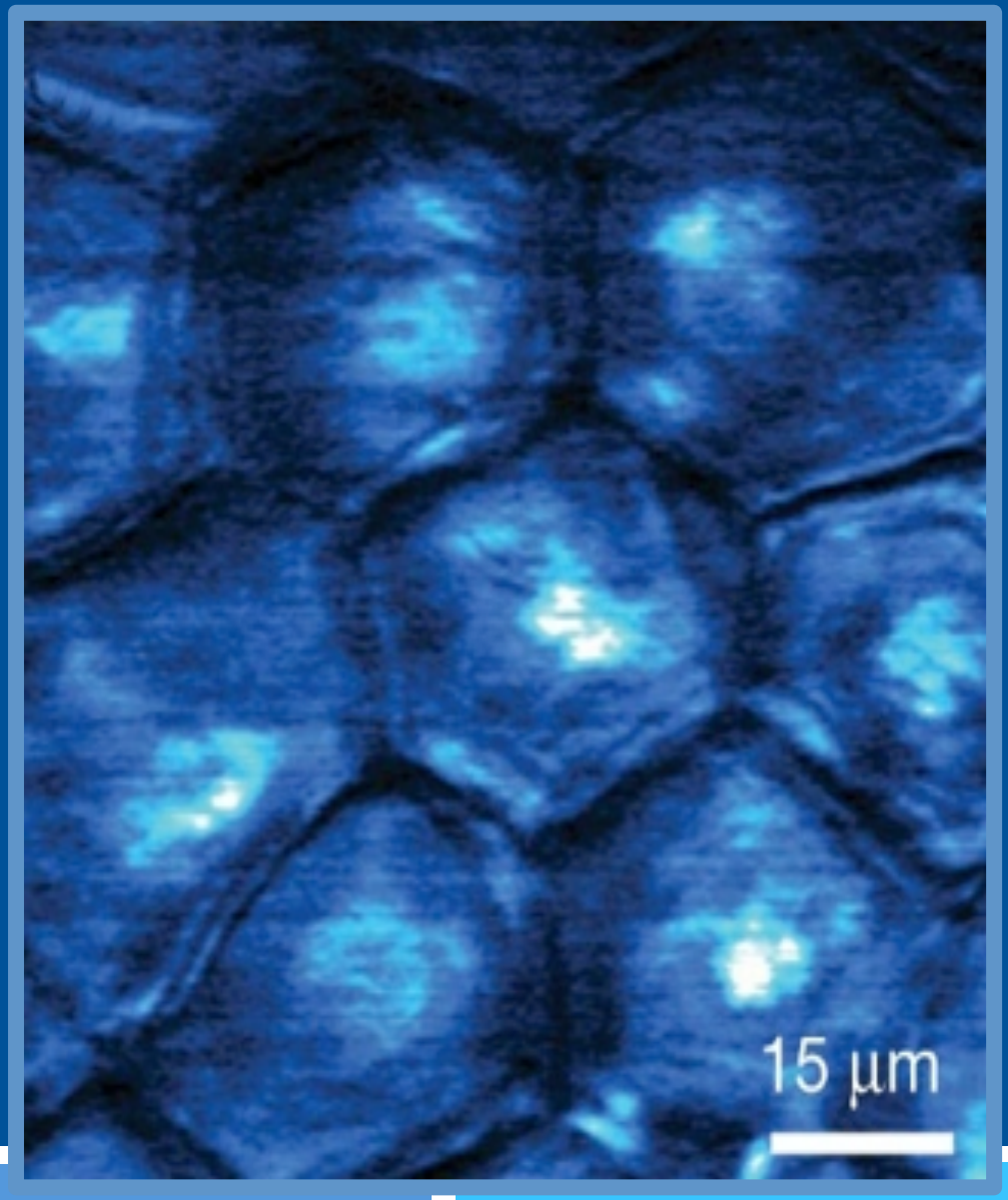

PROTEIN SORTING

Lecture 10

BIOL 266/4

2014-15

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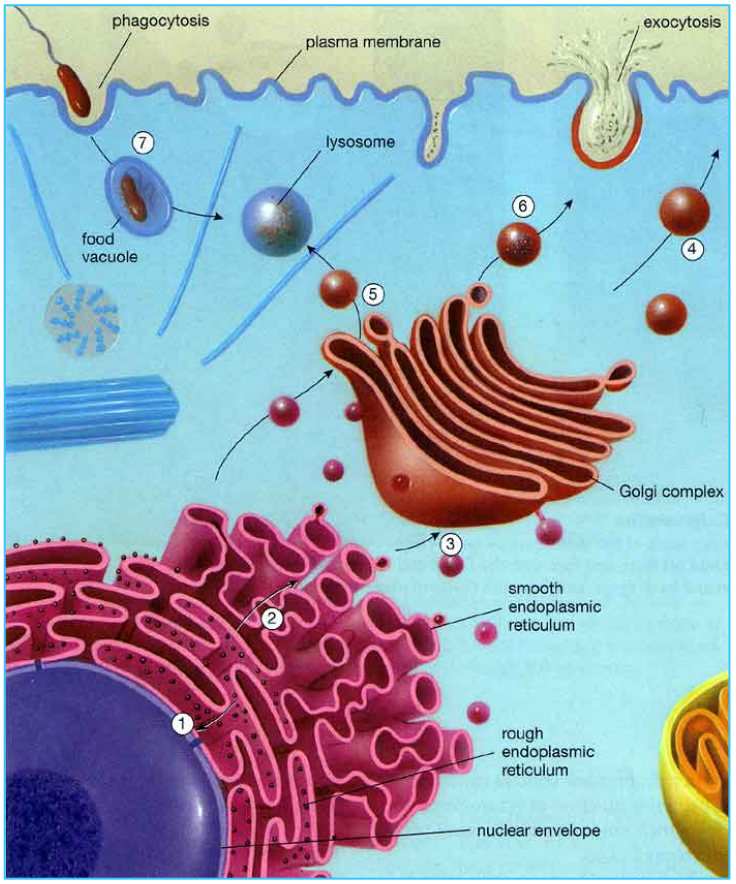
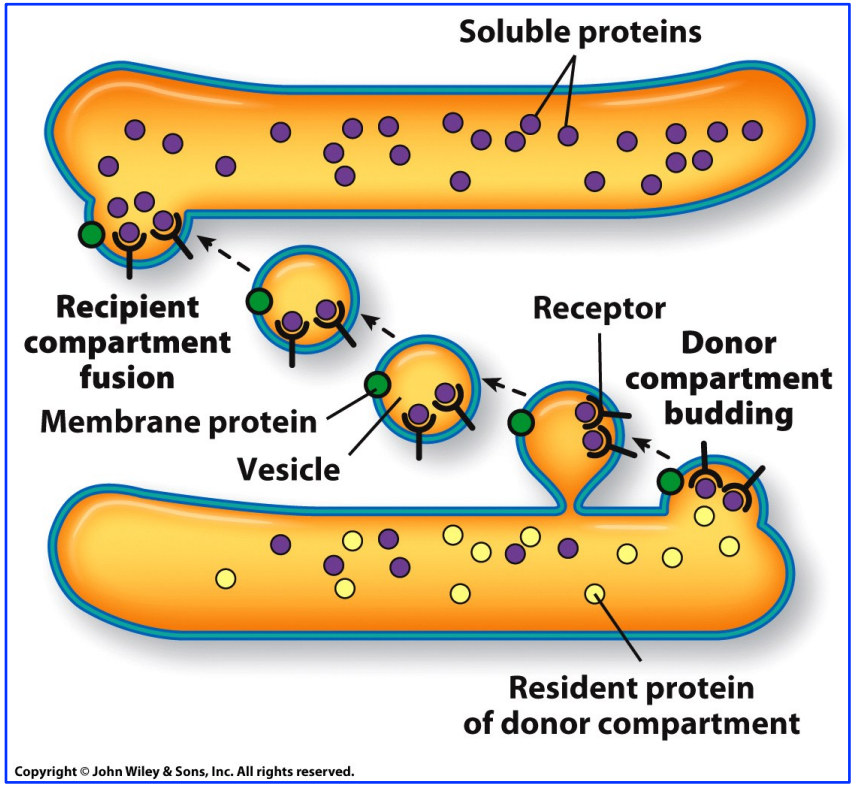
Introduction

- Membranes divide the cytoplasm of eukaryotic cells into distinct compartments.
- The **endomembrane system** includes the following organelles functioning as part of a coordinated unit.
  1. Endoplasmic reticulum
  2. Golgi complex
  3. Endosomes
  4. Lysosomes
  5. Vacuoles



Overview of the Endomembrane System

- Organelles of the endomembrane system are part of an integrated network in which materials are shuttled back and forth in membrane-bound **transport vesicles.**
- Upon reaching their destination, the vesicles fuse with the membrane of the acceptor compartment.



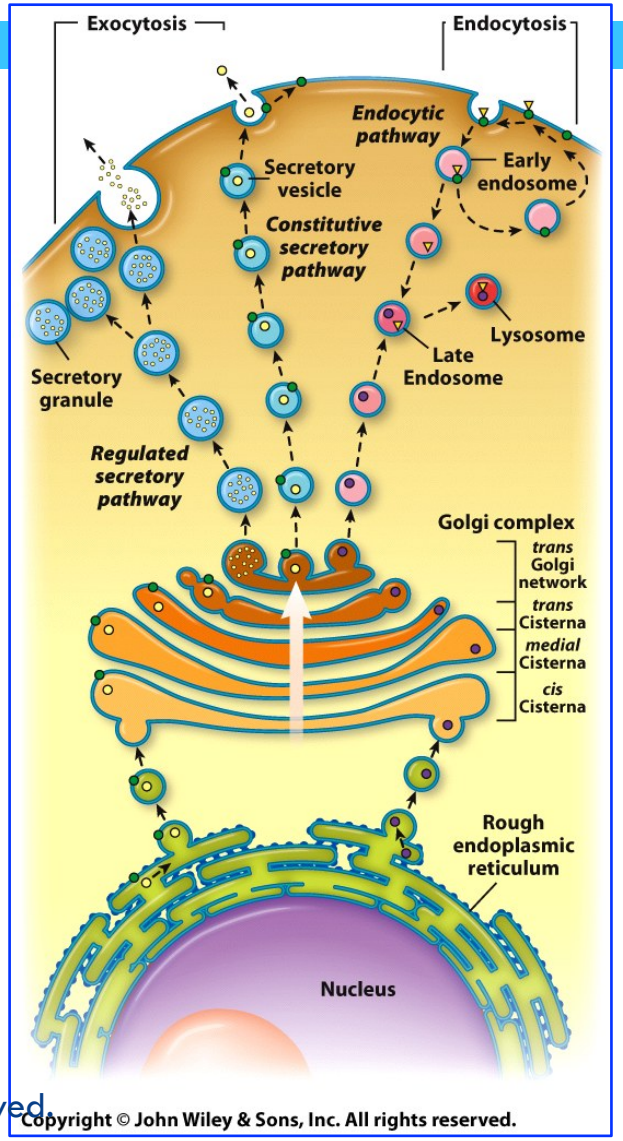
Overview of the Endomembrane System

Biosynthetic pathways

Biosynthetic pathway – synthesis in the ER, modification through the Golgi complex and transport of proteins to the plasma membrane and lysosomes etc.

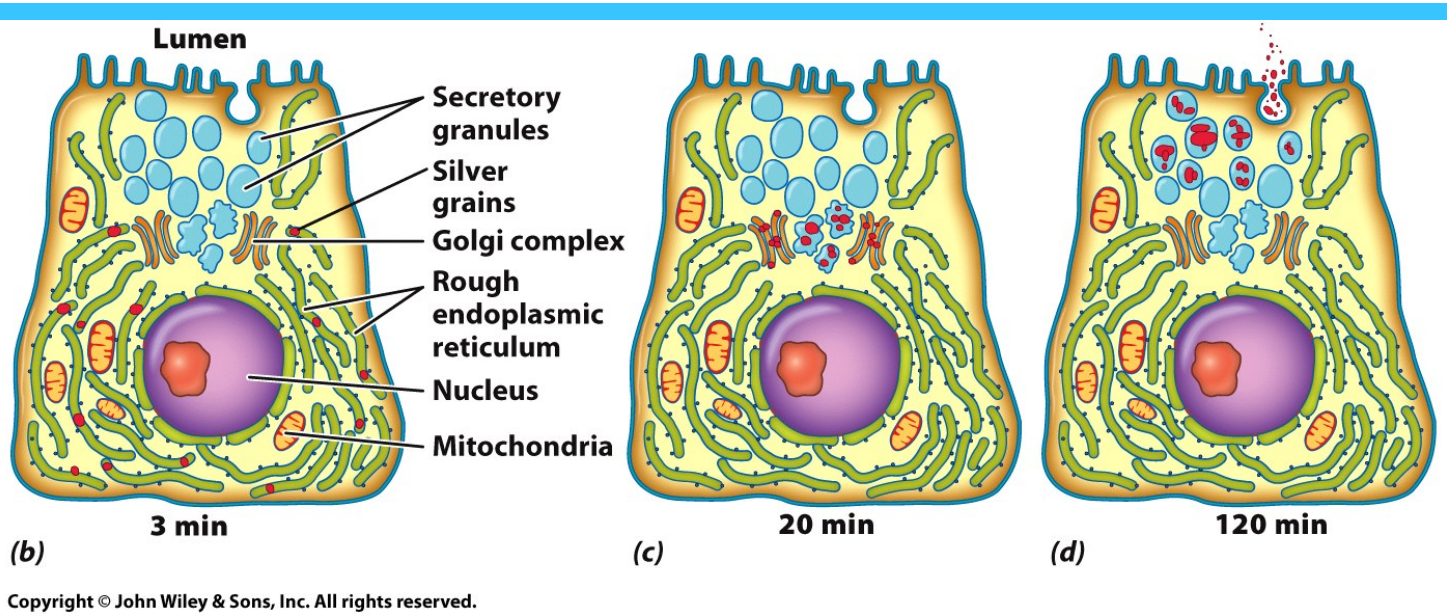
Also known as the secretory pathway – when proteins are discharged (**secreted**) from the cell.

- **Constitutive secretion** – in a continuous fashion.
- **Regulated secretion** – in response to a stimulus.



Overview of the Endomembrane System

Synthesis and transport of secretory proteins



3 minute pulse: radioactively labeled amino acid (red)

3 minute pulse: 17 minute chase

3 minute pulse: 117 minute chase

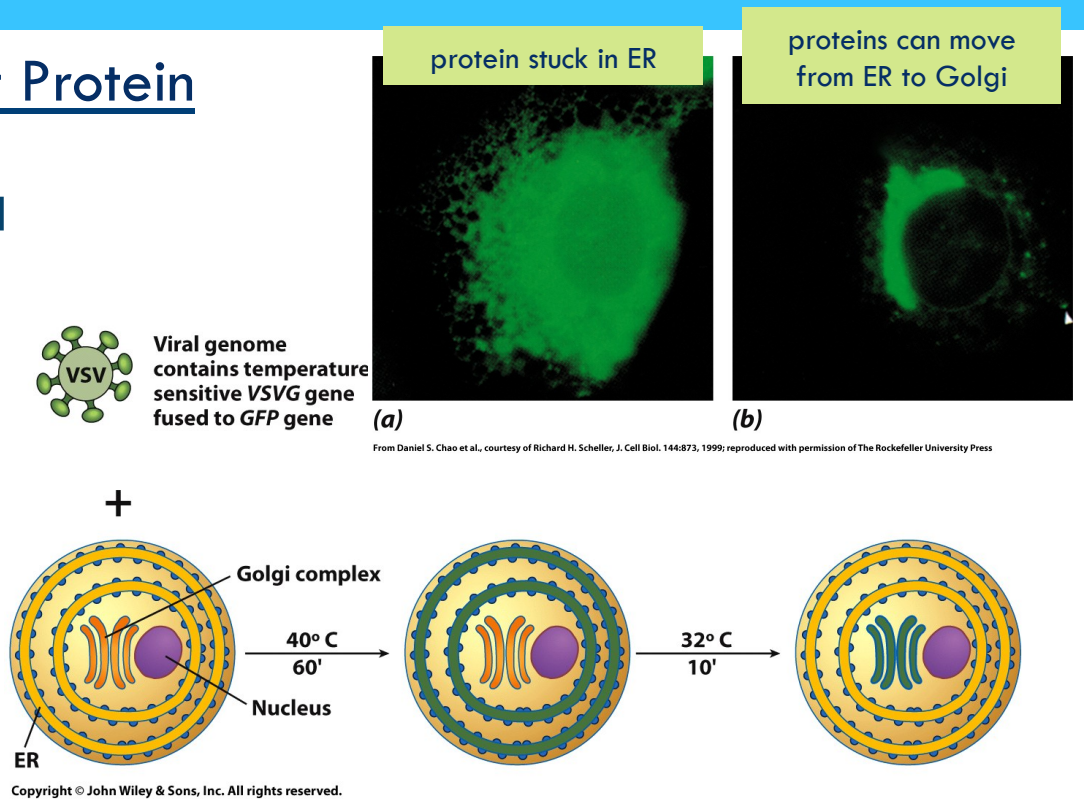
- Secretory pattern of the protein can be studied by using radioactive amino acids
- During regulated secretion, materials to be secreted are stored in large, membrane-bound **secretory granules.**

For example, secretion of digestive enzymes by pancreatic cells.

Study of Cytomembranes
GFP-based protein tracking

Use of Green Fluorescent Protein

- **Green fluorescent protein (GFP)** – a small protein isolated from jellyfish which emits green fluorescent light.
- A GFP-VSV gene chimera allows to observe the protein synthesis in the cell.
- By infecting a cell with VSV, massive amounts of VSVG protein are produced in the ER.
- Mutant VSVG is unable to leave the ER of the infected cell at 40 degrees

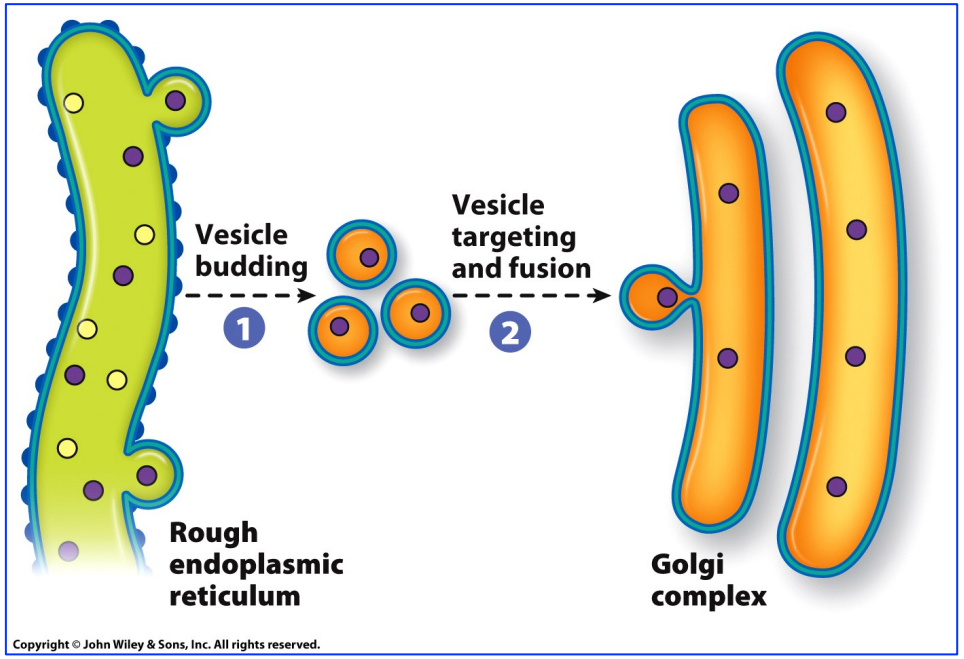


The use of green fluorescent protein (GFP) reveals the movement of proteins within a living cell.

Study of Cytomembranes
Utility of genetic mutants

Study of Mutants

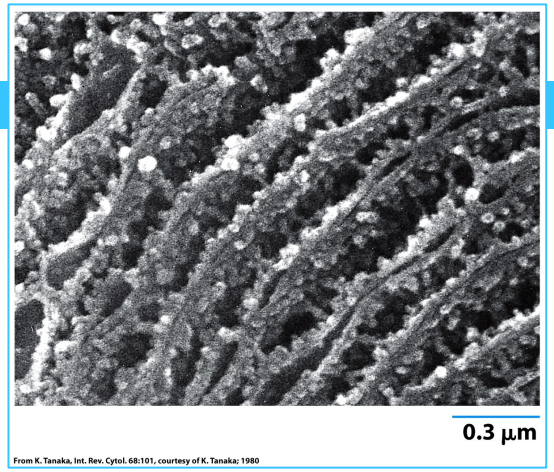
- Mutants provide insights about the function of normal gene products.
- Isolation of proteins from yeast has led to the identification of homologous proteins in mammals, pointing to the conserved nature of endomembrane systems.



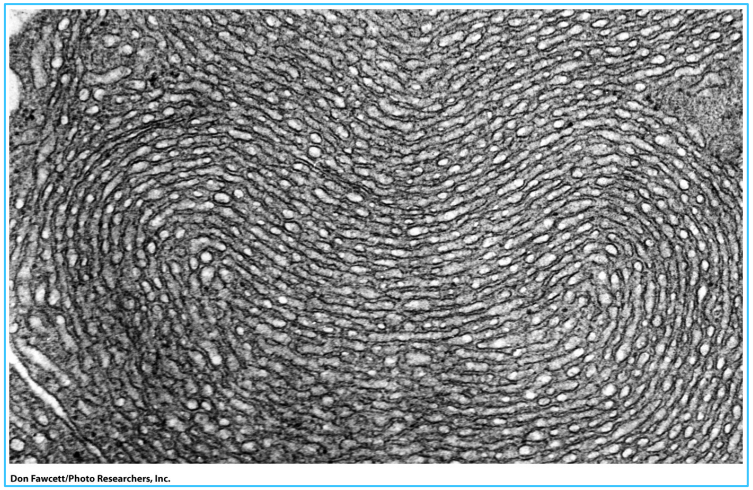
Mutations can be introduced at different steps (1 and 2)

1. The Endoplasmic Reticulum

- The **endoplasmic reticulum (ER)** comprises a network of membranes that penetrates much of the cytoplasm.
- Like other organelles, the ER is highly dynamic undergoing continual turnover and reorganization.
- Divided into two sub-compartments:
  1. **Rough endoplasmic reticulum (RER)**
  2. **Smooth endoplasmic reticulum (SER)**



Electron micrograph: rough ER

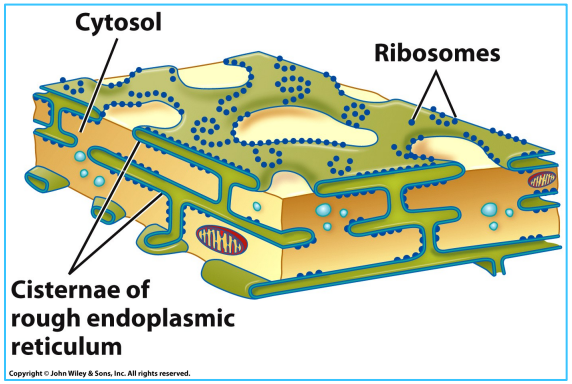


Electron micrograph: smooth ER

The Endoplasmic Reticulum

Rough Endoplasmic Reticulum (RER)

- Composed of a network of flattened sacs (**cistenae**).
- Continuous with the outer membrane of the nuclear envelope
- Has ribosomes on its cytosolic surface.
- Starting point of the biosynthetic pathway
- Site of discharge of the protein.
- Different types of cells have different ratios of the two types of ER, depending on activities of the cell.

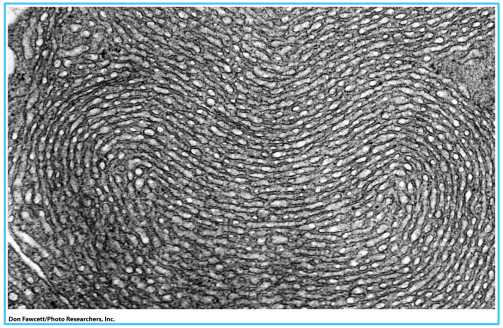


The Endoplasmic Reticulum

Smooth Endoplasmic Reticulum (SER)

- Extensively developed in a number of cell types;
- Functions include:
  - Synthesis of steroid hormones in endocrine cells of the gonad and adrenal cortex.
  - Detoxification of various organic compounds in the liver *(eg., by cytochrome P450 family)*
  - Contains a high concentration of calcium-binding proteins: sequestration of calcium ions from the cytoplasm of muscle cells.

Leydig cell (testes): extensive SER where steroid hormones are synthesized



The Endoplasmic Reticulum

- Functions of the RER

  Synthesis of Proteins on Membrane-Bound versus Free Ribosomes

  - Approximately 1/3 polypeptides encoded by the human genome are synthesized on ribosomes of RER include secretory proteins and integral membrane proteins
  - Polypeptides synthesized on "free" ribosomes include cytosolic proteins, peripheral membrane proteins, nuclear proteins, and proteins incorporated into chloroplasts, mitochondria and peroxisomes.

The Endoplasmic Reticulum

Synthesis of protein on membrane-bound ribosome

1. Synthesis of Secretory/ Lysosomal protein on Membrane-Bound Ribosomes

   - Messenger RNA binds to free ribosomes on cytosol
   - Movement through the membrane can occur as it is being synthesized (co-*translationally*) or post-translational.
   - Secretory proteins synthesized on membrane-bound ribosomes have their signal sequence recognized by a **signal recognition particle (SRP)**

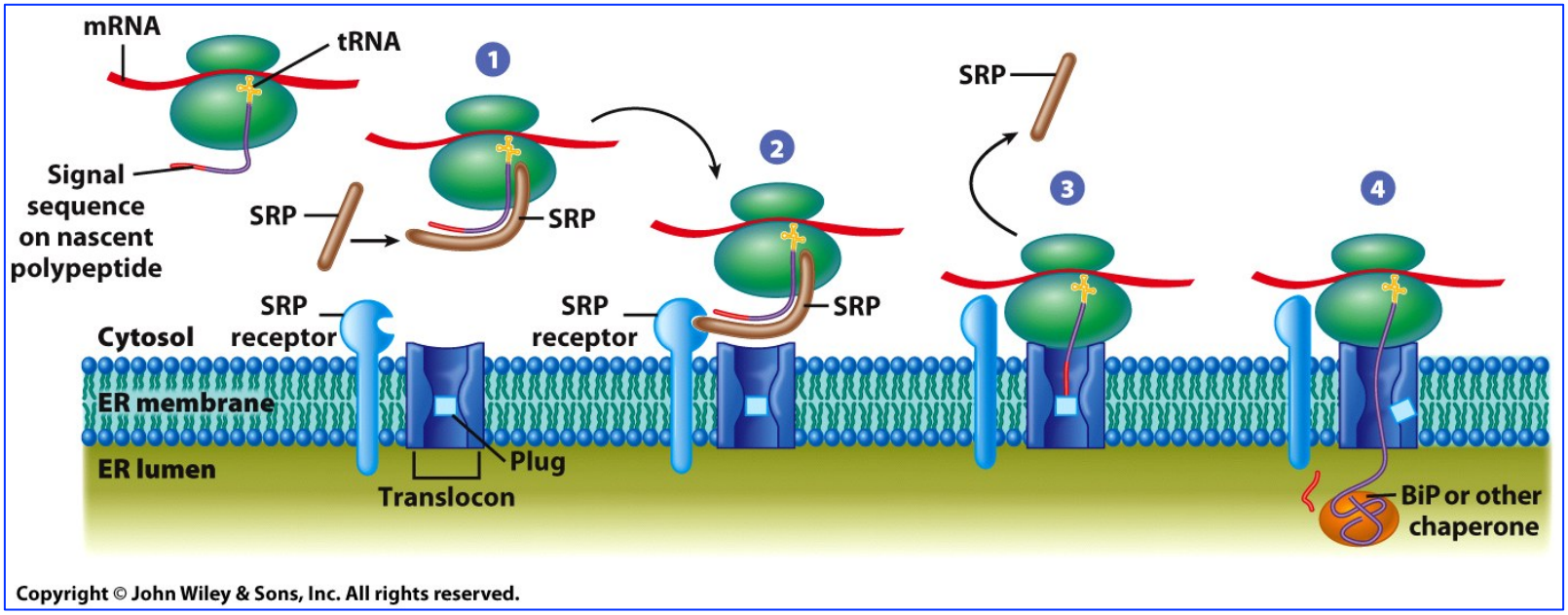
The Endoplasmic Reticulum

Synthesis of protein on membrane-bound ribosome

1. Synthesis of Secretory/ Lysosomal protein on Membrane-Bound Ribosomes
   - Binding to the ER occurs through two sequential interactions:
     1. SRP at N-terminus of the polypeptide must interact with a **SRP receptor** on the ER membrane.
     2. Ribosome interacts with the **translocon**, which is a protein-lined channel.
   - Once the SRP-ribosome-nascent peptide chain complex binds ER, SRP is released.
   - Release of SRP requires **GTP-binding proteins (G proteins)**.

The Endoplasmic Reticulum

Synthesis of protein on membrane-bound ribosome



A schematic model of the synthesis of a secretory protein (or a lysosomal enzyme) on a membrane-bound ribosome of the RER

The Endoplasmic Reticulum

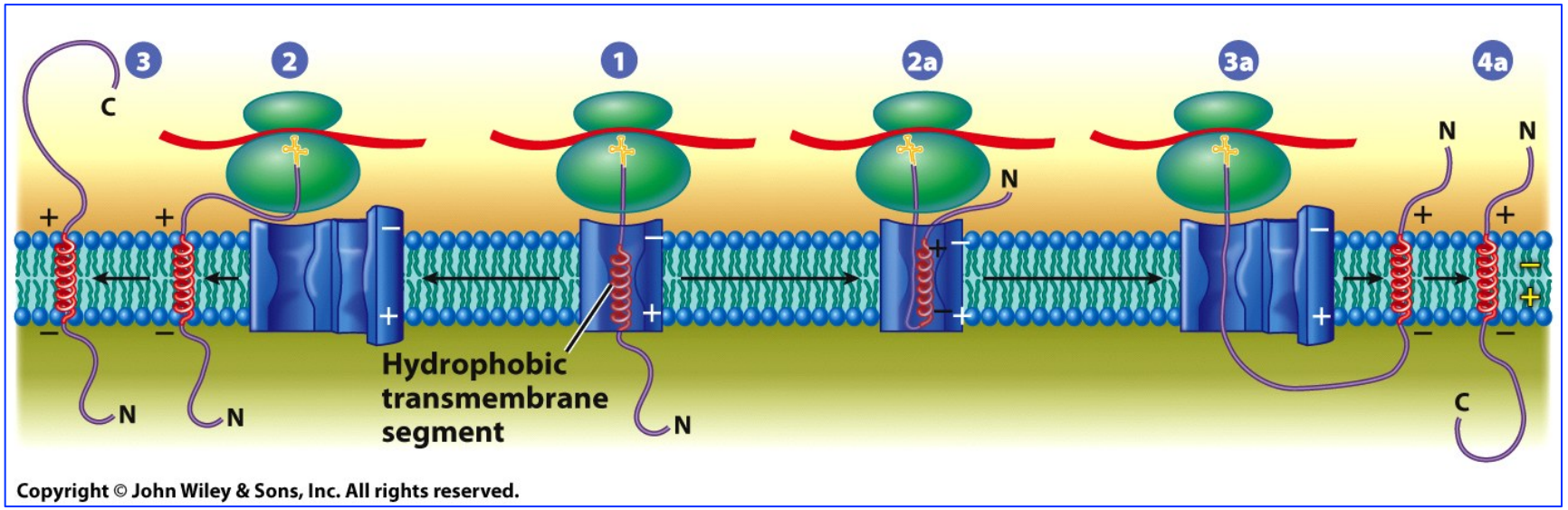
Synthesis of protein on membrane-bound ribosome

2. Synthesis of Integral Membrane Proteins on Membrane-Bound Ribosomes

- Integral proteins contain hydrophobic trans-membrane segments that interfere with transfer into the RER lumen.
- The two sequential events: SRP-SRP receptor binding and ribosome-translocon binding occurs similarly as for the secretory proteins.
- Translocon assists in the proper orientation of transmembrane sequences.
- The arrangement within the membrane is determined by the orientation of the first trans-membrane segment inserted.

The Endoplasmic Reticulum

Synthesis of protein on membrane-bound ribosome



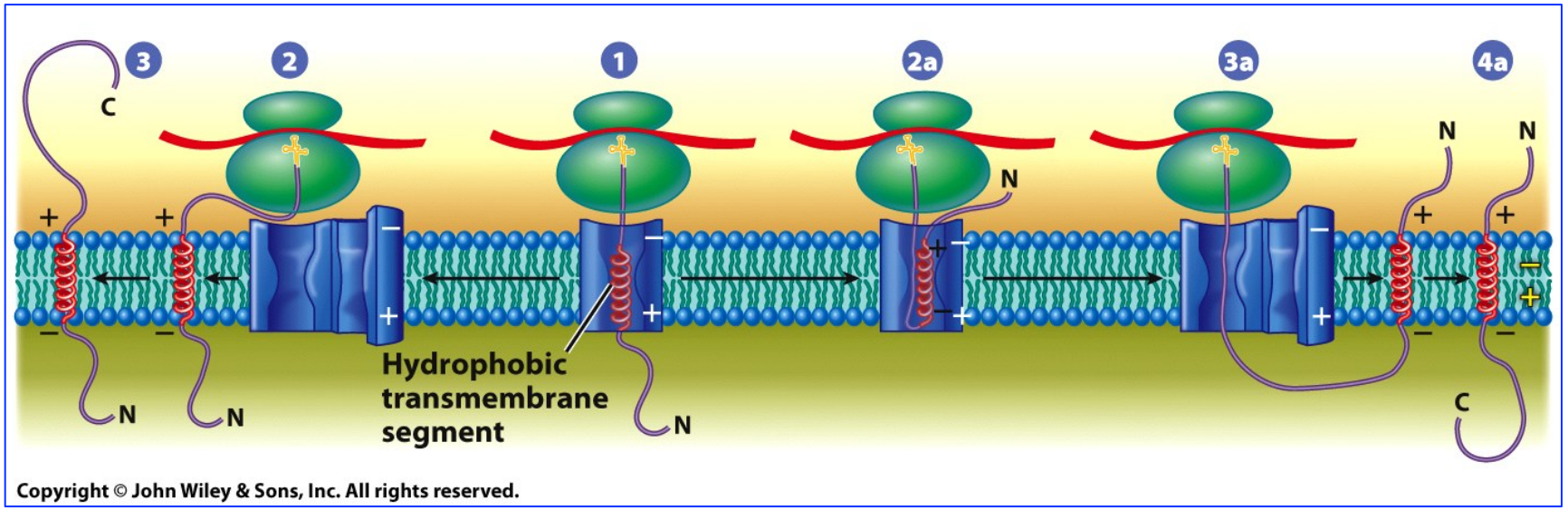
A schematic model for the synthesis of an integral membrane protein

Steps 1-3: N terminus is in the lumen and C terminus in the cytosol

Entry of the hydrophobic transmembrane sequence into the pore blocks further translocation of the polypeptide → Lateral gate of the transposon opens and the transmembrane segment is expelled in the lipid bilayer

The Endoplasmic Reticulum

Synthesis of protein on membrane-bound ribosome



A schematic model for the synthesis of an integral membrane protein

Steps 1-4a: C terminus is in the lumen and N terminus in the cytosol

Translocon assists in the proper orientation of transmembrane sequences→ difference in charge between phospholipids of the cytosolic and luminal leaflets of the bilayer is thought to play a role in the protein orientation.

The Endoplasmic Reticulum

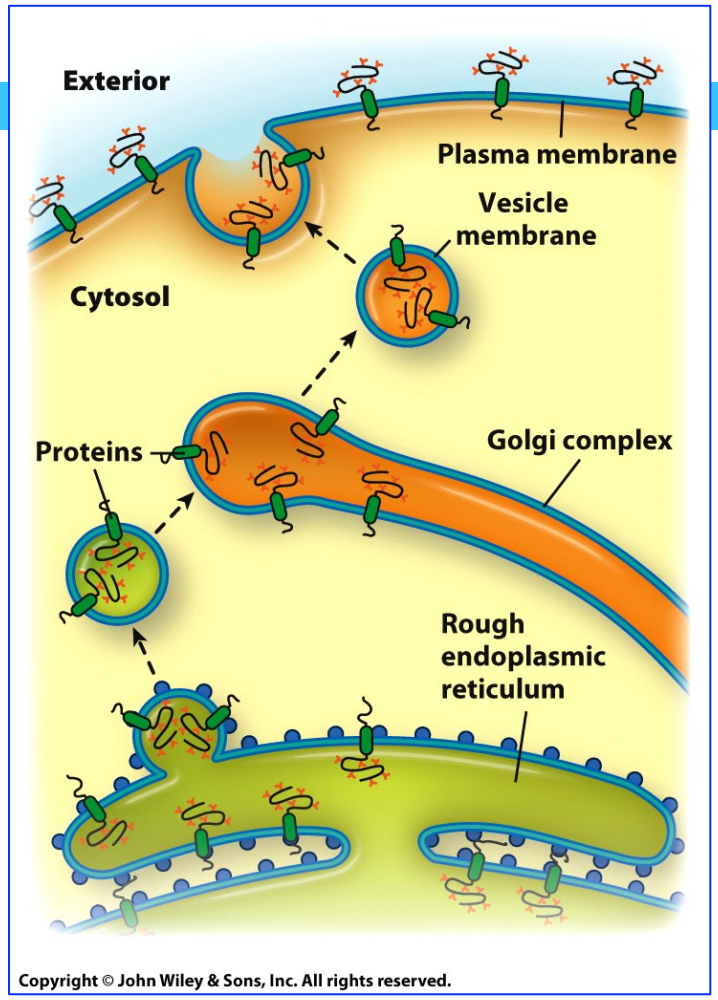
Synthesis of protein on membrane-bound ribosome

Processing of Newly Synthesized Proteins in the ER

- Upon entering the RER lumen, the signal sequence is cleaved by a **signal peptidase.**
- Carbohydrates are added by the enzyme *oligosaccharyltransferase.*
- The RER lumen is packed with chaperones to assist in folding, and also contains *protein disulfide isomerase* to add disulfide bonds to cysteines.

Membrane Biosynthesis in the ER

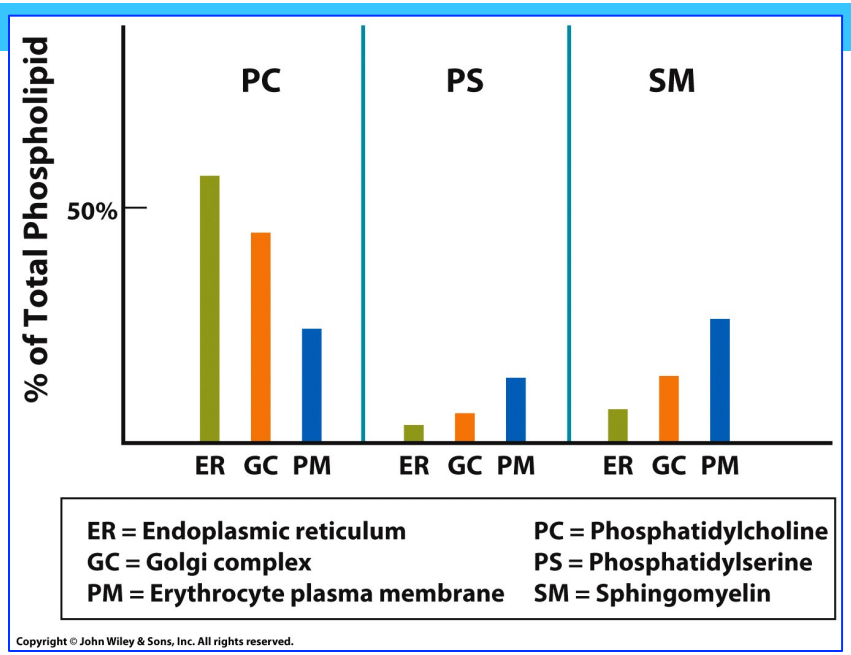
- Membranes arise from pre-existing membranes.
- Lipids are inserted into existing membranes.
- As the membrane moves from one compartment to the next, its proteins and lipids are modified.
- Membrane asymmetry is established initially and maintained during trafficking.



Maintenance of membrane asymmetry

The Endoplasmic Reticulum

Modifying the lipid composition of membranes



Histogram indicating percentage of each of three phospholipids in three different cellular membranes

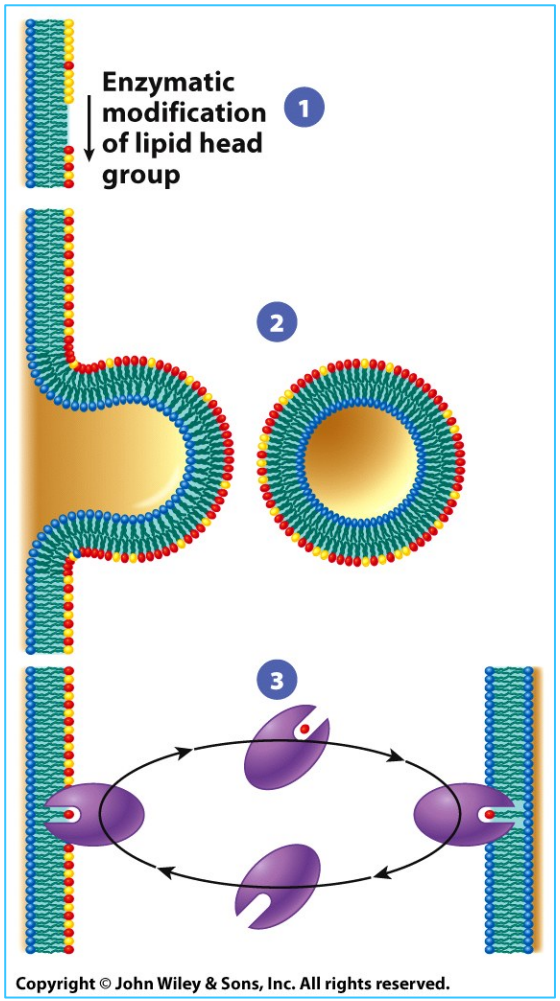
- Most membrane lipids synthesized within the ER except sphingomyelin and glycolipids and some unique lipids of mitochondria and chloroplasts.
- Newly synthesized phospholipids are inserted into half of bilayer facing the cytosol, and then flipped into opposite leaflet by *flippases*.
- There are enzymes that modify lipids already present within a membrane.

The Endoplasmic Reticulum

Modifying the lipid composition of membranes

Contributing factors to variation of organelle lipid composition

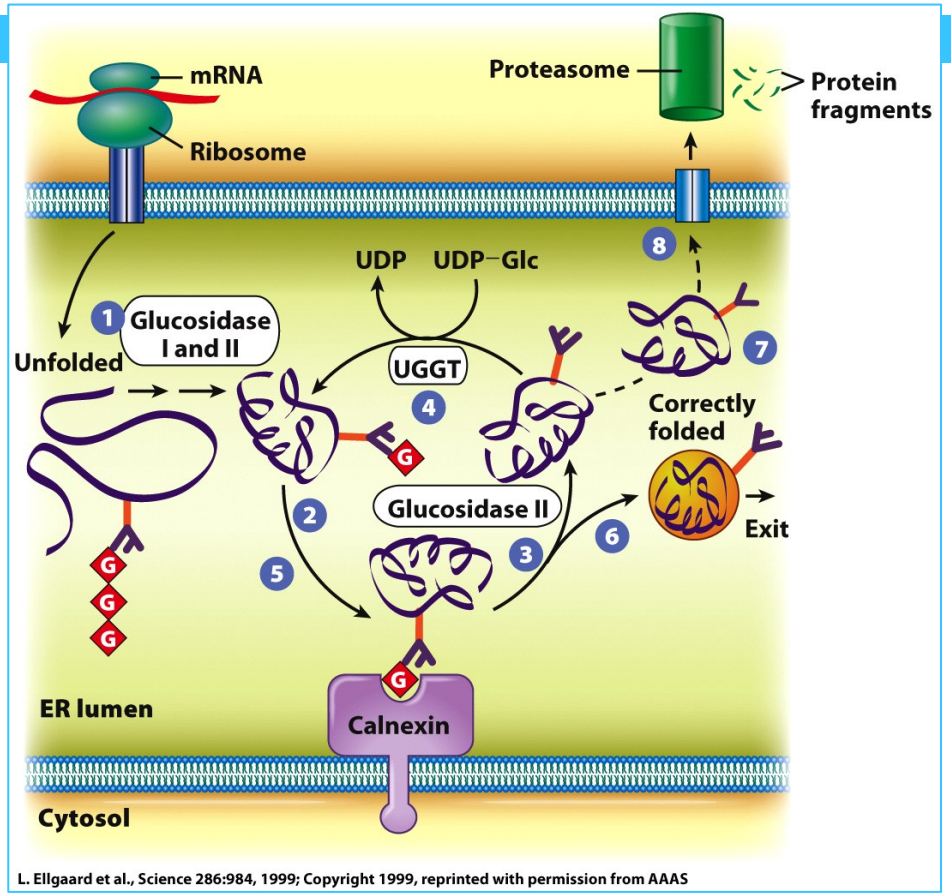
1. Enzymatic modification (head group): Organelle-specific enzymes for lipid conversion.
2. Modification during vesicle formation: Inclusion/exclusion process
3. Modification by phospholipid transfer proteins: **Lipid-transfer proteins** that bind and transport lipids without the use of vesicle transport



The Endoplasmic Reticulum

Quality control: ensuring misfolded proteins do not proceed forward.

- Soon after transfer to the polypeptide, the oligosaccharide is gradually modified.
- A glycoprotein goes through a system of **quality control** to determine its fitness for a specific compartment.
- Misfolded proteins are tagged by a terminal glucose and recognized by chaperones for refolding.
- If the protein does not fold correctly, it is translocated to the cytosol and destroyed.

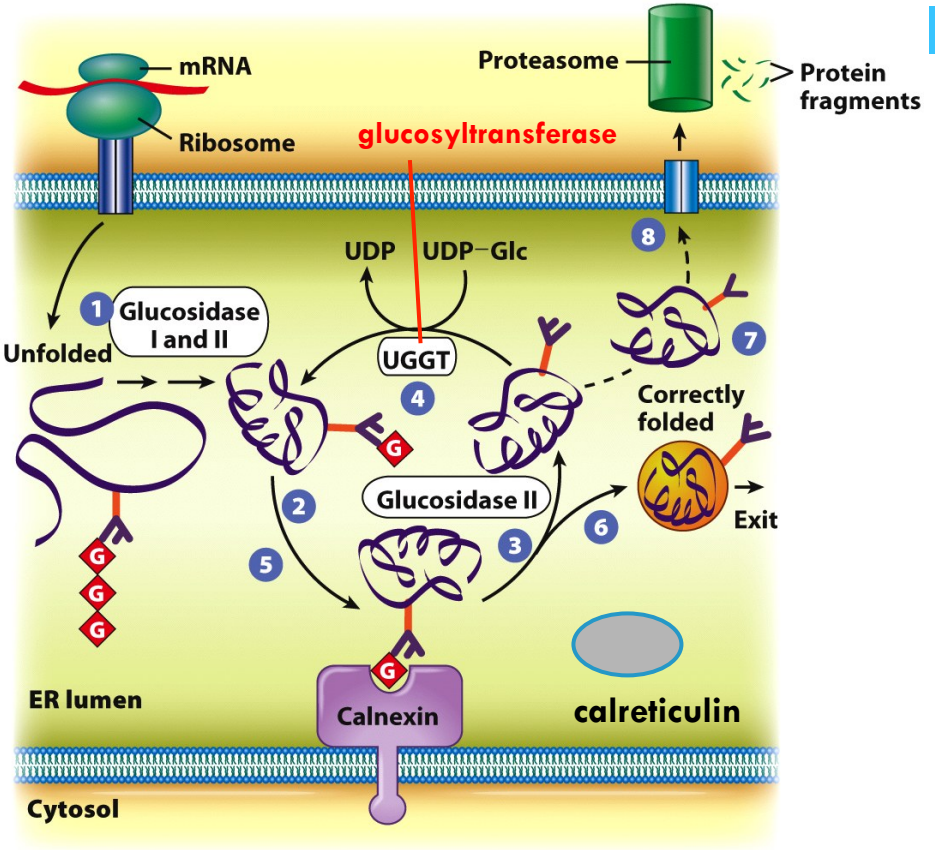


Quality control: ensuring that misfolded proteins do not proceed forward.

The Endoplasmic Reticulum

Mechanisms that ensure the destruction of misfolded proteins

- Misfolded proteins are not destroyed in the ER; instead they are transported into the cytosol where they are destroyed in proteasomes.
- This process is called *ER-associated degradation (ERAD)*, and ensures the misfolded proteins do not reach the cell surface.



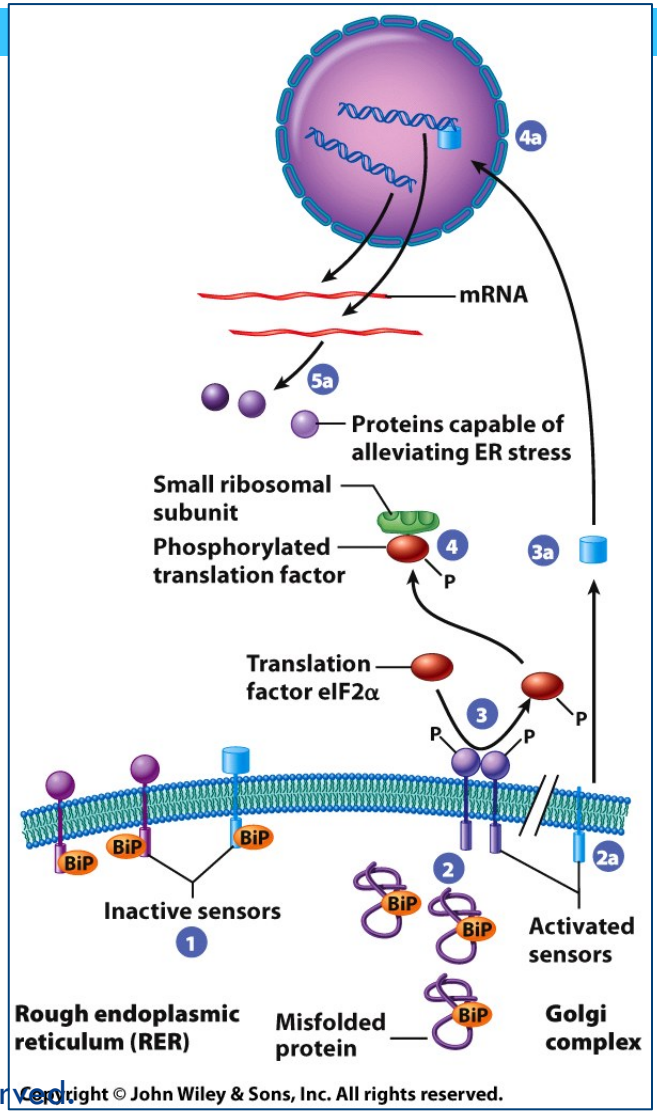
L. Ellgaard et al., Science 286:984, 1999; Copyright 1999, reprinted with permission from AAAS

Quality control: ensuring that misfolded proteins do not proceed forward.

A model of the mammalian unfolded protein response (UPR)

Destruction of misfolded proteins

- Accumulation of misfolded proteins triggers the **unfolded protein response (UPR)**.
- Sensors in the ER are kept inactive by the chaperone BiP but if misfolded proteins are accumulated, BiP molecules are incapable of inhibiting the sensors.
- Activated sensors send signals to trigger proteins involved in destruction of misfolded proteins.



Put your thinking cap on….

1. Which of the following cells would you expect to be engaged most heavily in bulk-phase endocytosis and exocytosis:

(a) an erythrocyte

(b) a pancreatic acinar cell

(c) a skeletal muscle cell?

And Why?

2. If you were to add a drug that interfered with the ability of ribosomes to bind to mRNA, what effect would this be expected to have on the structure of the RER?

Some interesting links

https://www.youtube.com/watch?v=eH5k8XYKycs
https://www.youtube.com/watch?v=IyBvn2iqI2M
https://www.youtube.com/watch?v=4qf1BSXn_tk