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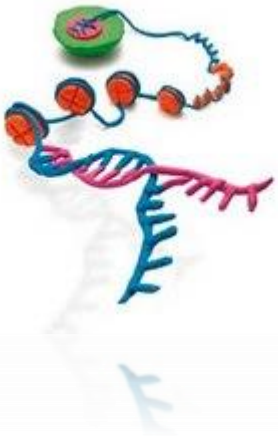
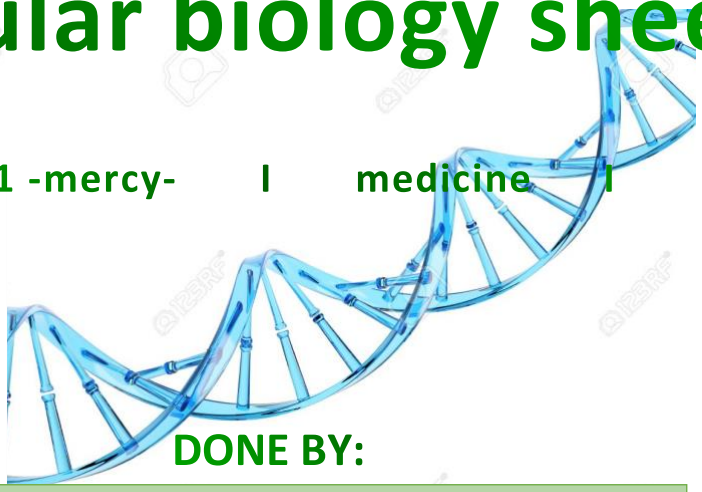
Molecular biology sheet

Doctor 2021 -mercy- I medicine I MU





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Charging the tRNA

1)Anchord

2)Charger protein

- **tRNA acts as a translator between mRNA and protein**
- **Each tRNA has a specific anticodon and an amino acid acceptor site.**
- **Each tRNA also has a specific charger protein (aminoacyl tRNA synthetases) which can only bind to that particular tRNA and attach the correct amino acid to the acceptor site.**
- **The energy to make this bond comes from ATP.**

The bind of tRNA & amino acid is irreversible

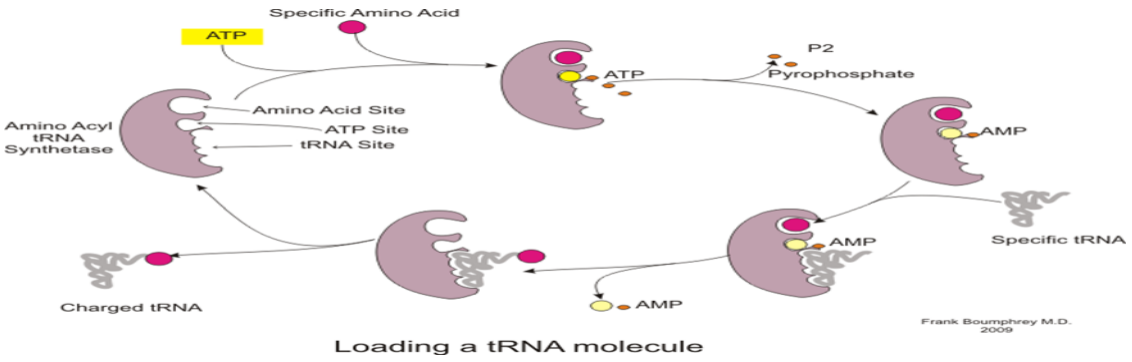
Synthetase : enzyme that need ATP to do it's function

Syntheses : enzyme that doesn't need ATP to do it's function

The first thing that binding of specific amino acid at the specific tRNA , then ATP (begins hydrolyzed into AMP and pyrophosphate to liberation energy that responsible of binding amino acid to acceptor arm of tRNA , that happened by two steps :

1- in the beginning occur reaction between amino acid and ATP to result aminoacyl AMP .

2- the sec reaction between the amino acid & tRNA ,the AMP exist to outside and replace it by tRNA to become finally aminoacyl tRNA



Loading a tRNA molecule

Aminoacyl tRNA synthetases: it works like regulatory protein which that the corrected amino acid it will be binding

- **There are 20 different synthetases one for each amino acid that can catalyze the covalent bond between the amino acid and tRNA**
- **A single synthetase may recognize multiple tRNAs for the same amino acid specified by the mRNA codon to which the tRNA anticodon binds**
- **Two classes of synthetases, differ in the 3-dimensional structures, which side of the tRNA they recognize and how they bind ATP**
- **Class I – monomeric(consist from one polypeptide), acylates the 2'OH on the terminal ribose**

Arg, Cys , Gln, Glu, Ile, Leu, Met, Trp Tyr, Val(10 amino acid)

- **Class II- dimeric,(consist from 2 polypeptide chain so it has quaternary structure) acylates the3'OH on the terminal ribose**
- **Ala, Asn, Asp, Gly, His, Lys, Phe, Ser, Pro, Thr (10A.A)**
- **When the mRNA binding with small subunit "in prokaryote , eukaryote "it must have anك orientation to start translation from '5-end and it's occurs by**

1) **ribosome scanning :**

- In eukaryote
- {no Shine Dalgarno sequence}

2) Shine Dalgarno sequence:

- In prokaryote
- recognized By complementary sequence that is 3'end in 16s rRNA

Initiation the only process that occurs in small subunit

- This phase of protein synthesis results in the assembly of a functionally competent ribosome in which an mRNA has been positioned correctly so that its start codon is positioned in the P (peptidyl) site and is paired with the initiator tRNA.
- The following ingredients are needed for this phase of protein synthesis:

1- Two ribosome subunits - 30S and 50S 2- The mRNA

3- Three Initiation Factors - IF1, IF2 (GTP){the ATP we Just use it in charged tRNA even in prokaryote, eukaryote after that all process need GTP} and IF3

4- The initiator fMet-$tRNA_f^{Met}$

The following steps take place:

A- Binding of the ribosome 30S subunit with initiation factor (IF3)

promotes the dissociation of the ribosome into its two component subunits. we dissociate the ribosome and separate the large subunit from the small subunit by IF3 factors because Initiation phase doesn't occur in the whole of ribosome so I separate the subunit to occur initiation

B-The presence of IF3 permits the assembly of the initiation complex

and prevents binding of the 50S subunit prematurely, IF1 assists IF3 in some way, perhaps by increasing the dissociation rate of the 30S

and 50S subunits of the ribosome. IF2 :In prokaryote

fMet-tRNAfMet :in the prokaryote cell

It is methionyl tRNA ATTACHED To formyl group to recognize the 5'end of the mRNA. However it is Met-tRNA without formyl group in eukaryotes due to the presence of cap in mRNA that determines the 5' end.

C- Binding of the mRNA and the fMet-tRNA$_f^{Met}$

- **IF3 assists the mRNA to bind with the 30S subunit of the ribosome so**

that the start codon is correctly positioned at the peptidyl site .{in prokaryote}of the ribosome.

- **The mRNA is positioned by means of base-pairing between the 3' end**

of the 16S rRNA with the Shine- Dalgarno sequence immediately upstream of the start codon. .{in prokaryote}

- **IF2(GTP) assists the fMet-tRNA$_f^{Met}$ to bind to the 30S subunit in the**

correct site - the P site.{in eukaryote}

- **At this stage of assembly, the 30S initiation complex is complete and**

IF3 can dissociate.

D- Binding of the ribosome 50S subunit and release of Initiation Factors

- **Three events now happen "simultaneously".**
- **As the 50S subunit of the ribosome associates with the 30S initiation**

complex, GTP hydrolysis occurs on IF2.

- **This hydrolysis may be helped by the L7/L12 ribosomal proteins(help in reassociate subunits) rather than by IF2 itself.**

✓ **The energy that produced from of GTP another 2 ribosomal protein it is the cause of helping the IF2 between 50s+30s to give 70s .**

✓ **30S initiation complex: it is small ribosome sub-unit binding with mRNA binding with genetic codon methionine that with IF2 with GTP (GTP hydrolyses into GDP +inorganic phosphate of the GTP) binding of the small substance with the large substance because the stage of protein synthesis in both prokaryotes and eukaryotes happen in the whole ribosome**

✓ **Initiation is the only stage in protein synthesis in cells that happen in the small sub-unit**

✓

Fig. 8.9

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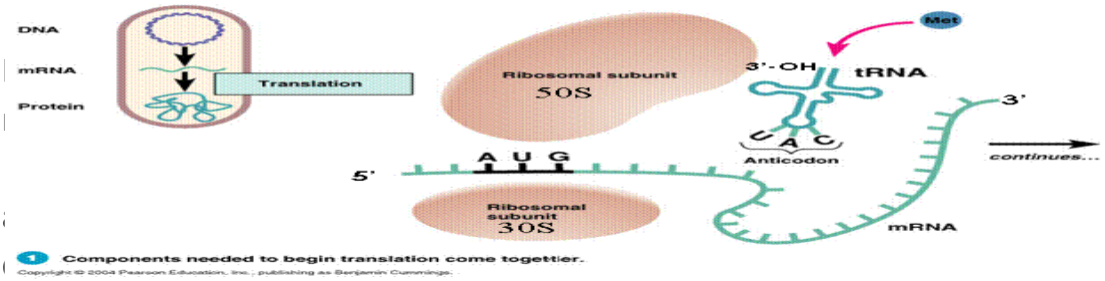
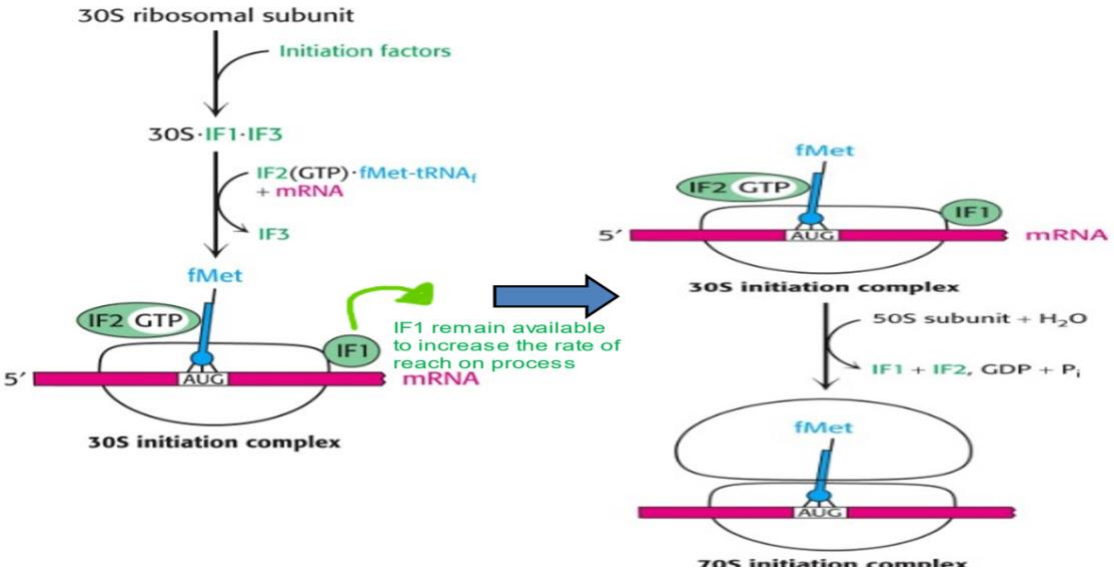
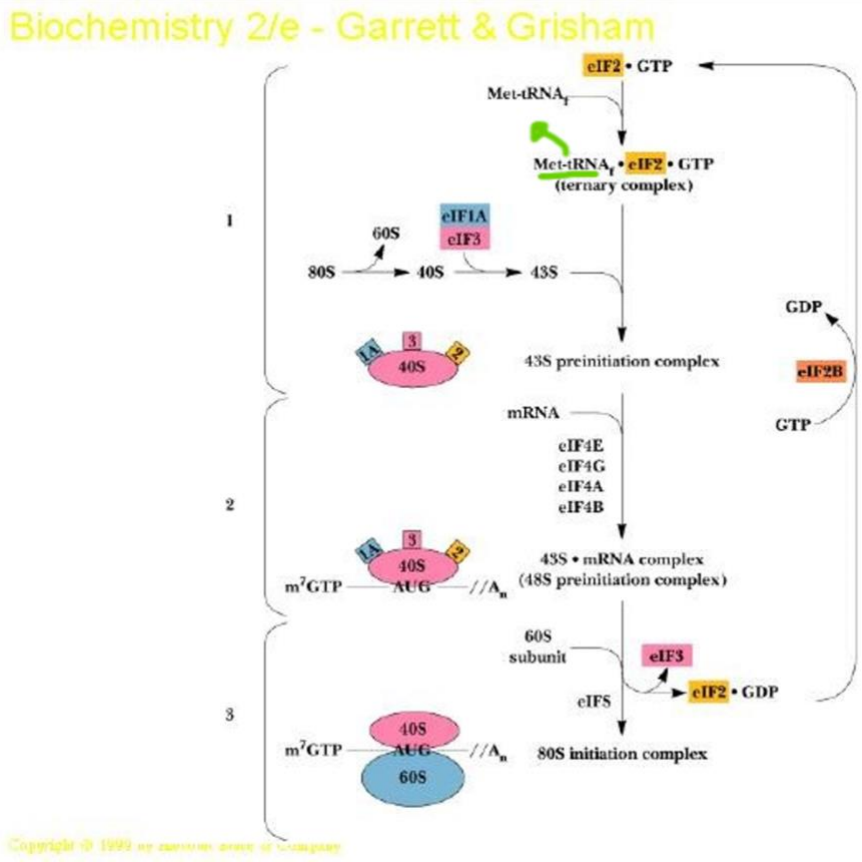


In addition to the APE sites there is an mRNA binding groove that holds onto the message being translated

- **The A site binds an aminoacyl-tRNA (a tRNA bound to an amino acid);**
- **P site binds a peptidyl-tRNA (a tRNA bound to the peptide being synthesized).**
- **The E site binds a free tRNA before it exits the ribosome.**





- **There is type of eIF**

eIF1A and eIF3: bind to the small substance to cause dissociation between large and small sub-units

eIF2 : bind to GTP with methionine on tRNA

eIF4A ,eIF4E, eIF4B, eIF4G: These four bind with mRNA

these 7 eIF for the formation of initiation complex 80S

80S :When the reassociation between small substance ribosome and large substance, all the type of initiation factor will be released and the GTP that binding to tRNA break down to give energy for reassociation between the small subunit and large sub unit in eukaryotic cells

- **There is two initiation factor eIF3 eIF1A and they do the to give 40s(small substance) where the intiation stage will happen on**
- **TERNARY Complex is methionion tRNA with e1f2 and GTP**
- **1.THERE IS SMALLSUBSTANCE BINDINGTO e1F1A and eIF3and tRNA with eIF2nd GTP and methionine that on the acceptor arm**

- **2. 43s preinitiation complex will bind with 4 eIF with mRNA and this will lead to increase the sedimentation give us preinitiation complex and bind to 60s to give 80s inanition complex**

- Eukaryotes use a scanning mechanism to initiate translation.
- Recognition of the AUG triggers GTP hydrolysis by eIF-2
- GTP hydrolysis by eIF2 is a signal for binding of the large subunit and beginning of translation

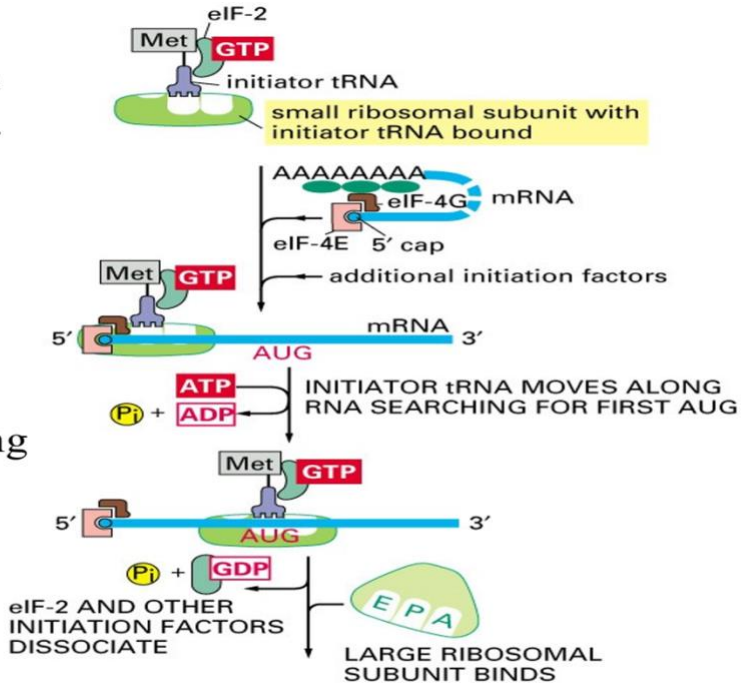


Figure 6–71 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

At the start of each cycle:

-The A (aminoacyl) site on the ribosome is empty

-The P (peptidyl) site contains a peptidyl-tRNA, and the E (exit) site contains an uncharged tRNA.

-The elongation factor, EF- Tu (GTP) binds with an aminoacyl-tRNA and brings it to the ribosome.

Once the correct aminoacyl-tRNA is positioned in the ribosome, GTP is hydrolyzed, EF-Tu (GDP) undergoes a conformational change and then dissociates away from the ribosome.

There are two ways that EF- Tu functions to ensure that the correct aminoacyl-tRNA is in place:

EF- Tu prevents the aminoacyl end of the charged tRNA from entering

the A site on the ribosome.

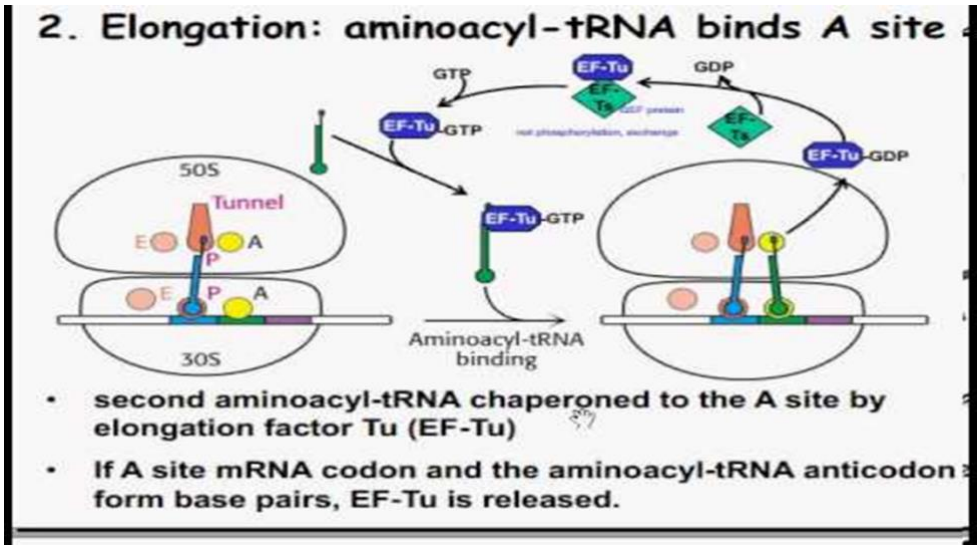
This ensures that codon- anticodon pairing is checked first before the charged tRNA is irreversibly bound in the A site and a new, potentially incorrect, peptide bond is made.

GTP hydrolysis is SLOW and EF- Tu cannot dissociate from the ribosome until it occurs.

The amount of time prior to GTP hydrolysis allows the final fidelity check to take place.

Hydrolysis is associated with a conformational change in EF-Tu.

Elongation step of protein synthesis



- **The Peptidyl transferase enzyme remove the amino acid from p site and transfer it to A site to form peptide bond ,here the p site has uncharged tRNA and the A site has charged tRNA by two amino acid linked to each other by peptide bond.**

Termination

-The final phase of protein synthesis requires that the finished polypeptide chain be detached from a tRNA.

- **This can only happen in response to the signal that a stop codon has been reached.**

***There no anti-codons for stop codons.**

Binding of Release factors

- **There are no tRNAs that recognize the stop codons.**
- **Rather stop codons are recognized by release factor RF1 (which recognizes the UAA and UAG stop codons) or RF2 (which recognizes the UAA and UGA stop codons).**
- **These release factors act at the A site of the ribosome.**
- **A third release factor, RF3 (GTP), stimulates the binding of RF1 and RF2.**

Hydrolysis of the peptidyl-tRNA

- **Binding of the release factors alters the peptidyltransferase activity with a nucleophilic effect.**
- **The result is hydrolysis of the peptidyl-tRNA and release of the completed polypeptide chain.**

  ***by adding H2O molecule rather than amino acid.**

- **The uncharged tRNA in the E site can dissociate as can the release factors.**
- **GTP is hydrolyzed without dissociating tRNA in the P site.**

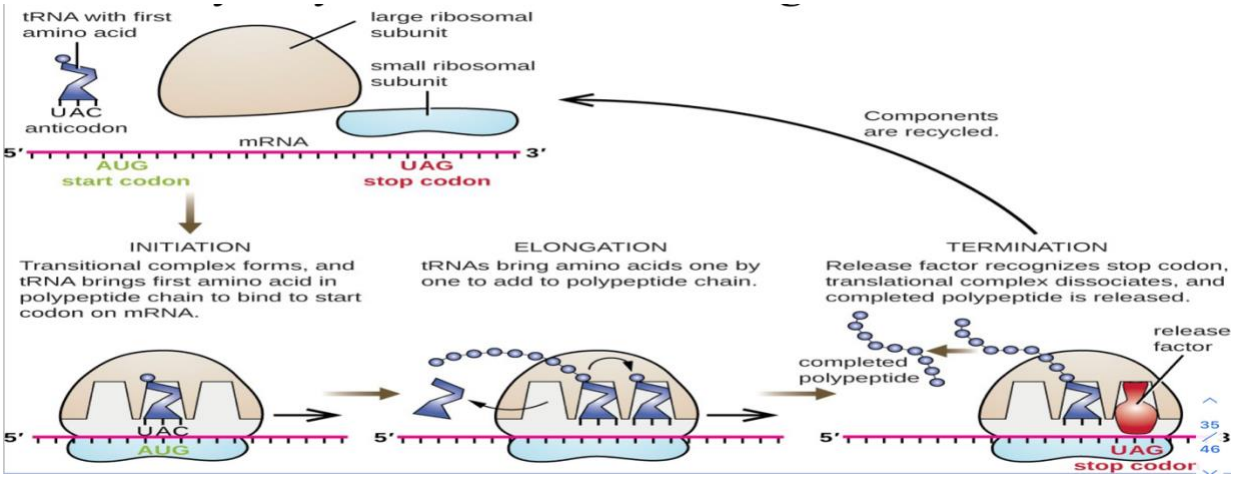


TABLE 29.4 Antibiotic inhibitors of protein synthesis

Antibiotic	Action
Streptomycin and other aminoglycosides	Inhibit initiation and cause misreading of mRNA (prokaryotes)
Tetracycline	Binds to the 30S subunit and inhibits binding of aminoacyl-tRNAs (prokaryotes)
Chloramphenicol	Inhibits the peptidyl transferase activity of the 50S ribosomal subunit (prokaryotes)
Cycloheximide	Inhibits the peptidyl transferase activity of the 60S ribosomal subunit (eukaryotes)
Erythromycin	Binds to the 50S subunit and inhibits translocation (prokaryotes)
Puromycin	Causes premature chain termination by acting as an analog of aminoacyl-tRNA (prokaryotes and eukaryotes)

The sec specified in (A)site

mRNA

it resembles the tyrosine ,No peptide amino acid

Diphtheria toxin it's not antibiotic

Inhibits eEF-2 by ADP-ribosylation of modified histidine in the factor

Antibiotics inhibiting translation

- **The bacterial ribosomal structure and the accessory functions differ in many respects from its eukaryotic equivalent. The translation reaction itself can be subdivided into three parts:**

1.Formation of the initiation complex, blocked by Streptomycin and Tetracyclins (the latter inhibiting binding of aminoacyl-tRNA to the ribosomal A- site at the 30S ribosomal subunit.

2.Introduction of aminoacyl-tRNA and synthesis of a peptide bond, inhibited by puromycin (leading to premature termination) and chloramphenicol (probably inhibiting the peptidyltransferase).

3.Translocation of the mRNA relative to the ribosome blocked by erythromycin and fusidic acid (the latter preventing release of EF-G/GDP.

Protein synthesis in eukaryotes

- **A major difference between eukaryotes and prokaryotes is ribosomal subunit that, in a typical eukaryotic cell, protein synthesis takes place in the cytoplasmwhile transcription and RNA processing take place in the nucleus.**
- **In bacteria, these two processes can be coupled so that protein synthesis can start even before transcription has finished.**
- **The steps of protein synthesis are basically the same in eukaryotic cells as in prokaryotes.**
- **The ingredients, however, can be different:**

1- Ribosomes are larger. 60s and 40s subunits combine to give 80s ribosomes which contain 4 RNAs: 28S,5.8S and 5S in the 60S subunit; 18S in the 40S subunit.

2- While the initiating amino acid in eukaryotic protein synthesis is still methionine, it is not formylated.

3- Eukaryotic mRNA is capped. This is used as the recognition feature for ribosome binding -- not the 18S rRNA.

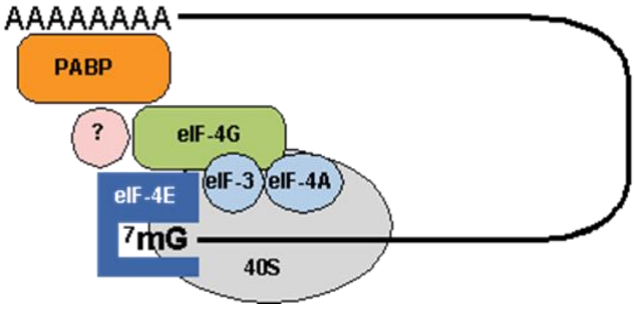
4- The initiation phase of protein synthesis requires over 10 eukaryotic initiation factors (eIFs) one of which is the cap binding protein

5- The eukaryotic elongation phase closely resembles that in prokaryotes.
- The corresponding elongation factors are eEF-1a (EF-Tu), eEF- 1bg (EF-Ts) need a GTP and eEF-2 (EF-G).

6- Eukaryotes require just a single release factor, eRF.

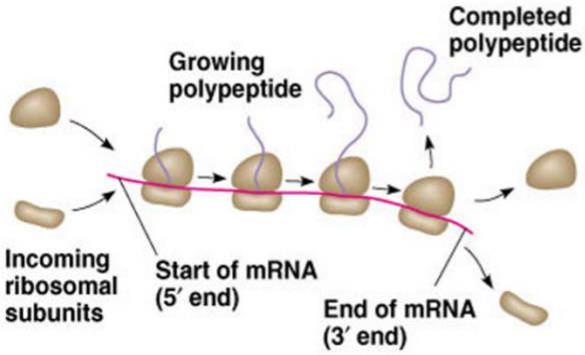
Coordinating protein synthesis with mRNA synthesis

- **It has recently been found that the eukaryotic initiation factor eEF-4G binds not only with other factors in the initiation complex but also with PABP (poly A binding protein) which binds to the poly A tail of mRNA.**



- It is thought that the binding of eEF-4G to PABP serves as a critical recruitment step for driving downstream translation.
- In another sense, however, the binding of eEF-4G to PABP represents a mechanism to ensure that only mature intact mRNAs are translated.
- Most mRNA are translated by more than one ribosome at a time; the result, a structure in which many ribosomes translate a mRNA in tandem, is called a polysomes. means the mRNA Can translate by more than one ribosome

*This process is done for long mRNAs for reducing the required time.



(a) An mRNA molecule is generally translated simultaneously by several ribosomes in clusters called polyribosomes.

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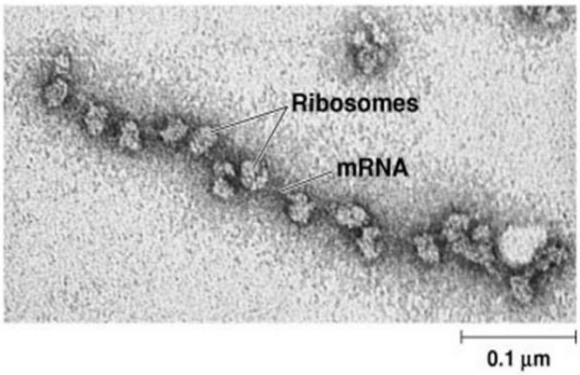

(b) This micrograph shows a large polyribosome in a prokaryotic cell (TEM).

Post-translational modifications

- They are the chemical modifications of a protein after its translation

- Characterized by:

1- Being numerous and diverse

2- Able to change the charge(add acidic or basic amino acid), conformation(outside) or size (number of amino acid)of protein molecule

Effects:

1. Stability of protein

2. Biochemical activity (activity regulation)

3. Protein targeting (protein localization)

4. Protein signaling

(protein - protein interaction

- **capping { it just found in eukaryote cell ,doesn't find in prokaryote cell}Provide or Protect the 5'end from 5' exonuclease, and polyadenylation of tail Provide or Protect the 3'end from 3'exonuclease**
- **the DNA modification**

1)methylation

2)Acetylation

3)organization {when the DNA wrap around histone it cant it form active enhance

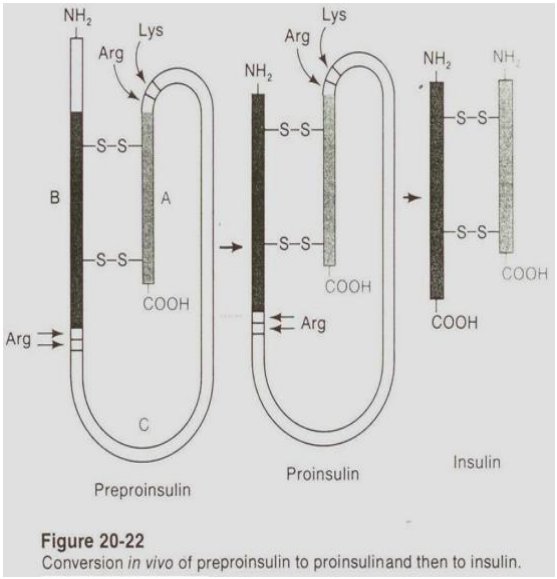

Figure 20-22
Conversion *in vivo* of preproinsulin to proinsulinand then to insulin.

insulin, which is a low molecular weight protein having two polypeptide chains which fold to allow interchain and

intrachain disulfide bridges.

A specific protease then clips out the segment that connects

the two chains which form the functional insulin molecule,

where a propeptide is removed from the middle of the chain;

the resulting protein consists of two polypeptide chains connected by disulfide bonds.

2- Also, most nascent polypeptides, the initial methionine {the first amino acid In protein synthesis } usually taken off during post-translational modification by specific aminopeptidases.

3- Other modifications, like phosphorylation, are part of common mechanisms for controlling the behavior of a protein, for instance activating or inactivating an enzyme.

4- Some animal viruses, as poliovirus and hepatitis A virus, synthesize long polycistronic proteins {the protein that is synthesized by: more than one gene with related functions are transcribed into one mRNA, the mRNA is then translated into one polypeptide chain, which will be separated into more than one protein (polycistronic proteins)}from one long mRNA molecule, these proteins must be cleaved at specific sites to provide the several specific proteins required for viral function.

5- Collagen, an abundant protein in the extracellular spaces of higher eukaryotes, is synthesized as procollagen three polypeptide molecules, that align themselves in a particular way that is dependent upon the existence of specific amino terminal peptides.

6- Specific enzymes then carry out hydroxylations and oxidations of specific amino acid residues within the procollagen molecules to provide cross-links for greater stability with cleavage of the NH2 terminal end to form a strong, insoluble collagen molecule.

Types of posttranslational modifications:

A- Trimming:

- Many proteins secretion from the cell are made as:

- large precursor molecules but functionally inactive Change of protein from non active for active molecule by removing portions of the protein chain by specialized endoproteases

Sites of the cleavage reaction:

- Endoplasmic reticulum

- Golgi apparatus

- Secretory vesicles

N.B. zymogens which are inactive enzymes, become activated through cleavage when they reach their proper sites of action

B- Covalent alterations:

- Proteins may be activated or inactivated by the covalent attachment of a variety of chemical groups

1- Phosphorylation :

- By adding phosphate group to the hydroxyl groups of (serine, threonine, tyrosine residues in a protein) which is catalyzed by protein kinases and reversed by protein phosphatases
- The phosphorylation may ↓ or ↑ the functional activity of protein

Phosphate group 1)some enzymes will be active by phosphorylation

2)some enzymes will be inactive by phosphorylation

2- Glycosylation :

- Many of proteins → become part of a plasma membrane, lysosomes or secreted from the cell have carbohydrate chains attached to serine or threonine hydroxyl groups (O-linked) or the amide nitrogen of asparagine (N-linked)
- Occurs: in the endoplasmic reticulum and golgi apparatus
- Used to : target protein to specific organelles

3- Hydroxylation :

As proline and lysine in endoplasmic reticulum by prolyl or lysyl hydroxylases (e.g. in collagen).

- In order to form cross linkages.

4- Other covalent modifications :

a-Carboxylation:

- Carboxyl groups can be added to glutamate residues by vitamin K
- The resulting carboxyglutamate residues are essential for the activity of several of the blood-clotting proteins

b- Biotinylated enzyme:

- Biotin is covalently bound to the amino groups of lysine residues of biotin-dependent enzymes that catalyze carboxylation reactions
- Such as: pyruvate carboxylase

c- Farnesylated protein:

- Help anchor proteins in membranes.
- Note : many proteins are acetylated.

* farnesyl group is lipid in nature (intermediate of cholesterol synthesis) so it helps in binding specific proteins with cell membrane.

لا يُخيّب الله من سعى، ثق بالشفاء مادُمت تستشفي، وبالرزق مادُمت تسعى، وبالوصول مادُمت تحاول، وبالفرح مادُمت ترضى، وبالزيادة مادُمت تشكر، وبالإجابة مادُمت تسأل وتلح.

لا تملّ ولا تبرح حتى تبلغ، لا تخذلك عجلتك في المنتصف، ولا تقف وقد بدا النور قريباً .

واصل المسير، فجر البشرى قد دنا بإذن الله.