say pant you do not fold it the same way, right. Each has got its own style of folding.

So, shirt and pant cannot be folded randomly either because although you can, but it will not look good. So, same way every protein has a particular way of folding it.

(Refer Slide Time: 24:13)

So, now let us see what happens finally, the polysomes and the ribosome recycling. So, polypeptide chain elongation proceeds at a rate of 3 to 5 amino acids per second. So, the efficiency of translation is increased via the binding of multiple ribosomes per mRNA and we call it as polysomes. Instead of one set of ribosome you can have a second, third,

fourth, fifth on same mRNA and we call it as polysomes to the mRNA at a given time. So, one RNA can continuously produce multiple polypeptides.

Every ribosome will give rise to one polypeptide production. But a second set of ribosomes on the same mRNA will produce another protein. So, like that like a conveyor belt like you can see in airports how conveyor belt will be bringing your suitcases. Belt is one, but you have multiple boxes on it, like that you can see the polysomes.

So, a given time it can produce multiple proteins from one mRNA. So, the translation efficiency is further increased due to the complex between poly a binding protein, PABP and the eLF4 mRNA at the 5 prime cap that occurs in the mRNA. So, this circular complex positions of ribosomes that have just terminated translation of the message it its an indicator that this RNA has just completed the one-round of translation of the this gives a message to the system also that you know this RNA has completed one-round of translation.

So, these ribosomes then they detach to one with the help of release factors are recycled and rapidly re-initiate another round of translation. That is what is mentioned in a pictorial manner because the 5 prime end and the 3 prime end, can come together and they stay together and this polysomes are present on the mRNA until it reached a stop codon.

So, stop codon will come somewhere here. So, until it is up to here and this is the 3 prime untranslated region, this is the poly a tail and this interaction will stay in a circular form. And once it is detached and if the RNA still stable it will continue to produce more and more protein or it will simply will be degraded as the life of the RNA is completed, no more protein will be produced from that RNA.

So, this is the end of the protein translation section. And if we have some doubts you know in future you can post me to my you know ID address. And so, this will conclude the RNA biology session and I request all of you to do well and study well for the exams all the best to all of you.

Thank you.