Review: Energy Generation in Mitochondria

- You are investigating ATP generation and discover **Compound X** that inhibits the movement of coenzyme Q and **Compound Y** that inhibits the movement of cytochrome c in the electron transport chain.

- Which compound would lead to less ATP generation per molecule of pyruvate that enters the mitochondria? Why?

- You next discover **Compound Z** that sterically prevents NADH from contacting Complex I of the electron transport chain. What would you expect to happen to cellular metabolism in the presence of Compound Z?

Review: Energy Generation in Mitochondria

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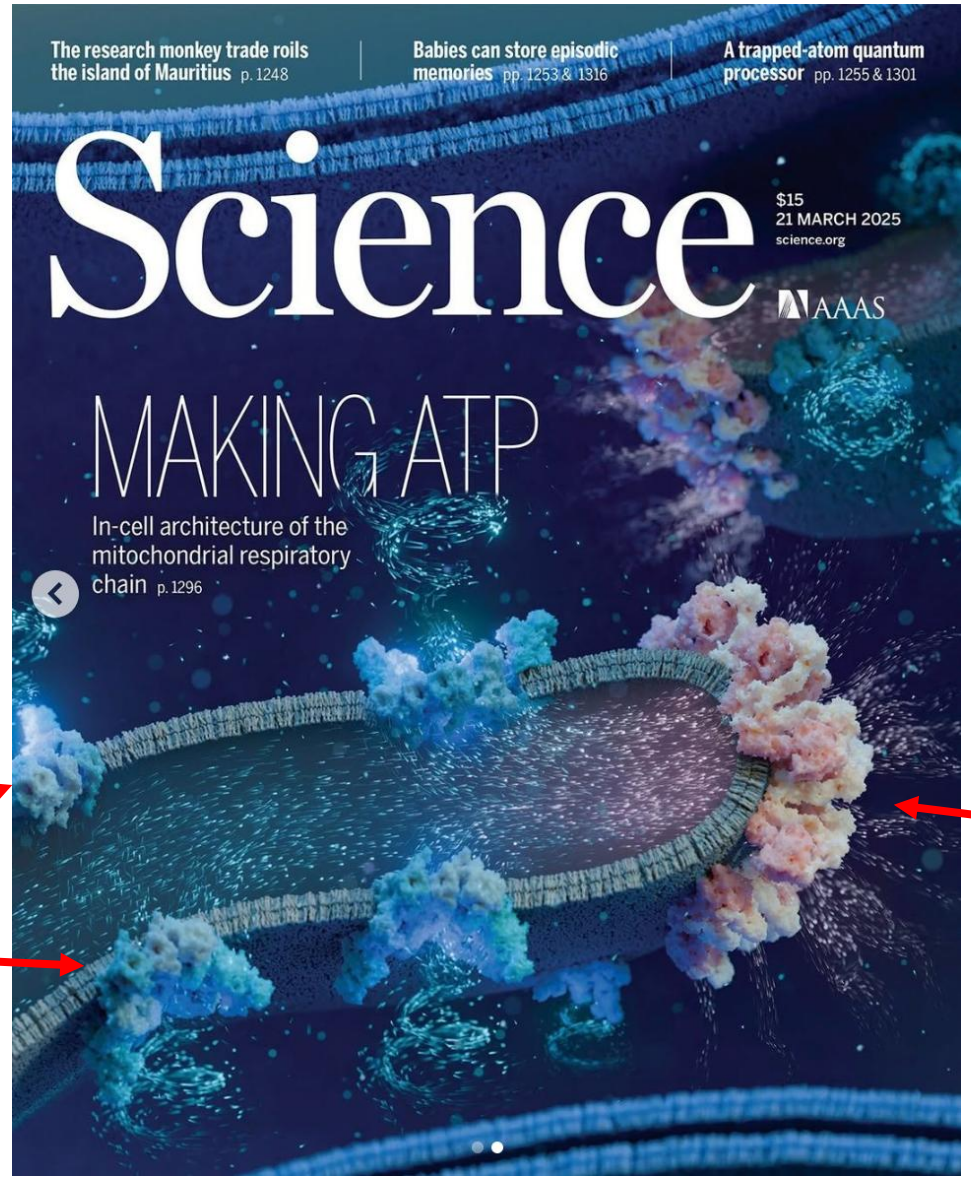
- Which compound would lead to less ATP generation per molecule of pyruvate that enters the mitochondria? Why?
  - Compound X would produce less ATP because an electrochemical proton gradient would only be able to form from Complex I whereas Compound Y would also incorporate protons pumped from Complex III

- You next discover **Compound Z** that sterically prevents NADH from contacting Complex I of the electron transport chain. What would you expect to happen to cellular metabolism in the presence of Compound Z?
  - In the presence of Compound Z, the electron transport chain would not function, and so the cell would not be able to produce ATP via oxidative phosphorylation. To produce ATP, compensatory mechanisms such as upregulation of glycolysis would likely occur, but depending on the energy demands of the cell would potentially be insufficient following prolonged exposure and lead to cell death

Most recent cover for Science magazine

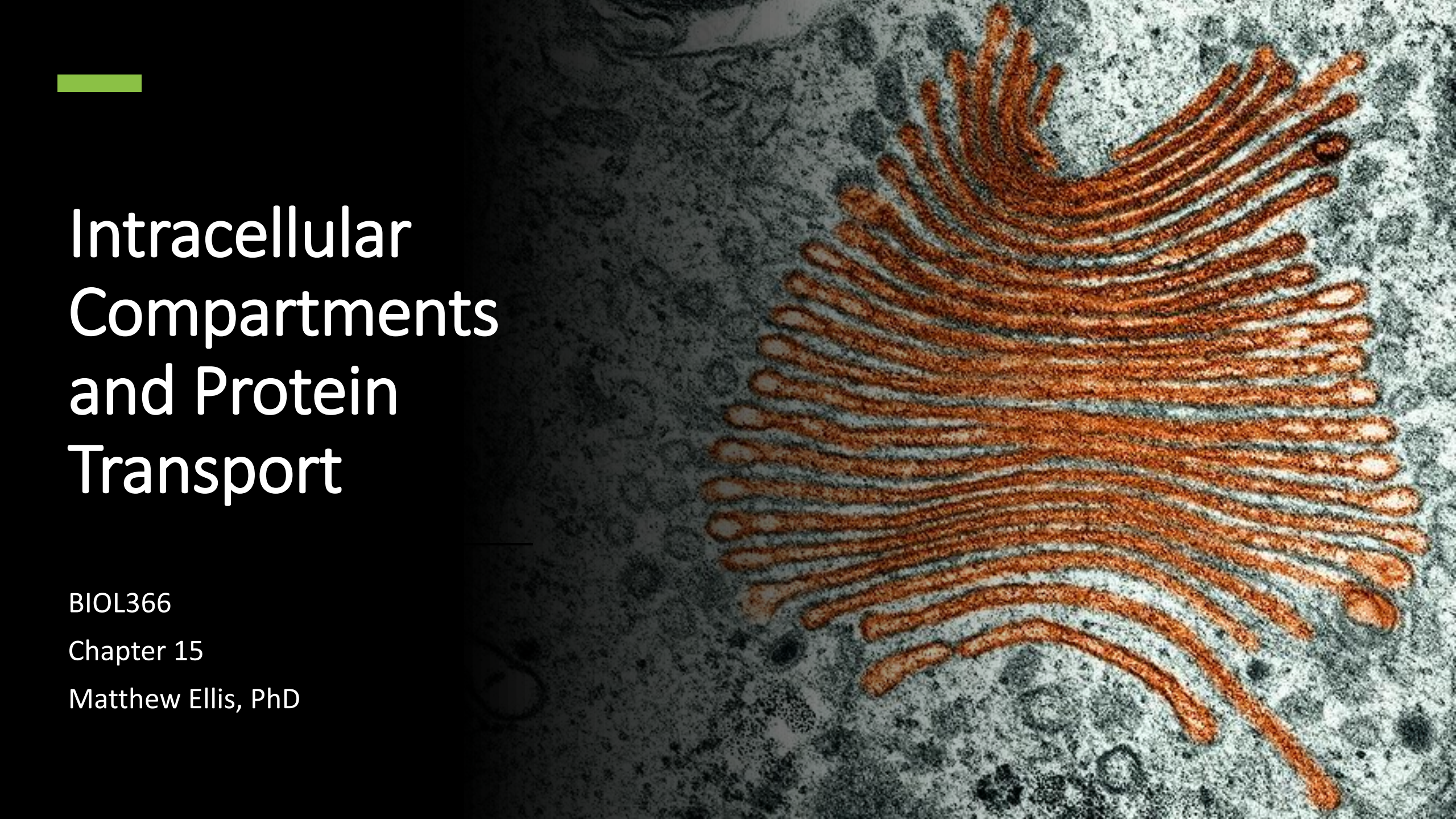


Electron Transport Chain Complexes

ATP Synthase

https://www.instagram.com/p/DHbkCAvNpxw/?igsh=NjZiM2M3MzIxNA%3D%3D&img_index=1

Intracellular Compartments and Protein Transport



BIOL366

Chapter 15

Matthew Ellis, PhD

Learning Objectives for Today's Lecture:

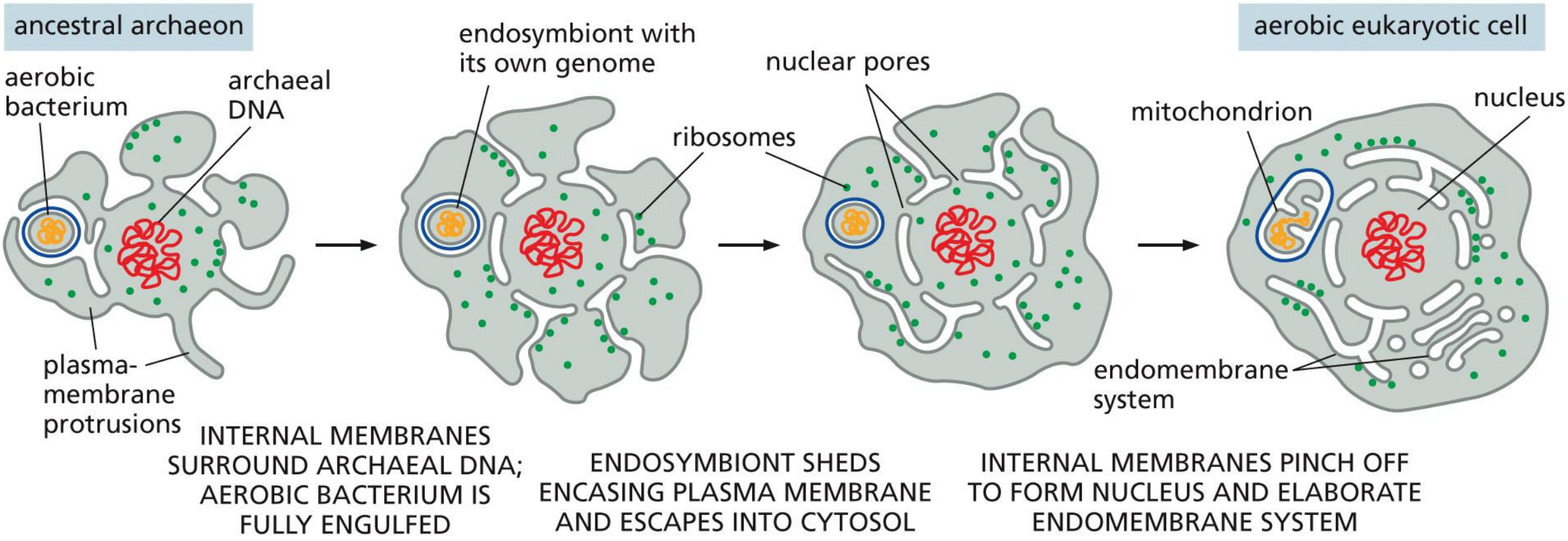
Upon completing this module, **you should be able to**:

- Understand the mechanisms cells use to distribute proteins within a cell (i.e., **signal sequences**, **GEFs** and **GAPs**)
- Describe how cells uptake and secrete molecules through **endocytosis** and **exocytosis**
- Apply knowledge of protein transport to interpret experimental findings

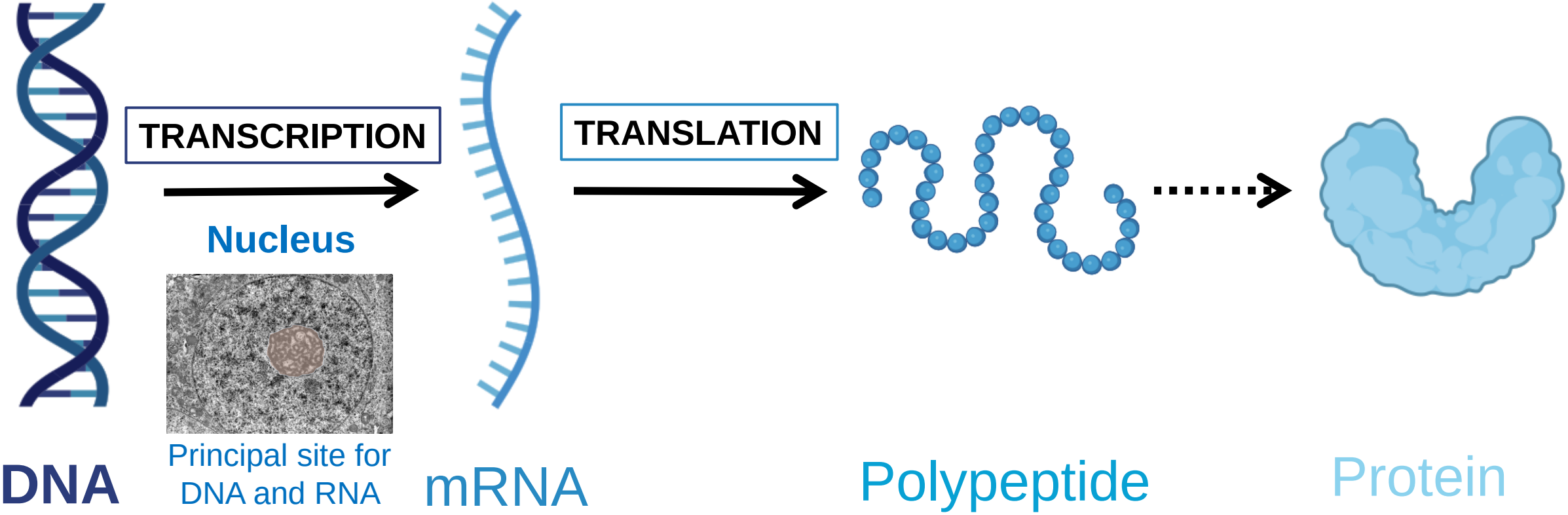
Key Terms

- **Organelle:** a specialized structure in the cell with multiple functions
- **Post-translational modification:** a chemical modification that occurs to a protein after it has been translated by a ribosome
- **Protein translocation:** a biological process where proteins move between cellular compartments
- **Guanine Exchange Factor (GEF):** proteins that stimulate release of GDP and binding of GTP
- **GTPase Activating Protein (GAP):** proteins that catalyze the hydrolysis of GTP to GDP
- **Chaperone protein:** A protein that helps other proteins fold properly during or after synthesis
- **Endocytosis:** Bringing material (proteins, molecules, etc) into the cell
- **Exocytosis:** Releasing material (proteins, molecules, etc) from the cell

Evolution of membrane enclosed organelles

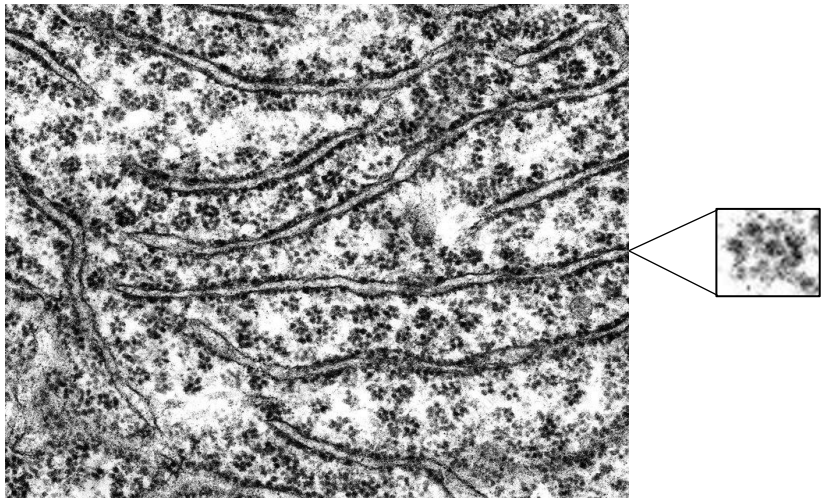


Recap: The Central Dogma of Molecular Biology

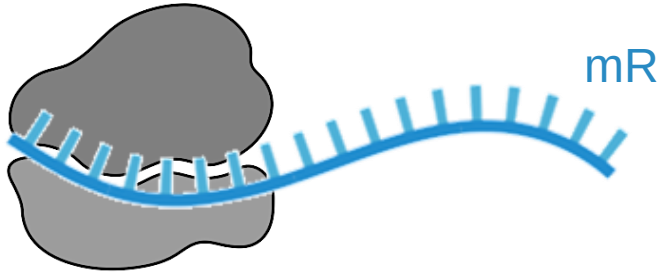


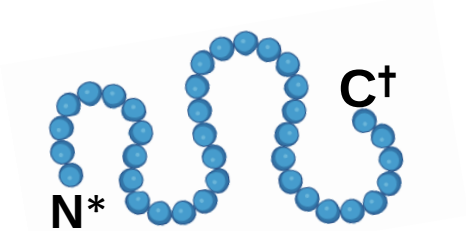
Proteins are synthesized on Ribosomes

Ribosomes



- Ribosomes are located in the cytosol (free-floating)
- Ribosomes are also attached Endoplasmic Reticulum (ER) ("bound" ribosomes)





Polypeptide

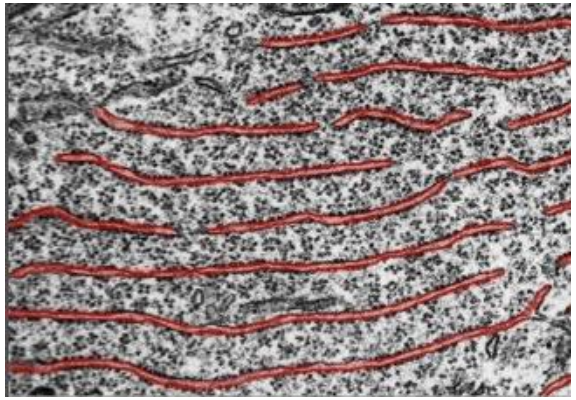
Protein

*N-terminus → 1st amino group

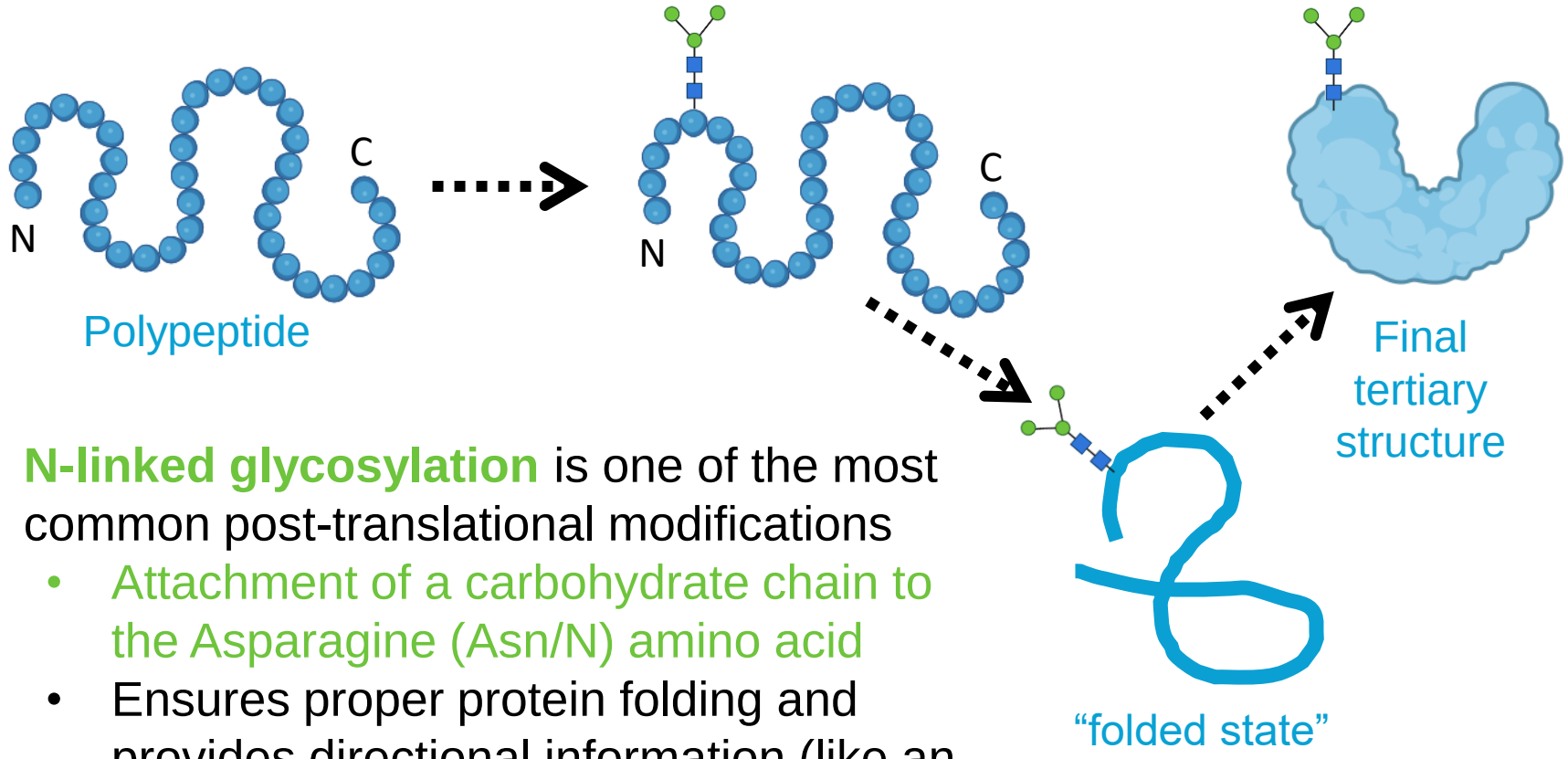
†C-terminus → last amino acid with Carboxyl group (COO^-)

Proteins undergo **post-translational modifications** and **folding in the rough ER**

Rough Endoplasmic Reticulum (ER)



Site for **protein synthesis**, **modification(s), folding and transport**



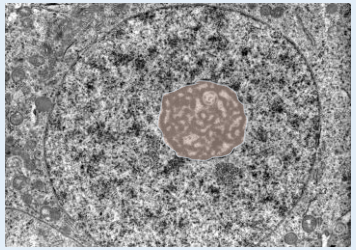
- **N-linked glycosylation** is one of the most common post-translational modifications
  - Attachment of a carbohydrate chain to the Asparagine (Asn/N) amino acid
  - Ensures proper protein folding and provides directional information (like an address on a letter)

Recap: The Central Dogma of Molecular Biology

DNA

TRANSCRIPTION

Nucleus

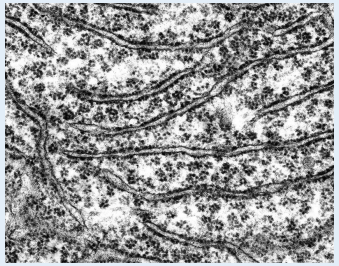


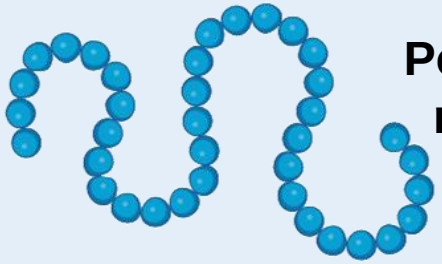
Principal site for DNA and RNA synthesis

mRNA

TRANSLATION

Ribosomes

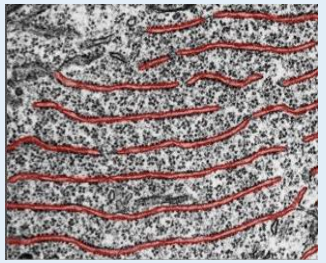




Polypeptide

Post translational modification(s)

Rough ER

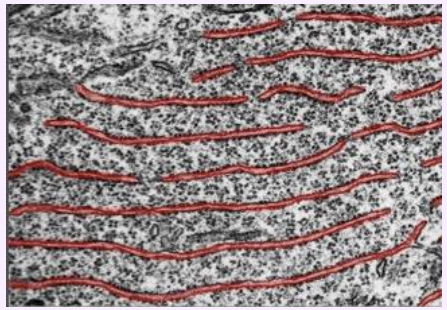


Protein

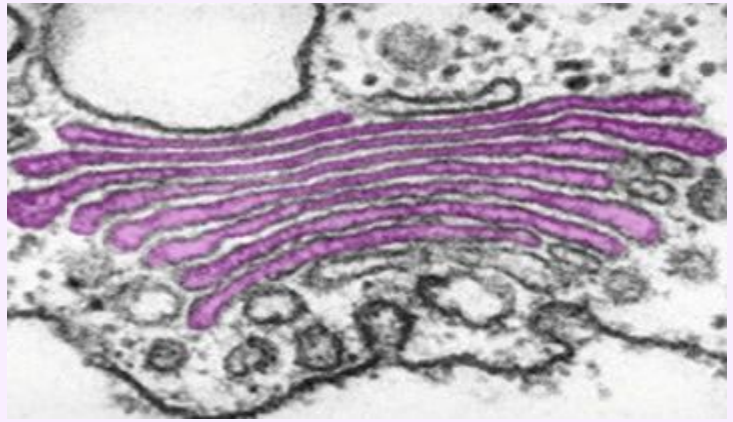
The Golgi apparatus sorts proteins received from the ER

Ribosomes

Rough Endoplasmic Reticulum (ER)



Golgi Apparatus



- Shipping/packaging center for proteins
- Further protein modifications can occur
  - **ER** + Golgi apparatus form the **endomembrane system**

Learning Objectives for Today's Lecture:

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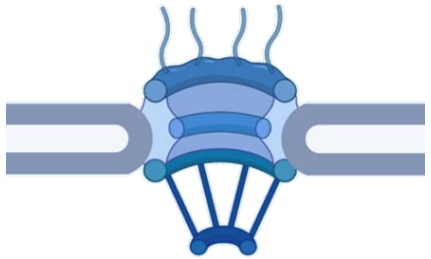
- Apply knowledge of protein transport to interpret experimental findings

Proteins are transported into membrane-bound organelles using 3 main mechanisms

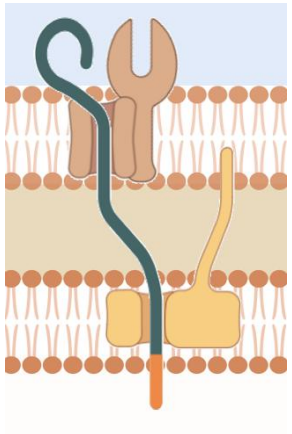
1

Nuclear Pore Complex transport



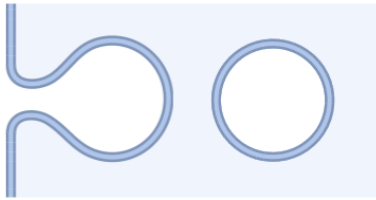
2

Transmembrane transport



3

Vesicular (Vesicle) transport



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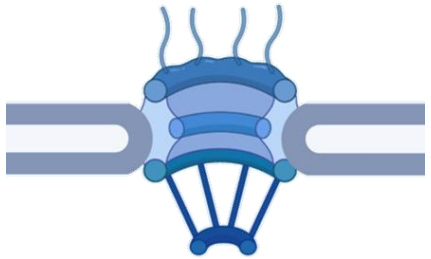

2

Transmembrane transport

3

Vesicular (Vesicle) transport

Signal sequences direct proteins to the correct cellular compartment

A signal sequence is comprised of a specific sequence of amino acids

Examples of signal sequences	Function of the signal sequence
-P-P-**K**-**K**-**K**-**R**-**K**-V-	Import into the Nucleus
M-E-E-**L**-S-Q-A-**L**-A-S-S-**F**-	Export from the Nucleus
H_3N-**M**-**M**-S-F-V-S-**L**-**L**-**L**-**V**-**G**-**I**-**L**-**F**- **A**-T-**E**-A-**E**-Q-L-T-K-C-**E**-V-F-Q-	Import into the ER
-**K**-**D**-**E**-**L**-COO^-	Retention in the ER

H_3N = N-terminus of protein Hydrophobic amino acids (-) charged amino acids (+) charged amino acids

COO^- = C-terminus of protein

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-K-D-E-L-COO^-	Retention in the ER

H_3N = N-terminus of protein Hydrophobic amino acids (-) charged amino acids (+) charged amino acids

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Proteins destined for the nucleus have a **nuclear localization signal (NLS)**

Extracellular

Cytoplasm

- Nuclear import receptor recognizes **NLS** signal and binds the protein

Nuclear import receptor

Cytosolic fibrils

Outer nuclear membrane

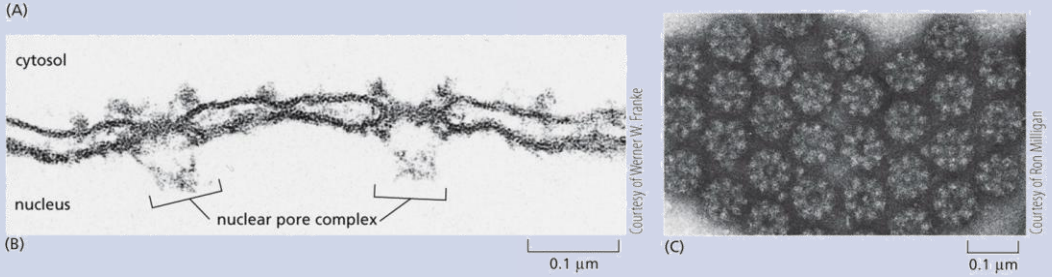
Lumen

Inner nuclear membrane

Nucleus

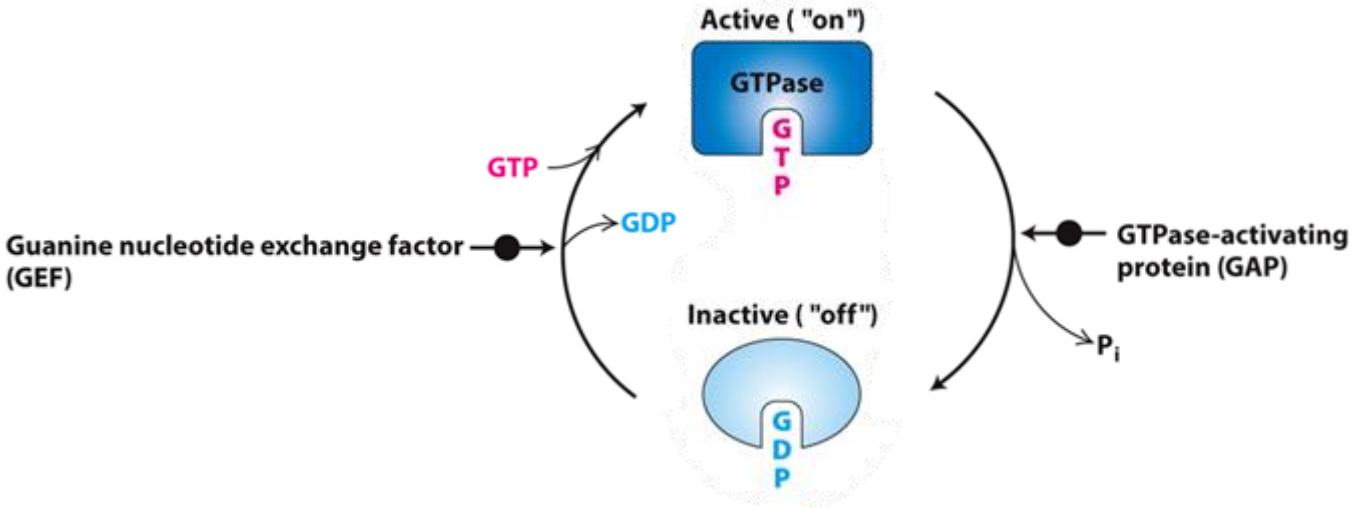
Nuclear basket

Nuclear Pore Complex

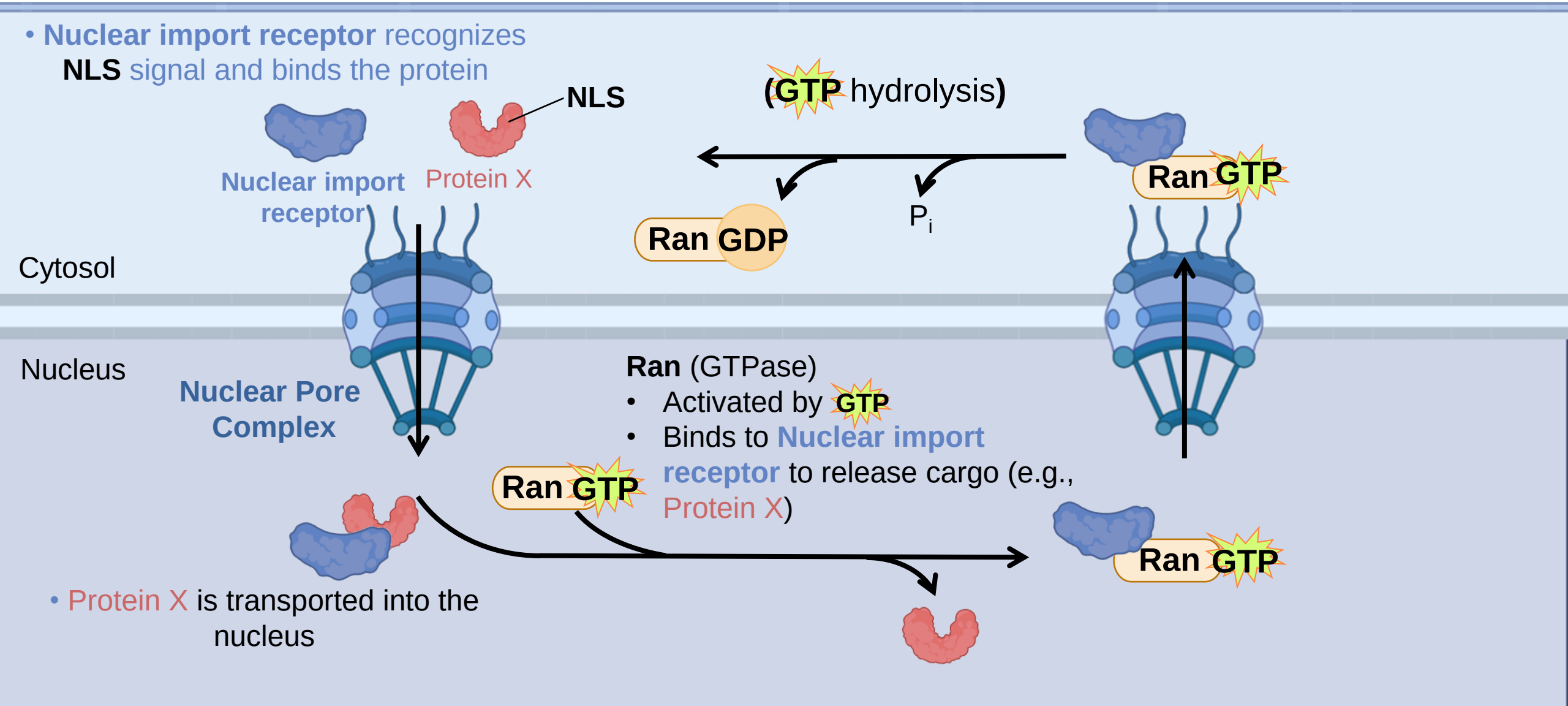


GTP hydrolysis drives nuclear transport

- GTP, like ATP, is an energy rich activated carrier. It's hydrolysis is used to drive reactions that are energetically unfavorable
- GTP or GDP are bound to hydrolyzing enzymes called *GTPases*. They are active when bound to GTP and inactive when bound to GDP
- These GTPases are *activated* by **guanine nucleotide exchange factors (GEFs)**, proteins stimulate release of GDP and binding of GTP
- They are *inhibited* by **GTPase activating proteins (GAPs)** that catalyze hydrolysis of GTP to GDP



GTP hydrolysis drives the transport of proteins from cytosol → nucleus

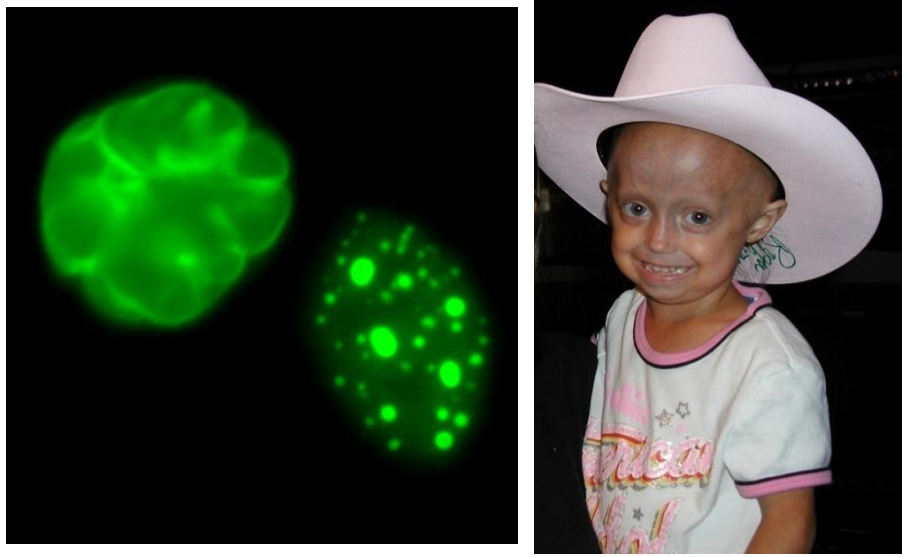


Hutchinson-Gilford Progeria Syndrome (HGPS)

Mutations in the nuclear lamin protein, essential to nuclear envelope formation and pore function lead to HGPS

HGPS patient develop the signs of premature aging (progeria), including hair loss, stiff joints, osteoporosis and atherosclerosis as early as 6 months of age due to defects in nuclear structure and function.

Nuclei are characterized by dysmorphic shape, increased DNA damage, and down-regulation of several nuclear proteins.



Progeria nucleus **Healthy nucleus**

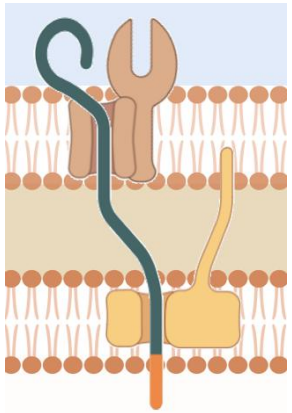
Proteins are transported into membrane-bound organelles using 3 main mechanisms

1

Nuclear Pore Complex transport

2

Transmembrane transport



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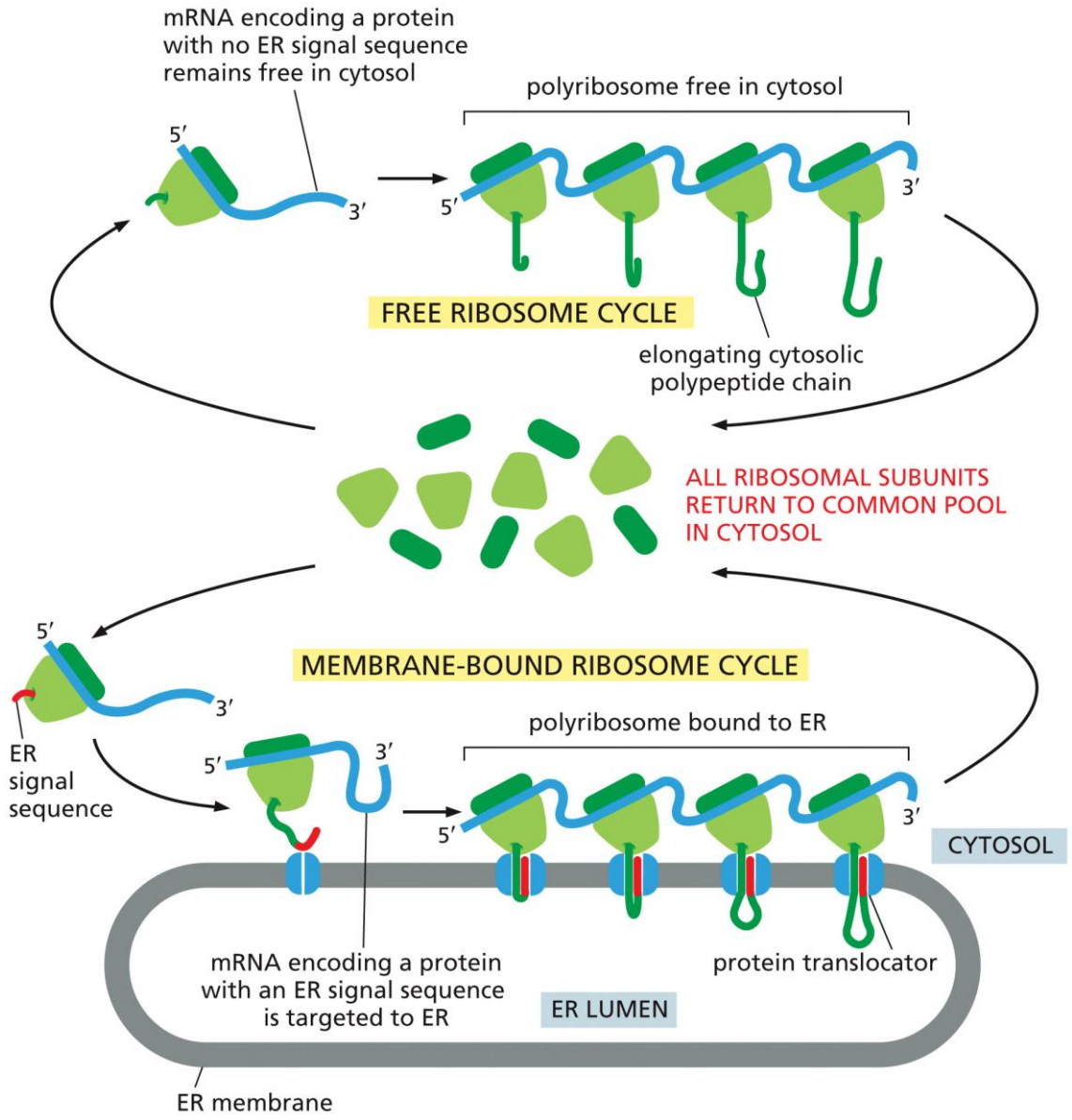
H_3N-M-M-S-F-V-S-L-L-L-V-G-I-L-F- A-T-E-A-E-Q-L-T-K-C-E-V-F-Q-	Import into the ER
-K-D-E-L-COO^-	Retention in the ER

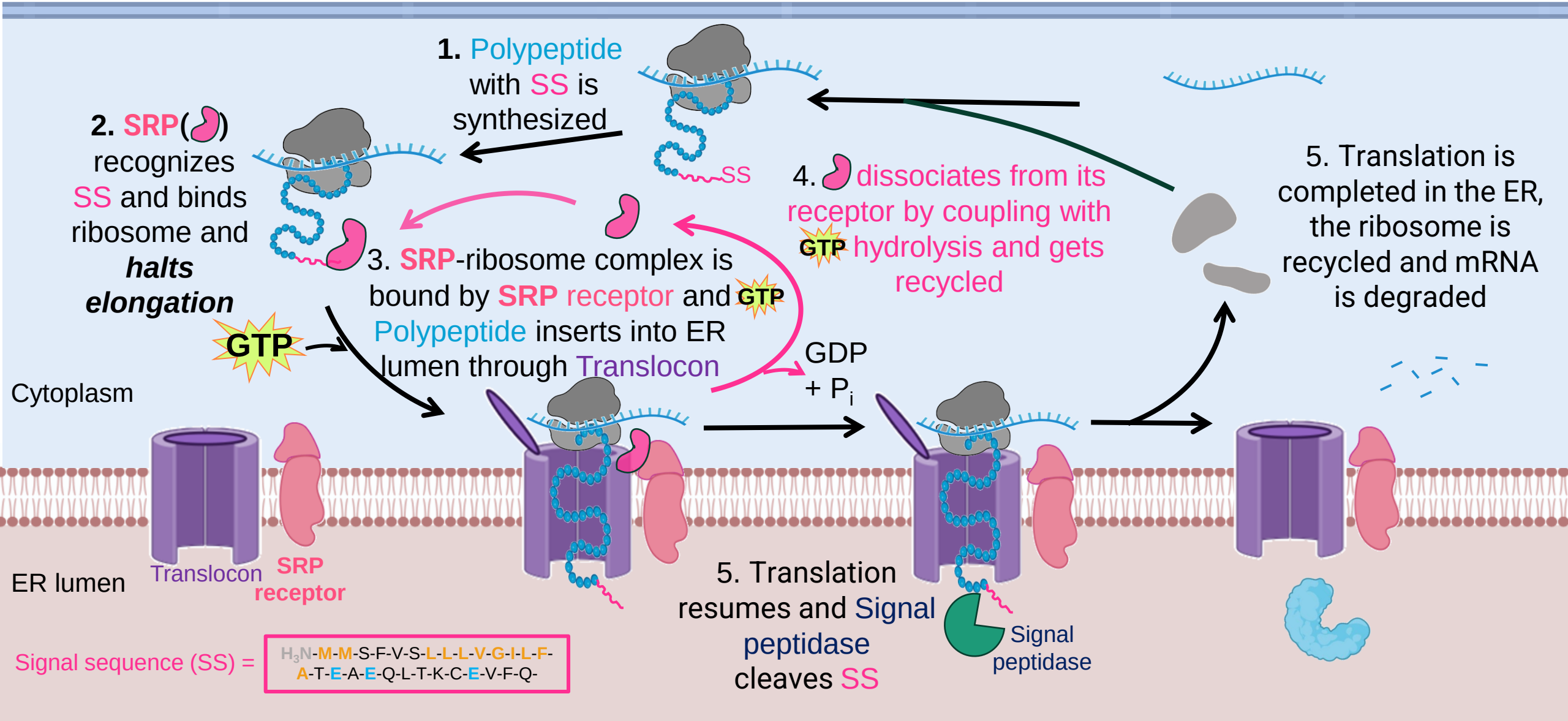
H_3N = N-terminus of protein Hydrophobic amino acids (-) charged amino acids (+) charged amino acids

COO^- = C-terminus of protein

Ribosomes attach to the endoplasmic reticulum in the presence of an ER signal sequence

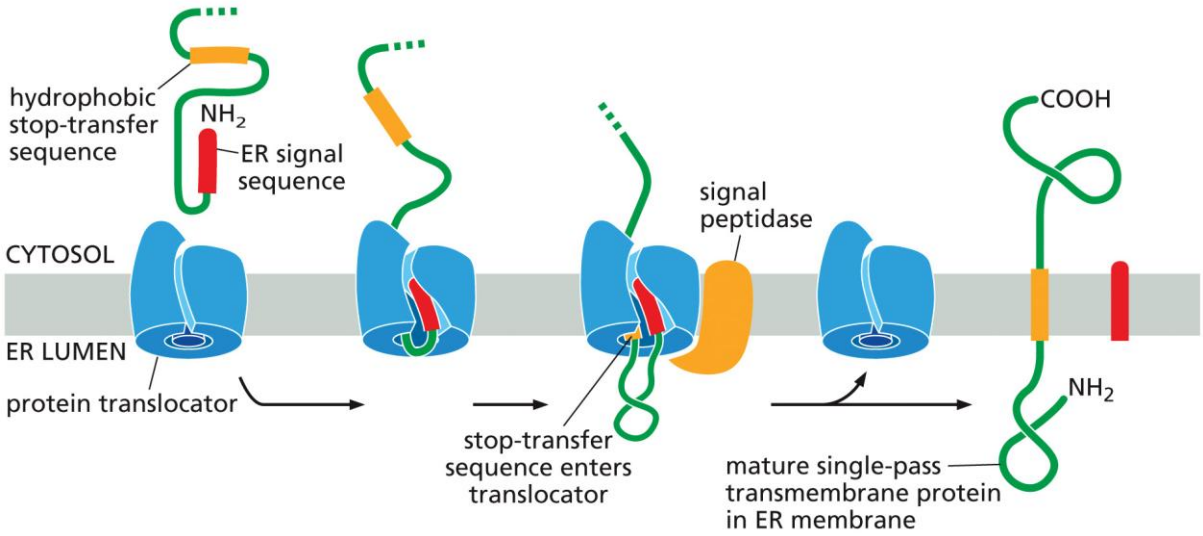


Targeting Proteins to the ER with Signal Recognition Particle (SRP)

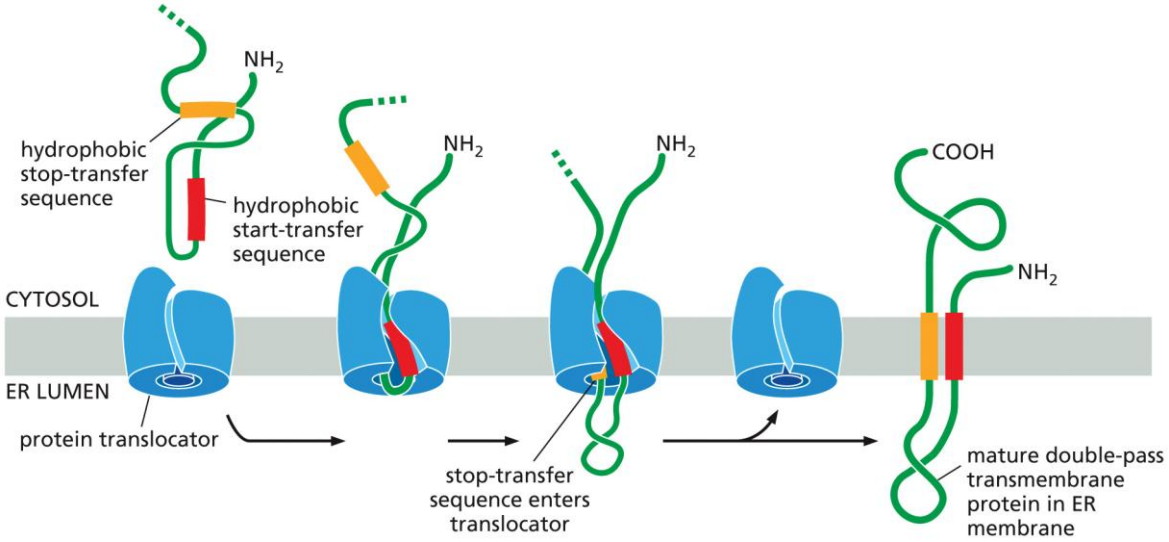


Start and stop signals dictate transmembrane localization in the lipid bilayer of ER

Scenario #1: Single pass transmembrane protein

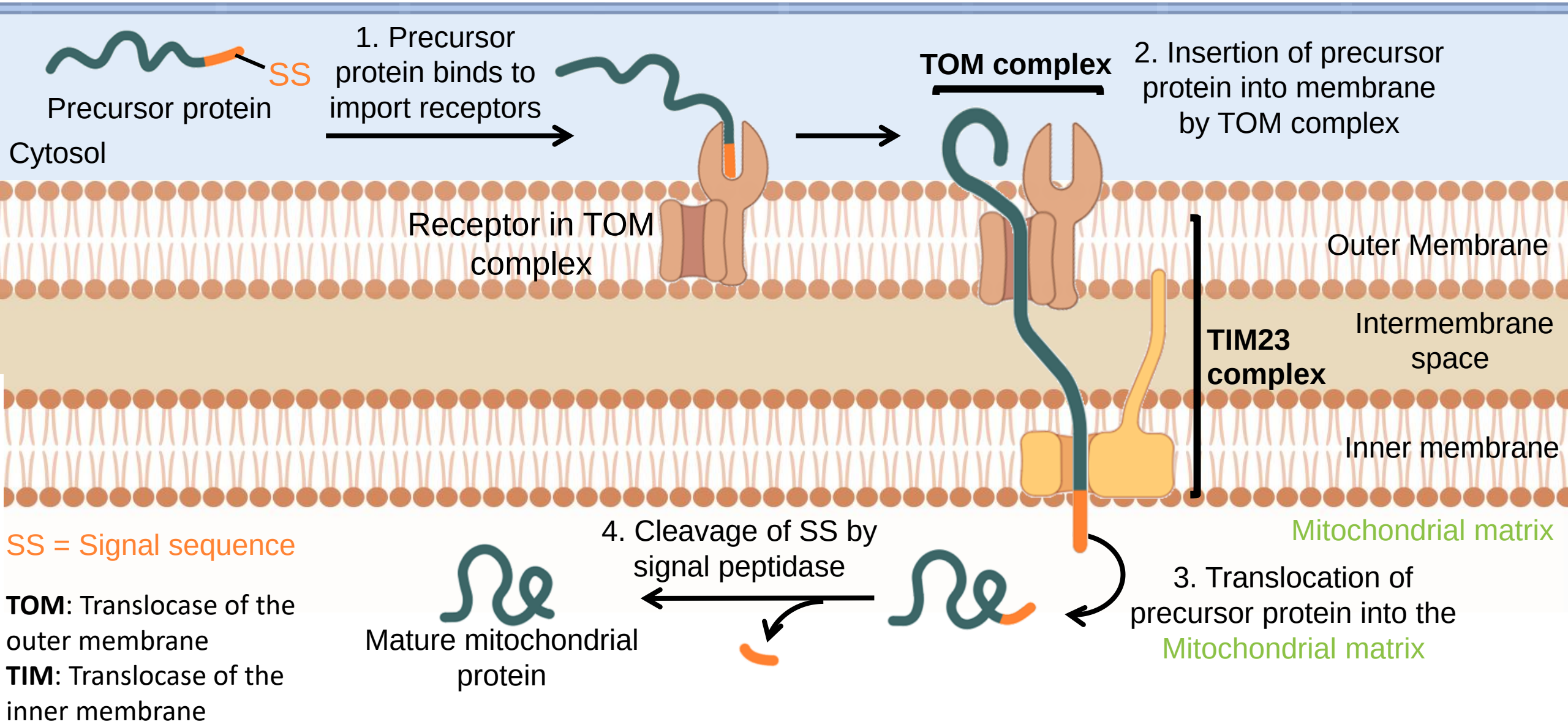


Scenario #2: Multiple transmembrane domains



- Translocator recognizes hydrophobic stop/start-transfer sequences (as with ER signal sequence)
- While the ER signal sequence is cleaved off to produce cytosolic ER proteins, internal transmembrane start and stop sequences are not cleaved
- Multiple start-stop combinations can occur for transmembrane proteins

TOM and TIM help import proteins into the mitochondria



SS = Signal sequence

TOM: Translocase of the outer membrane

TIM: Translocase of the inner membrane

Squarecap #1-2

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Transmembrane transport

3

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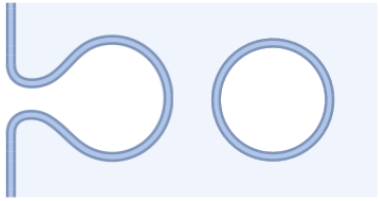
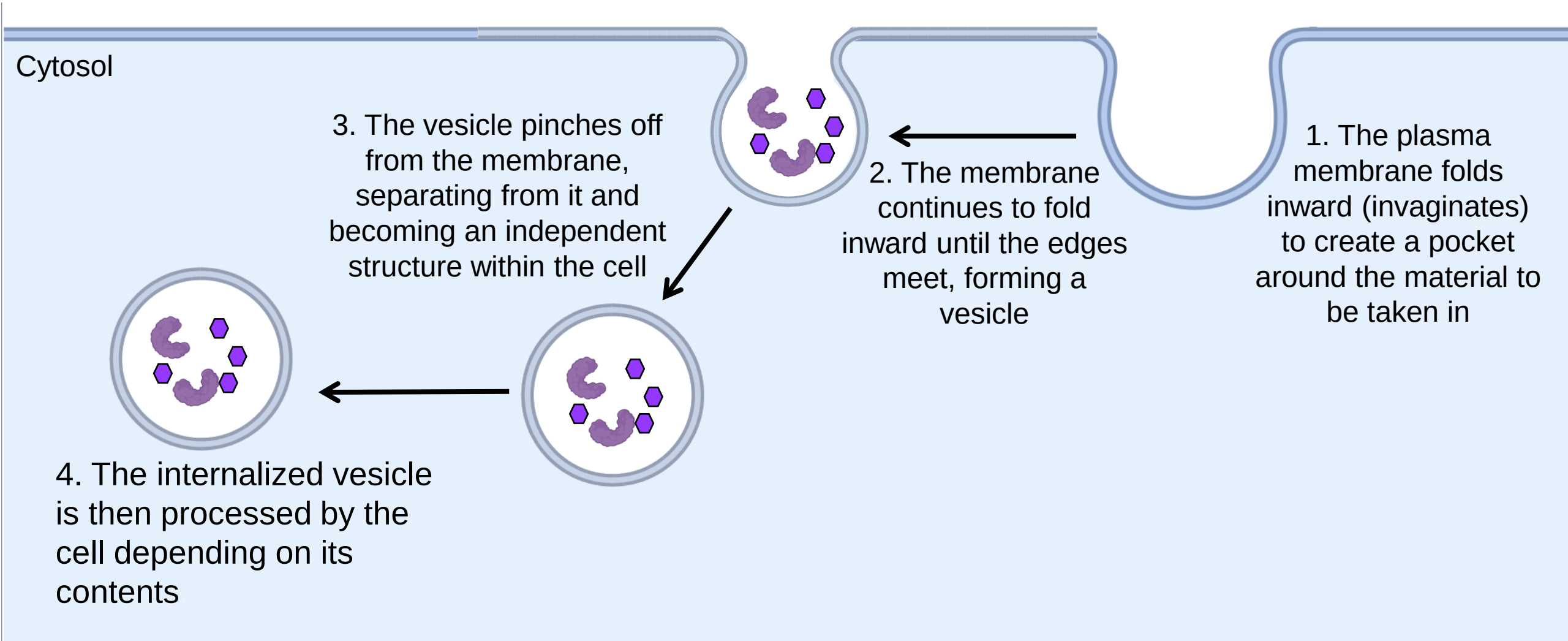
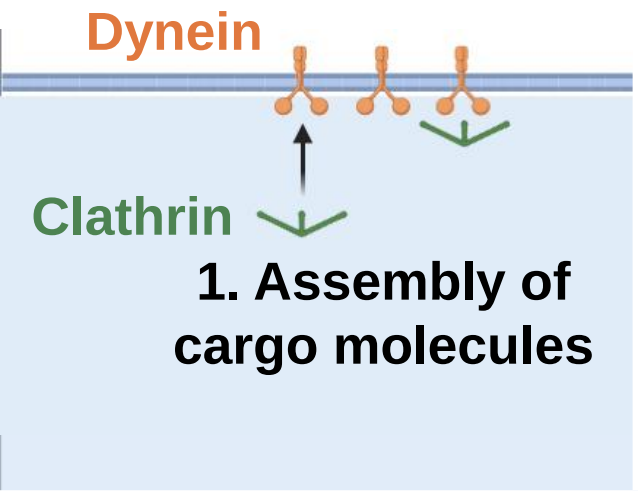


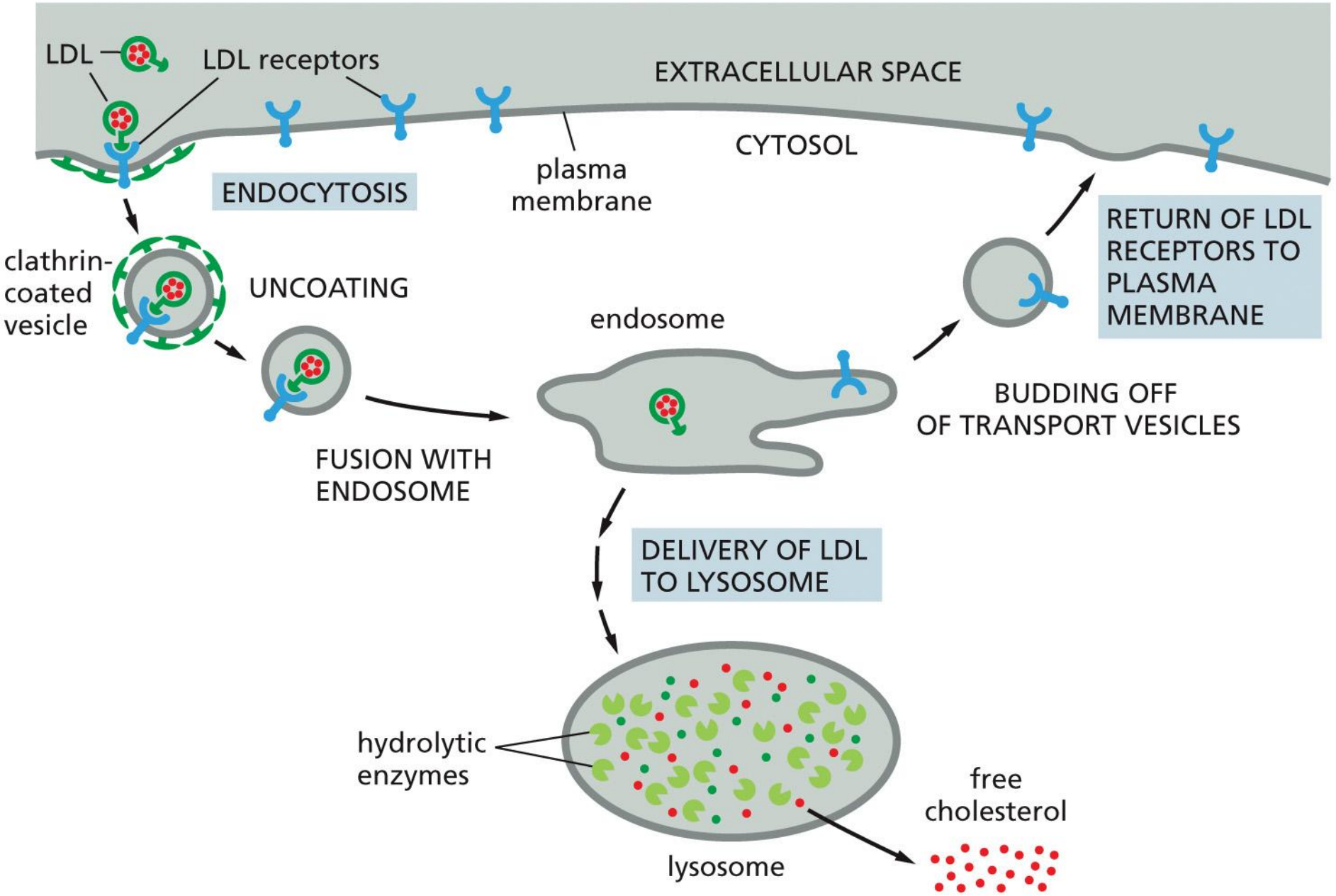
Endocytosis: Bringing material (proteins, molecules, etc.) into the cell



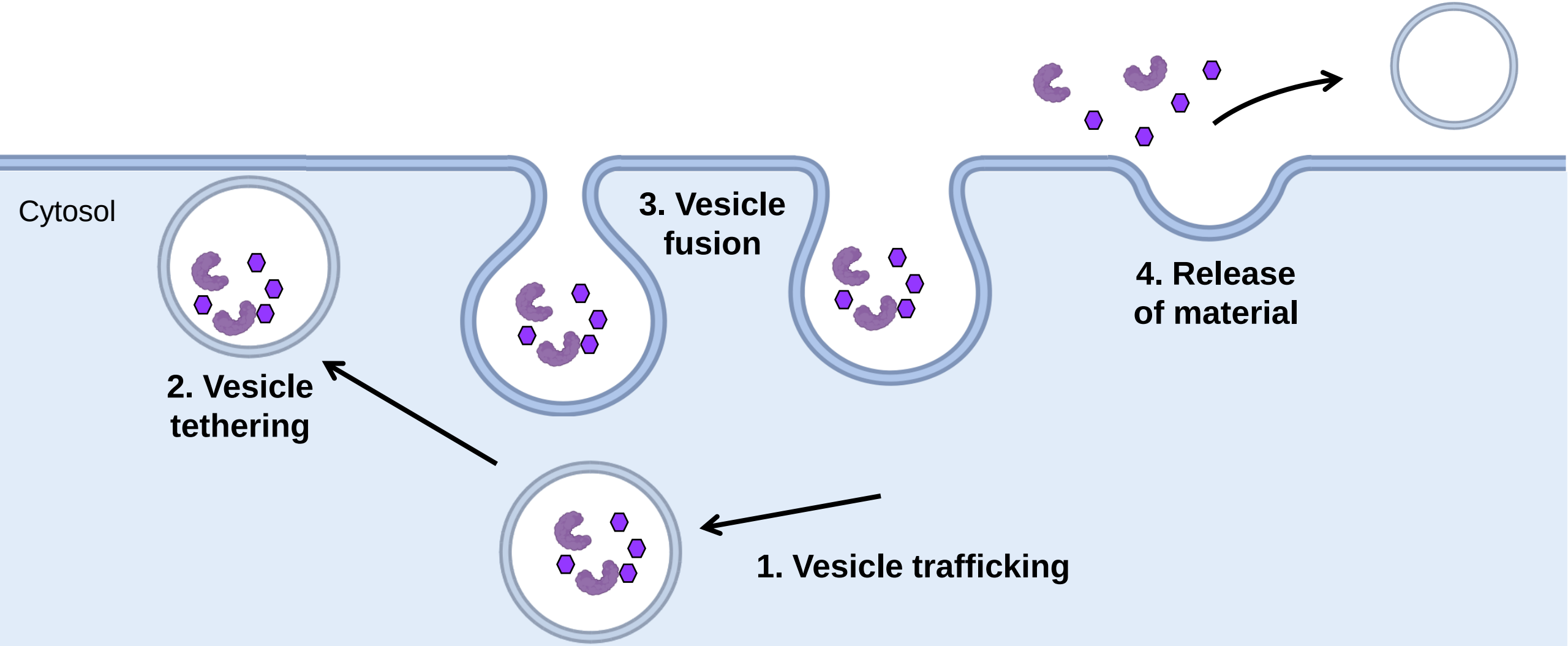
“Clathrin-mediated endocytosis” moves proteins and other molecules inside the cell



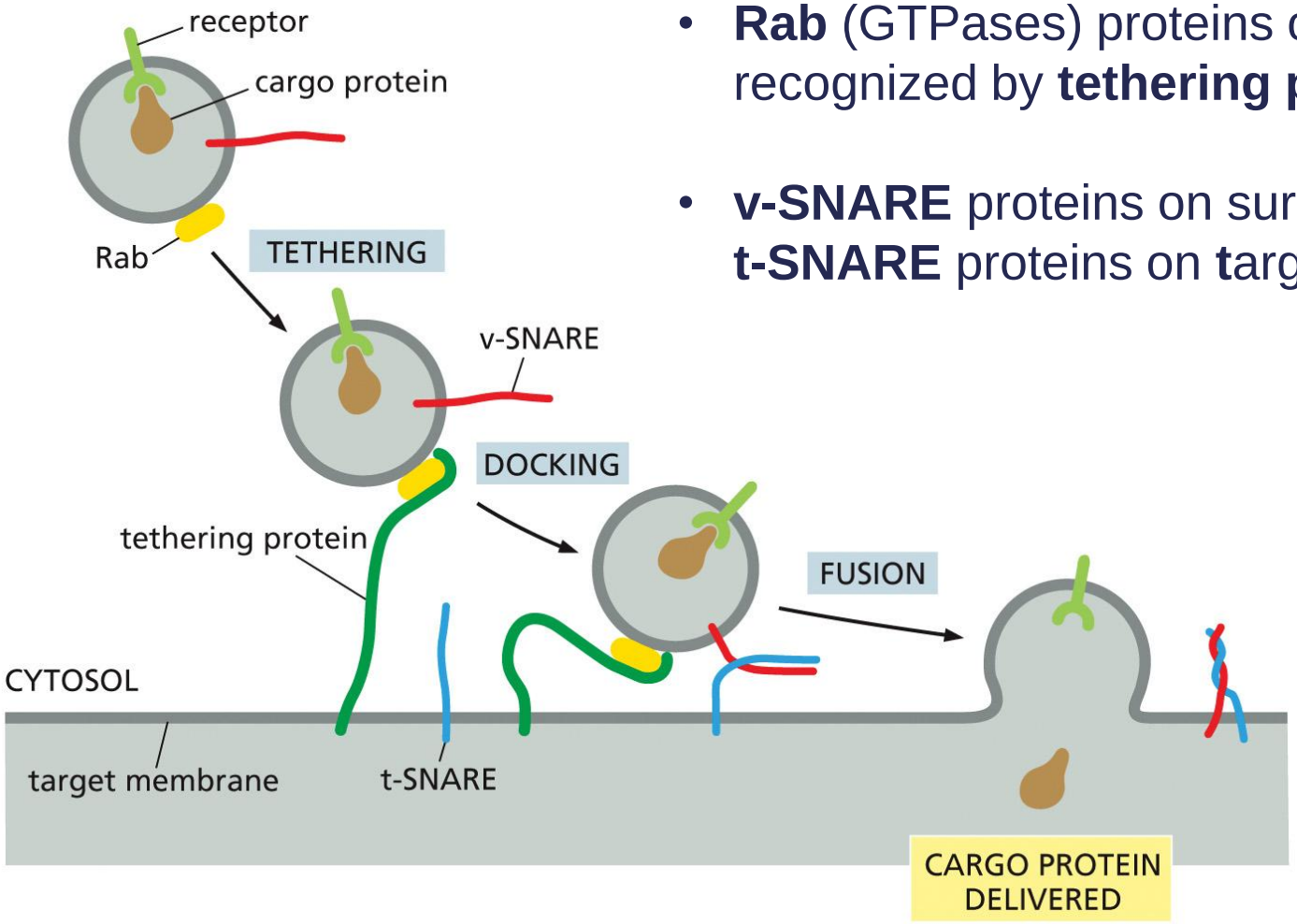
Receptor mediated endocytosis



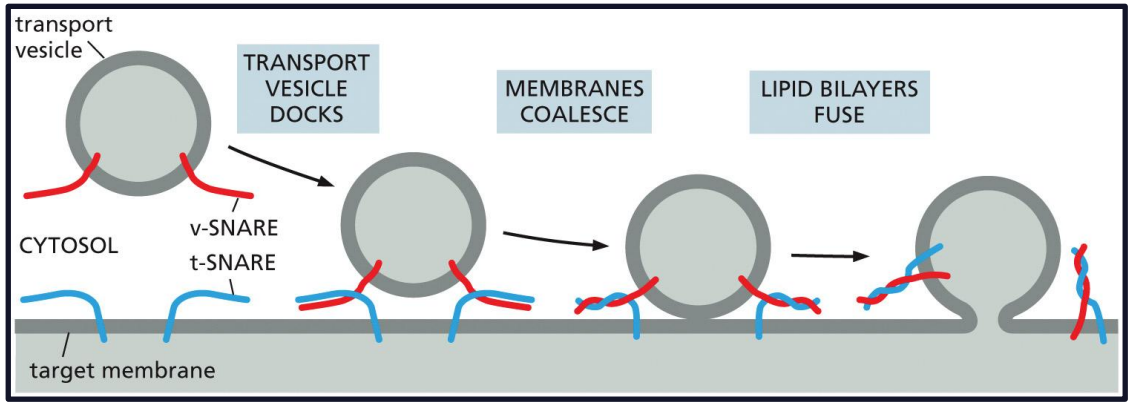
Exocytosis: Releasing material (proteins, molecules, etc.) from the cell



In general transport vesicle ‘docking’ requires distinct proteins

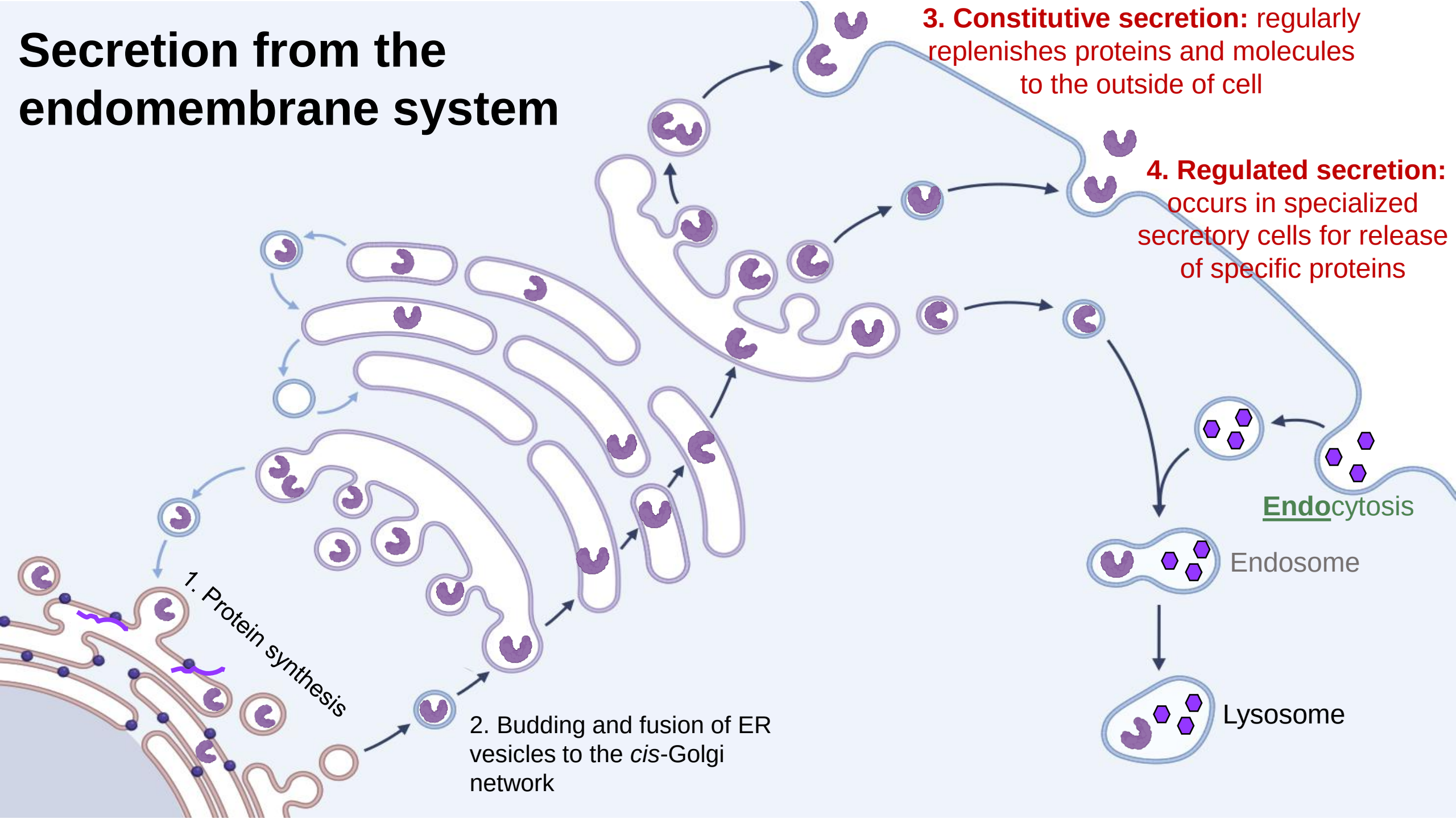


- **Rab** (GTPases) proteins on transport vesicle surface are recognized by **tethering proteins** on target membrane
- **v-SNARE** proteins on surface of **v**esicle can intertwine with **t-SNARE** proteins on **t**arget membrane

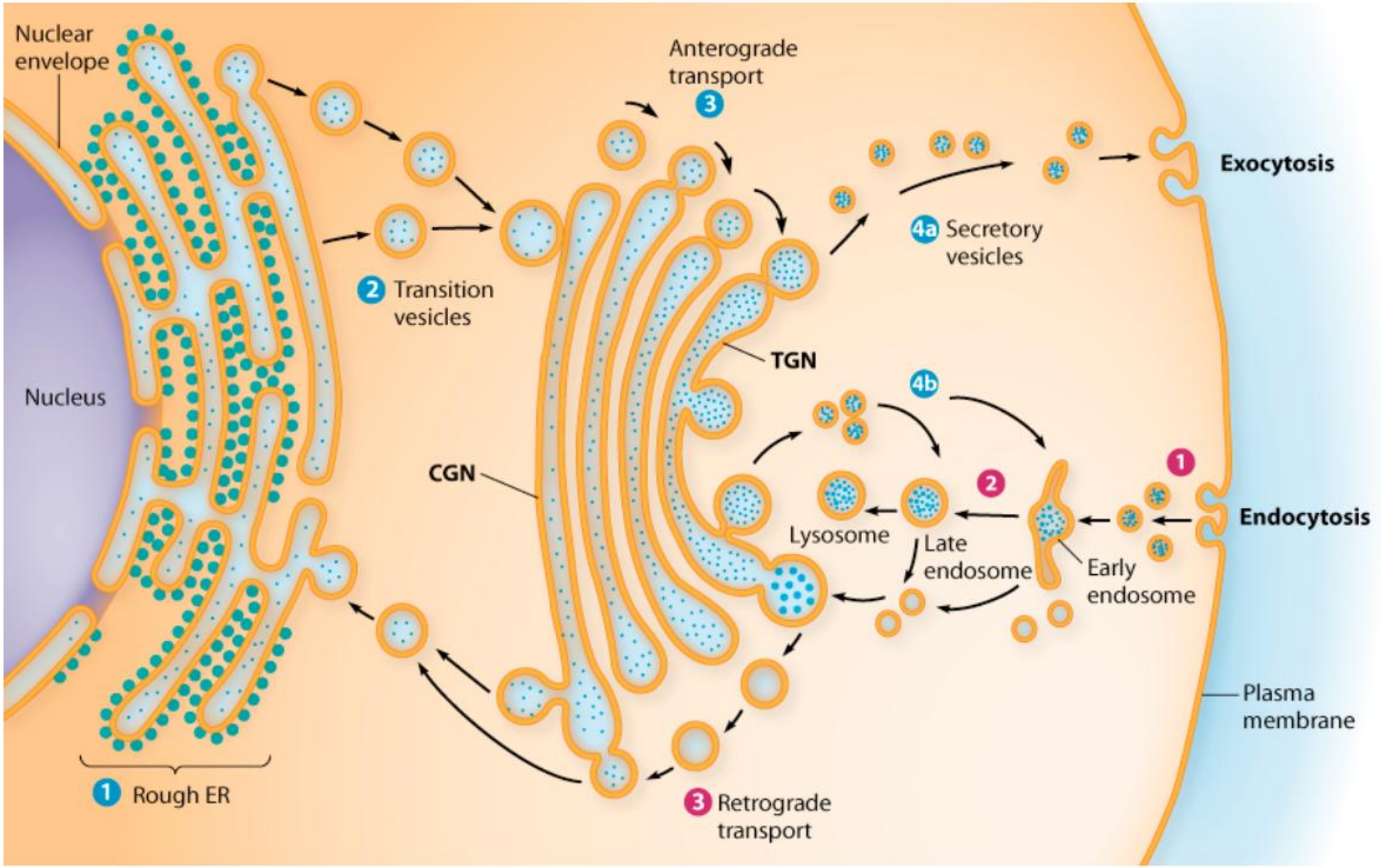


SNARE interactions drive vesicle fusion with target membrane through a “tug-of-war”

Secretion from the endomembrane system



Movement occurs both anterograde and retrograde



Endocytosed factors can thus be trafficked towards the ER and nucleus to affect gene expression and cell function

Learning Objectives for Today's Lecture:

Upon completing this module, **you should be able to**:

- Understand the mechanisms cells use to distribute proteins within a cell (i.e., **signal sequences**, **GEFs** and **GAPs**)
- Describe how cells uptake and secrete molecules through **endocytosis** and **exocytosis**
- Apply knowledge of protein transport to interpret experimental findings

Case Study

You are an ambitious graduate student who wants to engineer a new protein to express in the cytosol. You include the following sequence *on the C-terminus* of the protein: L-H-A-T-P-Y-A-S-H-K-D-E-L. You fluorescently label your protein with a GFP tag and introduce it into cells in a petri dish in the laboratory. The next day, when you check for the expression of your protein under a fluorescent microscope, you are surprised to find your protein is not localized to the cytosol.

1. **In which part of the cell would you see the GFP signal? How did your protein get there?**
2. **Given the sequence above, how would you engineer your protein so that it only localizes to the cytosol?**

Case Study

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1. **In which part of the cell would you see the GFP signal? How did your protein get there?**
   - It would appear in the ER, as the KDEL sequence at the C-terminus is the address label for resident ER proteins. Upon entering the cell, this signal sequence would be recognized and flagged for retrograde transport through the endomembrane system back to the ER.
2. **Given the sequence above, how would you engineer your protein so that it only localizes to the cytosol?**
   - Remove the KDEL sequence and be sure no other organelle-specific signal sequences exist

Case Study

A G-protein coupled receptor (GPCR) is an integral membrane protein with 7 transmembrane domains, an extracellular amino terminus and an intracellular carboxy terminus.

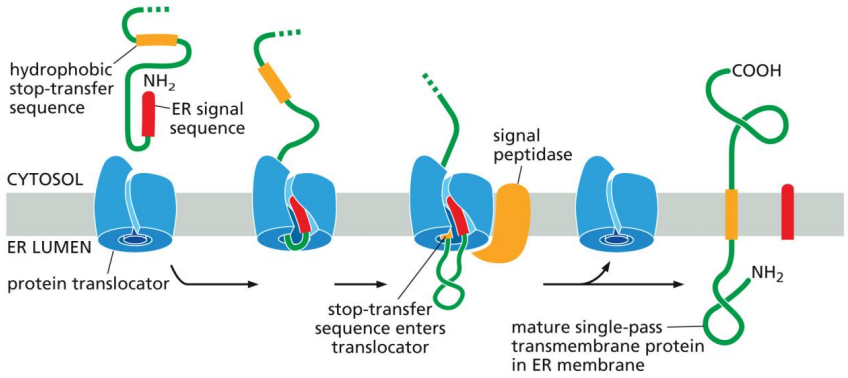
You are trying to design a novel GPCR to study cell biology. You design your GPCR with an ER signal sequence *at the N-terminus* and add multiple start- and stop-transfer sequences throughout. Upon visualization of your protein with Cryo-EM you are surprised to find your protein is inverted, with the amino and carboxy termini on the wrong sides.

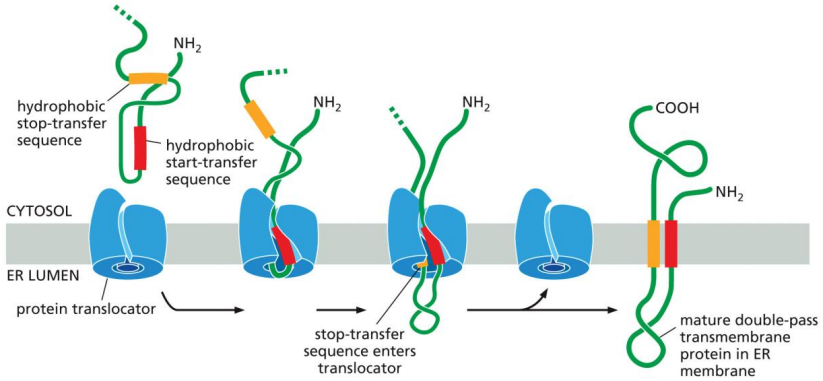
1. **Why would the protein be inverted?**

2. **How would you design the protein to be in the correct orientation?**

Case Study

1. **Why would the protein be inverted?**
   - With an ER signal sequence at the extreme N-terminus, this would be cleaved upon finishing translation, and so all “stop” and “start” transfer sequences would also be inverted
2. **How would you design the protein to be in the correct orientation?**
   - You would want the first hydrophobic sequence to occur later, more internally along the growing polypeptide chain, so that the N-terminus remains cytosolic while the SRP translocates to the ER, and would have 4 start transfer sequences and 3 stop transfer sequences for the C-terminus to remain in the ER lumen





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Metacognitive Reflection Form