Tutorial 5 . **Ribosome and protein synthesis**

1. DEFINITION

Ribosome, particle that is present in large numbers in all living cells and serves as the site of protein synthesis. Ribosomes occur both as free particles in prokaryotic and eukaryotic cells and as particles attached to the membranes of the endoplasmic reticulum in eukaryotic cells. The small particles that came to be known as ribosomes were first described in 1955 by Romanian-born American cell biologist George E. Palade, who found them to be frequently associated with the rough endoplasmic reticulum of eukaryotic cells..

2.ULTRASTRUCTURE

At the TEM and using the negative staining technique, the ribosomes appear as distinct electron-dense globular particles 14 to 23 nm in diameter (**Figure 1**). They exist in cells, free in the cytosol, in the form of two separate subunits when they are inactive or grouped in strings on the mRNA constituting polyribosomes (or polysomes) when they are active.

They are also attached as polyribosomes to the cytosolic surface of the cistern membrane of the endoplasmic reticulum (REG) and the outer nuclear membrane. They are also found in semi-autonomous organelles (mitochondria and chloroplast).

The negative staining technique, which consists of increasing the contrast with TEM by using an e-dense substance such as phosphotungstic acid (mimeograph p.31), has revealed that ribosomes are compact edifices made up of 2 sub-units of different shape and size, which adapt to each other thanks to the presence of an mRNA molecule during their activity (translation).

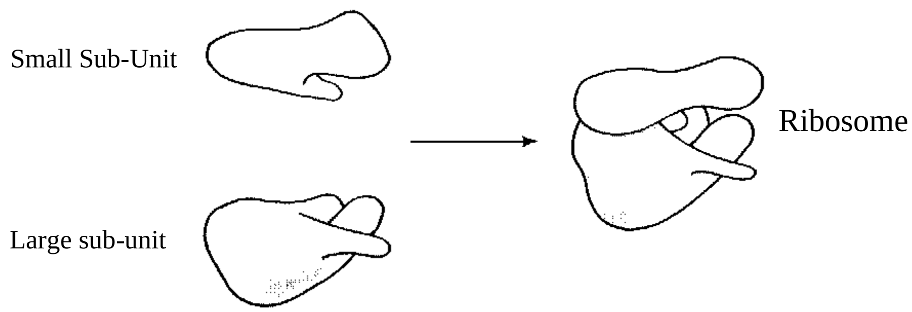

Figure 1 : Structural organization of the ribosome.

3.CHEMICAL COMPOSITION

Ribosomes are made of RNA and protein. In E. coli, they contain 34% protein and 66% RNA, while in eukaryotes, they contain 40% protein and 60% RNA. Chemical composition of large subunit is different from that of small subunit. The size and sedimentation coefficients of ribosomes and their subunits and the rRNAs vary in prokaryotic cytoplasm, eukaryotic cytoplasm, mitochondria and chloroplasts.

In prokaryotes, proteins in the small subunit (30S) are designated as S1, S2, …, S21, and each type is present in a single copy. In the large subunit (50S), the 31 specific proteins are designated as L1, L2, …. One protein L7/L12 is present in 4 copies ribosome, while the rest are present in single copies.

The ribosomes of prokaryotic and eukaryotic cells have structure and function similar. However, the length of the main rRNA molecules, the L and S protein content of each subunit, and the size of the elements differ between prokaryotes and eukaryotes (following table).

	Prokaryotic cell	**Eukaryotic cell**
Large sub-unit	-ARNr 23S et 5S -31 to 34 L proteins - sediment at 50S	- ARNr 28S, 5,8S et 5S 45 to 50 L proteins - sediment at 60S
Small Sub-Unit	- ARNr16S - 21 protéines S - sediment at 30S	- ARNr 18S -30 to 33 S proteins - sediment at 40S
Assembled and active ribosome	-Reduced size -Fewer - sediment at 70S	-Larger size -More numerous - sediment at 80S

Note : ribosomes are also found in chloroplasts and mitochondria, ribosomes in Chloroplasts are similar to prokaryotic ribosomes (70S) while mitochondrial ribosomes have smaller rRNAs and fewer proteins than prokaryotic ribosomes.

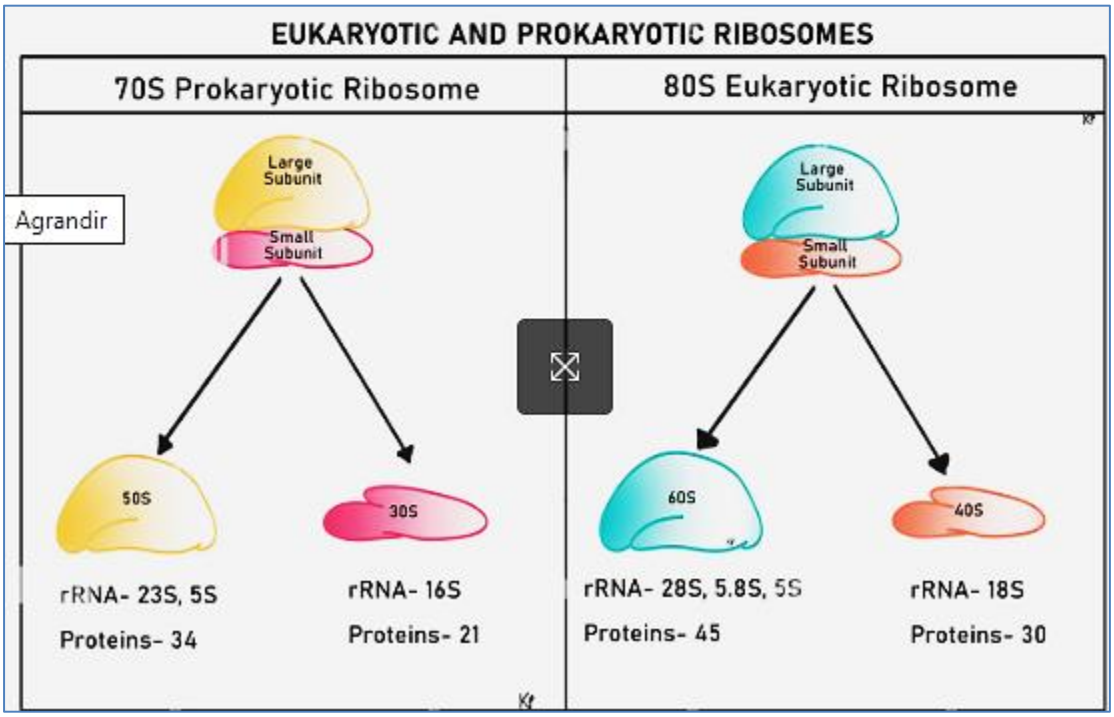

Figure 2 : Structure of the ribosome in prokaryotes and eukaryotes

4.ORGANIZATION AND LIAISON SITES

The ribosome has four binding sites located exclusively on rRNAs: a binding of the mRNA located on the rRNA of the small subunit and three tRNA binding sites located largely on the rRNA of the large subunit.

- The aminoacyl-tRNA binding site (A site), which binds the incoming tRNA molecule, carrying a new amino acid.
- The peptidyl-tRNA binding site (P site), which binds the tRNA molecule carrying the growing polypeptide, is the site where a new peptide bond between 2 amino acids is formed, this site is formed by 23S rRNA. Peptidyl transferase is therefore not a protein enzyme ribosomal but an rRNA playing the role of an enzyme (hence the name ribozyme for the ribosome).

3.And finally, the binding site of the outgoing empty tRNA (E or Exit site).

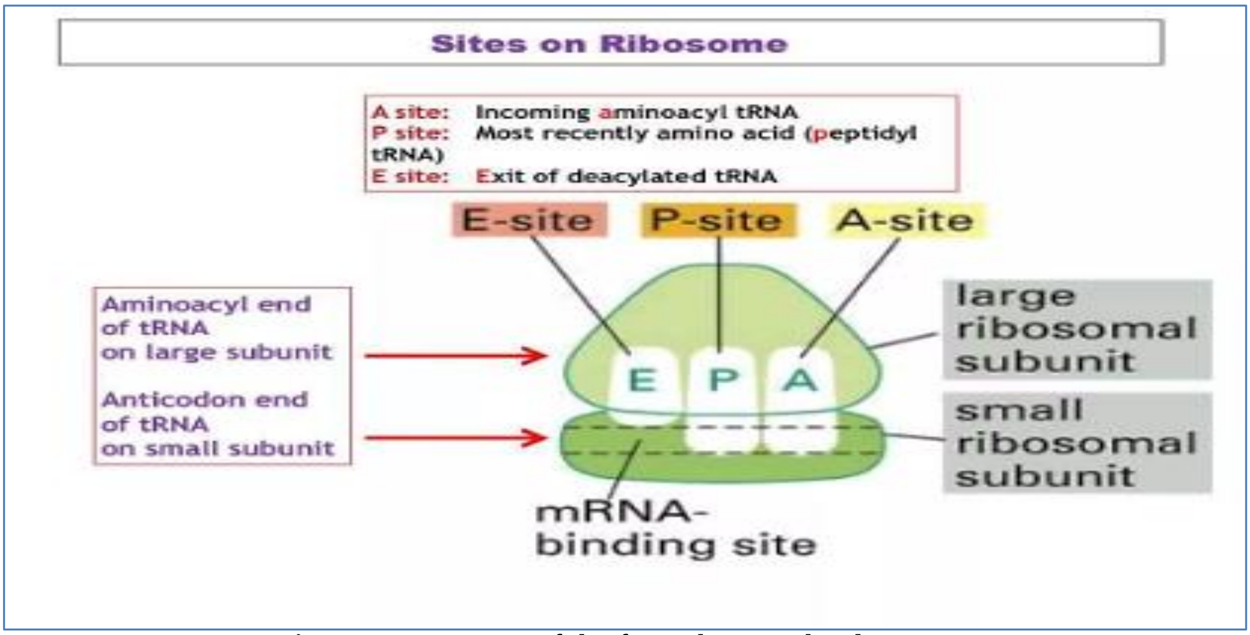

Figure 3 : Location of the four ribosome binding sites.

5.FUNCTIONS

The information transmitted by DNA is encoded in the form of a succession of groups of three bases (triplet or codon). After mRNA leaves the nucleus, it moves to a ribosome, which consists of rRNA and proteins. The ribosome reads the sequence of codons in mRNA. The tRNAs that carry amino acids recognize codons by their anticodons and bring them to the ribosome in the correct sequence. The reading of the mRNA is preceded by the formation of the initiation complex. Translation occurs in three stages: Initiation, Elongation and Termination.

Initiation:

After transcription in the nucleus, the mRNA exits through a nuclear pore and enters the cytoplasm. At the region on the mRNA containing the methylated cap and the start codon, the small and large subunits of the ribosome bind to the mRNA molecule at its 5' end to begin translation. These are then joined by a tRNA which contains the anticodons matching the start codon on the mRNA. This group of molecues (mRNA, ribosome, tRNA) is called an initiation complex.

Elongation:

tRNA keep bringing amino acids to the growing polypeptide according to complementary base pairing between the codons on the mRNA and the anticodons on the tRNA. As a tRNA moves into the ribosome, its amino acid is transferred to the growing polypeptide. Once this transfer is complete, the tRNA leaves the ribosome, the ribosome moves one codon length down the mRNA, and a new tRNA enters with its corresponding amino acid. This process repeats and the polypeptide grows.

Termination:

At the end of the mRNA coding is a stop codon which will end the elongation stage. The stop codon (UAA, UAG or UGA) doesn’t call for a tRNA, but instead for a type of protein called a release factor, which will cause the entire complex (mRNA, ribosome, tRNA, and polypeptide) to break apart, releasing all of the components.

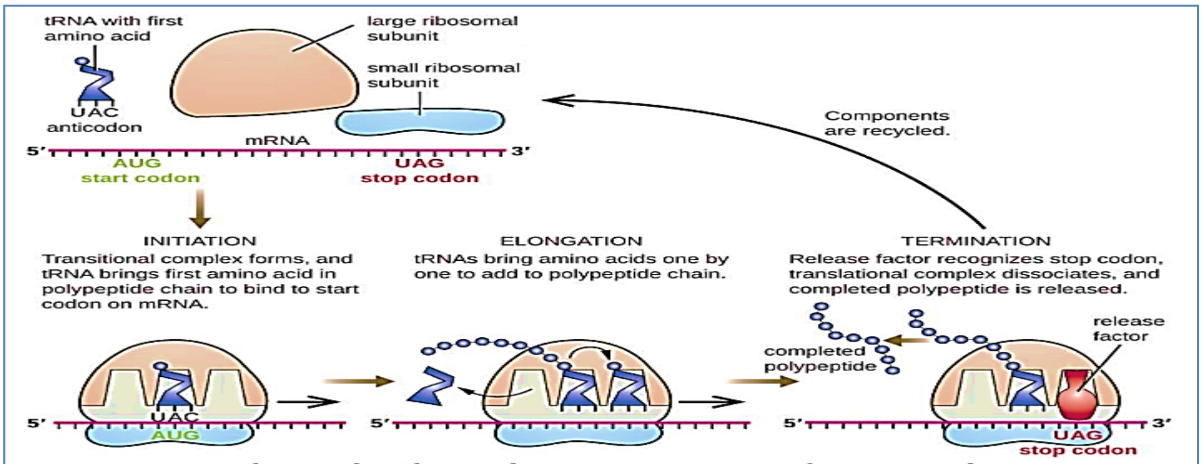

Figure 5.7.4 Translation takes place in three stages: Initiation, Elongation and Termination.

Figure 4 : Function of the ribosome

Molecules involved in translation : The molecules involved in the different stages of protein synthesis are succinctly:

- rRNAs that make up the ribosome, some of which act as enzymes,
- mRNA from the nucleus carrying genetic information in the form of codons (base triplets),
- tRNAs also from the nucleus with the anticodon and carrying the activated amino acids, from the cytosol,
- Amino acids present in the cytosol,
- Several types of cytosolic factors: factors responsible for the activation of amino acids and their attachment to different tRNAs between prokaryotes and eukaryotes, initiation, elongation and termination factors, ATP (Adenosine triphosphate) and GTP (guanosine triphosphate).

After a polypeptide chain is synthesized, it may undergo additional processes. For example, it may assume a folded shape due to interactions between its amino acids. It may also bind with other polypeptides or with different types of molecules, such as lipids or carbohydrates. Many proteins travel to the Golgi apparatus within the cytoplasm to be modified for the specific job they will do.

6.Protein synthesis locations

1. Secreted proteins, lysosomes and plasma membrane are synthesized in the granular endoplasmic reticulum (REG) by bound ribosomes. In particular, the secreted proteins follow the path:
   1) Endoplasmic reticulum
   2) Golgi apparatus
   3) Secretory vesicles
   4) Periplasmic space

2. Proteins from the nucleus, mitochondria, and peroxisomes are synthesized in the cytosol by free ribosomes.

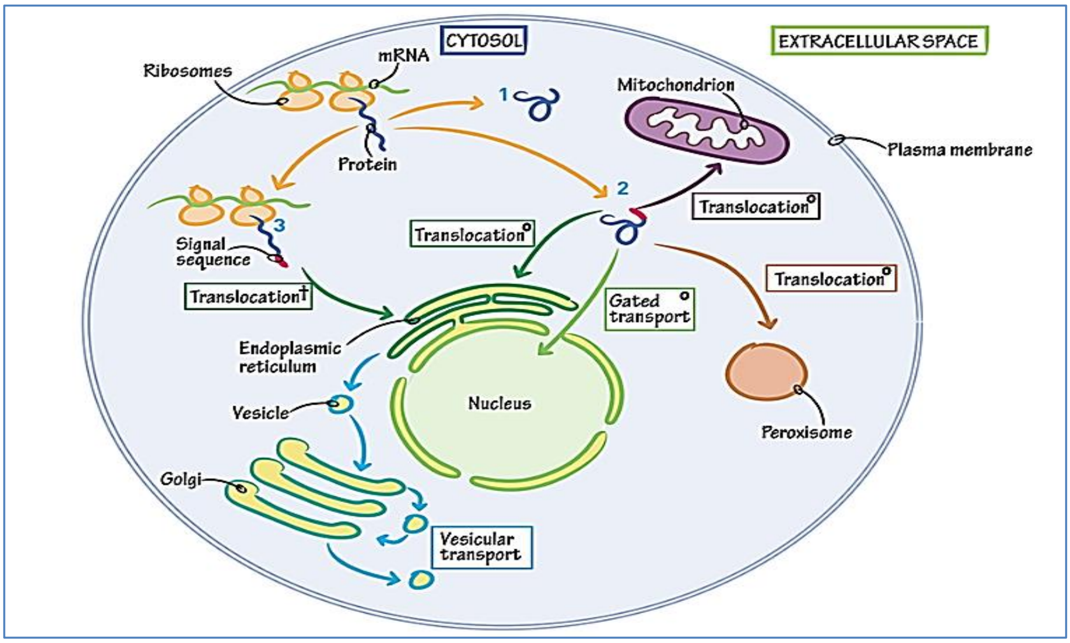

Figure 5 : Protein synthesis locations