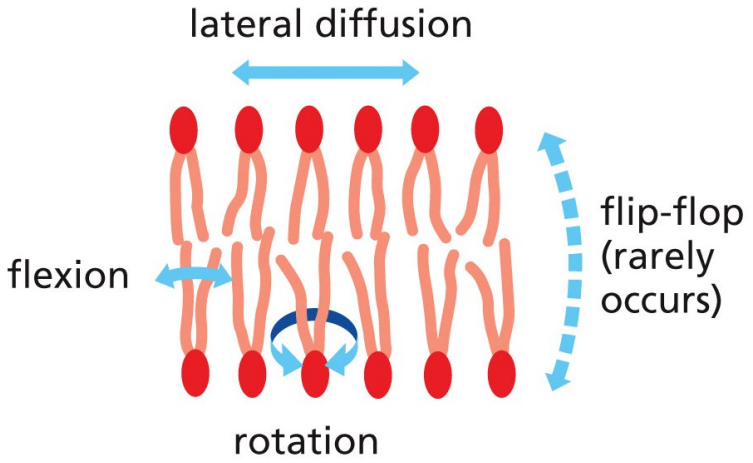
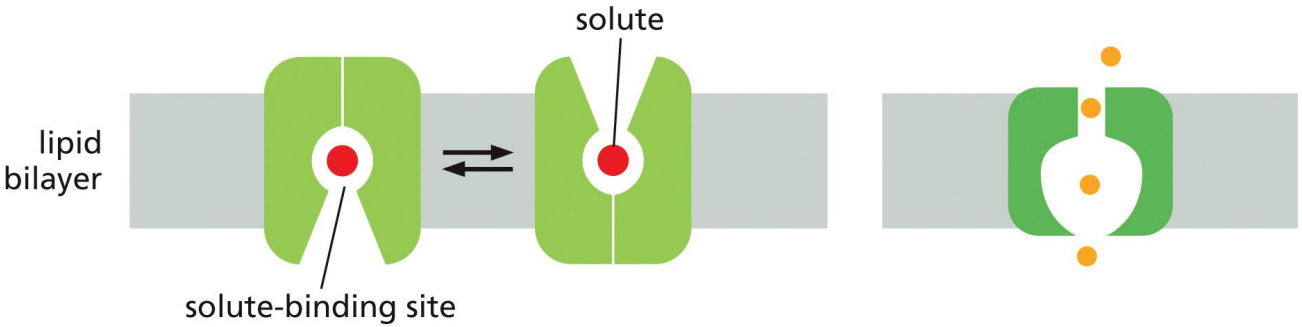
Recap, lecture 5

- Membranes are liquid, yet impermeable for most molecules (only small + hydrophobic pass freely)
- Allow cells to control the movement of molecules to the cytosol and between cytosol and different cell organelles



- Transporters and channels enable selective and controlled passing of molecules



Cell Biology
Lecture 6

Intracellular Compartments and Protein Sorting, Part I

Sesilja Aranko

10.11.2023

Alberts • Johnson • Lewis • Morgan • Raff • Roberts • Walter

Molecular Biology of the Cell

Sixth Edition

Chapter 12
Intracellular Compartments and Protein Sorting

Pages: 641-654, 669-688

Course overview – Tentative schedule

Date	Lecture		Chapters & Topics	Assignments
25.10.	1	Part 1	*Course overview,* DNA, Chromosomes, Genome, Ch. 4	
27.10.	2 -G		Replication, Repair, Recombination, Ch. 5	
1.11.	3		From DNA to protein, Ch. 6	
3.11.	4		Control of gene expression, Ch. 7	
8.11.	5	Part 2	Membrane structures, Ch. 10 Membrane transport, Ch. 11	**Assignment I (Essay) Draft I** (8.11.)
10.11.	6 -G		Intracellular compartments and protein sorting, Ch. 12	
15.11.	7		Intracellular compartments and protein sorting, Ch. 12 **Susanna Mäkinen, Solar Foods**	**Assignment II – Draft I (15.11.)**
17.11.	8		Membrane Traffic, Ch. 13 **iGEM team 2023**	*+iGEM intro*
22.11.	9	Part 3	Cell signalling, Ch. 15	**Assignment II – Peer review** (22.11.)
24.11.	10 -G		Cell signalling, Ch. 15	**Assignment I (Essay) Draft II** (24.11.)
29.11.	11		Cell cycle, Ch. 17 **Jere Weltner, Folkhälsan**	
1.12.	12		Apoptosis, Ch. 18	**Assignment II – final version** (1.12.)
7.12.	EXAM		December 7th	
8.12.	Final version essay		December 8th	**Assignment I (Essay) Final version** (8.12.) Aim at finishing before exam date. Use last days for polishing.

Learning outcomes

- Can appreciate the role of the compartments in organizing the cell and its functions
- Can describe transport mechanisms of proteins between cytosol and nucleus as well as to ER

THE COMPARTMENTALIZATION OF CELLS

- All eukaryotic cells have the same basic set of **membrane-enclosed organelles**

- Macromolecules can also be segregated *without* a surrounding membrane -> **condensates**

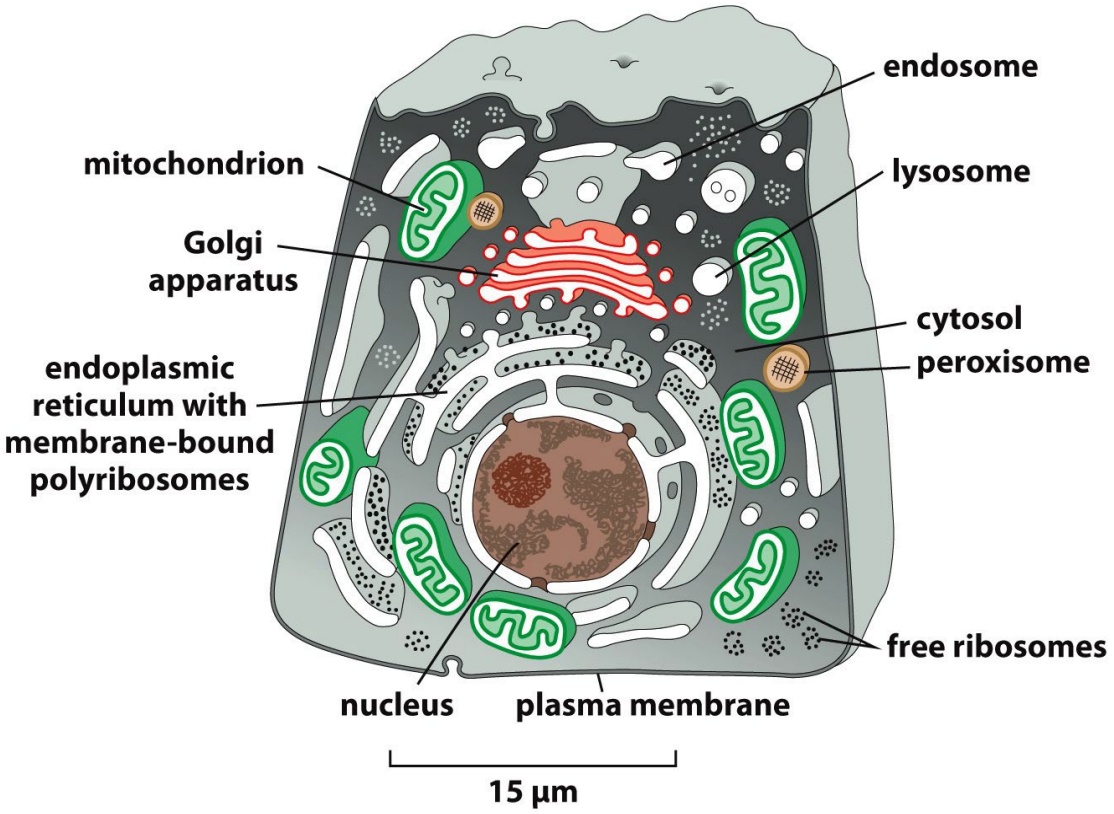
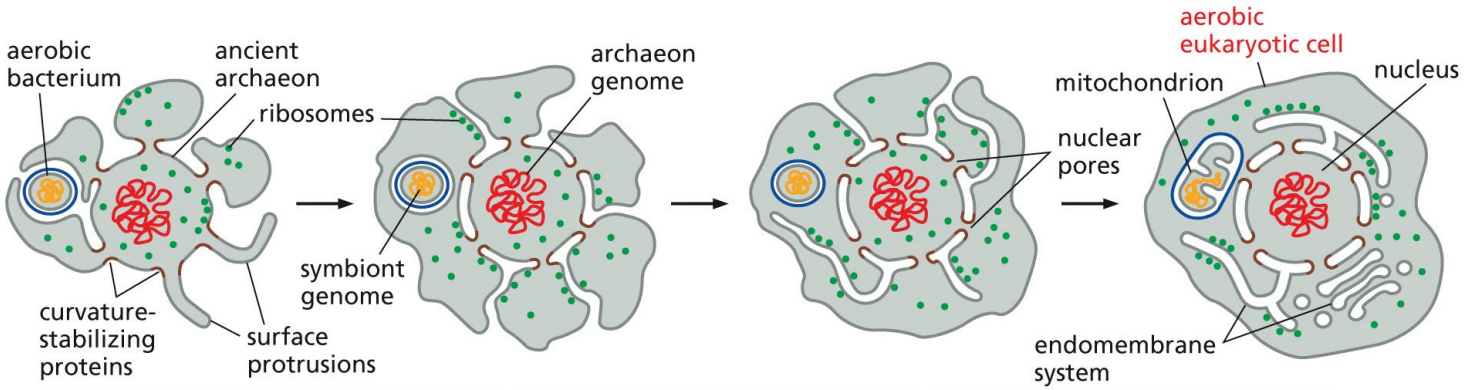


Figure 12-1 Molecular Biology of the Cell 6e (© Garland Science 2015)

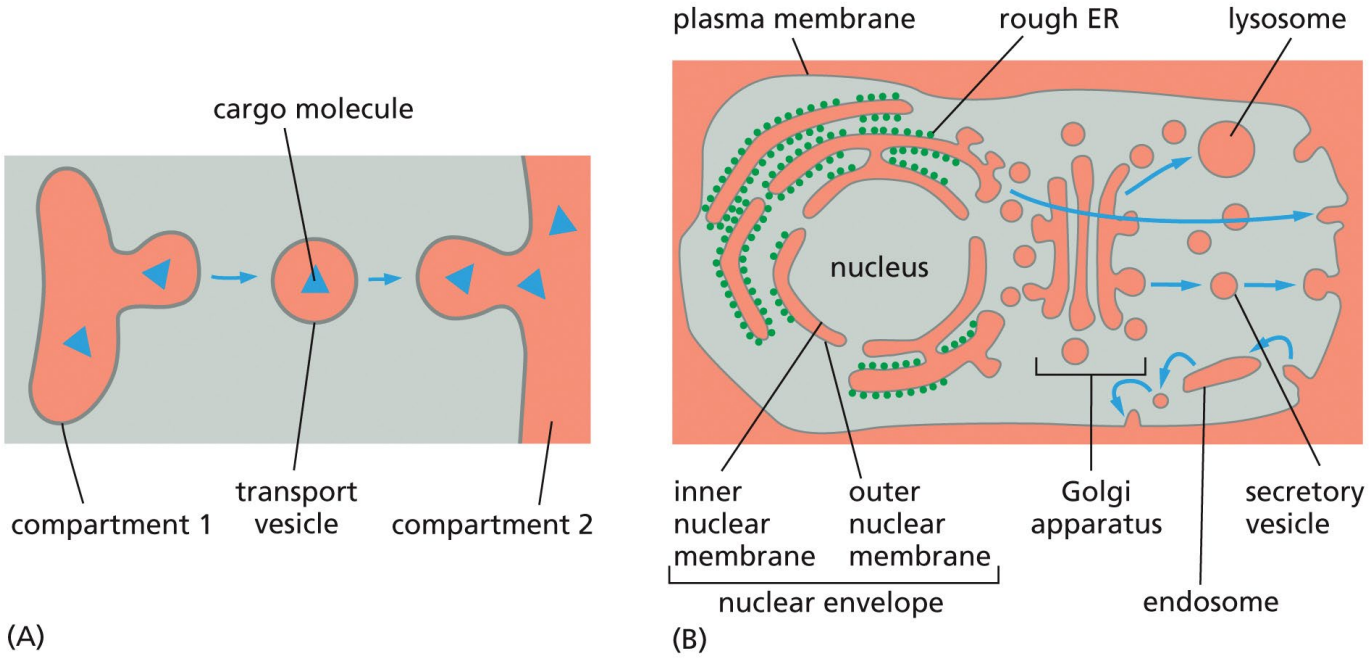
EVOLUTIONARY ORIGINS EXPLAIN THE TOPOLOGICAL RELATIONSHIPS OF ORGANELLES

- The lumen of the internal compartments is topologically equivalent to the extracellular space.
- The common origin of the internal compartments from a primordial intracellular compartment explains why all of these compartments can exchange material with each other through vesicular transport.
- The nucleus was formerly the cytosol in the ancient archaeon, explaining why the cytosol and nucleus are topologically equivalent compartments that can intermix during mitosis.



Adapted from J. Martijn and T.J.G. Ettema, *Biochem. Soc. Trans.* 41:451–457, 2013; D. Baum and B. Baum, *BMC Biol.* 12:76, 2014.

TOPOLOGICALLY EQUIVALENT COMPARTMENTS IN THE SECRETORY AND ENDOCYTIC PATHWAYS



Molecules can be carried from one compartment to another **topologically equivalent** compartment by **transport vesicles** that bud from one and fuse with the other. (lecture 8)

Note! Not mitochondria

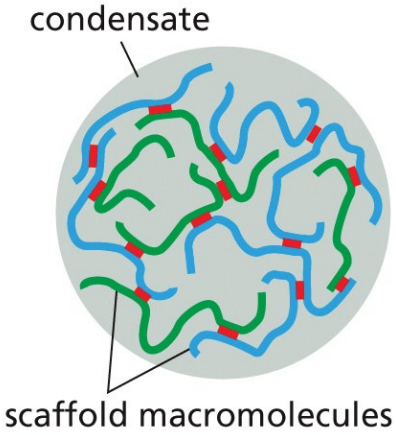
BIOMOLECULAR CONDENSATES

- Macromolecules can be segregated without a surrounding membrane

TABLE 12–3 Examples of Eukaryotic Biomolecular Condensates

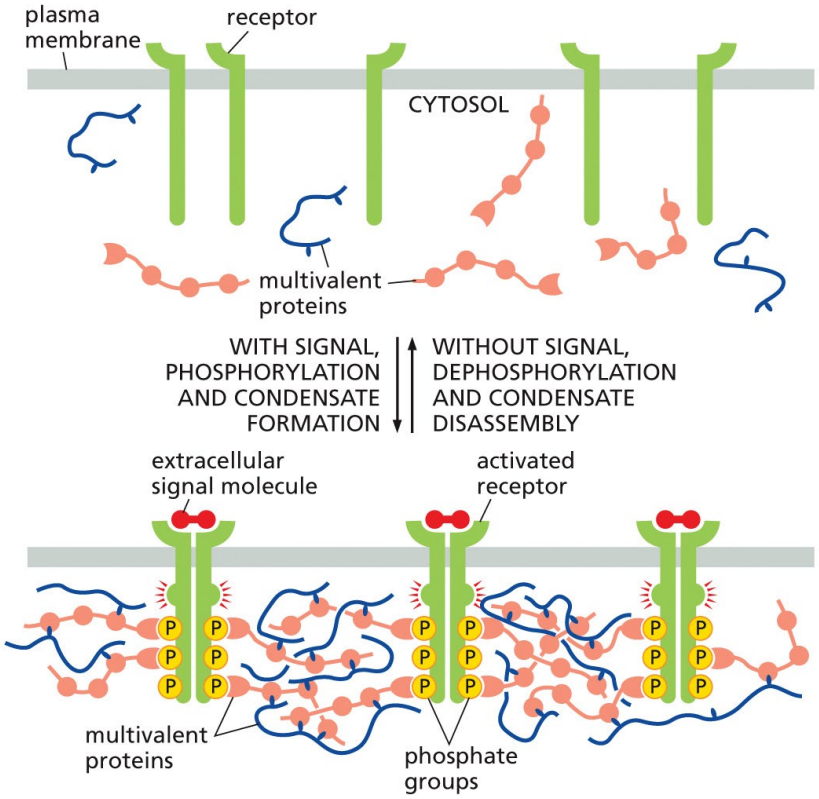
Biomolecular condensate	Location	Proposed associated function(s)
Nucleolus	Nucleus	rRNA transcription and ribosome assembly
Pyrenoid	Chloroplast	Carbon fixation from CO_2 in algae
Stress granules	Cytosol	Temporary storage, particularly of translation-related components
P-granules	Cytosol	RNA metabolism and inheritance
Balbiani body	Cytosol	Localization and inheritance of mRNAs and organelles
Cajal body	Nucleus	mRNA processing
Paraspeckles	Nucleus	Regulation of gene expression
RNA transport granule	Neuron	RNA localization to subcellular locations in development and in neurons
PML body	Nucleus	Storage of nuclear factors; regulation of gene expression
Postsynaptic density	Dendrite	Organization of macromolecules needed for neuronal transmission

BIOMOLECULAR CONDENSATES



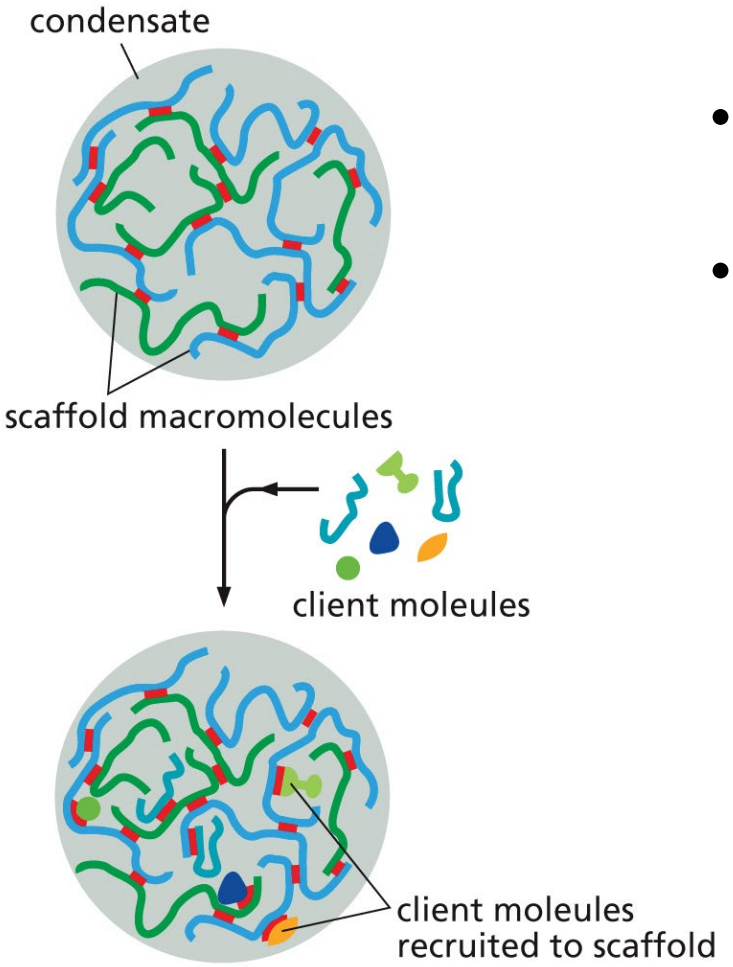
- Require at least one molecule to form **weak, multivalent interactions** with either itself or other constituents
- Typically, flexible and **intrinsically disordered**
- Weak interactions and unstructured molecules →**liquid** properties
- Liquid-liquid phase separation (LLPS)

BIOMOLECULAR CONDENSATES



- **Dynamic**:
  - Can assemble and disassemble
  - Molecules can move in and out
  - Can also e.g. solidify

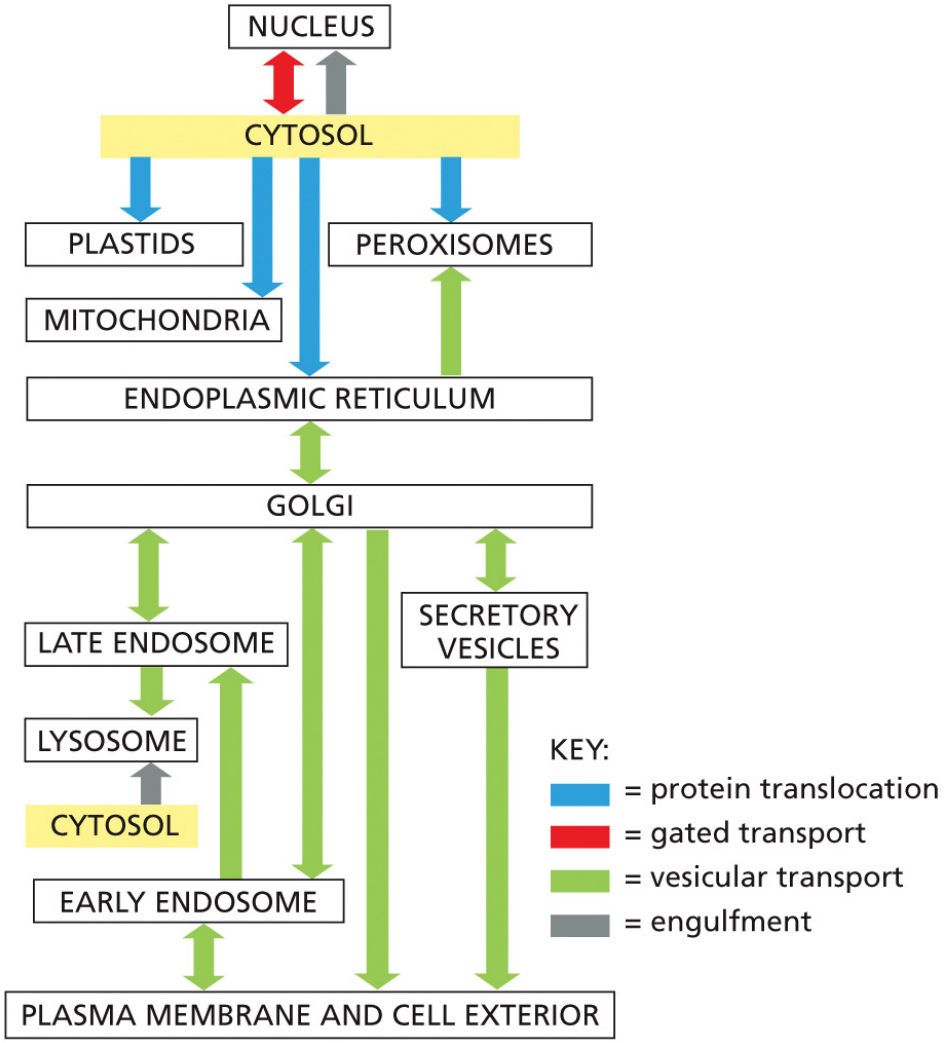
BIOMOLECULAR CONDENSATES



- Scaffold macromolecules can recruit clients
- For example, enzymes required for reaction cascades or enzymes and clients can be selectively concentrated

MOVING BETWEEN COMPARTMENTS

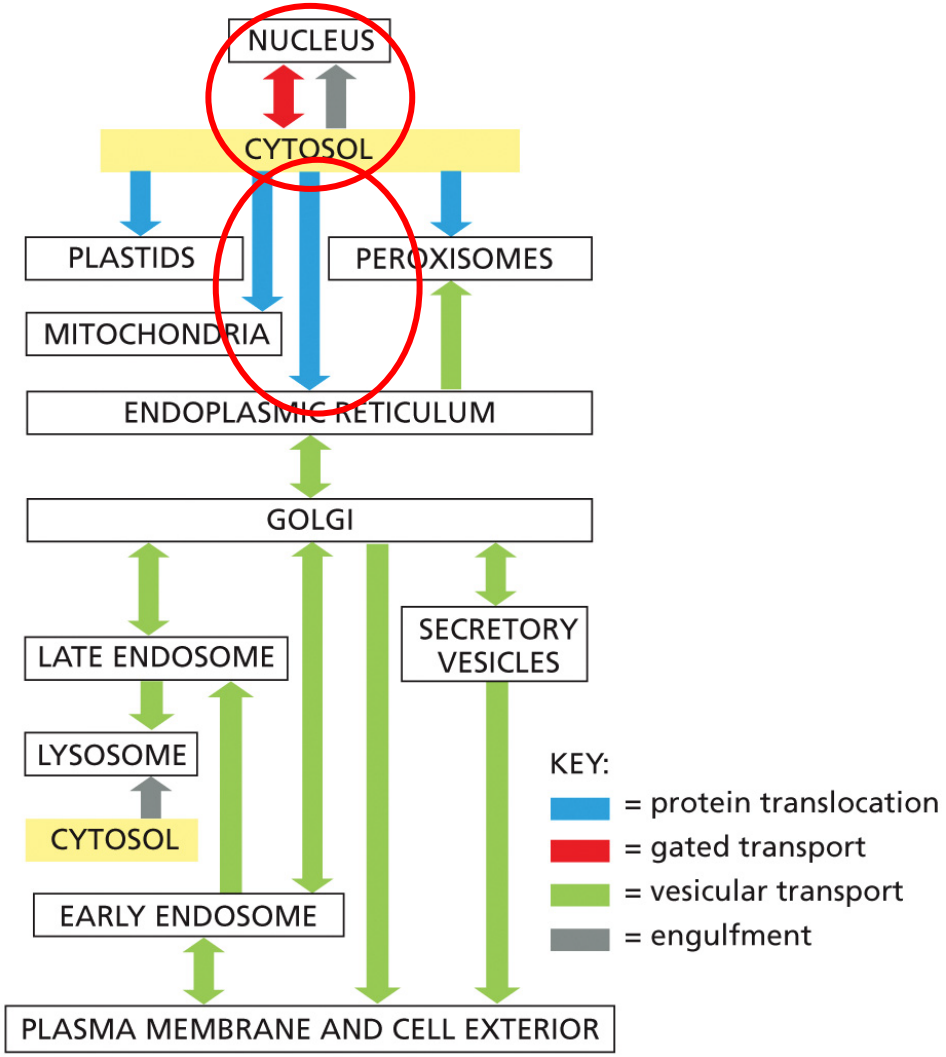
- Proteins can move between compartments in different ways:
  - **Gated transport**
  - **Protein translocation**
  - **Vesicular transport**
  - **Engulfment**



Note, these are all *compartments with membranes*!

MOVING BETWEEN COMPARTMENTS

- Proteins can move between compartments in different ways:
  - **Gated transport**
  - **Protein translocation**
  - **Vesicular transport**
  - **Engulfment**
- **Sorting signals** direct protein's movement and determine its location in the cell
- If *no signal* sequence, will *remain in cytosol*



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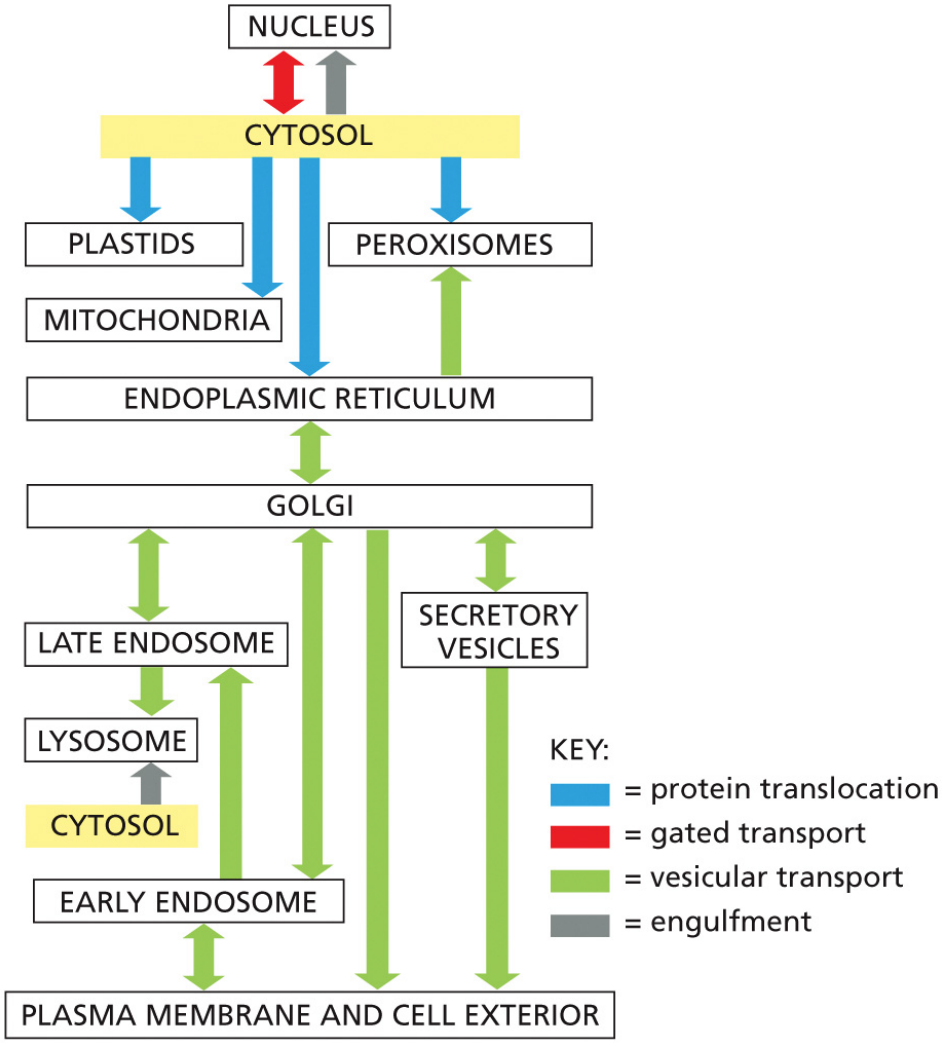

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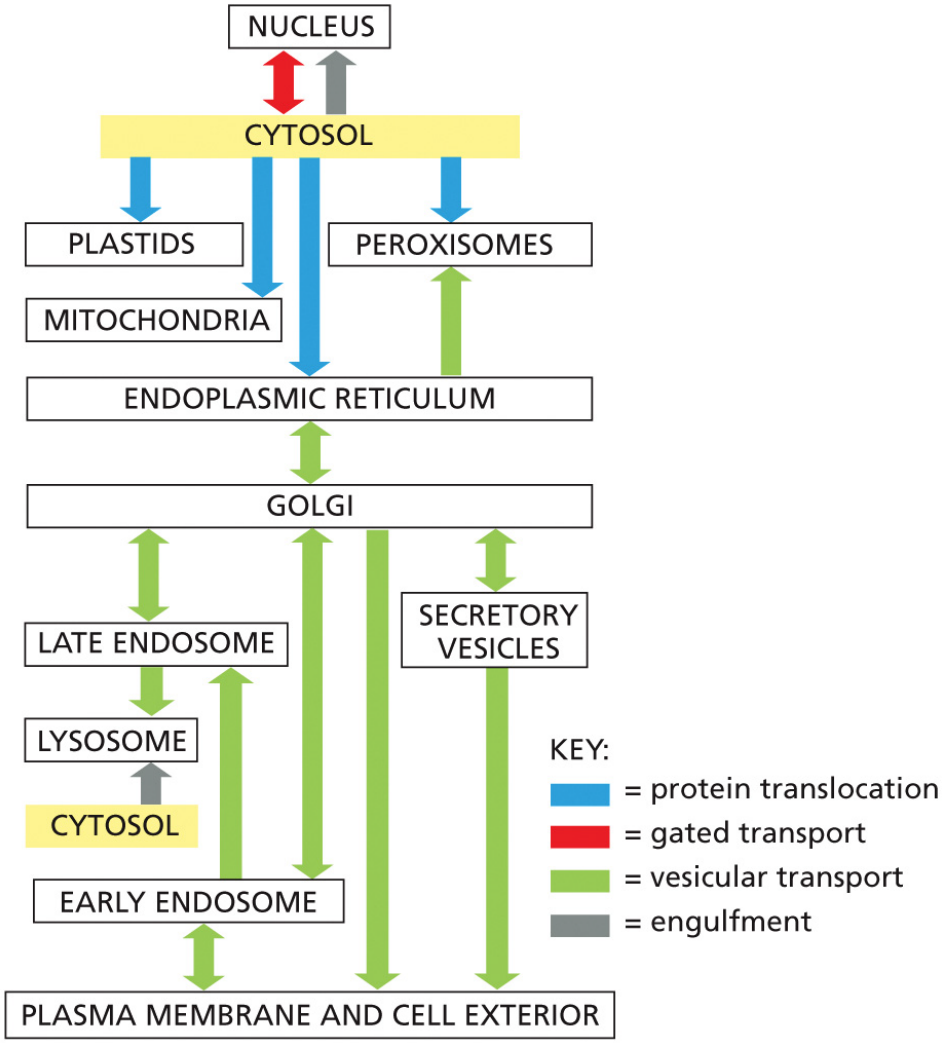
MOVING BETWEEN COMPARTMENTS

- **Gated transport**
  - Between cytosol and nucleus
  - Nuclear pore complexes = selective gates



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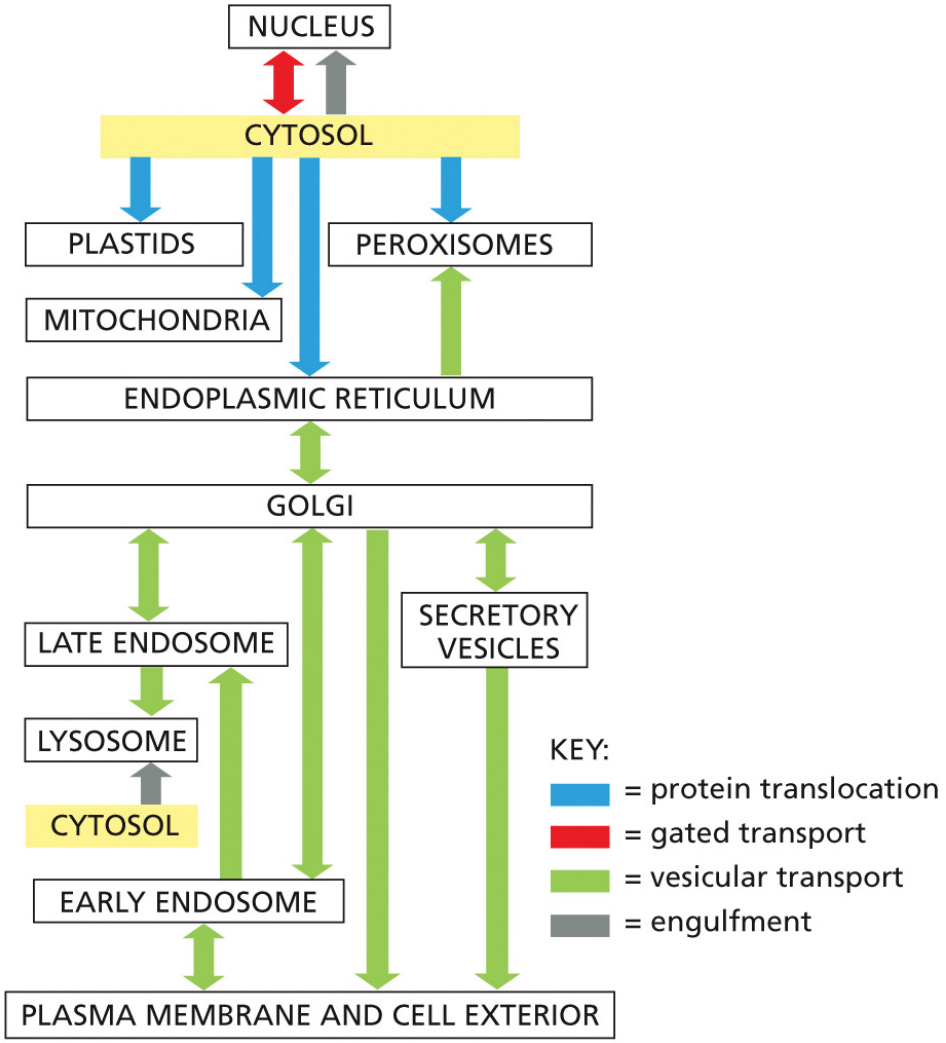
MOVING BETWEEN COMPARTMENTS

- **Gated transport**
  - Between cytosol and nucleus
  - Nuclear pore complexes = selective gates
- **Protein translocation**
  - From cytosol to topologically distinct compartments
  - By transmembrane protein translocators
  - Transported proteins typically unfolded



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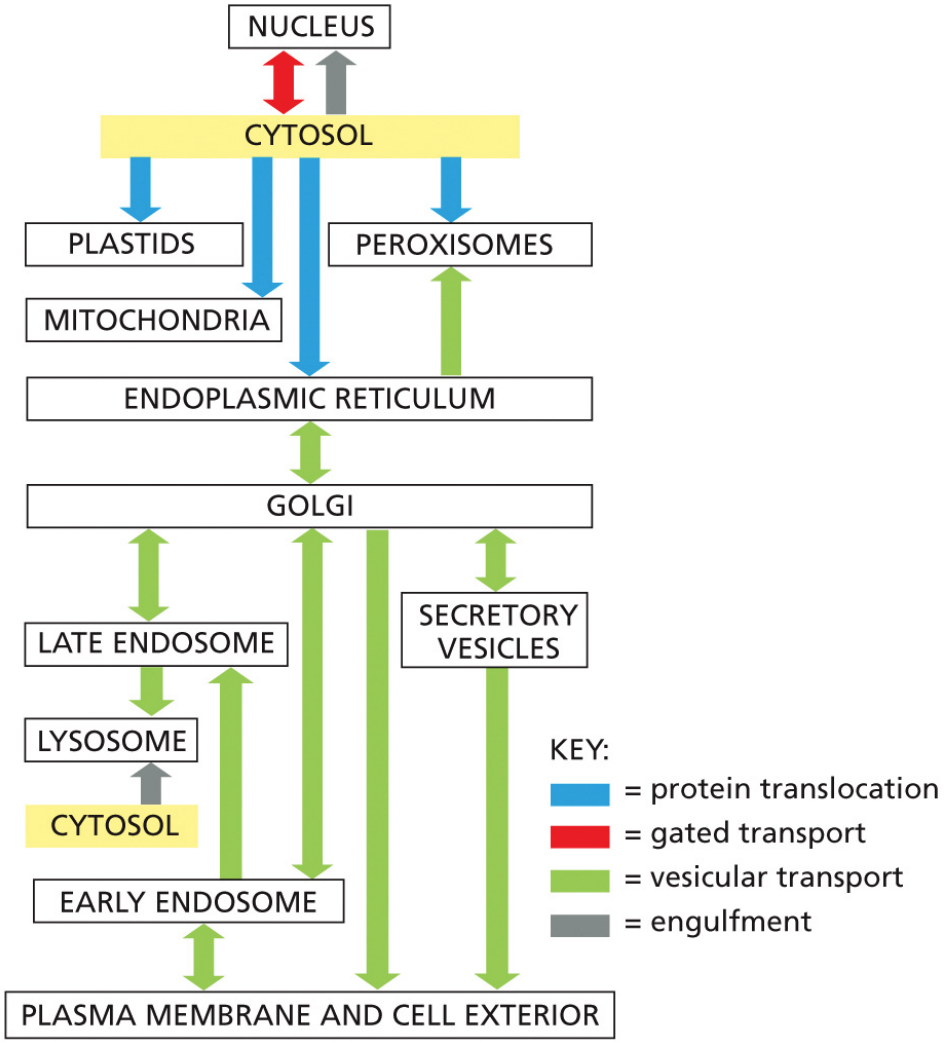
  - Nuclear pore complexes = selective gates
- **Protein translocation**
  - From cytosol to topologically distinct compartments
  - By transmembrane protein translocators
  - Transported proteins typically unfolded
- **Vesicular transport**
  - Between topologically similar compartments
  - By vesicles



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MOVING BETWEEN COMPARTMENTS

- **Gated transport**
  - Between cytosol and nucleus
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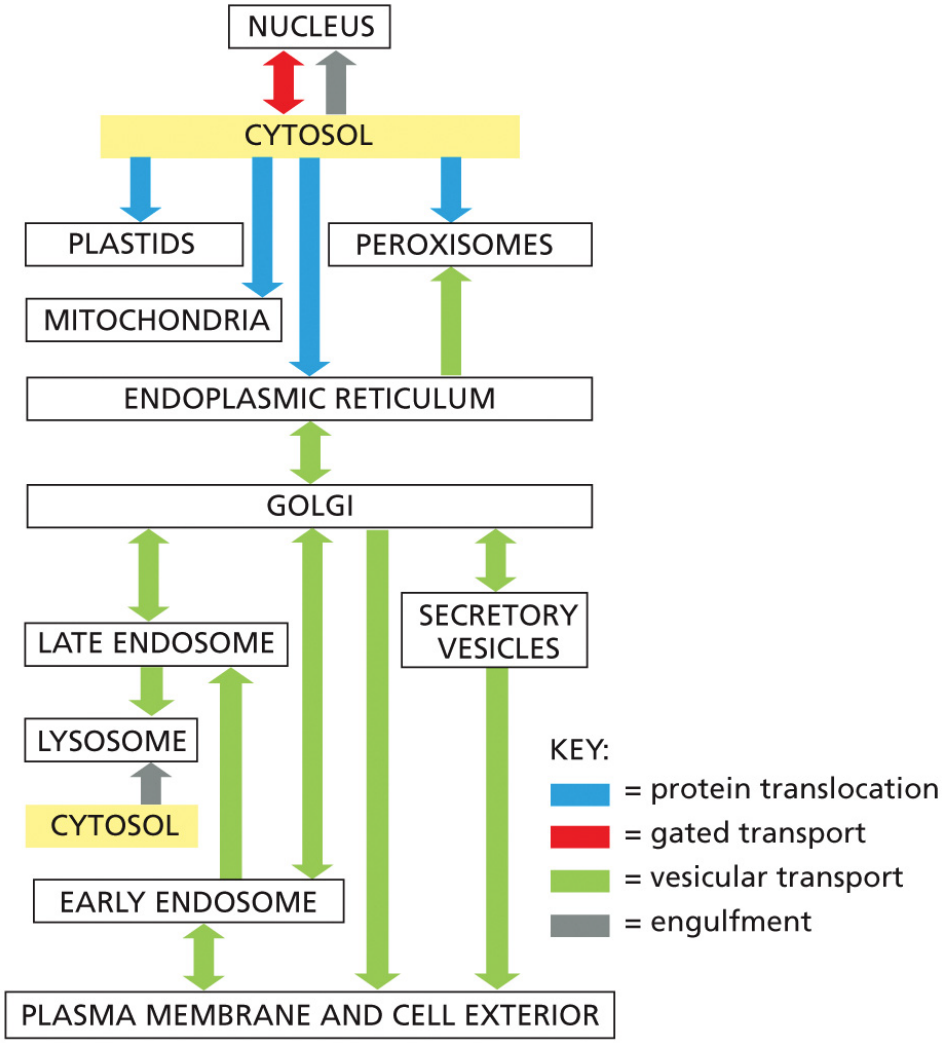
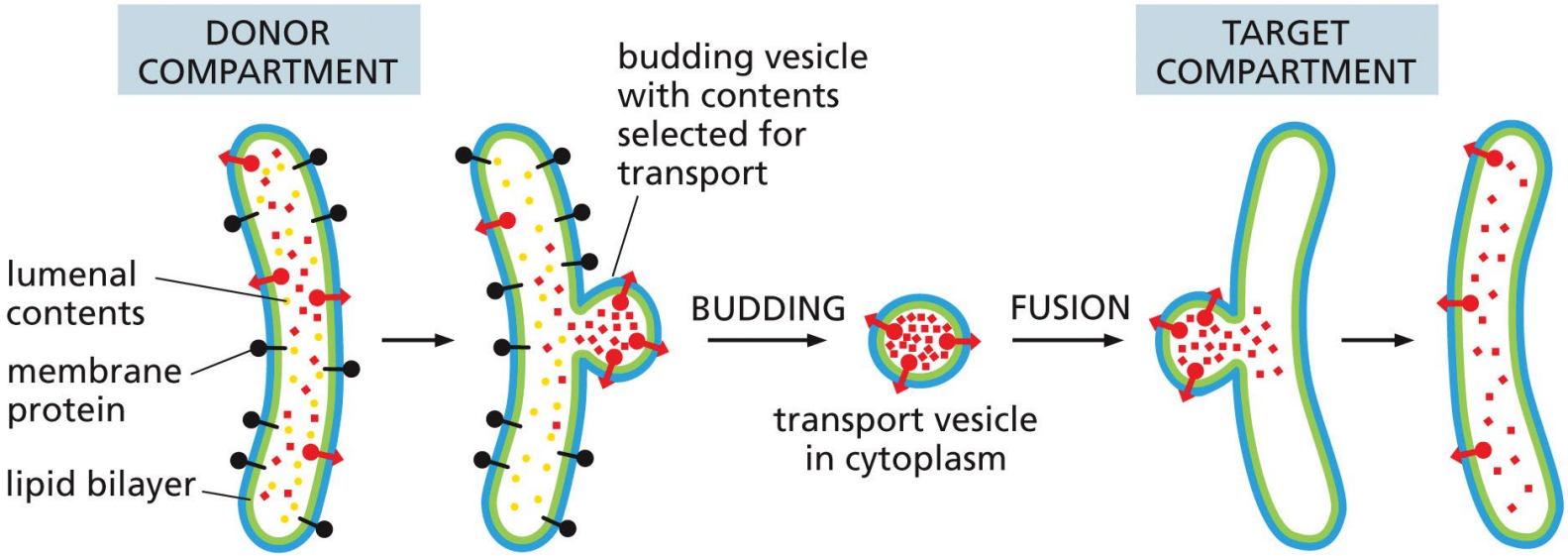
- **Protein translocation**
  - From cytosol to topologically distinct compartments
  - By transmembrane protein translocators
  - Transported proteins typically unfolded
- **Vesicular transport**
  - Between topologically similar compartments
  - By vesicles
- **Engulfment**
  - E.g., autophagy, from cytosol into lysosomes



Note, these are all *compartments with membranes*!

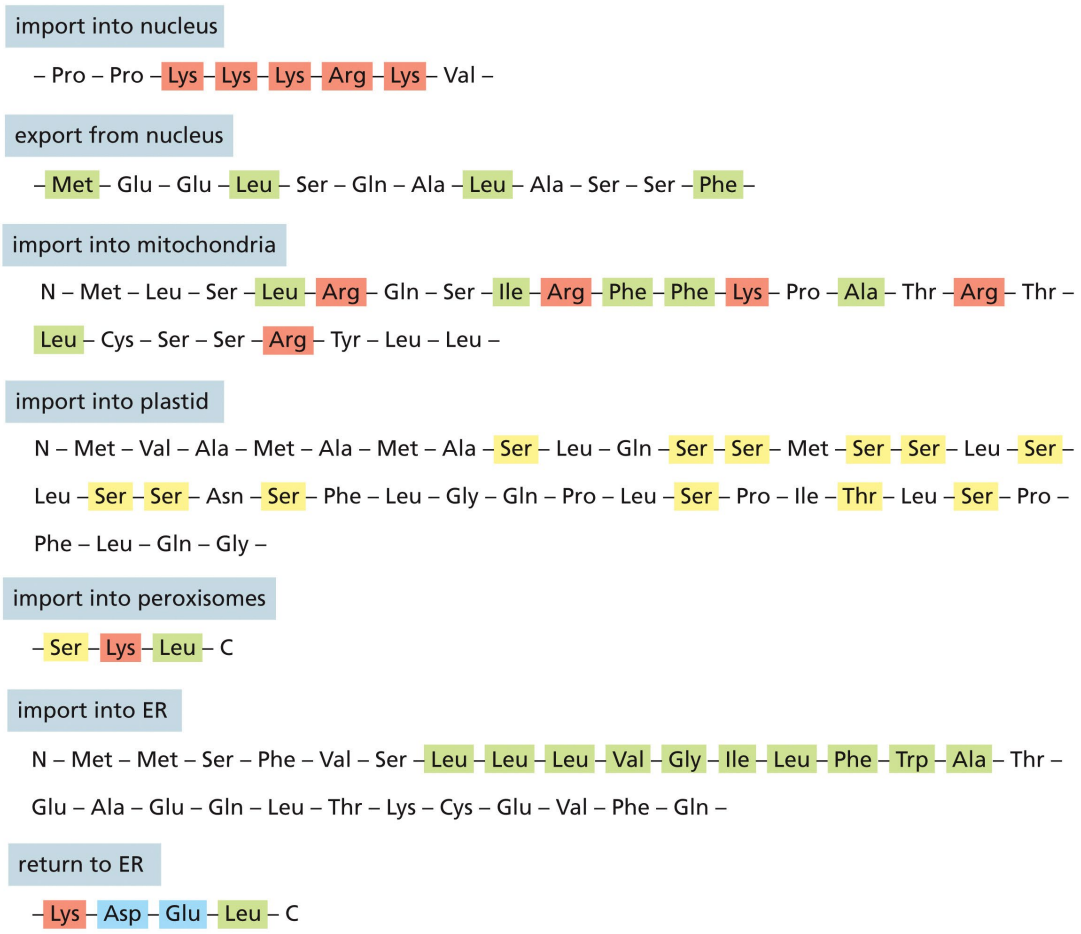
VESICULAR TRANSPORT



- Soluble components *(red dots)* are transferred from lumen to lumen
- Membrane is also transferred
- The orientation of both proteins and lipids will remain the same, the same domains always facing the cytosol
- Important in intracellular trafficking (lecture 8)

SORTING SIGNALS

- Typically, 15-60 aa in the N-terminus (but can also be in the middle or C-terminus)
- Usually removed by special peptidases
- Properties such as charge or hydrophobicity more important than exact sequence



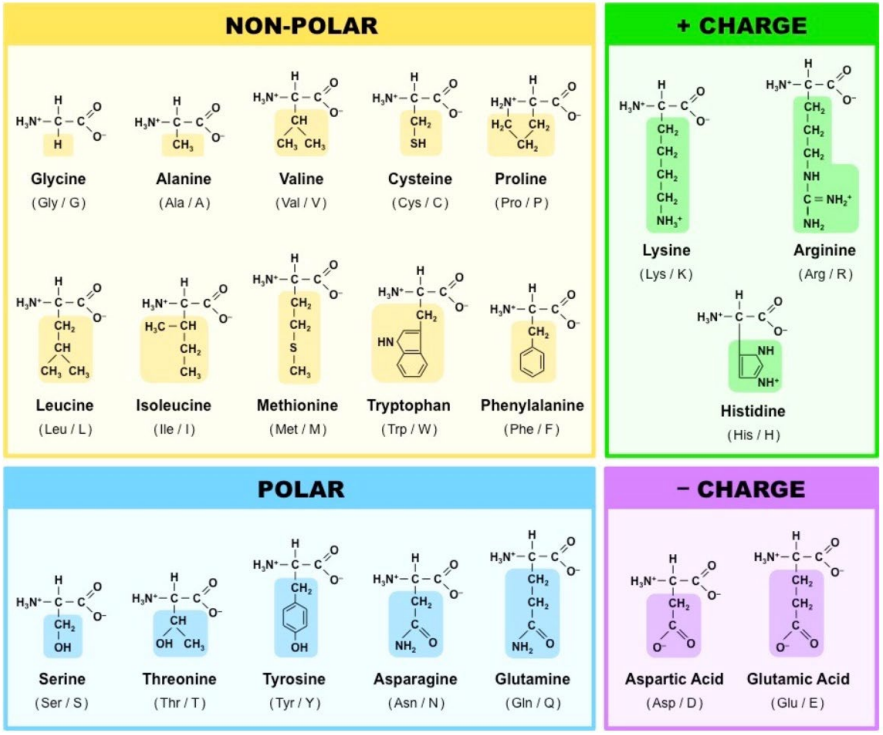
import into ER

N – 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 –

import into nucleus

– Pro – Pro – Lys – Lys – Lys – Arg – Lys – Val –

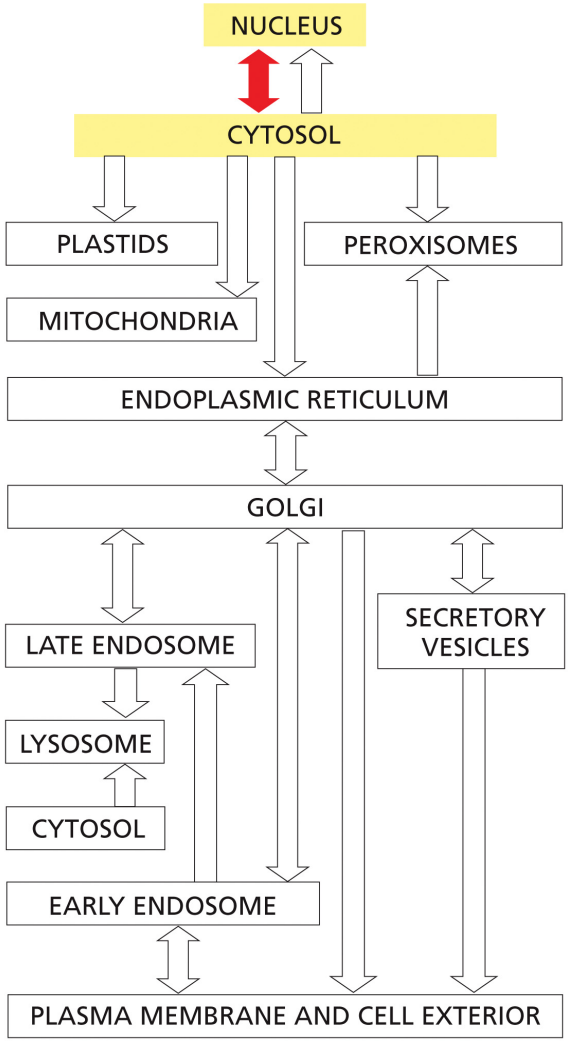


Which sequence would most likely be imported into nucleus? Which one imported into ER?

A. N-MATHPGITSSTLF-[200aa]-C
B. N-[100aa]-LTRRRKRKL-[100aa]-C
C. N-MATHIAIIVLLILF-[200aa]-C
D. N-[100aa]-LTTTTKTKL-[100aa]- C

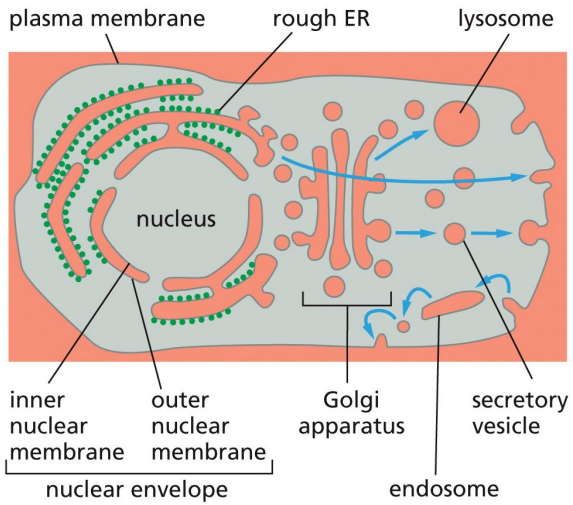
THE TRANSPORT OF MOLECULES BETWEEN THE NUCLEUS AND THE CYTOSOL

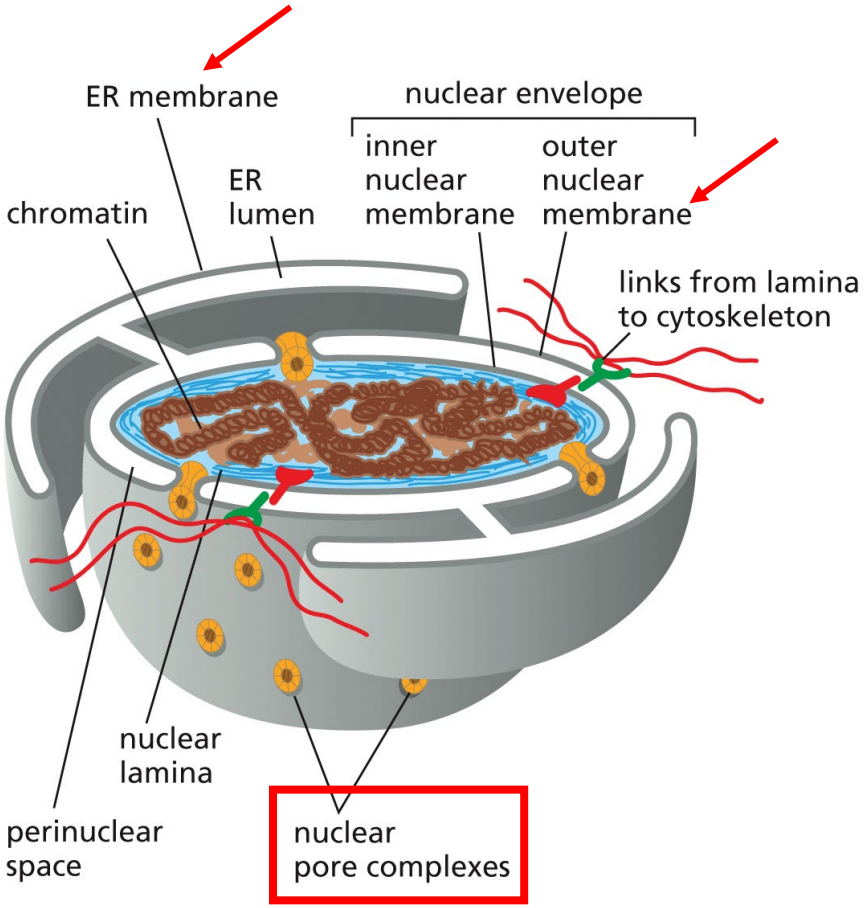
- **Gated transport**
  - Between cytosol and nucleus
  - Nuclear pore complexes = selective gates



THE TRANSPORT OF MOLECULES BETWEEN THE NUCLEUS AND THE CYTOSOL

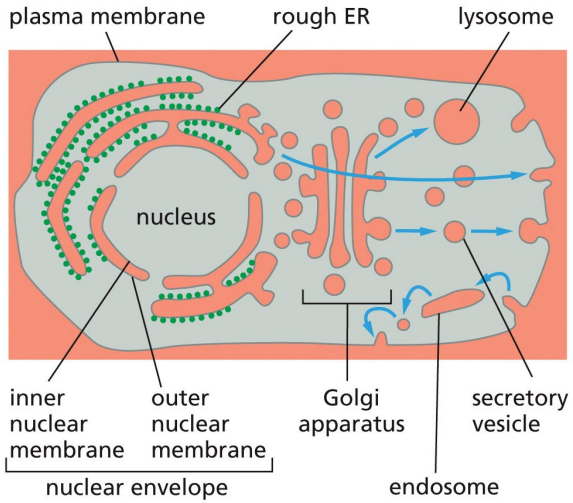
- Nuclear pore complexes perforate the nuclear envelope
- The outer nuclear membrane is continuous with the endoplasmic reticulum (ER).

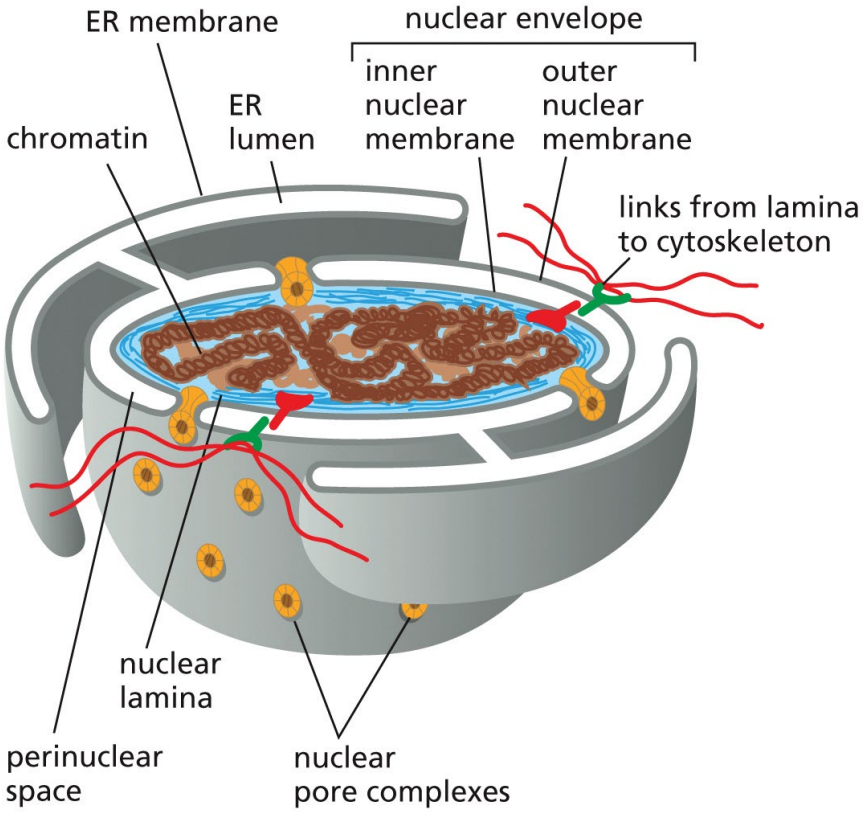




THE TRANSPORT OF MOLECULES BETWEEN THE NUCLEUS AND THE CYTOSOL

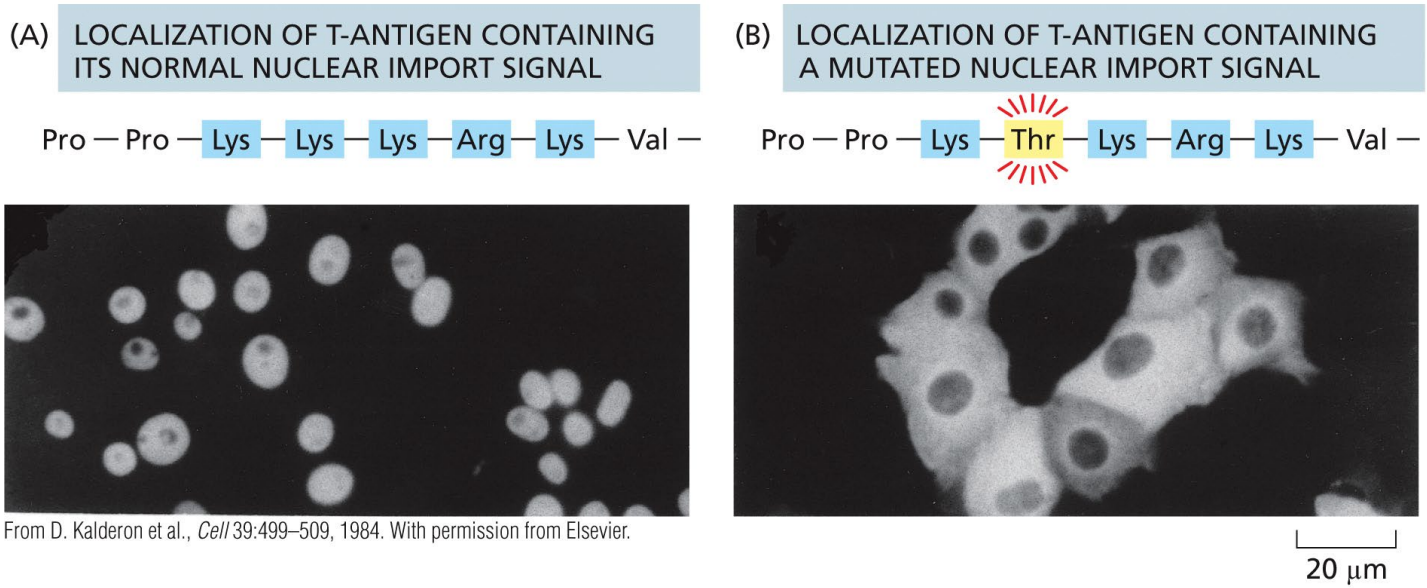
- Transport is **bidirectional** and **selective** (large molecules)





NUCLEAR LOCALIZATION SIGNALS

- Nuclear localization signals direct proteins to the nucleus

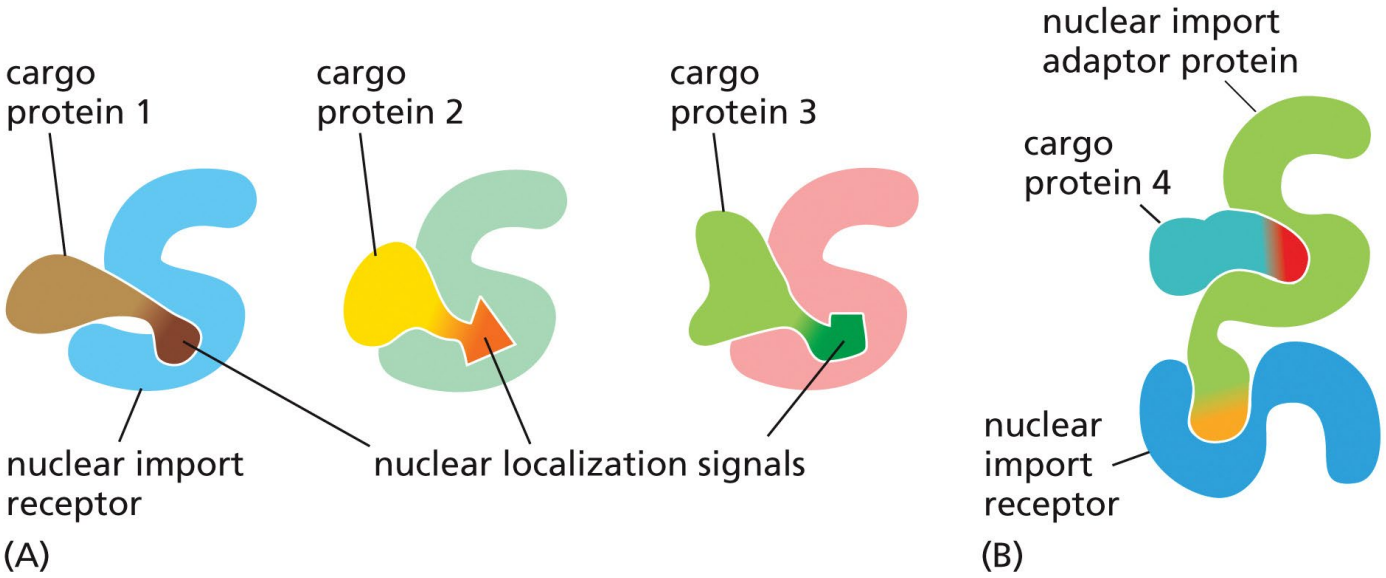


From D. Kalderon et al., *Cell* 39:499–509, 1984. With permission from Elsevier.

- Signals are typically **rich in positively charged residues**
- Can be anywhere in the protein, **often in the surface**, e.g. forming loops
- Proteins can be transported **in the folded form**

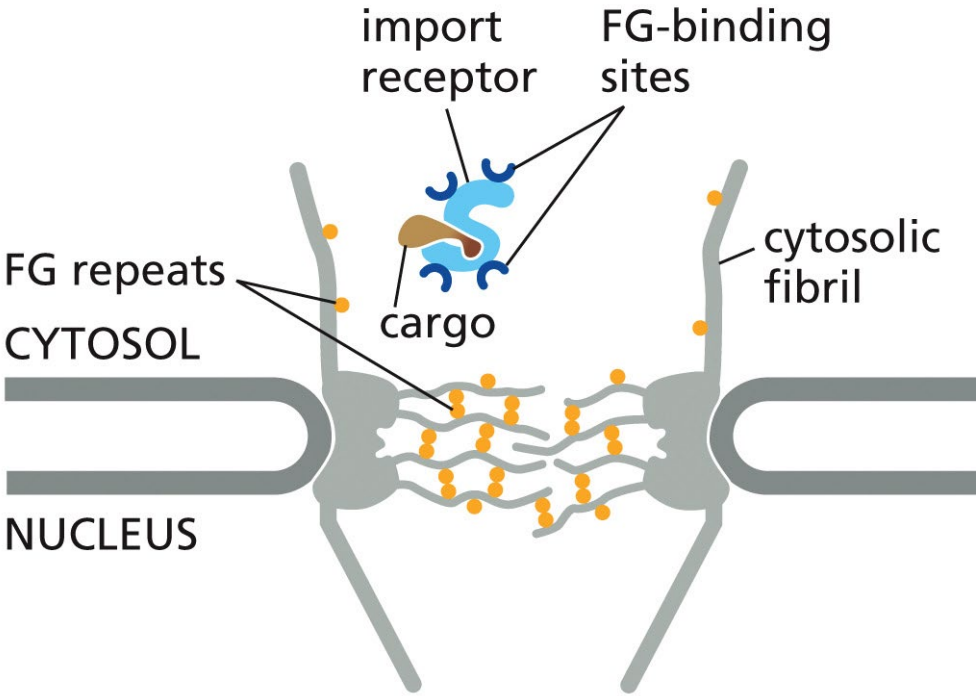
NUCLEAR IMPORT RECEPTORS

- Nuclear import receptors bind to both nuclear localization signals and NPC proteins
- A number of different nuclear import receptors available



- The adaptors also contain a nuclear localization signal
- This signal only becomes exposed when they are loaded with a cargo protein

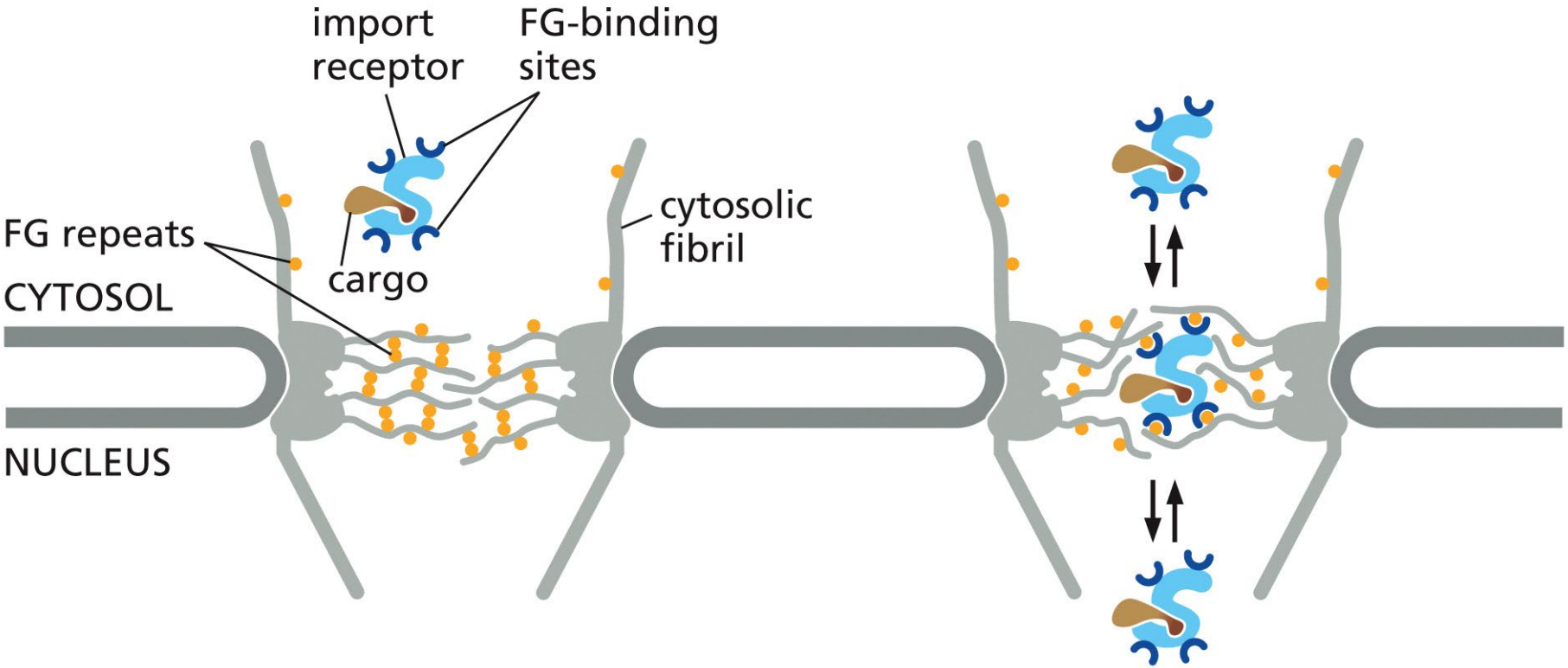
NUCLEAR IMPORT RECEPTORS



- The interior of the NPC is filled with a mesh of *FG repeat–containing proteins* whose weak interactions with each other *restrict nonspecific diffusion* of proteins and other macromolecules through the pore

- Nuclear import receptors contain **various low-affinity FG repeat–binding sites on their surface**

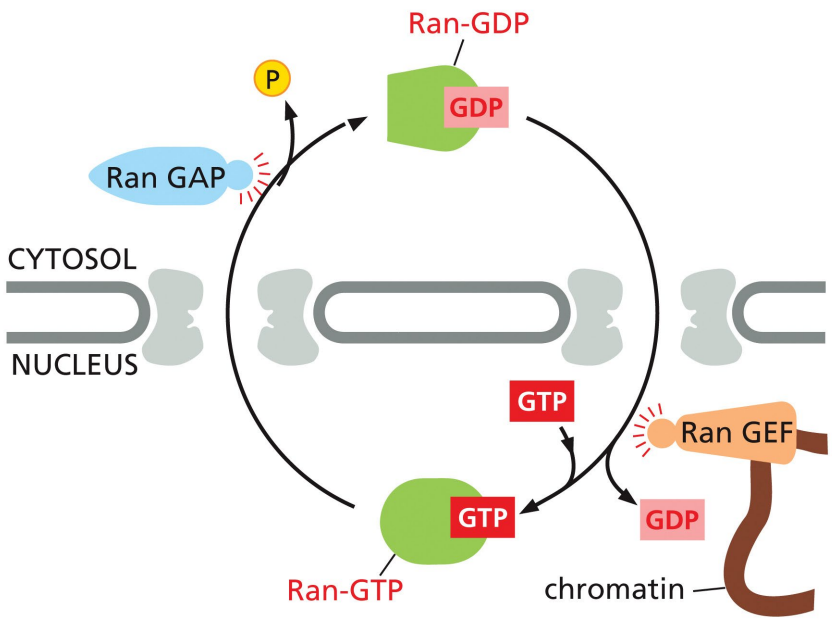
NUCLEAR IMPORT RECEPTORS



- **Cargo receptors** can rapidly partition into the FG repeat mesh by *interacting with the FG repeats and locally melting the mesh*
- Proteins without surface FG repeat–binding sites cannot melt the mesh, and their diffusion through the NPC is comparatively slow

DIRECTIONALITY ON NUCLEAR IMPORT

- The Ran GTPase imposes directionality on nuclear import



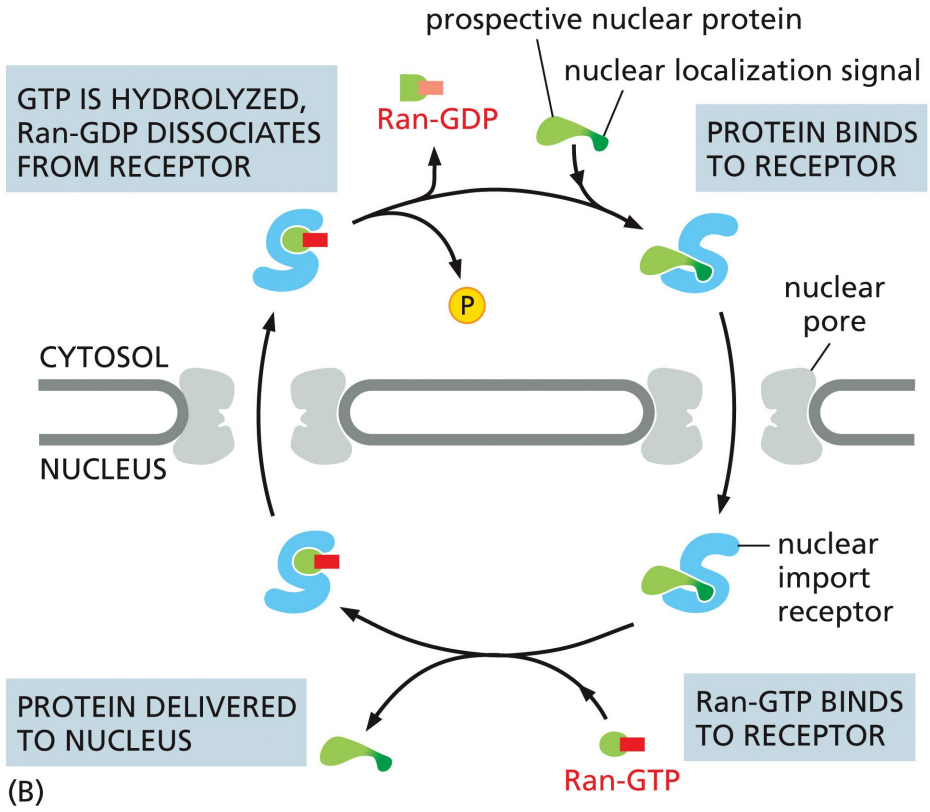
- Localization of **Ran-GDP in the cytosol** and **Ran-GTP in the nucleus** results from the *localization of two Ran regulatory proteins*
- Ran GTPase-activating protein (Ran GAP) is located in the *cytosol*
- Ran guanine nucleotide exchange factor (Ran GEF) binds to chromatin and is therefore located in the *nucleus*
- Ran-GDP is imported into the nucleus by an import receptor (not shown), which is specific for the GDP-bound conformation of Ran.

DIRECTIONALITY ON NUCLEAR IMPORT

- The Ran GTPase imposes directionality on nuclear import

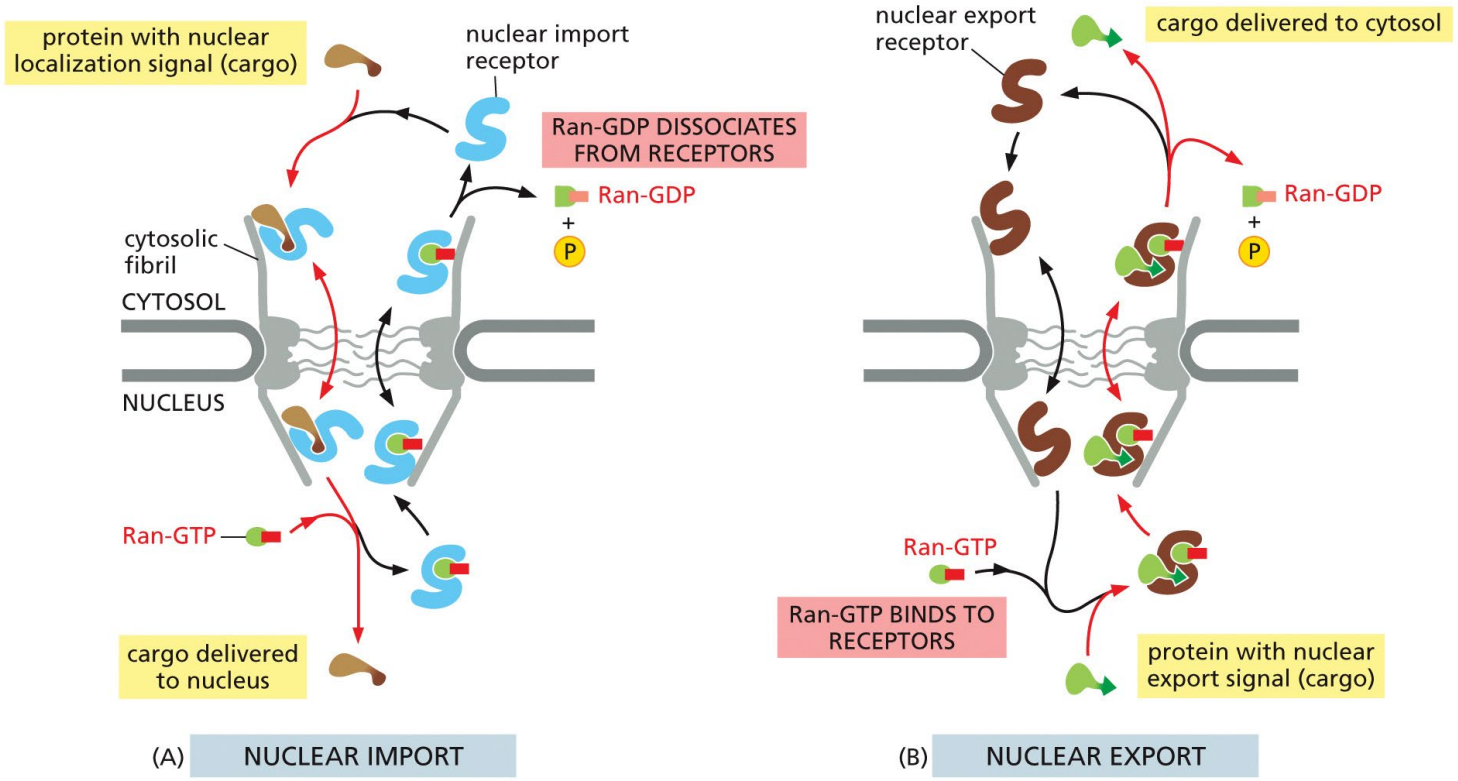
- The interaction between a nuclear import receptor and its cargo is reversed by **Ran-GTP**.
- Receptor–cargo interaction is favored in the cytosol but disfavored in the nucleus

->net cargo transport from the cytosol to the nucleus.



NUCLEAR EXPORT

- Nuclear export works like nuclear import, but in reverse



- In *nuclear import*, cargo *binding is mutually exclusive of Ran-GTP*; in *nuclear export*, cargo *binding requires Ran-GTP*

QUESTION

- What would happen if eucaryotes would not have nucleus separated from cytosol (like procaryotes)?

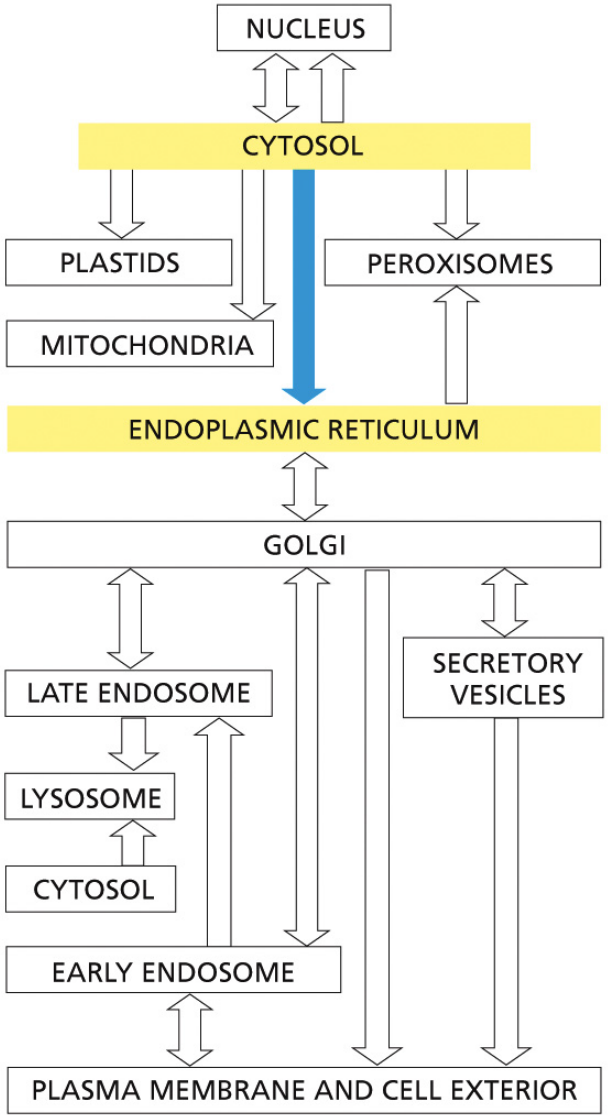
THE ENDOPLASMIC RETICULUM

Protein translocation

- From cytosol to topologically distinct compartments
- By transmembrane protein translocators
- Transported proteins typically unfolded

Vesicular transport

- Between topologically similar compartments
- By vesicles
- ***-> Lecture 8***



THE ENDOPLASMIC RETICULUM

- The ER is structurally and functionally diverse
  - Rough ER, Smooth ER
- Central role in biosynthesis of proteins and lipids
- Intracellular storage of Ca2+, used for signaling purposes

ER as protein factory

Protein folding, processing, and quality control in ER:

- *Translocation* of the protein into the ER
- Protein **folding** with the help of chaperones, protein disulfide isomerase, and calnexin-calreticulin cycle
- Protein **N-glycosylation**
- Protein **quality-control**
- ER-associated protein degradation
- Unfolded protein response

THE ENDOPLASMIC RETICULUM

- Proteins directed to membrane, to be secreted, ER, Golgi…
- Translocation to ER either co-translationally or post-translationally

- **A Signal-Recognition Particle (SRP)** directs the ER signal sequence to a specific receptor at the ER

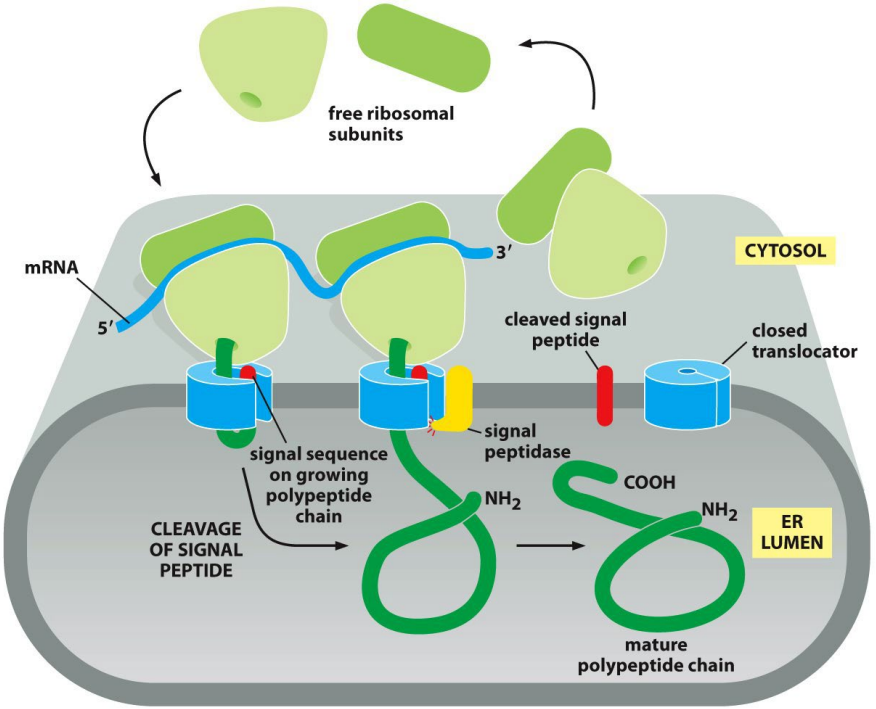
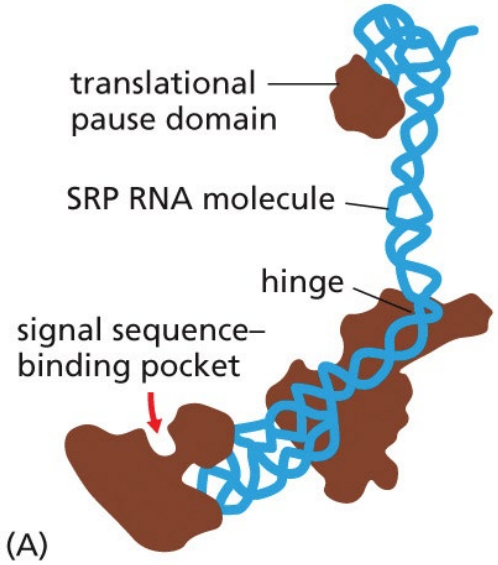


Figure 12-35 Molecular Biology of the Cell 6e (© Garland Science 2015)

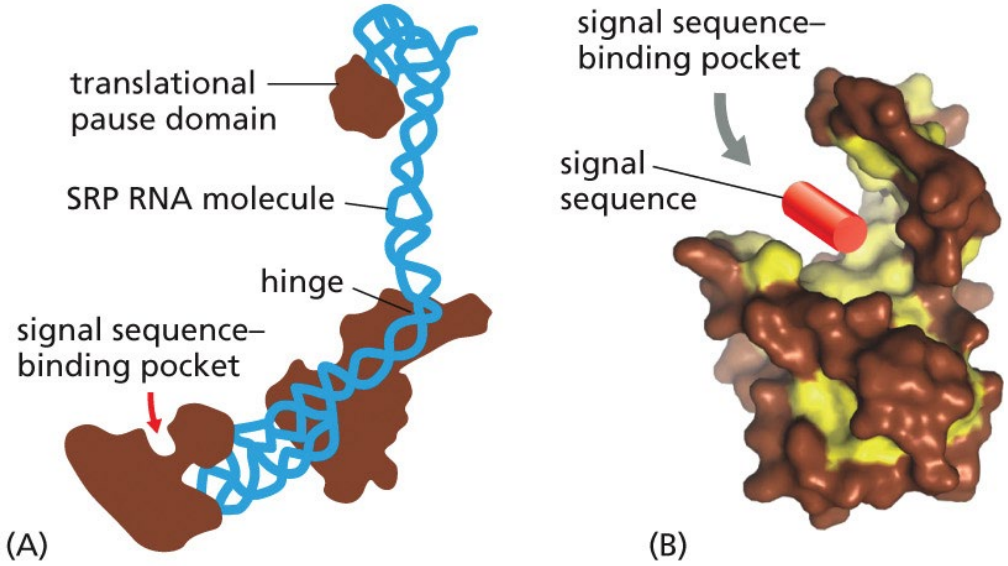
THE SIGNAL-RECOGNITION PARTICLE (SRP)

- Six protein subunits *(brown)* and one RNA molecule *(blue)*
- Recognizes signal peptides with **8 or more non-polar aa in middle**

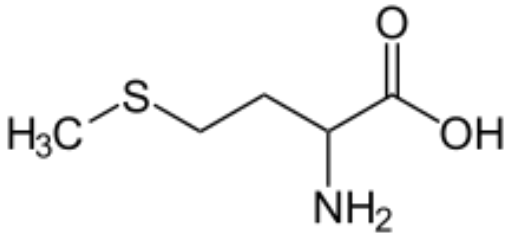


THE SIGNAL-RECOGNITION PARTICLE (SRP)

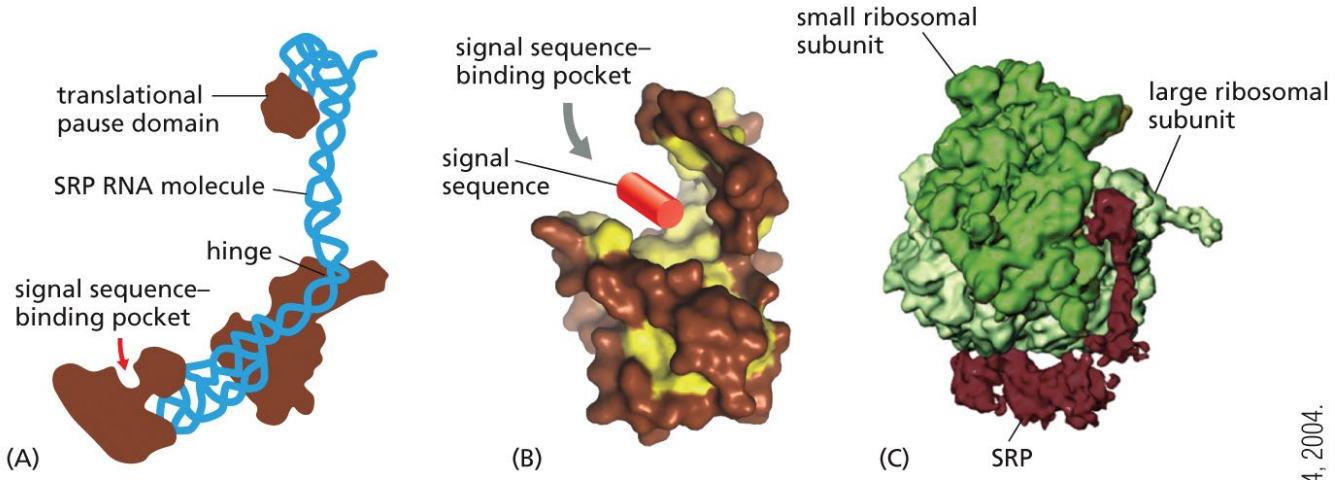
- Six protein subunits *(brown)* and one RNA molecule *(blue)*
- Recognizes signal peptides with **8 or more non-polar aa in middle**



- *Signal sequence bound into pocket rich in methionines*



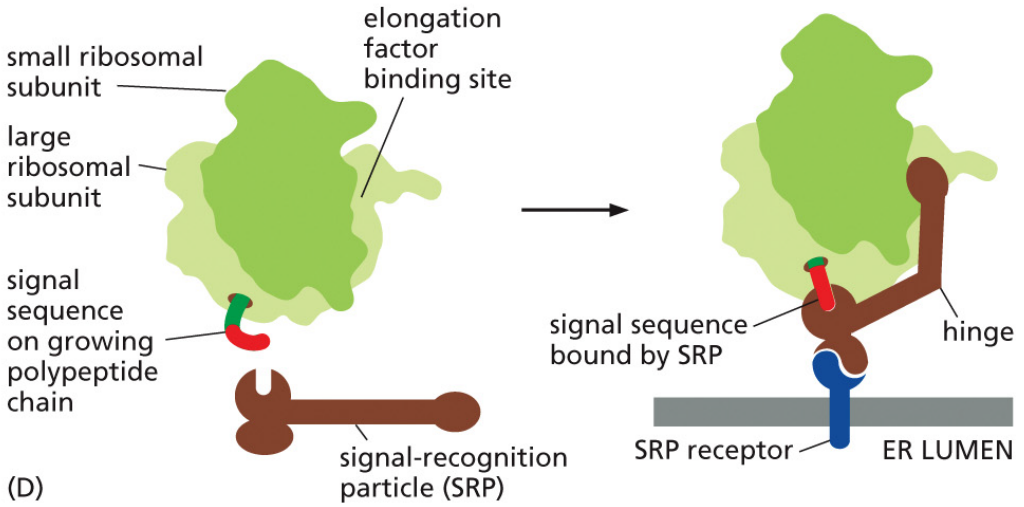
THE SIGNAL-RECOGNITION PARTICLE (SRP)



- Wrapped around the large ribosomal unit
- Binding of signal sequence -> another end blocks elongation factor binding site -> **synthesis halted**

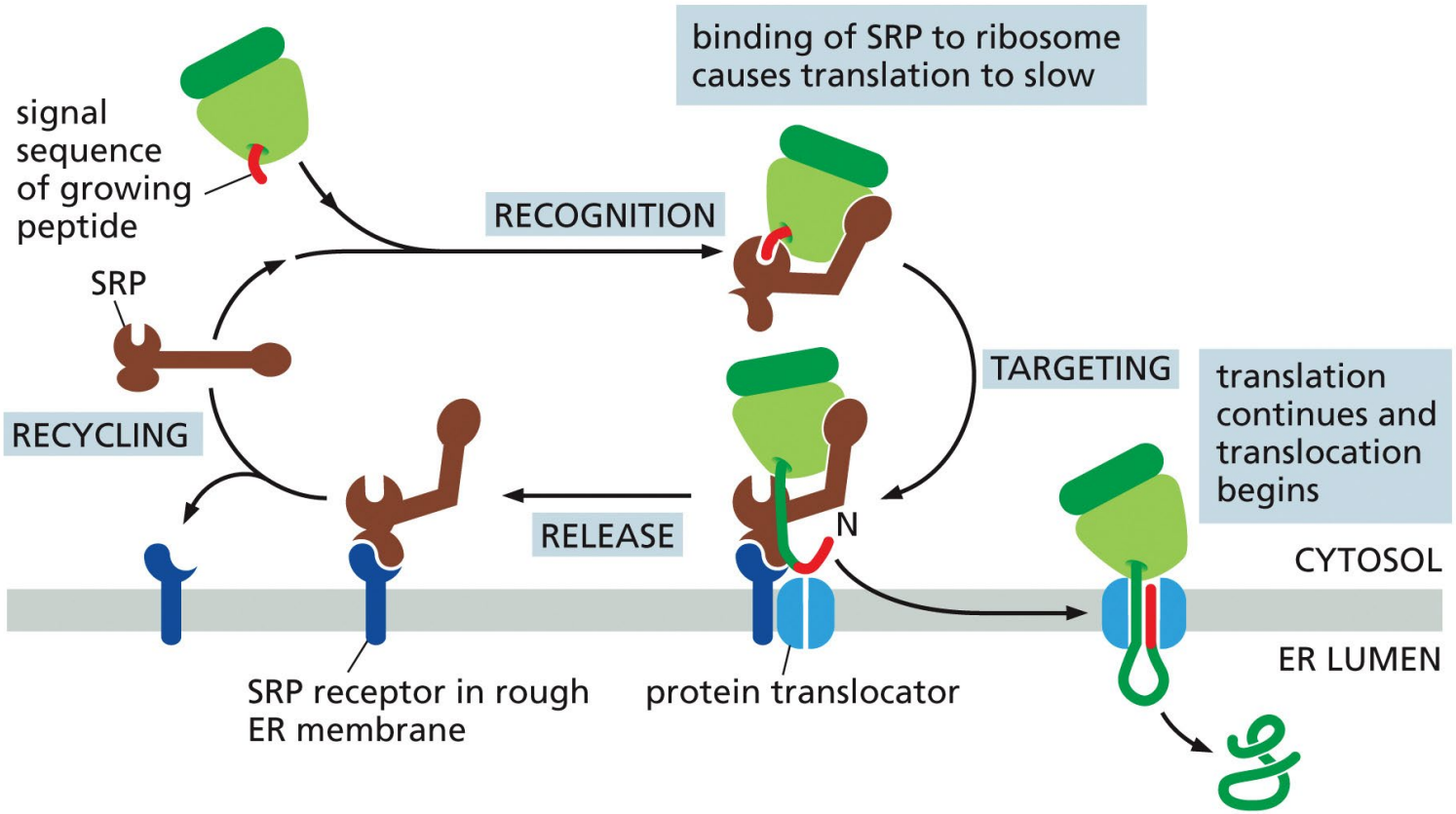
THE SIGNAL-RECOGNITION PARTICLE (SRP)

- SRP binding to signal sequence *exposes SRP receptor binding site*
- **SRP receptor** located in **rough ER**



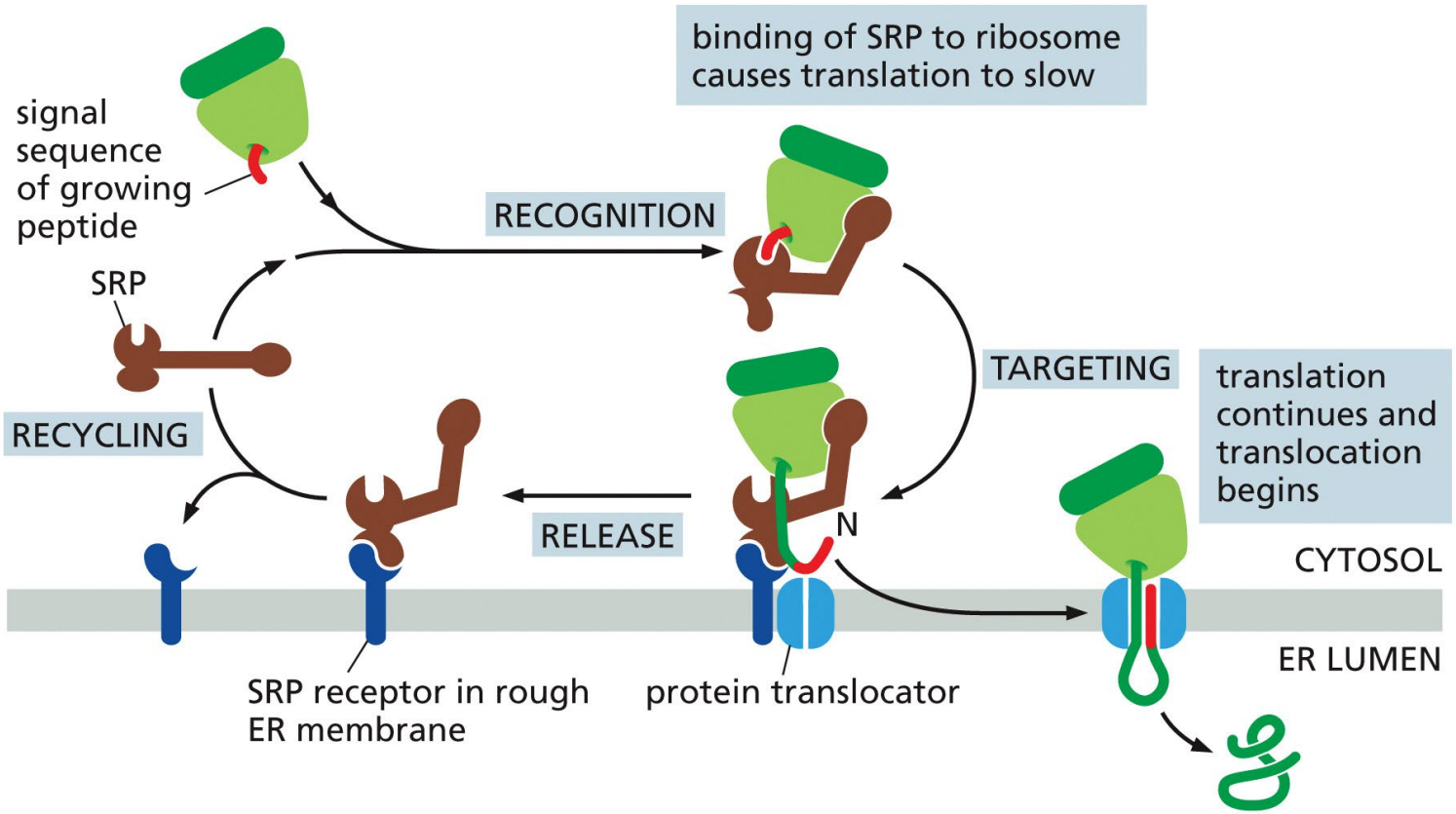
THE SIGNAL-RECOGNITION PARTICLE (SRP)

- Complex of SRP bound to SRP receptor + the protein to be translocated + ribosome is brought to **translocator**



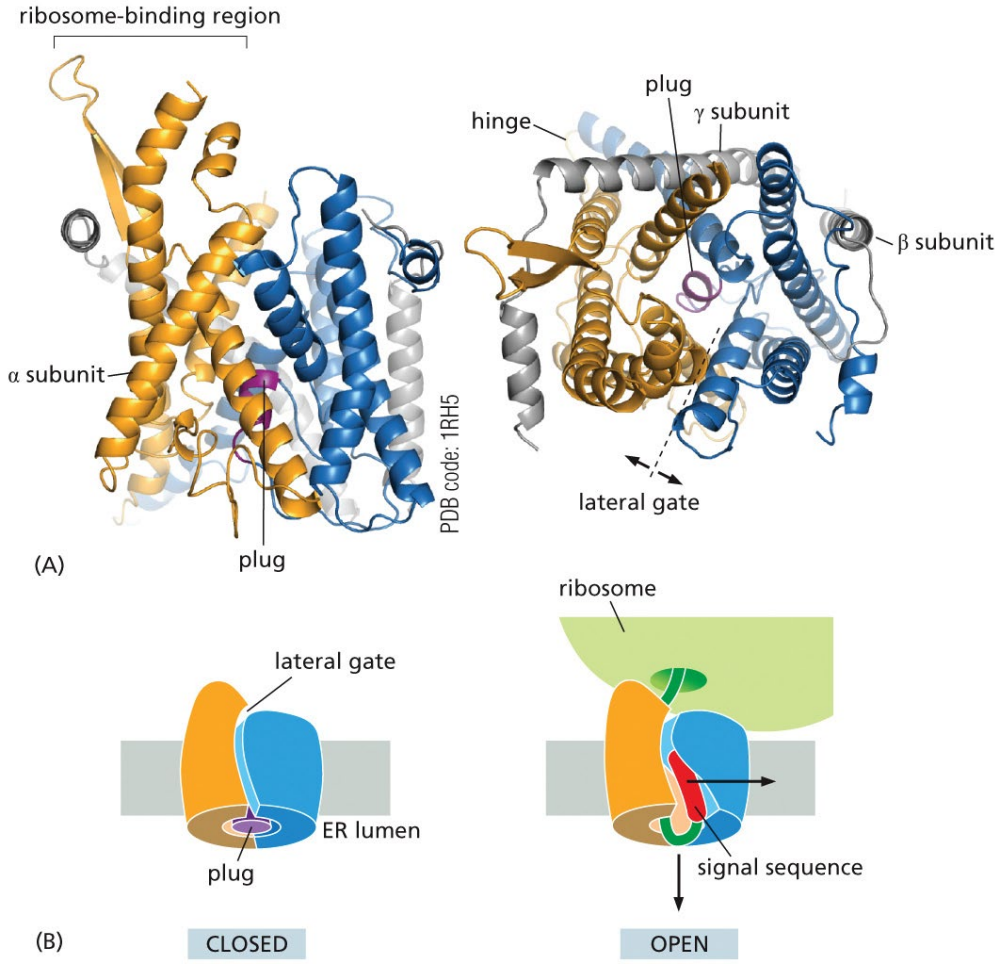
THE SIGNAL-RECOGNITION PARTICLE (SRP)

- SRP and SRP receptor are released
- Protein translation continues, now **translocated to ER lumen**



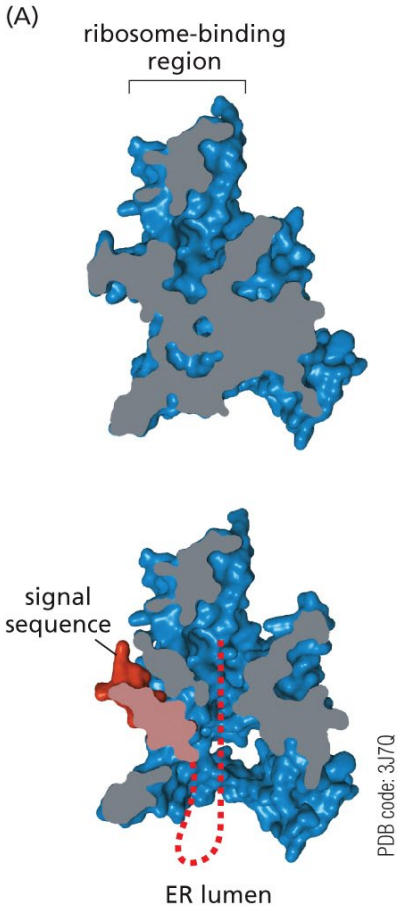
SEC61 TRANSLOCATOR

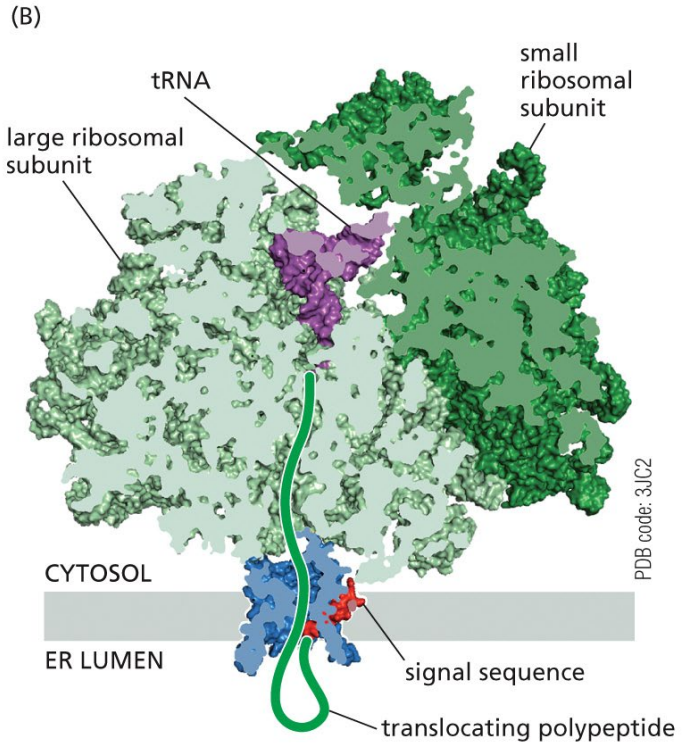
- The *dark blue* short helix forms a **plug** that seals the pore when the translocator is closed
- Some regions of the Sec61 a subunit that protrude into the cytosol *bind to the ribosome* during protein translocation



SIGNAL SEQUENCE–**GATED** TRANSLOCATOR

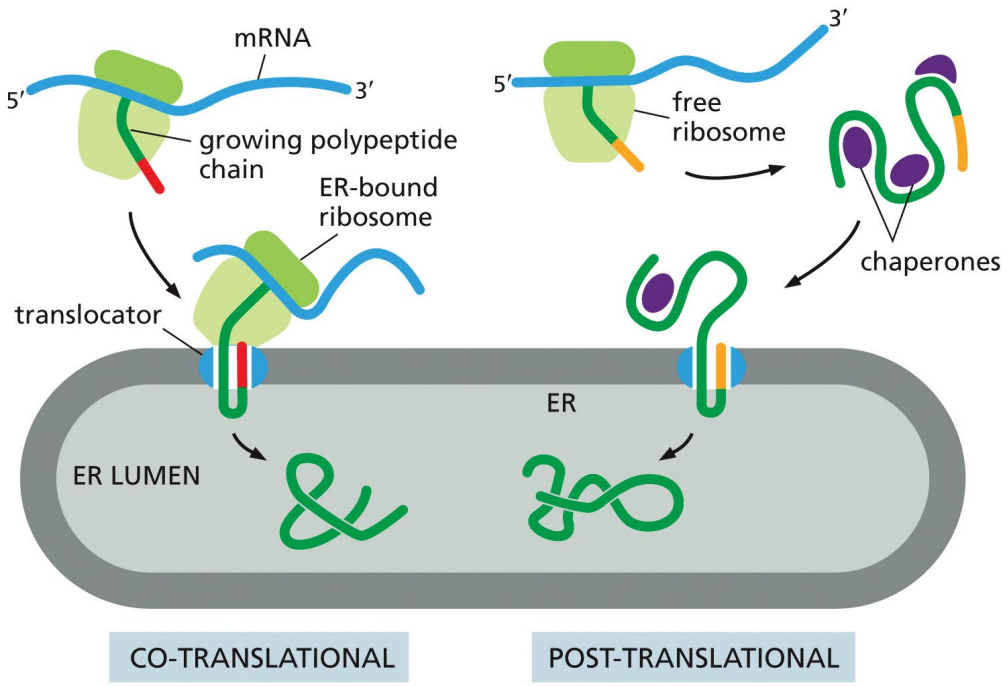
- Insertion of the *signal sequence* causes the central channel in the translocator to widen
- The *plug moves out* of the channel
- Continuous path across the membrane is now apparent





POST-TRANSLATIONAL TRANSLOCATION

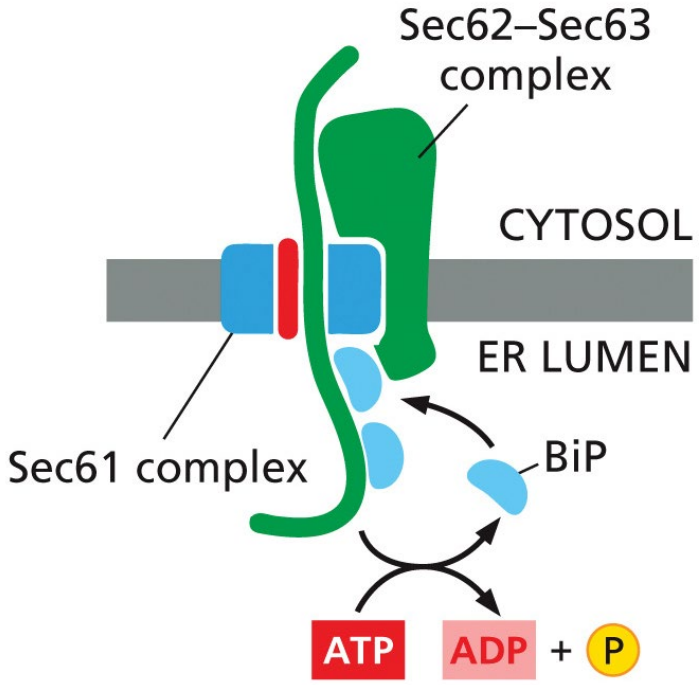
- Translocation across the ER membrane does not always require ongoing polypeptide chain elongation



- The released protein is kept unfolded in the cytosol by chaperones that dissociate before the protein is translocated across the membrane

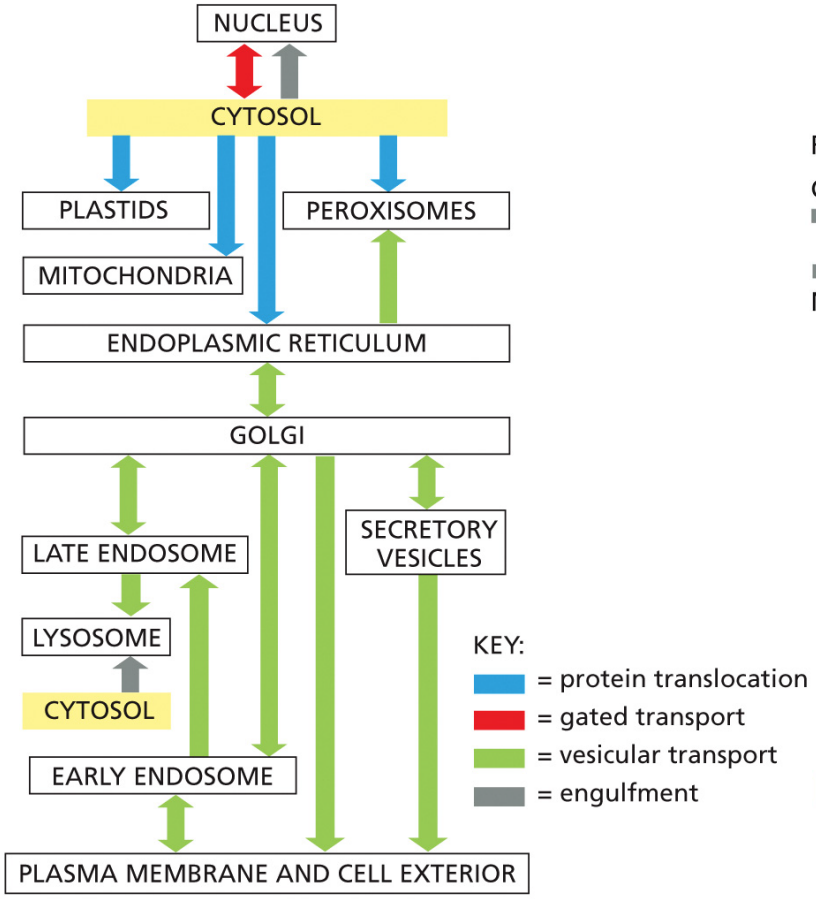
POST-TRANSLATIONAL TRANSLOCATION

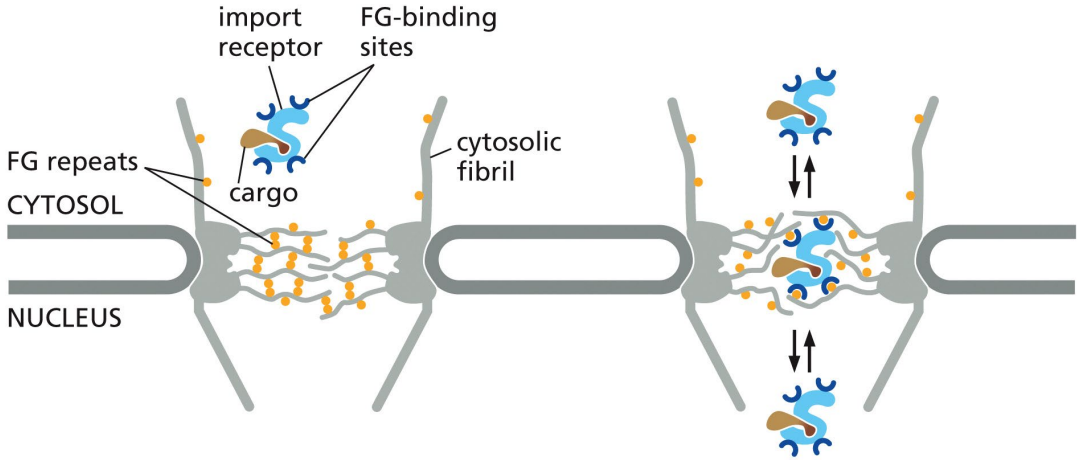
- Requires an additional complex composed of Sec62 and Sec63 proteins
- This complex is attached to the Sec61 translocator
- *Sec62-Sec63 positions BiP molecules* to bind to the translocating chain as it emerges from the translocator in the lumen of the ER
- **ATP-driven cycles of BiP binding and release pull the protein into the lumen.**

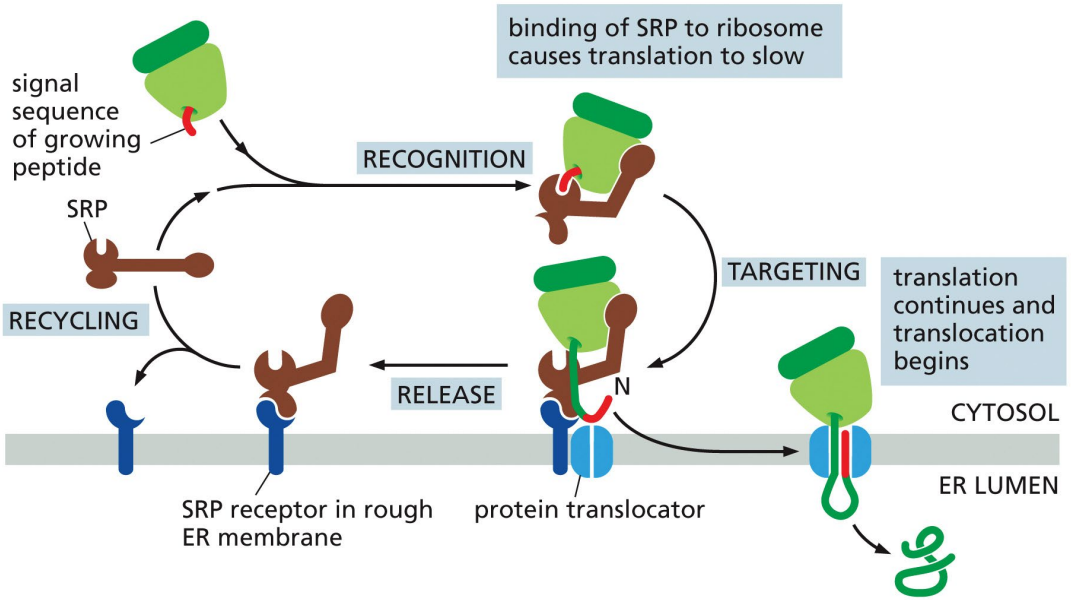


Also in prokaryotes, slightly different mechanism

SUMMARY


NUCLEUS
CYTOSOL
PLASTIDS
PEROXISOMES
MITOCHONDRIA
ENDOPLASMIC RETICULUM
GOLGI
LATE ENDOSOME
SECRETORY VESICLES
LYSOSOME
CYTOSOL
EARLY ENDOSOME
PLASMA MEMBRANE AND CELL EXTERIOR
KEY:
= protein translocation
= gated transport
= vesicular transport
= engulfment


import receptor
FG-binding sites
FG repeats
cargo
cytosolic fibril
CYTOSOL
NUCLEUS


binding of SRP to ribosome causes translation to slow
signal sequence of growing peptide
SRP
RECOGNITION
TARGETING
translation continues and translocation begins
RECYCLING
RELEASE
N
CYTOSOL
ER LUMEN
SRP receptor in rough ER membrane
protein translocator