

Basics of Biology
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Lecture - 30
Translation (Part-II)

Hello everyone. This is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering, IIT, Guwahati. And what we were discussing? We were discussing about the different properties of the living organisms, and in this context, so far what we have discussed, we have discussed about the classifications, evolutions, we have discussed about the prokaryotic as well as the eukaryotic cells.

And in the previous module, we have also discussed about the different types of biomolecules. And in the current module, we are discussing about the central dogma of life or the central dogma of molecular biology. And in that context so far what we have discussed, we have discussed about the DNA dependent, DNA synthesis and that process is called as the replications.

And while we were discussing about the replications, we have discussed about the different steps and how the replication is been responsible for the development of a technique which is called as the polymerase chain reactions. And then we have also discussed about the different types of applications of the polymerase chain reactions. In the previous lectures, we have discussed about the transcriptions, so DNA dependent RNA synthesis and that is being catalysed by the RNA polymerase and this process is known as the transcription.

And we have discussed about the detailed steps, what are involved in the transcription of the prokaryotes or the transcription of eukaryotes. Subsequent to that, we have also discussed about the post transcriptional modifications all the RNA species. We discussed about the capping, we have discussed about the tailing, and we have discussed about the splicing in the messenger RNA, and then we also discuss about the different types of post-transcriptional modifications into the tRNA and as well as in the ribosomal RNA.

So, in the today's lecture, we are going to discuss about the third topic of the central dogma of life or the central dogma of molecular biology, and that process is known as the RNA dependent protein synthesis or in general it is called as the protein synthesis. And this process is called as translations. So, let us start discussing about the translations, and we are also going to discuss about the post-translational modifications into the protein as well.

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TRANSLATION

- Structure of Translation Machinery
- Genetic Code
- Mechanism of Translation

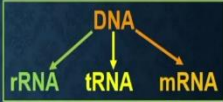
Activation of Amino acid $\xrightarrow{\text{1.}} \text{Complex and aminoacyl-tRNA} \xrightarrow{\text{2.}} \text{Protein synthesis}$

Initiation

Elongation

Termination

- Post-Translational Modification



So, what we have discussed, we had discussed about the structure of the translational machinery, we discussed about the genetic codes, and now we are going to start discussing about the mechanism of translations. And the first step in the mechanism of translation is that you want to activate the amino acids.

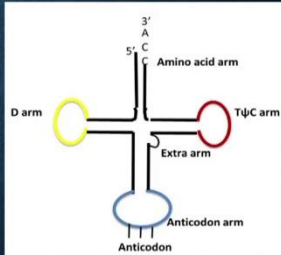
Which means you are actually going to couple the amino acyl to the tRNA, that is actually, you are going to couple it to the tRNA, as soon as you couple it to the tRNA and you are going to make the tRNA amino acyl or tRNA is acylated, it is going to be committed for the protein synthesis. So, let us see how we can do the amino acyl activations.

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TRANSLATION

- Mechanism of Translation

Activation of Amino acid: During this process amino acids are attached to the t-RNA in the presence of enzyme Amino acyl-t-RNA synthetase, this enzyme activate the amino acids by attaching covalently to the t-RNA, when t RNA get charged, its named as aminoacyl-t RNA. During this process amino acids are attached to t-RNA with high energy bond, so called as activated amino acids.



$\text{Amino Acids} + \text{tRNA} + \text{ATP} \xrightarrow{\text{Amino acyl t-RNA synthase}} \text{Amino acyl-tRNA} + \text{AMP} + \text{PPi}$

Handwritten notes in red: 'Activated amino acid' and 'AMP + PPi'.

So, amino acid activation, during this process, the amino acids are attached to the tRNA in the presence of enzyme which is called as amino acyl tRNA synthetase. This enzyme activates the amino acyl by attaching COOH entity to the tRNA, when the tRNA get charged, its name is called as a amino acyl tRNA.

So, during this process, the amino acids are attached to the tRNA with a high energy bond so that they are actually been called, so they are called as activated amino acids. So, what will happen is you have an amino acid which is going to be and then you have a tRNA and then you are actually going to utilize the energy in the form of ATP.

So, what happen is that the amino acyl tRNA synthetase is actually going to utilize the ATP and it is going to convert the ATP into the AMP and through into synthesize the pyrophosphate. And by breaking the ATP, it is actually going to generate the energy and that energy is going to utilize for the formation of the coupling of the amino acid to the tRNA.

And that is how you are going to have the amino acyl tRNA. This amino acyl tRNA is actually going to have is also called as the activated amino acid, and that activated amino acyl is going to participate into the protein synthesis.

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TRANSLATION

Met → Formyl
f-Met → (Met) + f-Met

- **Mechanism of Translation**

In eubacteria, first amino acid in the polypeptide chain is N-formyl-methionine which is specific to three codes as AUG, GUG, and UUG. Whenever these codes are present at the initiation point they code for N-formyl-methionine but if they are present in between the coding sequence then they code for methionine and valine respectively.

How does this happen?

This is because of difference in initiator tRNA and the one used in between the process of translation. Initiator tRNA has unique features that distinguish from elongating tRNA in eubacteria.

Amino Acids + tRNA + ATP $\xrightarrow{\text{amino acyl t-RNA synthase}}$ **Amino acyl-tRNA + AMP + PPi**

Once the amino acyl activation is over, that, then it is going to participate into the mechanism, into the translations. You know that we have already discussed that we have the initiation codon or we have the first codon which is actually going to be used as the initiation. So, the question is that what is the difference, will there be a difference between the tRNA?

What is going to be responsible for the initiation of the translational machinery or the it is actually the regular tRNA. So, there in the eubacteria the first amino acids in the polypeptide chain is N-formyl-methionine, which is specific to the three codes as the AUG GUG and UUG. Remember that we said that AUG, GUG and UUG can be a potential initiation codon.

So, these initial codes, initiation codons are coding, for not for the normal amino acids, they are not coding for the normal methionine, they are coding for the N-formyl-methionine. And that is why for the first tRNA, which is going to participate into the initiation reactions, it is not the methionine which is going to be tagged to that particular tRNA, it is the N-formyl-methionine is going to be used.

So, in this process what will happen is that methionine is actually going to be formulated to generate the F met or the N-formyl-methionine in and then this is actually going to coupled on to tRNA to generate the F met tRNA and then that is how the it is actually going to generate the F met tRNA and that F met tRNA is the tRNA species which is going to participate into the initiation steps.

So, wherever these codes are present in the initiation point, they code for the N-formyl-methionine, but they are present in between the coding sequence, they code for the methionine and valine respectively. which means if the AUG is present at the first codon, it is actually going to be code for the N-formyl-methionine but if the AUG is present in inside the codons, inside the sequence, then it is actually going to code for the normal methionine.

So, how does this happen? This happens because of the difference in the initiator tRNA and the one that you used in between the process of translation. So, initiator tRNA, has a unique feature that distinguish them from the elongating tRNA in the bacteria. So, you can have the two different types of tRNA molecules, the tRNA, which is responsible or the initiator tRNA and then you can also have the tRNA which are participating into the elongations steps.

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TRANSLATION

- Mechanism of Translation

Initiation ①

In the first step, small subunit of ribosome binds to mRNA such that initiation codon lies in partial P site. This gets possible due to activity of IF-3. It basically prevents untimely re-association of large and small subunit of ribosome. Moreover it promises accuracy of initiation site selection.

In mRNA there is ribosomal binding site which consists of **Shine-dalgarno sequence** and **initiation codon**. This Shine-dalgarno sequence which is **5'-AGGAGGU-3'** and is located 10bp upstream of initiation codon is complementary to region near 3' end of 16s rRNA, a component of small subunit of RNA.

The diagram shows a horizontal bar representing an mRNA strand with 5' on the left and 3' on the right. The Shine-Dalgarno sequence is labeled as 'AGGAGGU' and is circled in red. To its right is the 'Initiation codon' labeled 'AUG'. A red arrow points from the Shine-Dalgarno sequence to the initiation codon, with the text '3-10 Nucleotide' written below it. Above the Shine-Dalgarno sequence, a red structure represents the 'Small Subunit' of the ribosome, with a red circle around it.

Now, let us talk about the first step and the first step is called as the initiation. So, in the first step, the small subunit of the ribosome binds to the messenger RNA, such that the initiation codon lies into the partial P sites. This gets possible due to the activity of the initiation factor three. So, it basically prevents untimely re-association of the large and small subunit of the ribosome. Moreover, it promises the accuracy of initiation sites.

So, how that has been done? In the messenger RNA, there is a ribosomal binding site which consists of the Shine-dalgarno sequence, and initiation codon. This Shine-dalgarno sequence which is the 5 prime AGGAGGU and it is located 10 base pair upstream of the initiation codon is complementary to the region of the, near to the 3 prime end of the 16s ribosomal RNA, a component of the small subunit.

So, what happened is that you have the Shine-dalgarno sequences which are present on to the messenger RNA, and then these Shine-dalgarno sequences are actually having the affinity on to the small subunit of the ribosome. And when these two come together, the Shine-dalgarno sequence actually helps to align the two RNA species in such a way that the initiation codon actually is present on to the P site.

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TRANSLATION

- Mechanism of Translation

Initiation

In the next step, initiator tRNA carrying N-formyl methionine enters partial P site and binds to mRNA via its anti-codon loop. IF-2 is responsible for this activity. It directs initiator tRNA to its correct position in the initiation complex. It also exhibits ribosome dependent GTPase activity. Once GTP is hydrolysed then 50S subunit joins to form complete ribosome. Finally, when larger subunit also joins the complex it forms complete P site and A-site. Second charged tRNA (amino acid laden) enters A site. This tRNA as per the rule has anticodon corresponding to codon in mRNA.

The diagram illustrates the assembly of the 70S initiation complex. It shows an mRNA strand with a 5' AUG start codon and a 3' end. The 30S ribosomal subunit, along with initiation factors IF-1, IF-2, and IF-3, binds to the mRNA. IF-3 binds to the E site, IF-1 to the P site, and IF-2 to the A site. An initiator tRNA carrying N-formyl-methionine (Met-tRNA^{fMet}) with the anticodon UAC binds to the AUG codon in the P site. The 50S ribosomal subunit then joins the complex to form the 70S initiation complex. The diagram is annotated with red circles and arrows highlighting the AUG codon, the UAC anticodon, and the binding of IF-2 and the initiator tRNA.

So, this is what happened, you have the initiation codon, which is the AUG and before to this you are actually going to have the Shine-dalgarno sequence. So, in the next step your tRNA carrying the N-formyl-methionine is entered into the P site and binds to the messenger RNA why it is anti-codon loop. So, once the initiation codon and initiation codon, initiation factor one is actually going to present, and it is actually going to block the E and as well as the A side, so you have the 3 sides where you have the E, P and A,

P site, on P site, you have the starting codon, and whereas, on the E site and as well as A site, you are going to have the initiation factor 3 and 2, 3 and 1. Then what will happen is that the initiation tRNA is going to come along with the initiation factor which is actually a GTPase. And because of this the initiation codon is actually going to bind onto the P site with the help of the codon and anti-codon interaction.

So, you see these are the codon, AUG which is actually going to code for the N-formyl-methionine, whereas this is the anti-codon for the N-formyl-methionine. So, you are actually going to have the anti-codon which is going to be unique for this particular sequence. So, you see that it is actually complementary to each other. So, GC UA and AU, so this is that is how it is actually going to be recognized by the this particular initiation codon.

So, initiation factor 2 is responsible for this activity. It directs the initiation tRNA to its correct position in the initiation complex, it also exhibits the ribosome dependent GTPase activity. Once a GTPase is hydrolysed, then the 50s subunit joins to form the complete

ribosome. Finally, when the large subunit also joins the complex, it forms a complete P site and the A-site and the second charge tRNA enters into the A site.

This tRNA as per the rule has the anticodon corresponding to the codons into the messenger RNA. So, what happened is that, once this step is over, then you are actually going to have the entry of the 50s ribosomes and then the initiation factor 3 and 1 are actually going to be released along with the initiation factor 2 and the GTP.

And it is actually going to allow the binding of the large subunit and that is why it is actually going to assemble the complete ribosomal machinery. So, with this actually the initiation is going to be over in the case of prokaryotes.

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TRANSLATION

- Mechanism of Translation

Differences between initiation of prokaryotes and eukaryotes

In translation process, main difference between eukaryotes and prokaryotes is in the initiation process itself. Some major differences between Eukaryotic initiation and prokaryotic initiation are as follows.

In Eukaryotes, there is only one start codon for Eukaryotes AUG and it codes for methionine and not N-formyl-methionine. Eukaryotic cells need more initiation factors than prokaryotes.

Eukaryotic cells require 12 initiation factors. →

In Eukaryotes, process of association of mRNA with smaller subunit (40s) is more complex than prokaryotes. 40s first subunit identifies 5' methylated cap of m-RNA and then there is scanning process involved wherein initiation codon is recognized. This recognition is aided by ATP dependent helicases that hydrolyse ATP. This recognition of initiation codon is also aided by Kozak sequences 5'-ACCAUGG-3' similar to Shine-dalgarno sequence in prokaryotes

So, there are difference between the initiation of the into the prokaryotes and as well as the eukaryotes. So, all of those steps are almost the identical except that the initiation steps are different between the prokaryotes and eukaryotes. In the translation process, the main difference between the eukaryotes and prokaryotes is in the initiation process itself.

Some major difference between the eukaryotic initiation and the prokaryotic initiations are as follows. In the eukaryotes, there is only one start codon for the AUG and it codes for the methionine and not the N-formyl-methionine. Whereas, in the eukaryotic cell needs more initiation factor than the prokaryotes. So, there are two different, two major differences.

One, that the eukaryotic AUG codes for the methionine, whereas the prokaryotes, prokaryotic AUG code for N-formyl-methionine and the eukaryotic cell needs more initiation factors and then the prokaryotes. Eukaryotic cells requires the 12 initiation factors whereas the prokaryotic cells requires only the 3 initiation factor, initiation factor one, two and three.

In eukaryotes, the process of association of the messenger RNA with the small subunit, the 40s ribosomal subunit is more complex than the prokaryotes. The 40s ribosomal subunit identify the 5 prime methylated cap of messenger RNA and then there is a scanning process which is involved in, whereas, in the wherein initiation codon is recognized, this recognition is added by the ATP dependent helicase that hydrolyse the ATP.

This recognition of the initiation codon is also being helped by the Kozak sequences. And these Kozak sequences are almost similar as like the Shine-dalgarno sequences which are present in the prokaryotes. So, this is one of the major differences is that you have a scanning step in the case of the eukaryotic system, which means you are actually going to have the messenger RNA and this messenger RNA is actually going to scan by the small subunit for looking for the AUG sequence.

This scanning is being helped by the Kozak sequences, because the Kozak sequences which are present on to the ribosome, a small subunit is actually going to help the positioning of the small subunits. So, these are the some of the classical differences between the initiation of the messenger RNA or initiation of the translation between the prokaryotes and the eukaryotes.

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TRANSLATION

Elongation

- It is the cyclic process, elongation process start from formation of first peptide bond to addition of last of amino acid. The amino acid added to the chain one at a time to the nascent peptide chain. Addition of amino acid is very rapid process. The peptide sequence is in order of codon and anticodons in m-RNA, Rate of elongation is nearly 15 amino acids per second.
- There are some requirements regarding to elongation-
 - m-RNA and 70S ribosome
 - Amino acyl t RNA
 - Elongation factors

EF-Tu	It is G protein which binds to amino acyl t RNA and directs it to correct position at ribosome a site.
EF-Ts	Its main function is to regenerate to EF-Tu and hydrolysis of GTP.
EF-G	It is also a G protein which mediates translocation.

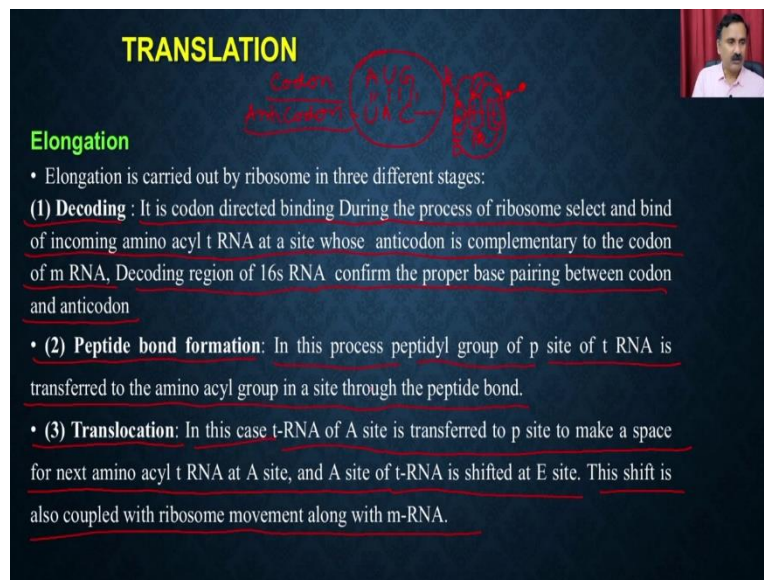
Then we steps within we entered into the elongation steps. So, elongation steps are actually it is it is the cyclic process, the elongation process starts from the formation of the first polypeptide peptide bond to addition of the last amino acids. The amino acyl added to the chain one at a time to the nascent polypeptide chain.

Addition of the amino acyl is very rapid process. The peptide sequence is in the order of codon and anticodon into the messenger RNA. The rate of elongation is nearly 15 amino acids per second. There are 3 some requirements regarding to the elongation. So, what are the requirements? You also require the messenger RNA and the 70s ribosomes.

You require the aminoacyl tRNA, for all the amino acids which are actually going to be present onto the messenger RNA in the form of codons and then you also require the elongation factor. So, what are the elongation factors are required? You require the elongation factor Tu, you require the integration factor Ts and then you require the elongation factor G. So, elongation factor Tu is a G protein which actually binds to the Amino acyl Trna and it is direct it to the correct position at the ribosome A site.

Whereas, the elongate Ts its main function is to generate the EF-Tu, regenerate the EF-Tu and the hydrolysis of GTP and EFG is it is also a G protein which actually mediates the translocations. So, elongation has the multiple steps. It requires this type of machinery, required the messenger RNA and the complete ribosome it required the Amino acyl rRNA, which are actually going to carry the amino acid, and they are also required the elongation factors.

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TRANSLATION

Elongation

- Elongation is carried out by ribosome in three different stages:
 - (1) **Decoding** : It is codon directed binding. During the process of ribosome select and bind of incoming amino acyl t RNA at a site whose anticodon is complementary to the codon of m RNA. Decoding region of 16s RNA confirm the proper base pairing between codon and anticodon.
 - (2) **Peptide bond formation**: In this process peptidyl group of p site of t RNA is transferred to the amino acyl group in a site through the peptide bond.
 - (3) **Translocation**: In this case t-RNA of A site is transferred to p site to make a space for next amino acyl t RNA at A site, and A site of t-RNA is shifted at E site. This shift is also coupled with ribosome movement along with m-RNA.

So, elongation is carried out by ribosome in the 3 stages one is decoding, so second is the peptide bond formation and the third is translocations. So, decoding - Decoding is being done by the interaction of the codon to the anticodon. And that I think we have already seen when, how the when we were talking about the initiation codon how the initiation codon is having the anti-codon for the codon onto the AUG.

So, it is codon directed binding during the process of ribosomes, select and bind to the incoming amino acyl tRNA at a site whose anti-codon is complementary to the codon of the messenger RNA. Decoding region of the 16s ribosomes confirm the proper base pairing between the codons as well as the anti-codon. So, you are going to have the codon and then you are going to have, for example, if you have the codon like AUG.

It is going to have the anti-codon like C A U. So, you are going to have the anticodon and that is going to make the base pairing as per the hydrogen bonding and that is why it is actually going to make the firm interaction between the tRNA and the messenger RNA what is present on the codon, what is present onto the messenger RNA.

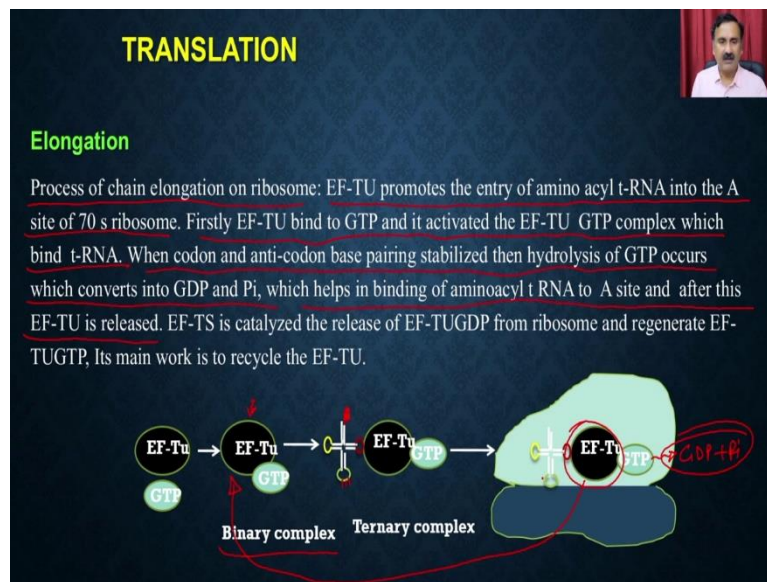
Once this is done, then you are going to have the second peptide bond formation. So, in this process, the peptidyl group of P site of tRNA is transferred onto the amino acyl group in a A site through the peptide bond. So, in this step what would happen is that you in the ribosomes, you are going to have the amino acyl tRNA, the initiator amino acyl and that is present onto the P site.

So, you are going to have the amino acid what is present onto the tRNA what is being present on to the P site. So, from here, it is actually going to be coupled onto this and so this amino acyl is actually going to have the amino acid and then it is going to have the initiation amino acid and that is how this is going to continue.

Then there will be a translocation. So, in the translocations the tRNA of the A site is transferred to the P site to make a space for the next amino acyl tRNA at A site and the A site of tRNA is shifted at the E site. This shift is also coupled with the ribosomal movement along with the messenger RNA. So, what is mean that translocation?

Translocation means that the this tRNA is going to move on to this and this deacylated tRNA is going to move into the E site and that is how it is actually going to be removed from the ribosome. So, let us see how this has been done.

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So, in the process of chain elongation on the ribosome, so EF-Tu promotes the entry of the amino acyl tRNA into the A site of the ribosomal RNA. First, the EF-Tu binds to the GTP and it activates the EF-Tu GTP complex, which binds the tRNA. When the codon and anticodon base pairing stabilizes, stabilized, then the hydrolysis of GTP occurs which converts into the GTP and PI which helps in the binding of eventual tRNA to the A site.

And after these, the EF-Tu is released, so this is what is going to happen. So, EF-Tu is actually going to combine with the GTP. So, that is how it is actually going to have the binary complex. Binary complex means the two molecules are coming together. And once the binary

complex is there, it is actually going to bind the tRNA, which is going to be different tRNA from the initiation tRNA.

So, this tRNA is going to contain the going to have the amino acid. Once the tRNA is, and this is going to have the anticodon. So once this anticodon is going to be matched onto the codon onto the messenger RNA, then this GTP is going to be hydrolysed and this GDP is going to be converted into GDP plus PI, which means and once this happens, then the EF-Tu is actually going to be released and it is going to be come back again.

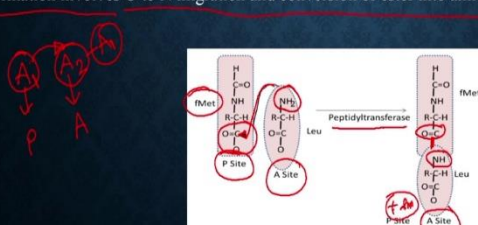
And then it is going its GTP, GDP is going to be released, and it is going to be replaced with another round of GDP, and that is how this cycle is going to continue. So that is how it will help the binding of the tRNA into the A site.

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TRANSLATION

Elongation

2) **Peptidyl Transfer:** it is peptide bond formation steps which the amino group of peptide bond are linked to t-RNA molecule in A site and carboxyl end of polypeptide chain uncoupled from t-RNA molecule in P site. This reaction is carried out by the enzyme peptidyl transferase. Peptidyl transferase is an enzyme which is associated with 23s r-RNA of 50S-ribosomal subunit, peptide bond formation involves O to N migration and conversion of ester into amide bond.



The diagram illustrates the peptidyl transferase reaction. On the left, a tRNA in the P site carries a polypeptide chain (fMet) and a tRNA in the A site carries a leucine (Leu) amino acid. The reaction is catalyzed by the enzyme Peptidyltransferase. On the right, the polypeptide chain has been transferred to the tRNA in the A site, and the tRNA in the P site is now empty. Handwritten red circles and arrows highlight the amino group of the Leu tRNA and the ester bond of the fMet tRNA, and the resulting amide bond and empty tRNA in the P site.

Once the tRNA is going to bind, then there will be a peptidyl transfer. So, it is the peptide bond formation step which the amino acid of the peptide bonds is linked to the tRNA molecule in A site and the carboxyl end of the peptide bond which is coupled, uncoupled from the tRNA molecule into the P site.

So, the peptide bond is going to be formed between the amino acid which is present on to the P site and the amino acid what is present onto the A site. So, from the, this amino acid is actually going to be transferred onto this, and it is going to be formed the peptide bond. So that is why if this is A1, then it is going to form the A2, A1 like this.

So, how this has been done. So, this reaction is carried out by the enzyme which is called as peptidyl transferase. Peptidyl transferase is an enzyme which is associated with the 23s ribosomes of the 50s ribosomal subunit, and the peptide bond formation involved the O to N migration and the conversion of ester into the amide bond.

So, this is what is going to happen. You have the F met, which is the initiation amino acid which is going to be present onto the P site and then it has the free carboxyl group, so you have a free carboxyl group and then there will be the attack from the amino group, from the second amino acyls, which is present onto the A site and that is how it is going to attack onto this carboxyl group.

And that is why the, this reaction is going to be catalysed by the peptidyl transferase. And then they will be amide linkage which is going to be formed between the amino acid what is present onto the A site. So, you have the, this is amino group, and this is going to be carboxyl group and there will be an amide linkage what is going to be formed and then this amino acid what is present on to the P site is going to be transferred onto this. And you still have the tRNA, the deacylated tRNA what is present onto the P site.

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TRANSLATION

Elongation

TRANSLOCATION: Three things are necessary for translocation.

- (1) Deacylated RNA remove from P site
- (2) Peptidyl t-RNA move from A to P site.
- (3) Ribosome should move on m-RNA, one codon down, so next codon can come at A site.

Translocation step carried out by EF-G factor, During translocation acceptor end of both t-RNA of A and P site are interact with peptidyl transferase centre of 23s t-RNA of 50 subunit. In translocation A and P t-RNA transfer to P and E site respectively, as ribosome move three nucleotide along m-RNA chain in 5' to 3' direction. During translocation step GTP is converted into GDP; and uncharged t-RNA released from P site to E site (exit site) and newly formed peptidyl t-RNA from A site to P site. Elongation process is nearly same in both prokaryotes and eukaryotes.

Then we have the translocation. So, translocation means you are actually going to move the molecules, so you are going to move the amino, the ribosomes onto the messenger RNA for the one more codons. There are the 3 things which are going to be necessarily for the translocations. The deacylated RNA moves from the P site, peptidyl transferase move from the A to P site, and the ribosome should move on to the messenger RNA one more codon.

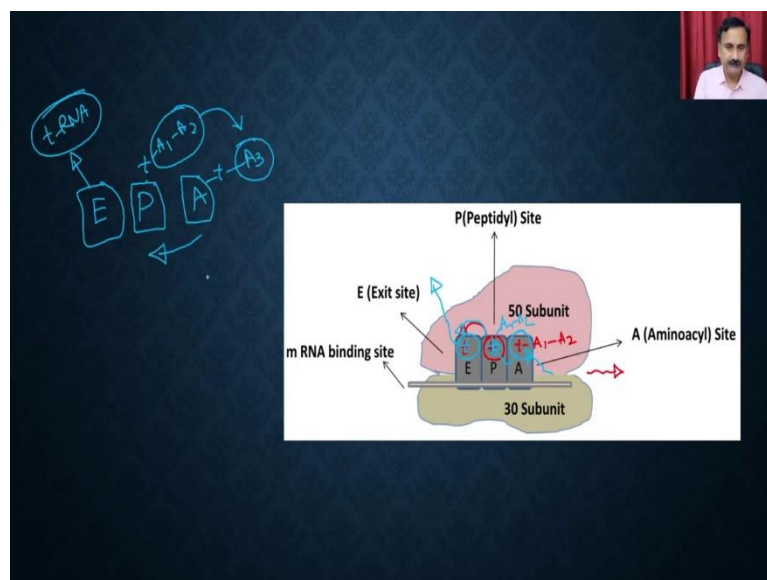
So that the next codon can come at the site of A site. So this means if you have a codon, like AUG, GG, GCG, initially the ribosome is going to be present onto the AUG, now it is going to move further one codon, and it is going to be present on this, so this codon is now going to be present onto the P site and that is how one another amino acyl is going to enter into the A site.

And the tRNA what was present onto the A site initially is going to be moved on to the P site, and that the deacylated tRNA what was present initially onto the P site is actually going to be present onto the E site and from this site, it is actually going to be released. Translocation step is carried out by the EF-G factors, elongation factor G. During the translocation acceptor end of the both tRNA of A and P sites are interact with the peptidyl transferase, centre of the 23s ribosomal RNA of s subunits.

In translocations, A and P tRNA transfer to the P and E sites respectively, as ribosomes moves three nucleotide along messenger RNA chain in the 5 prime to 3 prime direction. During the translocation steps, the GTP is converted into GDP; and the uncharged t-RNA is released from the P site to the E site and the newly formed, the peptidyl t-RNA is going to enter into the A site is going to P from the tRNA from the A site to the P site.

And the elongation process in nearly same in the both as the prokaryotes as well as the eukaryotes. So, this is what is exactly going to happen.

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You have the now, what we have is we have the A site, where we have the tRNA and that tRNA is going to contain the two amino acid, A1 and A2, right. And this side, you have the tRNA, which is actually does not contain any of the amino acyl, because this amino acyl is already been transferred on to another tRNA, and then you also have the M T E site.

So, when this drop, ribosome is going to shift into this direction. One codon, then what will happen is that this is going to move to here. And that is how you are going to have the deacylated tRNA. So, let me show you this with the different colour. So, this is what is going to happen. So, once this tRNA, once this ribosome is going to move further on one nucleotide, this is going to move to the here, so this is going to be present here.

And since this does not contain any of the amino acyls, it is going to be released from the ribosomes. Same is true for this one, so this is going to be moved on to this site. And it is actually going to have the, A1 and A2 on this. And when this happens, this site is going to take up the new amino, new codon, and that is going to have the new tRNA, and that is going to have the fresh amino acid according to the next codon.

And this process is going to continue which means the P is going to have the, P is going to have the tRNA which is going to have the a one and a two. And the A is actually going to have the new amino acid, so it is going to have the tRNA which is going to have the a 3 and then again, the same process is going to happen the A1 and A2 is going to be transferred onto the A3 and E is going to have those, that tRNA which is been does not contain the amino acid.

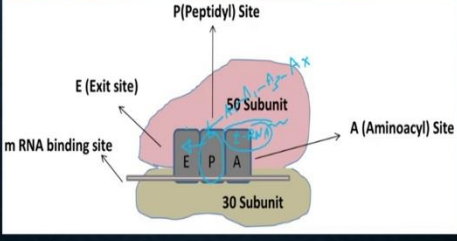
So, this process is going to continue like you are going to have deep coupling reactions, then you are going to have the translocation and that is how this will continue. So, this elongation steps continue until it reaches to the stop codon.

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TRANSLATION

Termination

Termination: Termination of translation occurs due to the stop codon. There are three stop codons (UAA, UAG and UGA) present. Out of these three when one of the stop codons appears in the A site of the ribosome it causes termination because there are no tRNAs present corresponding to these codons, so tRNA does not bind to the codon and causes termination. During termination release factors (RF) are involved. When UAA or UAG is in the A site, RF1 binds to the ribosome. When UAA or UGA is in the A site, RF2 binds to the ribosome. RF3 is a type of GTPase whose main function is to catalyze the release process through GTP binding and hydrolysis.



So, what happened at the stop codons? So, then it is going to have determinations. So, the termination of the translation occurs due to the stop codon. So, there are three stop codons UAA, UAG, and UGA which are present. So, out of these three, one of the stop codons appear in the A site of the ribosome. So, it causes the termination because there is no tRNA present corresponding to these codons.

So, tRNA is not going to bind the codon and it causes the terminations. So, during the termination, there are release factors which are involved. So, when the UAA or UGA is in the A site, the RF1 binds to the ribosomes and when the UAA or UGA is in the A site, the RF2 binds to the ribosome. And RF3 is a type of GTPase which has the main function to catalyze the release process through the GTP hydrolysis, GTP binding and the hydrolysis.

So, this is what exactly is going to happen. During the termination step, what will happen is that, you are actually going to have the fully synthesized protein, which is going to be present on the A site. So you are going to have A1, A2, A3, Axx like that. But this side, since this got moved to one more step, you are going to reach to the stop codon.

So, the stop codon is actually going to have the is allowing the entry of the tRNA, but this tRNA will not go to have any amino acid, so it is not going to have the amino acid and because of that, this is actually going to move on to this direction and it is actually going to be released. Because since there is no aminoacyl on this site, it is actually going to have the,

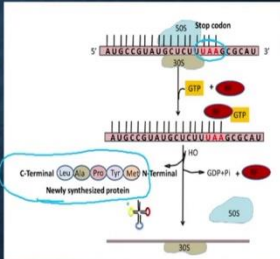
will not be able to do the coupling reaction and therefore it is actually going to be released from the ribosomes.

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TRANSLATION

Termination

1. Release factor (RF1) or Release factor (RF2) binds to the ribosome nearly to the A site.
2. Polypeptide chain are released from the ribosome by the peptidyl transferase complex, peptidyl transferase complex transfer the carboxy terminal residue of polypeptide chain from t-RNA of P site to water molecule.
3. Now the release factors (RF) and GDP released, and t RNA also freed.
4. Now 70S ribosome is unstable due the presence of initiation factors IF3 and IF1 and ribosome recycling factors. as a result, 70S ribosome disrupts into 30S and 50S subunits and prepared for initiation.

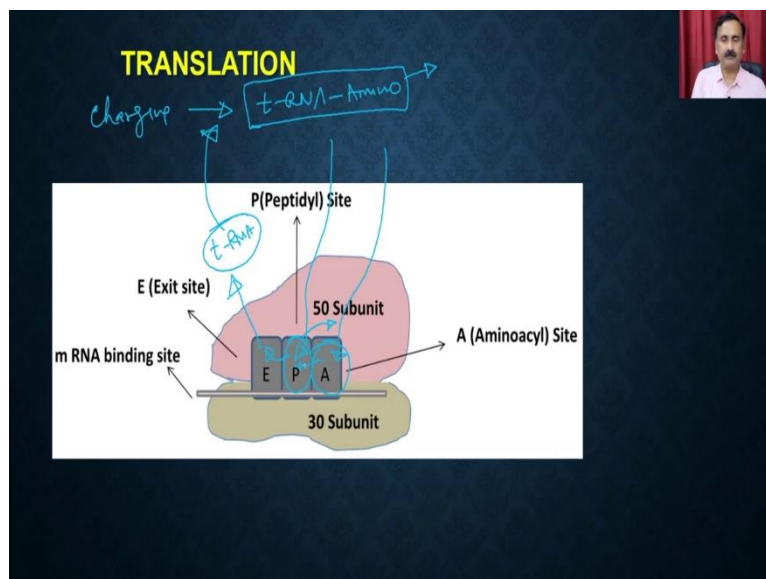


The release factors are RF1 or the release factor RF2 binds to the ribosome nearly to the A site. And polypeptide chains are released from the ribosome by the peptidyl transferase complex. The peptidyl transferase complex transfers the carboxyl terminal residue of the peptide chain from the tRNA, from the P site to the water molecule.

Now, the release factor RF and the GDP released, and the tRNA is also free from the ribosomes. And now the 70s ribosome is unstable due to the presence of the initiation, due to the presence of the initiation factor IF1 and IF2, and the ribosome is cycling factors. As a result, the 70s ribosome disrupt into the 30s and the 50s subunits and it is going to prepare it for the initiation.

So this is what exactly going to happen, once it reaches to this top codon the GTP and the RF factors are going to come and that is why they are actually going to release the newly formed polypeptide protein chain and they are also going to release the 30s as well as 50s subunits.

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So, this is all about the translations. What we have discussed so far? We have discussed about the charging of the amino acyls. So, we have discussed how the amino acids are going to be activated for the by the TR, by the help of the ATP and that is how it is going to couple on to the tRNA. So, first you are going to have the charging steps, where you are actually going to couple tRNA with the corresponding amino acid.

So, it is going to form the amino acyl tRNA. And once this is going to be synthesized, this is actually going to deliver the amino acids. So, what were the tRNA aminoacyl tRNA is going to bind, if it is the initiation codon then it is actually going to go and bind on to the P site, and then if it is a normal tRNA then it is actually going to go and bind to the A site.

And once it binds to the A site the tRNA what is present the initiation tRNA is going to be the, there will be a peptide bond which is going to be formed onto the tRNA what is present on to the P site to the tRNA what is present on to the A site, and then this cycle will going to continue. Whereas the tRNA is, when it is getting deacylated, it is going to be transferred on to the E site and from here the A site, this tRNA is going to be released into the cytosol.

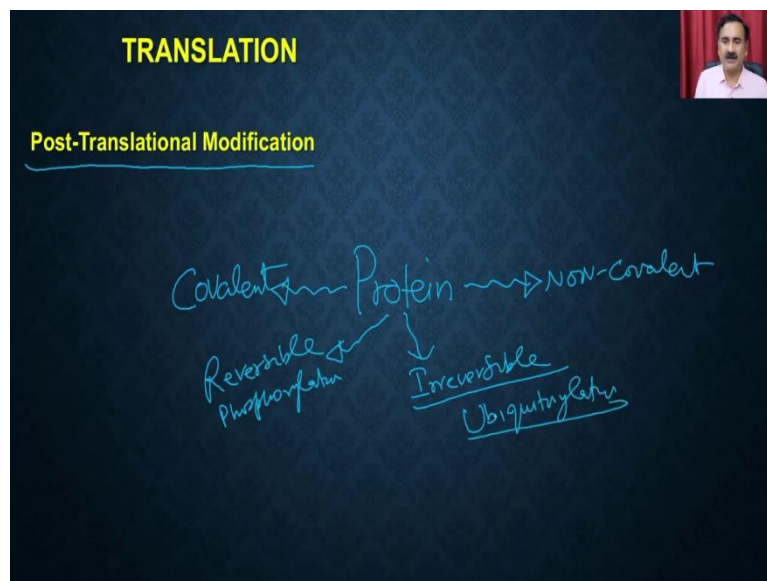
And then this tRNA is again going to participate into the charging reactions. Whereas the, during the translocation, the tRNA which is actually going to have the dye peptide is going to be moved on to the P site and then another amino acid, another tRNA is going to come onto

the A site and that is how this cycle is going to continue, until the ribosome is going to reach onto the stop codon.

And at the stop codon the release factors are going to come and that is how they are actually going to release the polypeptide and they are also going to disintegrate the small subunit as well as the large subunit. Because since at the stop codon there will be no, there will be no tRNA, which is actually going to have the amino acid bound.

So, they are actually going to activate, allow the release factor to release the machinery from the messenger RNA. So, that is how the messenger RNA is all that small subunit as well as a large subunit of the ribosome is going to be dismantled from the protein synthesis machinery.

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So, this is all about the translation what we have discussed. Now, we are going to discuss about post translational modifications. So, when we talk about the post translational modifications, the protein can be synthesized as a native protein and then this actually can be converted into the different types of modifications.

These modifications could be the covalent modifications, these could be non-covalent modifications, these modifications could be the reversible conversion modifications, or it could be irreversible modifications. So, one of the classical examples of the reversible post-translational modification is the phosphorylations.

And the non-irreversible post-translational modification is the ubiquitylations. So, let us discuss about the post-translational modifications.

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TRANSLATION

Post-Translational Modification

Phosphorylation: Phosphorylation is an important post-translational modification. It is prevalent from bacteria to higher eukaryote sustaining as mainly two types. First it acts to functionally regulate the catalytic activity of the protein by defining a rigid and permanent 3-D protein structure. Secondly, temporarily phosphorylate proteins serve as anchors for other protein substrates in signal transduction pathways. As, such it acts as a key-player in the regulation of many cellular processes like cell-cycle, cell growth, apoptosis and regulation of signal transduction pathways.

Protein + ATP → Protein-Poi (high energy)

Protein-Poi → Str. Modification, Activity, Association → Protein, Substrate

So, post-translational modification, the first post-translational modification we want to discuss is the phosphorylation. So, phosphorylation is actually being done by the ATP. So, when you have a protein, the protein is actually going to take up the phosphate from the ATP and that is why it is actually going to form the protein phosphate. And you know that the phosphate is actually going to have the high energy bond.

So, it is actually going to provide the energy into the protein molecules and that is why it is actually going to induce different types of modifications. It is actually going to make the modifications is going to have the structural modifications, so it is going to have the structural modifications, it can have the activity modifications like it is going to increase or decrease the activity of the proteins.

And it also can actually have the modification in terms of the association of the proteins with another protein or another or the substrate also. So, when you have the phosphorylation, it is actually going to induce the multiple types of changes into the protein and changes could be advantageous or it could be disadvantageous, for example, sometime it may actually increase the activity and sometime it is actually going to decrease activity.

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TRANSLATION

Post-Translational Modification

Phosphorylation: Serine, Threonine, Tyrosine

Mechanism of phosphorylation: In eukaryotic cells, phosphorylation is known to occur only at the side chains of three amino acids, serine, threonine and tyrosine. This is because these amino acids harbour a nucleophilic (-OH) group. The terminal phosphate group (γ -PO₃²⁻) on the universal phosphoryl donor adenosine triphosphate (ATP) serves as the point of nucleophilic attack from that -OH group, which results in the transfer of the phosphate group to the amino acid side chain. Magnesium (Mg²⁺) ions acts as catalyst by chelating the γ - and β -phosphate groups resulting in lowering of the threshold for phosphoryl transfer to the nucleophilic (-OH) group

The diagram shows the chemical reaction of serine phosphorylation. On the left, a serine molecule (with a hydroxyl group on its side chain) and an ATP molecule (with three phosphate groups) are shown. A blue circle highlights the hydroxyl group of serine and the gamma-phosphate of ATP. An arrow indicates the nucleophilic attack of the hydroxyl group on the gamma-phosphate. A blue box labeled 'phosphoserine' is shown as the product, with the phosphate group now attached to the serine side chain. Another blue box labeled 'ADP' is shown as the other product. The reaction is catalyzed by a 'Serine Kinase' enzyme, and Mg²⁺ ions are shown chelating the gamma and beta phosphate groups of ATP.

So, how the phosphorylation is going to happen? The phosphorylation is always been done on to the amino acid. So, you have the classically you have the three amino acid on which the phosphorylation can be done the threonine, serine and you can also have the tyrosine. So, all these amino acids are actually containing the free hydroxyl group and all these free hydroxyl group, you can actually be able to attach the phosphate.

So, how this happened? For example, I have shown you a one reaction where I have used a threonine. You have the OH which is actually going to contain the lone pair electrons and these lone pair electrons are attacking onto the phosphate molecule and then that is why with this high energy intermediate, this phosphate is going to be transferred onto the phosphate, onto the serine and therefore it is actually going to form the phosphoserine molecules.

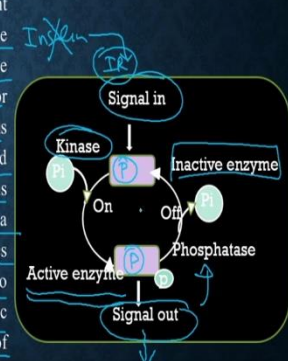
You require some of the cofactors like magnesium and then this reaction is always been catalyzed by an enzyme which is called as the kinase. So, in this case, if this is the serine which is getting phosphorylated then it this is can be called as the serine kinase.

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Post-Translational Modification

Phosphorylation:

These conformational changes can affect the protein in two different ways; (1) Phosphorylation causing conformational changes in the phosphorylated proteins, These conformational changes stimulate the catalytic activity of protein, so any protein can be activated or inactivated by the phosphorylation. (2) Phosphorylated proteins employ the neighbouring proteins which have structurally conserved domains that distinguish and bind to phosphomotifs. These domains are specific for diverse amino acids. Protein phosphorylation is a reversible Post translation modification which is carried by kinases which phosphorylate and phosphatases which dephosphorylate to substrates. These two type of enzymes make possible the dynamic nature of phosphorylated proteins. So the balance concentration of kinase and phosphatase is very important for the cell and it is also important for the catalytic efficiency of a particular phosphorylation site.



The diagram illustrates the reversible cycle of protein phosphorylation. It shows an 'Inactive enzyme' (represented by a blue box) being converted to an 'Active enzyme' (represented by a blue box with a 'P' in a circle) by the action of a 'Kinase' (represented by a green circle). This process is labeled 'On' and 'Signal in'. The reverse process, where the 'Active enzyme' is converted back to an 'Inactive enzyme' by the action of a 'Phosphatase' (represented by a green circle), is labeled 'Off' and 'Signal out'. The diagram also shows the release of inorganic phosphate (Pi) during the dephosphorylation step. A handwritten note 'Inducer' with an arrow points to the 'Signal in' input.

How the phosphorylation, I think we have already discussed that the phosphorylation is actually going to have the conformational changes into the protein. So, phosphorylation causes a conformational change in the phosphorylated protein. These conformational changes stimulate the catalytic activity of the protein. So many proteins can be activated or inactivated by the phosphorylation.

The phosphorylated proteins employ the neighbouring protein which have the structurally conserved domain that does distinguish and bind to the phosphomotifs. These domains are specific for the diverse amino acids. The protein phosphorylation is a reversible post translational modification, which is carried out by the kinase, which phosphorylates and the phosphatases which dephosphorelate to the substrate.

There are two types of enzymes make possible to the dynamic nature of the phosphorylated proteins. So, the balance concentration of the sinus and the phosphatase is very important for the cell and it is important for the catalytic efficiency of the particular phosphorylation site. So, you can imagine that you have the two different types of proteins. So, protein can be present as a P or it can be present as like this.

So, if when you have the, you can have the active enzyme. So, when you if you add the help of the kinase, you can take the ATP and you can actually add the phosphate and you actually go into form the phosphorylated enzyme, so it could be an active enzyme and once the signal

is over, so if once you get the signal from the, from some kind some kind of stress, some kind of ligand.

So, for example, you can have the insulin, so if insulin is actually go and bind to the insulin receptor, then it is that will be going to constitute a signal. Once the signal is there, then that signal is going to be perceived by the protein kinase and that protein kinase is going to say, phosphorylate this particular protein, and because there will be a phosphorylation of this protein, it is actually going to activate that protein.

But once the signal is over, which means once you have removed the insulin from the vicinity, then the signal is going to die. And once the signal is over, then that is actually going to activate the corresponding phosphatase. And that corresponding phosphatase is actually going to remove the phosphate molecule from the protein.

And that is how it is actually going to generate the inactive enzyme or to the native enzyme back. So this is the one of the classical example how the phosphorylation actually can be able to use as a tool to modulate the protein activity. And that is how you actually can respond to the external stimuli.

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TRANSLATION

Post-Translational Modification

Glycosylation: Glycosylation is a direct function of the biosynthetic-secretory pathway in the endoplasmic reticulum (ER) and Golgi apparatus. Approximately 50% proteins characteristically expressed in a cell go through this alteration, which involves the covalent addition of sugar moieties to specific amino acids. Mostly, soluble and membrane-bound proteins expressed in the endoplasmic reticulum undergo glycosylation, including all secreted proteins, surface receptors and ligands. Moreover, some proteins that are transferred from the Golgi to the cytoplasm are also glycosylated.

Protein + Carbohydrate → Glycoprotein

Now, apart from the phosphorylation, you can also have the glycosylation. So, glycosylation is a direct function of the biosynthetic secretory pathway in the endoplasmic reticulum and Golgi apparatus, approximately 50 percent of the protein characteristically expressed in a cell

grow through, through this alteration which involves the covalent addition of the sugar moieties to the specific amino acids.

Mostly soluble and membrane bound protein expressed in the endoplasmic reticulum, undergo the glycosylation, including the all-secreted protein surface receptor and the login. Moreover, some proteins that are transferred from the Golgi to the cytoplasm are also glycosylated. So, glycosylation is a topic which we are actually going to discuss in detail when we are actually going to talk about the vesicular transport.

So I am not going to discuss the glycosylation mechanism. But what is mean by the glycosylation is that the protein is going to be connected with the carbohydrate molecules or carbohydrate, a combination of the carbohydrate molecule. And that is why it is actually going to form the glycoprotein, and this glycoprotein is actually going to have the different types of sugar molecules which are been attached.

And based on these sugar molecules, it actually going to provide the signal into the cell and that is how these protein molecules are going to be distributed to the different organelles. So, that we are actually going to discuss in detail when we are going to discuss about the vesicular transport.

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TRANSLATION

Post-Translational Modification

Ubiquitination: This is one another post-translational modification where ubiquitin is added to protein. Ubiquitin is the eukaryotic protein coded by 4 different genes in mammals UBA52, RPS27A, UBB, and UBC. Protein is made of 76 amino acids and has a molecular mass of about 8.5 kDa. It is characterized by presence of C-terminal tail and 7 lysine residues. In ubiquitination, basically, carboxylic acid of the terminal glycine from the di-glycine motif in the activated ubiquitin forms an amide bond to the epsilon amine of the lysine in the modified protein. It marks the cellular protein for the process of degradation via proteasome, changes protein's location, prevent or promote protein-protein interaction.

Protein → Ubiquitin →

Apart from this, we can also have the ubiquitination. So, ubiquitination is a covalent modification of the protein and is a irreversible modifications. So, this is another post translational modification where the ubiquitin which is a protein is added to another protein.

So, ubiquitin is the eukaryotic protein coded by the four different gene in the mammalian cells like the UBA52, RPS27, AUBB, and the UBC.


And the ubiquitin is made up off of the 76 amino acids and it has a molecular weight of 8.5kDa. It is characterized by the presence of C-terminal tail and seven lysine residues. In ubiquitnization basically, the carboxylic acid of the terminal glycine from the di-glycine motif in the activated ubiquitin forms an amide bond to the epsilon amino of the lysine in the modified protein.

It marked the cellular protein for the process of degradation by the proteasome, changes of protein locations, prevent or promote the protein-protein interaction. So, ubiquitination is required for the protein degradation. So, when the protein is actually going through the aging process, when the protein is non-functional, it will go for the ubiquitination.

And this ubiquitination is also going to be sometime altered it is the localization or it is also sometime alter the Protein-Protein interaction, which means if you actually going to mask some of the sites which are responsible for the protein-protein interactions by the ubiquitination, you can also be able to disrupt the protein-protein interaction as well.

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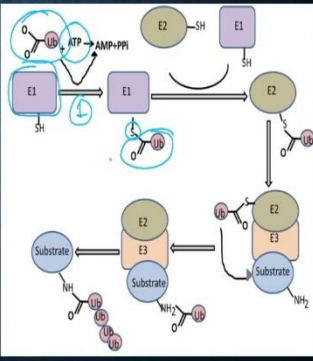
TRANSLATION



Post-Translational Modification

Steps

1. Activation of Ubiquitin: It occurs in a two-step reaction process. At first, ubiquitin interacts with ATP and forms ubiquitin-adenylate intermediate. In the next step, ubiquitin is transferred to E1 active site containing cysteine residue. This causes formation of thioester linkage between the C-terminal carboxyl group of ubiquitin and the E1 cysteine sulfhydryl group.



So, what are the different steps which are involved in the ubiquitination, in the step one you are going to have the activation of the ubiquitin. So, it occurs in the two-step reaction process, at first the ubiquitin interacts with the ATP and form the adenylated ubiquitin or

ubiquitin-adenylate intermediate. In the second step, the ubiquitin is transferred to the E1 active site containing the cysteine residues.

So, you can actually have the activation of the ubiquitin. So, that is the first step, where you are actually going to have the ubiquitin, it is going to attach to the ATP and it is actually going to form the adenylated ubiquitin. And that diluted ubiquitin is actually going to be coupled on to the ubiquitin enzyme, the E1 enzyme and with the help of the sulfur.

So, this is going to form up. So, E1 is actually going to contain the cysteine, and that is how it is going to utilize that cysteine to couple the ubiquitin.

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TRANSLATION

Post-Translational Modification

Steps

Transfer of Ubiquitin from E1 active site to E2 active site via trans-esterification reaction occurs.

In the last step of the ubiquitylation cascade there is formation of an isopeptide bond between a lysine of the target protein and the C-terminal glycine of ubiquitin via activity of one of the hundreds of E3 ubiquitin-protein ligases. In E1-E2-E3 cascade, one E1 molecule causes binding to several E2 which in turn bind to hundreds of E3 in hierarchical fashion.

The diagram illustrates the ubiquitylation cascade. It starts with E1 (purple) and E2 (green) enzymes, both containing a cysteine residue (-SH). Ubiquitin (Ub) is activated by ATP, forming an adenylated intermediate (Ub-AMP) and releasing AMP+PPi. Ubiquitin is then transferred from E1 to E2 via a trans-esterification reaction, forming an E2-Ub conjugate. This E2-Ub conjugate then binds to E3 (orange), which in turn binds to a substrate protein (blue). The diagram shows multiple E2 molecules binding to a single E3 molecule, which then binds to a single substrate protein, illustrating the hierarchical nature of the cascade. Handwritten notes in blue ink highlight the formation of an isopeptide bond between a lysine of the target protein and the C-terminal glycine of ubiquitin via the activity of E3.

Then we have the ubiquitin a to enzymes. So, transfer of the ubiquitin is going to be done from the E1 to the E2 site via trans-esterification reactions. From the E1 the ubiquitin is going to be transferred onto the E2 by a trans-esterification reaction. Now, this E2 and as well as the substrate protein is actually going to bind into the E3.

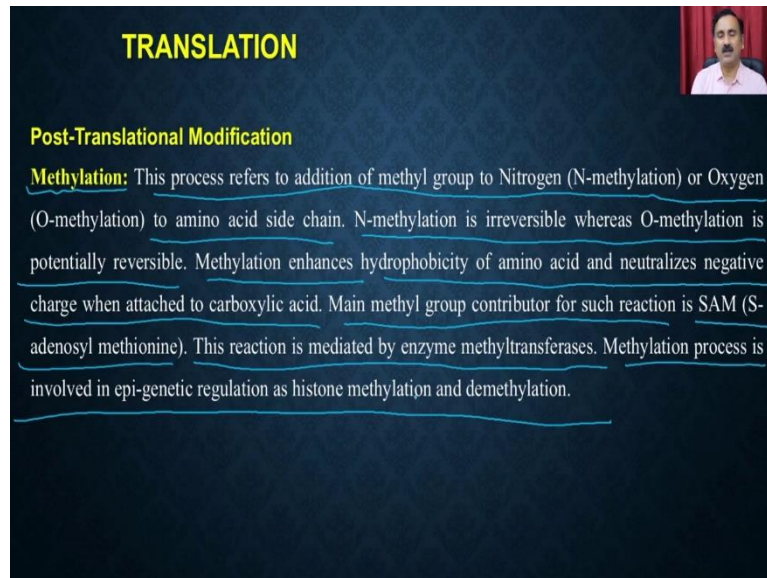
And in the last step that ubiquitin cascade there is a formation of a isopeptide bond between the lysine of the target protein and the C-terminal glycine of the ubiquitin via the activity of one of the hundreds of the E3 ubiquitin ligase. So, this is going to be E3, which is going to be called as a E3 ubiquitin ligase and it is actually going to couple the ubiquitin.

What is present onto the E2 onto the substrate protein which is actually going to have the free amino group and this free amino group, the ubiquitin is going to be transferred. And you can

imagine that this process if you continue for several rounds, then you are actually going to have the multiple ubiquitin could be transferred onto the substrate molecule. So, the substrate molecules you can have the mono ubiquitination or you can have the poly ubiquitination.

And depending on the mono or the poly ubiquitylation, the protein can be, the proteins properties can be modulated.

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TRANSLATION

Post-Translational Modification

Methylation: This process refers to addition of methyl group to Nitrogen (N-methylation) or Oxygen (O-methylation) to amino acid side chain. N-methylation is irreversible whereas O-methylation is potentially reversible. Methylation enhances hydrophobicity of amino acid and neutralizes negative charge when attached to carboxylic acid. Main methyl group contributor for such reaction is SAM (S-adenosyl methionine). This reaction is mediated by enzyme methyltransferases. Methylation process is involved in epi-genetic regulation as histone methylation and demethylation.

Apart from that, we can also have another kind of method post translational modification that is called as the methylation. So, this process refers to the addition of methyl group to the nitrogen or oxygen or to amino acid site. The N-methylation is irreversible, whereas the O-methylation is a potentially irreversible. Methylation causes the hydrophobicity of the amino acid and it neutralizes the negative charge when attached to the carboxyl acid.

Main methyl group transfer for such a reaction is the SAM or S-adenosyl methionine. This reaction is mediated by the enzyme methyl transferase. Methylation process is involved in epi-genetic regulation as the histone methylation and the demethylation. So, methylation is a process which actually is responsible for the opening and closing of the chromatins and that is how it is actually going to be responsible for activation or the deactivation of the transcription processes.

So it is a kind of a mechanism which has been used for the regulatory purposes. So, for example, there are heterochromatin and euchromatin, and the chromatin can be fractionated into the heterochromatin and euchromatin. And so, the methylation and the demethylation

can be a process through which you can actually be able to convert the heterochromatin into euchromatin and euchromatin into heterochromatin.

So, with this, I would like to conclude my lecture here and what we have discussed, we have discussed about the translation in the different steps of the translations. We discussed about the protein synthesis machinery, where we have discussed about the messenger RNA, tRNA or ribosomal RNA. And then we have also discussed about the different steps of the protein synthesis like the, we have discussed about the amino acid's activations.

Then we have discussed about the initiation elongation and terminations. And ultimately at the end we have also discussed about the post translational modifications into the protein. So, with this I would like to conclude my lecture here. In our subsequent lecture, we are going to discuss some more aspects related to the living organism. Thank you.