Chapter 5: **RIBOSOMES**

I. DEFINITION

Ribosomes are small, compact particles present in all cells, in very large numbers. They are the major ribonucleoprotein complexes in prokaryotic and eukaryotic cells; they catalyzing the assembly of amino acids (AA) in a predetermined order and thus the elongation of polypeptides or protein synthesis.

II. ULTRASTRUCTURE

Using the TEM and negative staining technique, ribosomes appear as distinct globular electron-dense particles 14 to 23 nm in diameter (**figure 1**). They exist in cells, either free in the cytosol, as two separate subunits when inactive, or grouped in strings on mRNA forming polyribosomes (or polysomes) when active.

They are also attached as polyribosomes to the cytosolic face of the membrane of the granular endoplasmic reticulum (GER) 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 in the TEM using an e-dense substance such as phosphotungstic acid (handout 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).

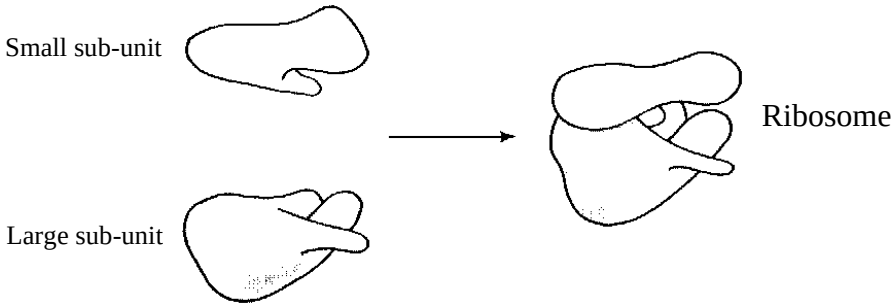

Figure 1: Structural organization of the ribosome.

III. CHEMICAL COMPOSITION

1. Isolement

The third DUC pellet of a cell homogenate contains microsomes (small rough vesicles obtained by fragmentation of the (GER). By adding a mild detergent, the ribosomes are detached from the membranes. At very high speed, DUC separates the free ribosomes in pellet 4. The sucrose concentration gradient is used to characterize ribosomes and ribosomal subunits according to their sedimentation coefficient expressed in Svedberg units (S).

2. Analysis results

The ribosomes of all cells comprise a large subunit (large element) and a small subunit (small element). These contain ribosomal RNA (rRNA) molecules (around 65%) and a number of proteins (around 35%). The proteins and rRNAs differ from one subunit to another. The large subunit comprises a main rRNA molecule and the small subunit a small rRNA molecule. Large (L) proteins characterize the large subunit and Small (S) the small subunit.

Ribosomes in prokaryotic and eukaryotic cells have a similar structure and function. 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 (see table below).

	Prokaryotic cell	**Eukaryotic cell**
Large sub-unit	- 23S and 5S rARN - 31 to 34 proteins L - sedimented at 50S	- 28S, 5,8S and 5S rARN - 45 to 50 proteins L - sedimented at 60S
Small sub-unit	- rARN16S - 21 proteins S - sedimented at 30S	- rARN 18S - 30 to 33 proteins S - sedimented at 40S
Ribosome assembled and active	- Reduced size - Fewer - sedimented at 70S	- Larger size - More of them - sedimented at 80S

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

IV. ORGANIZATION AND CONNECTION SITES

The ribosome has four binding sites located exclusively on rRNAs: an mRNA binding site located on the rRNA of the small sub-unit and three tRNA binding sites located mainly on the rRNA of the large sub-unit.

- The aminoacyl-tRNA binding site (site A), which binds the incoming tRNA molecule, carrying a new amino acid.

- The peptidyl-tRNA binding site (site P), which binds the tRNA molecule carrying the growing polypeptide; this is the site where a new peptide bond is formed between two amino acids; this site is formed by the 23S rRNA. Peptidyl transferase is therefore not a ribosomal protein enzyme but an rRNA playing the role of an enzyme (hence the name ribozyme for the ribosome).
- And finally, the empty tRNA binding site (E or Exit site).

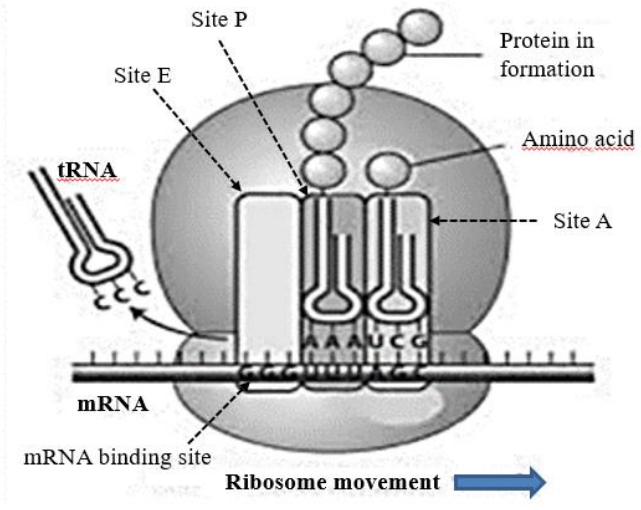

Figure 2: Location of the four-ribosome binding sites.

V. FUNCTIONS

The function of ribosomes is translation or protein synthesis, the various stages of which can be summarized as follows: the small sub-unit matches tRNAs to mRNA codons, the large sub-unit

catalyses the formation of peptide bonds that link amino acids together to form a polypeptide chain.

The two elements come together on an mRNA molecule at its 5' end to begin translation. The ribosome moves along the mRNA translating the nucleotide sequence codon by codon in the 5'-3' direction into an amino acid sequence, using the tRNAs as adapters to add the correct order of each amino acid to the end of the growing polypeptide chain. The 2 sub-units separate when the synthesis of the protein or polypeptide is complete.

Molecules involved in translation: the molecules involved in the various stages of protein synthesis are briefly:
- rRNAs making up the ribosome, some of which act as enzymes,
- mRNA from the nucleus carrying genetic information in the form of codons (triplets of bases),
- tRNAs also coming from the nucleus with the anticodon and carrying the activated amino acids, coming 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 tRNAs, which differ between prokaryotes and eukaryotes, initiation, elongation and termination factors, ATP (adenosine triphosphate) and GTP (guanosine triphosphate).

For more information

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4. Lodish, Baltimore, Berk, Zipursky et Matsudaira, 2000- Biologie moléculaire de la cellule. Deboeck université édition. pp : 128-140.
5. Cooper G., 1999- La cellule. Deboeck université édition, pp. 273-311.
6. Campbell et Reece, 2004- Biologie. Deboeck université édition, pp : 111-122 et pp : 337-353.
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