

rough-
Endoplasmatic
Reticulum
rER

Sorting
of
proteins

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MD., PhD.
Professor

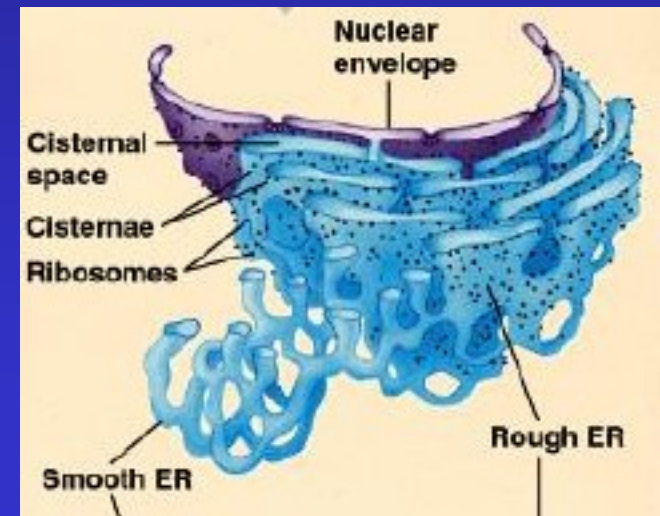
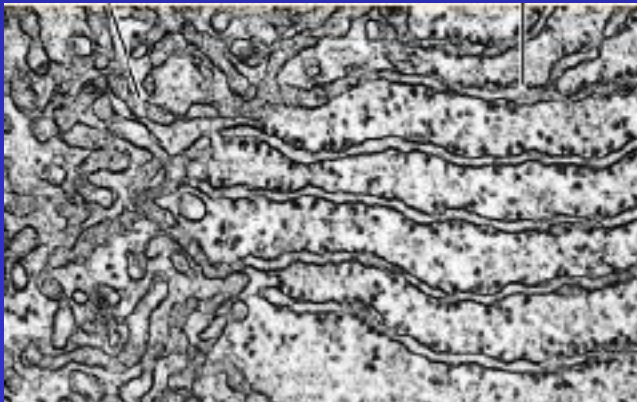
Department of Genetics, Cell- and
Immunobiology
Semmelweis University
Sept 26/ 2023



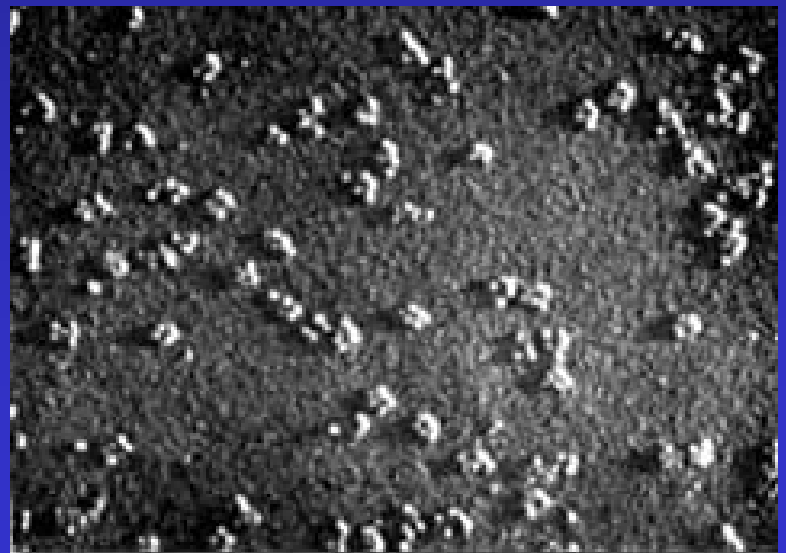
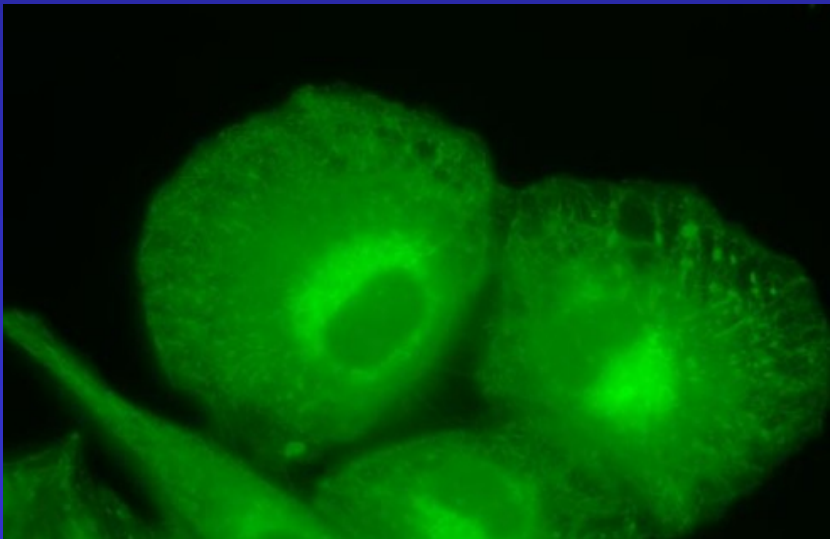
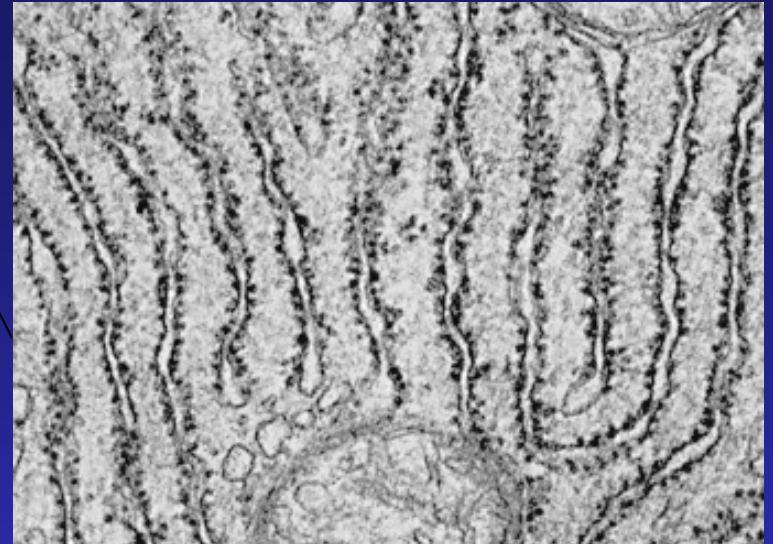
Endoplasmic = inside the cell; reticulum = network



- Extensive membrane system
- Includes up to half of membrane of cell
- Tubules and sacs = cisternae
- Continuous with the nuclear envelope
- Two types: rough ER (ribosomes)
 smooth ER



rER

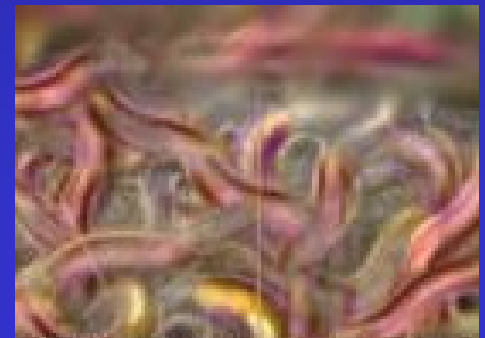
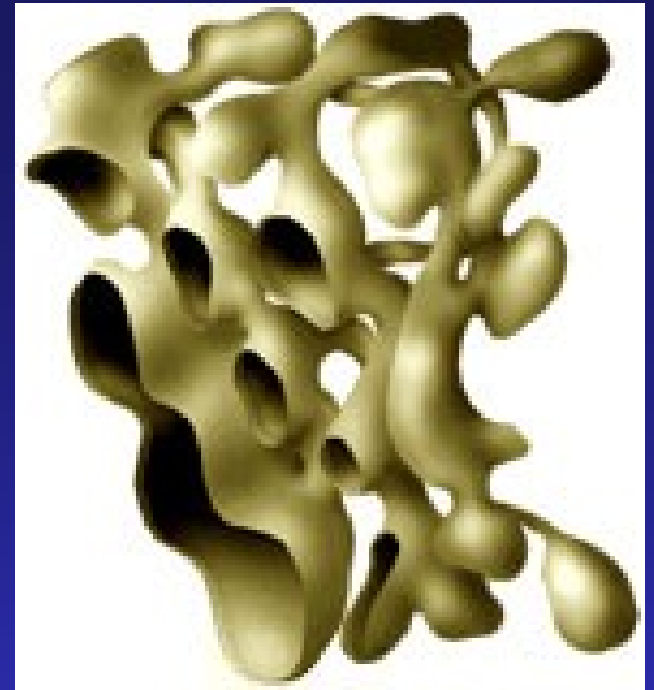


! s-ER (smooth ER)

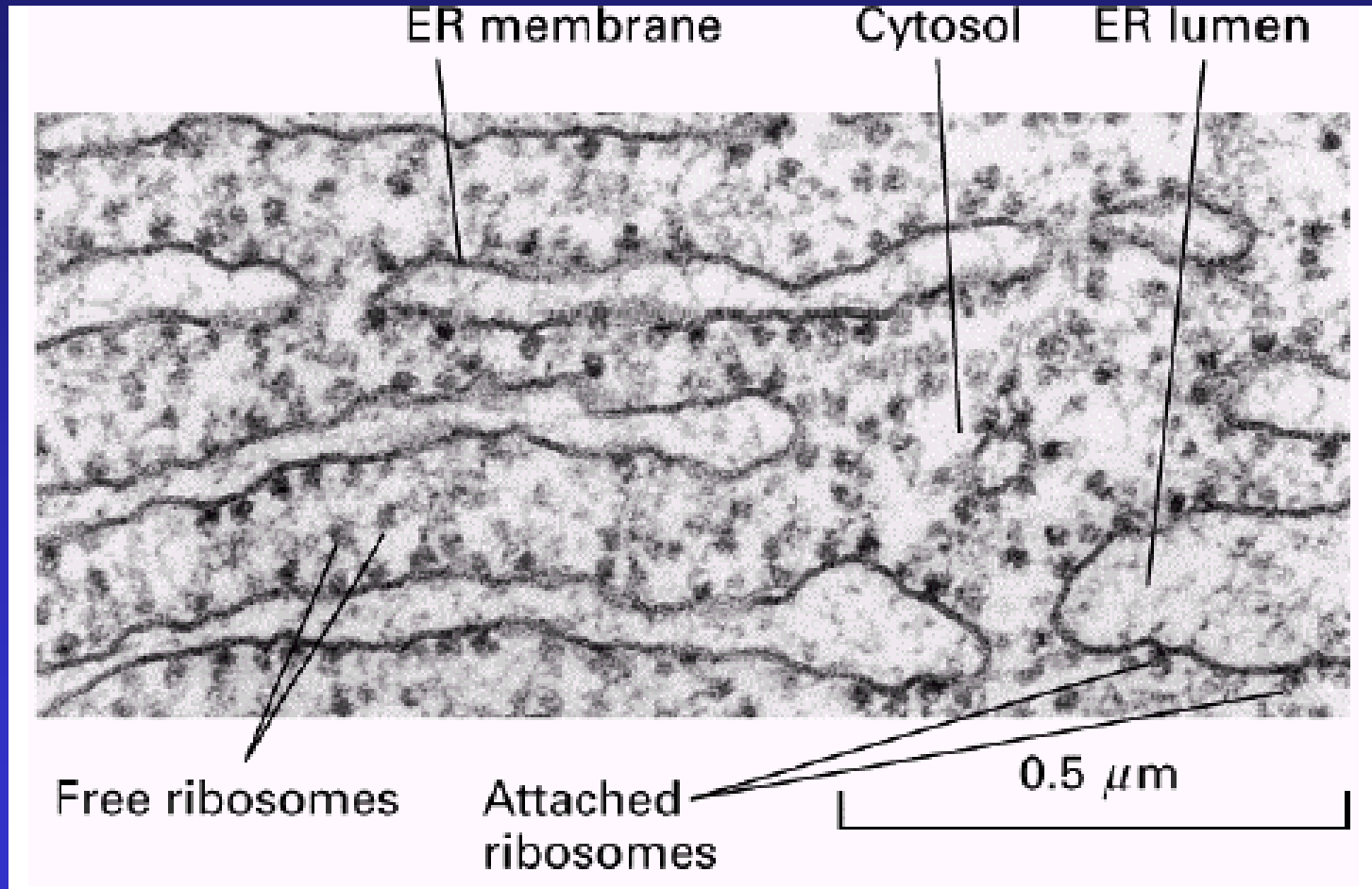
Structure: tubular

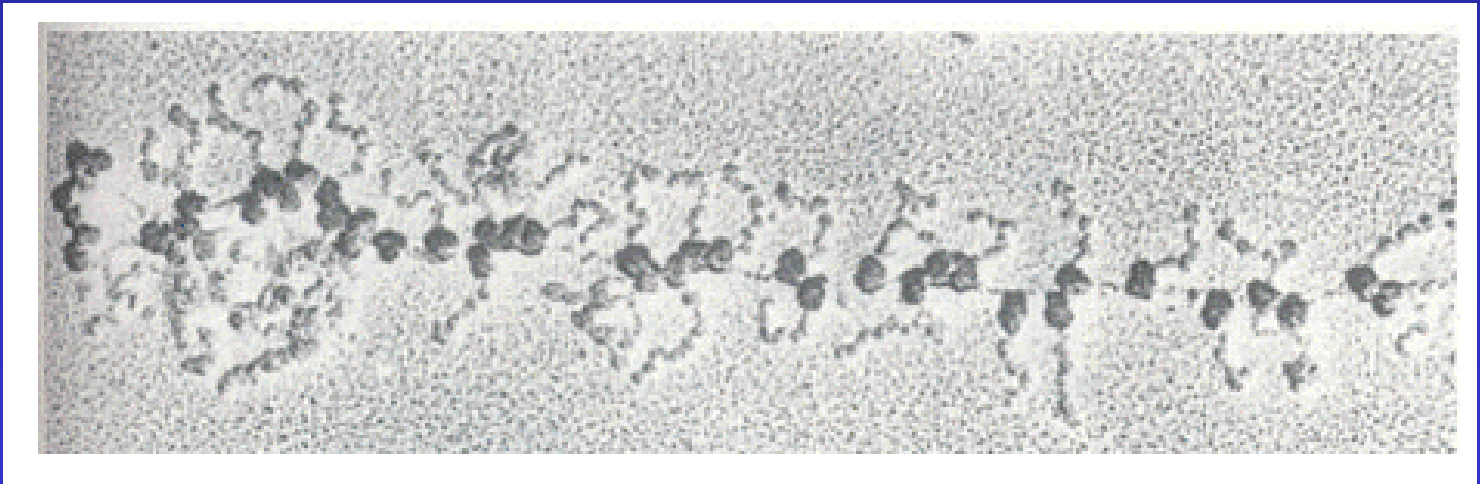
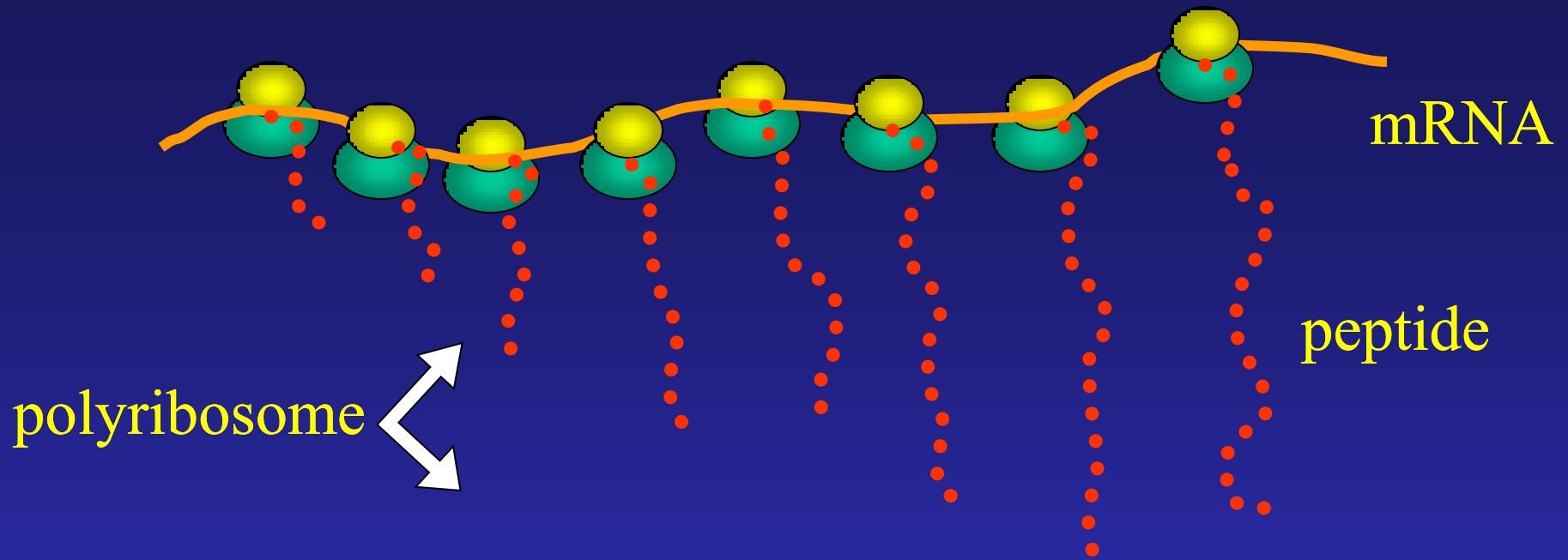
Function:

- synthesis of phospholipids, cholesterol, ceramide
- synthesis of steroids
- storage and regulation of Ca^{2+}
- detoxification – cyt P450

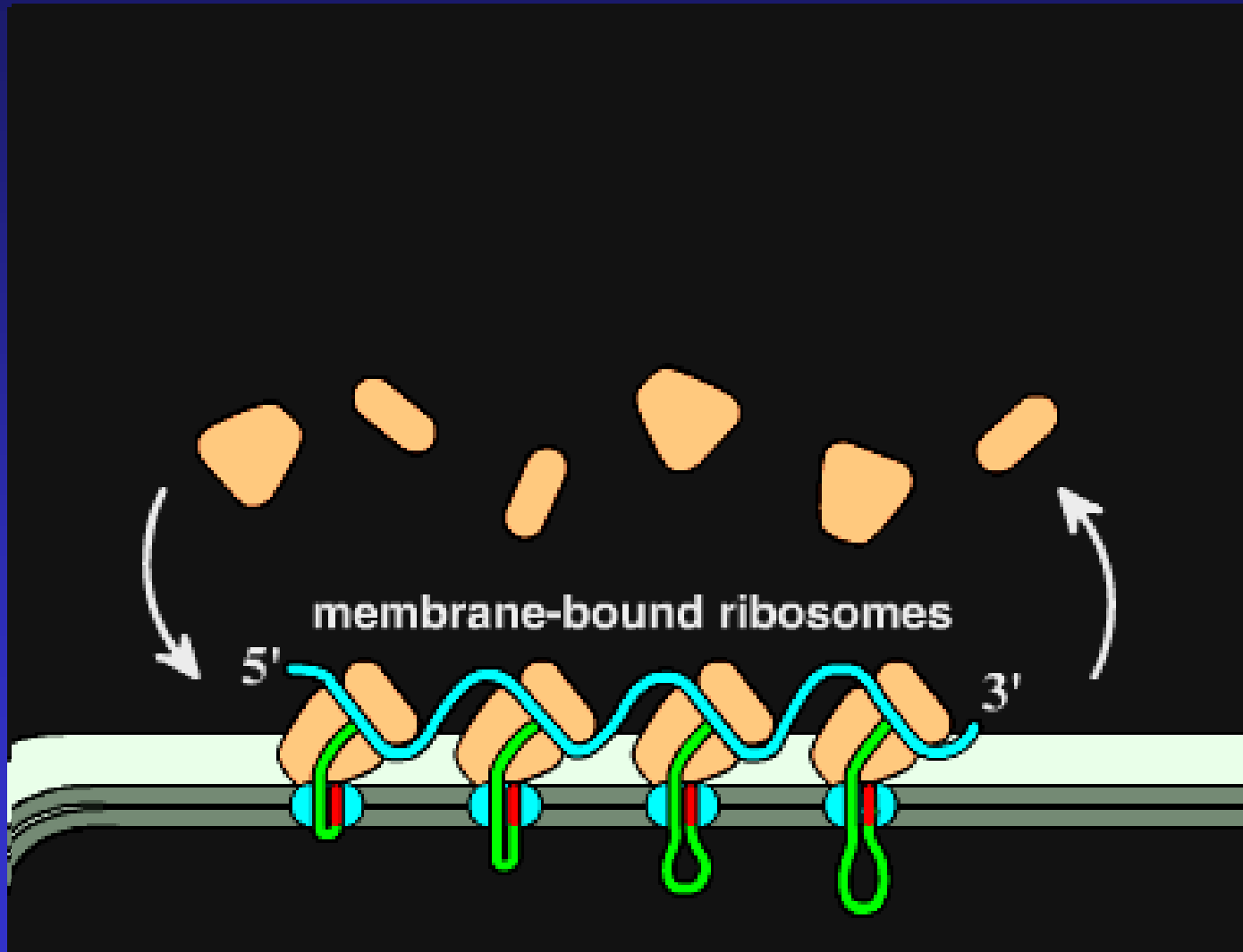


TEM of ribosomes attached to the rER in a pancreatic exocrine cell

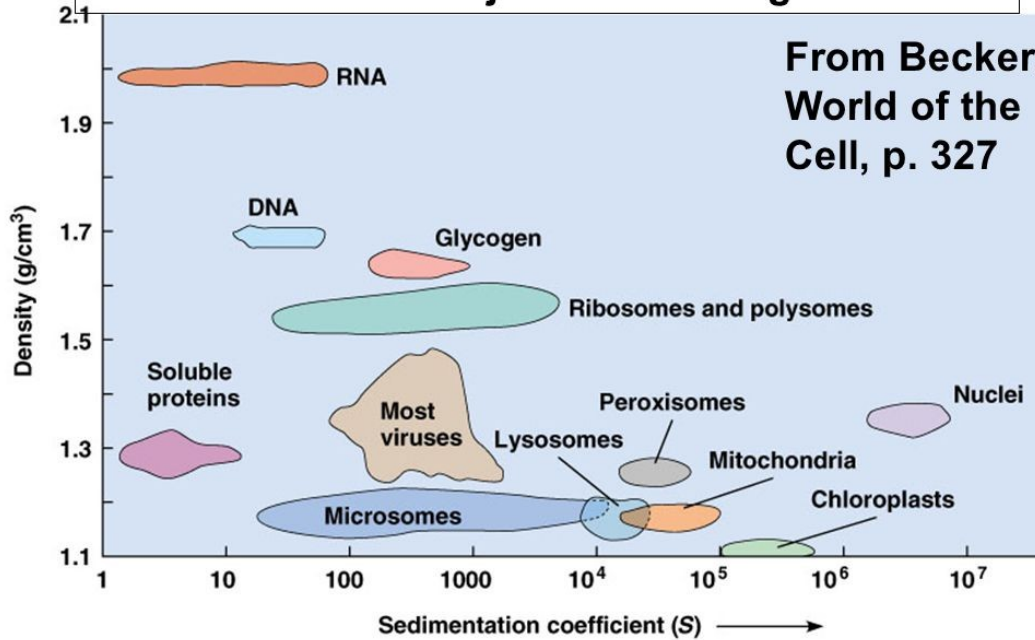




Ribosomes – mRNA – Polyribosome



Svedberg unit, S, shows how fast a particle sediments when subject to centrifugal force.



From Becker,
World of the
Cell, p. 327

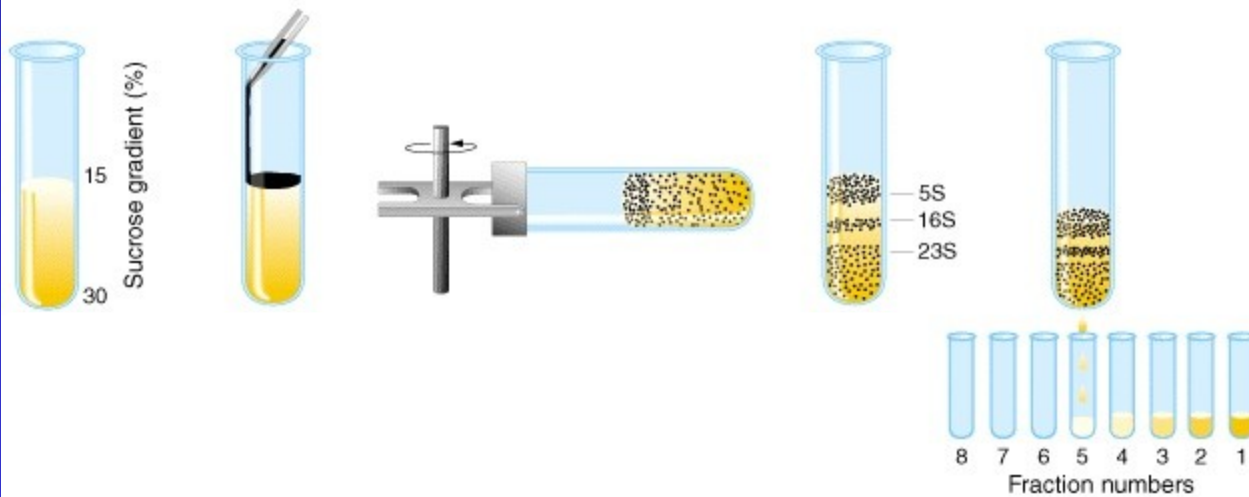
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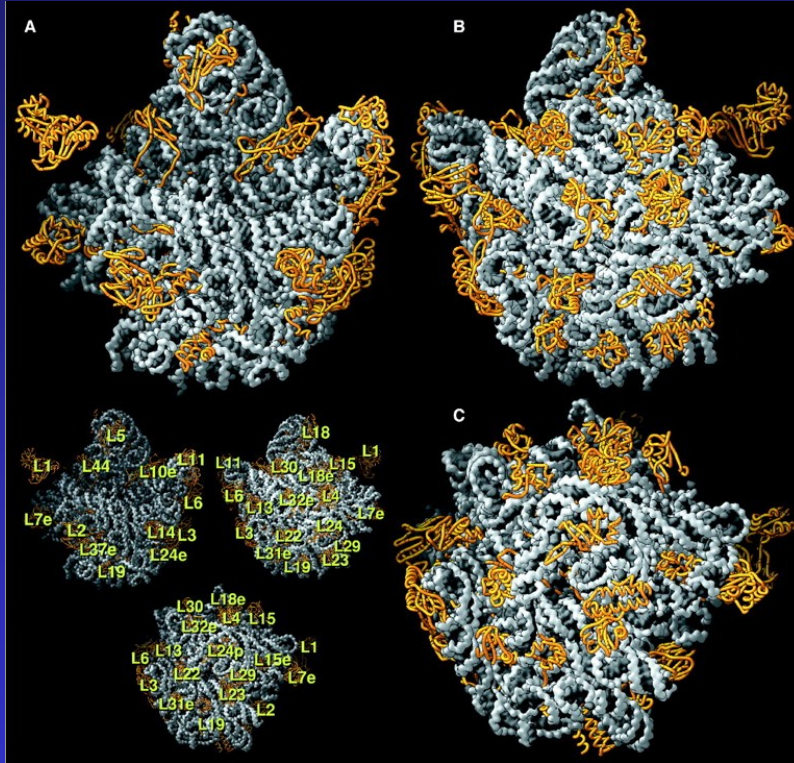
Svedberg, Theodore
1884 - 1971



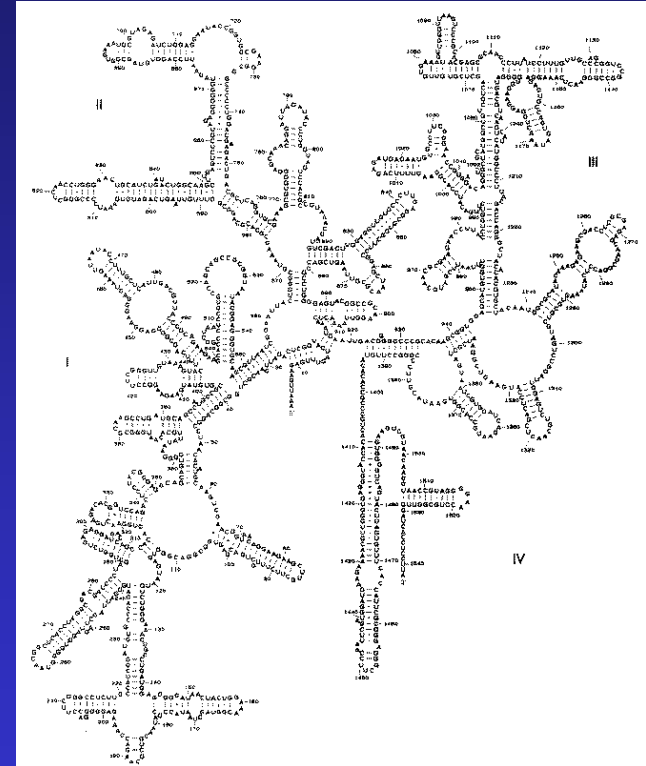
Nobel prize
1926



Molecular composition of ribosome

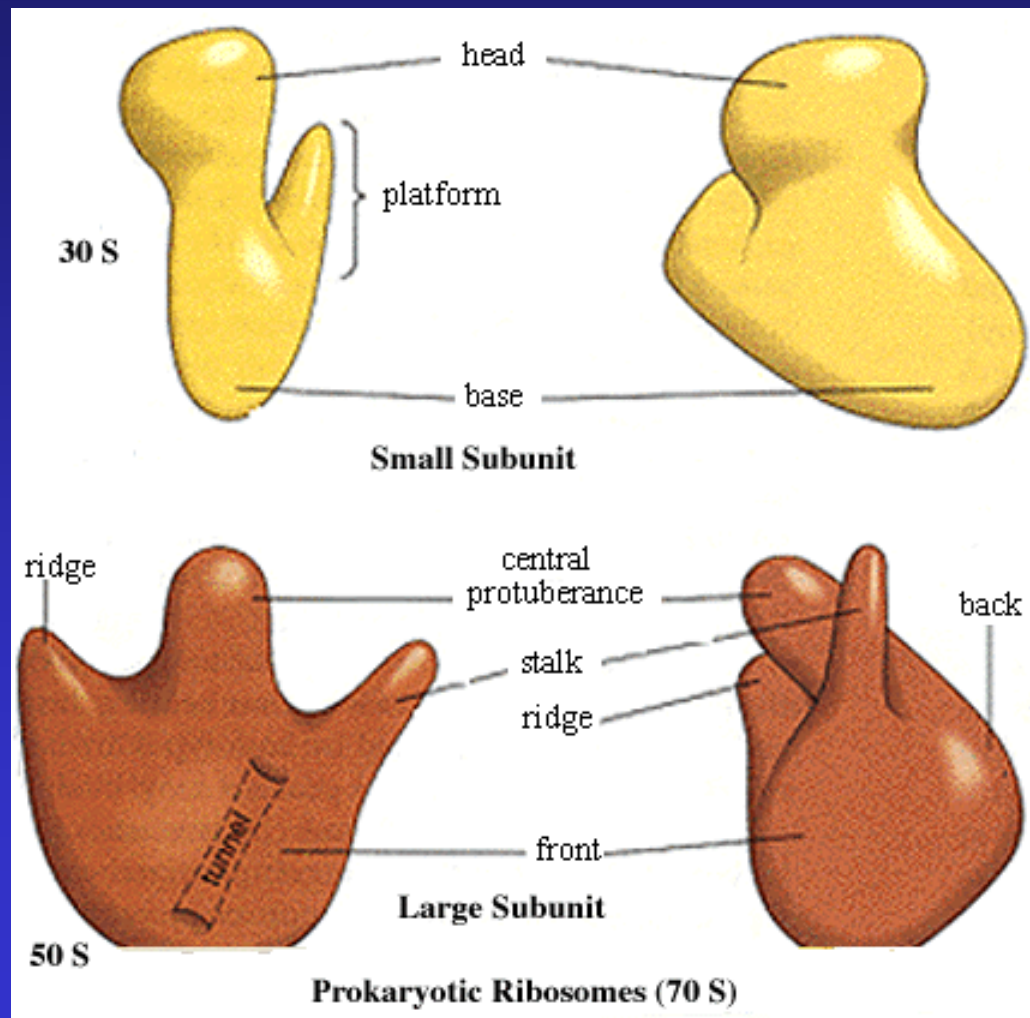


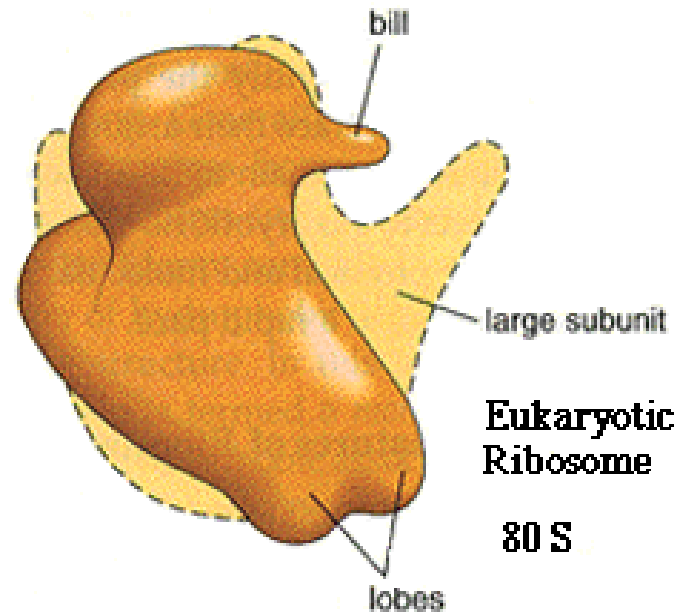
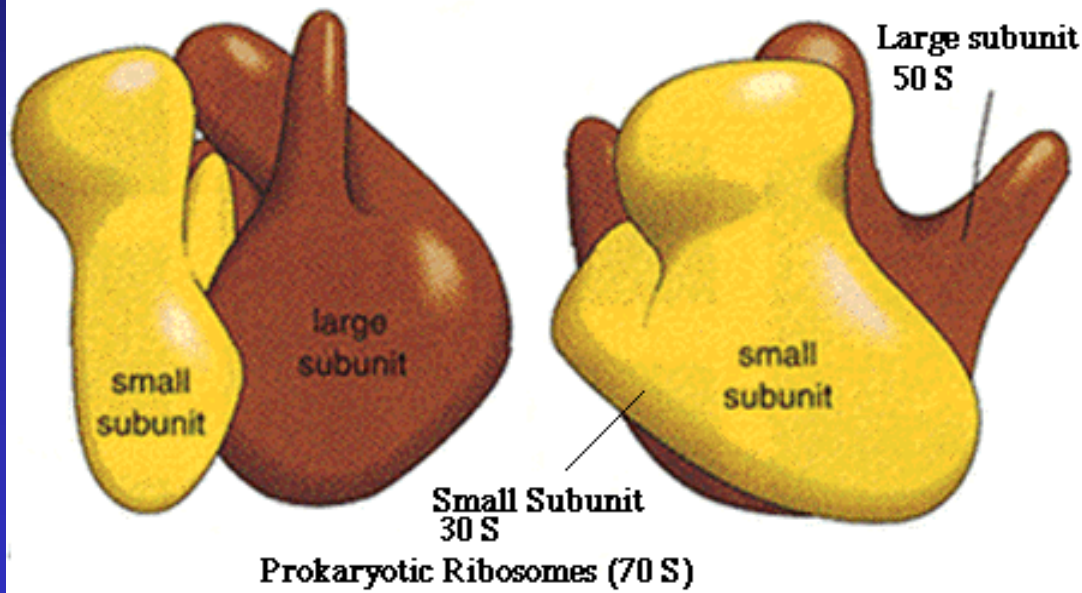
60S rRNA + peptides

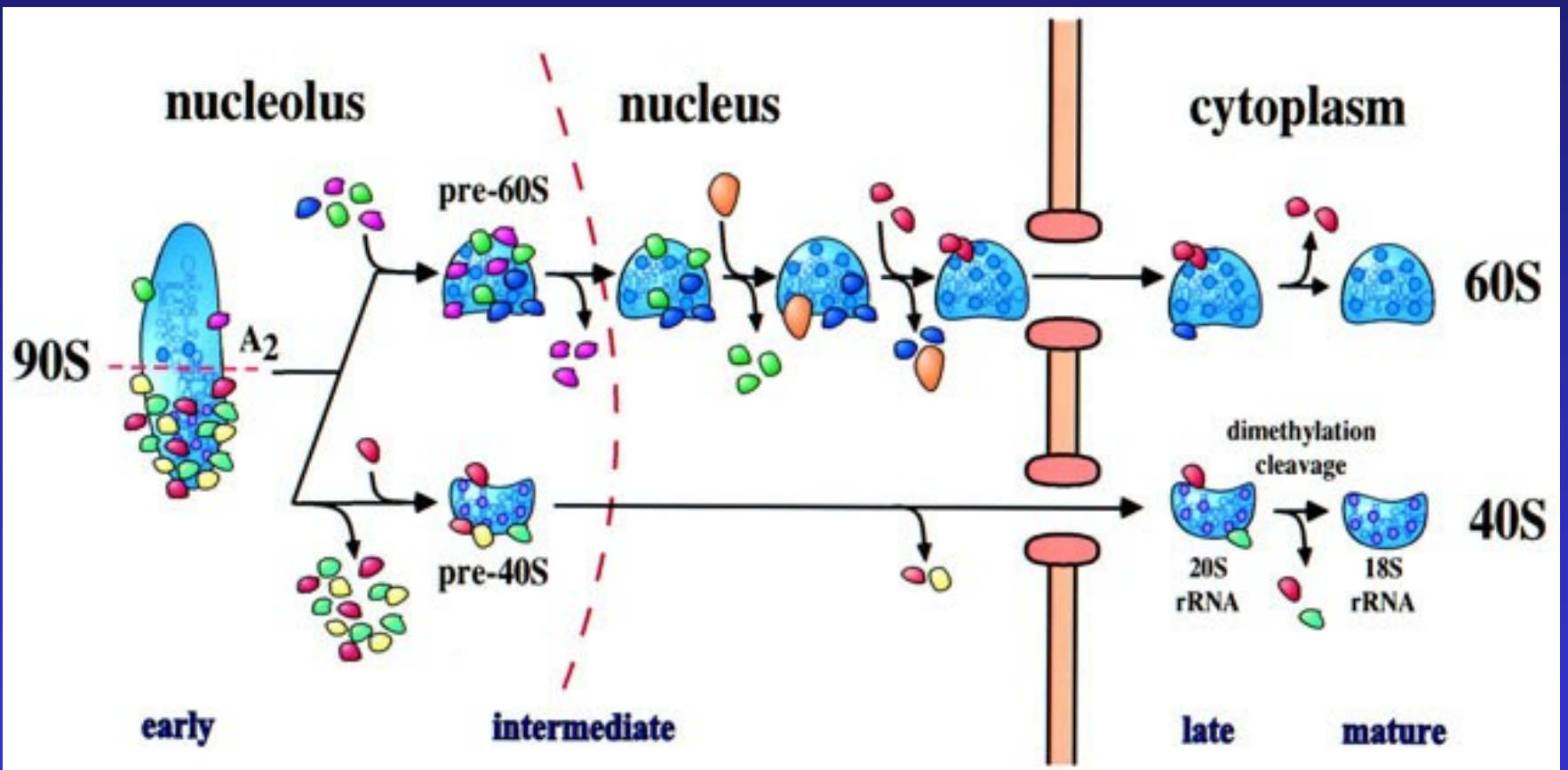


rRNA

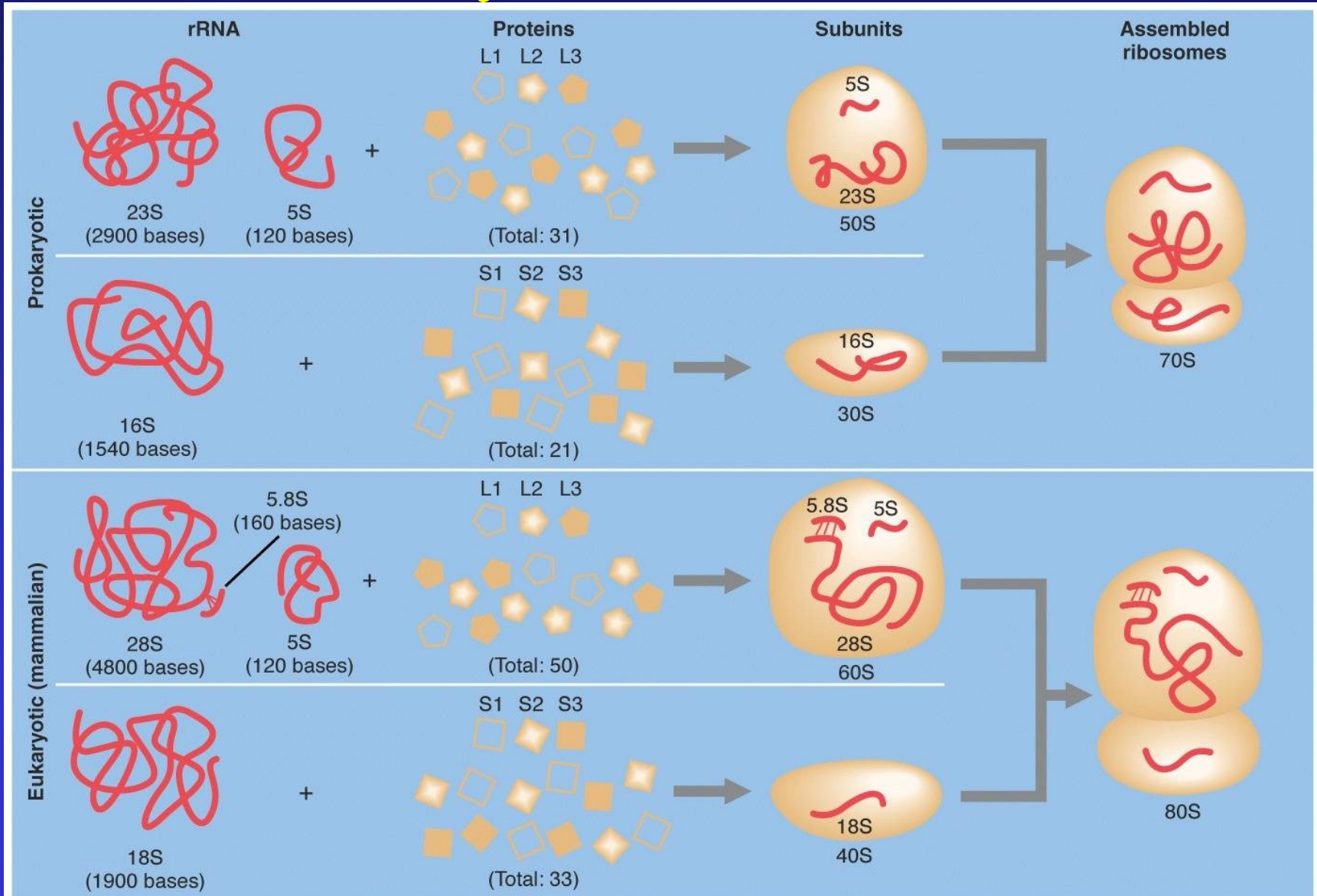
Ribosome subunits



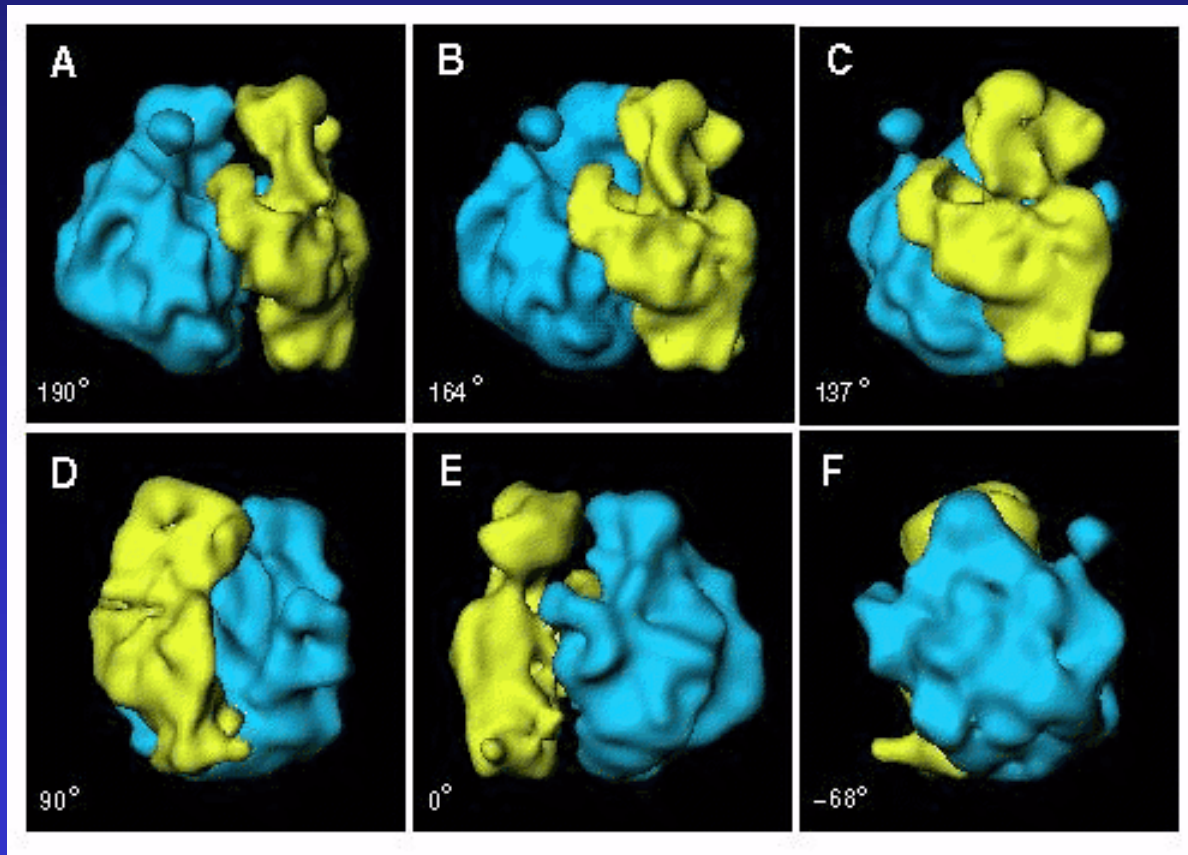
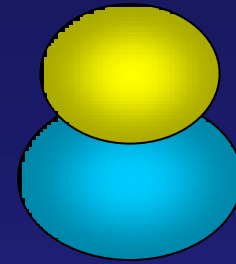




Comparison of prokaryotic and eukaryotic ribosomes



Structure of ribosome





The Nobel Prize in Chemistry 2009

"for studies of the structure and function of the ribosome"



Photo: MRC Laboratory of
Molecular Biology

**Venkatraman
Ramakrishnan**

🕒 1/3 of the prize

United Kingdom



Credits: Michael
Marsland/Yale University

Thomas A. Steitz

🕒 1/3 of the prize

USA



Credits: Micheline
Pelletier/Corbis

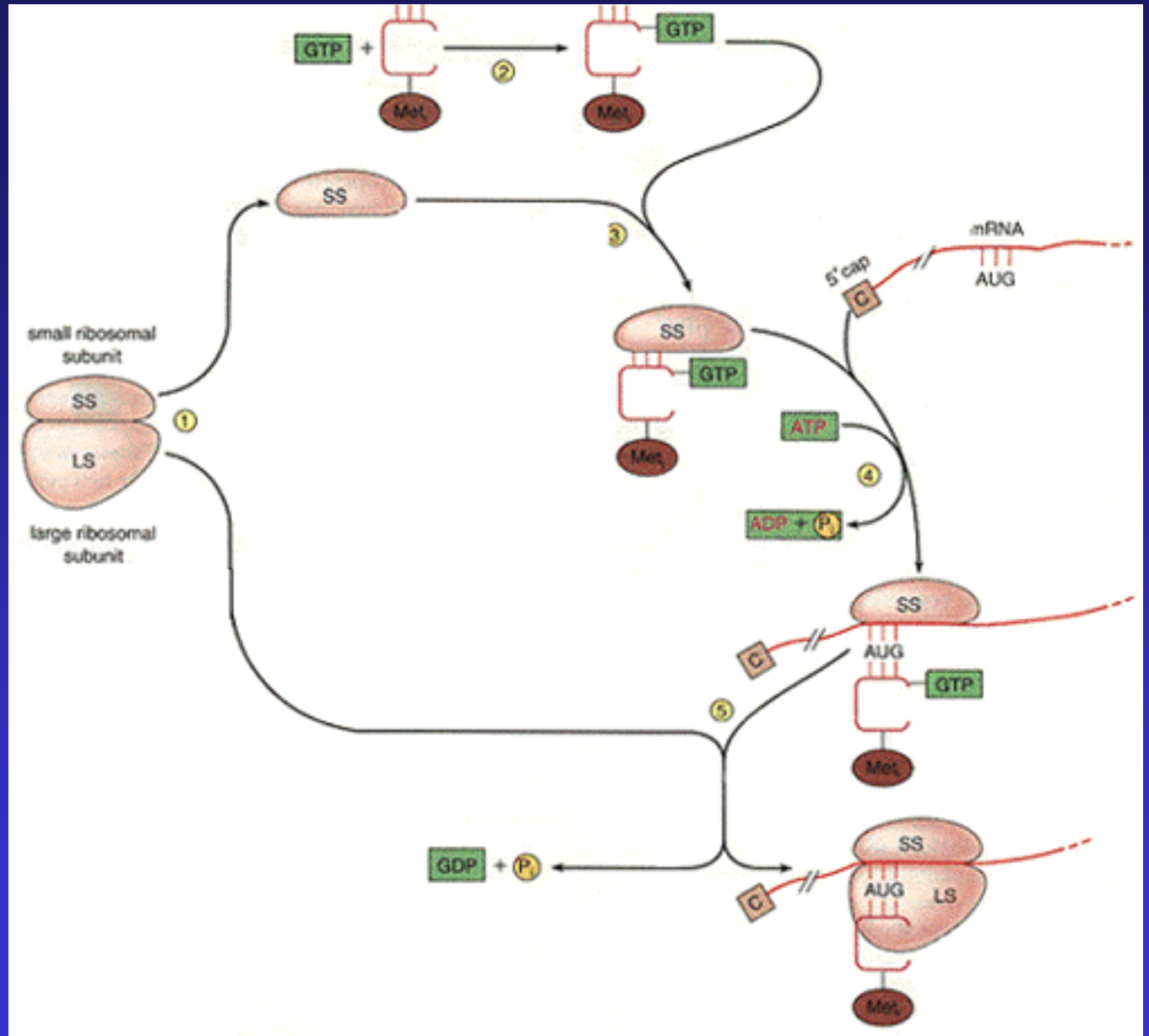
Ada E. Yonath

🕒 1/3 of the prize

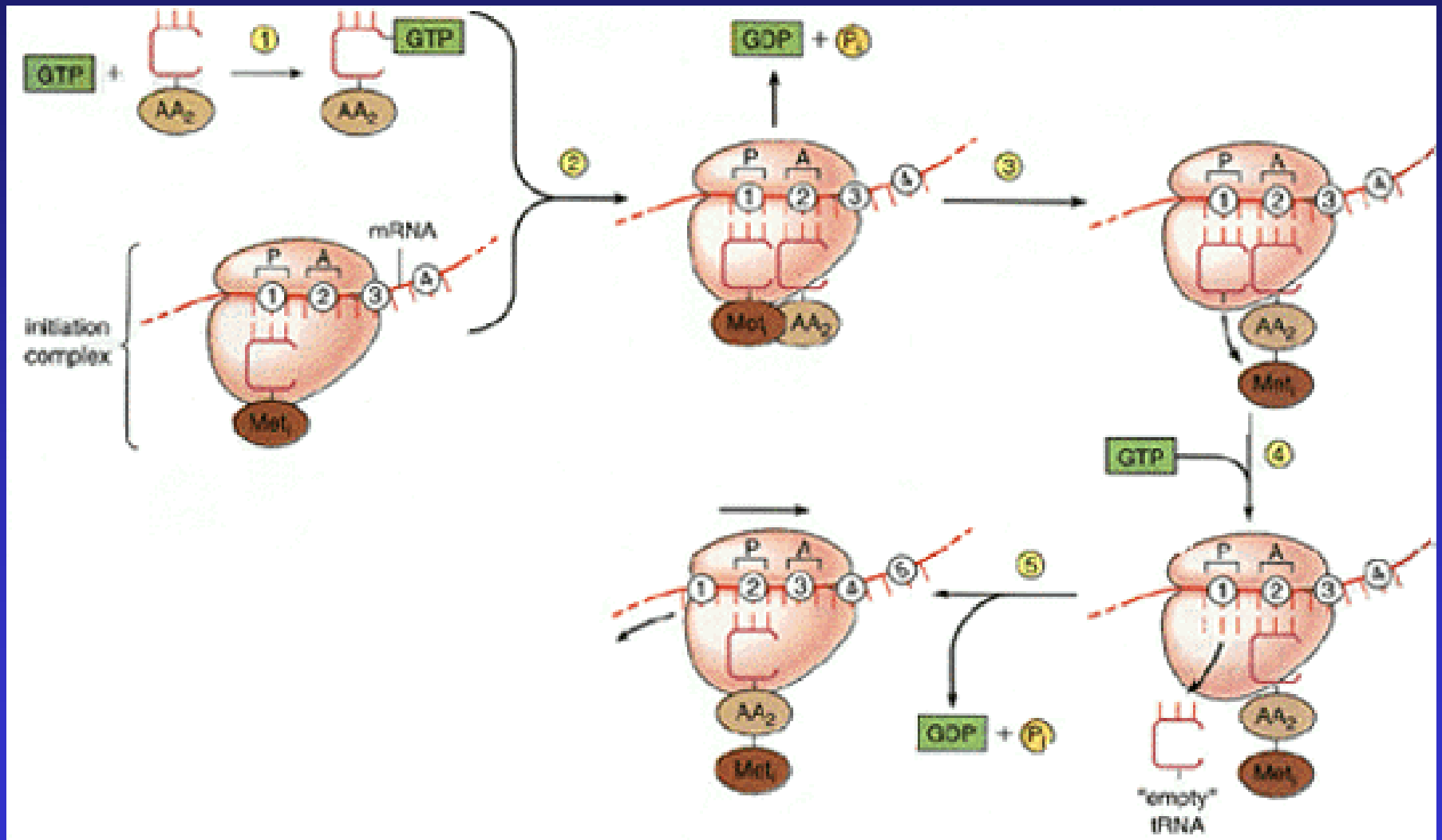
Israel

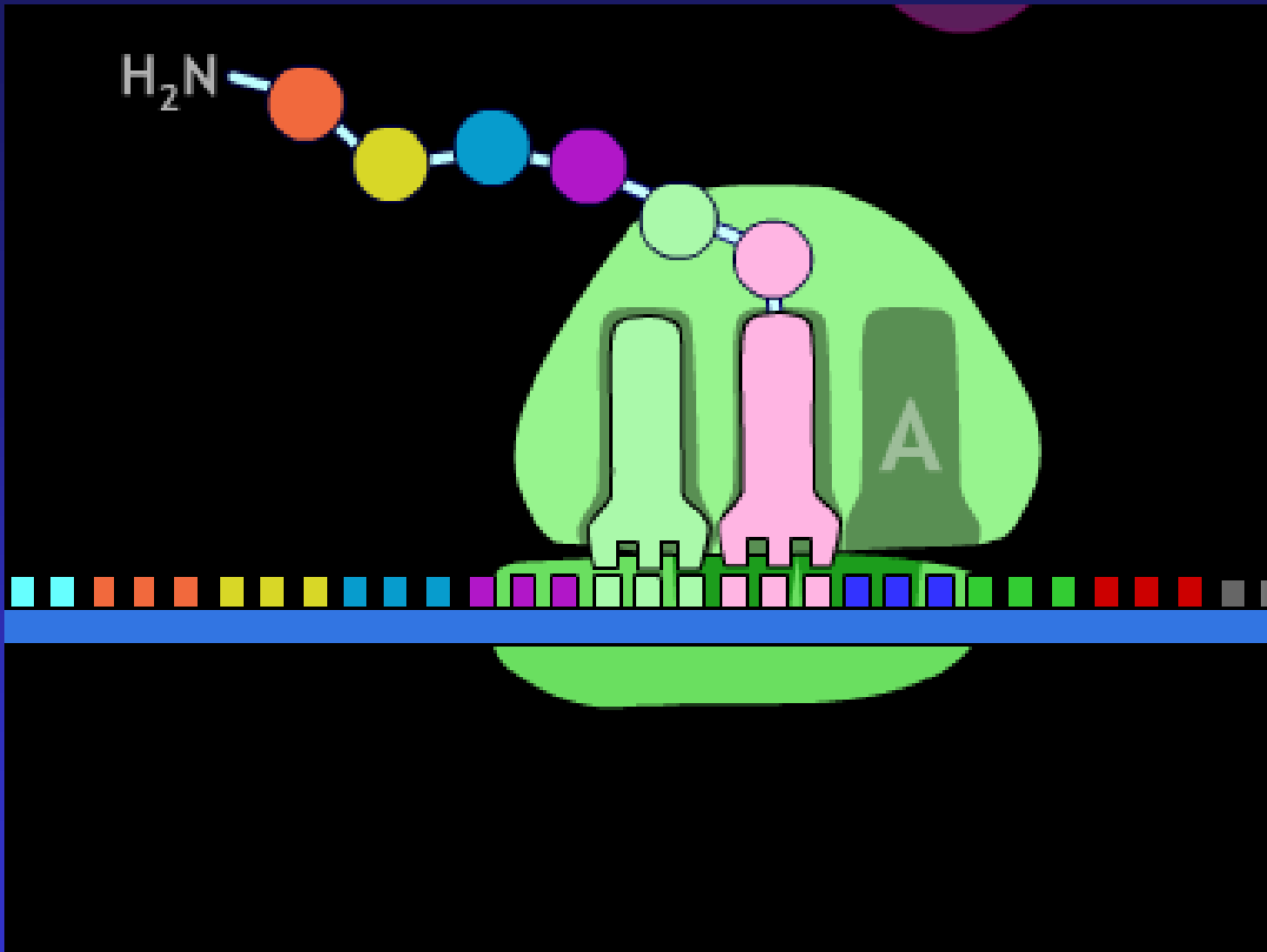


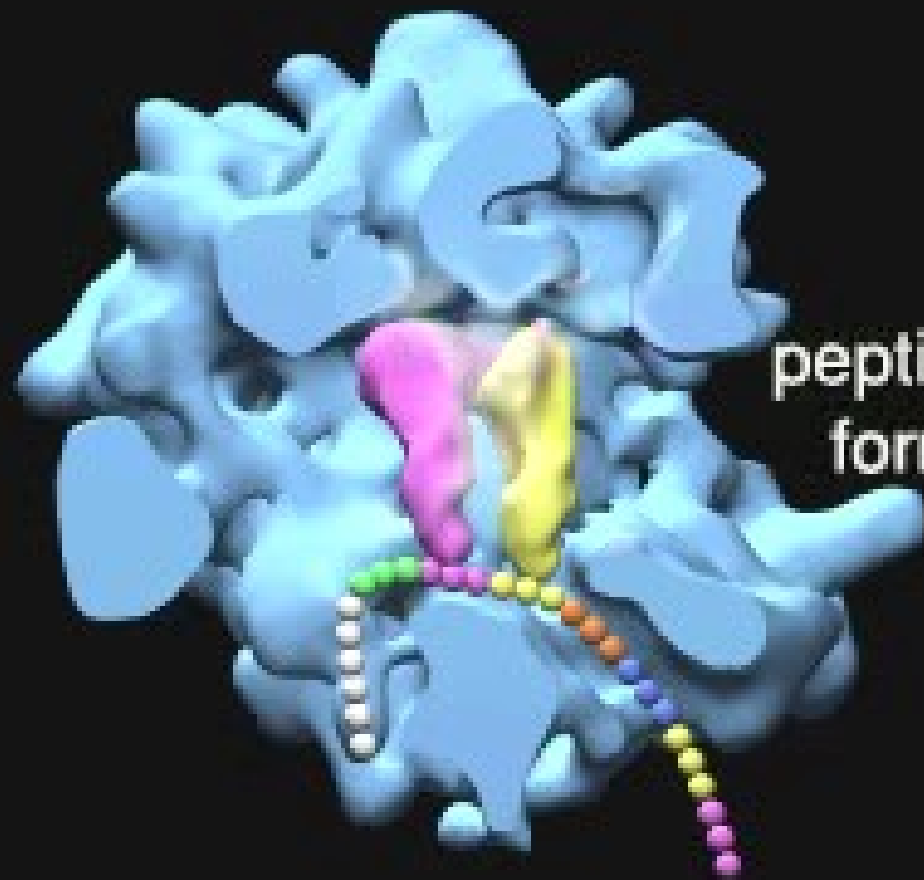
Initiation



Elongation







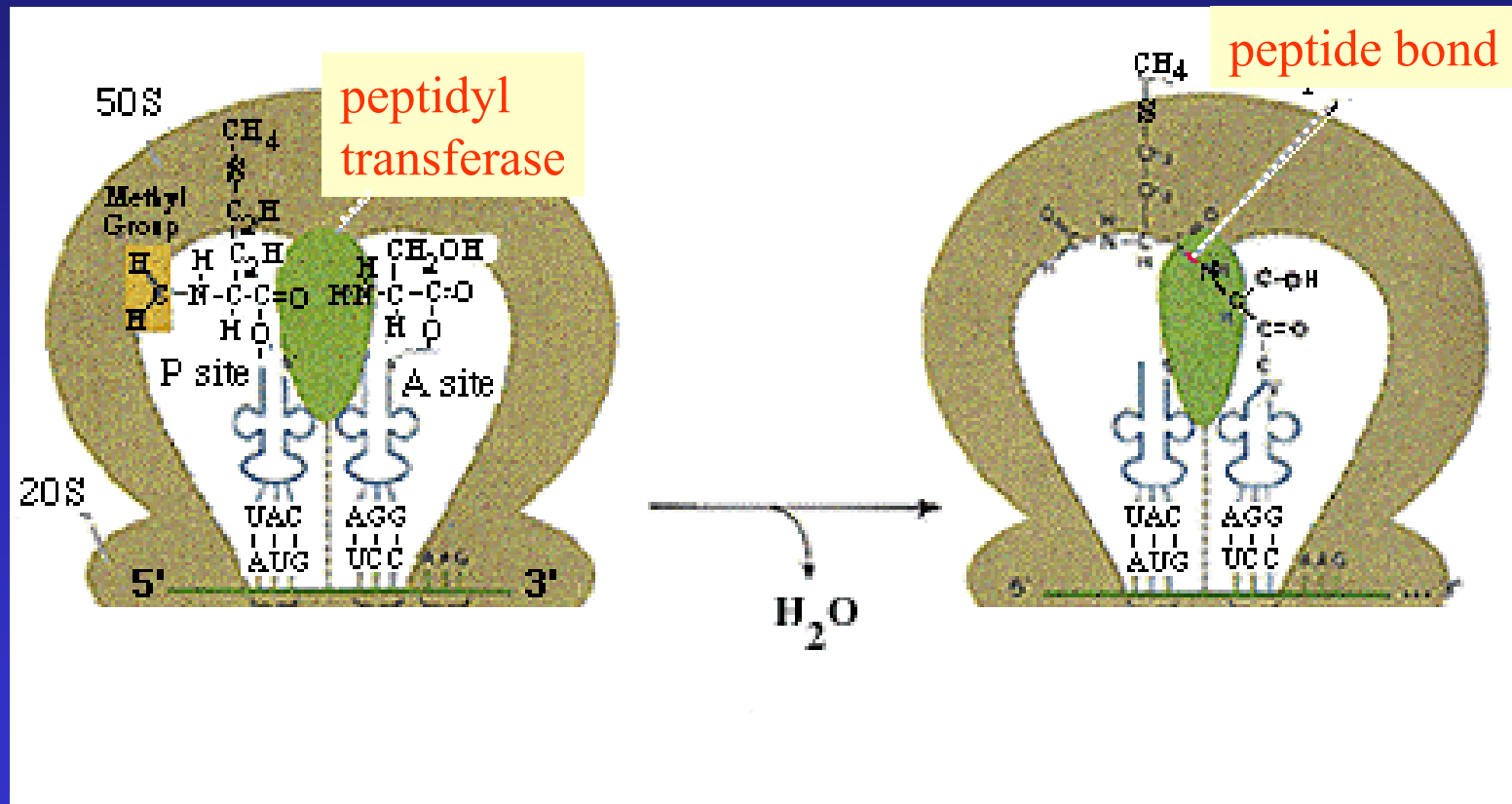
peptide bond
formation

Second base of codon

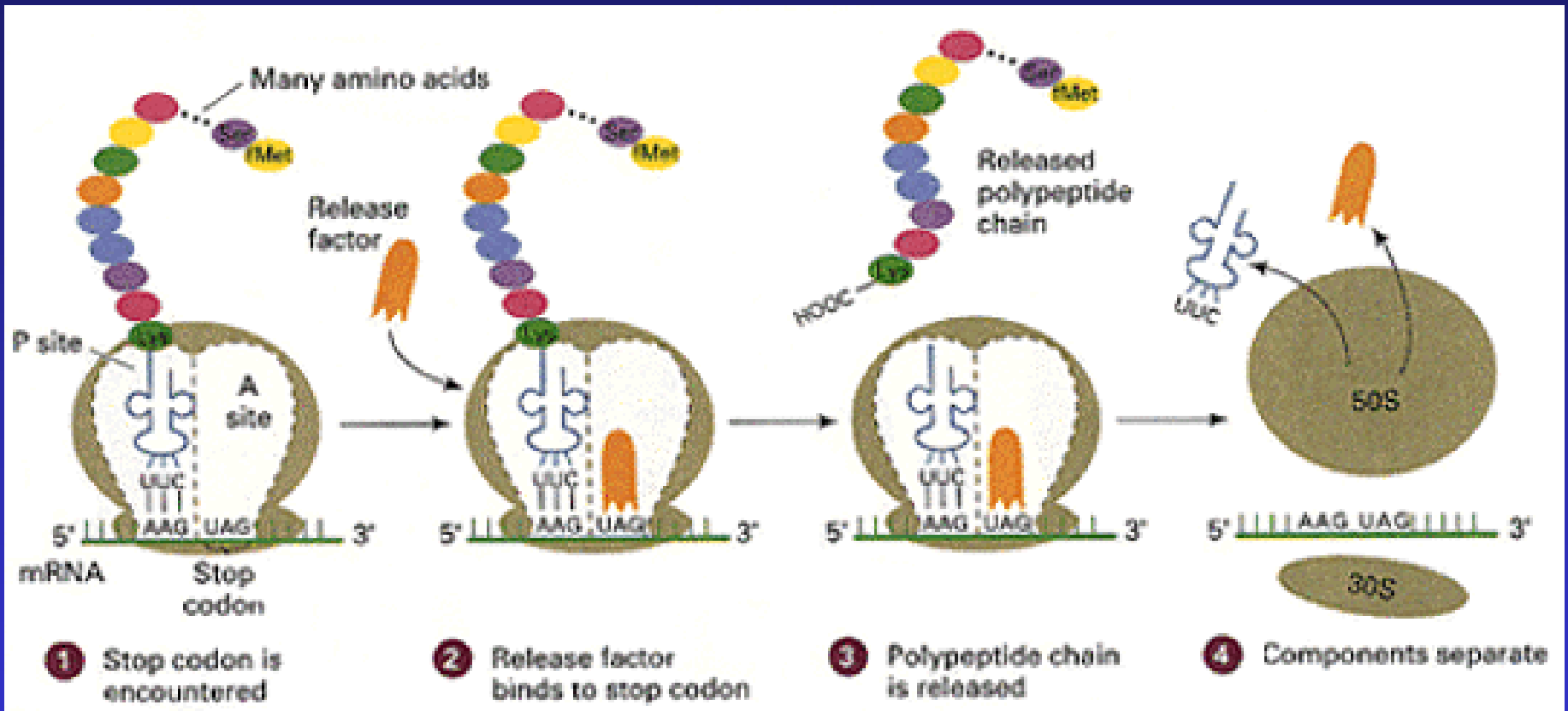
		U	C	A	G		
First base of codon	U	UUU } Phe UUC } UUA } Leu UUG }	UCU } UCC } SER UCA } UCG }	UAU } Tyr UAC } UAA UAG	UGU } Cys UGC } UGA UGG } Trp	U C A G	Third base of codon
	C	CUU } Leu CUC } CUA } CUG }	CCU } CCC } Pro CCA } CCG }	CAU } His CAC } CAA } Gln CAG }	CGU } Arg CGC } CGA } CGG }	U C A G	
	A	AUU } Ile AUC } AUA } AUG Met	ACU } ACC } Thy ACA } ACG }	AAU } Asn AAC } AAA } Lys AAG }	AGU } Ser AGC } AGA } Arg AGG }	U C A G	
	G	GUU } Val GUC } GUA } GUG }	GCU } GCC } Ala GCA } GCG }	GAU } Asp GAC } GAA } Glu GAG }	GGU } Gly GGC } GGA } GGG }	U C A G	

The genetic code, written by convention in the form in which the Codons appear in mRNA. The three terminator codons, UAA, UAG, and UGA, are boxed in red; the AUG initiator codon is shown in green.

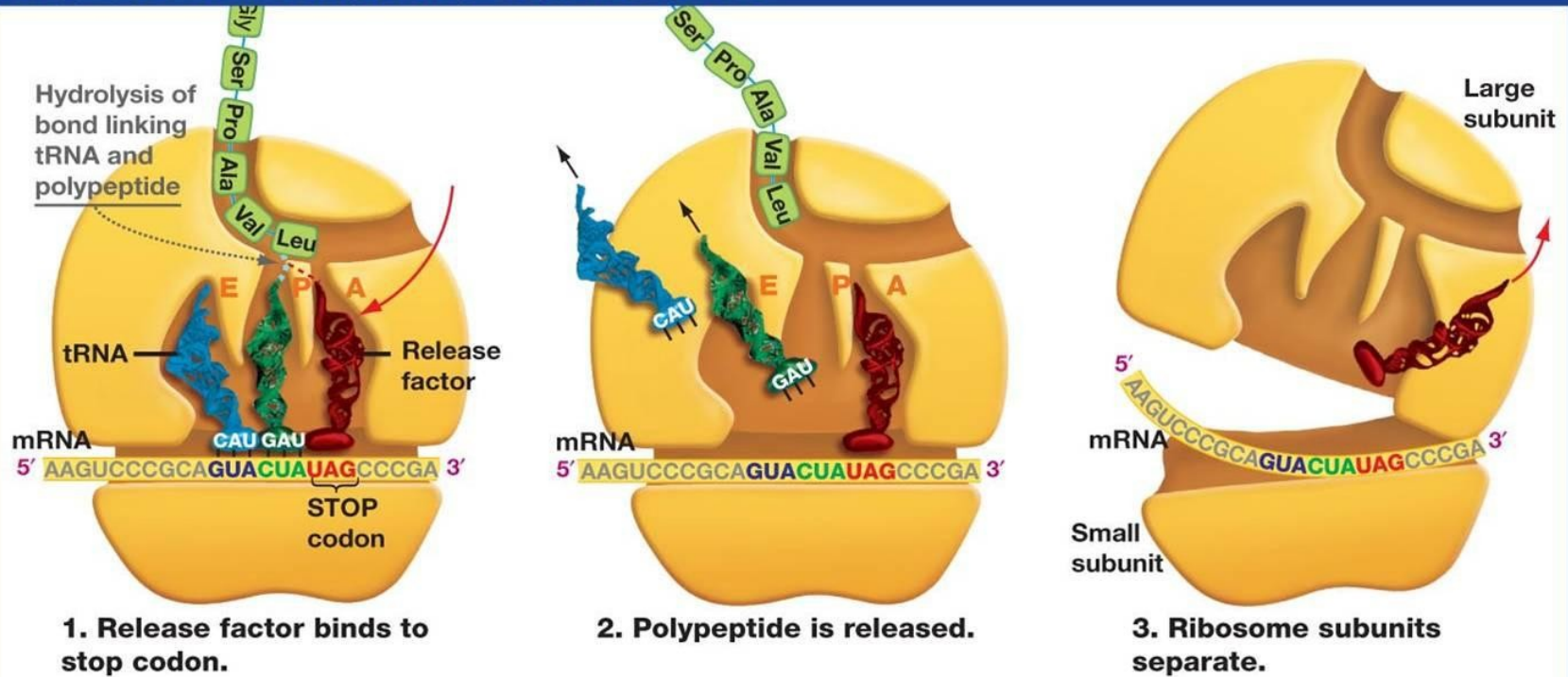
Peptide bond formation



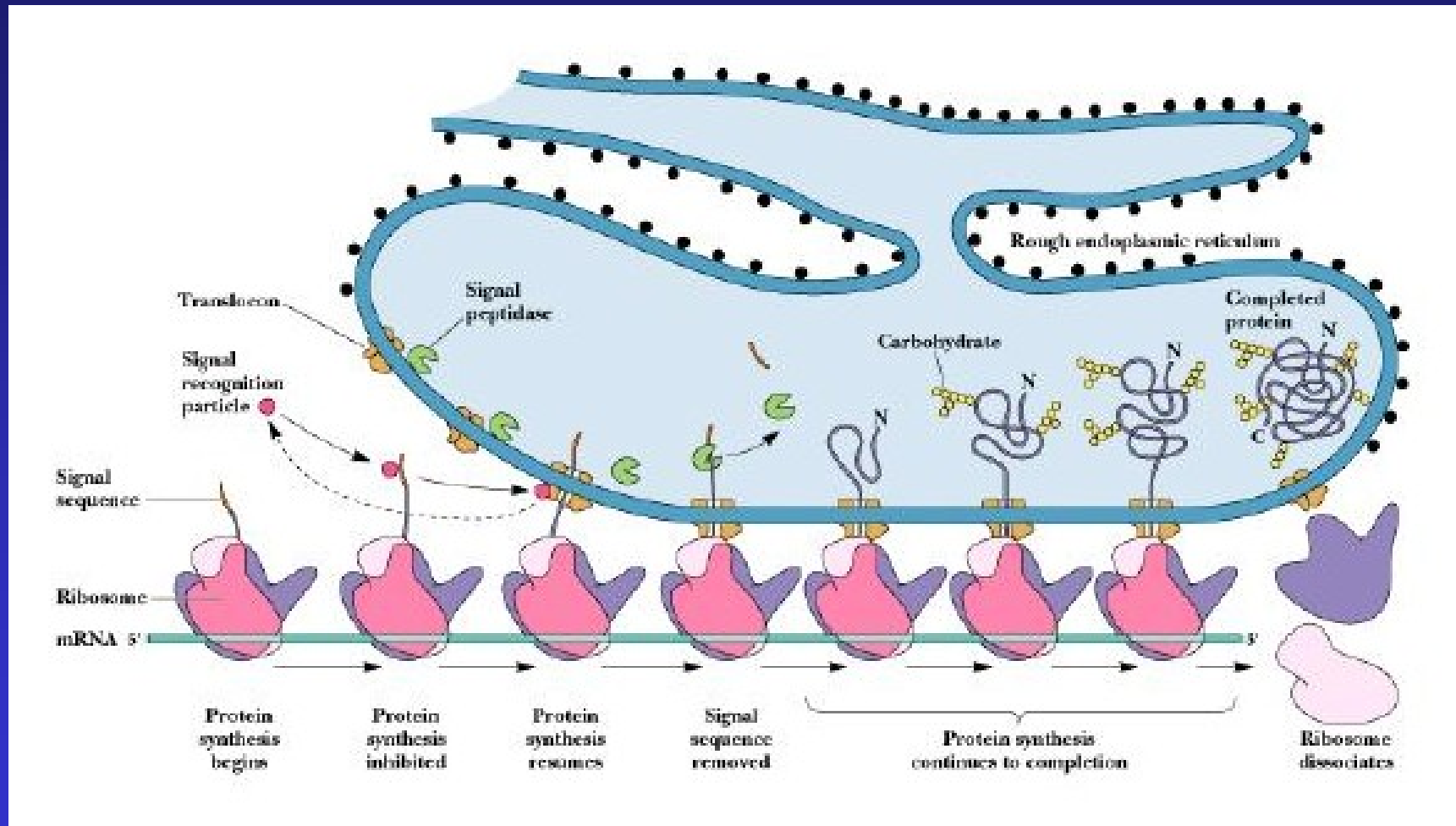
Termination

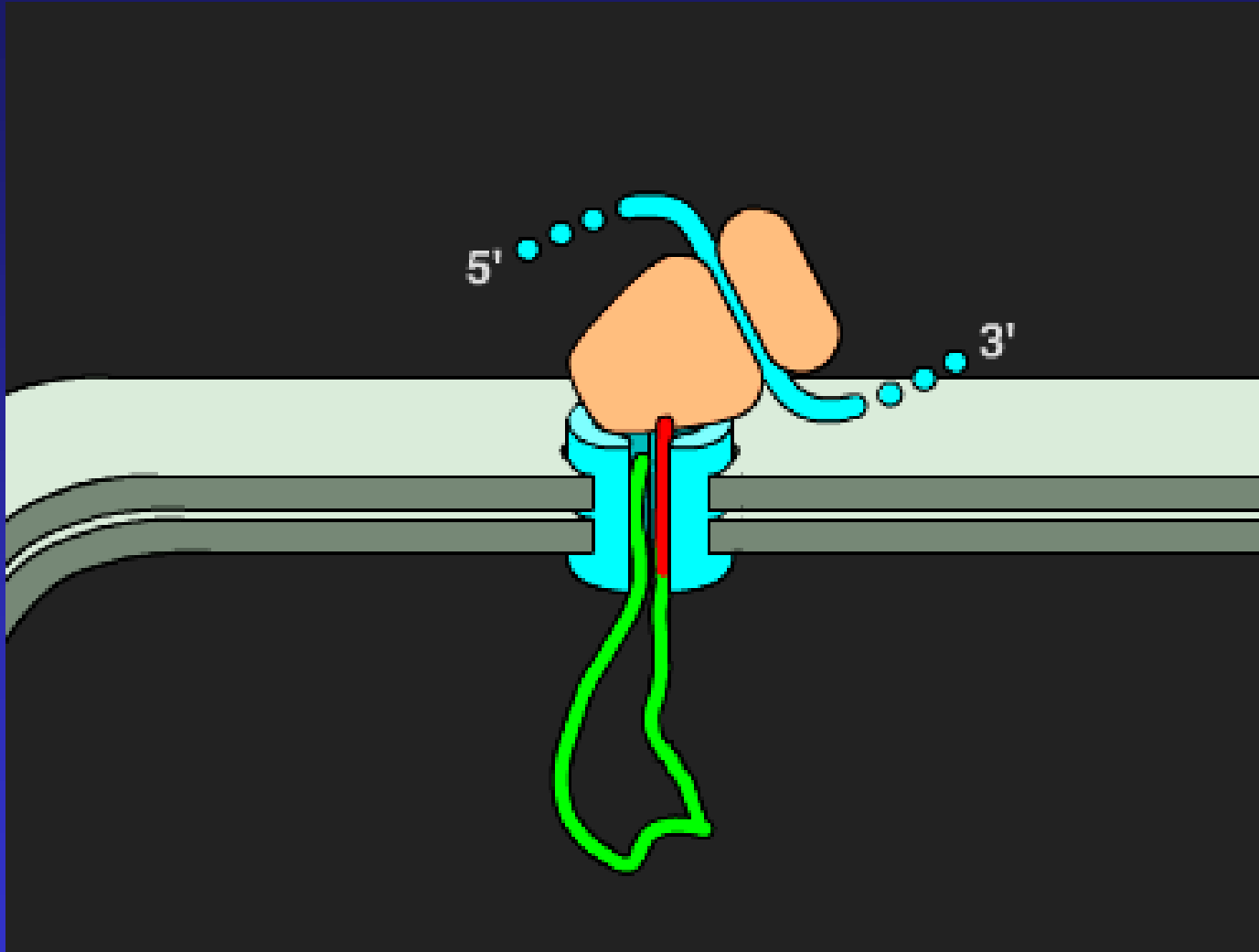


PROCESS: TERMINATING TRANSLATION IN BACTERIA

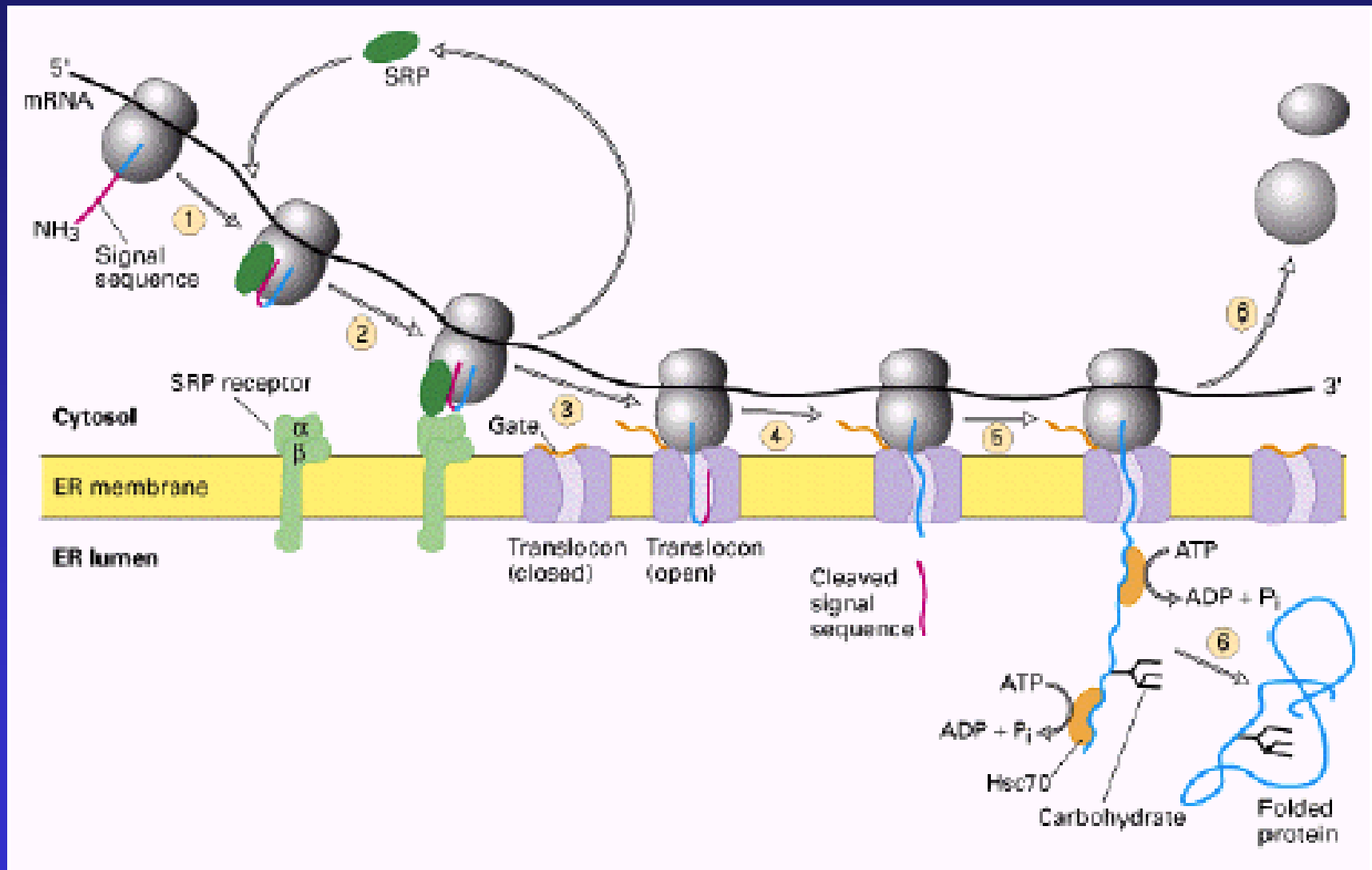


Internalization of peptides into the rER

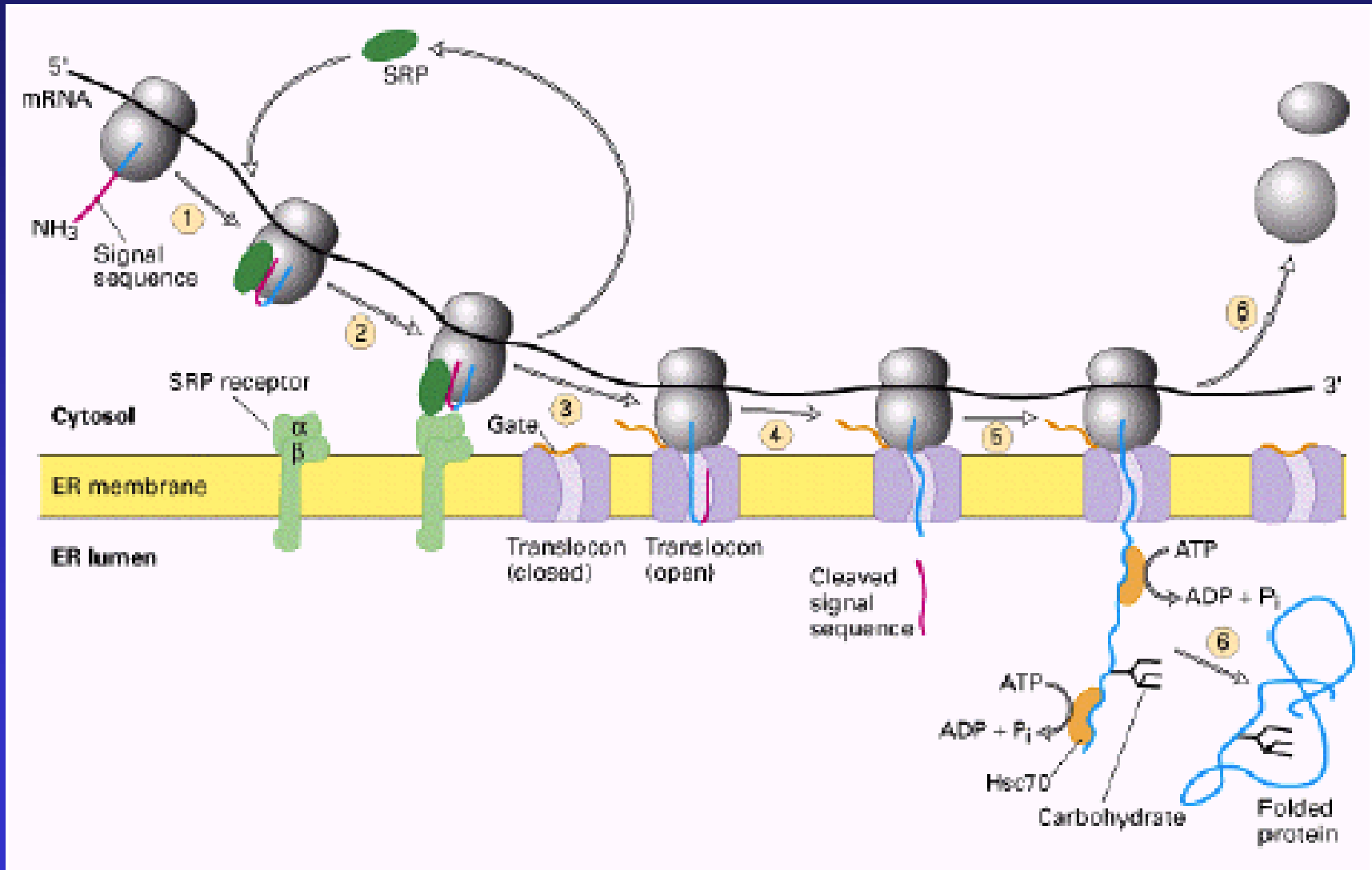


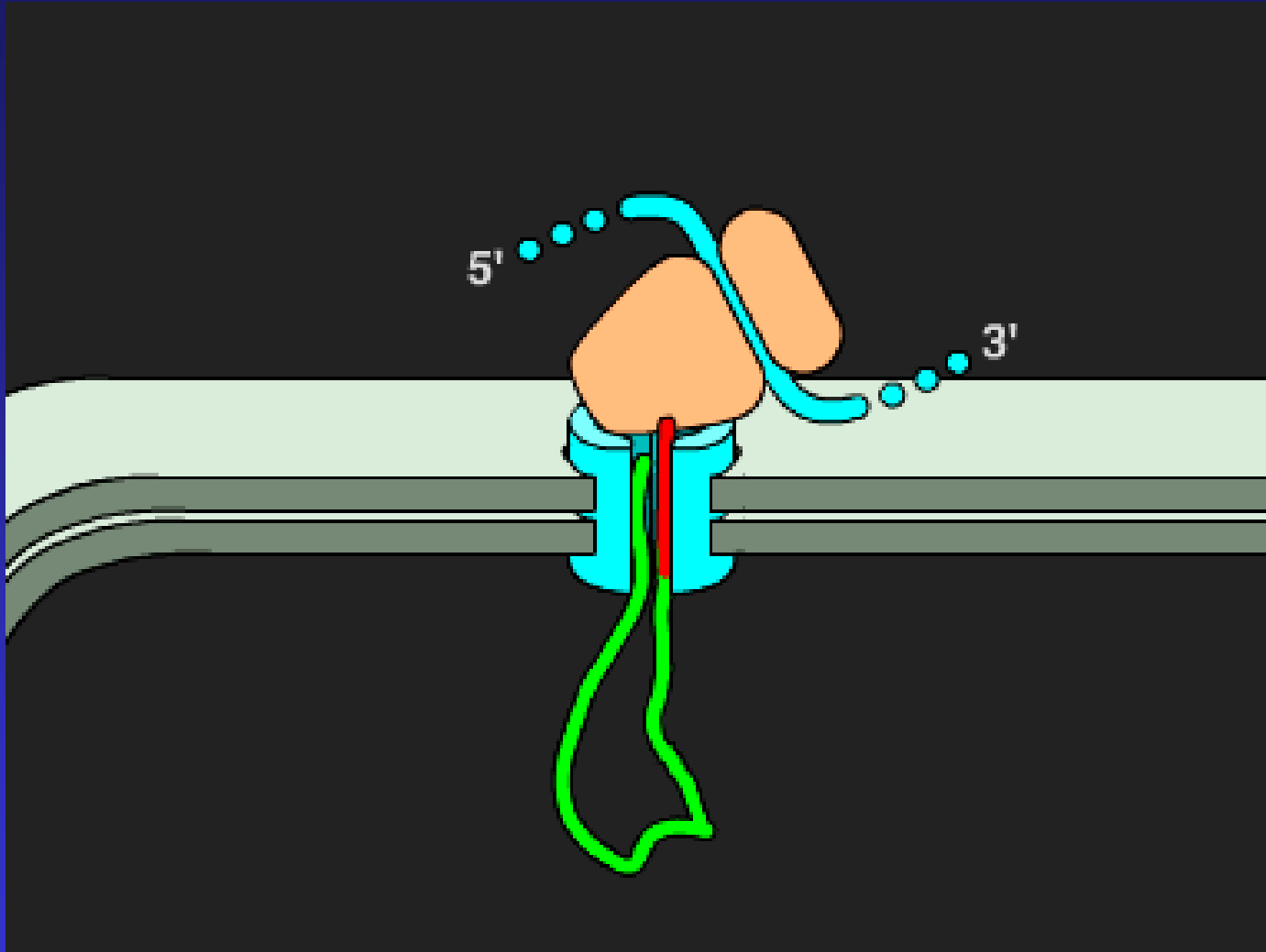


Synthesis of secretory proteins on the rER



Synthesis of secretory proteins on the rER



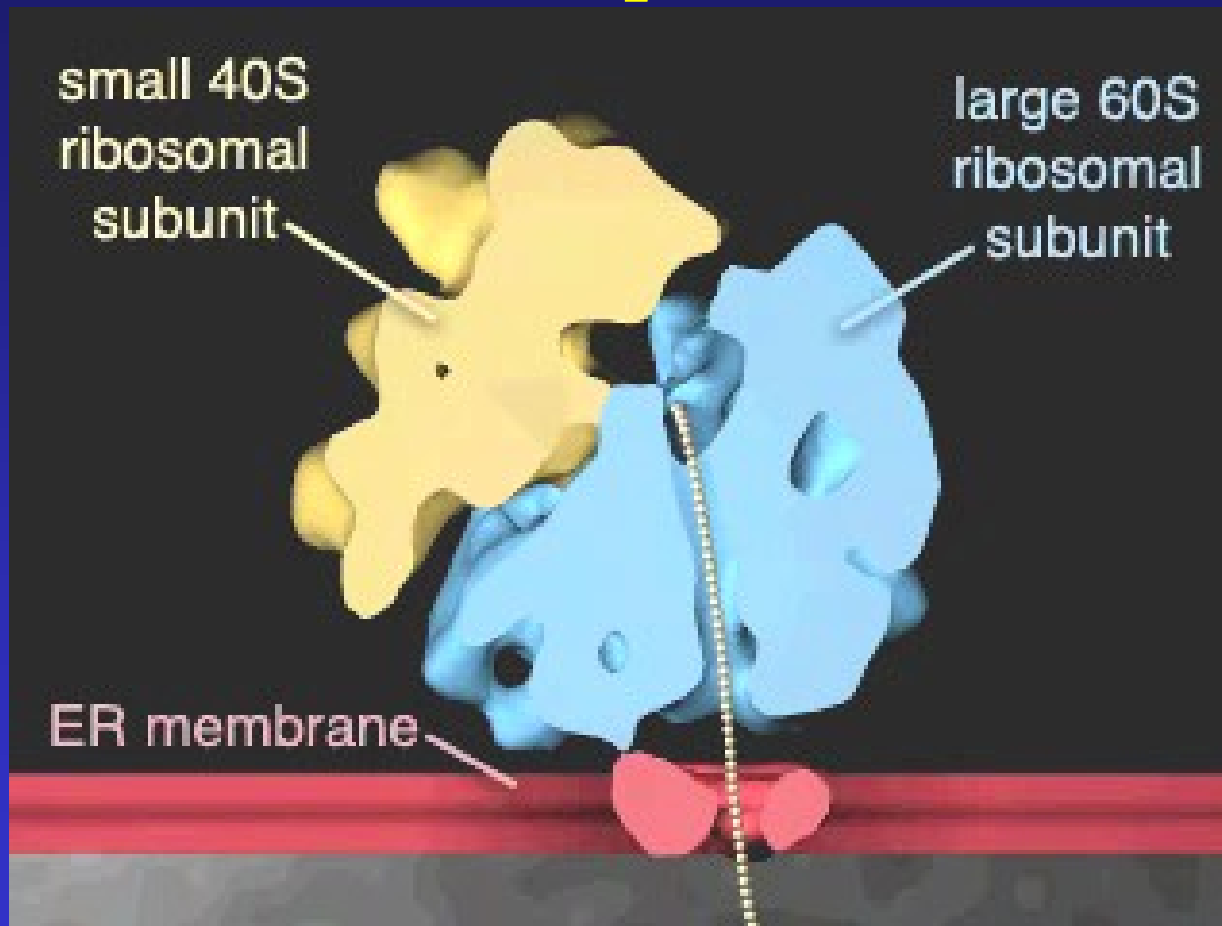


Electron microscopic view of a translocon channel

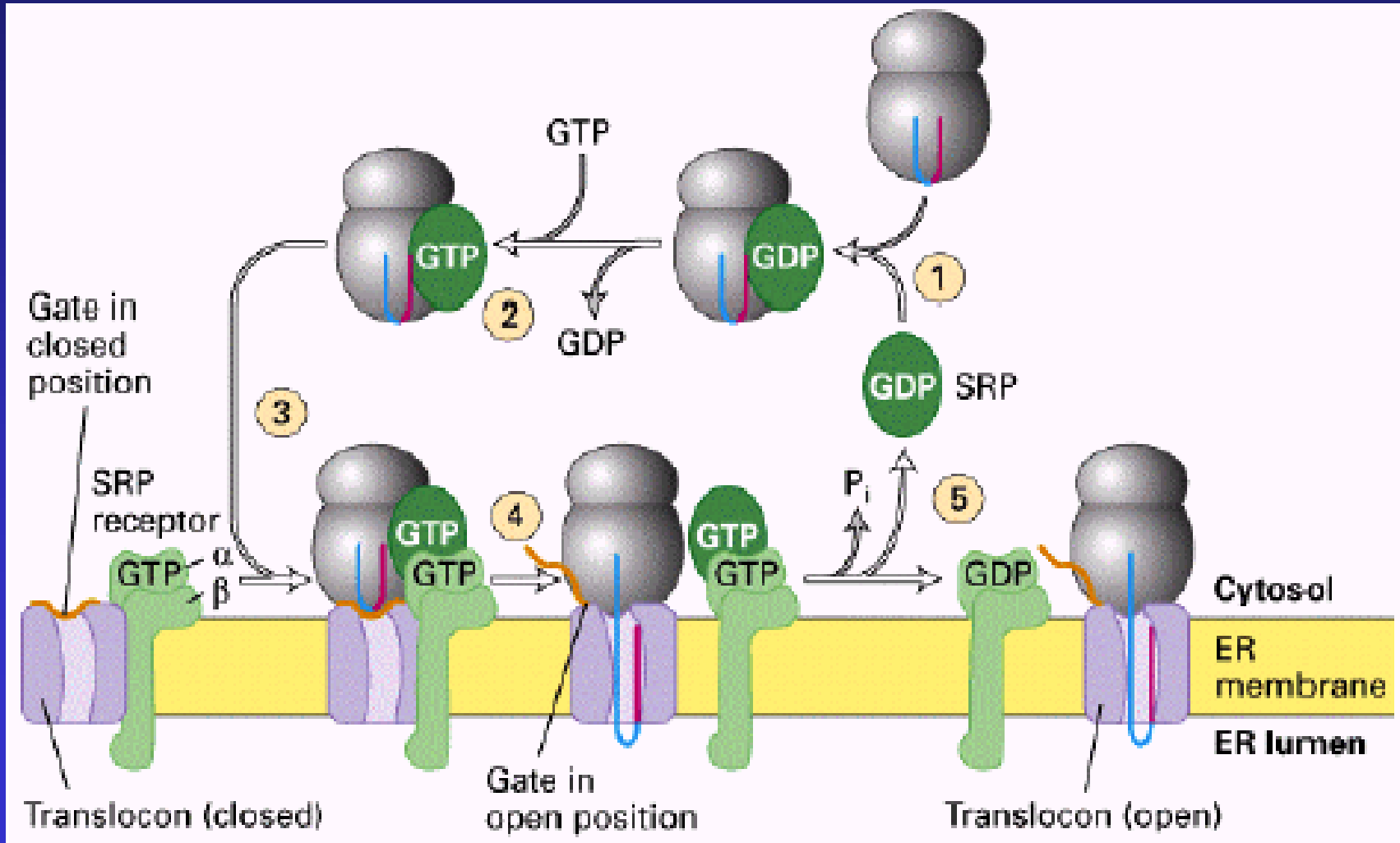


- donut-like structure
- binds the signal sequence
- binds the ribosome
- participates the transmembrane transfer

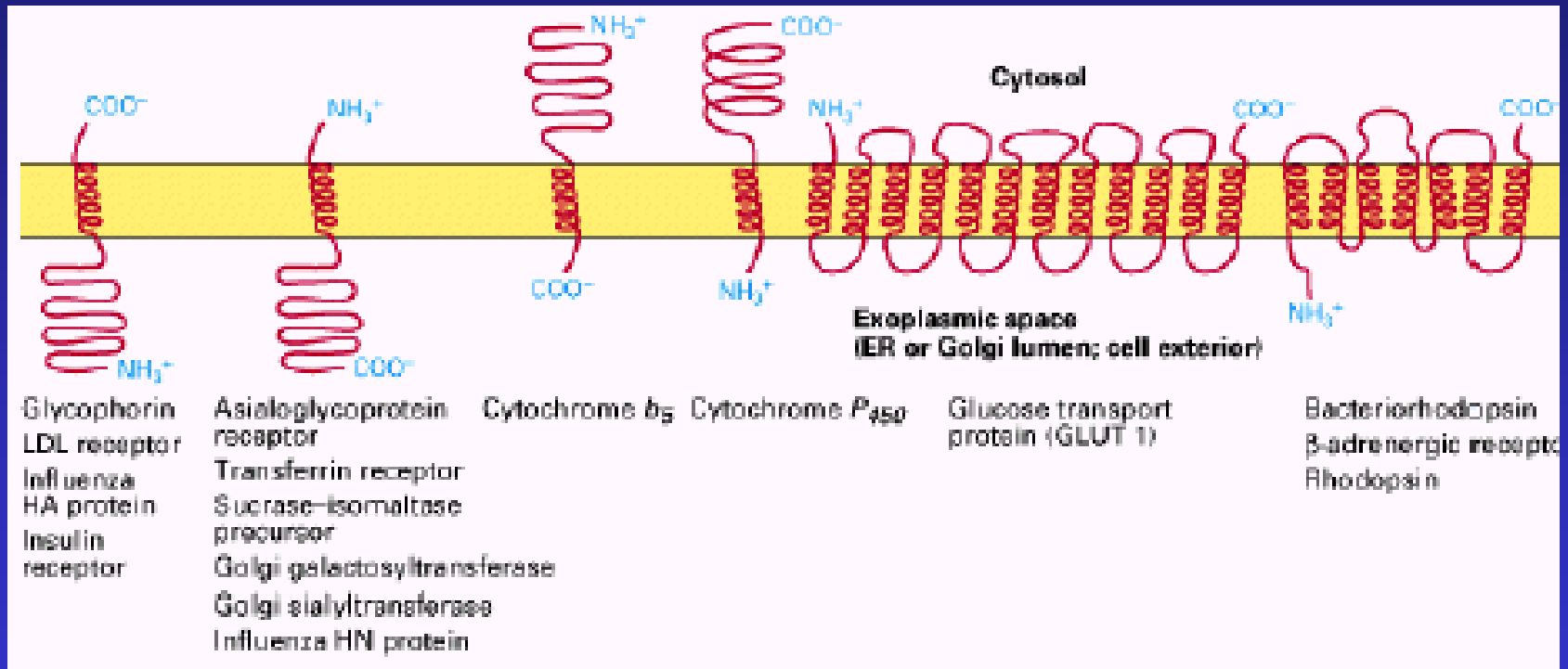
The ribosome-translocon-ER membrane complex

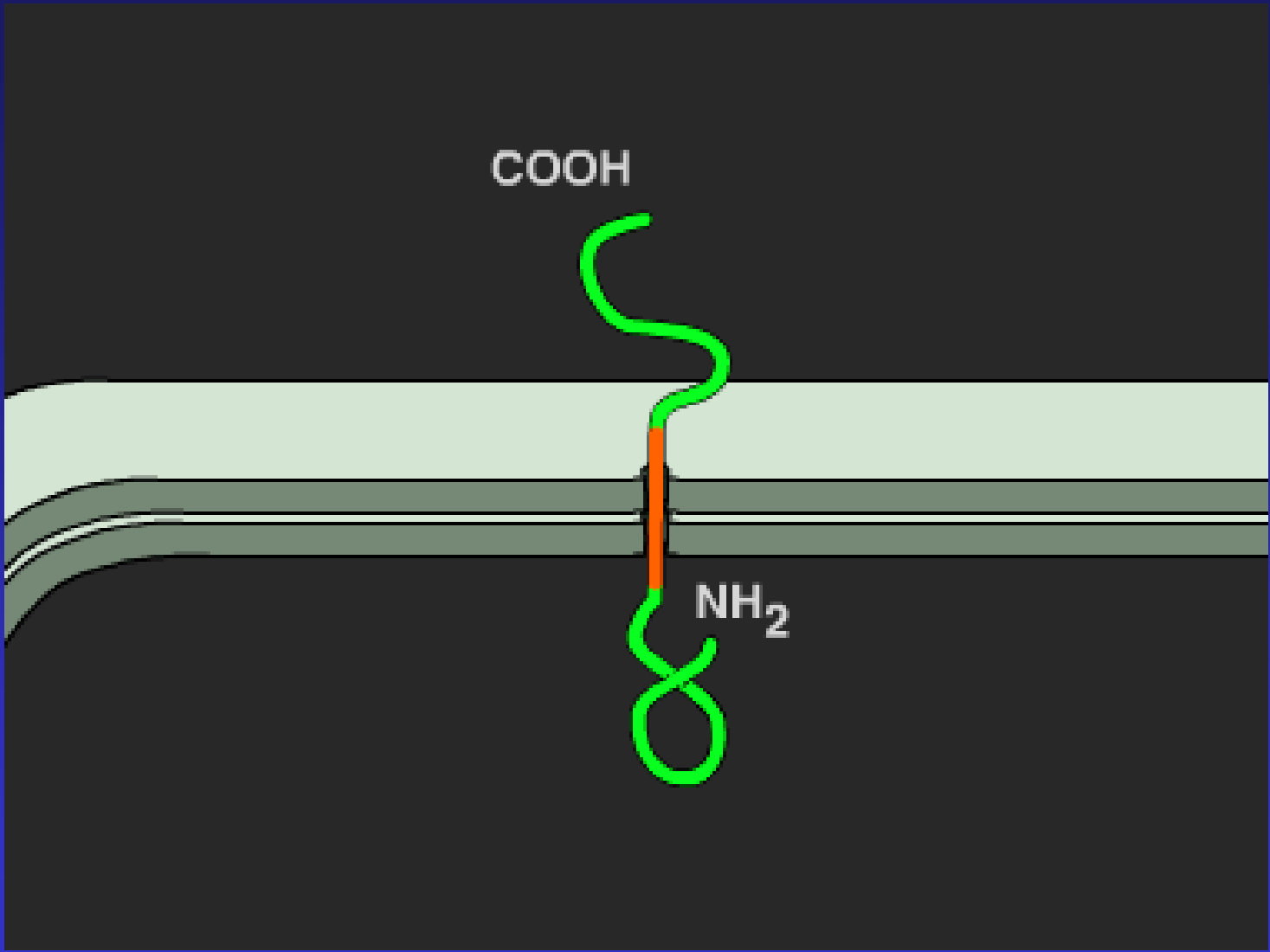


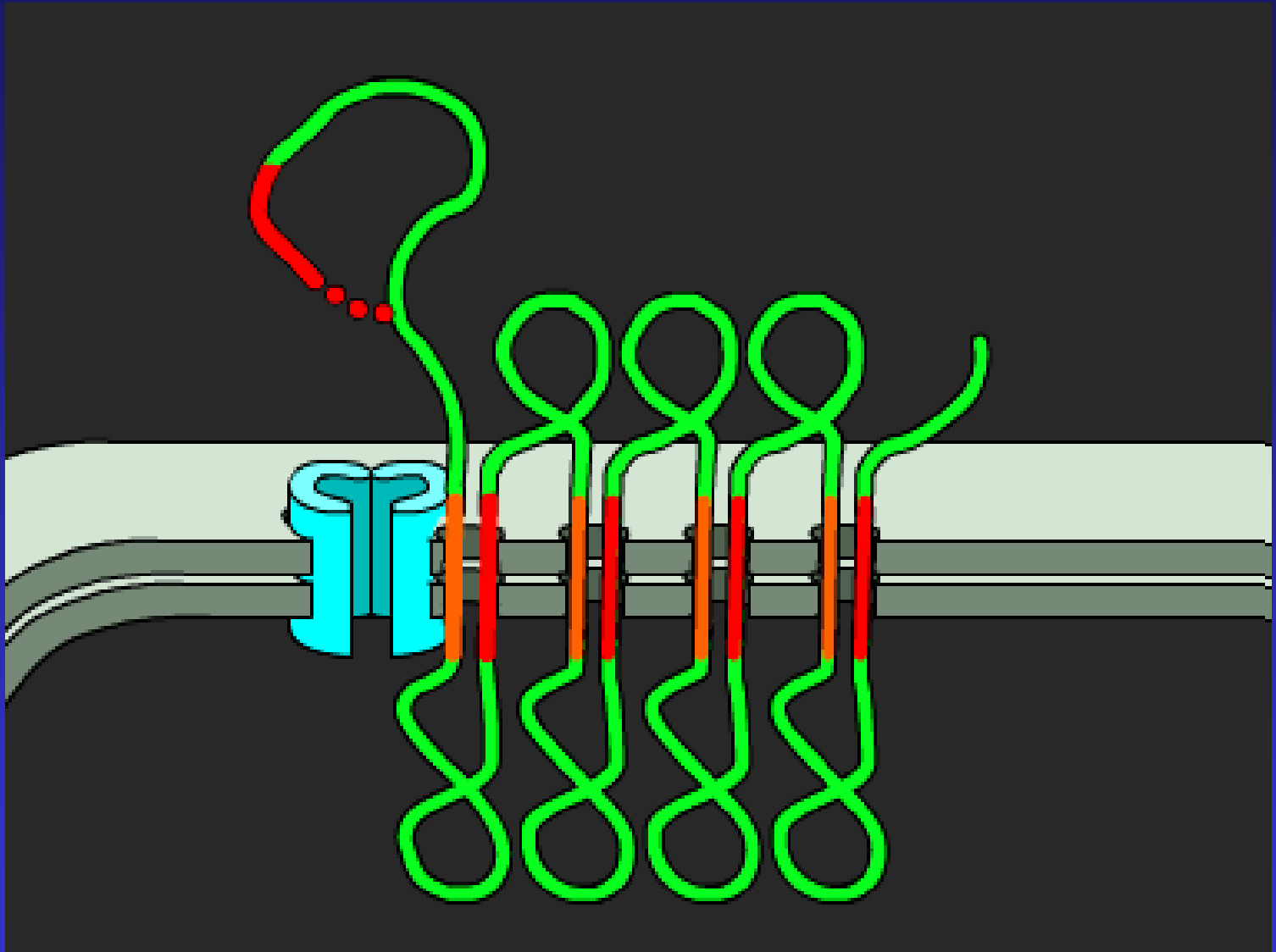
Cycles of GDP/GTDP exchange and GTP hydrolysis that drive insertion of nascent secretory protein into the translocon



Topologies of some integral membrane proteins synthesized on the rER

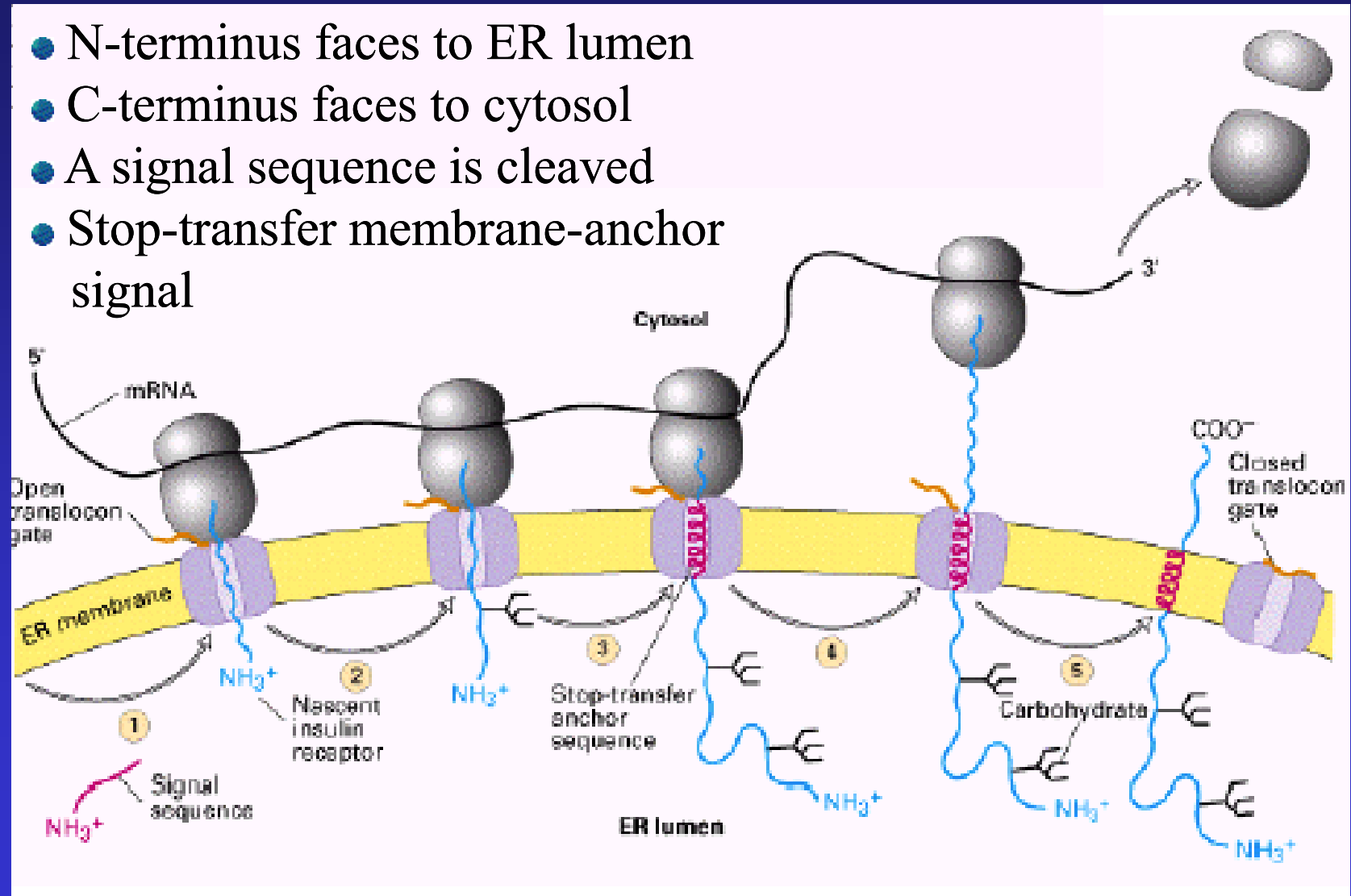






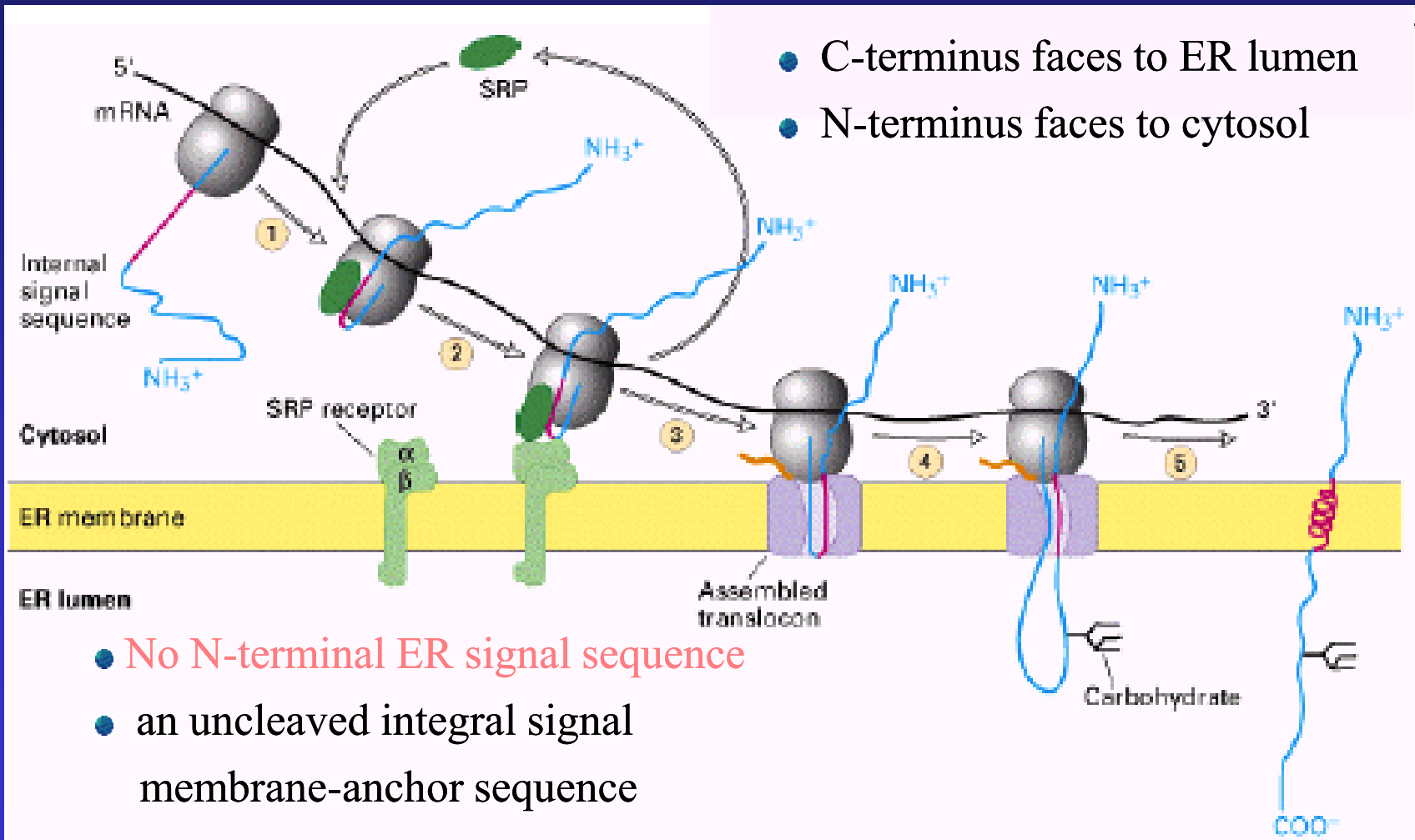
Synthesis and insertion into the ER membrane of the insulin receptor and similar proteins

- N-terminus faces to ER lumen
- C-terminus faces to cytosol
- A signal sequence is cleaved
- Stop-transfer membrane-anchor signal



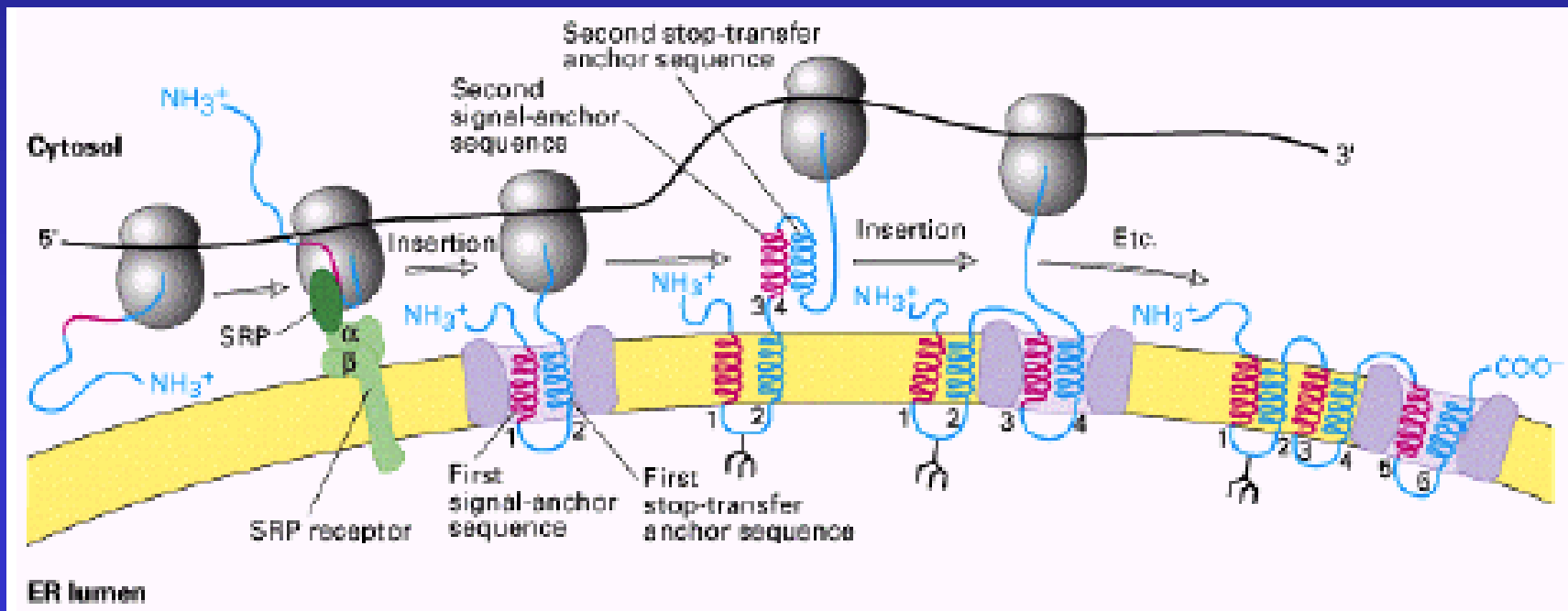
Synthesis and insertion into the ER membrane of the asialoglycoprotein receptor and similar proteins

- C-terminus faces to ER lumen
- N-terminus faces to cytosol

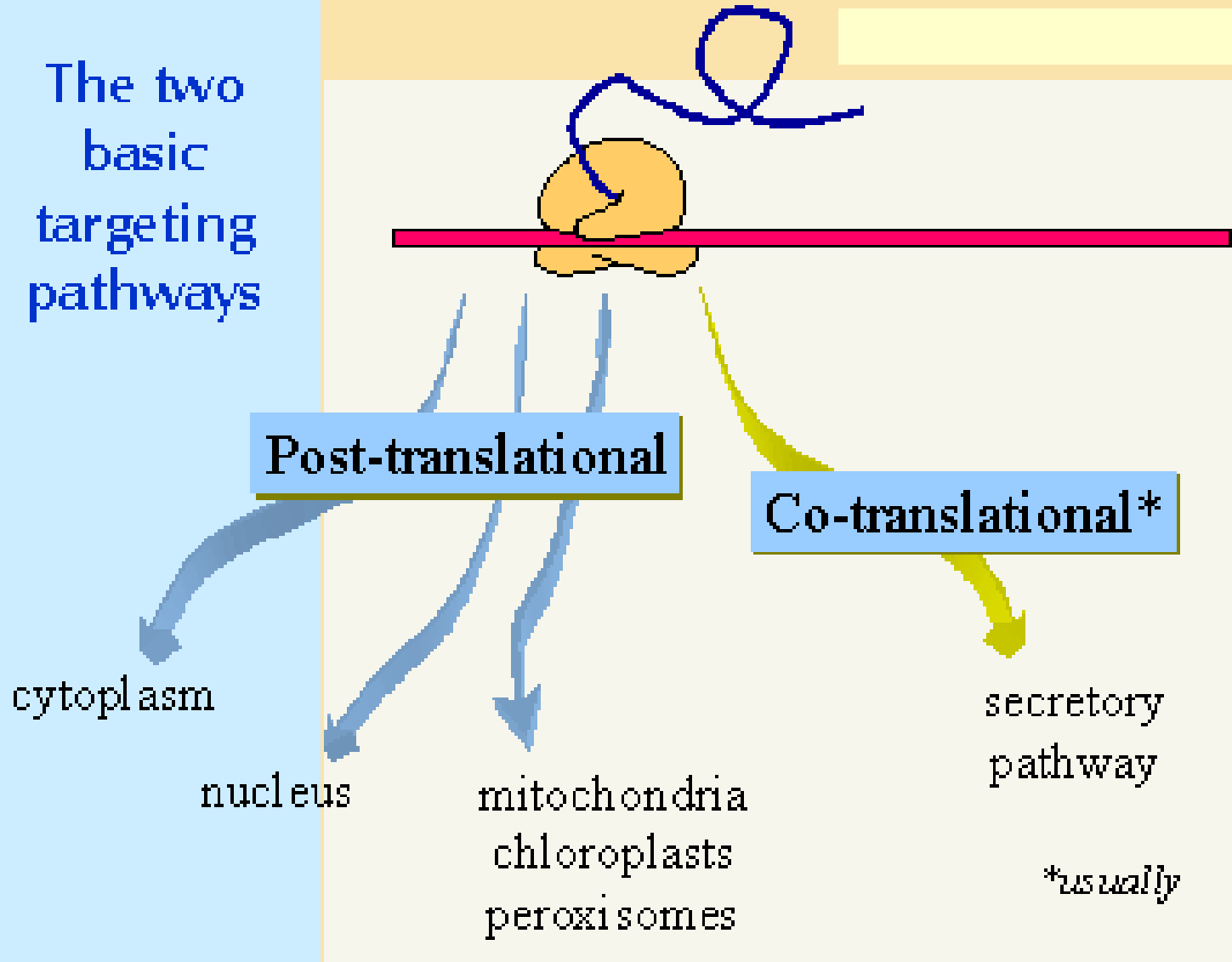


Synthesis and insertion into the ER membrane of proteins with multiple transmembrane α -helical segments

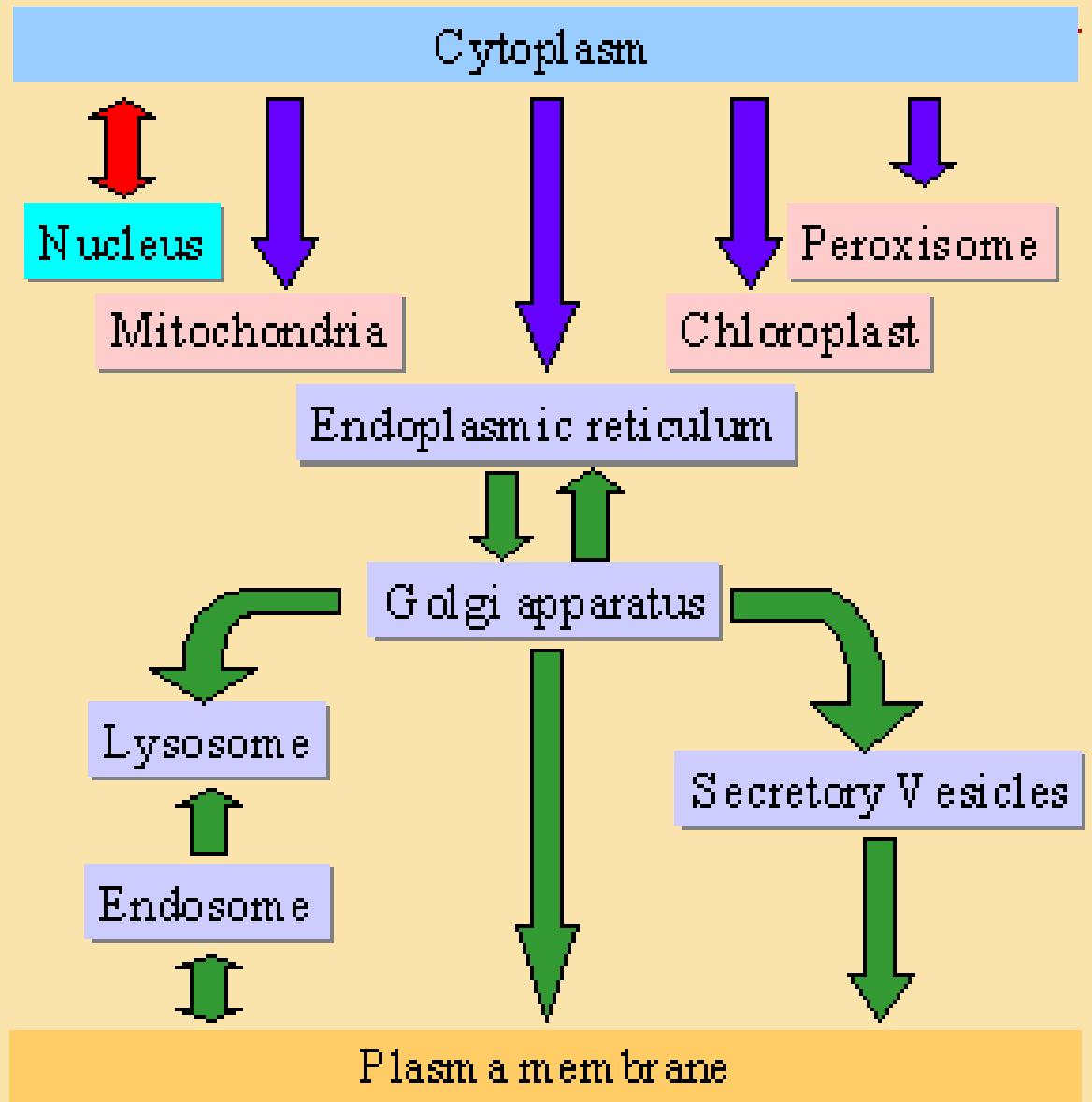
- An uncleaved internal signal membrane-anchor sequence
- A stop-transfer membrane-anchor sequence
- An uncleaved internal signal membrane-anchor sequence
- Etc.



The two basic targeting pathways



The basic targeting pathways



Post-translational modification



- Proteolytic cleavage of proteins
- Glycosilation

- Acylation
- Methylation
- Phosphorylation
- Sulfation
- Prenylation
- Vitamin C-dependent modifications
- Vitamin K-dependent modifications
- Selenoproteins

Proteolytic cleavage



- Removal of signal peptide from
preproproteins
preproteins
- Signal peptidase

Properties of uptake-targeting signal sequences



Target organelle	Usual signal location within protein	Signal removal	Nature of signal
rER	N-terminal	+	„core” of 6-12 mostly hydrophobic amino acids, often preceded by one or more basic amino acids
Mitochondrion	N-terminal	+	3-5 nonconsecutive Arg or Lys residues often with Ser and Thr ; no Glu or Asp
Chloroplast	N-terminal	+	No common motives, generally rich in Ser, Thr , poor in Glu and Asp
Peroxisome	C-terminal	-	Ser-Lys-Leu
Nucleus	Internal	-	Cluster of 5 basic amino acids or two smaller clusters separated by 10 amino acids

Glycoproteins



Predominant sugars are:

glucose, galactose, mannose, fucose,
GalNAc, GlcNAc, NANA

O-glycosidic linkage –

hydroxyl group of Ser, Thr, hydrLys

N-glycosidic linkage – consensus sequence **N-X-S(T)**
(BUT No P)

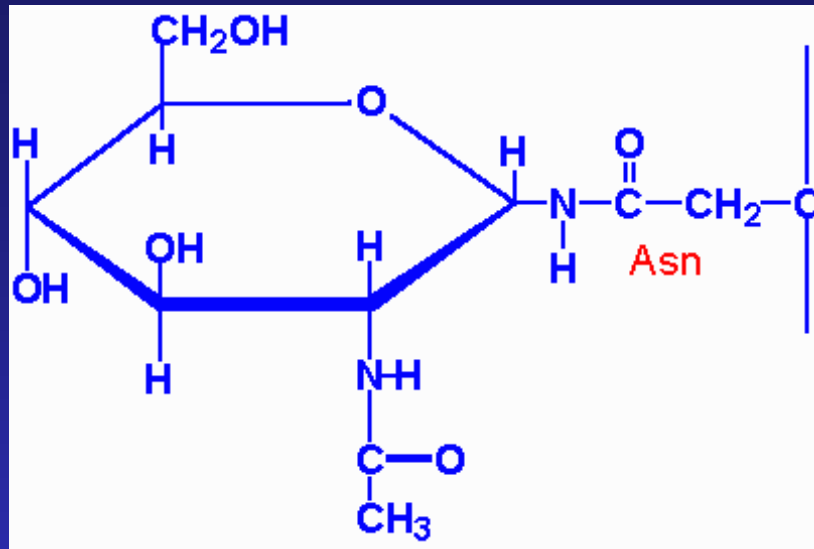
Major N-linked families:

high mannose type,

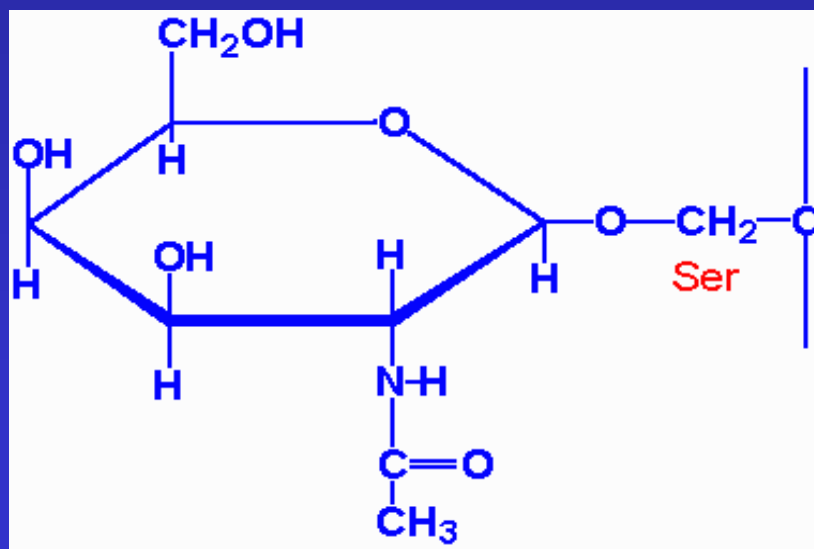
hybride type,

complex type (sialic acids)

Glycosylation



rER
N-linkage to GlcNAc



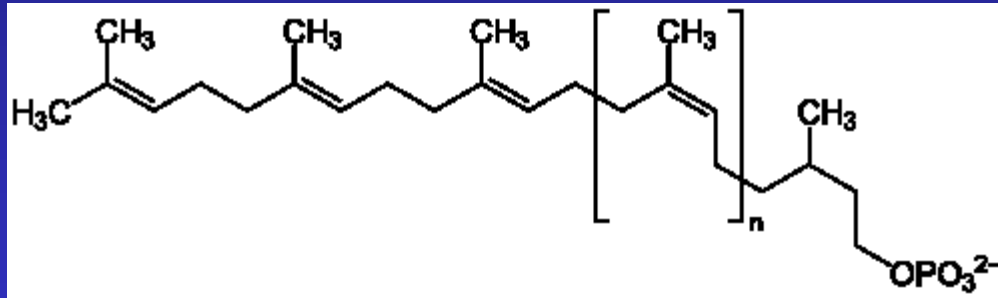
rER
O-linkage to GalNAc

O-linked sugars:

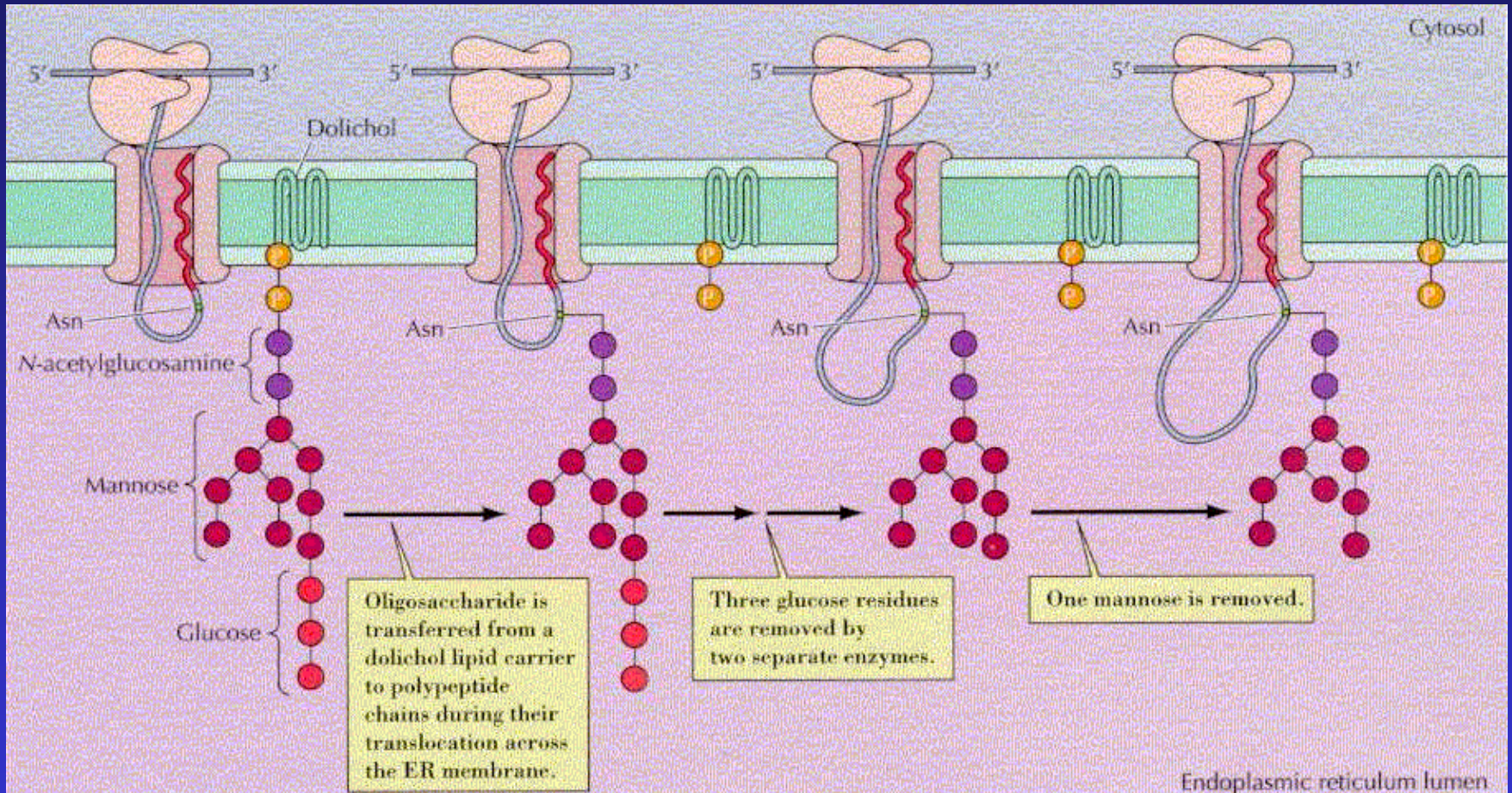
sugars coupled to UDP, GDP (mannose), CMP (NANA)
glycosyltransferase

N-linked sugars:

Requires a lipid intermediate **dolichol phosphate**



N-Glycosylation



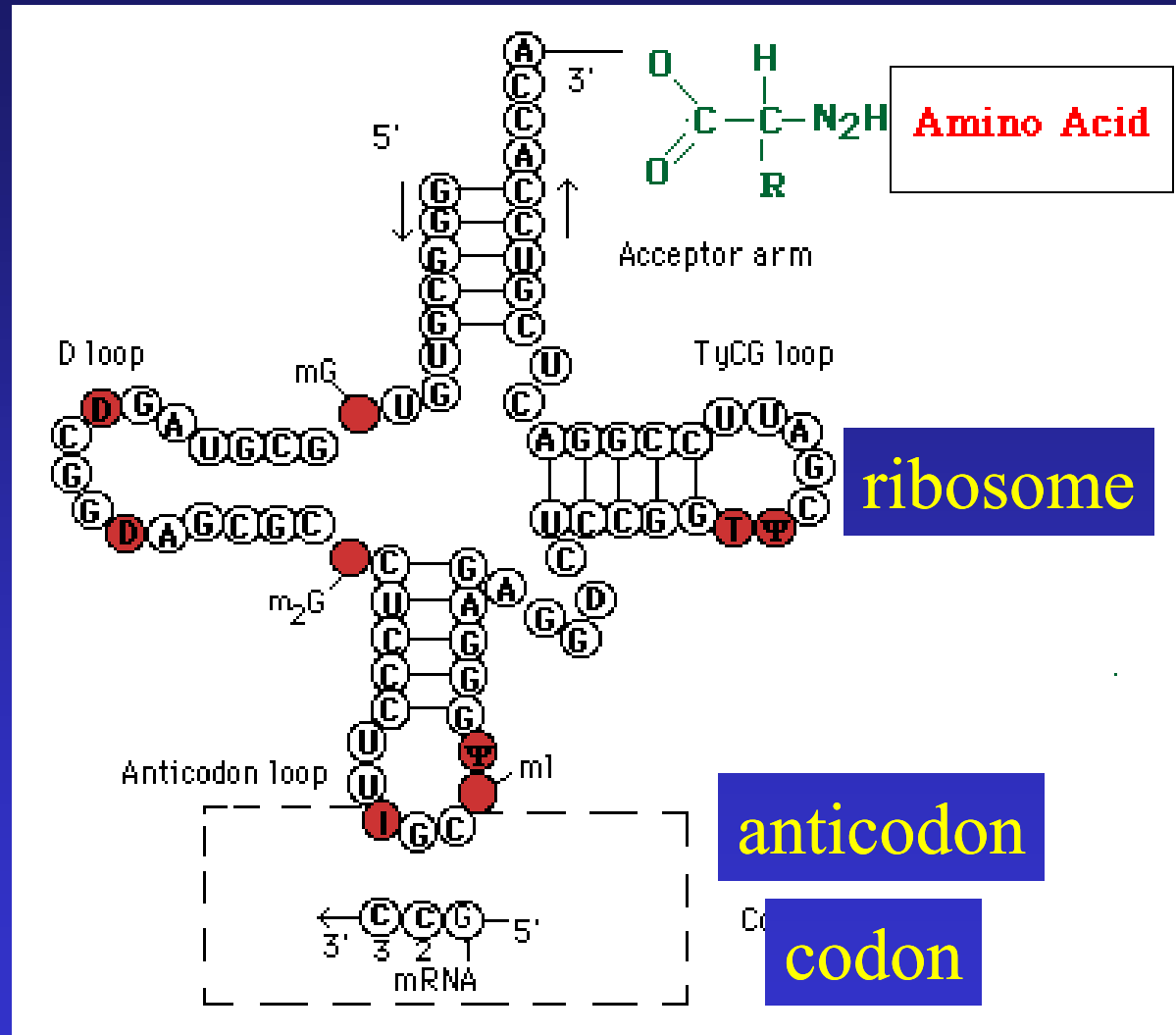
Appendix

Information on the next slides are only to inform students about the subject, they do not belong to the core subjects of Cell Science.

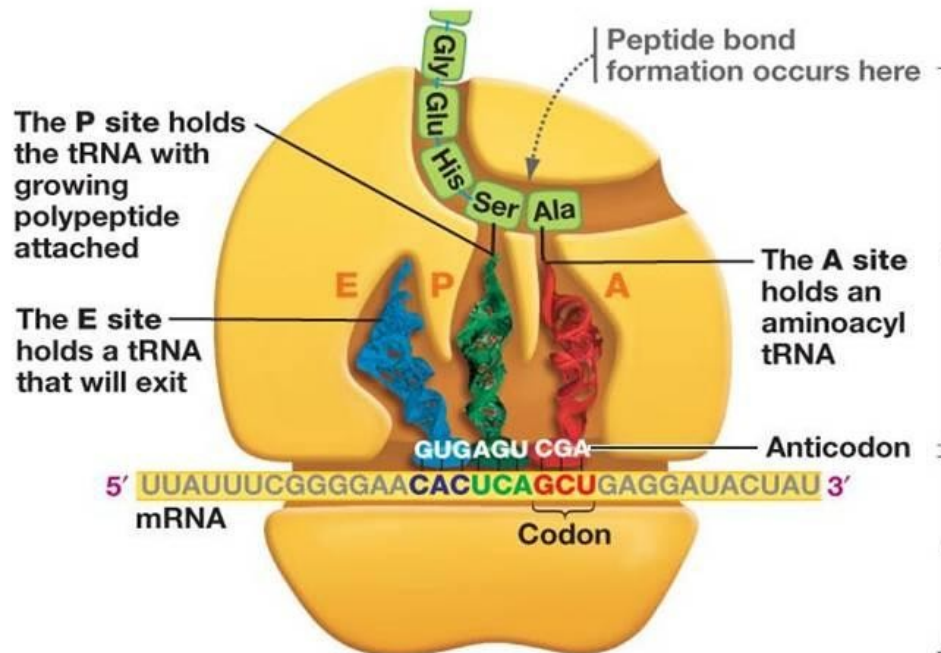
t-RNA



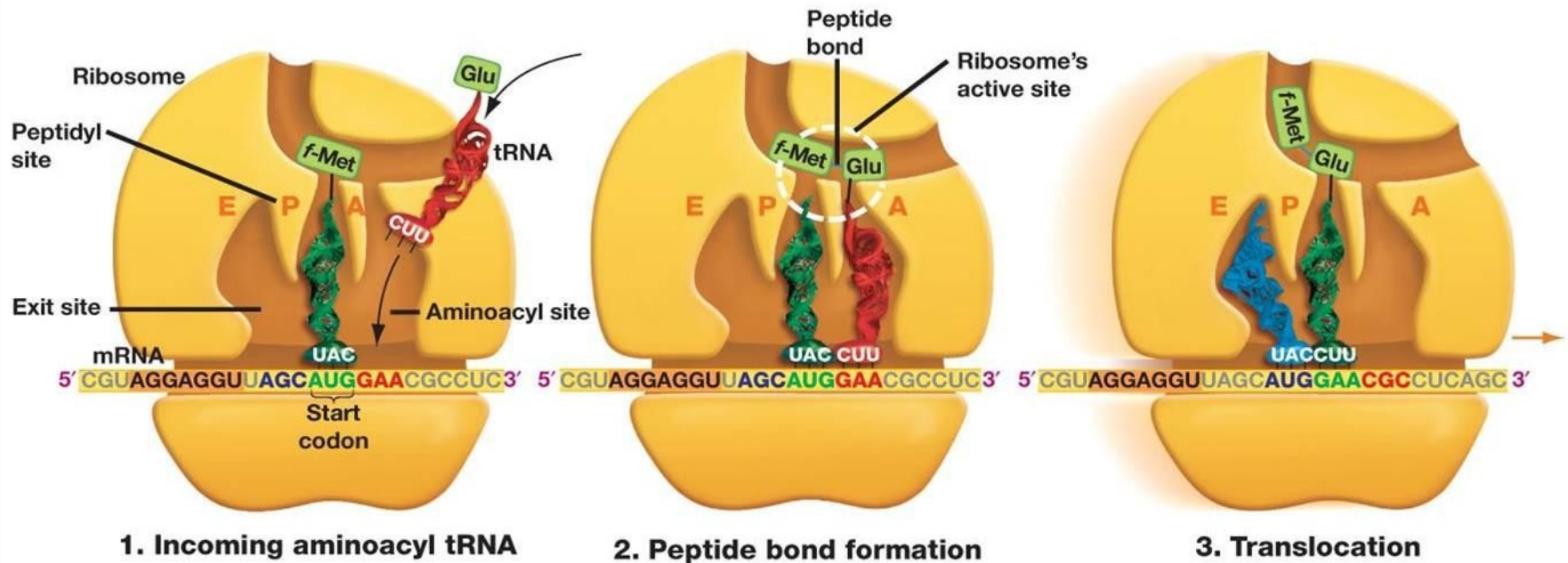
activator
enzyme
of AA



(a) Diagram of ribosome during translation (interior view)

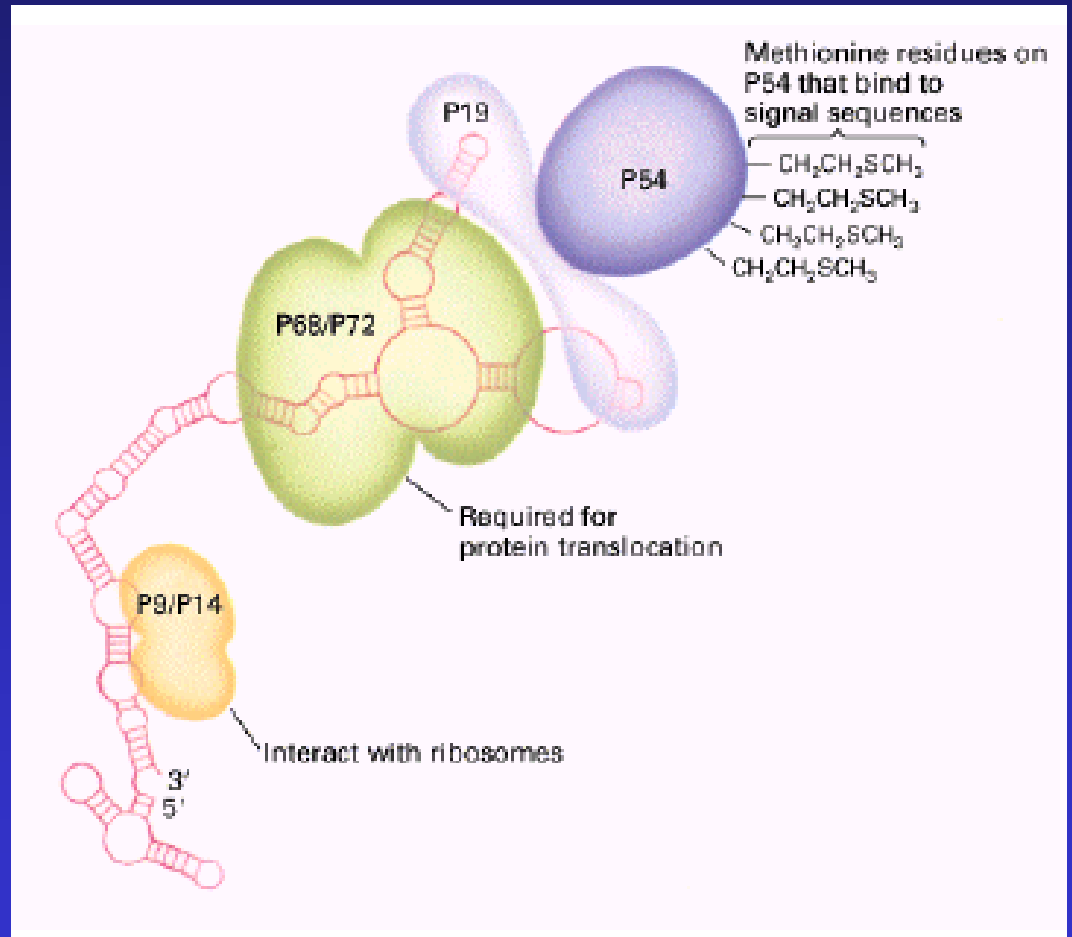


PROCESS: ELONGATION OF POLYPEPTIDES DURING TRANSLATION



Structure of SRP

- Universal
- 300 base RNA
- Six proteins
- P54 - signal peptide
- P9, P14 - ribosome
- P68, P72 move the peptide

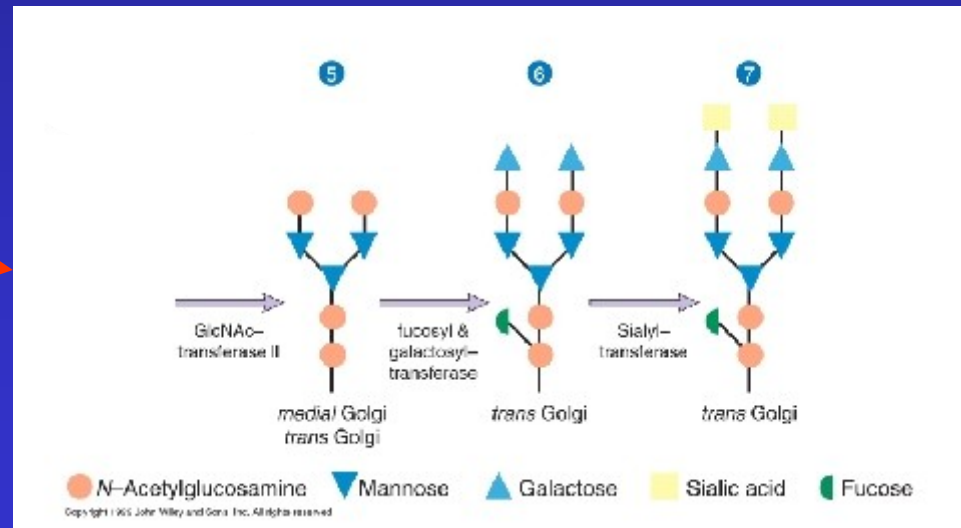
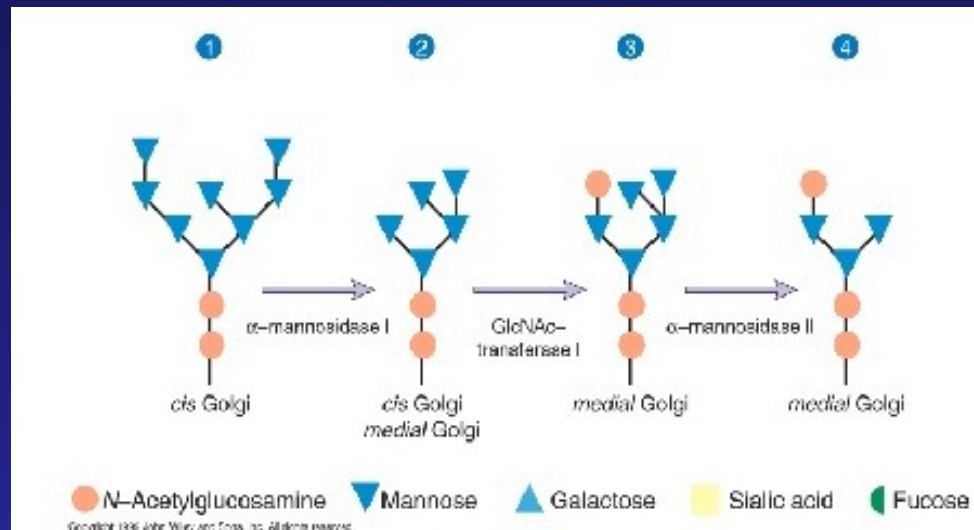


Translocon complex



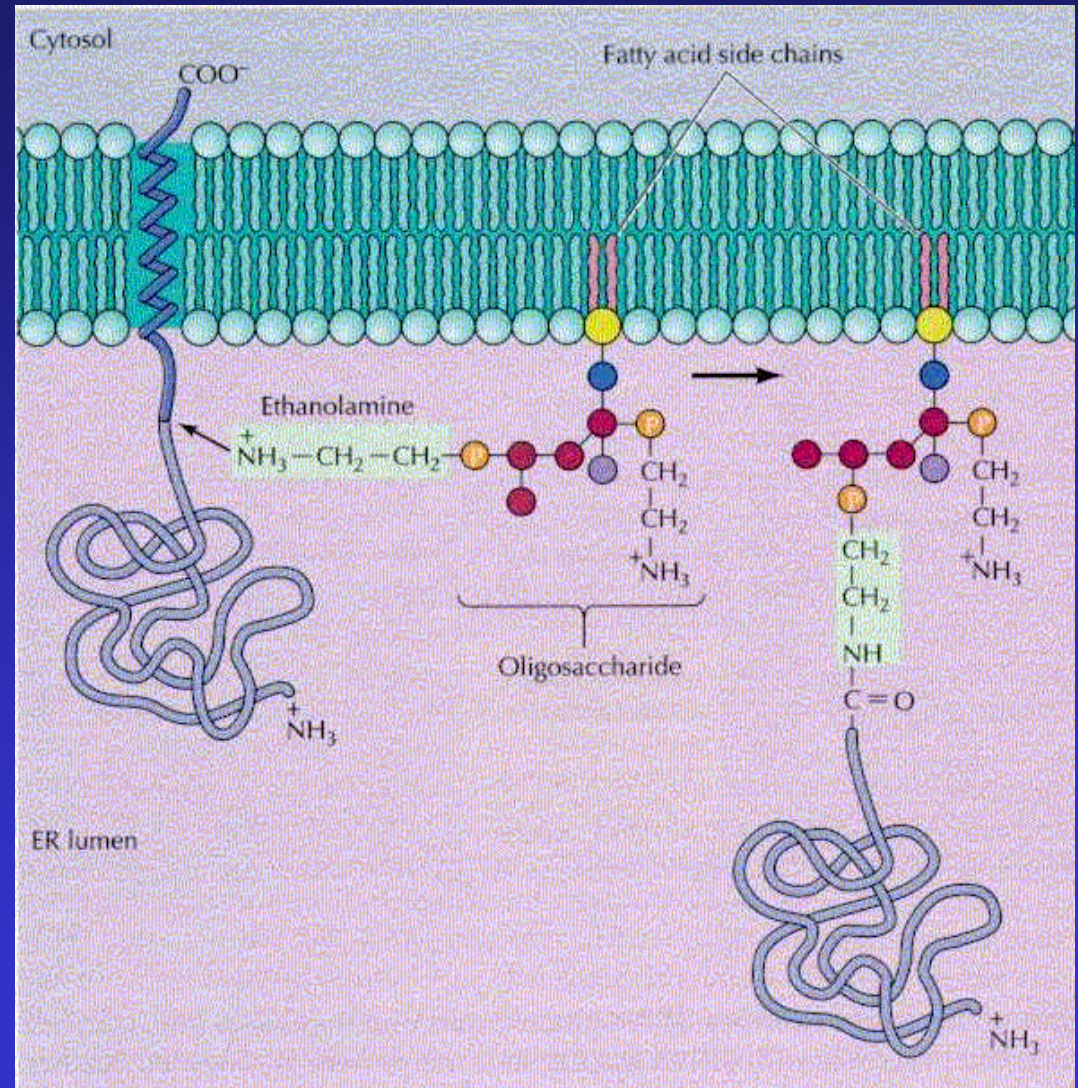
- **TRAM** – (= translocating chain-associated membrane protein) binds the **signal sequence**
- **Sec61p** – major constituent of the translocon channel; assembles into a **donut-like structure**
- The **Sec 61 complex** binds the ribosome, participates the transmembrane transfer





Glycosylphosphatodyl inositol (GPI) -anchored peptides

GPI-anchored peptides become the outer surface of the surface membrane



Protein signals:



- Integral, soluble proteins of ER, Golgi retrieved by the **KDEL-receptors**. They recognize the **KDEL** signal (Lys-Asp-Glu-Leu at C-terminus).
- ER membrane proteins have a **KKXX** (dilysine motif) on the C-terminus.
- Other ER membrane proteins possess **di-arginine** motif on the N-terminus.

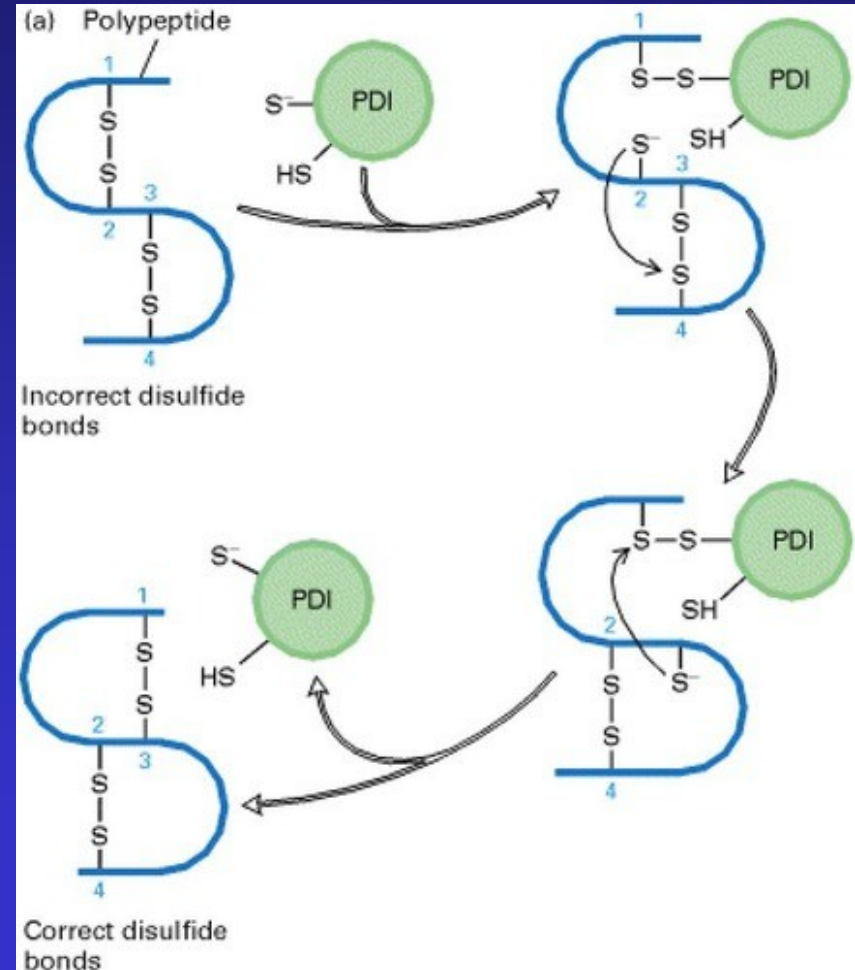
Protein folding:



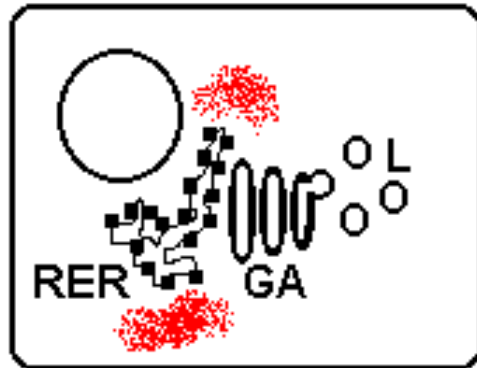
- **Peptidyl-prolyl isomerase:**
accelerates **rotation** about peptidyl-prolyl bonds
- **Oligosaccharide protein transferase:**
transfers **carbohydrate chains** to the nascent polypeptide as they enter the lumen of ER
- **Calnexin, calreticulin:**
interact with **CHO groups** of glycoproteins

Protein folding: Protein Disulfide Isomerase (PDI) !

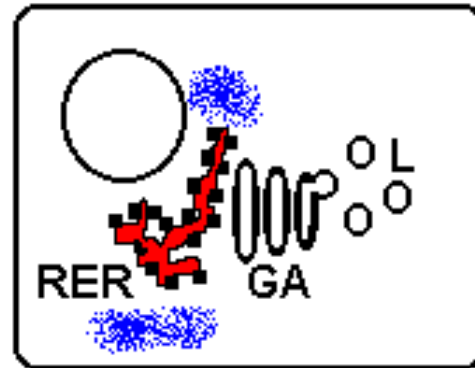
- Provides mechanism for breaking incorrectly paired disulfide bonds.
- The most stable folded state is reached



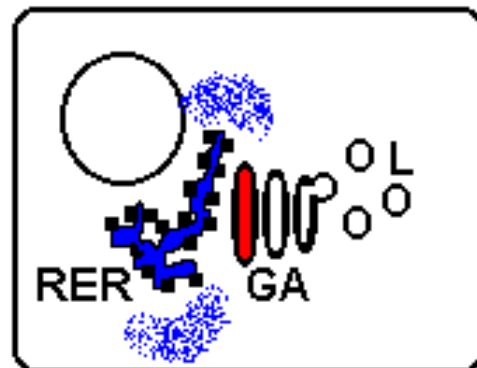
Chase-pulse technique



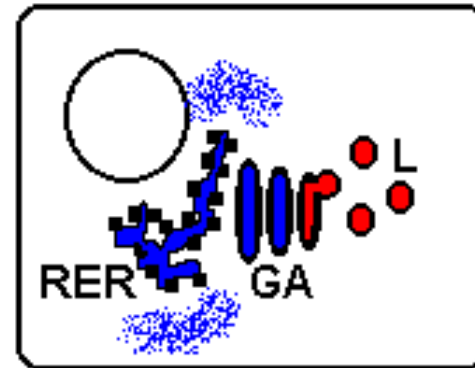
Pulse



Chase 3 h



Chase 6h



Chase 12h

Antibiotics

They inhibit different steps of protein synthesis

- Actinomycin D - transcription (complex with DNA)
 - Rifamycin - transcription (RNA polymerase)
 - Amanitin - transcription (RNA polymerase II)
 - Streptomycin - initiation
 - Tetracycline - aminoacyl-tRNA - A locus interaction
 - Erythromycin - translocation of tRNA from A to P locus
 - Cycloheximide - “ (only in eukaryotes)
 - Chloramphenicol - peptide bond formation
 - Puromycin - termination
- Penicillins and Cephalosporins - synthesis of bacterial cell wall (proteoglycans)

