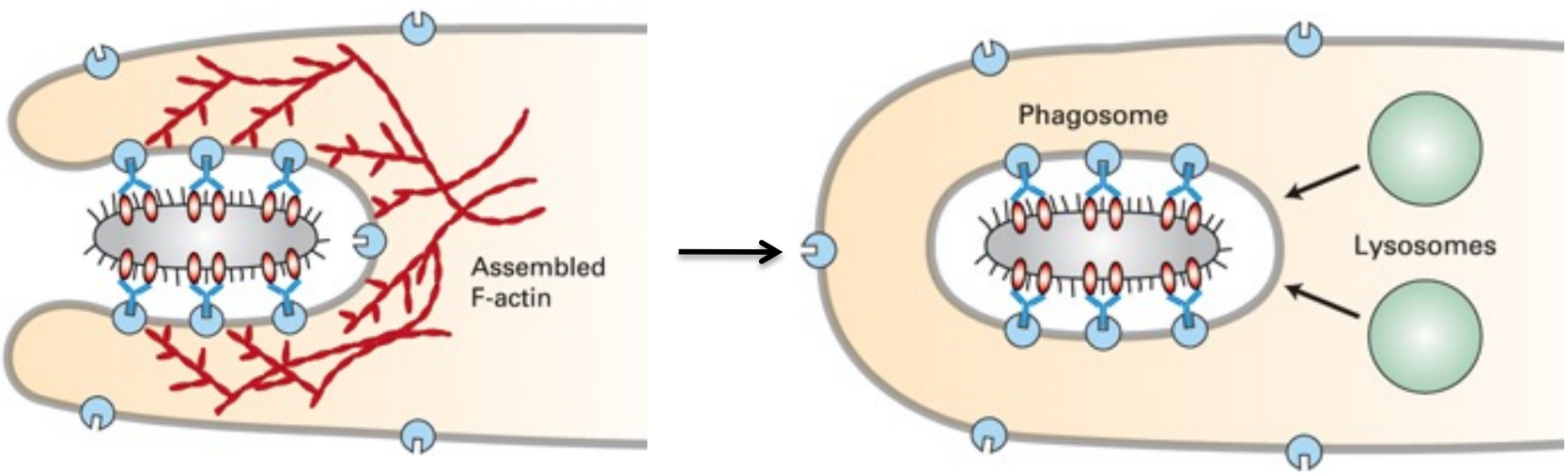
# Lecture 13: Lysosomal breakdown

October 24th, 2022

BICD 110

# Directing membrane proteins and cytosolic materials to lysosome for degradation

- The main function of a lysosome is to degrade extracellular materials taken up by the cell and intracellular components under certain conditions
- Materials to be degraded must be delivered to the lumen of the lysosome where enzymes reside
- **Phagosomes** – large vesicles in which extensions of the plasma membrane envelop ingested material; can fuse with lysosomes and release their contents for degradation (ex. bacteria)




**Lodish Figure 17.19** **Phagocytosis and actin dynamics.**

# Two pathways deliver materials to the lysosomal lumen for degradation

- To degrade endocytosed membrane proteins – utilizes an unusual type of vesicle that buds into the lumen of the endosome to produce a **multivesicular endosome**
- To degrade cytosolic materials – involves the *de novo* formation of a double membrane organelle known as **autophagosome** that envelops cytosolic material (autophagy)

- Both pathways lead to fusion of either the multivesicular endosome or autophagosome with the lysosome, depositing the contents of these organelles into the lysosomal lumen for degradation

# Multivesicular endosomes

- Endocytosed membrane proteins are transferred in their entirety to the interior of the lysosome by **multivesicular endosomes**
- These multivesicular endosomes contain numerous vesicles in their interior that have budded ***inwards*** from the surface into the lumen
- The surface membrane of a multivesicular endosome fuses with the lysosome, thereby delivering its internal contents for degradation

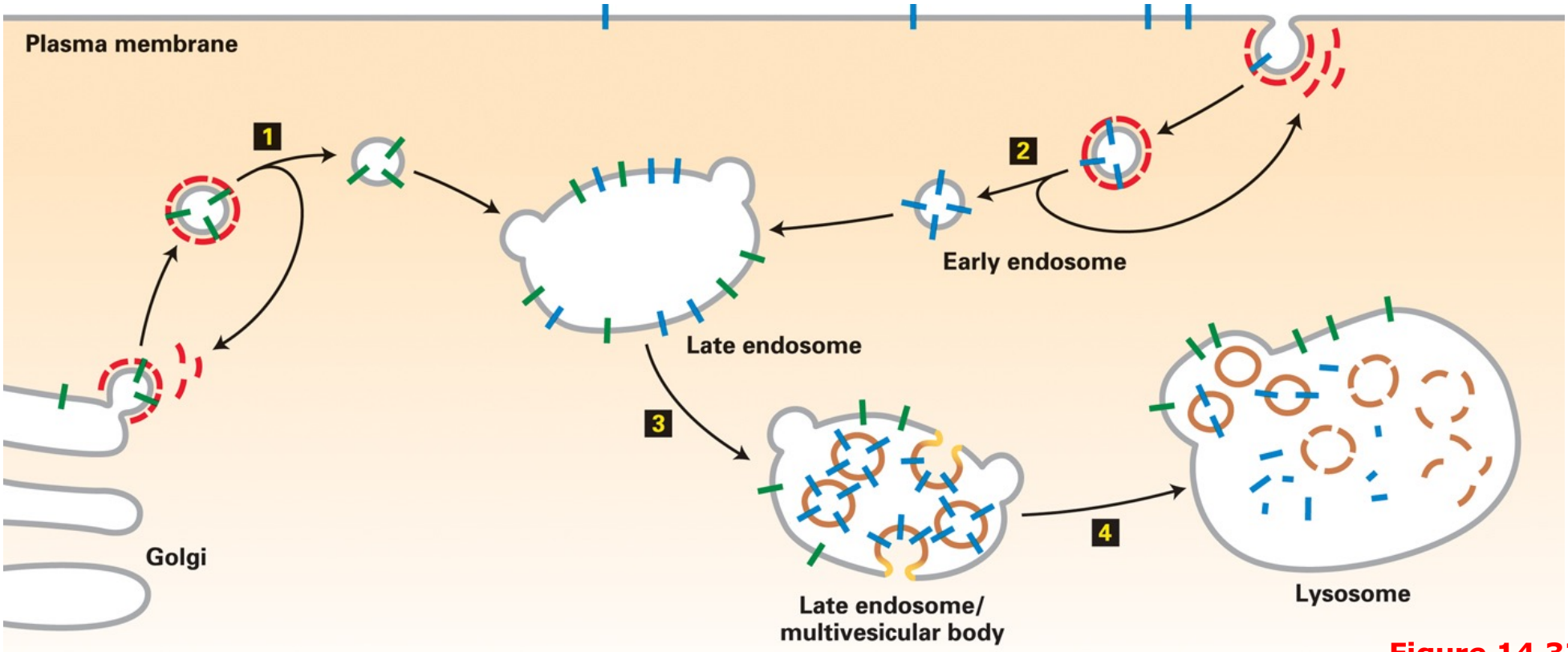



Figure 14.32

# ESCRT proteins

- **Hrs** is a ubiquitin-tagged peripheral membrane protein in the endosome membrane; facilitates recruitment of a set of protein complexes to the membrane
  - **ESCRT** (endosomal sorting complexes required for transport) proteins (this includes ubiquitin-binding protein Tsg101)
- ESCRT proteins pinch off bud, releasing it into the interior of the endosome
- ESCRT proteins are disassembled by the ATPase **Vps4**

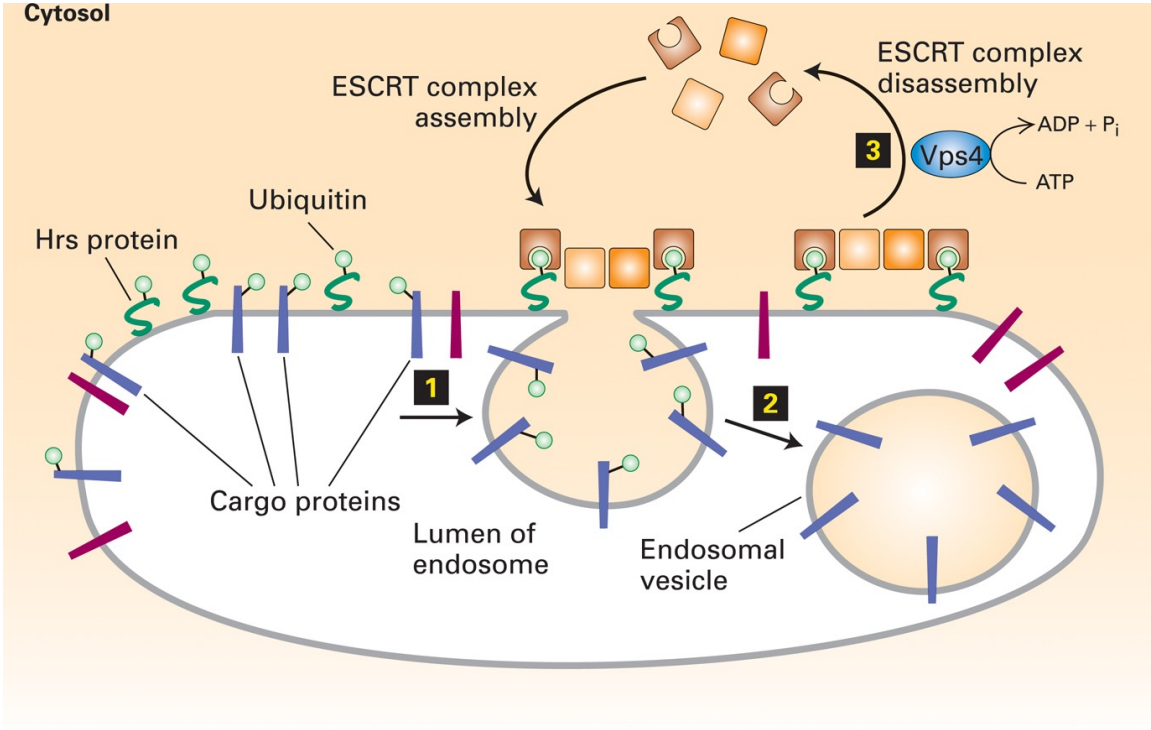
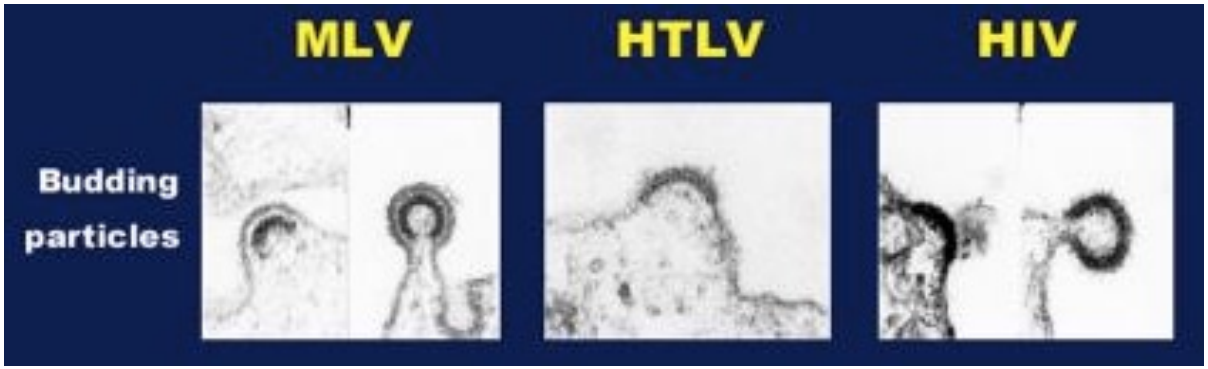



Figure 14.33

# Medical relevance of formation of multivesicular endosomes

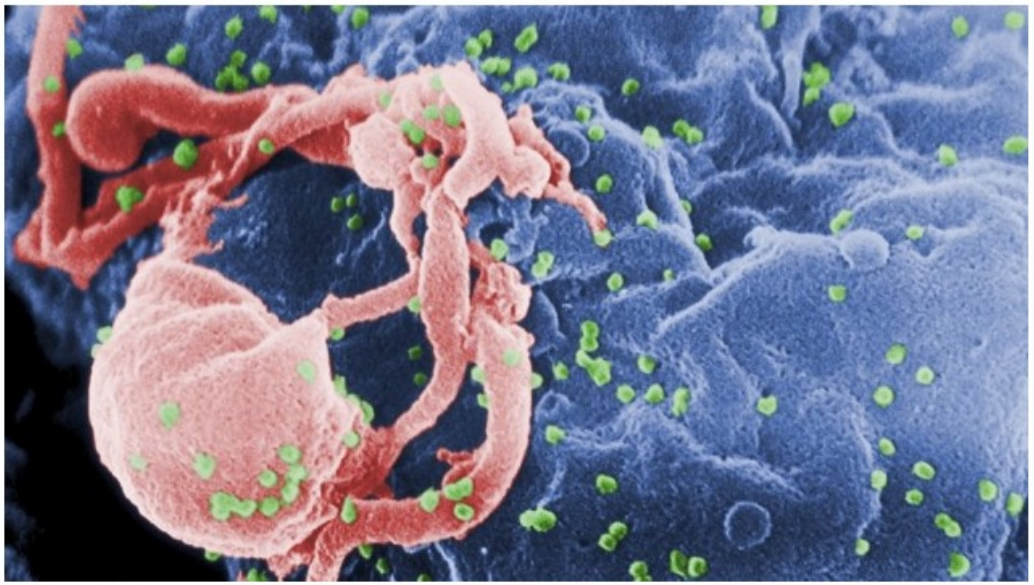
- Retroviruses bud from the plasma membrane by a process similar to the formation of multivesicular endosomes
- A common set of proteins is required for both multivesicular endosome budding and enveloped virus particles that bud from the plasma membrane of virus-infected cells
- Two processes so closely parallel each other that it suggests that enveloped viruses have evolved mechanisms to recruit the cellular proteins used in inward endosome budding for their own purpose




Nisole and Saïb *Retrovirology 2004*

# HIV is an enveloped retrovirus that buds from the plasma membrane of infected cells

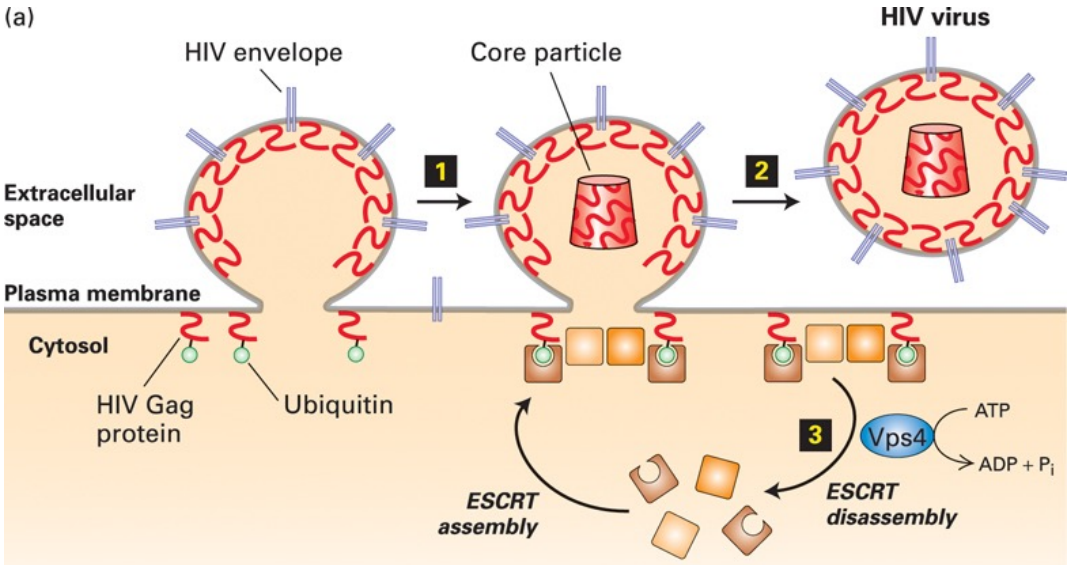
- This process is driven by the viral Gag protein
  - N-terminal segment is required for association with the plasma membrane
  - C-terminal segment is required for pinching off complete HIV particles
- Gag binds to the cytosolic face of the plasma membrane in infected cells and 4,000 Gag molecules polymerize into a spherical shell producing a structure that looks like a vesicle but protruding outward from the plasma membrane



Scanning electron micrograph of HIV-1 budding (in green) from cultured lymphocyte. (photo credit: CDC/public domain)

# HIV budding employs the same molecular machinery as vesicle budding into endosomes

- Tsg101 (ESCRT protein) binds to the C-terminal of Gag protein
- Gag is ubiquitinated as part of the virus budding
  - What does this mimic the function of?
- Vps4 is an ATPase that disassembles ESCRT proteins



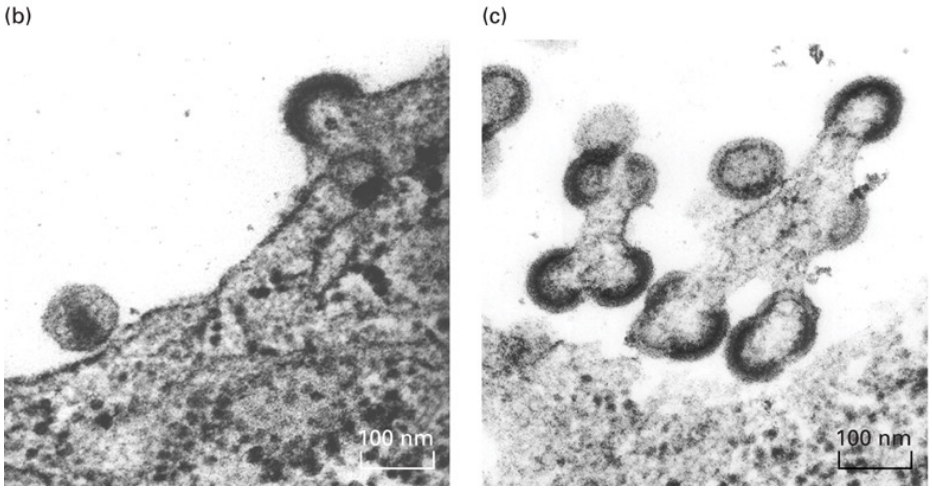
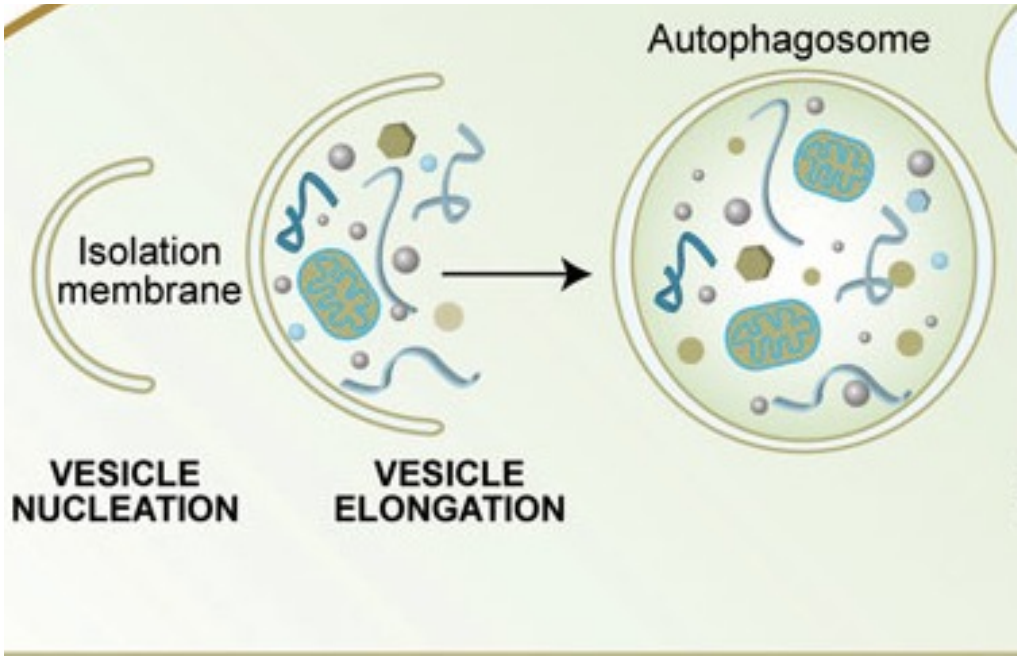




Figure 14.34

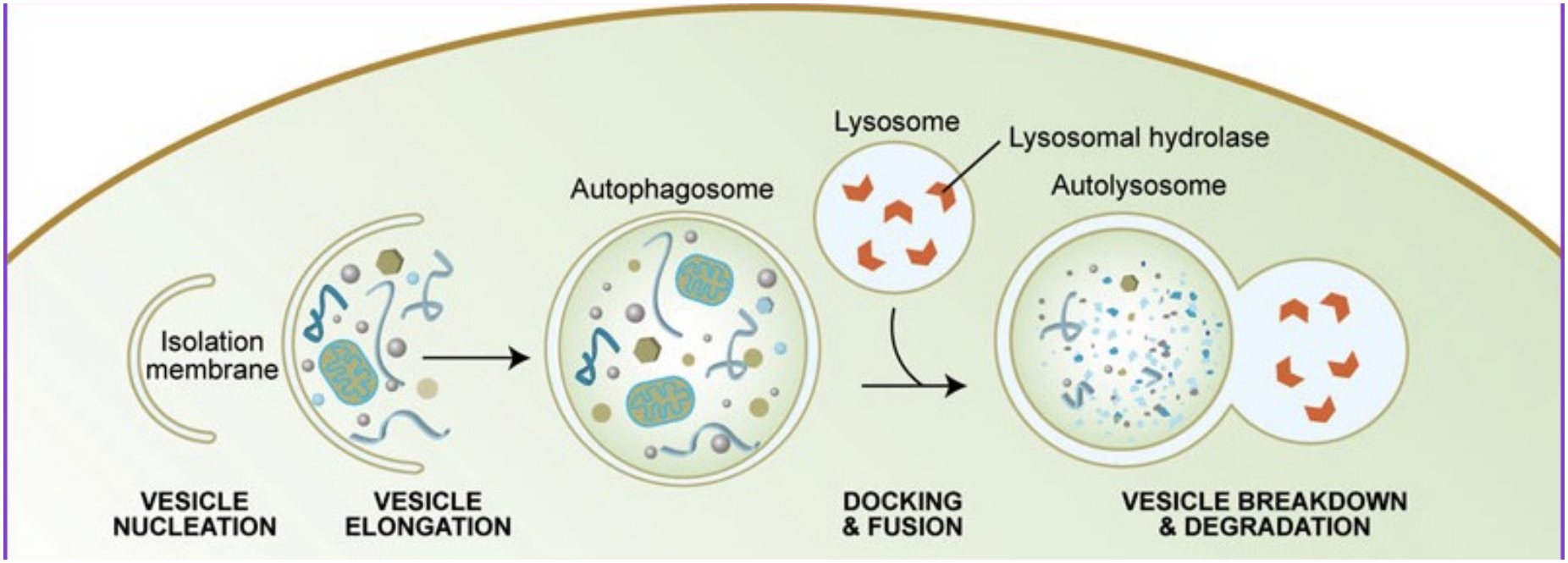
# Autophagy delivers cytosolic proteins or entire organelles to lysosomes

- Cell stresses (ex. starvation) can lead to autophagy “eating oneself” to recycle macromolecules for use as nutrients in a process of lysosomal degradation
- The autophagic pathway involves the formation of a flattened double-membrane cup-shaped structure that envelops a region of the cytosol or an entire organelle (ex. mitochondrion) forming an **autophagosome** or autophagic vesicle




Alicia Meléndez and Beth Levine; Wormbook

- Outer membrane of autophagic vesicle can fuse with the lysosome, delivering a large vesicle, bounded by a singe membrane bilayer, to the interior of the lysosome
- Lipases and proteases within the lysosome will degrade the autophagic vesicle and its contents
- In addition to recycling components under stress conditions, can also target for destruction dysfunctional mitochondria that have lost their integrity and have stopped functioning properly



# How and where the autophagic vesicle forms is unclear

- **Autophagic vesicle nucleation** – origin of vesicle is thought to be from a fragment of a membrane-bounded organelle
  - Origin difficult to trace because no known integral membrane proteins seem to be required for formation of the vesicle

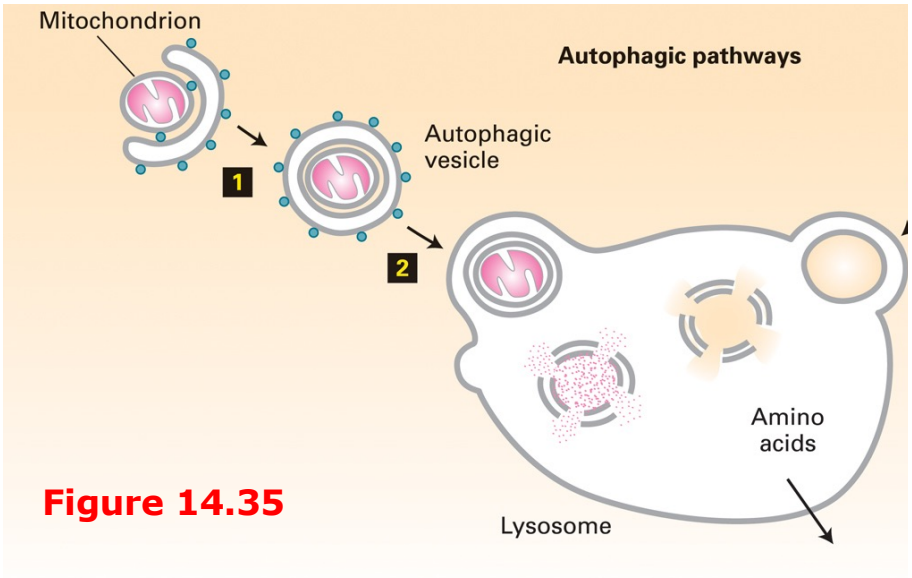


Figure 14.35

Starvation causes a <u>non-specific response</u> in which a random portion of the cytoplasm (and organelles) is enveloped by an autophagosome

Random nucleation site in this case

# When things go wrong – lysosomal storage diseases

- GM2-Gangliosidosis Type I (Tay Sachs Disease)
  - Caused by *HexA* deficiency (enzyme called beta-hexosaminidase A)
  - Progressively destroys nerve cells in brain and spinal cord
- Gaucher Disease (Types I, II, and III):
  - Most common type of lysosomal storage disorder
  - Type I shows absence of neurological complications; most affected individuals have type I, and they may experience easy bruising, chronic fatigue, and an abnormally enlarged liver and/or spleen (hepatosplenomegaly)
  - Mutation in *GBA* gene (beta-glucocerebrosidase)

**Figure 13.1** **Overview of major protein-sorting pathways in eukaryotes.**

- Proteins without an ER-targeting signal sequence are synthesized in the cytosol on free ribosomes
- Proteins with an organelle specific targeting sequence (mitochondria, chloroplast, peroxisome) are first released into the cytosol, then imported