Vesicular trafficking and cellular Transport

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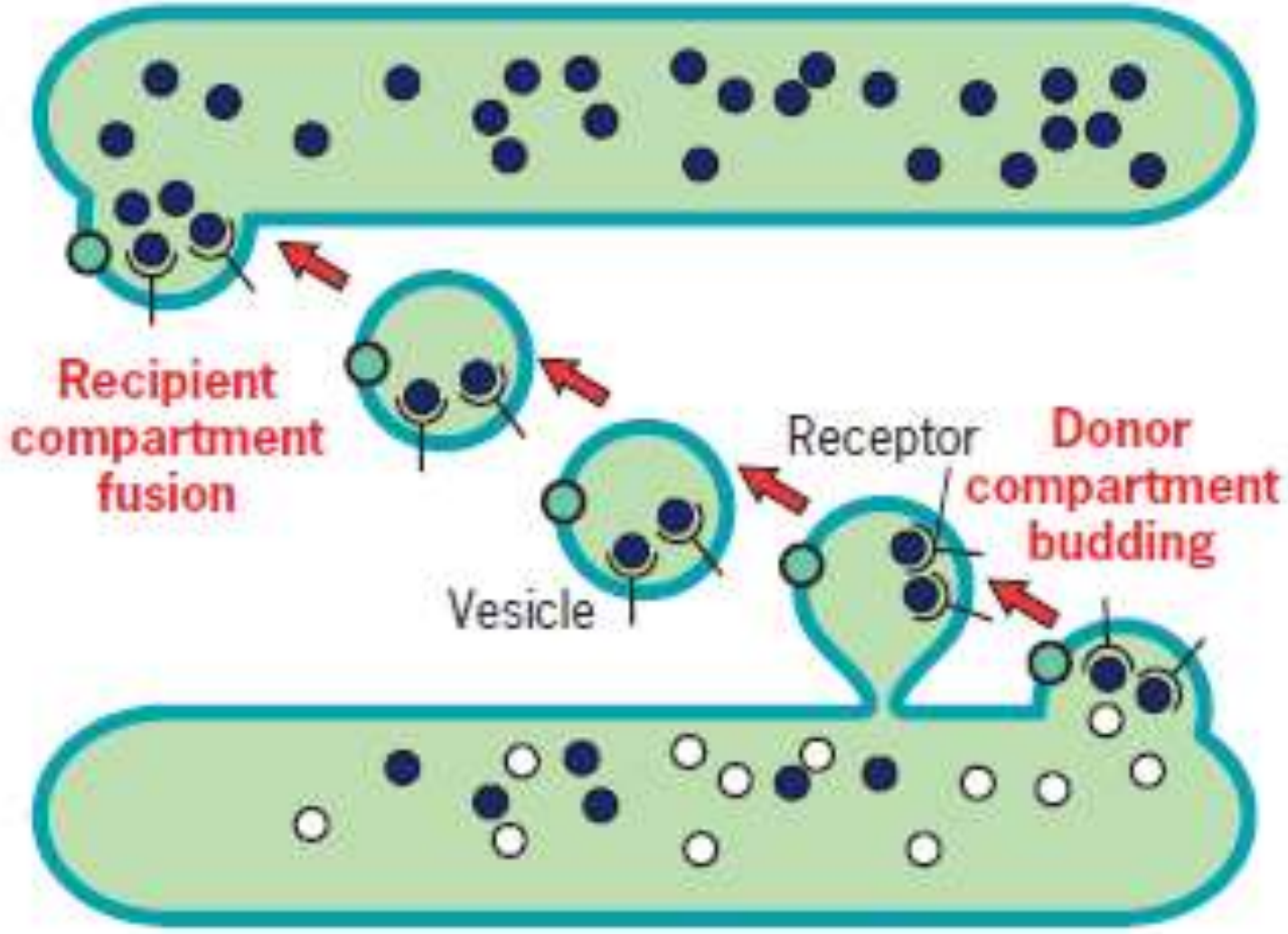
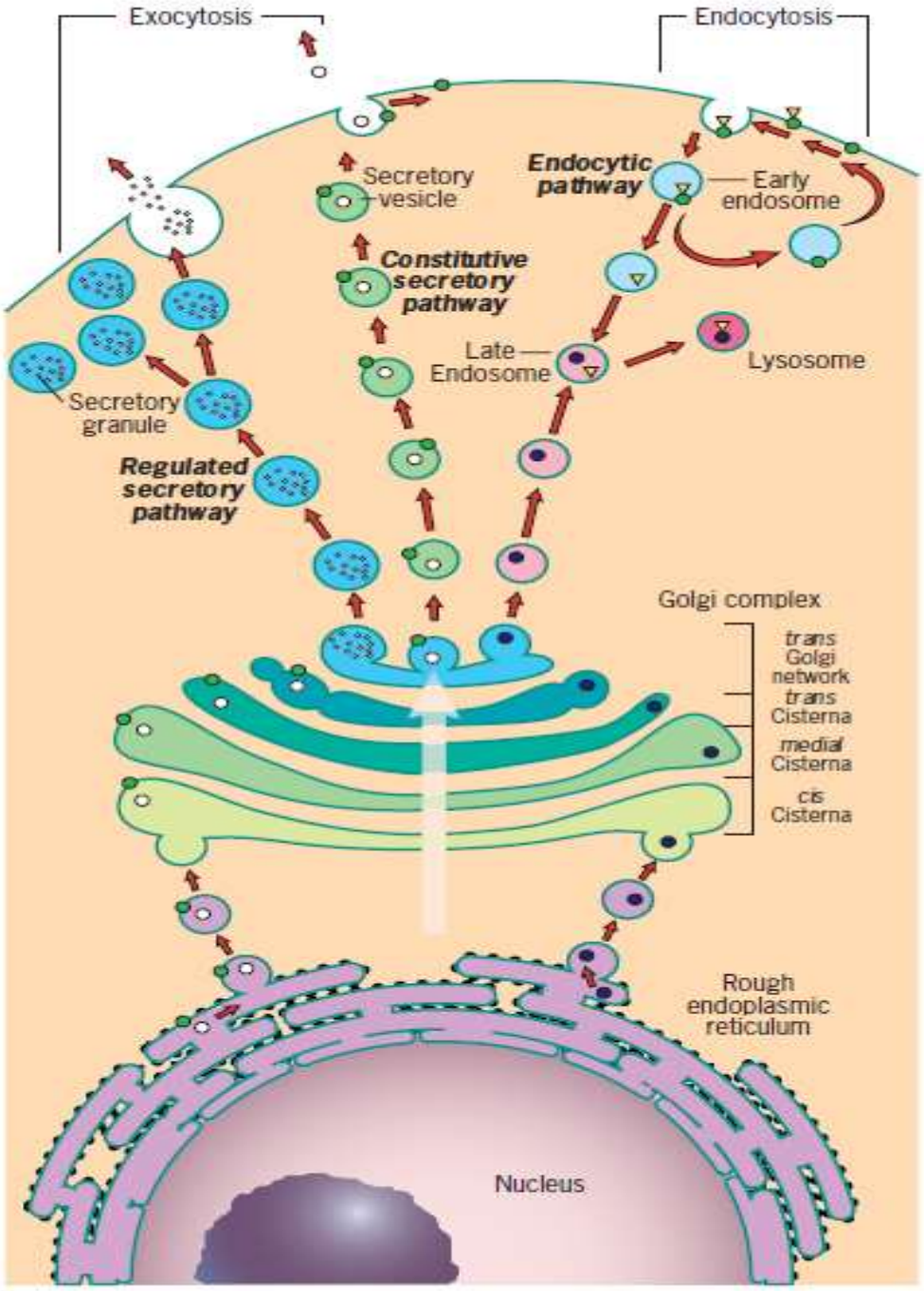
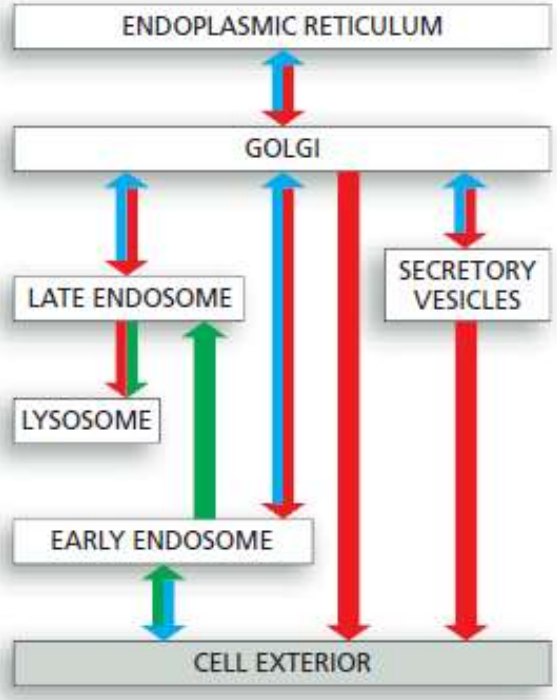

Recipient compartment fusion
Vesicle
Receptor
Donor compartment budding

(a)

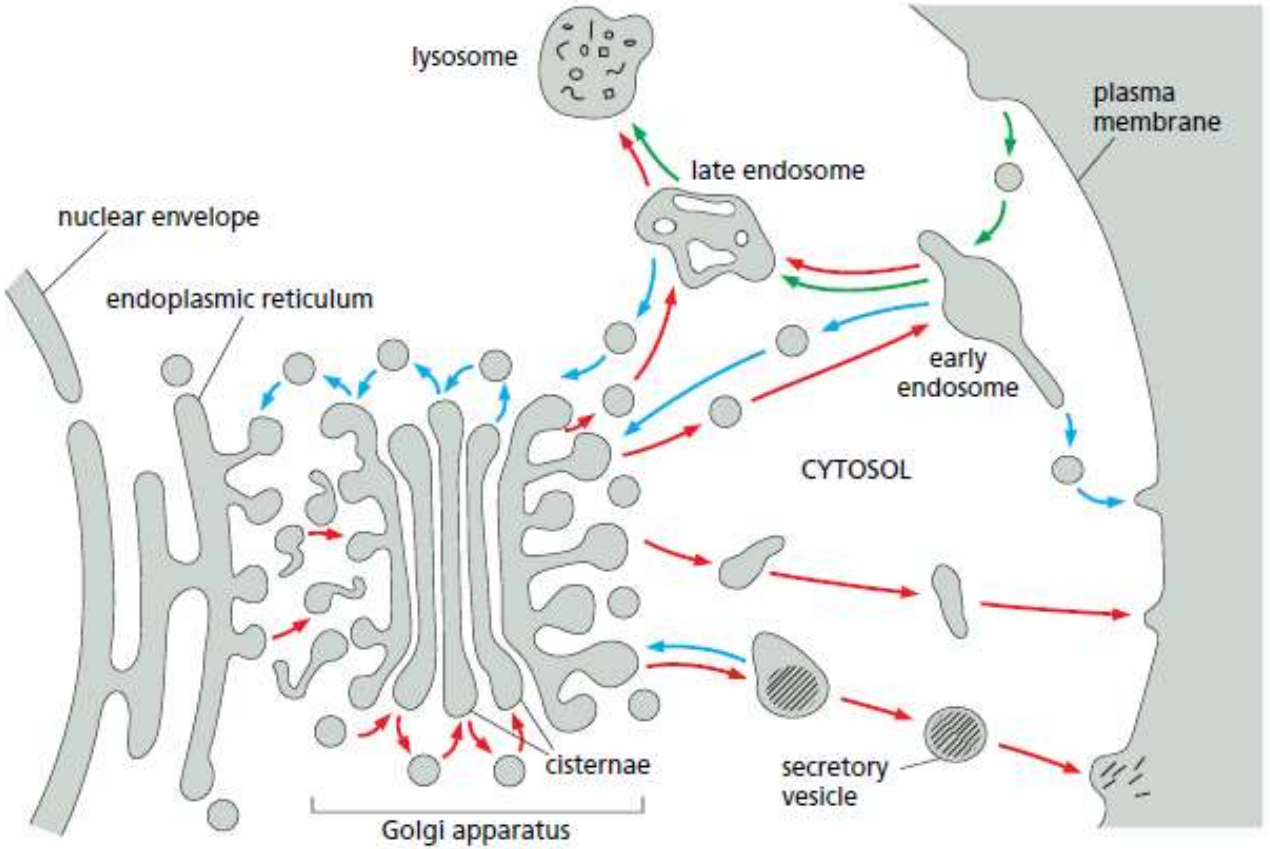
Endomembrane system
Biosynthetic pathway
Secretory pathway
Constitutive secretion
Regulated secretion


Exocytosis
Endocytosis
Secretory vesicle
Endocytic pathway
Early endosome
Constitutive secretory pathway
Late Endosome
Lysosome
Secretory granule
Regulated secretory pathway
Golgi complex
trans Golgi network
trans Cisterna
medial Cisterna
cis Cisterna
Rough endoplasmic reticulum
Nucleus

(b)

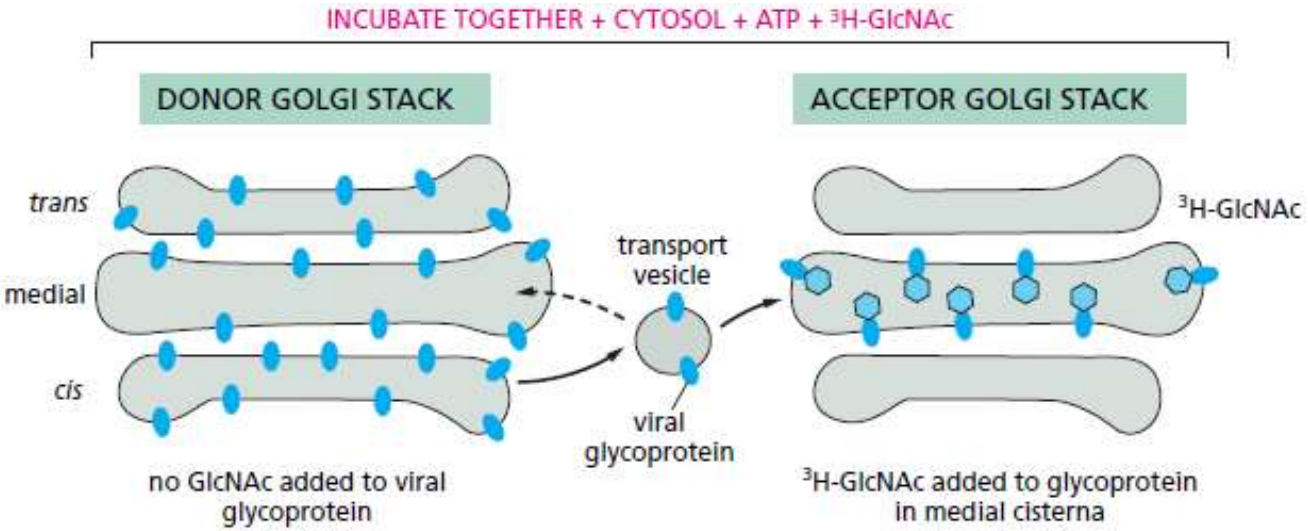

ENDOPLASMIC RETICULUM
GOLGI
LATE ENDOSOME
SECRETORY VESICLES
LYSOSOME
EARLY ENDOSOME
CELL EXTERIOR

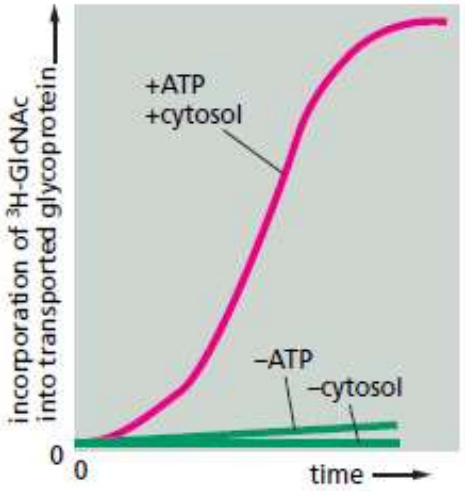
(A)


lysosome
plasma membrane
late endosome
nuclear envelope
endoplasmic reticulum
early endosome
CYTOSOL
cisternae
secretory vesicle
Golgi apparatus

(B)

CELL-FREE SYSTEMS FOR STUDYING THE COMPONENTS AND MECHANISM OF VESICULAR TRANSPORT





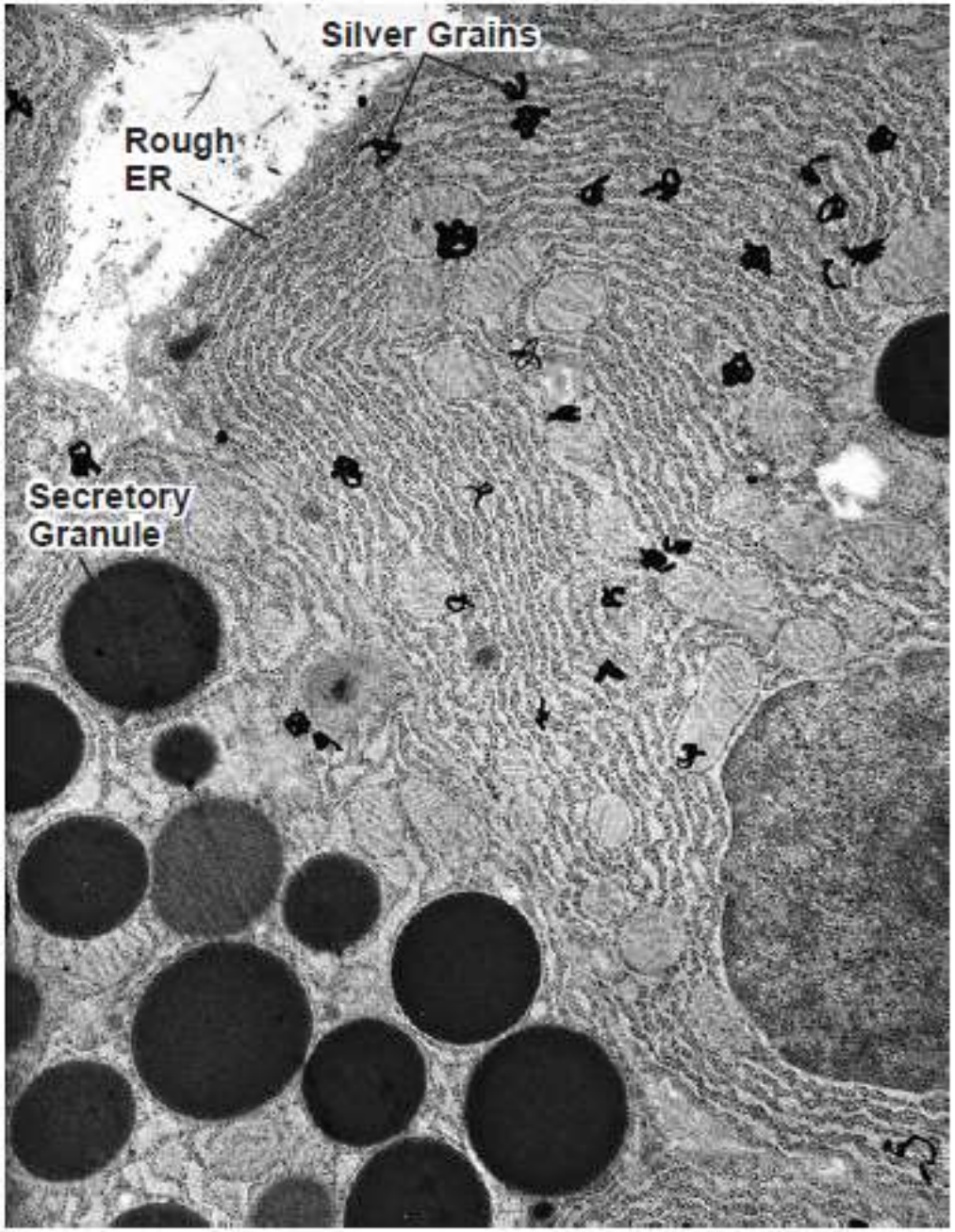
GlcNAC transferase 1 mutant and infected

Wt but uninfected

GENETIC APPROACHES FOR STUDYING VESICULAR TRANSPORT

Multi copy suppression

Autoradiography And Pulse Chase labelling



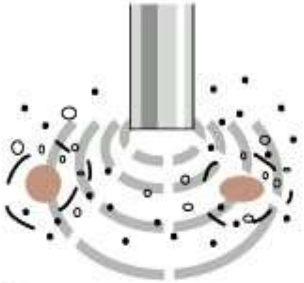
(a)

BREAKING CELLS AND TISSUES

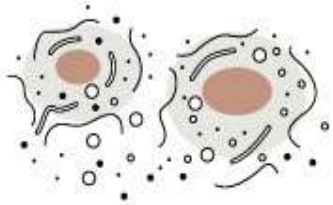
The first step in the purification of most proteins is to disrupt tissues and cells in a controlled fashion.

Using gentle mechanical procedures, called homogenization, the plasma membranes of cells can be ruptured so that the cell contents are released. Four commonly used procedures are shown here.

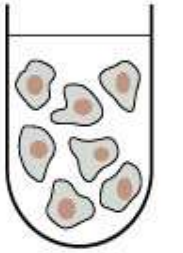
The resulting thick soup (called a homogenate or an extract) contains large and small molecules from the cytosol, such as enzymes, ribosomes, and metabolites, as well as all the membrane-bounded organelles.



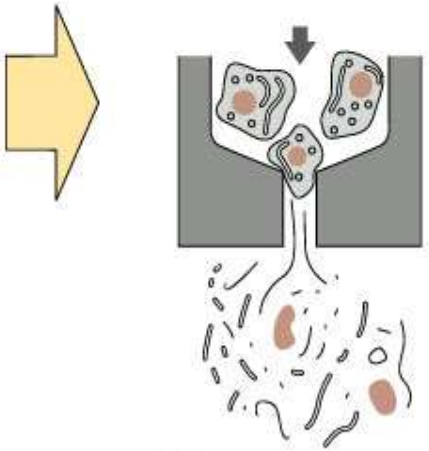
1 break cells with high frequency sound



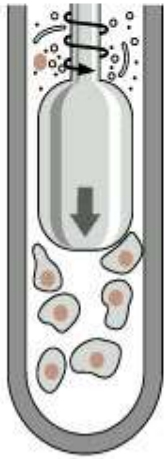
2 use a mild detergent to make holes in the plasma membrane



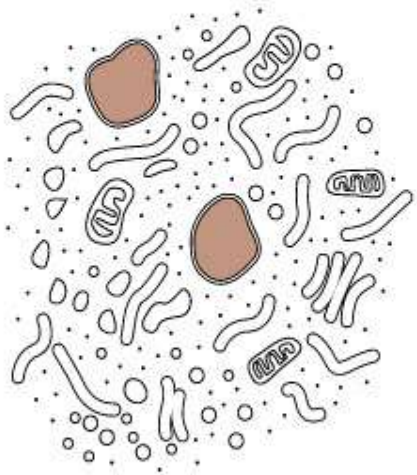
cell suspension or tissue



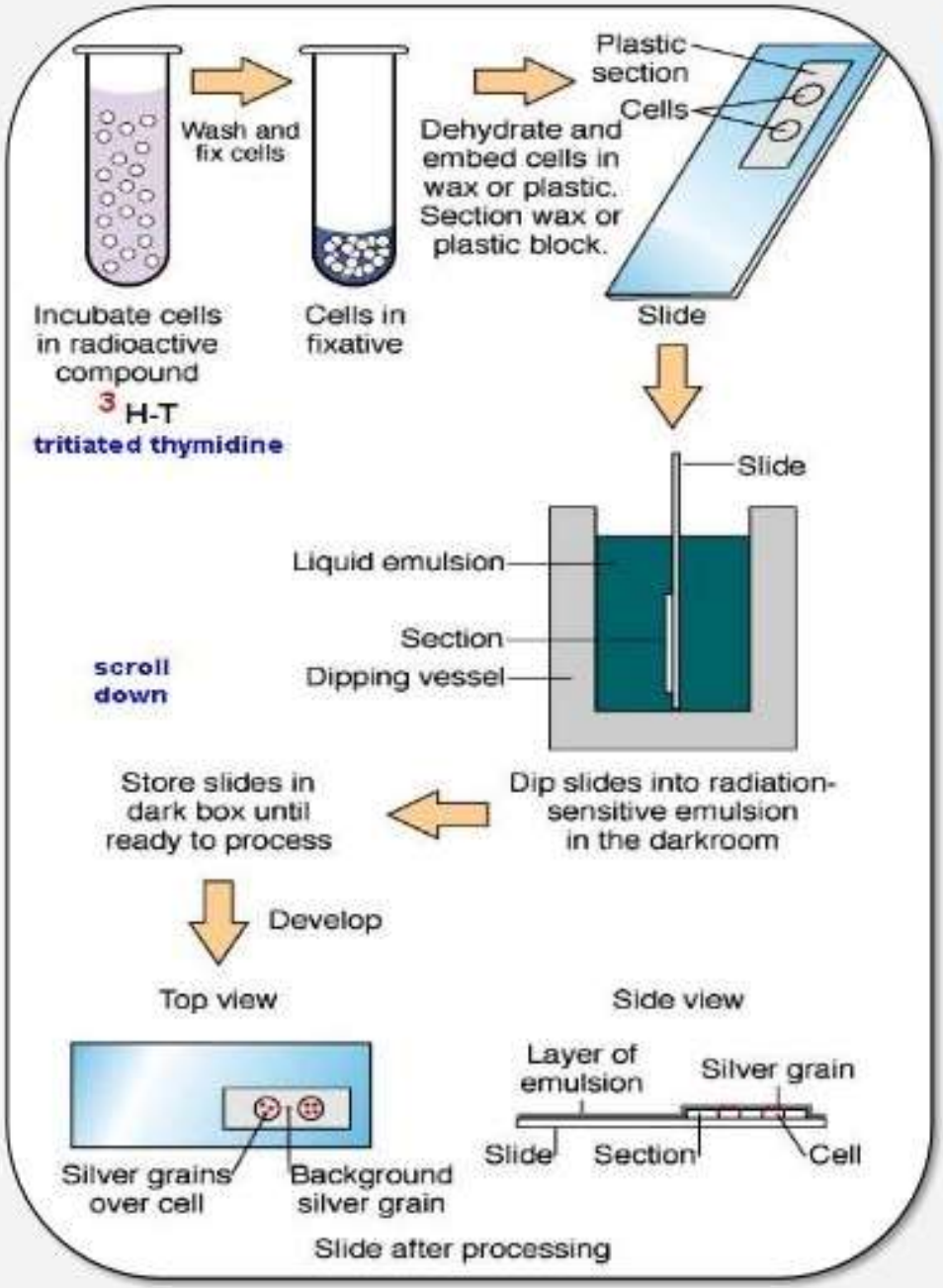
3 force cells through a small hole using high pressure



4 shear cells between a close-fitting rotating plunger and the thick walls of a glass vessel

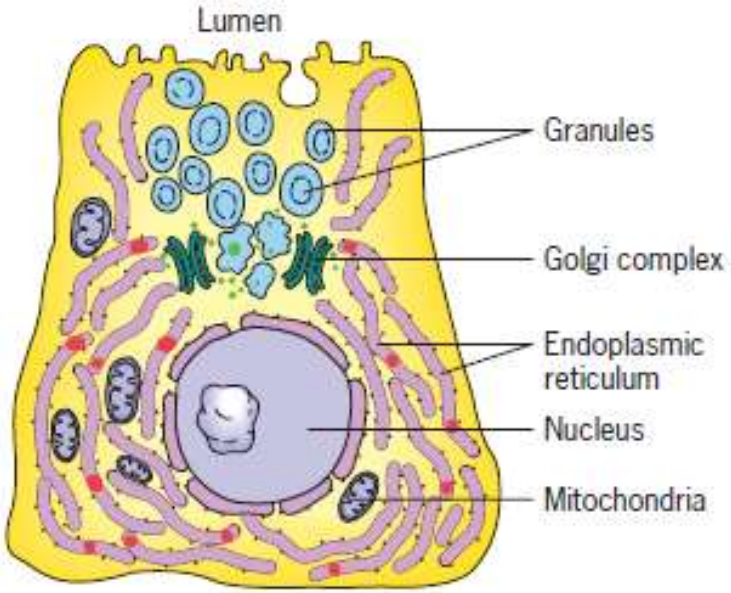


When carefully applied, homogenization leaves most of the membrane-bounded organelles intact.



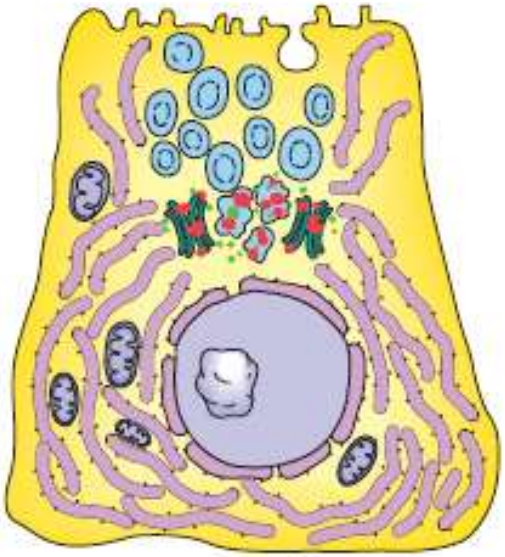
AUTORADIOGRAPHY

- Radioisotopes are taken up selectively by cells to be studied
- Exposure of photographic film to their emitted radiation reveal presence of such isotopes in the vicinity of these target cells
- Silver bromide crystals in emulsion detect radiation, that reduce them to visible black granules.



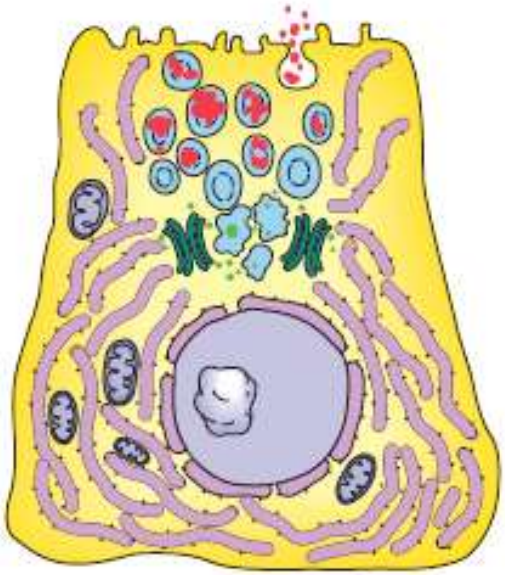
3 min

(b)



20 min

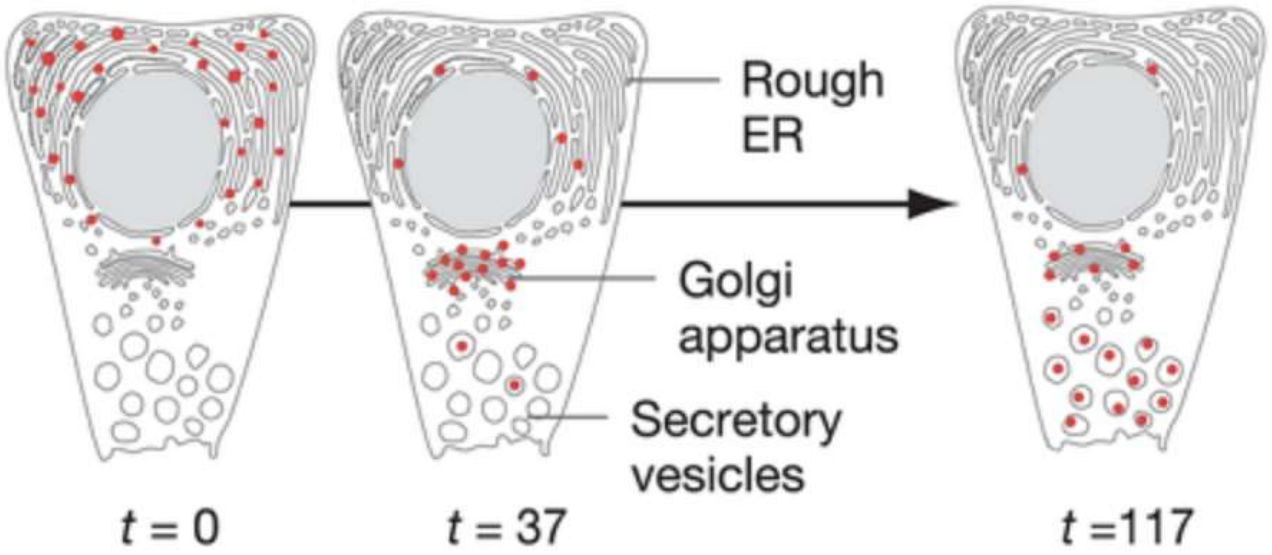
(c)



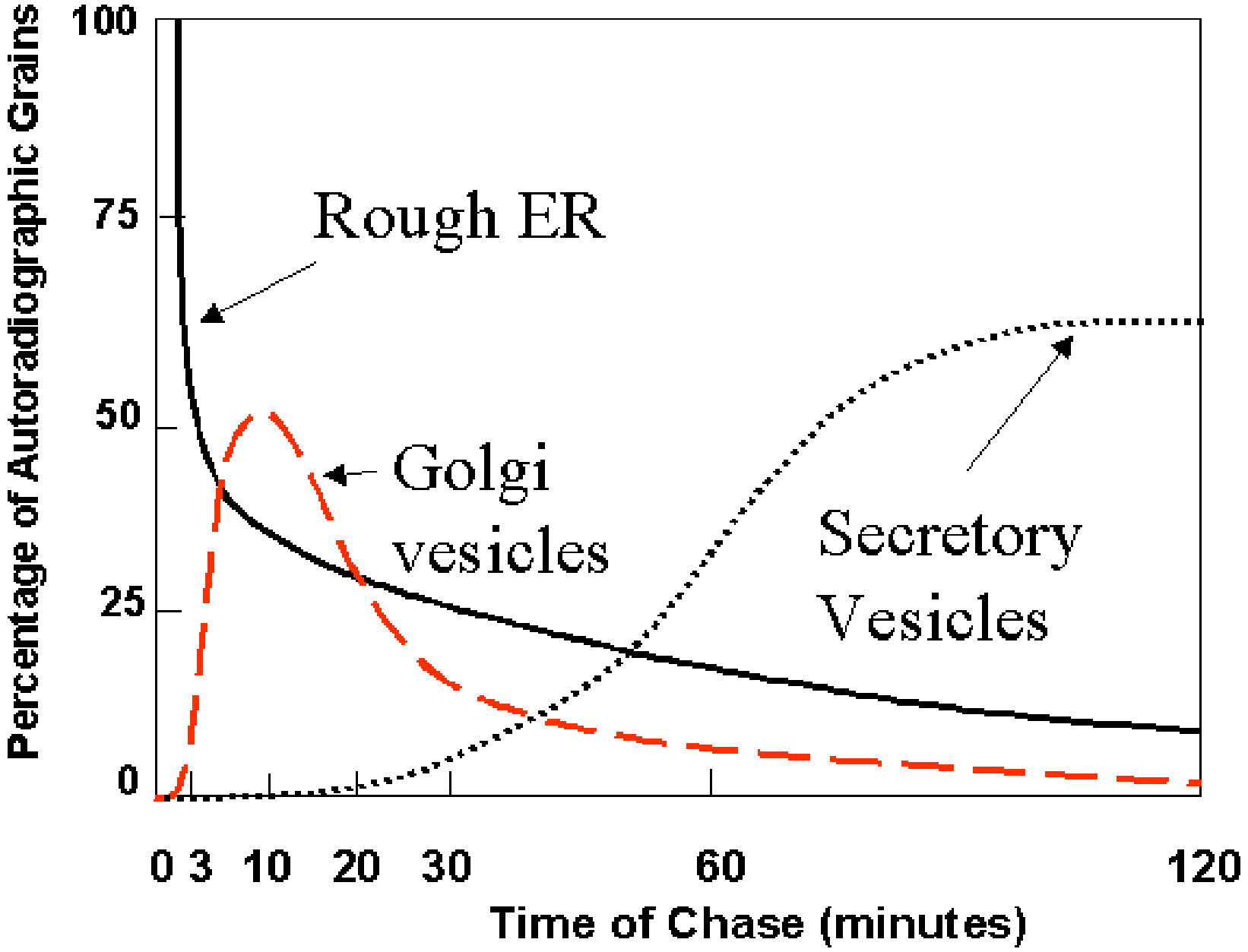
120 min

(d)

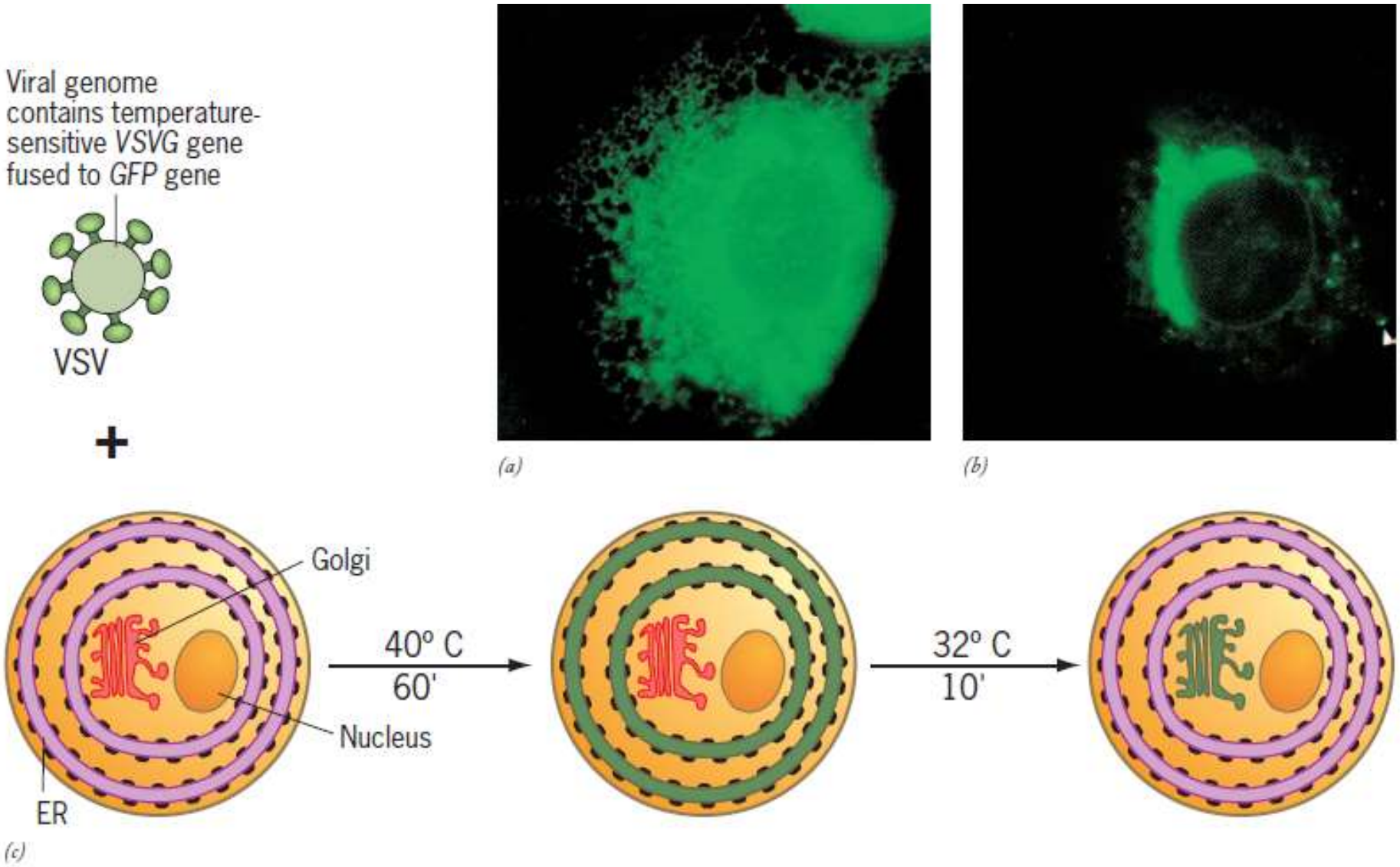
Based on the results from this pulse-chase experiment, where are most secreted proteins synthesized?

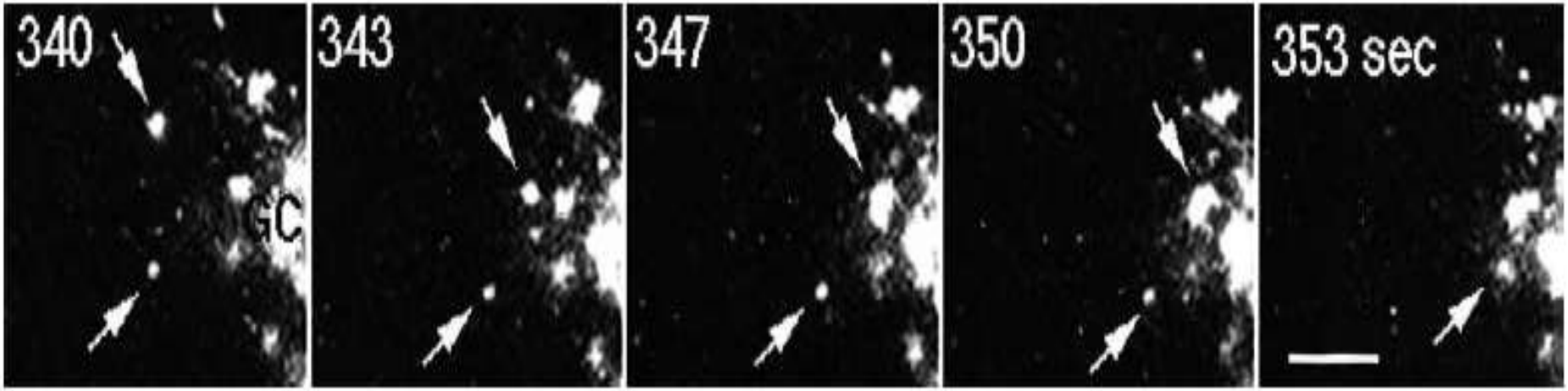


- in the rough ER
- in the Golgi apparatus
- in the secretory vesicles
- The answer cannot be determined using this assay.

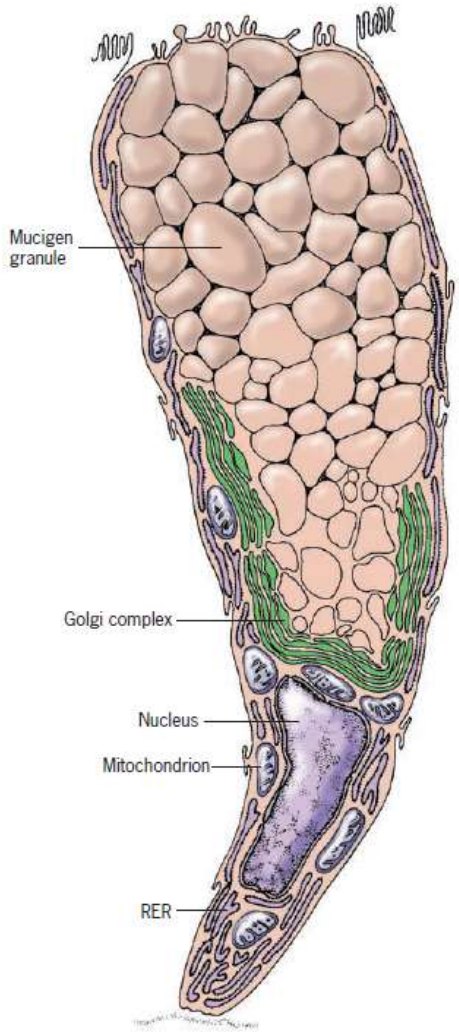

Percentage of Autoradiographic Grains
100
75
50
25
0
Rough ER
Golgi
vesicles
Secretory
Vesicles
0 3 10 20 30 60 120
Time of Chase (minutes)

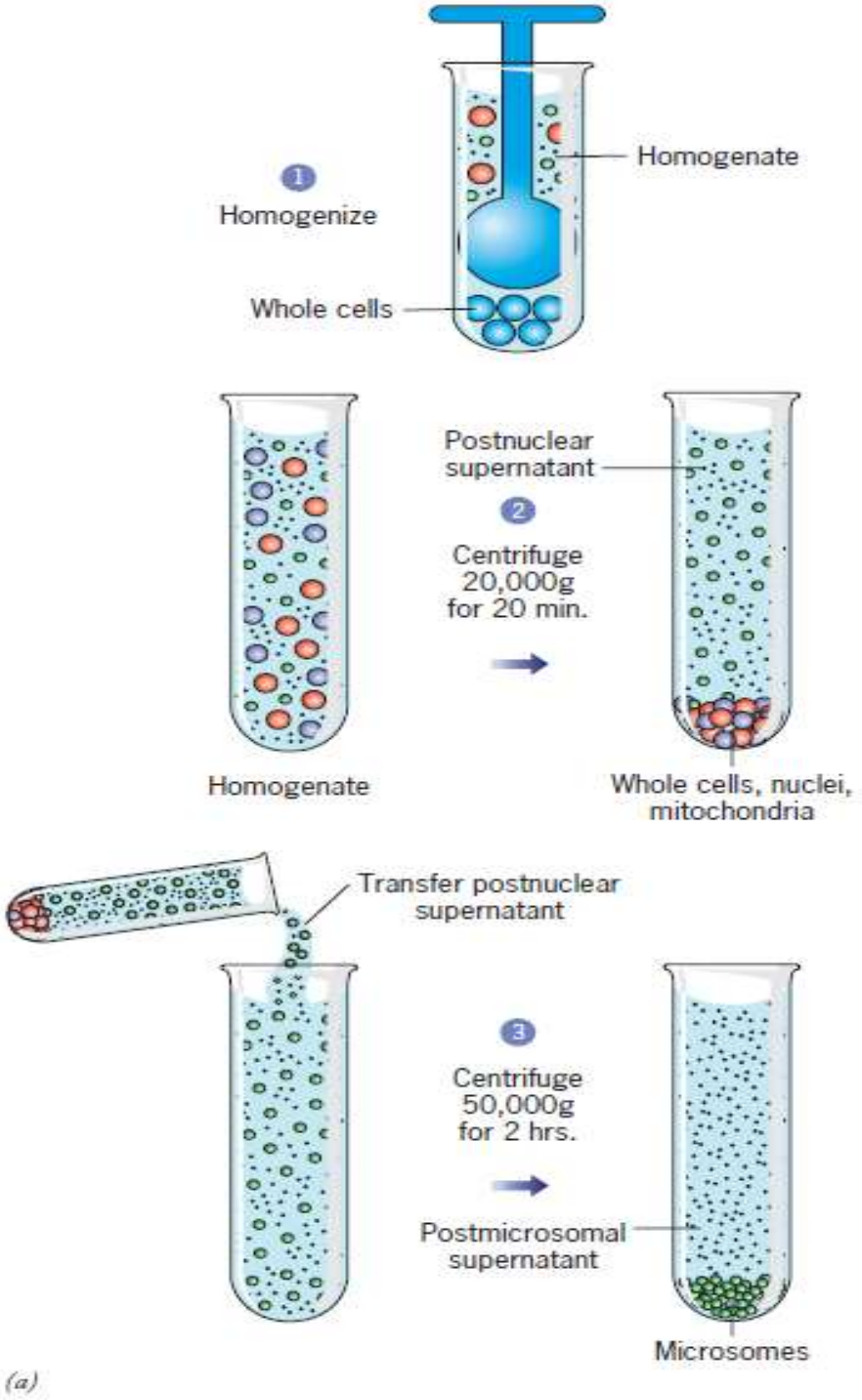
GFP protein


Viral genome contains temperature-sensitive *VSVG* gene fused to *GFP* gene
VSV
+
(a)
(b)
Golgi
Nucleus
ER
40° C
60'
32° C
10'
(c)

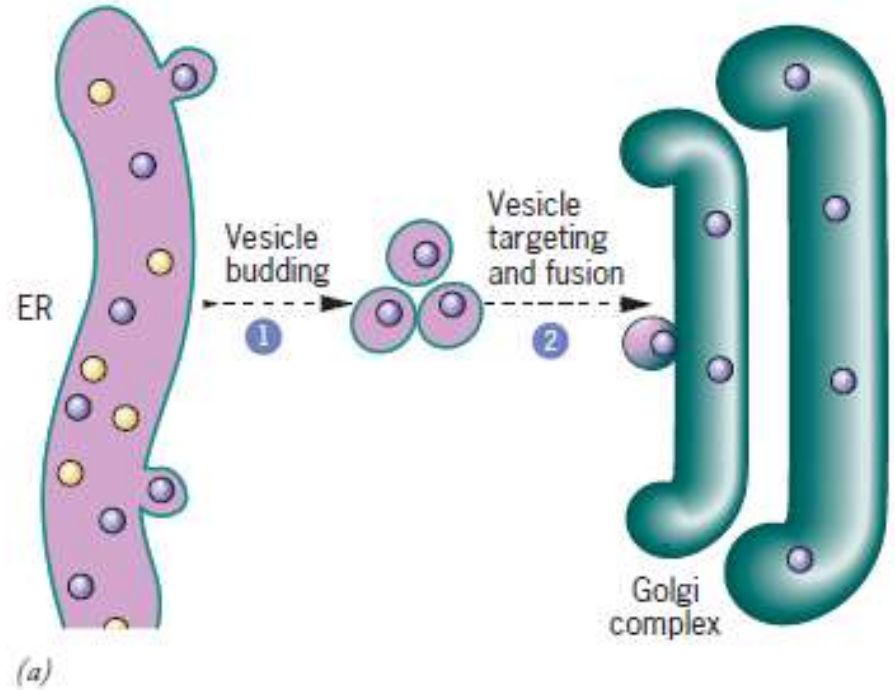

340
343
347
350
353 sec
GC

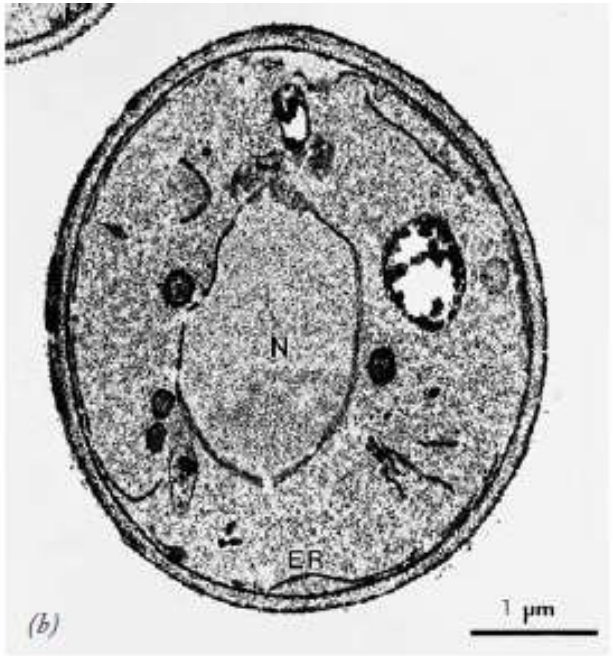
Subcellular Fractionation

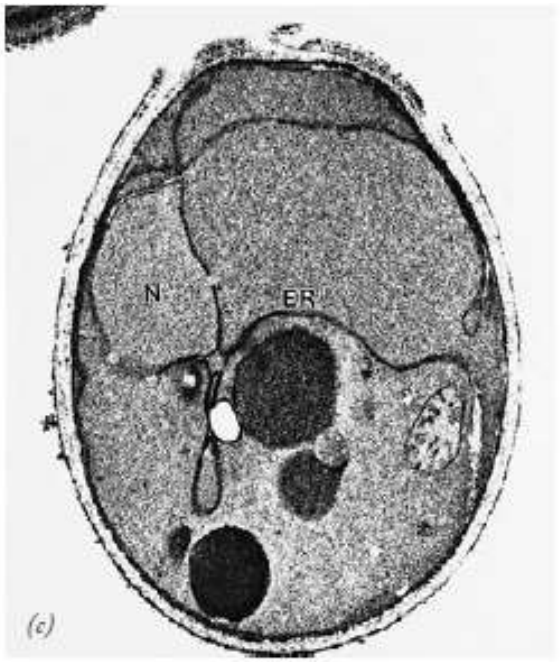


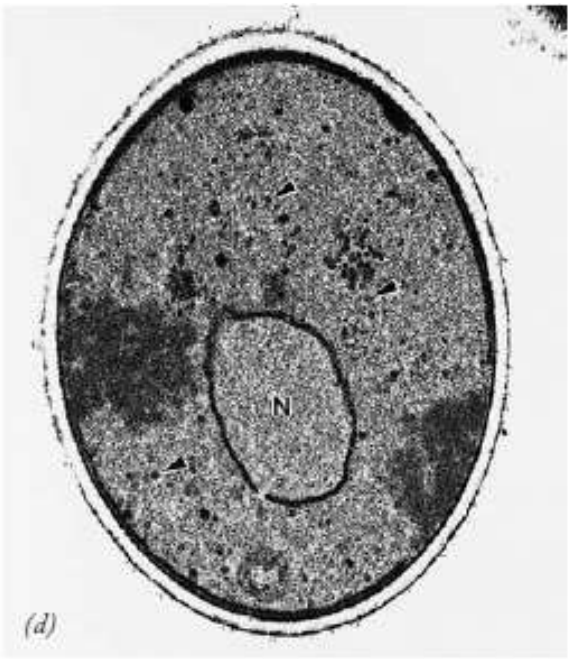


The use of genetic mutants in the study of secretion

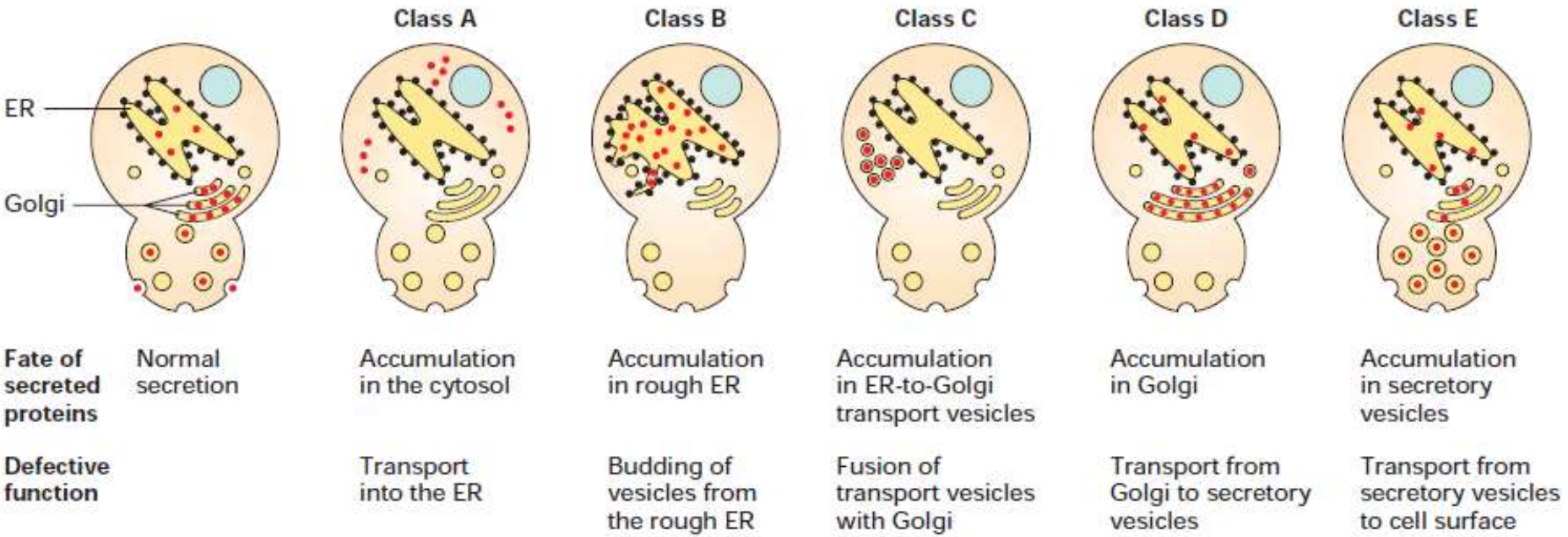


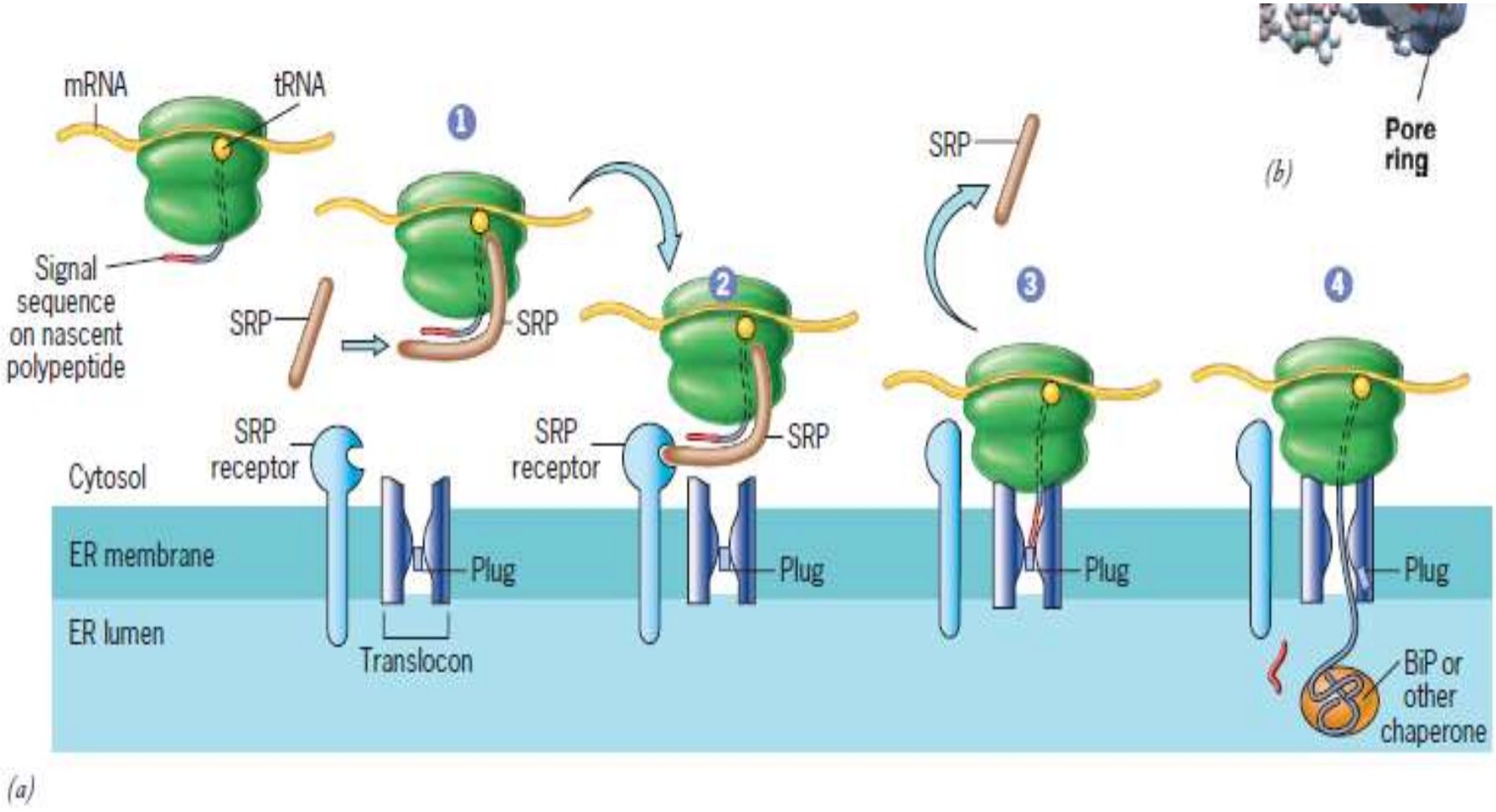


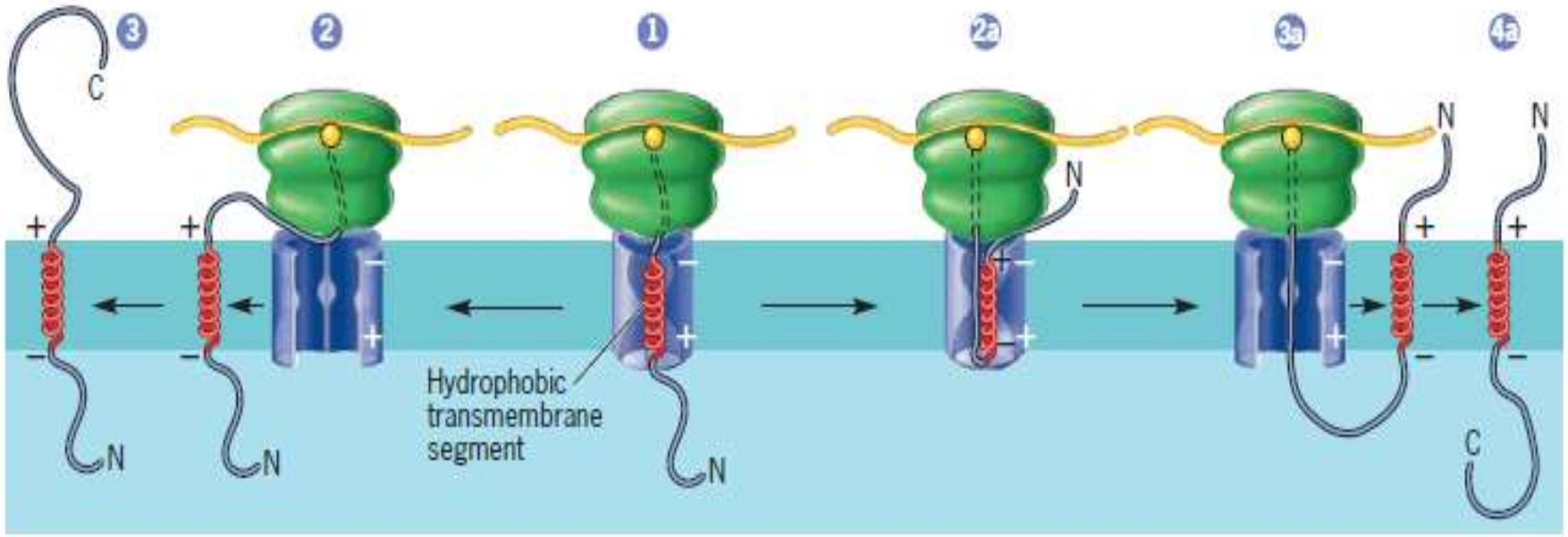


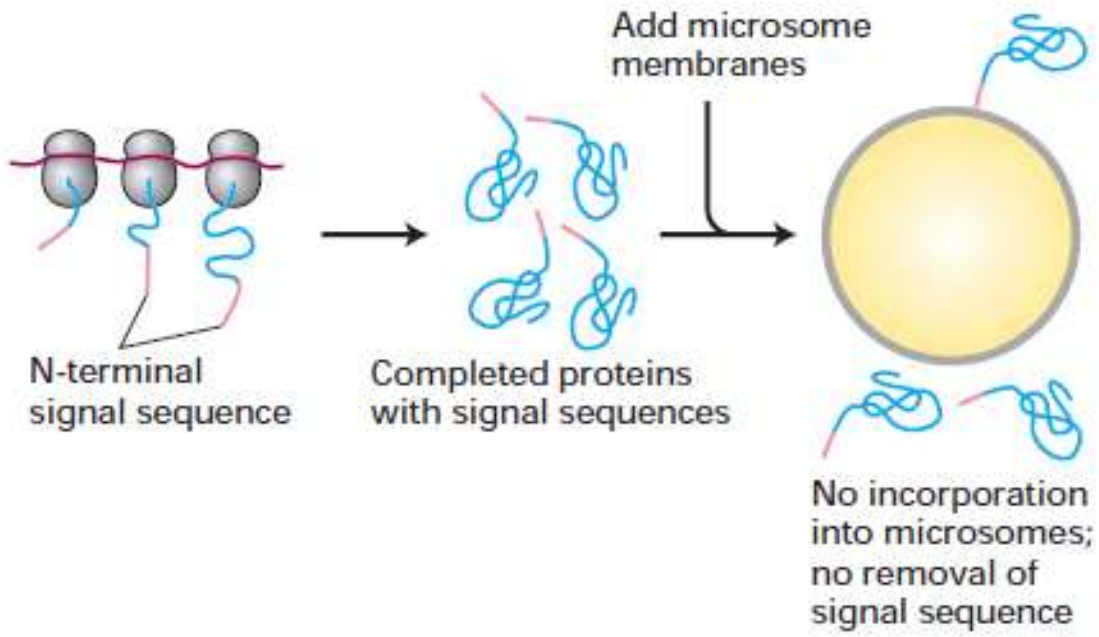


Phenotypes of yeast *sec* mutants identified stages in the secretory pathway

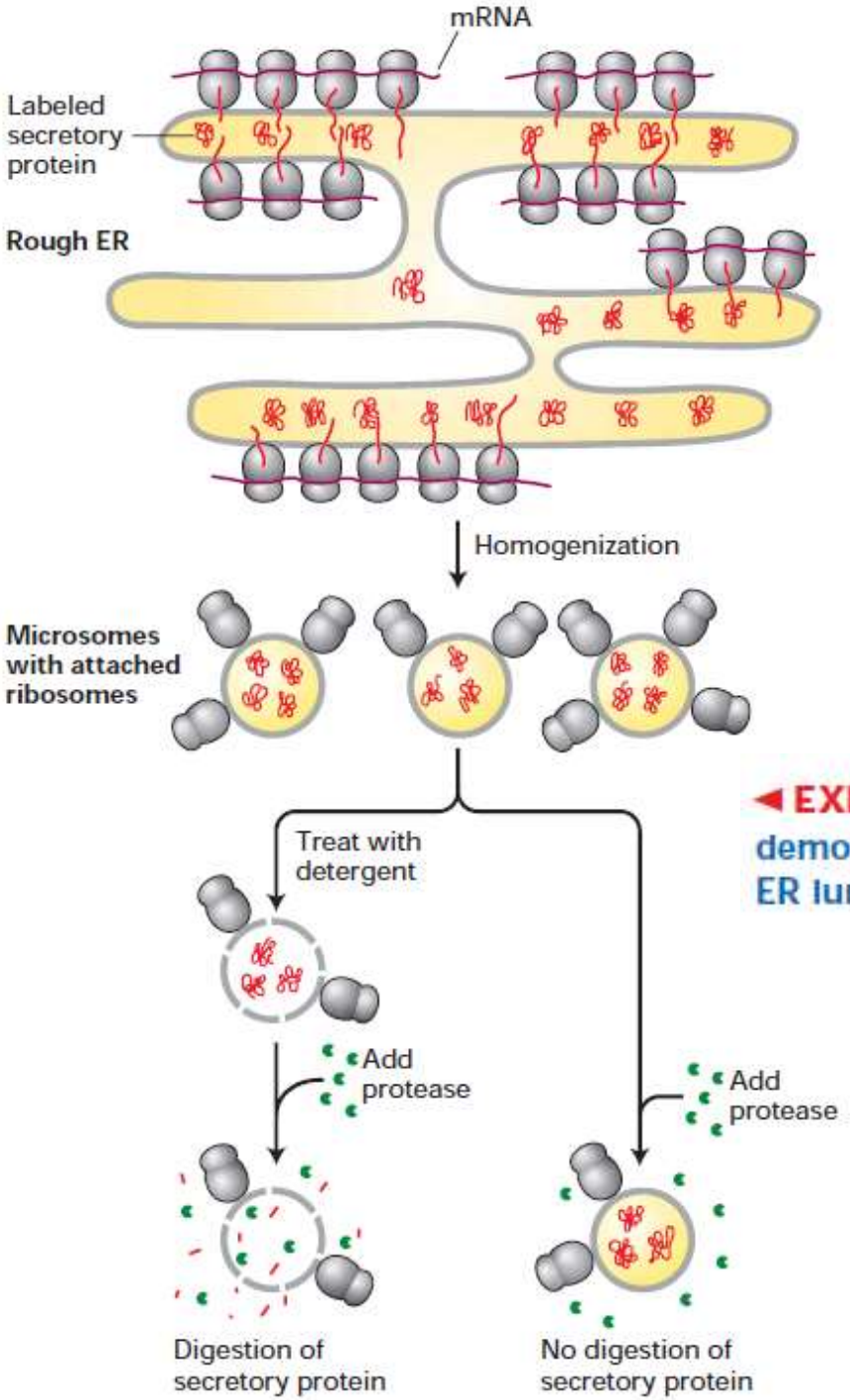



mRNA
tRNA
Signal sequence on nascent polypeptide
SRP
SRP receptor
Cytosol
ER membrane
ER lumen
Plug
Translocon
1
2
3
4
BiP or other chaperone
(a)
(b)
Pore ring


3
2
1
2a
3a
4a
C
N
Hydrophobic
transmembrane
segment

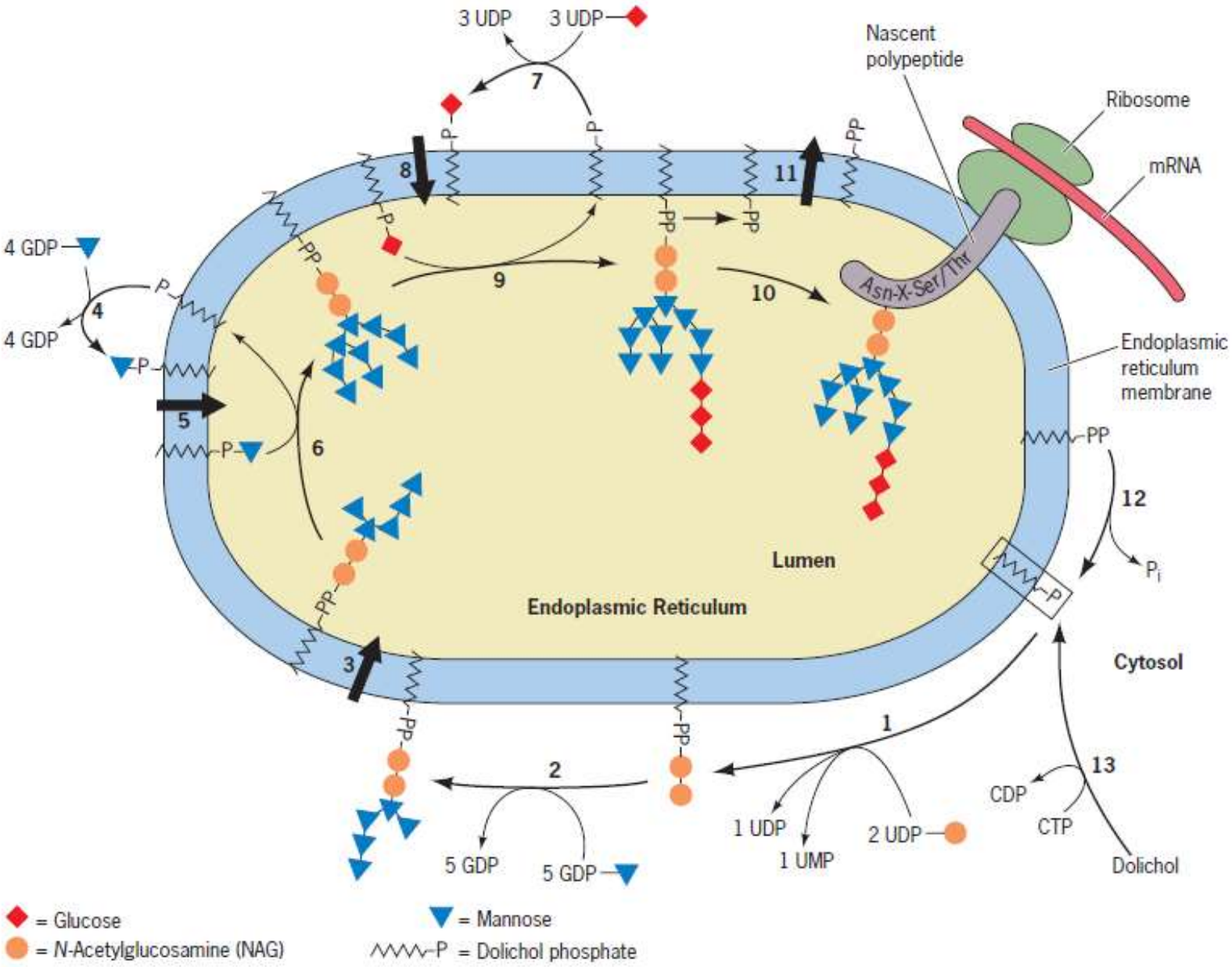


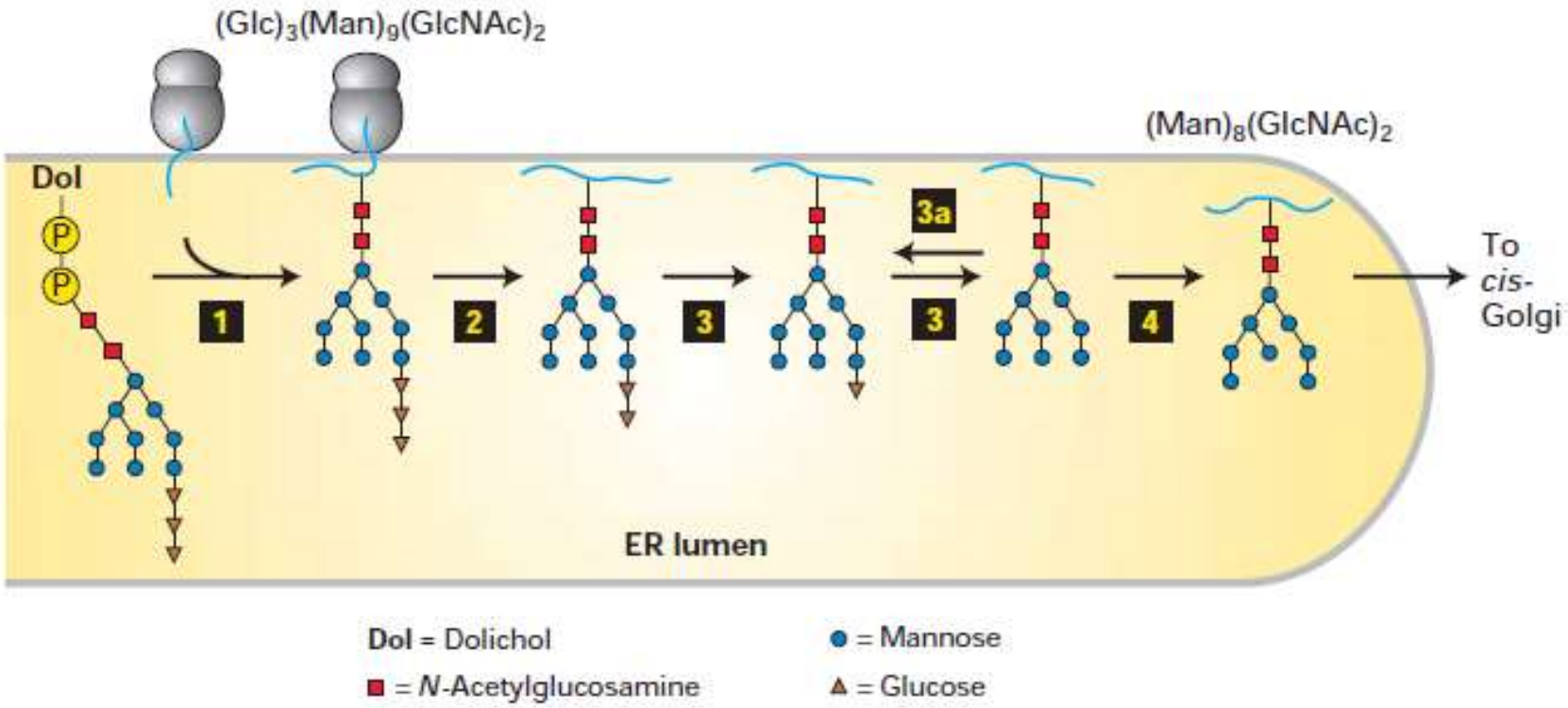
◀ EXPERIMENTAL FIGURE 16-4 Cell-free experiments demonstrate that translocation of secretory proteins into microsomes is coupled to translation. Treatment of



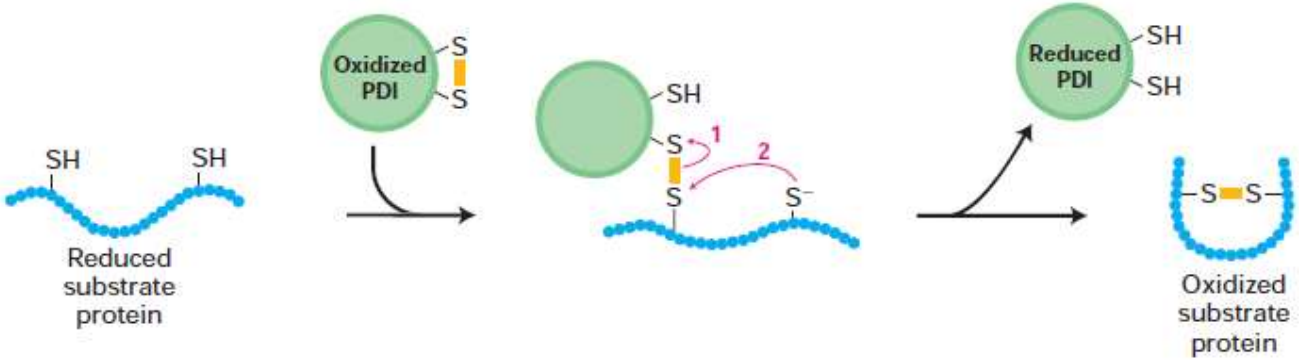
◀ EXPERIMENTAL FIGURE 16-3 Labeling experiments demonstrate that secretory proteins are localized to the ER lumen shortly after synthesis. Cells are incubated for a

Steps in the synthesis of the core portion of an *N-linked* oligosaccharide in the rough ER

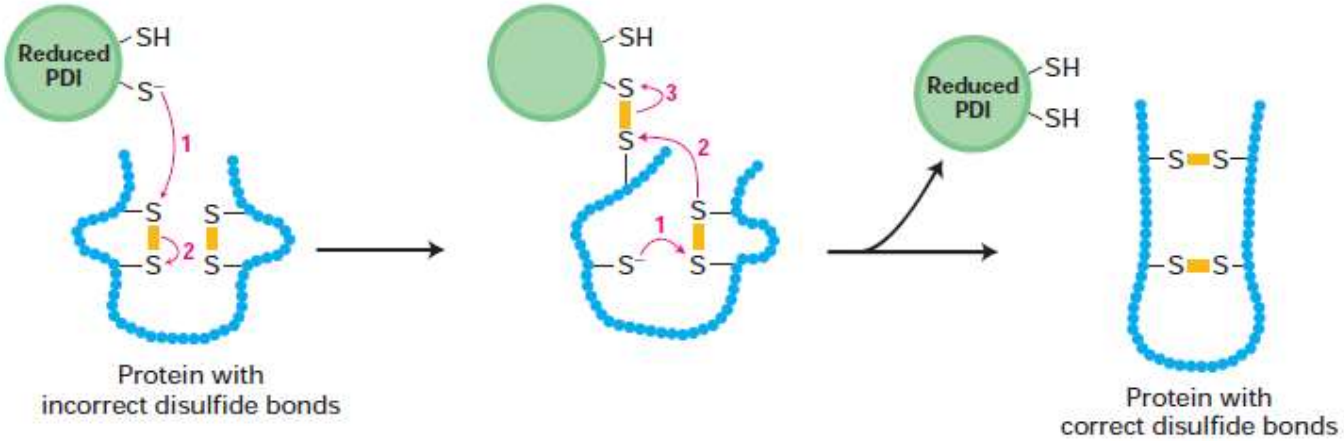


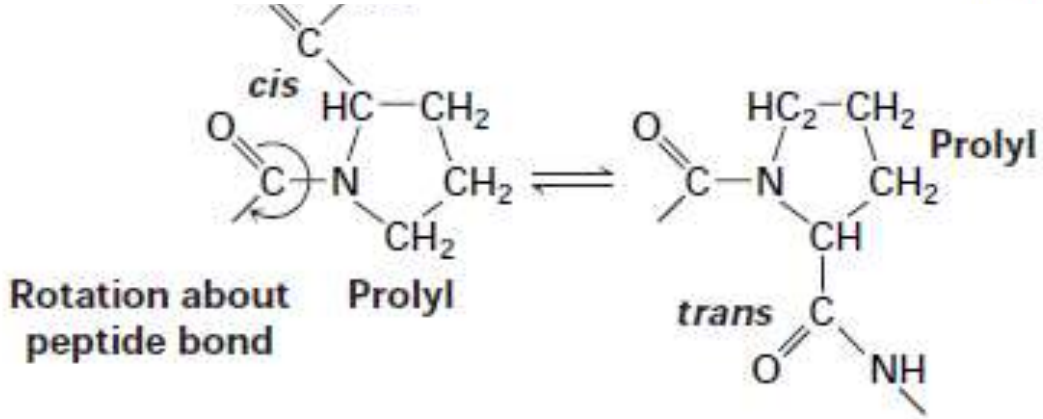

(Glc)3(Man)9(GlcNAc)2
(Man)8(GlcNAc)2
Dol
P
P
1
2
3
3a
3
4
To
cis-
Golgi
ER lumen
Dol = Dolichol
= N-Acetylglucosamine
= Mannose
= Glucose

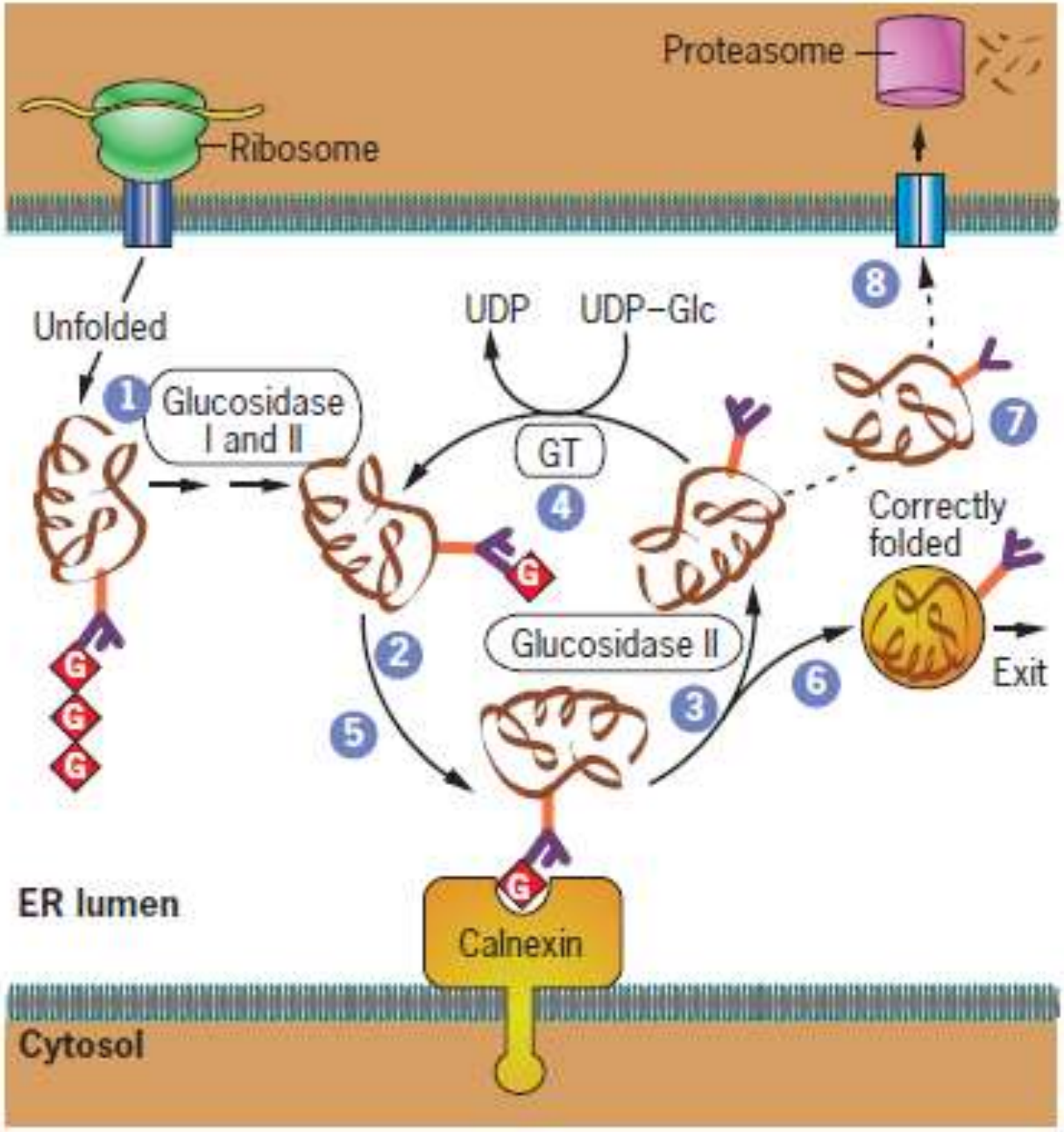
(a) Formation of a disulfide bond



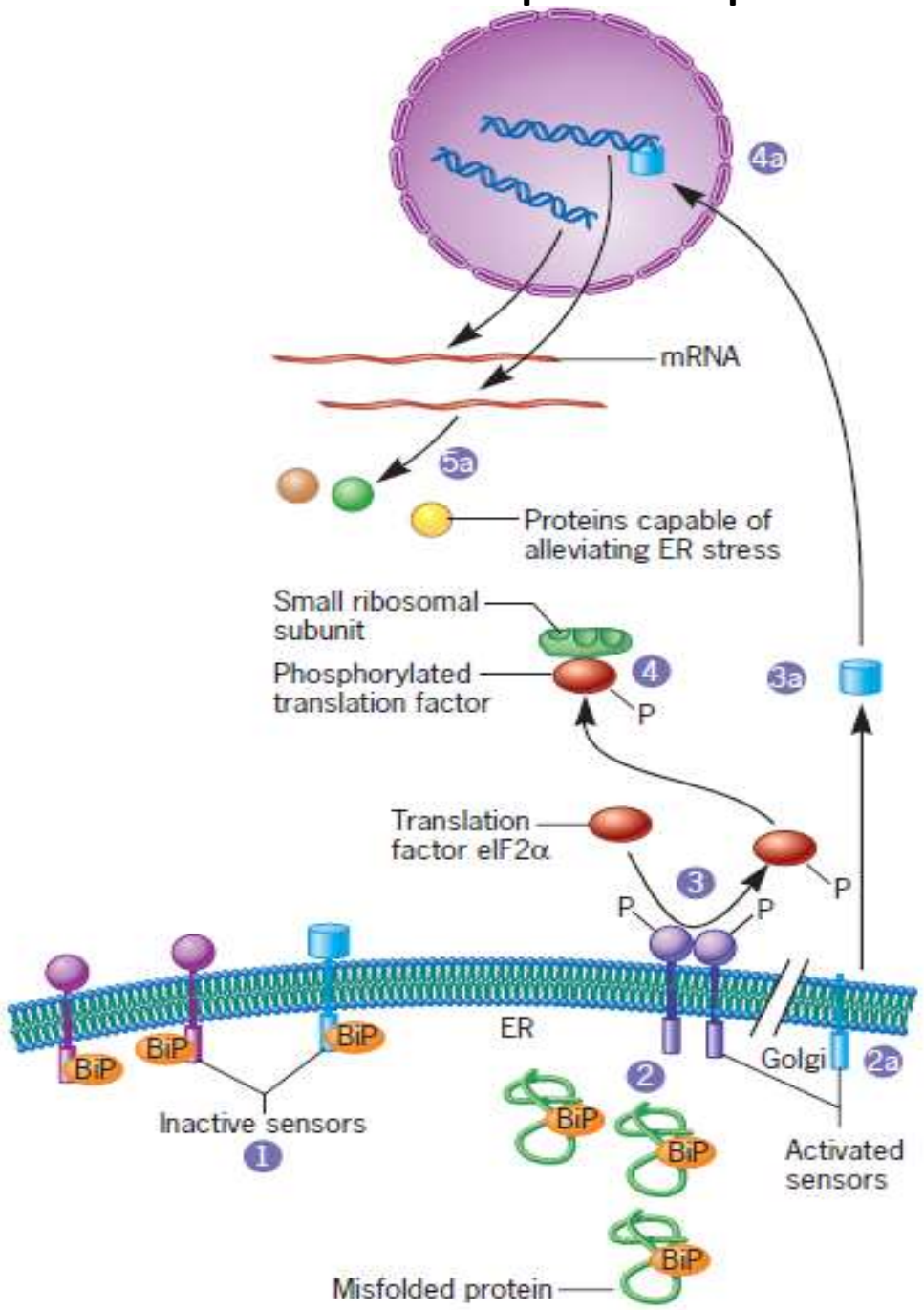
(b) Rearrangement of disulfide bonds






Proteasome
Ribosome
ERAD
Unfolded
UDP
UDP–Glc
1
Glucosidase I and II
GT
4
8
7
Correctly folded
G
2
Glucosidase II
6
Exit
5
3
ER lumen
Calnexin
Cytosol

A model of the mammalian unfolded protein response



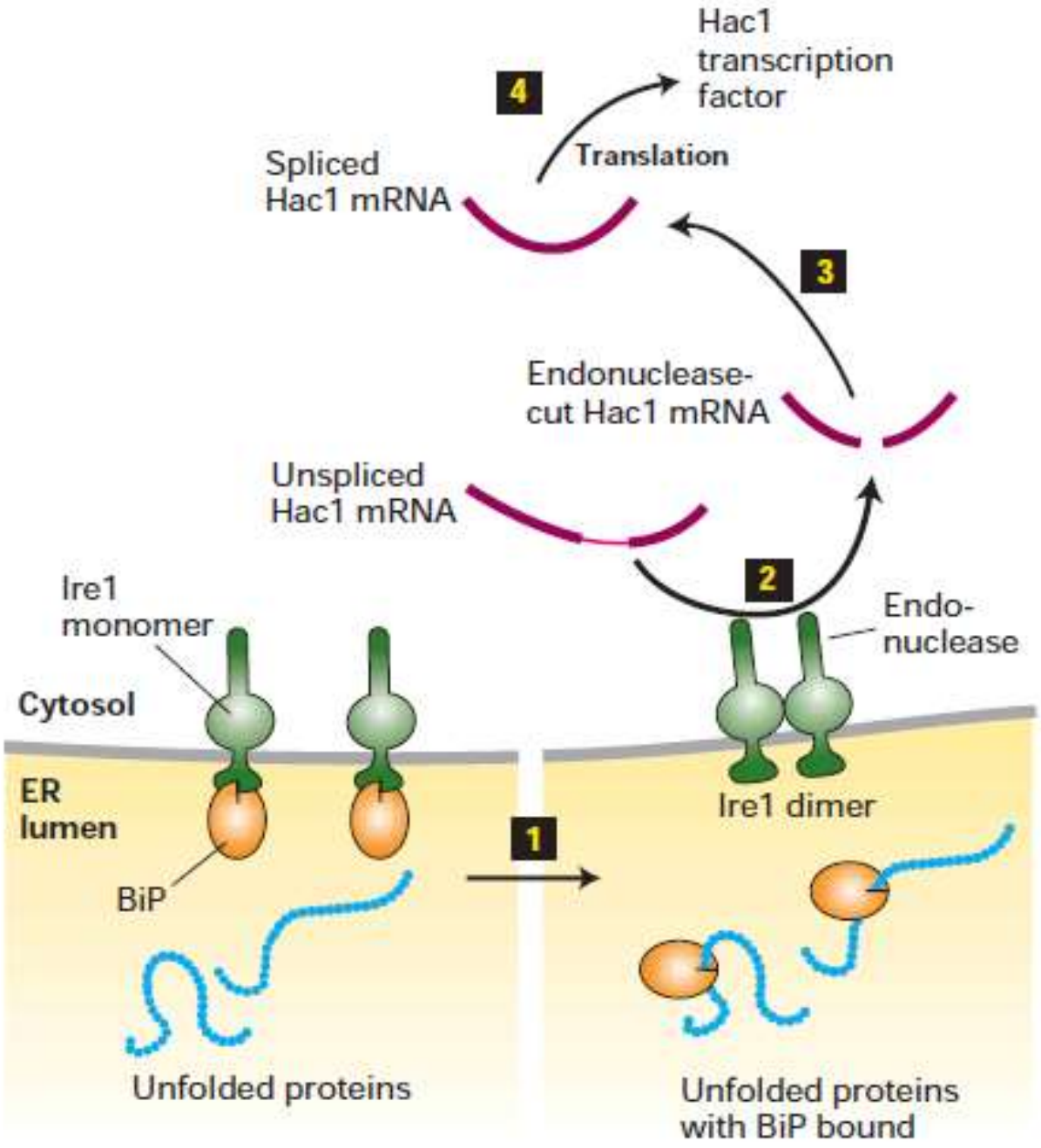
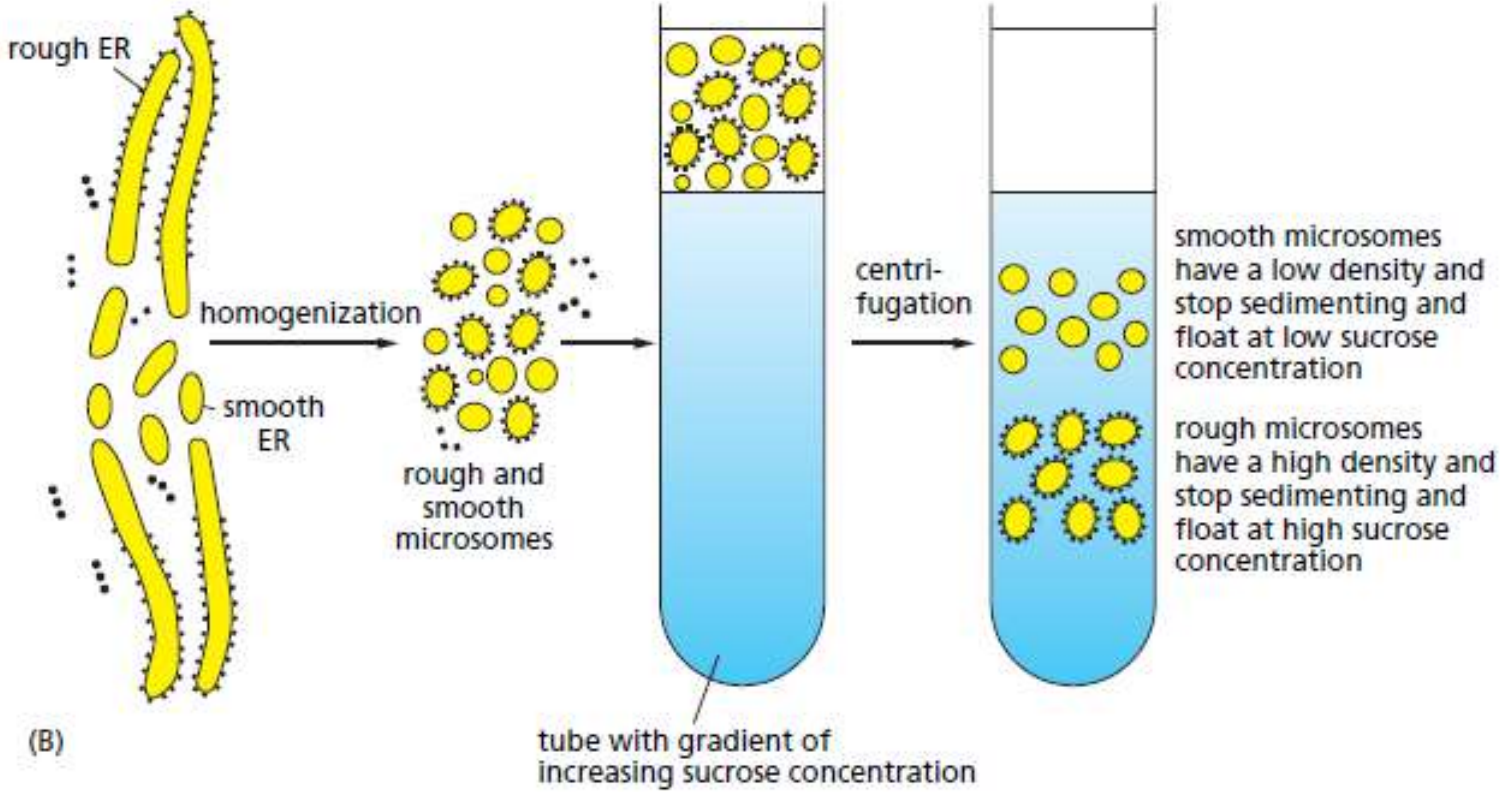
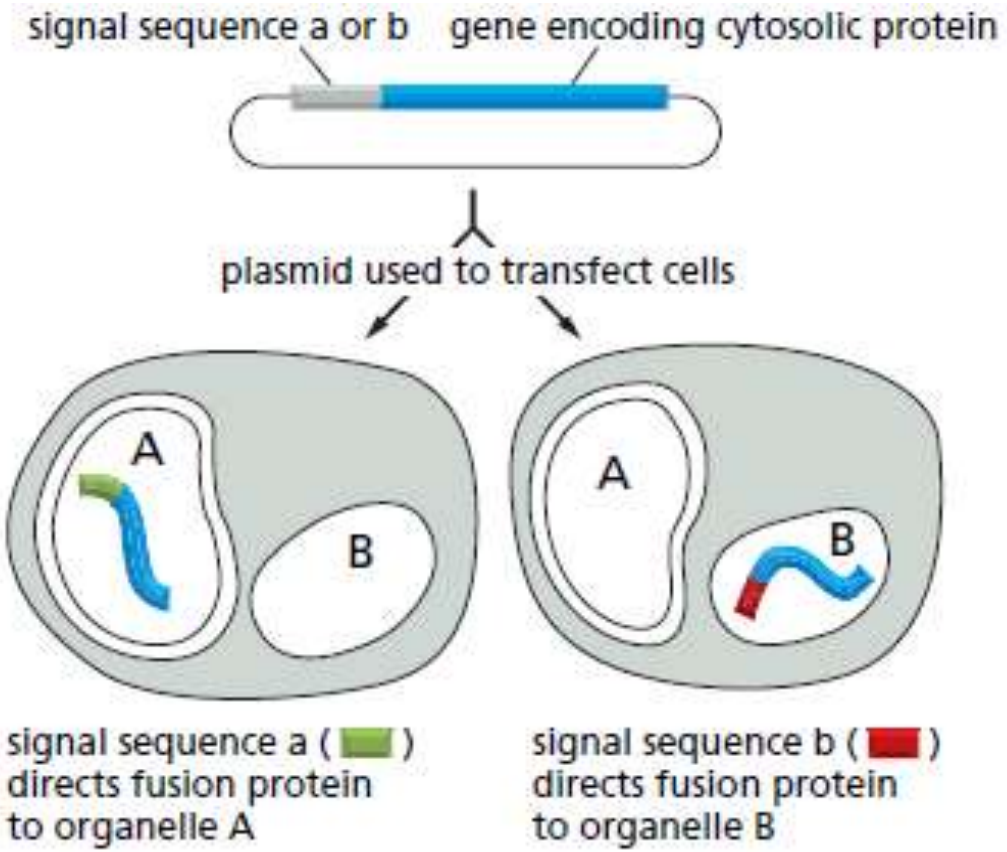

Hac1
transcription
factor
4
Translation
Spliced
Hac1 mRNA
3
Endonuclease-
cut Hac1 mRNA
Unspliced
Hac1 mRNA
2
Ire1
monomer
Endo-
nuclease
Cytosol
ER
lumen
Ire1 dimer
1
BiP
Unfolded proteins
Unfolded proteins
with BiP bound

Table 12–3 Some Typical 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^{-}



A TRANSFECTION APPROACH FOR DEFINING SIGNAL SEQUENCES



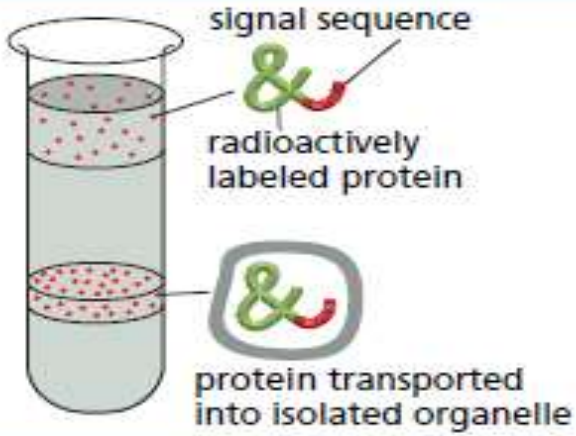
By altering the signal sequence using site-directed mutagenesis, we can determine which structural features are important for its function.

A BIOCHEMICAL APPROACH FOR STUDYING THE MECHANISM OF PROTEIN TRANSLOCATION

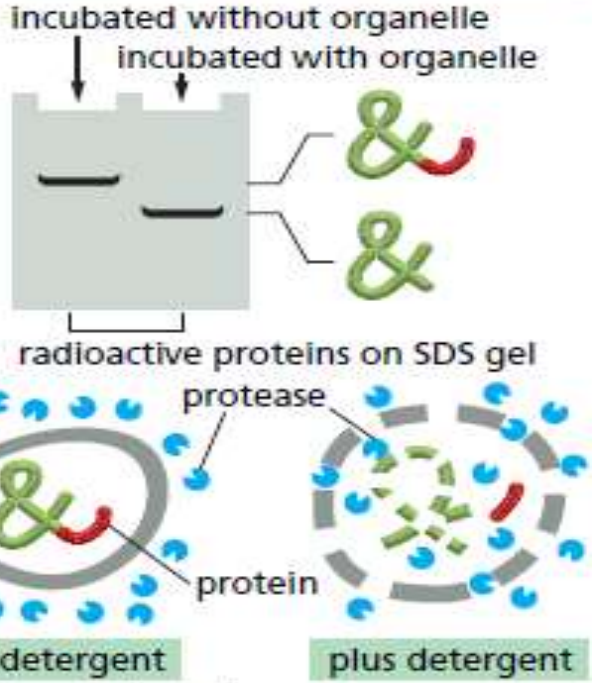
In this approach, a labeled protein containing a specific signal sequence is transported into isolated organelles *in vitro*. The labeled protein is usually produced by cell-free translation of a purified mRNA encoding the protein radioactive amino acids are used to label the newly synthesized protein 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.



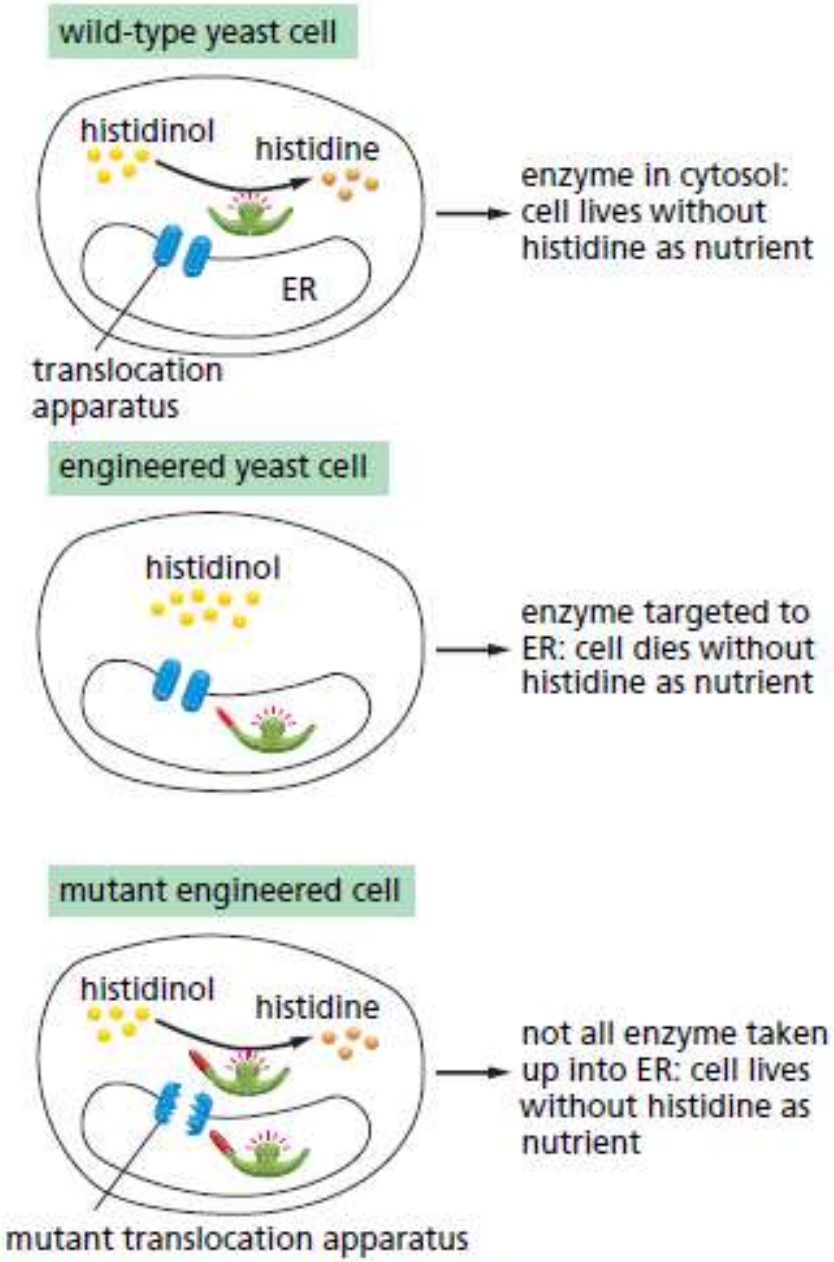
2. The signal sequence is removed by a specific protease that is present inside the organelle.

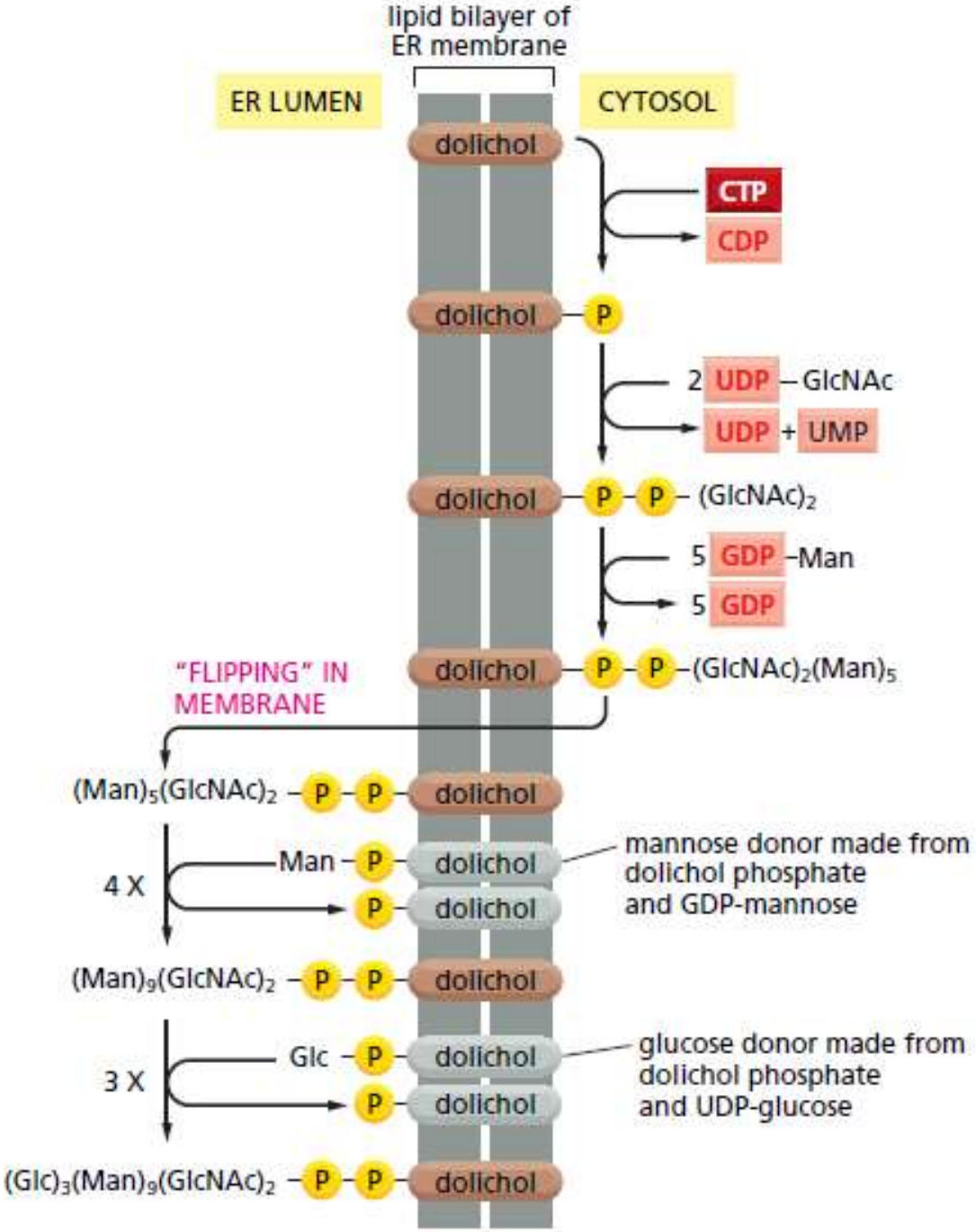


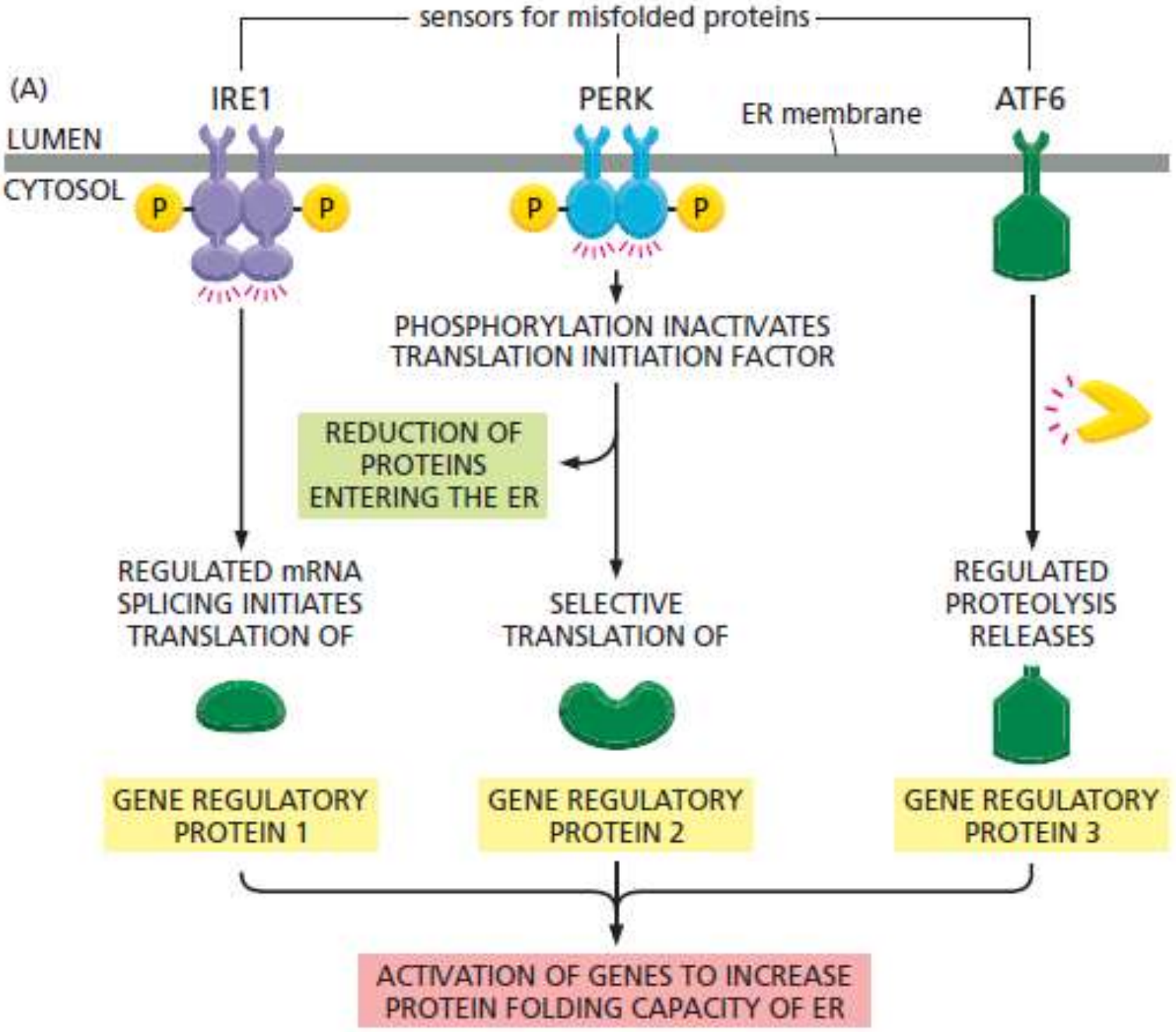
3. The protein is protected from digestion when proteases are added to the incubation medium, but is susceptible if a detergent is first added to disrupt the organelle membrane.

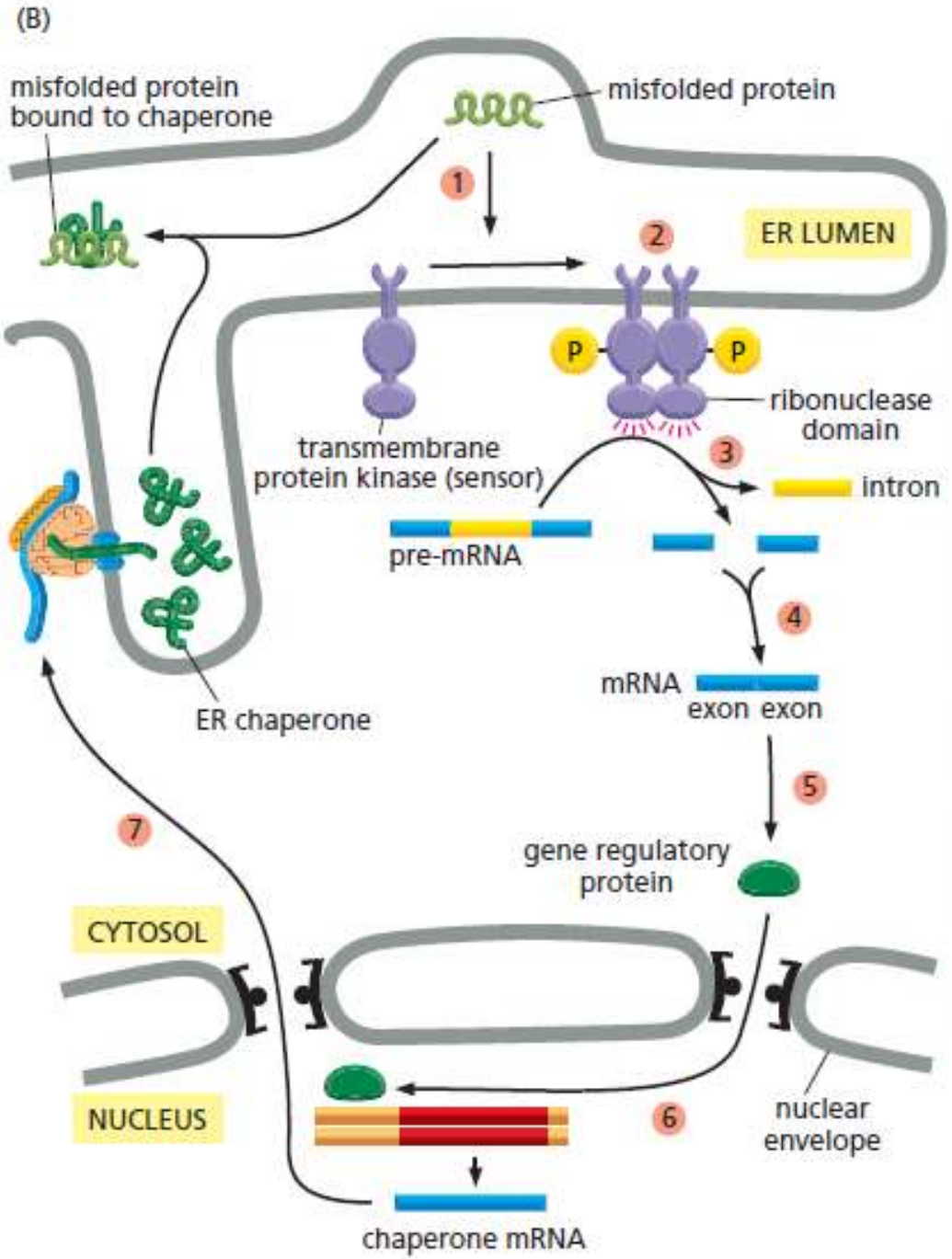
By exploiting such *in vitro* assays, one can determine what components (proteins, ATP, GTP, etc.) are required for the translocation process.

GENETIC APPROACHES FOR STUDYING THE MECHANISM OF PROTEIN TRANSLOCATION



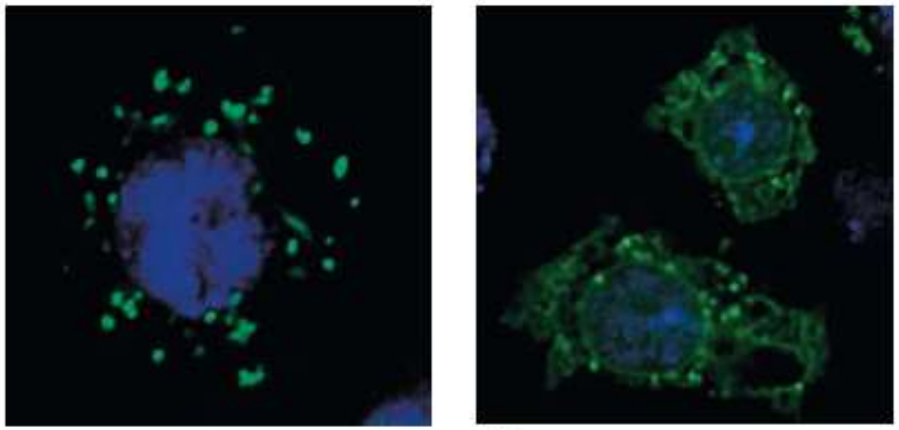

lipid bilayer of ER membrane
ER LUMEN
CYTOSOL
dolichol
CTP
CDP
dolichol P
2 UDP – GlcNAc
UDP + UMP
dolichol P P – (GlcNAc)2
5 GDP – Man
5 GDP
dolichol P P – (GlcNAc)2(Man)5
"FLIPPING" IN MEMBRANE
(Man)5(GlcNAc)2 P P dolichol
4 X
Man P dolichol
P dolichol
mannose donor made from dolichol phosphate and GDP-mannose
(Man)9(GlcNAc)2 P P dolichol
3 X
Glc P dolichol
P dolichol
glucose donor made from dolichol phosphate and UDP-glucose
(Glc)3(Man)9(GlcNAc)2 P P dolichol


sensors for misfolded proteins
(A)
IRE1
PERK
ATF6
ER membrane
LUMEN
CYTOSOL
P
P
P
P
PHOSPHORYLATION INACTIVATES TRANSLATION INITIATION FACTOR
REDUCTION OF PROTEINS ENTERING THE ER
REGULATED mRNA SPLICING INITIATES TRANSLATION OF
SELECTIVE TRANSLATION OF
REGULATED PROTEOLYSIS RELEASES
GENE REGULATORY PROTEIN 1
GENE REGULATORY PROTEIN 2
GENE REGULATORY PROTEIN 3
ACTIVATION OF GENES TO INCREASE PROTEIN FOLDING CAPACITY OF ER



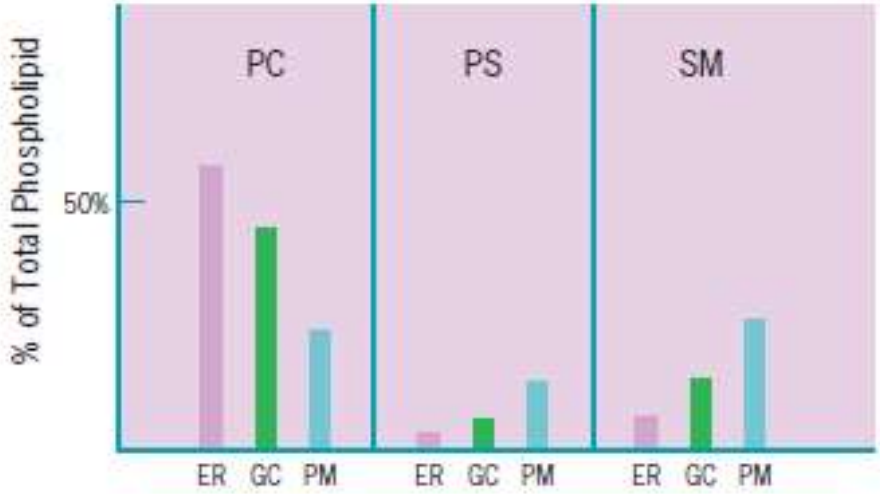
1. MISFOLDED PROTEINS IN ER SIGNAL THE NEED FOR MORE ER CHAPERONES BY ACTIVATING A TRANSMEMBRANE KINASE
2. ACTIVATED KINASE TURNS INTO AN ENDORIBONUCLEASE
3. ENDORIBONUCLEASE CUTS SPECIFIC RNA MOLECULES AT TWO POSITIONS, REMOVING AN INTRON
4. TWO EXONS ARE LIGATED TO FORM AN ACTIVE mRNA
5. mRNA IS TRANSLATED TO MAKE A GENE REGULATORY PROTEIN
6. GENE REGULATORY PROTEIN ENTERS NUCLEUS AND ACTIVATES GENES ENCODING ER CHAPERONES
7. CHAPERONES ARE MADE IN ER, WHERE THEY HELP FOLD PROTEINS

Inhibition of gene expression with RNA interference

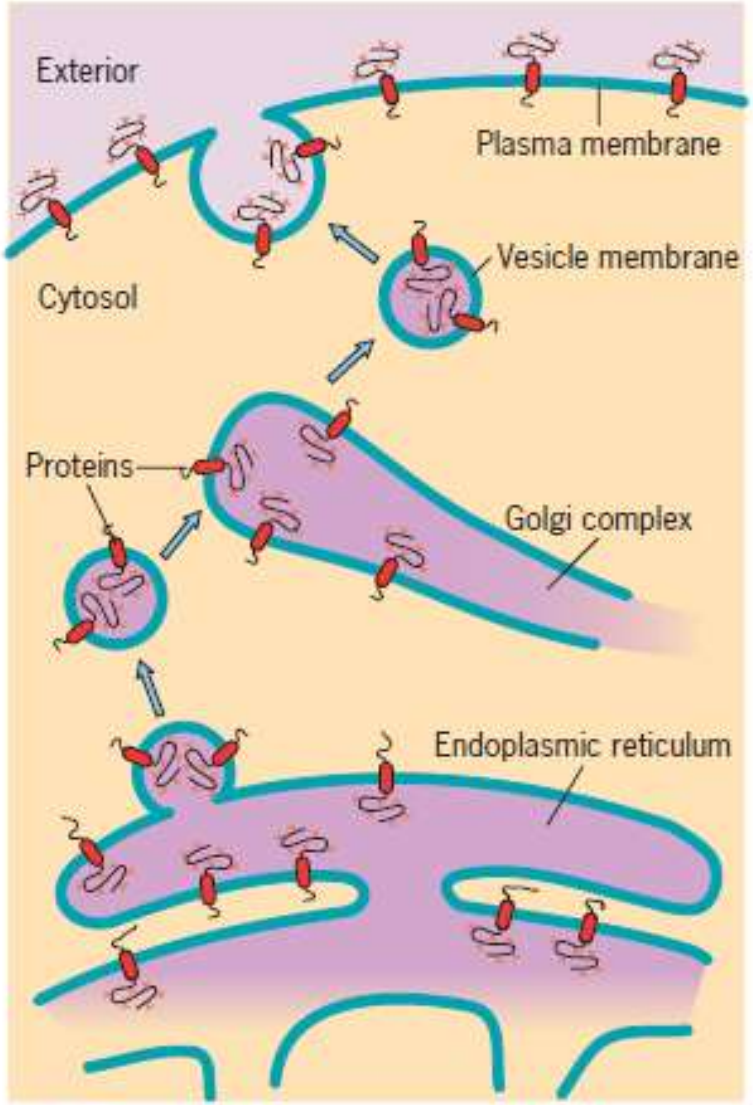


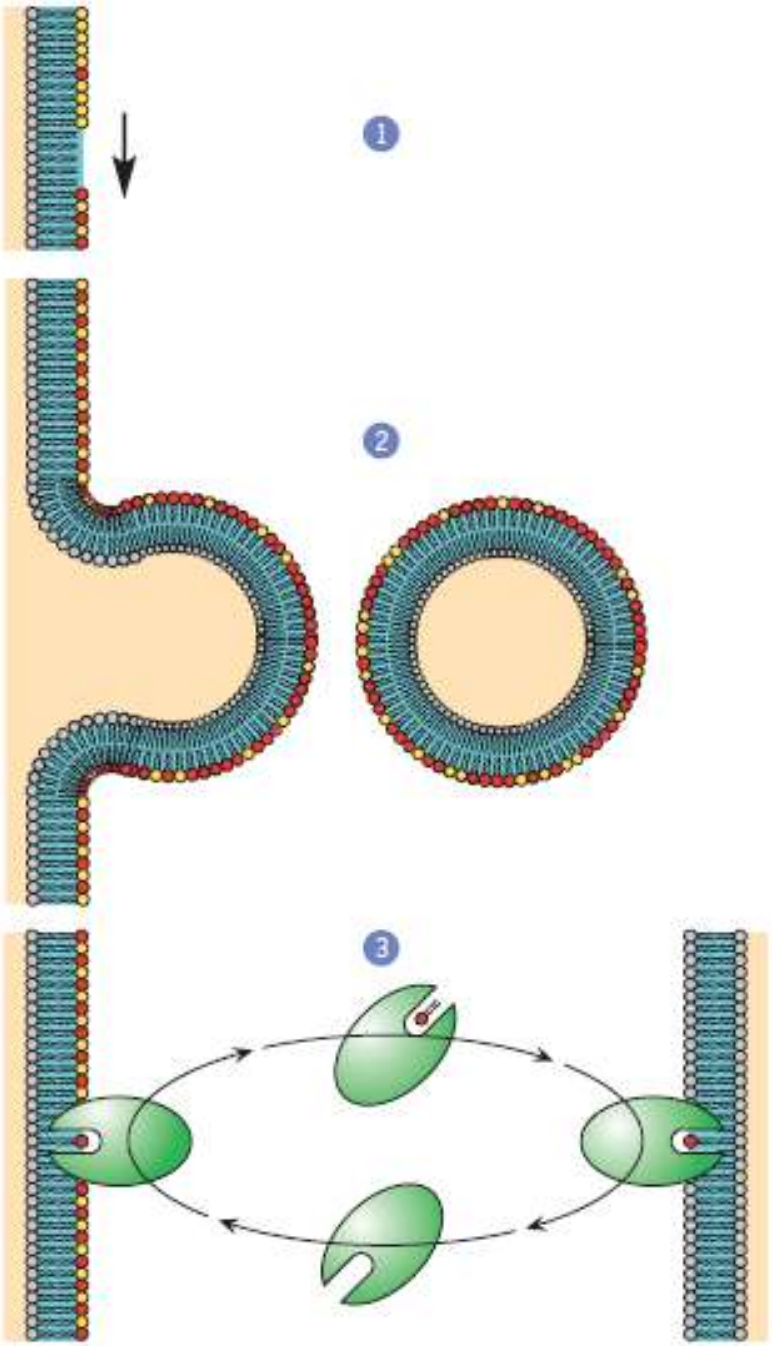
(a) (b)

Maintenance of membrane asymmetry.



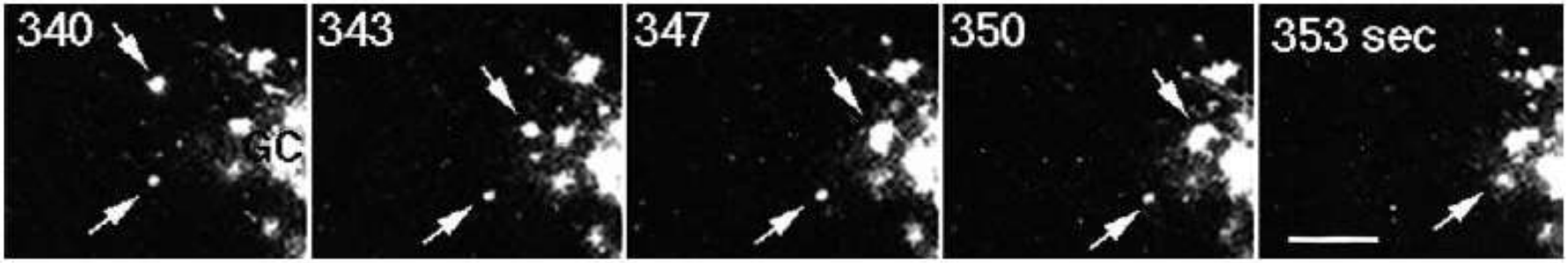
(a)

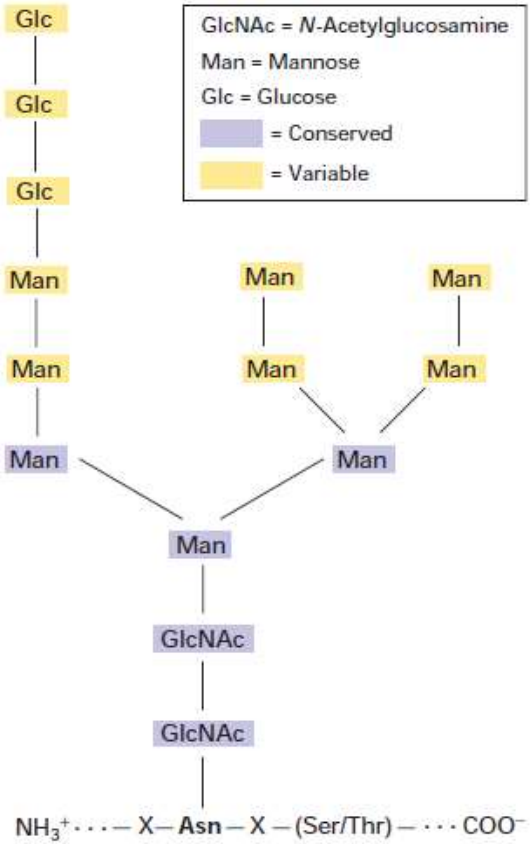




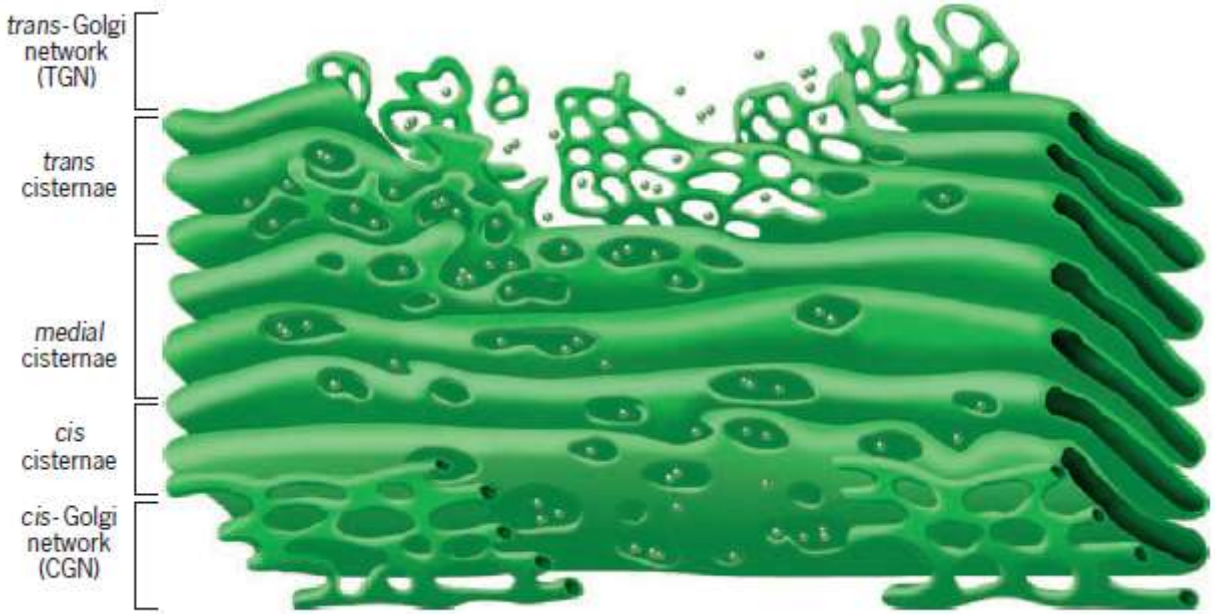
(b)

Visualizing membrane traffic with the use of a fluorescent tag

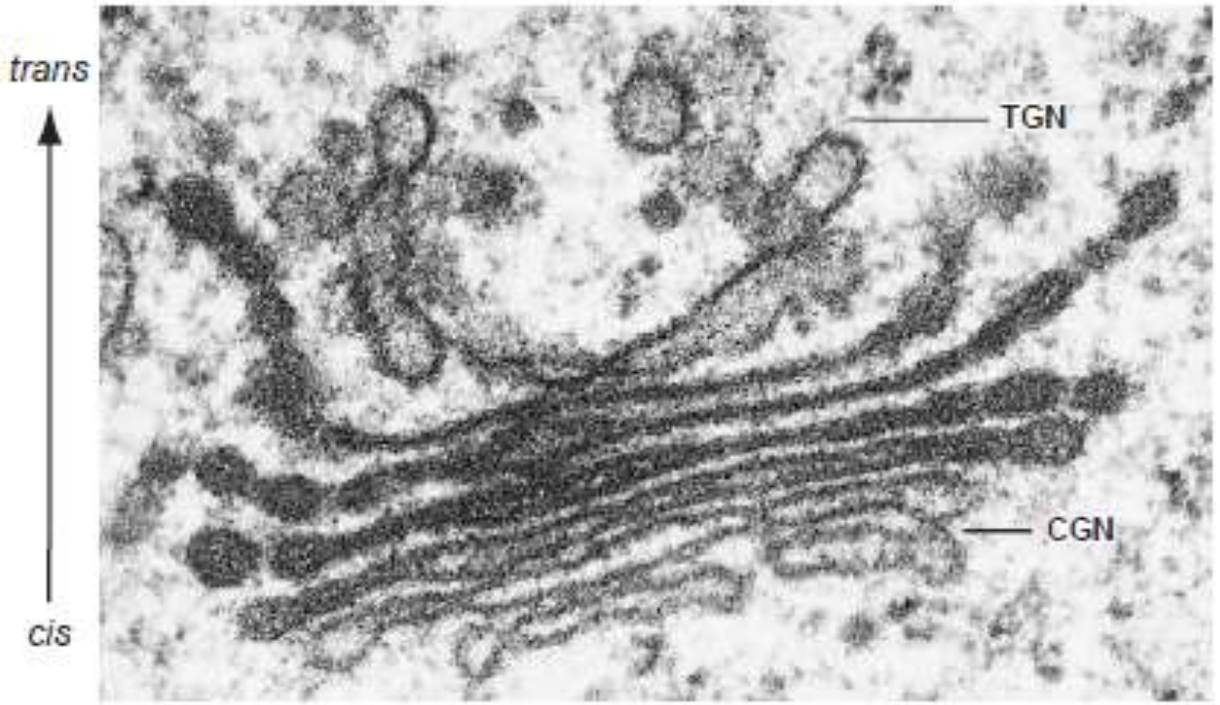




GlcNAc = *N*-Acetylglucosamine
Man = Mannose
Glc = Glucose
= Conserved
= Variable

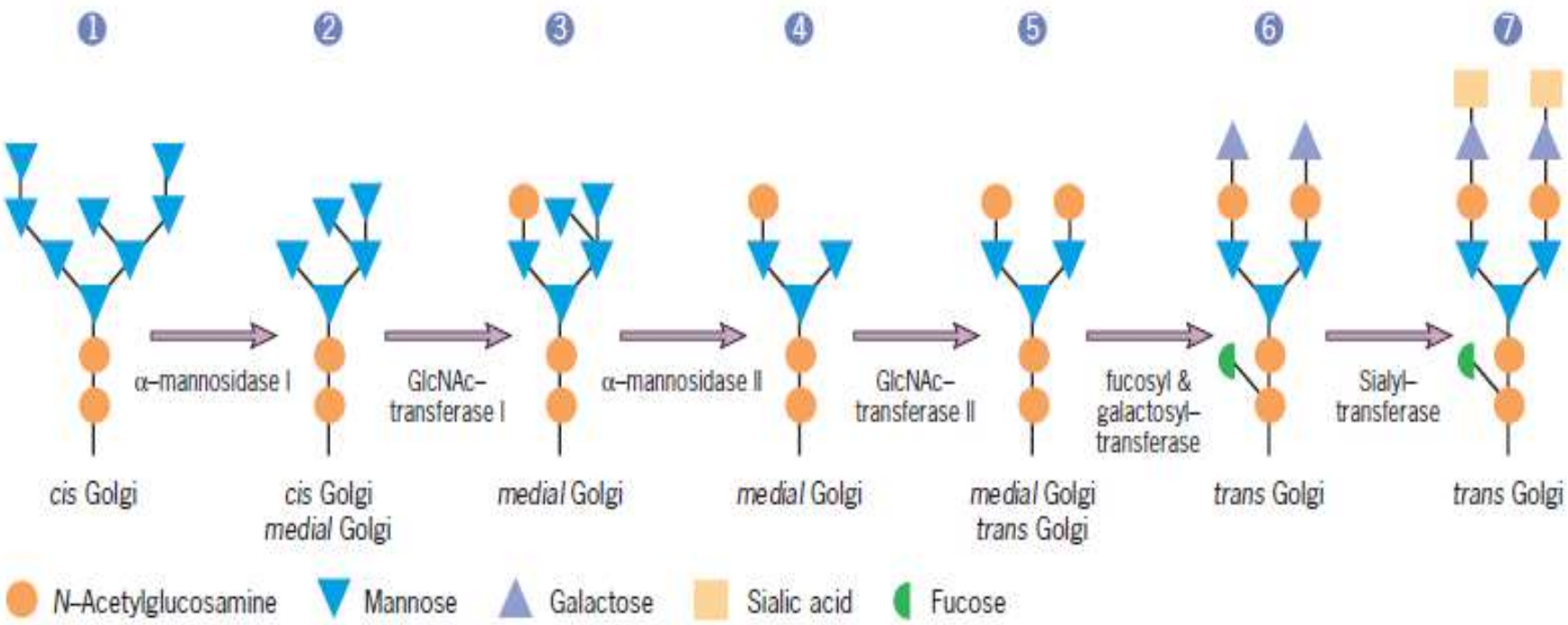

trans-Golgi network (TGN)
trans cisternae
medial cisternae
cis cisternae
cis-Golgi network (CGN)

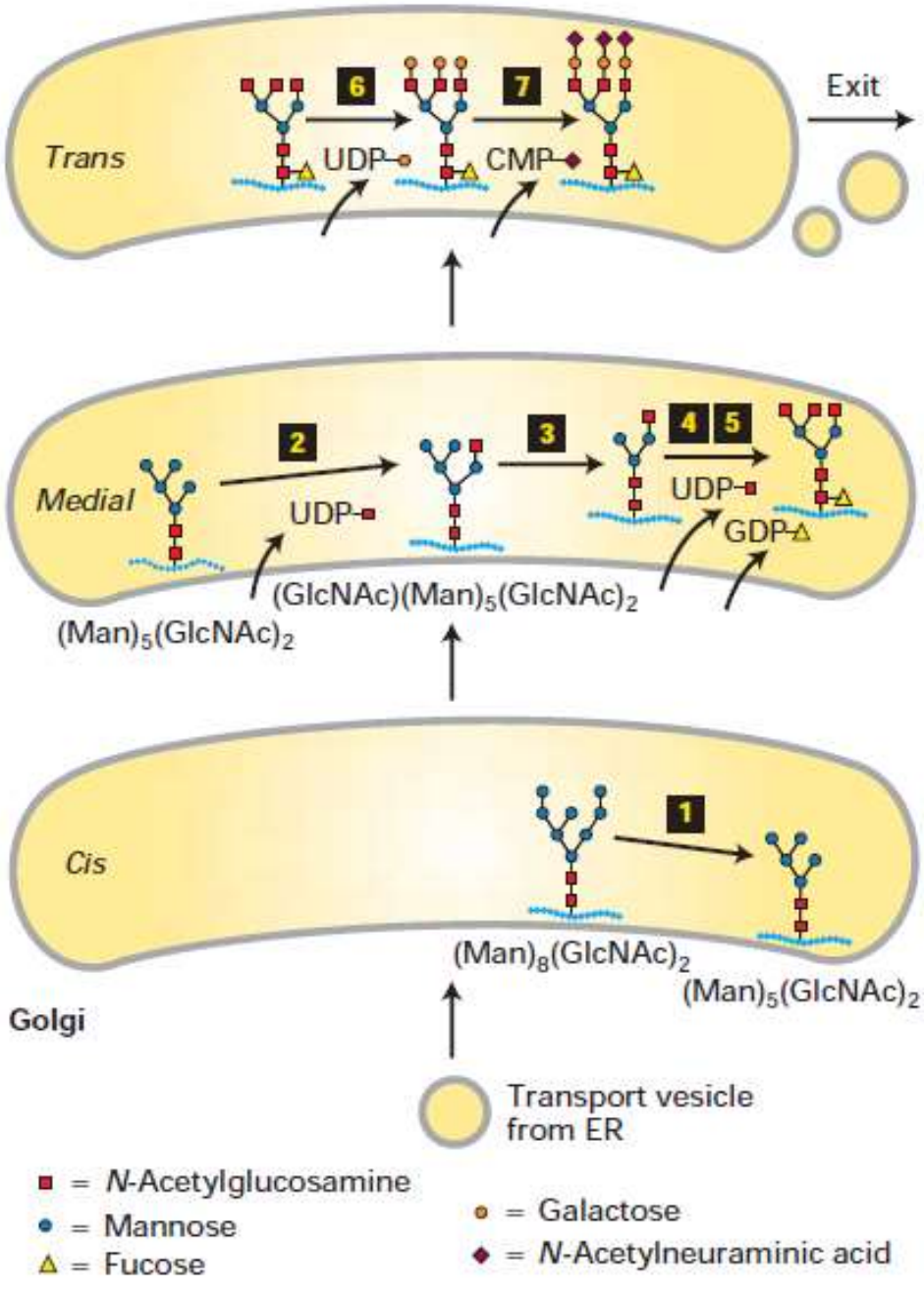
(a)


trans
cis
TGN
CGN

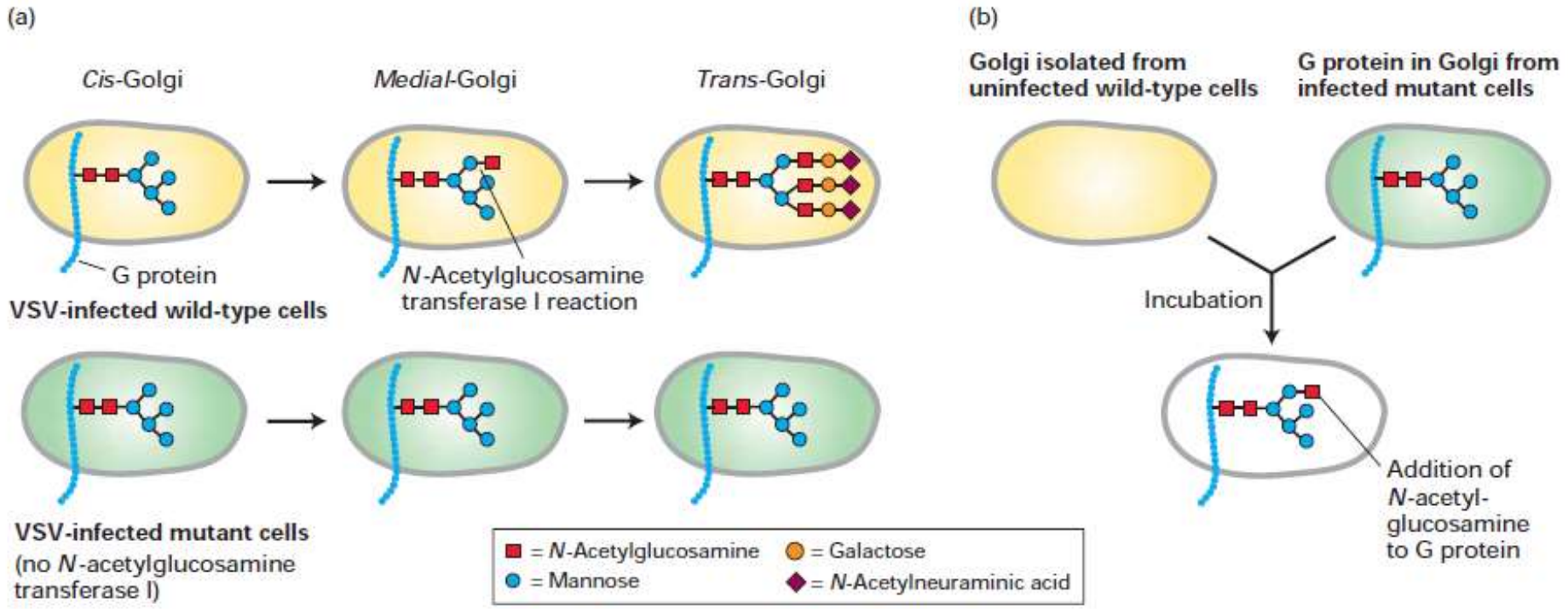
(b)

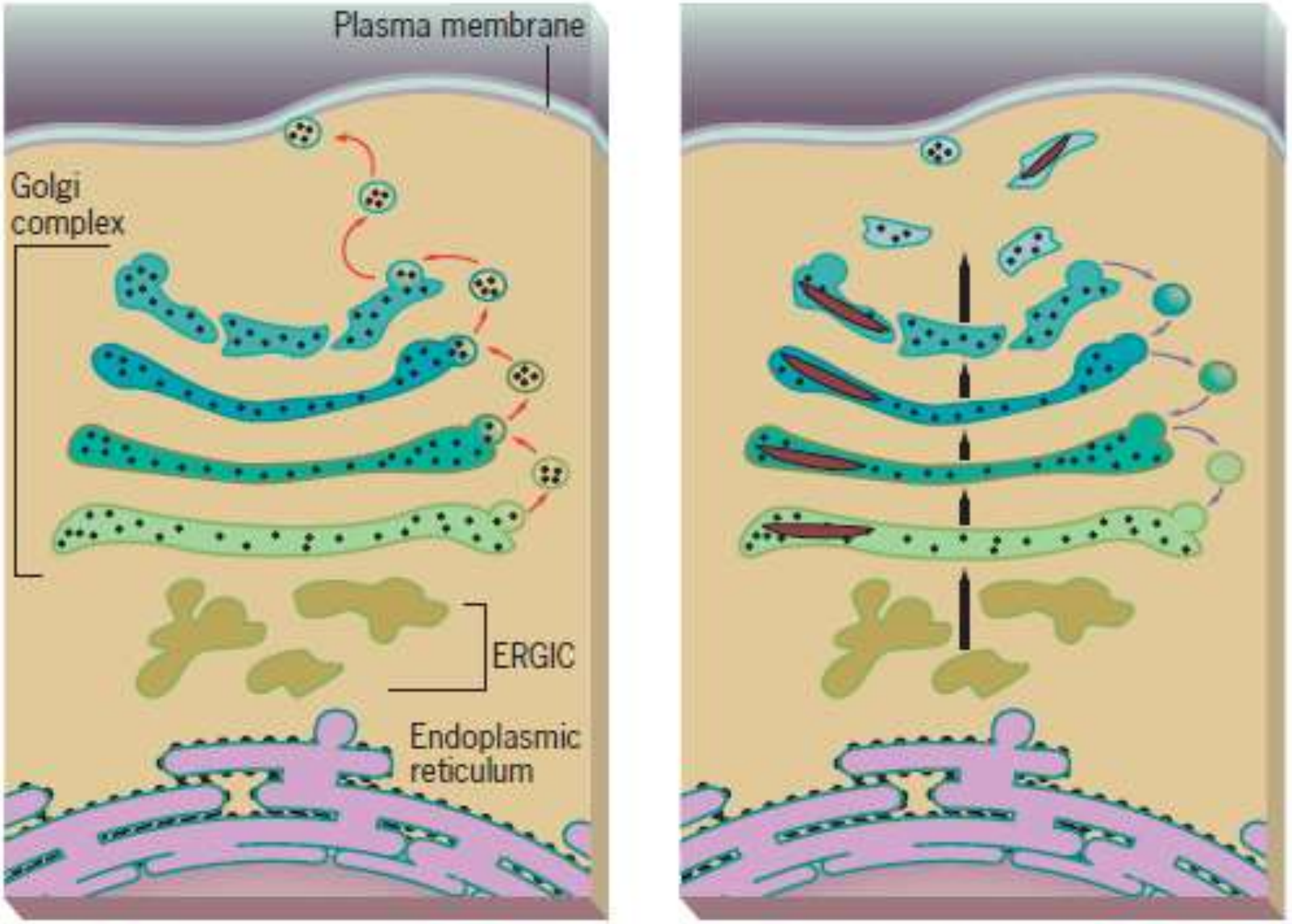
Steps in the glycosylation of a typical mammalian *N-linked oligosaccharide in the Golgi complex*




Trans
6
7
UDP
CMP
Exit
Medial
2
3
4
5
UDP
GDP
(GlcNAc)(Man)5(GlcNAc)2
(Man)5(GlcNAc)2
Cis
1
(Man)8(GlcNAc)2
(Man)5(GlcNAc)2
Golgi
Transport vesicle
from ER
= N-Acetylglucosamine
= Mannose
= Fucose
= Galactose
= N-Acetylneuraminic acid

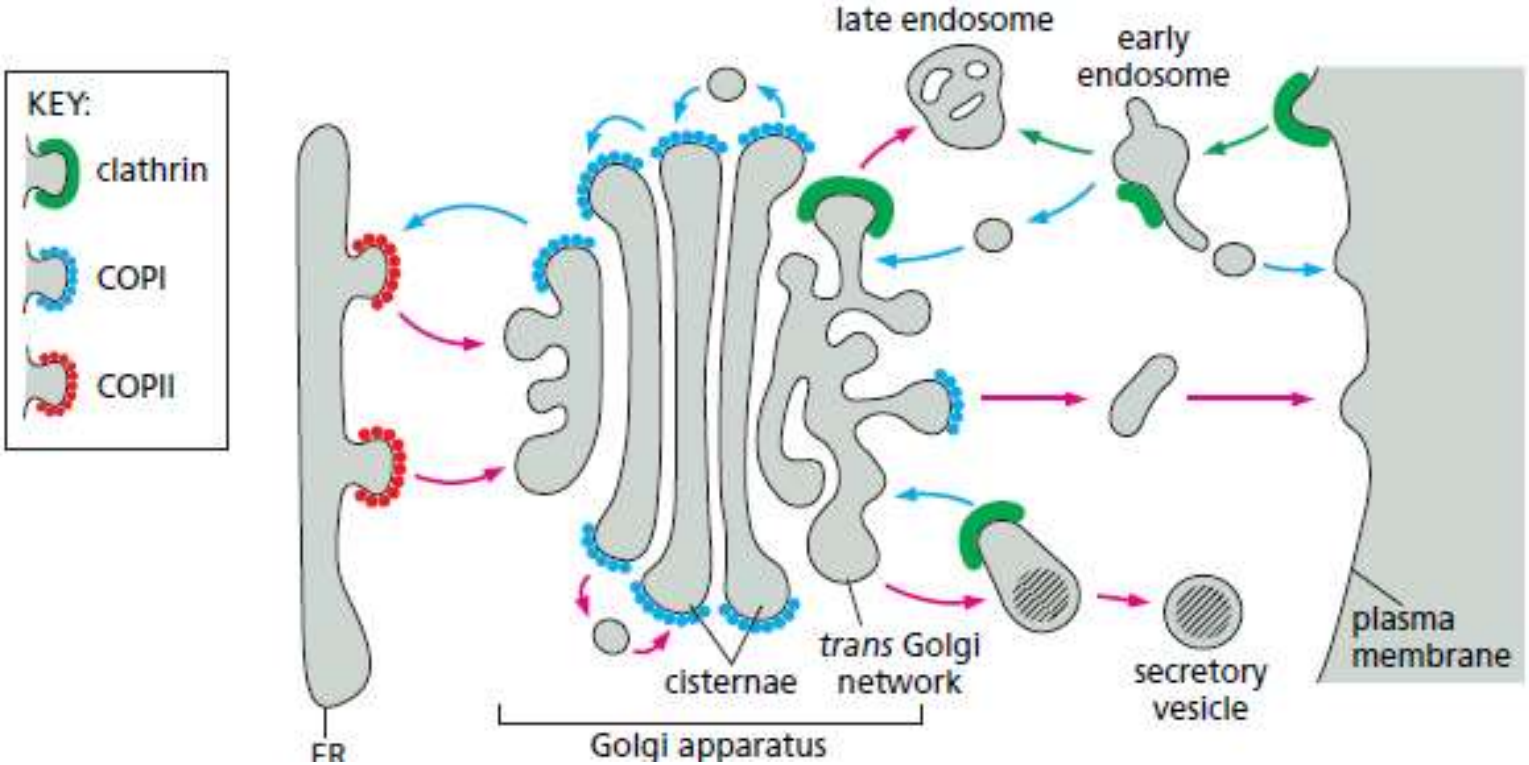
Protein transport from one Golgi cisternae to another can be assayed in a cell-free system

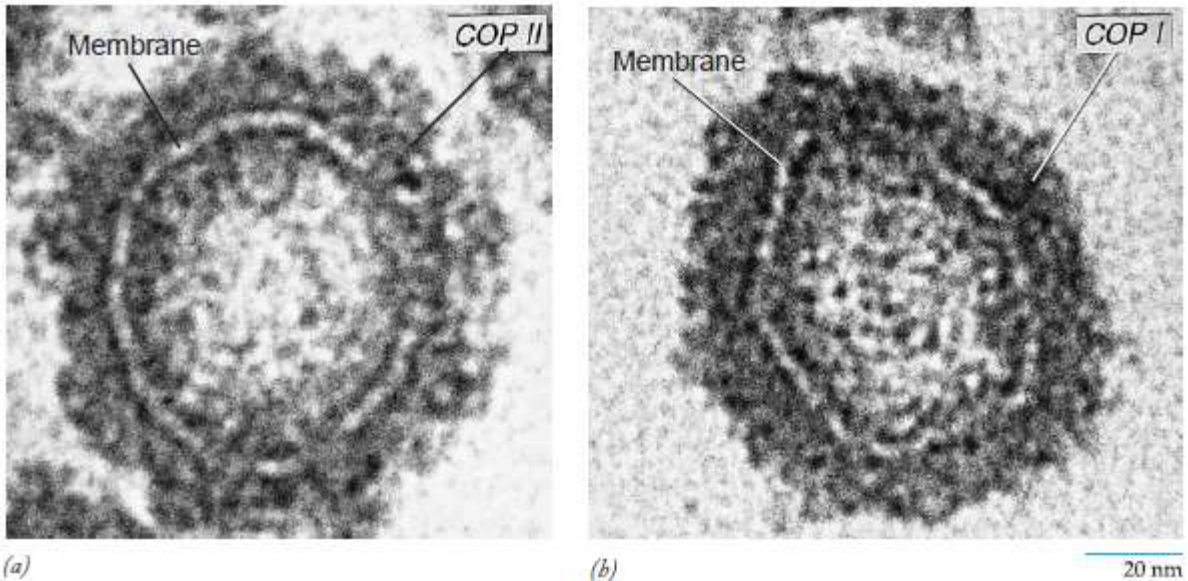


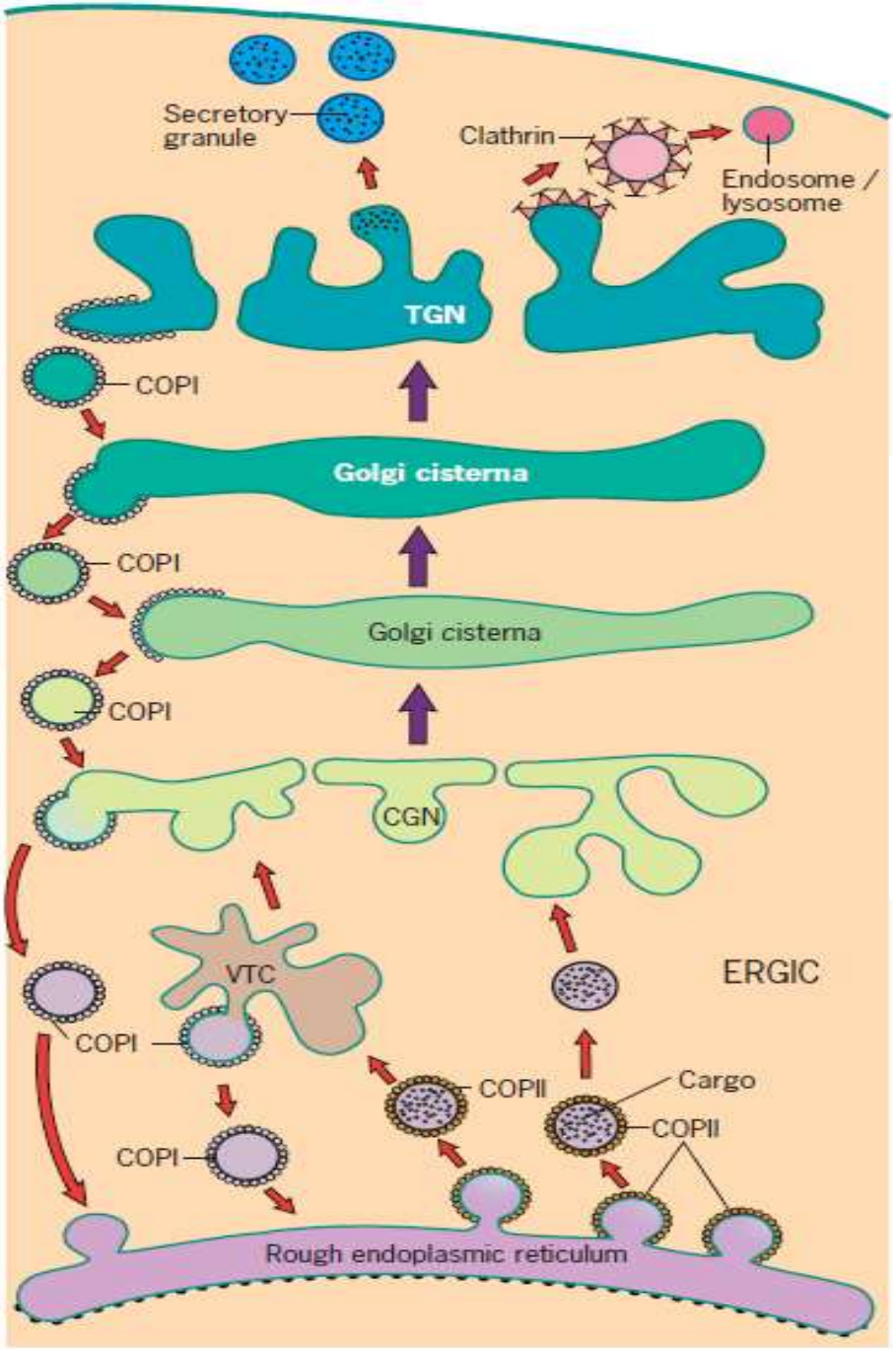


(a) Vesicular transport model

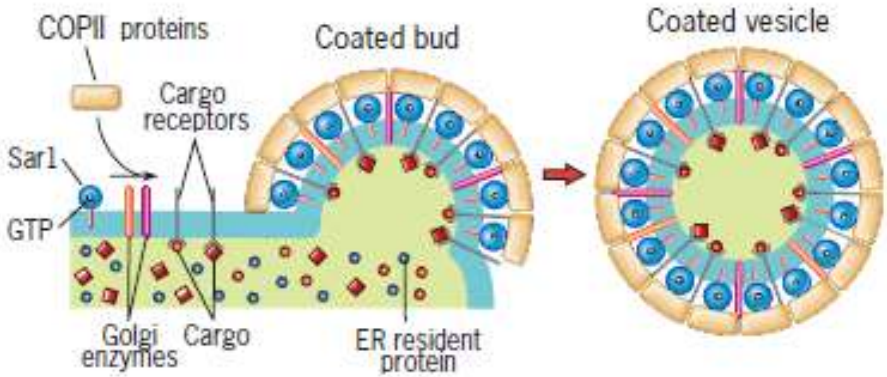
(b) Cisternal maturation model


KEY:
clathrin
COPI
COPII
late endosome
early endosome
plasma membrane
trans Golgi network
cisternae
secretory vesicle
ER
Golgi apparatus


Membrane
COP II
Membrane
COP I
(a)
(b)
20 nm

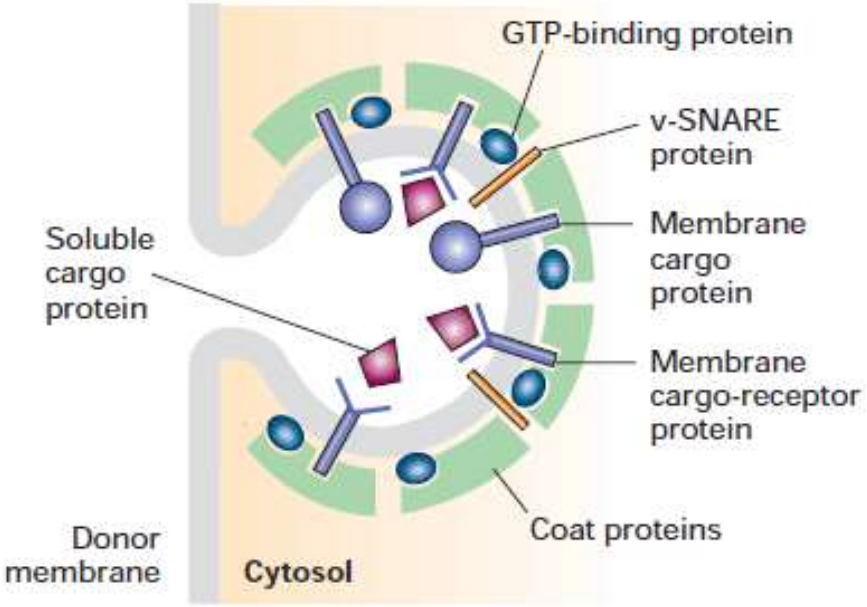

Secretory granule
Clathrin
Endosome / lysosome
TGN
COPI
Golgi cisterna
COPI
Golgi cisterna
COPI
CGN
VTC
ERGIC
COPI
COPII
Cargo
COPII
COPI
Rough endoplasmic reticulum

(a)


COPII proteins
Coated bud
Coated vesicle
Cargo receptors
Sar1
GTP
Golgi enzymes
Cargo
ER resident protein

(b)

(a) Coated vesicle budding



(b) Uncoated vesicle fusion

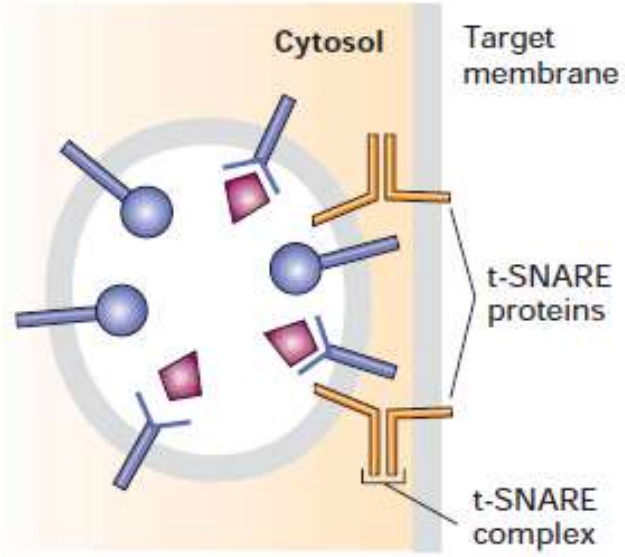
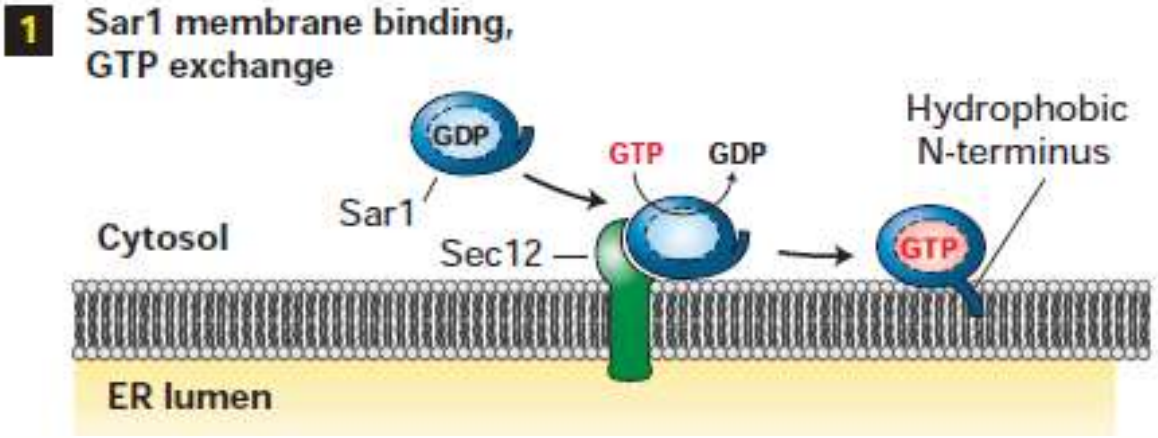
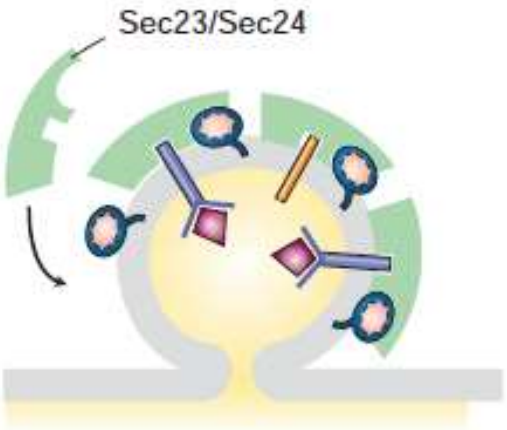


TABLE 17-1 Coated Vesicles Involved in Protein Trafficking

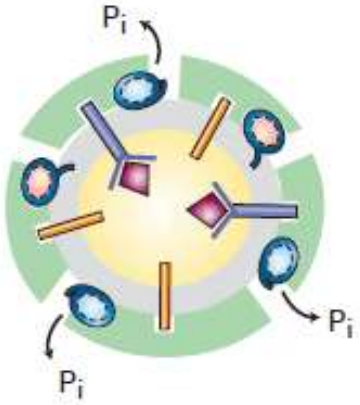
Vesicle Type	Coat Proteins	Associated GTPase	Transport Step Mediated
COPII	Sec23/Sec24 and Sec13/Sec31 complexes, Sec16	Sar1	ER to *cis*-Golgi
COPI	Coatomers containing seven different COP subunits	ARF	*cis*-Golgi to ER Later to earlier Golgi cisternae
Clathrin and adapter proteins*	Clathrin + AP1 complexes	ARF	*trans*-Golgi to endosome
	Clathrin + GGA	ARF	*trans*-Golgi to endosome
	Clathrin + AP2 complexes	ARF	Plasma membrane to endosome
	AP3 complexes	ARF	Golgi to lysosome, melanosome, or platelet vesicles

*Each type of AP complex consists of four different subunits. It is not known whether the coat of AP3 vesicles contains clathrin.

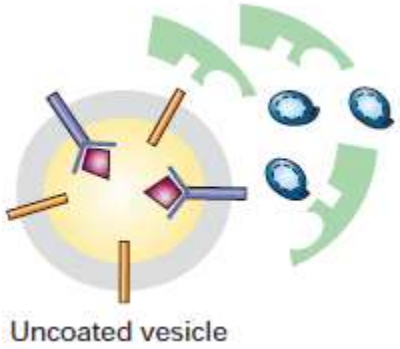




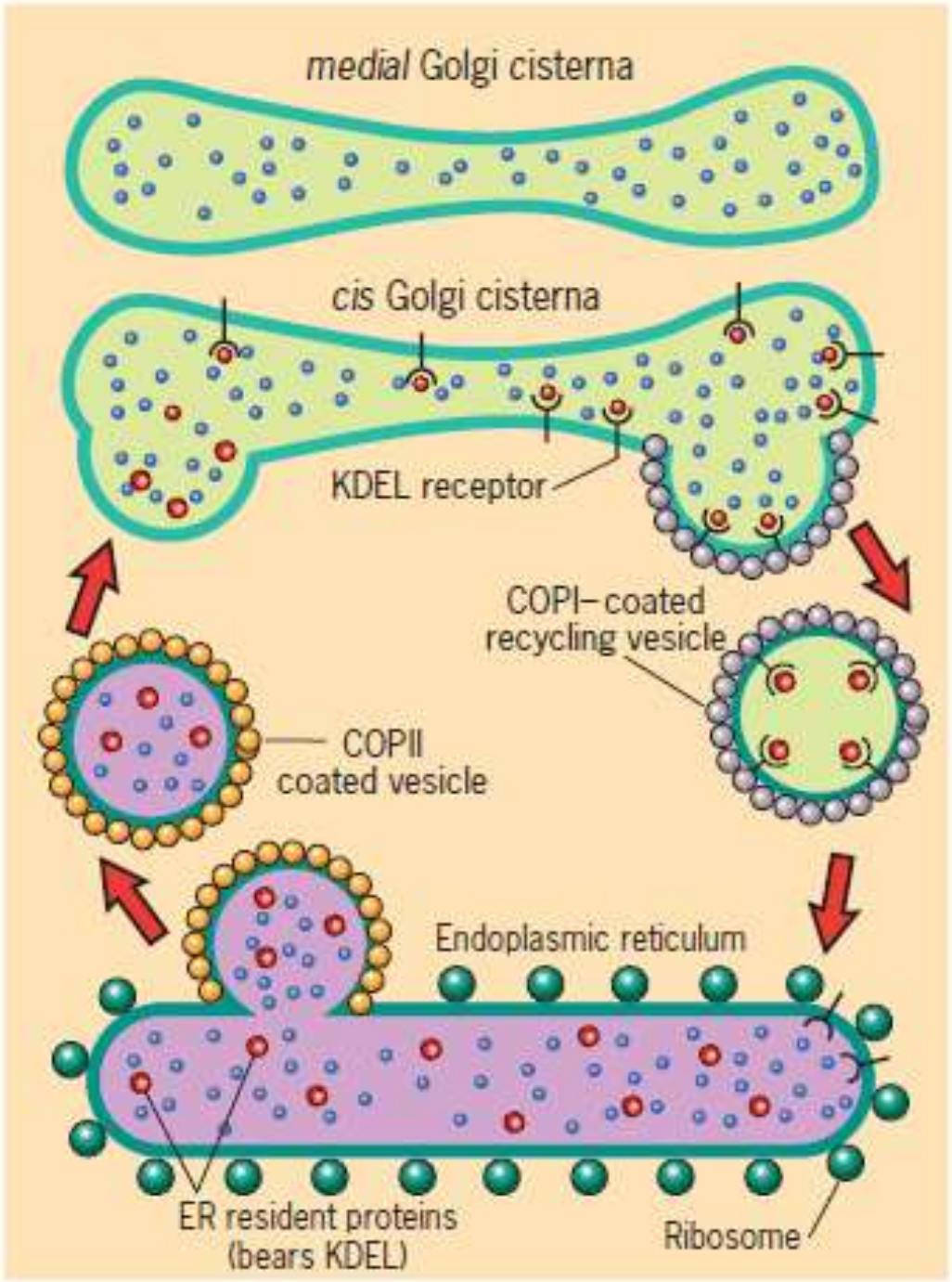
2 COPII coat assembly

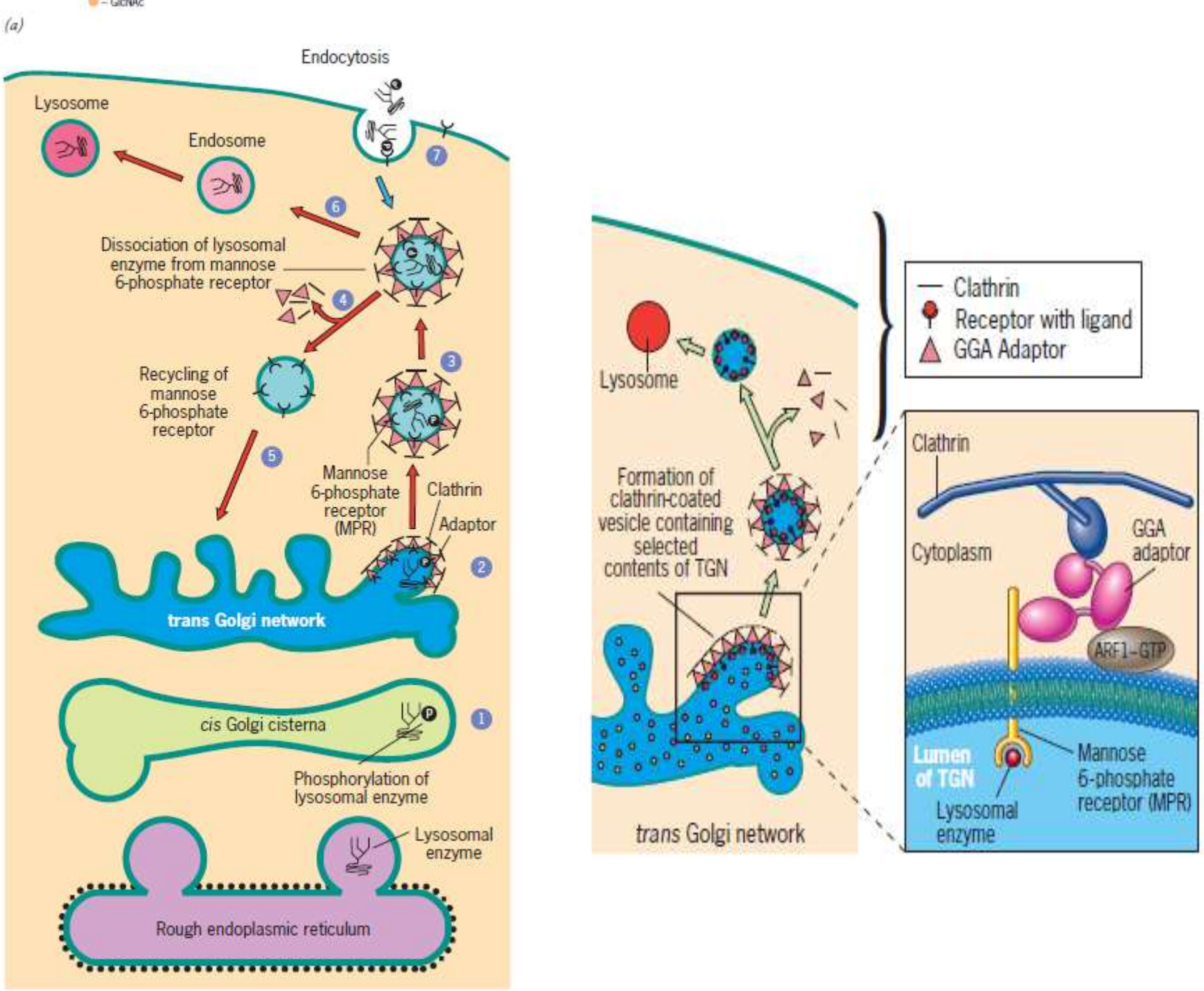


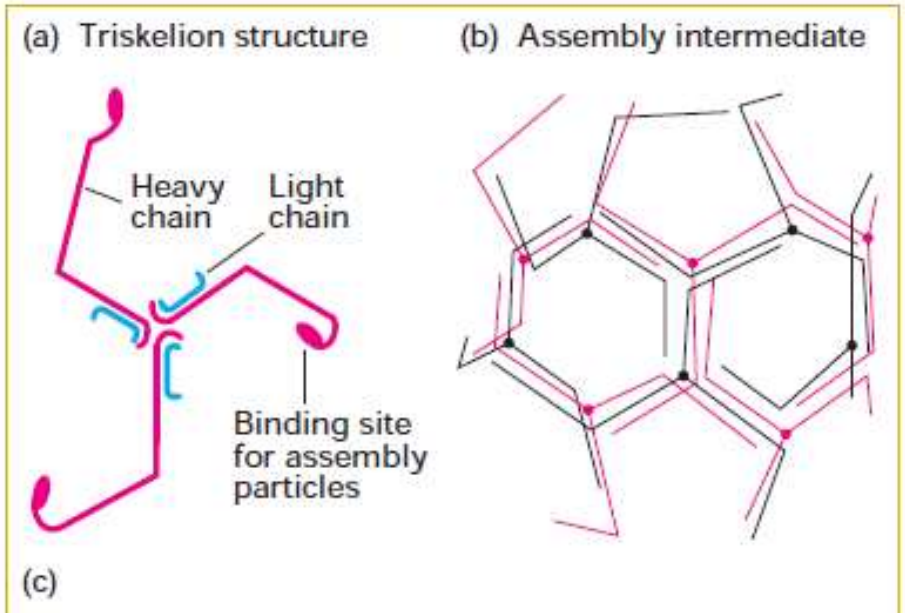
3 GTP hydrolysis

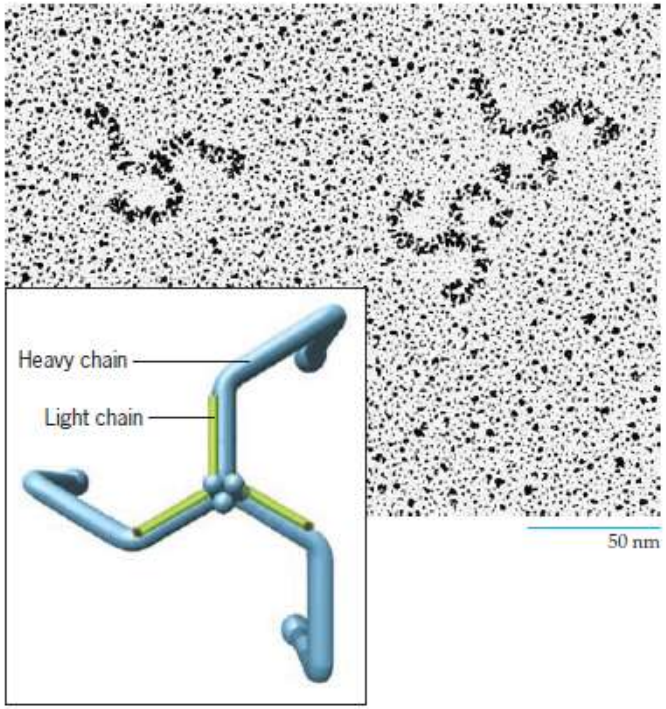


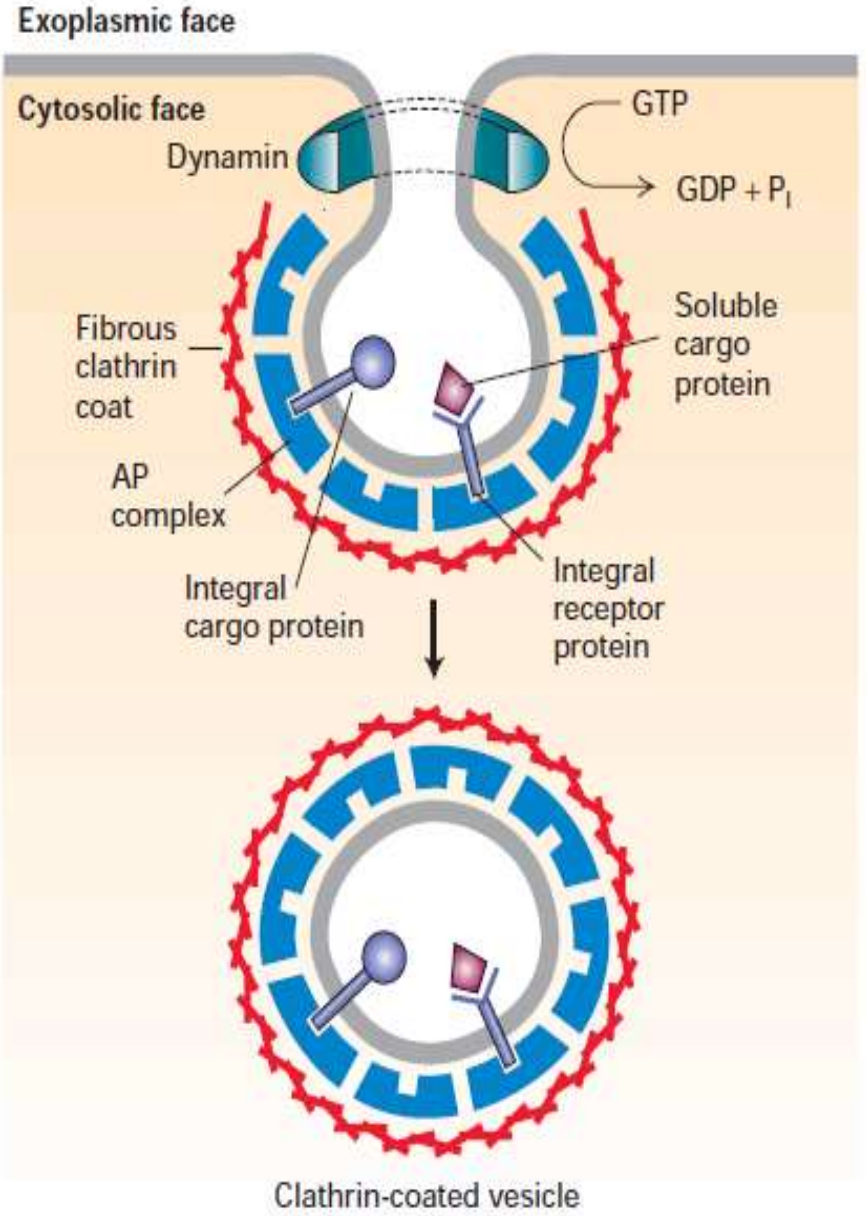
4 Coat disassembly

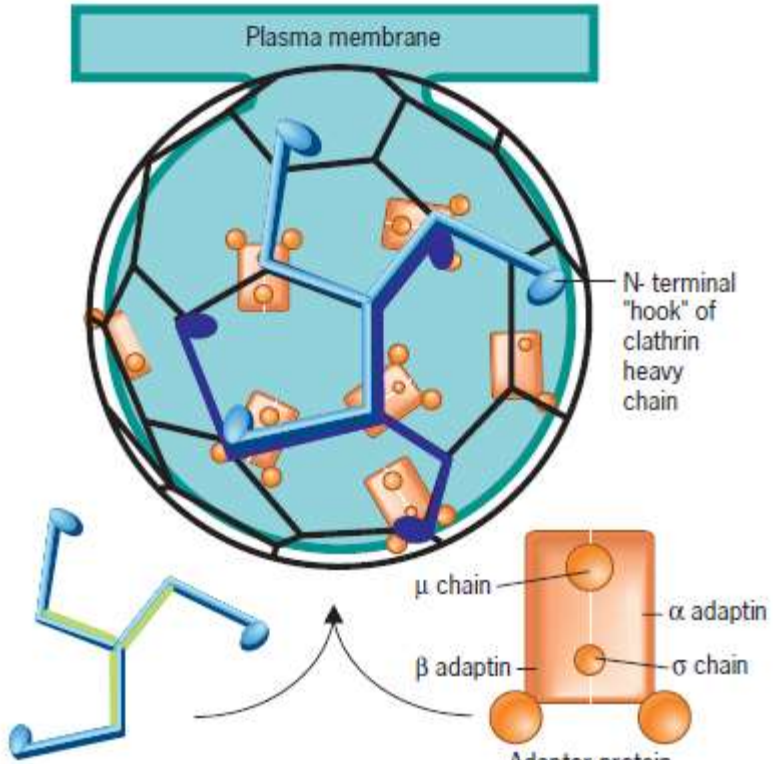

medial Golgi cisterna
cis Golgi cisterna
KDEL receptor
COPI–coated
recycling vesicle
COPII
coated vesicle
Endoplasmic reticulum
ER resident proteins
(bears KDEL)
Ribosome


(a)
Endocytosis
Lysosome
Endosome
7
6
Dissociation of lysosomal enzyme from mannose 6-phosphate receptor
4
3
Recycling of mannose 6-phosphate receptor
5
Mannose 6-phosphate receptor (MPR)
Clathrin
Adaptor
2
trans Golgi network
cis Golgi cisterna
1
Phosphorylation of lysosomal enzyme
Lysosomal enzyme
Rough endoplasmic reticulum
Lysosome
Formation of clathrin-coated vesicle containing selected contents of TGN
trans Golgi network
Clathrin
Receptor with ligand
GGA Adaptor
Clathrin
Cytoplasm
GGA adaptor
ARF1-GTP
Lumen of TGN
Lysosomal enzyme
Mannose 6-phosphate receptor (MPR)

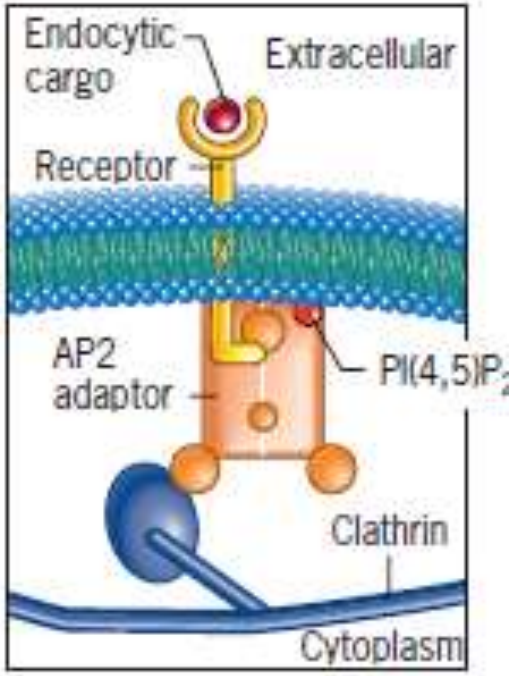

(a) Triskelion structure
(b) Assembly intermediate
Heavy chain
Light chain
Binding site for assembly particles
(c)


Heavy chain
Light chain
50 nm

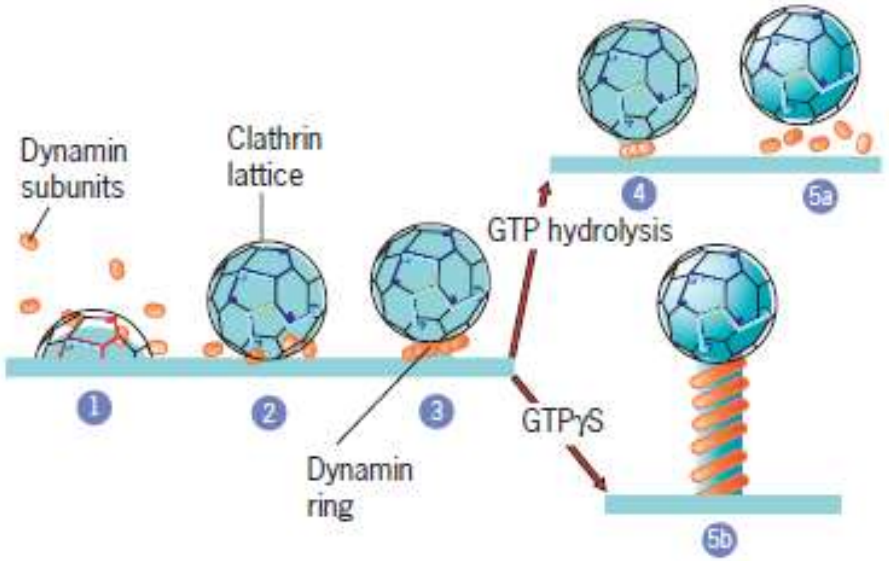

Exoplasmic face
Cytosolic face
Dynamin
GTP
GDP + P$_i$
Fibrous clathrin coat
Soluble cargo protein
AP complex
Integral cargo protein
Integral receptor protein
Clathrin-coated vesicle


Plasma membrane
N- terminal "hook" of clathrin heavy chain
μ chain
α adaptin
β adaptin
σ chain
Clathrin triskelion

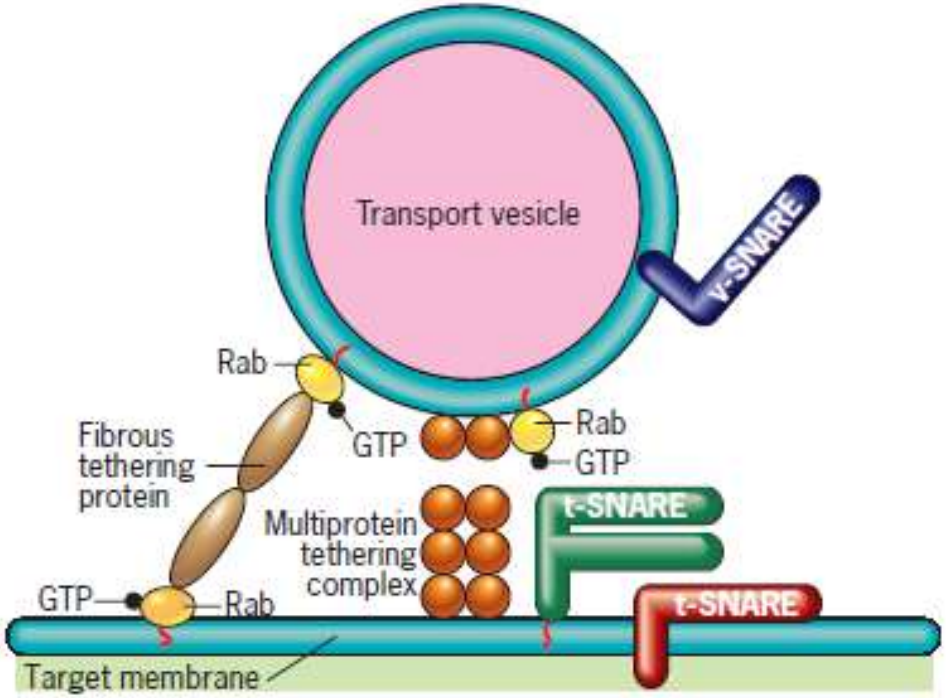
(a)


Endocytic cargo
Extracellular
Receptor
AP2 adaptor
PI(4,5)P2
Clathrin
Cytoplasm

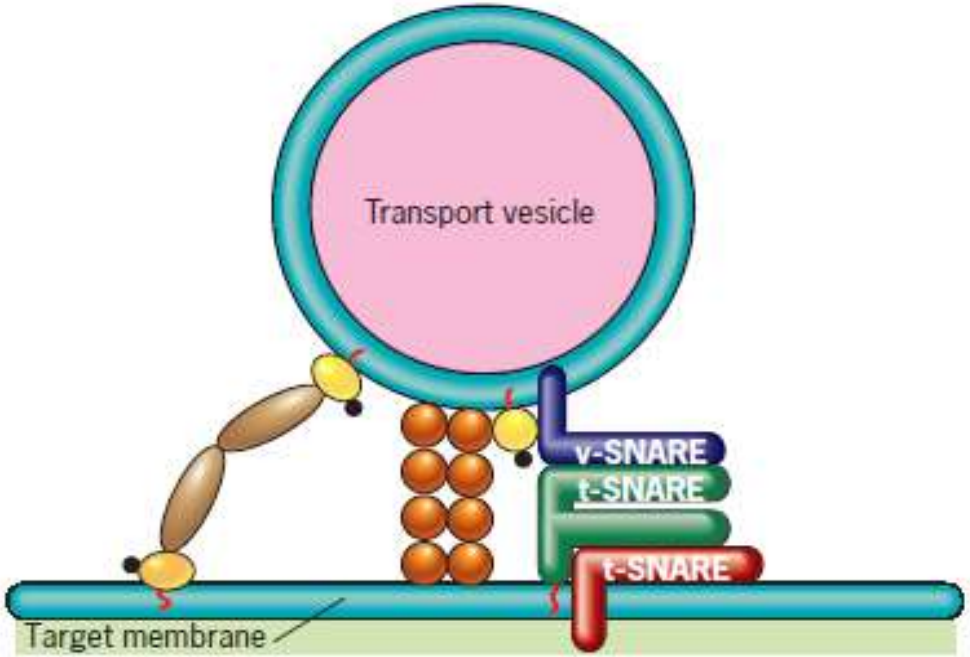
(b)


Dynamin subunits
Clathrin lattice
GTP hydrolysis
GTPγS
Dynamin ring
1
2
3
4
5a
5b

(a)



(a) Tethering



(b) Docking

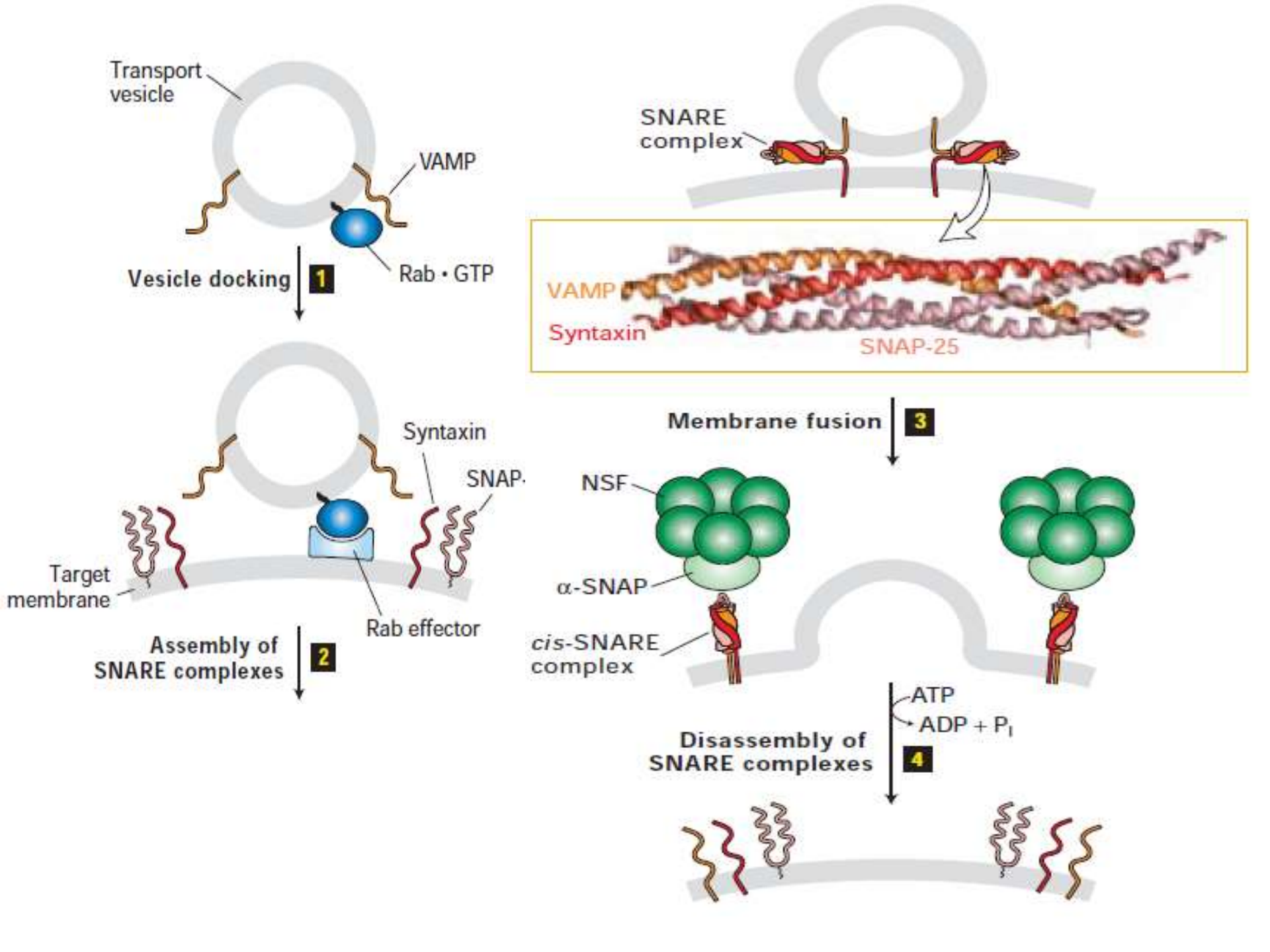
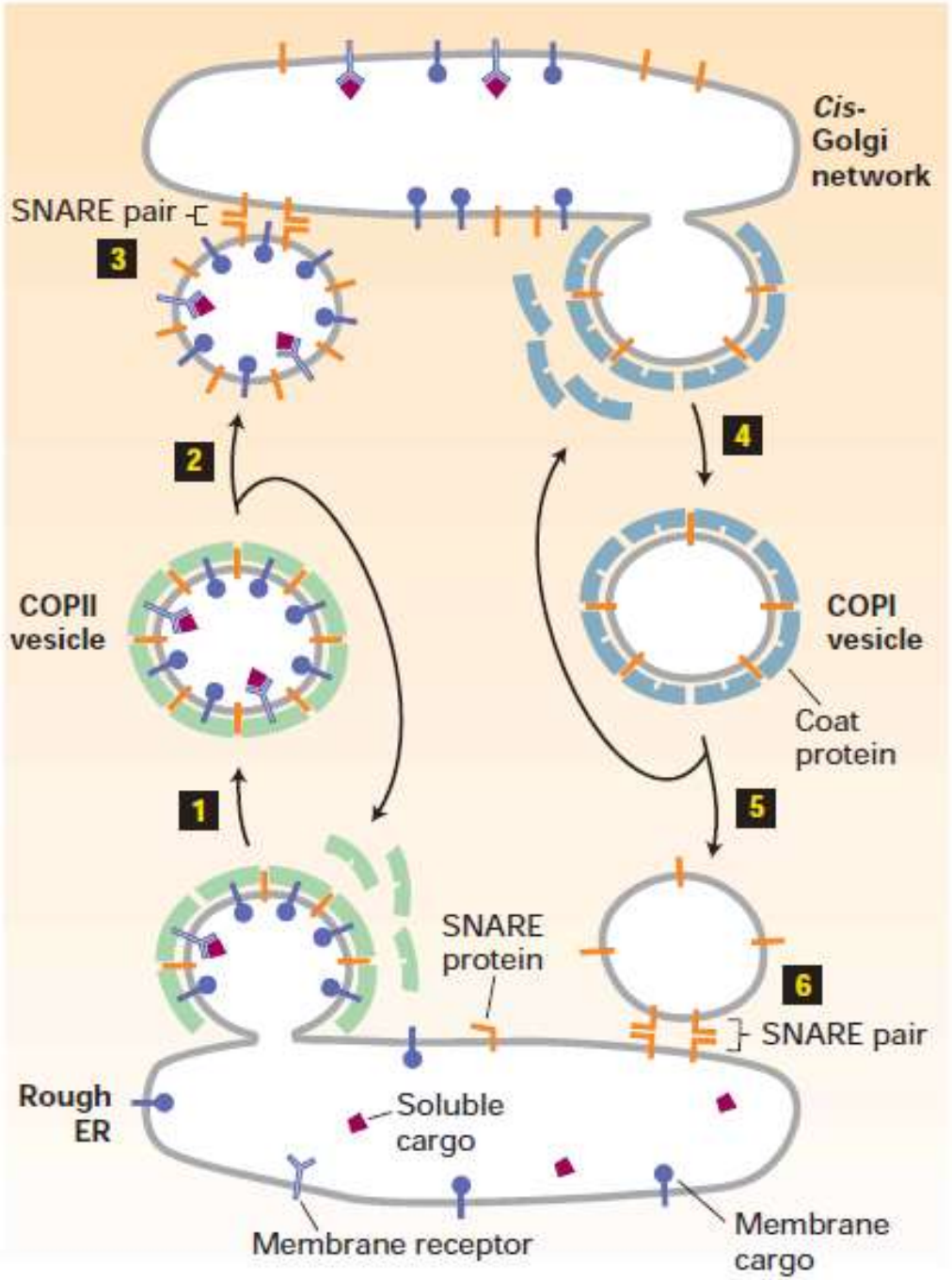
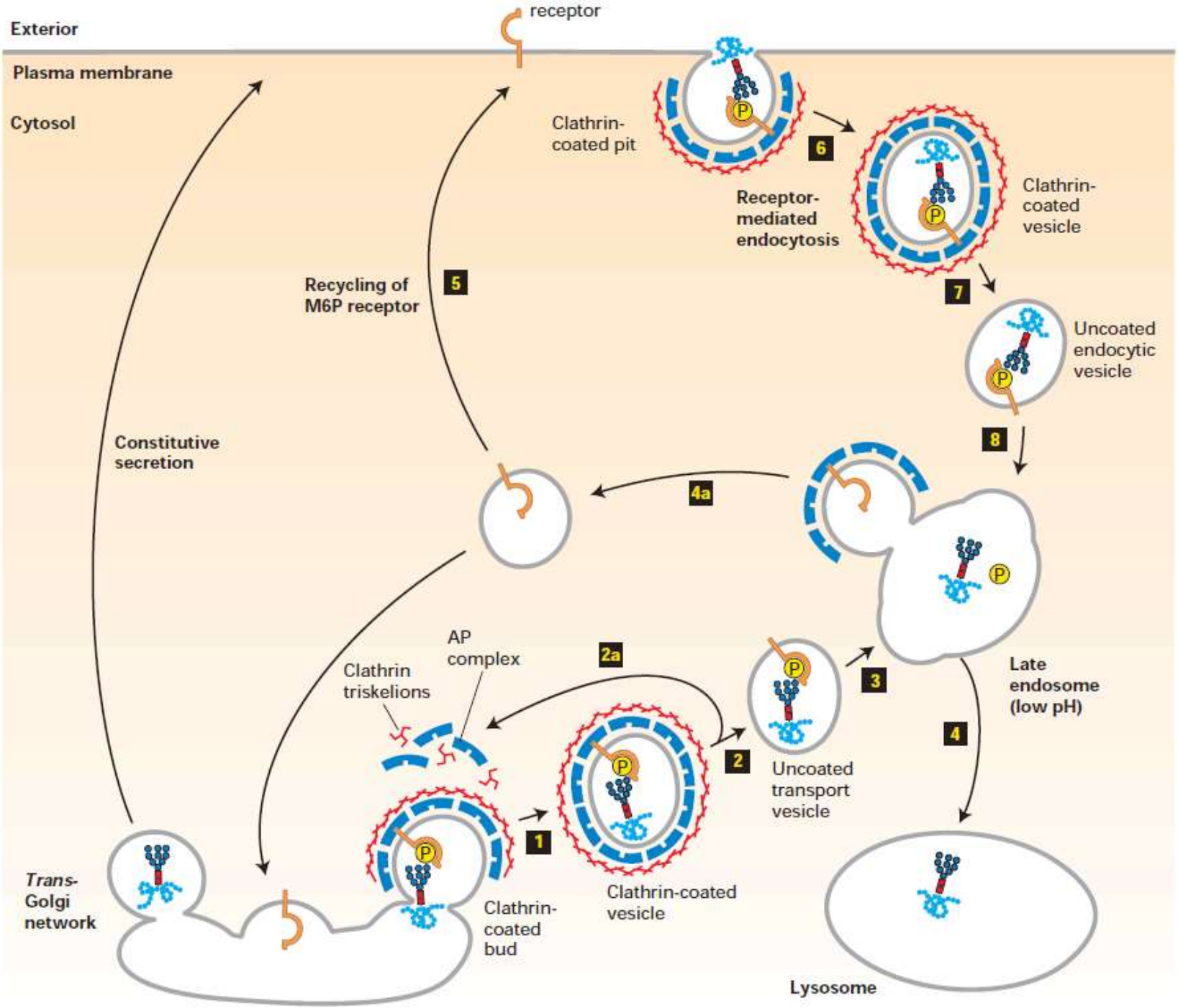

Transport vesicle
VAMP
Rab • GTP
Vesicle docking 1
Syntaxin
SNAP-25
Target membrane
Rab effector
Assembly of SNARE complexes 2
SNARE complex
VAMP
Syntaxin
SNAP-25
Membrane fusion 3
NSF
α-SNAP
cis-SNARE complex
ATP
ADP + P$_i$
Disassembly of SNARE complexes 4

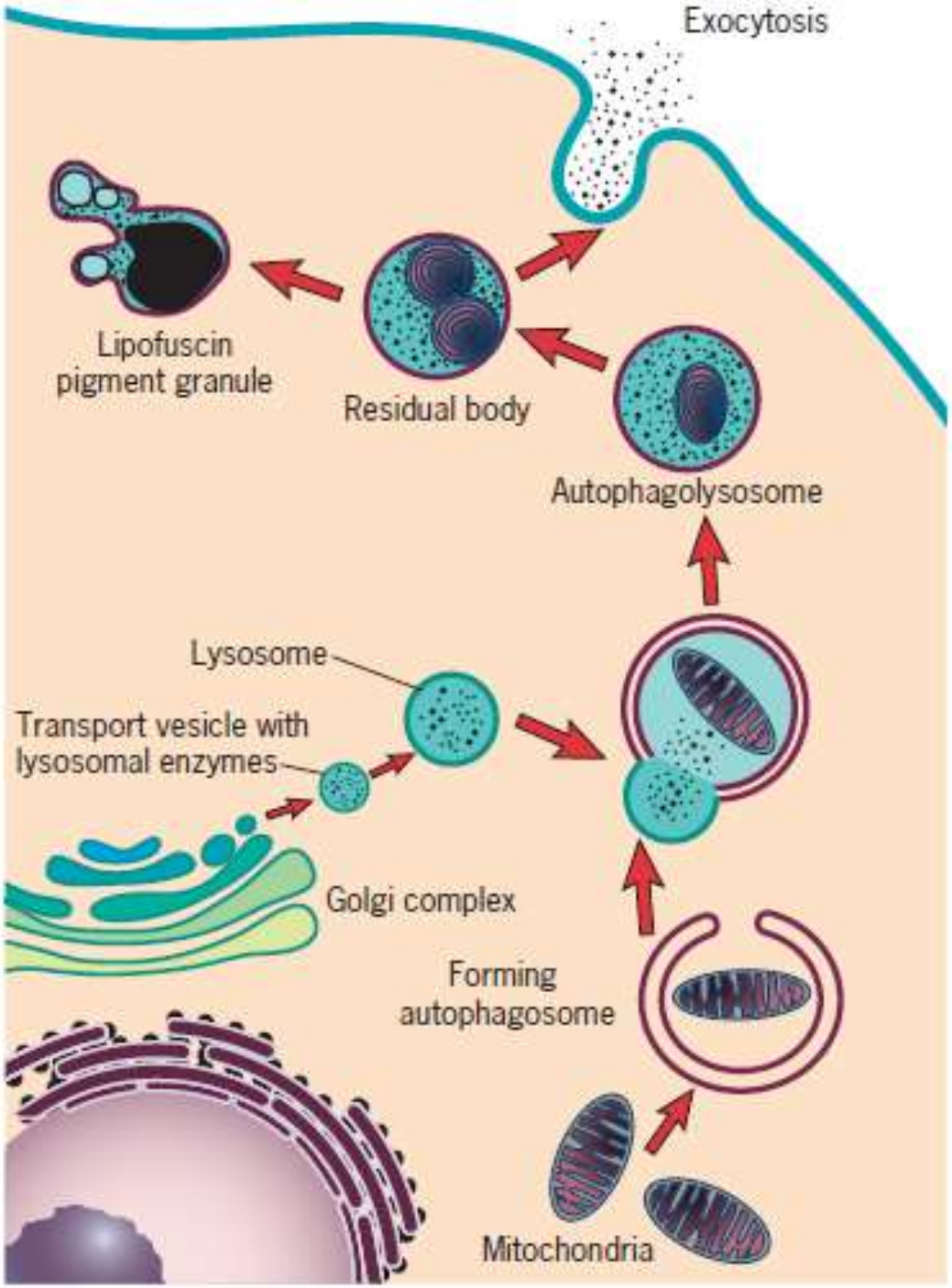
TABLE 17-2 Known Sorting Signals That Direct Proteins to Specific Transport Vesicles

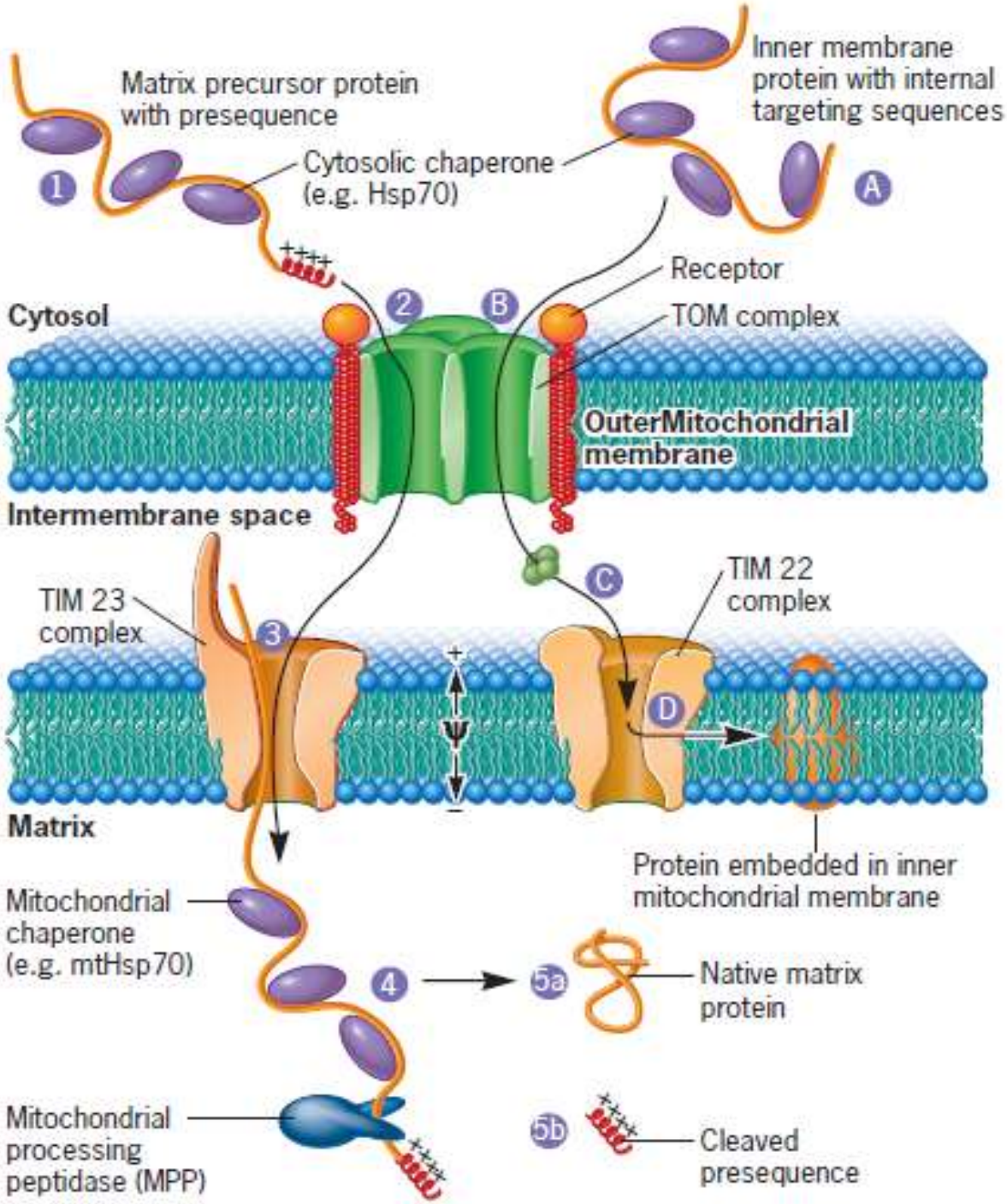
Signal Sequence*	Proteins with Signal	Signal Receptor	Vesicles That Incorporate Signal-bearing Protein
Lys-Asp-Glu-Leu (KDEL)	ER-resident luminal proteins	KDEL receptor in *cis*-Golgi membrane	COPI
Lys-Lys-X-X (KKXX)	ER-resident membrane proteins (cytosolic domain)	COPI α and β subunits	COPI
Di-acidic (e.g., Asp-X-Glu)	Cargo membrane proteins in ER (cytosolic domain)	COPII Sec24 subunit	COPII
Mannose 6-phosphate (M6P)	Soluble lysosomal enzymes after processing in *cis*-Golgi	M6P receptor in *trans*-Golgi membrane	Clathrin/AP1
	Secreted lysosomal enzymes	M6P receptor in plasma membrane	Clathrin/AP2
Asn-Pro-X-Tyr (NPXY)	LDL receptor in the plasma membrane (cytosolic domain)	AP2 complex	Clathrin/AP2
Tyr-X-X-Φ (YXXΦ)	Membrane proteins in *trans*-Golgi (cytosolic domain)	AP1 (μ1 subunit)	Clathrin/AP1
	Plasma membrane proteins (cytosolic domain)	AP2 (μ2 subunit)	Clathrin/AP2
Leu-Leu (LL)	Plasma membrane proteins (cytosolic domain)	AP2 complexes	Clathrin/AP2

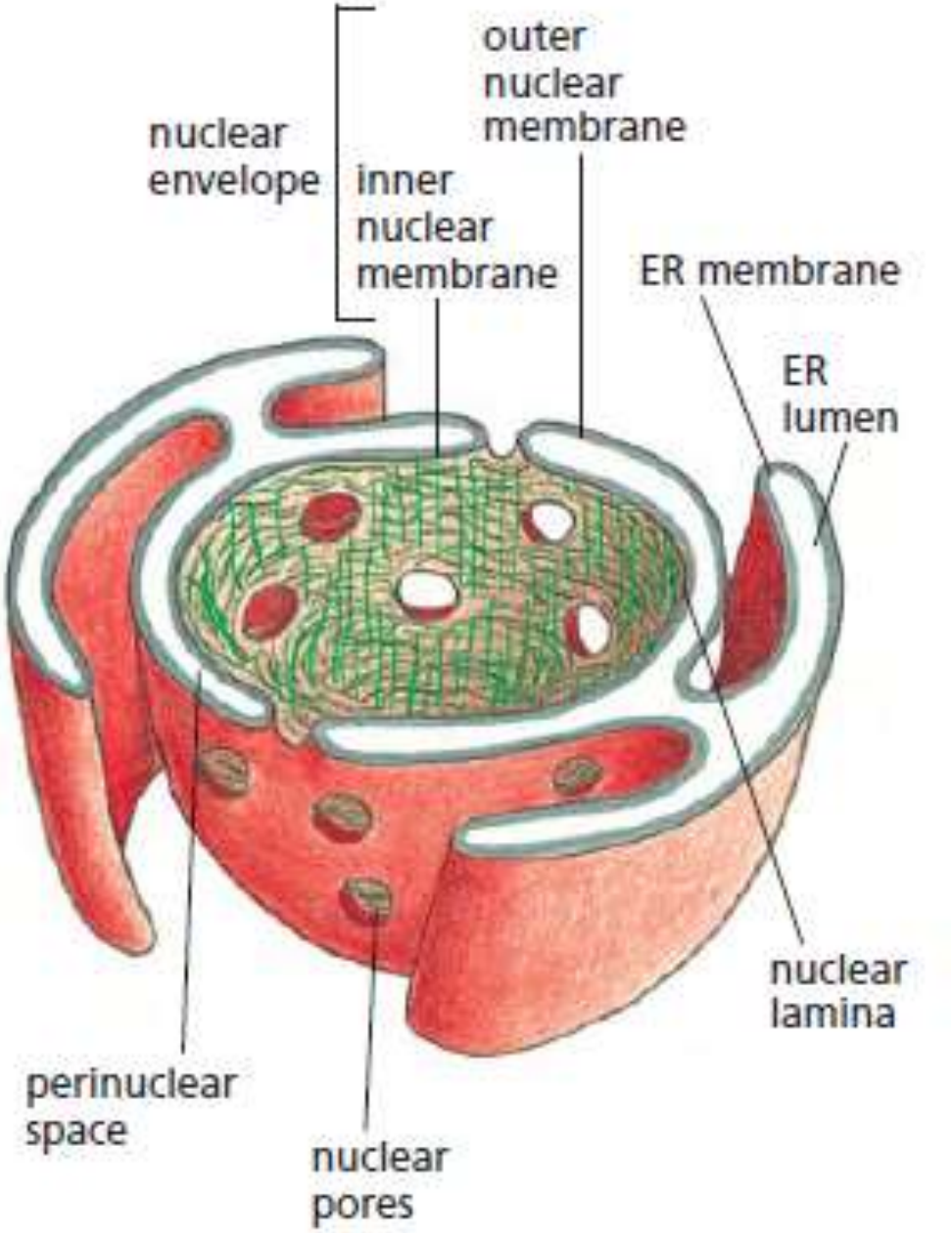
*X = any amino acid; Φ = hydrophobic amino acid. Single-letter amino acid abbreviations are in parentheses.

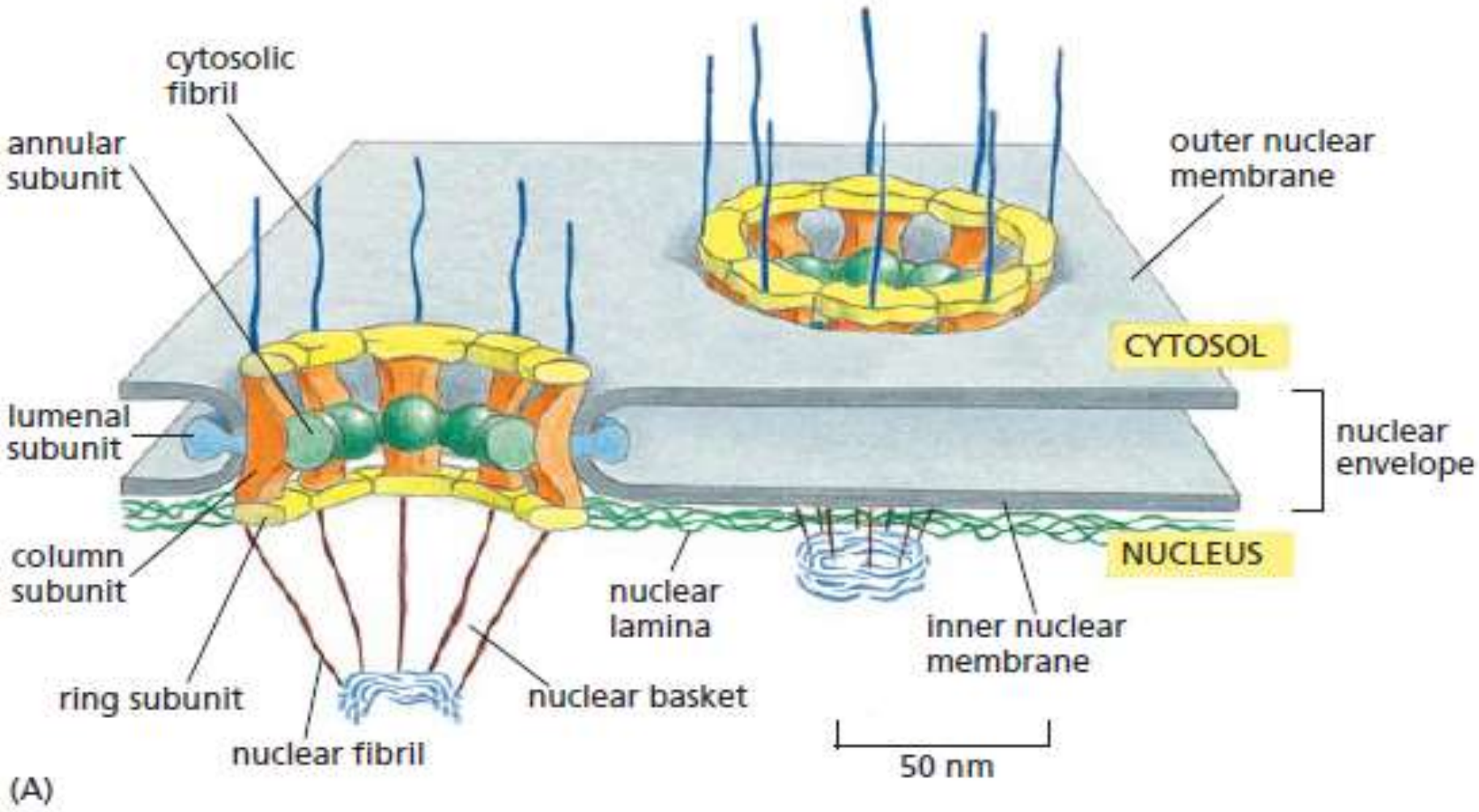

Cis-
Golgi
network
SNARE pair
3
2
4
COPII
vesicle
COPI
vesicle
Coat
protein
1
5
SNARE
protein
6
SNARE pair
Rough
ER
Soluble
cargo
Membrane receptor
Membrane
cargo

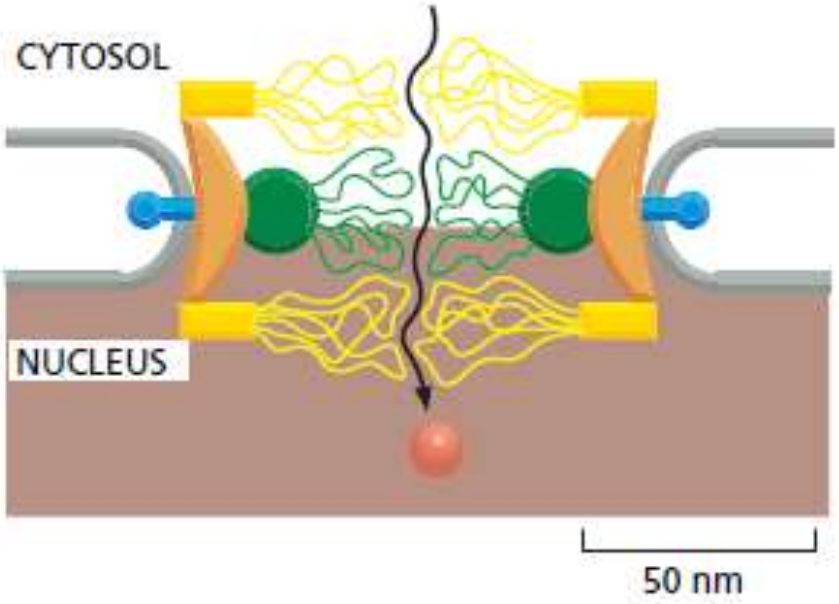

Exterior
receptor
Plasma membrane
Cytosol
Clathrin-coated pit
6
Receptor-mediated endocytosis
Clathrin-coated vesicle
7
Uncoated endocytic vesicle
8
Recycling of M6P receptor
5
Constitutive secretion
4a
AP complex
Clathrin triskelions
2a
3
Late endosome (low pH)
4
2
Uncoated transport vesicle
1
Clathrin-coated bud
Clathrin-coated vesicle
Trans-Golgi network
Lysosome


Exocytosis
Lipofuscin pigment granule
Residual body
Autophagolysosome
Lysosome
Transport vesicle with lysosomal enzymes
Golgi complex
Forming autophagosome
Mitochondria


Matrix precursor protein with presequence
Cytosolic chaperone (e.g. Hsp70)
Inner membrane protein with internal targeting sequences
1
A
Receptor
TOM complex
2
B
Cytosol
OuterMitochondrial membrane
Intermembrane space
TIM 23 complex
TIM 22 complex
C
3
D
Matrix
Protein embedded in inner mitochondrial membrane
Mitochondrial chaperone (e.g. mtHsp70)
4
5a
Native matrix protein
Mitochondrial processing peptidase (MPP)
5b
Cleaved presequence


outer
nuclear
membrane
nuclear
envelope
inner
nuclear
membrane
ER membrane
ER
lumen
nuclear
lamina
perinuclear
space
nuclear
pores


cytosolic fibril
annular subunit
outer nuclear membrane
CYTOSOL
lumenal subunit
nuclear envelope
column subunit
NUCLEUS
nuclear lamina
inner nuclear membrane
ring subunit
nuclear basket
nuclear fibril
50 nm
(A)

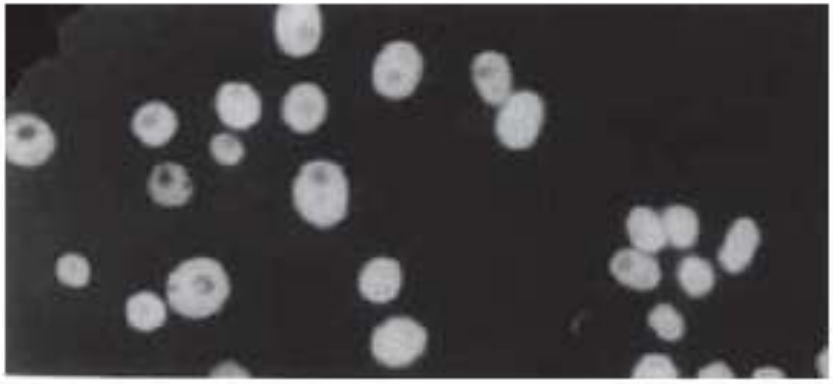


size of molecules
that enter nucleus
by free diffusion

size of macromolecules
that enter nucleus
by active transport

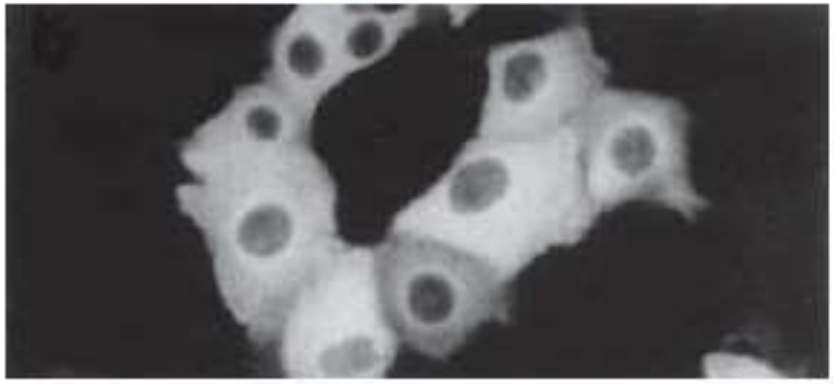
(A) LOCALIZATION OF T-ANTIGEN CONTAINING ITS NORMAL NUCLEAR IMPORT SIGNAL

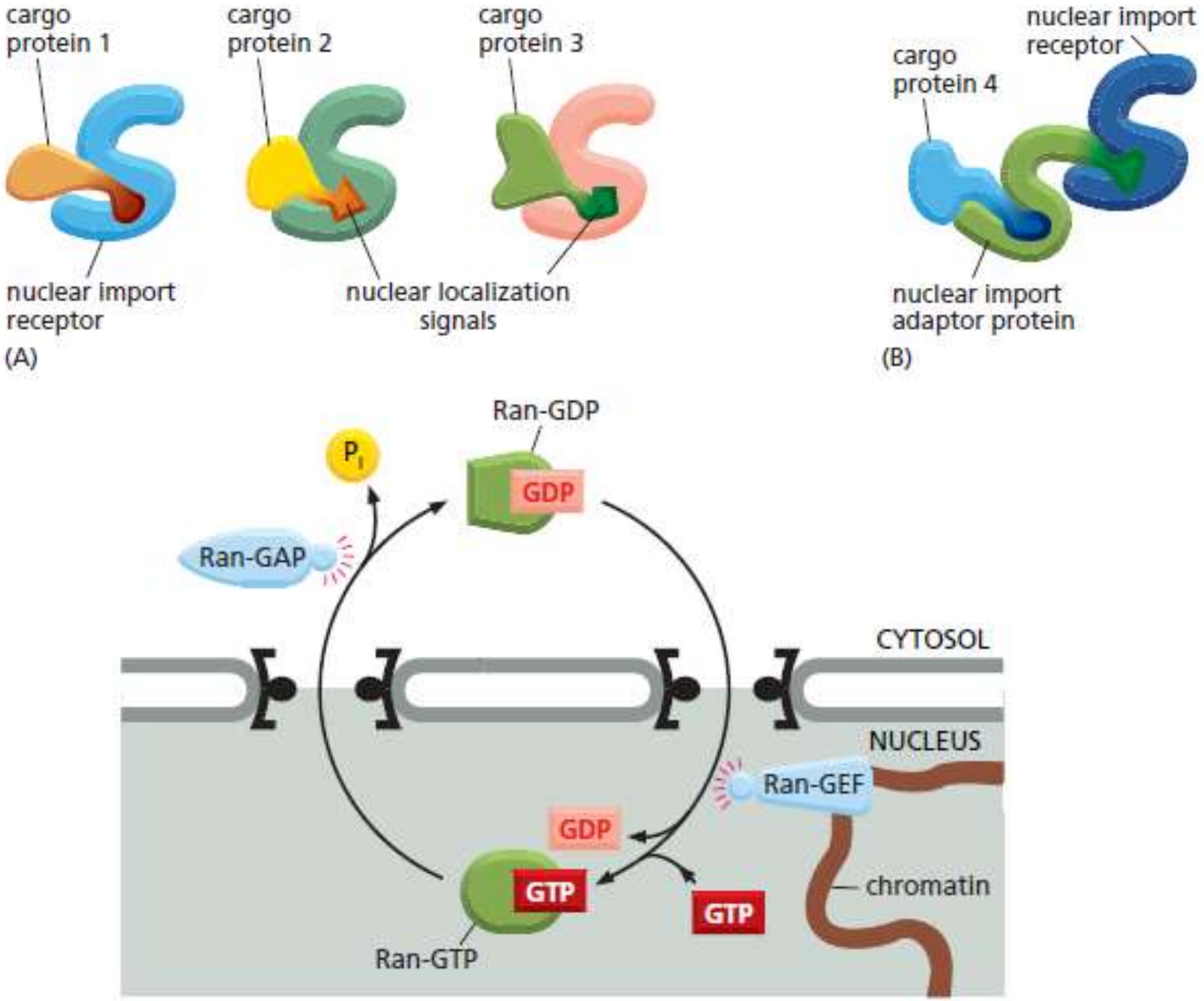
Pro — Pro — Lys — Lys — Lys — Arg — Lys — Val —

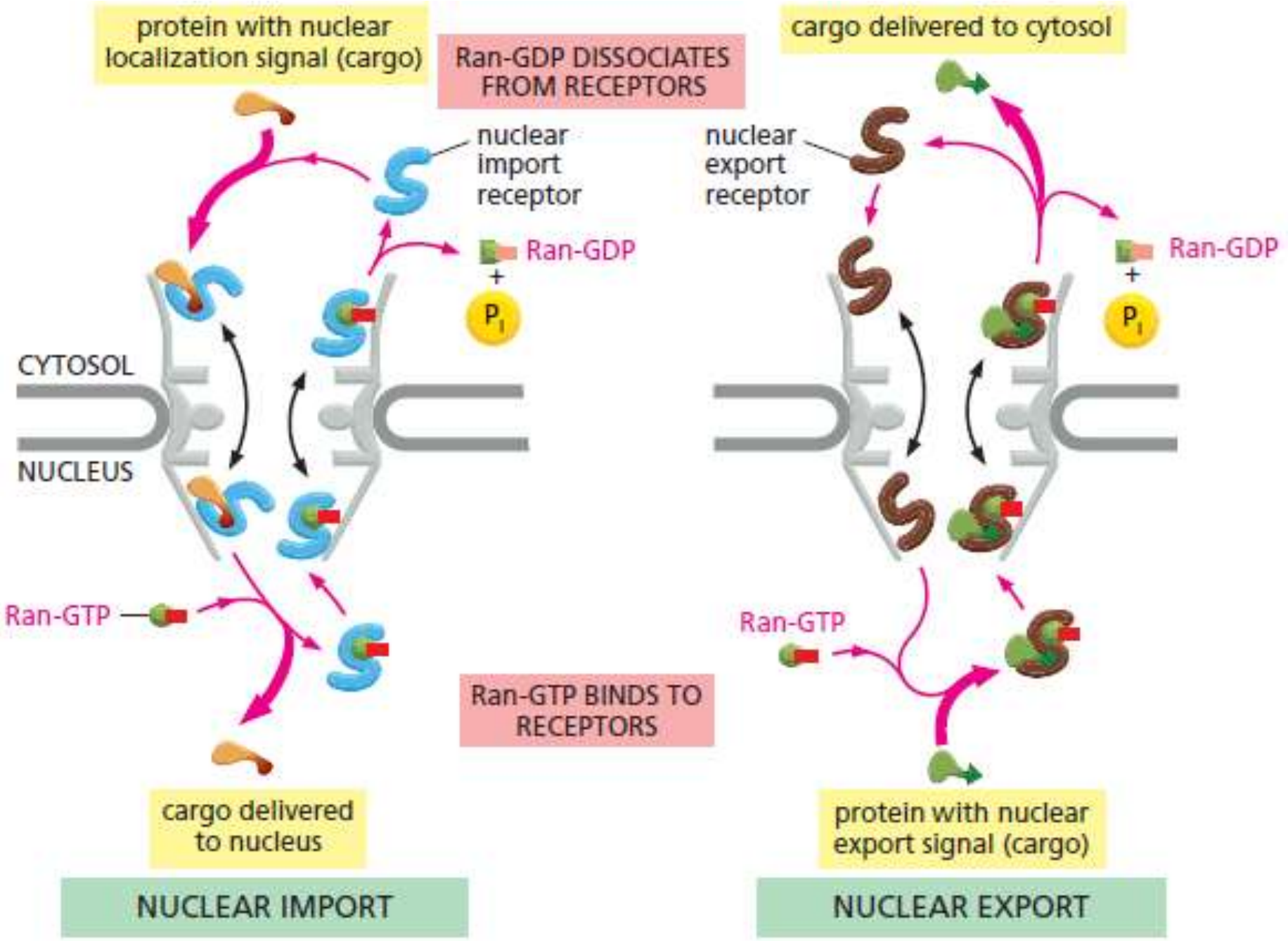


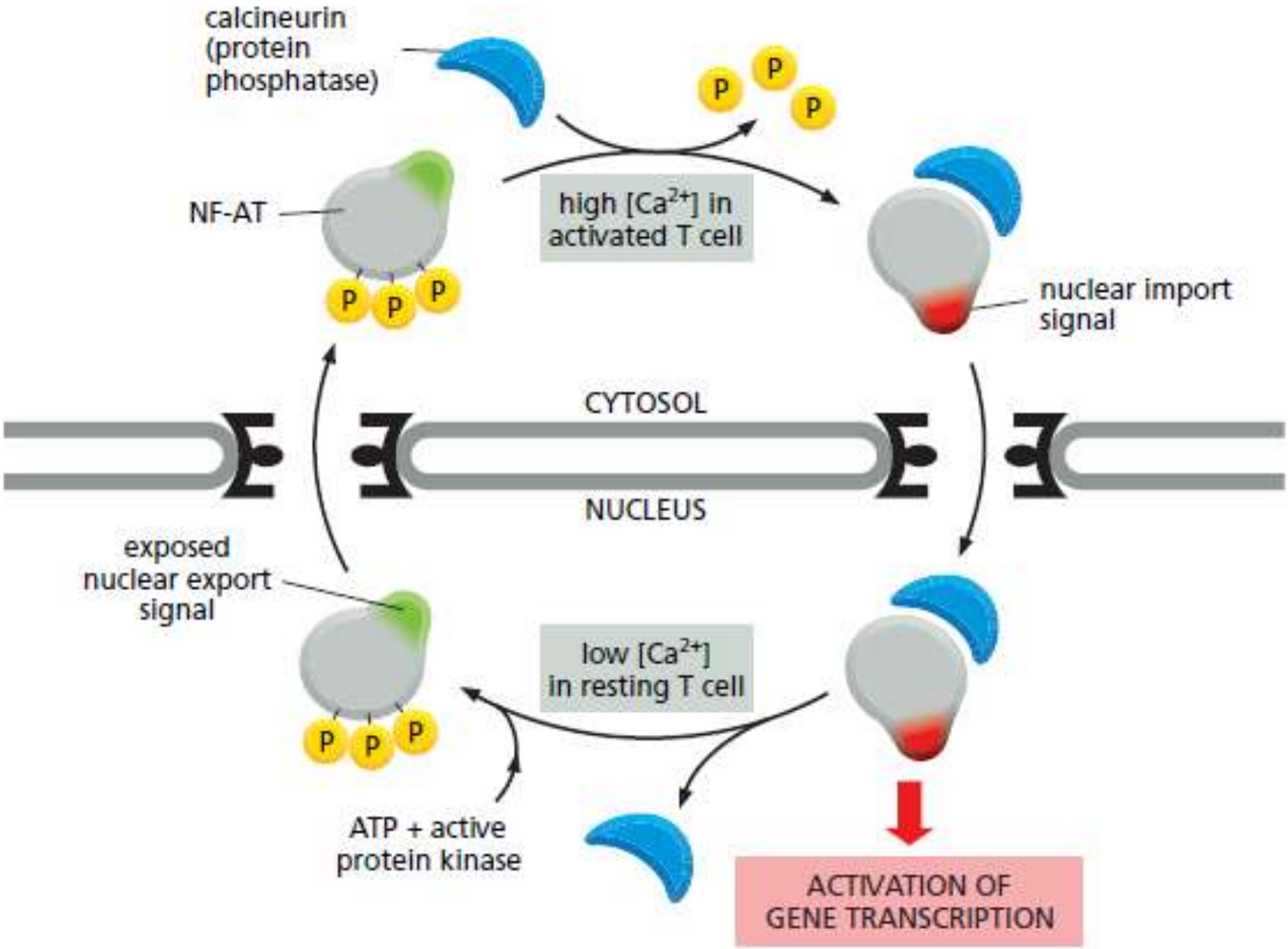
(B) LOCALIZATION OF T-ANTIGEN CONTAINING A MUTATED NUCLEAR IMPORT SIGNAL

Pro — Pro — Lys — Thr — Lys — Arg — Lys — Val —




cargo protein 1
cargo protein 2
cargo protein 3
nuclear import receptor
nuclear localization signals
(A)
nuclear import receptor
cargo protein 4
nuclear import adaptor protein
(B)
Ran-GDP
P_i
GDP
Ran-GAP
CYTOSOL
NUCLEUS
Ran-GEF
GDP
GTP
GTP
chromatin
Ran-GTP


protein with nuclear localization signal (cargo)
Ran-GDP DISSOCIATES FROM RECEPTORS
cargo delivered to cytosol
nuclear import receptor
nuclear export receptor
Ran-GDP
+
P_i
CYTOSOL
NUCLEUS
Ran-GTP
Ran-GTP BINDS TO RECEPTORS
cargo delivered to nucleus
protein with nuclear export signal (cargo)
NUCLEAR IMPORT
NUCLEAR EXPORT


calcineurin
(protein
phosphatase)
P
P
P
NF-AT
high [Ca2+] in
activated T cell
P
P
P
nuclear import
signal
CYTOSOL
NUCLEUS
exposed
nuclear export
signal
low [Ca2+]
in resting T cell
P
P
P
ATP + active
protein kinase
ACTIVATION OF
GENE TRANSCRIPTION