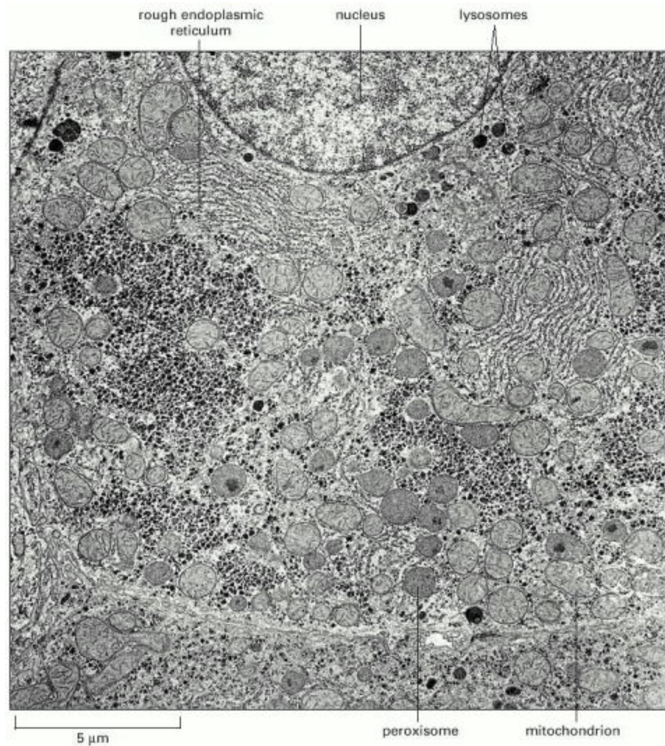
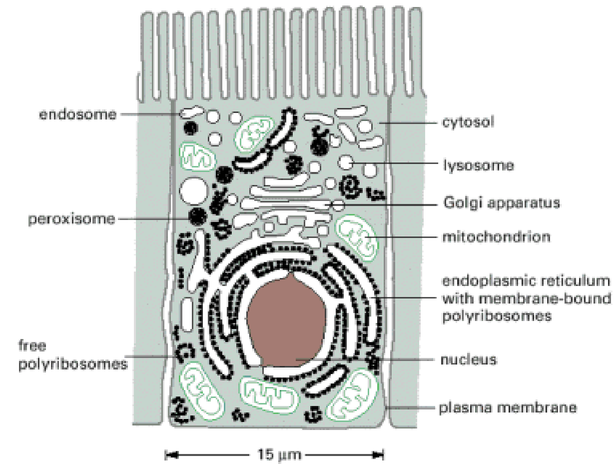
Ενδοκυττάρια διαμερίσματα και μεταφορά

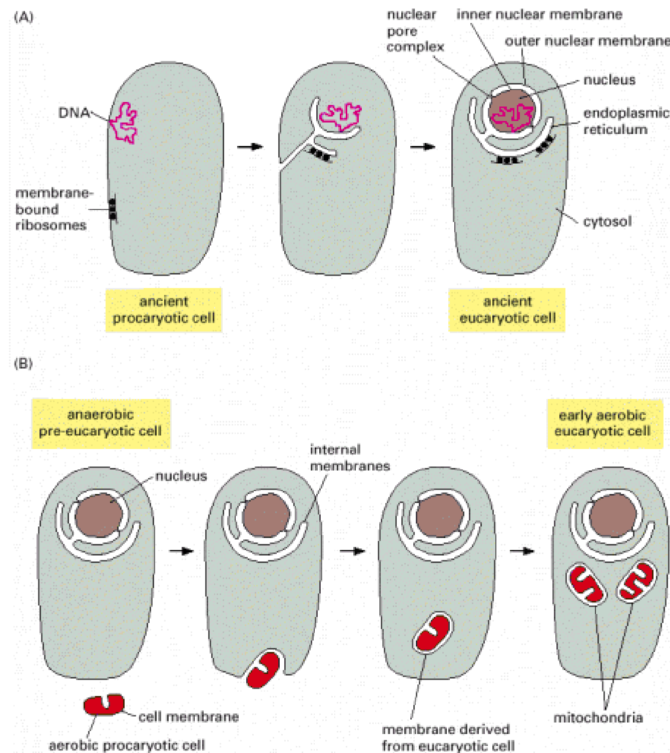
•Μεμβρανικά οργανίδια
•Διαλογή των πρωτεϊνών
•Μεταφορά με κυστίδια
•Εξωκυττάρωση
•Ενδοκυττάρωση

Μεμβρανικά οργανίδια

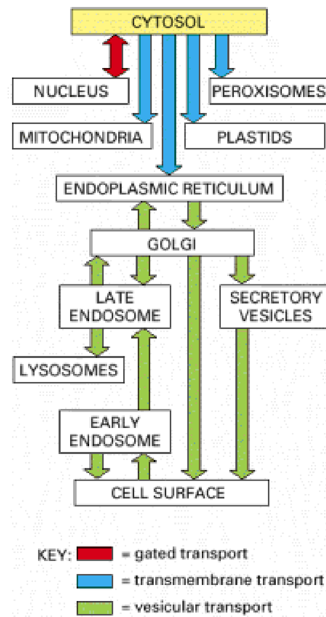




Πώς εξελίχθηκαν τα μεμβρανικά οργανίδια



Διαλογή πρωτεϊνών (protein sorting)

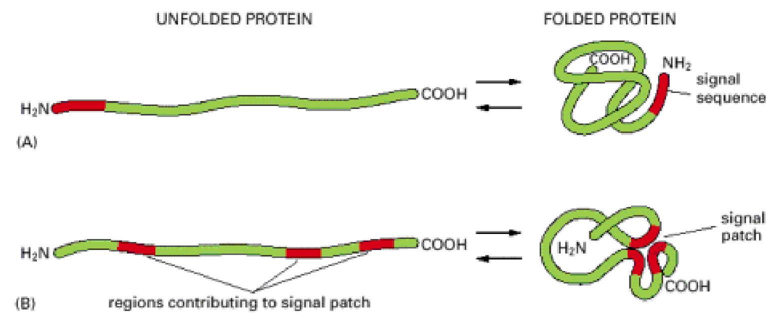


Μερικές χαρακτηριστικές σηματοδοτικές αλληλουχίες

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 *yellow* and hydroxylated amino acids are shown in *blue*. $^{+}H_3N$ indicates the N-terminus of a protein; COO^- indicates the C-terminus.

Δύο τρόποι με τους οποίους φτιάχνονται οι σηματοδοτικές αλληλουχίες



Τρόποι μελέτης σημάτων διαλογής και μετάθεσης πρωτεϊνών διαμέσου μεμβρανών

A TRANSFECTION APPROACH FOR DEFINING SIGNAL SEQUENCES

One way to show that a signal sequence is required and sufficient to target a protein to a specific intracellular compartment is to create a fusion protein in which the signal sequence is attached by genetic engineering techniques to a protein that is normally resident in the cytosol. After the cDNA encoding this protein is transfected into cells, the location of the fusion protein is determined by immunostaining or by cell fractionation.

signal sequence a or b
gene encoding cytosolic protein
plasmid used to transfect cells
A
B
signal sequence a ( ) directs fusion protein to organelle A
signal sequence b ( ) directs fusion protein to organelle B

By altering the signal sequence using site-directed mutagenesis, one can determine which structural features are important for its function.

A BIOCHEMICAL APPROACH FOR STUDYING THE MECHANISM OF PROTEIN TRANSLOCATION

In this approach a labeled protein containing a specific signal sequence is transported into isolated organelles *in vitro*. The labeled protein is usually produced by cell-free translation of a purified mRNA encoding the protein; radioactive amino acids are used to label the newly synthesized protein so that it can be distinguished from the many other proteins that are present in the *in vitro* translation system.
Three methods are commonly used to test if the labeled protein has been translocated into the organelle:

1. The labeled protein co-fractionates with the organelle during centrifugation.

signal sequence
radioactively labeled protein
protein transported into isolated organelle

2. The signal sequence is removed by a specific protease that is present inside the organelle.

incubated without organelle
incubated with organelle
radioactive proteins on SDS gel

3. The protein is protected from digestion when proteases are added to the incubation medium but is susceptible if a detergent is first added to disrupt the organelle membrane.

protease
protein
no detergent
plus detergent

By exploiting such *in vitro* assays, one can determine what components (proteins, ATP, GTP, etc.) are required for the translocation process.

GENETIC APPROACHES FOR STUDYING THE MECHANISM OF PROTEIN TRANSLOCATION

wild-type yeast cell
histidinol
histidine
ER
translocation apparatus
enzyme in cytosol: cell lives without histidine as nutrient

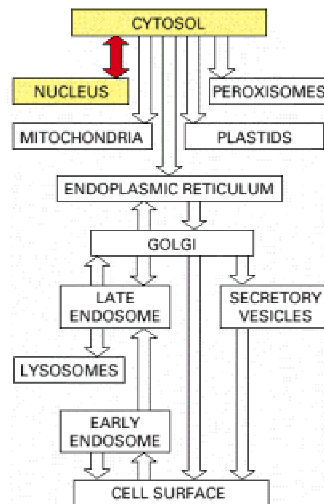
engineered yeast cell
histidinol
enzyme targeted to ER: cell dies without histidine as nutrient

mutant engineered cell
histidinol
histidine
mutant translocation apparatus
not all enzyme taken up into ER: cell lives without histidine as nutrient

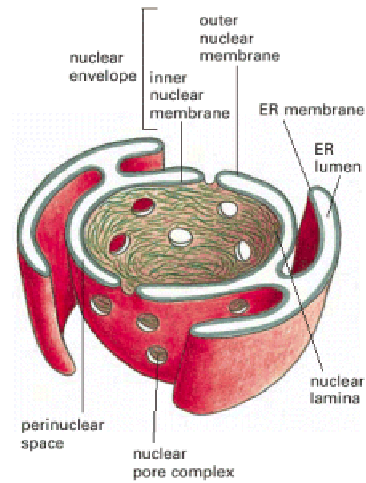
Yeast cells with mutations in genes that encode components of the translocation machinery have been useful for studying protein translocation. Because mutant cells that cannot translocate proteins across their membranes will die, the trick is to design a strategy that allows weak mutations that cause only a partial defect in protein translocation to be isolated.

One way uses genetic engineering to design special yeast cells. The enzyme histidinol dehydrogenase, for example, normally resides in the cytosol, where it is required to produce the essential amino acid histidine from its precursor histidinol. A yeast strain is constructed in which the histidinol dehydrogenase gene is replaced by a re-engineered gene encoding a fusion protein with an added signal sequence that misdirects the enzyme into the endoplasmic reticulum (ER). When such cells are grown without histidine, they die because all of the histidinol dehydrogenase is sequestered in the ER, where it is of no use. Cells with a mutation that partially inactivates the mechanism for translocating proteins from the cytosol to the ER, however, will survive because enough of the dehydrogenase will be retained in the cytosol to produce histidine. Often one obtains a cell in which the mutant protein still functions partially at normal temperature but is completely inactive at higher temperature. A cell carrying such a temperature-sensitive mutation dies at higher temperature, whether or not histidine is present, as it cannot transport any protein into the ER. This allows the normal gene that was disabled by the mutation to be identified by transfecting the mutant cells with a yeast plasmid vector into which random yeast genomic DNA fragments have been cloned: the specific DNA fragment that rescues the mutant cells when they are grown at high temperature should encode the wild-type version of the mutant gene.

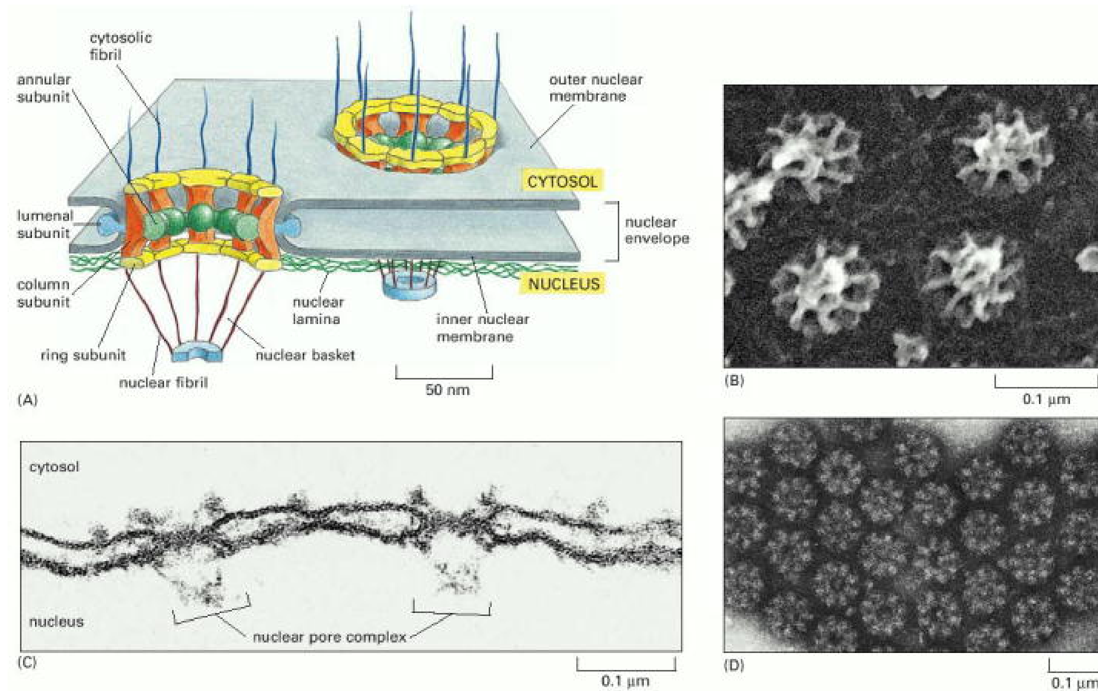
Μεταφορά μορίων μεταξύ πυρήνα-κυτταροπλάσματος



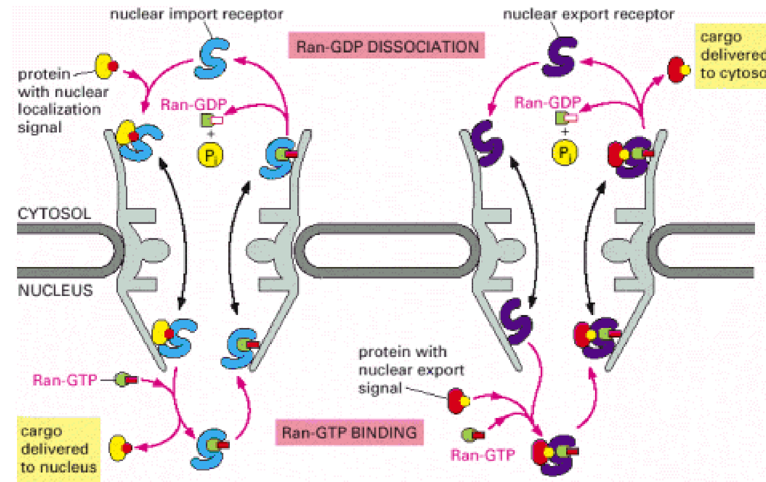
Το πυρηνικό περίβλημα



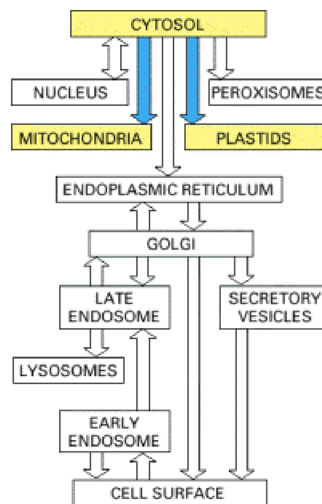
Το σύμπλοκο του πυρηνικού πόρου



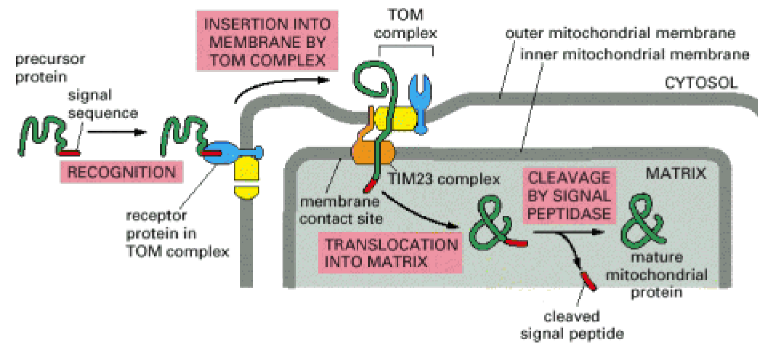
Σχηματική αναπαράσταση του μηχανισμού της ενεργής μεταφοράς μέσω πυρηνικών πόρων



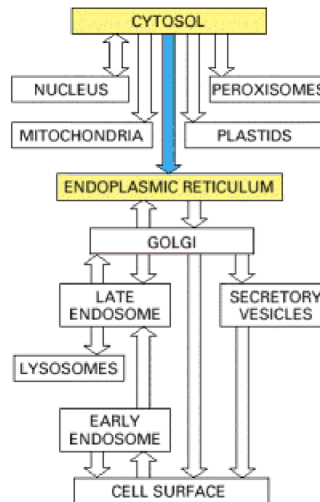
Μεταφορά πρωτεϊνών στα μιτοχόνδρια και στους χλωροπλάστες



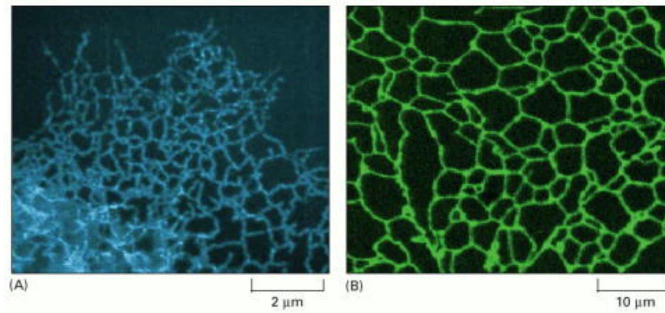
Εισαγωγή πρωτεϊνών στα μιτοχόνδρια

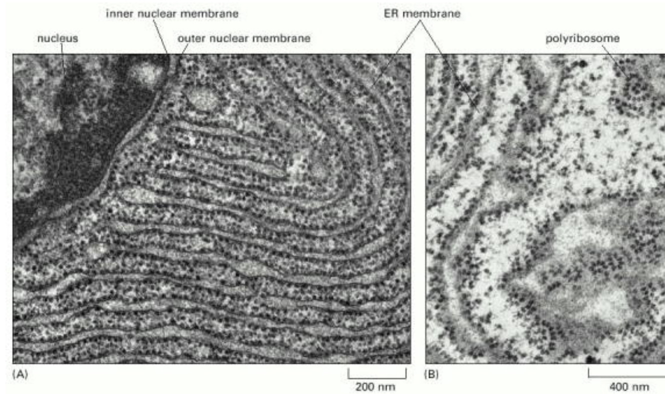


Οι πρωτεΐνες εισέρχονται στο ενδοπλασματικό δίκτυο κατά τη διάρκεια της σύνθεσής τους

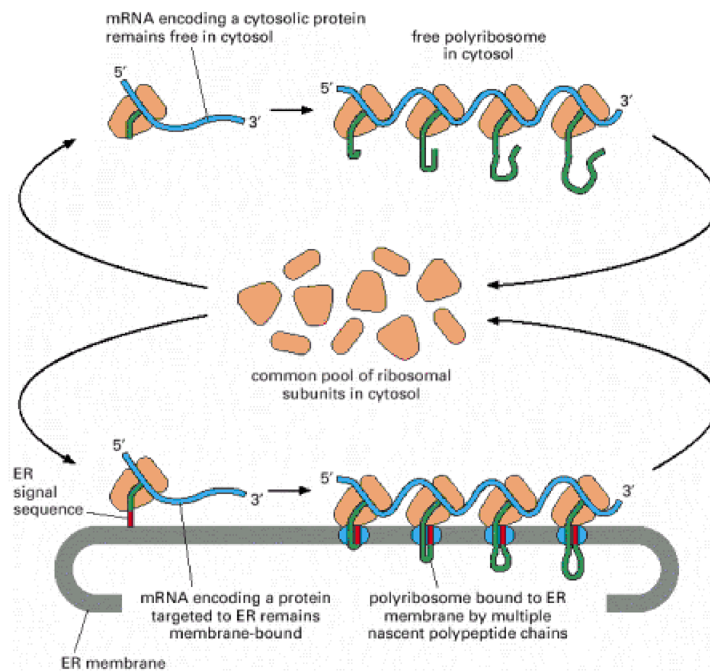


Ενδοπλασματικό δίκτυο

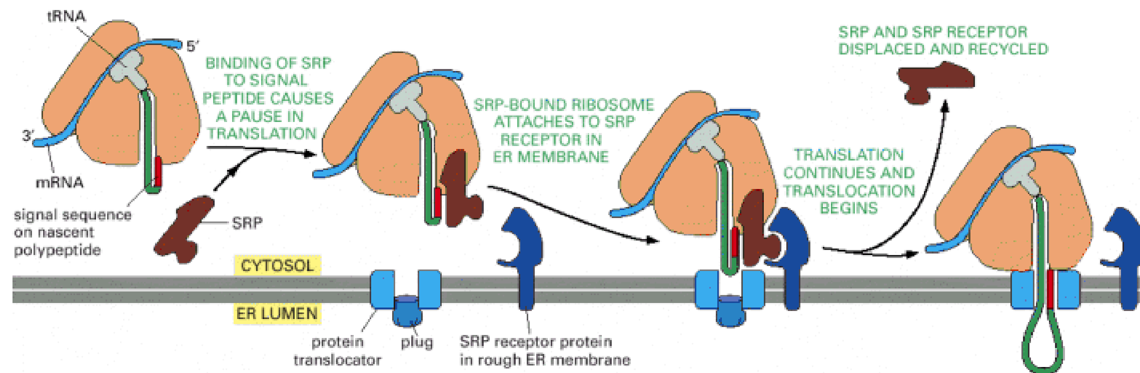




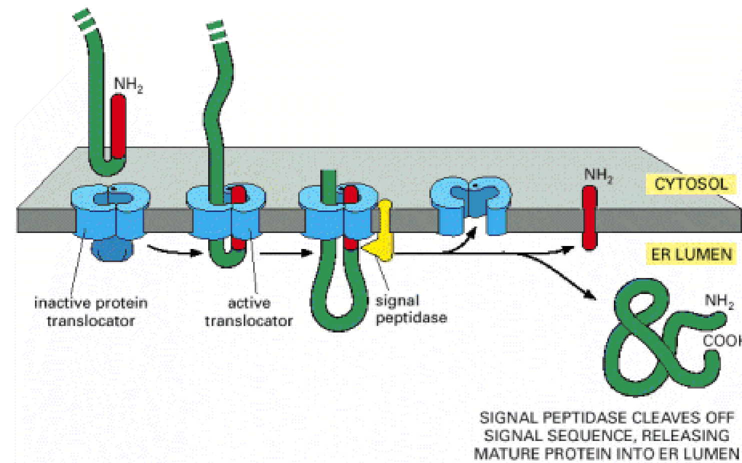
Ελεύθερα και μεμβρανικά ριβοσωμάτια



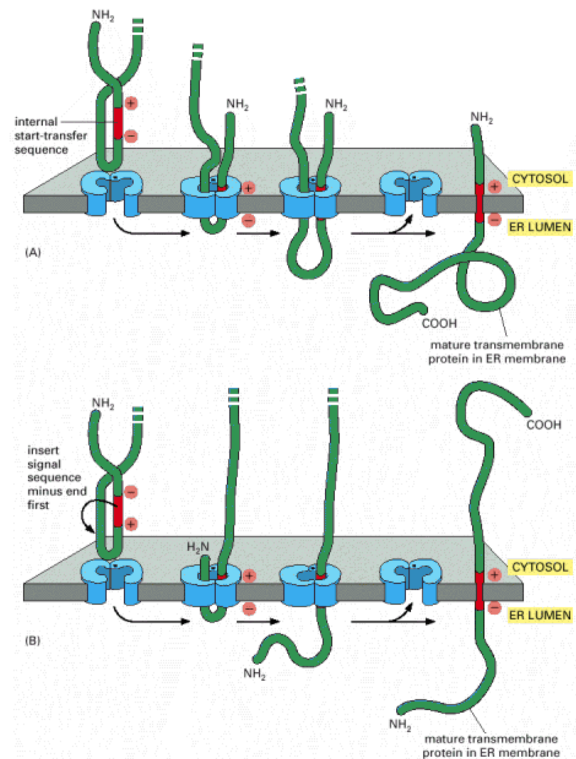
Μια σηματοδοτική αλληλουχία για το ΕΔ και ένα σωματίδιο αναγνώρισης σήματος (signal-recognition particle, SRP) καθοδηγούν το ριβοσωμάτιο στη μεμβράνη του ΕΔ



Πώς μια διαλυτή πρωτεΐνη περνάει τη μεμβράνη του ΕΔ



Η ενσωμάτωση μιας διαμεμβρανικής πρωτεΐνης στη μεμβράνη του ΕΔ



Η ενσωμάτωση μιας διαμεμβρανικής πρωτεΐνης που διαπερνά τη μεμβράνη του ΕΔ δύο φορές