Sheet no13 (part 2)

Molecular biology

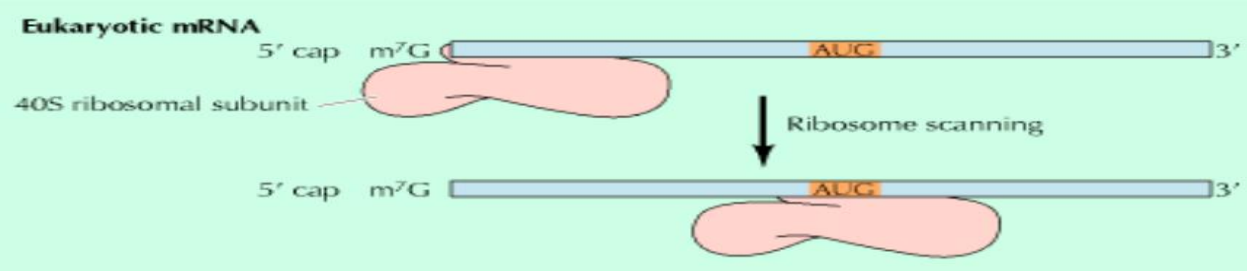
Writer: Mamoun Ahram
Corrector:
Doctor: doctor 2020

ملاحظه صغيره حتلاقييي كم معلومه من كيسي ك توضيح وشكرا الشيت بسيطه بالتوفيق

THE general mechanism for translation:

WE talked about prokaryotic cells we well continue in this sheet about eukaryotic cells .

Eukaryotic ribosomes recognize mRNAs by binding to the 7- methyl guanosine cap at their 5´ terminus, and then it starts scanning for the initiation regions to start translation.

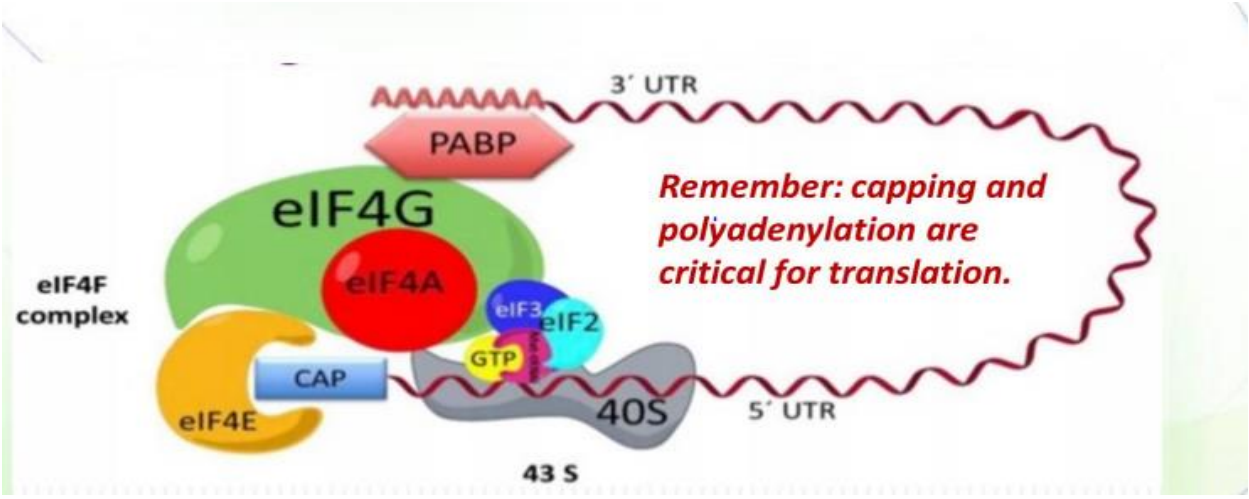


*** Notice here in the figure the translation **does not start** directly first the 40s subunit recognizes the cap and scans to find the initiation region.

eIF4 initiation

1.The eIF4 initiation factors form a complex that **links** the poly-A tail to the CAP via poly-A binding protein (PABP) to the cap, so it look like a bridge between the 3' and 5' of mRNA..

2.The eIF4 initiation factors then bring the mRNA to the 40S ribosomal subunit.



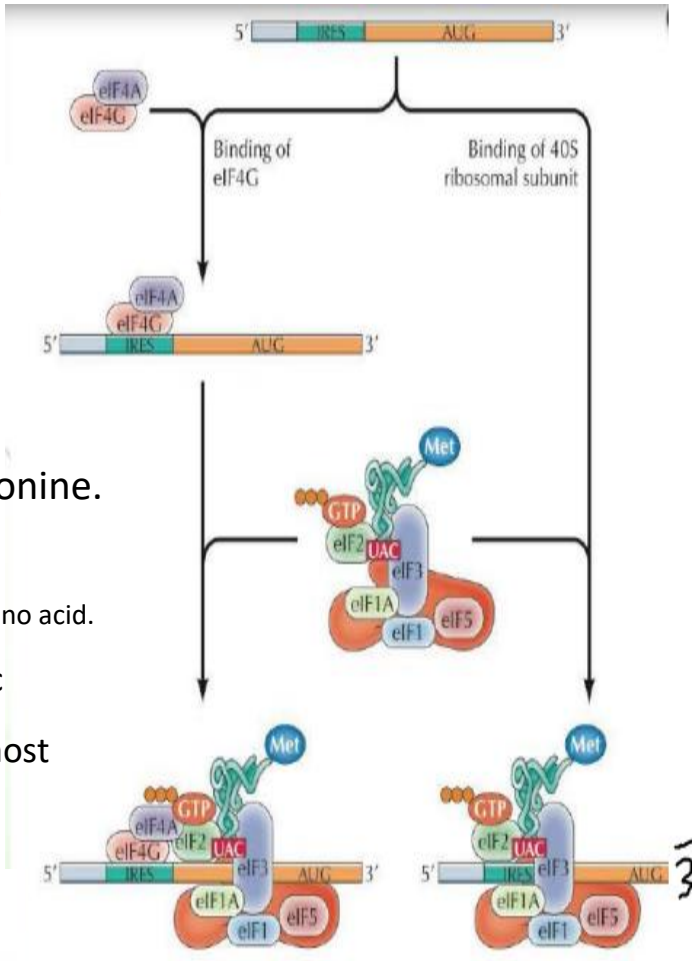
Internal ribosome entry site (IRES):

The 40s subunit can recognize mRNA by two methods:

1-5'cap (we have talked about it in the figure above)

2-IRES So, what is IRES: it is a small mRNA element found in 5' UTR and easily recognize by 40s subunit or first it bound to eIF4G and then recognize by 40s. (5' cap independent way)

- So again alternatively, internal ribosome entry site (IRES) exist in some other mRNAs and is recognized by the 40S ribosome or eIF4G protein followed by recruitment of the 40S ribosome.



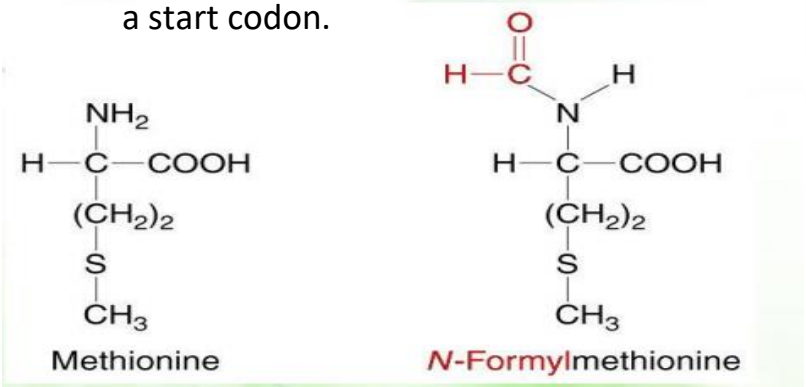
The first amino acid (start codon):

- Translation always initiates with the amino acid methionine, usually encoded by AUG.

*In most bacteria, it is N-formyl methionine.

(توظيحح) N-formyl methionine is simply consists of methionine and formaldehyde in the N terminus of amino acid.

So again, in both prokaryotic and eukaryotic we have methionine as start codon but in most bacteria, we have N-formylmethionineas a start codon.



NOW WE WILL TAKE ABOUT stages of translation, protein factors that are involved in the translation process, regulation of translation and some models that regulate synthesis of proteins.

Building a Polypeptide: -

Translation contains **three** stages:

1- Initiation 2- Elongation 3- Termination

All three stages require protein "**factors**" that aid in the translation process (these protein factors help in regulating this process)

Translation initiation:

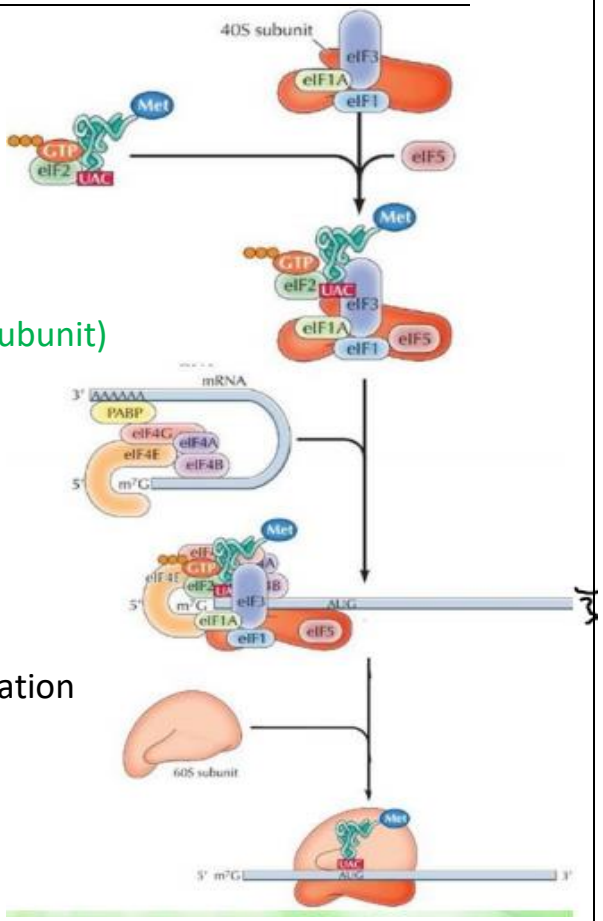
-Remember that we have a start site in the mRNA which is AUG codon, a codon for methionine amino acid.

1-tRNA forms a complex with 40S ribosomal subunit: at the beginning the tRNA that carries the methionine amino acid should assemble to the 40S ribosomal subunit (the small ribosomal subunit)

2- mRNA joins the complex (tRNA complex)

3- After that, the 40S ribosomal subunit scans for the first AUG.

4- The large ribosomal subunit joins them all (the small subunit and mRNA) to start the translation



#During all these steps we have protein factors that aid and help to regulate translation initiation (A large group of initiation factors facilitate every step), in this example eIF2 (eukaryotic initiation factor 2) which is very important in bringing the tRNA with the methionine amino acid with the small ribosomal subunit (eIF2 brings tRNA to small ribosomal subunit)

-Also, we have eIF2 and eI4G which play very important rule in bringing mRNA to tRNA/40S ribosomal subunit. (NOTE: you just need to know the general factors involved 'those mentioned in the sheet' because it's very complicated)

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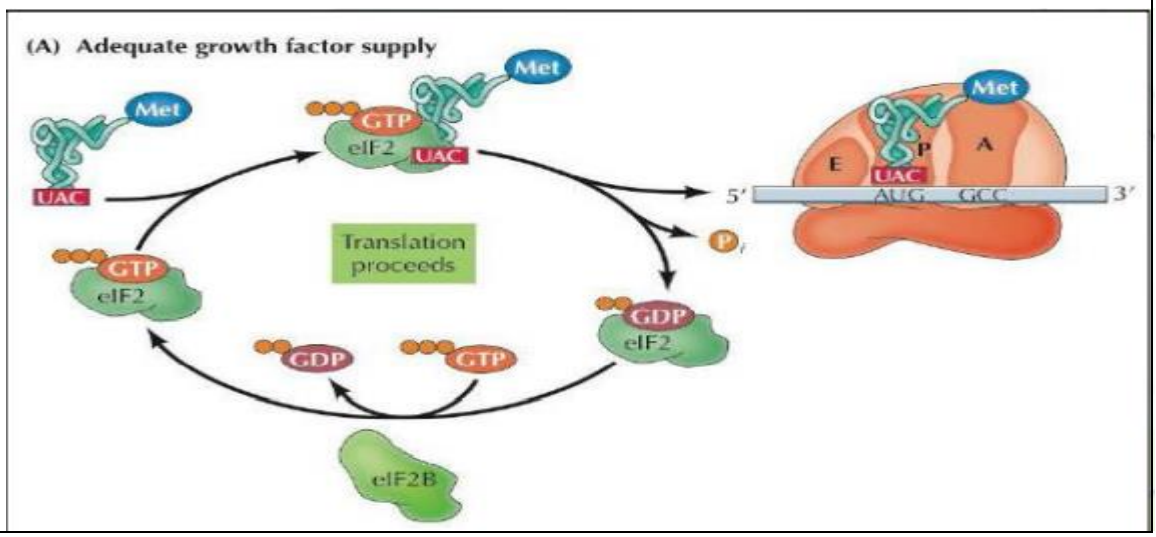
Regeneration of eIF2:

-Important information: eIF2 can be regenerated and recycled WITH Eif 2B so it can be used several times in translation initiation.

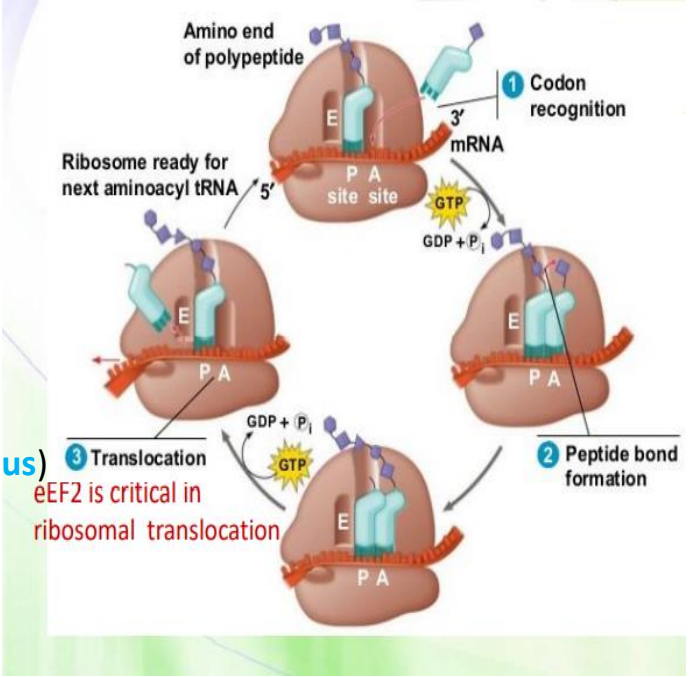
At the beginning eIF2 should be complexed to GTP to be active, and then when the correct tRNA (with methionine) is inserted, GTP is hydrolyzed to GDP.

At this moment the GDP will be dissociated from the eIF2and the eIF2 will be inactive in this stage.

After that the eIF2 will bind again to new GTP and becomes active, the active eEF2/GTP complex must be regenerated by exchanging of the bound GDP for GTP, and this cycle can be repeated several times.



Translation elongation I:



It contains three steps:

1-Aminoacyl-Trna binding by eEF1α:

aminoacyl-tRNA bind to the A site.

2- Peptide bond formation: between the growing polypeptide (**the carboxy terminus**) and the coming amino acid (N- terminus)

3. translocation with the help of elongation factors (**eEF2**): the (P) and the (A) sites are translocated, and uncharged tRNA will exit the ribosomes so it can be used for another amino acid and the growing polypeptide linked with tRNA stays in the (P) site.

-So as the translation occurs, the polypeptide chain draws again and again.

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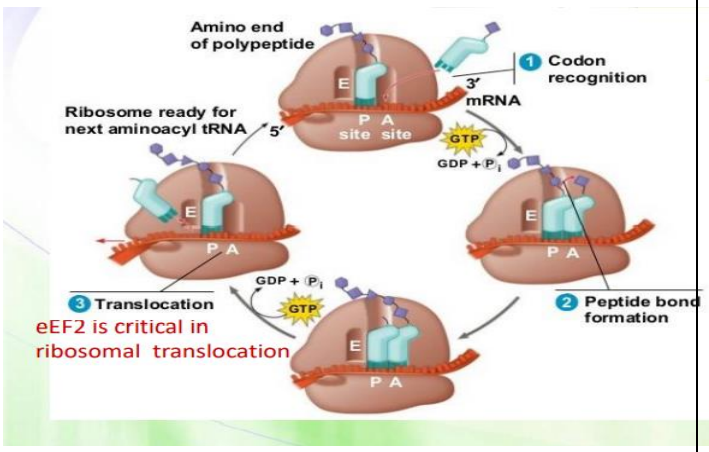
IN more details or information or illustration call it what you like



1- First the new amino acyl tRNA will come and bind to the (A) site and in the A site we have the codon that is complementary to the anticodon

2- After binding, a peptide bond will start forming between the growing polypeptide (carboxy We have different eukaryotic elongation factors so you need to distinguish between initiation and elongation factors, so for now we have: 1- eEF1α brings next aminoacyl-tRNA to the A chamber in the ribosome 2- eEF2 that is critical in ribosomal translocation (tRNA translocation and the exit of

uncharged tRNA from the ribosomes)4 | Page terminus) and the coming amino acid (N- terminus).

3- After that, the tRNA in the (A) site will catch and carry the polypeptide and the tRNA in the (P) and (A) sites will be translocated. So, the uncharged tRNA will be removed from the ribosome through the exit site and the tRNA in the (A) site will be translocated to the (P) site, and now the ribosome is ready for the next amino acyl tRNA to come and so on.

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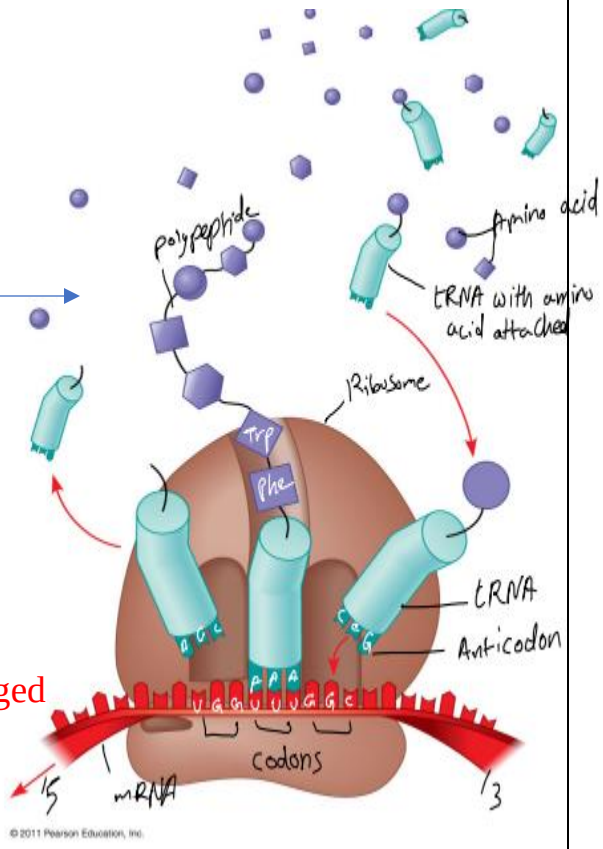
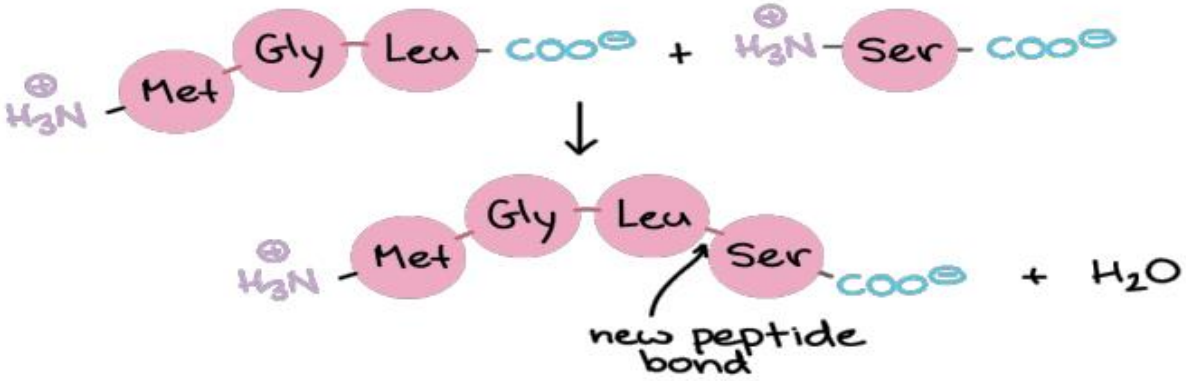


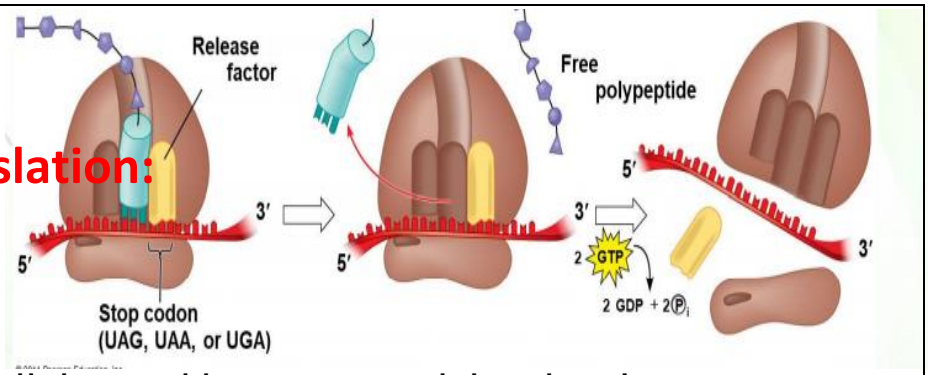
Remember that reading of mRNA is from 5' to 3'

-you can see the entrance of the aminoacyl tRNA, binding to its codon in the (A) site, and then the formation of a peptide bond between the growing polypeptide and the new amino acid, then the translocation of tRNA in the (A) and (P) sites and the exit of the uncharged amino acid (actually the uncharged amino acid will be again charged and used in the next protein synthesis)

- During the elongation stage, amino acids are added one by one to the preceding amino (N)-terminus (of the coming tRNA) to the carboxy (C)-terminus of the growing chain.



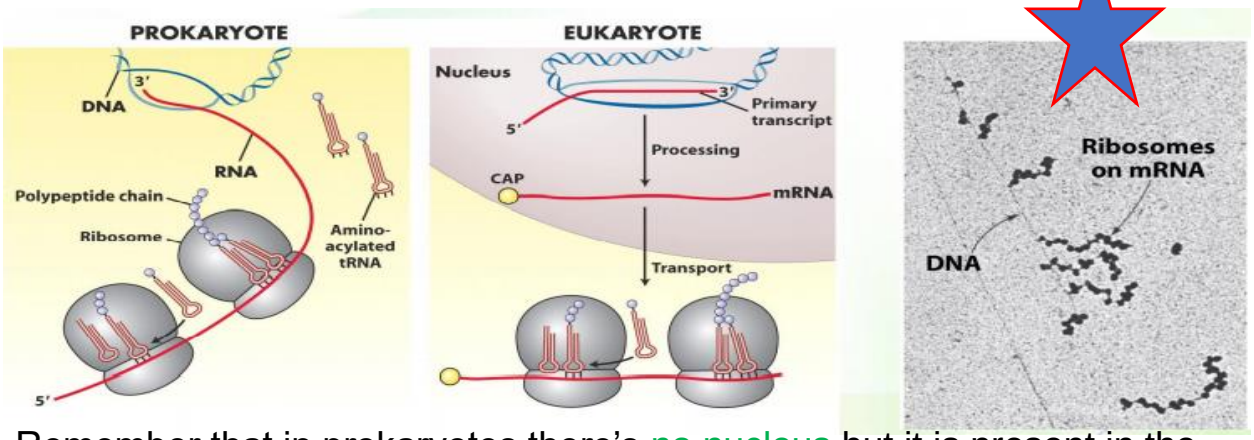
Termination of Translation:



It is the last stage of the translation process, so we need a signal that will tell the machine to stop and that there's no more synthesis of proteins.

The codons UAA, UAG, and UGA are the stop signals (when the ribosome reaches one of these codons, that's a signal for termination). They are not recognized by any tRNAs (because they are stop codons and not codons for any amino acid), but a release factor protein.

The A site accepts the release factor, which causes the release of the polypeptide, and the translation assembly then comes apart (the release of the ribosomal units from the mRNA).

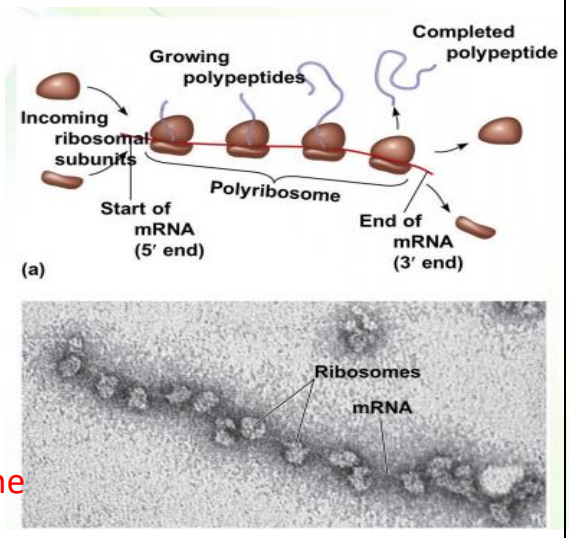


-Remember that in prokaryotes there's no nucleus but it is present in the eukaryotic cells, so that translation and transcription are coupled in space and time in prokaryotes (occur at the same time and place), BUT it's not the same story for eukaryotic cells because the transcription occurs in the nucleus then the primary transcription undergoes processing to produce mRNA and this mRNA should be transported to the cytoplasm where the translation occurs.

-So, the transcription and translation are directly coupled in prokaryotes while they're indirectly coupled in eukaryotes.(الخلاااااااااصه)

Polyribosomes (polysomes): A single mRNA molecule is translated by several ribosomes simultaneously (simply polyribosomes used to describe the translation of a single mRNA molecule by several ribosomes simultaneously to produce several copies of the polypeptide chain), and these polyribosomes exist in both prokaryotes and eukaryotes. Each ribosome produces one copy of the polypeptide chain specified by the mRNA. When the protein has been completed, the ribosome dissociates into subunits that are used in further rounds of protein synthesis.

-Here for example (the pic above *) in this figure, which is for a prokaryotic cell, you see electron microscopy for the DNA sequence and we have several mRNA copies and we have several ribosomes bond to single mRNA molecule.



Polysomes (in eukaryotes): We have a little difference between prokaryotes and eukaryotes cells by means of translation and transcription coupling, and in eukaryotic cells mRNA is located in the cytoplasm where the translation takes place. A number of ribosomes can translate a single mRNA simultaneously, forming a polyribosome (or polysome). Polyribosomes enable a cell to make many copies of a polypeptide very quickly.

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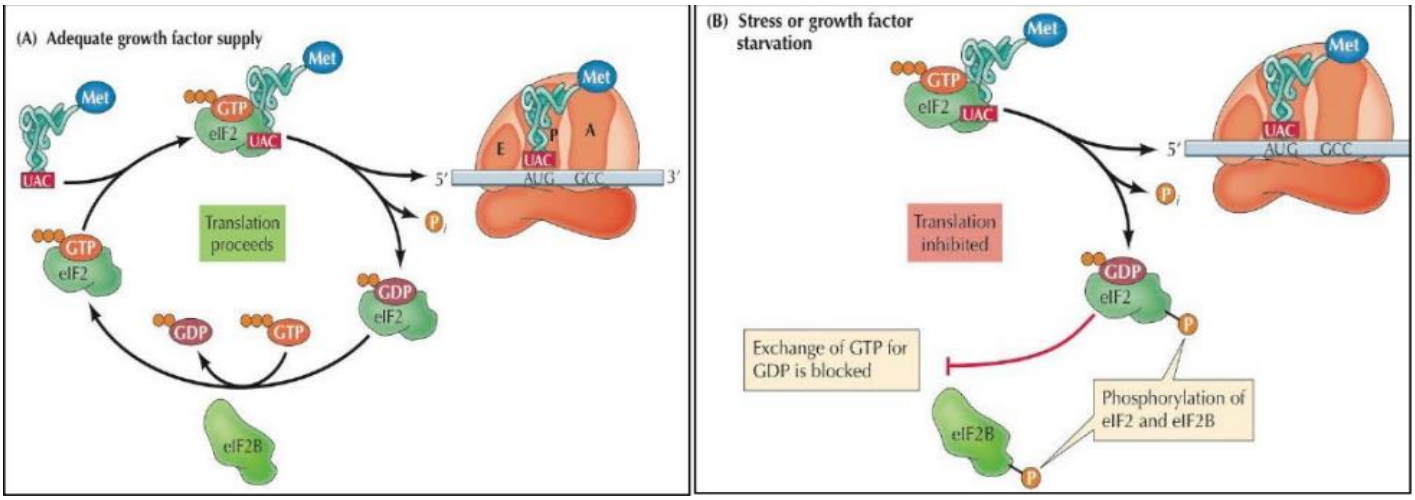
Regulation of translation: -The translation process id regulated process and can be regulated at different levels: global level and specific level.

Global regulation:



Previously we talked about degeneration and recycling of eIF2 and this is very important in the initiation of translation, the function of eIF2 of bringing tRNA with the methionine amino acid to the small ribosomal subunit to initiate the translation, so here we have adequate and enough supply of essential amino acids and growth factors, so continuous activation and deactivation of eIF2 lead to continuous translation process while stress and low essential amino acids and growth factors supply will lead into inactivation of the eIF2 by phosphorylation of it and eif2b and there's no more initiation of translation and then translation is inhibited.

Heme and protein synthesis: (specific example of global regulation) -
Heme: part of hemoglobin (which is kind of protein found in our red blood cells and responsible for transporting of oxygen from the lung to the tissues and CO2 from the tissues to the lung)

1-First, in reticulocytes(immature erythrocytes which are type of cells in our bone marrow),heme stimulates protein synthesis -synthesis of hemoglobin occurs in these cells.

2-The mRNA is translated only if adequate heme is available to form functional hemoglobin molecules.

3-This is done via regulating the activity of eIF-2, which is responsible for escorting initiator methionyl tRNA to the ribosome.

4-eIF-2 must be bound to GTP to be active. When it is released from the ribosome, GTP is hydrolyzed to GDP, which must be exchanged with GTP for eIF-2 to be active again.

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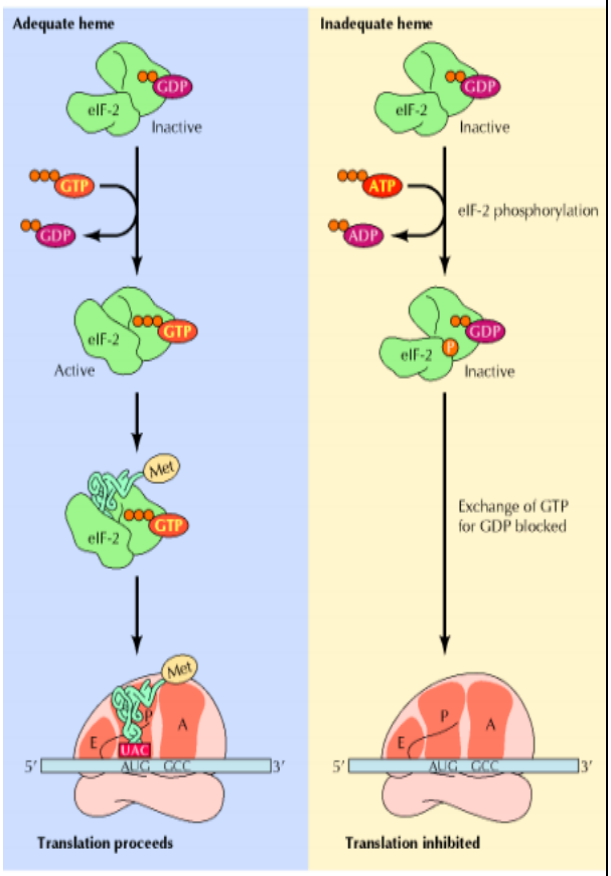
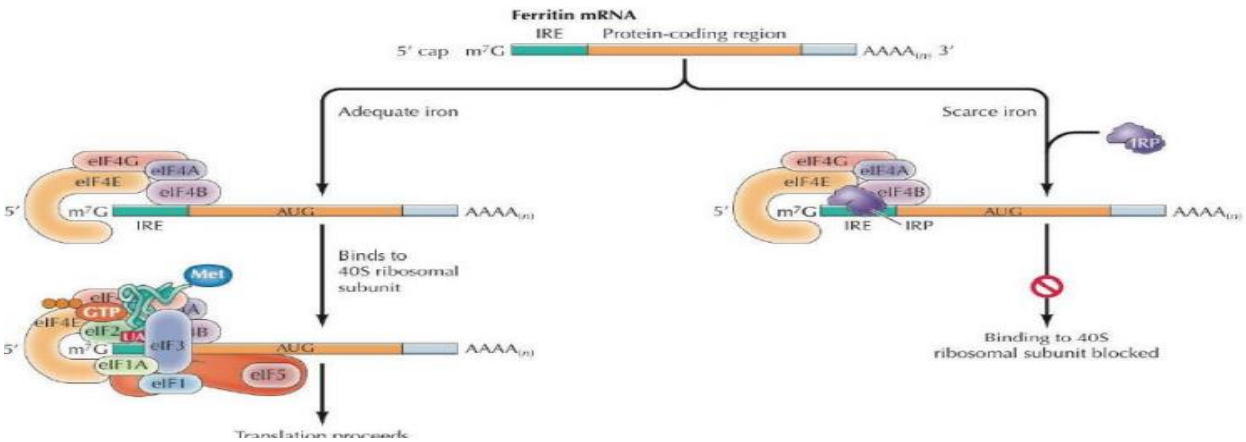
1- If adequate heme is available eIF2 can be activated, deactivated and recycled, GDP-GTP exchange occurs and translation is able to proceed. (Initiation of translation)

2- If heme supplies are inadequate translation will be inhibited because there's no activation and recycling of eIF2, a protein kinase that phosphorylates eIF-2 is activated. Phosphorylation of eIF-2 blocks the exchange of GTP for GDP, so eIF 2/GTP cannot be regenerated and translation is inhibited. (NO initiation of translation)





FERRITIN

There's a relationship between the iron conc. and ferritin expression, in case we have low amounts of iron in our body that means that there's no iron to bind to a specific protein called iron responsive binding protein and this protein once it's free without iron, it can go to iron responsive element at the untranslated region at the 5' end of the ferritin mRNA and once it binds to the iron responsive element it can stop the initiation of the translation while when we have adequate amount of iron then the iron will bind to the iron responsive binding protein and inhibit its binding to the iron responsive element at the 5' end of the ferritin mRNA so the translation can proceed and produce more ferritin protein.

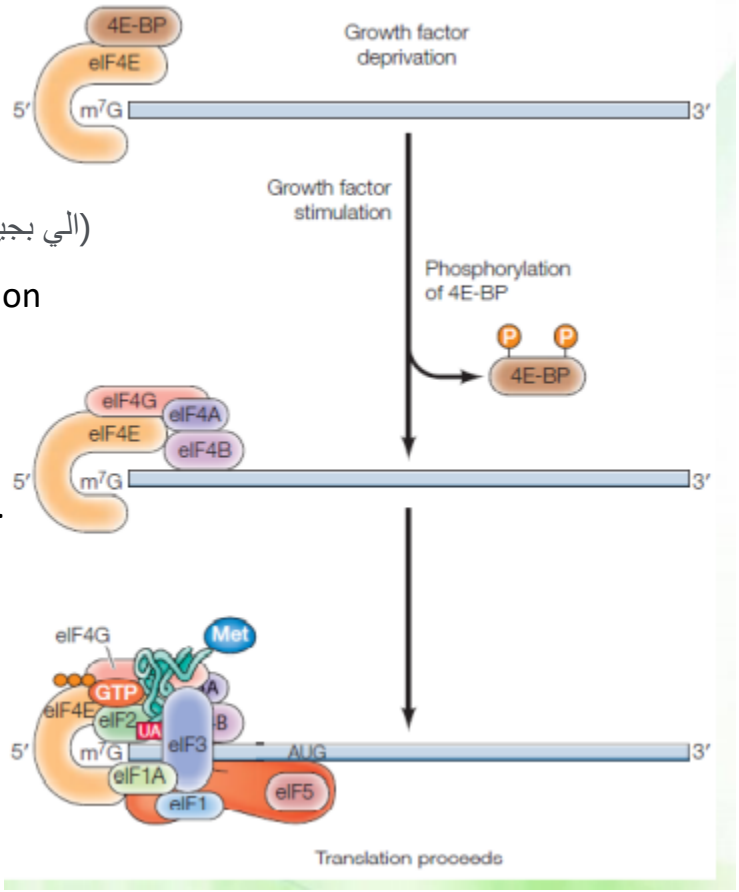
EIF4E

eukaryotic translation initiation factor involved in directing ribosomes to the cap structure of mRNAs.

(الي بجيب الكاب ع البولي اي تيل حكينا عنه هان بنخربه وبس)

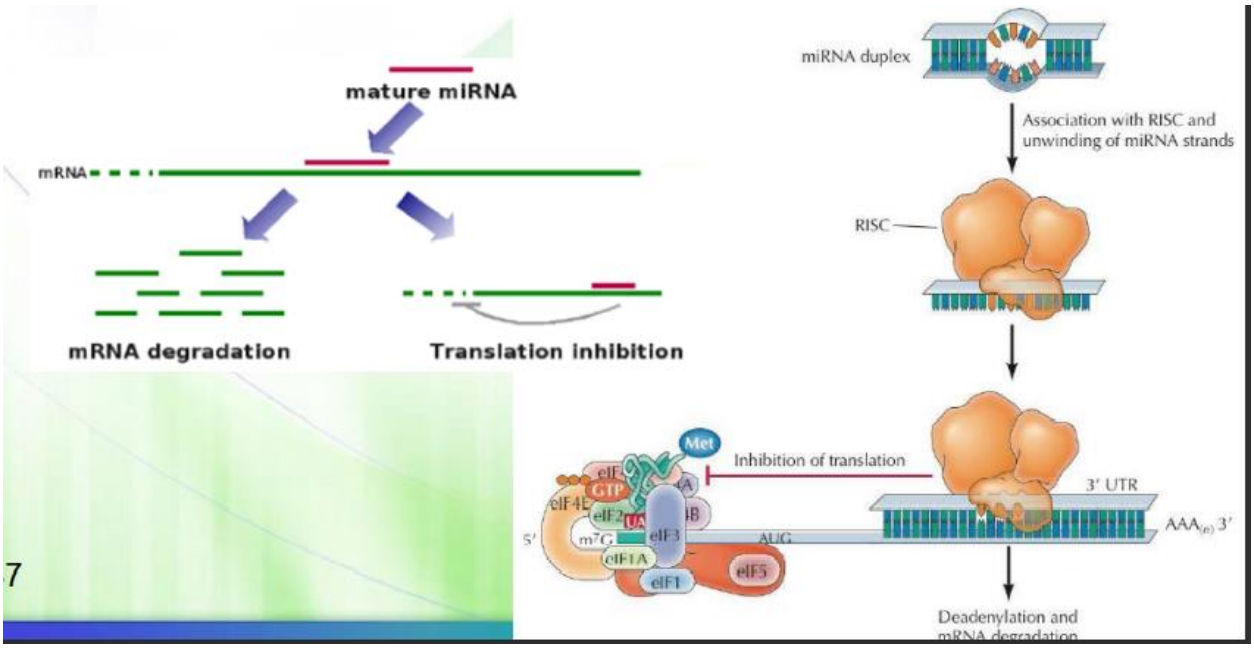
In the absence of growth factors, translation is inhibited by eIF4E binding proteins (4E-BPs), which bind to eIF4E and block its interaction with eIF4G.

Growth factor stimulation leads to the phosphorylation of 4E BPs, which then dissociate from eIF4E, allowing translation to proceed



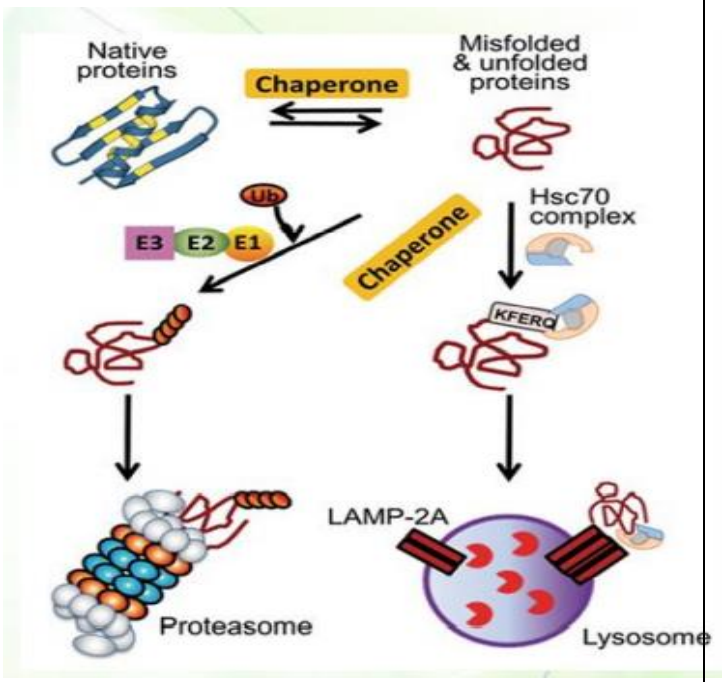
Regulation by microRNA (miRNA)

miRNA: a short non-coding RNA synthesized by RNA Pol II into single-stranded, primary miRNA (pre-miRNA) transcript which gets processed and one strand loaded into RISC (RNA induced silencing complex) complex where miRNA is targeted to the 3'-UTR of mRNA and the miRNA can be complementary to mRNA -once it finds this complementary sequence- then the RISC complex can induce the degradation of the mRNA or inhibit the translation process without degrdation.



Fate of (mis)- and (un)-folded proteins:

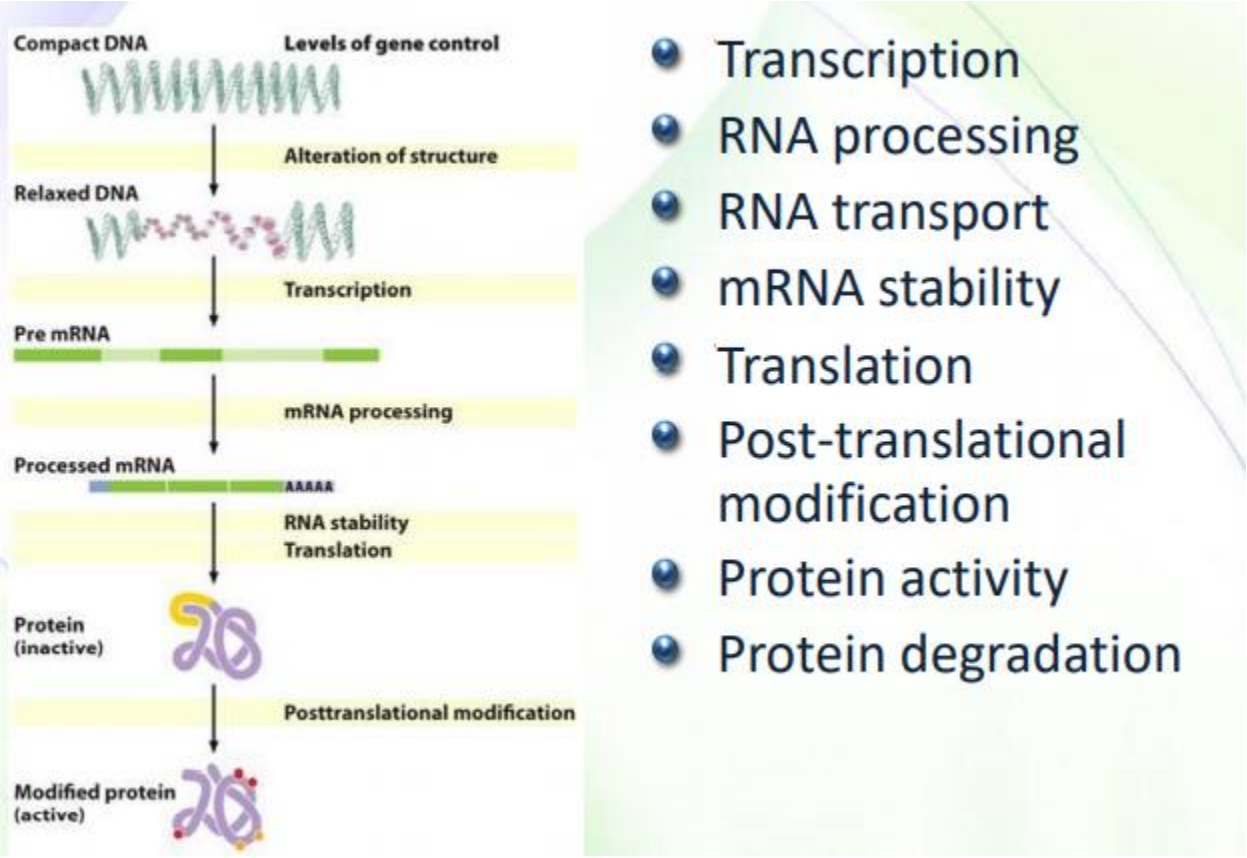
You need to remember that there's relationship between the structure and function of any protein which means that a protein should have a specific structure folded to do the proper and right function which should require proper folding, so in our cells there's a



kind of proteins that regulate the proper folding of proteins called chaperons but in case we have mis and unfolded proteins that need to be removed by cells through the degradation by proteases.

NOTE: any protein in the cell wont last for ever like humans in earth all will be degraded .

this process occurs in either in degradative subcellular organelles like lysosomes (rich in proteases and have very low PH) or by the macromolecular proteasomes (they are complex of proteins that can degrade the unneeded of mis folded proteins by a process called ubiquitinylation which involves labeling mis folded proteins by small polypeptides known as ubiquitin).



Just summery of all what we have taken in this course in regulation

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