II. Biosynthesis of membrane proteins and secretory proteins

II.1. Sites of protein synthesis

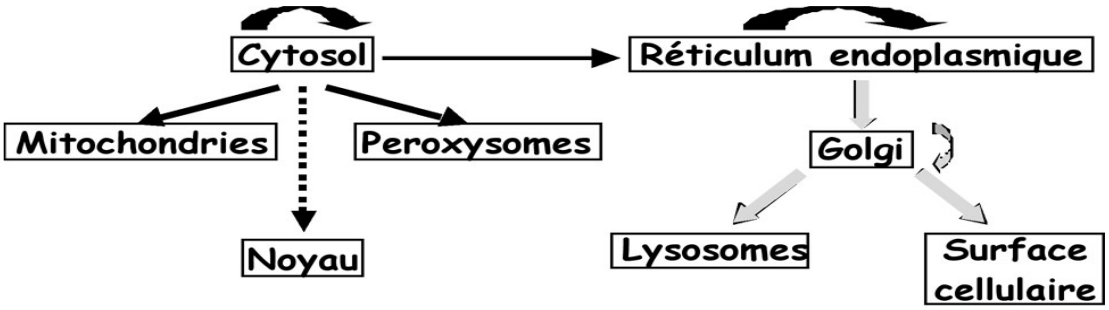
The presence of ribosomes on the surface of the RER indicates that it plays a major role in the synthesis of proteins (membrane and exported), as opposed to free ribosomes in the cytosol, which are responsible for the synthesis of cytosolic proteins. The synthesis of these proteins takes place at the level of polysomes (polyribosomes). We can distinguish between :

Table III.1. Proteins synthesized by different categories of ribosomes

Location of ribosomes	Types of proteins synthesized
Mitochondria	-All proteins encoded by mitochondrial DNA: certain intrinsic proteins of the inner membrane.
Free cytosolic ribosomes	-Cytoskeletal proteins and those attached to the cytosolic face of intrinsic proteins (spectrin and ankyrin). -Proteins anchored by a lipid to the PM (GPI, etc.). -Mitochondrial proteins encoded by nuclear DNA. -Chloroplast proteins encoded by nuclear DNA. -Peroxisomal and nuclear proteins (histones, lamin proteins, etc.)
Cytosolic ribosomes attached to membranes	Intrinsic proteins and glycoproteins (PM, nuclear, Golgi, ER, lysosomes, and endosomes). -Secreted proteins and those of the extracellular matrix: fibronectin and collagen, which are frequently attached to intrinsic PM proteins, lysosomal enzymes, ER, Golgi complex.

II.2. The different pathways of protein trafficking

All proteins originate on the ribosomes of the cytosol and are then directed to two main branches.



Pore nucléaire sélectif

Transport membranaire (protéine membranaire de translocation)

Transport vésiculaire

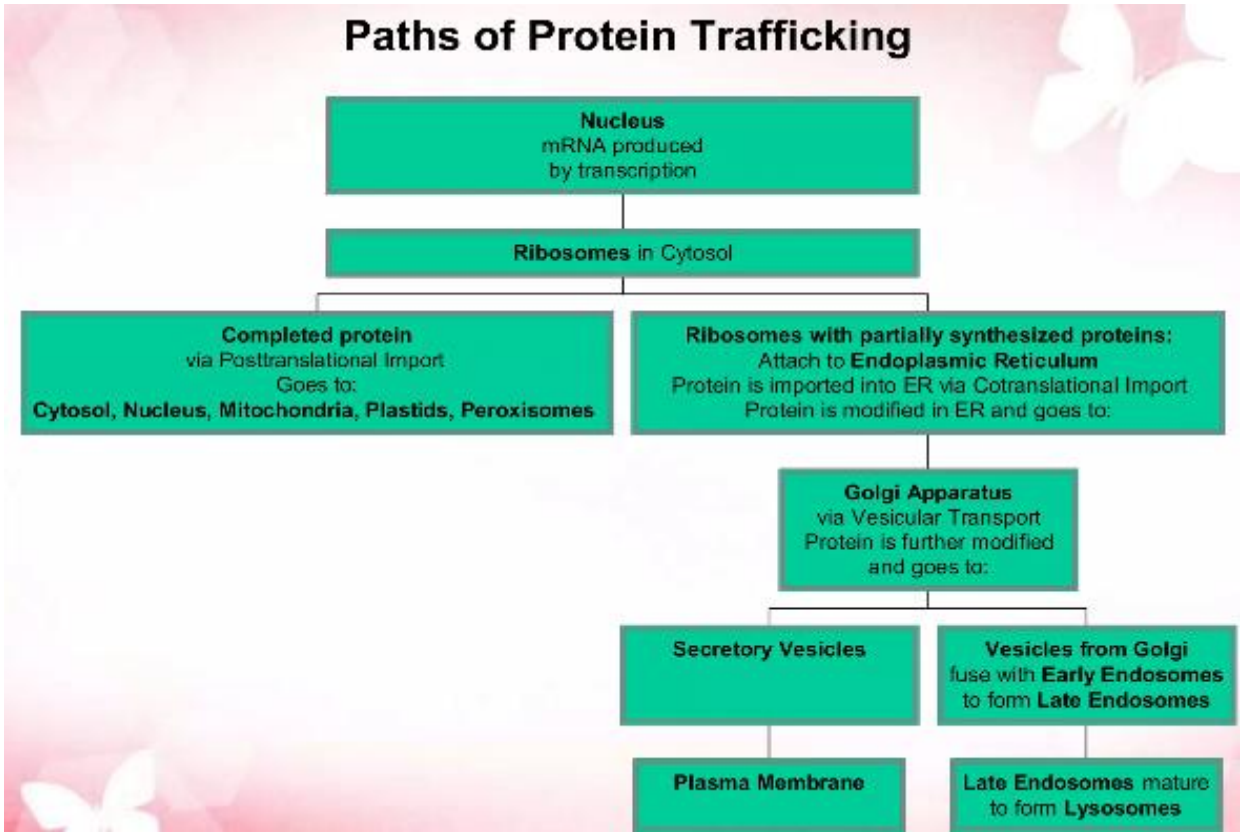


Figure III.3. Different pathways of protein trafficking **[23].**

In the first branch, proteins are initially released into the cytosol after synthesis. Most of these proteins remain in the cytosol, while others are exported to the mitochondria, nucleus, or peroxisomes. Proteins pass through the mitochondrial or peroxisomal membrane thanks to a membrane translocation protein. Proteins pass into the nucleus through nuclear pores, which have selective permeability.

In the second pathway, proteins are initially transferred to the endoplasmic reticulum. These proteins are transferred to the endoplasmic reticulum after the ribosomes that synthesize them attach to the reticulum. Translocation requires a transport protein. Translocated proteins can remain in the

endoplasmic reticulum or be directed to the Golgi apparatus. In the Golgi apparatus, proteins can again remain in this compartment or be directed either to the lysosomes or to the plasma membrane. The transfer of proteins from the reticulum to other organelles (Golgi, lysosomes) and the plasma membrane is carried out by transport vesicles.

II.3. Protein addressing

Addressing is a set of biochemical mechanisms that enable proteins to be sorted from their place of production to their place of function (their intracellular or extracellular destination). The sorting mechanism is complex and depends in part on sorting (or addressing) signals present in proteins, which are recognized by specific receptor proteins. There are two types of sorting signals:

II.3.1. The signal peptides

They consist of a continuous sequence of amino acids (15 to 60 residues). When the sorting process is complete, these signal peptides are usually cleaved from the mature protein by a signal peptidase. Signal peptides are used to transport proteins from the cytosol to the endoplasmic reticulum, mitochondria, peroxisomes, and nucleus, and are also involved in protein retention in the endoplasmic reticulum.

II.3.2. The signal regions

Amino acids that form a signal region may be very distant from each other on the unfolded molecule. Such signal regions are involved in the transport of proteins from the Golgi apparatus to lysosomes.

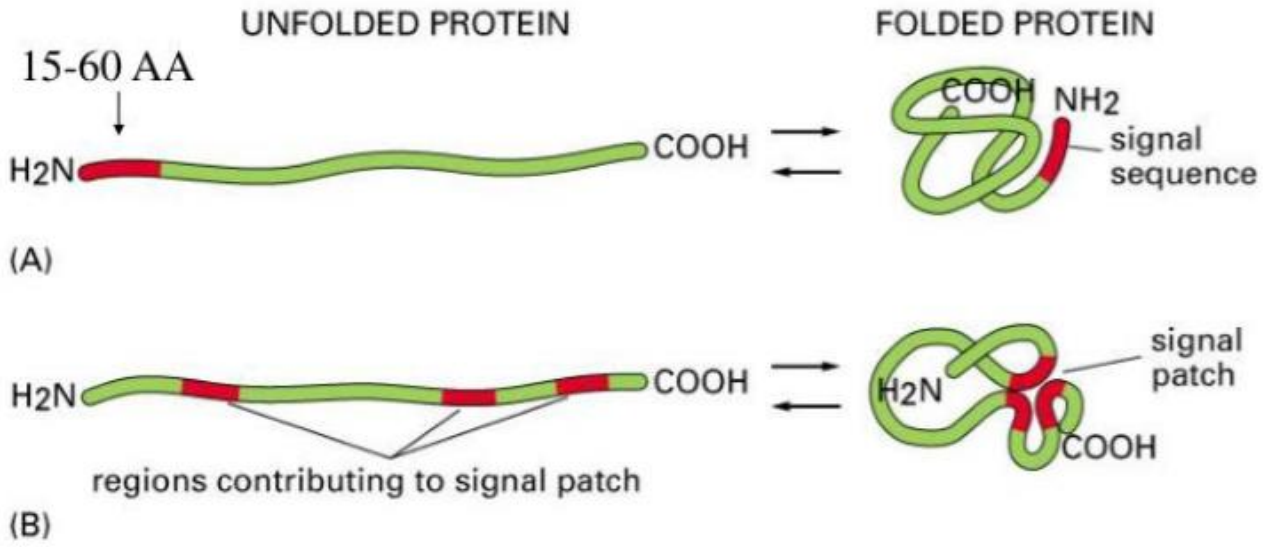


Figure III. 4. Types of sorting signals **[23].**

Signal Sequences

FUNCTION OF SIGNAL SEQUENCE	EXAMPLE OF SIGNAL SEQUENCE
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Export from nucleus	-Leu-Ala-Leu-Lys-Leu-Ala-Gly-Leu-Asp-Ile-
Import into mitochondria	$^{+}H_3N$-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu-
Import into plastid	$^{+}H_3N$-Met-Val-Ala-Met-Ala-Met-Ala-Ser-Leu-Gln-Ser-Ser-Met-Ser-Ser-Leu-Ser-Leu-Ser-Ser-Asn-Ser-Phe-Leu-Gly-Gln-Pro-Leu-Ser-Pro-Ile-Thr-Leu-Ser-Pro-Phe-Leu-Gln-Gly-
Import into peroxisomes	-Ser-Lys-Leu-COO^-
Import into ER	$^{+}H_3N$-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-
Return to ER	-Lys-Asp-Glu-Leu-COO^-

Some characteristic features of the different classes of signal sequences are highlighted in color. Where they are known to be important for the function of the signal sequence, positively charged amino acids are shown in *red* and negatively charged amino acids are shown in *green*. Similarly, important hydrophobic amino acids are shown in *white* and hydroxylated amino acids are shown in *blue*. $^{+}H_3N$ indicates the N-terminus of a protein; COO^- indicates the C-terminus.

Table 12-3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

II.4. Mechanisms of co-translational insertion (translocation) of proteins into the RER

The entry of proteins into the ER occurs concurrently with translation. The signal recognition particle (SRP) complex is a structure that recognizes the signal sequence of the protein and transports it to the ER, but also acts as a signal that allows the RNA to be translated. This complex is made up of six proteins and a messenger RNA. When the SRP domain recognizes a particular domain of the protein, it surrounds the ribosome with its structure, preventing it from continuing translation. This SRP complex is recognized by receptors in the ER membrane. When they bind, translation starts again and the protein gradually enters the reticulum through a pore. This phenomenon is called a co-translational translocation mechanism. This complex is also GTPase-dependent, meaning it hydrolyzes itself to detach from the receptor. Once the sequence is found in the ER, if the protein is soluble, a signal peptidase protein will cut the signal sequence from the ER address when it finishes translation, and the protein will be released into the cytosol. Once it is free in the cytosol, it will adopt a tertiary and quaternary conformation thanks to chaperone proteins. The protein can also be found in a non-soluble form, in which case it is a transmembrane protein.

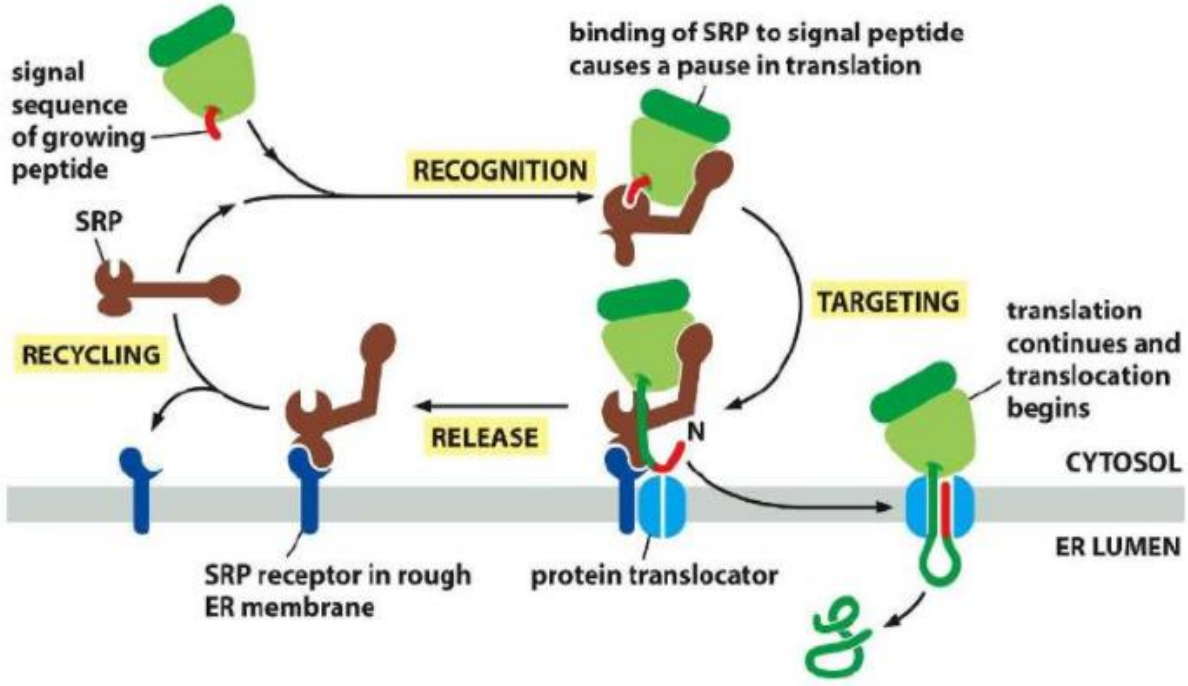


Figure III.5. Ribosome targeting to the RER membrane **[23].**

II.5. Destiny of proteins synthesized in the ER

Most proteins produced in the ER reach the Golgi apparatus via transport vesicles that sprout from the RER.

- **Soluble proteins**

- Secreted to the outside by exocytosis
- Directed to the lumen of lysosomes

- **Transmembrane proteins**

- Plasma membrane
- Lysosome membrane

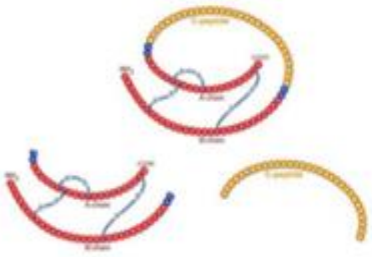
- **Other proteins are resident in the ER**

Post Translational Modification of Proteins

For proteins to be functional they must often be modified after translation.

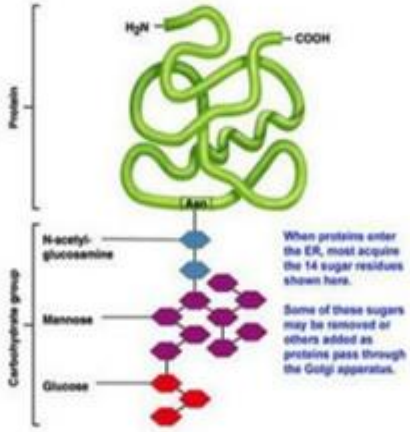
Cleaving

- Initiator aa (Methionine) is cleaved off during translation
- Signaling aa are cleaved off in ER, Golgi, or target organelle.
- Some proteins must be cleaved into smaller pieces to be function (e.g. insulin); Most cleaving takes place in the Golgi



Glycosylation

- Glycosylation: attachment of a carbohydrate to a Lipid or Protein
- Occurs in ER and Golgi Apparatus



Chaparones

- Most proteins can fold on their own into the 'right' (i.e. active) conformation
- Chaperones are a class of proteins that help some proteins to fold
- Sometimes Chaperones prevent folding until the polypeptide reaches its target organelle (e.g. mitochondria)