Sheet no.13 (part 1)

Molecular biology

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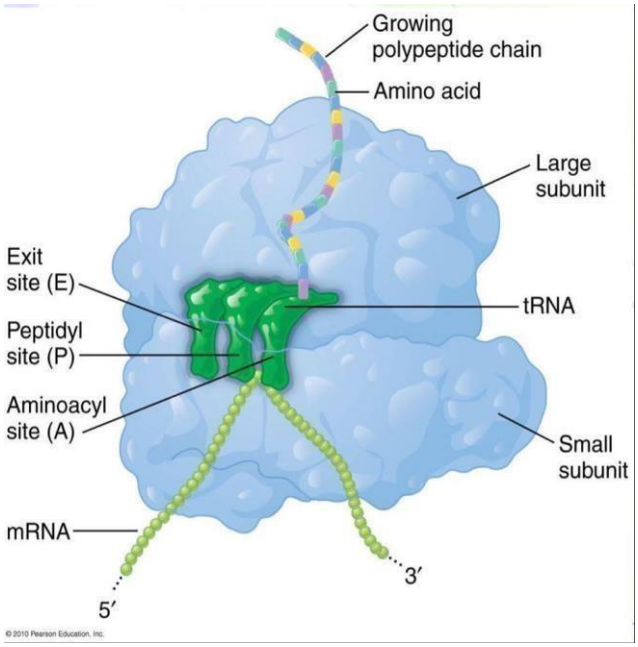
General Information

Protein synthesis involves interactions between 3 types of RNA molecules:

*tRNAs (Transfer RNA), this type is responsible for transferring amino acids to the growing polypeptide.

*rRNAs, which exist in ribosomes(the factories of protein synthesis), so their function is structural. Also they are involved in catalyzing peptide bond formation.

*mRNA templates, this type is the RNA sequence which is a code for protein synthesis.



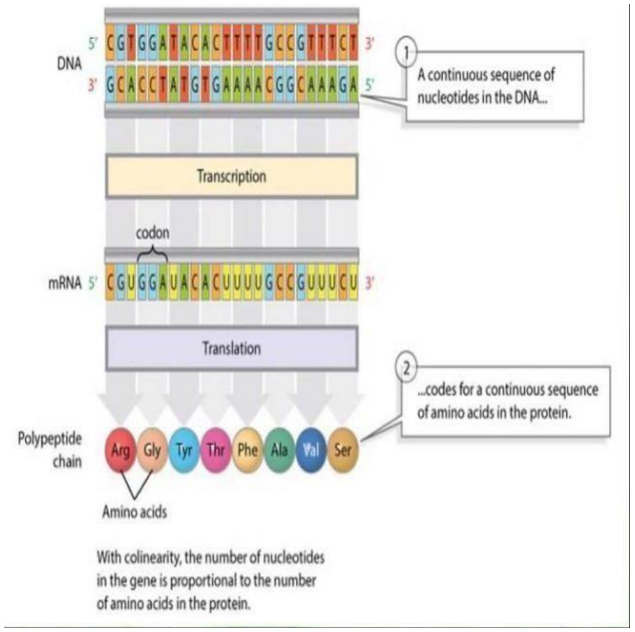
In this figure you can see the components of translation complex, we have the ribosome which is composed of **small ribosomal subunit** (responsible for binding of messenger RNA) and **large ribosomal subunit** (responsible for to the tRNA and the small ribosomal subunit).

Note that we have three separate sites:

1-A site (Aminoacyl site):responsible for receiving or accepting the tRNA charged with amino acids.

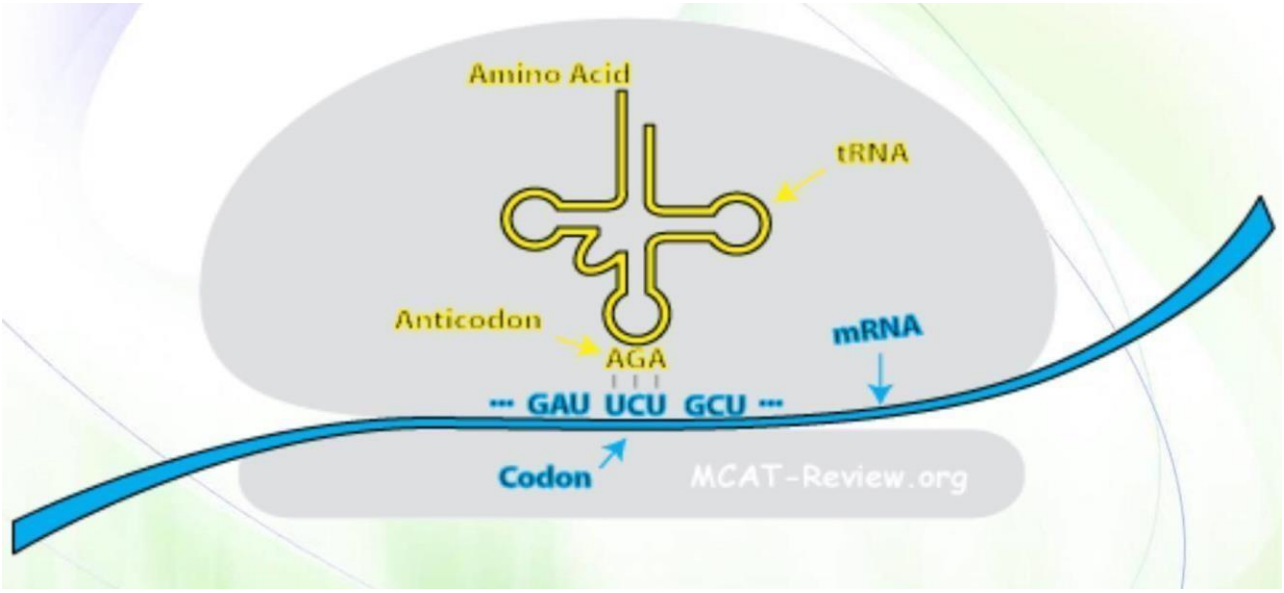
2-**P site(Peptidyl site)**: is the site where polypeptide synthesis occurs and the growing polypeptide started.

3-E site(**Exit site**): here tRNA is removed and again used for another amino acid.

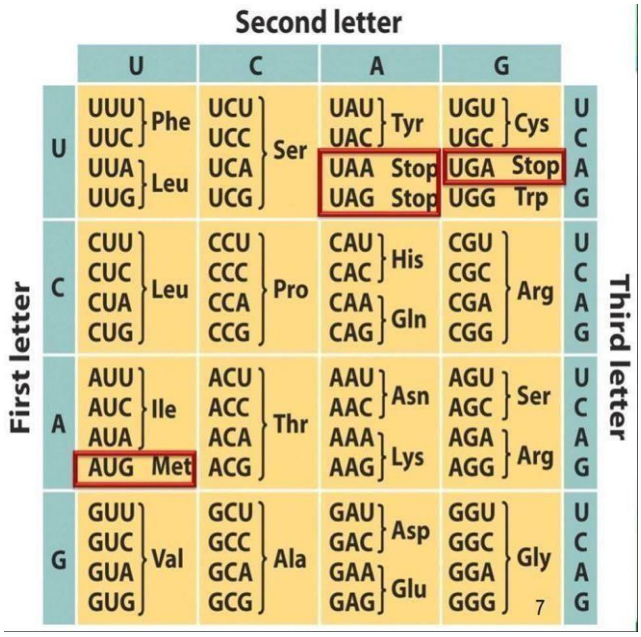


Scientists noticed a collinearity between DNA, mRNA & polypeptide chain. Even though they are structurally different, one made from nucleotides and one from A.a, any change happens to the beginning of mRNA will correspond to a change in the beginning of polypeptide chain. Also long mRNA will correspond to long peptide chain.

This shows that mRNA is read and translated to polypeptide chain.



Here in this figure you can see that the mRNA is read by tRNA in triplets.
(Cells decode the mRNA by reading their nucleotides in groups of three nucleotides , 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.



If every codon contains 3 nucleotides then how many possible codons can we get?
Each nucleotide have 4 possibilities thus 64 different codon.

Each codon codes for a specific function, 61 codon code for 21 amino acids (thus each A.a can have more than one codon)

Met is an A.a that have only on codon coding for it. Met is the beginning of any polypeptide.

Other 3 codons UAA, UAG, UGA are not represented by any A.a; However, key code translation termination.

Features of the genetic codon.

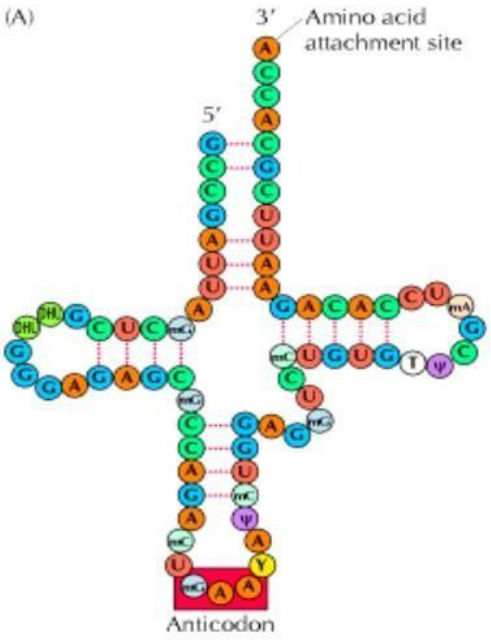
-All 64 possible codons of the genetic code and the amino acid specified by each, as read in the 5' to 3' direction from the mRNA sequence.

-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.

tRNA structure

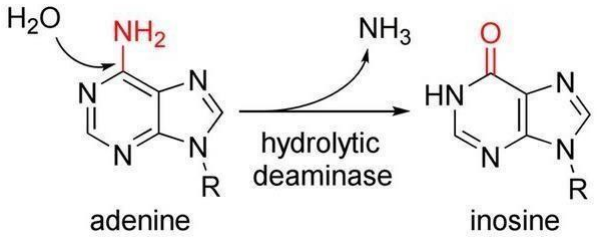


- tRNAs are short single-stranded RNA molecules (80 bases long).
- Like any other RNA, it has 5' & 3' ends. There is a consensus sequence on the 3' end which is CCA,
- "Charged" or "activated" tRNA carries one amino acid and this amino acid attached to ribose of the terminal adenosine at CCA in the 3' end of tRNA as we can see in the figure , and it is attached and mediated by **aminoacyl-tRNA synthetases**.
- There are many types of **aminoacyl-tRNA synthetases,** they look at tRNA sequences, mainly the anticodon, and add a specific Amino acid at the terminal A
- tRNA structure is due the internal hydrogen bonds between complimentary bases.
- tRNAs contain stem loop structures, modified bases, and unusual bases (example: inosine).

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.

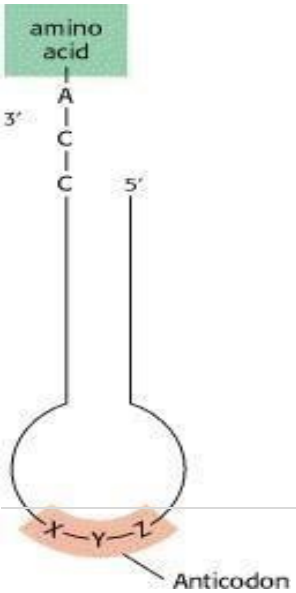
What are the benefits of stem loop structures, modified bases, and unusual bases?

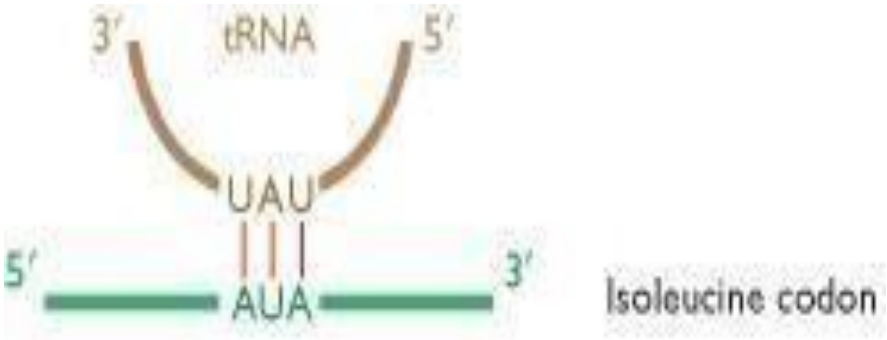
They are very important in:

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

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.





After pairing, the amino acid carried by the tRNA will be added to the growing polypeptide chain.

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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.

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

Must the anticodon match completely to the codon in mRNA?

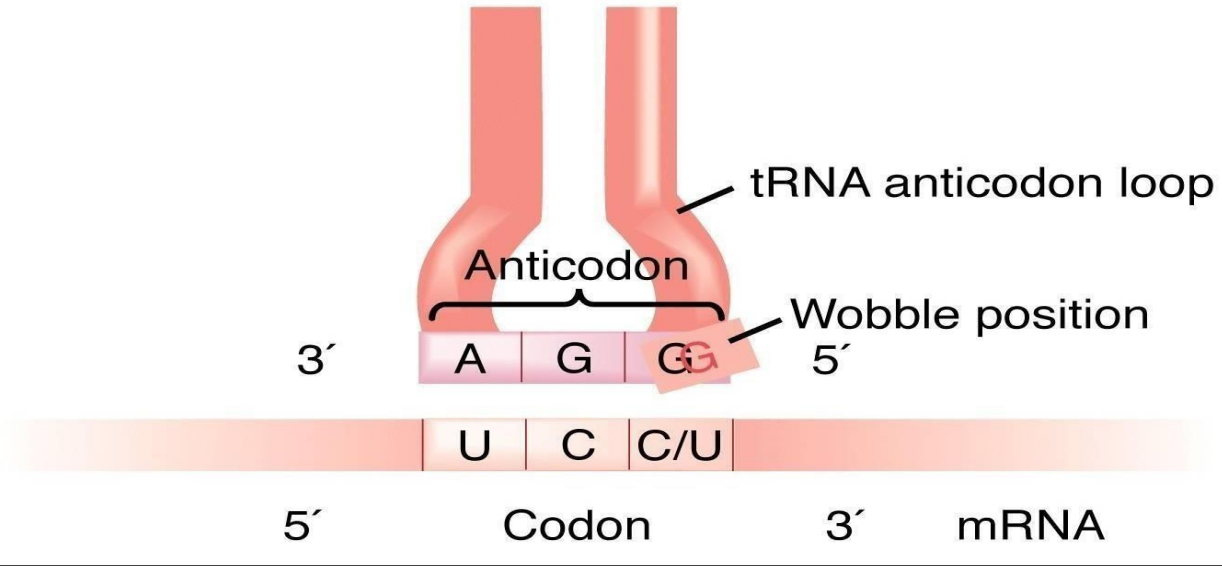
Actually NO, let's know why?

- 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

If a mutation occur and Phe is replaced with Leu, the major structure of protein is not totally affected, because both are hydrophobic Amino acids.

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

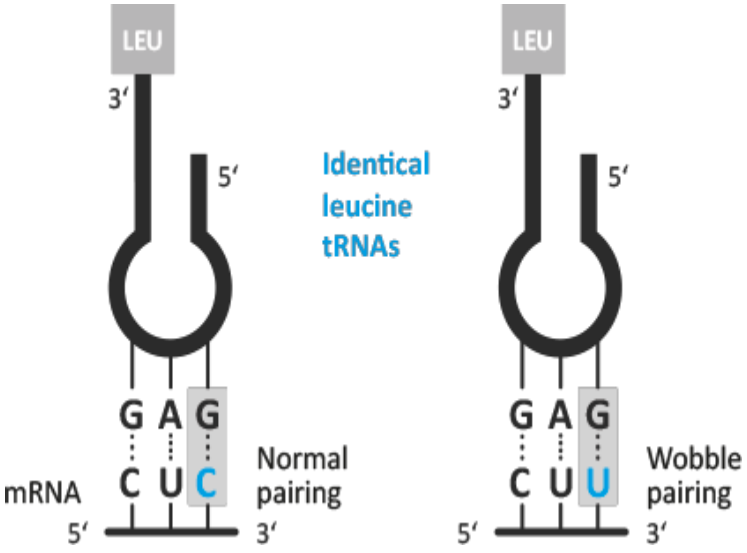
How the wobble base pairing applied to this different codon for the amino acid?

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 in the third place in the codon in mRNA for the same amino acid.



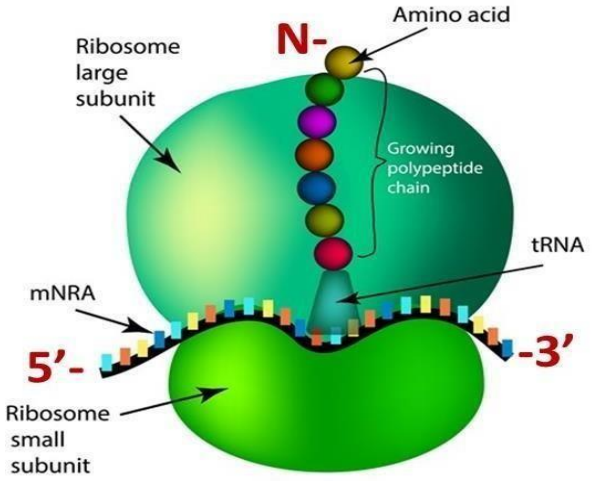
What does the benefit of wobble base pairing provide?

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

If a mutation occurs in the third base of the codon, nucleic acid sequence will be affected; However; amino acids sequences may not change.

Ribosomes

- Ribosomes are the sites of protein synthesis, and because cells need large quantities of proteins , ribosomes are found in large quantities in prokaryotes and eukaryotic cells
- *E. coli* contain about 20,000 ribosomes (~25% of the dry weight of the cell).
- Rapidly growing mammalian cells contain about 10 million ribosomes.

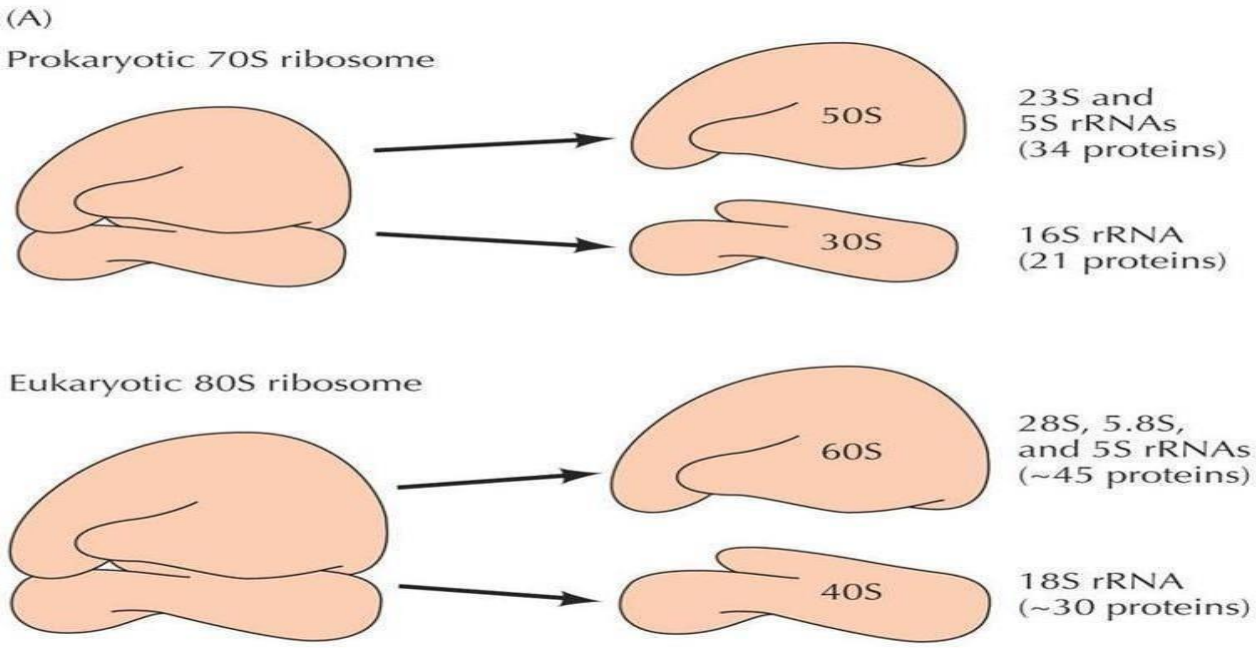


Ribosomes are made from large subunits and small subunits.

The polypeptide is synthesized on the . large subunit.(**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.

<u>Study this figure well.(don't memorize number of proteins, just rRNA types.)</u>



Notice that the ribosomes found in prokaryotes are similar to eukaryotic ribosomes (large and small subunits). This shows that they didn't evolve through millions of years, and this reflects their importance.

Notice that ribosome S is not equal to the sum of large and small subunits S , this is because S is determined by shape and size

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 primarytranscript (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 differentgenes transcribed by RNA polymerase III.

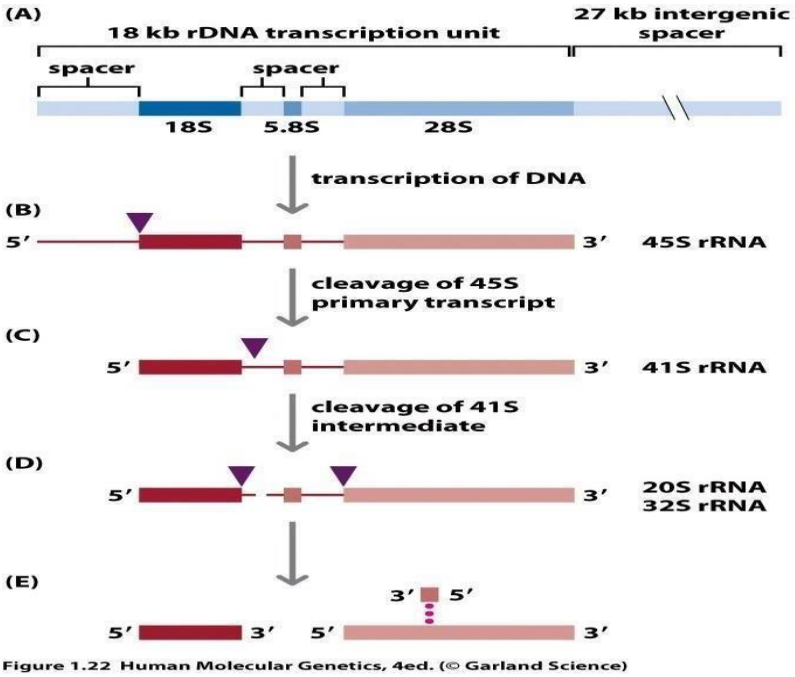
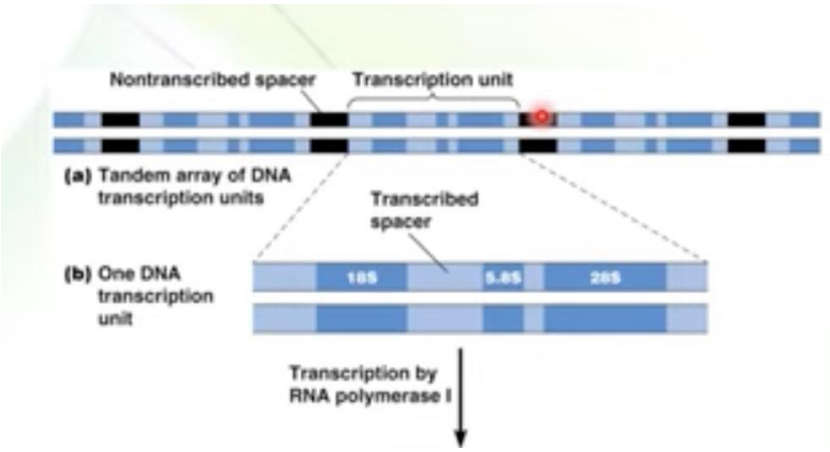


Figure 1.22 Human Molecular Genetics, 4ed. (© Garland Science)

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



Notice that genes that code for rRNA are tandemly ordered(repeated over and over) to produce as much rRNA. Similar thing to histones

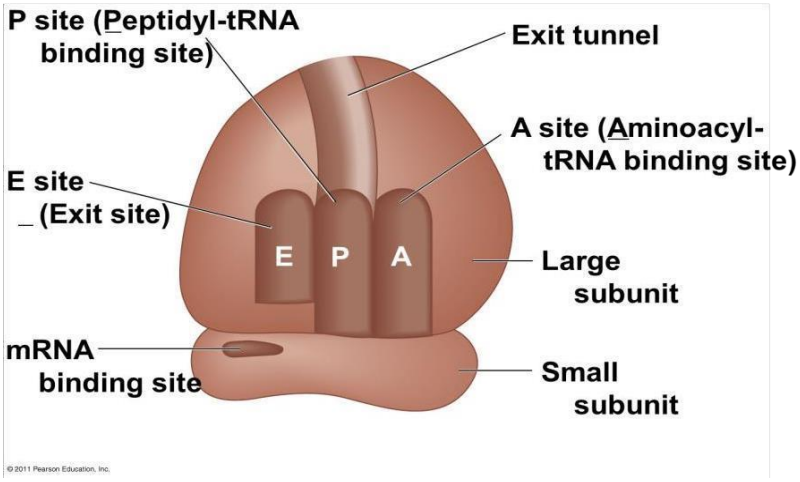
Functional & structural components of ribosomes

*Ribosomes are responsible for associating tRNA and mRNA together and initiates translation

*The RNA components acts as enzymes, they catalyze amino acids linking reaction
This was known when 90% of proteins are removed and ribosomes still function

Also, and evidence on their catalytic activity is that they are located at polypeptides synthesis site.

The chambers of ribosomal subunits:



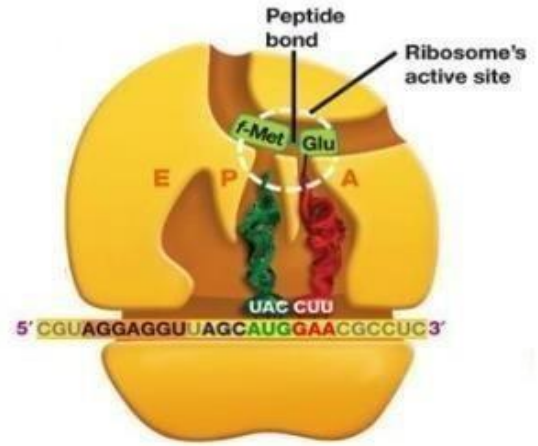
The figure above shows the 3 champers of ribosome:
1-**A site**: aminoacyl-tRNA binding site holds the tRNA that carries the next amino acid tobe added to the chain.
2-**p site**: peptidyl-tRNA binding site which holds the tRNA that carries the growing polypeptide chain.
3-**E site**: exit site, where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

NOTE: there are other chambers in the ribosome, such as mRNA binding site or exit tunnel, but the doctor didn't mention them, so we will stick to what the doctor said.

THE general mechanism for translation:

- Three stages: initiation, elongation, and termination.(each stage has its specific proteins responsible for it)

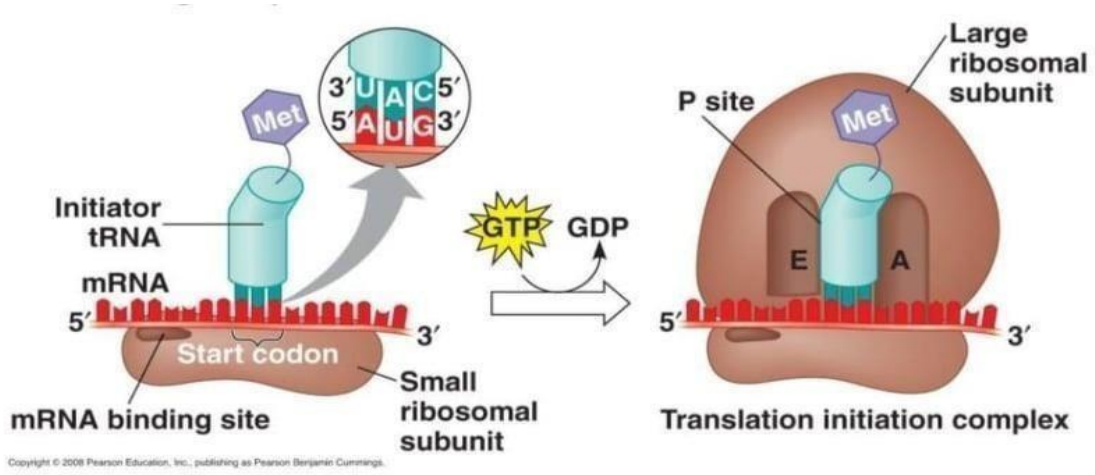
*The direction for reading mRNA is from 5' → 3'.
*Protein synthesis begins at the amino terminus and extends toward the carboxyl terminus.(This means that A.a are added on the carboxyl terminus of the growing polypeptide)



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 AUG (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).



First, small subunit binds to mRNA, then tRNA binds to AUG codon(notice that this happens before the complete assembly of ribosome, then using energy from GTP large subunits binds to form the translation initiation complex.

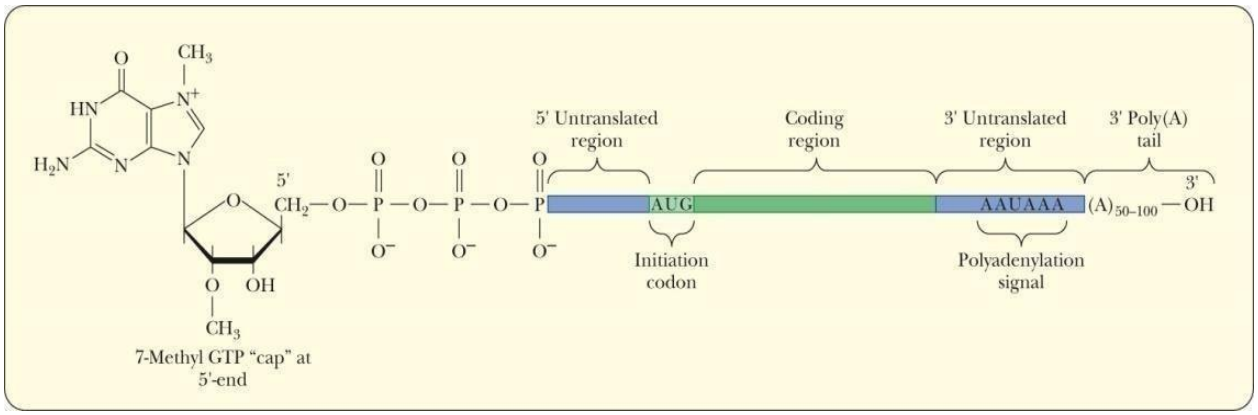
Untranslated regions (UTRs):

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.

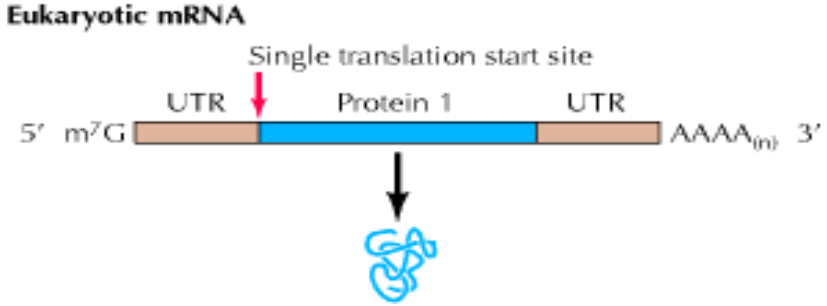
- 3'untranslated region, which follows any of the three stop codons.

*the UTRs have an importance in regulation translation.



-Remember:

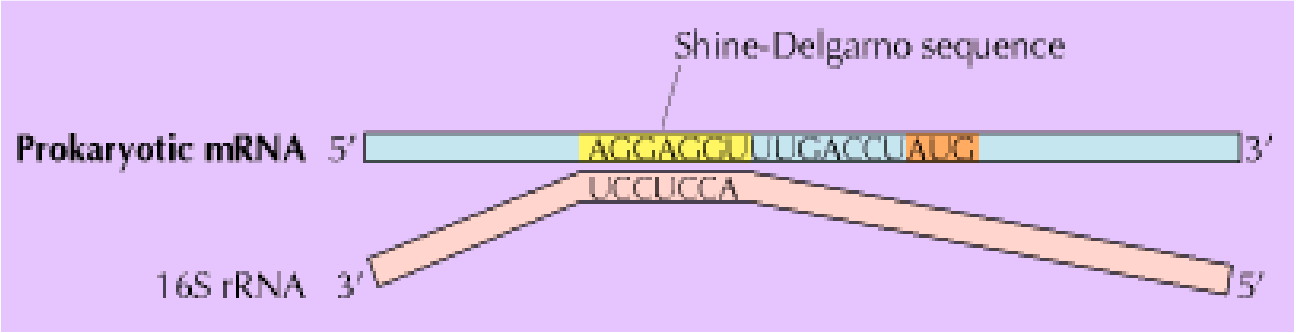
- Bacterial mRNA is polycistronic: many polypeptides from the same mRNA.
- Eukaryotic mRNA is monocistronic: one polypeptide from each mRNA.



*Shine-Dalgarno sequence:

A good question to ask is how do ribosomes know that this certain area is a region and should be translated, specially that there is more that on AUG, then how do prokaryotic ribosomes identify which AUG is the translation starting site?

In prokaryotic:



The figure shows a shine-Dalgamo sequence, a consensus sequence, which is found 8 bases upstream the initiation site

This sequence is complementary to a region in the 16S rRNA, which is part of the small subunit.

Thus translation starts at the first AUG after the shine-Dalgano sequence

* if a bacterial gene codes for 3 different polypeptides that use in the same process, then gene have 3 different start codons(AUG with a with shine-Dalgamo sequence before it) & 3 different stop codons.