Membranous organelles:

Lysosomes and Peroxisomes

Nonmembranous organelles: Ribosomes

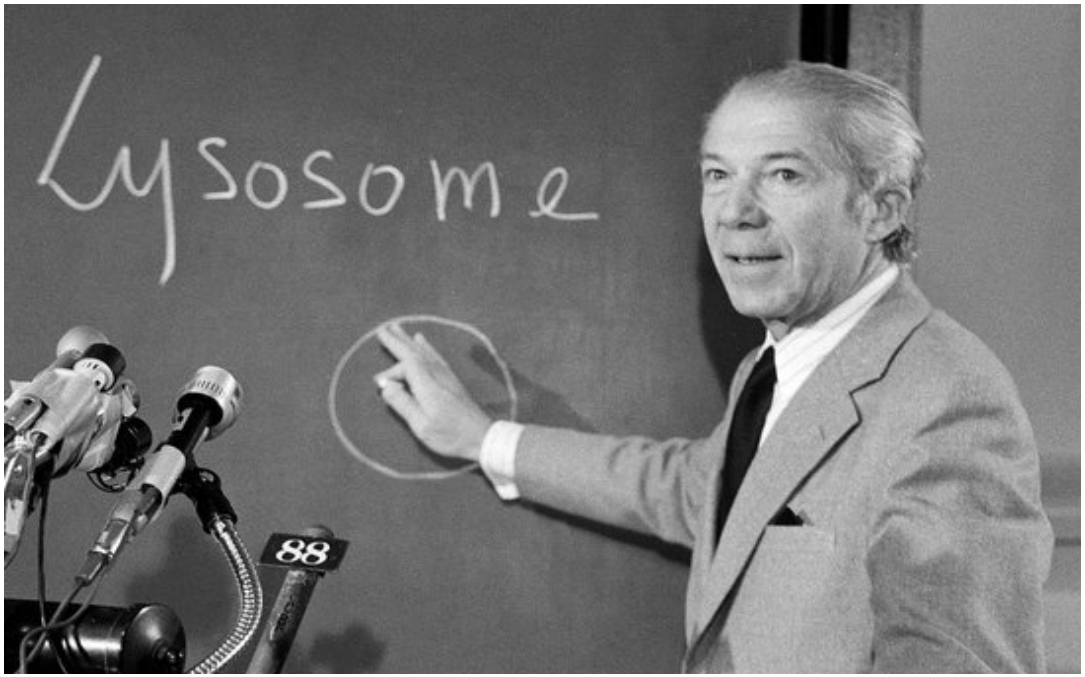
"Cytology, Histology and Embryology"
Stefan Trifonov MD, PhD
27.03.2019

1. Lysosomes – biogenesis, structure and functions:
    a. Primary lysosomes.
    b. Secondary lysosomes.
2. Proteasomes.
3. Peroxisomes – biogenesis, structure and functions.
4. Ribosomes – structure and role in protein synthesis.

Lysosomes

- The term **lysosome** is derived from the Greek words *lysis*, “a loosing” and *soma*, “body”.

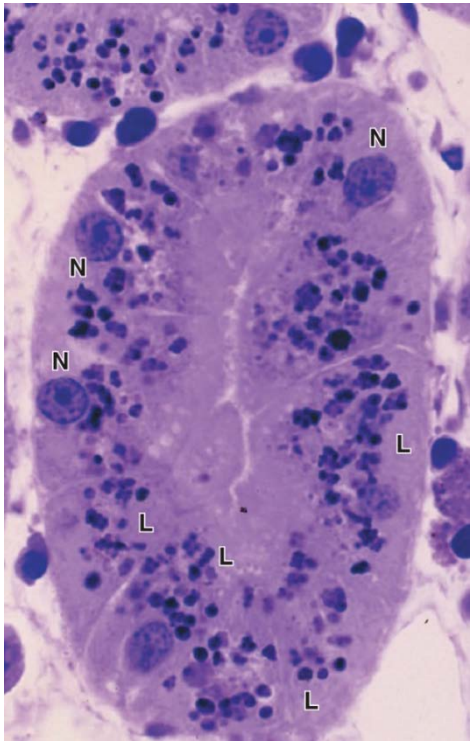
- They were discovered and named by Belgian biologist **Christian de Duve in 1955**, who eventually received the Nobel Prize in Physiology or Medicine in 1974.

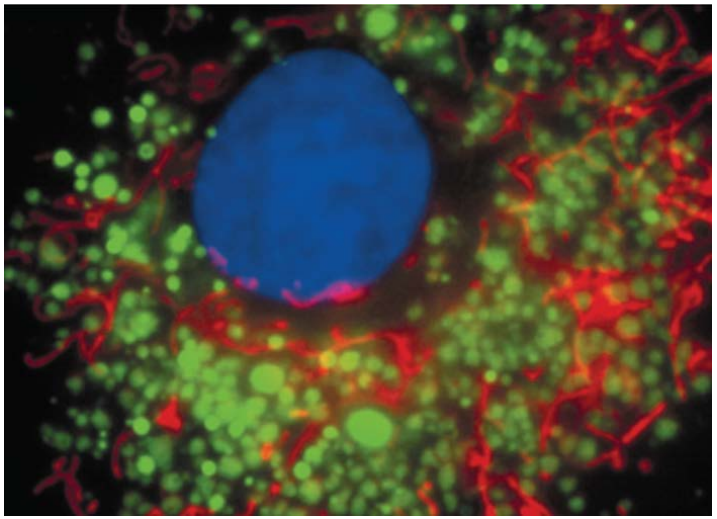


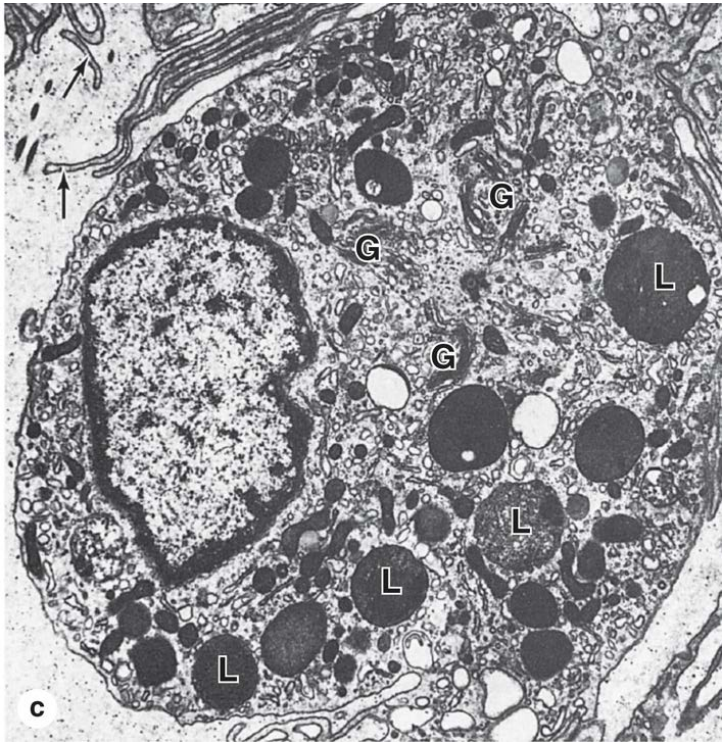
Christian de Duve
1917 – 2013

Lysosomes

- Lysosomes are membrane-bound cell organelles found in all animal cells (except in red blood cells).
- The size of lysosomes varies from 0.1 – 1.2 µm.
- Lysosomes are not well shown on HE-stained cells but can be visualized by light microscopy after staining with toluidine blue and after special histochemical procedures to demonstrate lysosomal enzymes.

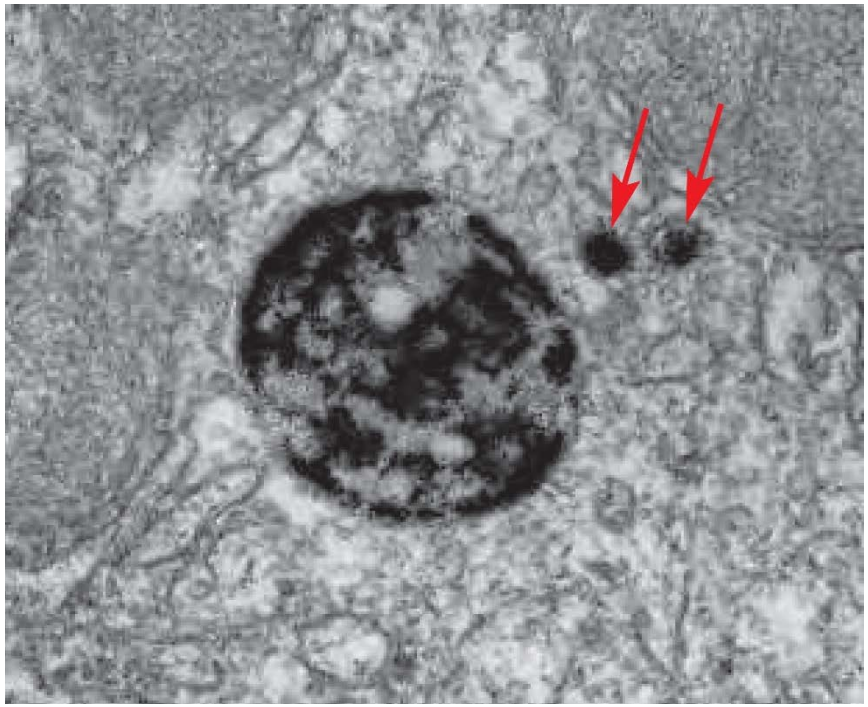


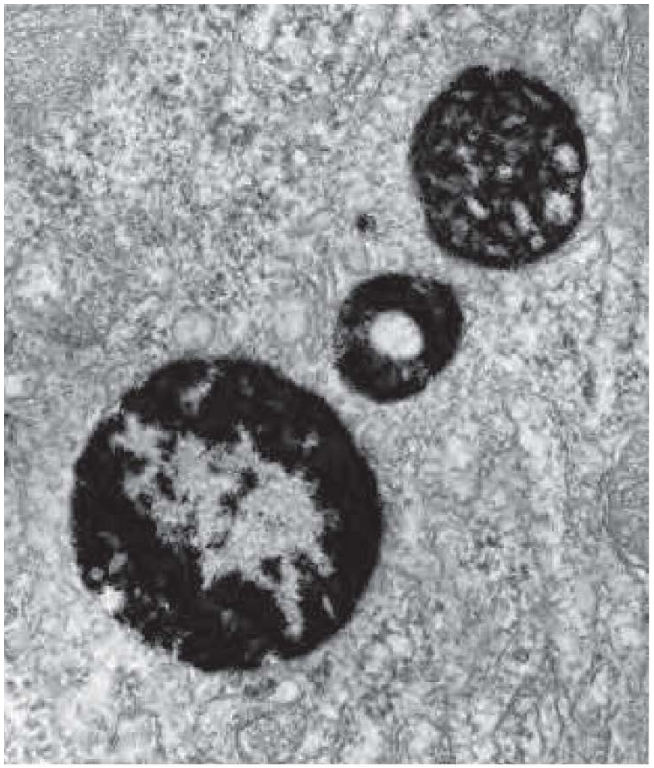




Lysosomes are Heterogeneous

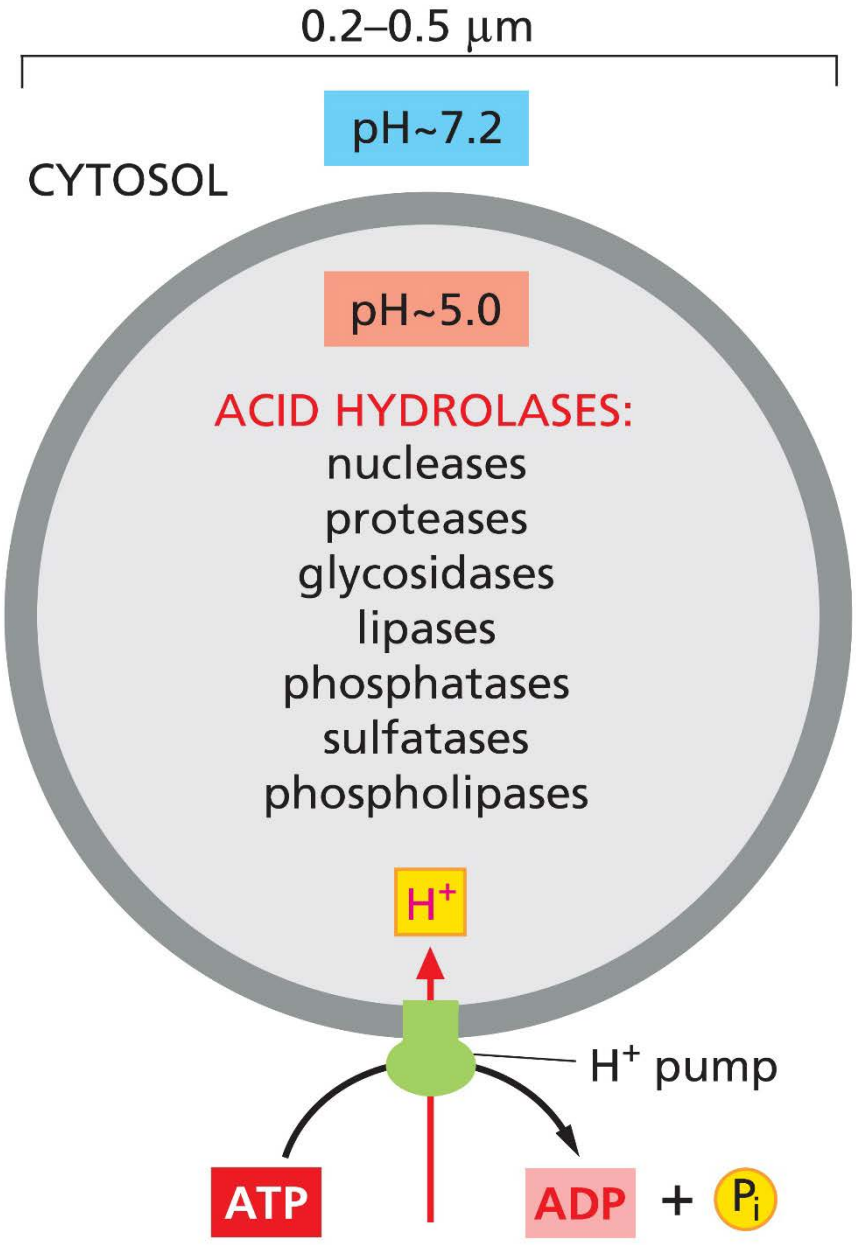
- Histochemical visualization of lysosomes. The cells are stained to reveal the location of **acid phosphatase**, a marker enzyme for lysosomes.





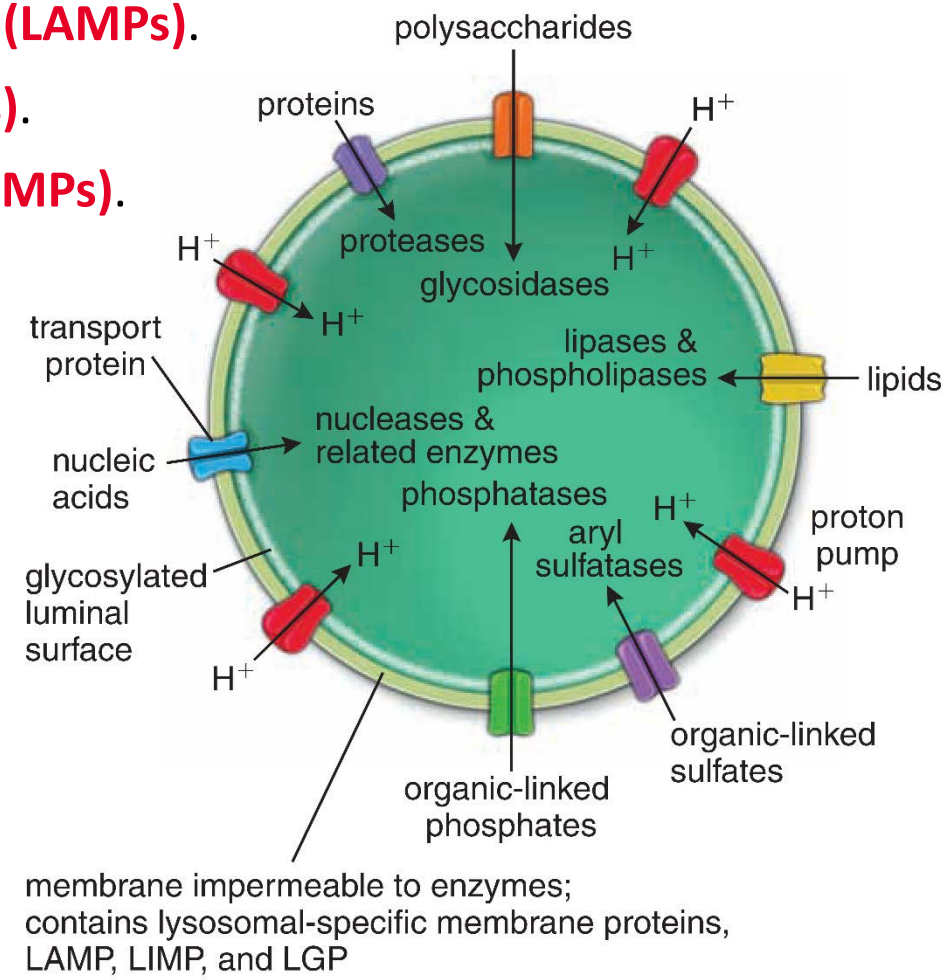
Lysosomes are the Principal Sites of Intracellular Digestion

- Lysosomes contain about 40 types of hydrolytic enzymes (**acid hydrolases**), including:
  - ✓ Proteases.
  - ✓ Nucleases.
  - ✓ Glycosidases.
  - ✓ Lipases.
  - ✓ Phospholipases.
  - ✓ Phosphatases.
  - ✓ Sulfatases.
- They need to be activated by **proteolytic cleavage**.
- Acid hydrolases work best at **acidic pH from 4.5 to 5**.



Lysosomes Have a Unique Surrounding Membrane

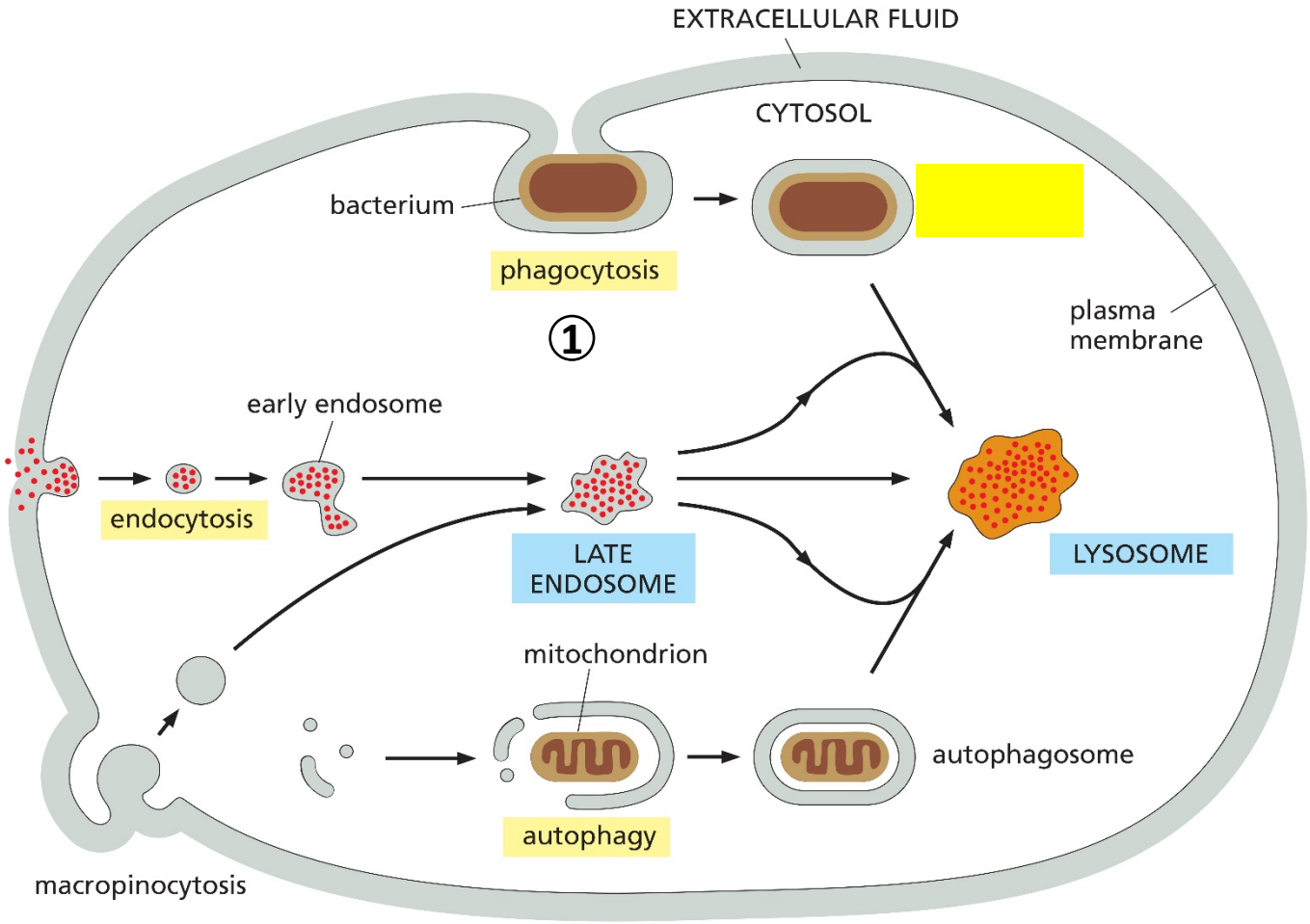
- It has an unusual phospholipid structure that contains cholesterol and a unique lipid called **lysobisphosphatidic acid**.
- The structural lysosomal membrane proteins are classified into:
  - ✓ **Lysosome-associated membrane proteins (LAMPs)**.
  - ✓ **Lysosomal membrane glycoproteins (LGPs)**.
  - ✓ **Lysosomal integral membrane proteins (LIMPs)**.
- The lysosomal membrane also contains **transport proteins**.
- A ***vacuolar H^+ ATPase*** in the lysosome membrane uses the energy of ATP hydrolysis to pump H^+ into the lysosome, maintaining the acidic pH.



Multiple Pathways Deliver Materials to Lysosomes

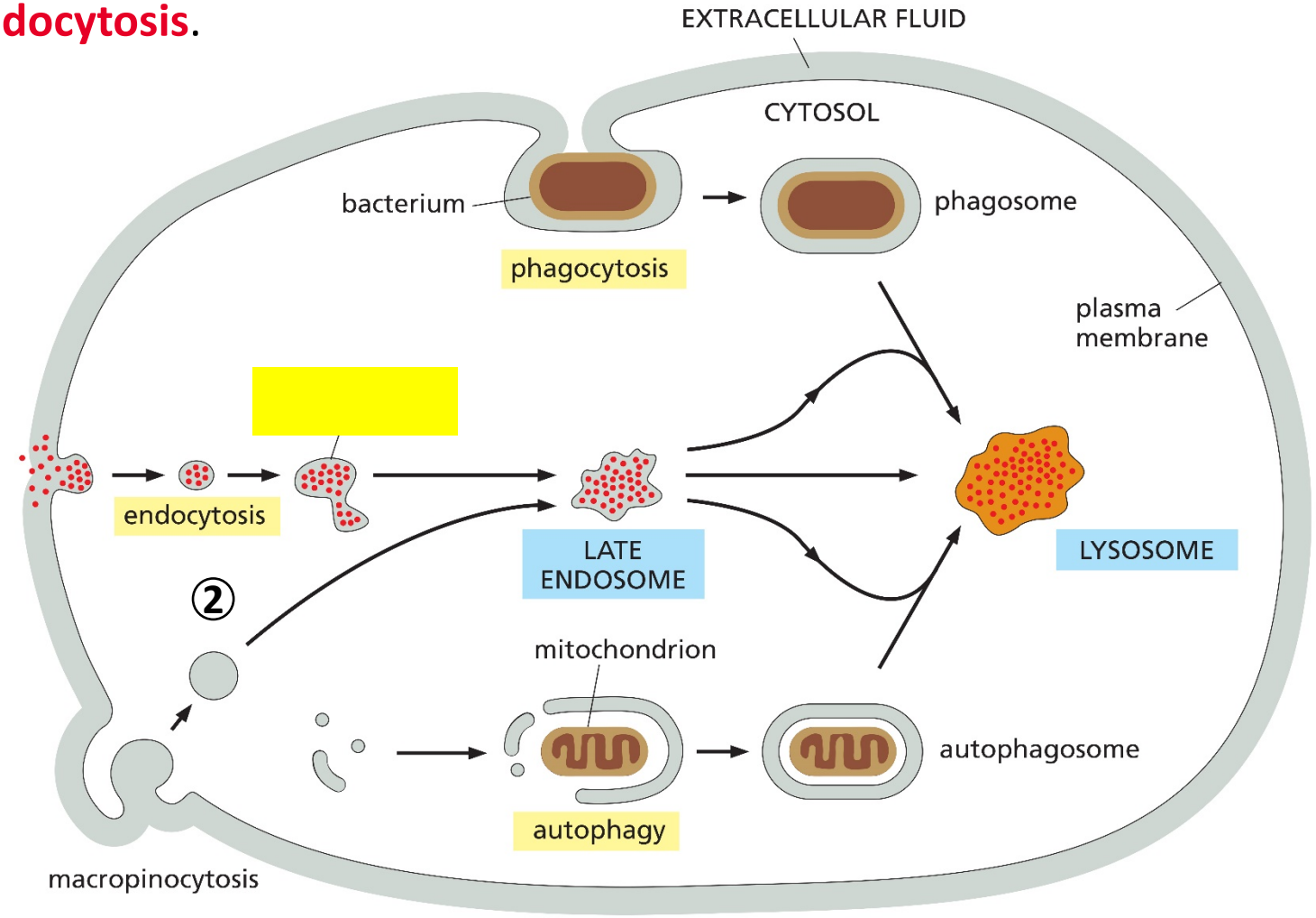
- Lysosomes are meeting places where several streams of intracellular traffic converge.
- Three different pathways deliver material for intracellular digestion in lysosomes.

1. **Extracellular large particles** such as bacteria, cell debris, and other foreign materials are engulfed in the process of **phagocytosis**.



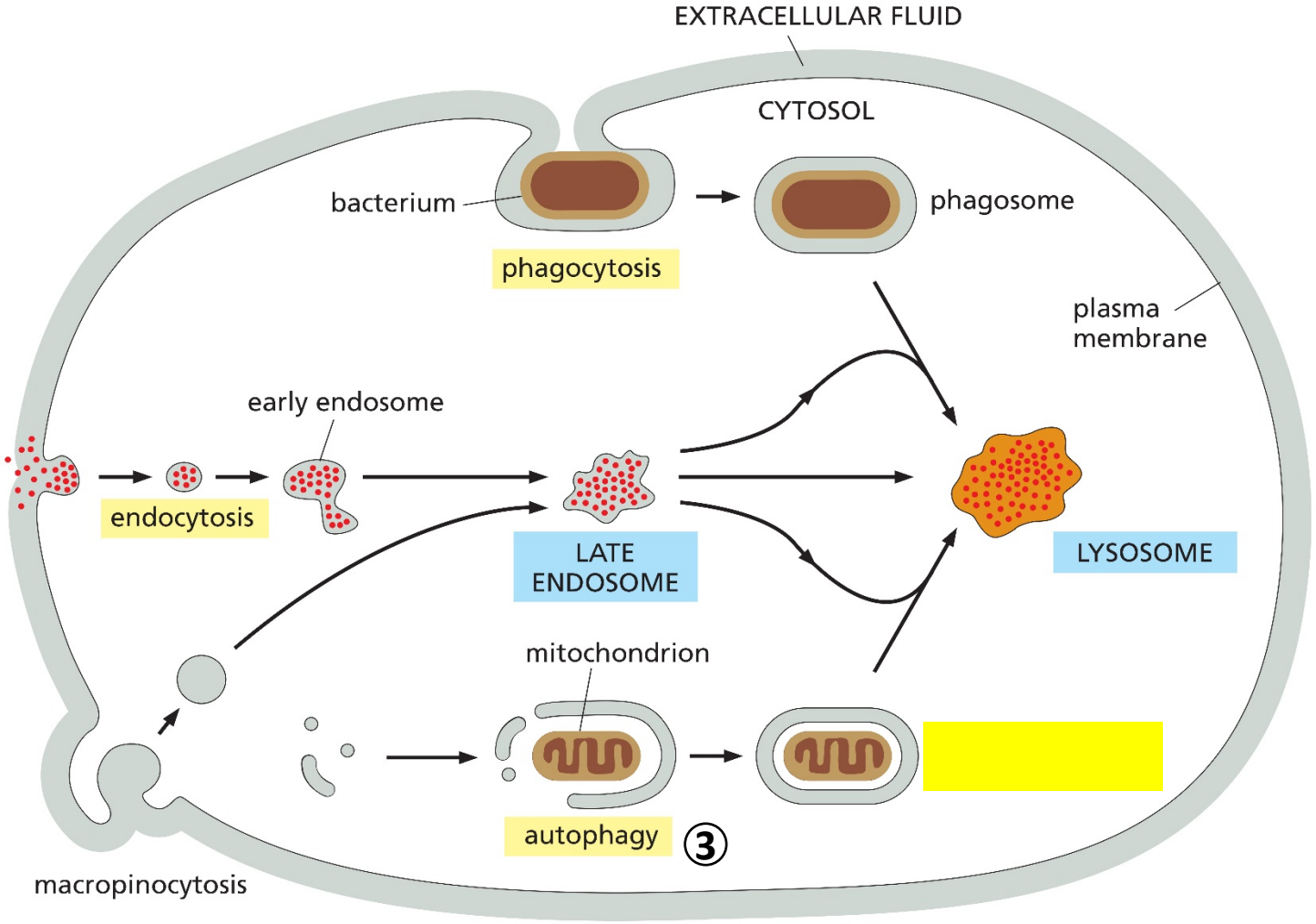
Multiple Pathways Deliver Materials to Lysosomes

2. **Extracellular small particles** such as extracellular proteins, plasma membrane proteins, and ligand-receptor complexes are internalized by **pinocytosis** and **receptor-mediated endocytosis**.



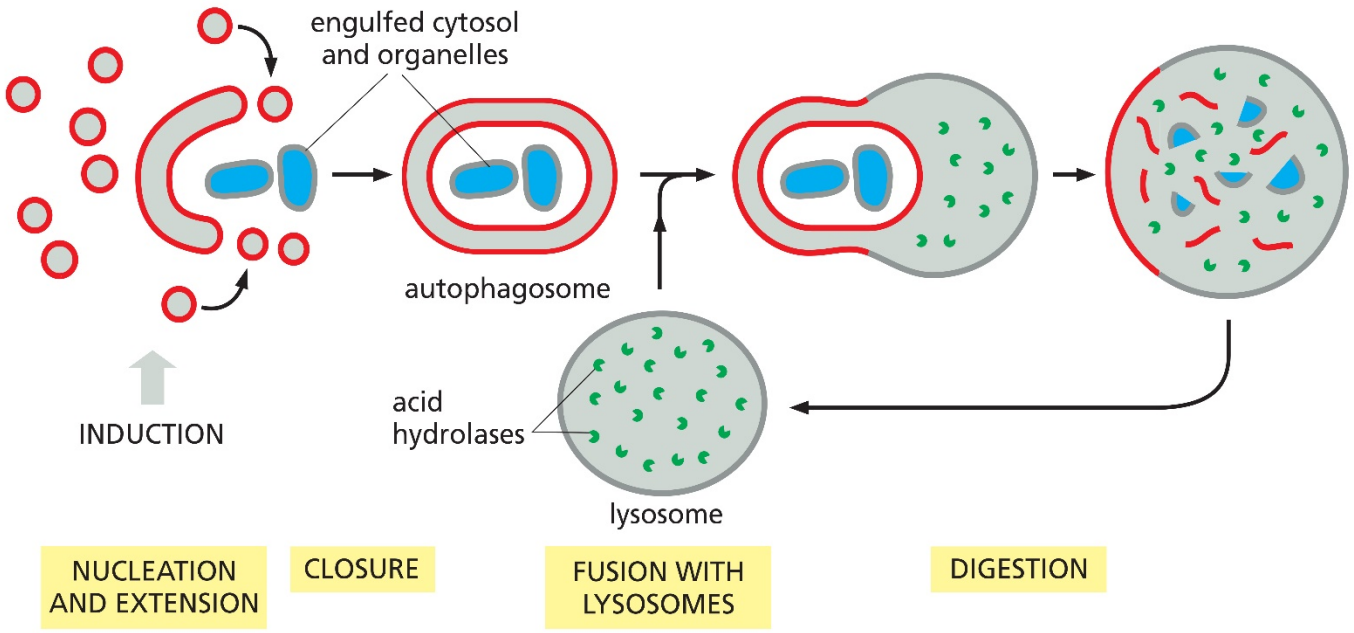
Multiple Pathways Deliver Materials to Lysosomes

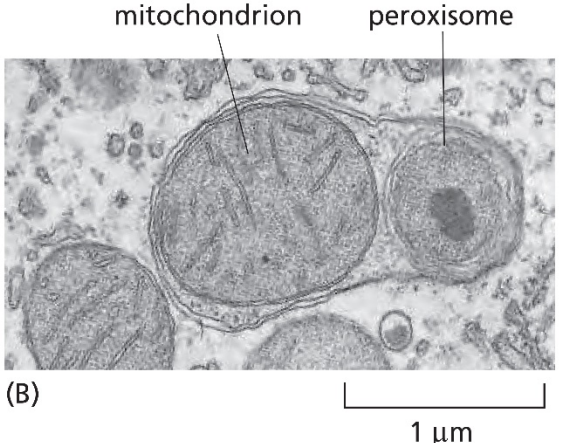
3. **Intracellular particles** such as entire organelles, cytoplasmic proteins, and other cellular components are isolated from the cytoplasmic matrix by endoplasmic reticulum membranes, transported to lysosomes and degraded. This process is called **autophagy.**



Autophagy Degrades Unwanted Proteins and Organelles

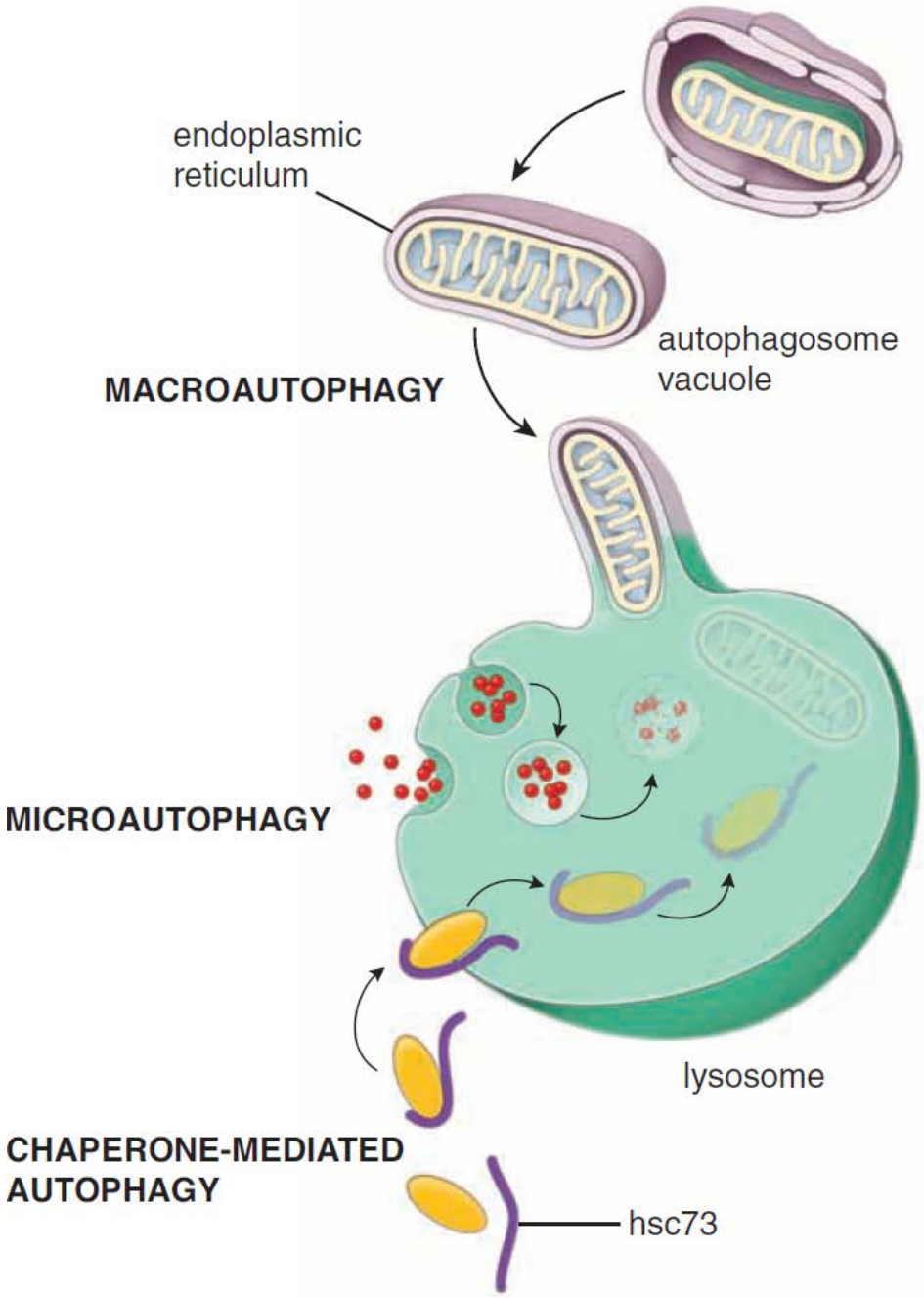
1. Induction by activation and inactivation of **signaling molecules** (mTOR complex 1).
2. **Nucleation** and extension of a delimiting membrane into a crescent-shaped cup.
3. **Closure** of the membrane cup around the target to form a sealed double-membrane-enclosed **autophagosome**.
4. **Fusion** of the autophagosome with lysosomes, catalyzed by SNAREs.
5. **Digestion** of the inner membrane and the lumenal contents of the autophagosome.





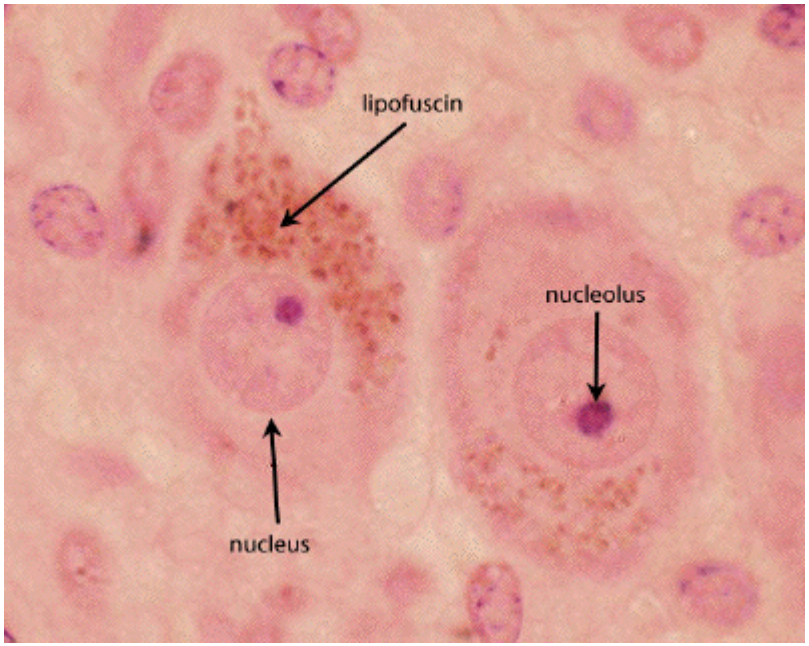
Autophagy Degrades Unwanted Proteins and Organelles

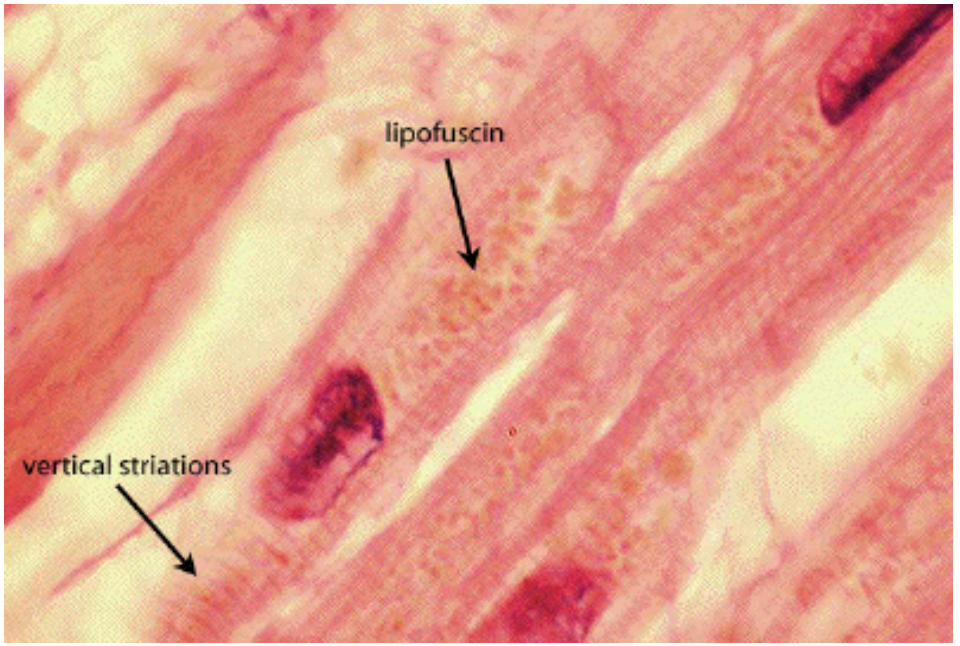
- Autophagy can be divided into three well-characterized pathways:
  - ✓ **Macroautophagy** – nonspecific process in which a portion of the cytoplasm or an entire organelle is being degraded.
  - ✓ **Microautophagy** – nonspecific process in which cytoplasmic proteins are degraded.
  - ✓ **Chaperone-mediated autophagy** – the only selective process of protein degradation and requires assistance from specific cytosolic chaperones.



Lysosomes

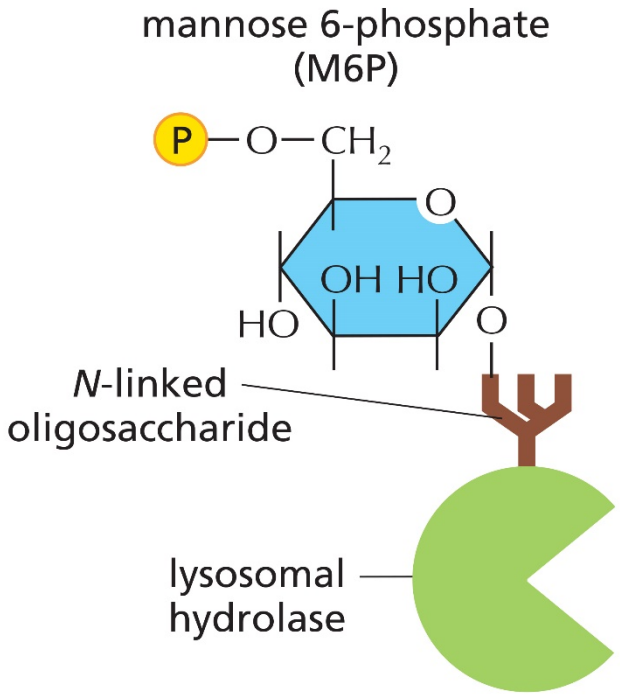
- Hydrolytic breakdown of the contents of lysosomes often produces a debris-filled vacuole called a **residual body** that may remain for the entire life of the cell. For example, in neurons, residual bodies are called **age pigment** or **lipofuscin granules**. Residual bodies are a normal feature of cell aging.





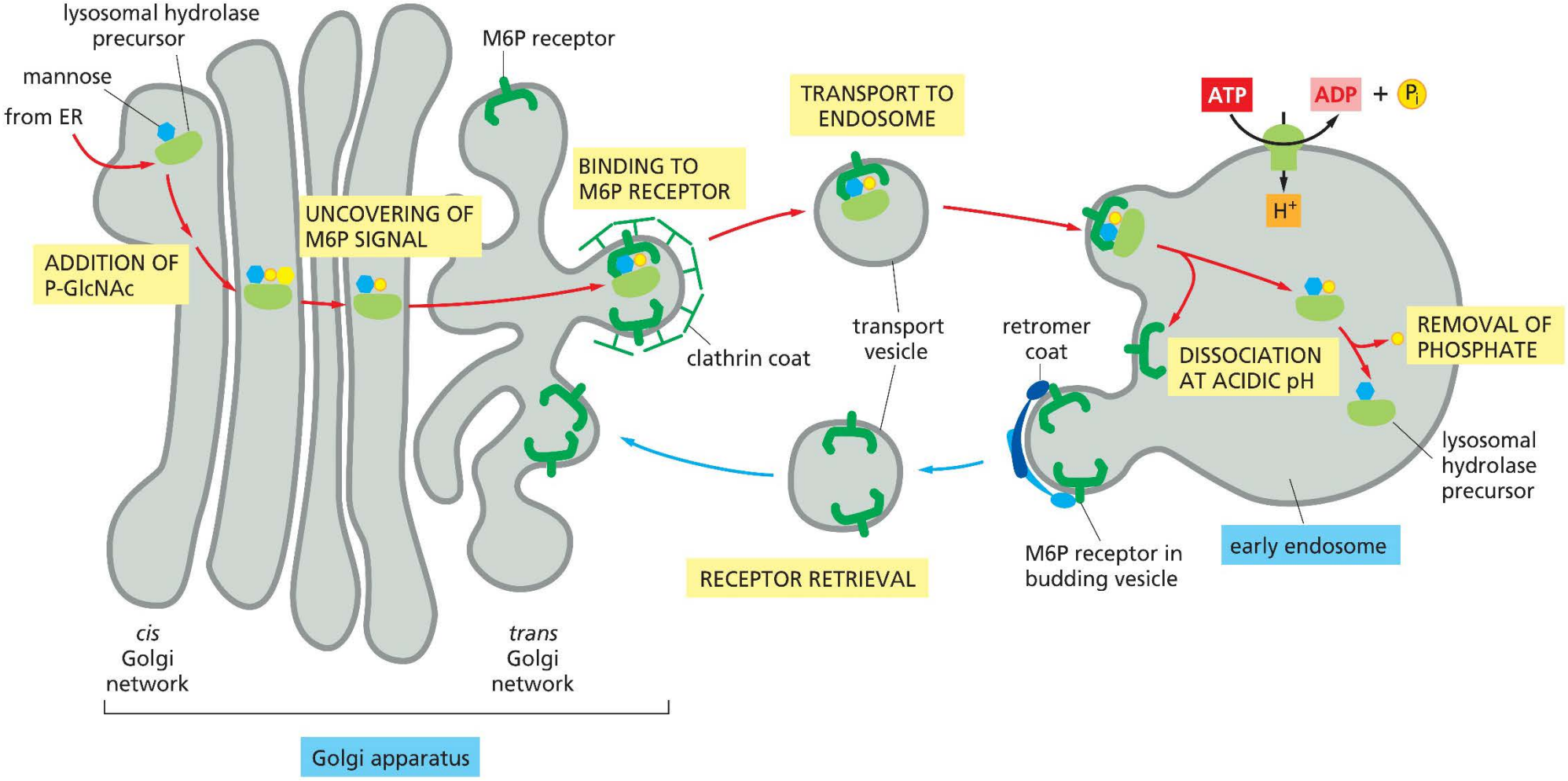
How are Lysosomal Hydrolases Recognized and Selected in the TGN with the Required Accuracy?

- ***M6P*** groups are added exclusively to the *N*-linked oligosaccharides of the soluble lysosomal enzymes as they pass through the lumen of the *cis* Golgi network.



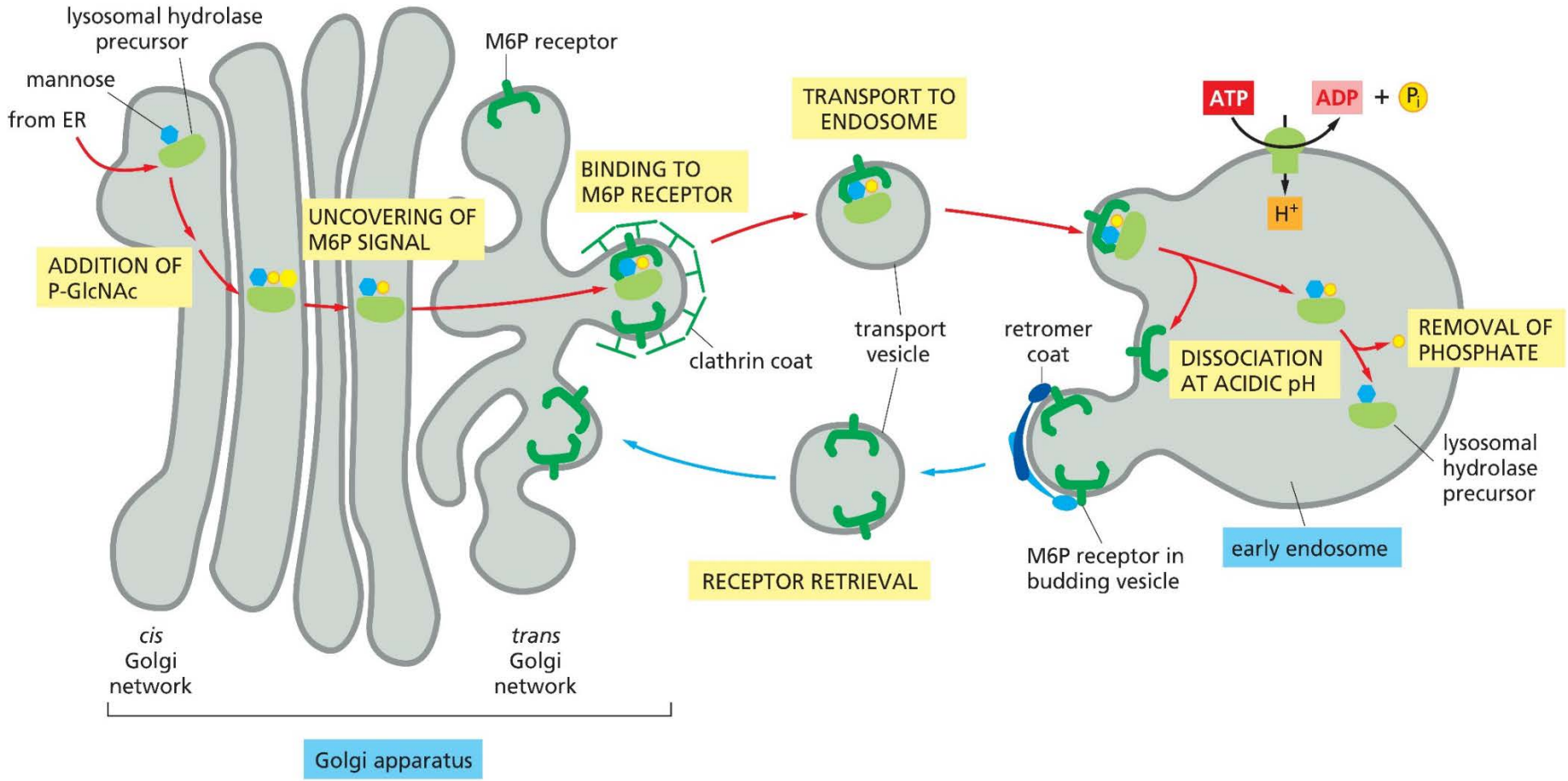
How are Lysosomal Hydrolases Recognized and Selected in the TGN with the Required Accuracy?

- Transmembrane **M6P receptor** proteins, which are present in the TGN, recognize the M6P groups and bind to the lysosomal hydrolases on the lumenal side of the membrane and to adaptor proteins in assembling clathrin coats on the cytosolic side.



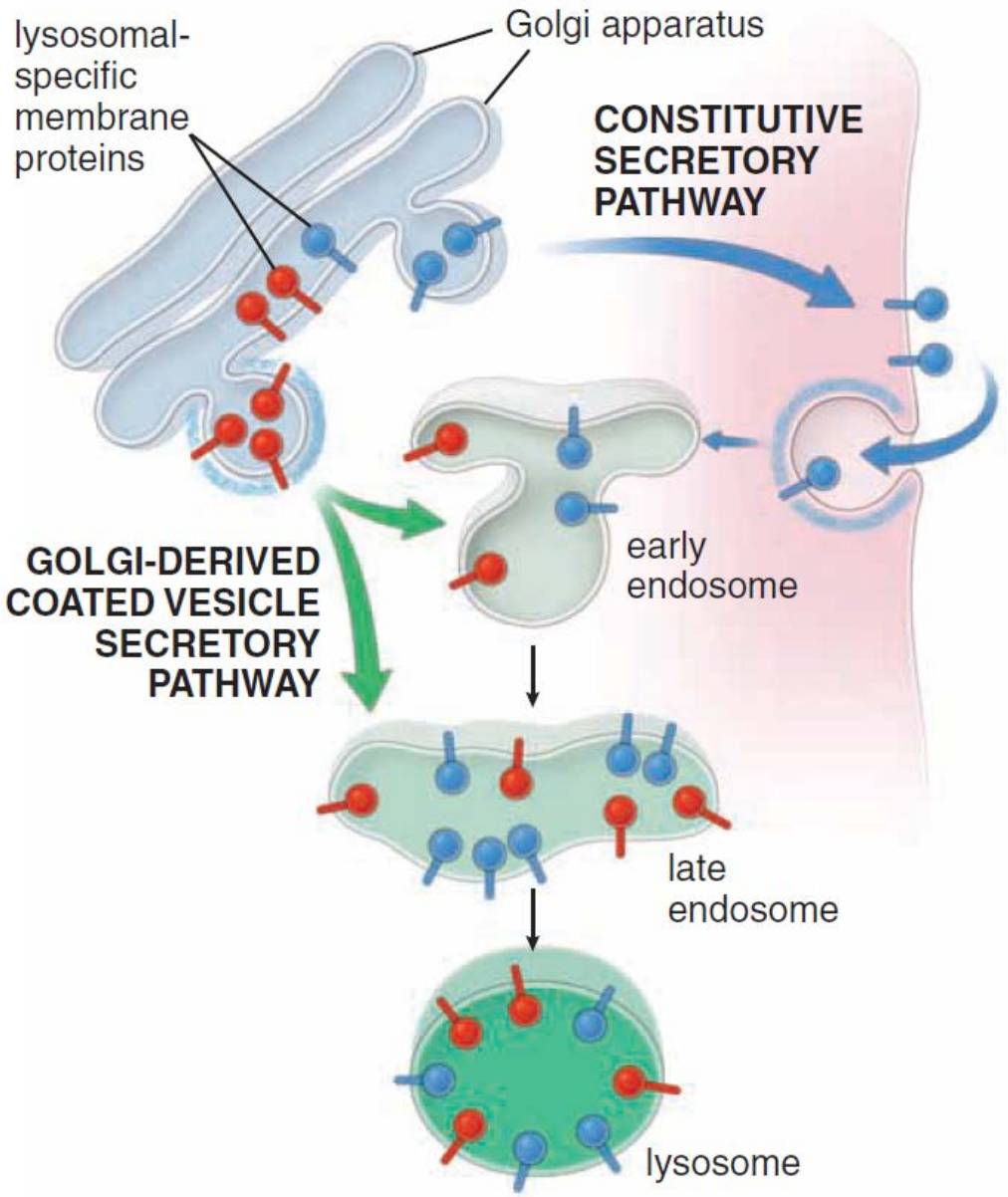
How are Lysosomal Hydrolases Recognized and Selected in the TGN with the Required Accuracy?

- The **M6P receptor** protein binds to **M6P** at pH 6.5–6.7 in the TGN lumen and releases it at pH 6, which is the pH in the lumen of endosomes.
- After the receptor is delivered, the lysosomal hydrolases dissociate from the **M6P receptors**, which are retrieved into transport vesicles that bud from endosomes.



Lysosomal Membrane Proteins are Synthesized in the rER and Have a Specific Lysosomal Targeting Signal

- These proteins reach their destination by one of two pathways:
  - ✓ **Constitutive secretory pathway**.
  - ✓ **Golgi-derived coated vesicle secretory pathway**.



Lysosomal Storage Disease

- The absence of certain lysosomal enzymes can cause the pathologic accumulation of undigested substrate in residual bodies. This can lead to several disorders collectively termed **lysosomal storage diseases**.

Examples of lysosomal storage diseases caused by defective lysosomal enzymes.

Disease	Faulty Enzyme	Main Organs Affected
Hurler syndrome (MPS I)	α-L-Iduronidase	Skeleton and nervous system
McArdle syndrome	Muscle phosphorylase	Skeletal muscles
Tay-Sachs	GM_2-gangliosidase	Nervous system
Gaucher	Glucocerebrosidase	Liver and spleen
I-cell disease	Phosphotransferase for M6P formation	Skeleton and nervous system

Proteasome

- There are two major intracellular structures in which damaged or unneeded proteins are being degraded:
  - ✓ **Lysosomes**.
  - ✓ **Proteasomes**.

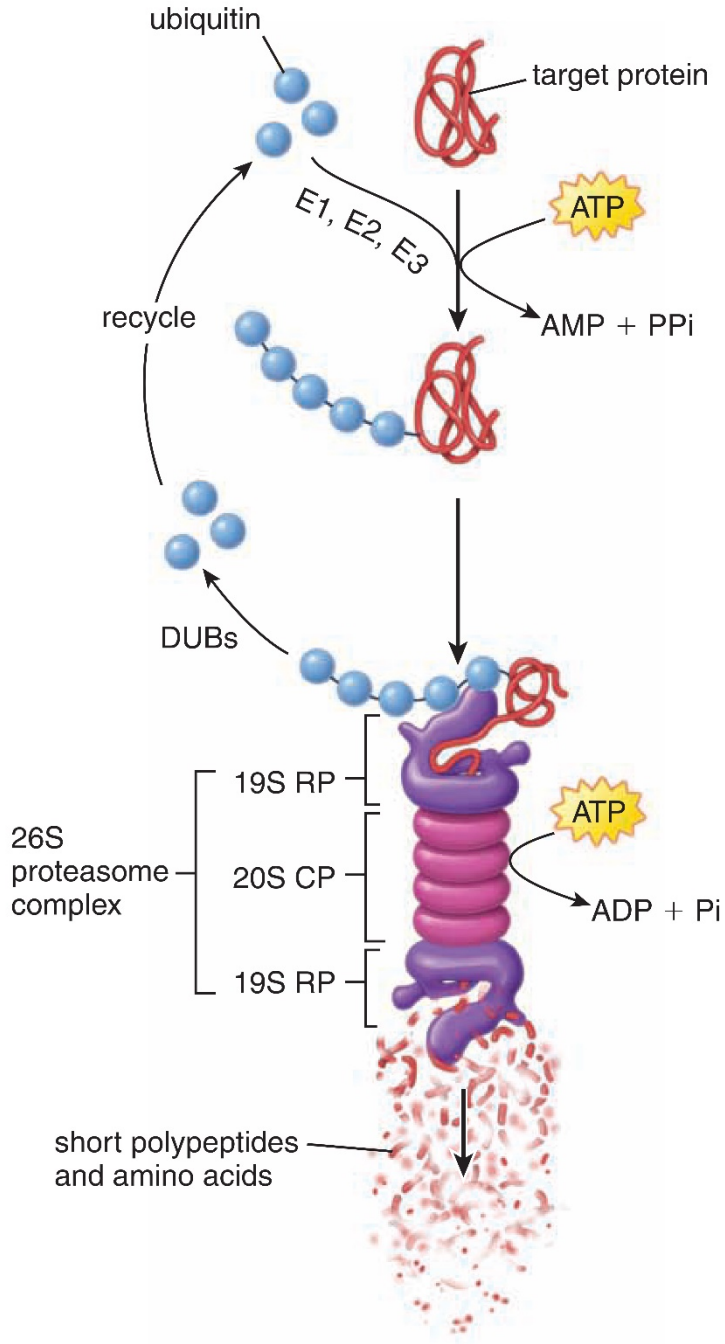
- The apparatus that deliberately destroys aberrant proteins is the proteasome, an abundant ATP-dependent protease that constitutes nearly 1% of cell protein.

- **Proteasome mediated degradation** is used by cells to destroy:
  - ✓ **Abnormal proteins** that are misfolded, denatured, or contain abnormal amino acids.
  - ✓ **Normal short-lived regulatory proteins** that need to be rapidly inactivated and degraded, such as mitotic cyclins that regulate cell cycle progression, transcriptional factors or tumor suppressors.

Proteasome

Each proteasome consists of:

- A hollow cylinder, containing a **20S core particle (CP)** in which polyubiquitinated proteins are degraded into small polypeptides and amino acids.
- On both ends of the CP cylinder are two **19S regulatory particles (RPs):**
  - ✓ One RP recognizes and cleaves the polyubiquitin tags, unfolds the protein, and regulates its entry into the destruction chamber.
  - ✓ RP on the opposite side releases short peptides and amino acids after degradation of the protein is completed.

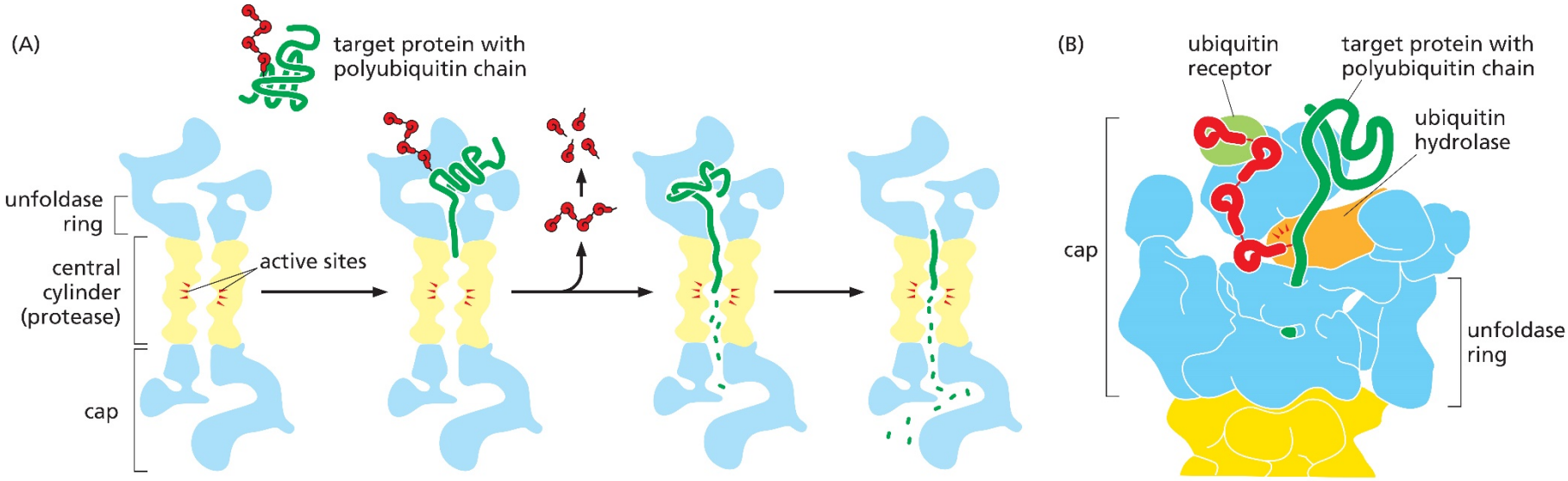


Proteasome

Degradation of a protein in the proteasome-mediated pathway involves two successive steps:

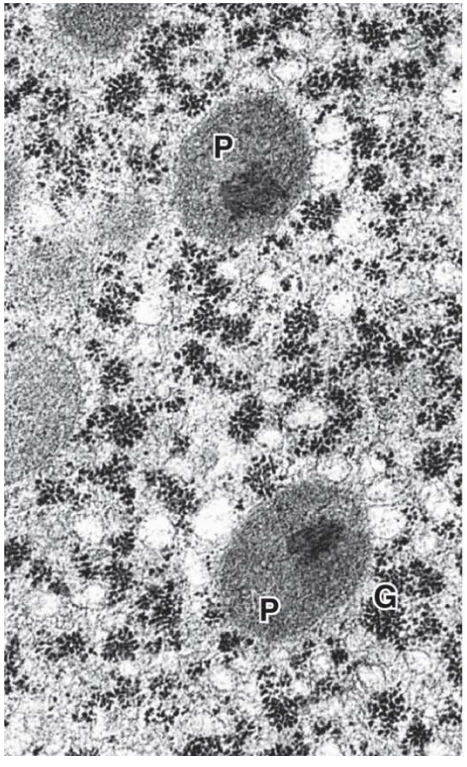
1. **Polyubiquitination** – proteins targeted for destruction are repeatedly tagged by covalent attachments of a small (8.5 kDa) protein called **ubiquitin** by **ubiquitin-activating enzymes E1, E2, and E3**.

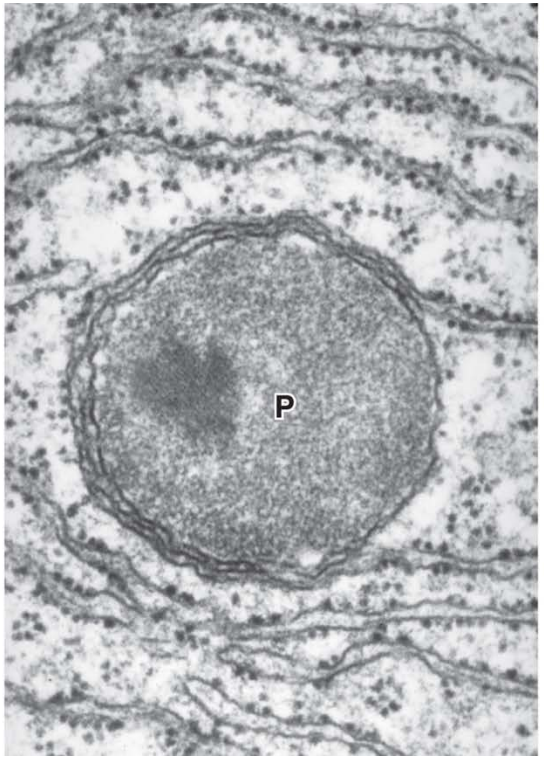
2. **Degradation of the tagged protein by the 26S proteasome complex**.

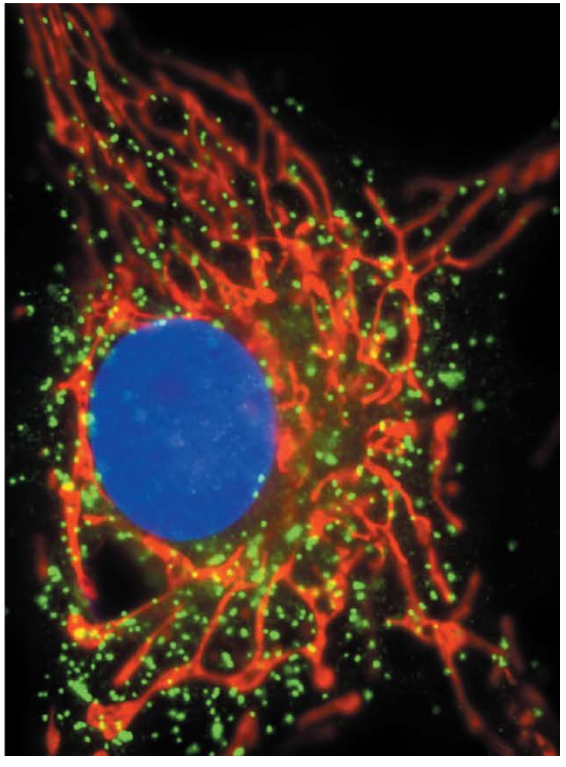


Peroxisomes

- Peroxisomes were identified as organelles by **Christian de Duve in 1967** after they had been first described by a Swedish doctoral student J. Rhodin in 1954 (microbodies).
- *Gr.* peroxide + *soma,* body
- Spherical organelles (70–100 per cell), size – **macroperoxisomes** (0.5 to 3 µm) and **microperoxisomes** (0.1 to 0.3 µm).
- They have homogeneous **matrix**, ***crystalloid core (nucleoid)*** and **single layer membrane**.







Peroxisomes

- Peroxisomes contain more than 50 enzymes:
  - ✓ Catalase – 40%.
  - ✓ β-oxidase of very long-chain fatty acids.
  - ✓ D- and L-amino oxidases.
  - ✓ *Urate oxidase (not in humans).*

Functions

- Breakdown of very long chain fatty acids through **β-oxidation**.
- The first reactions in the formation of **plasmalogen** in animal cells also occur in peroxisomes. Plasmalogen is the most abundant phospholipid in **myelin**.

Peroxisomes

- Oxidative enzymes within the peroxisome, by using molecular oxygen, remove hydrogen atoms from specific organic substrates (labeled as R), in an **oxidative reaction**, producing hydrogen peroxide (H_2O_2, itself toxic):

$$RH_2 + O_2 \rightarrow R + H_2O_2$$

- ***Catalase*** uses the H_2O_2 generated by other enzymes in the organelle to oxidize a variety of other substrates (formic acid, formaldehyde, and alcohol) by the "peroxidation" reaction:

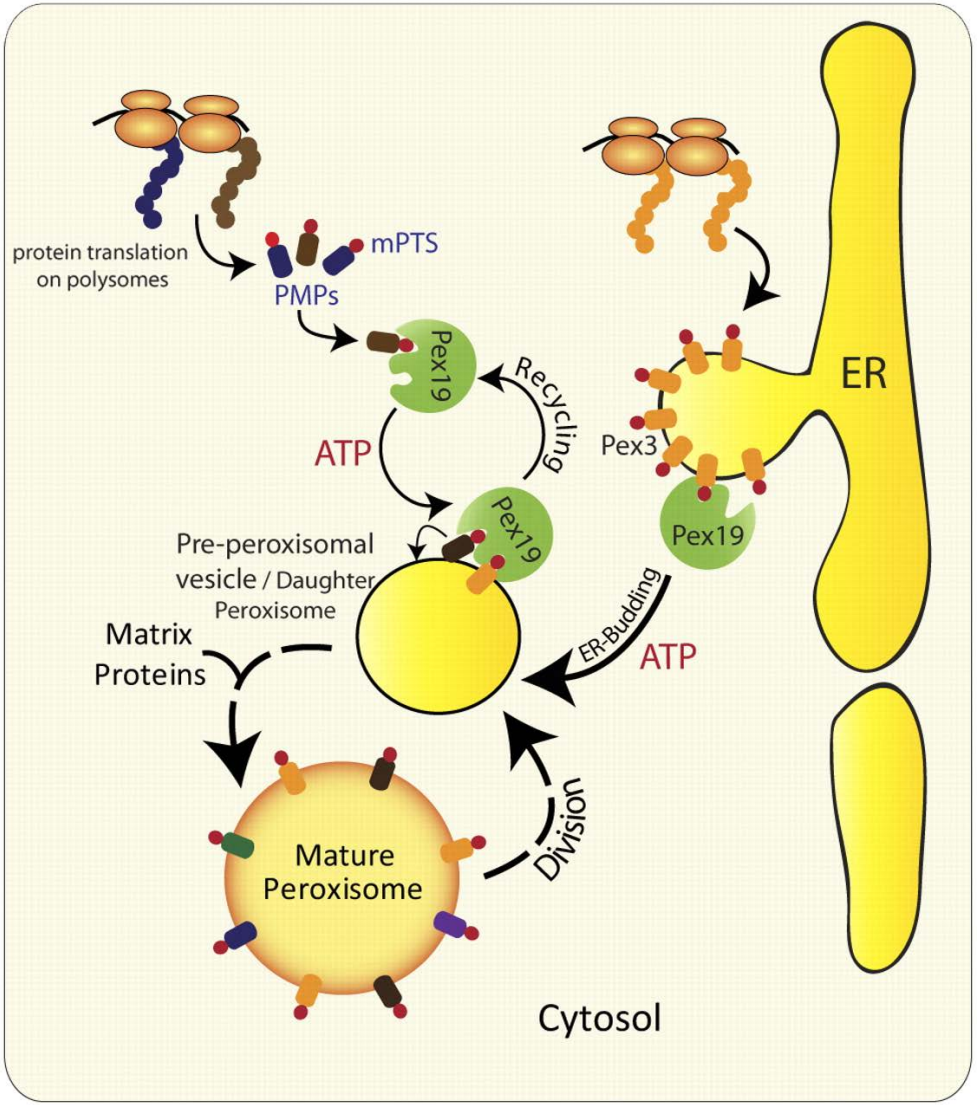
$$H_2O_2 + R'H_2 \rightarrow R' + 2H_2O$$

- About 25% of the ethanol we drink is oxidized to acetaldehyde in this way.
- In addition, when excess H_2O_2 accumulates in the cell, catalase converts it to H_2O through the reaction:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Biogenesis of Peroxisomes

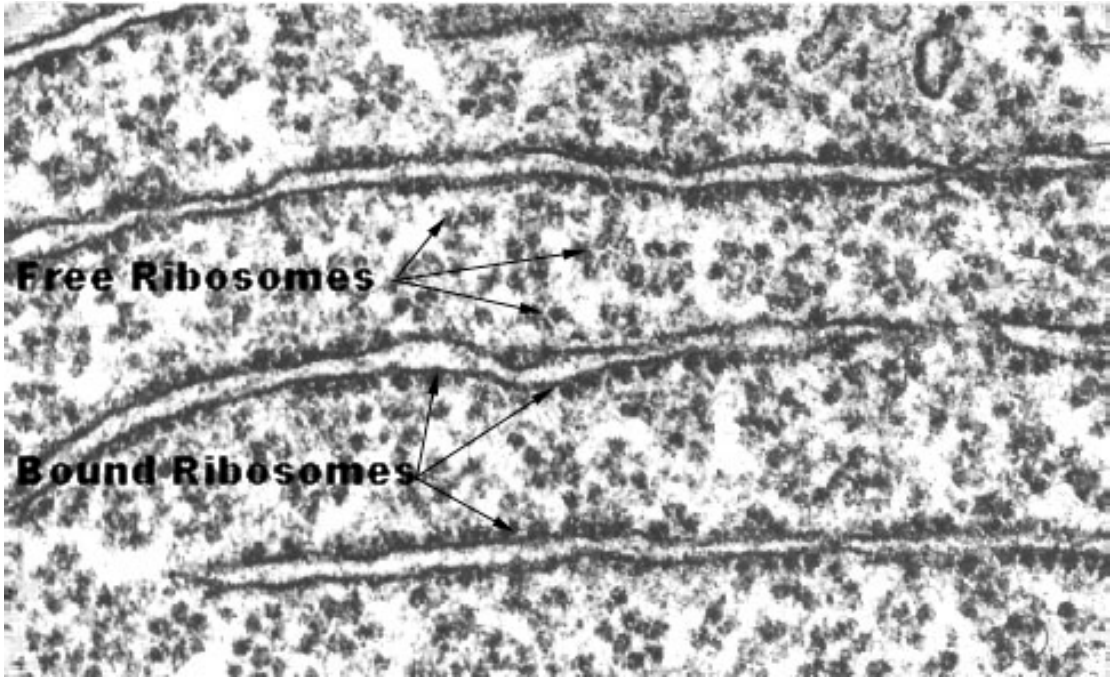
- Peroxisomes can be derived **from the ER** and **replicate by fission**.
- Peroxisome matrix proteins are translated in the cytoplasm prior to import in peroxisomes.
- Specific amino acid sequences (peroxisomal targeting signal, PTS) at the *C-terminus* (PTS1) or *N-terminus* (PTS2) of peroxisomal matrix proteins signals them to be imported into the organelle.
- Numerous distinct proteins, called **peroxins (PEX5 and PEX7)**, participate in the import process, which is driven by ATP hydrolysis.



Ribosomes

- Ribosomes were first observed in the **mid 1950s** by Romanian cell biologist **George Emil Palade** using an electron microscope as dense particles or granules for which, in 1974, he won a Nobel Prize.

- The term "ribosome" was proposed by scientist Richard B. Roberts in 1958.

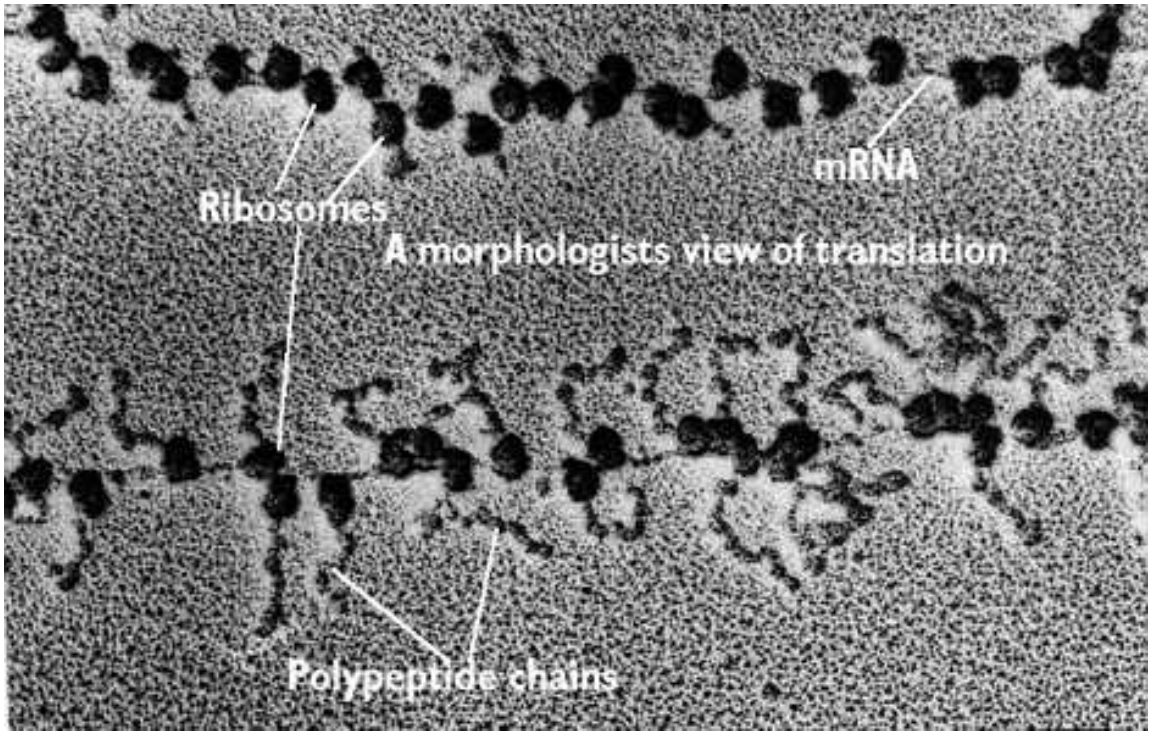


George Palade
1912 – 2008

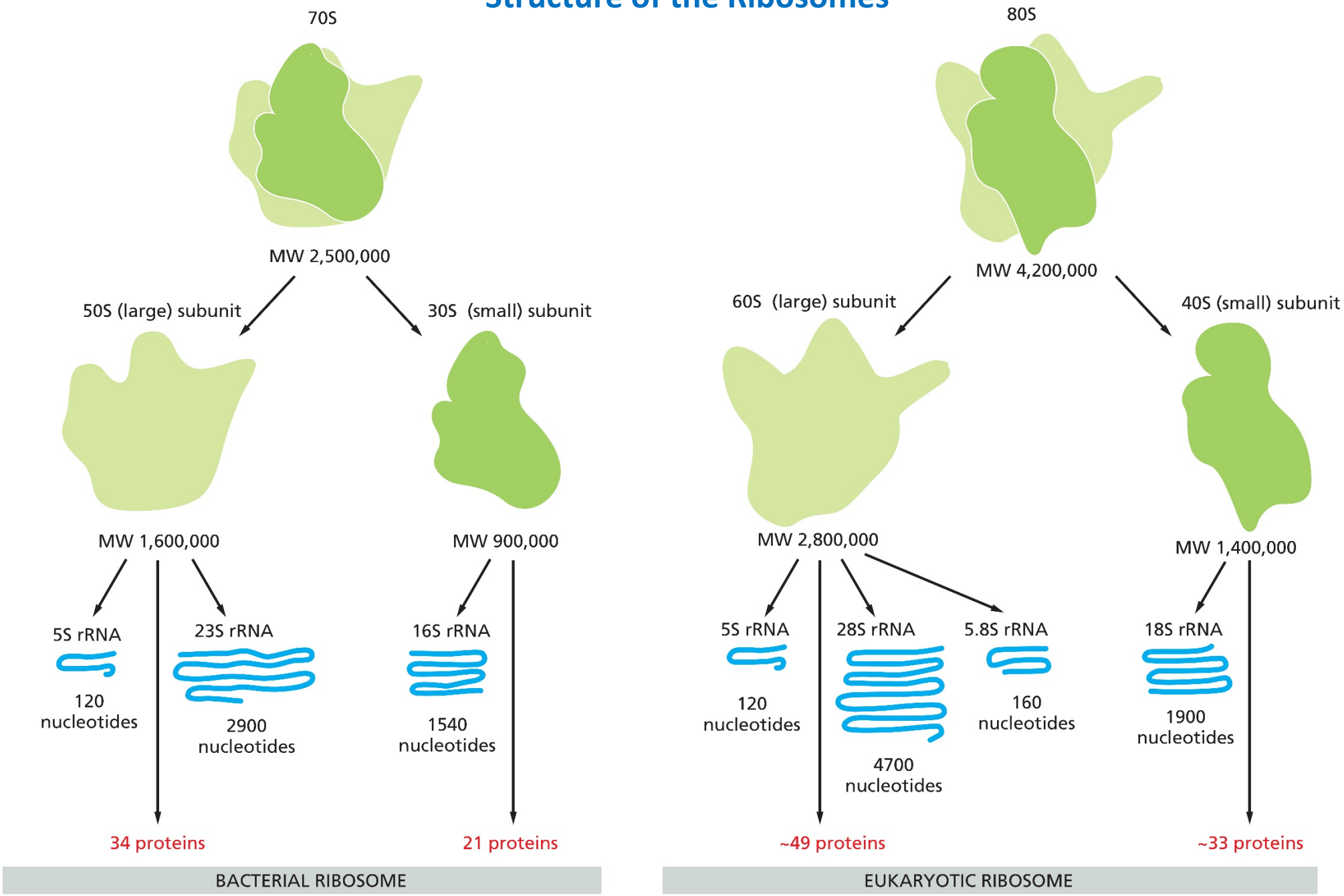
Ribosomes

- The synthesis of proteins is guided by information carried by mRNA molecules.

- To maintain the correct reading frame and to ensure accuracy (about 1 mistake every 10 000 amino acids), protein synthesis is performed in the **ribosome**, a complex catalytic machine made from more than 50 different proteins (***ribosomal proteins***) and several RNA molecules, the **ribosomal RNAs** (rRNAs).

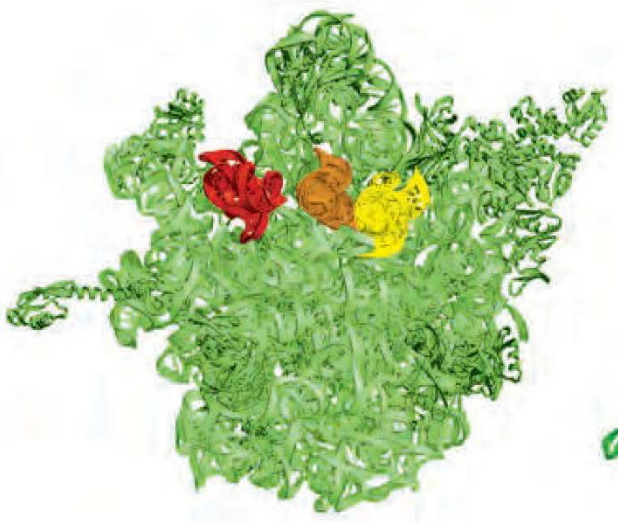


Structure of the Ribosomes



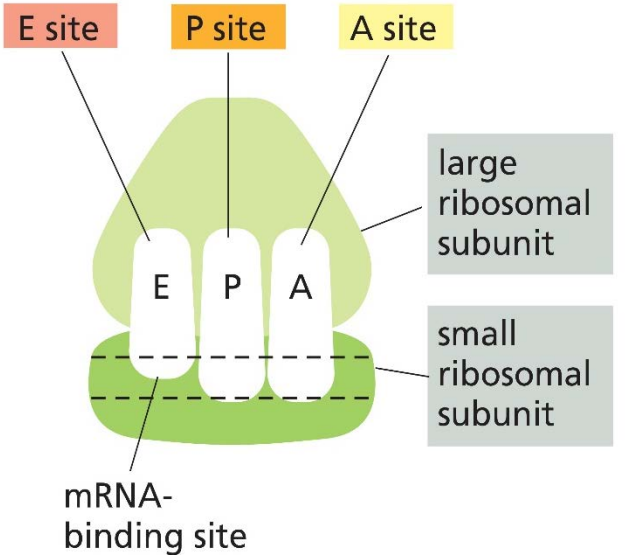
Structure of the Ribosomes

- To choreograph the many coordinated movements required for efficient translation, a ribosome contains **four binding sites** for RNA molecules:
  - ✓ One is for the mRNA.
  - ✓ **A site**, the **P site**, and the **E site** are for tRNAs. A tRNA molecule is held tightly at the A and P sites only if its anticodon forms base pairs with a complementary codon on the mRNA molecule that is threaded through the ribosome.



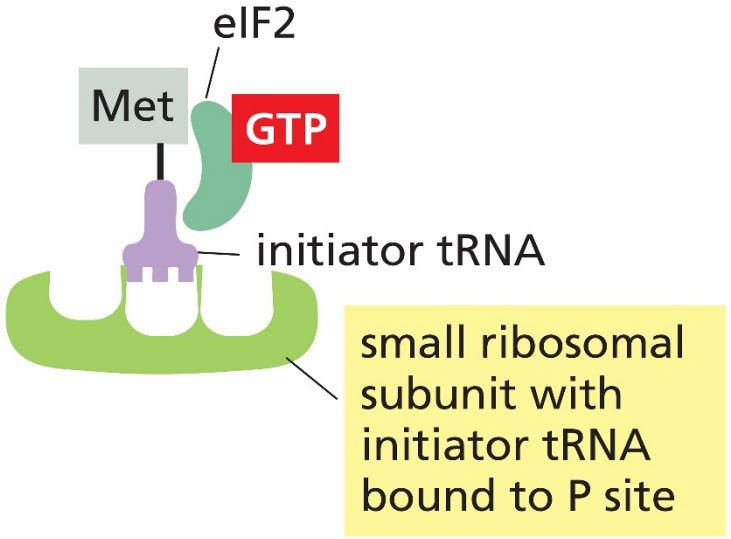
large subunit

small subunit



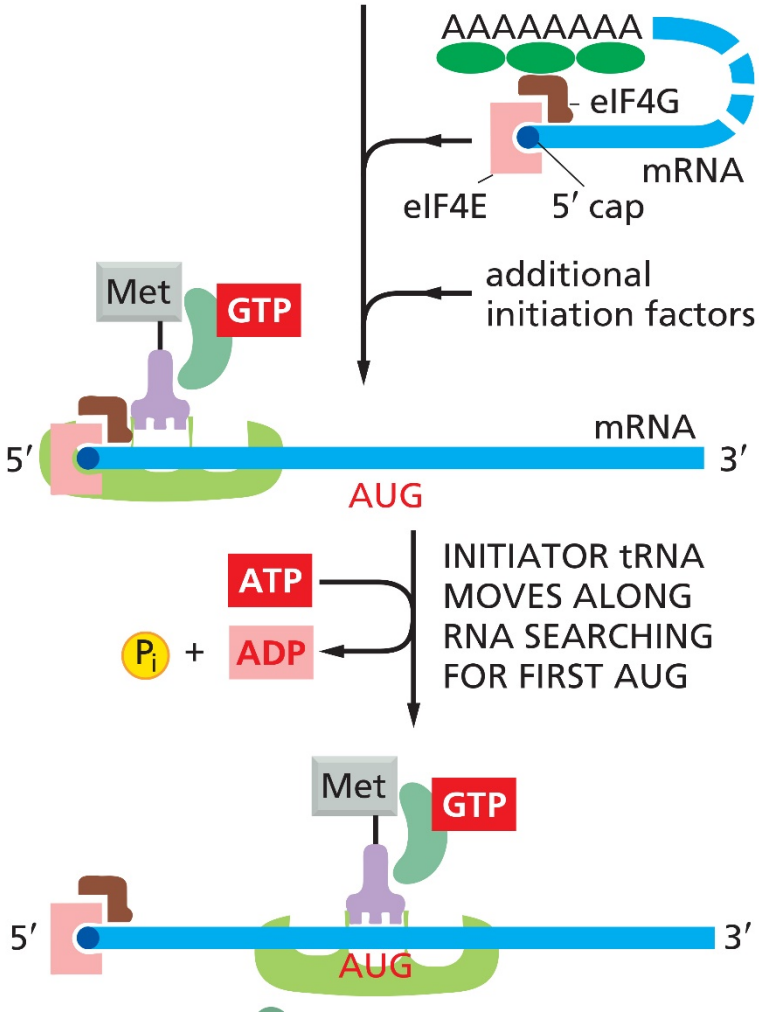
Function of the Ribosomes – Initiation

- The translation of an mRNA begins with the codon **AUG**, and a special tRNA is required to start translation.
  - ✓ This initiator tRNA always carries the amino acid **methionine.**
  - ✓ The initiator tRNA is specially recognized by **initiation factors** because it has a nucleotide sequence distinct from that of the tRNA that normally carries methionine.
- In eukaryotes, the initiator tRNA-methionine complex (Met-tRNAi) is first loaded into the small ribosomal subunit along with the **eukaryotic initiation factors**.



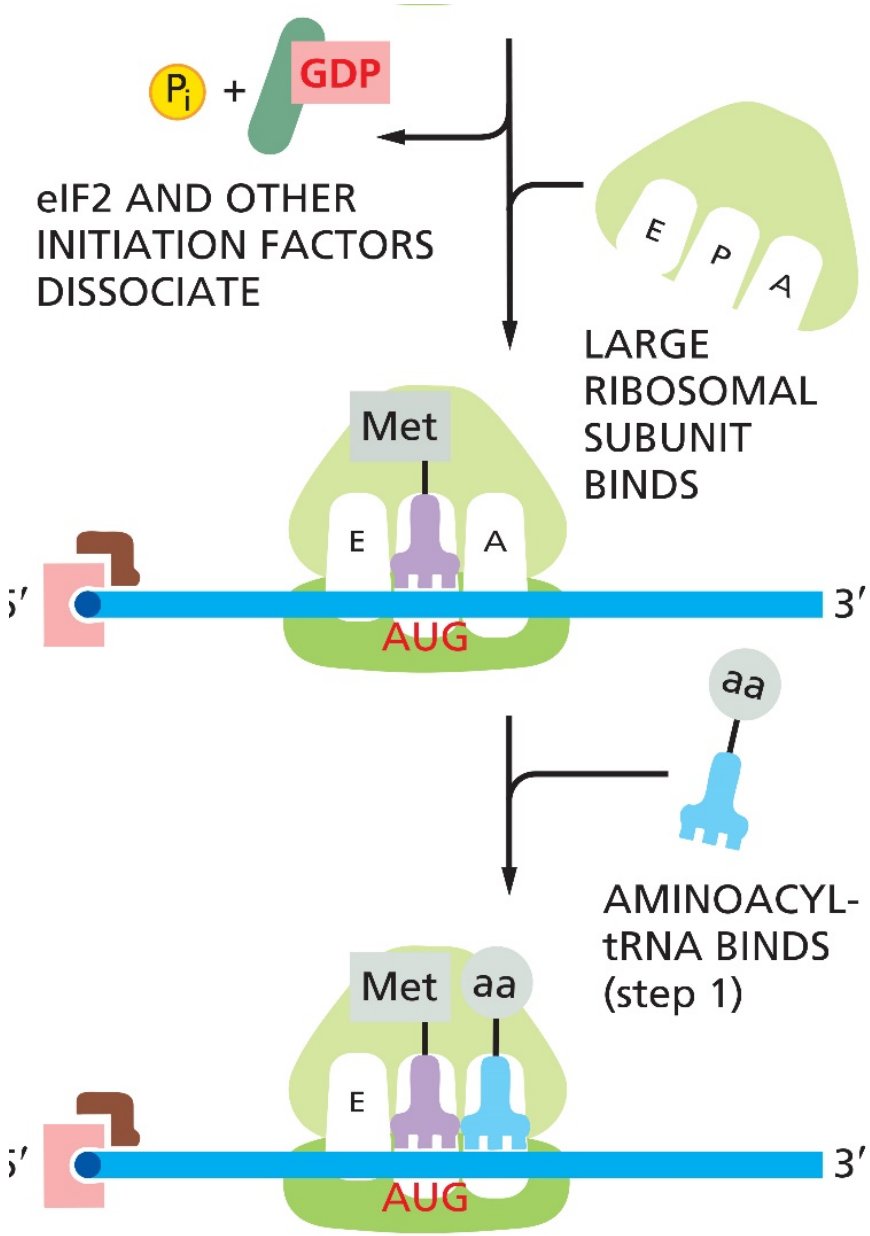
Function of the ribosomes – Initiation

- The small ribosomal subunit binds to the 5′ end of an mRNA molecule, which is recognized by virtue of its 5′ cap that has previously bound **two initiation factors, eIF4E and eIF4G.**



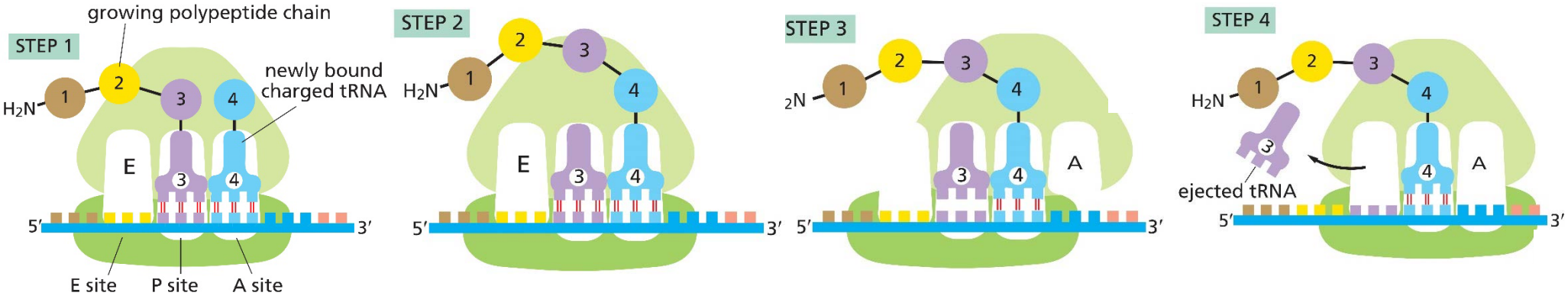
Function of the ribosomes – Initiation

- In 90% of mRNAs, translation begins at the first **AUG** encountered by the small subunit. At this point, the initiation factors dissociate, allowing the **large ribosomal subunit** to assemble with the complex and complete the ribosome. The initiator tRNA remains at the **P site**, leaving the **A site** vacant.
- Protein synthesis is therefore ready to begin.



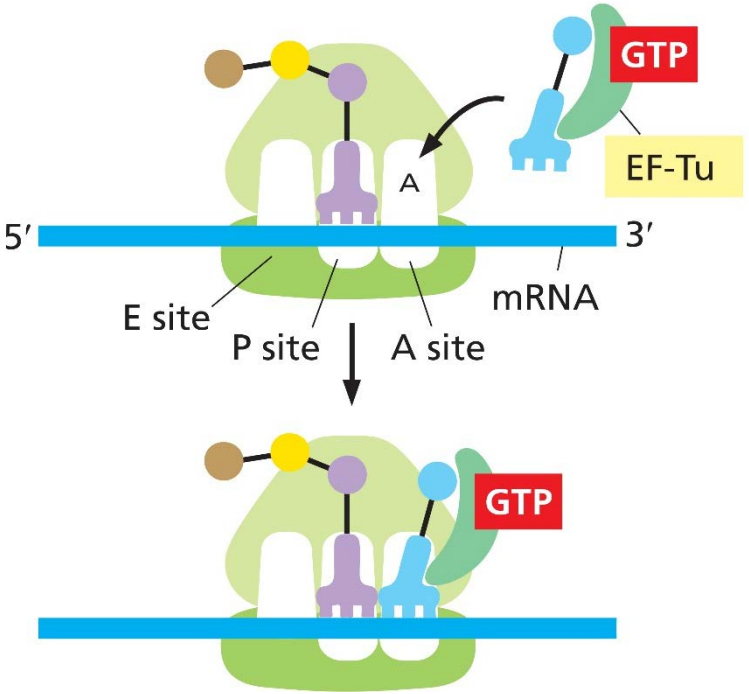
Function of the ribosomes – Elongation

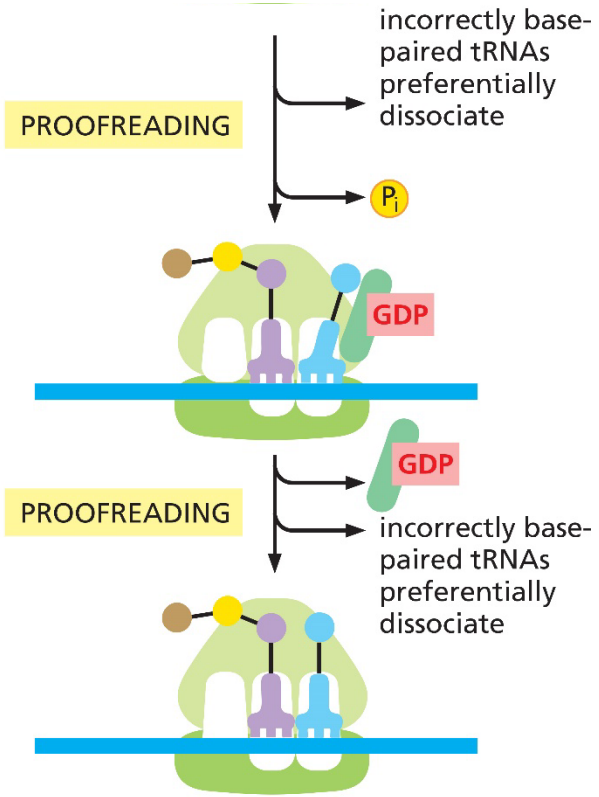
- Once protein synthesis has been initiated, each new amino acid is added to the elongating chain in a cycle of reactions containing four major steps:
  1. tRNA binding
  2. Peptide bond formation (***peptidyl transferase***)
  3. Large subunit translocation
  4. Small subunit translocation
- As a result of the two translocation steps, the entire ribosome moves three nucleotides along the mRNA and is positioned to start the next cycle.



Function of the ribosomes – Elongation

- ***Elongation factors*** drive translation forward and improve its accuracy.
- **Two *elongation factors*** enter and leave the ribosome during each cycle, each hydrolyzing GTP to GDP and undergoing conformational changes in the process. These factors are called EF-Tu and EF-G in bacteria, and EF1 and EF2 in eukaryotes.





Function of the ribosomes – Termination

- The end of the protein–coding message is signaled by the presence of one of three ***stop codons* – UAA, UAG, or UGA**.