CYTOLOGY

Premed 2018 - JU

◉ Sheet ○ Slides

Number

20

Done by:

Horani & Rabei

Corrected by:

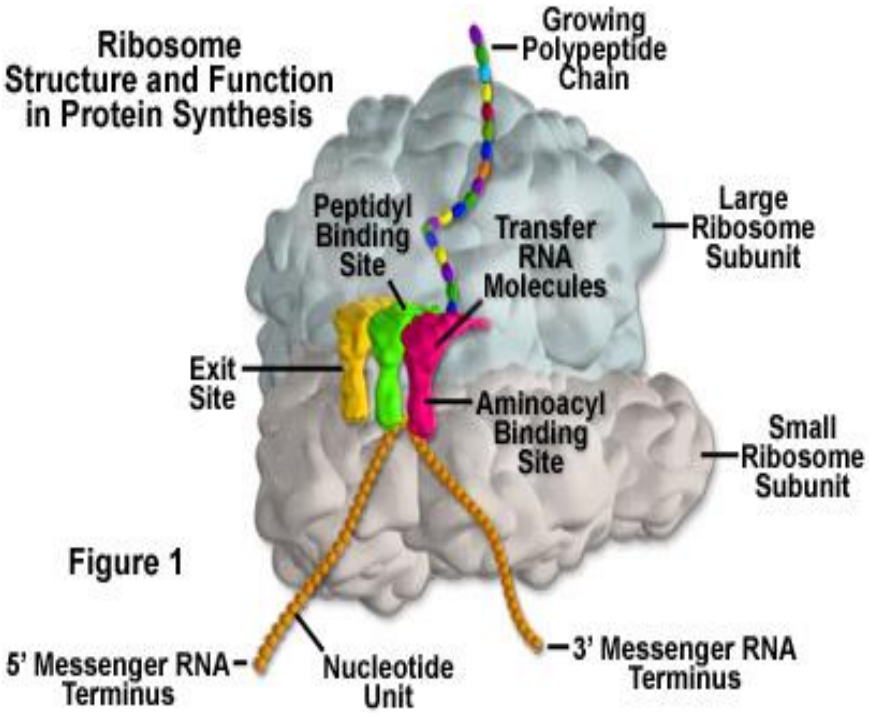
Anas Mahseeri & Rabei

Doctor

Mamoon Ahram

A brief revision:

A the figure shows this is a ribosome; which consists of small subunit and large subunit, the large ribosomal subunit is responsible for the formation of peptide bond between two amino acids , and has three champers where the tRNA molecules bind, and these champers are designated as A,P and E.



A → Aminoacyl-tRNA binding site

B → Peptidyl-tRNA binding site

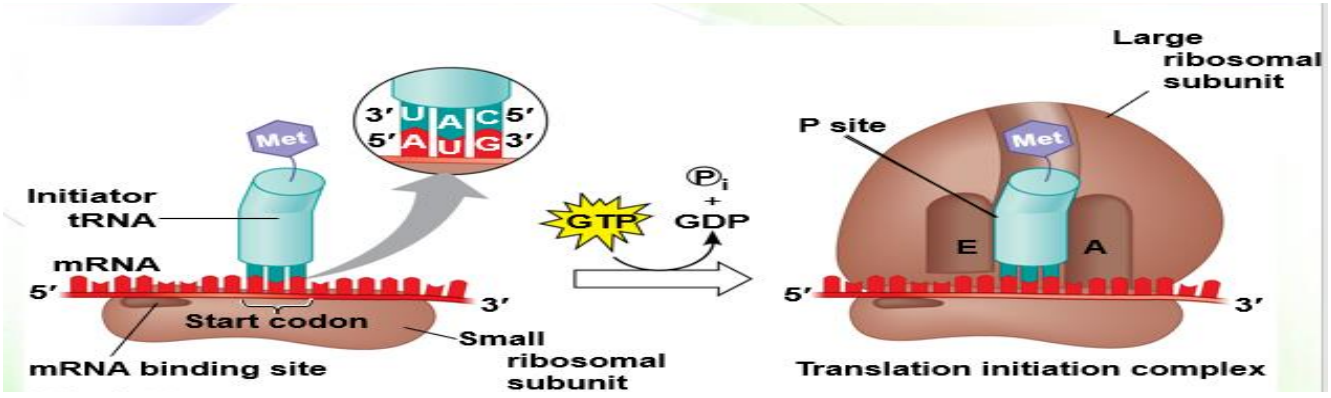
E → exit-tRNA binding site

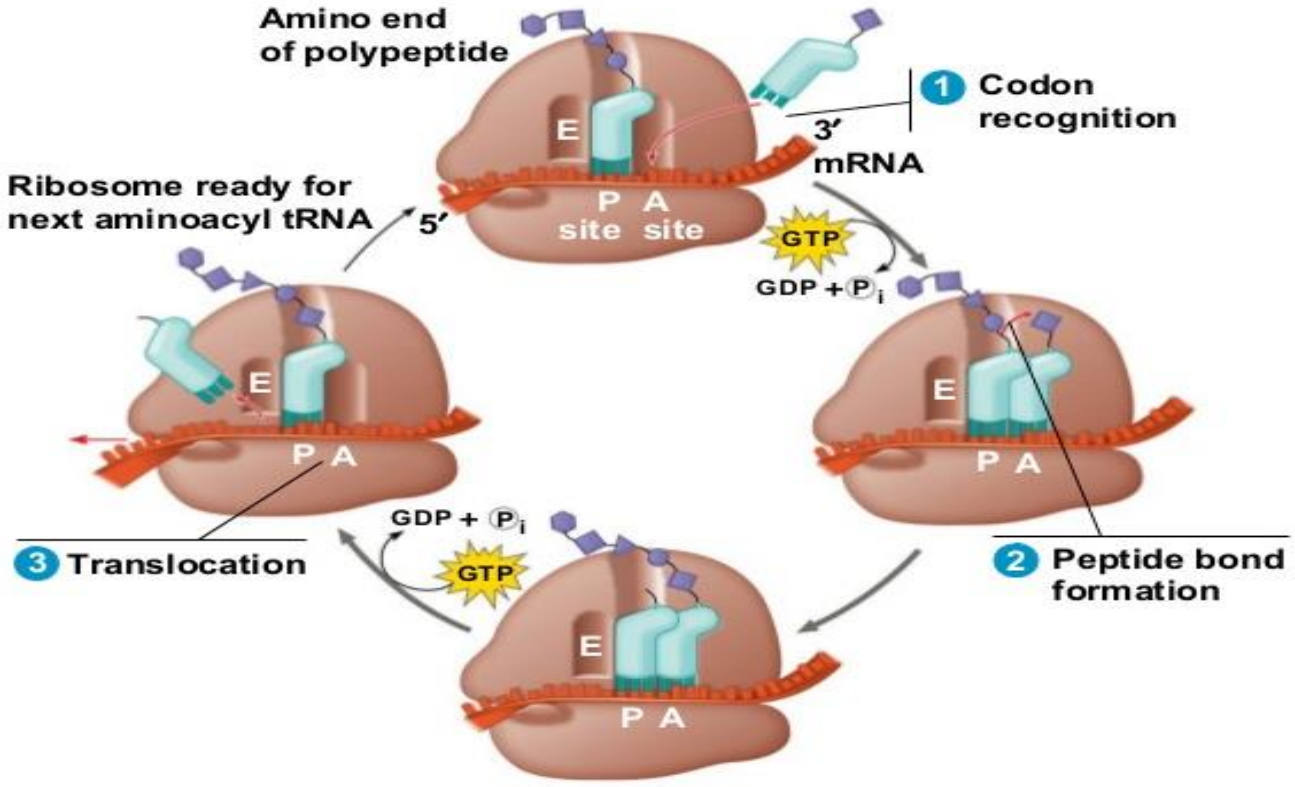
Let's begin:

So what happens in order to form a polypeptide is that there is a sequence of events takes place:

1- Binding of small ribosomal subunit with the mRNA followed by the binding of the first tRNA (containing the anti-codon) to the start codon (AUG). the first tRNA usually carries methionine residue.

2- Entry of the large ribosomal subunit and formation of ribosome; the first tRNA fits into the P site.





3- As the figure above shows (notice the energy requirement in the figure above) , this is followed by the entry of the second tRNA molecule (binds to the A site) ; so the two tRNA molecules are next to each other, after that the peptide bond is formed…. When they are next to each other….. the amino acid on the first tRNA (located in the **P SITE**) jumps onto the amino acid carried by the second tRNA (located in the **A SITE**).

*4- then the first tRNA is empty (uncharged) so the ribosome shifts (moves the mRNA and changes the codon inside), Simultaneously the uncharged tRNA enters the E site and leaves the whole compound and the other tRNA moves to P site (was in A SITE), also a new tRNA enters the A site, and so on until we hit a stop codon.

The general mechanism of translation:

- Contains Three Stages: initiation, elongation and termination; each stage needs a set of proteins. → → → → -------→
- The direction of mRNA reading : 5` →3`
- The protein is synthesized from the N terminus to the C terminus.

For initiation: initiation factors

For elongation: elongation factors

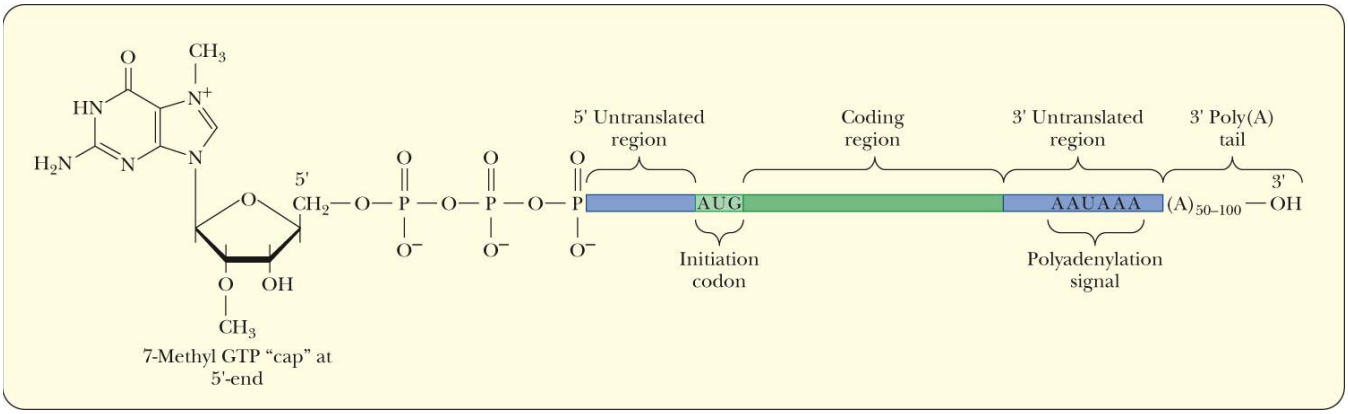
For termination: one single protein will be discussed later

So now Where do ribosomes start translation at?

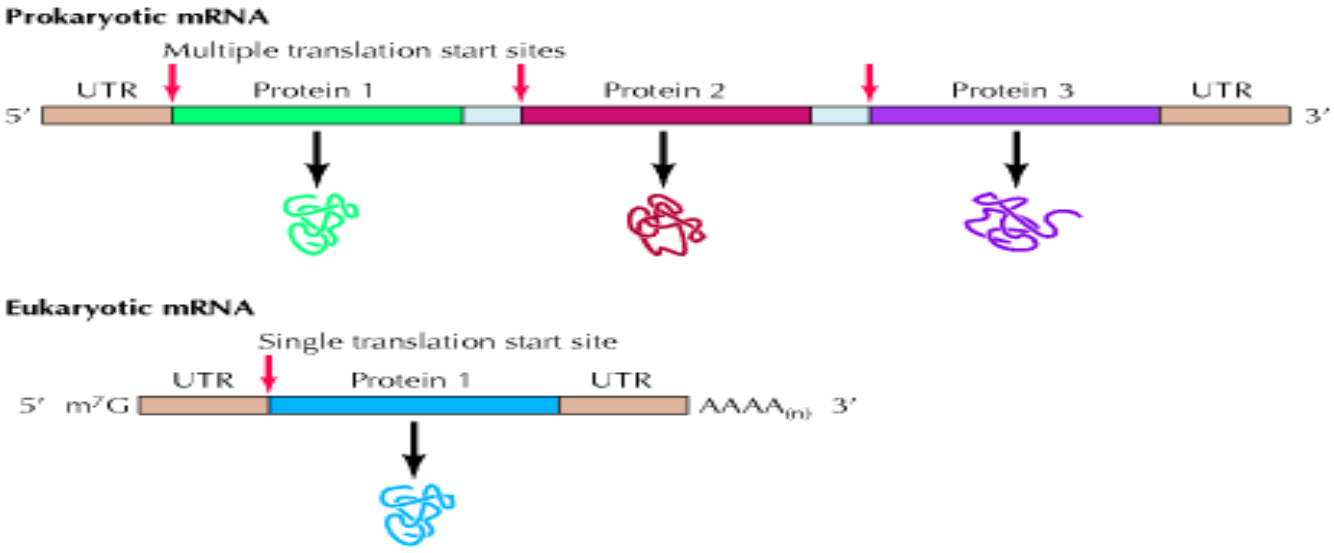
They Start at the codon (AUG), **But** not necessarily the first AUG they read.

The 5´ terminal portions upstream of the initiation sites of both prokaryotic and eukaryotic mRNAs contain noncoding sequences, referred to as 5´ untranslated regions (UTRs).

There is also a 3'-untranslated region.



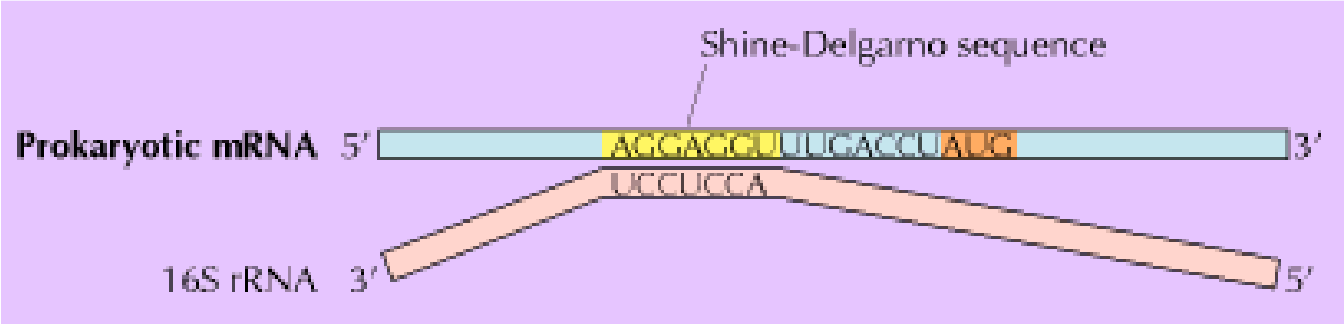
Now, let's have a look on this polycistronic mRN



There is more than one AUG; some present at the start sites and some AUGs inside the mRNA sequence. but the question is how the ribosomes know where to start if there are multiple AUGs?

The answer is;

By the presence of Shine-Dalgarno sequence; the sequence is located before the AUG and is complementary to a sequence on 16S rRNA,
Remember that the polycistronic sequence is only existed on prokaryotes, so in prokaryotes the 16S rRNA is part of the small subunit.



What happens is that the small ribosomal subunit binds to the mRNA and scans it, if the Shine-Delgarno sequence (since it's complementary to the 16S) exists, the small ribosomal subunit binds tightly to the mRNA and makes itself ready to start translation and then it reads the first AUG it faces.

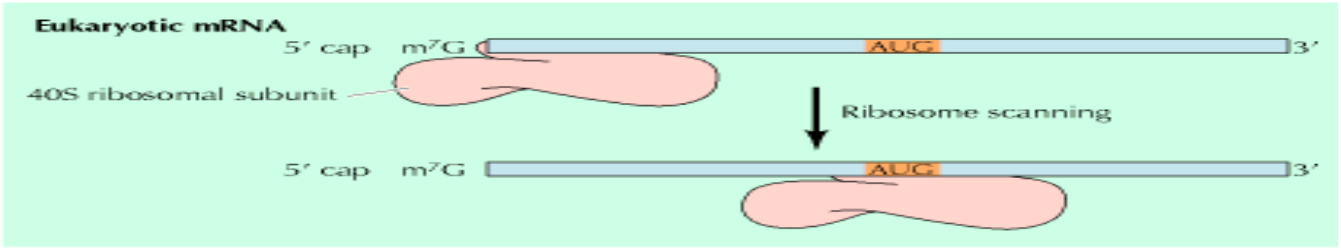
For clarification; the Shine-Delgarno sequence is complementary to a sequence found on the 16S, since that the small ribosomal subunit in prokaryotes uses the Shine-Delgarno sequence to know where to start specifically while the presence of multiple AUGs.

Rule: In prokaryotes, before each AUG that must be used as start codon the the Shine-Delgarno sequence exists. (shine-delgarno is a consensus sequence located in the UTR)

After the binding to the AUG the very first tRNA comes and binds to the small subunit and then the same mechanism mentioned before occurs.

But in eukaryotes, there are two mechanisms for the ribosome to recognize the first AUG to initiate translation,

A) The first one is recognizing mRNAs by binding to the 7-methylguanosine cap at their 5′ terminus so that is how the cap is important for translation.



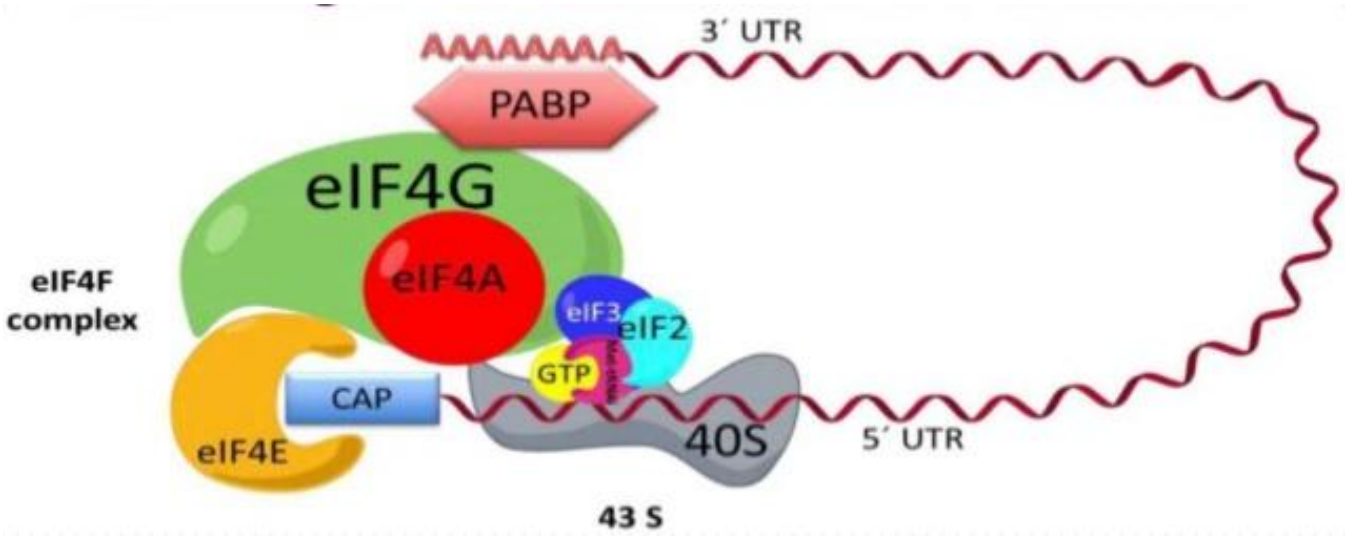
For that mechanism to be done we need the poly-A tail also, that exists after the region that will be translated.

Wait a minute!!!, the poly-A tail exists at the end of the mRNA. how is it important to translation????

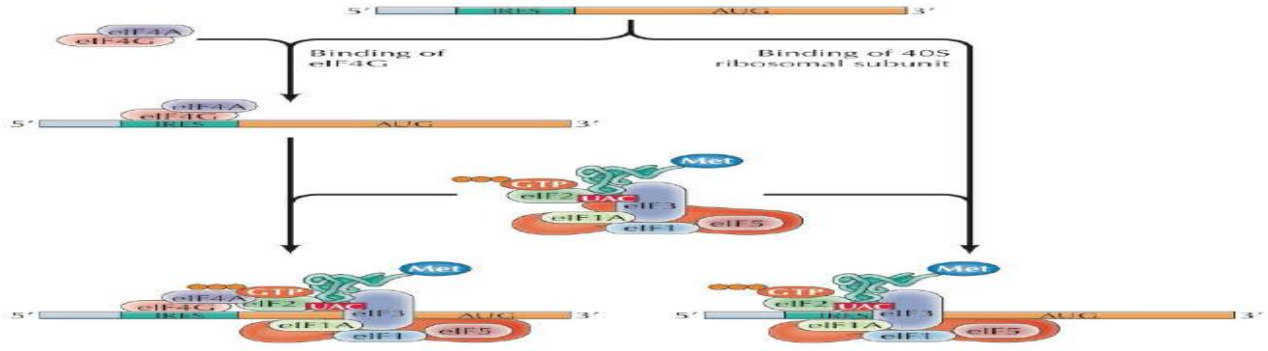
There is a protein known as Poly-A tail binding protein (PABP) -that from its name- binds to the poly-A tail.

The PABP is at the end of the mRNA, so the initiation factor, eIF4G, is member of a complex that links the poly-A tail to the CAP via poly-A binding protein (PABP) and the CAP-binding protein eIF4E,so now the mRNA is folded via a complex of several proteins (eIF4E,eIF4G,eIF4A, etc…) after that the mRNA is now ready to be translated.

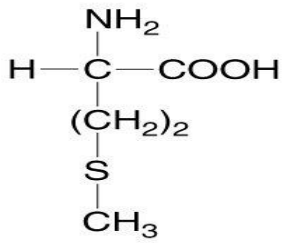
If this set of reactions didn't happen, translation will never begin in some mRNAs.



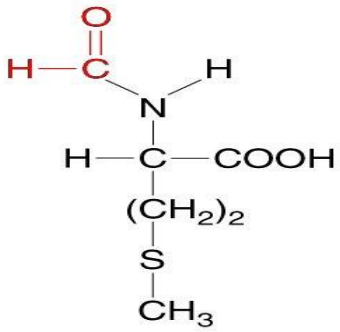
B) Alternatively, internal ribosome entry site (IRES) exists in some other mRNAs and is recognized by the 40S ribosome or eIF4G protein followed by recruitment of the 40S ribosome, IRES is similar to Shine-Delgarno sequence, this is the other mechanism of initiation of translation in eukaryotes



In bacteria the start codon is AUG and translated into methionine, a special form of methionine known as N-formyl methionine.



Methionine



N-Formylmethionine

So the first amino acid in most bacteria formyl methionine. And that isn't true in eukaryotic cells.

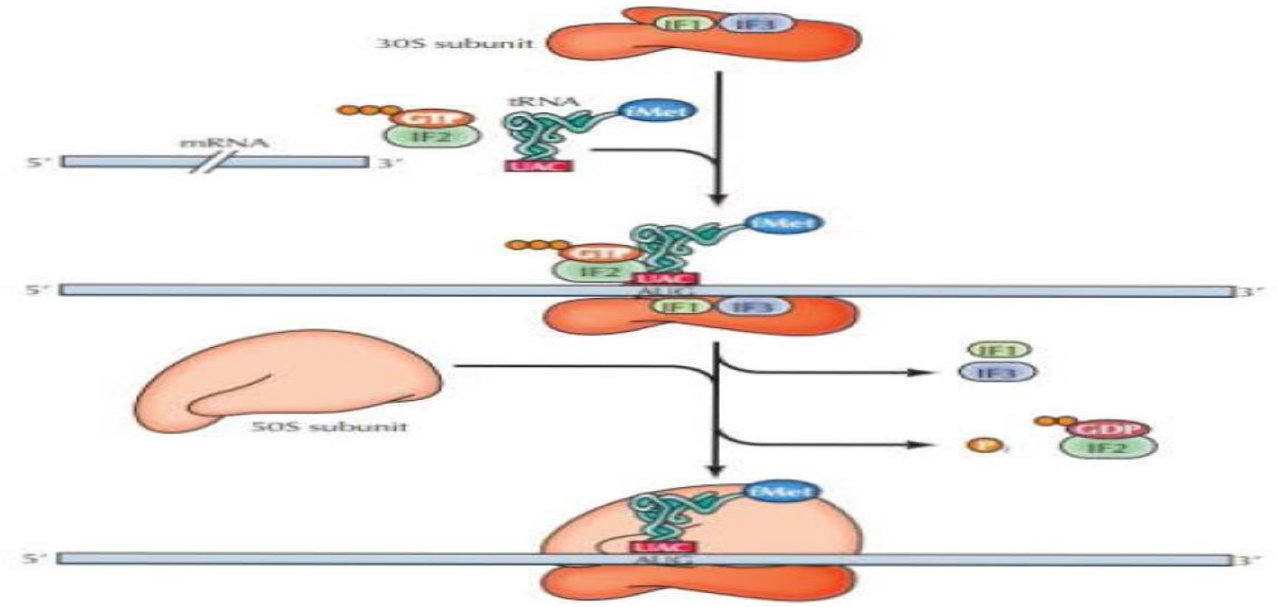
(be familiar with the differences between eukaryotic and prokaryotic cells because it is abundant in D. mamoon`s questions)

Let's Rediscuss the stages of translation but with more details:

In prokaryotes,

Translation initiation: The 30S (small) ribosomal subunit binds to mRNA and fmet-tRNA in the presence of GTP and the three initiation factors, IF-1, IF-2, and IF-3, forming the 30S initiation complex.

The 50S (large) ribosomal subunit is added, forming the 70S initiation complex (the ribosome).



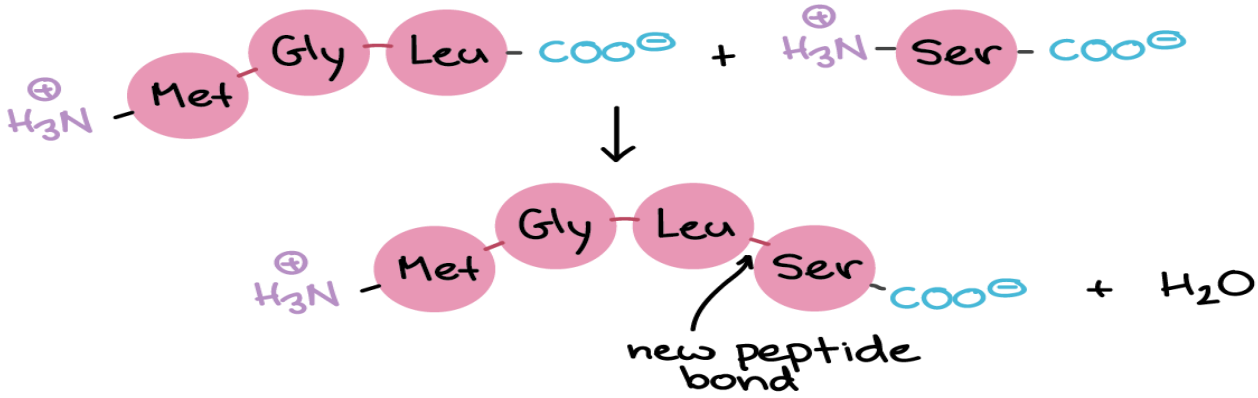
Elongation, Step 1: An aminoacyl-tRNA is bound to the A site on the ribosome. Elongation factor EF-Tu (Tu) and GTP are required. The P site on the ribosome is already occupied.

Step 2: Elongation factor EF-Tu is released from the ribosome and regenerated

Step 3: The peptide bond is formed, leaving an uncharged tRNA at the P site.

Step 4: the uncharged tRNA is released. The peptidyl-tRNA is translocated to the P site, leaving an empty A site. The uncharged tRNA is translocated to the E site and subsequently released.

Additionally, in elongation stage, amino acids are added one by one to the preceding amino acid at the C-terminus of the growing chain, and we need GTP.

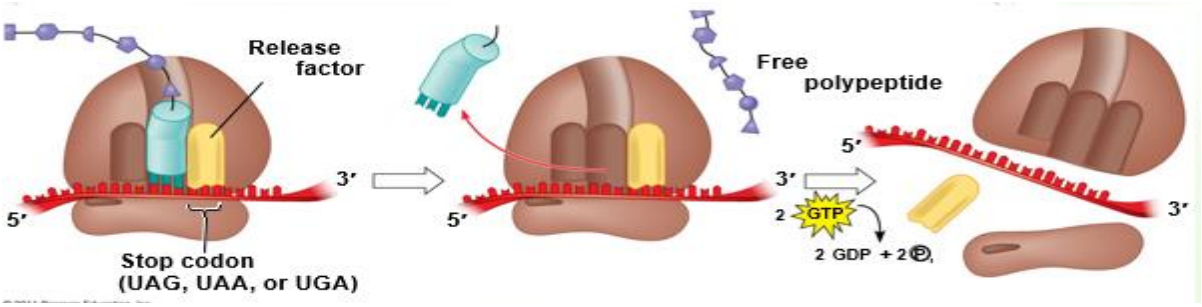


Termination,

The codons UAA, UAG, and UGA are the stop codons (we need to memorize them). They are not recognized by any tRNAs, but a release factor protein.

The A site accepts the release factor, which causes the addition of a water molecule instead of an amino acid and the dissociation of the whole ribosomal complex.

This reaction releases the polypeptide, and the translation assembly then comes apart (Take a look on the animation in the slides)



After that the doctor revised the mutations, but everything was in previous sheets, so we won't talk about it, we urge you to back to video and watch from 24:55 till 31:00 to make sure that you have got everything.

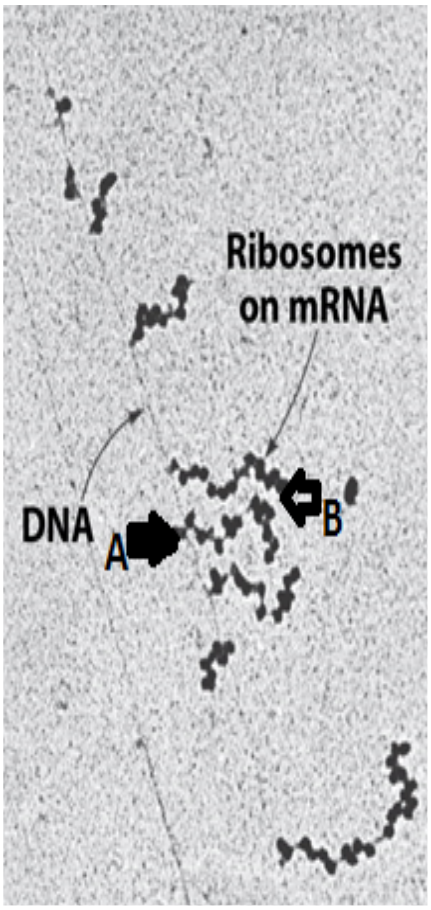
Transcription/translation coupling

** In bacteria translation and transcription are coupled, they happen at the same time and place. In eukaryotic cells this can never happen, the transcription happen then translation due to several reasons:

A) the existence of the nucleus. B) The mRNA processing.

Polyribosomes (polysomes): A single mRNA molecule is translated by several ribosomes simultaneously. Each ribosome produces one copy of the polypeptide chain specified by the mRNA, this phenomenon **occurs in prokaryotes and eukaryotes**.
(make sure that you know that this term (**polysomes)** differs from **polysomes** we disused in transcription)

Look at this EM image, the thin long line is the DNA, the black dense dots represents the ribosomes that are bound to the mRNA.



(very important to understand what we will discuss next)

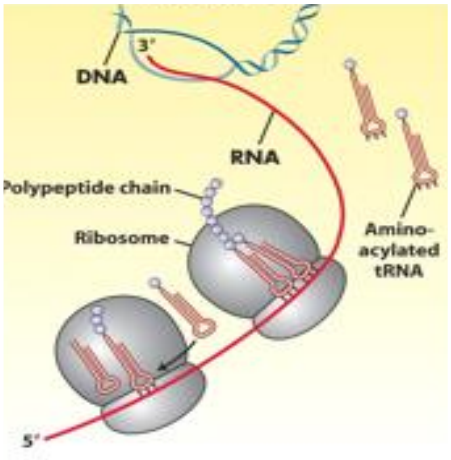
The beginning of the gene is at the top of the picture; so the mRNA is small at the beginning because it hasn't been fully transcribed, but at the bottom of the picture mRNA is long because it's terminating and has been fully transcribed.

Now look at the mRNA between A and B, A represents the 3` end and B represents the 5` end; this refers to transcription direction from 5` → 3`.

The very first ribosome (we mean the first ribosome to bound to mRNA) is found near to A, near to 3` end, this refers to translation direction 5` →3` so the first one will be the nearest to the 3` end and so the longest polypeptide exists at A. "for better understanding to this point look at the next picture"

The first ribosome bound before and is making the polypeptide chain, so its polypeptide chain is longer and it has translated more codons →so it will be nearer to the 3` end.

The last two picture exhibited is about translation in prokaryotes because the translation and transcription occur at the same time and place.



Polyribosomes enable a cell to make many copies of a polypeptide very quickly.

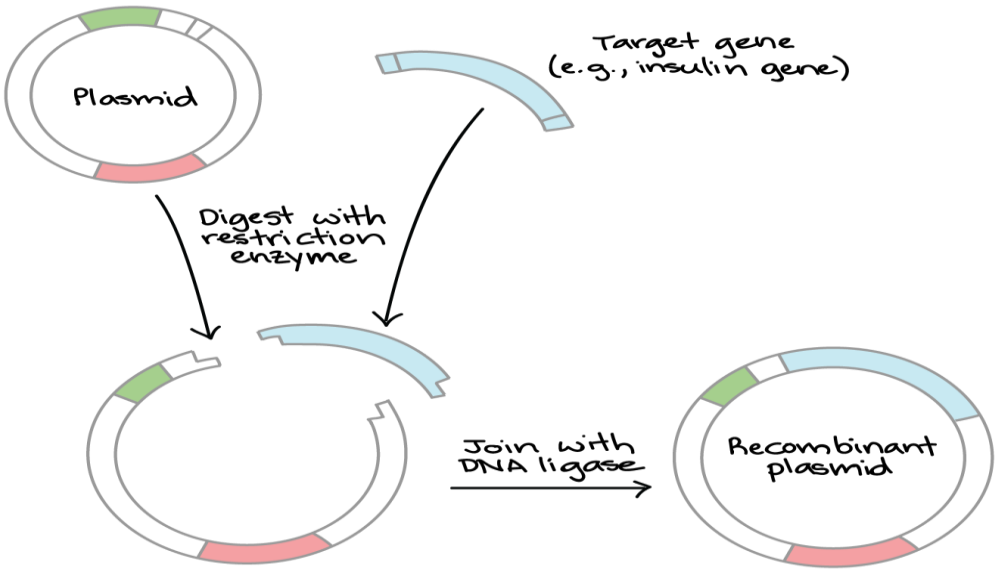
Cloning: making copies of certain DNA fragments (genes)

The question is How we do cloning?

in this technique we use bacteria as our copier to synthesize multiple copies of our gene of interest .So we take plasmid from bacteria (extrachromosomal molecule, so it is independent from bacterial chromosomes and it has genes but it's extrachromosomal), then we cut it by endonucleases, after that we add our gene (the gene that we want to do our experiment on) and then we close the circle of plasmid by ligases.

(the plasmid produced is called recombinant plasmid)

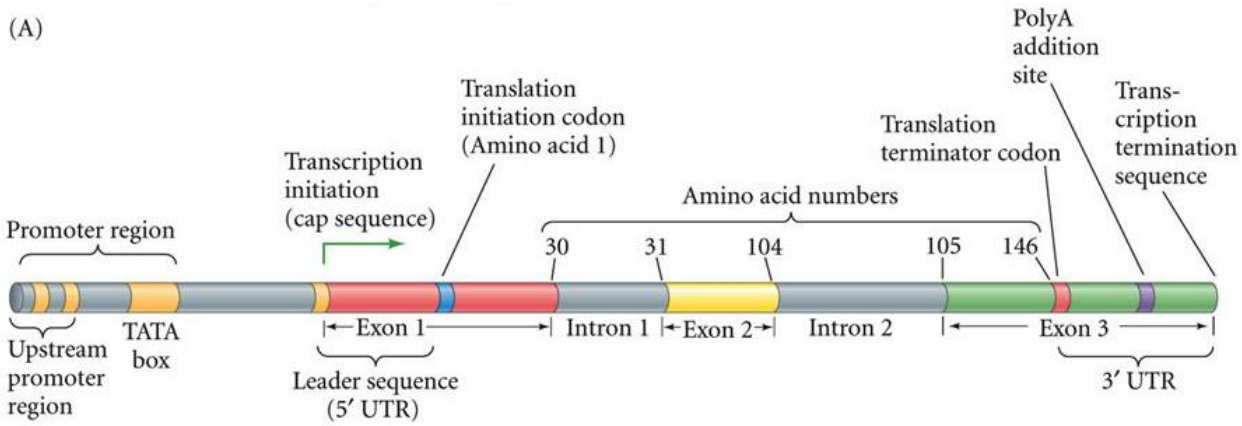
Note: if our gene of interest is a polypeptide synthesizing gene (e.g. hormones) we add a bacterial promoter upstream of our gene so that it will be transcripted then translated.



The bacterial division takes 20 minutes, and after adding the edited plasmid, we make a culture for bacteria to grow and proliferate, the bacteria will make new copies of that plasmid with each division. And so we have new copies of our target gene.

We can use this process to make proteins like insulin, we put the gene that makes that protein after being transcribed and the mRNA produced being translated. We can purify the proteins from bacteria.

Anatomy of eukaryotic gene



(REVIEW)

-We have a eukaryotic gene which has a protmoter region that contains basal promotor/core (TATA box, BRE, Intiator…) and we might have PPE or enhancer or silencer.

-Then we have a transcription start site where the synthesis of mRNA (Introns and Exons) begins until we reach a termination sequence at poly A signal where it stops transcription.

-After the mRNA finishes its transcription it undergoes processing (splicing / capping/ PolyAdenylation) producing a MATURE mRNA.

-Finally, the mRNA undergoes translation beginning with start codon (AUG) and terminating with a stop codon (UGA/UAG/UAA).

Example : Synthesis of β-globin

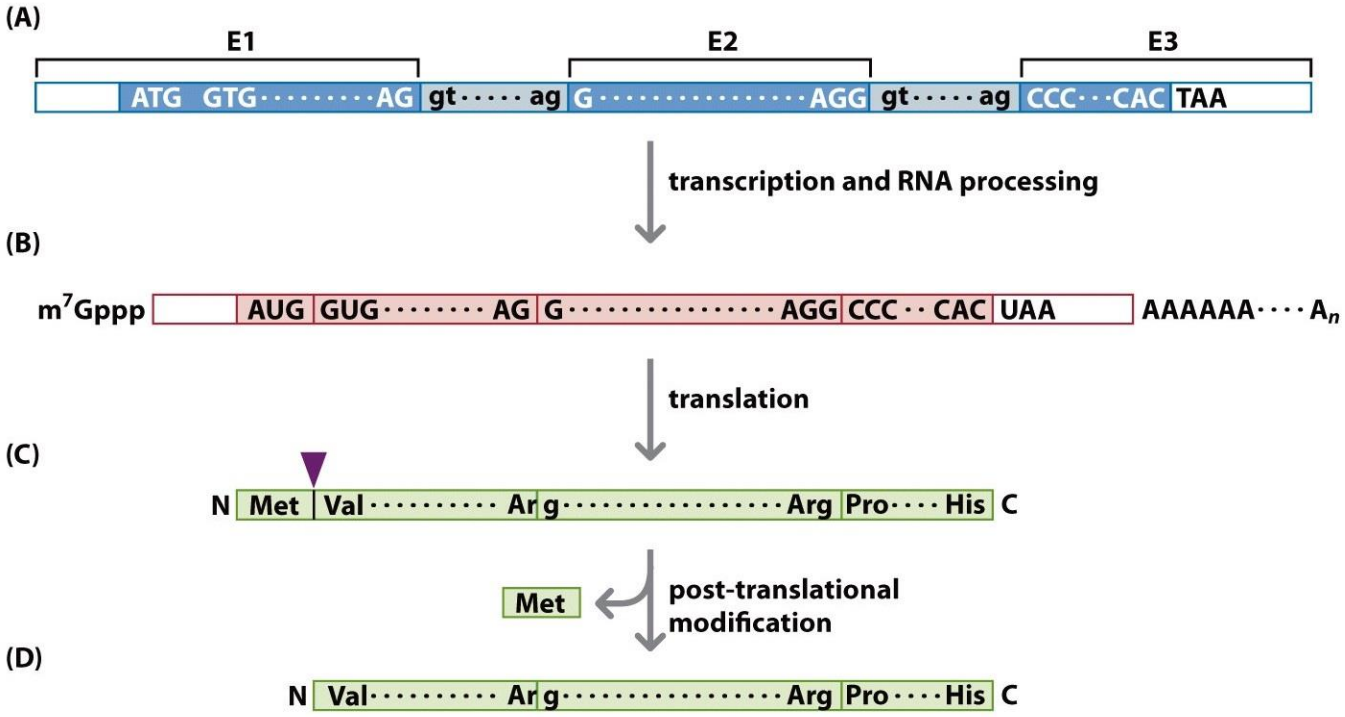


Figure 1.23 Human Molecular Genetics, 4ed. (© Garland Science)

The end