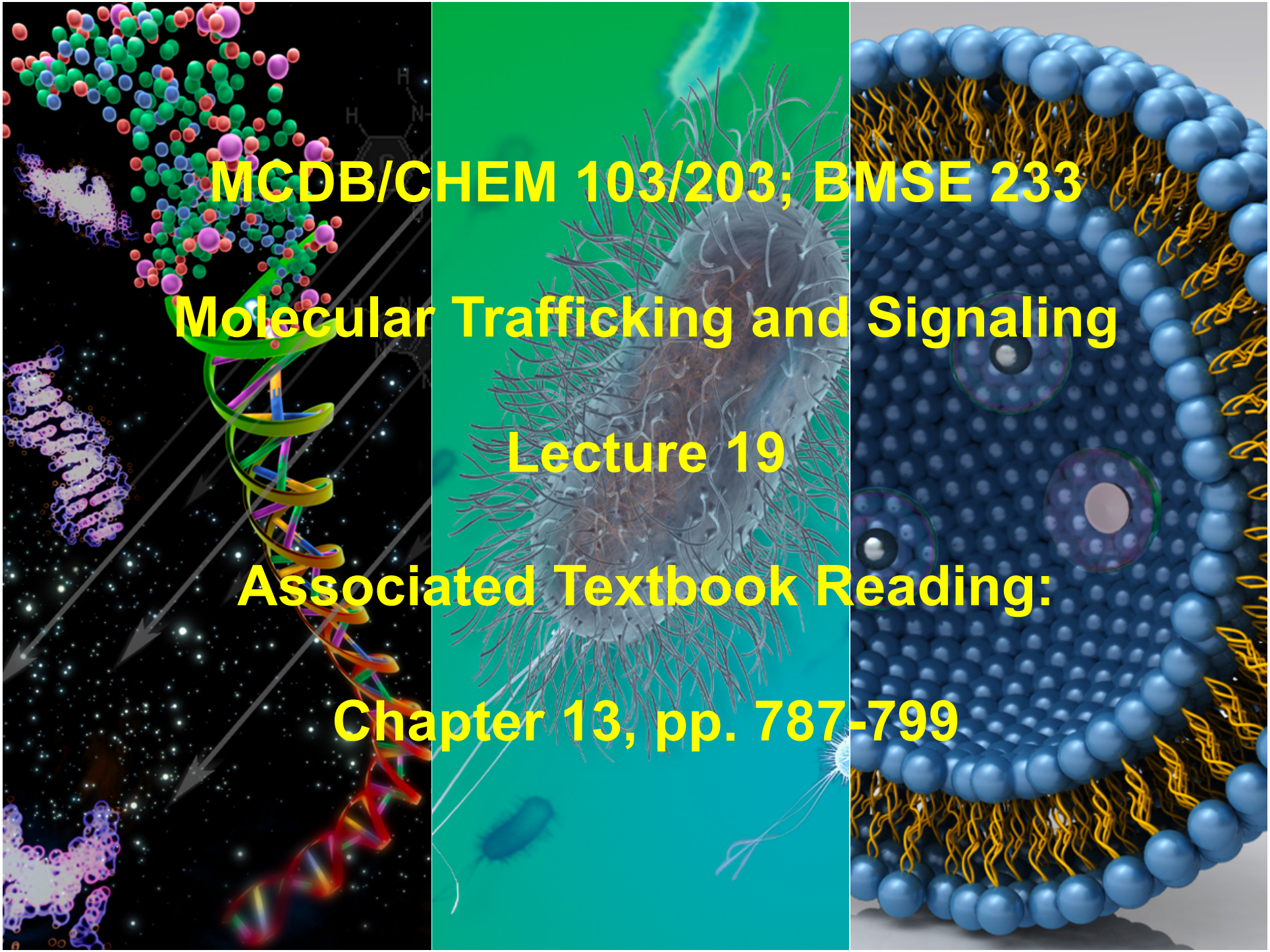


MCDB/CHEM 103/203; BMSE 233

Molecular Trafficking and Signaling

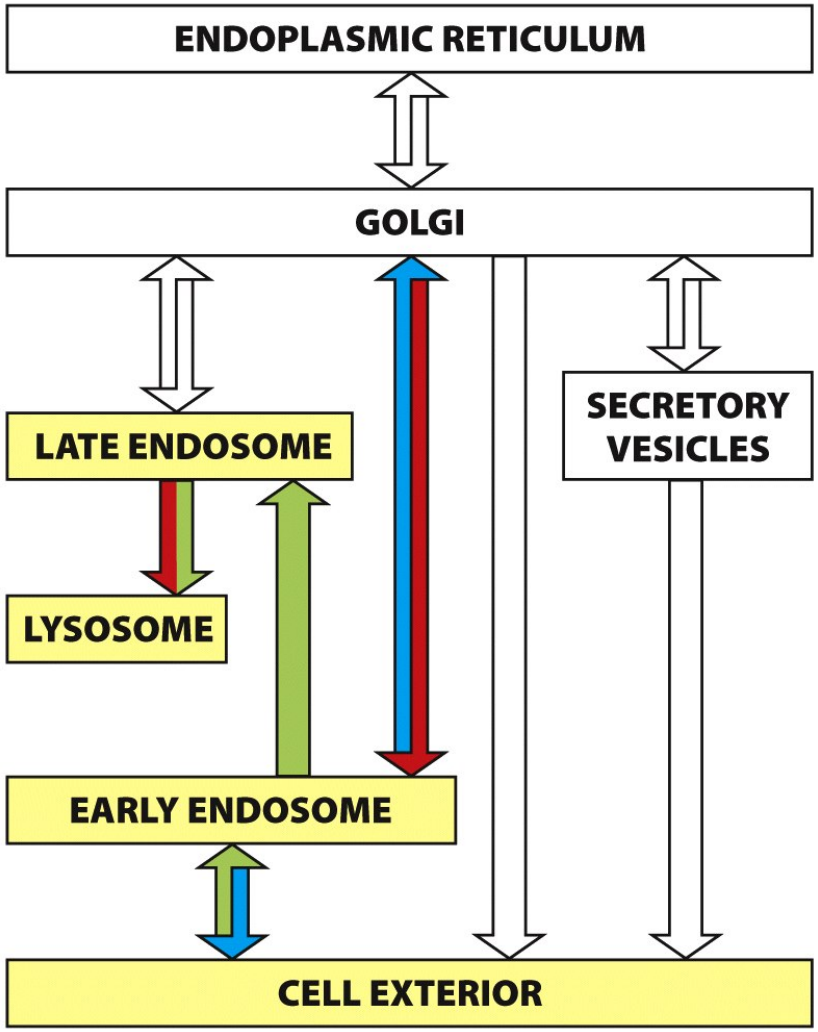
Lecture 19

Associated Textbook Reading:

Chapter 13, pp. 787-799

The Endocytic / Endosomal Pathway

Functions of the Endocytosis/Endosomal Pathways



Nutrient uptake

Homeostasis of molecules (e.g. blood glucose or cholesterol)

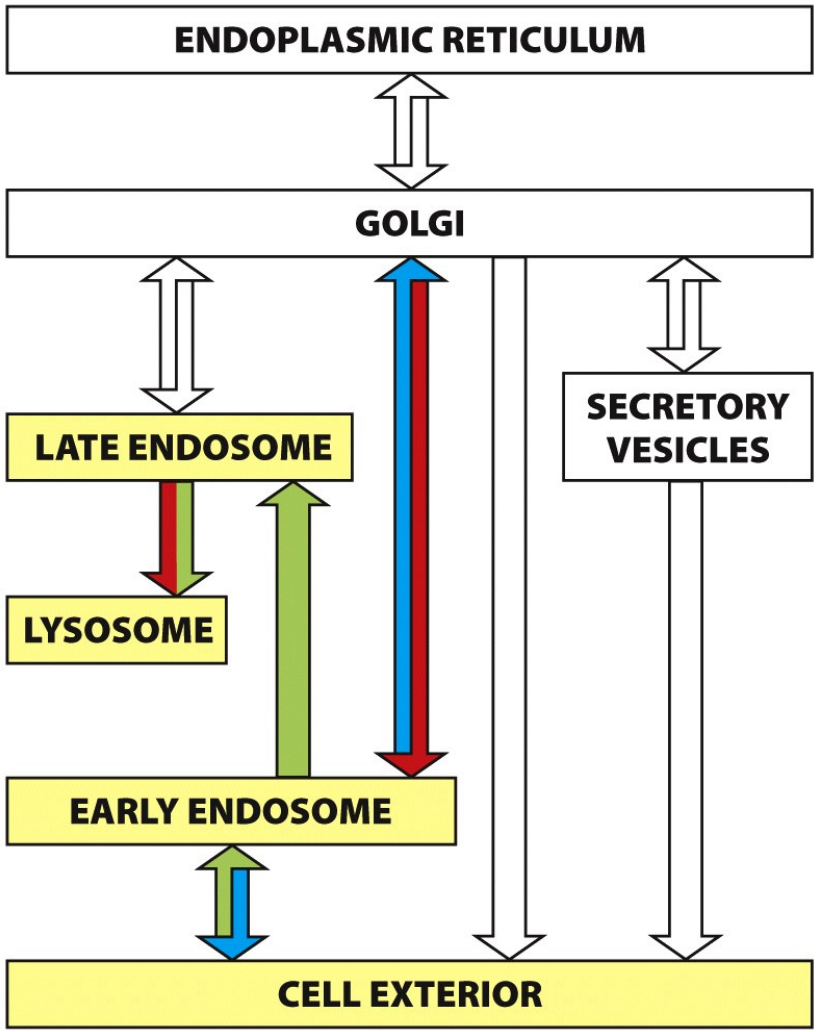
Regulation of surface proteins and lipids

Immunity

Entry of drugs

Entry of pathogens

The Endocytosis/Endosomal Pathways



endocytosis pathway (internalization)

a process by which eukaryotic cells internalize extracellular fluid, macromolecules, and particles into membrane-bound vesicles. Endocytosis also removes proteins and lipids from the plasma membrane (PM).

endosomal pathway (post-internalization fate)

degradative pathway (green):
delivering an internalized molecule to lysosomes for its degradation. (PM to early endosome to late endosome to lysosome)

recycling pathway (blue):
delivering an internalized molecule back to the TGN or PM via endosomes (mostly from early endosome, but some from late endosome or lysosome)

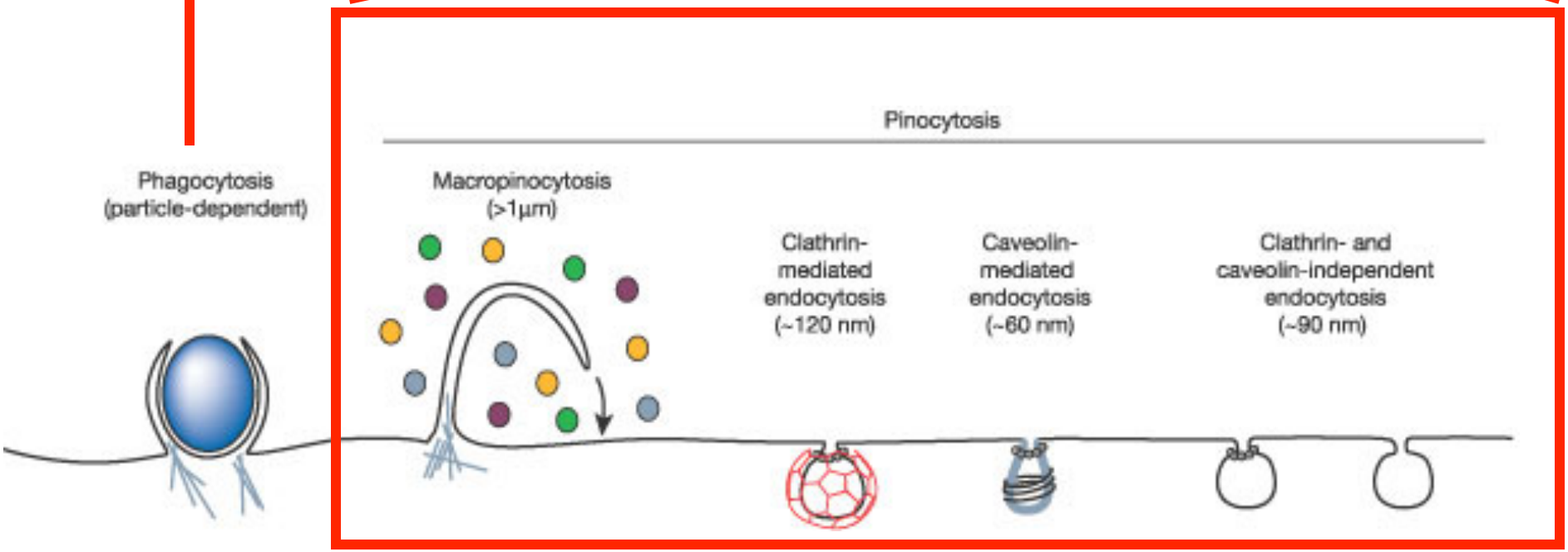
Endocytosis (Internalization)

Endosomal (Endocytic) Trafficking

There are at least 5 different endocytosis pathways in mammals

phagocytosis (particle eating)

pinocytosis (fluid drinking)



membrane moving outward to form large vesicles

~ 1 um diameter

membrane moving inward to form small vesicles

~ 50-100 nm diameter

Phagocytosis

Properties:

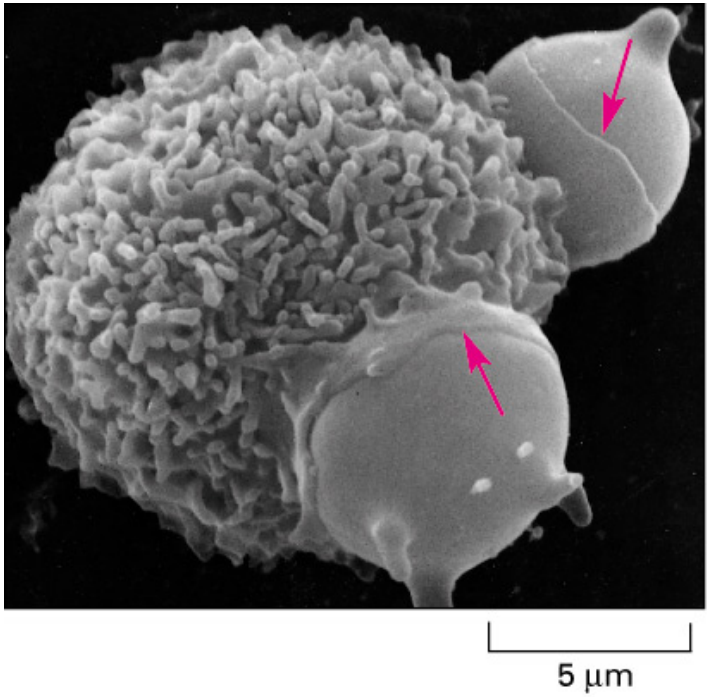
1. engulfment of large particles such as microorganism and dead cells via large endocytic vesicles called phagosomes
2. performed by professional phagocytes such as protozoa or macrophages, neutrophils & dendritic cells in mammals
3. specific (occurs only after binding of particles)

Functions:

1. a way of feeding (for protozoa)
2. scavenging senescent or dead cells (e.g. macrophages phagocytose more than 100 billion senescent red blood cells each day)
3. protect against infection (by ingesting invading microorganisms)

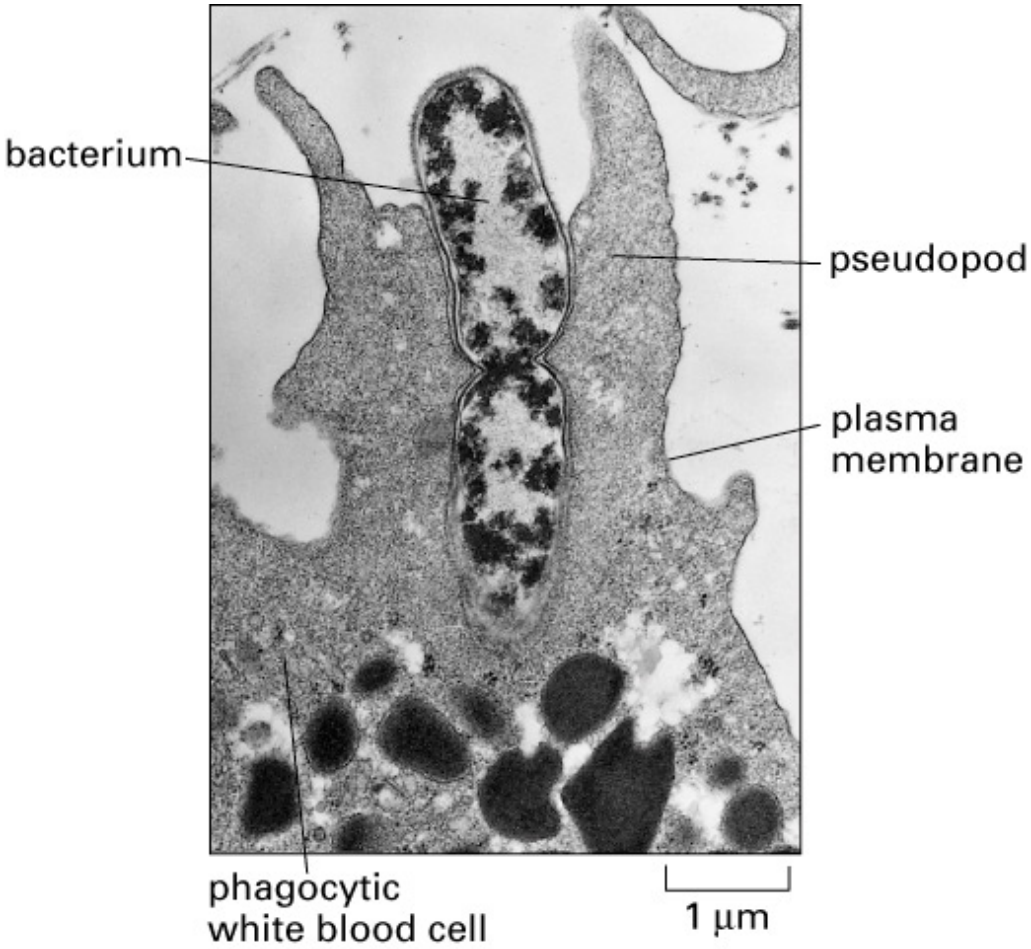
scavenging

phagocytosis of red blood cells by a macrophage

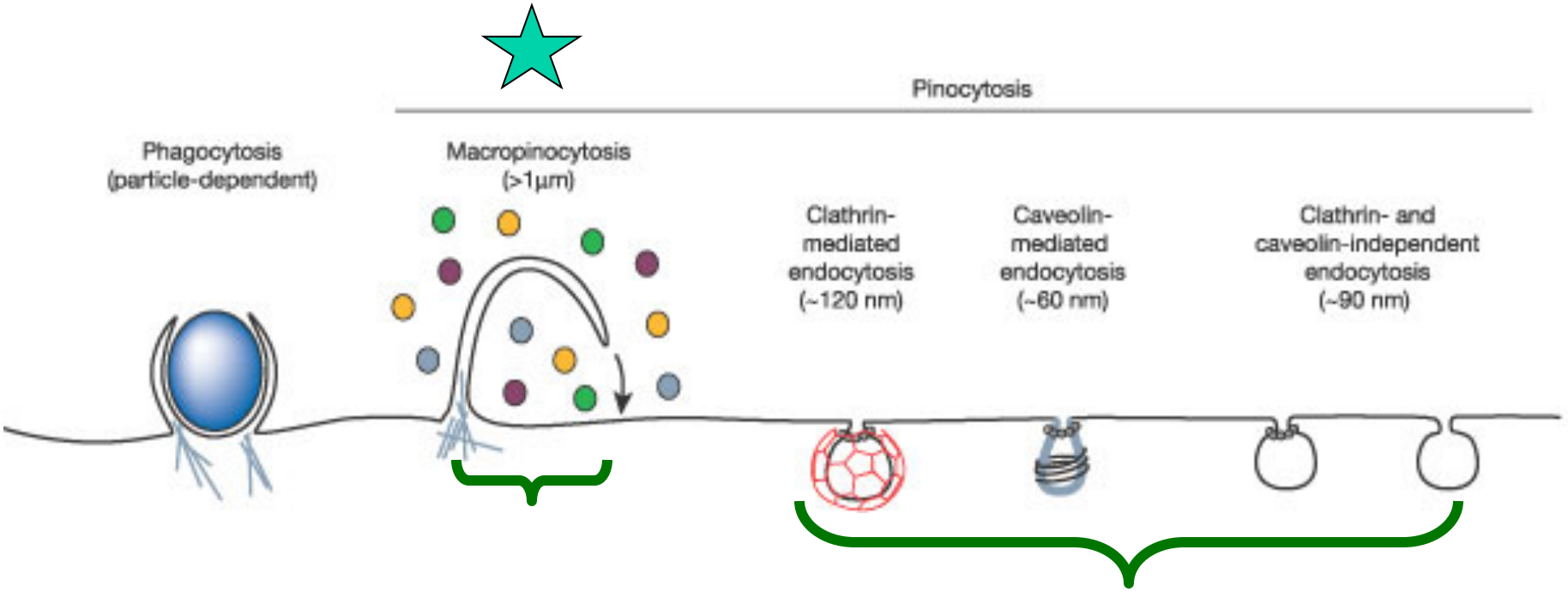


fighting infection

phagocytosis of a bacterium by a neutrophil



Macropinocytosis as a powerful way to capture exogenous antigens



Diameter: **>1 um (1000nm)** **~100 nm**

a constitutive and non-specific uptake process in antigen-presenting cells

Macropinocytosis

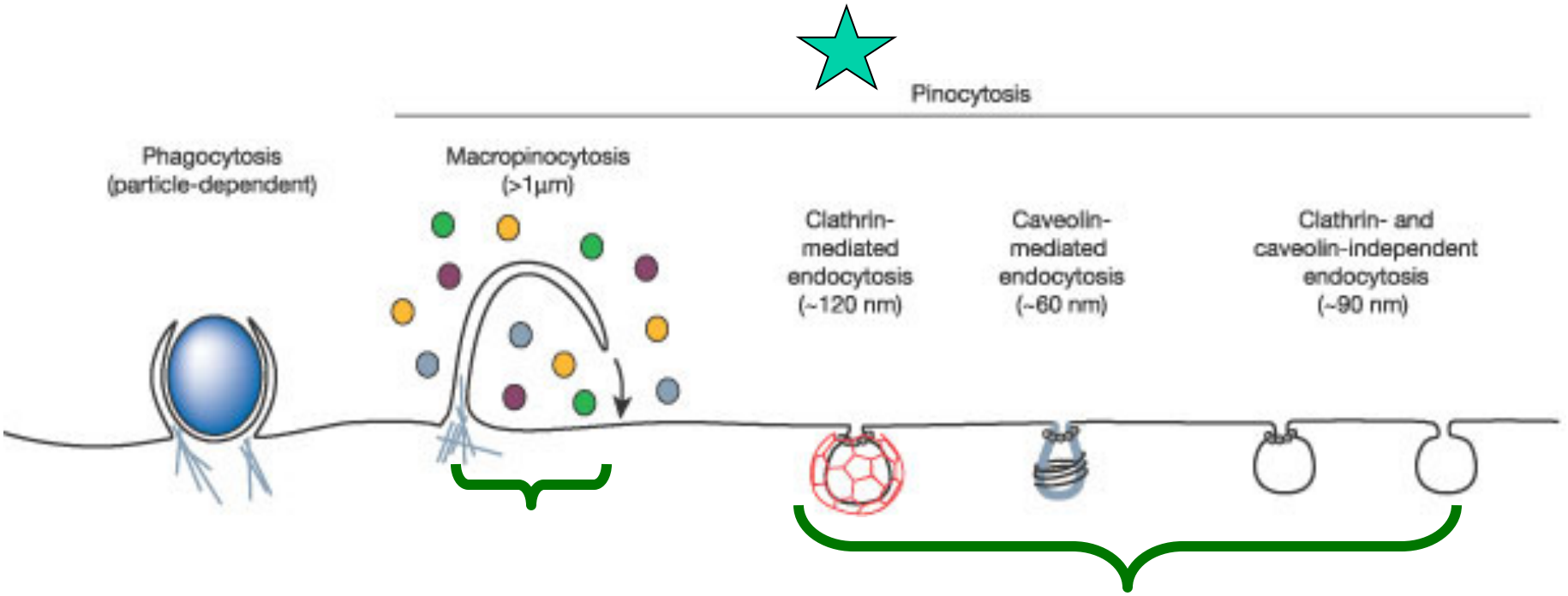
Formations:

1. a non-selective form of endocytosis via large endocytic vesicles called macropinosomes (> 1 um)
2. occurring in many cell types at a low level and triggered by growth factors, but constitutive in antigen presenting cells such as dendritic cells
3. non-specific

Functions:

1. a way of feeding
2. antigen presentation (dendritic cells)
3. cell migration

Clathrin-mediated Endocytosis



Diameter: **>1 um (1000nm)** **~100 nm**

a constitutive and non-specific uptake process in antigen-presenting cells

Clathrin-mediated endocytosis

Formation:

1. a form of endocytosis by forming clathrin-coated vesicles (~ 120 nm)
2. present in eukaryotic cells from yeasts to mammals
3. highly efficient and subject to regulation
4. highly specific – sorting signals of cargo proteins required

Functions:

1. a way of feeding
2. molecular homeostasis
3. regulation of the cell surface density of a specific plasma membrane protein (due to the signal-mediated nature)

Clathrin-dependent endocytosis is a major internalization pathway in most cell types

While phagocytosis & macropinocytosis generate large vesicles,

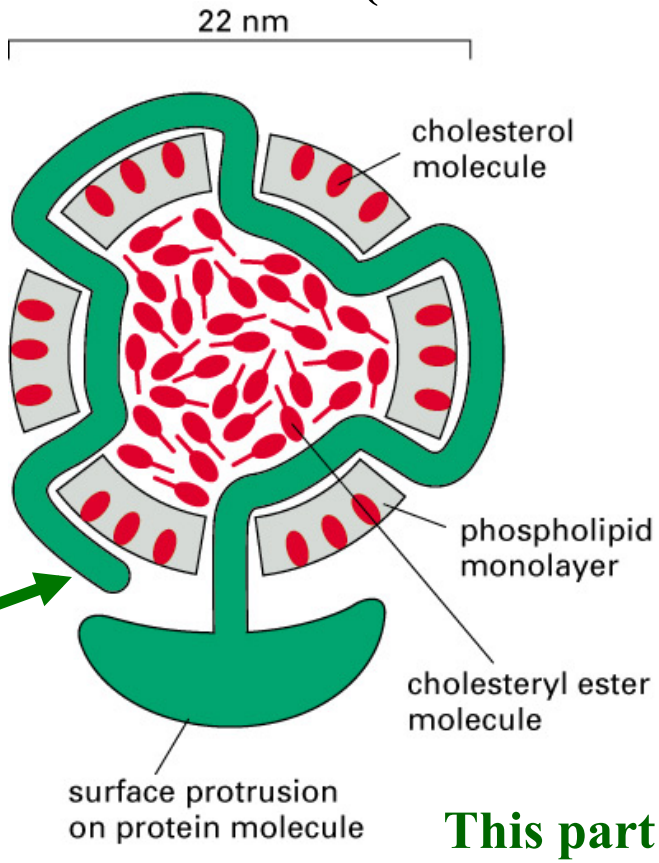
Phagocytosis only occurs in specific professional cells
Macropinocytosis is only transiently triggered in most cell types

While clathrin-dependent endocytosis generates small vesicles, clathrin-coated pits occupy 2% of the PM area in most cells and they have a fast turnover rate. As a result, this mode of internalization constitutes one major internalization mode in most cell types (1% PM internalization per min in fibroblasts)

Clathrin associates with adaptor protein complex 2 (AP2) at the PM to induce endocytosis

Internalization of LDL and LDLR as one example

LDL (low density lipoprotein particle) is the major cholesterol carrier in the blood: carrying ~2000 cholesterol molecules (free or ester forms) per particle



Apolipoprotein (ApoB in most cases)

This part interacts with LDLR

LDL binds to the LDL receptor (LDLR) on the plasma membrane and is cleared from the blood by clathrin-mediated internalization.
Which one, LDL or LDLR, contains the internalization signal?

Describing the Regulation of Cholesterol Metabolism

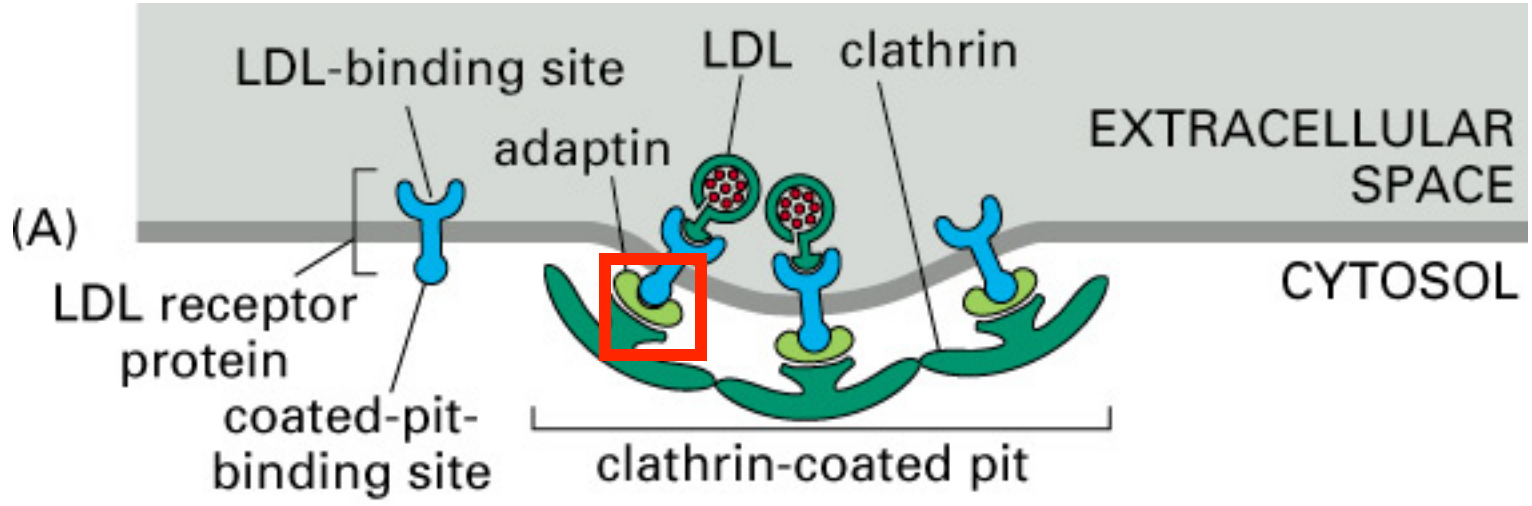
Michael S. Brown **Joseph L. Goldstein**

1985 Nobel Prize in Physiology or Medicine

Brown and Goldsten discovered that human cells have low-density lipoprotein (LDL) receptors that remove cholesterol from the bloodstream. The lack of sufficient LDL receptors is implicated in familial hypercholesterolemia, which greatly predisposes for cholesterol-related diseases. In addition to explaining the underlying pathology of this disease, their work uncovered a fundamental aspect of cell biology - Receptor-mediated endocytosis.

Their findings led to the development of statin drugs, the cholesterol-lowering compounds that today are used by 16 million Americans and are among the most widely prescribed medications in the United States.

A sorting signal on the cytoplasmic domain of LDL receptor (NPVY) directs the LDL-LDLR complex to the clathrin-coated pits on the PM via interacting with the adaptor protein complex 2 (AP2)



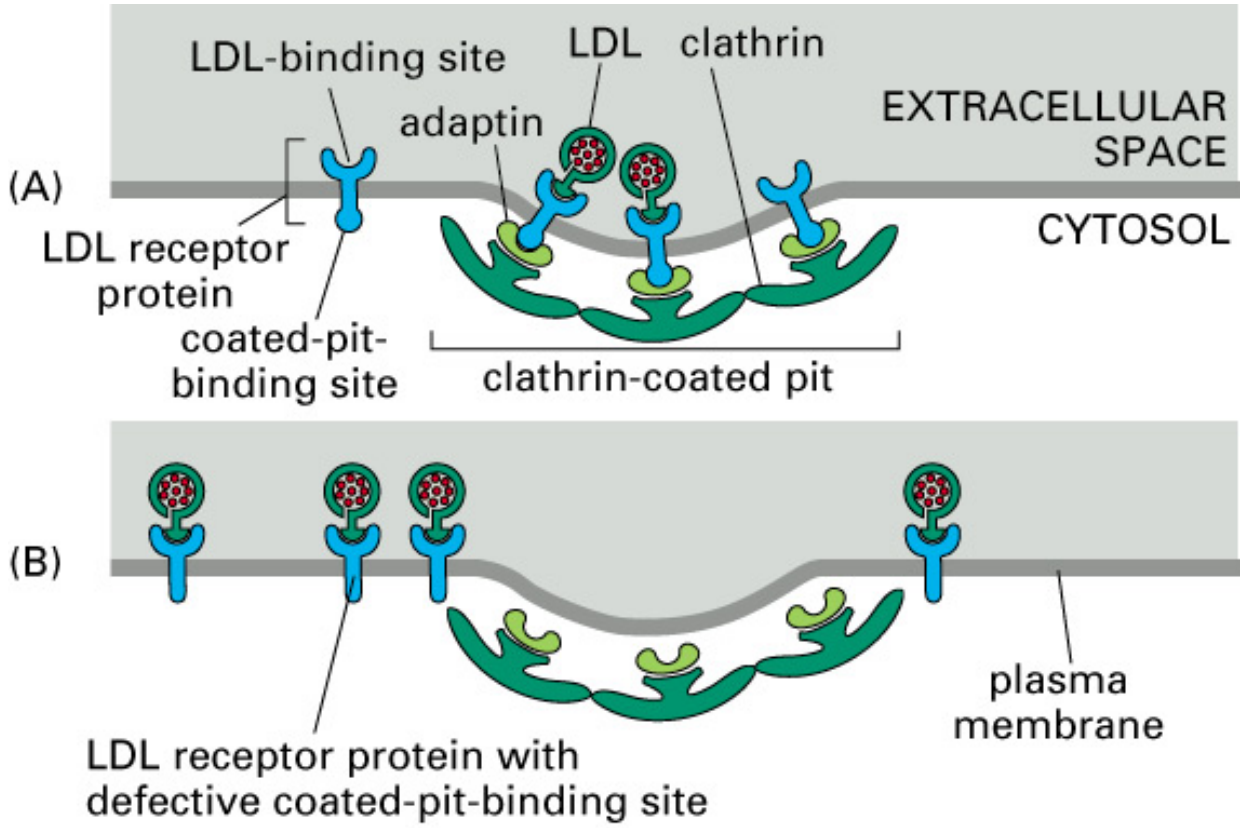
"NPVY" → **AP2** → **Clathrin**

AP2 complex interacts with both cargo and clathrin and thus functions as an adaptor in clathrin-mediated internalization

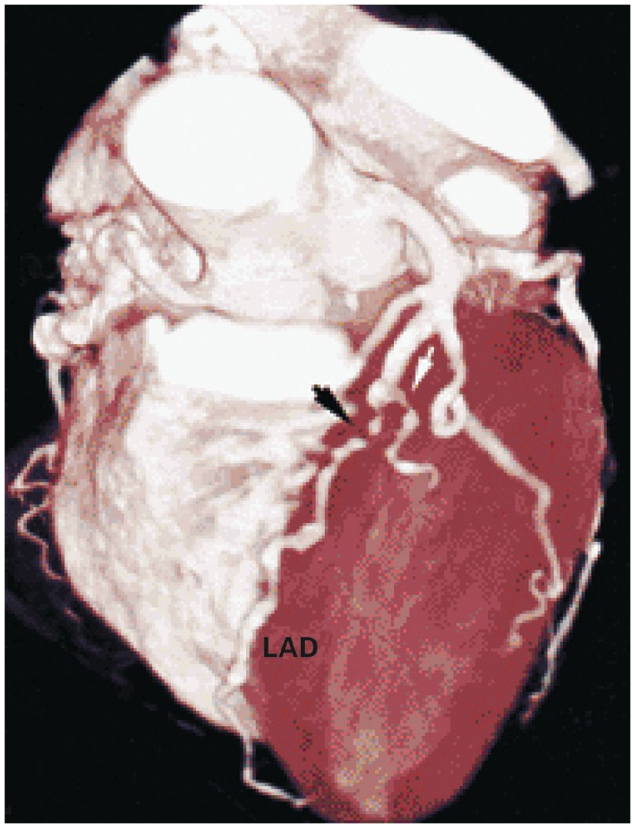
Recent studies suggest that LDLR binds to another adaptor associated with AP2 complex rather than AP2 itself

Improper regulation of clathrin-dependent internalization & diseases

Mutant LDLR with a defective endocytosis rate leads to increased blood cholesterol & heart attack



Atherosclerosis



Formation of a clathrin-coated vesicle (CCV) from the PM

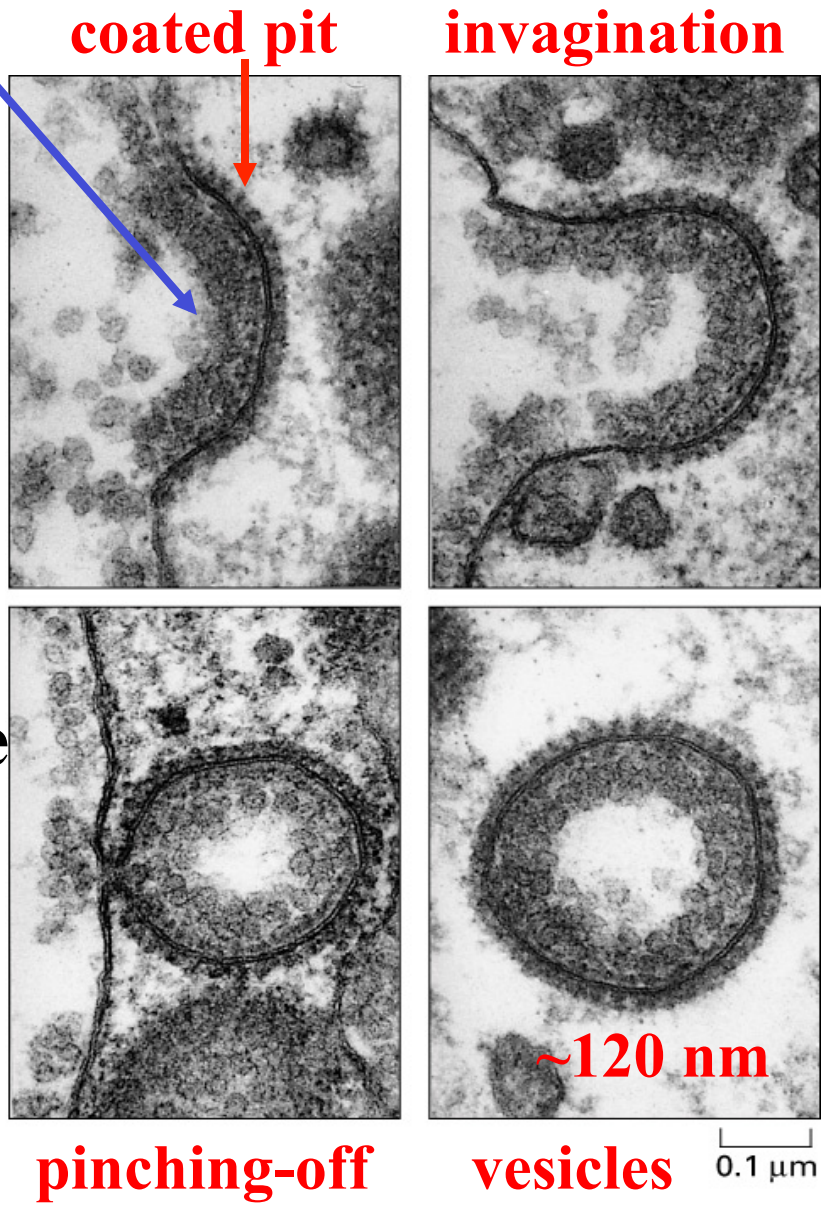
cargo recruitment
(mediated by AP2 and associated proteins)

membrane deformation
(mediated by clathrin, AP2 and associated proteins)

pinching-off of the coated vesicle
(dynamin and associated proteins are required)

shedding of the coat
(cytoplasmic chaperone needed)

no GTPase involved but chaperones are ATPases



Multiple Signals Exist for Clathrin-Dependent Internalization

NPXY: X can be any amino acid residue

YXXØ: X is any polar residue, Ø is a hydrophobic residue (often bulky)

di-leucine: LL (sometimes one Leu can be replaced by Val or Ile)

Ubiquitin: mono-ubiquitin or multiple mono-ubiquitin

(different from the poly-ubiquitin signals for proteasomal degradation)

Clathrin Associates with Different AP Complexes at Different Sites

AP2: PM

AP180: PM (neuron-specific form)

AP1, 3, and 4: Endosomes or TGN

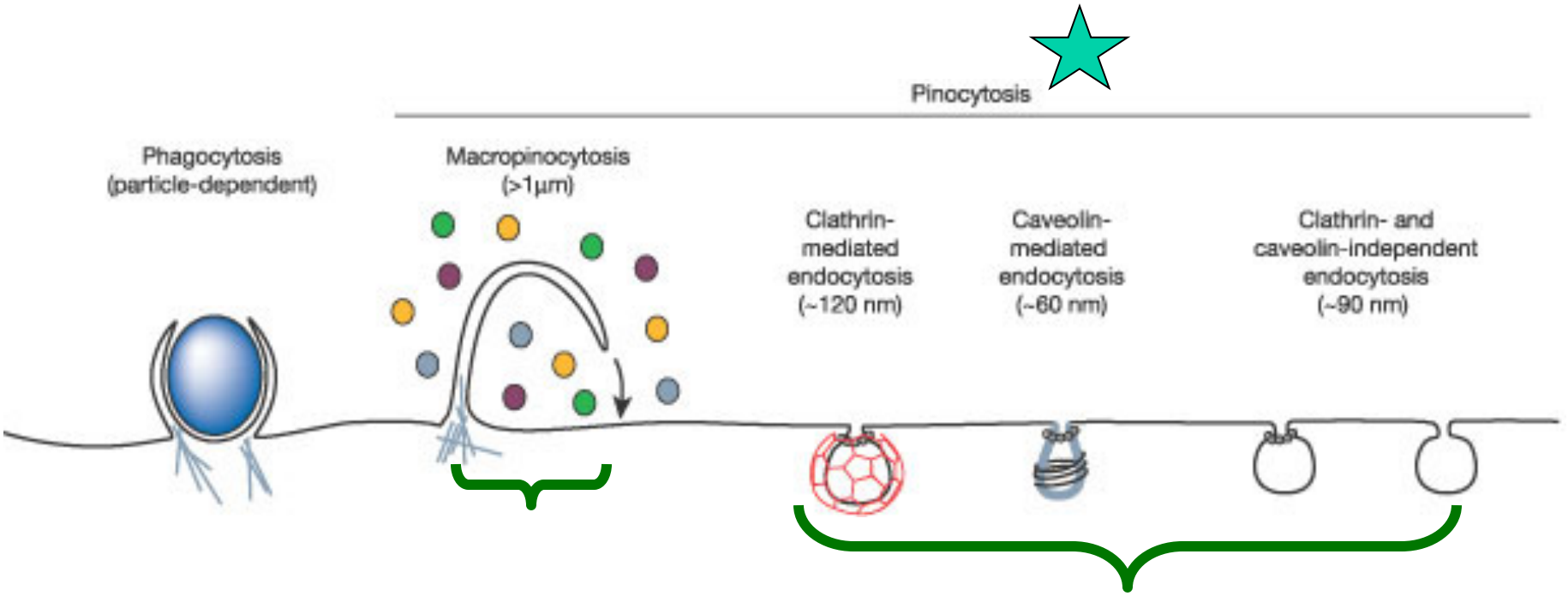
GGA family: TGN, … (?)

While adaptor complexes often directly bind cargo and clathrin, some clathrin-dependent trafficking signals are linked to adaptor complexes indirectly via additional cargo-specific proteins

Why are so many AP complexes and adaptors involved?

The combination of multiple clathrin-dependent signals, multiple adaptor complexes, and the presence of cargo-specific adaptors allow a cell to use the clathrin-dependent internalization to regulate the surface density of one or few specific plasma membrane proteins.

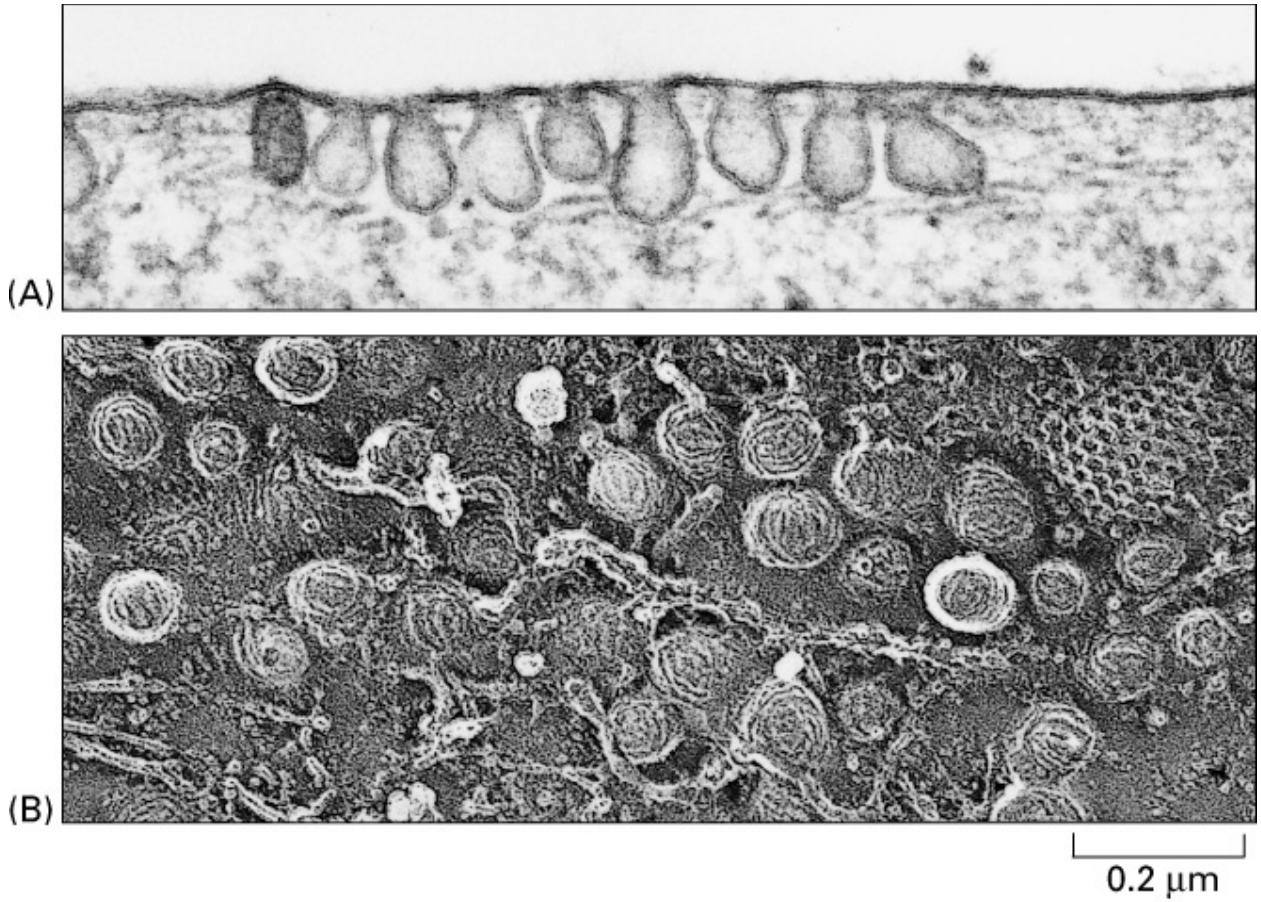
Caveolin-mediated Endocytosis



Diameter: **>1 um (1000nm)** **~100 nm**

a constitutive and non-specific uptake process in antigen-presenting cells

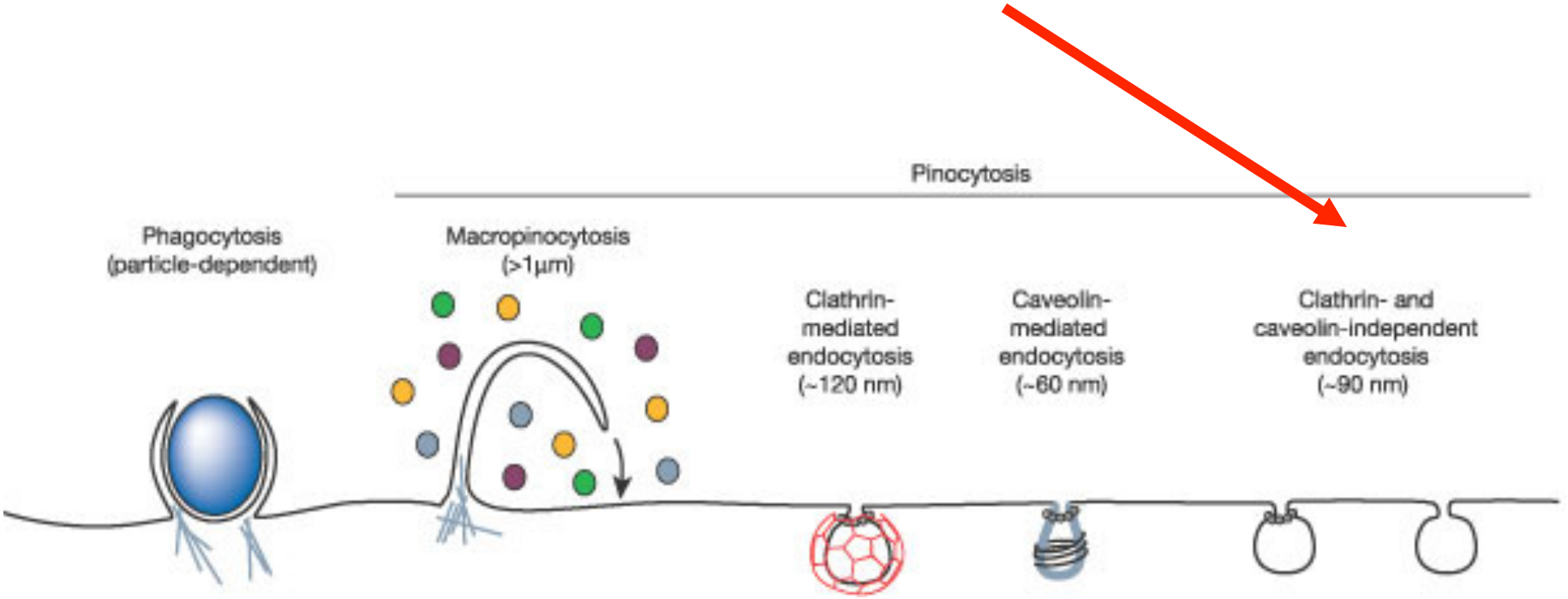
Caveolae are flask-shaped invaginations of the PM



1. one type of lipid rafts includes caveolin as a major component
2. found in many cell types, especially abundant in endothelial cells
3. a signaling platform (as are forms of lipid rafts)
4. a role in the endocytosis of viruses as well as transcytosis in endothelial cells
5. may also function in endocytosis in other cells

Clathrin- and Caveolae-Independent Endocytosis

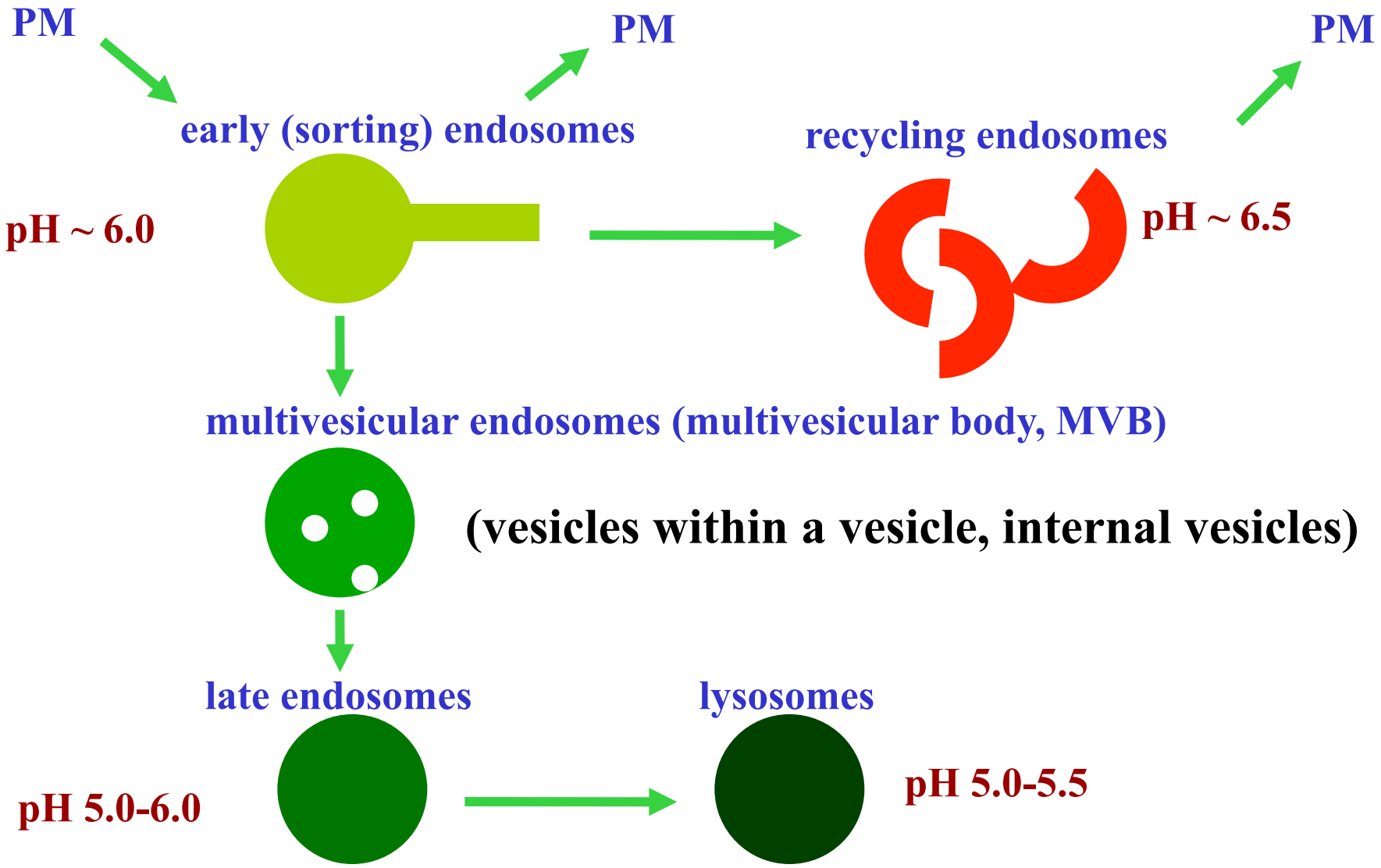
little is known & much to be learned



Endocytosis (Internalization)

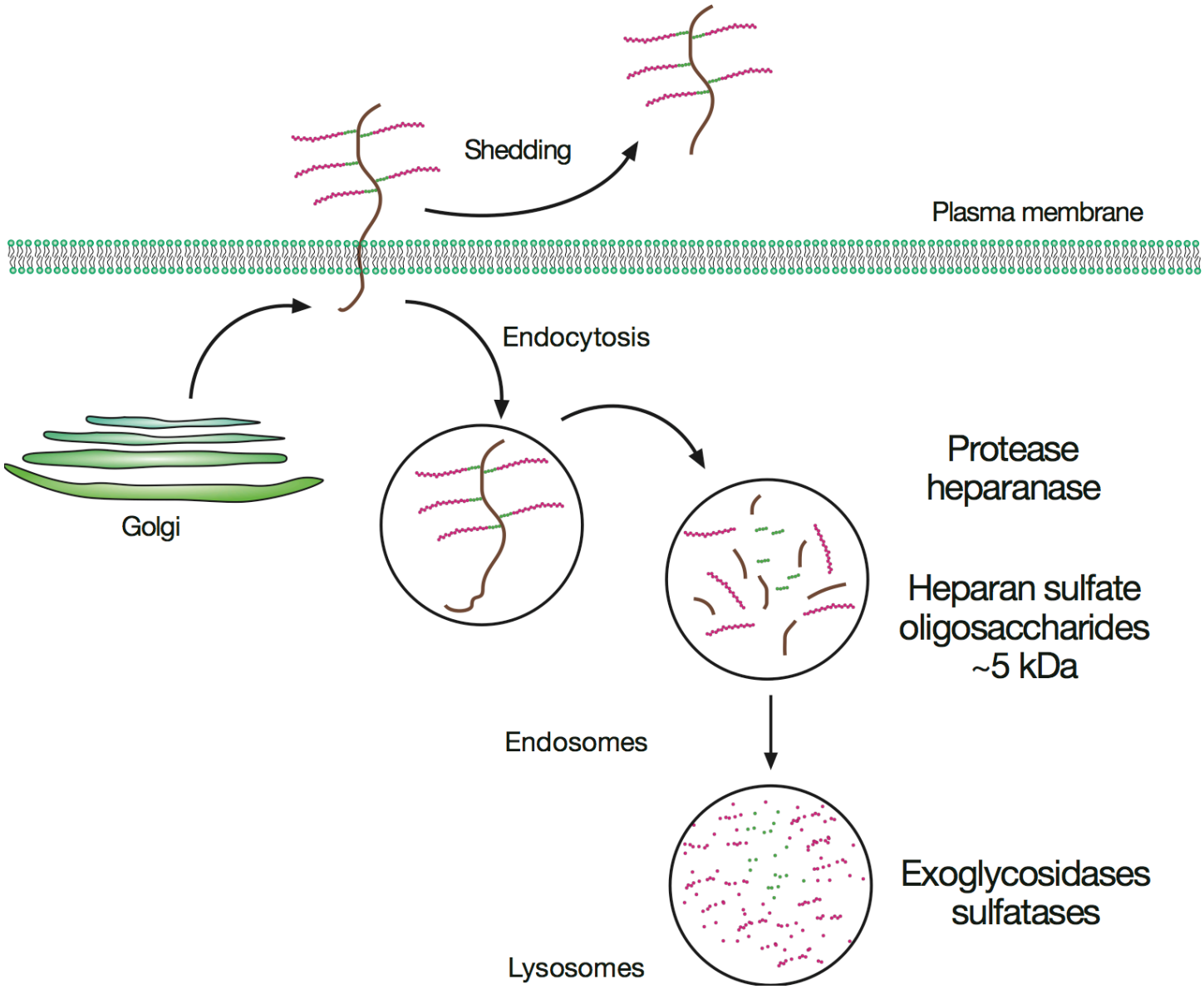
Endosomal (Endocytic) Trafficking

Major compartments of the endosomal pathway

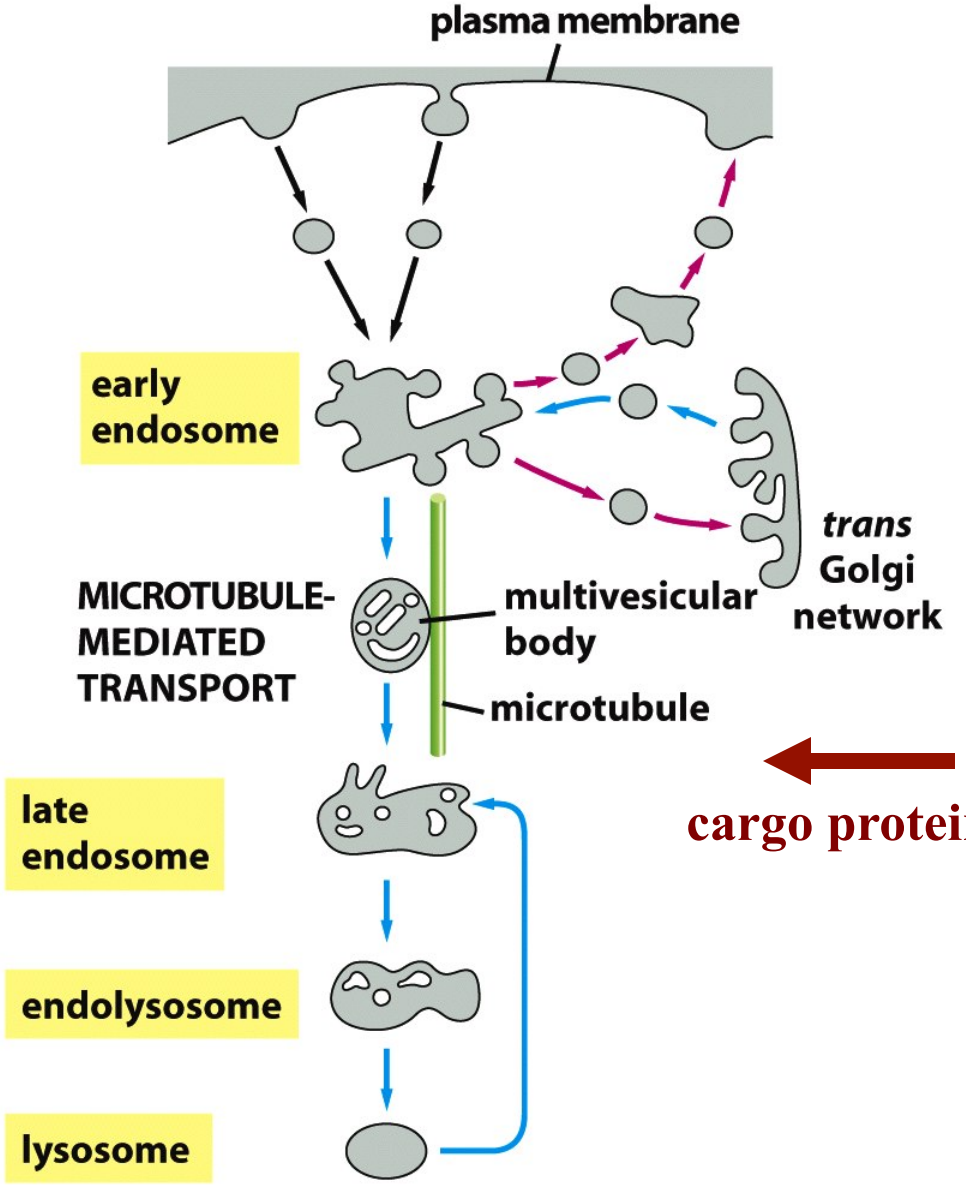


A major function of endosomes (esp. early endosomes) is sorting (different pH and morphology)

Like Proteins many Glycans are degraded in lysosomes by glycosidases:
Example: Proteoglycans



Cargo proteins to be degraded are targeted to the internal vesicles of a MVB from early endosomes in a signal-dependent manner

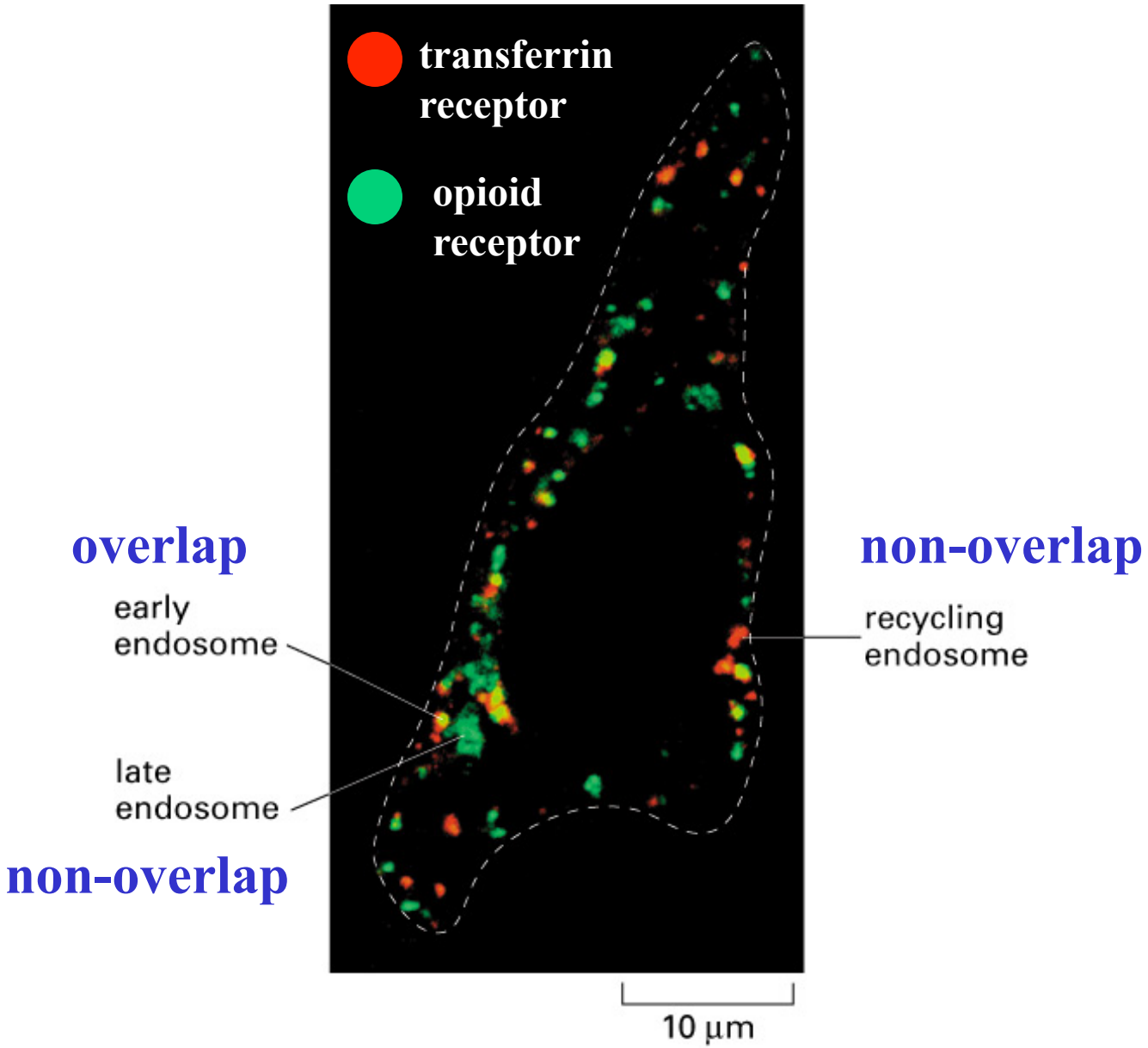


cargo proteins to be degraded contain signals

Proteins trafficking via early endosomes can be recycled or degraded

Nutrient receptors (e.g. LDLR or TfR) are rapidly recycled to the PM (no signals required)

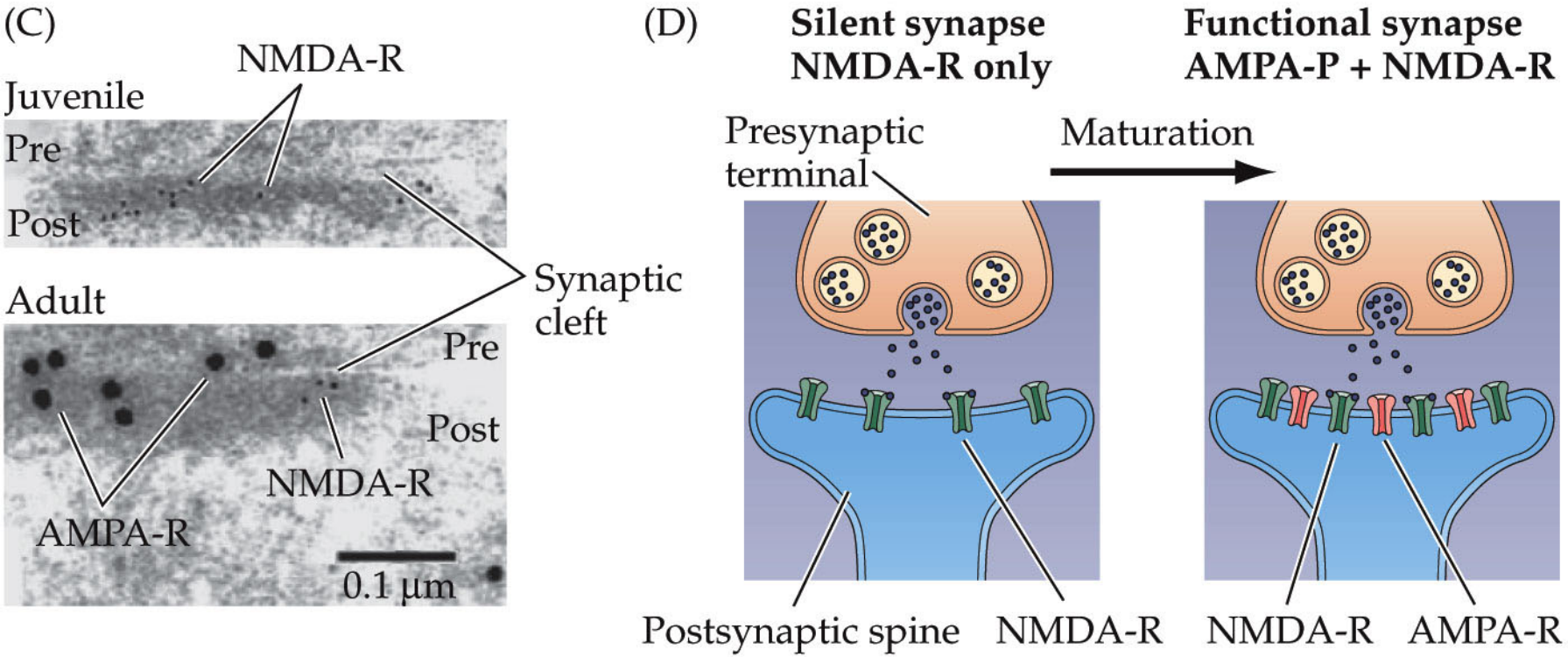
Signaling receptors (e.g. EGFR) are often sorted to the late endosomes/lysosomes to be degraded (signal-mediated)



Some membrane proteins are sequestered in endosomes

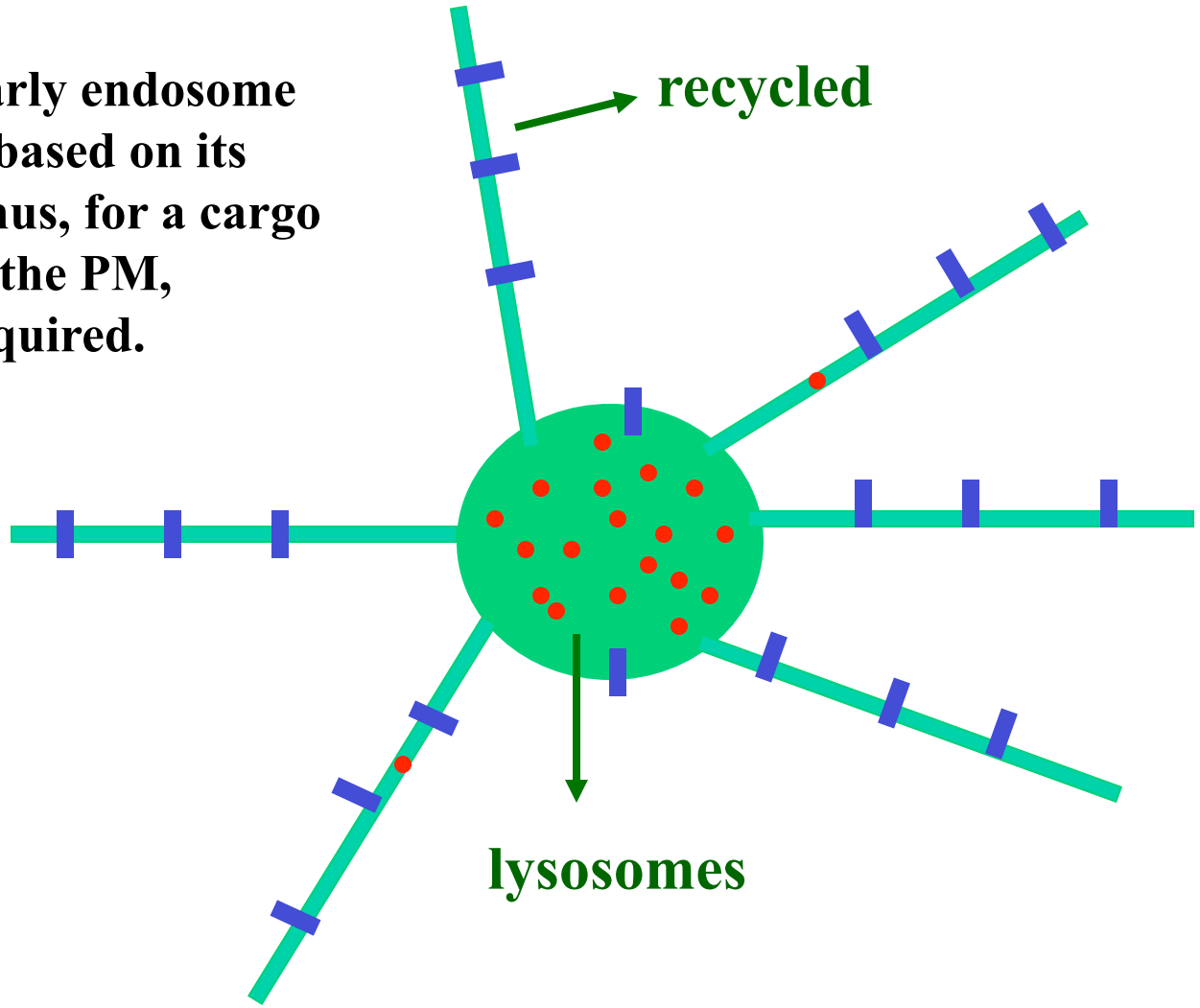
endosomal trafficking and brain function

Insertion of AMPA receptor from endosomes to PM enhances synaptic strength, which is thought to mediate learning and memory

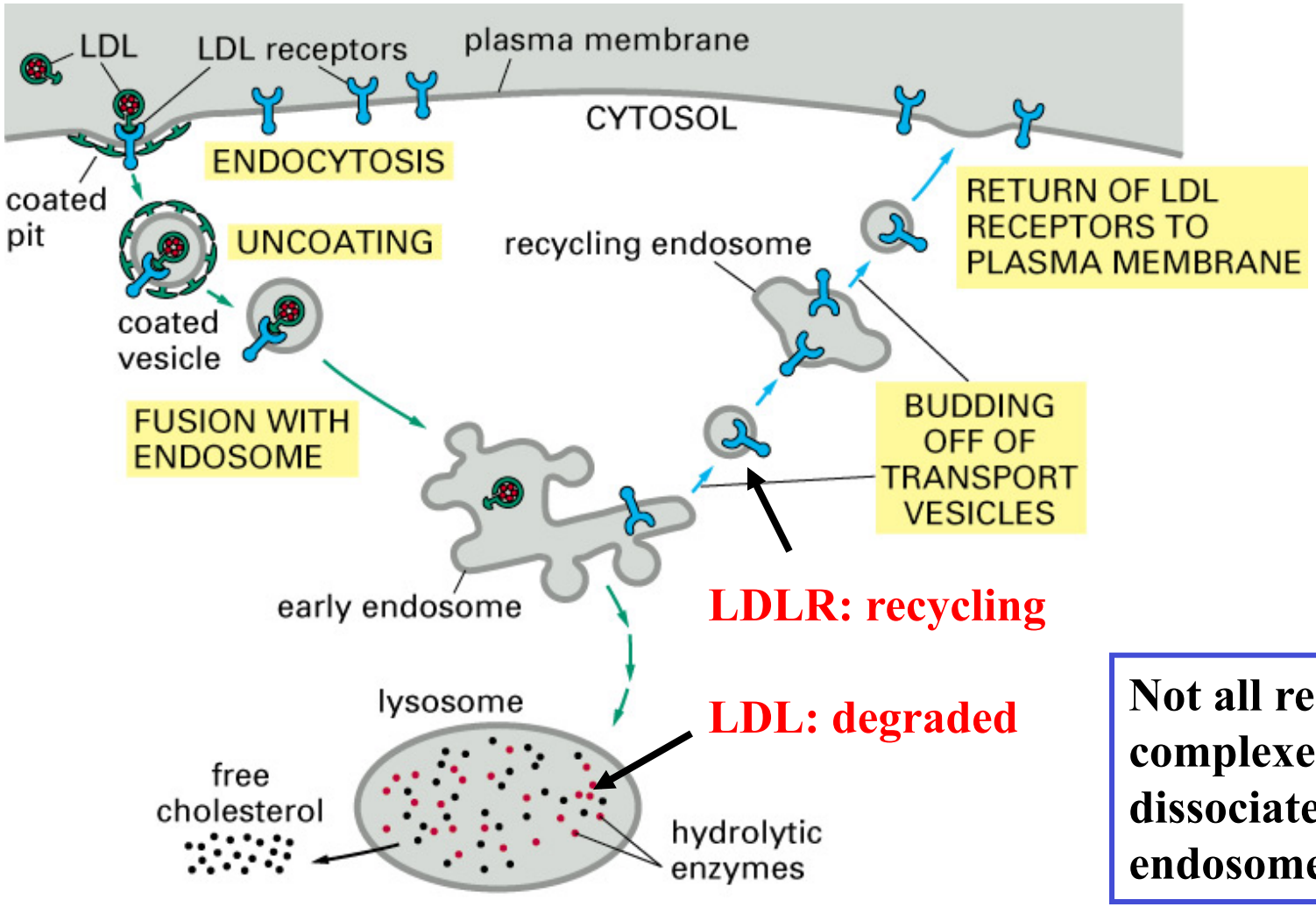


Geometric consideration of sorting at the early endosomes

The morphology of an early endosome can sort a cargo protein based on its surface/volume ratio. Thus, for a cargo protein to be recycled to the PM, no specific signals are required.



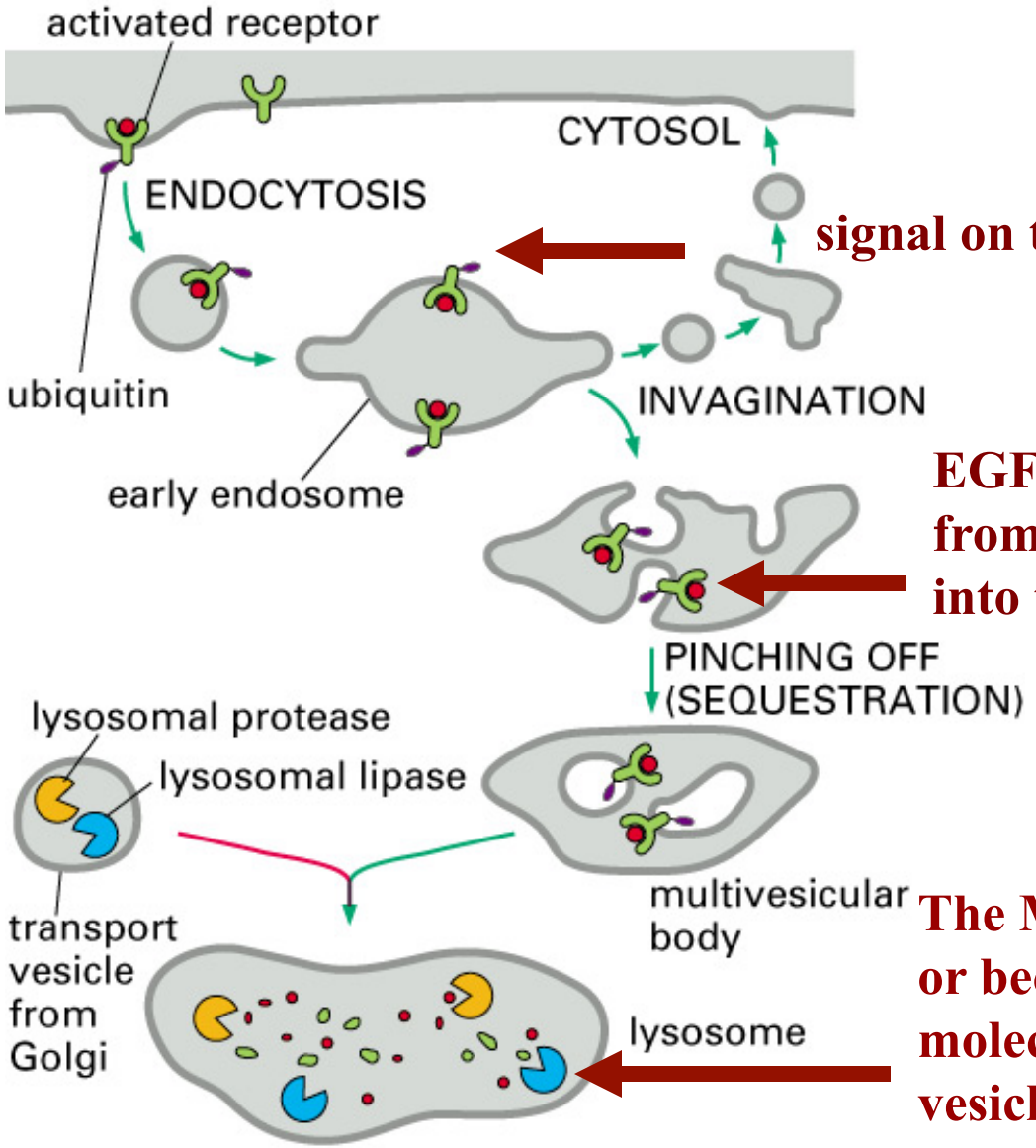
Early endosomes are the major sorting station of the endocytic pathway



Not all receptor-ligand complexes become dissociated in early endosomes

dissociation of LDL & LDLR is due to a lower pH

The MVBs fuses with late endosomes/lysosomes or with themselves to form late endosomes/lysosomes leading to the cargo degradation

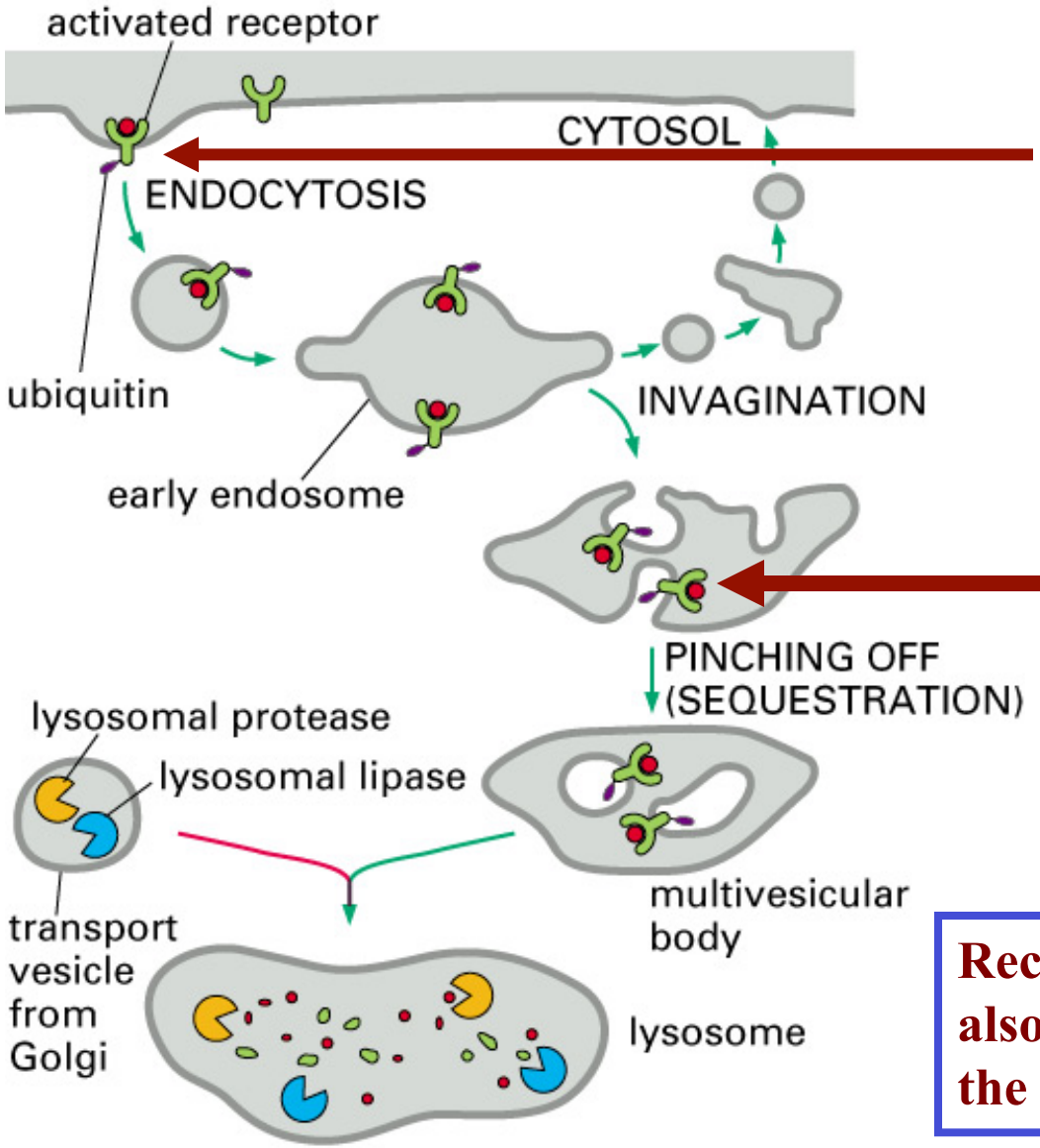


signal on the cytoplasmic domain of EGFR

EGF-bound EGFR are first sorted from the membrane of early endosomes into the internal vesicles of a MVB

The MVB is then fused with a lysosome or becomes a lysosome and the cargo molecules associated with its internal vesicles are then degraded in the lumen of lysosomes

Ubiquitin as a sorting signal at the PM & early endosomes to direct specific cargo proteins for lysosomal degradation



ubiquitination of EGFR as an internalization signal at the PM

ubiquitination of EGFR also required for its entering the internal vesicles of a MVB

Recent studies suggest that ubiquitin also involves cargo sorting at the TGN.

Multiple Functions of Ubiquitination

Signals for proteasomal degradation (UPR) (K48 poly-ubiquitinated)

Signals for internalization (EGFR)

Signals for sorting at the early endosomes (EGFR)

Signals for proper functioning of sorting machinery (not discussed)

Signals for PM outward budding (HIV)

} **mono- or multi-ubiquitination**

Many other functions

The actual function of ubiquitination depends on:

1. **the identity of the target protein (soluble or TM)**
2. **the number of ubiquitin molecules added at the lysine of the target protein**
3. **the linking pattern of the ubiquitin chain of the target protein**

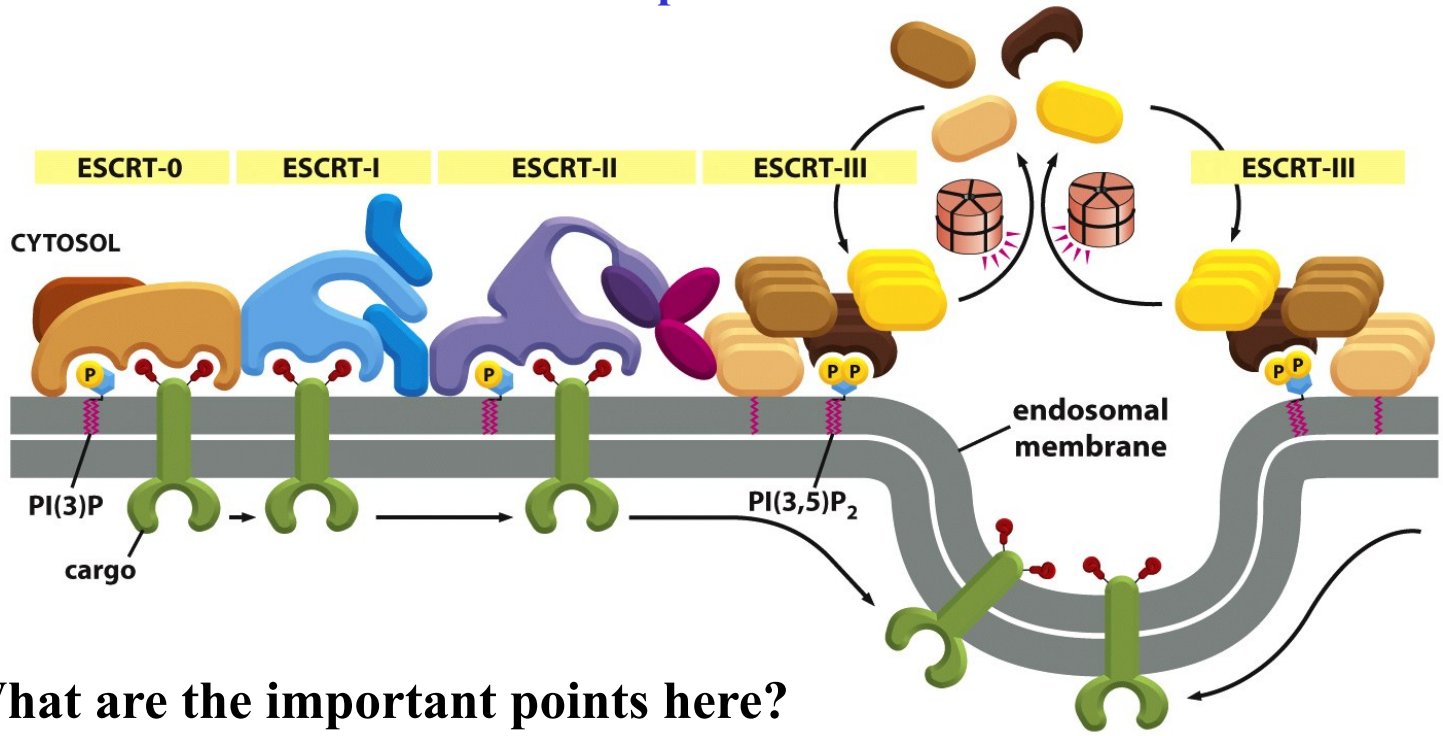
Endocytosis (Internalization)

Endosomal (Endocytic) Trafficking

How is a cargo sorted into the internal vesicles of a MVB?
(MVB is also an endosomal sorting station)

PI3P, ESCRT Complex and Ubiquitin Tag work together to pack a cargo into the internal vesicles of a MVB

1. Ubiquitin tag on the cargo as a signal
2. PI3P is on the endosomal membrane to recruit ESCRT complexes
3. ESCRT Complexes (0-II) recognize ubiquitinated cargo proteins
4. ESCRT Complex III confines and enriches ubiquitinated cargo proteins within a budding membrane
5. An ATPase disassemble ESCRT complexes for re-use

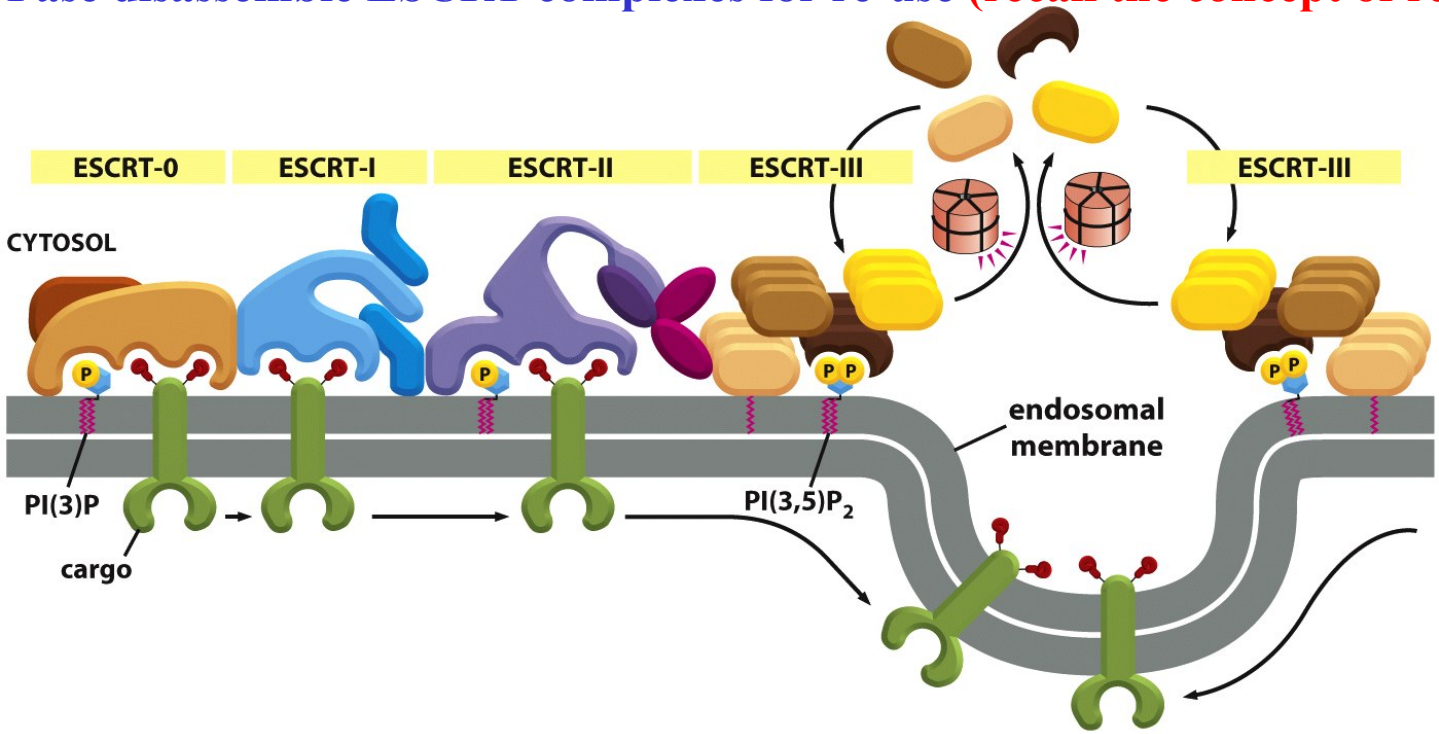


What are the important points here?
Ubiquitin, PI3P, recycle, budding morphology

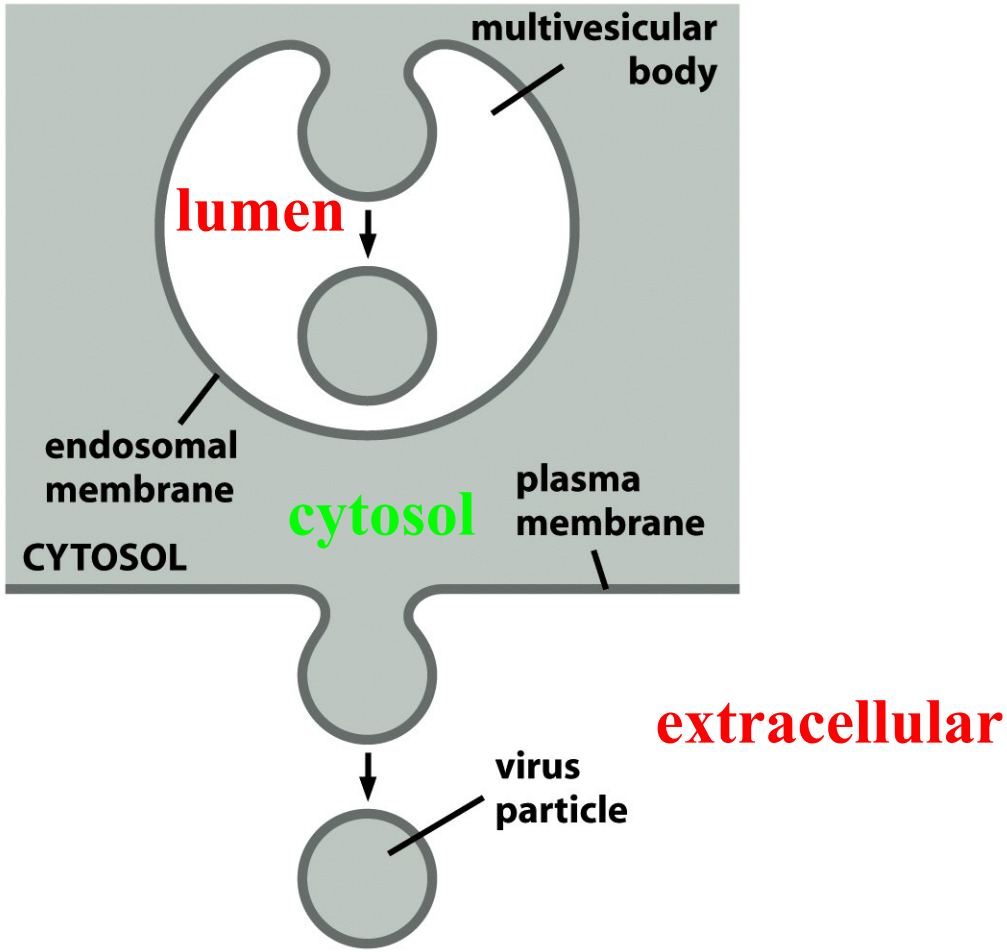
What is the machinery which sorts cargo into the internal vesicles of a MVB?

PI3P, ESCRT Complex and Ubiquitin Tag work together

1. Ubiquitin tag on the cargo as a signal
2. PI3P is on the endosomal membrane to recruit ESCRT complexes
(recall how PtdIns works)
3. ESCRT Complexes (0-II) recognize ubiquitinated cargo proteins
4. ESCRT Complex III confines and enriches ubiquitinated cargo proteins within a budding membrane
5. An ATPase disassemble ESCRT complexes for re-use (recall the concept of recycling)

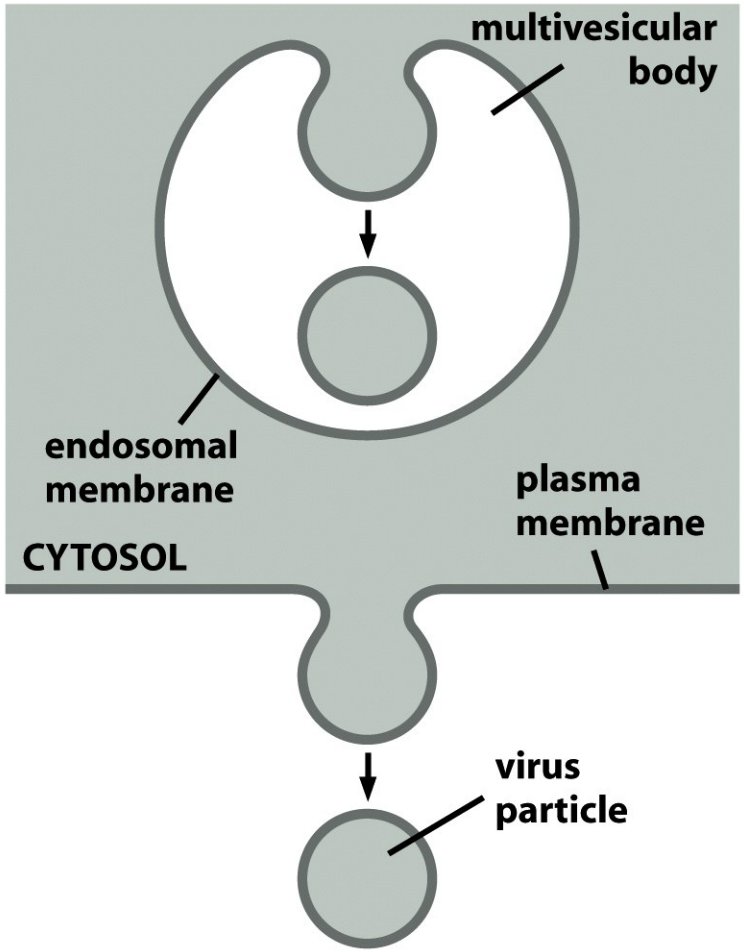


Budding into a MVB
is topologically similar to the virus budding at the PM



This type of budding is different from the budding of COPII, COPI & clathrin vesicles

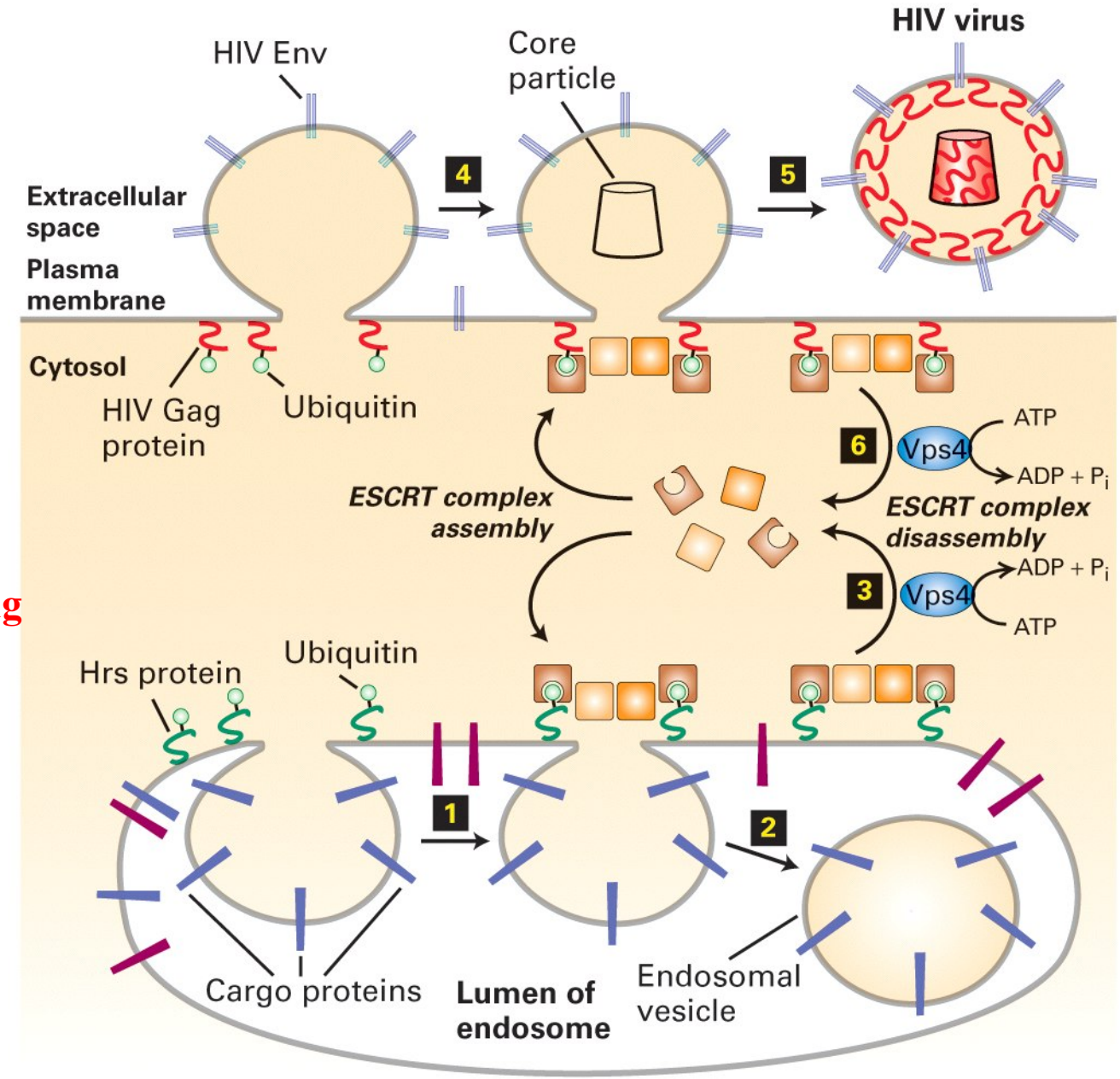
Budding into a MVB
is topologically similar to the virus budding at the PM



Both use the same ESCRT budding machinery which is very different compared to the COPI, COPII, and clathrin machinery in terms of their budding topology.

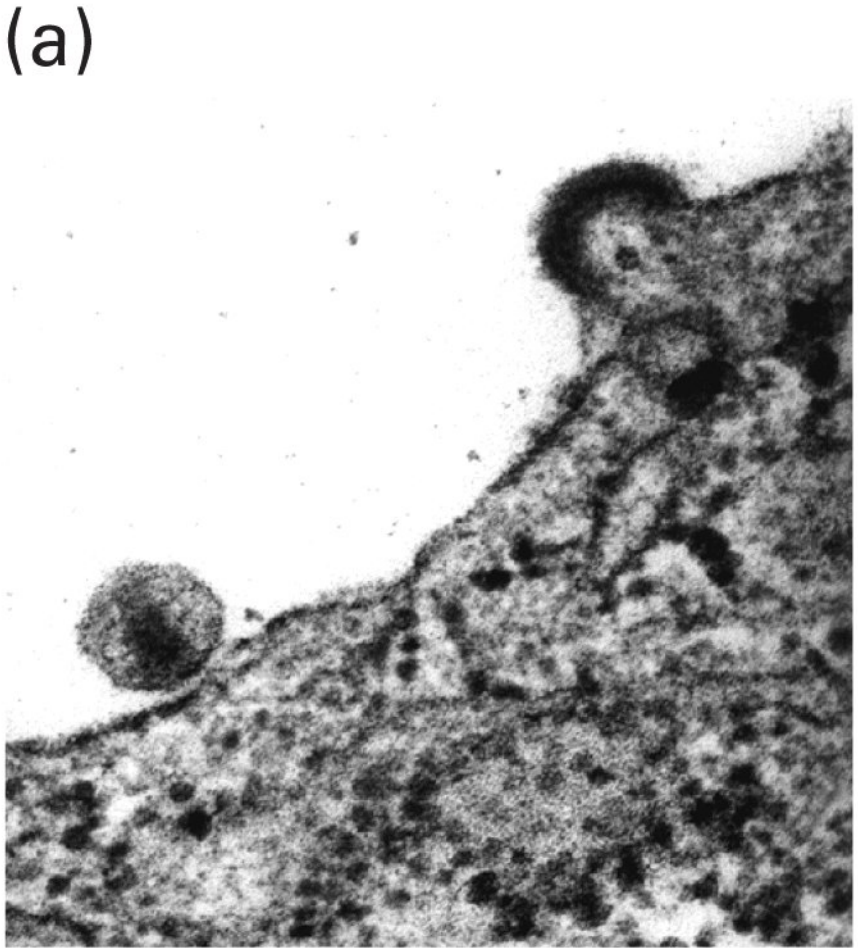
HIV Gag proteins are ubiquitinated

The ubiquitinated HIV Gag protein functions in a way similar to an ubiquitnated cargo to recruit the ESCRT complexes for the budding of HIV viruses

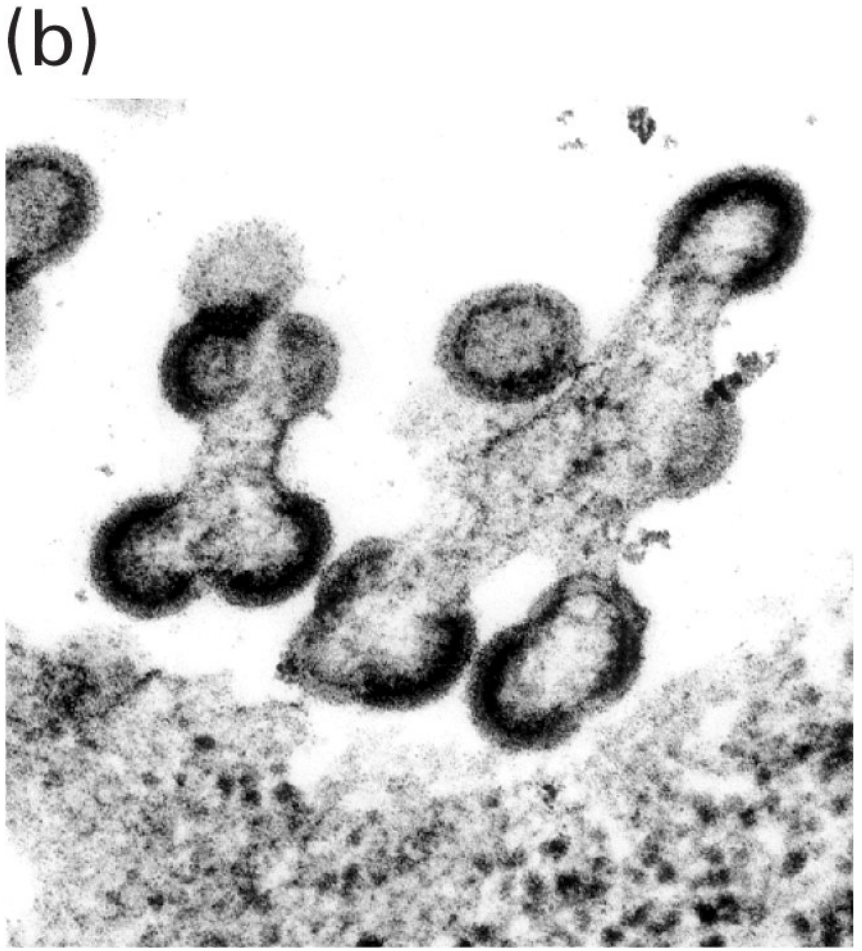


Budding of HIV virus particles from the PM

Wild-type cells



ESCRT-deficient cells



HIV viral particles still form but fail to pinch off from the PM

Multiple types of signals for sorting cargo

Short trafficking motifs (everywhere)

Transmembrane domains (ER and Golgi)

Glycan modifications (Golgi and TGN)

Ubiquitin tag (TGN, PM, endosomes)

Lipid microdomains (TGN, PM)

Geometric sorting (Endosomes)

Aggregation and passive sorting (secretory vesicles)