16

# Molecular Biology

Doctor 2019 | Medicine | JU

Sheet Slides


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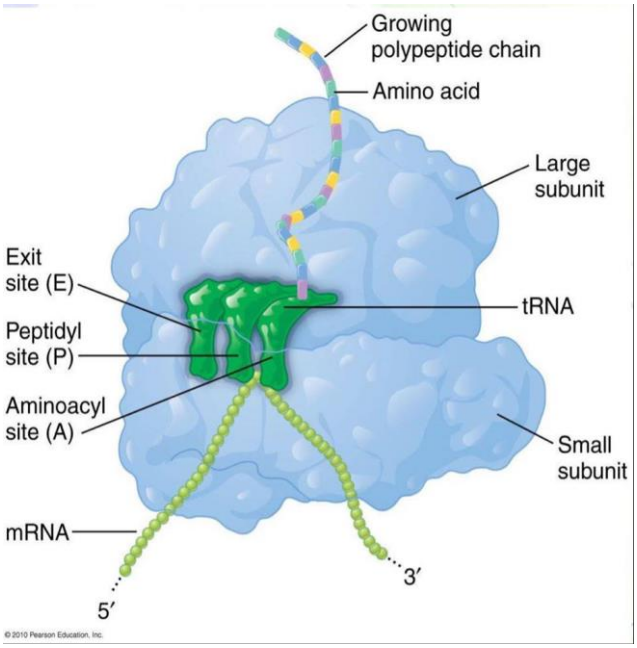
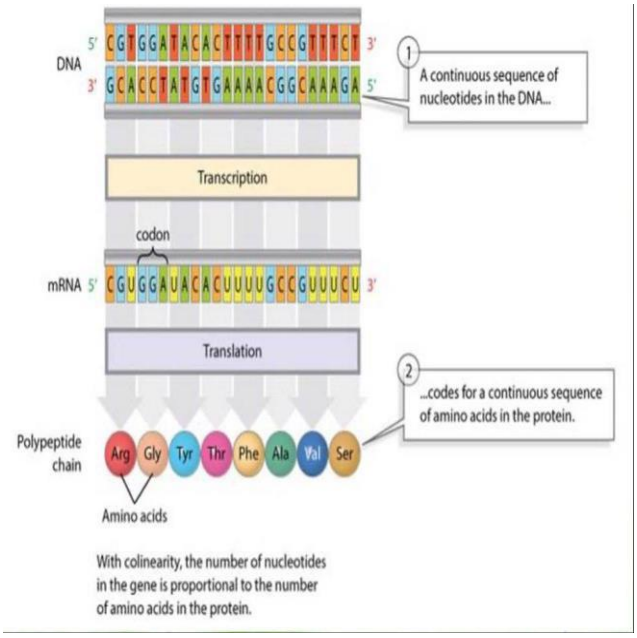
-Let's talk about the last topic in this course which is translation which means protein synthesis.
Refer to Cooper textbook chapter 9 for additional reading.

## ***General Information***

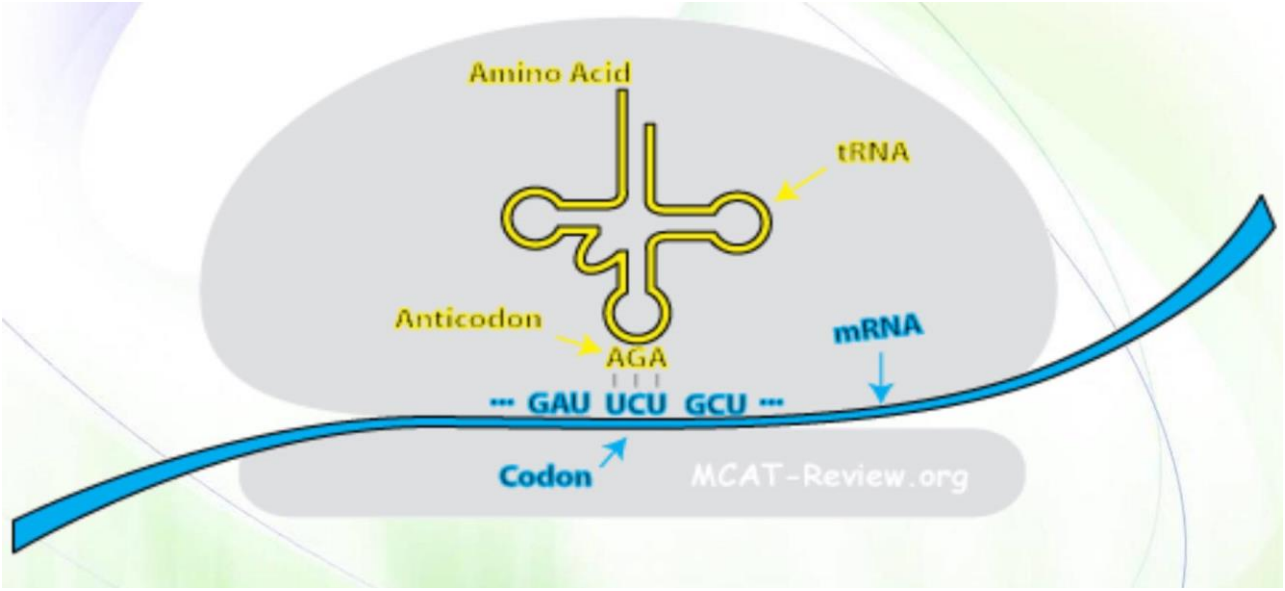
**Protein synthesis involves interactions between 3 types of RNA molecules**:
*tRNAs (Transfer RNA), this type is responsible for decoding the messenger RNA sequence into a protein.
*rRNAs, which exist in ribosomes(the factories of protein synthesis), so these are parts of ribosomes.
*mRNA templates, this type is the RNA sequence which is a code for protein synthesis.



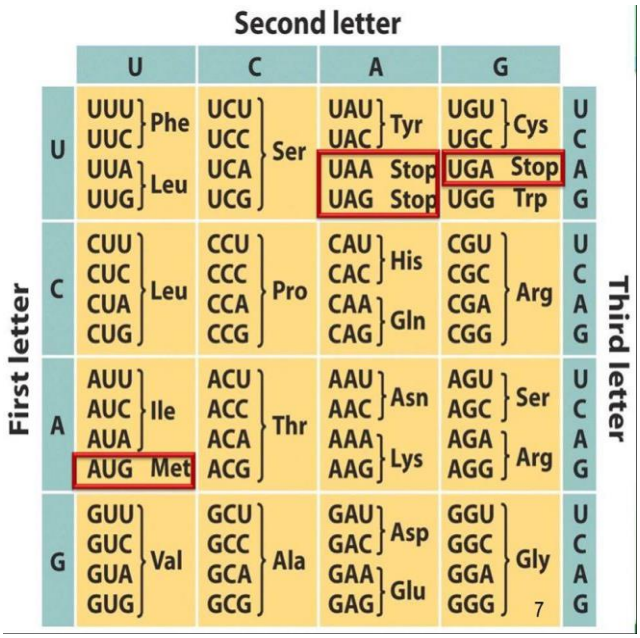

In this figure you can see the components of translation complex, we have the ribosome which is composed of **small ribosomal subunit** (responsible for binding of messenger RNA) and **large ribosomal subunit** (responsible for to the tRNA and the small ribosomal subunit).
**Note that we have three separate sites:**
**1-A site (Aminoacyl site):**responsible for receiving or accepting the tRNA charged with amino acids.
2-**P site(Peptidyl site)**: is the site where polypeptide synthesis occurs and the growing polypeptide started.
**3-E site**(**Exit site**): here tRNA is removed and again used for another amino acid.




Here are some important concepts you need to know, In transcription the DNA sequence of a gene is rewritten (transcribed) in an RNA sequence.
In the translation the sequence of nucleotides in mRNA are translated into an amino acid sequence or a polypeptide which can form a protein later on.
Note that the number of nucleotides in gene is proportional to the number of amino acids in the protein, we call that **Colinearity of genes, mRNA& proteins.** So usually when you have a longer DNA sequence or a gene then you'll have longer mRNA and polypeptide.




Here in this figure you can see that the mRNA is read by tRNA in triplets.
What do we mean by triplets? Cells decode the mRNA by reading their nucleotides in groups of three nucleotides in each one, we call each group a **codon**. So, for each amino acid we have a codon and a codon consists of three nucleotides from the mRNA.



| First letter | Second letter: U | Second letter: C | Second letter: A | Second letter: G | Third letter |
|---|---|---|---|---|---|
| U | UUU, UUC: Phe; UUA, UUG: Leu | UCU, UCC, UCA, UCG: Ser | UAU, UAC: Tyr; UAA Stop; UAG Stop | UGU, UGC: Cys; UGA Stop; UGG Trp | U C A G |
| C | CUU, CUC, CUA, CUG: Leu | CCU, CCC, CCA, CCG: Pro | CAU, CAC: His; CAA, CAG: Gln | CGU, CGC, CGA, CGG: Arg | U C A G |
| A | AUU, AUC, AUA: Ile; AUG Met | ACU, ACC, ACA, ACG: Thr | AAU, AAC: Asn; AAA, AAG: Lys | AGU, AGC: Ser; AGA, AGG: Arg | U C A G |
| G | GUU, GUC, GUA, GUG: Val | GCU, GCC, GCA, GCG: Ala | GAU, GAC: Asp; GAA, GAG: Glu | GGU, GGC, GGA, GGG: Gly | U C A G |


So we talked about how our cells decode mRNAs by reading their nucleotides in a group of three.
If every codon contains 3 nucleotides then how many possible codons can we get?
Look at the table, if the first nucleotide is U for example then there are 4 different possibilities for the second one (U, C, A OR G) and for each possibility we have other 4 different possibilities for the third nucleotide. So, four by four by four we have 64 possible different codons.
But the thing is not all of these 64 codons specify amino acids, however some of these specify a stop codon (3 of them) used for terminating the synthesis of the protein. We also have a start codon (AUG) -methionine amino acid- which is a signal for the translation machine to begin the synthesis.
You remember that we have only 21 amino acids in our bodies (Eukaryotic cells) so for each amino acid we can have mor than 1 codon.

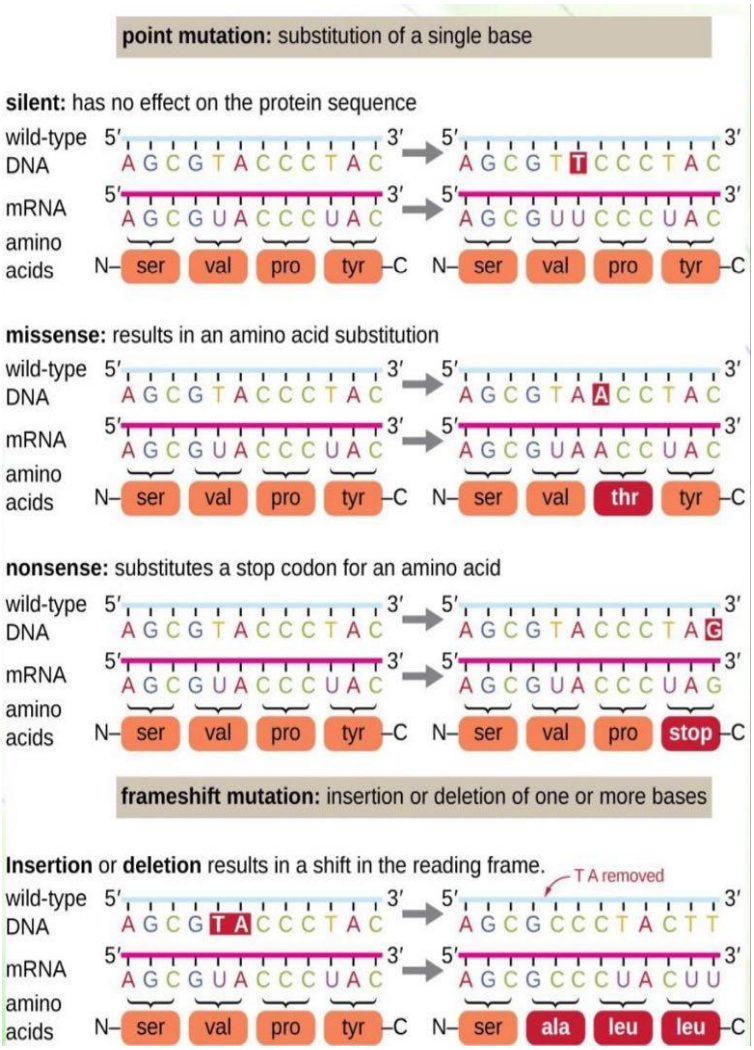
## *Features of the genetic codon.*

-All 64 possible codons of the genetic code and the amino acid specified by each, as read in the 5' to 3' direction from the mRNA sequence.

-Sixty-one codons specify an amino acid.

*Three STOP codons (UAA, UAG, and UGA) do not encode any amino acid so they are just a signal for the translation machine to stop the protein synthesis.

-The genetic code for mitochondrial mRNA (mtRNA) conforms to the universal code (follows the previous table) except for a few variants.




Recall that **Point mutation** is the substitution of a single base so we change a base from one to another.

*and they are divided into different types*:

A-***Silent Mutation***: this type has no effect on the protein sequence, meaning that the substitution in the DNA sequence will change the mRNA sequence and will also affect the codon but in this case the mutation changed the codon which codes for a specific amino acid into another codon which codes for the same specific amino acid. Recall that each amino acid has 4 different codons. That is why we call it silent mutation because it has no effect on the amino acid sequence.

B-***Missense***: here this type results in an amino acid substitution, so we have a change in the amino acid sequence. Take an example from the figure. An important question is raised here, will this substitution cause a disease in this case? will it harm the function of the protein? Actually that depends on the importance of the amino acid that has been substituted.

So, if the amino acid has an important function in the protein and a mutation occurs in it this will affect the function of the protein such as in the substrate binding site or catalytic site etc... if a mutation occurs in these sites the function of the protein will be affected. But if the amino acid change occurs in the body of the protein not harming the function of it the protein will continue to do its job.

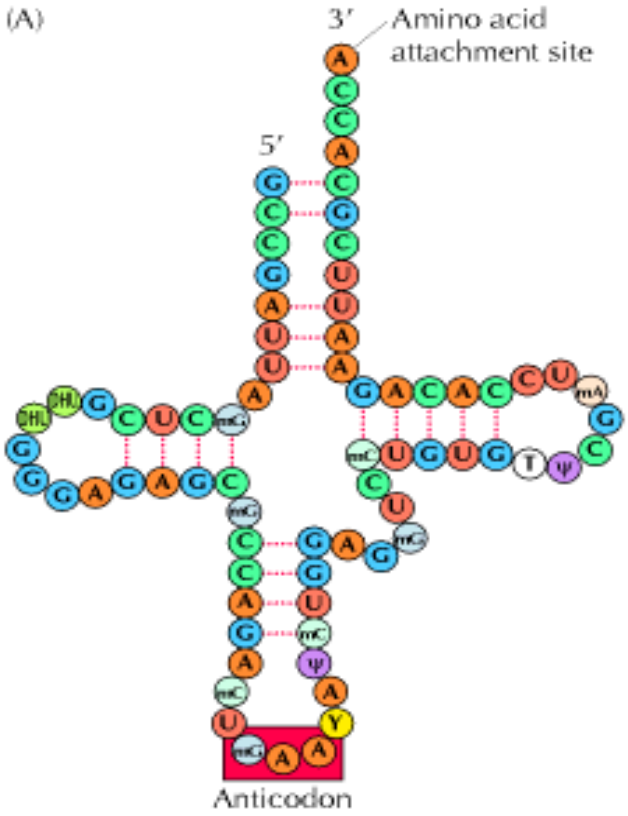
A concept that you should understand is ***structural and functional relationship*** and this means that structure fits the function, for example, the wheels of a car are rounded which fits their function and makes it easier for the car to move. So, if the structure changes then possibly the function will be affected.

C- ***Nonsense mutation***: Here, a normal codon for a certain amino acid can be changed into a stop codon.

This will result in an incomplete polypeptide chain and immature protein and mostly the resulting protein will not do its proper job. So, the substitution of a normal codon into a stop codon is called a nonsense mutation.

D- ***Insertion*** or ***Deletion mutation***: we get this type of mutation if delete or insert a nucleotide and this will result in a frame shift in the protein synthesis or a frame shift mutation. Look at the figure for an example of this type. The difference might result in an abnormal sequence of amino acids resulting in a protein that doesn't do its proper function.

Now, we'll talk about structure of tRNA, let's enjoy together.




- tRNAs are short single-stranded RNA molecules (80 bases long).
- "Charged" or "activated" tRNA carries one amino acid and this amino acid attached to ribose of the terminal adenosine at CCA in the 3' end of tRNA as we can see in the figure , and it is attached and mediated by **aminoacyl-tRNA synthetases**.
- The specific attachment of the tRNA is not specified by only the anticodon, also we can identify it by **identifier sequences** within tRNA can also specify the attachment of specific amino acid to the tRNA.
- tRNAs contain stem loop structures, modified bases, and unusual bases (example: inosine).

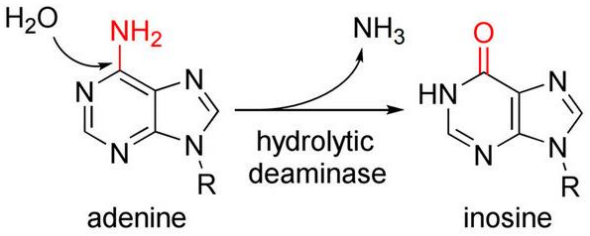
## The role for aminoacyl-tRNA synthetases.

1- Linking of the amino acid with its proper
2- Correct tRNA

As we said, tRNAs contain bases stem loop structures, modified bases, and unusual bases (example: inosine), inosine Is unusual bases other than normal ones (G C A U).

**The question is, how can inosine produce?**

Look at this figure:




Inosine produced **by hydrolytic deaminase** which means we will remove **amino group** from adenine and the inosine will be produced.

**What are the benefits of stem loop structures, modified bases, and unusual bases?**

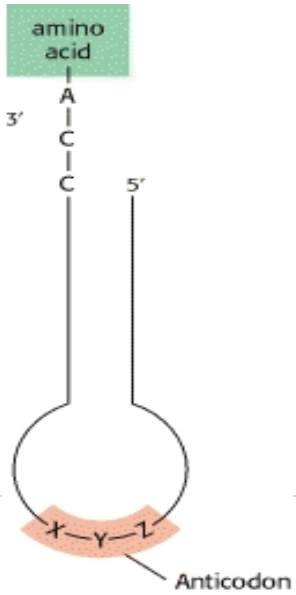
They are very important in:

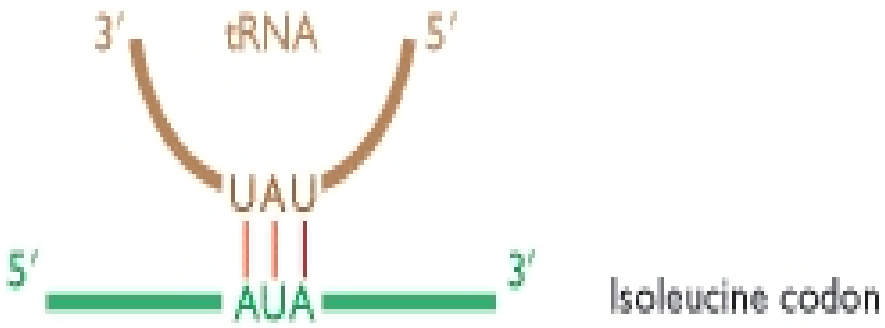
1- They give stability of tRNA.
2- Binding tRNA to proper codon in the mRNA.

Doctor said we didn't need to know exactly what kind of modified bases and the position and what they did to tRNA, just simply know that we have stem loop structures, modified bases, and unusual bases, and the structure of tRNA.

**Codon vs. anticodon**

- tRNAs contain a three-nucleotide sequence known as "anticodon" that pairs with the "codon' or "triplet" mRNA molecules (note the anti-parallel alignment of mRNA-tRNA complex).
  5' to 3' in mRNA and 3' to 5' in tRNA.



**Fidelity of translation**

**What makes sure the accurate translation does have any mistakes in the translation?**

We have 2 steps required for the accurate translation.

- First: accurate association of amino acid to tRNA by **aminoacyl-tRNA synthetase.**

**Remember** that we have **aminoacyl-tRNA synthetase** for each amino acid and this **aminoacyl-tRNA synthetase** responsible for linking between amino acid and its correct tRNA.

Sometimes **aminoacyl-tRNA synthetase** do mistakes, and can bind to the wrong amino acid, BUT it has own proofreading site, so if there is a wrong binding between amino acid and **aminoacyl-tRNA synthetase** it will pops up (show and recognize) the wrong amino acid back off and allowed correct amino acid to bind to correct tRNA.

**aminoacyl-tRNA synthetase** has own proofreading capacity. بتصلح حالها من حالها.

- Second: a correct match between the tRNA anticodon and an mRNA codon.

**Must the anticodon match completely to the codon in mRNA?**

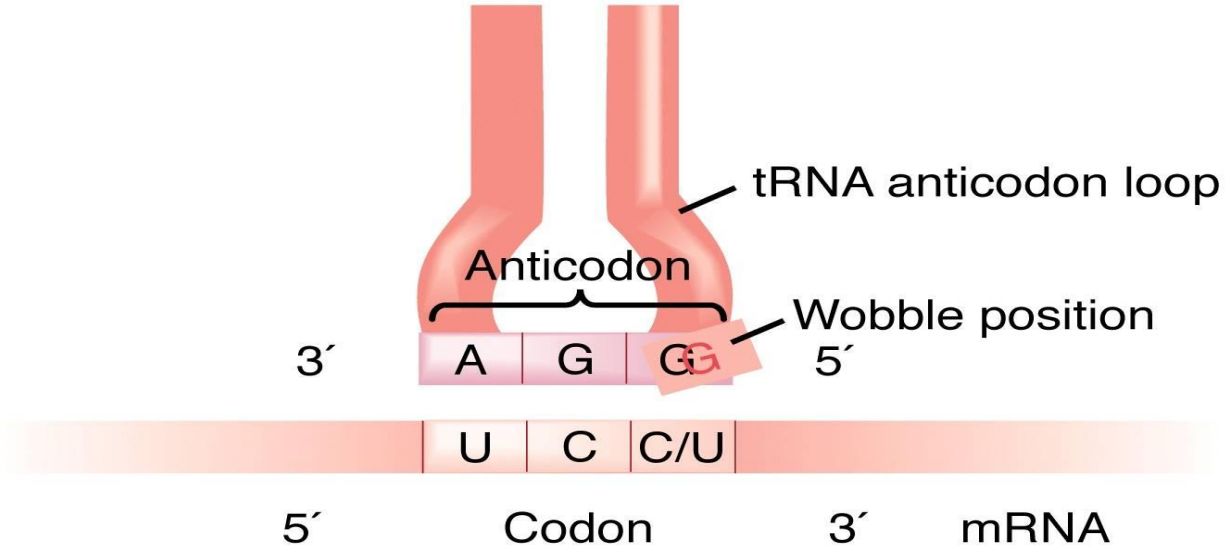
Actually NO, let's know why?

- There is flexible pairing at the third base of a codon to the anticodon allowing some tRNAs to bind to more than one codon.

It is called **wobble** base pairing.

The bases that are common to several codons are usually the first and second bases, with more room for variation in the third base.

It acts as a buffer against deleterious mutations.




**The first and second bases in anticodon should match and complementary to the codon but the third one has some flexibility.**

**Examples of wobble base pairing.**

Both are hydrophobic amino acids

| | U | C | A | G | |
|---|---|---|---|---|---|
| U | UUU, UUC } Phe | UCU, UCC, UCA, UCG } Ser | UAU, UAC } Tyr | UGU, UGC } Cys | U |
| | UUA, UUG } Leu | | UAA Stop, UAG Stop | UGA Stop, UGG Trp | C A G |
| C | CUU, CUC, CUA, CUG } Leu | CCU, CCC, CCA, CCG } Pro | CAU, CAC } His; CAA, CAG } Gln | CGU, CGC, CGA, CGG } Arg | U C A G |

**We know that each amino acid can have more than one codon.**

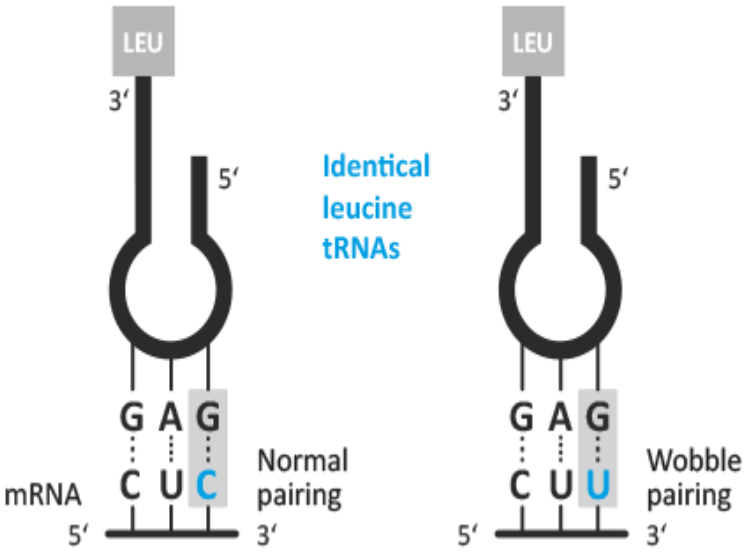
**How the wobble base pairing applied to this different codon for the amino acid?**

**We'll know in the next page. ^-^**

Let us taka leucine as an example.

We have identical leucine tRNA with anticodon GAG, normally the anticodon in tRNA or leucine tRNA should pair with codon CUC as the left figure.

Sometimes the same tRNA can pair with CUU codon through **wobble base pairing** and this is can occur for the third place in the codon in mRNA for the same amino acid.




## What does the benefit of wobble base pairing provide?

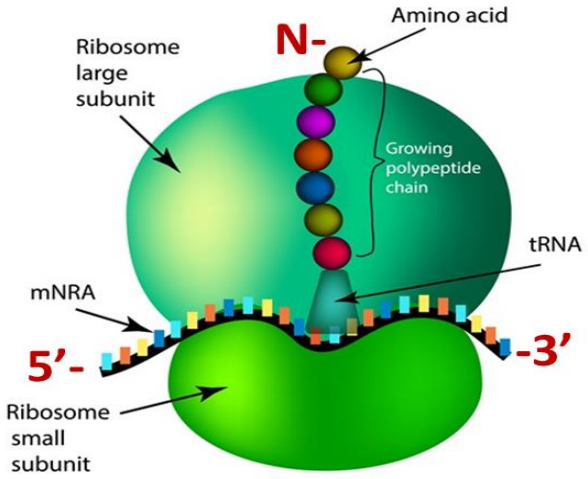
**Remember that we talked about silent mutation, sometimes if there is mutation occurs in the third base of the codon that will change the nucleic acid sequence but will not change the codon amino acid sequence.**

So, wobble base pairing can work as a buffer against deletion mutations also provide flexibility for the synthesis of polypeptide chains.

### Ribosomes

- Ribosomes are the sites of protein synthesis.
- *E. coli* contain about 20,000 ribosomes (~25% of the dry weight of the cell).
- Rapidly growing mammalian cells contain about 10 million ribosomes.

**We have large quantities of ribosomes in prokaryotes and eukaryotic cells because we need the synthesis of protein and these ribosomes involved in all protein synthesis.**




**There is an enzymatic activity:**

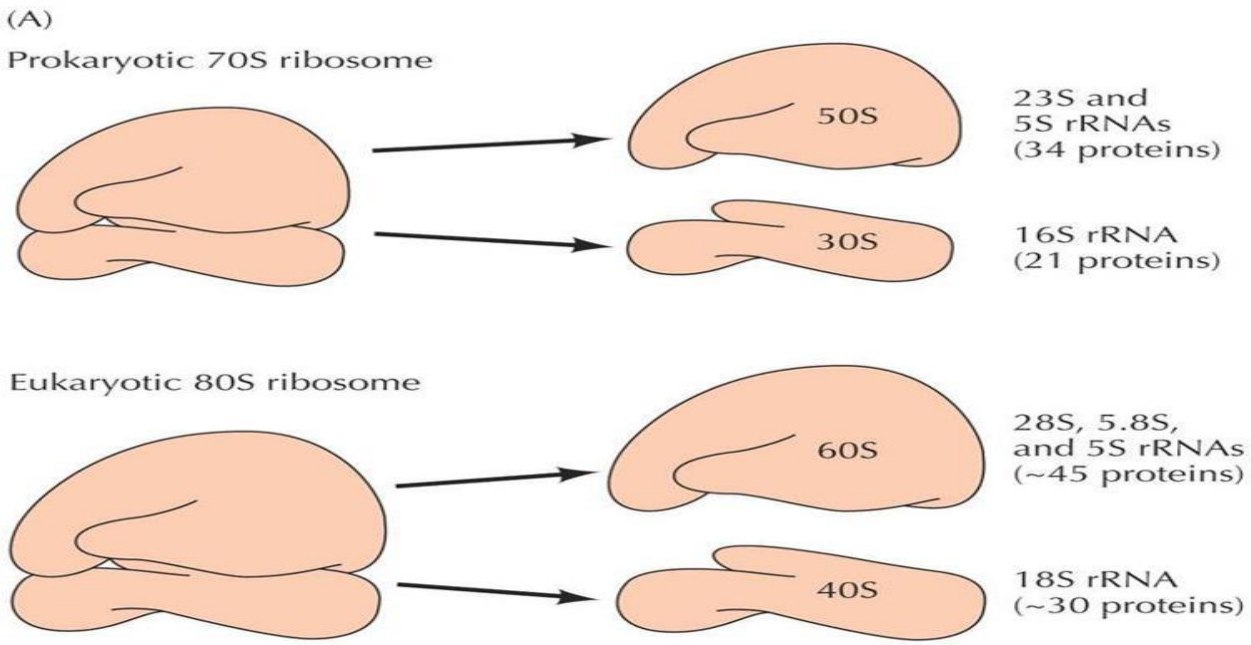
**The peptidyl transferase reaction of a peptide bond is catalyzed by the rRNA of the large ribosomal subunit.**

**And it's responsible for establishing the hold between the growing amino acid sequence and the coming amino acid.**

**We have ribosomal RNA in the ribosome, rRNA in ribosomal large subunit playing very important role in the catalyzing of peptidyl transferase activating.**

Now, let us go deep into the ribosome's structures and the difference between prokaryote and eukaryote.

**Study this figure well.**




## But what dose (s) unit mean?

S unit is a spread unit, a unit for sedimentation coefficient (معامل الترسيب ), it measures the sedimentation rate of the particles. بالعربي يعني بتحسب سرعة ترسب الجزيئات أو المركبات

The larger particle sediment first and the smaller one will sediment later. بالعربي تاني الجزيئات الأكبر رح تترسب أسرع من الجزيئات الأصغر.

So, we notice that eukaryotes (80s) are a bit larger than prokaryotes (70s).

**The sedimentation depends on the size, density and the shape of the particle.**

## rRNA synthesis and processing

- The 18S, 5.8S, and 28S rRNAs are encoded by a single gene (13 kb long).
- Transcription by **RNA polymerase I (18S, 5.8S, and 28S rRNA)** produces a primary transcript (45S rRNA) that then undergoes post-transcriptional cleavages producing individual 18S, 28S, and 5.8S rRNA molecules.

The 18S rRNA associates with the small ribosomal subunit.

The 5.8S 28S rRNAs associates with the large ribosomal subunit.

- The large ribosomal subunit also contains 5S rRNA, which is encoded by different genes transcribed by RNA polymerase III.

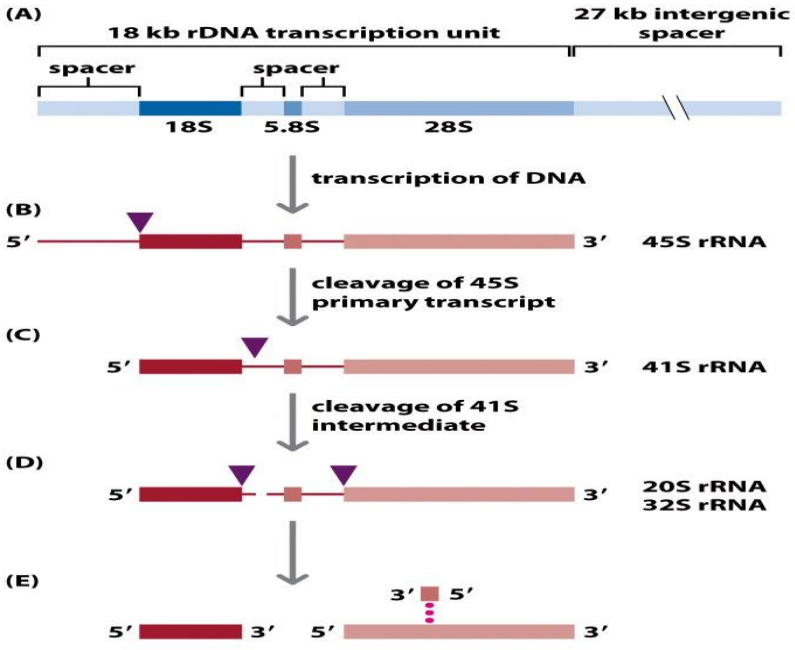


Figure 1.22 Human Molecular Genetics, 4ed. (© Garland Science)

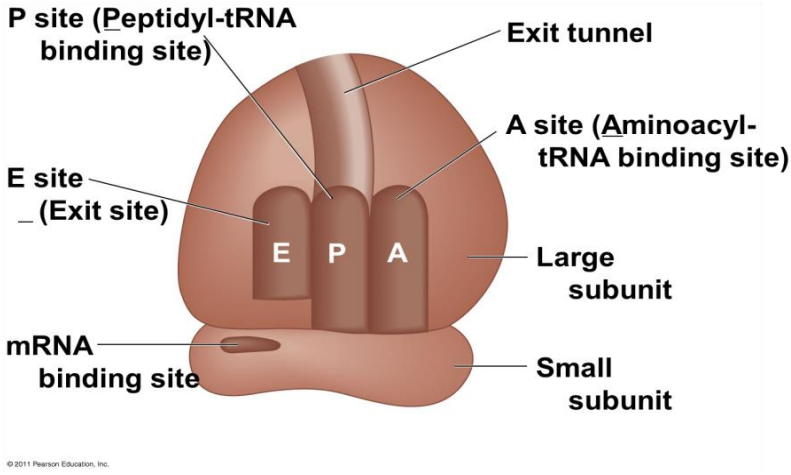
**We have some intergenic DNA spacers in the transcription unit (the blue one)**

Now let us talk about general structure and components of Ribosomes:

*Ribosomes facilitate specific coupling of tRNA anticodons with mRNA codons in protein synthesis.

*The RNA components are predominantly responsible for the catalytic function of the ribosome, the protein components enhance the function of the rRNA molecules.

## The chambers of ribosomal subunits:



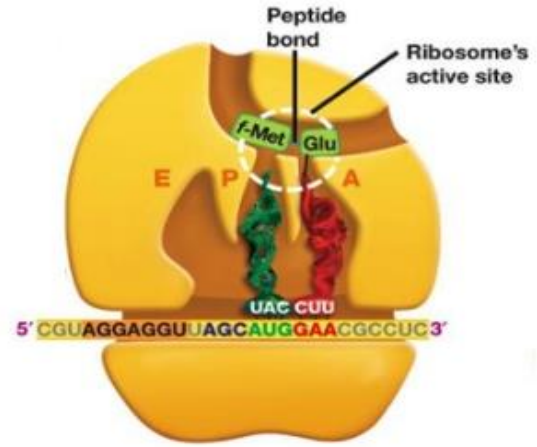

The figure above shows the champers which are:
1-mRNA binding site: where mRNA can bind to the small subunit.
2-A site: aminoacyl-tRNA binding site holds the tRNA that carries the next amino acid to be added to the chain.
3-p site: peptidyl-tRNA binding site which holds the tRNA that carries the growing polypeptide chain.
4-E site: exit site, where discharged tRNAs leave the ribosome.
5-THE exit tunnel: for exit the synthesized polypeptide.

*So, know how these champers integrate with each other in the synthesis process?
First the charged (activated)tRNA enter the A site and form peptide bond with the growing polypeptide chain in P site so the growing peptide chain moves to A site and the tRNAs translocate and makeshift in their positions.
Note: this may not be clear now but in the next sheet will become clearer.

# THE general mechanism for translation:

- Three stages: initiation, elongation, and termination.

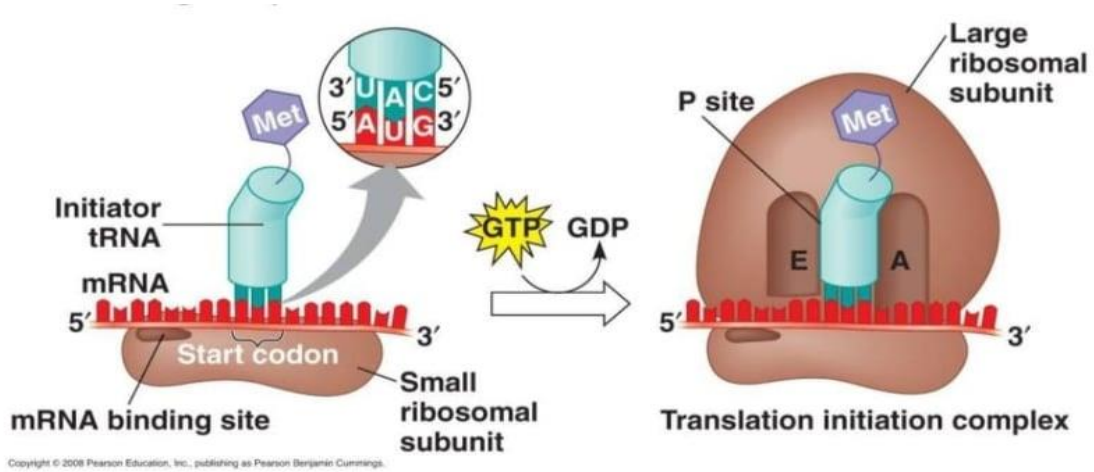
*The direction for reading mRNA is from 5’ → 3’.
*Protein synthesis begins at the amino terminus and extends toward the carboxyl terminus.
-Remember that in protein sequence we have N terminus (amino terminus) and C terminus (carboxyl terminus).




**In this figure you can notice the peptide bond between the new amino acid and the growing polypeptide chain**

## *start of translation:

In both prokaryotes and eukaryotes, translation starts at specific initiation sites, which is UAG (methionine), and not from the first codon of the mRNA.
So the translation does not start when the ribosome find mRNA,but there is a start codon that ribosome need to notice (AUG).
*Note: the start codon called the initiator tRNA.
-The translation process needs energy which came from GTP.



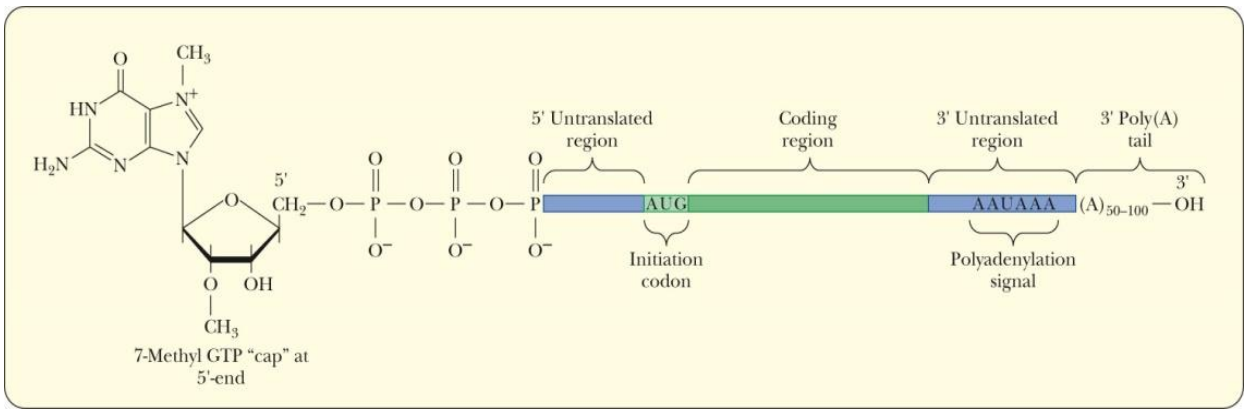
# Untranslated regions (UTRs):

We have 2 UTR in mRNA:

- 5´untranslated regions (UTRs) which found upstream of the initiation sites of both prokaryotic and eukaryotic mRNAs contain noncoding sequences.

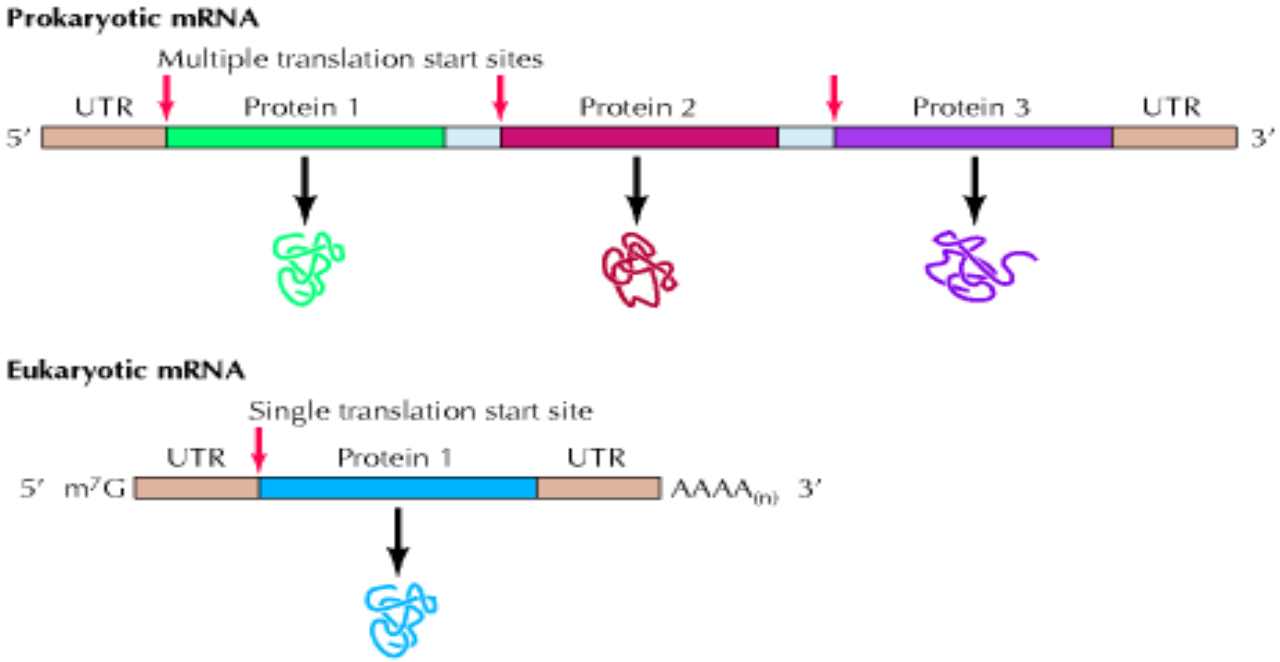
- 3'untranslated region, which follows any of the three stop codons.

*the UTRs have an importance in regulation translation.




-Remember:

- Bacterial mRNA is polycistronic: many polypeptides from the same mRNA.
- Eukaryotic mRNA is monocistronic: one polypeptide from each mRNA.

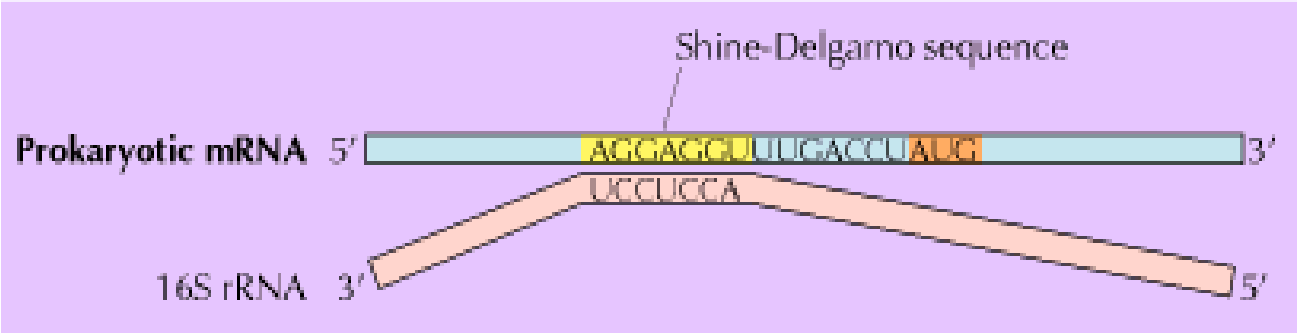


# *Shine-Dalgarno sequence:

*Now how ribosomes identify and bind to mRNA?

The ribosome can't bind directly to mRNA it first binds to 3' UTR and start scanning for the initiation site and when find it know it can start translation.

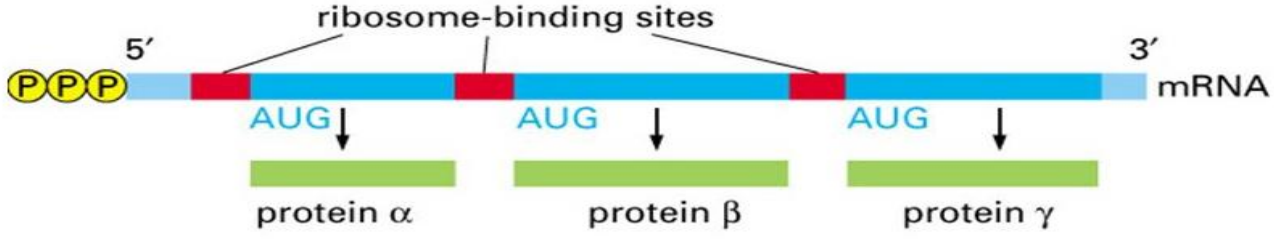
In prokaryotic:



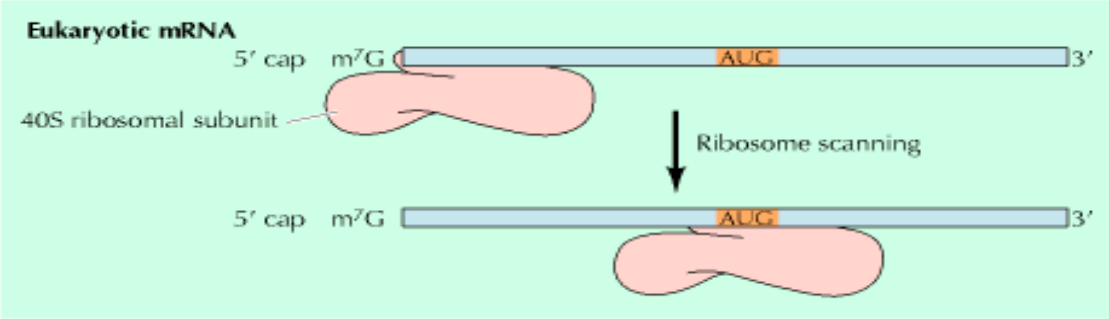

The figure shows a shine -Dalgamo sequence which is found 8 bases upstream the initiation site and this sequence is identified by 16s rRNA (small ribosomal subunit).

*We said that prokaryotic can produce different polypeptides from the same gene but that does not mean that we have the same start codon and stop codon, so for example if we have a bacterial gene codes for 3 different polypeptides that use in the same process then we have 3 different start codons with 3 different stop codons.

*so always remember that to have the same mRNA does not mean to have the same start and stop codon for each polypeptide going to be synthesized to use in the same process. (polycistronic)



- But how 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.

## Internal ribosome entry site (IRES):

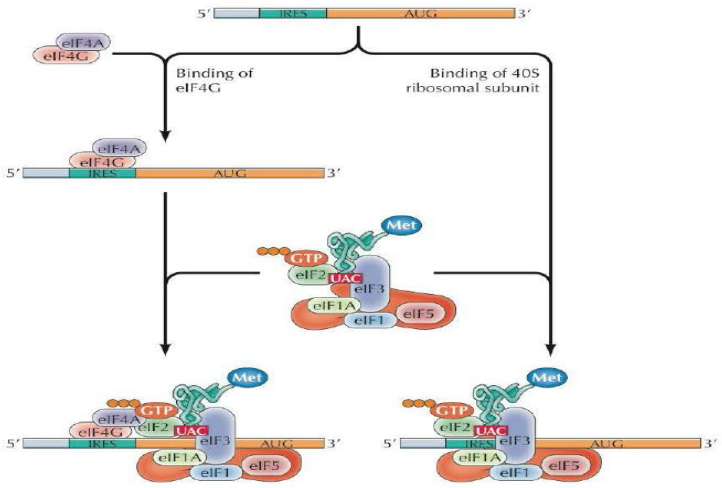
The 40s subunit can recognize mRNA by two methods:

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

2-IRES

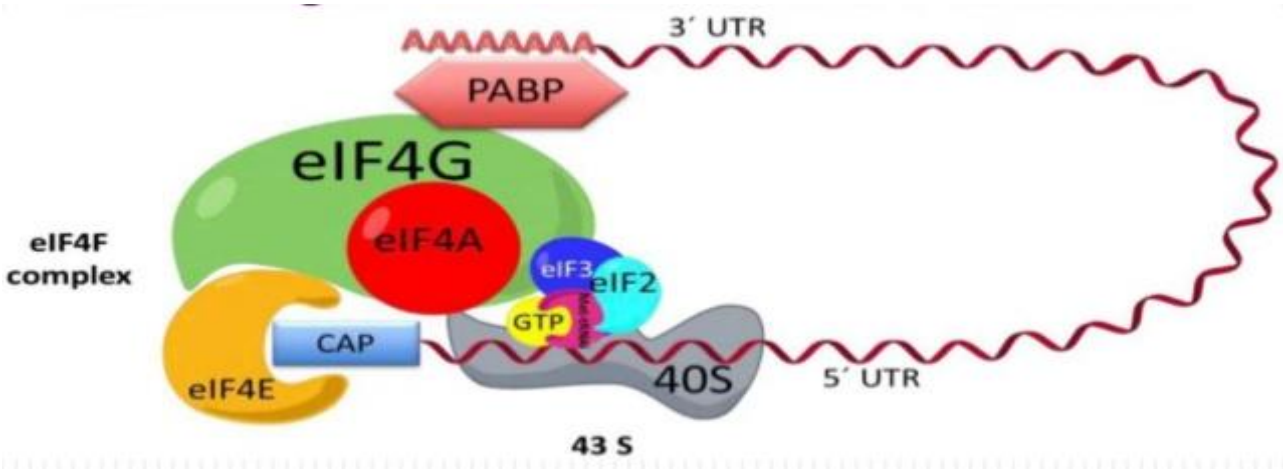
So, what is IRES: it is a small mRNA element found in 5' UTR and easily recognize by 40s subunit or first it bound to eIF4G and then recognize by 40s. (5' cap independet way)

- 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.




Note: IRES method of recognition found mainly in RNA viruses because they do not have 5’ cap but also we can have this method in eukaryotic too.

## Translation initiation in eukaryotes.



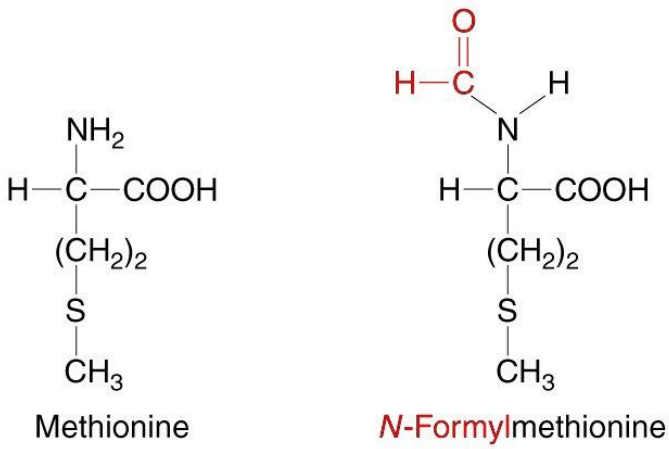

The figure above shows the translation initiation complex in eukaryotic which is composed from the cap and eIF4E and eIF4G which is bounded to PABP (poly A binding protein) so it look like a bridge between the 3’ and 5’ of mRNA.

## Finally, the first amino acid (start codon);

- Translation always initiates with the amino acid methionine, usually encoded by AUG.
- In most bacteria, it is N-formylmethionine.

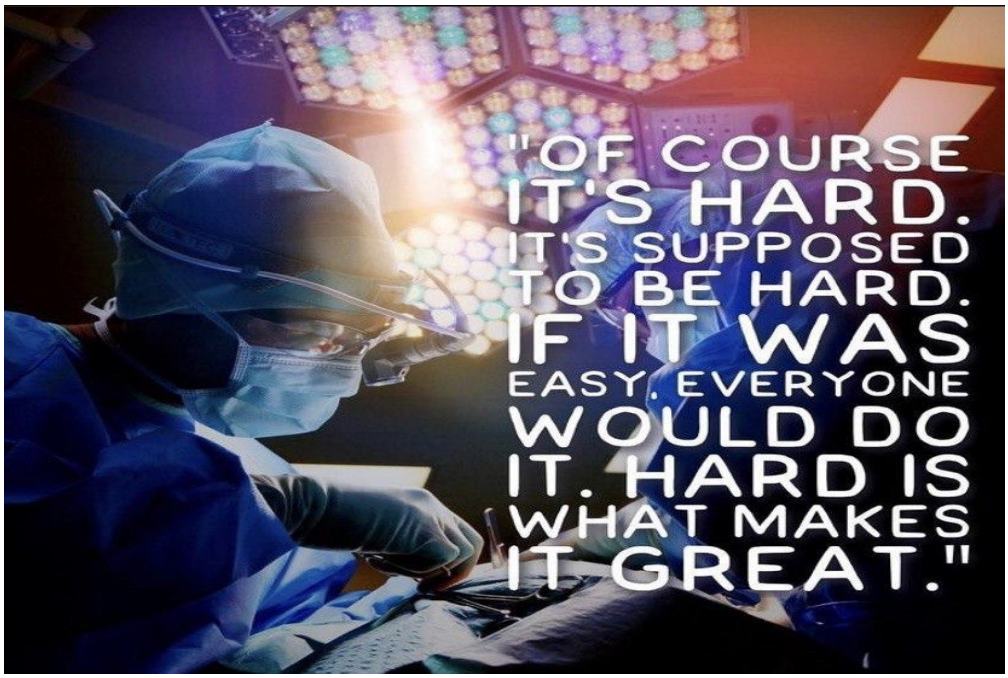
*Note: N-formylmethionine is simply consists of methionine and formaldehyde in the N terminus of amino acid.

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




## Building of polypeptide: (will be discussed in next sheet)

- The three stages of translation
  - Initiation
  - Elongation
  - Termination
- All three stages require protein “factors” that aid in the translation process




#كل_الحب ^-^