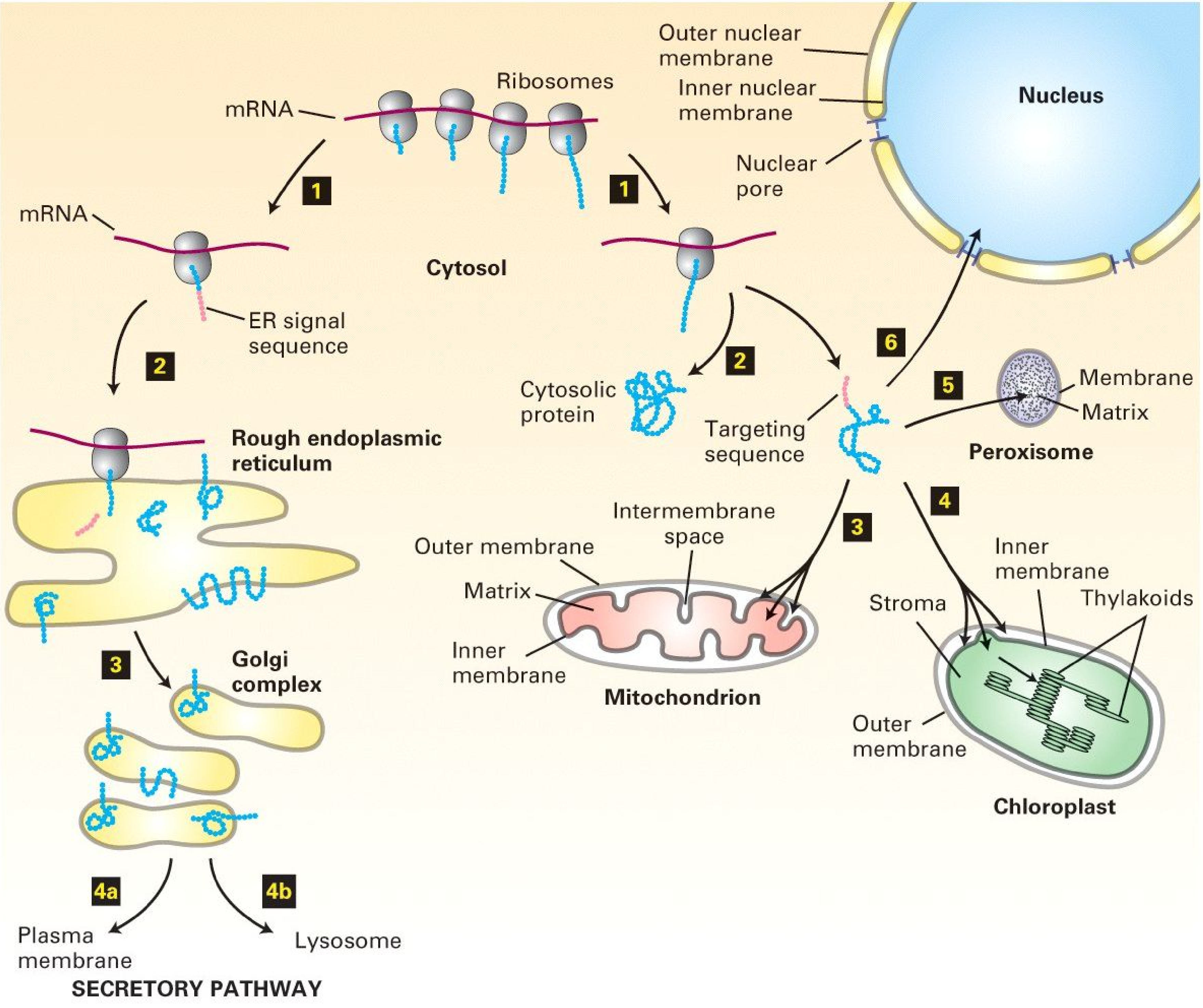
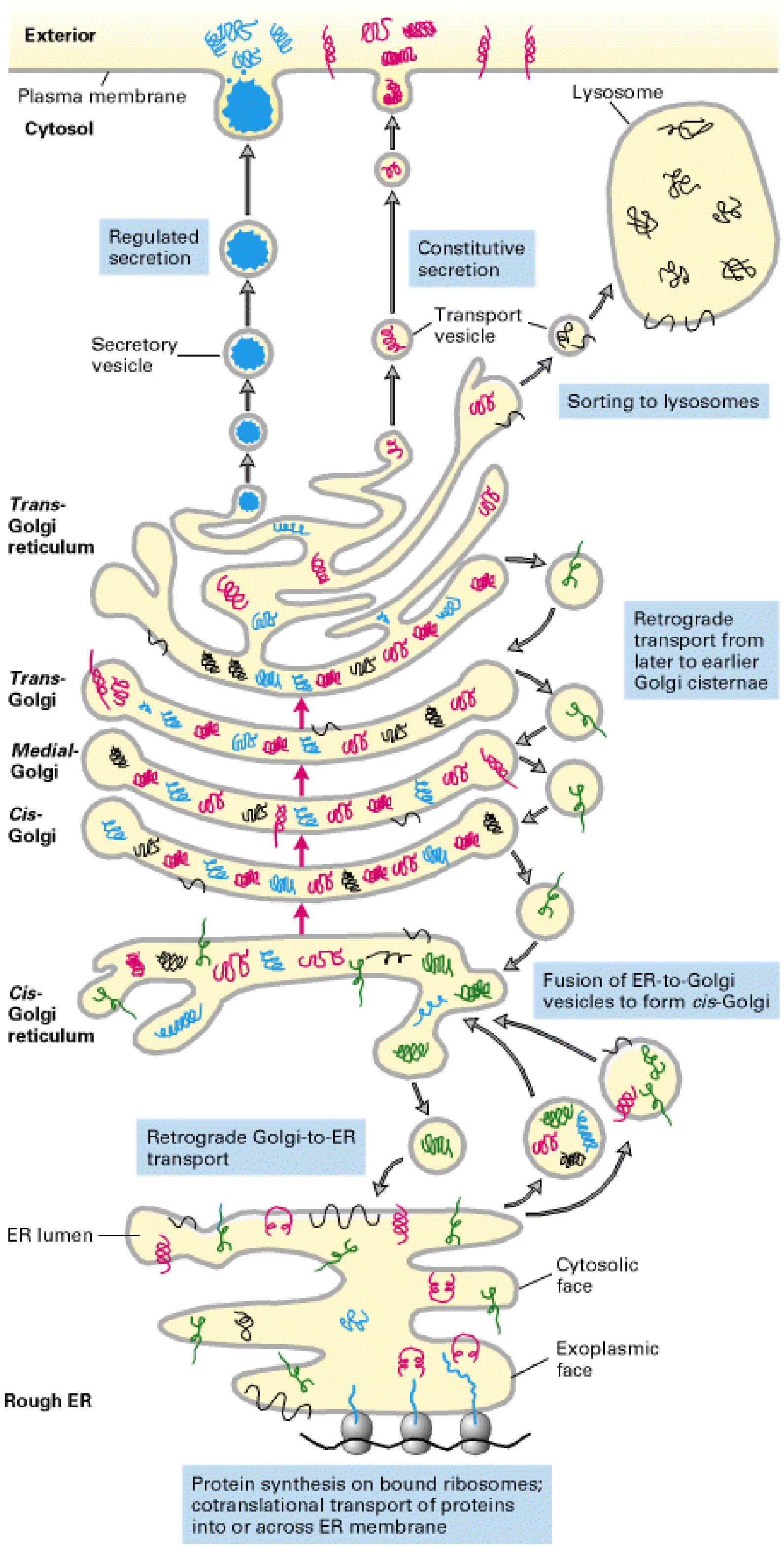
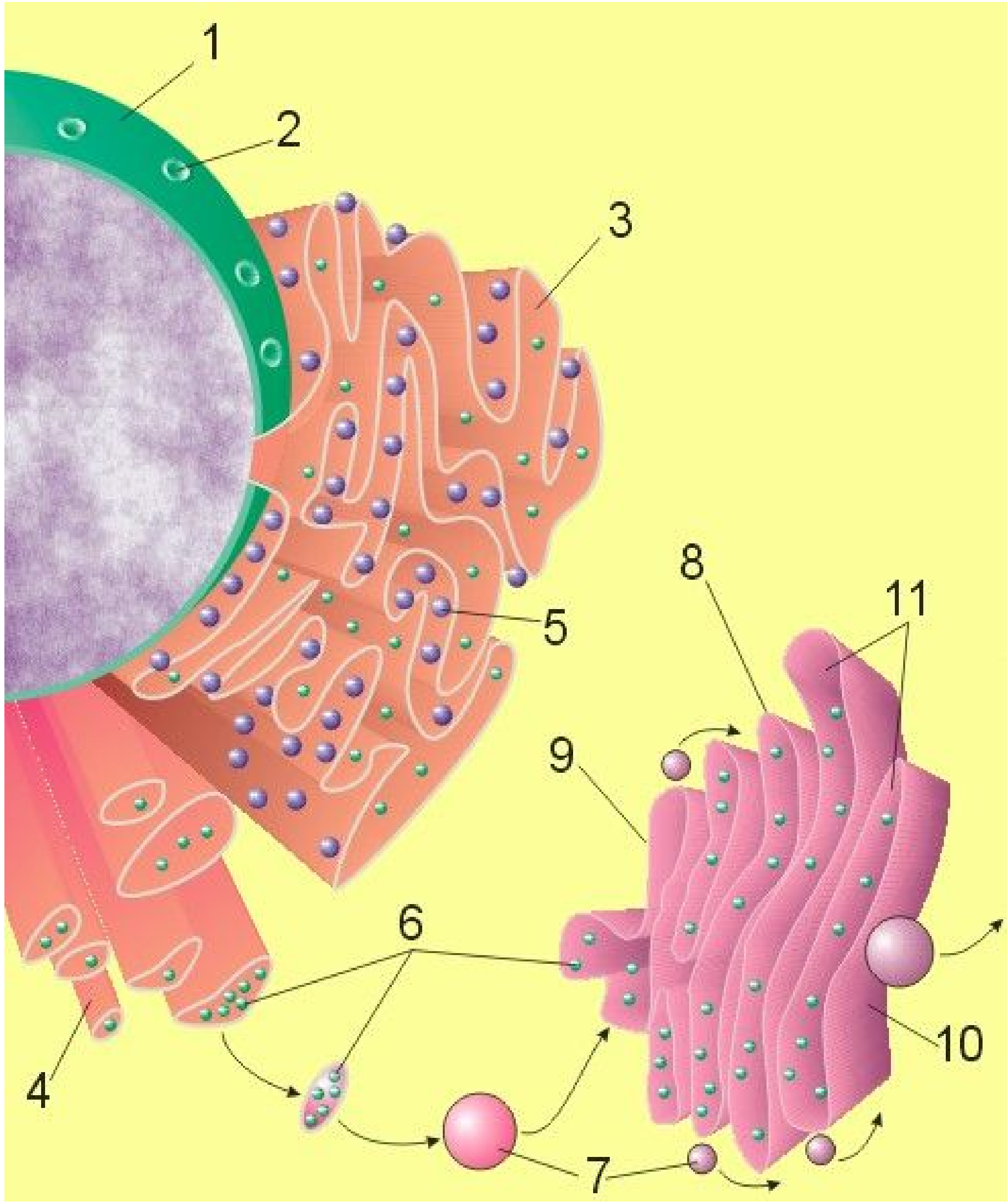
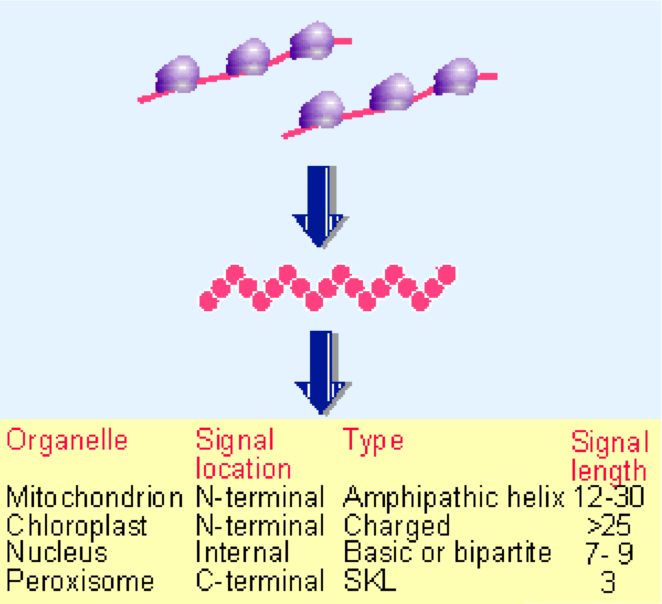
Protein localization

- Cytosol
- Cytoplasmic Membrane
- Nucleus
- Organelles
- Endoplasmic reticulum
- Extracellular environment


Outer nuclear membrane
Inner nuclear membrane
Nuclear pore
Nucleus
Ribosomes
mRNA
1
1
mRNA
Cytosol
ER signal sequence
2
Cytosolic protein
2
Targeting sequence
6
5
Membrane
Matrix
Peroxisome
Rough endoplasmic reticulum
4
3
Intermembrane space
Outer membrane
Matrix
Inner membrane
Mitochondrion
Inner membrane
Thylakoids
Stroma
Outer membrane
Chloroplast
3
Golgi complex
4a
4b
Plasma membrane
Lysosome
SECRETORY PATHWAY


Exterior
Plasma membrane
Cytosol
Lysosome
Regulated secretion
Constitutive secretion
Secretory vesicle
Transport vesicle
Sorting to lysosomes
Trans-Golgi reticulum
Retrograde transport from later to earlier Golgi cisternae
Trans-Golgi
Medial-Golgi
Cis-Golgi
Cis-Golgi reticulum
Fusion of ER-to-Golgi vesicles to form cis-Golgi
Retrograde Golgi-to-ER transport
ER lumen
Cytosolic face
Exoplasmic face
Rough ER
Protein synthesis on bound ribosomes; cotranslational transport of proteins into or across ER membrane


1
2
3
5
8
11
9
6
10
4
7



Organelle	Signal location	Type	Signal length
Mitochondrion	N-terminal	Amphipathic helix	12-30
Chloroplast	N-terminal	Charged	>25
Nucleus	Internal	Basic or bipartite	7- 9
Peroxisome	C-terminal	SKL	3

Proteins synthesized on free ribosomes in the cytosol are directed after their release to specific destinations by short signal motifs.

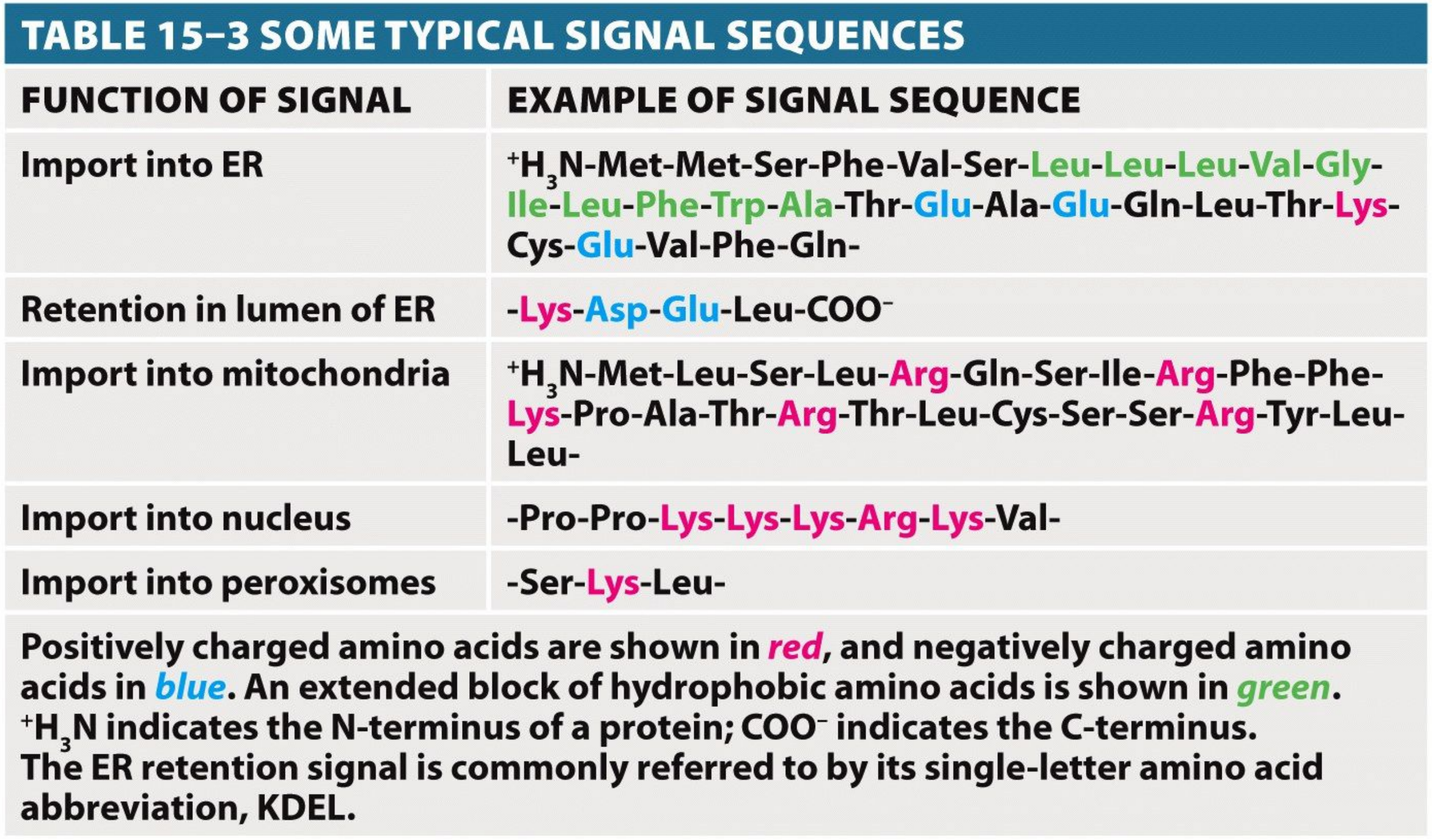


TABLE 15–3 SOME TYPICAL SIGNAL SEQUENCES

FUNCTION OF SIGNAL	EXAMPLE OF SIGNAL SEQUENCE
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-
Retention in lumen of ER	-Lys-Asp-Glu-Leu-COO^-
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 nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Import into peroxisomes	-Ser-Lys-Leu-

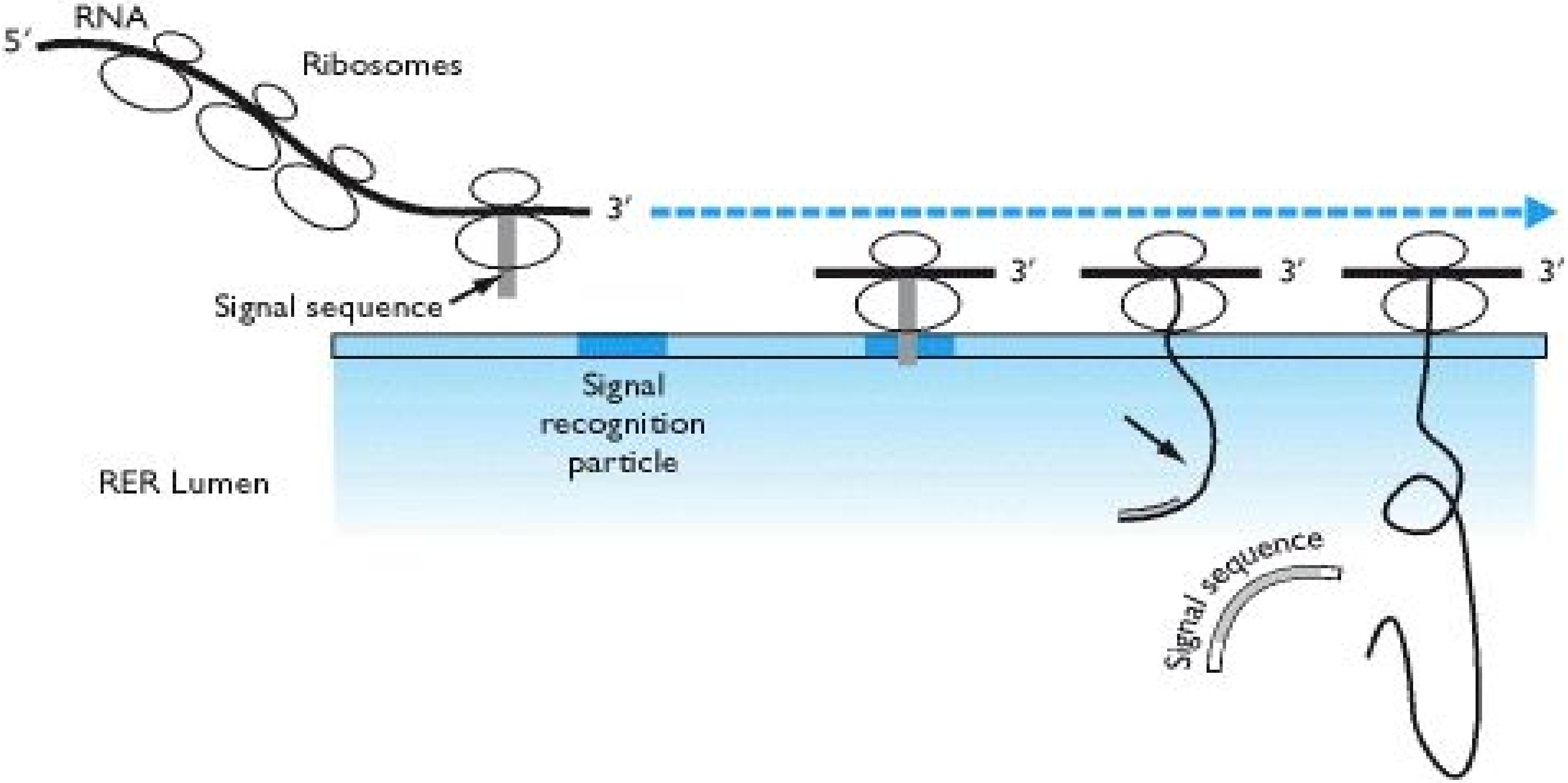
Positively charged amino acids are shown in *red*, and negatively charged amino acids in *blue*. An extended block of hydrophobic amino acids is shown in *green*. ^+H_3N indicates the N-terminus of a protein; COO^- indicates the C-terminus. The ER retention signal is commonly referred to by its single-letter amino acid abbreviation, KDEL.

Table 15-3 Essential Cell Biology 3/e (© Garland Science 2010)

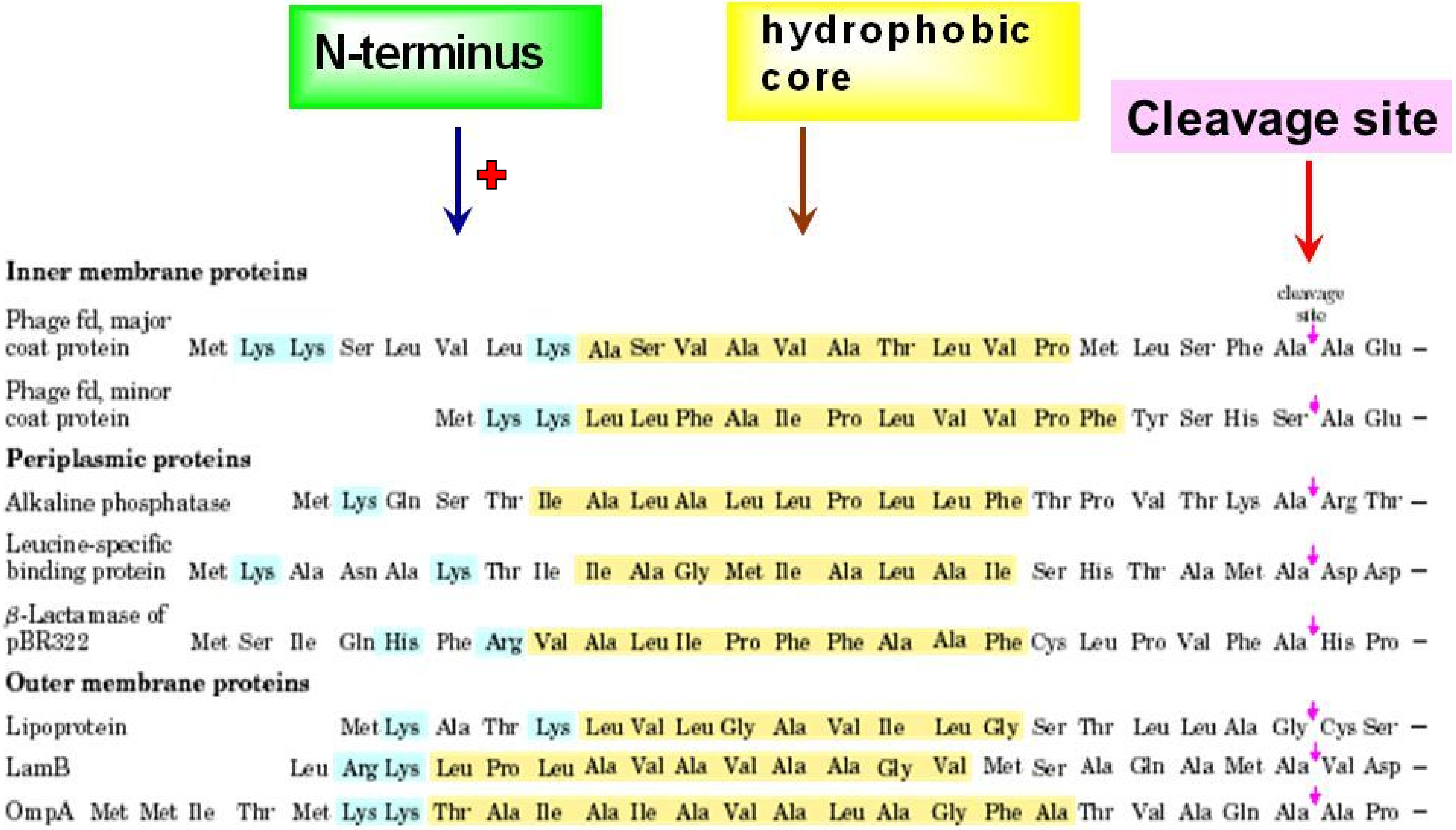
Protein localization

- ***Co-translational***
- ***Post-translational***

Proteins can enter the ER only during translation

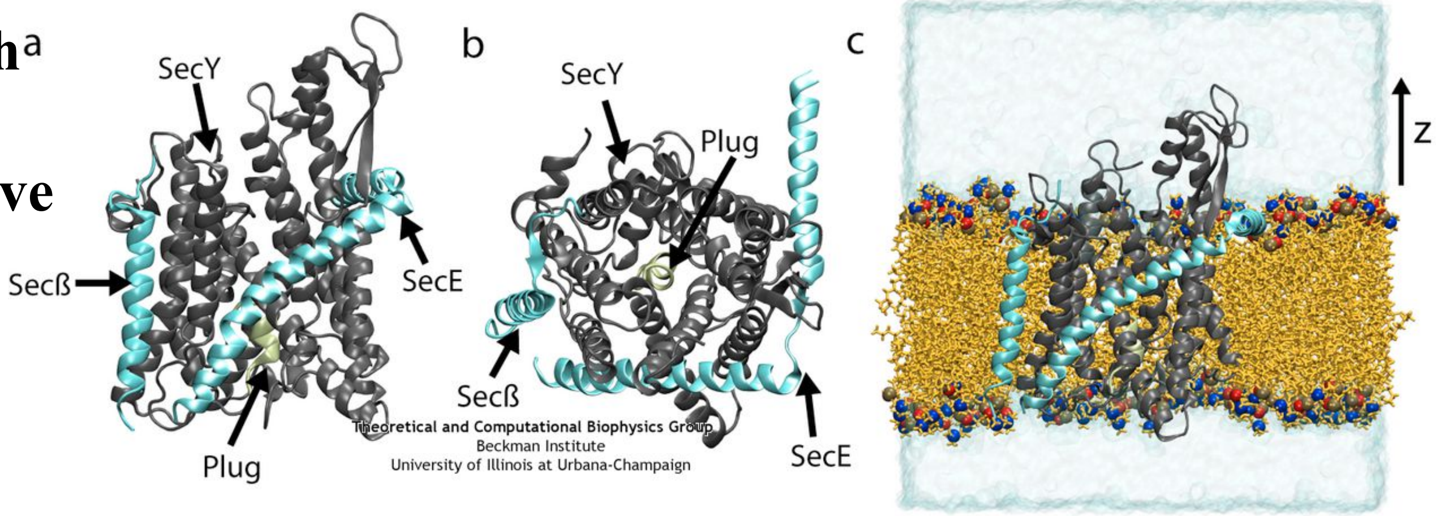


Signal sequence for ER

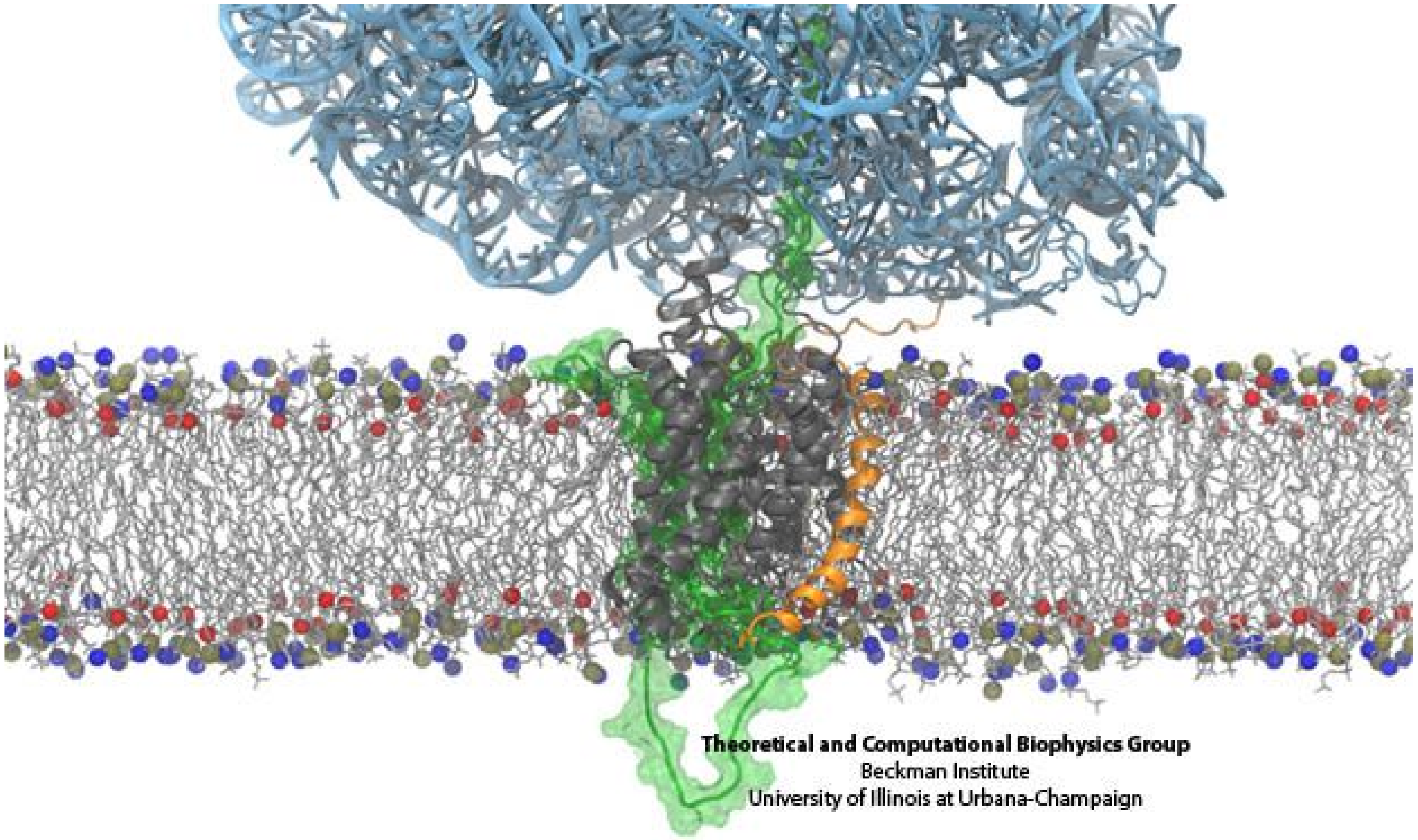


Inner membrane proteins	
Phage fd, major coat protein	Met Lys Lys Ser Leu Val Leu Lys Ala Ser Val Ala Val Ala Thr Leu Val Pro Met Leu Ser Phe Ala ↓ Ala Glu –
Phage fd, minor coat protein	Met Lys Lys Leu Leu Phe Ala Ile Pro Leu Val Val Pro Phe Tyr Ser His Ser ↓ Ala Glu –
Periplasmic proteins	
Alkaline phosphatase	Met Lys Gln Ser Thr Ile Ala Leu Ala Leu Leu Pro Leu Leu Phe Thr Pro Val Thr Lys Ala ↓ Arg Thr –
Leucine-specific binding protein	Met Lys Ala Asn Ala Lys Thr Ile Ile Ala Gly Met Ile Ala Leu Ala Ile Ser His Thr Ala Met Ala ↓ Asp Asp –
β-Lactamase of pBR322	Met Ser Ile Gln His Phe Arg Val Ala Leu Ile Pro Phe Phe Ala Ala Phe Cys Leu Pro Val Phe Ala ↓ His Pro –
Outer membrane proteins	
Lipoprotein	Met Lys Ala Thr Lys Leu Val Leu Gly Ala Val Ile Leu Gly Ser Thr Leu Leu Ala Gly ↓ Cys Ser –
LamB	Leu Arg Lys Leu Pro Leu Ala Val Ala Val Ala Ala Gly Val Met Ser Ala Gln Ala Met Ala ↓ Val Asp –
OmpA	Met Met Ile Thr Met Lys Lys Thr Ala Ile Ala Ile Ala Val Ala Leu Ala Gly Phe Ala Thr Val Ala Gln Ala ↓ Ala Pro –

SecYEβ, shown here both from the side and from above and also in its native membrane/water.



Theoretical and Computational Biophysics Group
Beckman Institute
University of Illinois at Urbana-Champaign



Theoretical and Computational Biophysics Group
Beckman Institute
University of Illinois at Urbana-Champaign

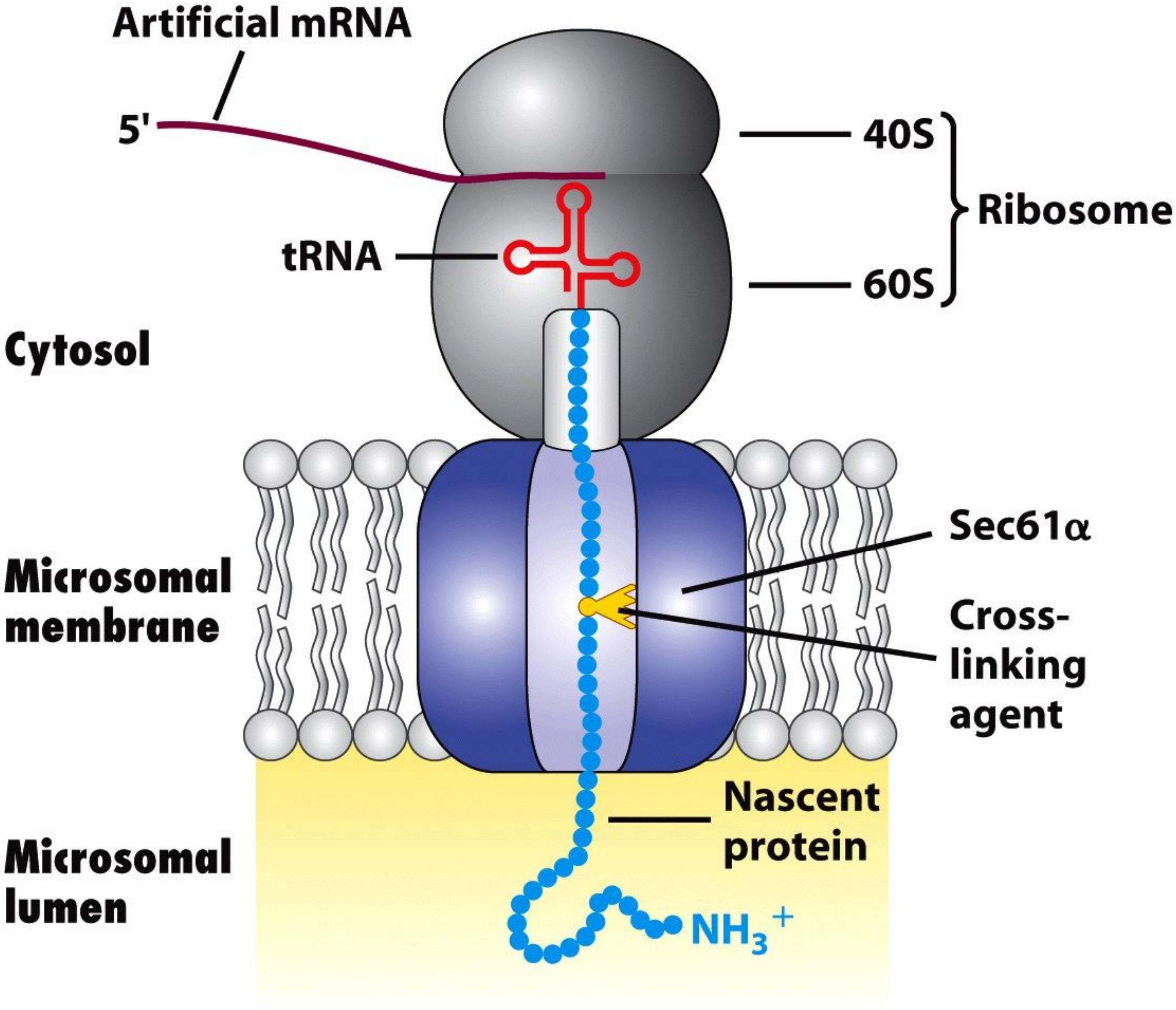


Figure 13-7
Molecular Cell Biology, Sixth Edition

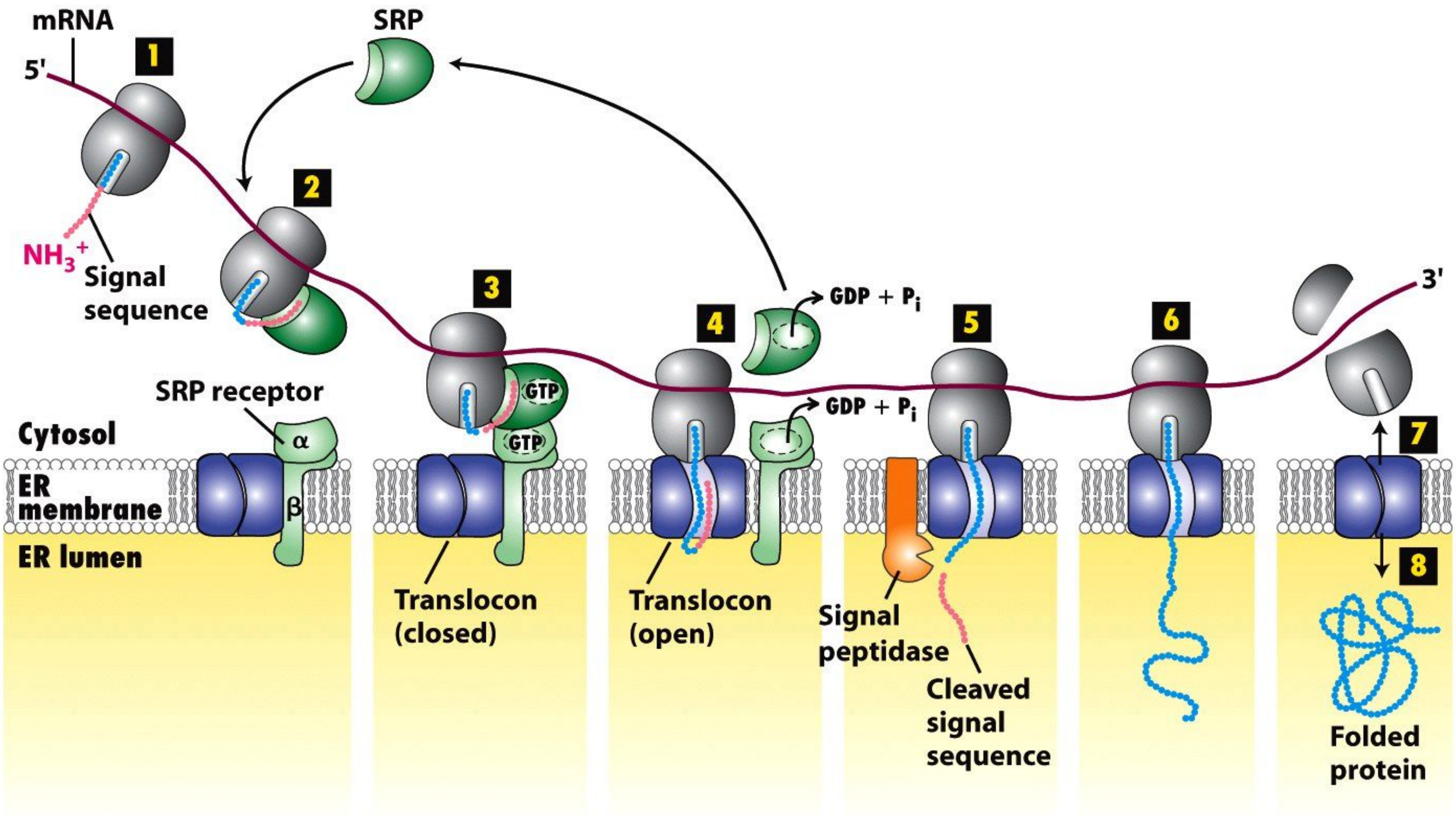
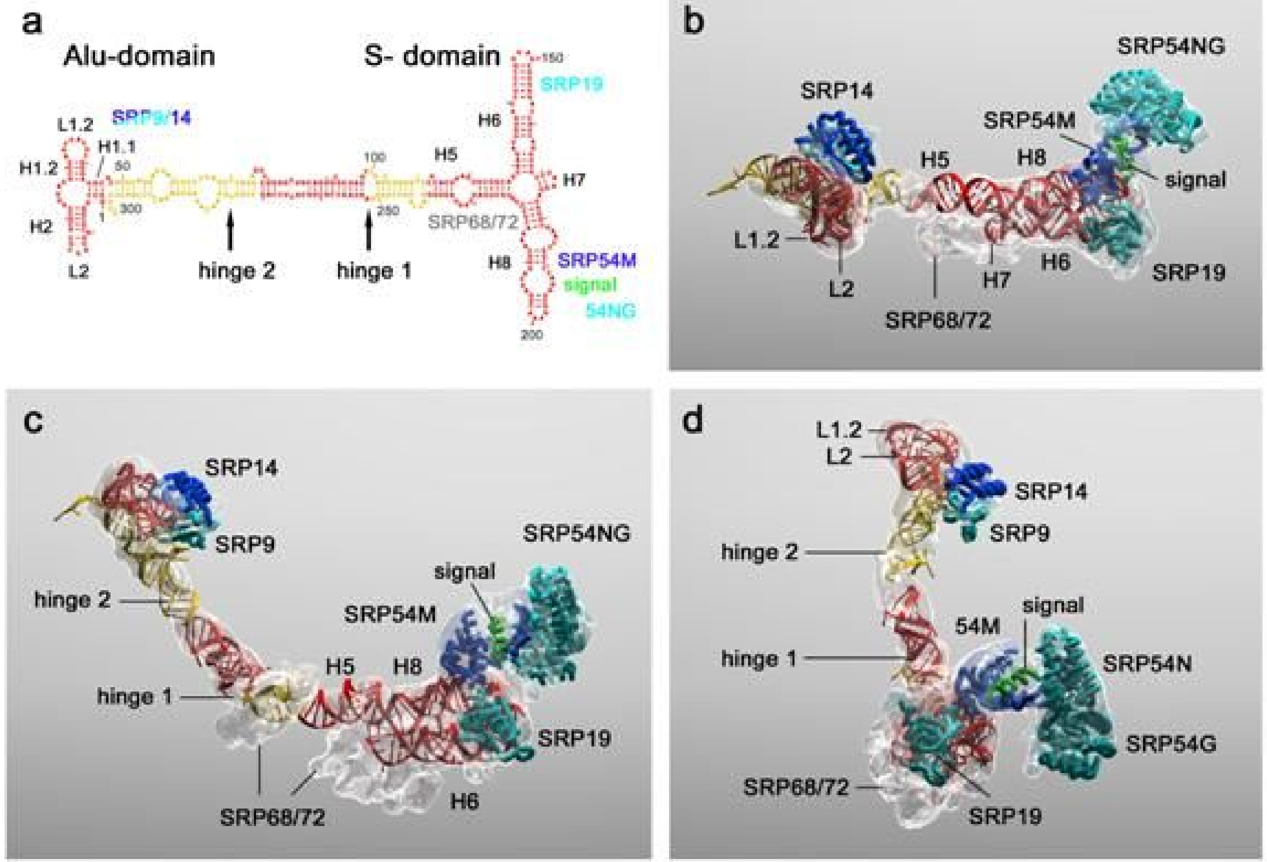
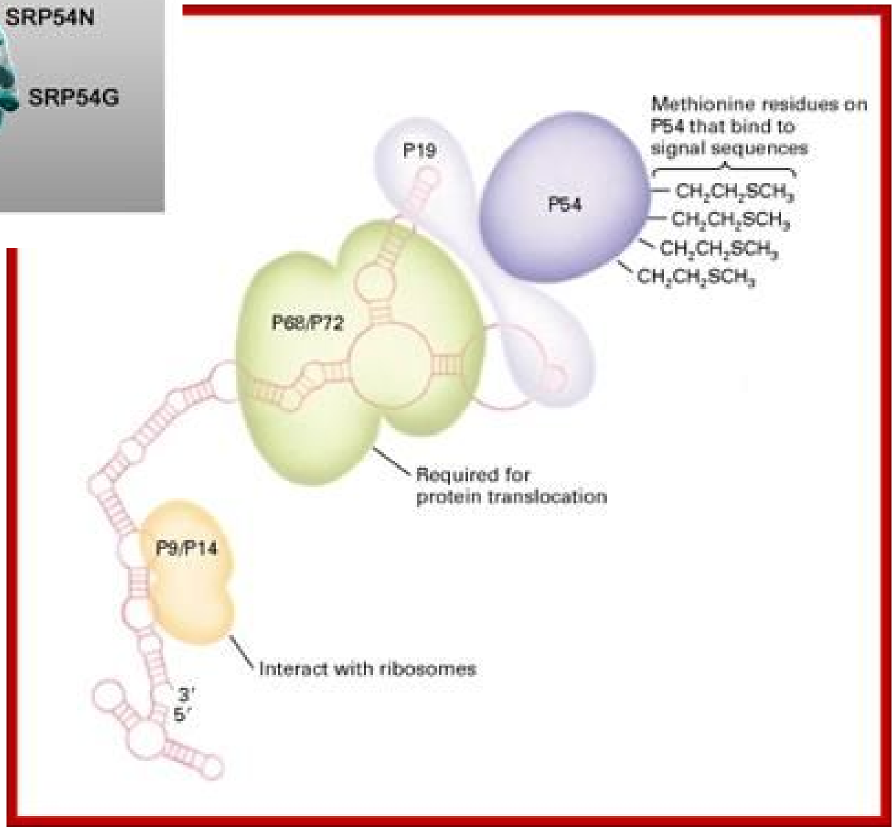
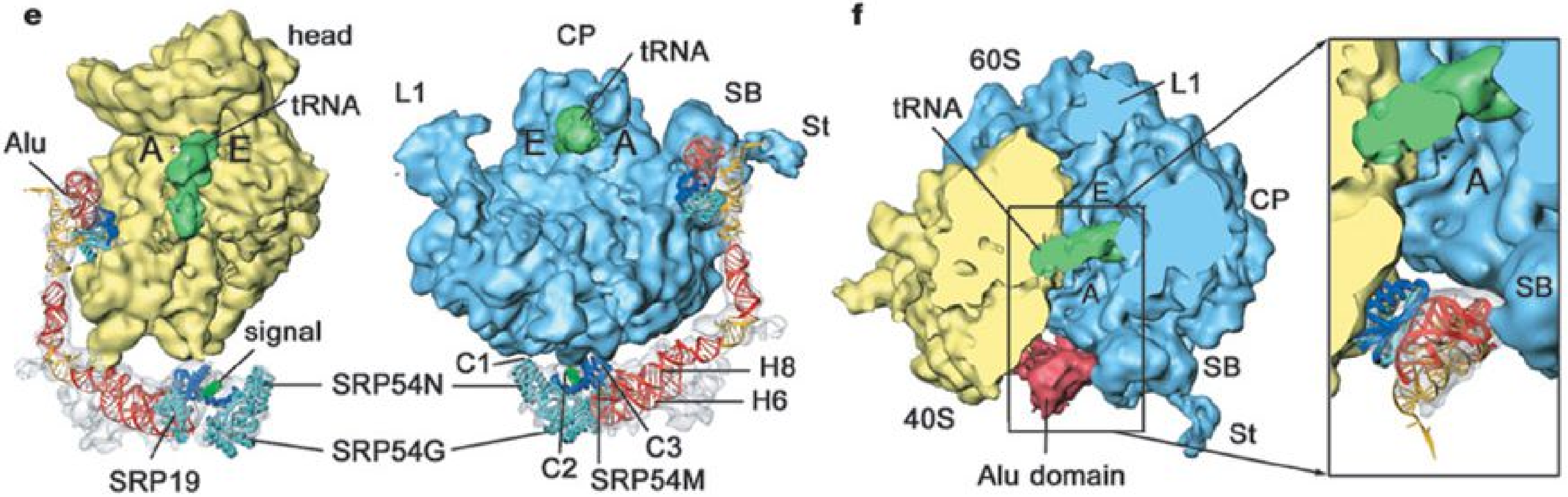
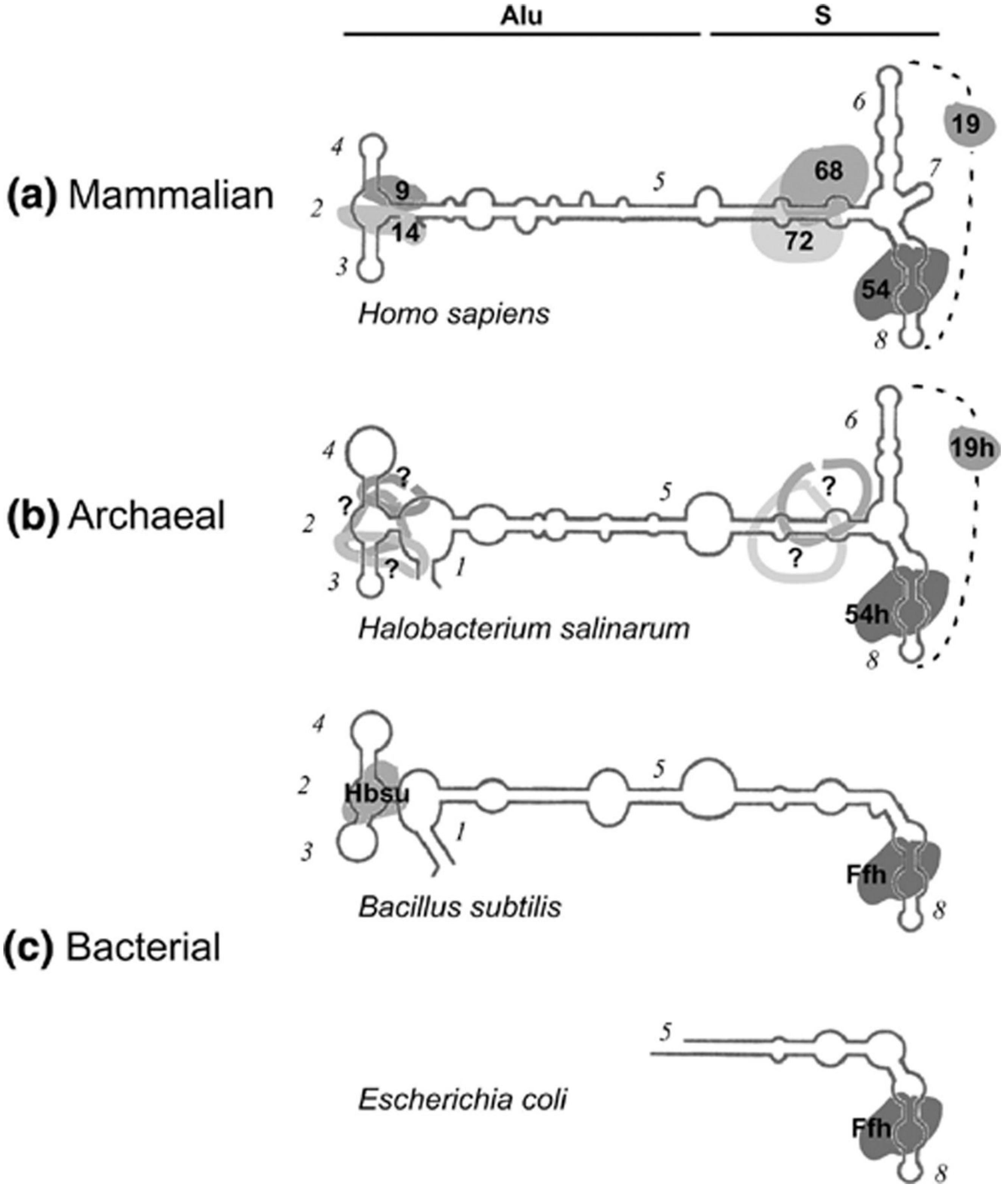


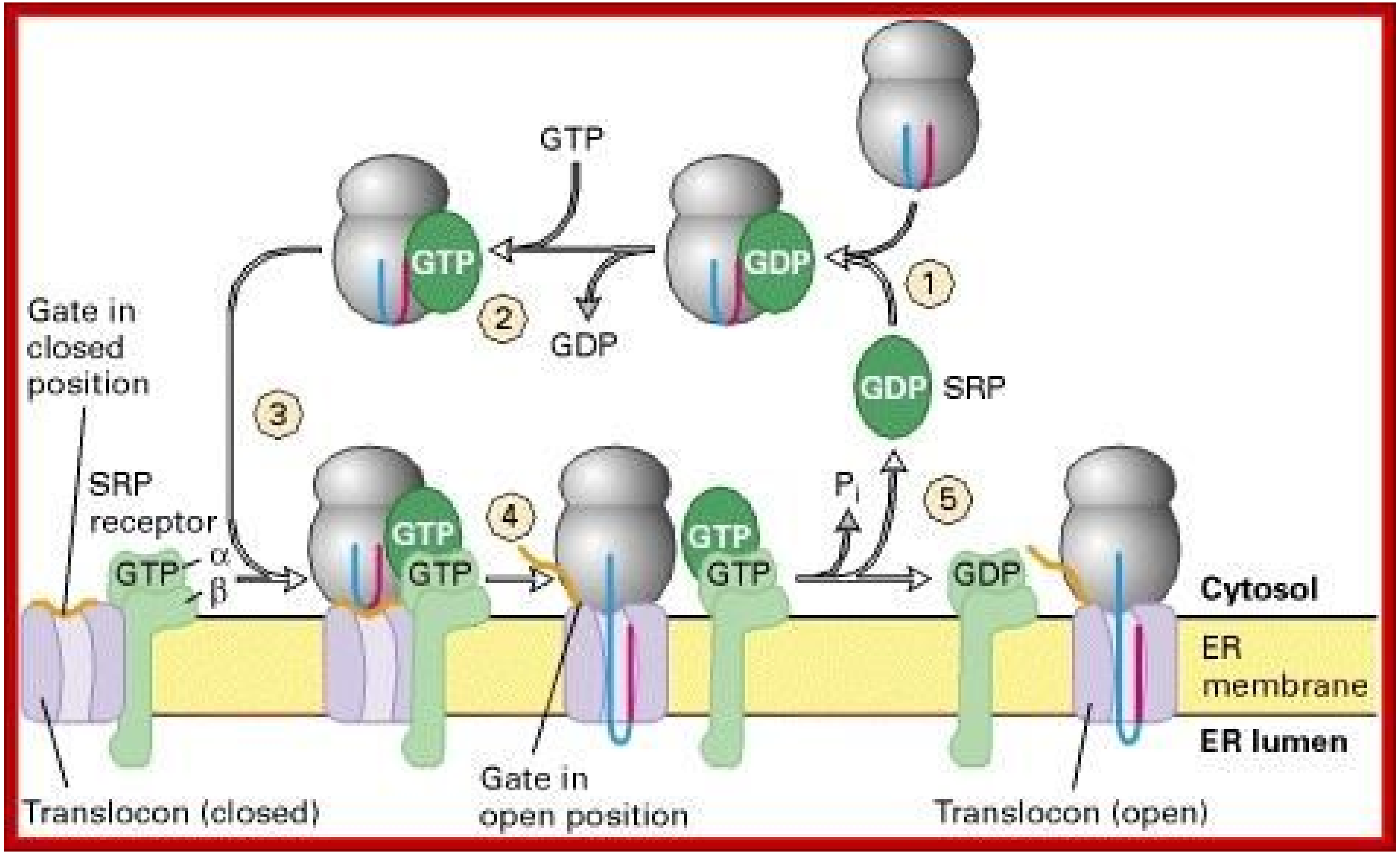
Figure 13-6
Molecular Cell Biology, Sixth Edition


a
Alu-domain
S- domain
L1.2
SRP9/14
H1.1
H1.2
50
300
1
H2
L2
hinge 2
hinge 1
100
280
H5
150
SRP19
H6
H7
SRP68/72
H8
SRP54M
signal
54NG
200
b
SRP54NG
SRP14
SRP54M
H5
H8
signal
L1.2
L2
H7
H6
SRP19
SRP68/72
c
SRP14
SRP9
SRP54NG
signal
hinge 2
SRP54M
H5
H8
hinge 1
SRP19
SRP68/72
H6
d
L1.2
L2
SRP14
SRP9
hinge 2
signal
54M
hinge 1
SRP54N
SRP54G
SRP68/72
SRP19

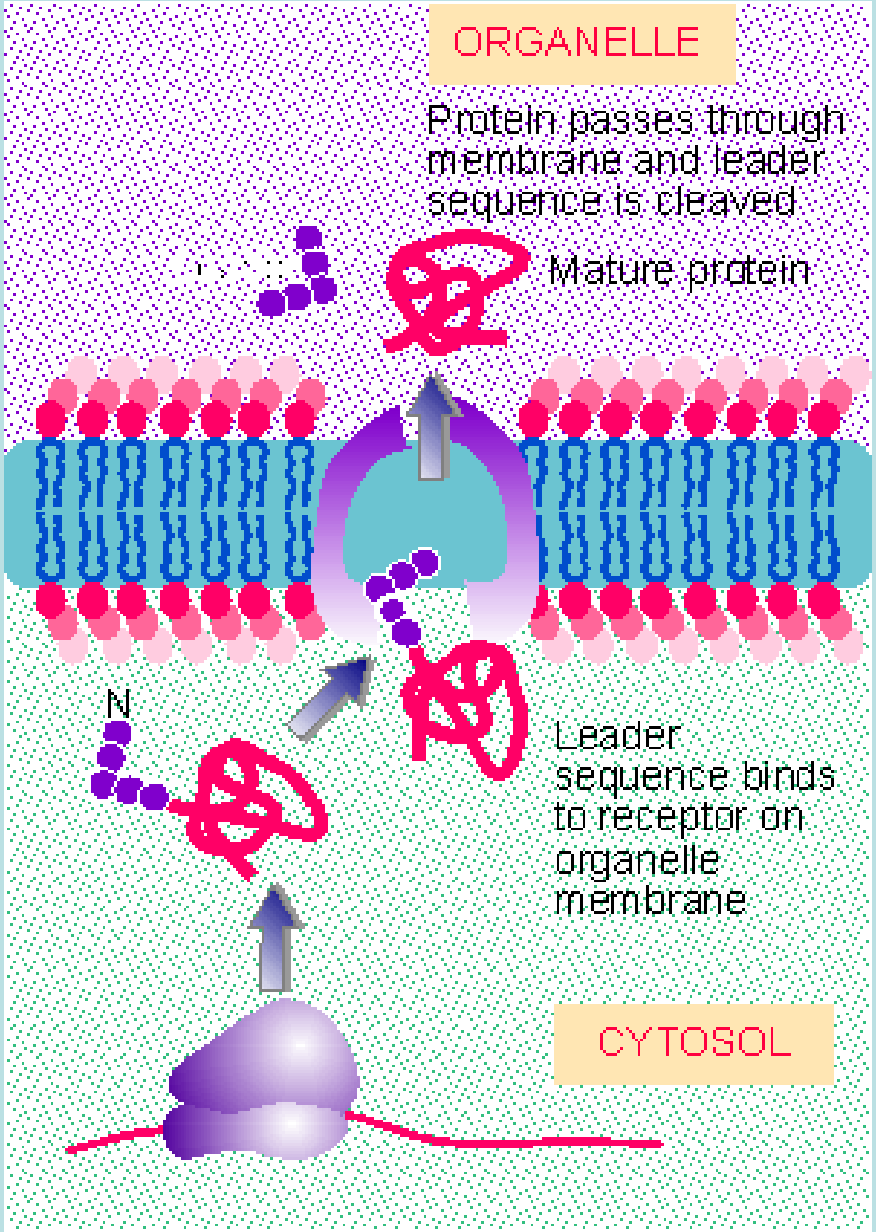

Methionine residues on P54 that bind to signal sequences
P19
P54
$CH_2CH_2SCH_3$
$CH_2CH_2SCH_3$
$CH_2CH_2SCH_3$
$CH_2CH_2SCH_3$
P68/P72
Required for protein translocation
P9/P14
Interact with ribosomes
3′
5′


e
head
tRNA
Alu
A
E
signal
SRP54N
SRP54G
SRP19
CP
tRNA
L1
SB
St
E
A
C1
C2
C3
H8
H6
SRP54M
f
60S
L1
tRNA
E
CP
A
SB
St
40S
Alu domain
A
SB


Alu
S
(a) Mammalian
4
9
2
14
3
5
6
19
68
7
72
54
8
Homo sapiens
(b) Archaeal
4
?
?
2
?
1
3
5
6
19h
?
?
54h
8
Halobacterium salinarum
(c) Bacterial
4
2
Hbsu
3
1
5
Ffh
8
Bacillus subtilis
5
Escherichia coli
Ffh
8


GTP
GTP
GDP
GDP
②
①
Gate in closed position
③
SRP
GDP
SRP receptor
α
β
GTP
GTP
GTP
④
GTP
GTP
P_i
⑤
GDP
Cytosol
ER membrane
ER lumen
Translocon (closed)
Gate in open position
Translocon (open)

Translocation to organelles



Leader sequences allow proteins to recognize mitochondrial or chloroplast surfaces by a post-translational process.

Signal sequence on Mitochondrial proteins

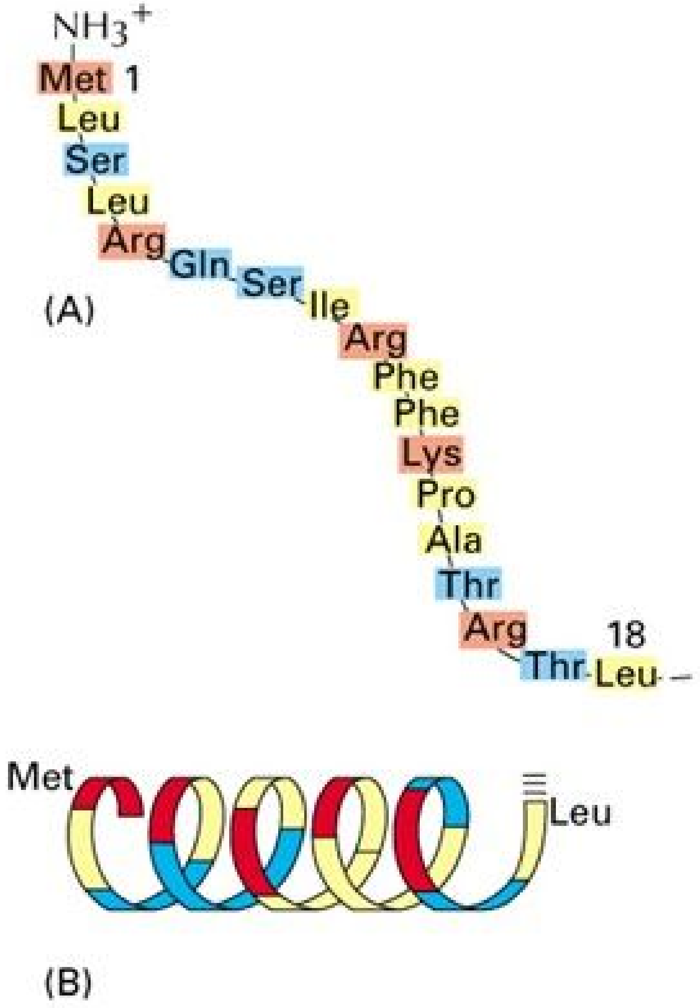


Figure 12–23. Molecular Biology of the Cell, 4th Edition.

- ➢ All mitochondrial precursor proteins have a signal sequence at their N terminus that is rapidly removed after import by a protease (the signal peptidase) in the mitochondrial matrix.

- ➢ These signal sequences are actually an amphipathic α helix, in which positively charged residues are clustered on one side of the helix, while uncharged hydrophobic residues are clustered on the opposite side. This configuration rather than a precise amino acid sequence is recognized by specific receptor proteins that initiate protein translocation.

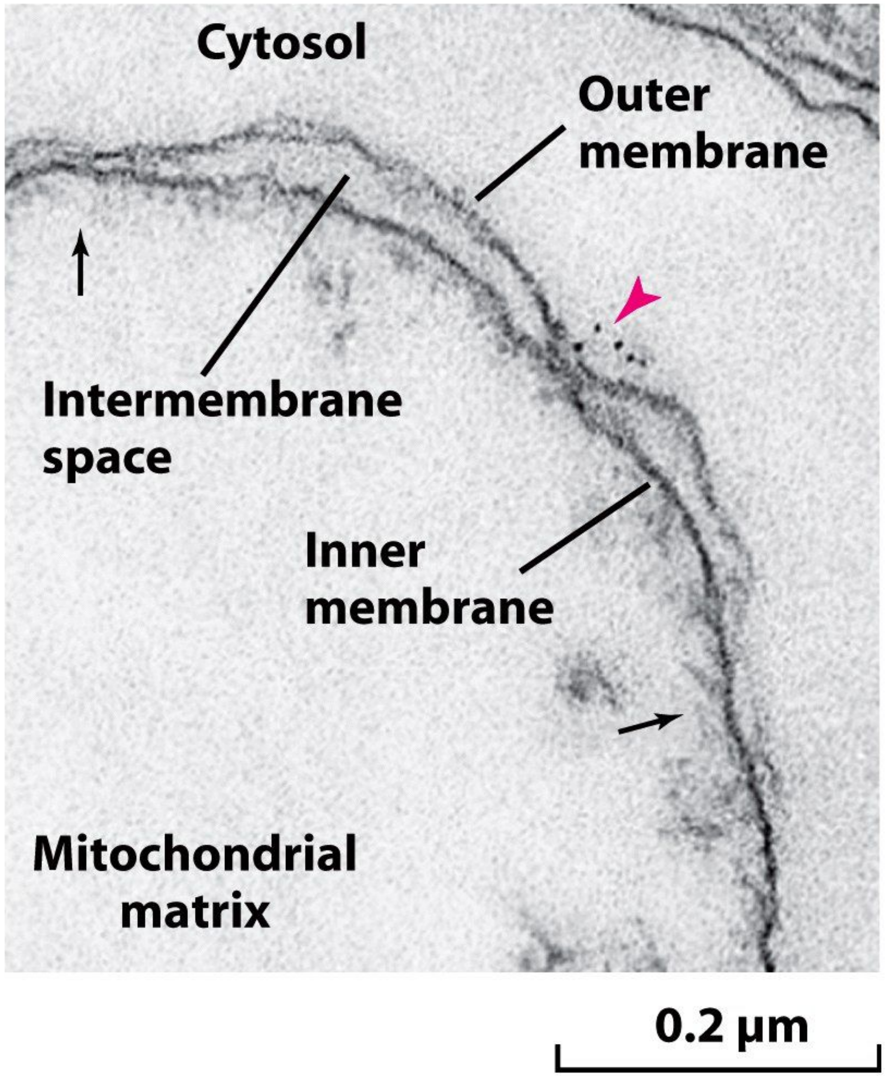
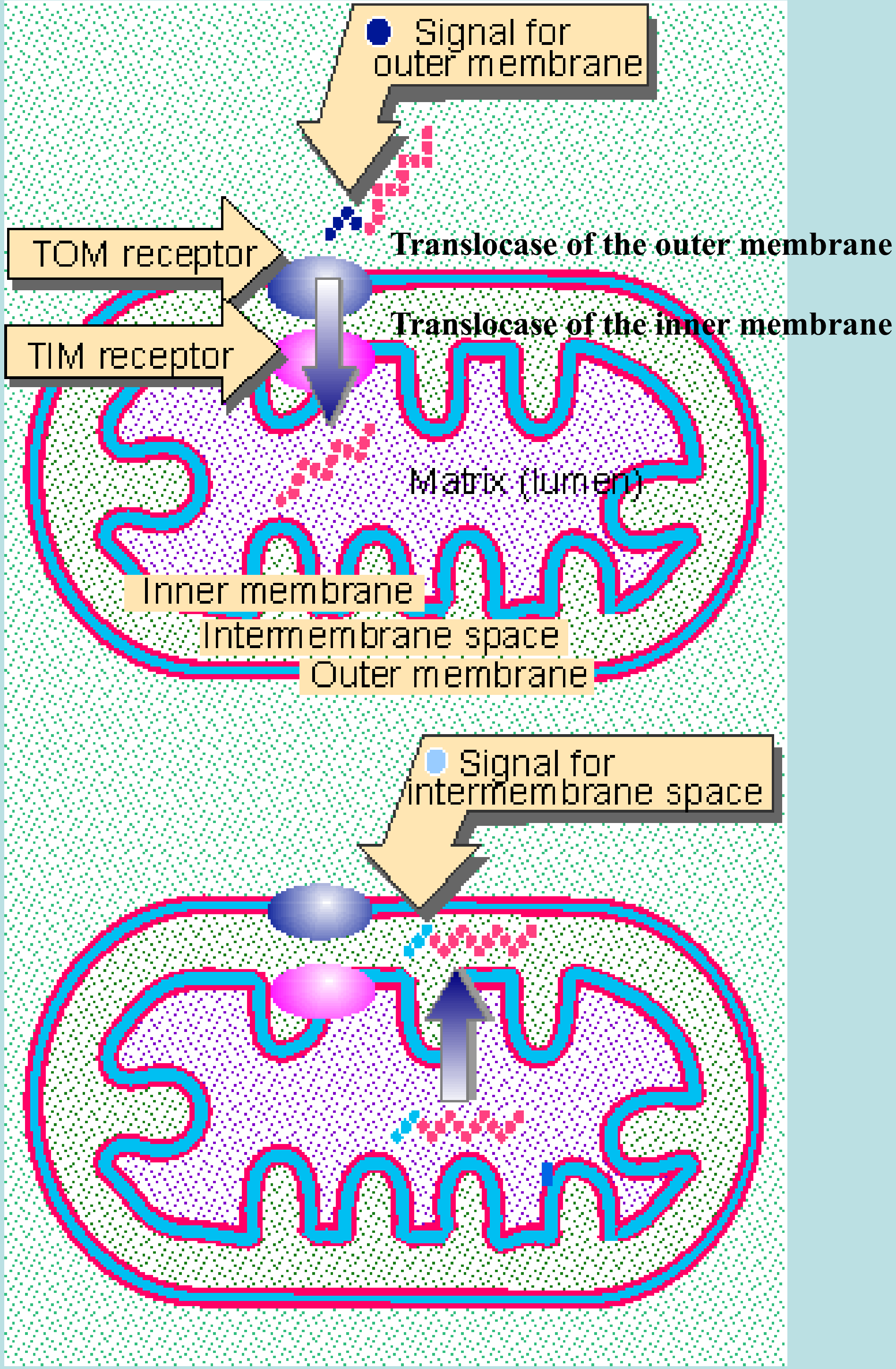
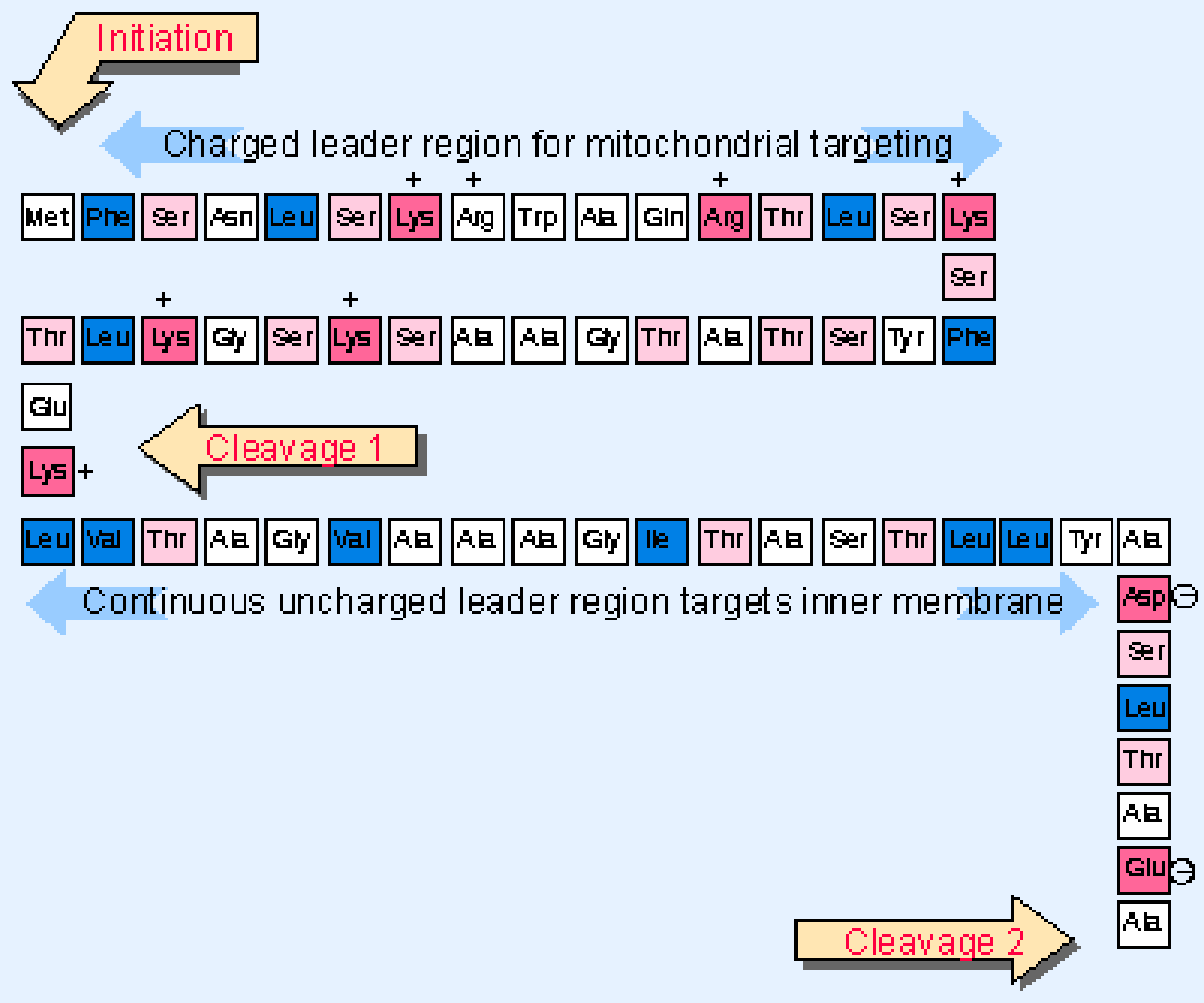
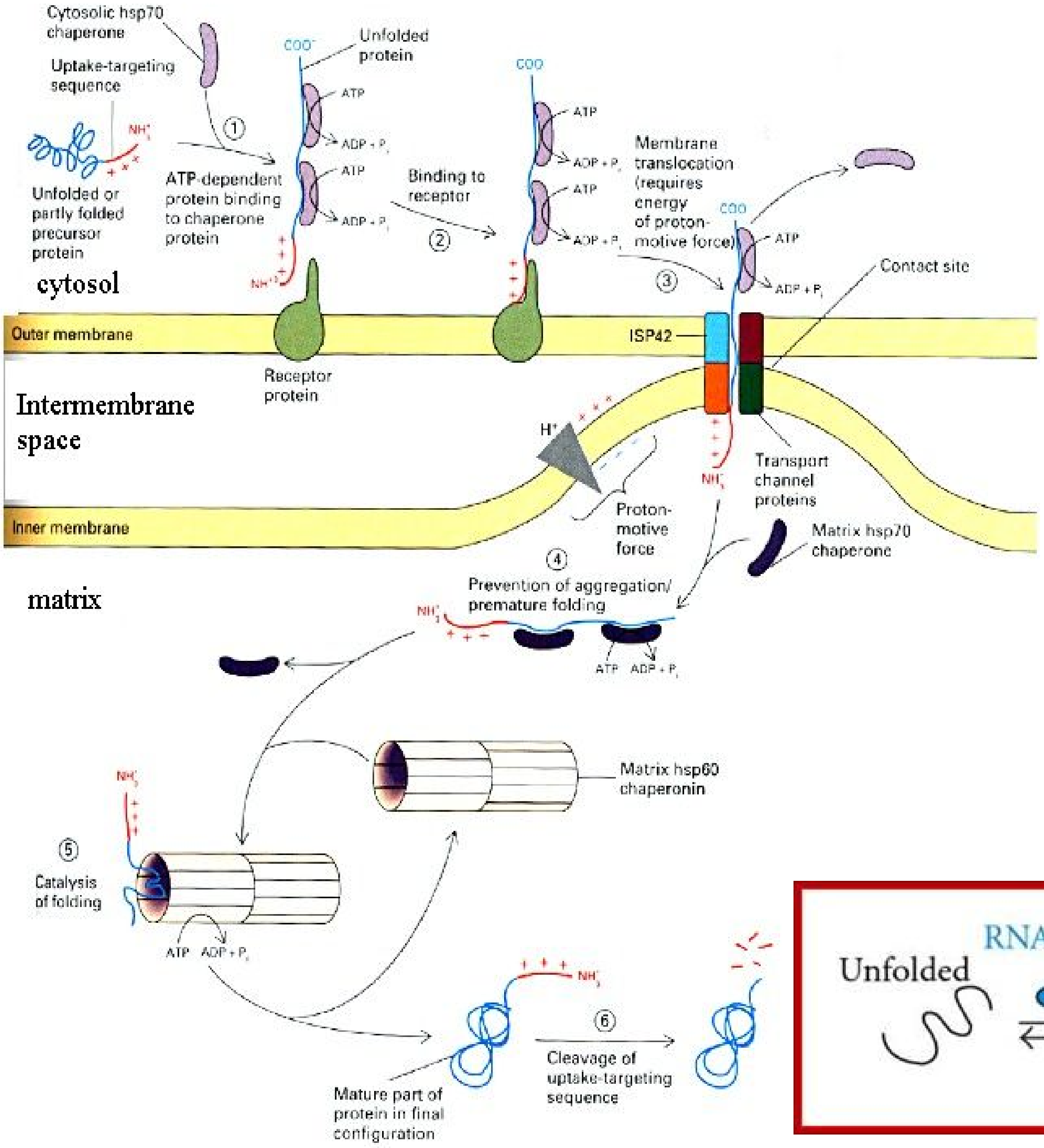


Figure 13-24c
Molecular Cell Biology, Sixth Edition
© 2008 W. H. Freeman and Company

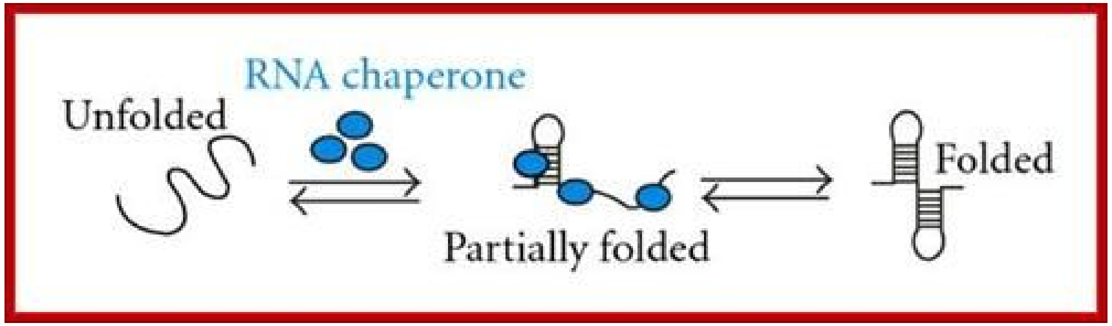




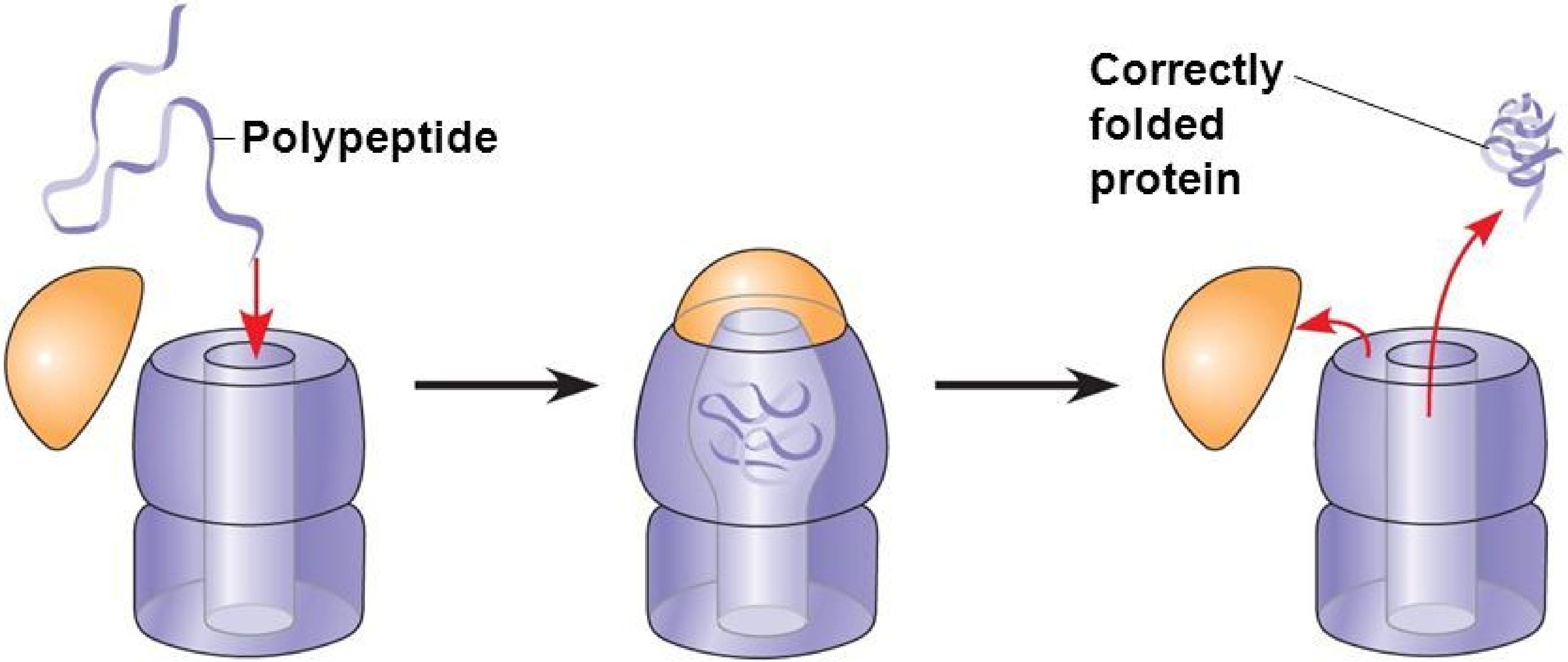
The leader of yeast cytochrome *c*1 contains an N-terminal region that targets the protein to the mitochondrion, followed by a region that targets the (cleaved) protein to the inner membrane. The leader is removed by two cleavage events.



Proteins are synthetized in cytosol as unfolded or partly folded precursors. There are some proteins table to interact with these precursors and make them soluble even if in an unfolded state; they act before and after translocation (chaperons). Other proteins help to reach the correct fold once precursors are inside the matrix (chaperonins).



LE 5-23b

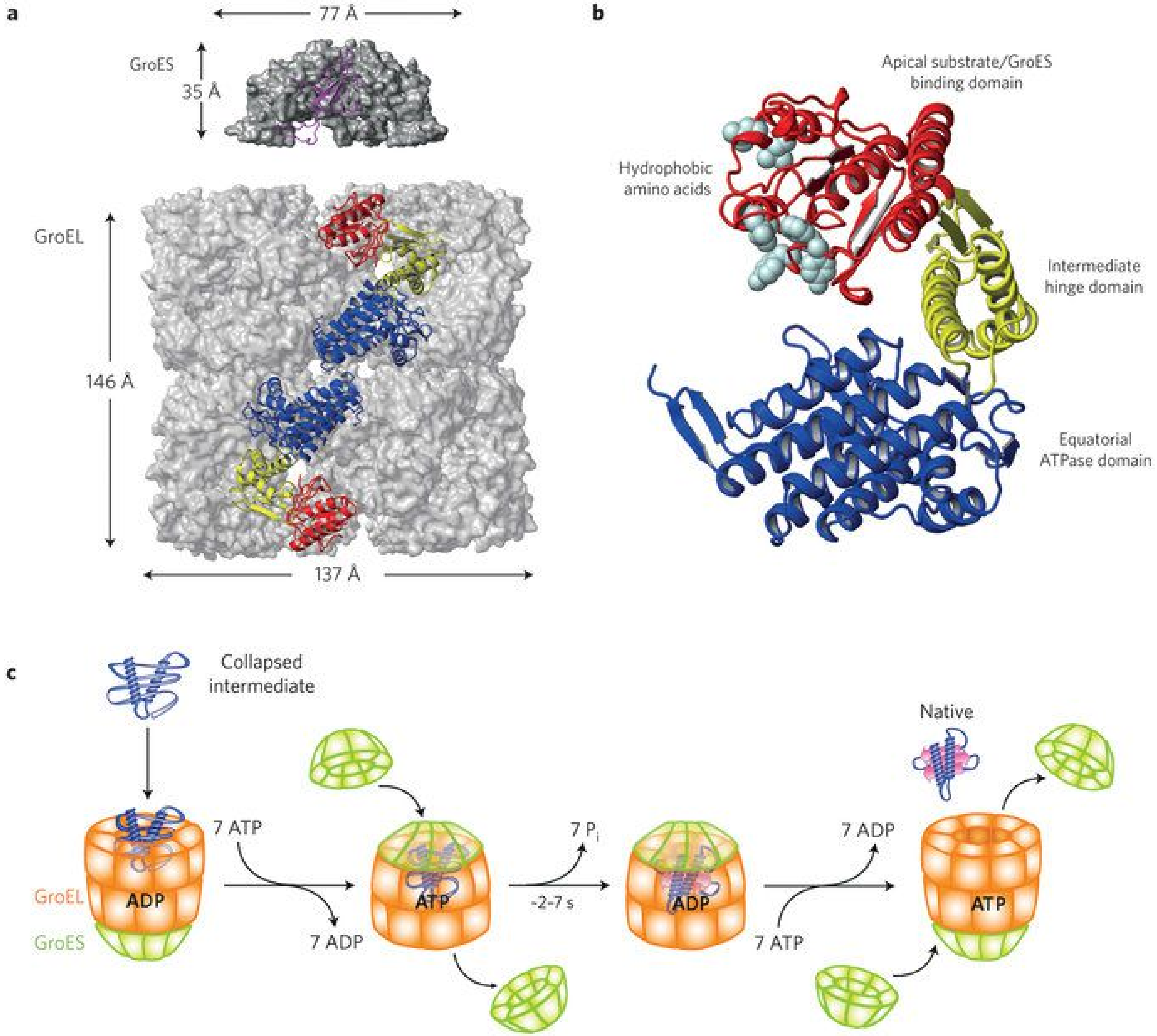


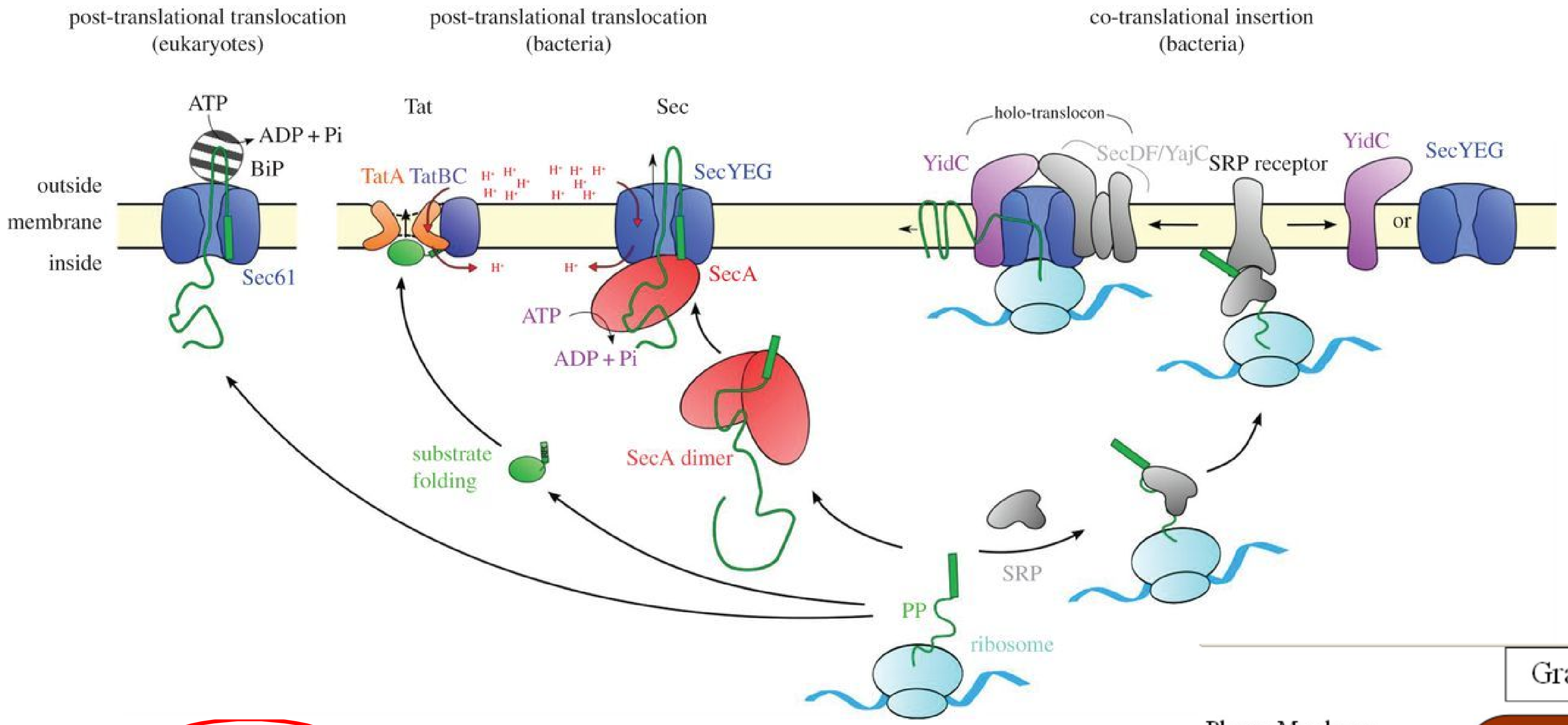
Steps of Chaperonin Action:

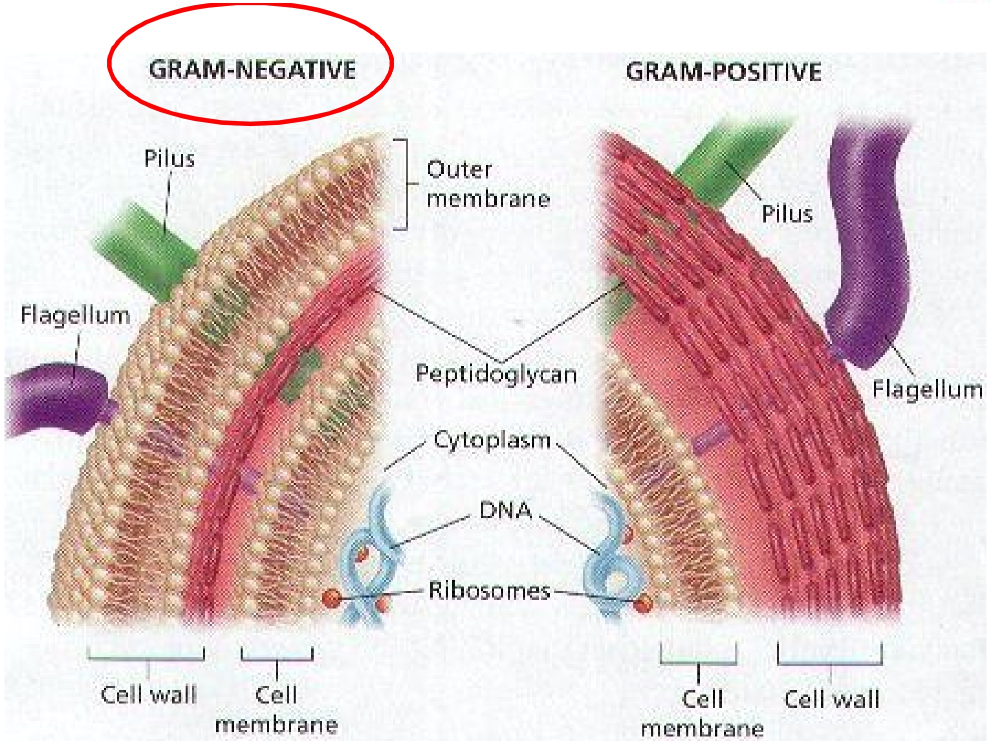
① An unfolded polypeptide enters the cylinder from one end.

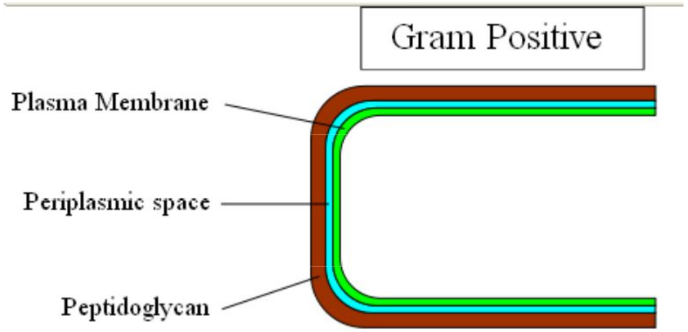
② The cap attaches, causing the cylinder to change shape in such a way that it creates a hydrophilic environment for the folding of the polypeptide.

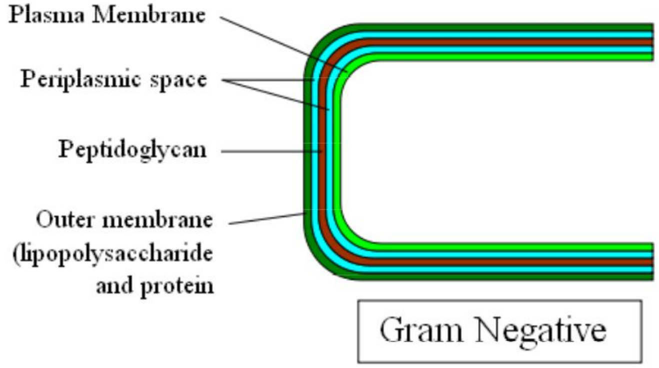
③ The cap comes off, and the properly folded protein is released.

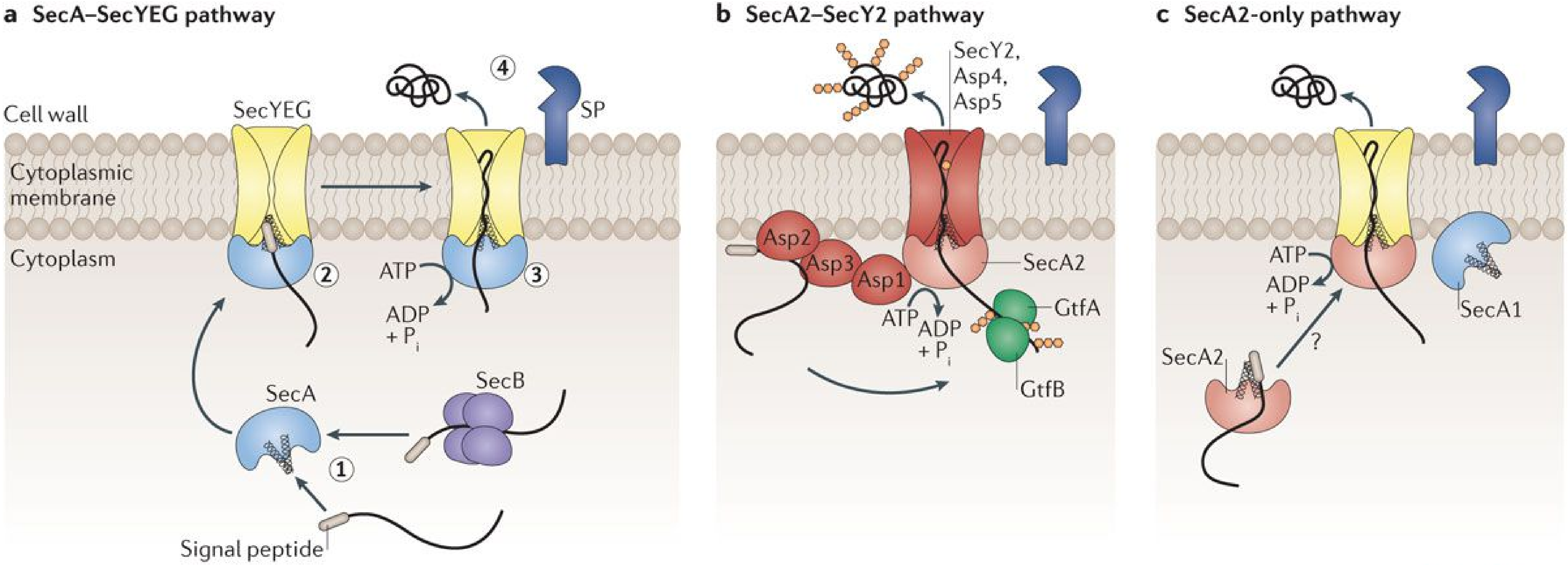

a
77 Å
GroES
35 Å
GroEL
146 Å
137 Å
b
Apical substrate/GroES binding domain
Hydrophobic amino acids
Intermediate hinge domain
Equatorial ATPase domain
c
Collapsed intermediate
7 ATP
GroEL
ADP
GroES
7 ADP
ATP
7 P_i
-2-7 s
ADP
7 ATP
7 ADP
Native
ATP


post-translational translocation
(eukaryotes)
post-translational translocation
(bacteria)
co-translational insertion
(bacteria)
ATP
ADP + Pi
BiP
outside
membrane
inside
Sec61
Tat
TatA TatBC
H+
Sec
SecYEG
SecA
ATP
ADP + Pi
SecA dimer
substrate
folding
holo-translocon
YidC
SecDF/YajC
SRP receptor
YidC
SecYEG
or
SRP
PP
ribosome

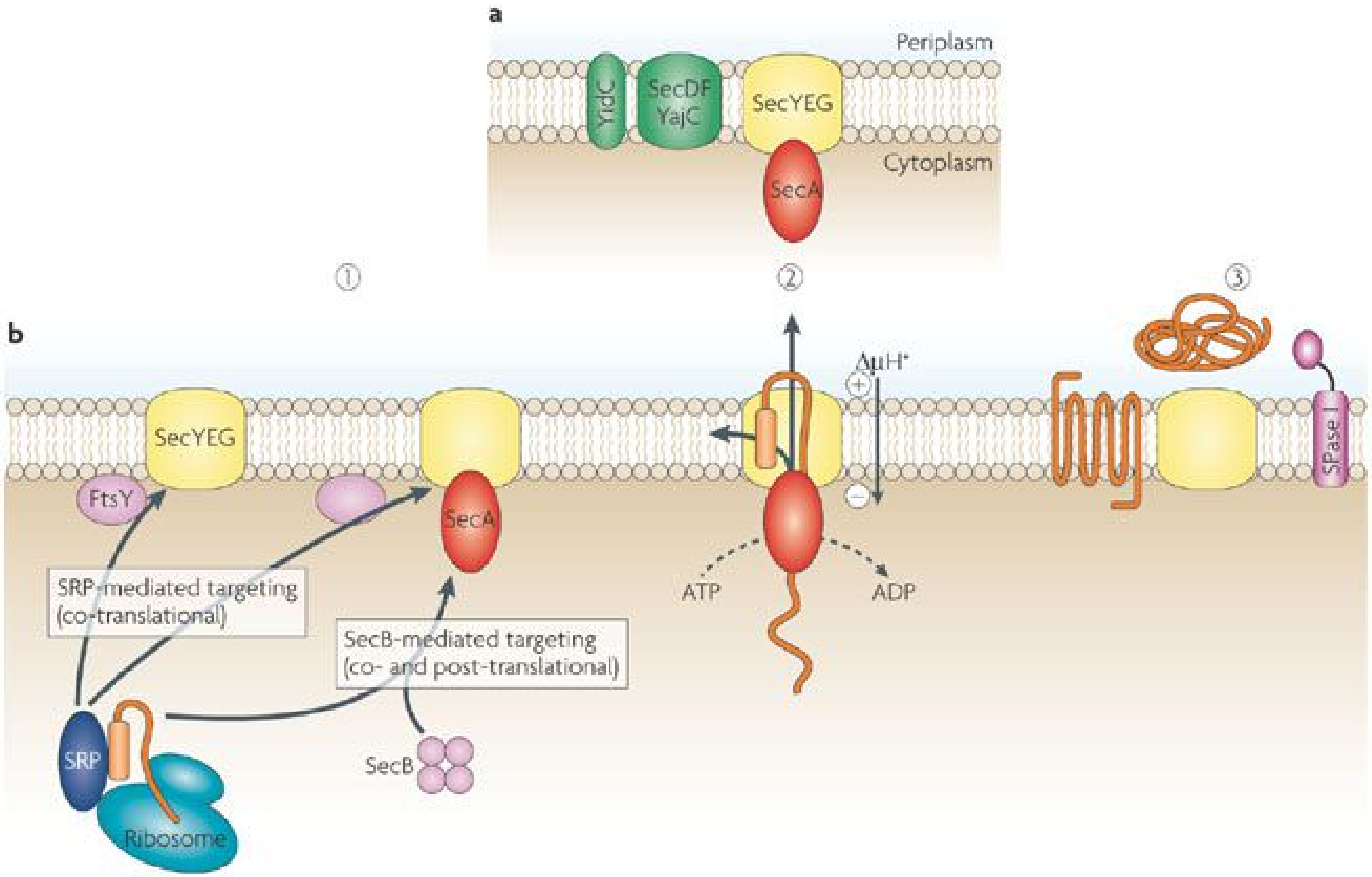

GRAM-NEGATIVE
GRAM-POSITIVE
Pilus
Outer
membrane
Flagellum
Peptidoglycan
Pilus
Flagellum
Cytoplasm
DNA
Ribosomes
Cell wall
Cell
membrane
Cell
membrane
Cell wall


Gram Positive
Plasma Membrane
Periplasmic space
Peptidoglycan

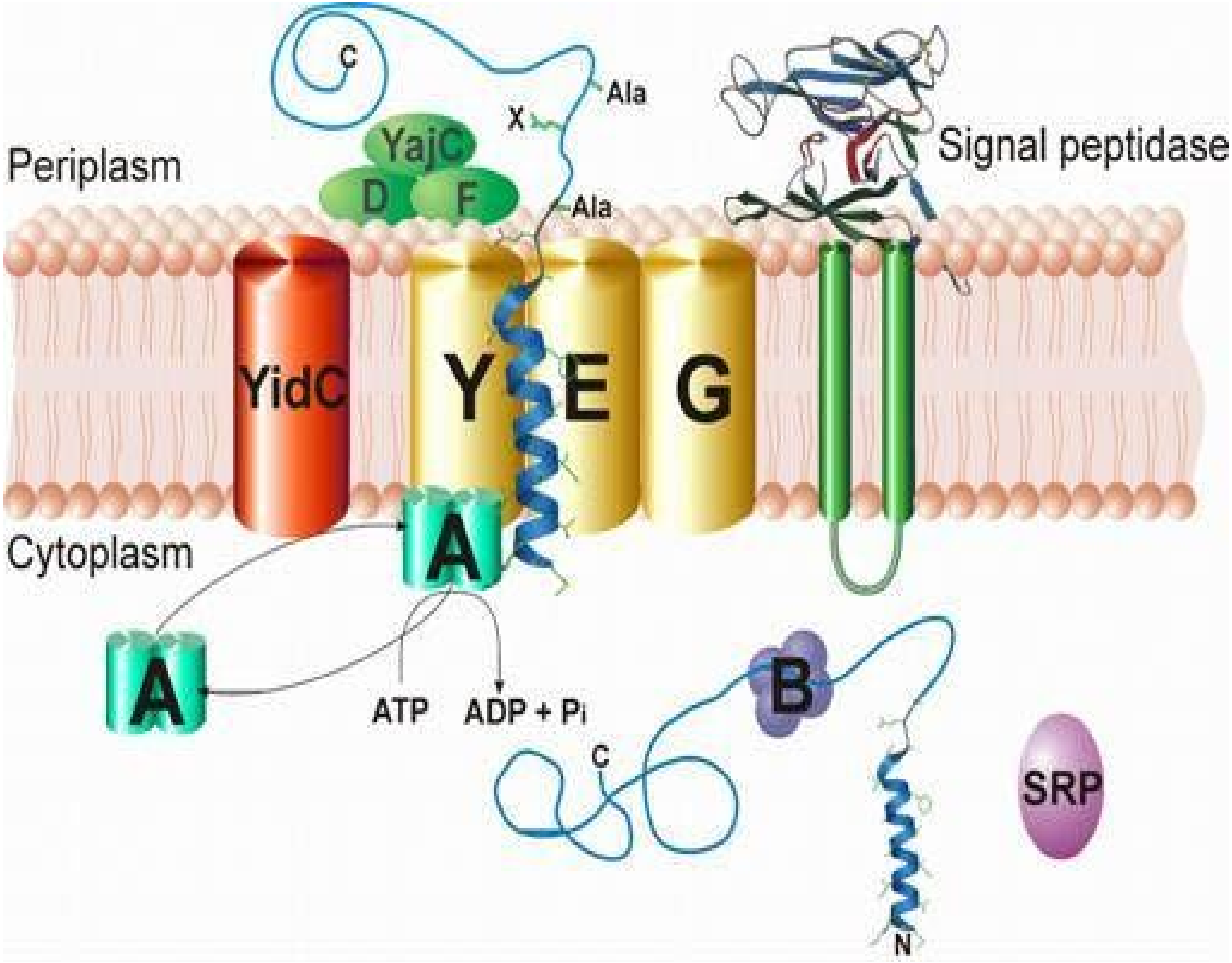

Plasma Membrane
Periplasmic space
Peptidoglycan
Outer membrane
(lipopolysaccharide
and protein
Gram Negative



Nature Reviews | Microbiology

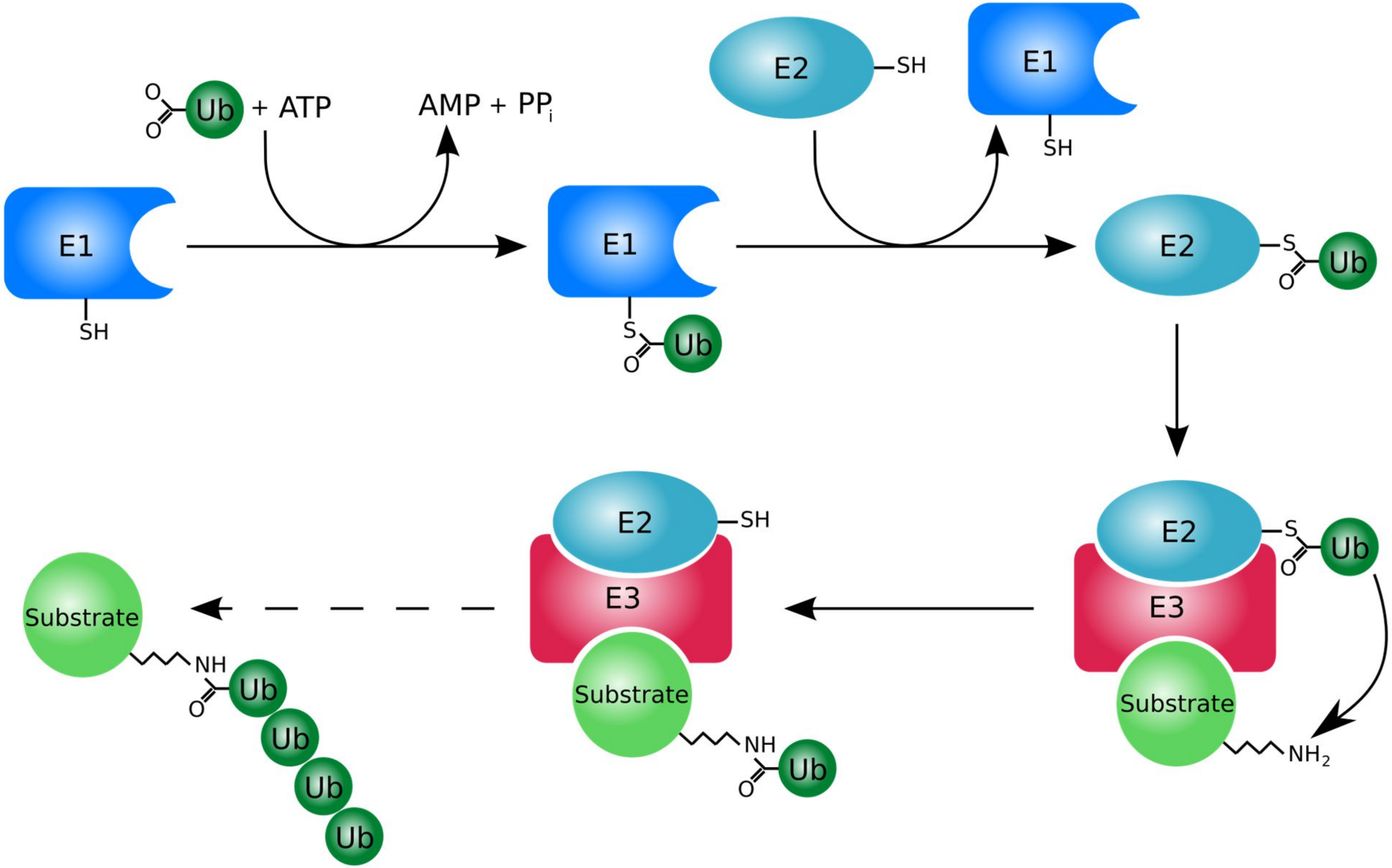


Nature Reviews | Microbiology


C
Ala
X
YajC
Periplasm
D
F
Ala
Signal peptidase
YidC
Y
E
G
Cytoplasm
A
A
ATP
ADP + Pi
B
C
SRP
N

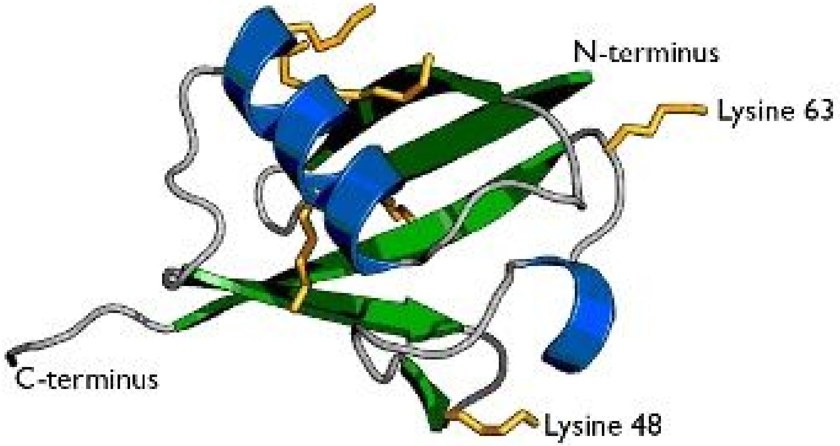
Degrade unneeded or damaged proteins by proteolysis

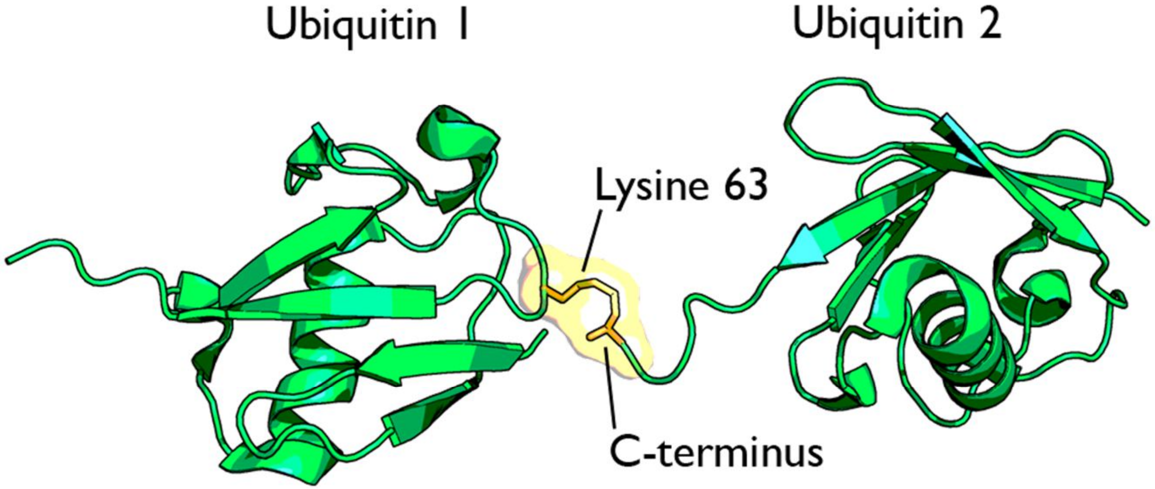
Ubiquitin is a highly-conserved regulatory protein that is *ubiquitously* expressed in eukaryotes. **Ubiquitination** (or **ubiquitylation**) refers to the post-translational modification of a protein by the covalent attachment (via an isopeptide bond) of one or more ubiquitin monomers. The most prominent function of ubiquitin is labeling proteins for proteasomal degradation. Besides this function, ubiquitination also controls the stability, function, and intracellular localization of a wide variety of proteins.

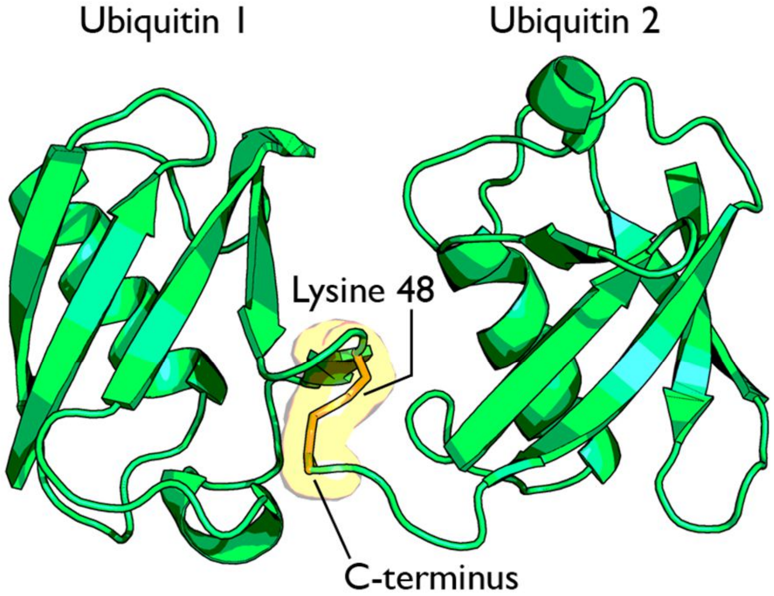


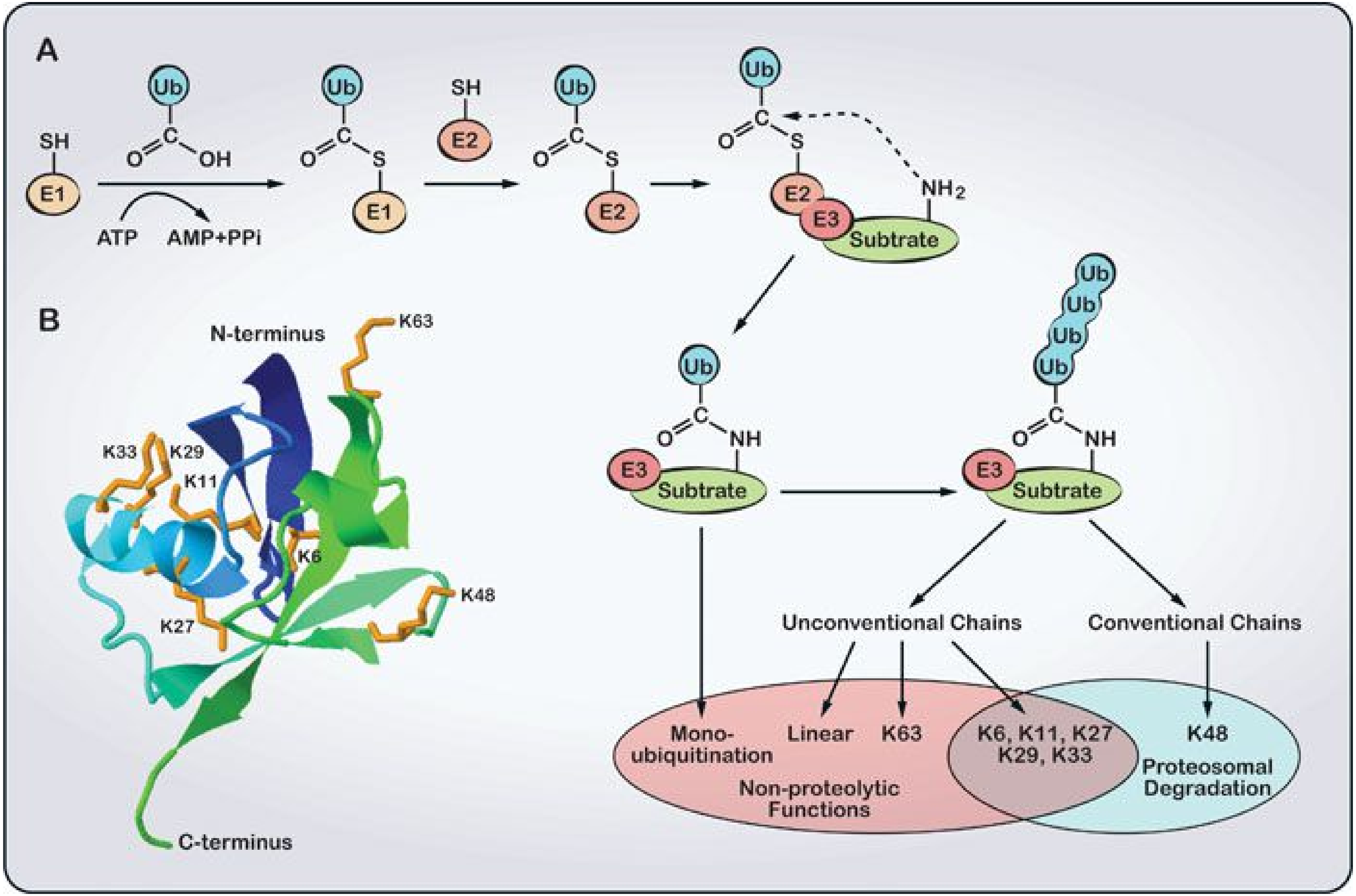
Ubiquitin

- Consists of 76 amino acids, 8.5 kDa
- Found in all eukaryotic cells (ubiquitously)
- Highly Conserved
- Used in post-translational modification

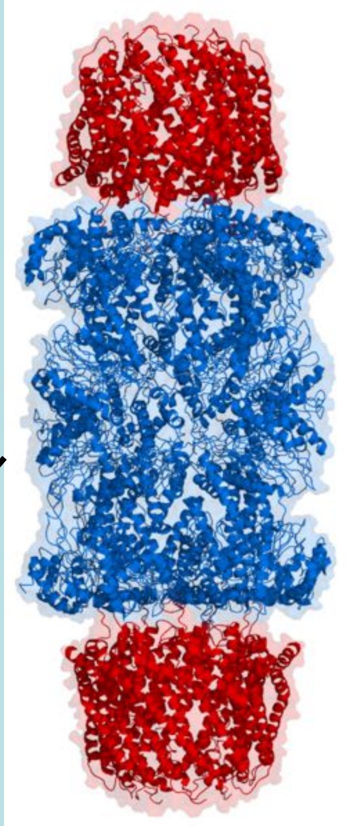


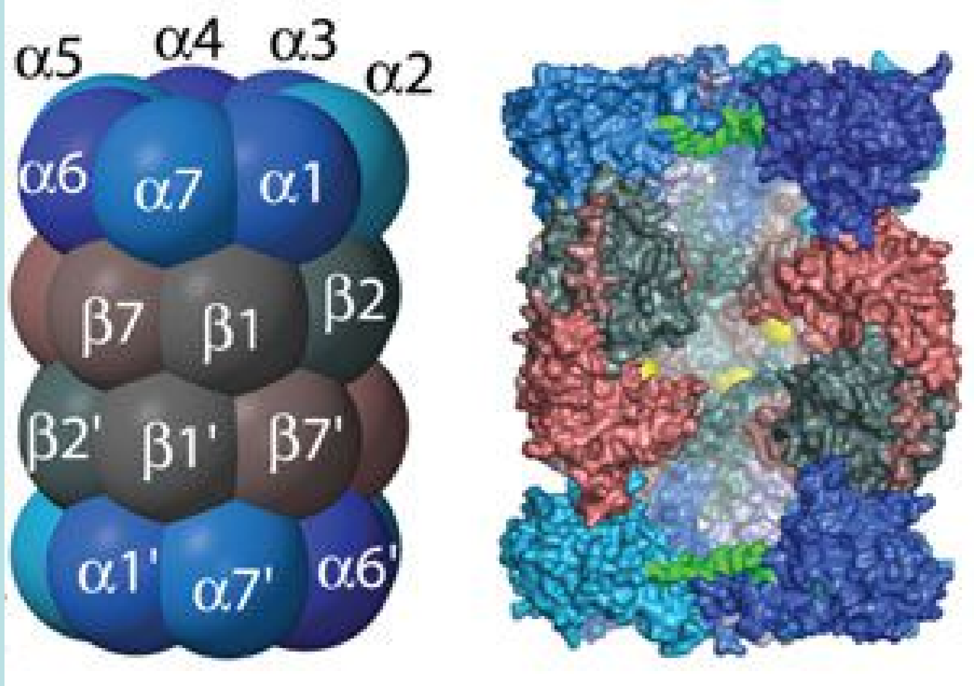


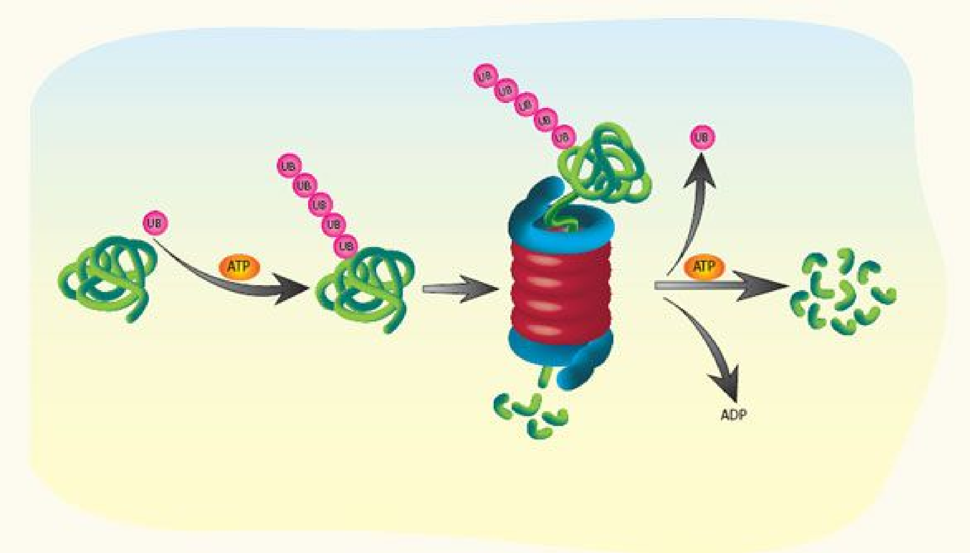



A
SH
E1
Ub
C
O
OH
ATP
AMP+PPi
S
E1
SH
E2
E2
E3
Subtrate
NH2
NH
Ub
Ub
Ub
Ub
Unconventional Chains
Conventional Chains
Mono-ubiquitination
Linear
K63
K6, K11, K27
K29, K33
K48
Proteosomal Degradation
Non-proteolytic Functions
B
N-terminus
K63
K33
K29
K11
K6
K48
K27
C-terminus

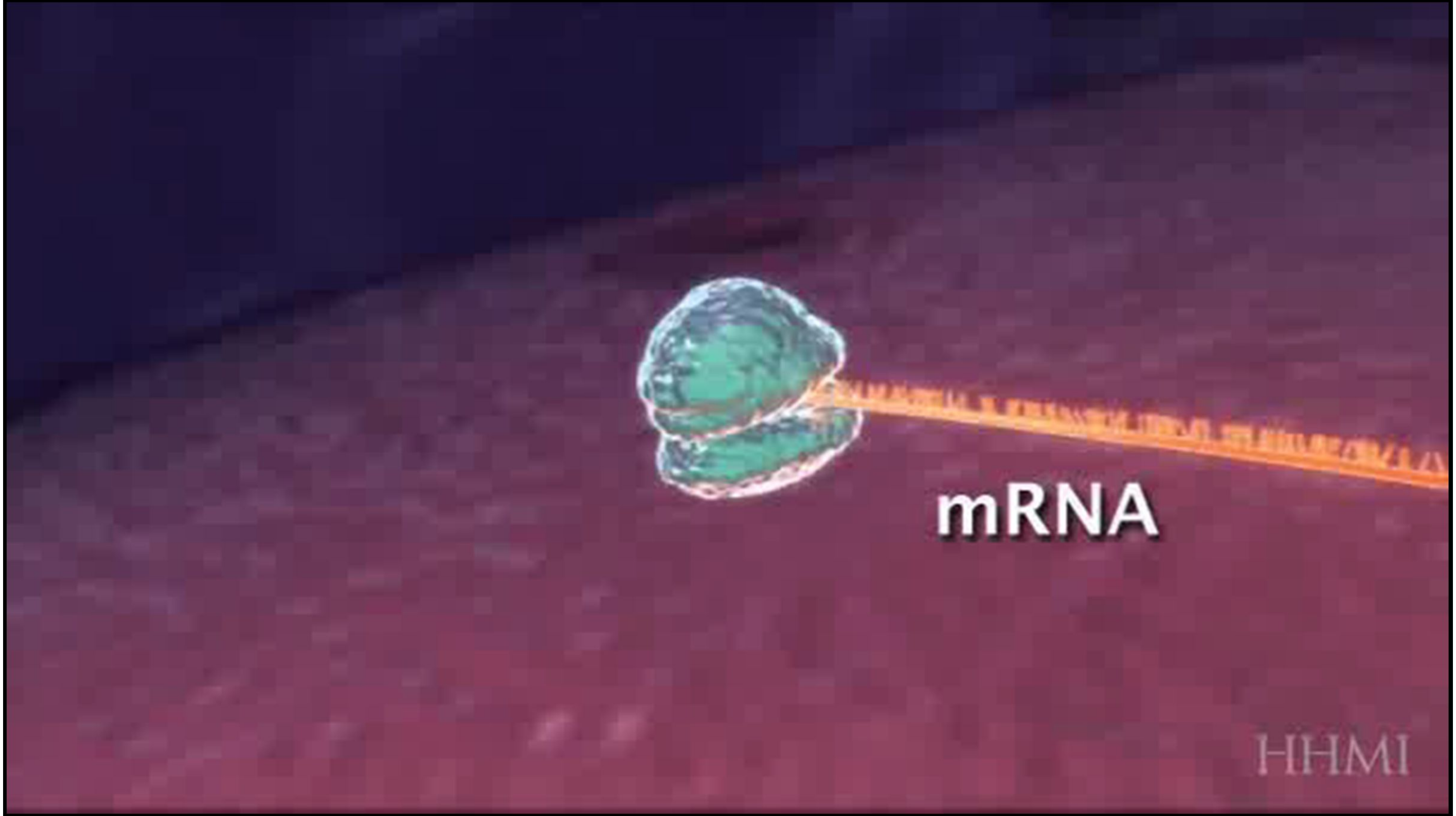
Proteasomes are large protein complexes inside all eukaryotes and archaea, as well as in some bacteria. In eukaryotes, they are located in the nucleus and the cytoplasm. The main function of the proteasome is to degrade unneeded or damaged proteins by proteolysis, a chemical reaction that breaks peptide bonds.







HHMI


mRNA
HHMI