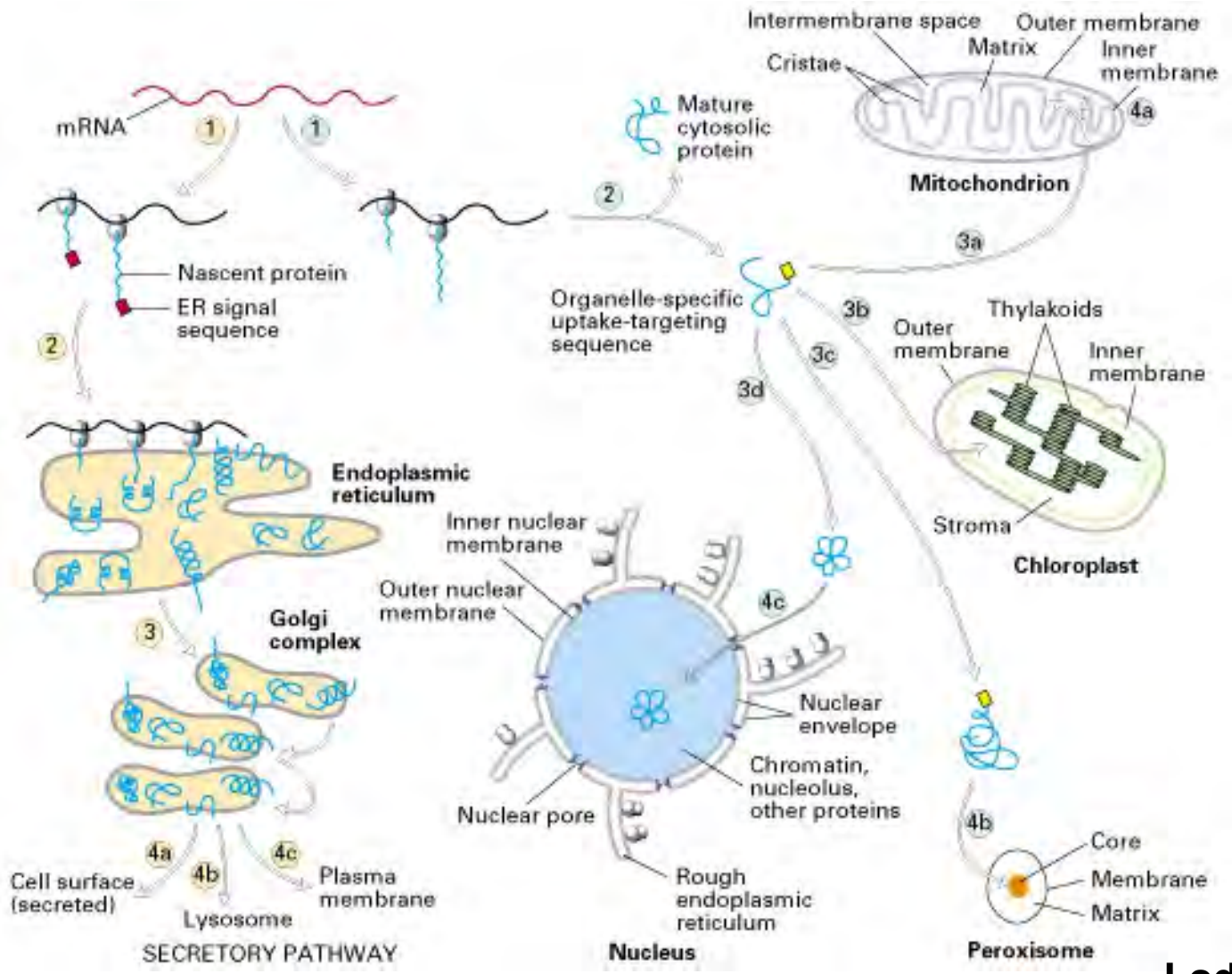
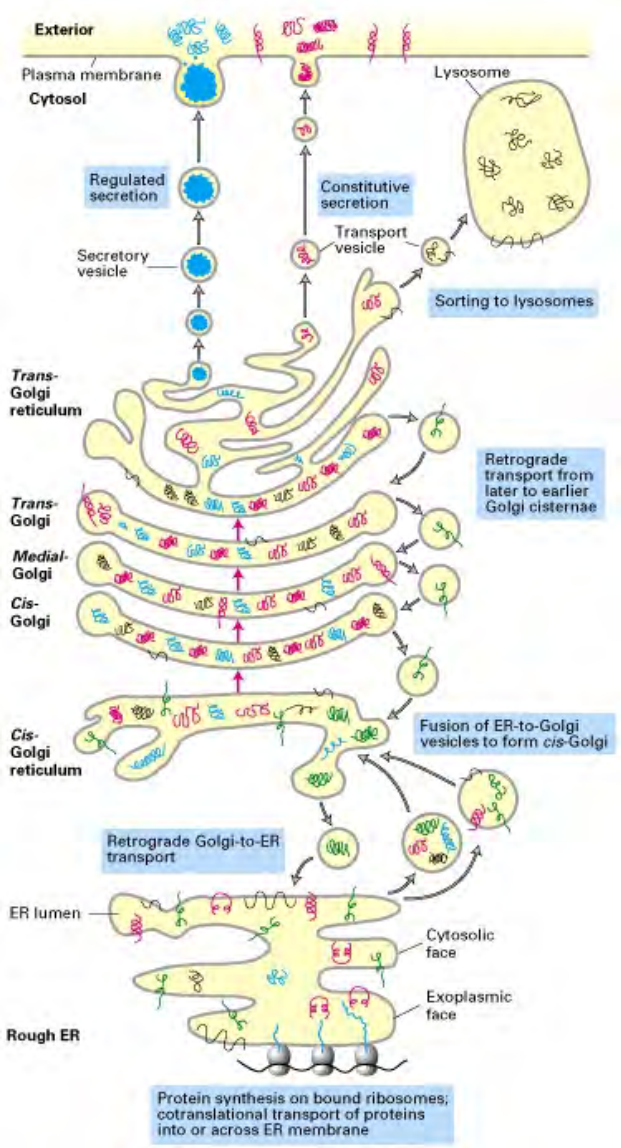
Protein and membrane trafficking



Lodish et al, 2000

The secretory pathway

cisternal progression



Lodish et al, 2000

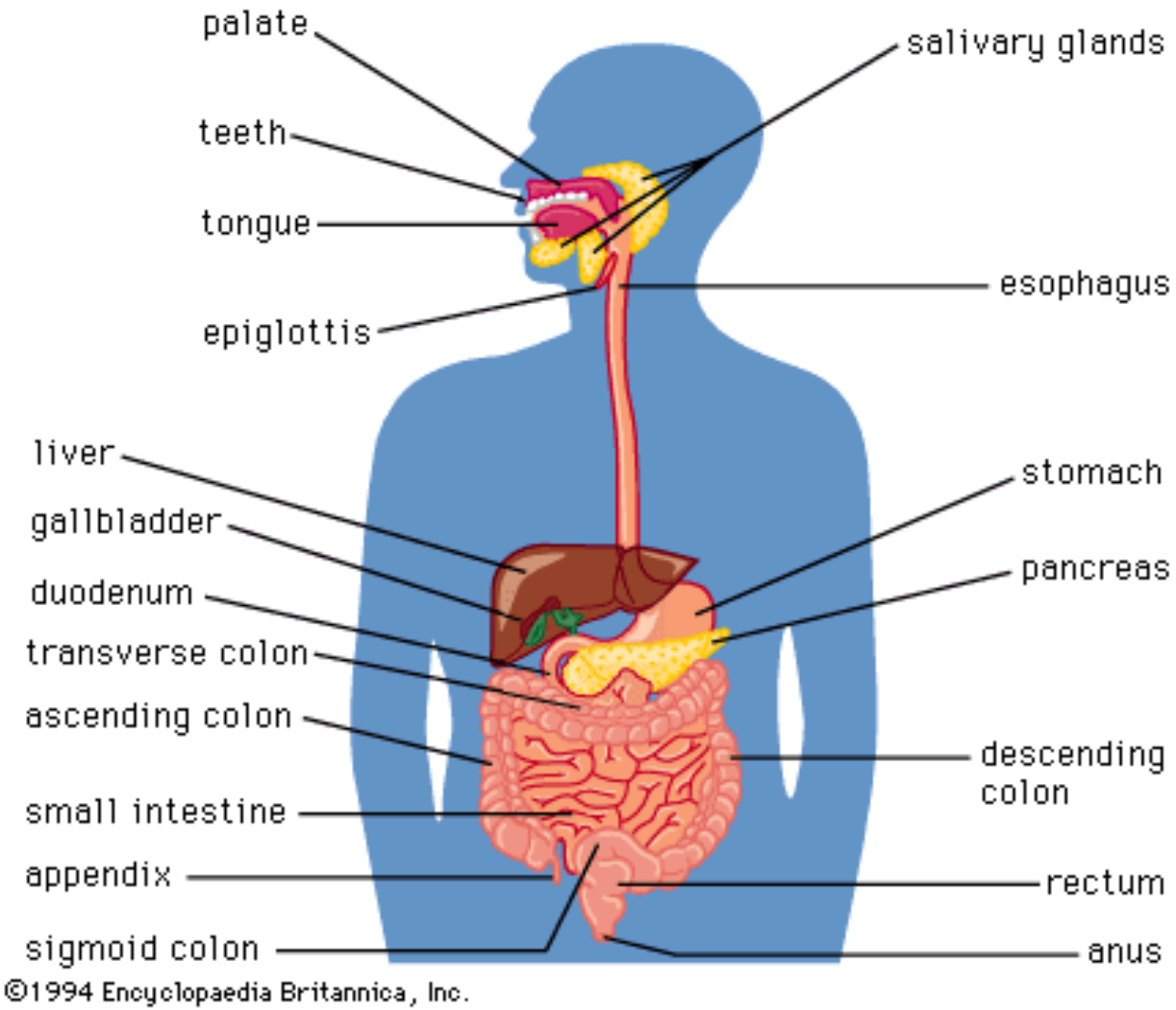
Cells can be specialized for the secretion of specific proteins

TABLE 17-3 Classes of Secretory Proteins in Vertebrates

Protein Type	Example	Site of Synthesis
	Constitutive Secretory Proteins	
Serum proteins	Albumin	Liver (hepatocyte)
	Transferrin (Fe transporter)	Liver
	Lipoproteins	Liver, intestine
	Immunoglobulins	Lymphocytes
Extracellular matrix proteins	Collagen	Fibroblasts, others
	Fibronectin	Fibroblasts, liver
	Proteoglycans	Fibroblasts, others
	Regulated Secretory Proteins	
Peptide hormones	Insulin	Pancreatic β-islet cells
	Glucagon	Pancreatic α-islet cells
	Endorphins	Neurosecretory cells
	Enkephalins	Neurosecretory cells
	ACTH	Anterior pituitary lobe
Digestive enzymes	Trypsin	Pancreatic acini
	Chymotrypsin	Pancreatic acini
	Amylase	Pancreatic acini, salivary glands
	Ribonuclease	Pancreatic acini
	Deoxyribonuclease	Pancreatic acini
Milk proteins	Casein	Mammary gland
	Lactalbumin	Mammary gland

Lodish et al, 2000

The pancreas is an organ of the digestive tract

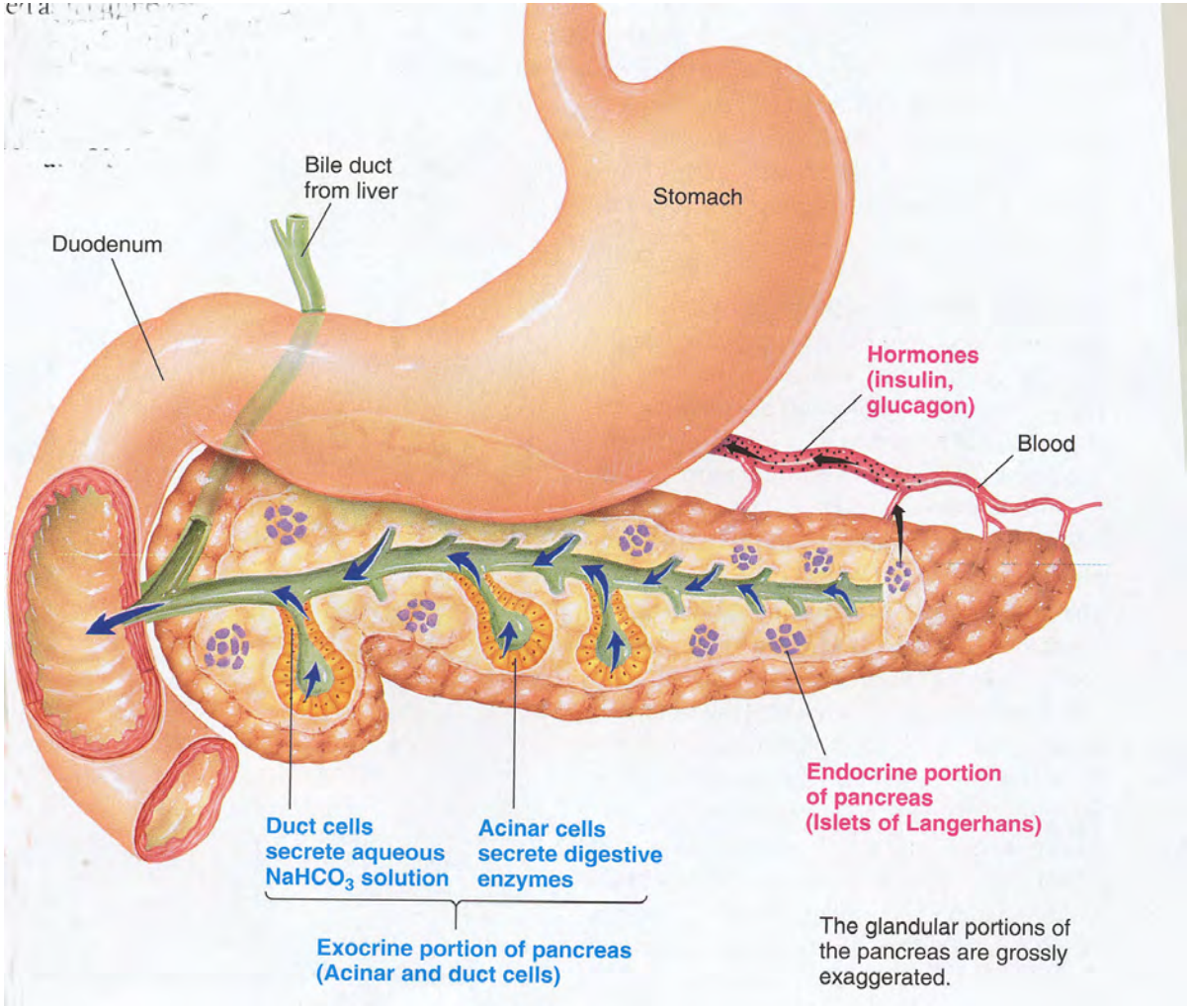


<u>Small intestine</u> (10-15 ft)

- Duodenum
- Jejunum
- Ileum

- The pancreatic duct goes into the duodenum.

The pancreas secretes into the duodenum



Pancreatic acinar cells as a model system for secretion

Endocrine organ: regulation of blood glucose

1% of pancreatic cells= islets of Langerhans, secretes insulin, glucagon, somatostatin

Exocrine organ

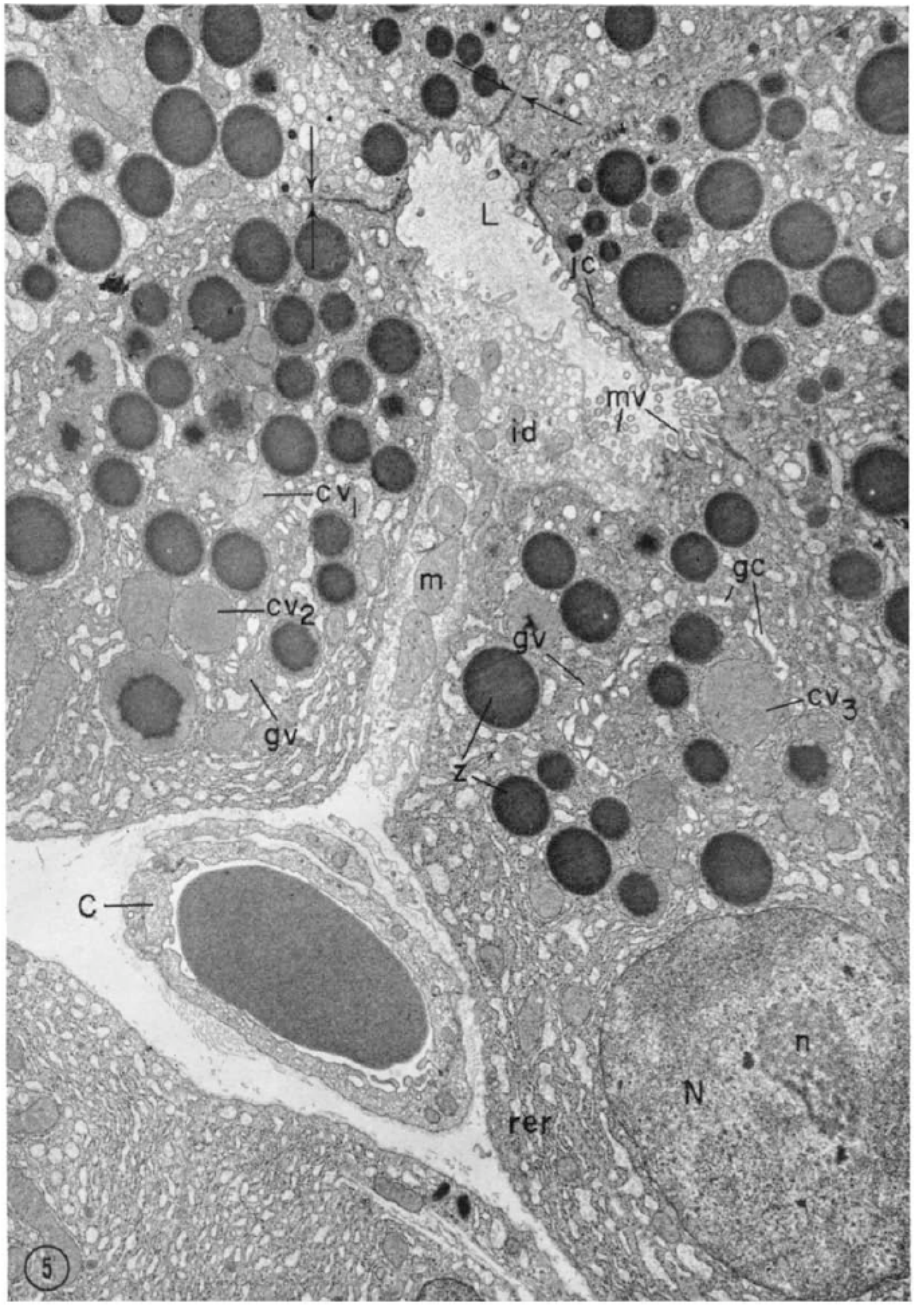
99% of cells=acinar cells, secrete 1.2-1.5 liters of pancreatic juice per day

Pancreatic juice enzymes
- -amylase
- -trypsinogen>trypsin
- -chymotrypsinogen>chymotrypsin
- -procarboxypeptidase>carboxypeptidase
- -lipase
- -ribonuclease/deoxyribonuclease

EM of pancreatic slice

Slices were cut from guinea pigs were incubated in culture media 3 hr.

L=lumen
mv=microvilli
jc=junctional complex
id=intercalated duct cell
rer=rough ER
gc=golgi cisternae
cv=condensing vesicles
N=nucleus
n=nucleolus
C=capillary
n=mitochondria



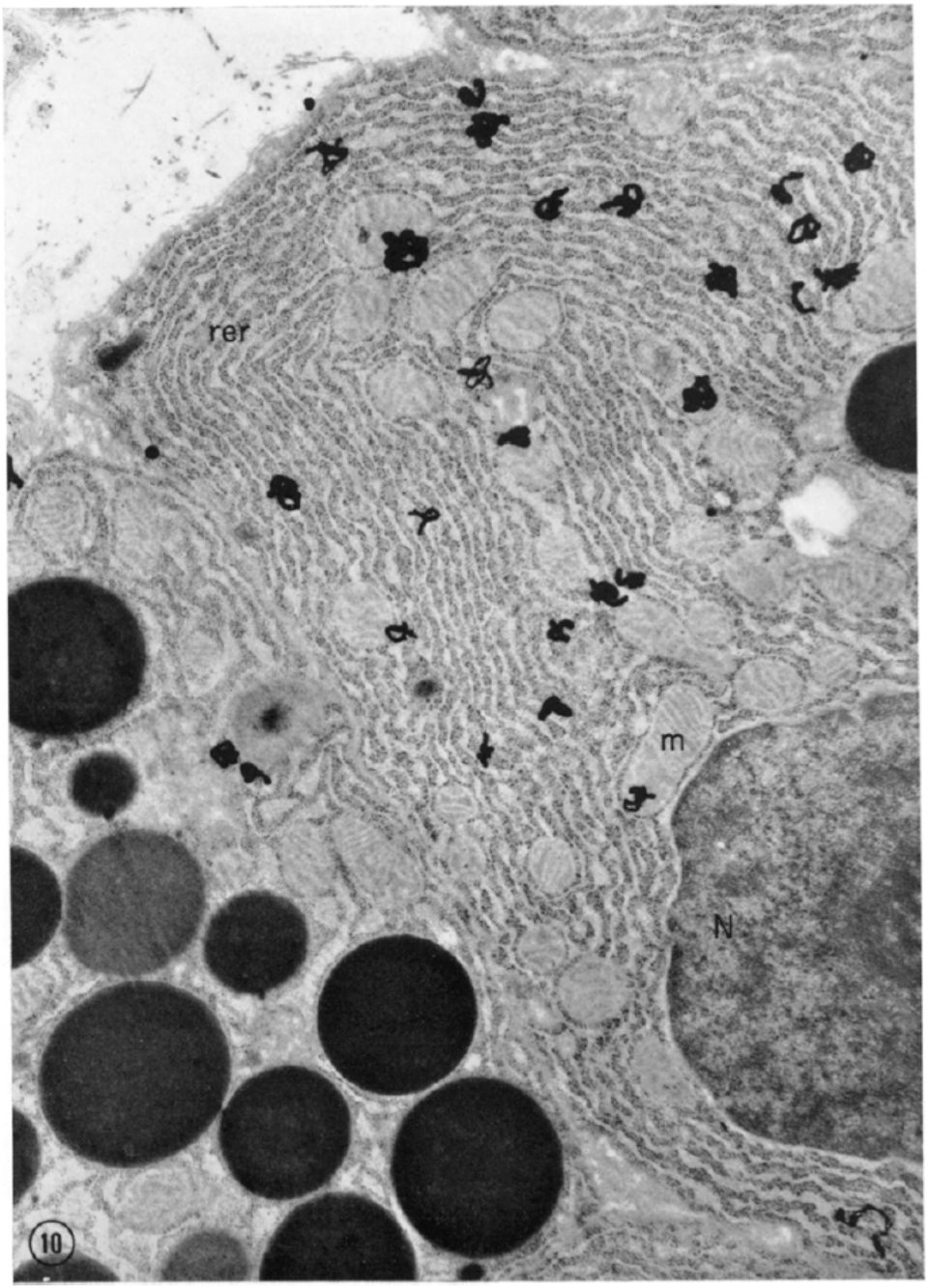
Jamieson and Palade (1967) JCB

Pulse labeling for 3 minutes

Pancreatic slices incubated in media containing ^{3}H leucine for 3 minutes. Transfer to media with unlabeled leucine. Process for EM and autoradiography.

Radioautographic grains located over rough ER.

rer=rough ER
N=nucleus
n=mitochondria



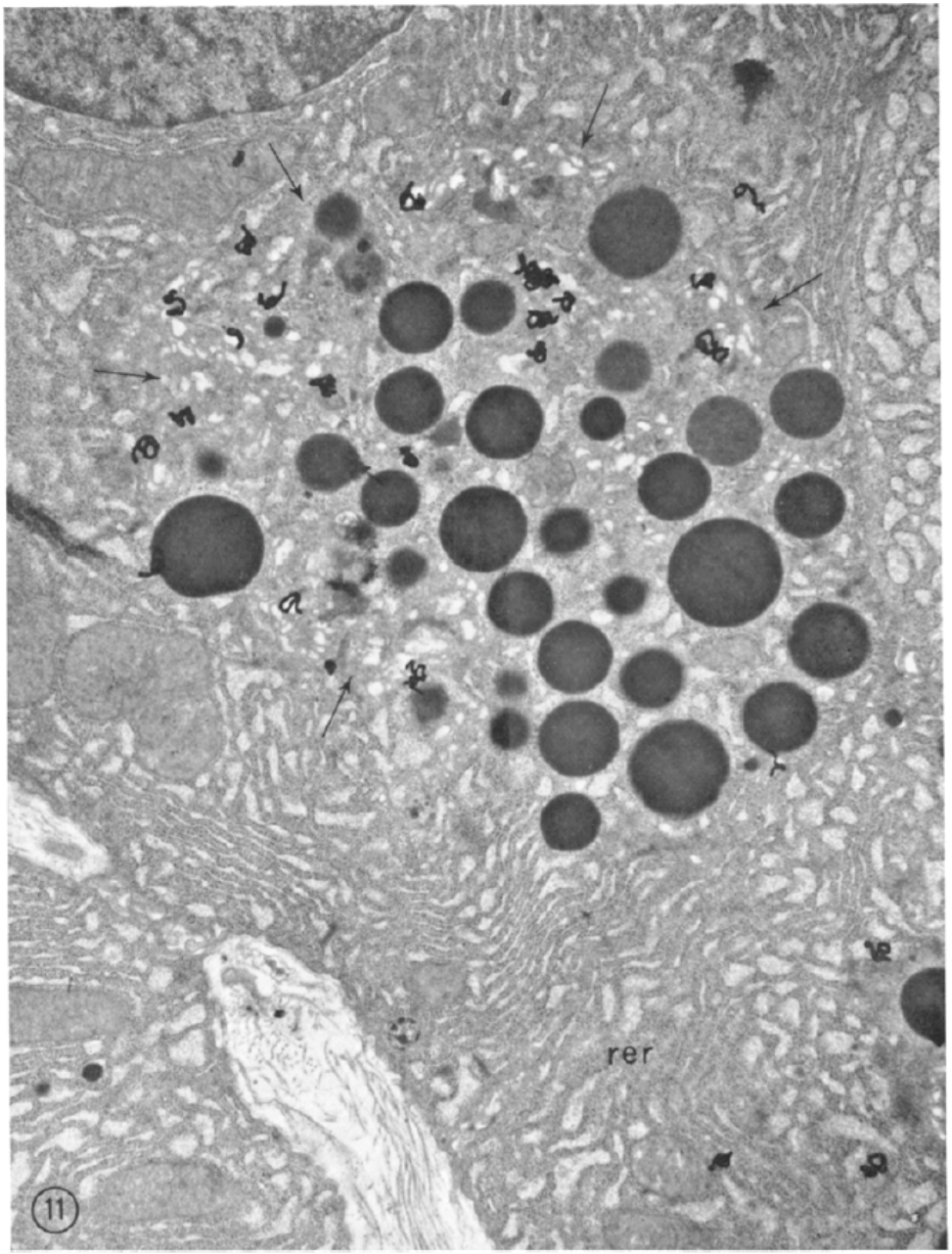
Jamieson and Palade (1967) JCB

Post-pulse: +7 minutes

7 minute chase.

Radioautographic grains located over Golgi complex.

rer=rough ER

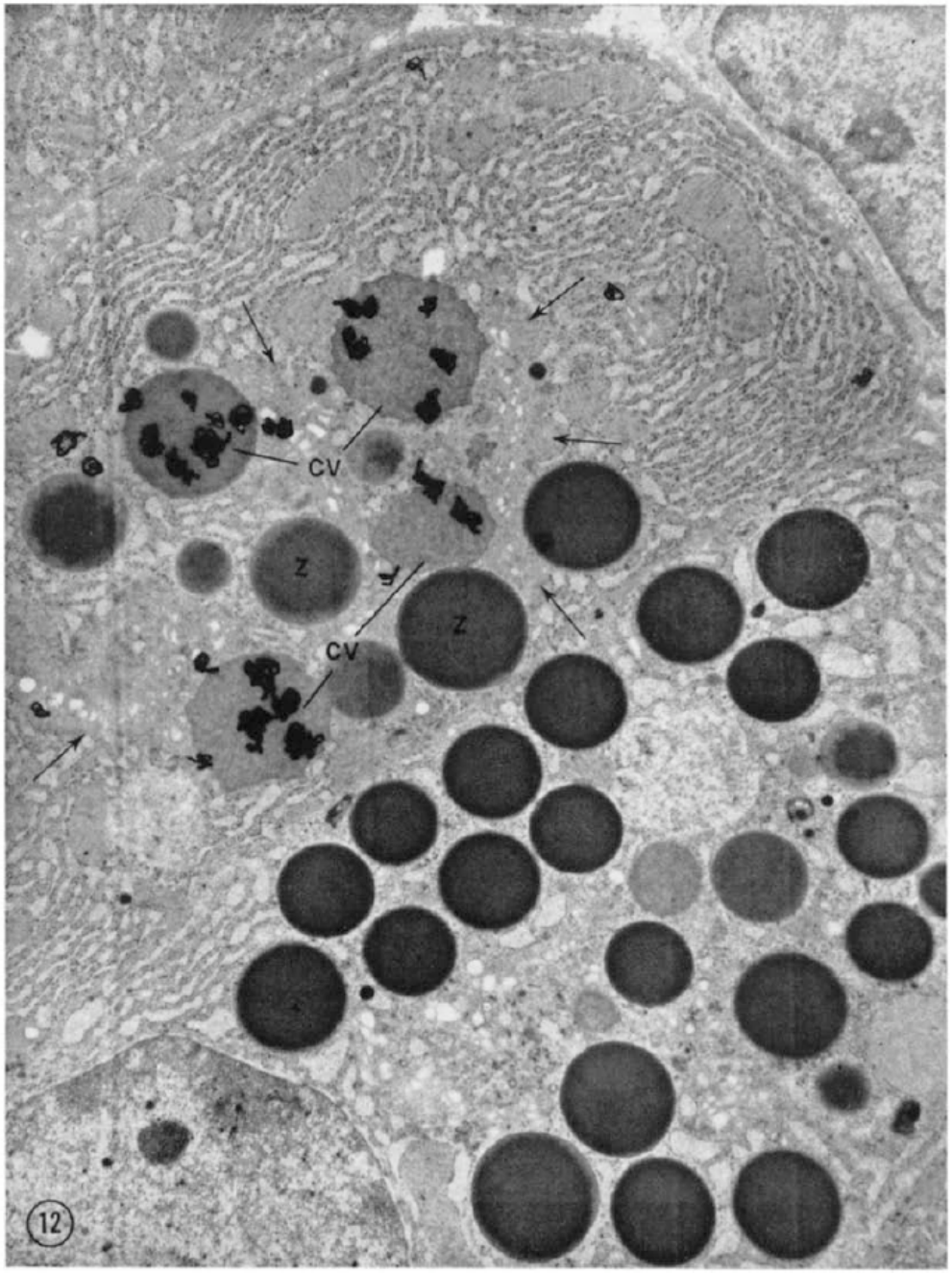


Jamieson and Palade (1967) JCB

Post pulse: +37 minutes

Radioautographic grains located over condensing vacuoles. Zymogen granules unlabeled.

cv=condensing vacuoles

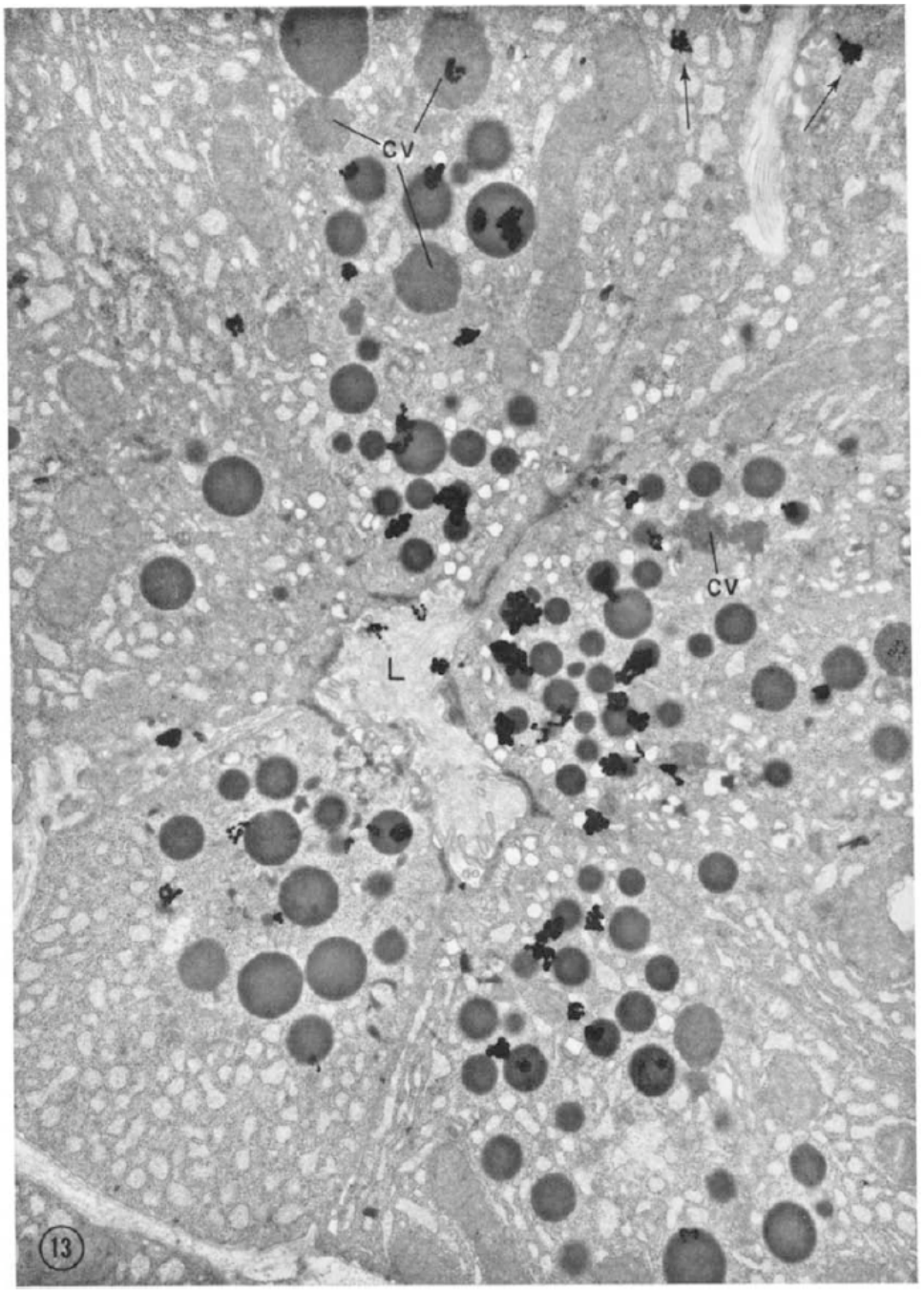


Jamieson and Palade (1967) JCB

Post-pulse: +117 minutes

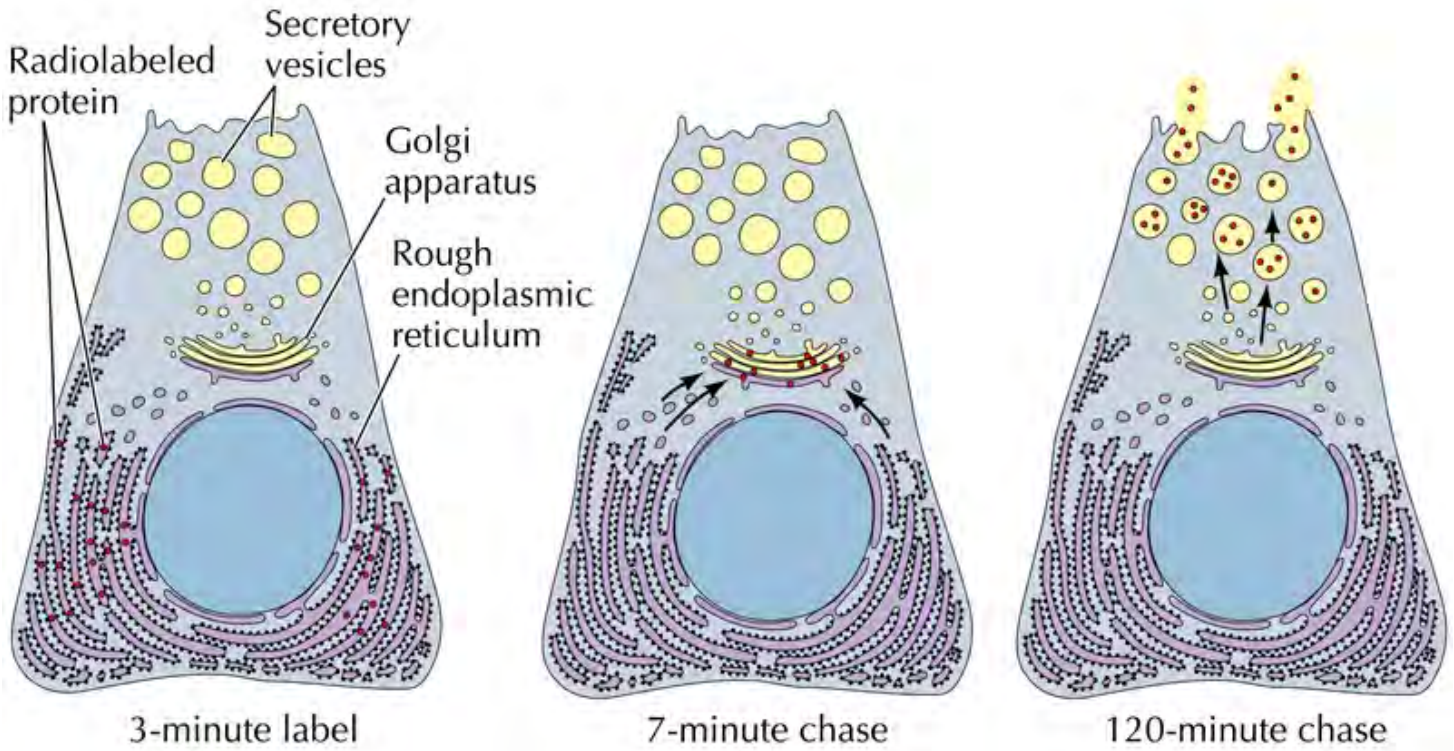
Radioautographic grains located over zymogen granules, not condensing vacuoles.

L=lumen
cv=condensing vacuoles



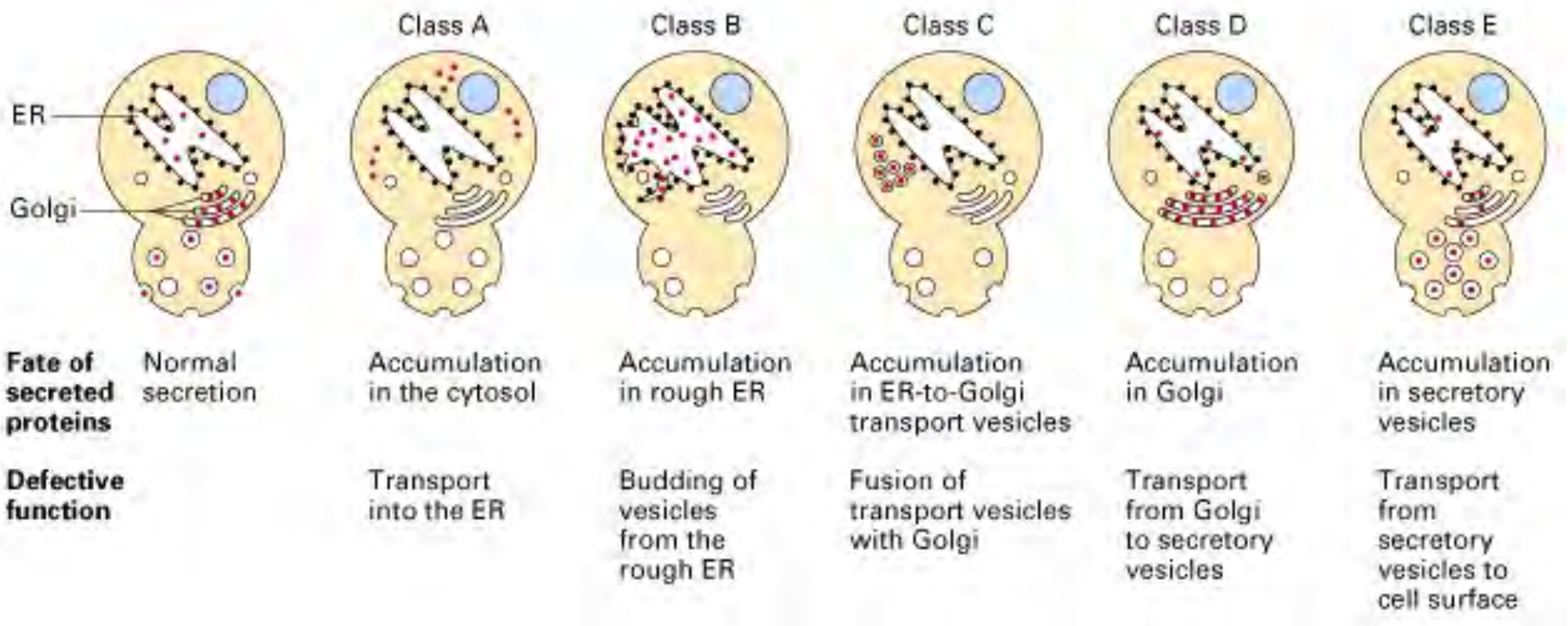
Jamieson and Palade (1967) JCB

Secretion pathway revealed by pulse-chase labeling of pancreatic acinar cells



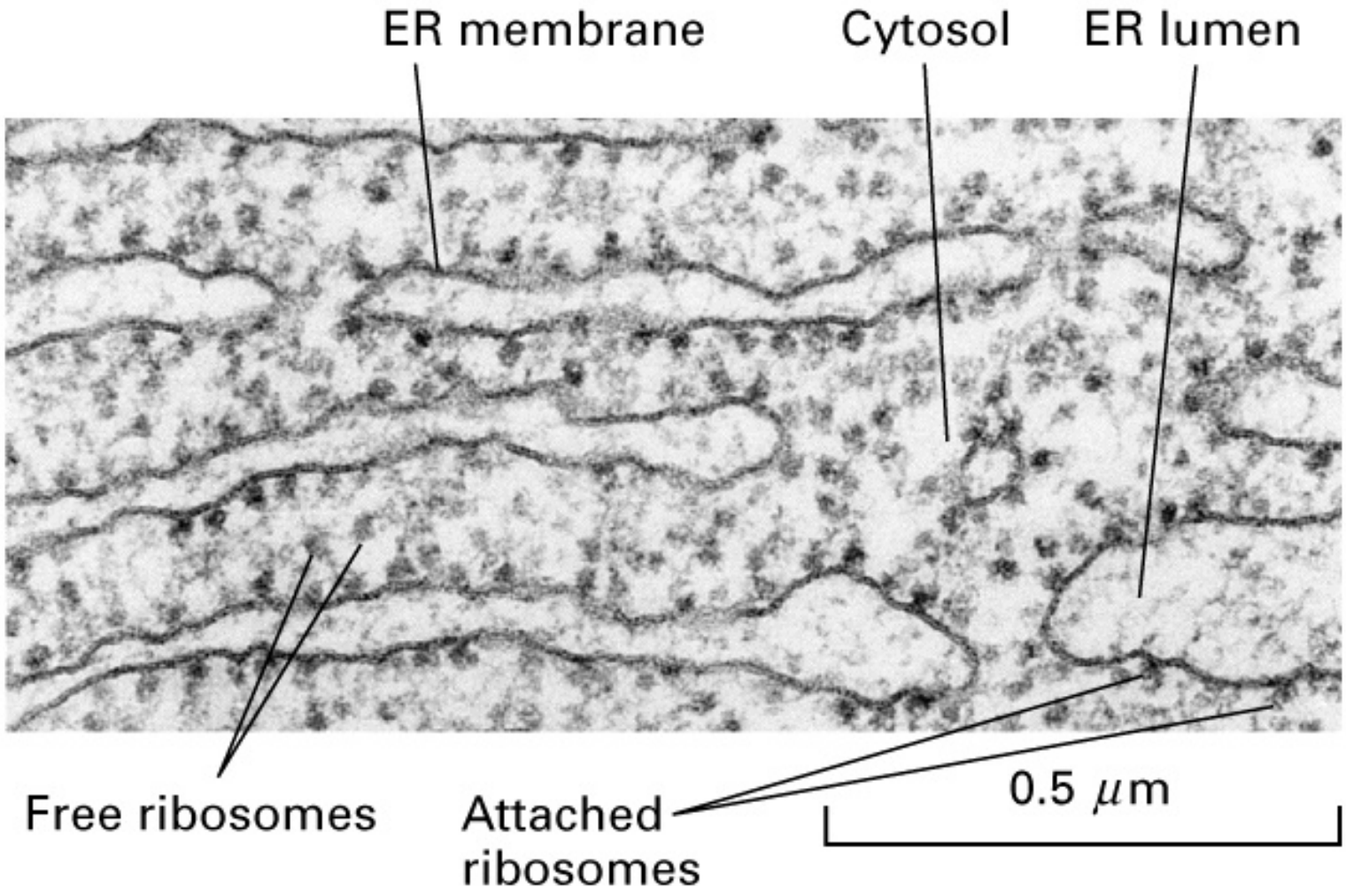
Cooper, 2000

Yeast secretion mutants can be classified and ordered by epistasis analysis



Lodish et al, 2000

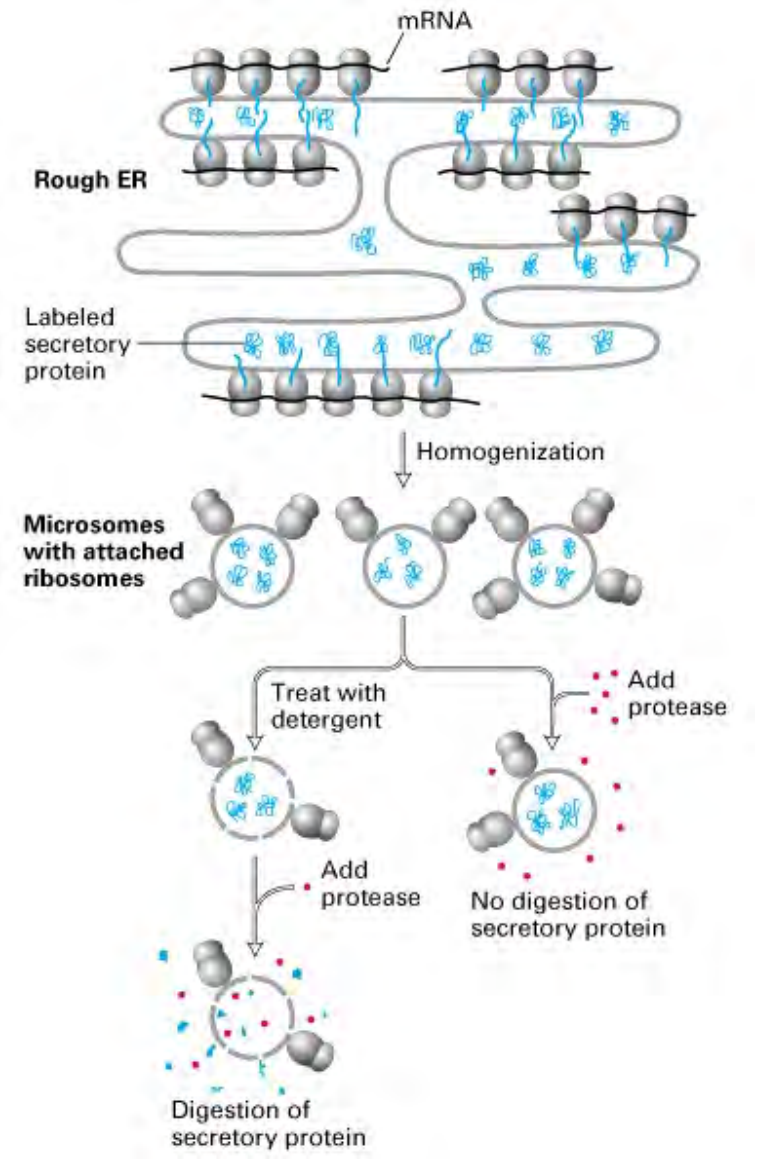
The rough ER is an extensive interconnected series of flattened sacs



Lodish et al, 2000

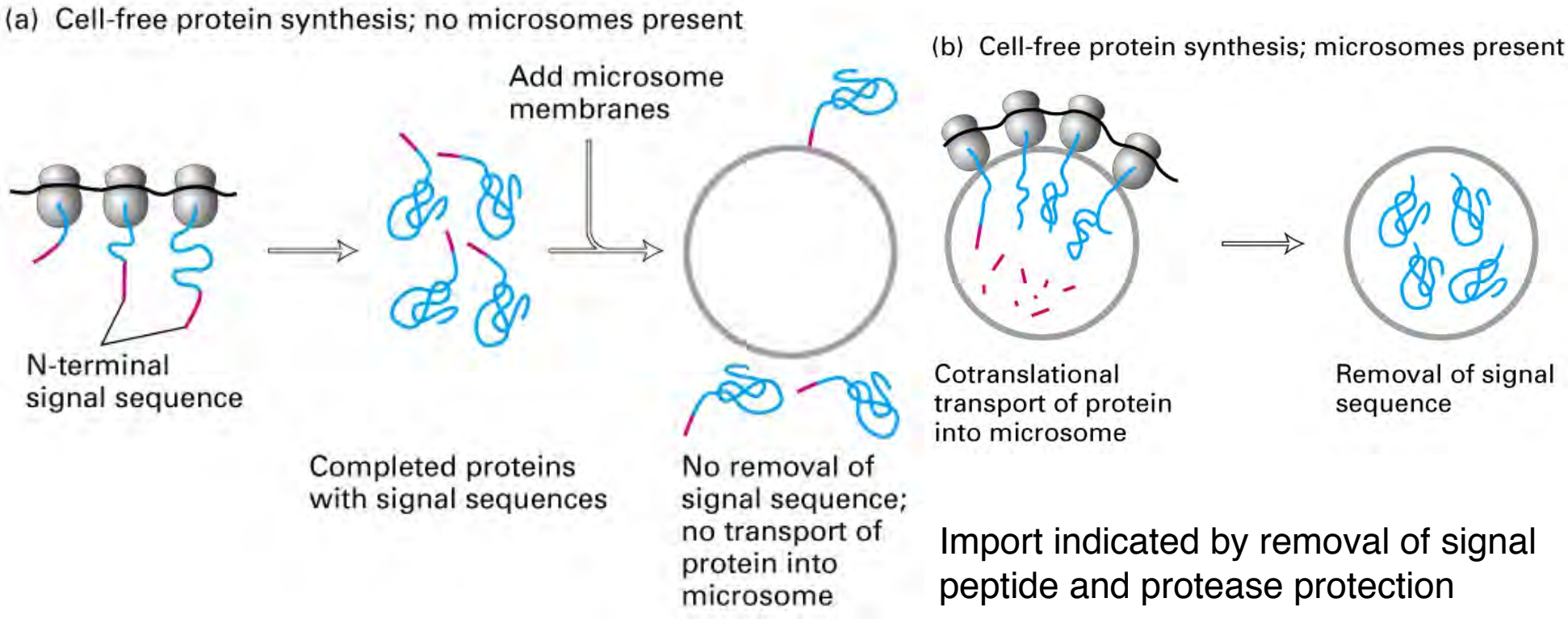
Secretory proteins are found in the ER lumen immediately after synthesis

Microsome lumen
equivalent to ER lumen



Lodish et al, 2000

Microsome in vitro assay: most eukaryotic proteins are imported cotranslationally and the signal peptide cleaved



Import indicated by removal of signal peptide and protease protection

Lodish et al, 2000

Signal sequences of nascent secretory proteins

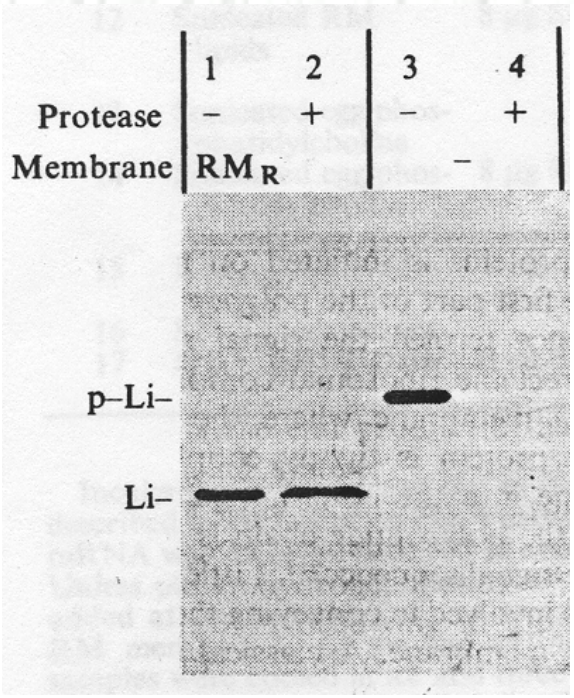
TABLE 17-4 Amino Acid Sequences of ER Signal Peptides in Three Eukaryotic Proteins

Protein	Amino Acid Sequence*
Preproalbumin	**Met-Lys-Trp-Val-Thr-Phe-Leu-Leu-Leu-Leu-Phe-Ile-Ser-Gly-Ser-Ala-Phe-Ser** ↓ Arg . . .
Pre-IgG light chain	**Met-Asp-Met-Arg-Ala-Pro-Ala-Gln-Ile-Phe-Gly-Phe-Leu-Leu-Leu-Leu-Phe-Pro-Gly-**Thr-Arg-Cys ↓ Asp . . .
Prelysozyme	**Met-Arg-Ser-Leu-Leu-Ile-Leu-Val-Leu-Cys-Phe-Leu-Pro-Leu-Ala-Ala-Leu-Gly** ↓ Lys . . .

**Hydrophobic residues are in boldface; arrows (↓) indicate the site of cleavage by signal peptidase.*

SOURCE: D. P. Leader, 1979, *Trends Biochem. Sci.* **4**:205 and T. A. Rapoport, 1985, *Curr. Topics Membrane Transport* **24**:1.

- Precursor protein has a leader sequence that is cleaved on import
- Import protects mature protein from proteolysis



RM_R: RNAse-treated rough microsomes

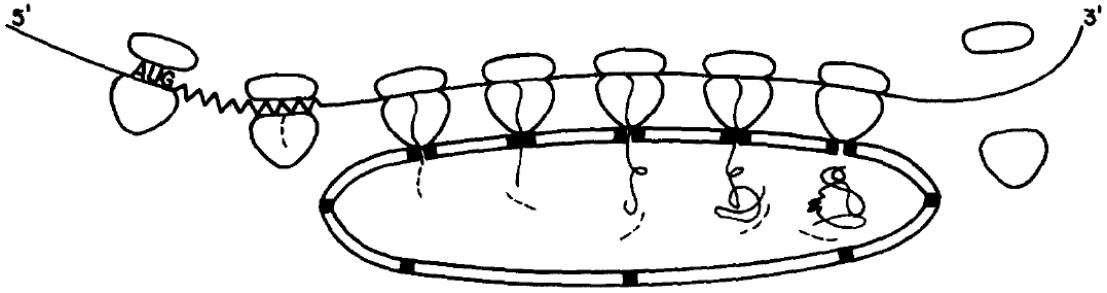
pLi=light chain precursor

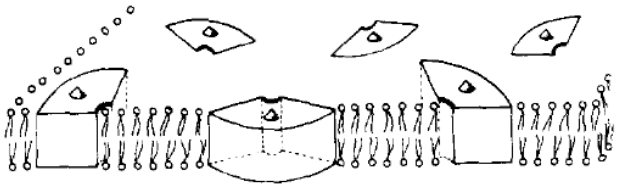
Li=mature light chain

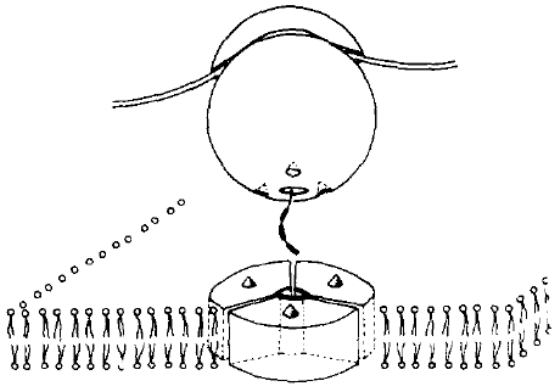
Warren & Dobberstein

(1978) Nature 273:569

Signal hypothesis





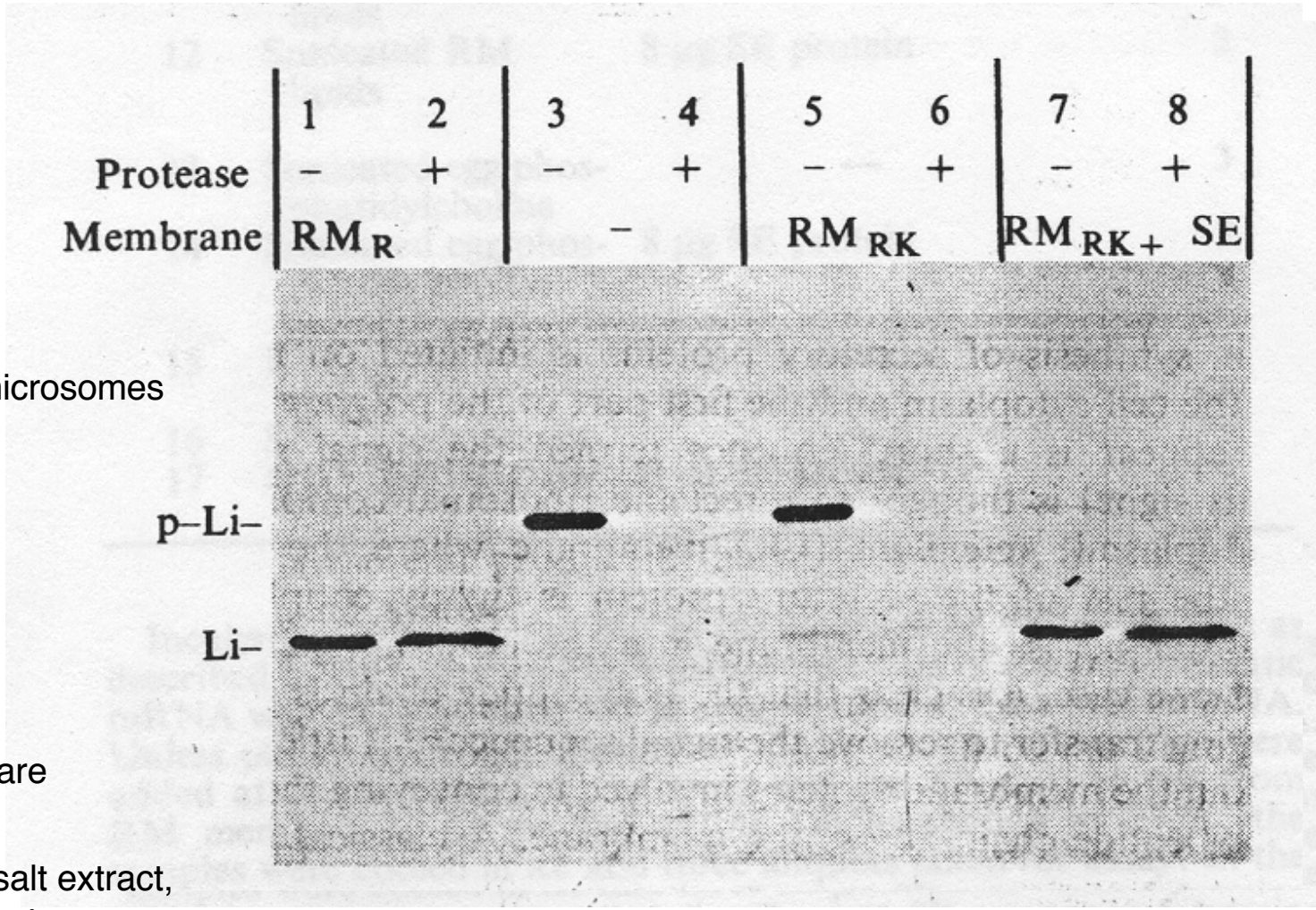


Based on proteolytic processing of immunoglobulin light during co-translational import into microsomes.

1) Translation of all mRNAs begins on free ribosomes
2) Nascent chains containing a signal peptide trigger attachment of the ribosome to the ER membrane.
3) Signal peptide triggers formation of a hypothetical transient tunnel made of protein. Polypeptide chain threaded vectorially into ER. Signal peptide is cleaved. After completion of translation, tunnel dissociates.

From Dobberstein and Blobel (1975) JCB; also see Blobel and Sabtini (1971) Biomembranes.

Reconstitution of microsomal translocation led to SRP identification



RM_R=RNAse-treated rough microsomes

RM_{RK}=0.5M KCl treated

SE= salt extract

Salt extract contains SRP

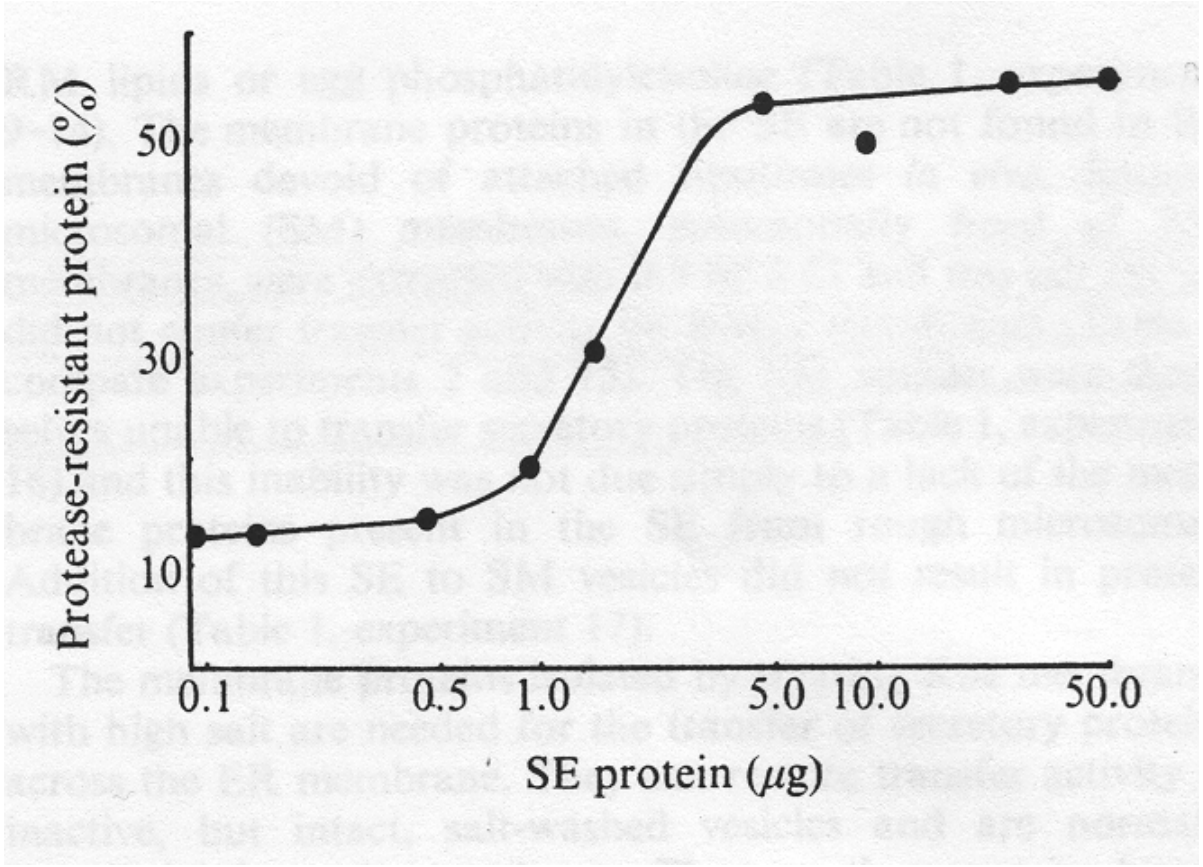
A. Salt treated microsomes are nonfunctional

B. They can be rescued by salt extract, providing a purification strategy.

A B

Warren & Dobberstein (1978) Nature 273:569

SRP activity is saturable

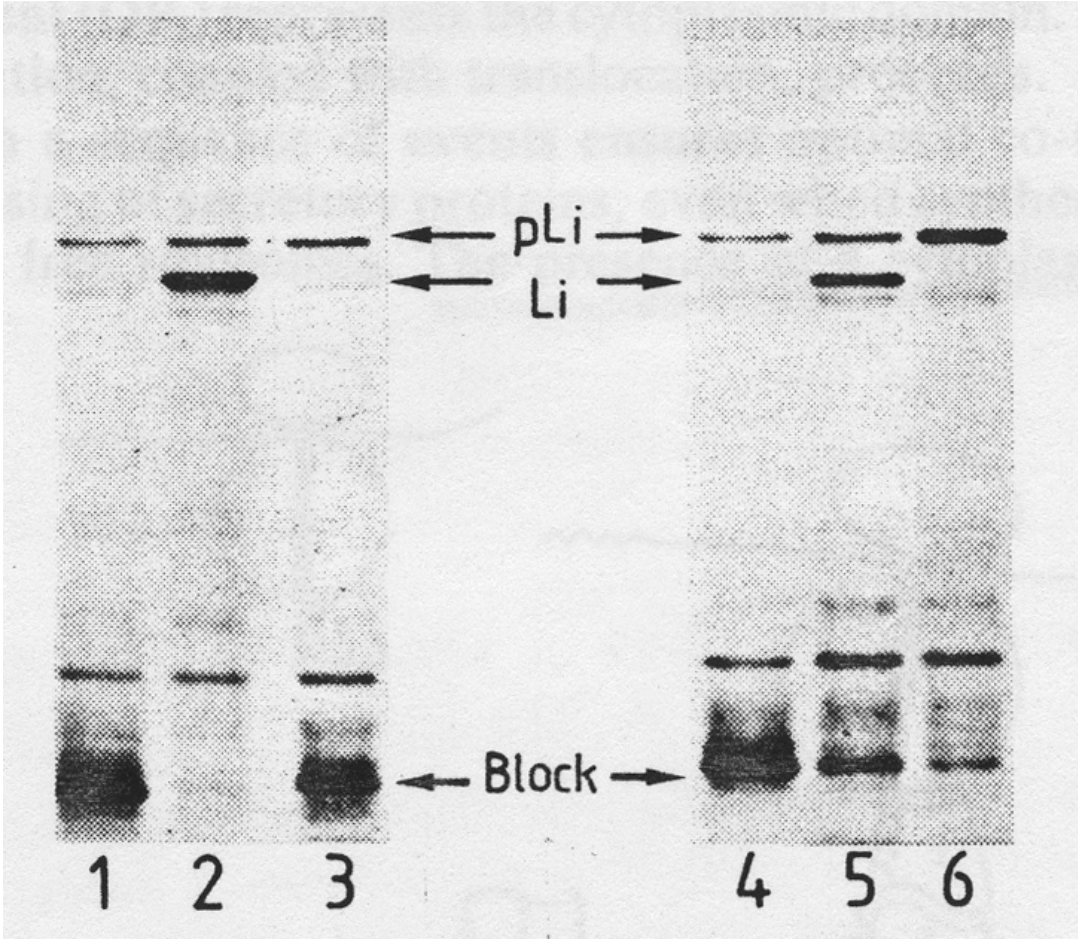


Warren & Dobberstein (1978) Nature 273:569

SRP mediates translational arrest;

SRP receptor (DP_f) relieves arrest

1. SRP	translational arrest
2. SRP, RM_K	relief
3. SRP, RM_{EK}	no relief
4.same as 1	
5. SRP, RM_{EK}, DP_f	relief*
6. SRP, DP_f	relief, no transloc



pLi=light chain precursor

Li=light chain

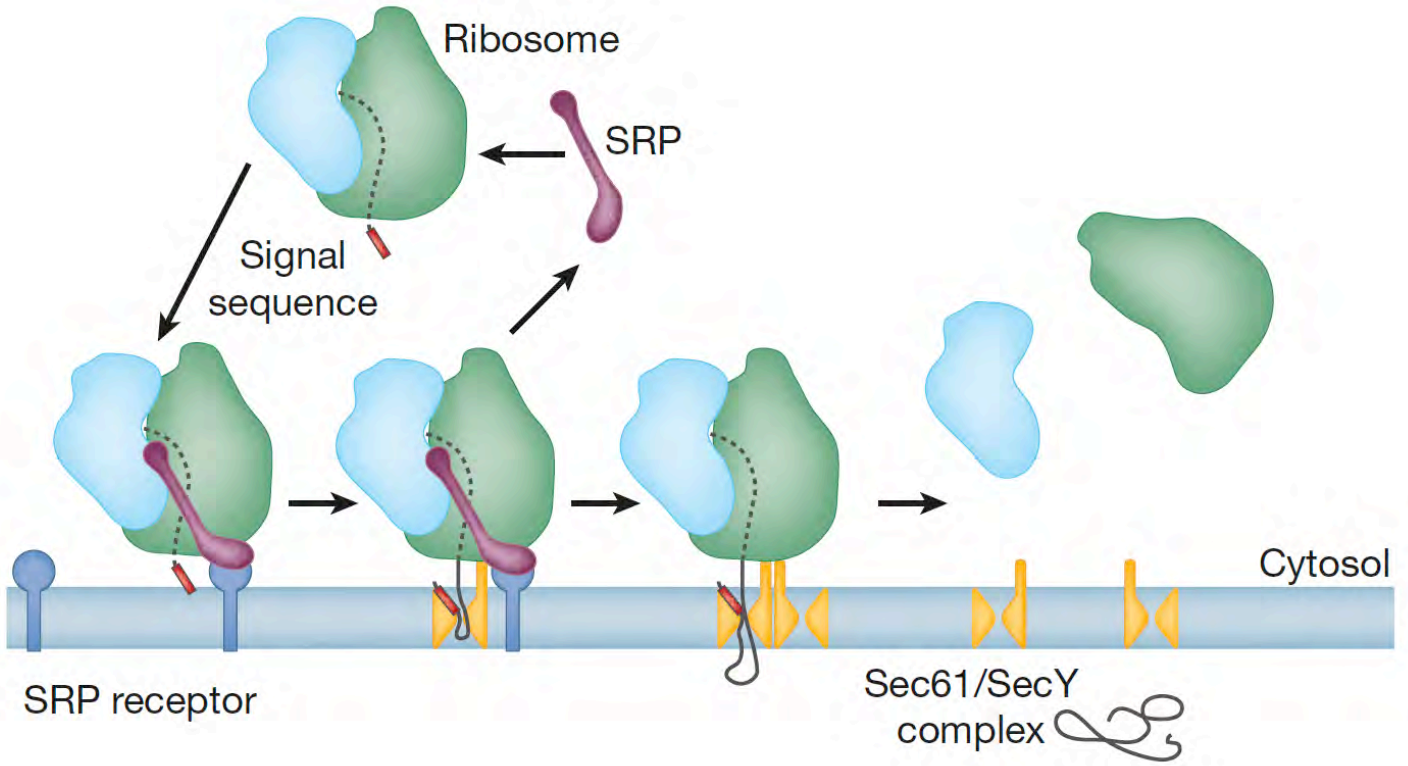
RM_{EK}= KCl/elastase-treated

DP_f=docking protein fragment,
a 60 kD cytoplasmic domain

Meyer et al (1982). Nature 297:647

Co-translational import into the ER

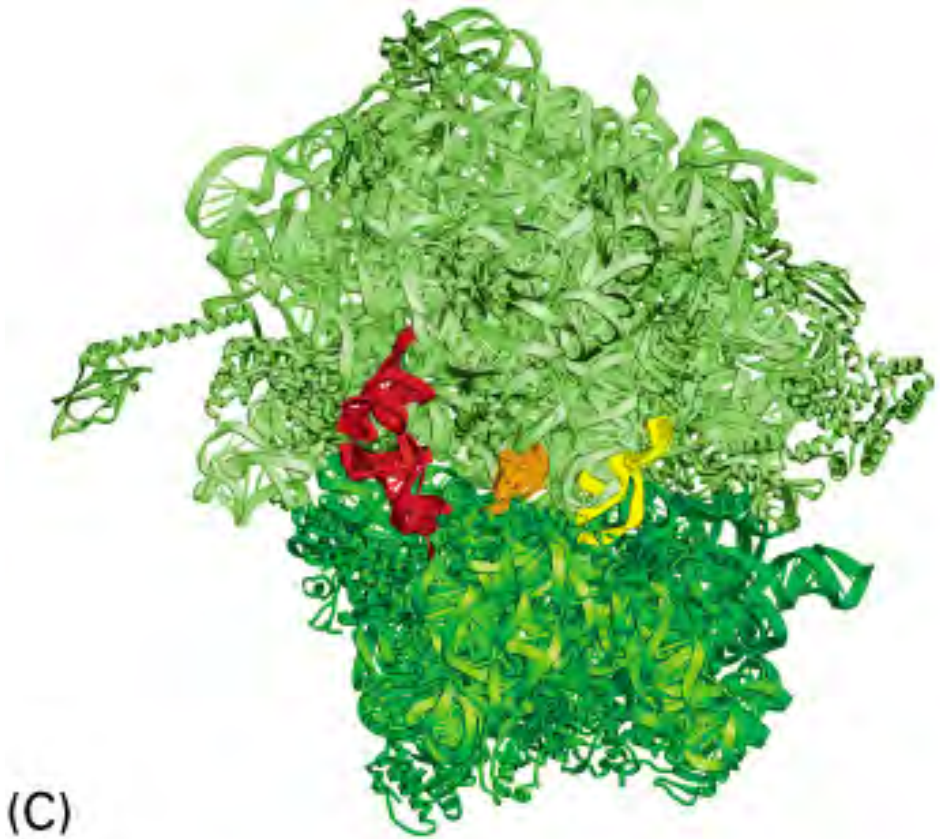
- signal sequence recognized by SRP
- translational arrest
- targeting to membrane: SRP-SRP receptor; then ribosome and Sec61
- translational elongation: exiting peptide goes through channel



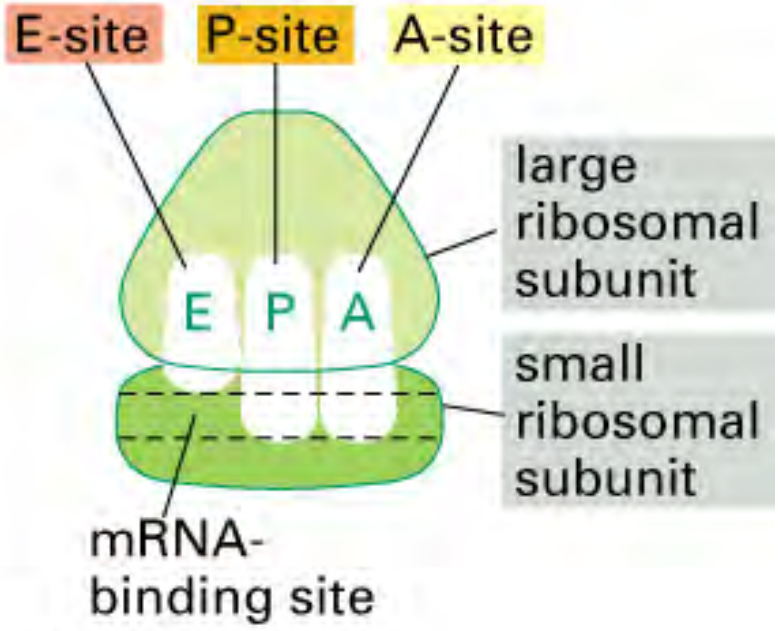
Rapoport (2007) Nature

3-site model of ribosome

E (exit), P (peptidyl), A (aminoacyl)



(C)



(D)

Figure 6–64 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

3-site model of elongation

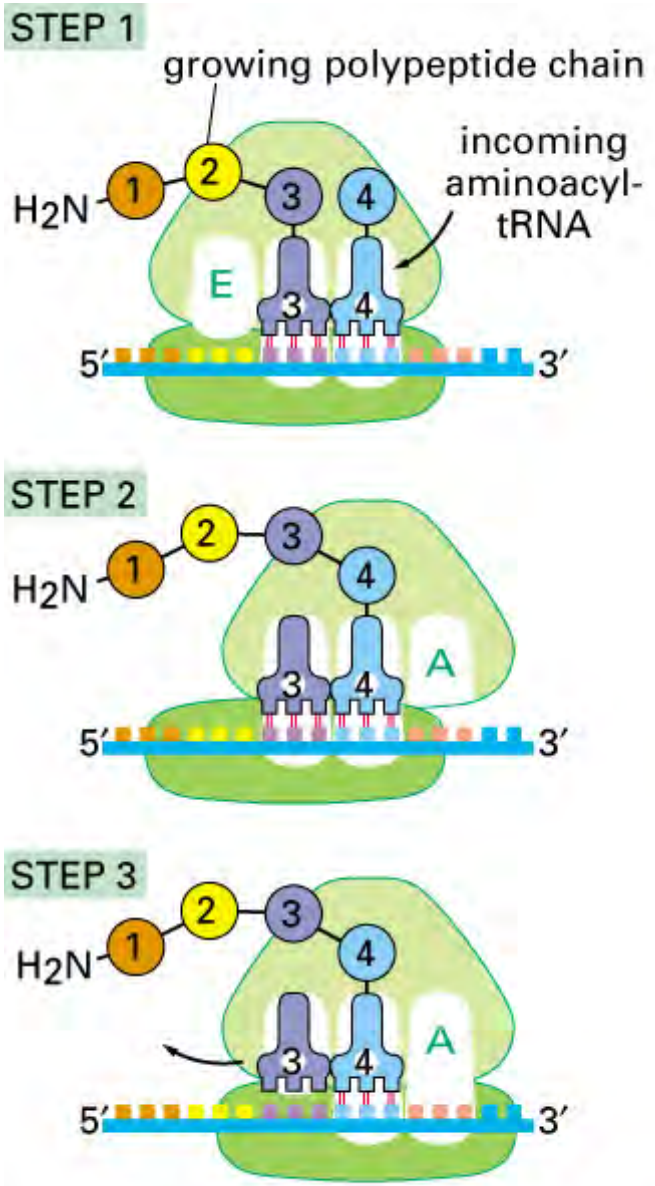


Figure 6–65 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

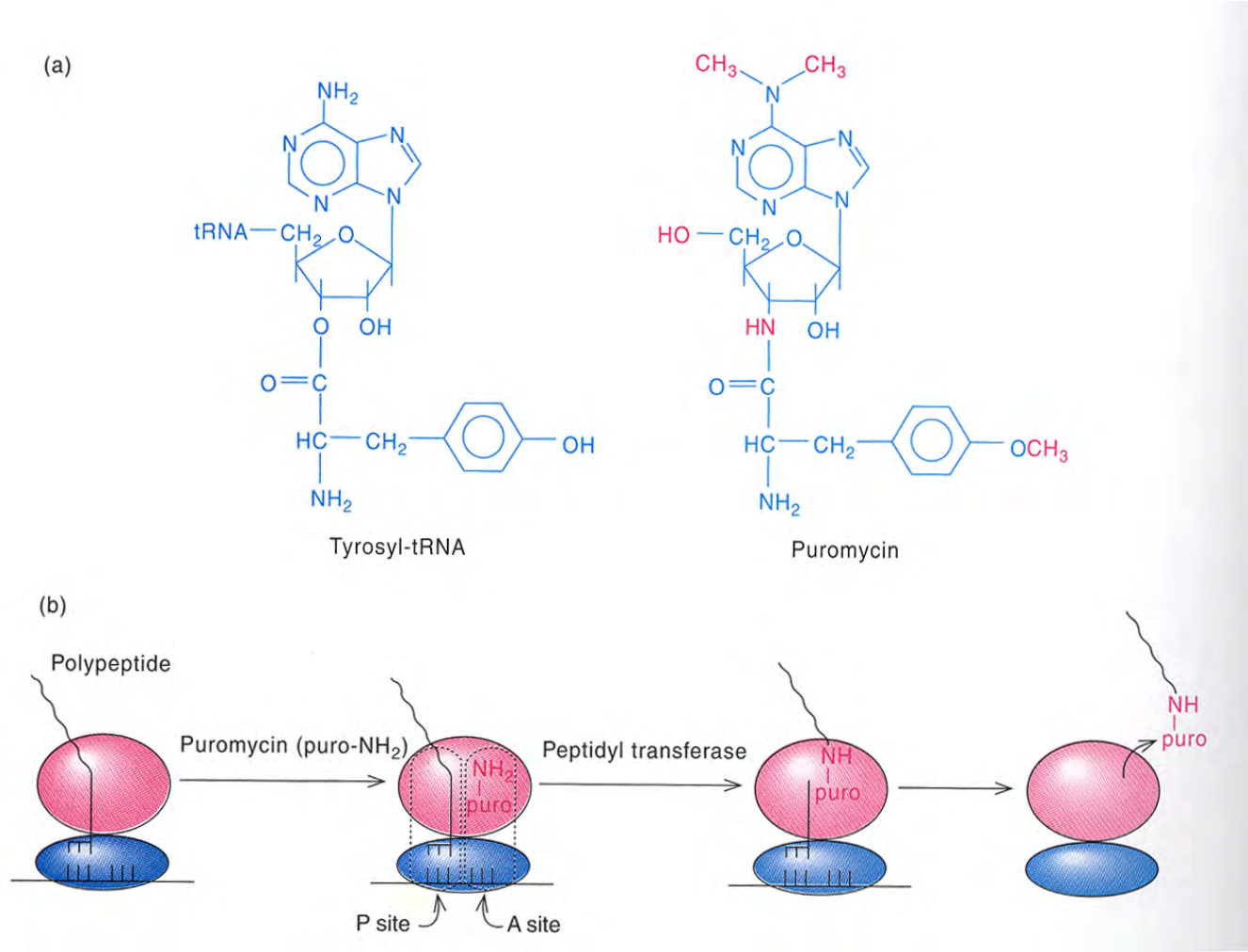
A (aminoacyl), P (peptidyl) , E (exit) sites

A) Aminoacyl tRNA (determined by anticodon) binds to vacant A site

B) Large subunit catalyzes peptide bond, transferring polypeptide chain from its tRNA in P site to the aminoacyl tRNA in the A site; P site now contains deacylated tRNA

C) Translocation: mRNA moves one codon; the attached peptidyl-tRNA in A site moves to P site; the deacylated tRNA moves to E site, where it leaves ribosome

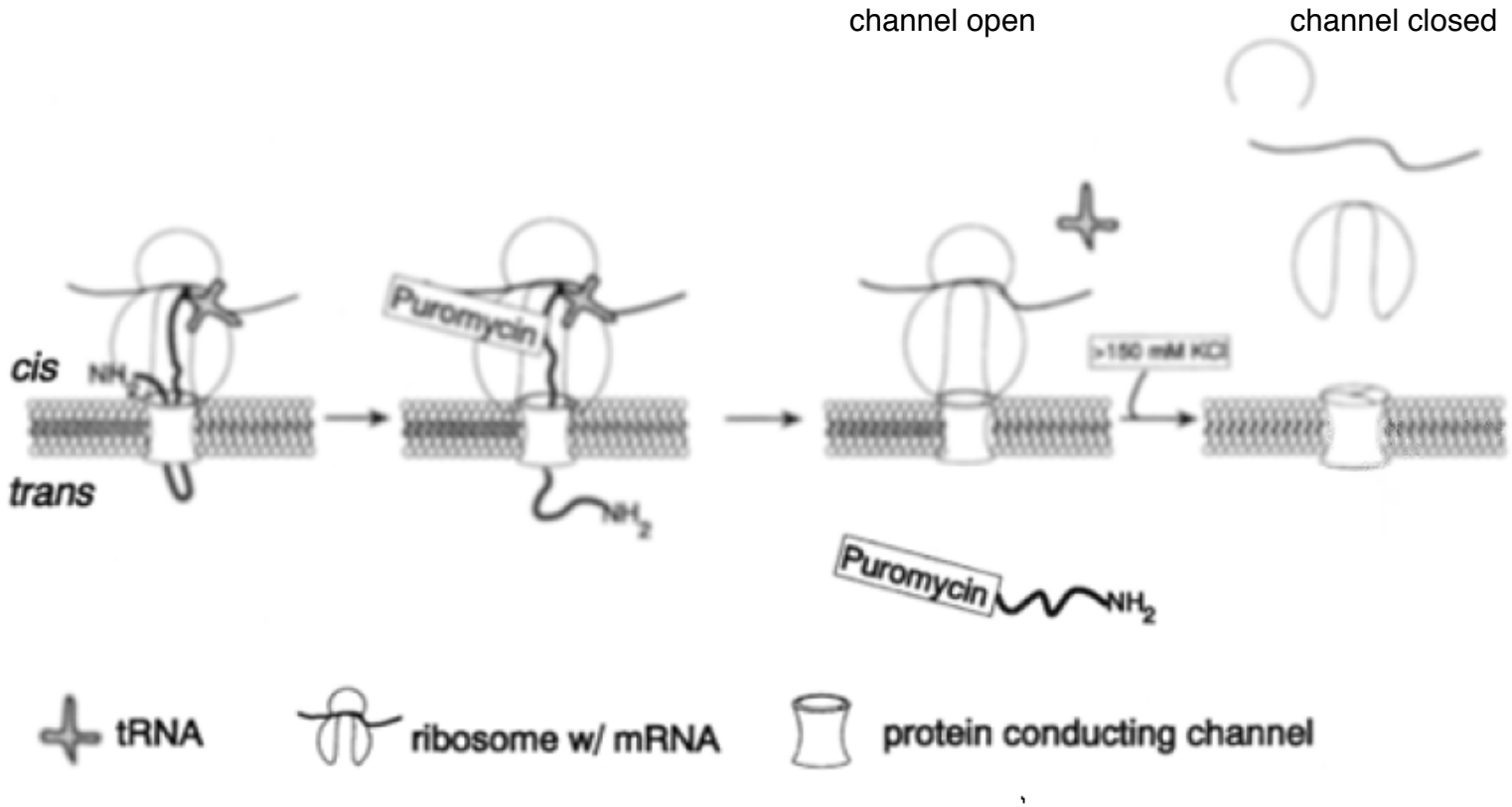
Puromycin is an analog of aminoacyl-tRNA



- Puromycin: antibiotic that inhibits protein synthesis.
- Recognized by ribosome as an incoming aminoacyl-tRNA.
- Polypeptide is transferred to NH2 group of puromycin.
- The polypeptide is then released from the ribosome.
- During normal translation, release of polypeptide occurs at stop codons and is mediated by release factors (RFs).

Weaver (1999) Molecular Biology

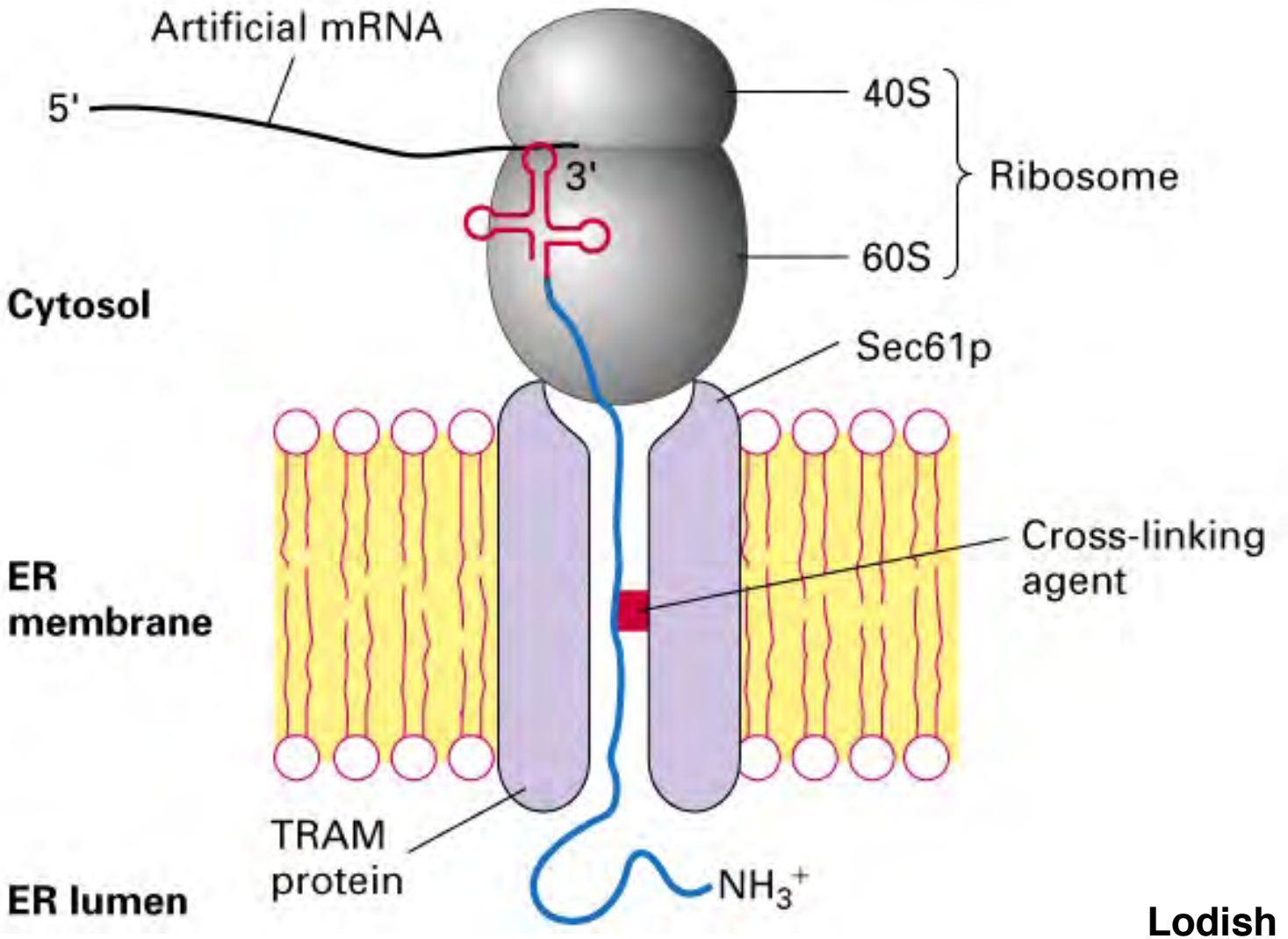
Model for puromycin-mediated opening of ER channel



- During translocation, the Sec61 channel remains impermeable to ions.
- During translational termination, ribosome subunits dissociate.
- Artifical condition: Puromycin + experimental conditions release polypeptide but prevent ribosome dissociation.

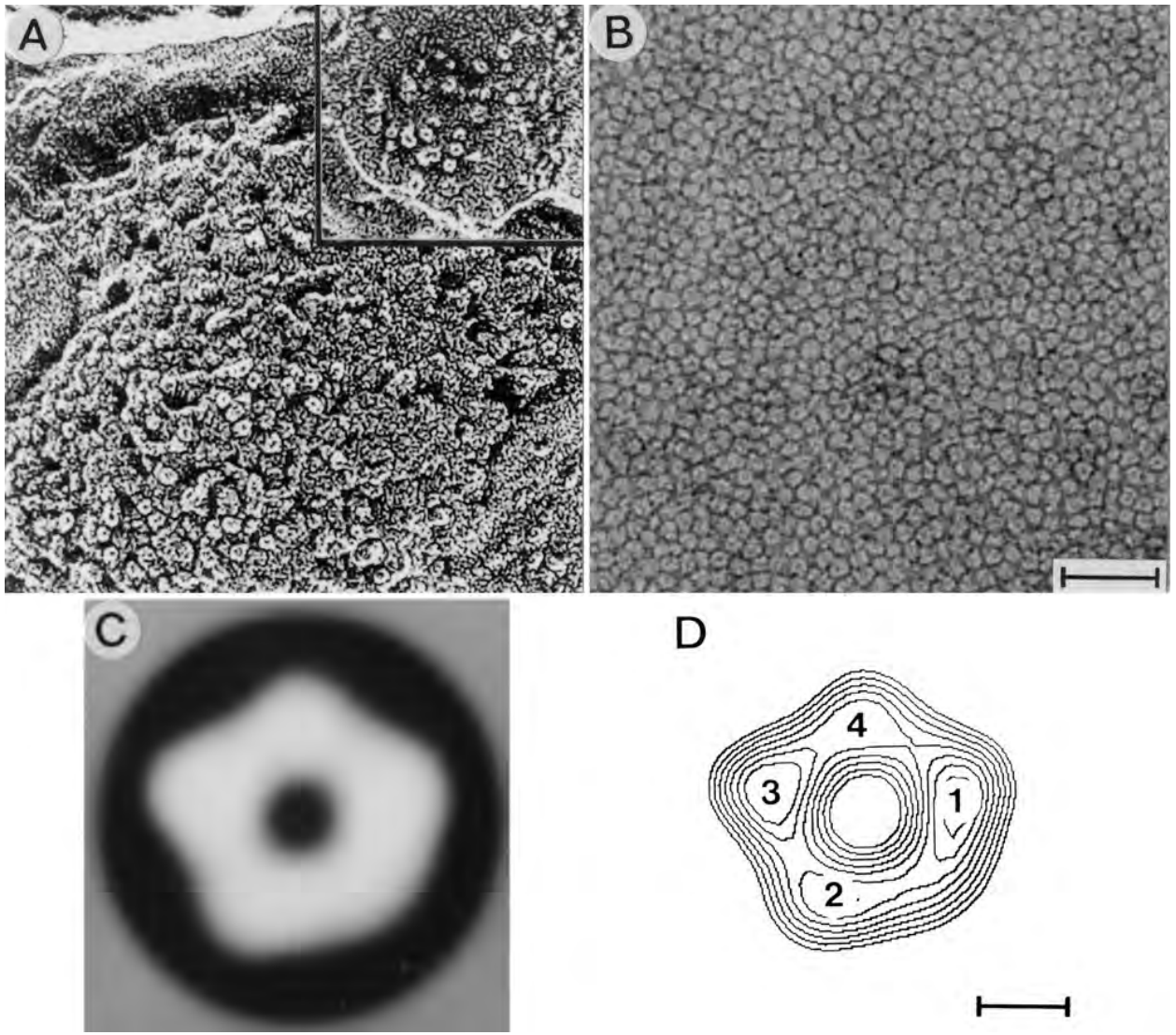
Simon and Blobel (1991) Cell

Polypeptides move through the translocon into the ER lumen



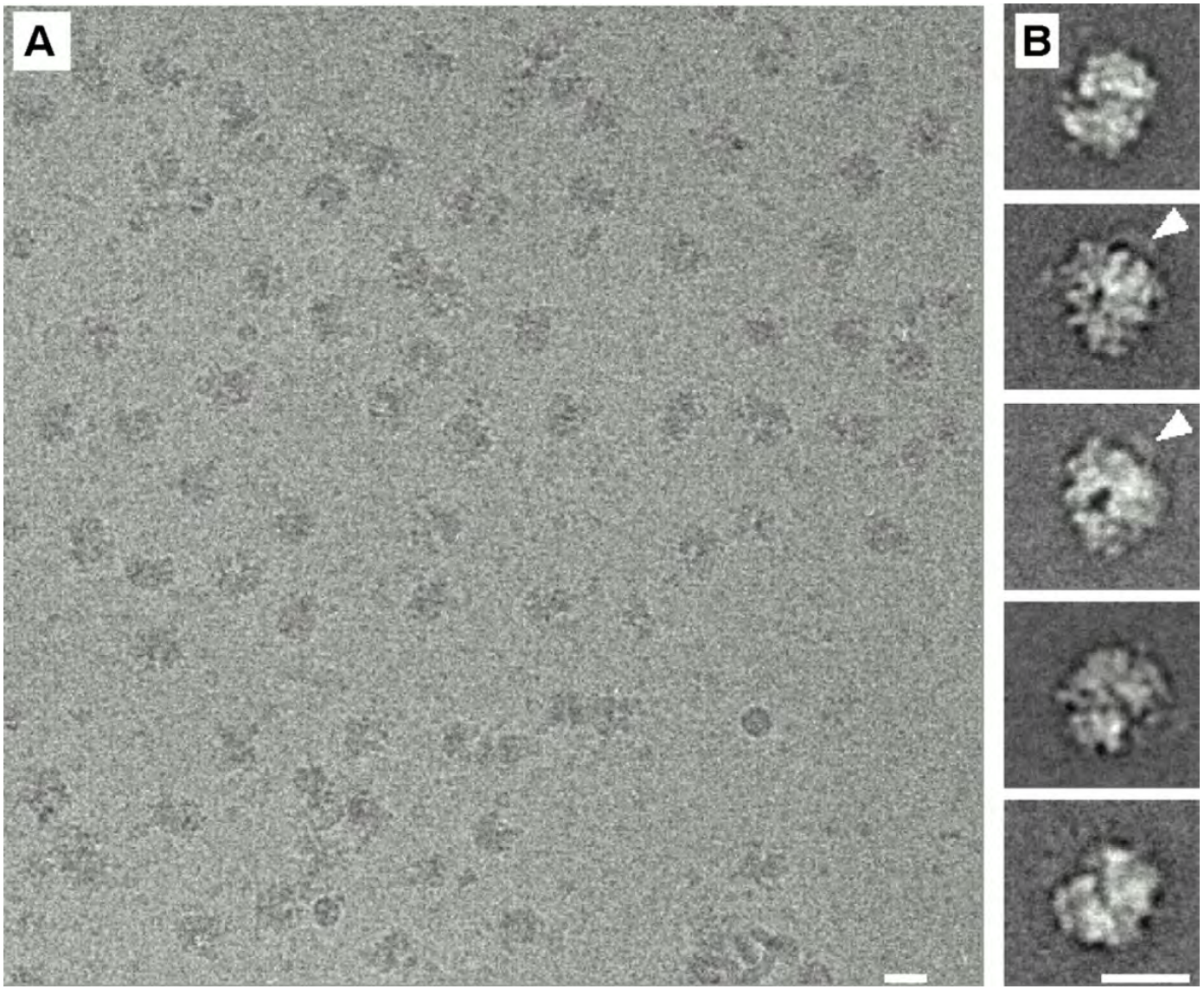
Lodish et al, 2000

Sec61 forms a channel



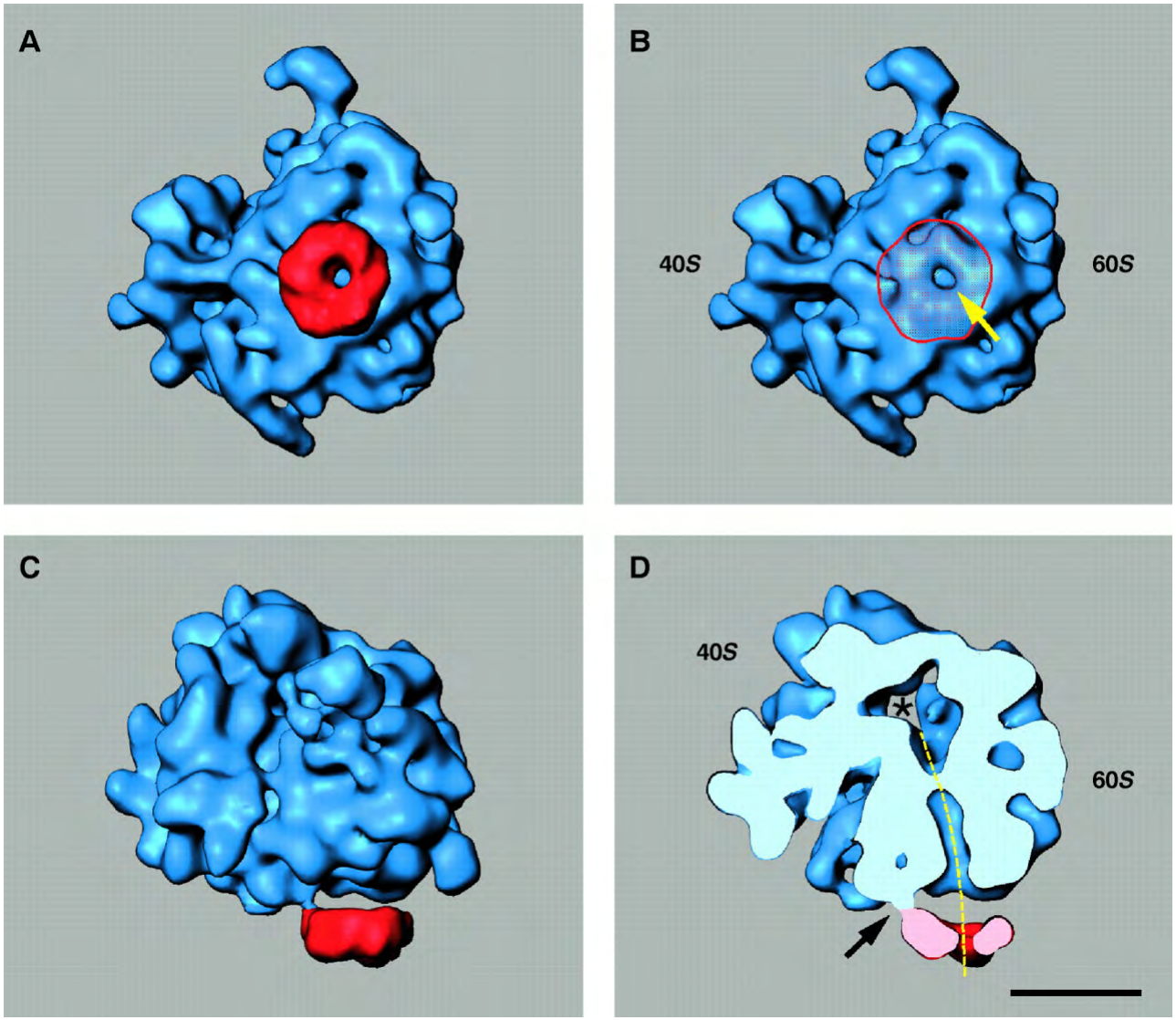
Matlack et al (1998) Cell 92:381

Cryo-EM images of ribosomes complexed with Sec61



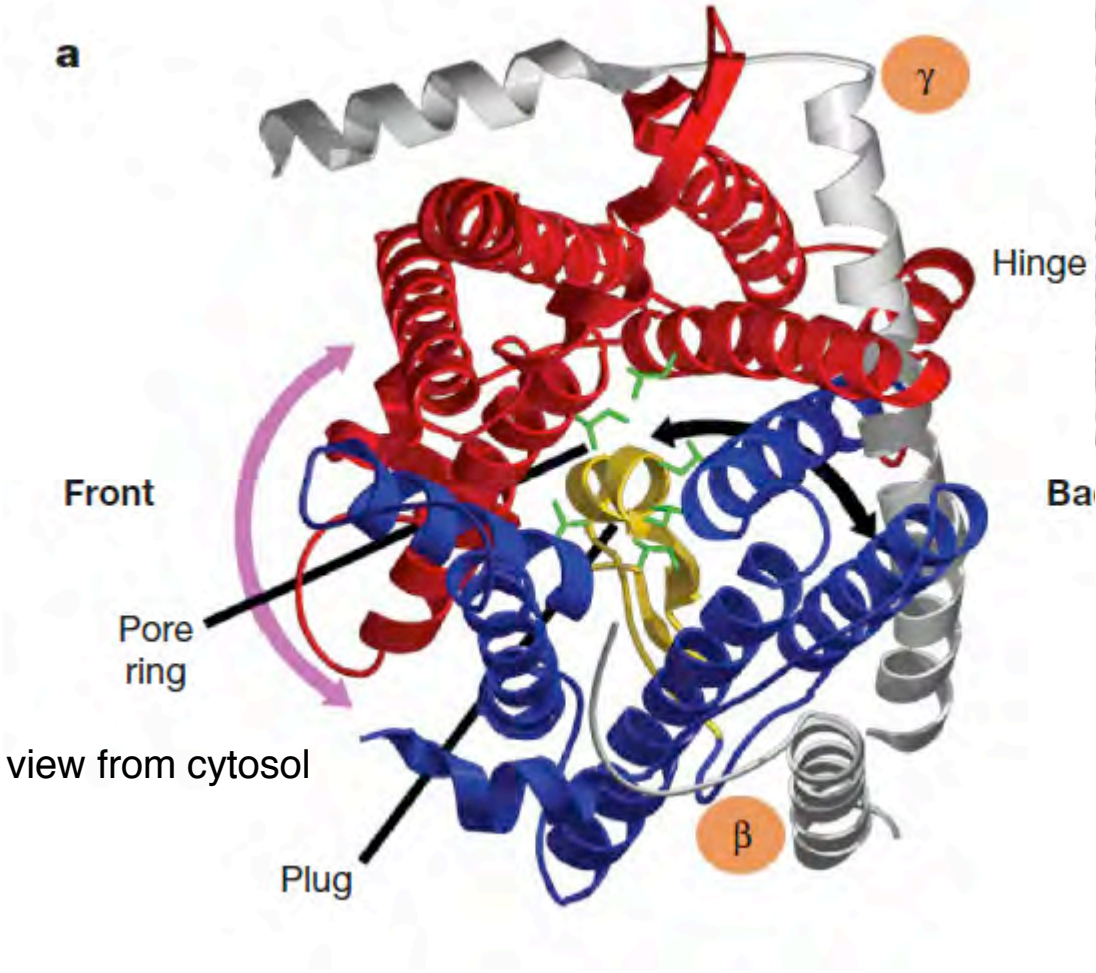
Beckmann et al. (1997) Science 278: 2123.

Reconstructed Cryo-EM structure of ribosome complexed with Sec61

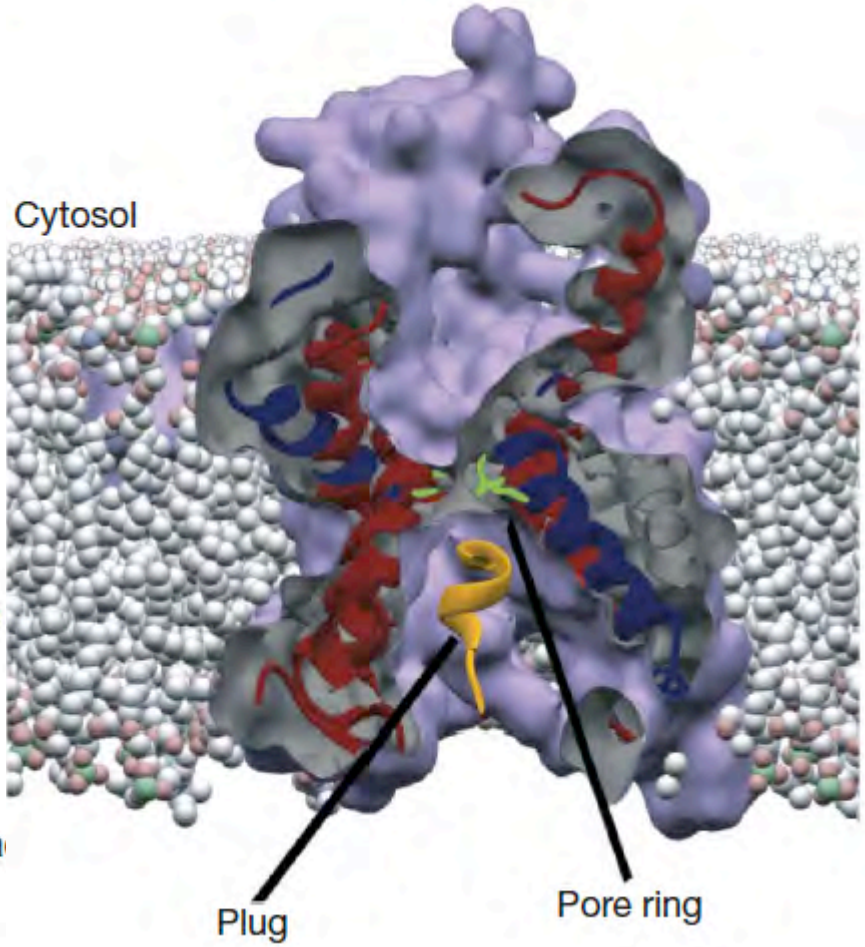


Beckmann et al. (1997) Science 278: 2123.

X-ray structure of translocation channel SecY



view from cytosol

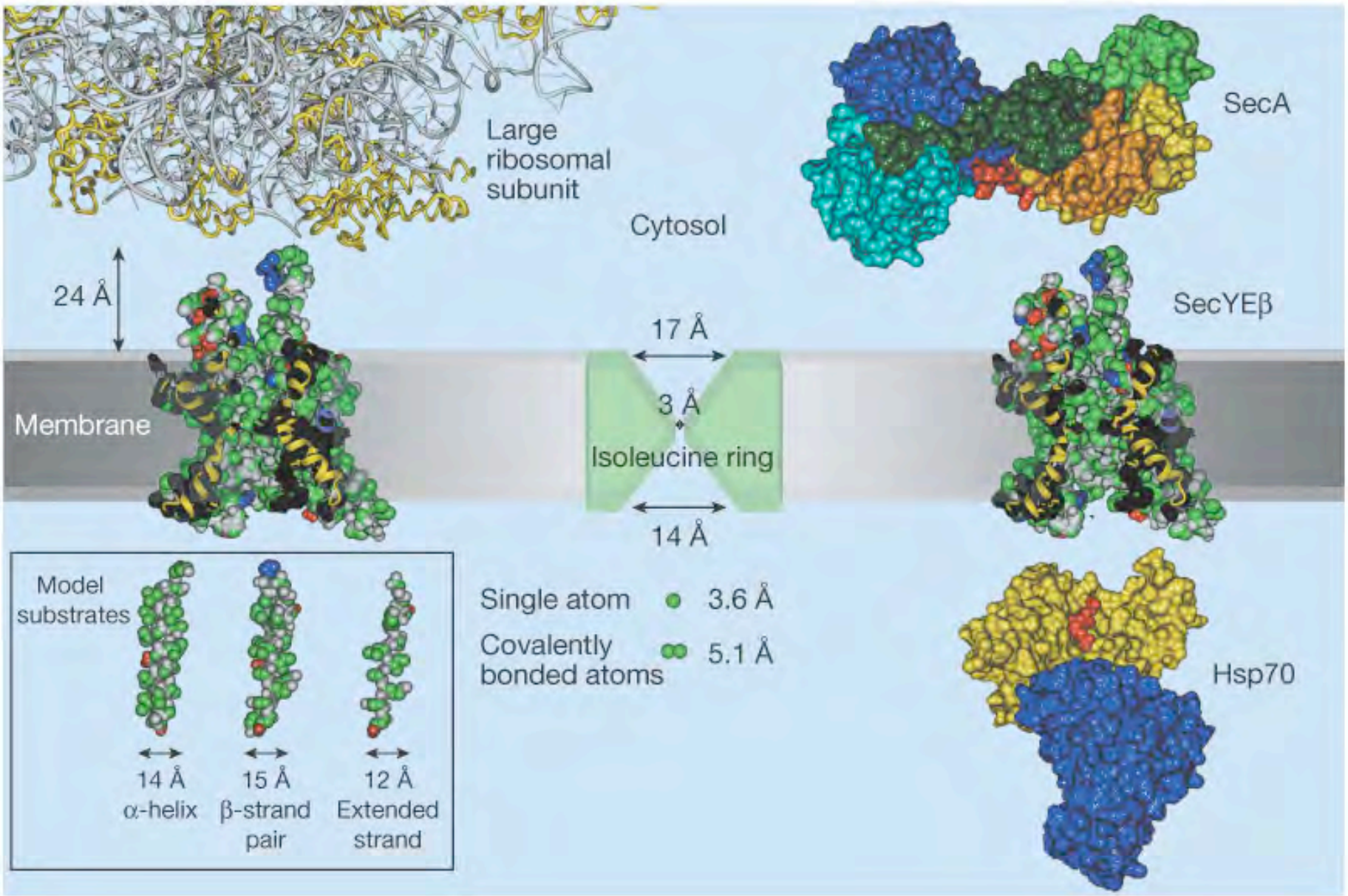


In archae, the polypeptide (post-translationally) moves from cytosolic funnel, through pore ring, into external funnel (which contains a plug in the presumed closed conformation.

10 helices form hourglass pore.

Rapoport (2007) Nature

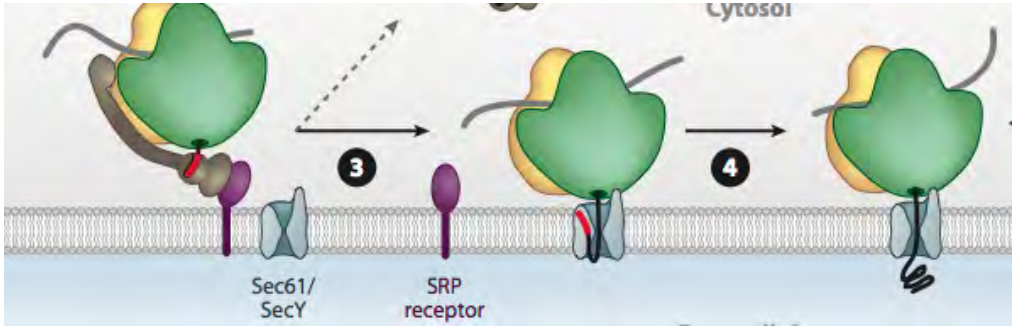
Space consideration of translocation channel SecY



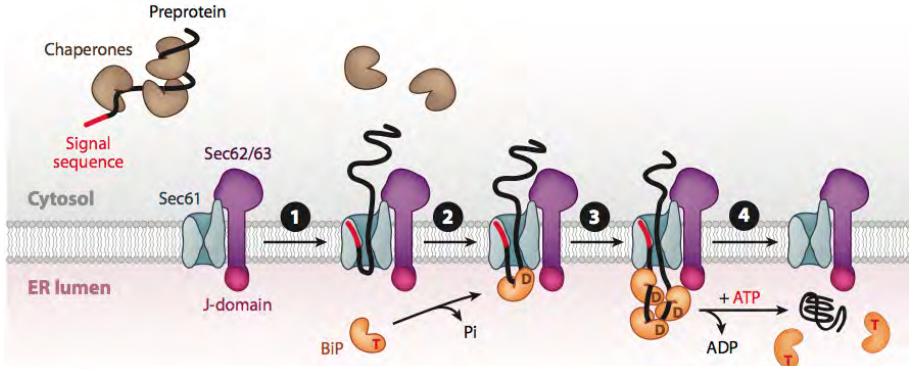
Benach and Hunt (2004) Nature

Mechanisms to get proteins through the channel

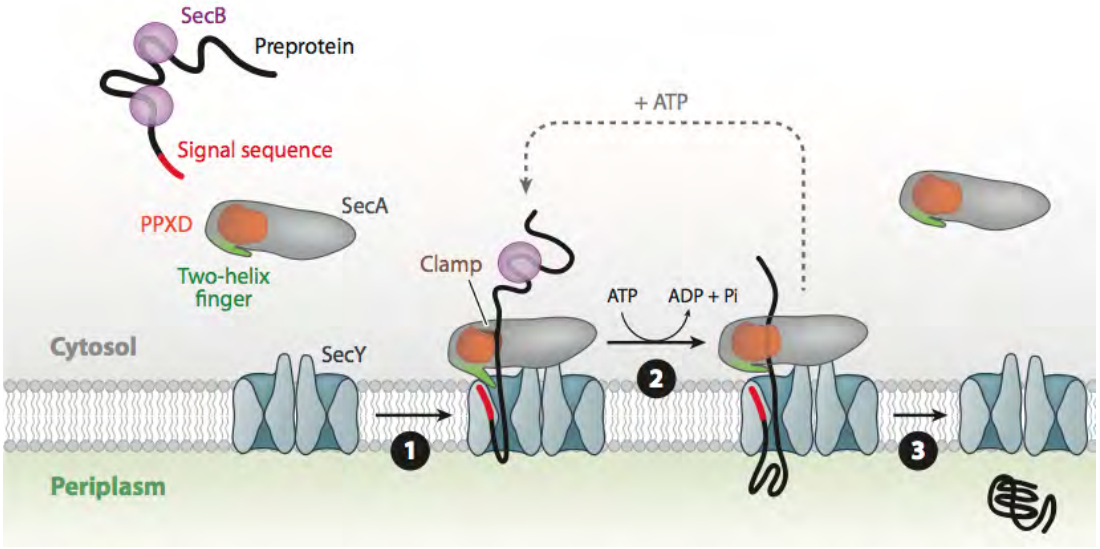
• Eukaryotic, co-translational: polypeptide elongation



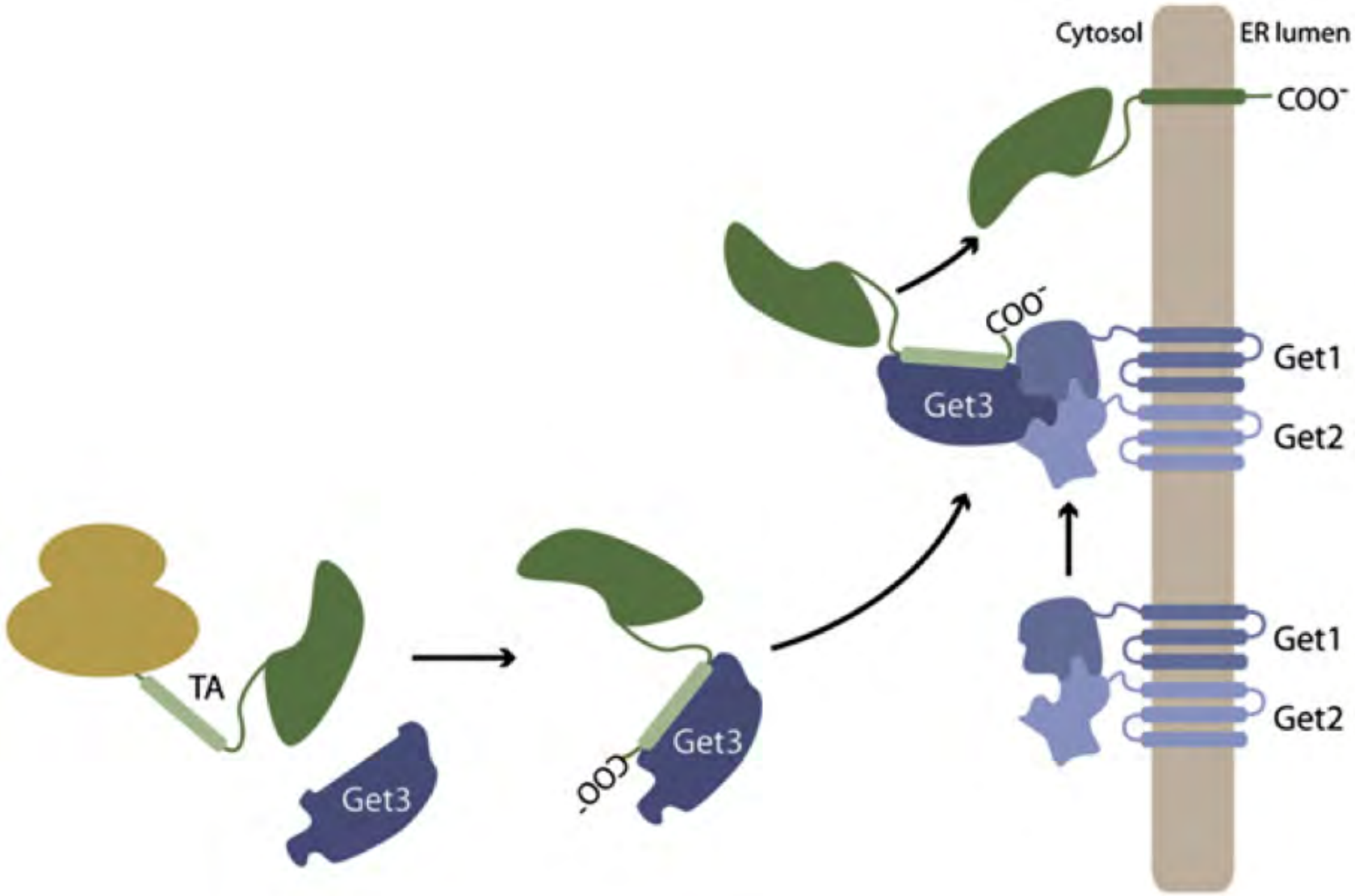
• Eukaryotic, post-translational: Brownian rachet



• Bacterial, post-translational: ATP hydrolysis by SecA pushes polypeptide through channel

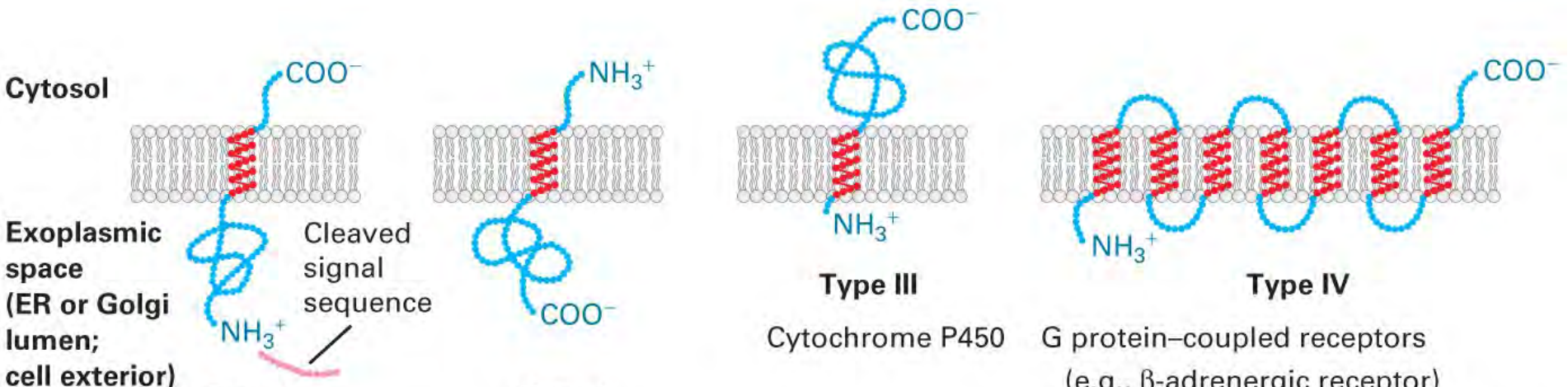


Post-translational insertion of tailed-anchored proteins into the ER



Schuldiner et al (2007) Cell

Topologies of some integral membrane proteins synthesized on the rough ER



Type I

Glycophorin
LDL receptor
Influenza HA protein
Insulin receptor
Growth hormone receptor

Type II

Asialoglycoprotein receptor
Transferrin receptor
Sucrase–isomaltase precursor
Golgi galactosyltransferase
Golgi sialyltransferase
Influenza HN protein

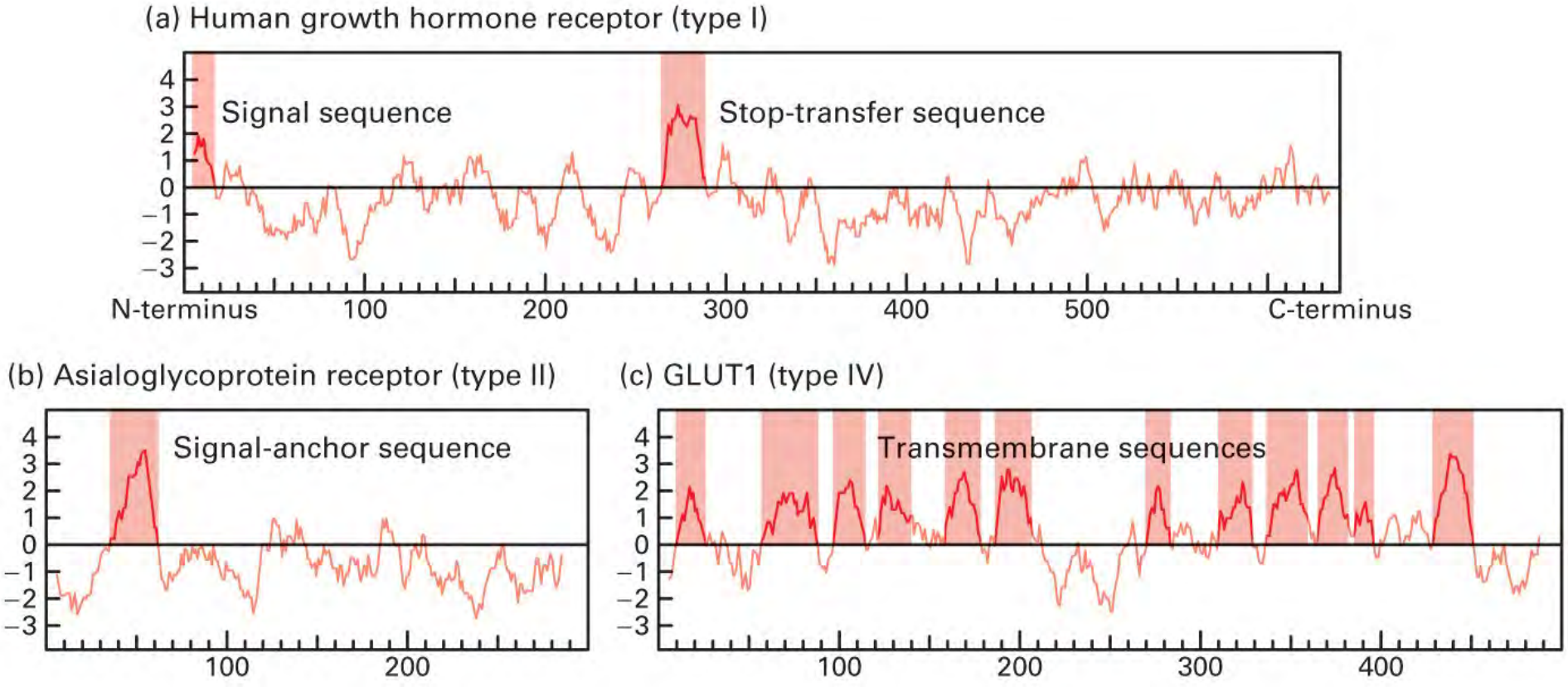
Type III

Cytochrome P450

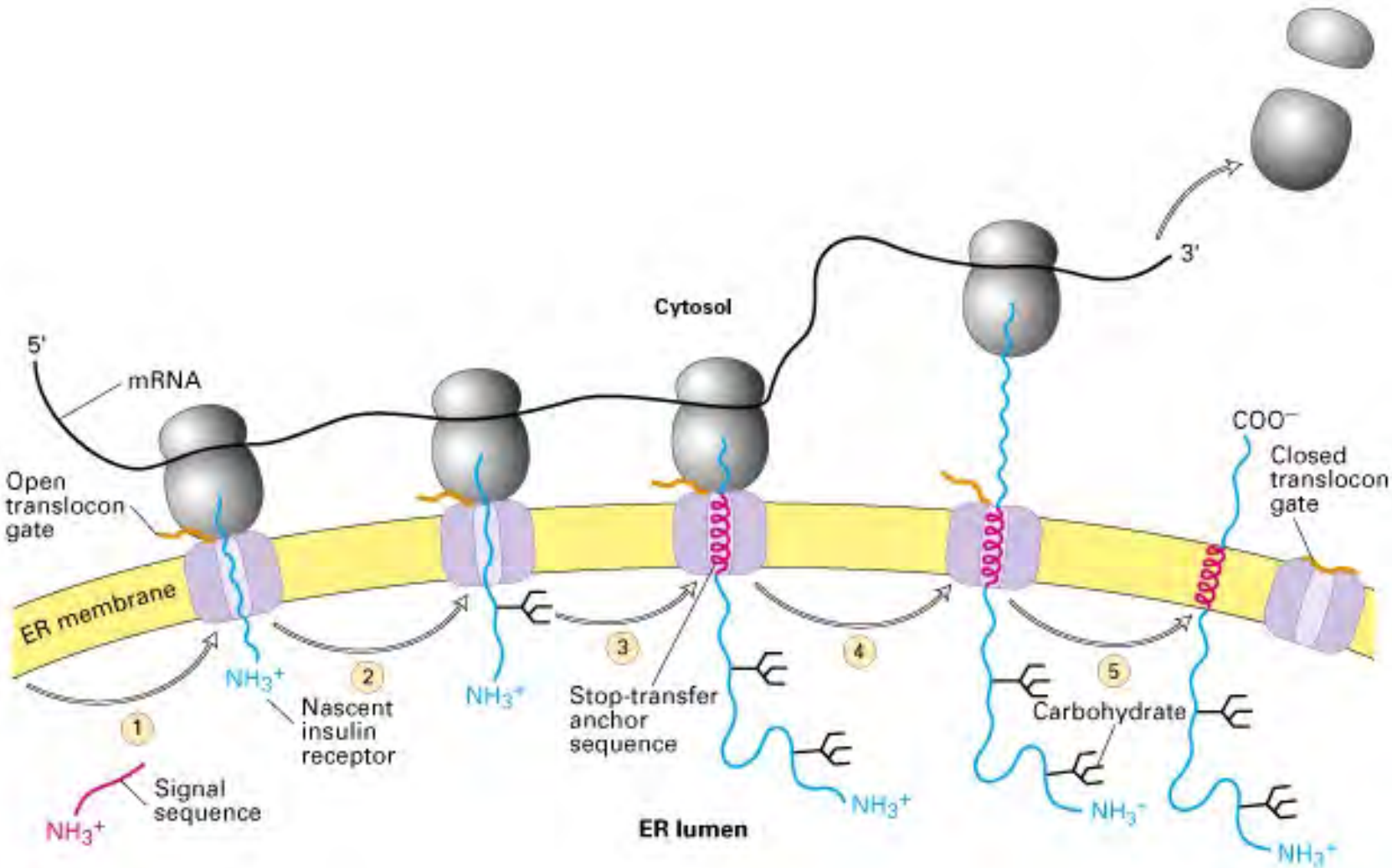
Type IV

G protein–coupled receptors (e.g., β-adrenergic receptor)
Glucose transporters (e.g., GLUT1)
Voltage-gated Ca_2^+ channels
ABC small molecule pumps
CFTR (Cl^-) channel
Sec61
Connexin

Lodish et al, 2004

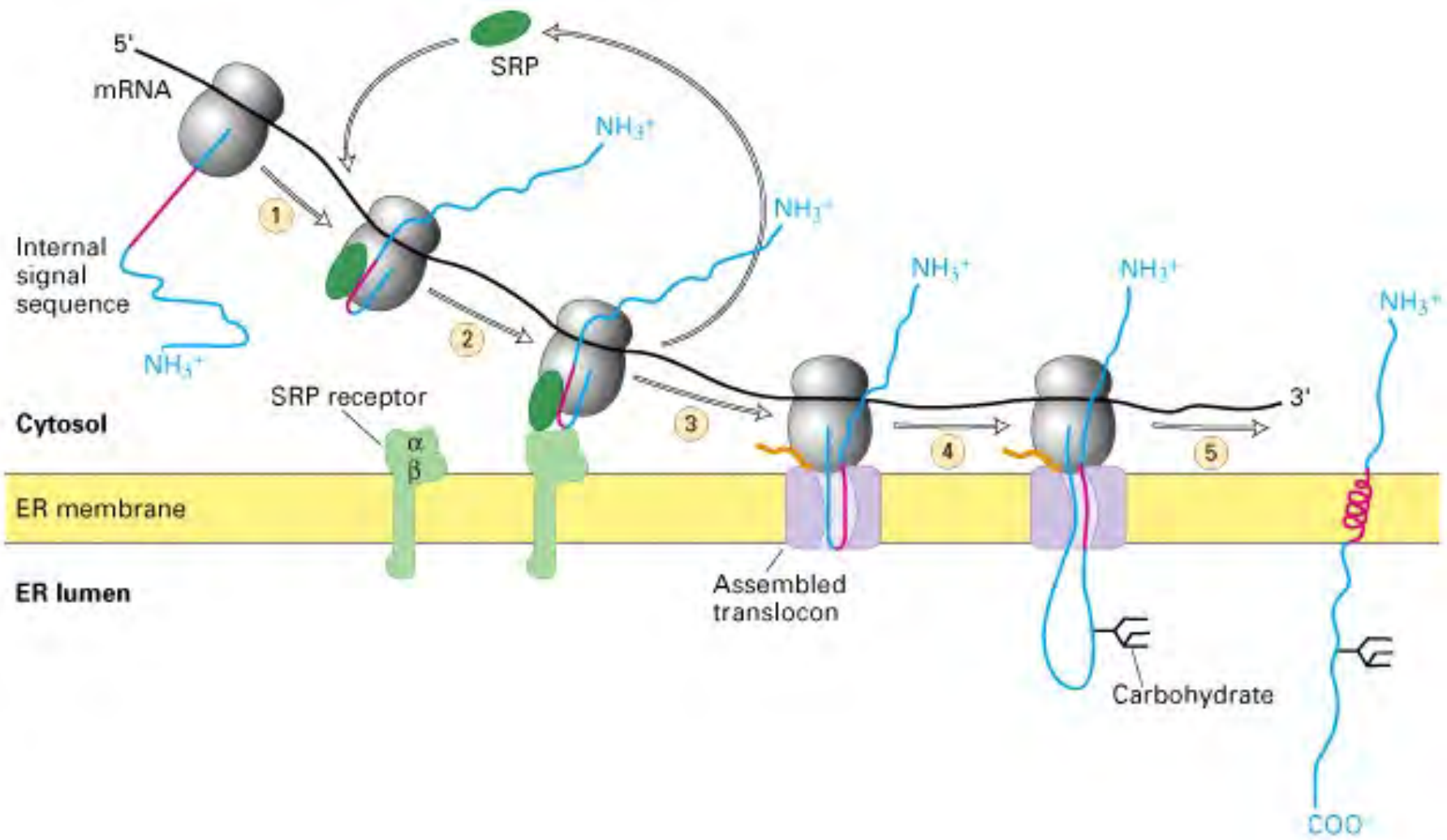

(a) Human growth hormone receptor (type I)
Signal sequence
Stop-transfer sequence
4
3
2
1
0
−1
−2
−3
N-terminus
100
200
300
400
500
C-terminus
(b) Asialoglycoprotein receptor (type II)
Signal-anchor sequence
100
200
(c) GLUT1 (type IV)
Transmembrane sequences
100
200
300
400

Most transmembrane proteins (Type I) have an N-terminal signal sequence and an internal topogenic sequence



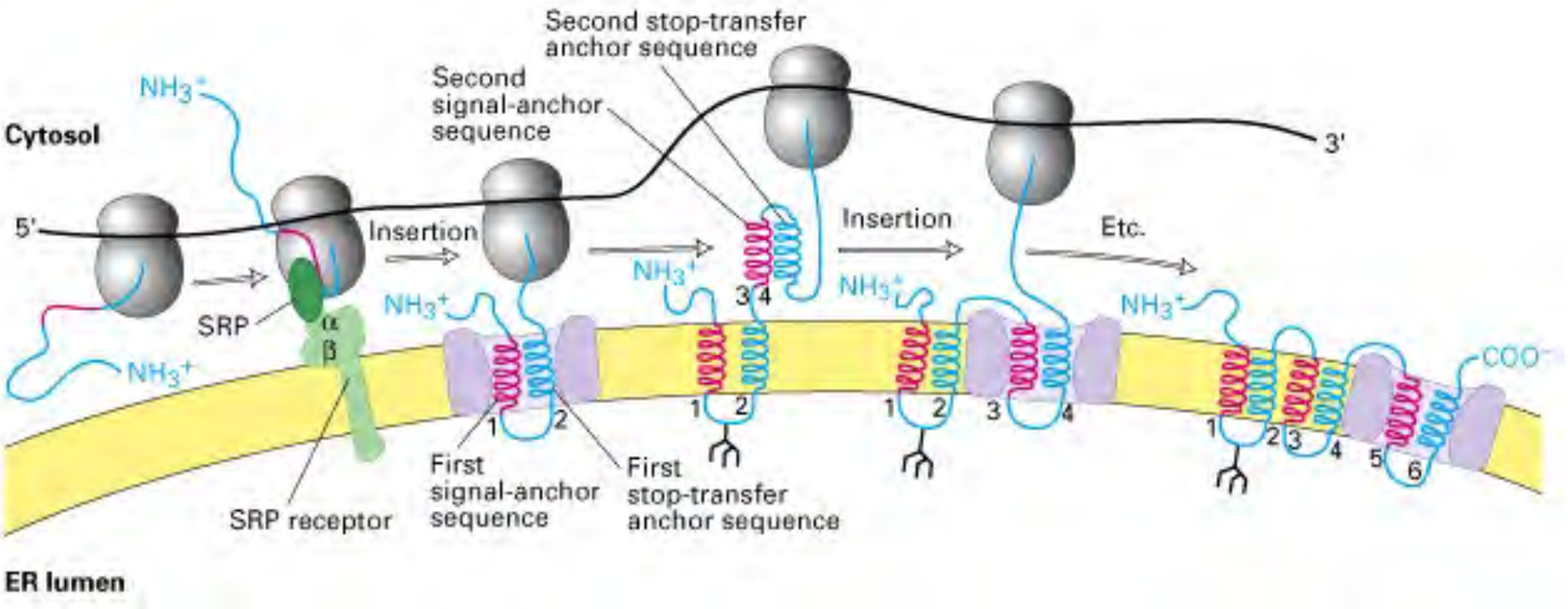
Lodish et al, 2000

A single internal topogenic sequence directs insertion of some single-pass transmembrane proteins (Type II)



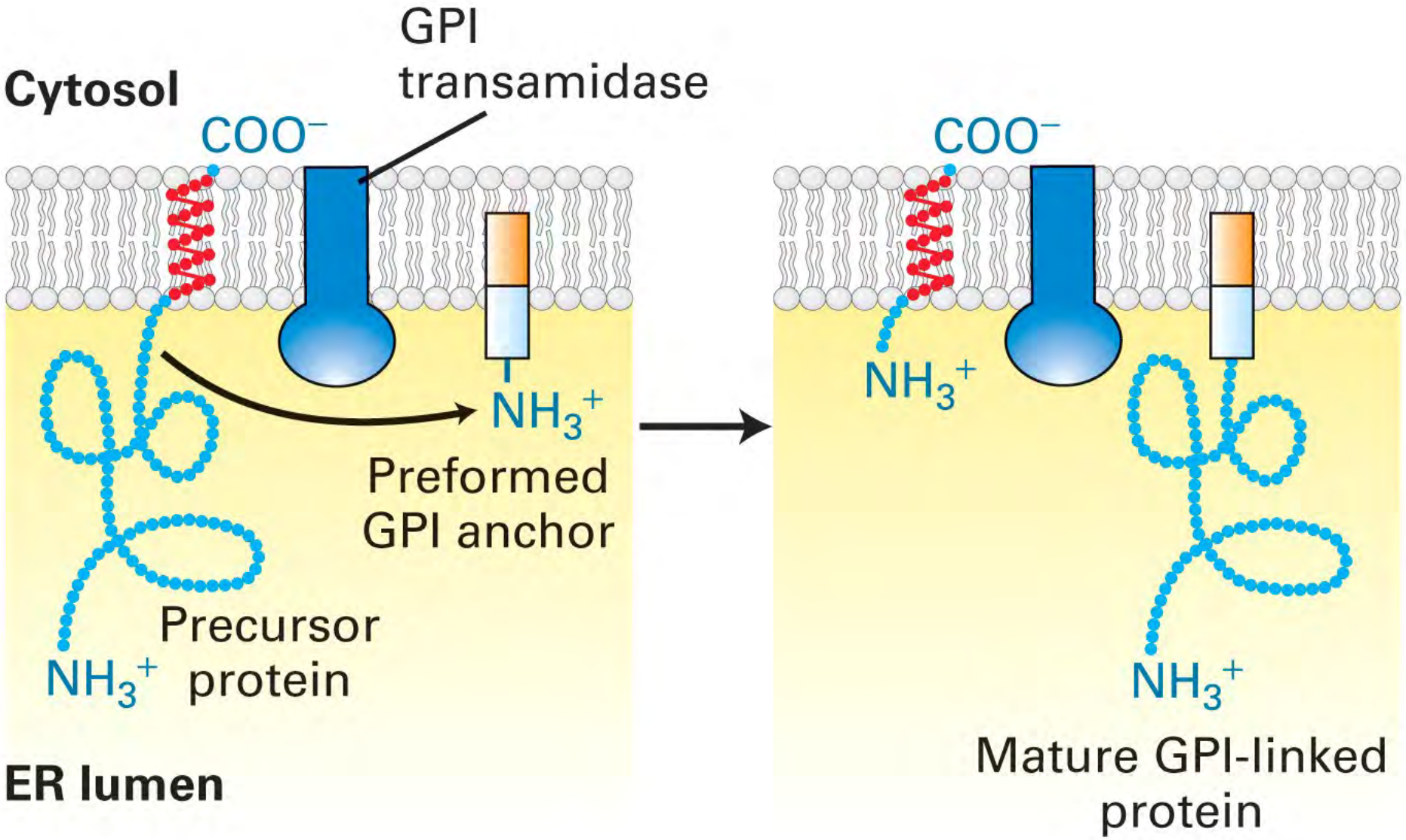
Lodish et al, 2000

Multipass transmembrane proteins have multiple topogenic sequences



Lodish et al, 2000

After insertion into the ER membrane, some proteins are transferred to a GPI anchor



Lodish et al, 2004

The glycosylphosphatidylinositol (GPI) anchor is a glycolipid