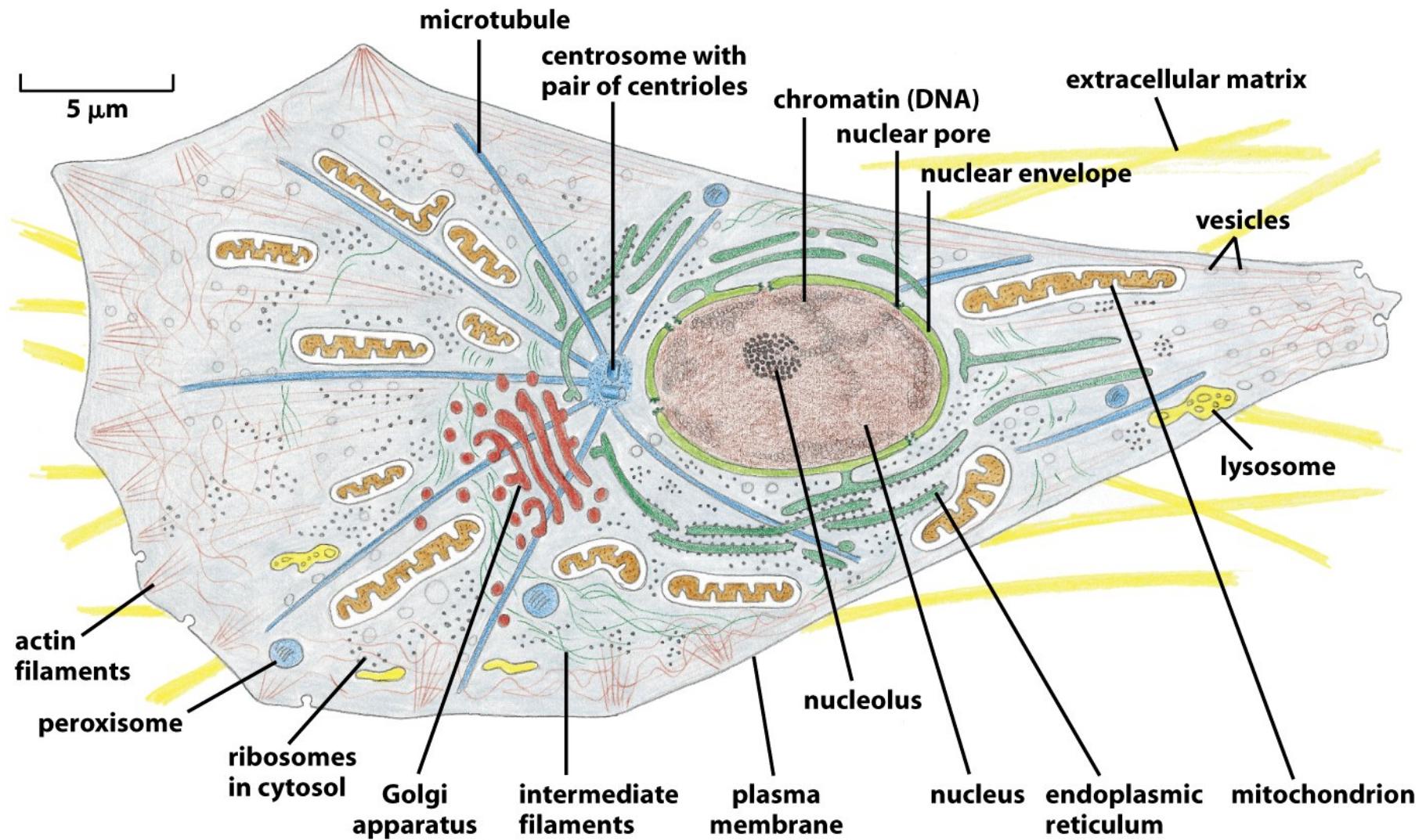


# Main Intracellular Organelles or Compartments

- Nucleus:** Contains nuclear genome, DNA and RNA synthesis (has two membranes)
- Peroxisomes:** Oxidizes toxic molecules and fatty acids
- Mitochondria:** ATP synthesis via oxidative phosphorylation, has its own genome (has two membranes)
- Chloroplasts (plants):** Site of photosynthesis, has its own genome (has two membranes)
- Endoplasmic Reticulum (ER):** Lipid and membrane protein synthesis (ribosomes), secretory pathway, intracellular  $\text{Ca}^{2+}$  storage for signaling (cytosolic, nuclear, mitochondrial and peroximal proteins are synthesized in the cytosol)
- Golgi:** Receives and modifies membrane proteins & lipids from the ER, then sorts them into endosomes, lysosomes, and the plasma membrane, the secretory pathway
- Endosomes:** Storage and sorting compartments of internalized plasma membrane proteins and lipids
- Lysosomes:** Degradation site of cellular organelles and macromolecules, as well as internalized foreign particles.

# Various Major Anatomical Features of the Cell



# **Endoplasmic Reticulum**

**The Endoplasmic Reticulum was Discovered by Albert Claude.  
Ribosomes were discovered by George Emil Palade.**



**Albert Claude**

**1898-1983  
1974 Nobel prize in  
Physiology or Medicine**

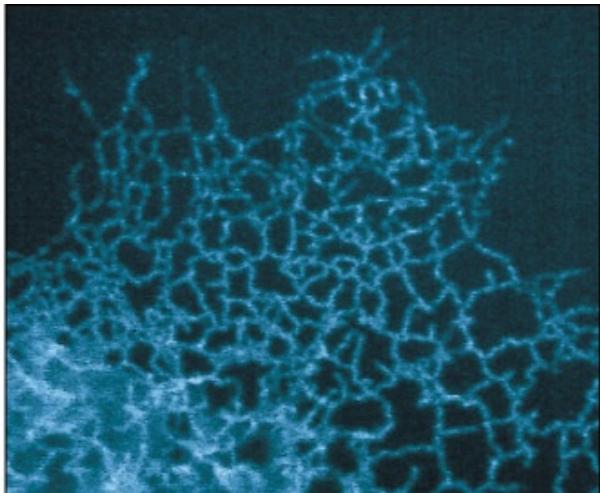


**George Palade**

**1912-2008  
1974 Nobel prize in  
Physiology or Medicine**

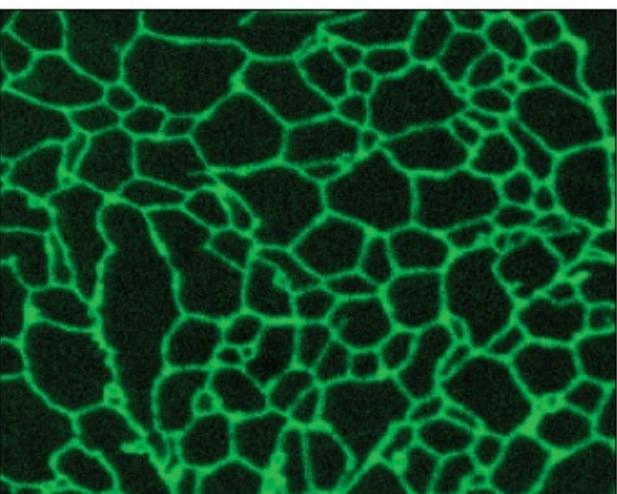
**Innovations in electron microscopy and cell fractionation which together  
laid the foundations of modern molecular cell biology.**

# Features of the ER



(A)

2 μm



(B)

10 μm

**A lipid bilayer membrane enclosing the internal space (the lumen) of the ER**

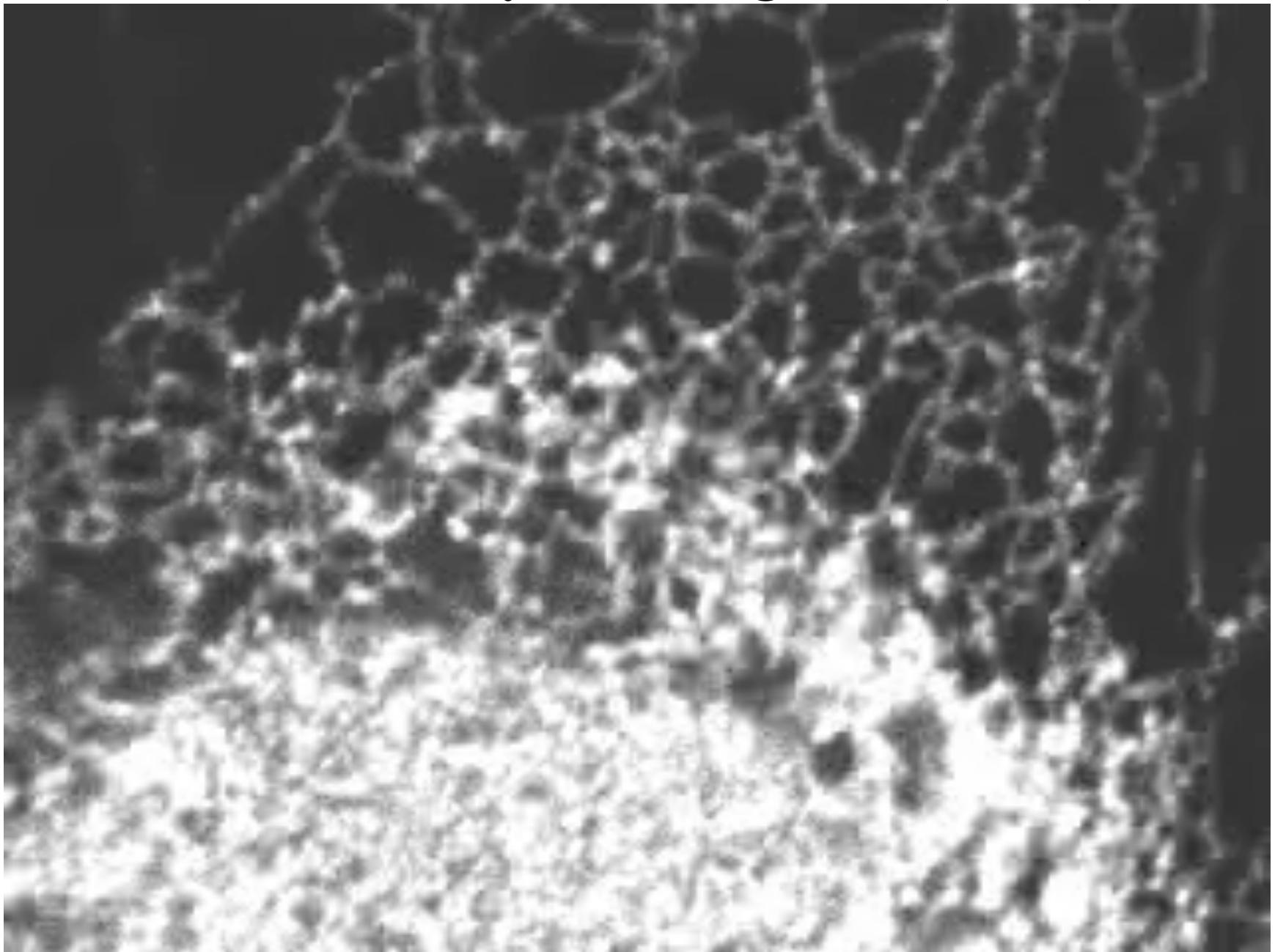
**Existing in the forms of tubes and sacs throughout the cytosol**

**Tubes are interconnected with three-way junctions**

**Constitutes more than 50% of the total membrane of most cells (synthesis of most membrane proteins except for those found in mitochondria and peroxisomes)**

**A dynamic structure**

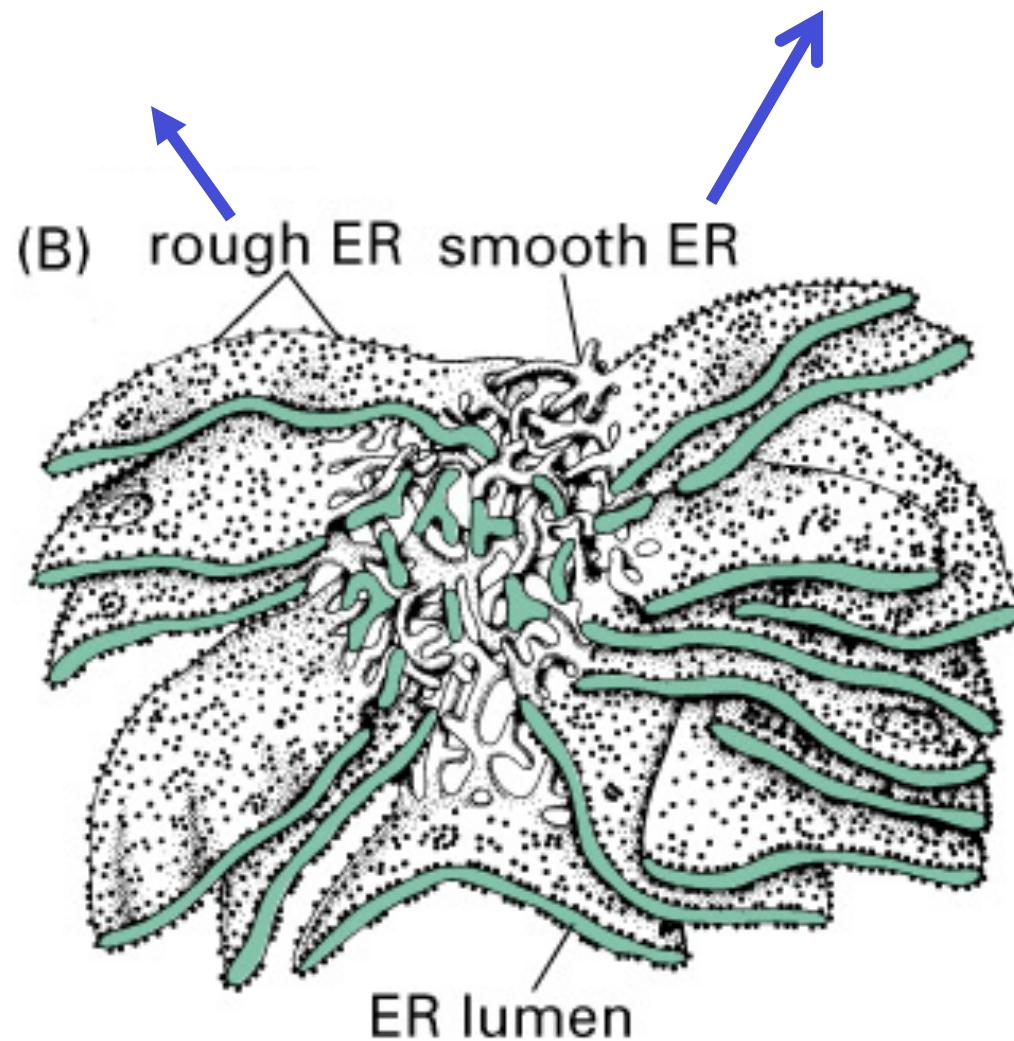
## The ER is a Dynamic Organelle (movie)



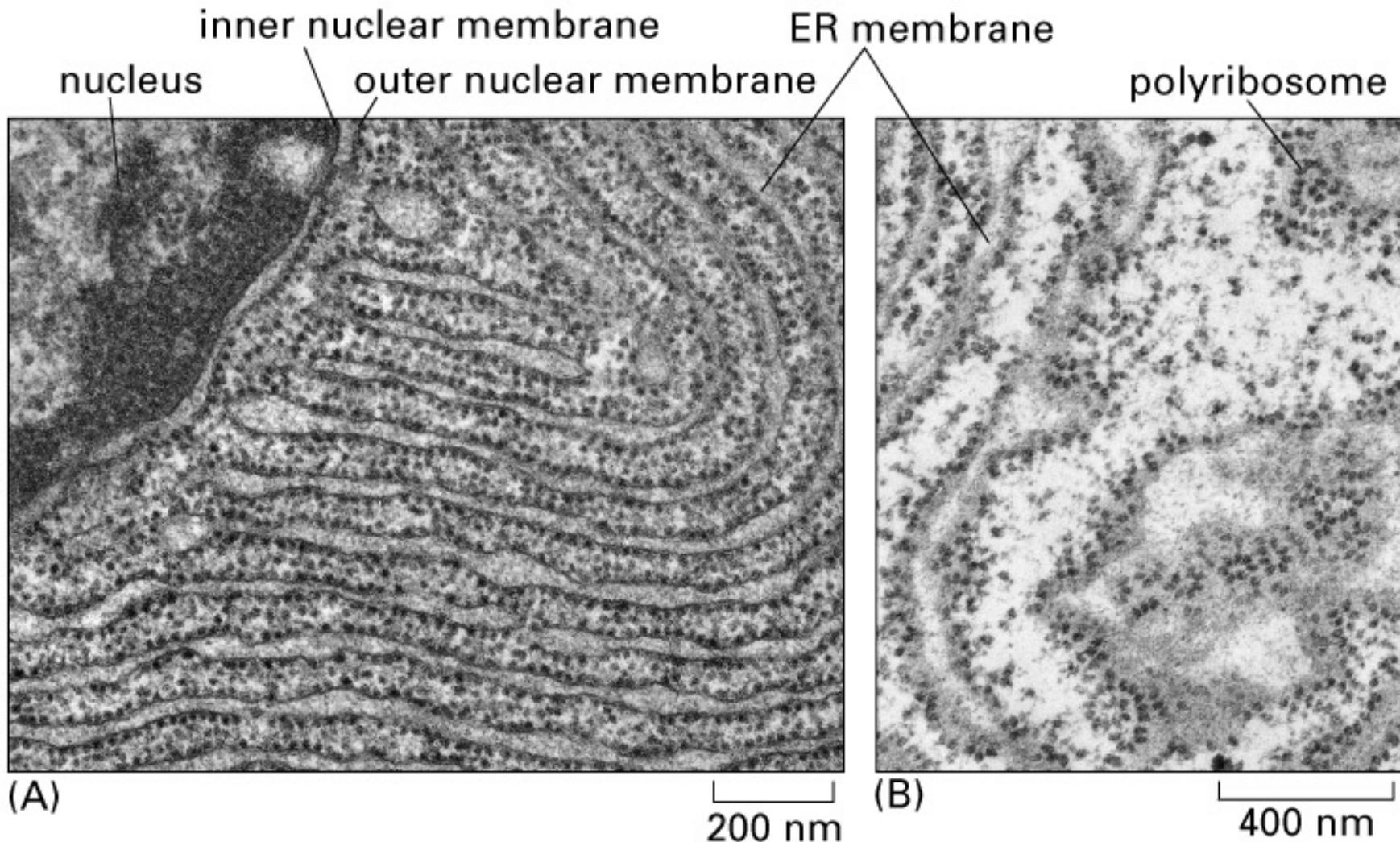
## Two Types of ER Membrane Domains

**polyribosomes attached where the biosynthesis of proteins takes place**

**no ribosomes attached where the biosynthesis of lipids takes place**



**“Rough” appearance of the rough ER is due to the presence of ribosomes on the ER membrane from the cytoplasmic side**

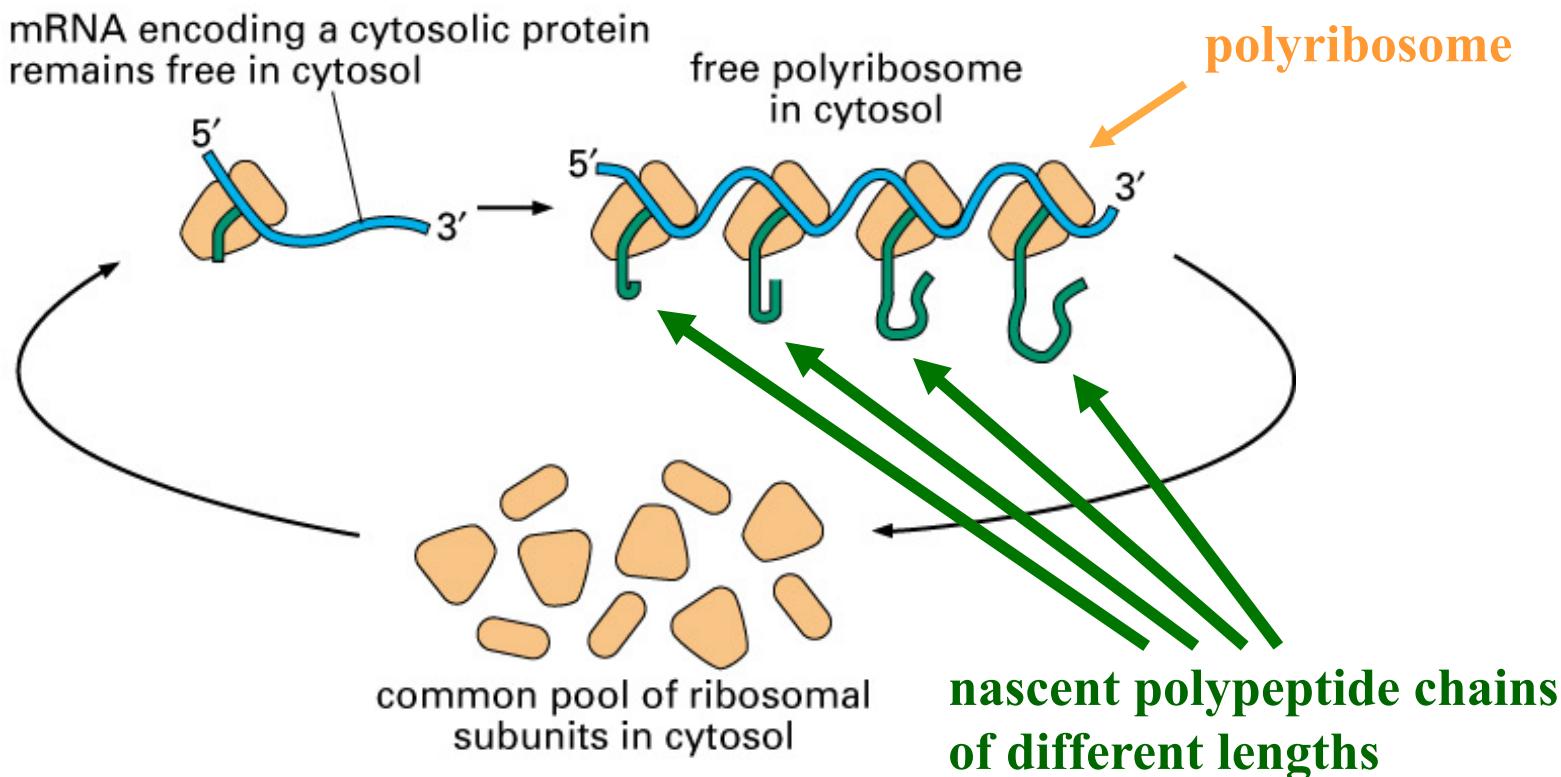


**Note the presence of ribosomes on the nuclear outer membrane as well  
(remember that the nuclear outer membrane is connected to the ER)**

# Polyribosomes

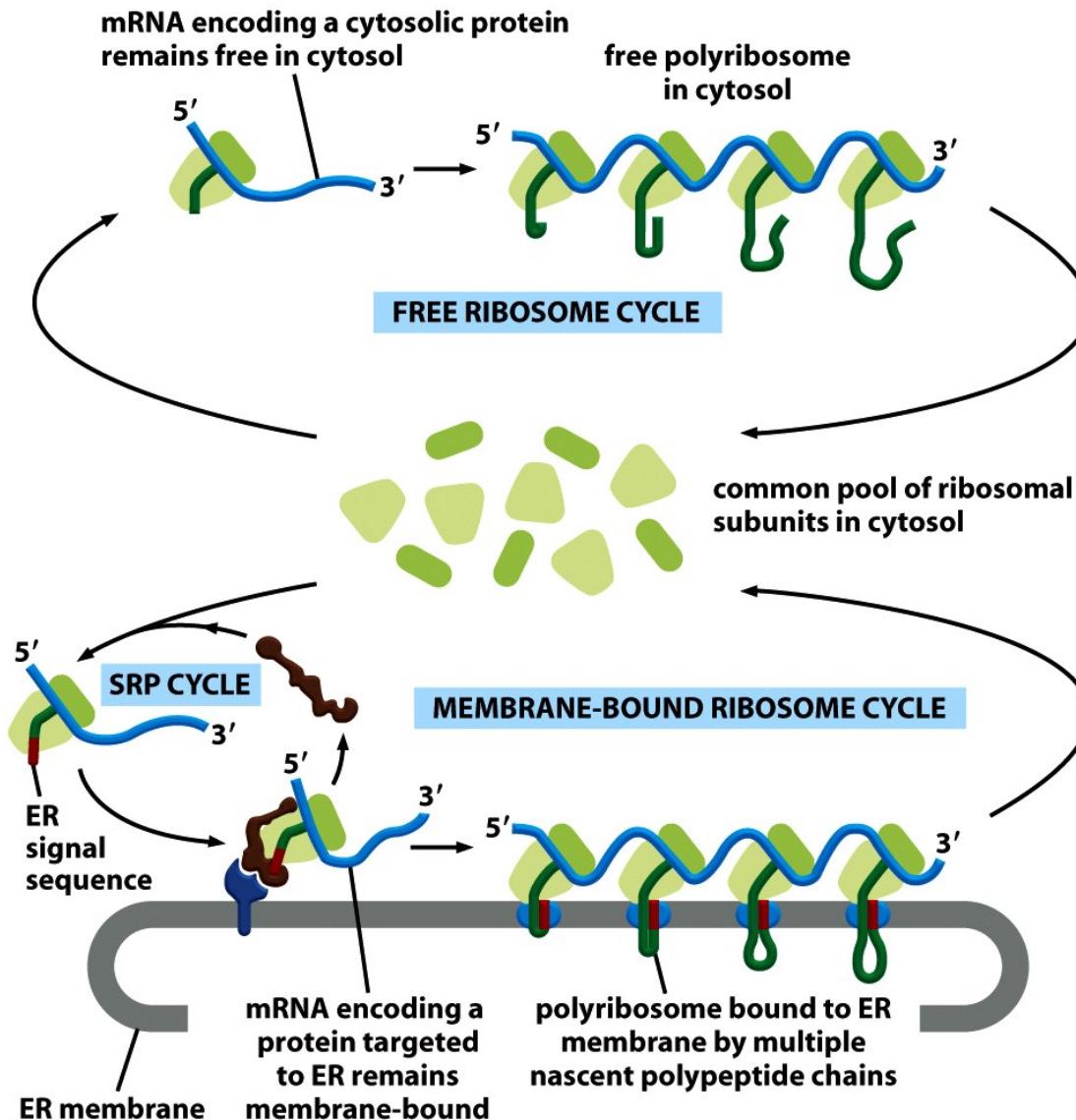
**Polyribosomes: multiple ribosomes translating a single mRNA at the same time.**

**Polyribosomes are generated because another ribosome can start translation of a mRNA molecule before the previous ribosomes finish the translation (a common feature of mRNA translation)**



# Different Localization of Polyribosomes

Depending on the nature of the translated proteins,  
there are two pools of polyribosomes in a cell.



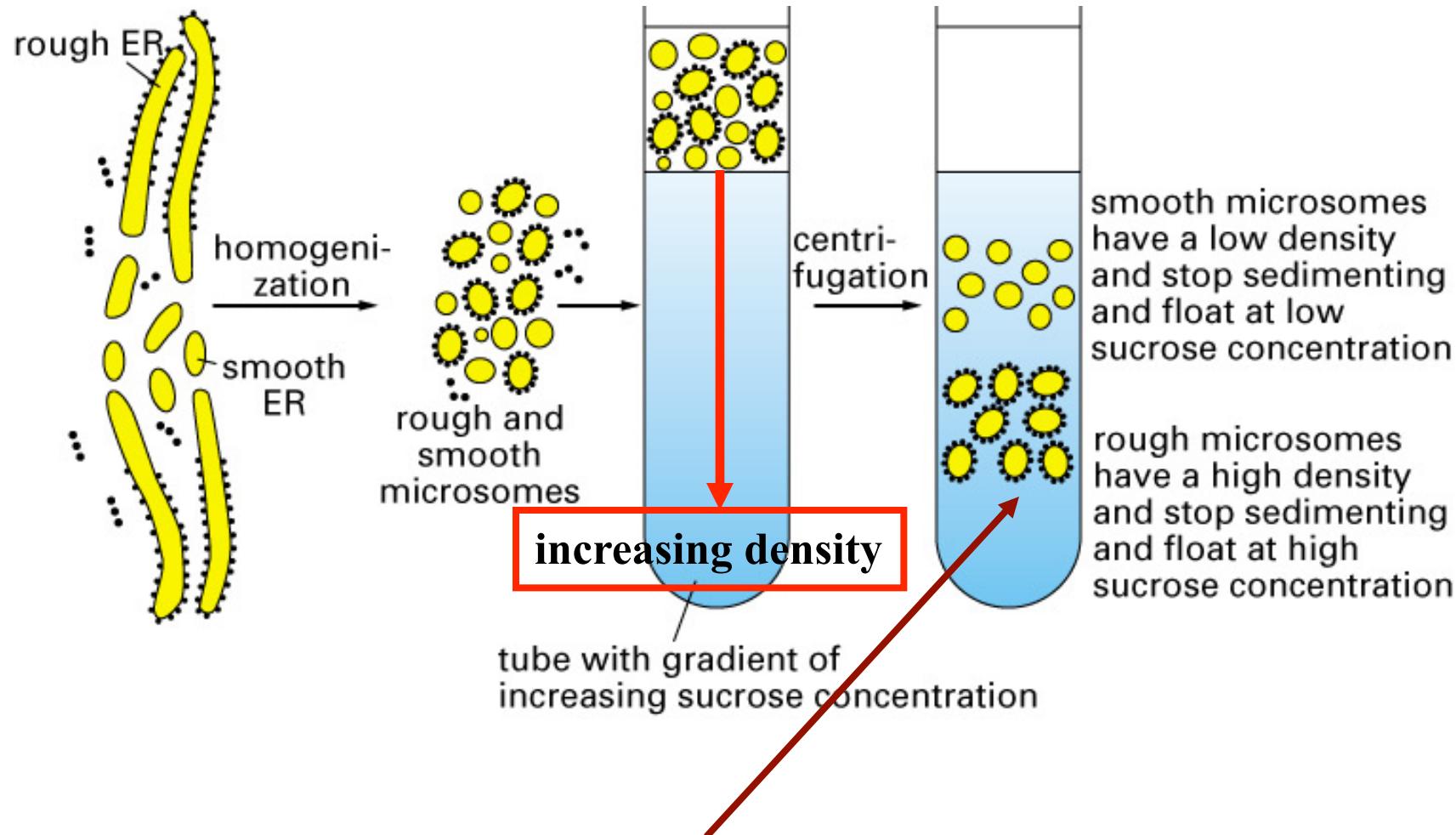
**Cytoplasmic polyribosomes:**  
Polyribosomes translating  
cytosolic proteins remain  
in the cytosol

**ER-associated polyribosomes:**  
Polyribosomes translating  
a protein targeted to ER are  
recruited to the ER membrane  
from the cytosol  
(to be discussed later)

# Rough & Smooth ER can be Separated by Their Different Densities

Start with cell homogenization: the process of breaking up a cell & making the lysate uniform microsomes: small vesicles generated from fragmented ER following homogenization.

Then, sucrose gradient centrifugation: a type of centrifugation used to separate subcellular structures based on their densities.

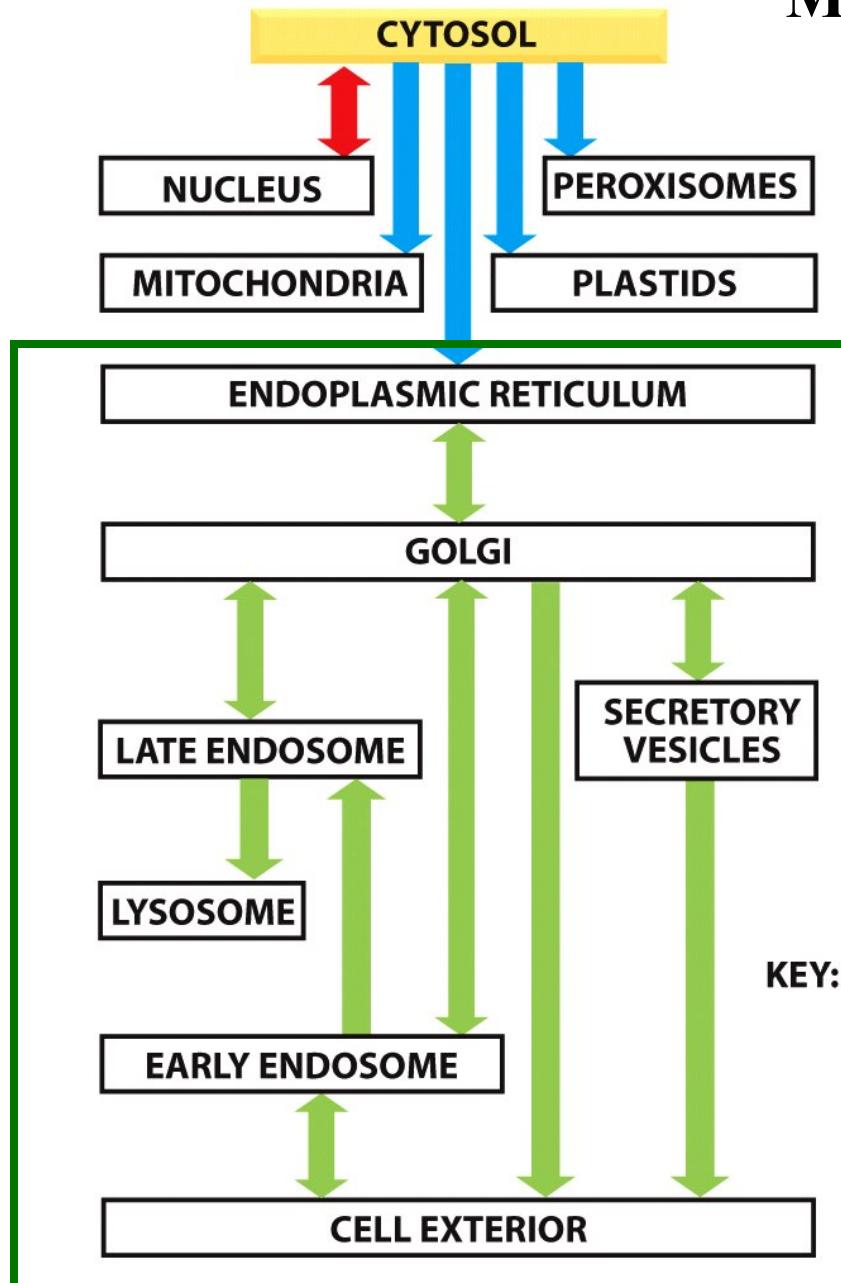


**Rough ER has a higher density due to its associated polyribosomes**

# **Functions of the ER**

- 1. Biosynthesis of many proteins including**
  - a. Proteins secreted to the cell surface/exterior - secretory path.**
  - b. Transmembrane proteins on the plasma membrane.**
  - c. Transmembrane or luminal proteins of vesicular transport organelles, including ER, Golgi, secretory vesicles, endosomes, & lysosomes.**
- 2. Biosynthesis of most lipids**
- 3. Intracellular calcium storage**  
**(The ER lumen is a major reservoir of  $\text{Ca}^{2+}$  used in signaling)**

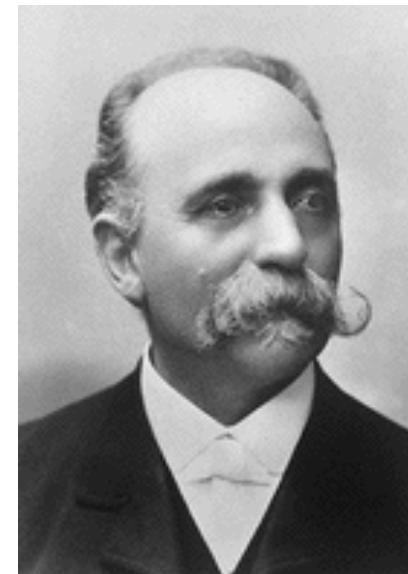
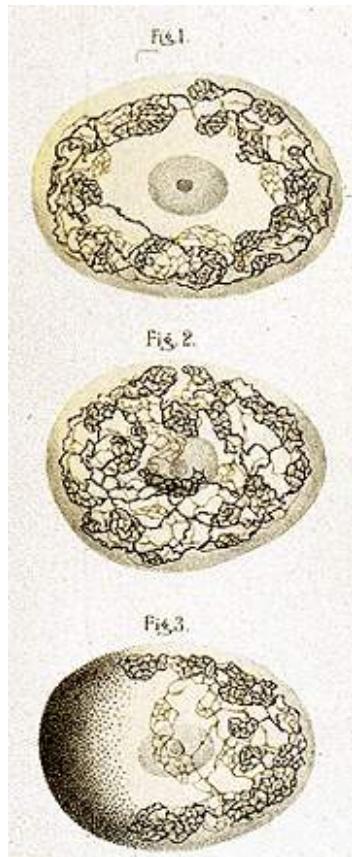
# Molecular Traffic Patterns in the Cell



All proteins of these compartments, regardless whether integral membrane or luminal proteins, are first synthesized in the ER and delivered to their final destinations by vesicular transport pathways.

Plastids are organelles of plants and algae.

# The Golgi Apparatus



**1843-1926  
1906 Nobel prize in  
Physiology or Medicine**

**First drawings of the Golgi**

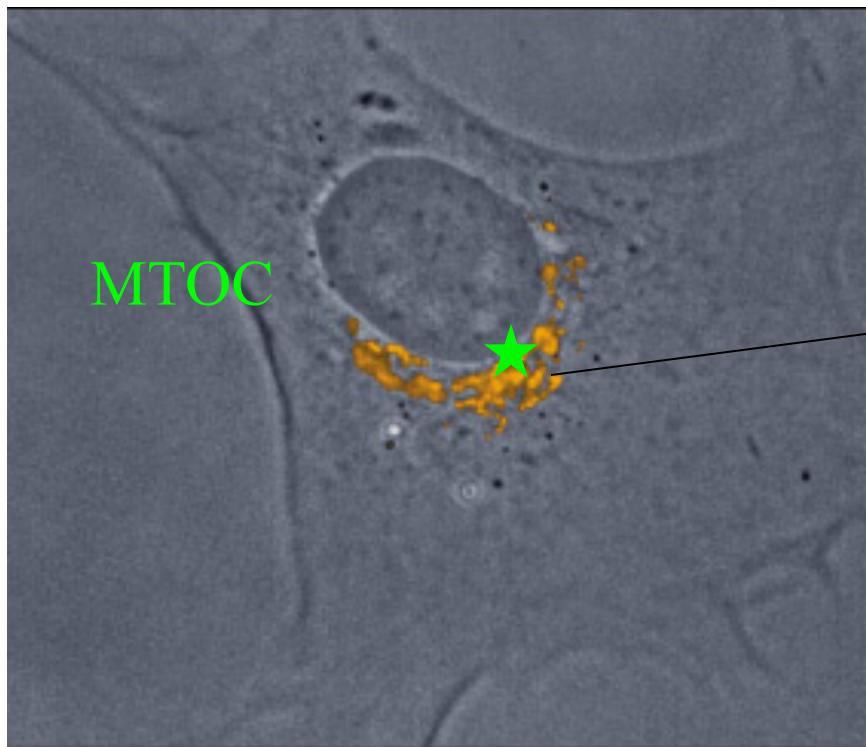
# The Golgi in an Animal Cell is Often Polarized

The Golgi often appears as a ribbon-like compact structure located on one side of nucleus (near the microtubule organization center, MTOC)

Microtubules are required for maintaining the perinuclear localization of the Golgi



a type of cytoskeleton component (to be discussed later)

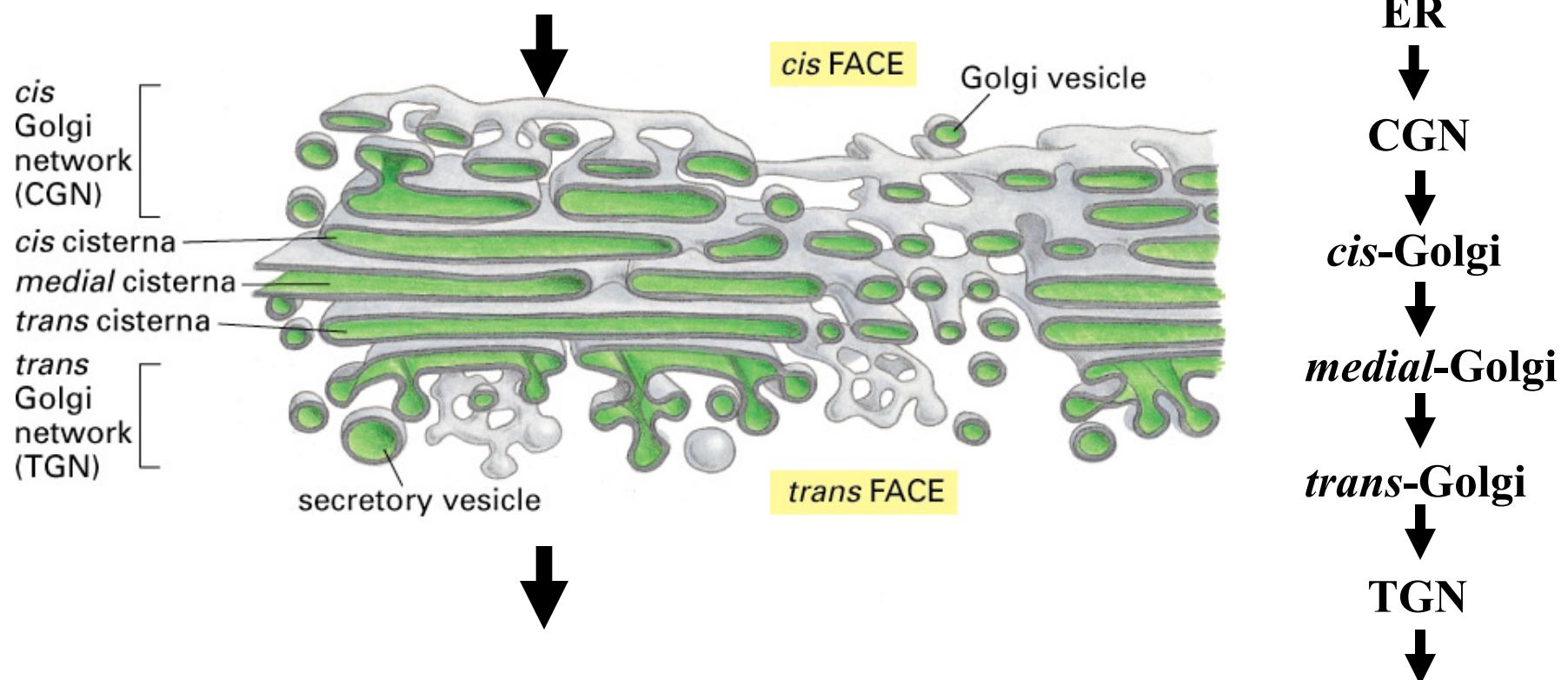


Golgi marker

# The Golgi apparatus can be Divided into Five Parts

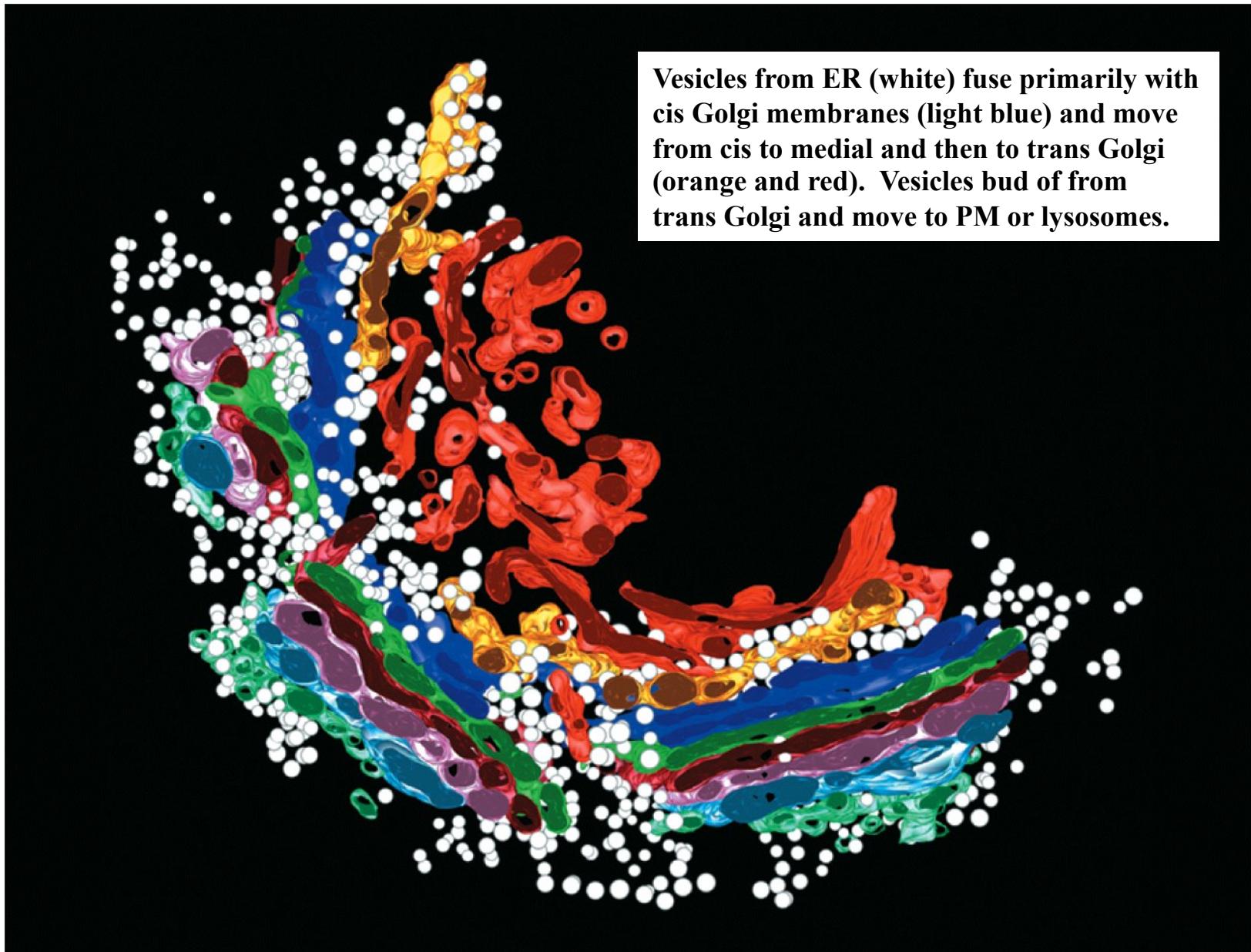
**CGN, *cis*-Golgi, *medial*-Golgi, *trans*-Golgi, and TGN  
(packed together into a compact ribbon-like structure)**

**The CGN faces the nucleus and receives the cargo from the ER**



**The TGN faces the PM and sorts the cargo into various compartments, including PM, endosomes, and specialized secretory vesicles**

# The Golgi Apparatus



Vesicles from ER (white) fuse primarily with cis Golgi membranes (light blue) and move from cis to medial and then to trans Golgi (orange and red). Vesicles bud off from trans Golgi and move to PM or lysosomes.

Figure 9-6

*Molecular Cell Biology, Sixth Edition*

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# Functions of the Golgi Apparatus

biosynthesis of glycolipids  
(fatty acids are made in ER)

covalent modifications of proteins  
(occurring throughout the Golgi apparatus)

mature protein sorting  
(occurring at the later Golgi compartments)

targeted secretion  
(occurring in special types of cells)

# **Major Types of Protein Modifications in the Golgi Lumen**

## **Glycosylation (examples below):**

**N-link glycosylation (Asn-X-Ser/Thr)**

**(begins co-translationally in the ER but completes in the Golgi)**

**O-linked glycosylation (Ser or Thr)**

**(a post-translational modification beginning in ER and Golgi)**

**Glycosaminoglycans (Ser)**

**(a post-translational modification beginning in Golgi)**

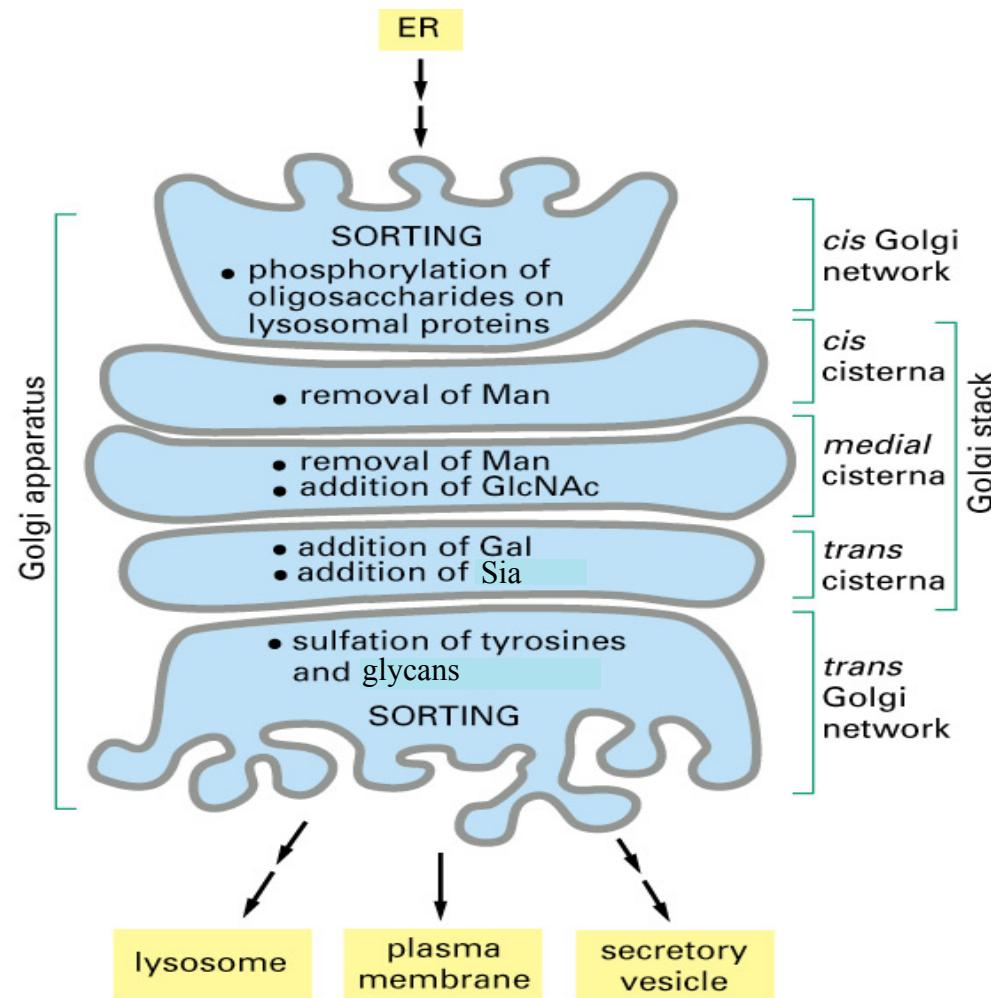
## **Sulfation**

**(on select glycan linkages, and tyrosine amino acids of proteins in the Golgi)**

## **Phosphorylation (rare in the lumen of the ER and Golgi)**

**(some secretory proteins & as a sorting signal to lysosomes, discussed later)**

# Compartmentalization of Protein Modifications within the Golgi



## General feature 1:

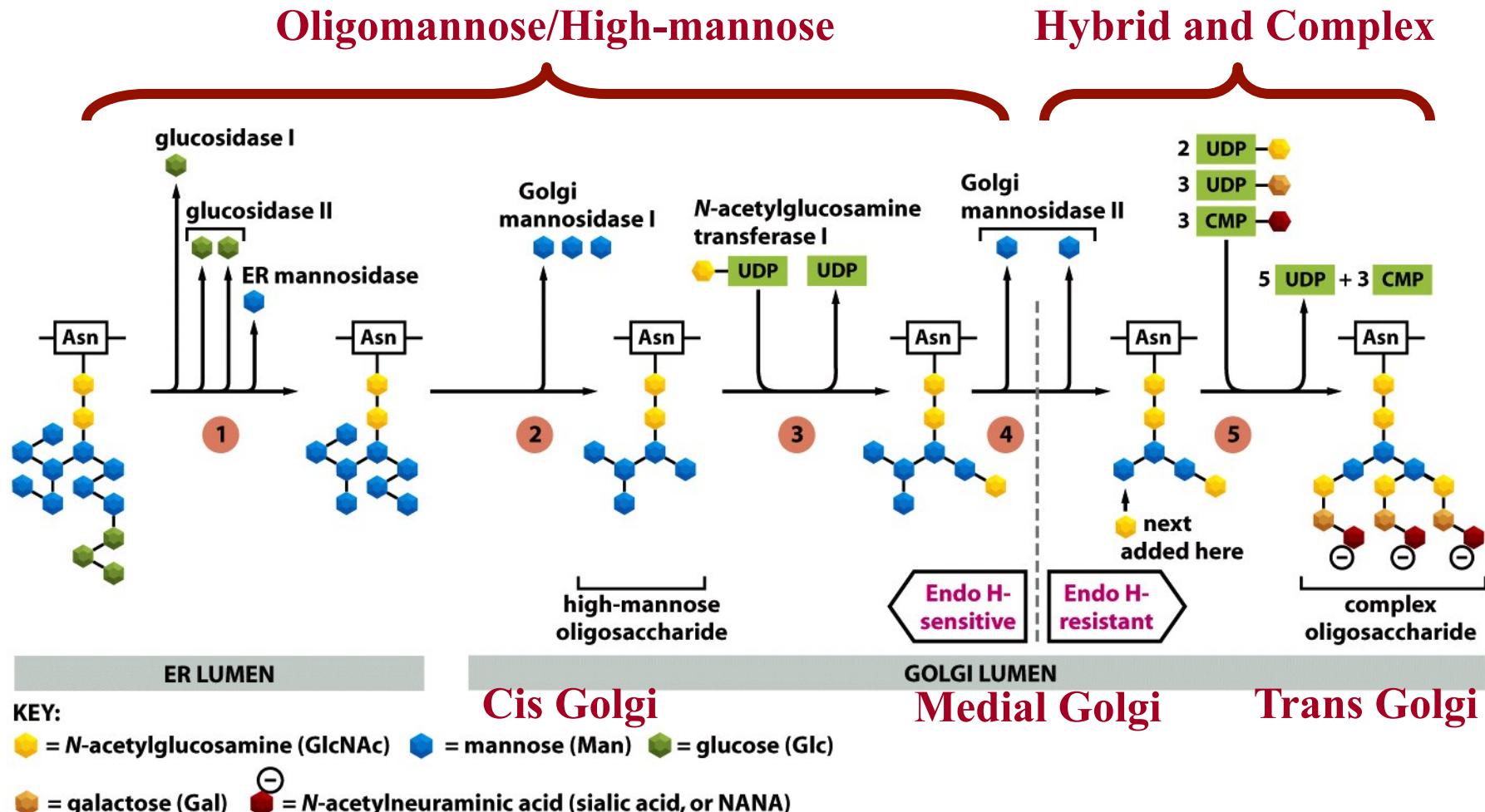
The distribution of a modifying enzyme is graded (i.e. enriched if not restricted to a specific subcompartment).

## General feature 2:

For a specific modification event, the completion of the early modification is usually required for the late modification step to take place (stepwise/sequential)

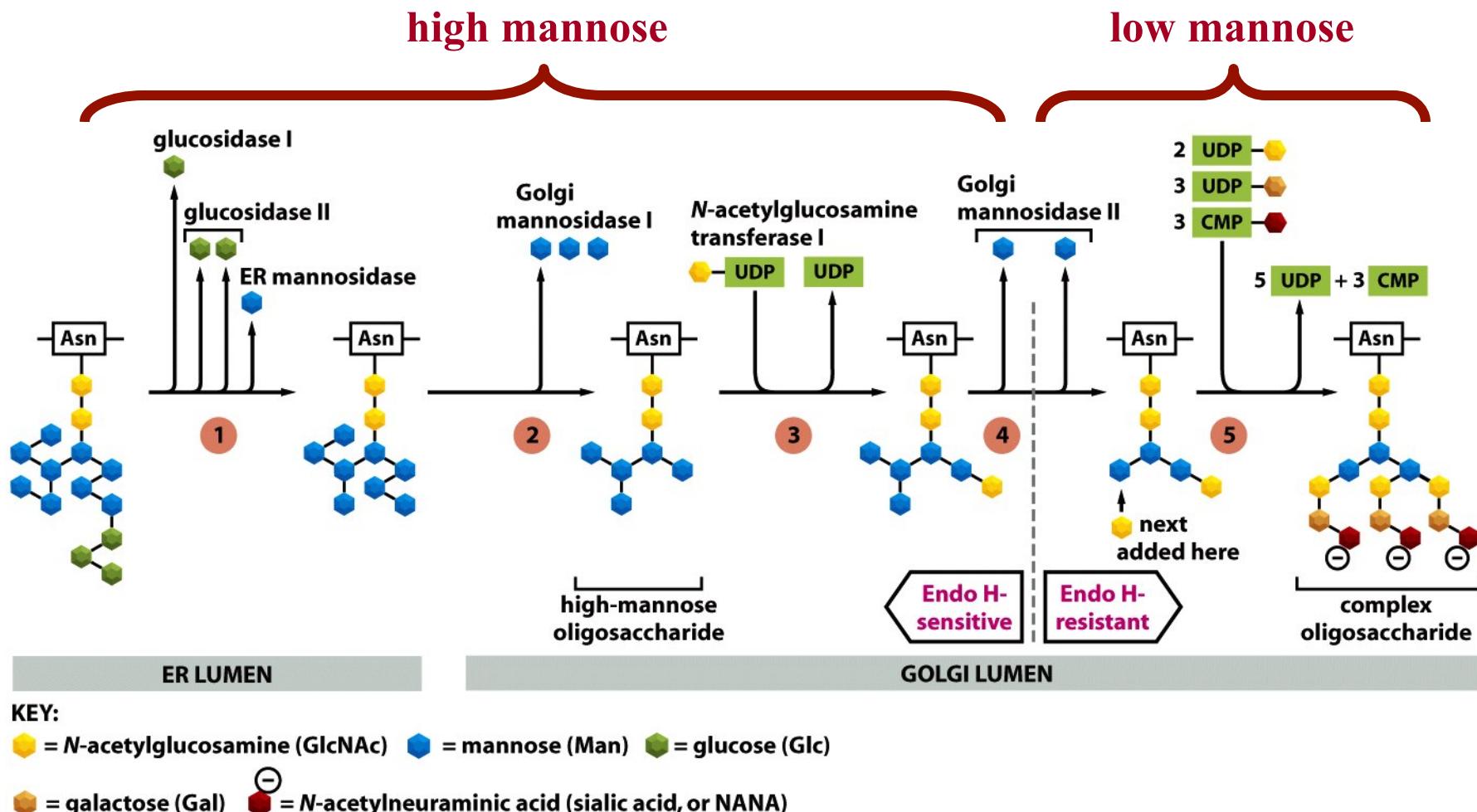
# One Example: “Sequential” N-linked Glycosylation

(note different/incorrect monosaccharide symbology is used in this slide)



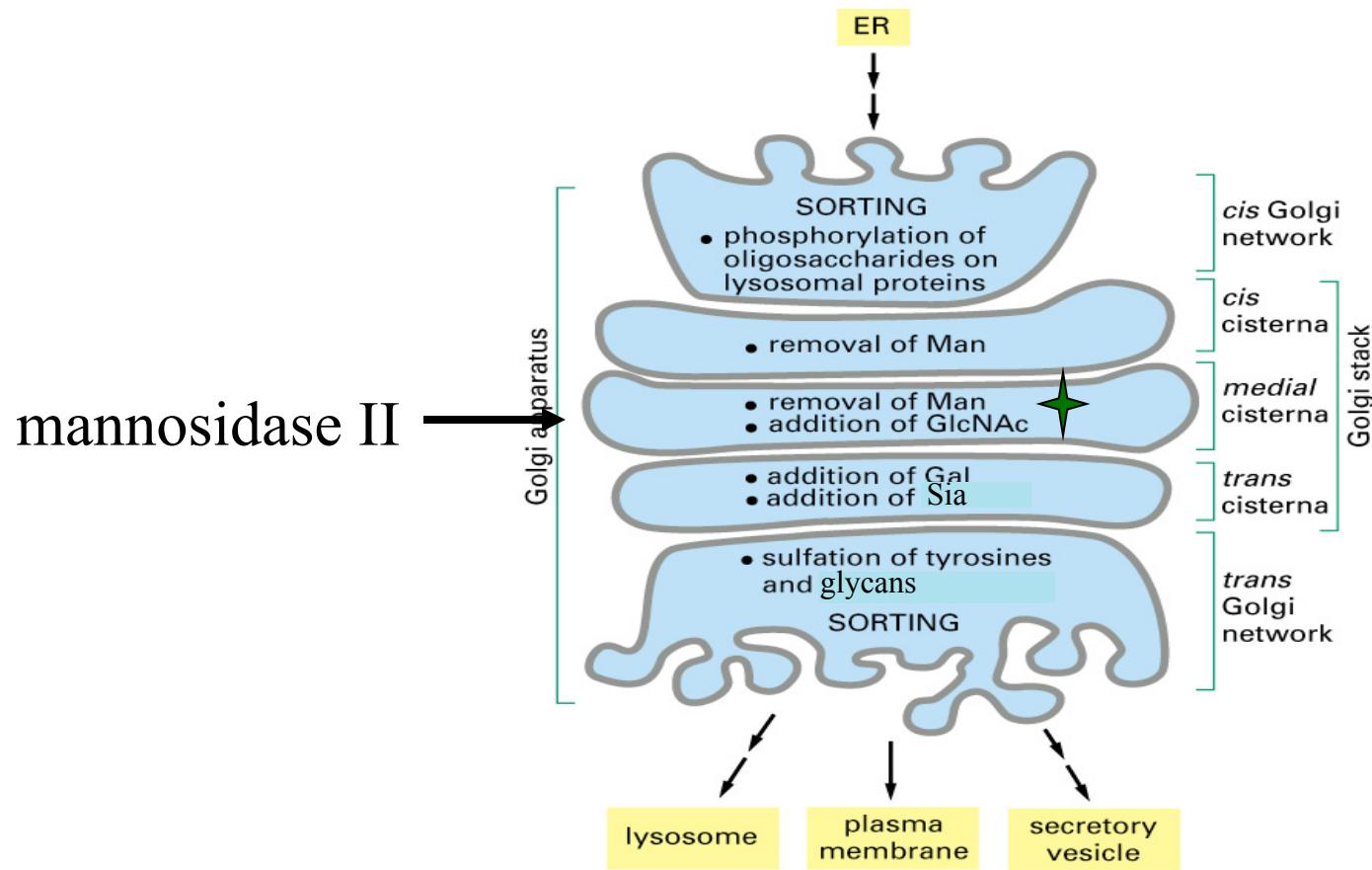
Mannosidase II resides in the *medial-Golgi* compartment

**Endo H is a Specific Endoglycosidase which Recognizes an Unprocessed Hybrid Form of N-Glycans (but not the Complex Form of N-Glycans) and Cleaves it**



**Golgi mannosidase II removes two mannoses leading to the resistance of this intermediate form of the hybrid N-glycan to the digestion of Endo H.**  
**Note the localization of the mannosidase II within the Golgi....**

# Endo H sensitivity as an Indicator of ER-to-Golgi Transport



Since the mannosidase II is localized in the *medial*-Golgi, N-glycans of a glycoprotein become resistant to the Endo H when and only when the cargo reaches the *medial*-Golgi.

How is the graded distribution of a modifying enzyme maintained?

# **Retention/Retrieval of Golgi Resident Proteins**

## **Retention mechanism:**

**Transport (vesicle) carriers leaving Golgi destined for plasma membrane have a higher content of cholesterol and thus a thicker lipid bilayer.**

**Most of the transmembrane alpha-helix domains of plasma membrane proteins are longer (>20~25 a.a.), while those of resident Golgi proteins are usually shorter (~15 a.a.).**

**Due to the short transmembrane domain, many Golgi resident proteins are excluded from export vesicle carriers by this type of retention.**

## **Retrieval mechanisms:**

**to be discussed later**

**Both retention and retrieval mechanisms work together to keep Golgi proteins in the correct subcompartments.**

# **Functions of the Golgi Apparatus**

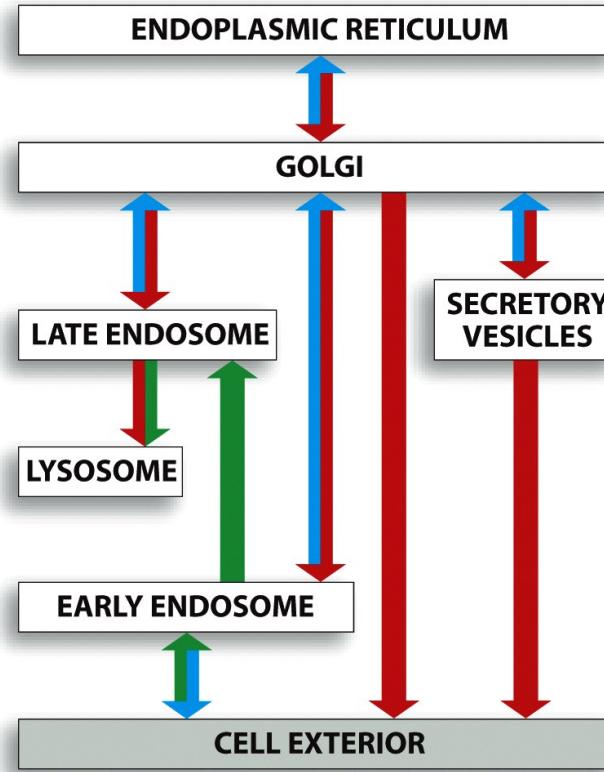
**biosynthesis of glycolipids and sphingomyelin  
(derived from ceramide which is made in ER)  
(not to be further discussed)**

**covalent modifications of proteins  
(occurring throughout the Golgi apparatus)**

**protein sorting  
(occurring at the late Golgi compartments)**

**targeted secretion  
(occurring in special types of cells)**

# The Golgi apparatus lies in the crossroad of both biosynthetic and recycling pathways



The Golgi is the major sorting station of biosynthetic pathways  
The Golgi is also involved in recycling/retrieving cargo

# **Functions of the Golgi Apparatus**

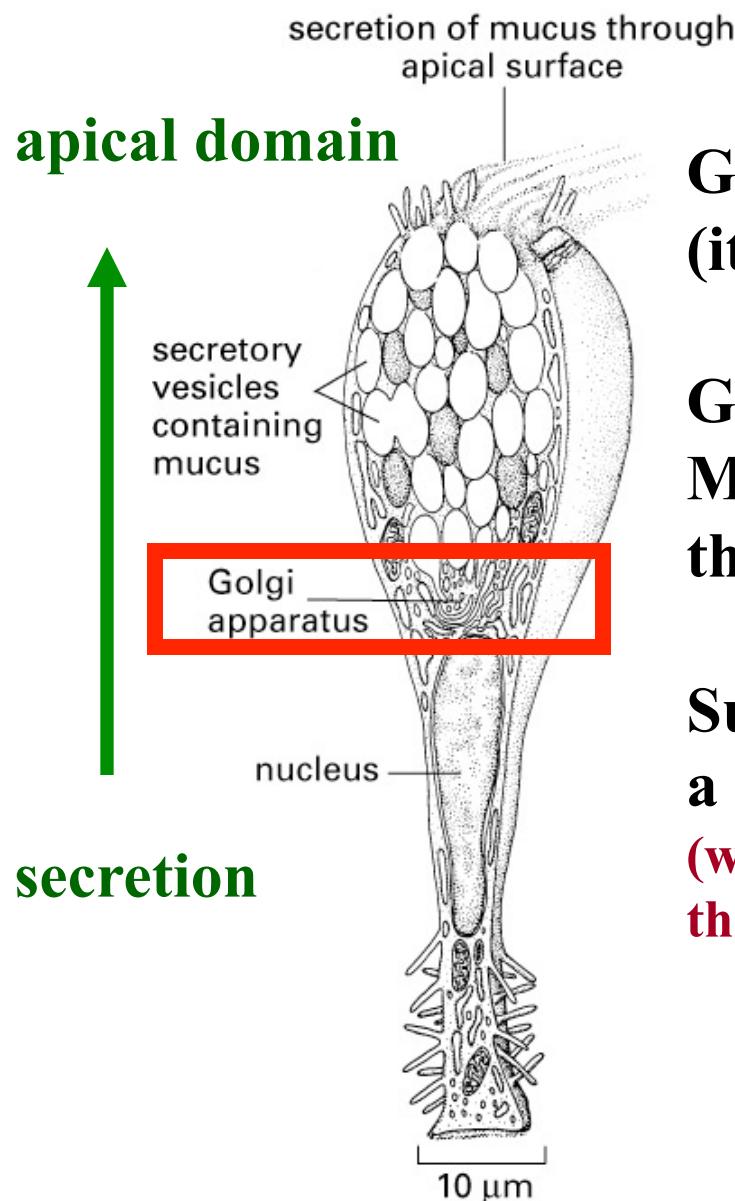
**biosynthesis of glycolipids and sphingomyelin  
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**covalent modifications of proteins  
(occurring throughout the Golgi apparatus)**

**protein sorting  
(occurring at the late Golgi compartments)**

**targeted secretion  
(occurring in special types of cells)**

# The Golgi & Targeted Secretion



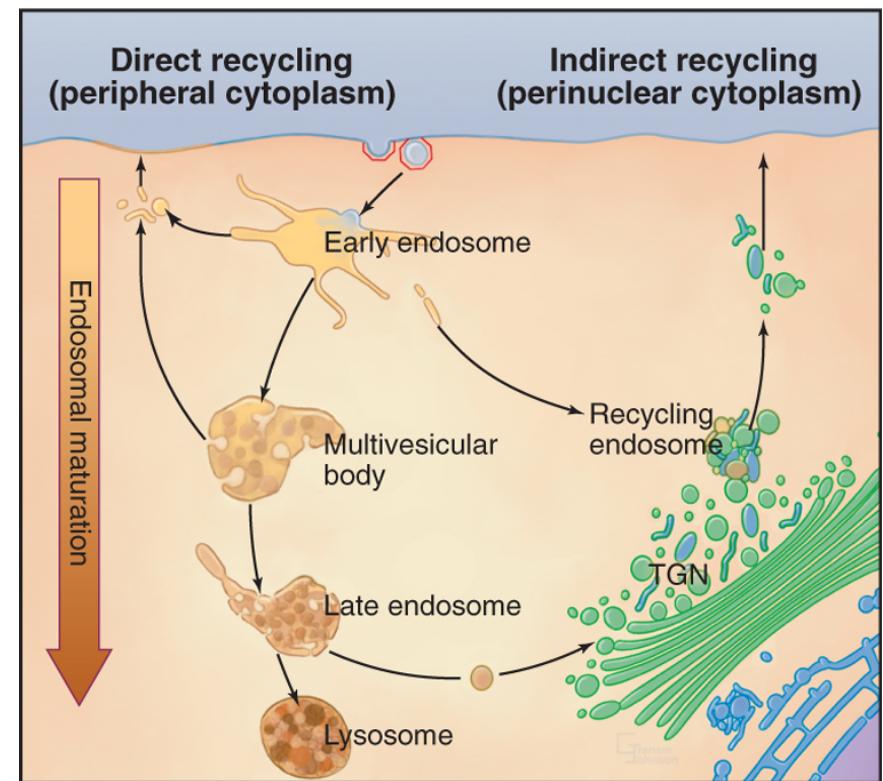
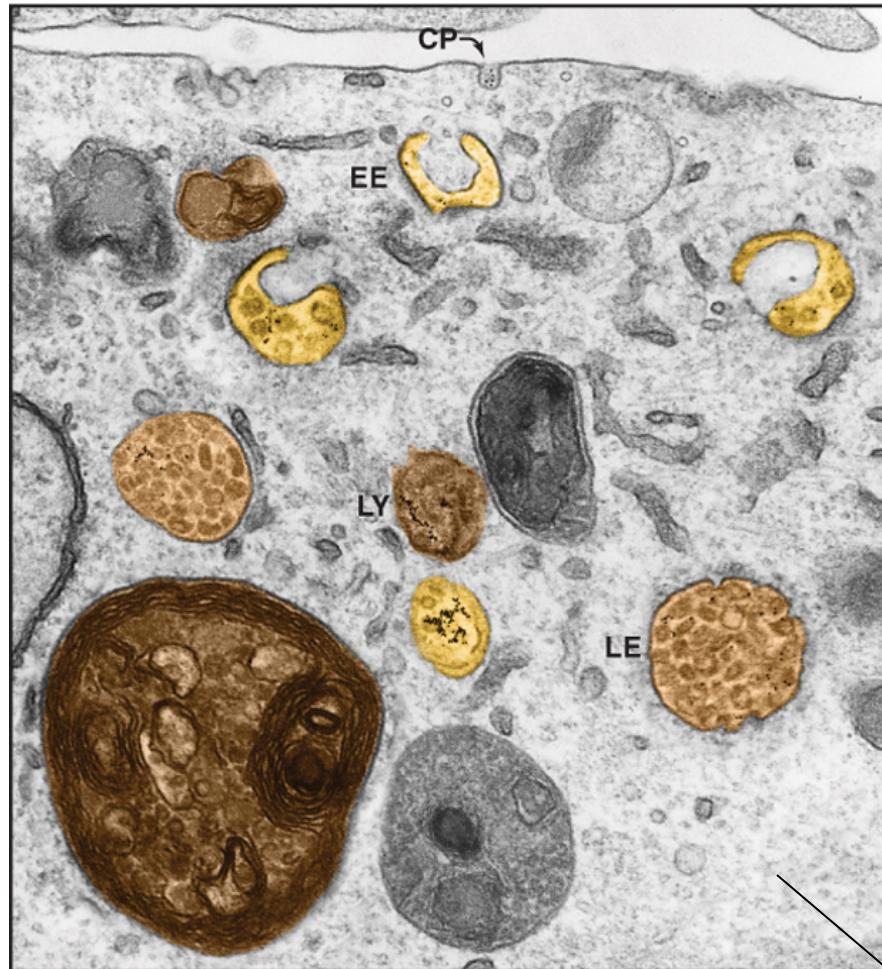
**Goblet cells are a type of epithelial cells (its apical domain facing the gut lumen)**

**Goblet cells secrete mucins that comprise Mucus from their apical domain into the lumen of small intestine**

**Such directional secretions is facilitated by a highly polarized Golgi apparatus (which is localized to the side of the nucleus facing the apical membrane)**

# **Endosomes**

# Endosomes are Vesicles for Transport of Contents To and From the Plasma Membrane

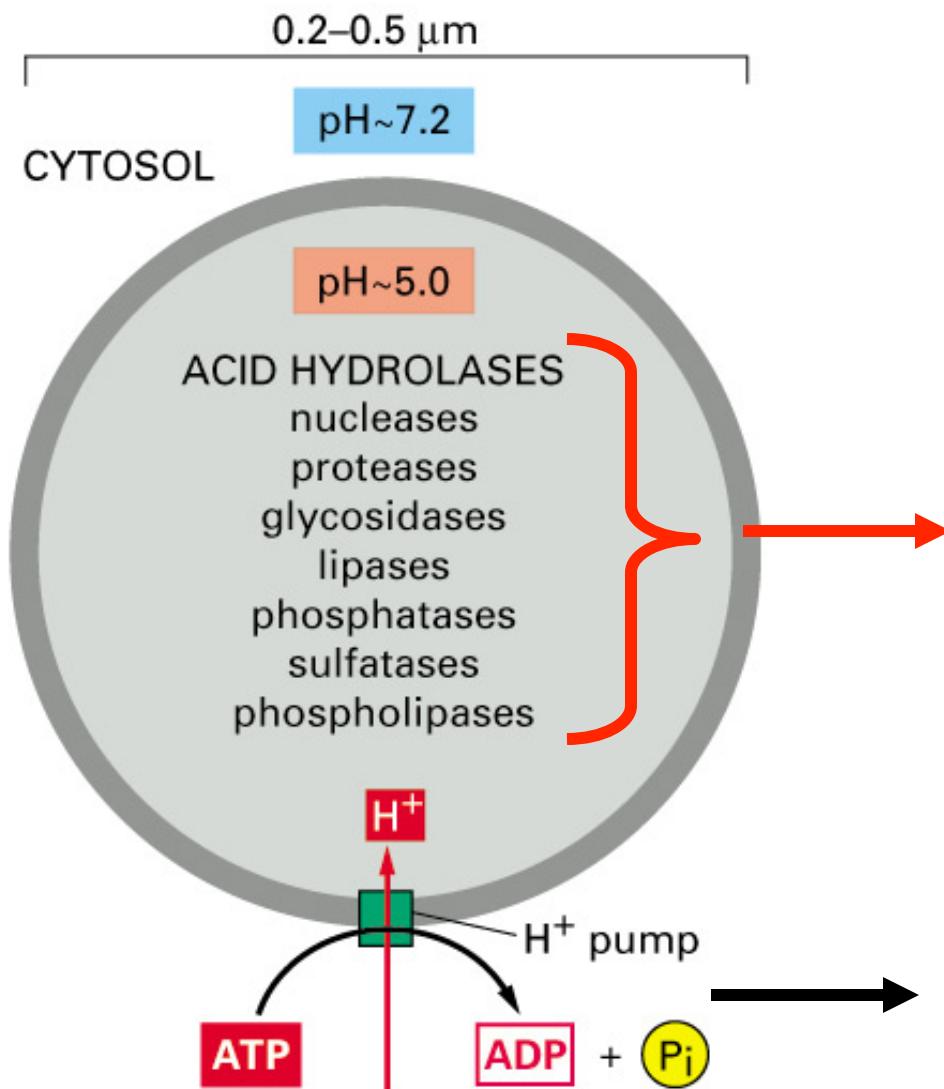


CP: Coated Pit (discussed later)  
EE: Early Endosome  
LE: Late Endosome  
LY: Lysosome

**Lysosomes (Animals)**

**Vacuoles (Plants)**

# Lysosomes are a Collection of Heterogeneous Organelles with a High Content of Hydrolytic Enzymes (Hydrolases)



Hydrolases are luminal enzymes

Different types of hydrolases have different substrate specificities

Hydrolases display optimal enzymatic activity at the acidic pH

Acid hydrolases are often heavily glycosylated

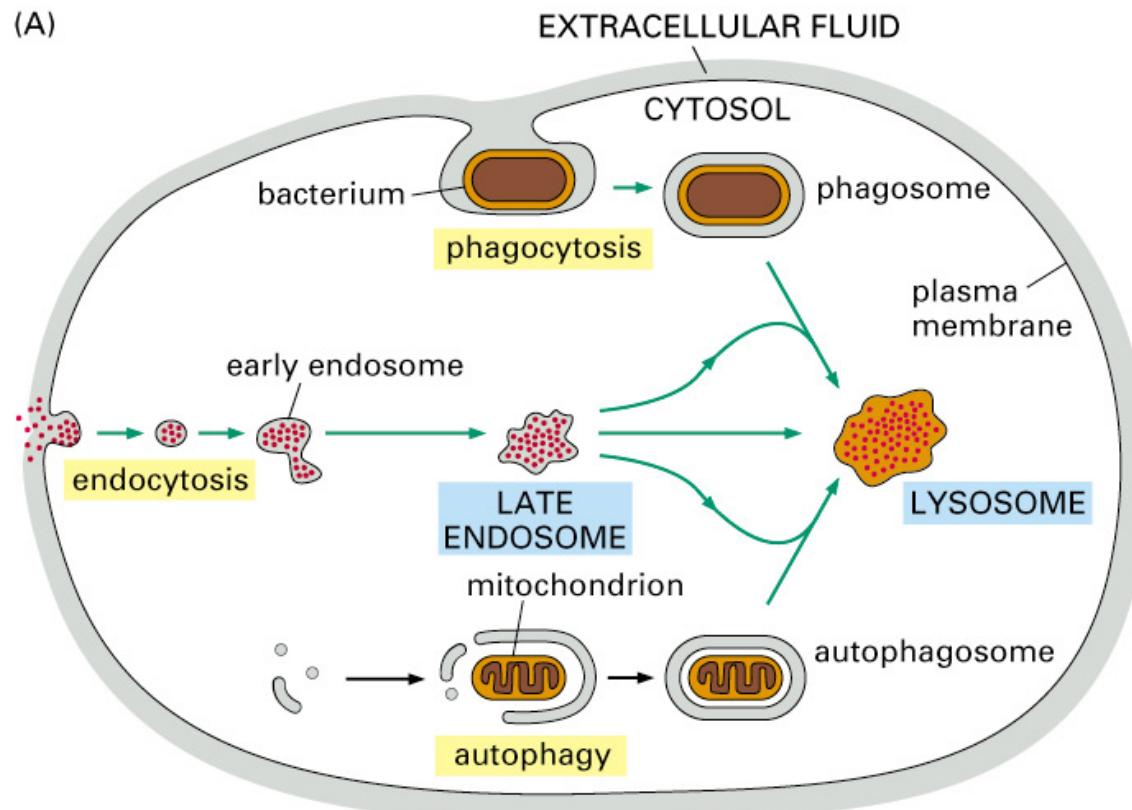
to generate the acidic pH

# **Functions of Lysosomes**

**Conventional Functions:  
the principle site of intracellular digestion in animal cells**

**Nonconventional Functions  
“secretory” lysosomes**

# Three Pathways Leading to Lysosomal Degradation



**Endocytosis:** the process by which eukaryotic cells internalize extracellular fluid, macromolecules, and proteins into membrane bound vesicles

**Phagocytosis:** a ligand-induced process responsible for the uptake of large extracellular particulate ligands (>150-200 nm in diameter such as bacterium) into vesicles (**a special form of endocytosis**)

**Autophagy:** a process by which the cytoplasmic organelles are selectively destroyed (for starvation survival or programmed cell death-apoptosis)

# Lysosomal Storage Diseases

A group of genetic disorders caused by the missing of one or more lysosomal enzymes. As a consequence, molecules normally degraded become accumulated in the lysosomes.

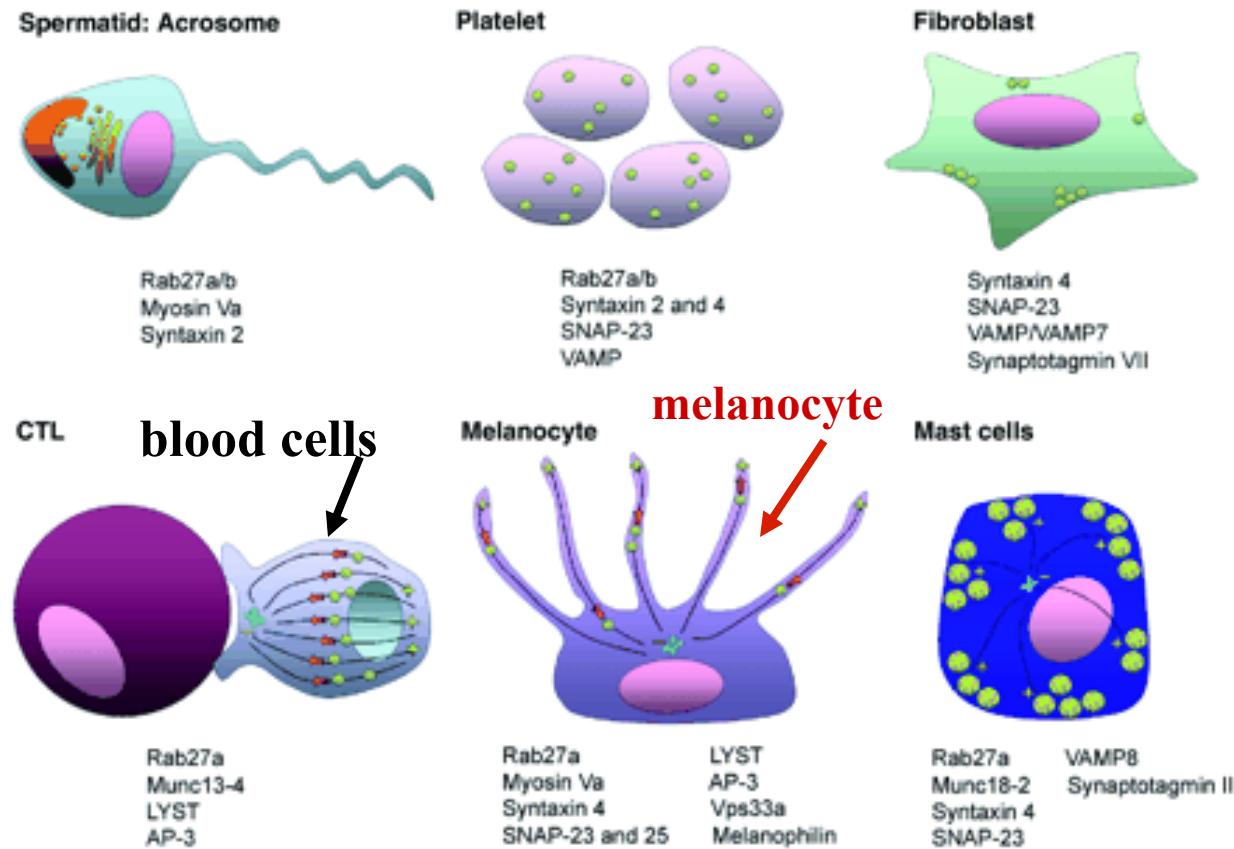
Inclusion Cell (I-Cell) disease is a severe form of lysosomal storage disorder caused by the lack of the GlcNAc phosphotransferase enzyme (as a result, lysosomal enzymes accumulate in the TGN).

# **Functions of Lysosomes**

**Conventional Functions:  
the principle site of intracellular digestion in animal cells**

**Nonconventional Functions:  
“secretory” lysosomes**

# Secretory Lysosomes are Found in Multiple Cell Types with Diverse Functions



A melanocyte is a cell which produces melanosomes.

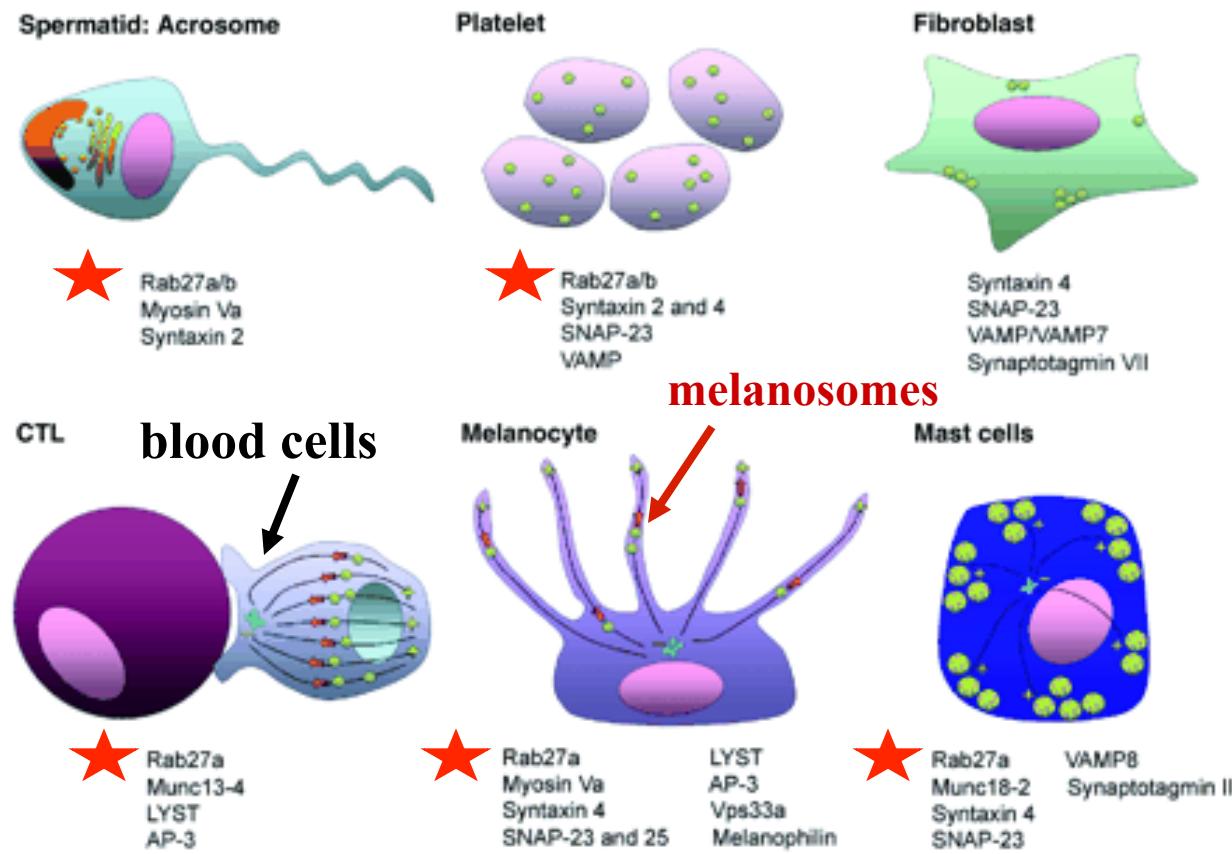
A melanosome is a lysosome-derived organelle containing the melanin pigment.

Melanosome secretion may be regulated in some animals (fish skin color change).

A CTL is a cytotoxic T lymphocyte.

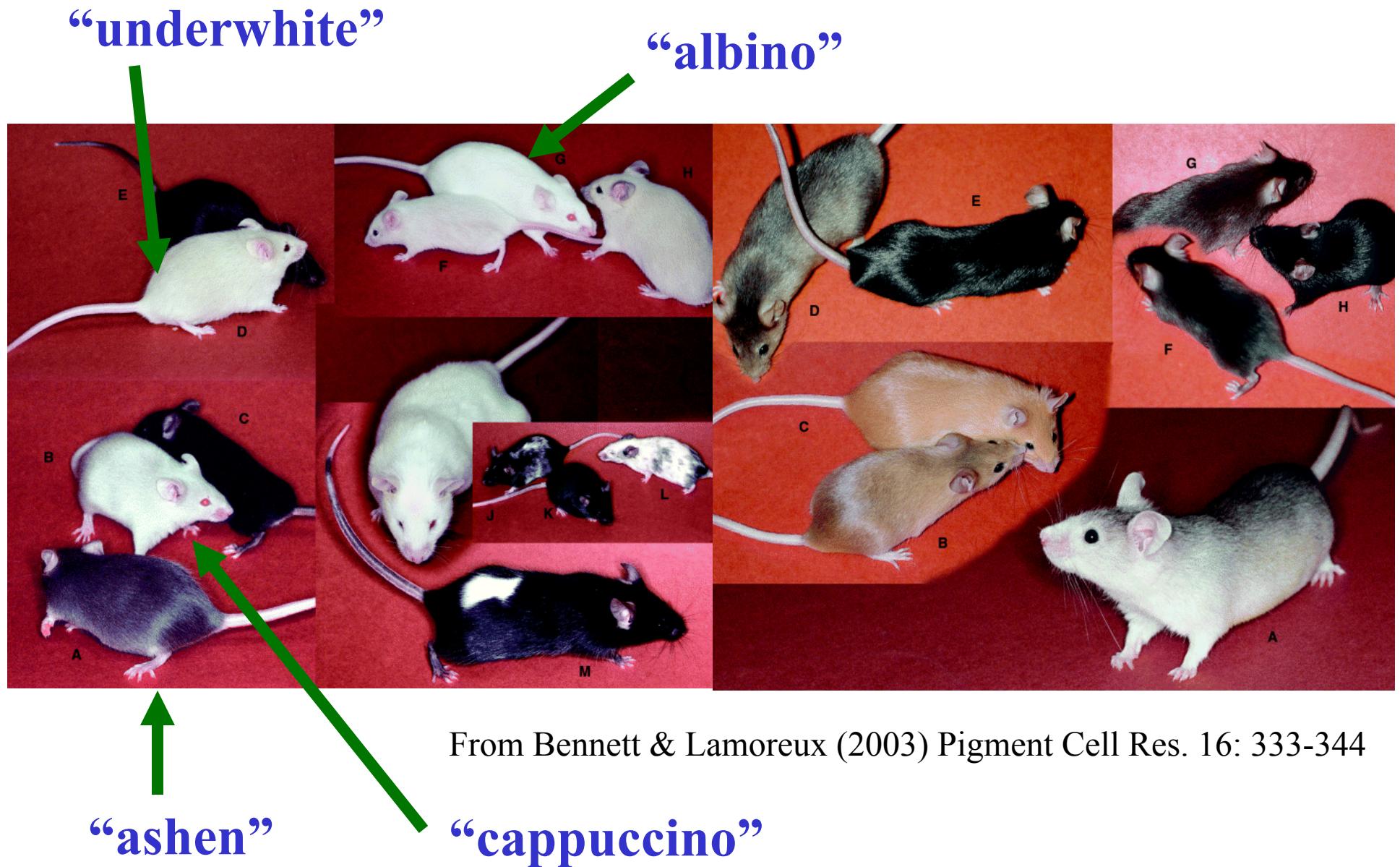
A CTL recognizes and kills the virus-infected or malignant cells by secreting toxic molecules stored in their lysosomes.

# Rab27a is involved in the secretion of lysosomes or lysosome-derived organelles in several cell types



Rab27a modulates transport of melanosomes along the cytoskeleton

# Rab27a (*ashen*) Mutant Mice Display Hypopigmentation



**Secretory lysosomes provides a clue to the missing link  
between albinism and immunodeficiency**

**Rab27a mutations**



**Griselli syndrome in human**

Hypopigmentation  
Immunodeficiency