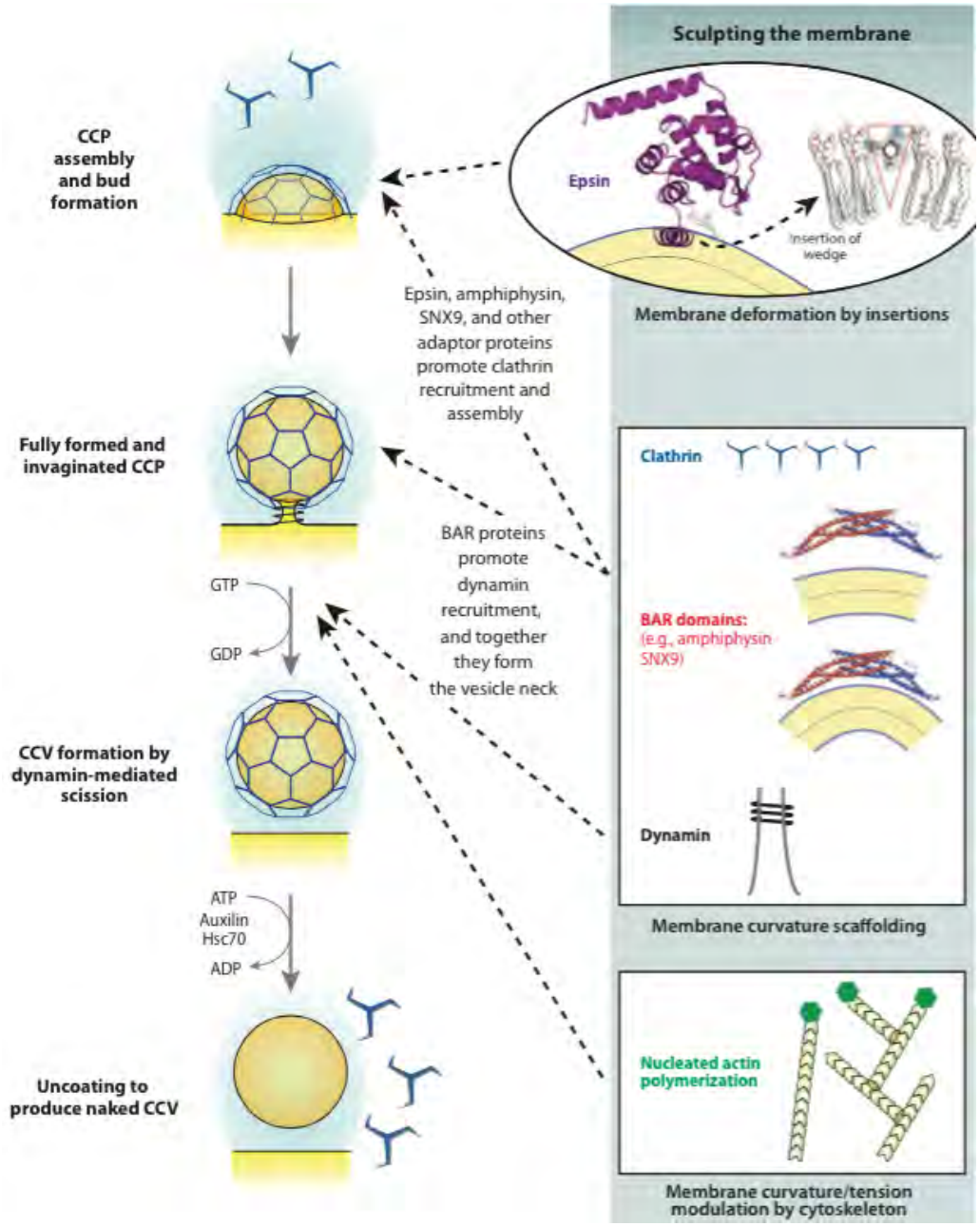
Overview of clathrin-mediated endocytosis

Accessory and adaptor proteins promote clathrin nucleation on the plasma membrane and some help deform membrane.

Clathrin assembly into lattices stabilize the membrane curvature.

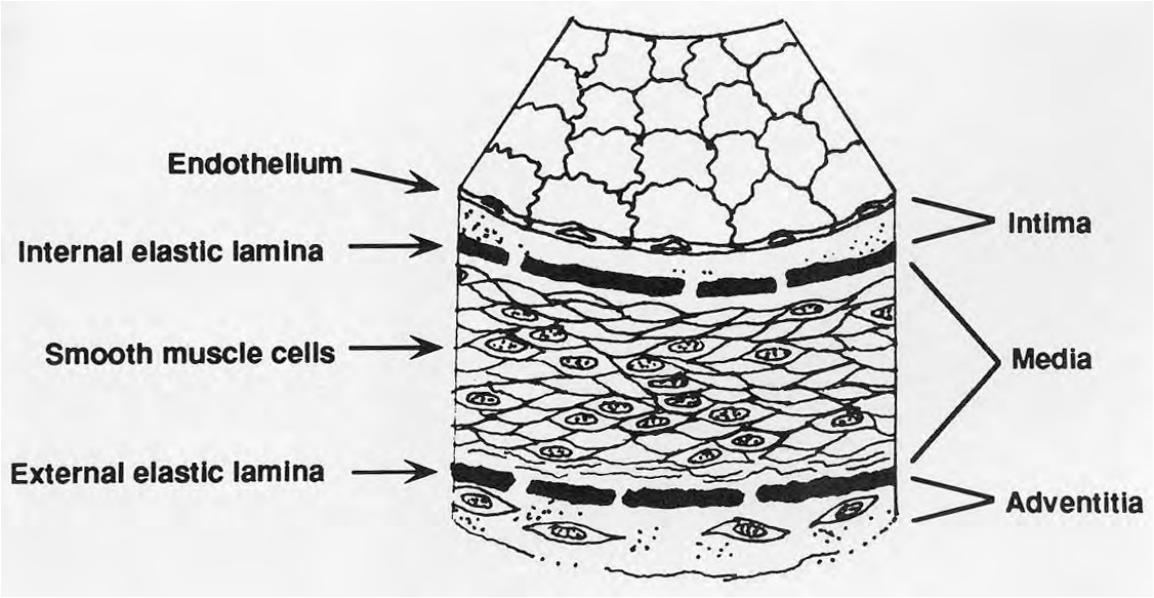
The dynamin GTPase forms a polymerize collar around the endocytic neck and mediates scission of the vesicle.

The clathrin coat is disassembled.



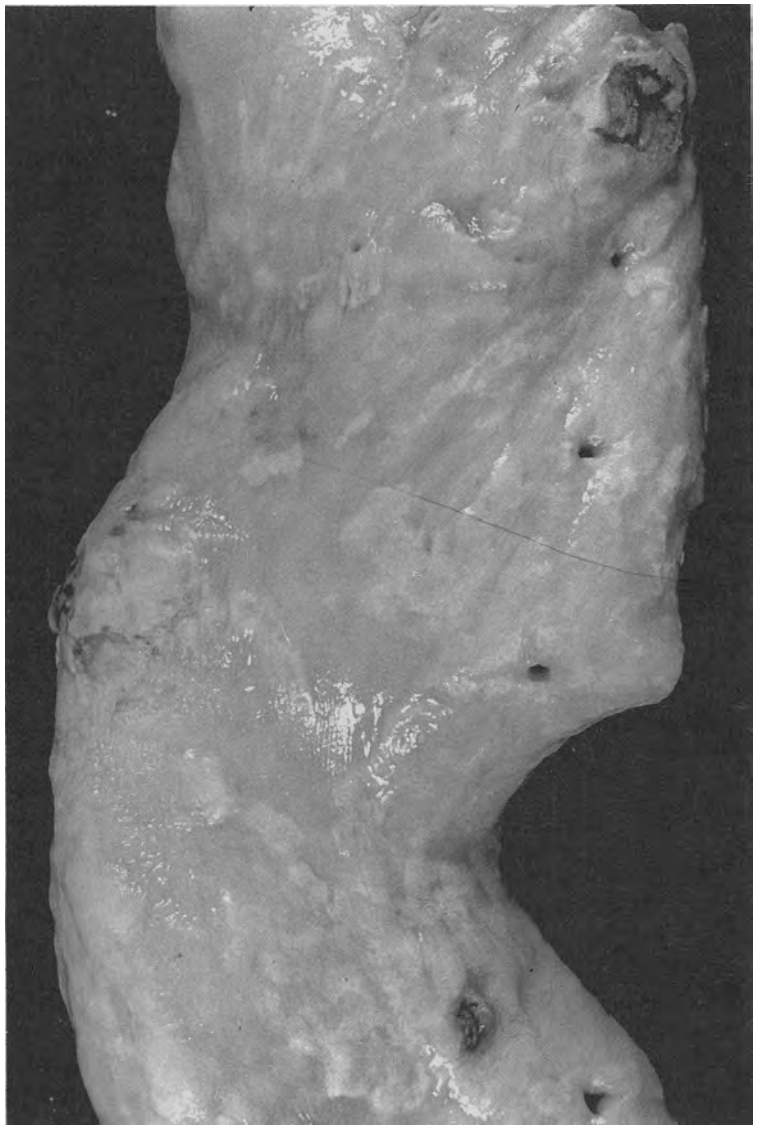
Doberty & McMahon (2009)
Ann Rev Biochem

A single layer of endothelial cells lines arteries



Lilly (1993) Pathophysiology of Heart Disease

Fatty streaks: the earliest visible sign of atherosclerosis



Yellowish discoloration
on endothelial surface

Lilly (1993) Pathophysiology of Heart Disease

Fatty streaks contain macrophage cells with lipid deposits

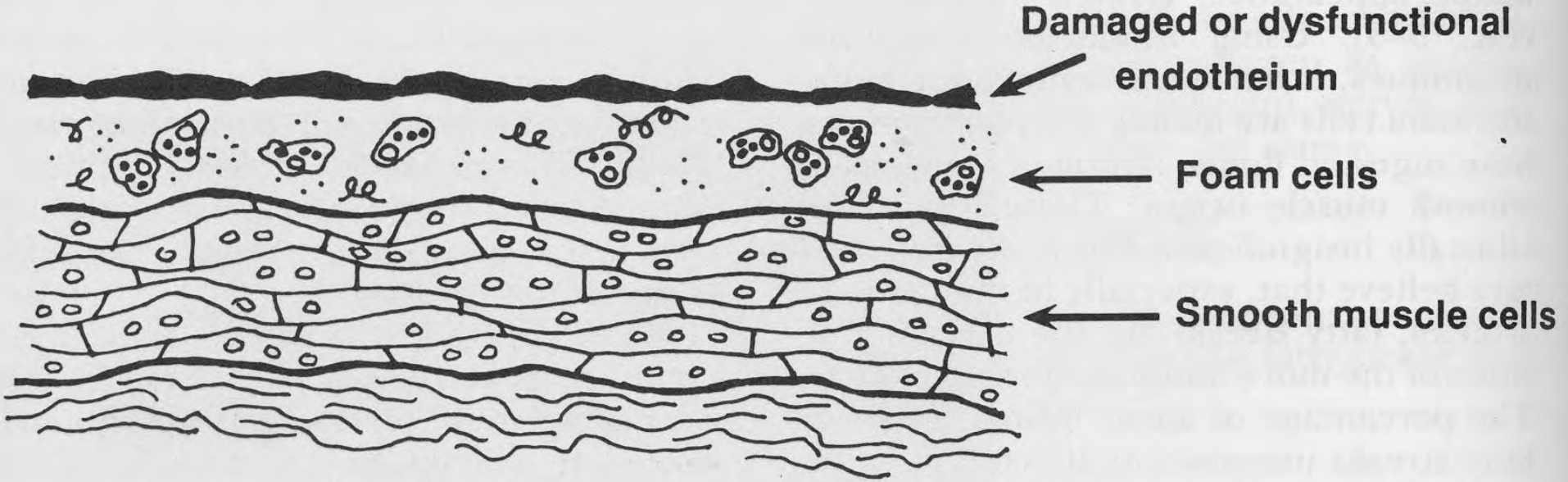
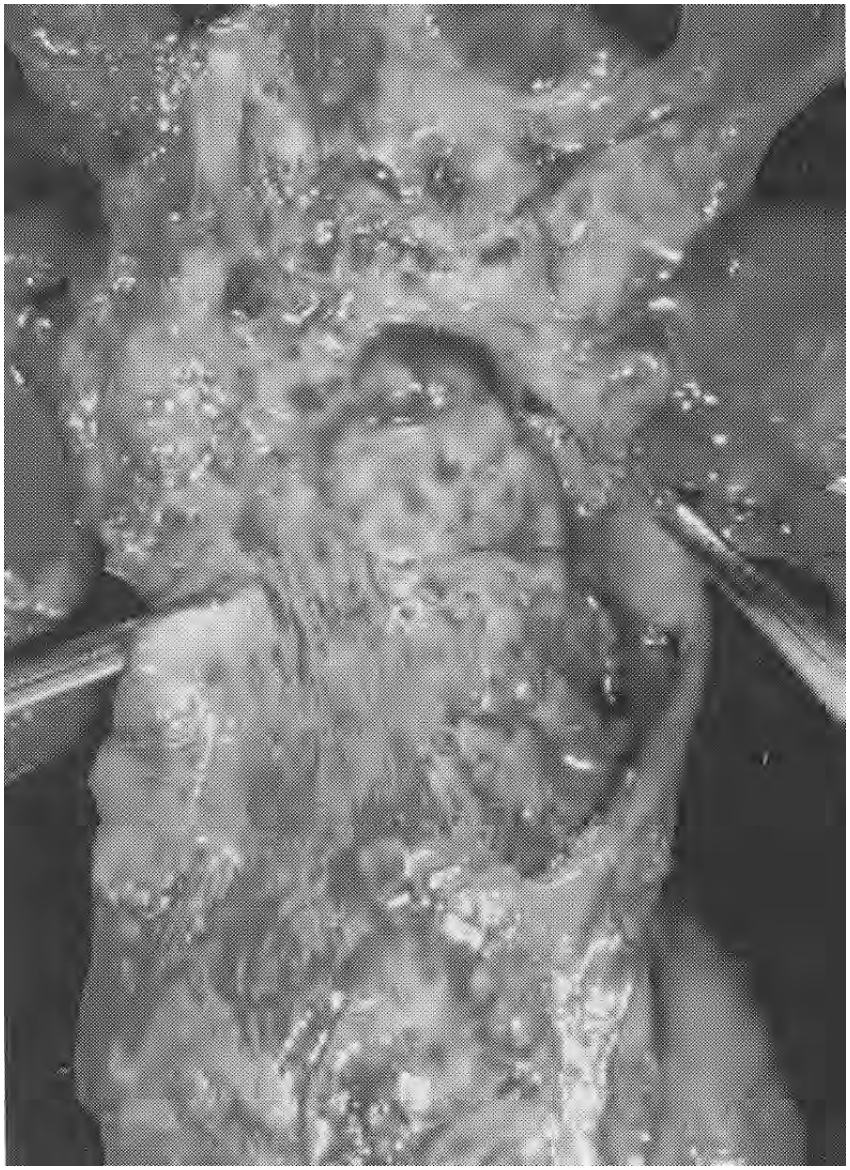


Fig. 5–3. Components of the fatty streak. There is the subendothelial accumulation of large lipid-laden cells, known as foam cells, of macrophage and smooth muscle origin.

- Foam cells=macrophages that have engulfed fatty material on endothelial layer.
- Their presence indicates chronic hyperlipidemia.

Lilly (1993) Pathophysiology of Heart Disease

Fibrous plaque: pathologic atherosclerosis



A swelling in the artery wall made of macrophage cells, debris, lipids, calcium, and connective tissue.

Complications:

1) Calcification-rigid and fragile

2) Thrombosis

3) Hemorrhage

4) Aneurysm (dilatation)

- Myocardial infarction
- Stroke
- Claudication

Lilly (1993) Pathophysiology of Heart Disease

Fibrous plaques damage the endothelial layer

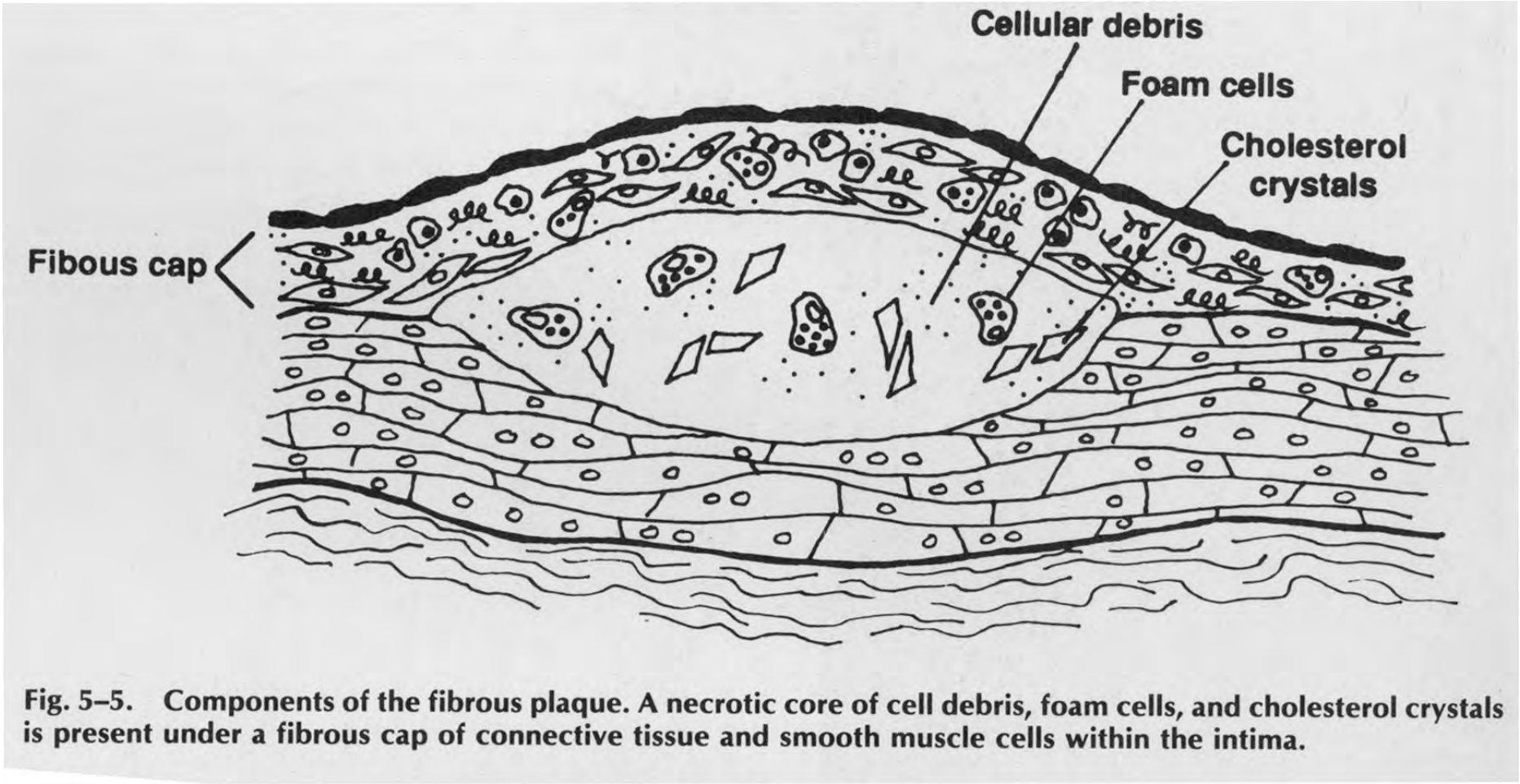


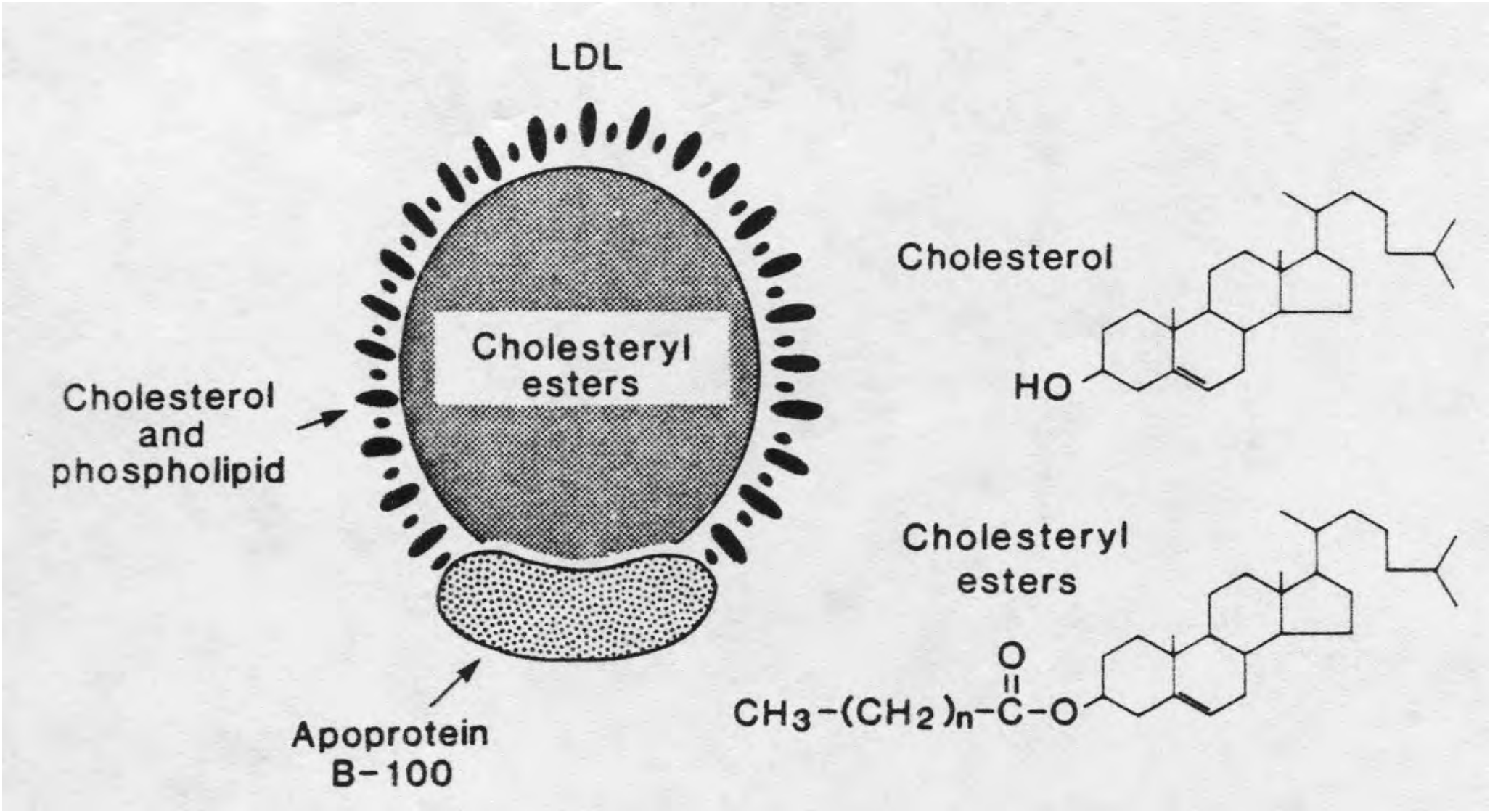
Fig. 5–5. Components of the fibrous plaque. A necrotic core of cell debris, foam cells, and cholesterol crystals is present under a fibrous cap of connective tissue and smooth muscle cells within the intima.

Lilly (1993) Pathophysiology of Heart Disease

Risk factors for cadiovascular disease

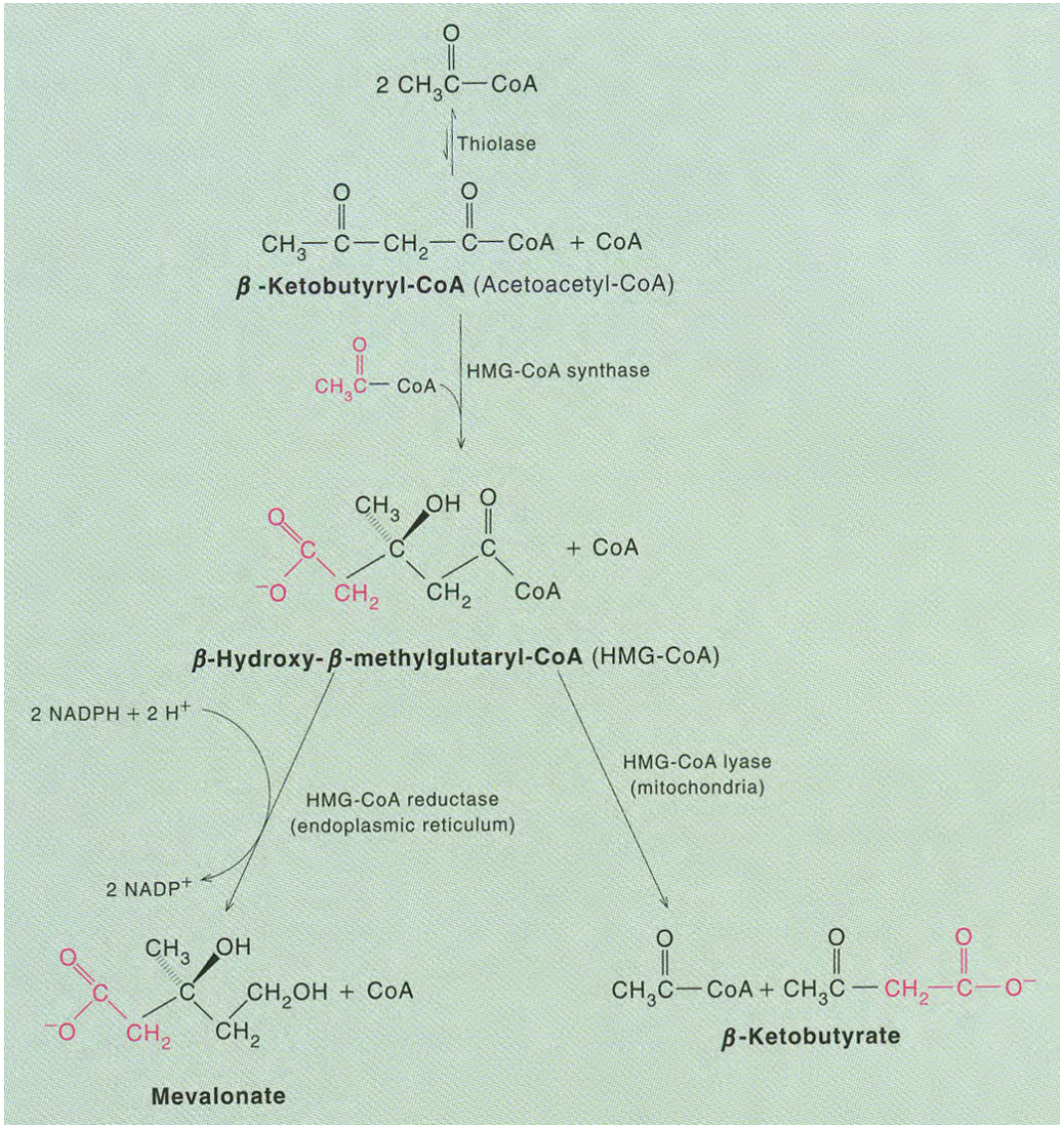
- hyperlipidemia
- hypertension
- smoking
- diabetes
- age
- male
- family history

Cholesterol is carried mostly as low density lipoprotein (LDL) particles



Brown & Goldstein (1986) Science 232:34

HMGCoA controls the rate limiting step in cholesterol synthesis

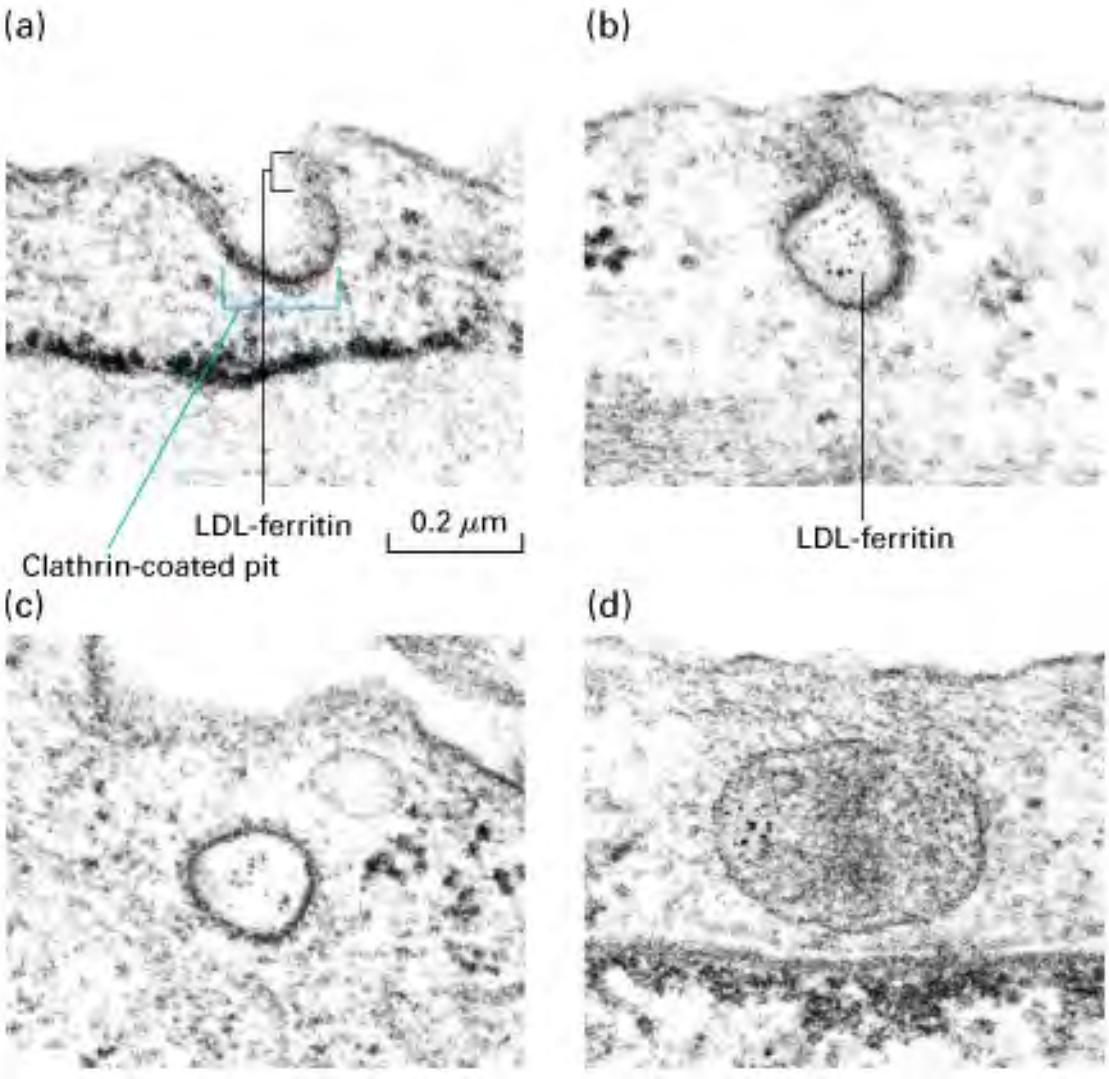


- Cholesterol is sterol lipid.
- Essential component (30%) of animal cell membrane.
- Precursor for biosynthesis of steroid hormones, bile acid, vitamin D.
- Synthesized by ER of hepatocytes.
- Two thirds of body's cholesterol is internally synthesized.

Mevalonate is early precursor to cholesterol.

Zubay, 1998

Receptor-mediated endocytosis via clathrin-coated pits and vesicles

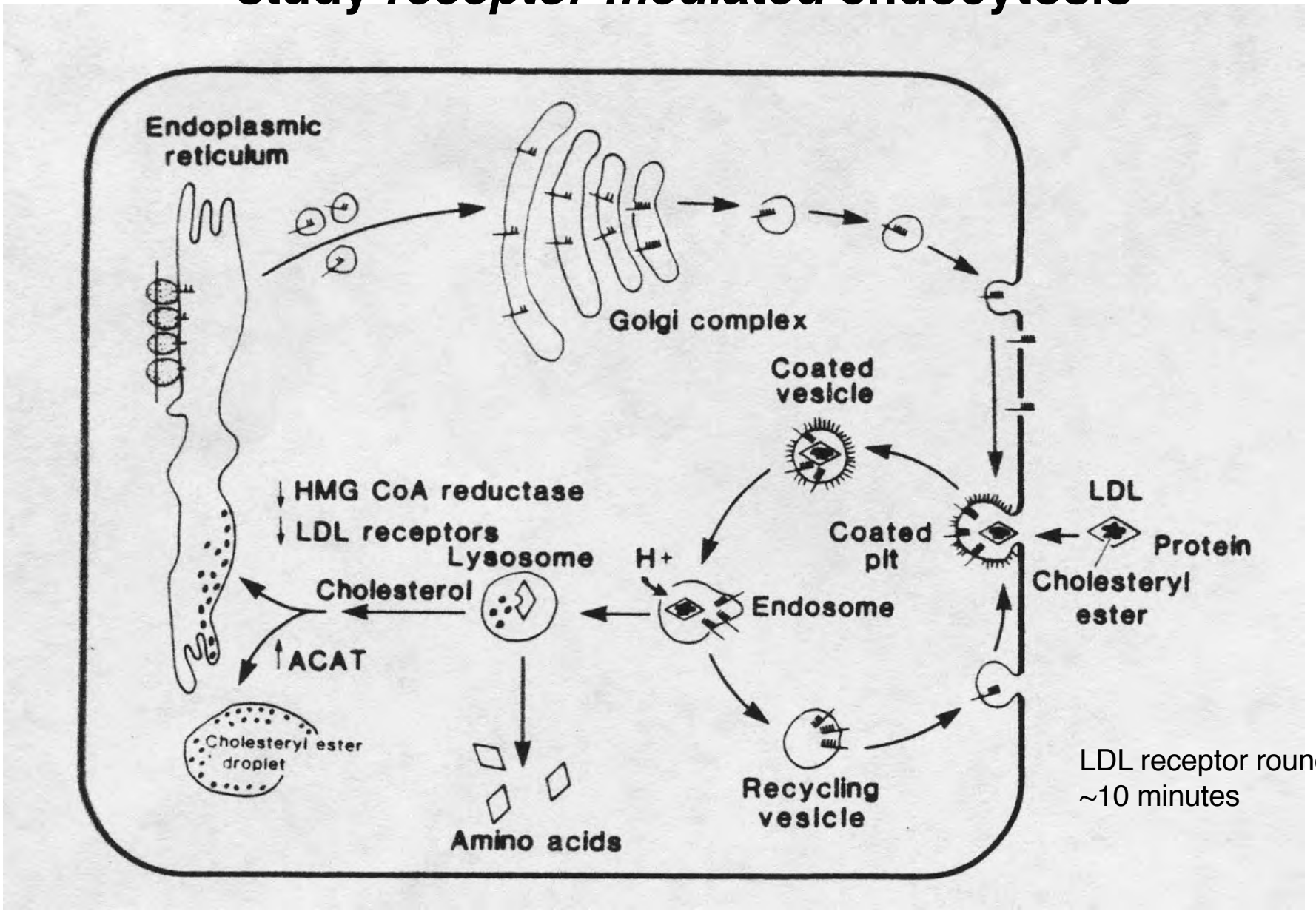


Endocytosis:

- Internalization of lipids, integral membrane protein, and extraceullar material via membrane vesicles.
- Allows interaction of cell with its environment.
- The most well-studied form is clathrin-mediated endocytosis; the clathrin coat is visible by EM.
- But note: a large proportion of endocytic events is clathrin-independent.

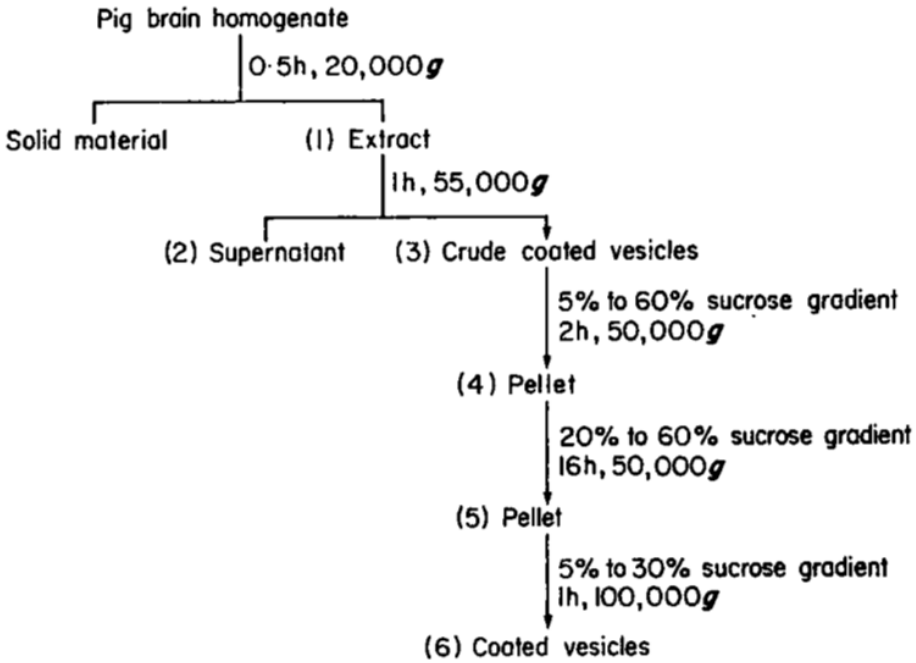
Lodish et al, 2000

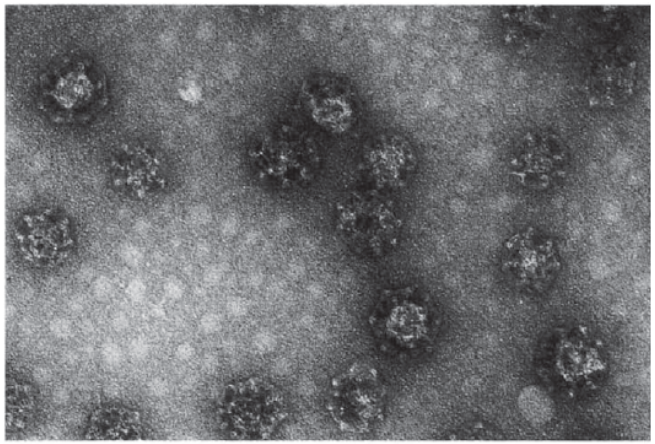
LDL receptor recycling pathway: a model system to study *receptor-mediated* endocytosis



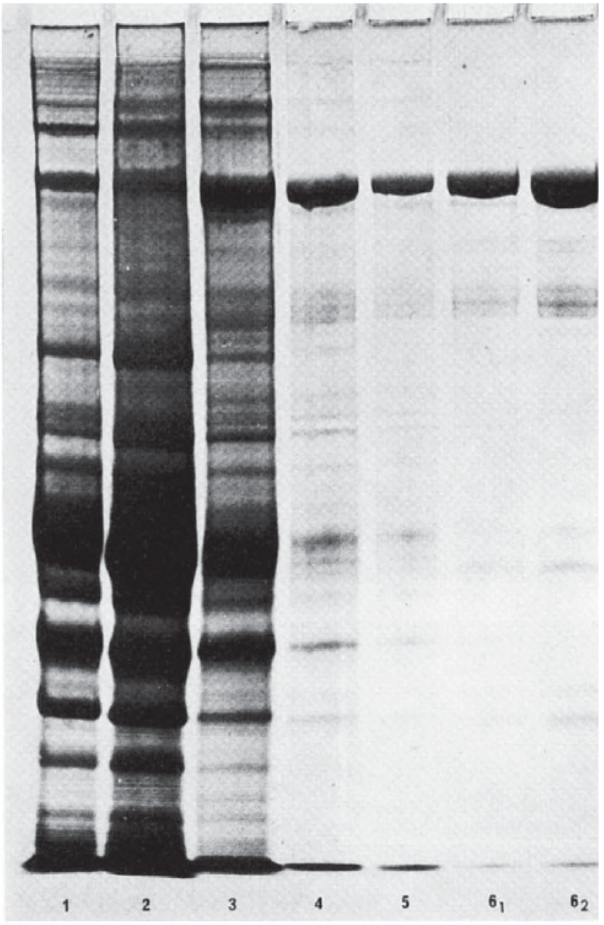
Brown & Goldstein (1986) Science 232:34

Clathrin is a major protein in purified coated vesicles





Negative stain of prep

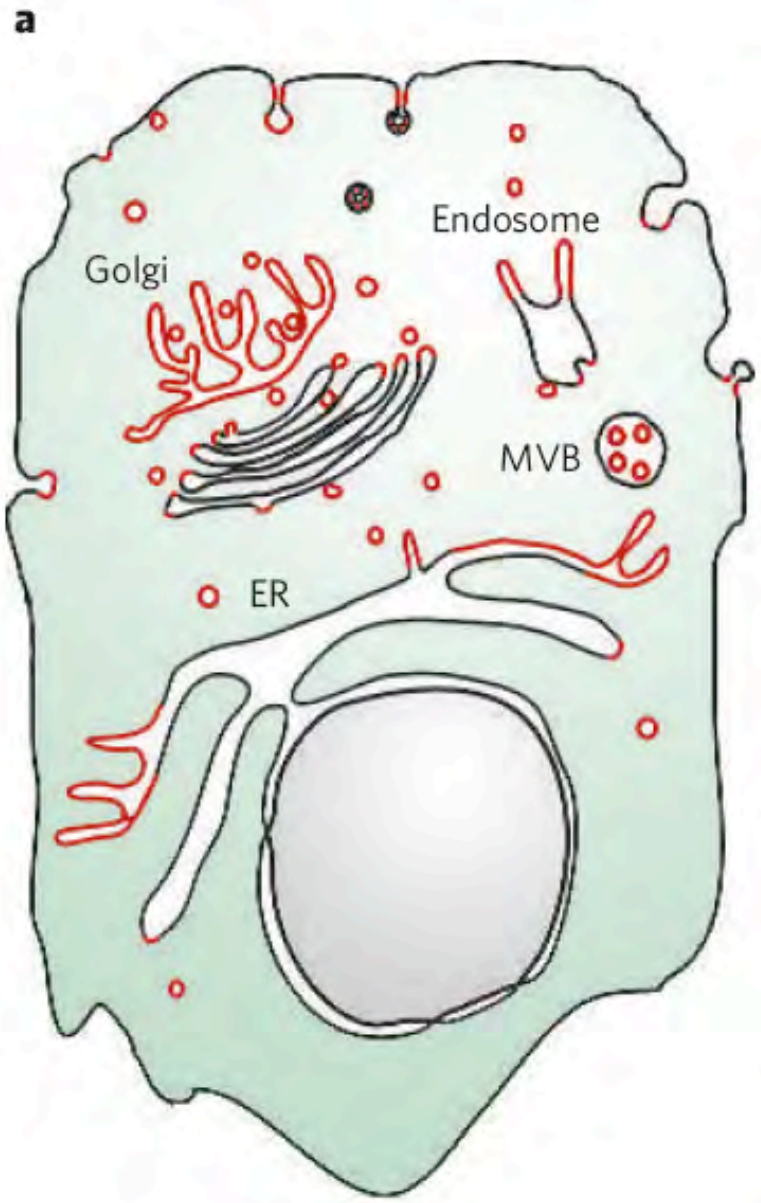


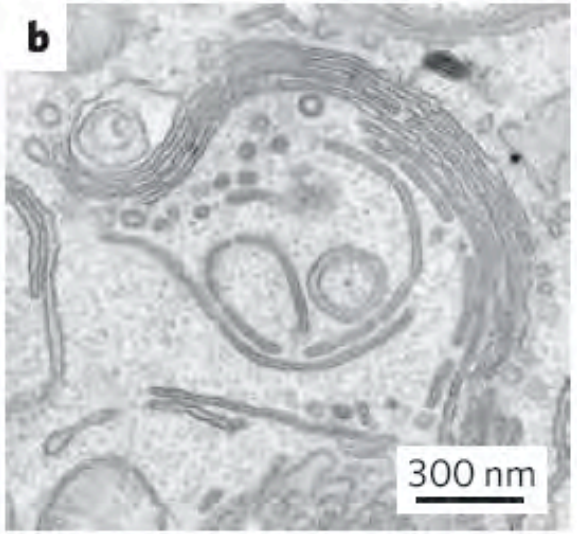
Proteins present at various stages of purification. Major protein=180 kD. Termed “clathrin”.

- In 1975, coated vesicles were purified from pig brain.
- In highly purified preps, the most abundant protein is ~180 kD.

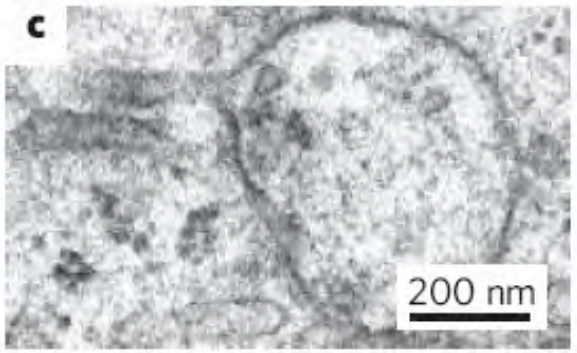
Pearse, B (1975) J Mol Biol

Curved membranes in cells

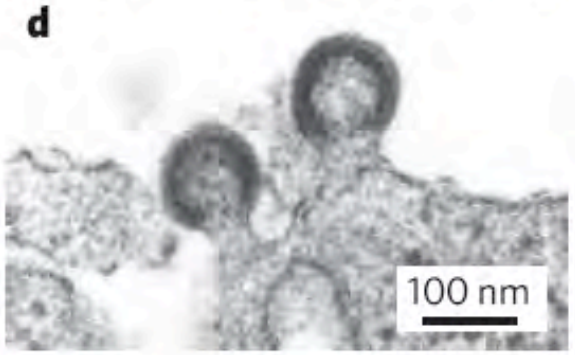




Golgi



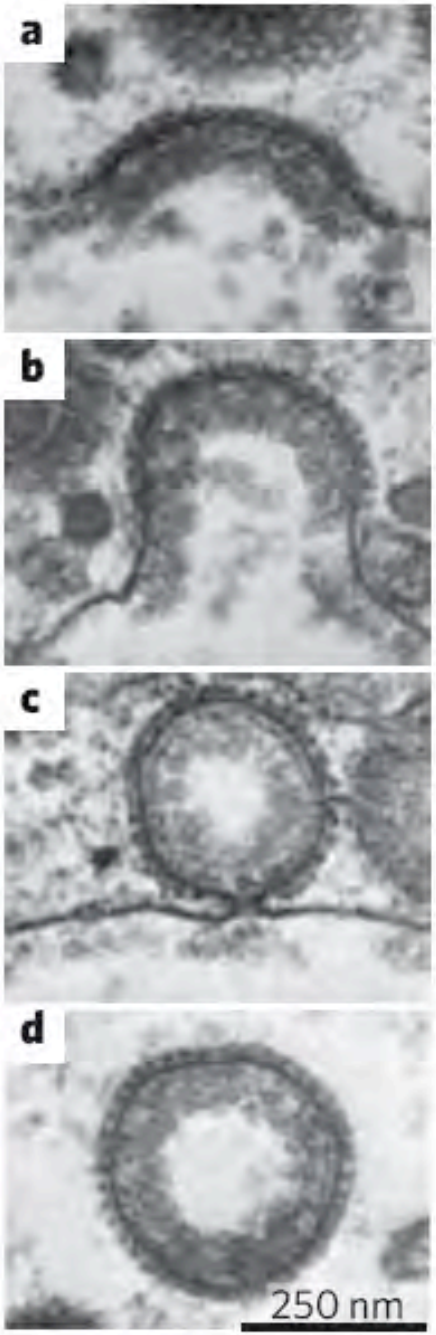
Endoso
me

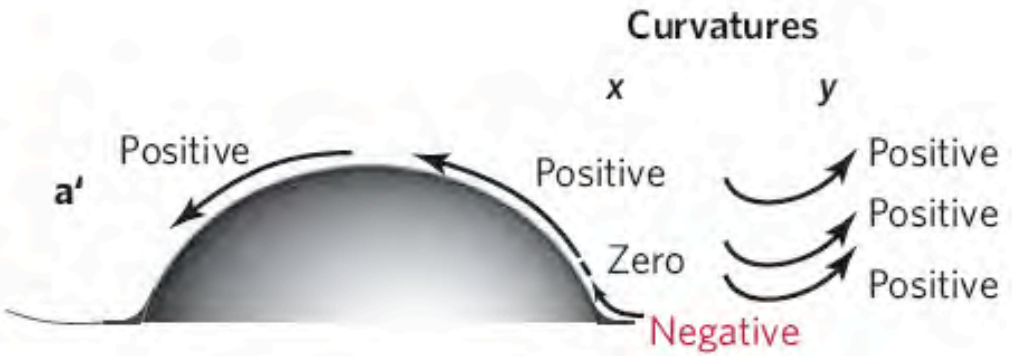


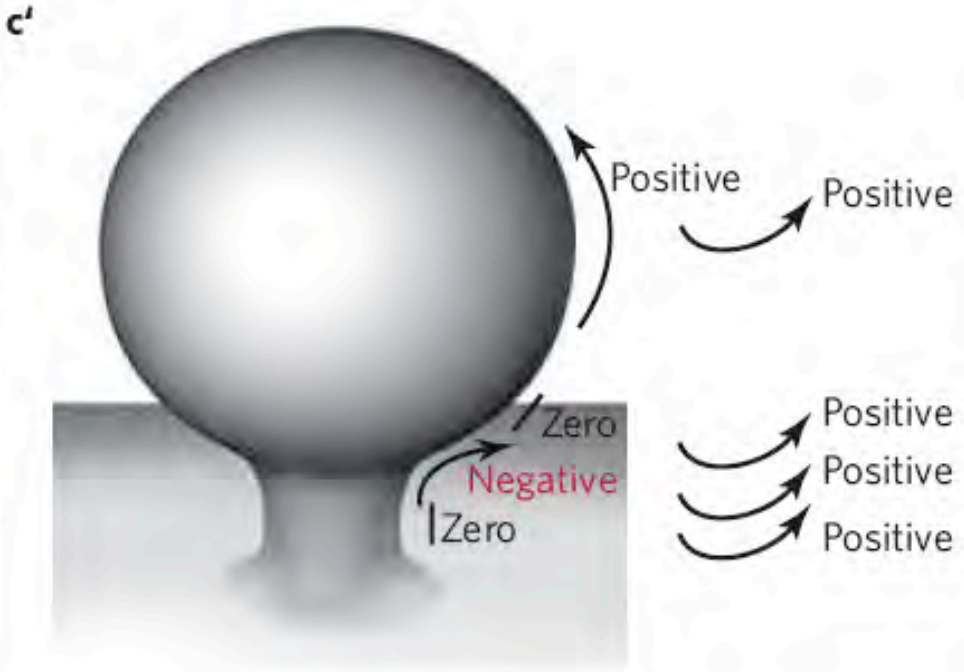
HIV
budding

McMahon, 2005

Biological membranes often show high curvature







What are the mechanisms to deform lipid membranes?

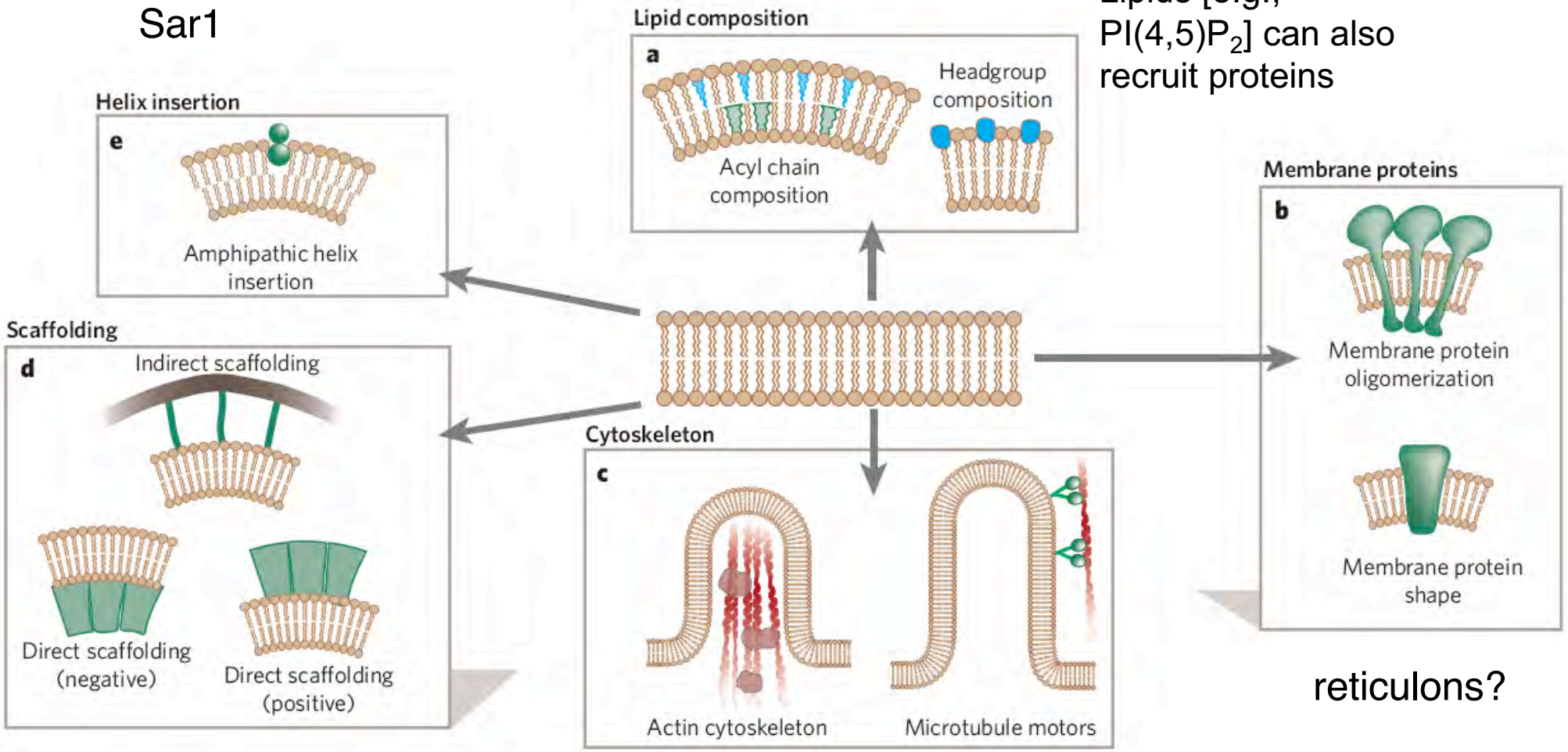
In diagram, positive curvature indicates regions of membrane that curve inwards towards the cytoplasm (like dome of invaginating vesicle).

McMahon, 2005

Ways to impose membrane curvature

Sar1

Lipids [e.g., $PI(4,5)P_2$] can also recruit proteins

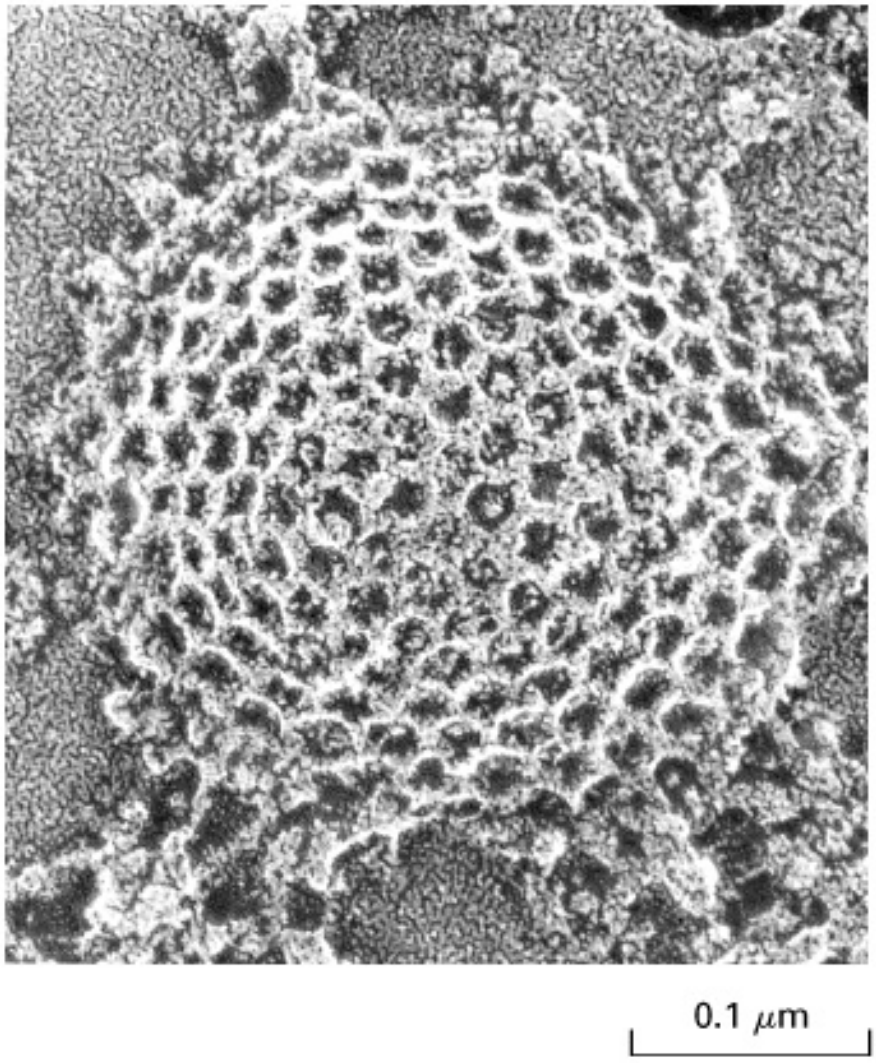


reticulons?

clathrin and other vesicle coats
dynamin
BAR domains

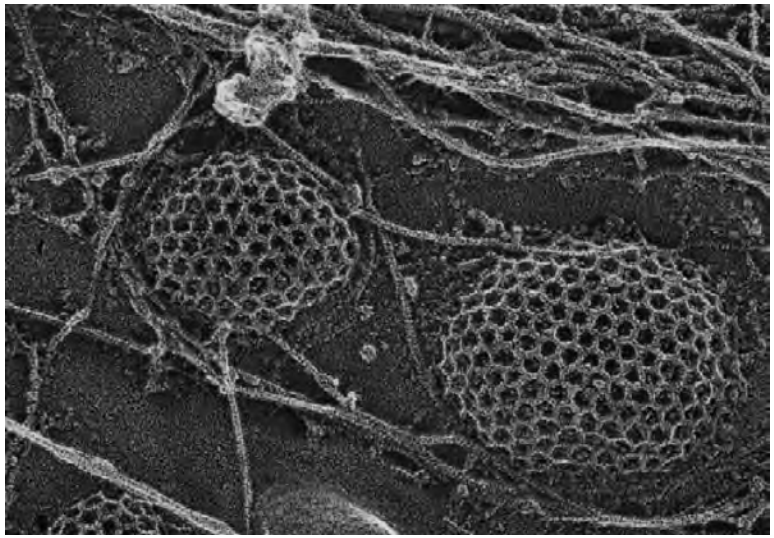
McMahon, 2005

EM image of clathrin-coated pits from cytosolic side



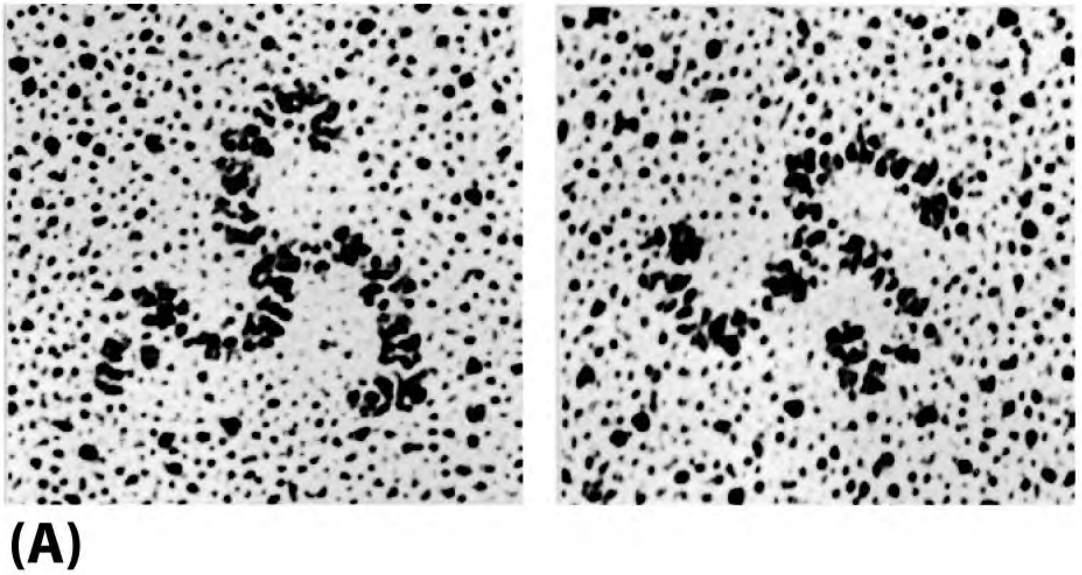
Lodish et al, 2000

- Budding of vesicles is driven by the polymerization of coat proteins onto the membrane.
- The coat proteins control curvature of the membrane.
- The coat protein helps determine what cargo is carried within the vesicle.

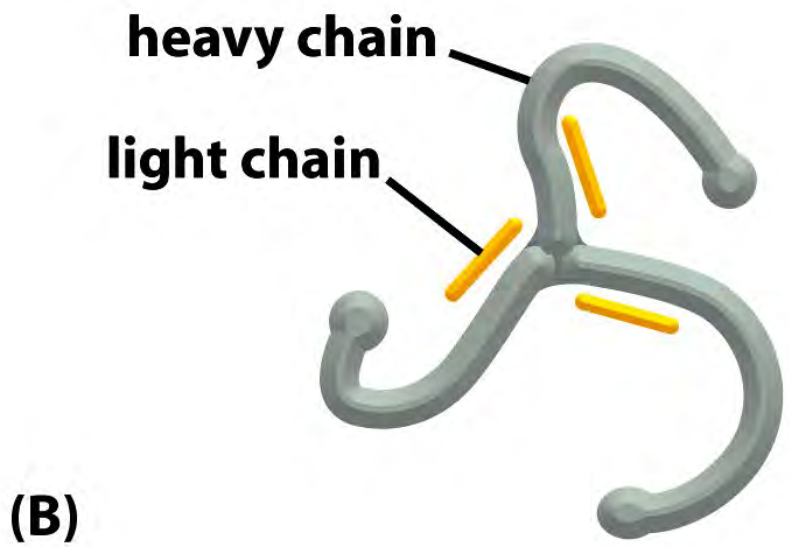


Heuserlab.wustl.edu

Clathrin is a major vesicle coat protein



(A)

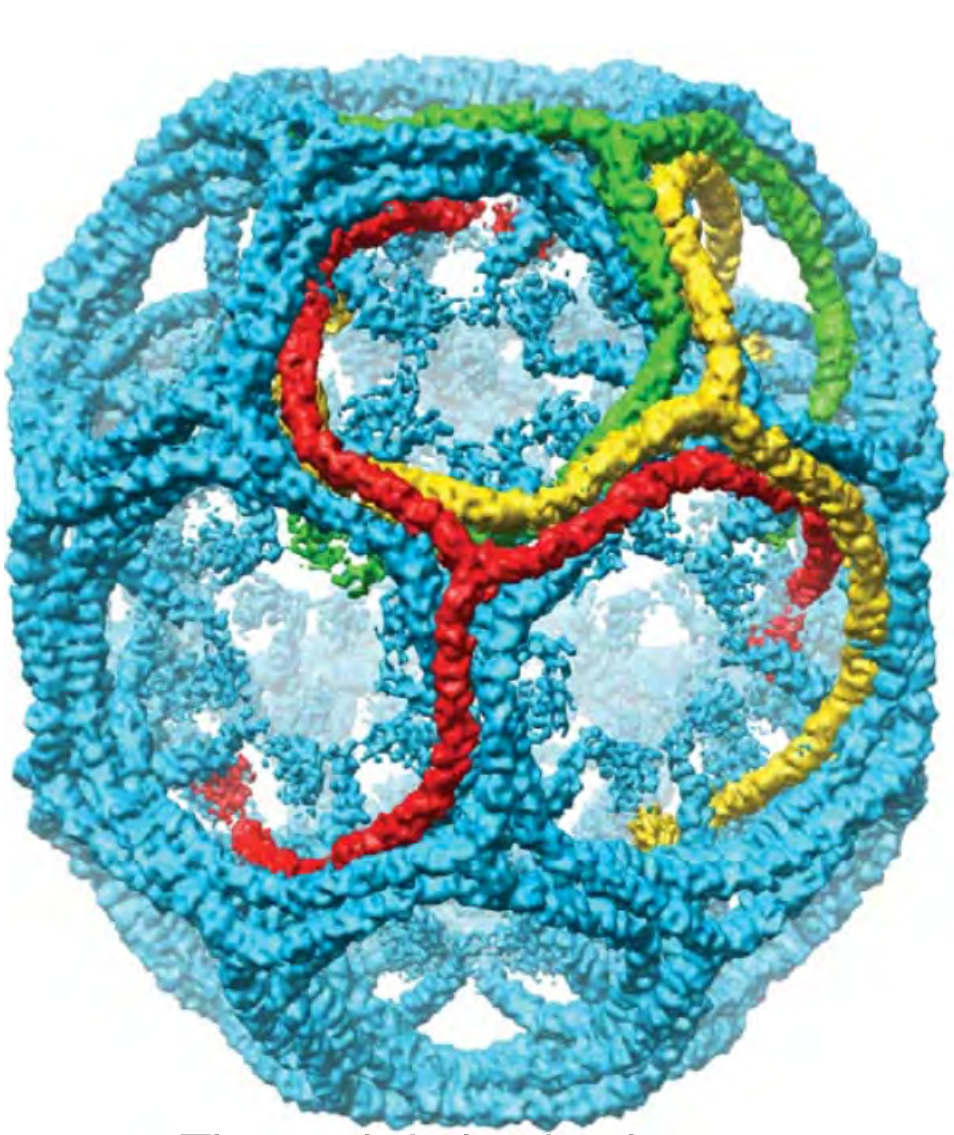


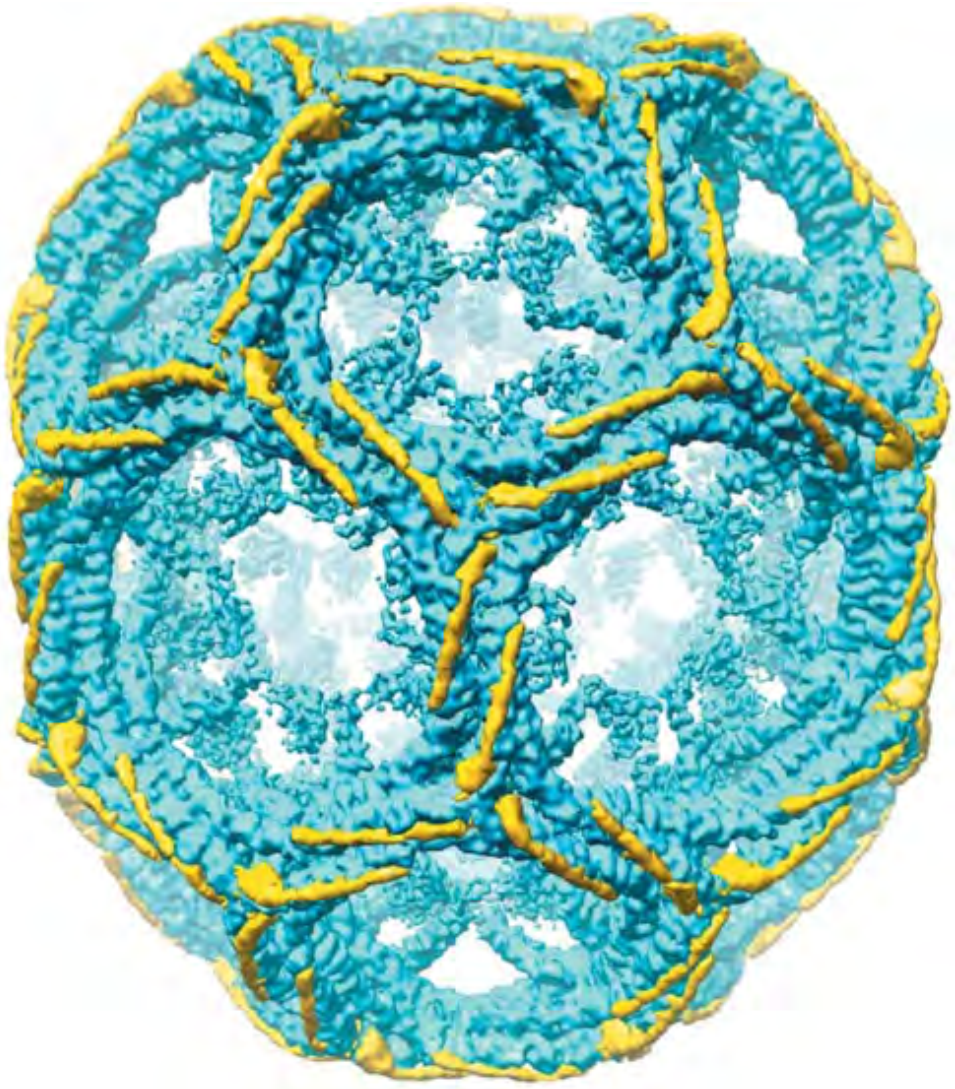
(B)

- Forms a triskelion shape made of 3 heavy chains and 3 light chains.
- The triskelion is a basic unit forming the polyhedral lattice on the outside of a coated vesicle.
- Triskelions can assembly into a basket.

Figure 13-7a, b *Molecular Biology of the Cell* (© Garland Science 2008)

Assembly of clathrin into cages

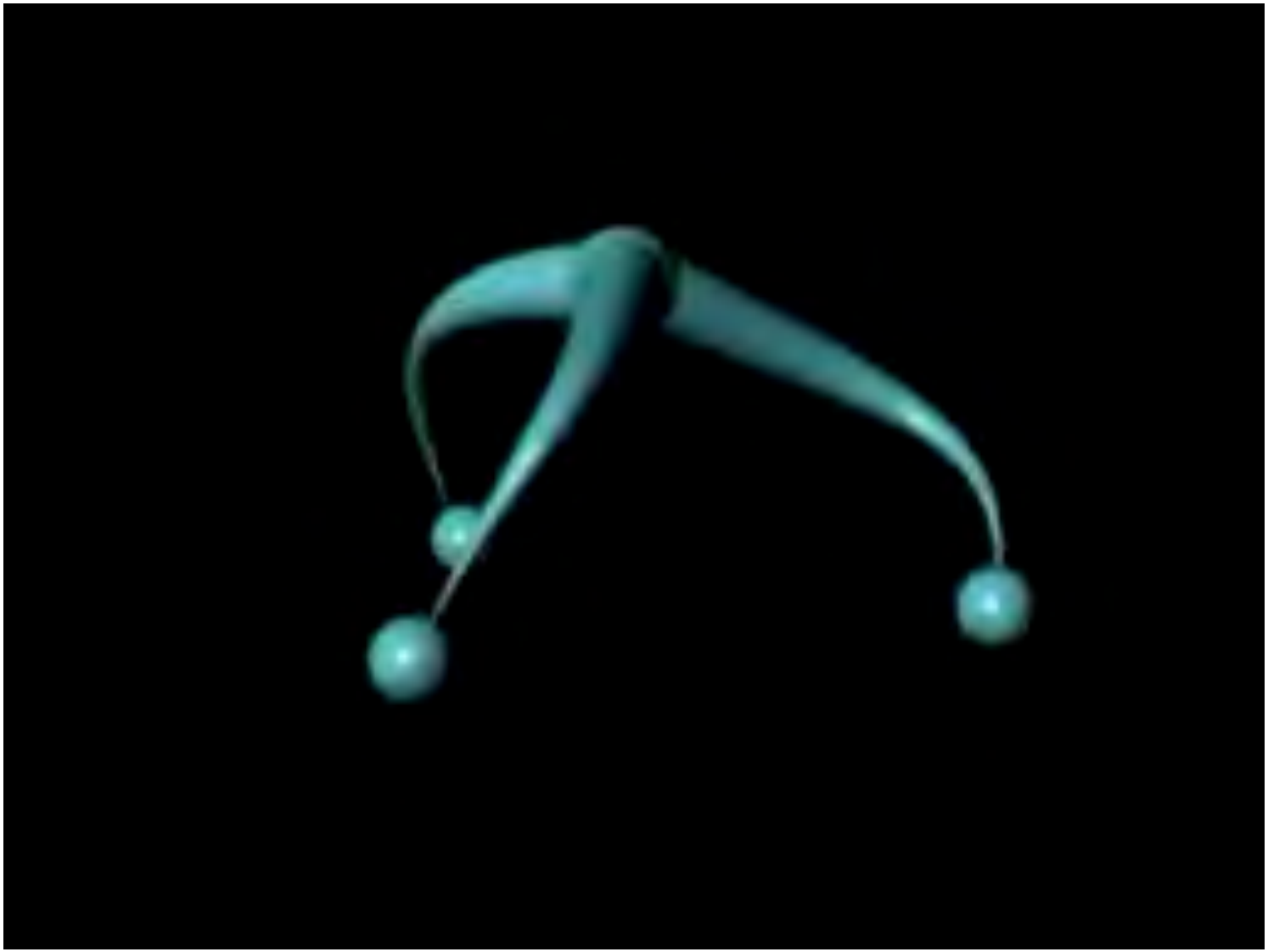




- The clathrin basket has pentagons and hexagons.
- Different size vesicles are possible.

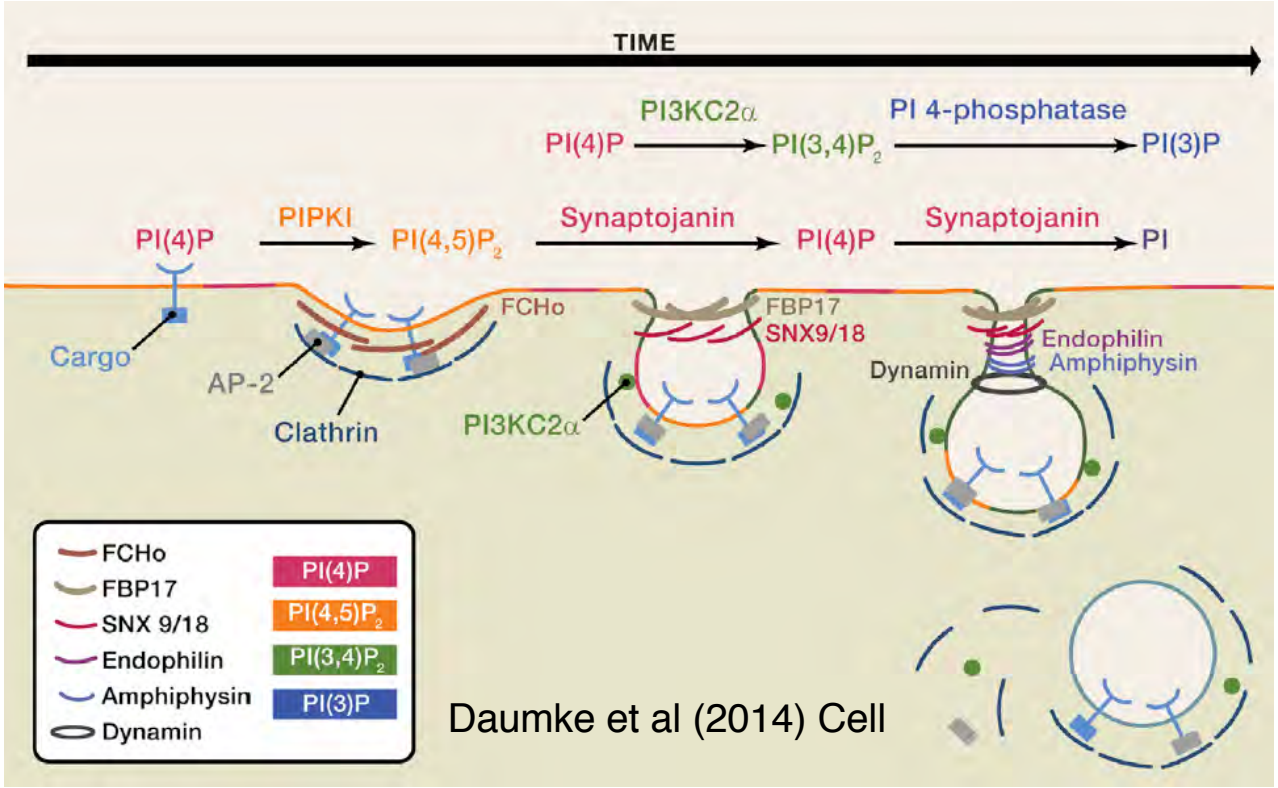
Fotin et al (2004) Nature

Assembly of clathrin coated vesicle

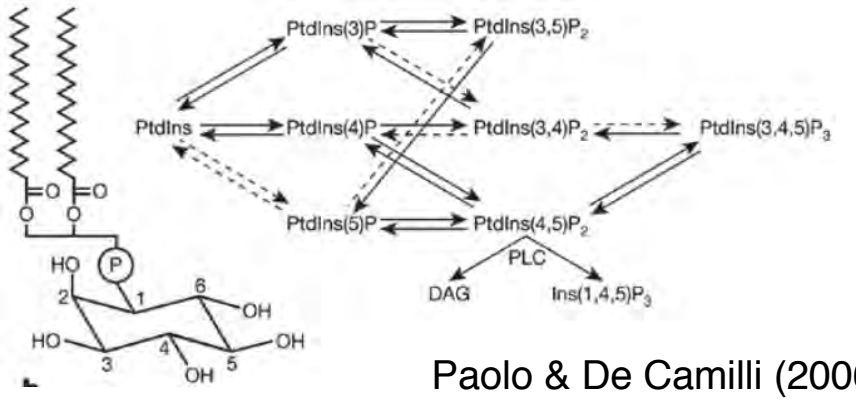


movie by Allison Bruce

Lipids play important roles in endocytosis



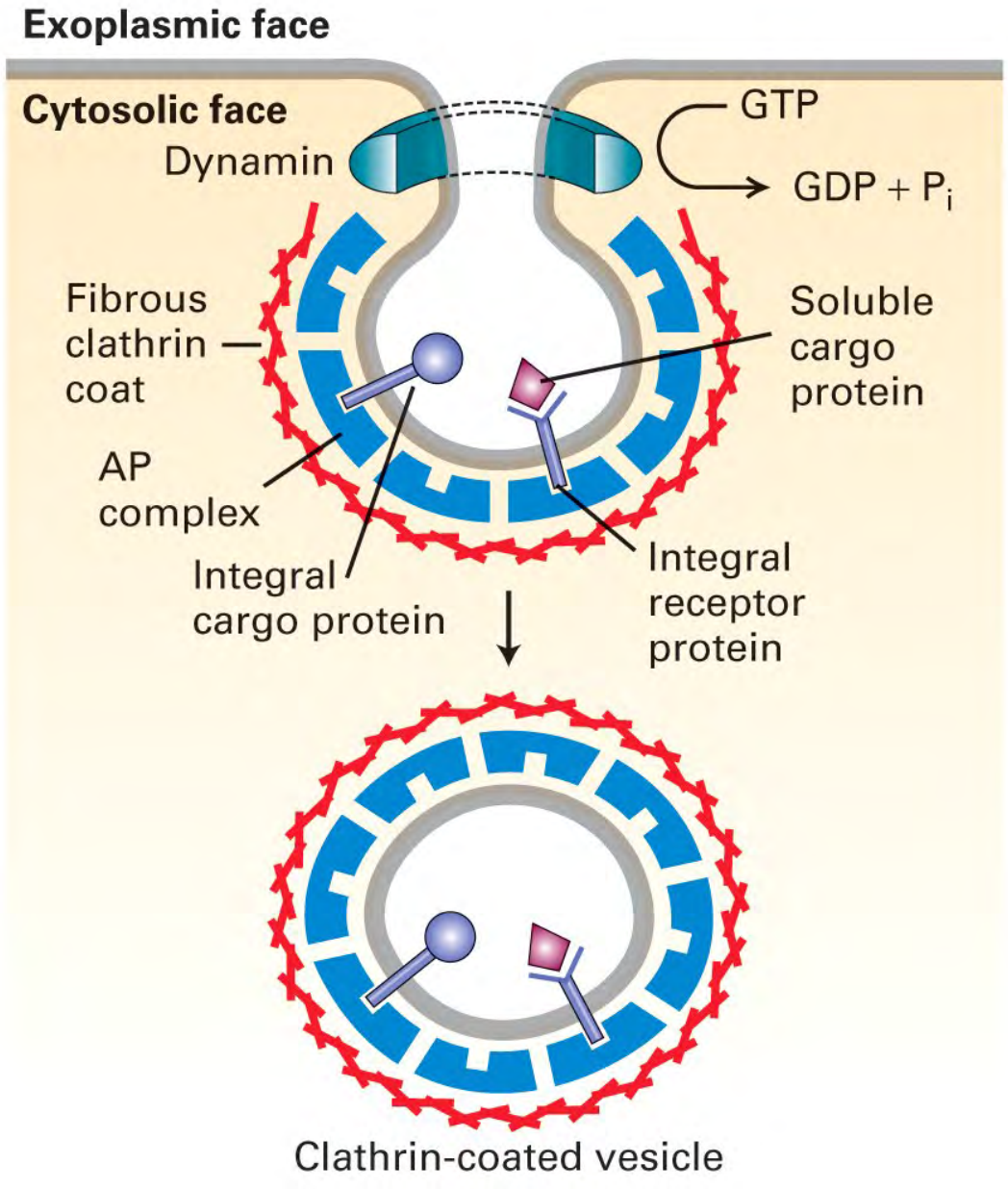
Daumke et al (2014) Cell



Paolo & De Camilli (2006) Nature

- Local lipid composition is dynamically changed during CME.
- Phosphoinositides, phosphorylated products of phophatidylinositol (PtdIns/PI), play important roles.
- Pits are initiated at sites containing $PI(4,5)P_2$ on the cytoplasmic leaflet.

Adaptor proteins bind membranes, clathrin, and cargo



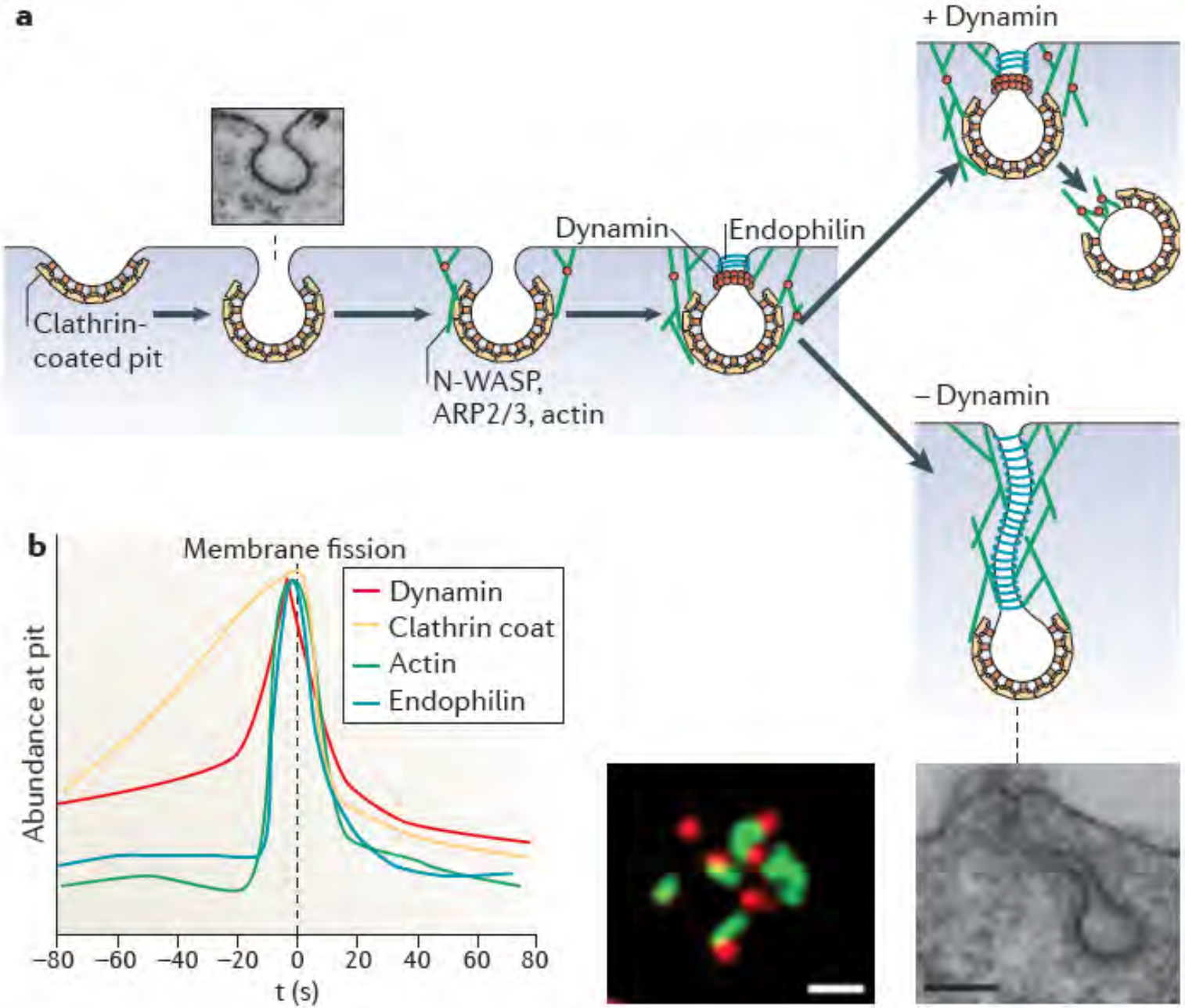
1) AP-2 (Adaptor Protein 2) complex is the major clathrin adaptor.
2) Nucleation function: mediates binding of clathrin to membrane.
3) Contains phospholipid-interacting motifs
4) Cargo selection function: Involved in selective recruitment of transmembrane proteins (cargo) into coated pits.
5) Heterotetramer: α, β2, μ2, and σ2

6) cargo recruitment motifs:
•YxxΘ (Θ=bulky hydrophobic)
•FxNPxY (in cytoplasmic tail of LDL receptor)
•(DE)xxxLL/I) "di-leucine motif")

7) Crystal structure of μ2 subunit bound to YxxΘ peptide; not accessible in large core structure of core AP-2. Requires conformational change to expose cargo binding domain of μ2.
σ2 binds dileucine motifs; also requires conformational change.

Lodish et al, 2004

Many proteins involved in clathrin-mediated endocytosis

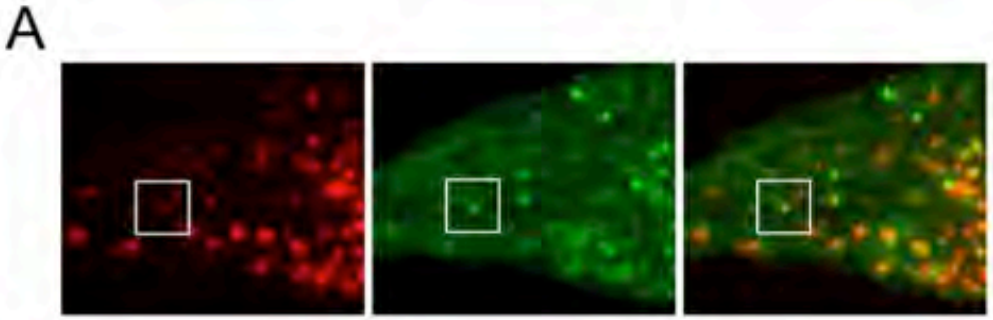


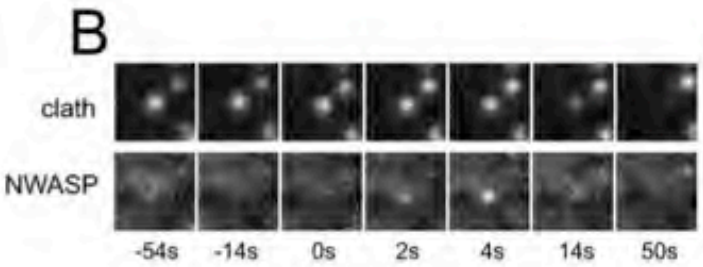
in dynamin ko cells, elongation of endocytic necks is actin-dependent

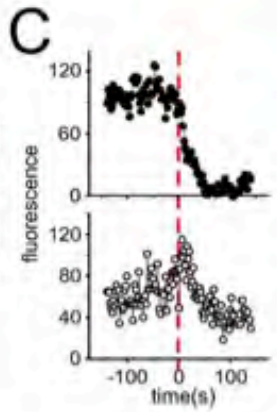
endophilin is a BAR-domain containing protein

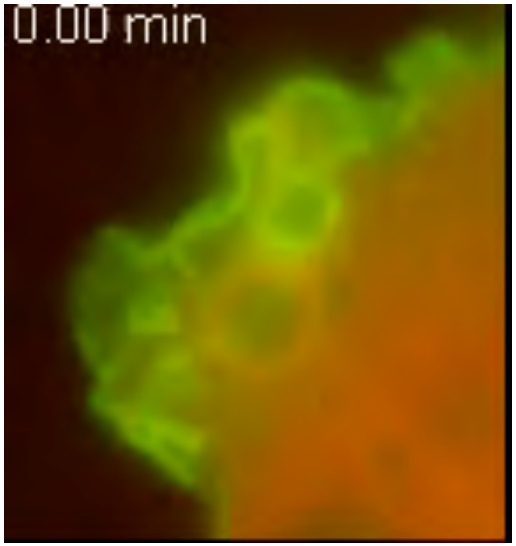
Ferguson & De Camilli (2012) NRMCB

Studying the timing of endocytic proteins

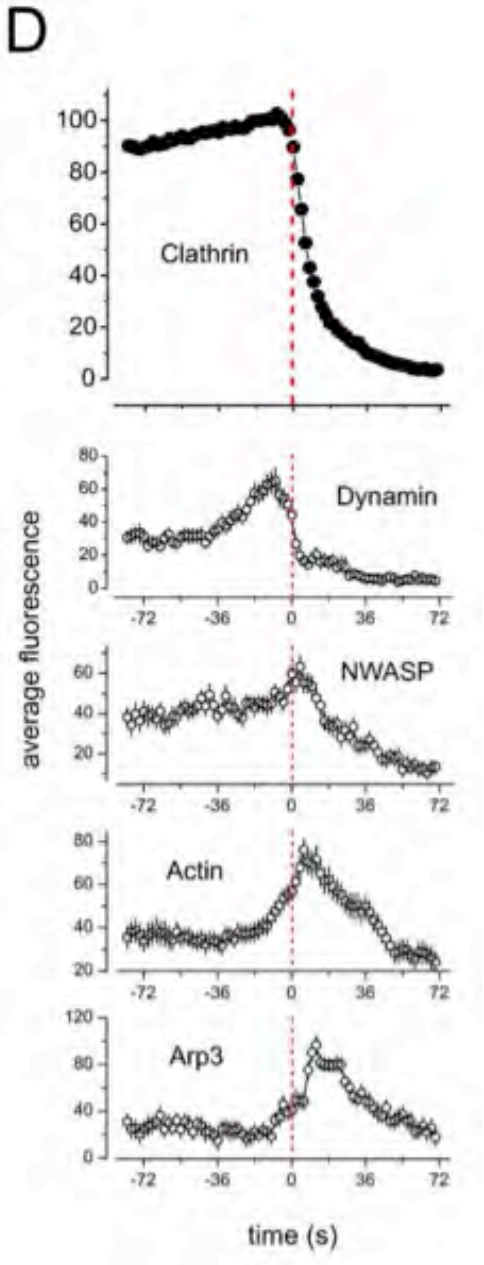








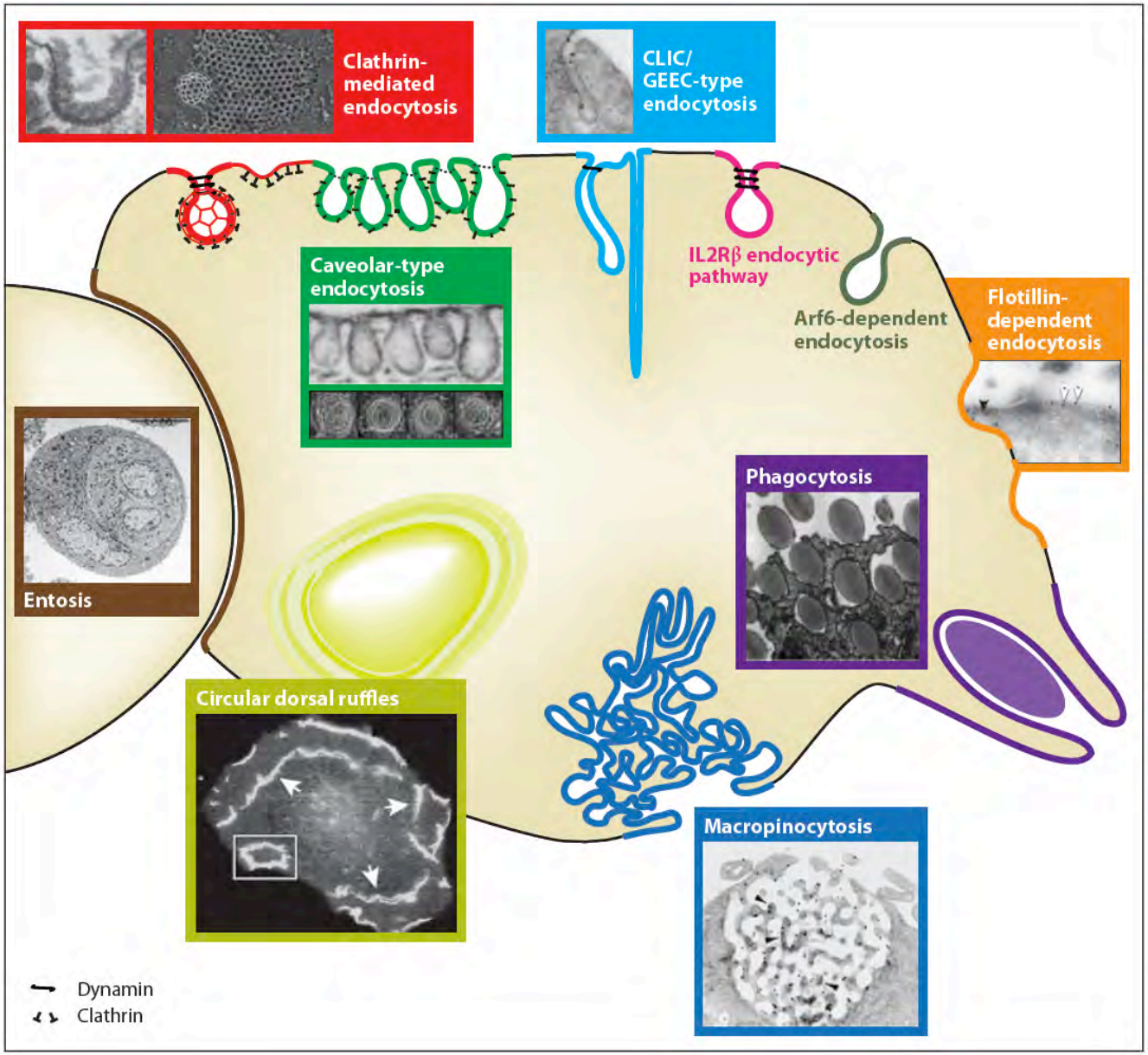
GFP fusion has PH domain that binds a specific phosphoinositide. RFP fusion has a Rab5 effector.



Zoncu et al (2009) Cell

Merifield, 2004

In addition to clathrin-mediated endocytosis, other types of endocytic events



Caveolae ("little cavities")

•Flask-shaped invaginations (60-80 nm diameter).

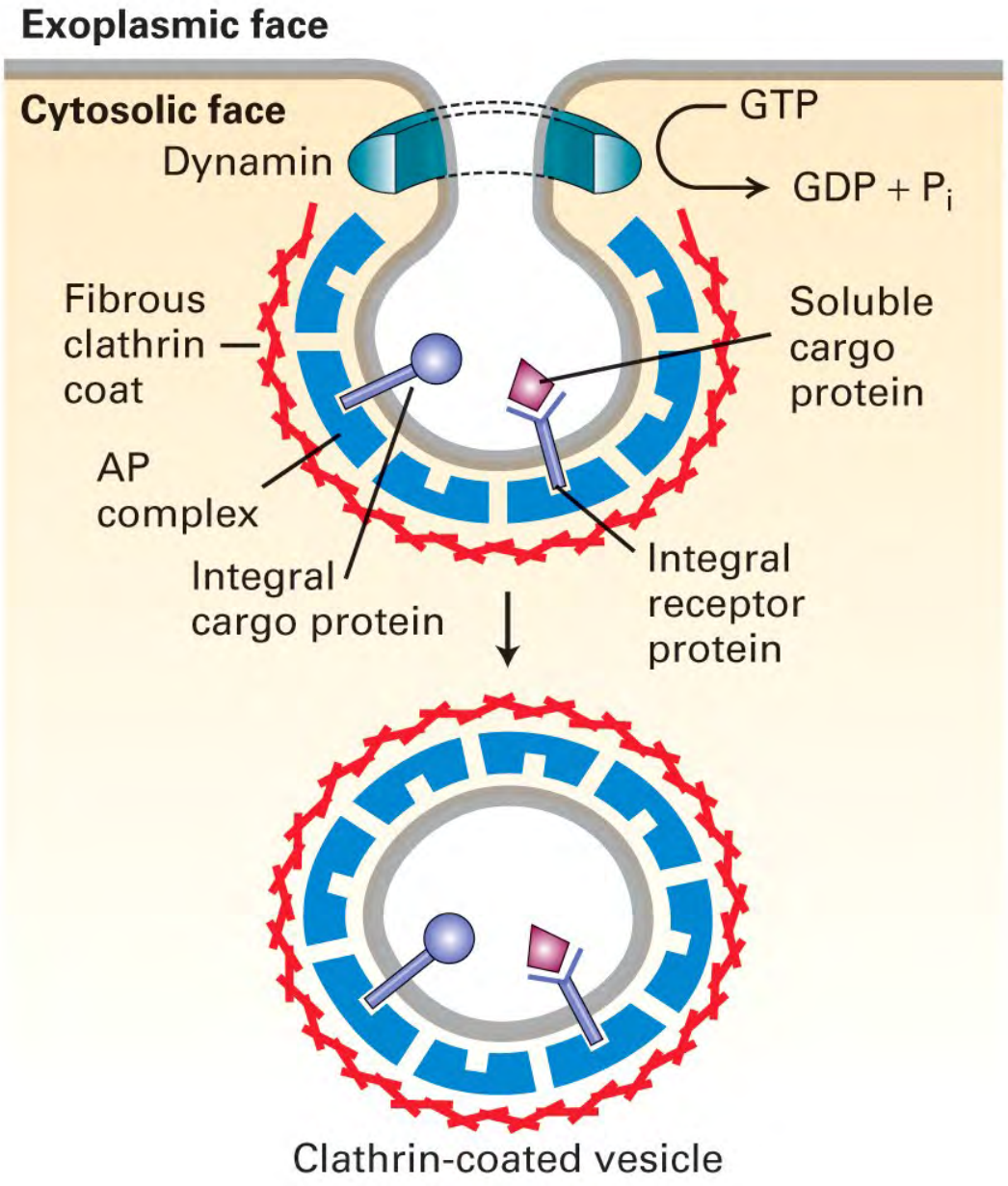
•Spike-like coat.

•Caveolins are the major structural component; inserted into the cytosolic leaflet; forms large assemblies.

•Invaginations associated with lipid rafts (enriched for cholesterol, glycosphingolipids, GPI-anchored proteins).

Doberty &McMahon (2009) Ann Rev Biochem

What causes scission of the endocytic vesicle?



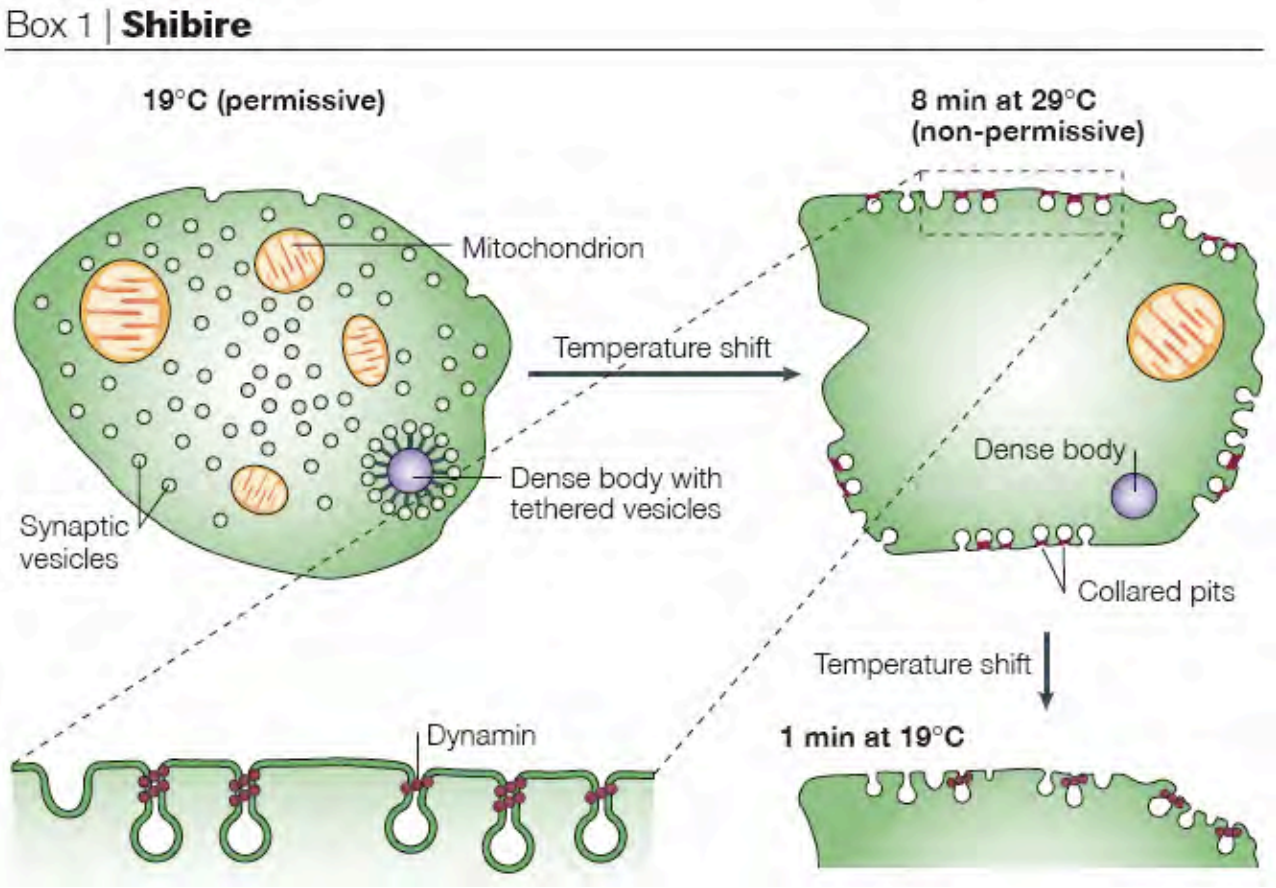
Genetic and biochemical studies implicate the dynamin GTPase

Lodish et al, 2004

Shibire mutants are paralyzed at high temperatures

Bing Zhang, UT Austin

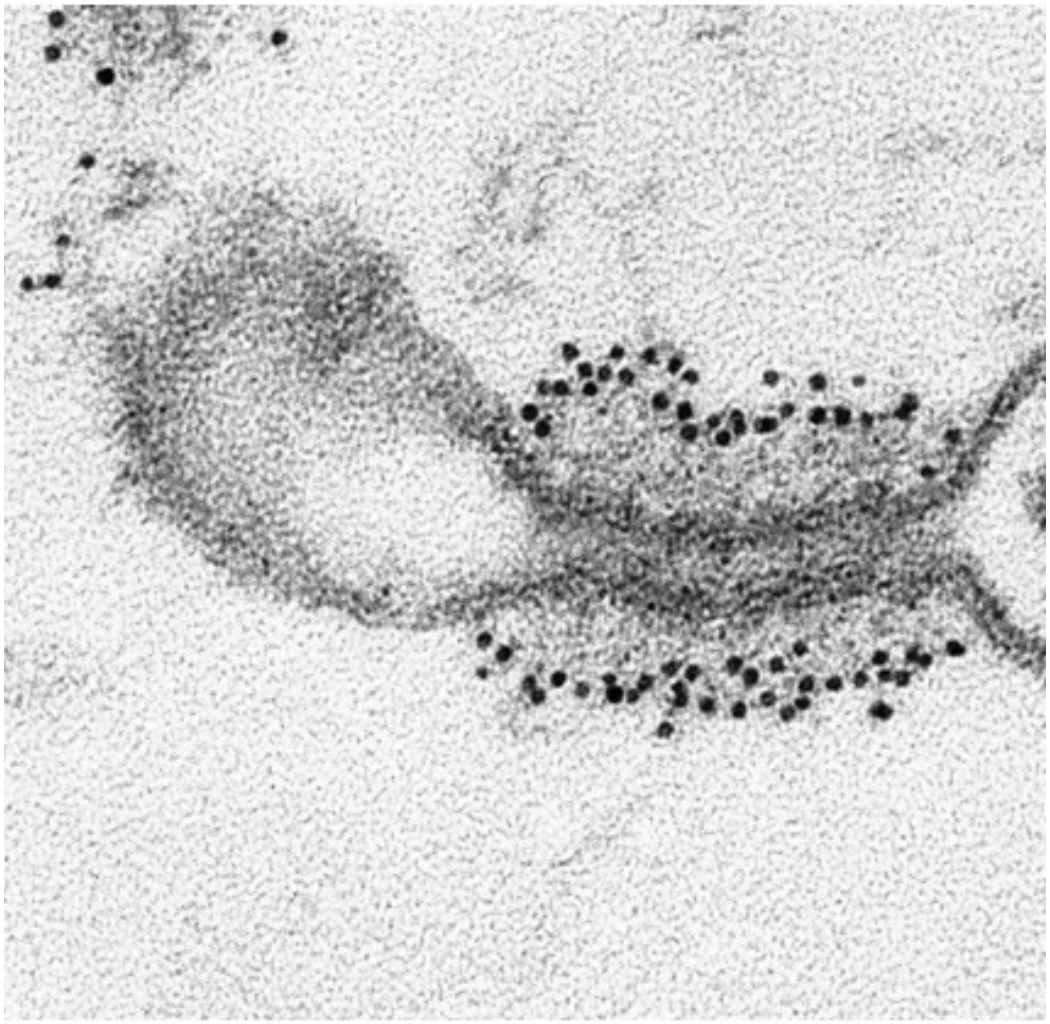
Fly shibire mutants are paralyzed due to defect in synaptic vesicle recycling



- At non-permissive temperature, Shibire flies have sudden paralysis and depletion of synaptic vesicles upon activity.
- They accumulate endocytic intermediates at synapse that are protein coated
- Shibire means “numbness”.

Praefcke and McMahon, 2004

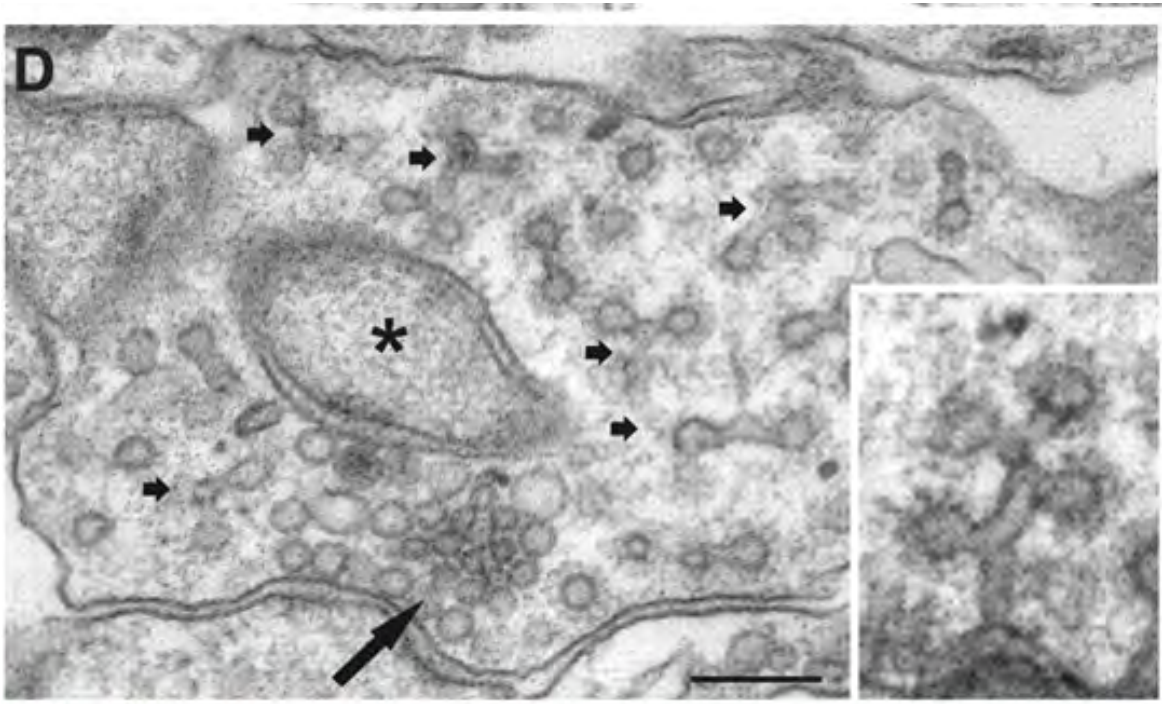
Dynamin: a GTPase enriched on the necks of endocytic vesicles

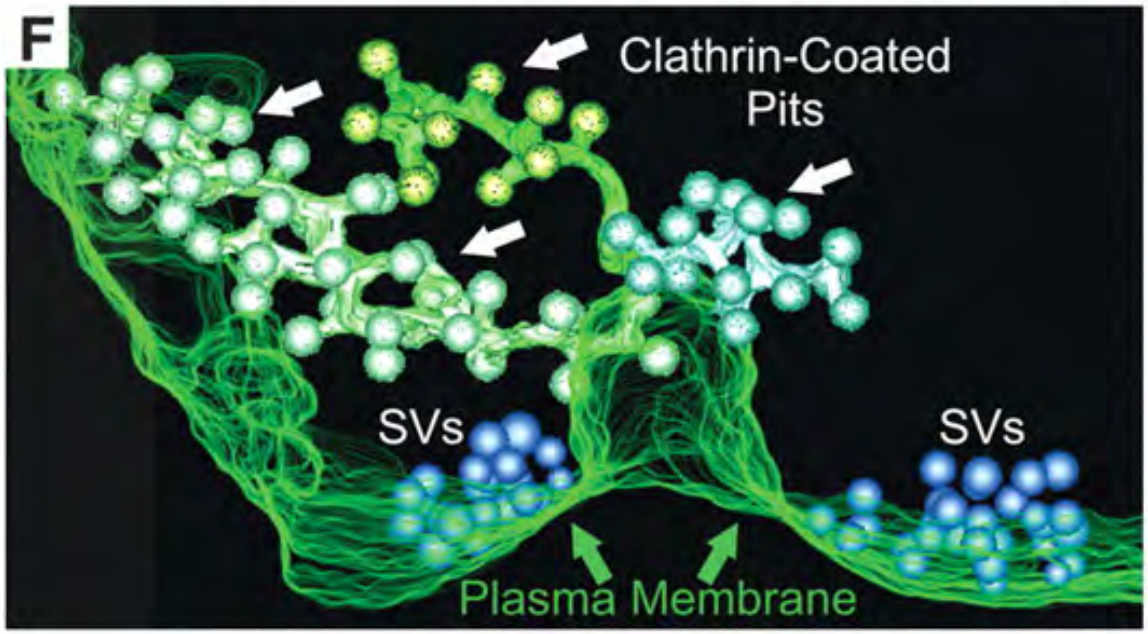


- Dynamin 1: major isoform in brain; dynamin 2: ubiquitous; dynamin 3: brain, testes, lung.

Lodish et al, 2000

Role of Dynamin 1 in synaptic vesicle recycling



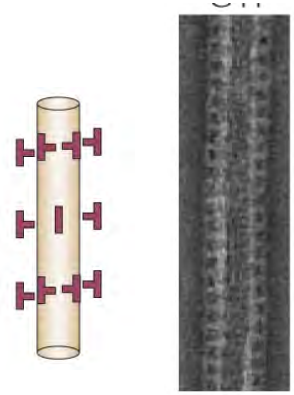


- Dynamin 1 mutant mice feed poorly after birth and die within 2 weeks.
- Neurons have functional synapses, but they have limited recycling capacity.
- With high stimulation, synaptic vesicles are depleted.
- EM shows accumulation of interconnected clathrin-coated buds.
- In contrast, expression of mutant Dyn1 or Dyn2 show much greater defects.

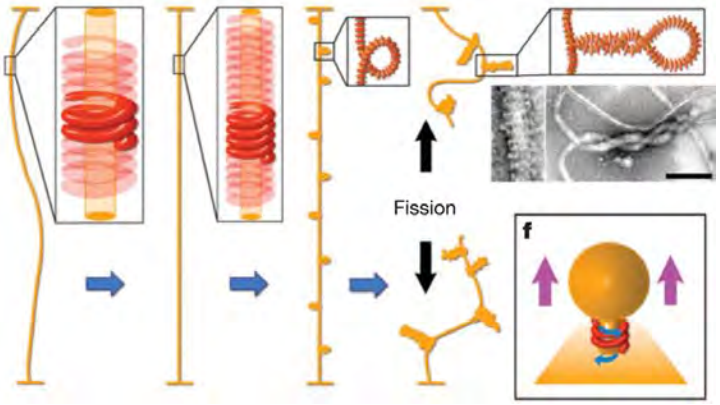
Ferguson et al. Science 2007

Models of the molecular mechanism of dynamin

Model 1: molecular constriction



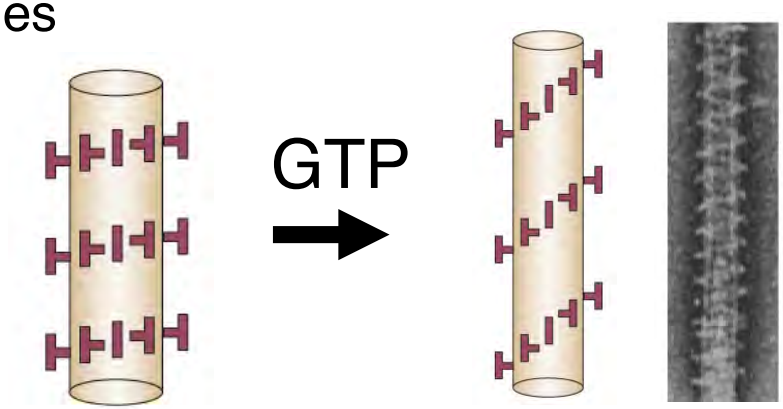
Model 3: molecular twisting



Roux et al., Nature (2006)

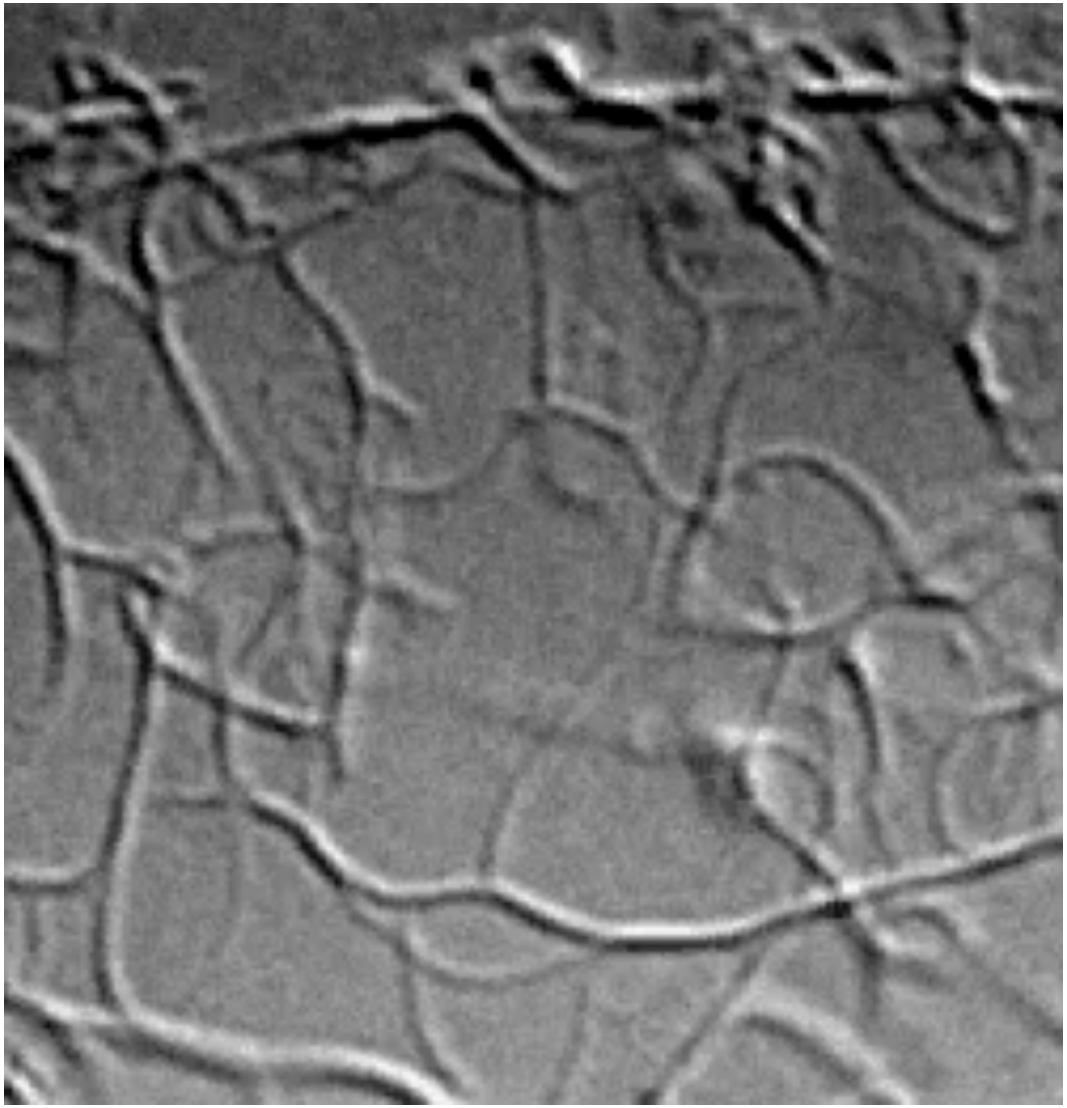
Model 2: molecular expansion

assembly on liposomes



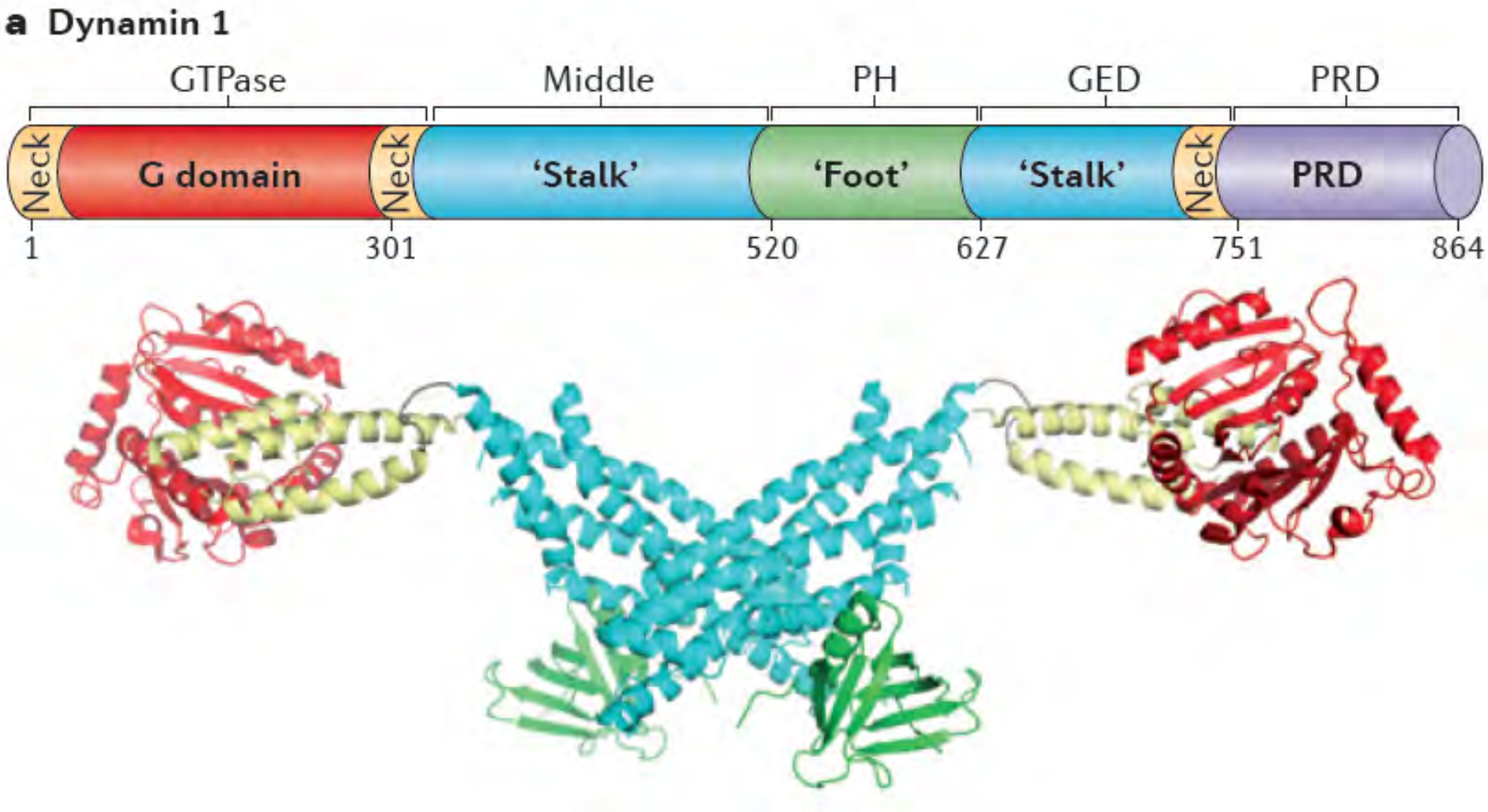
Praefcke & McMahon Nature reviews (2004)

Dynamin fragments lipid tubules upon addition of GTP



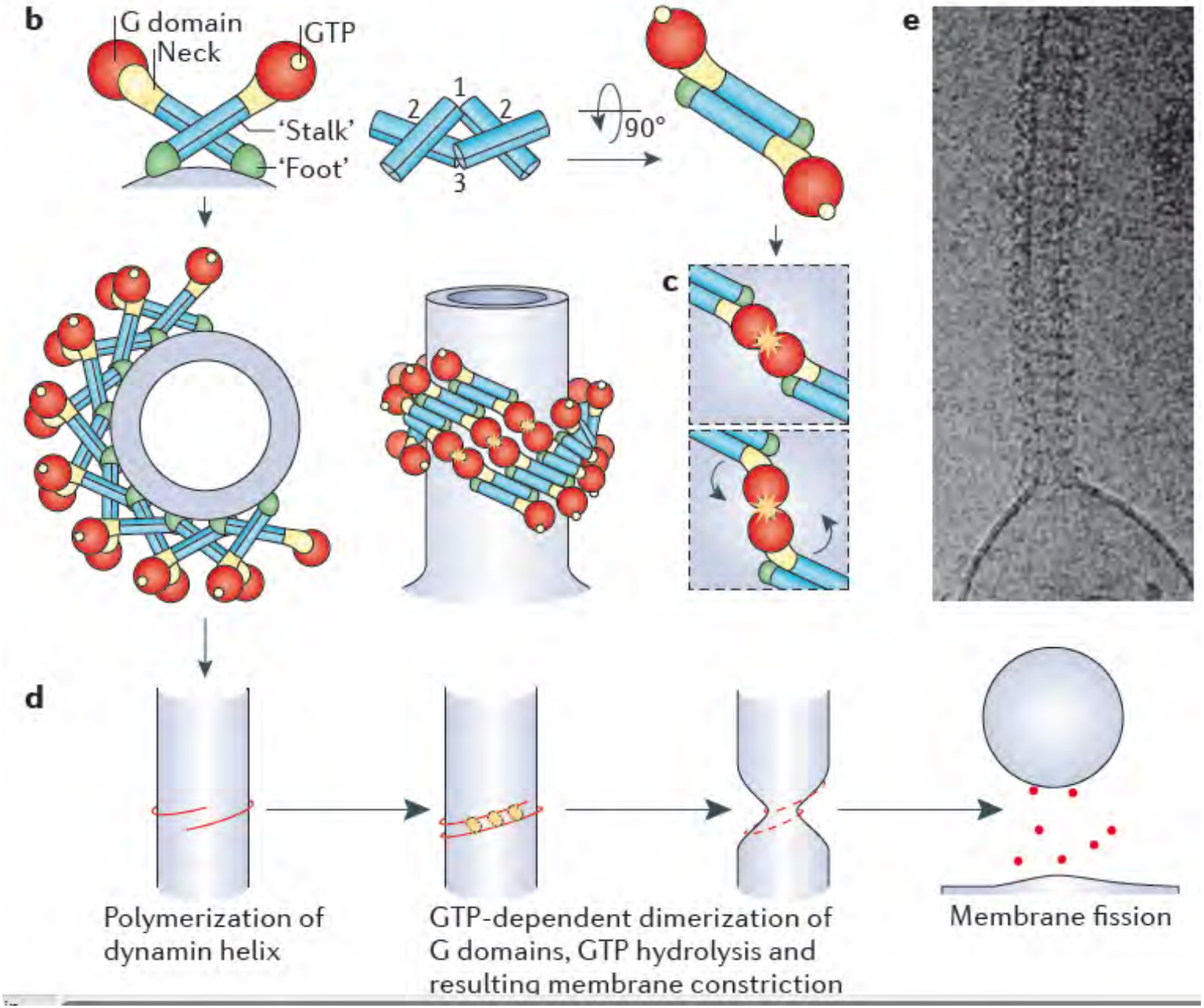
Effect of 1 mM GTP on a netwrk of dynamin-coated lipid tubules.
From P. De Camilli

Crystal structure of dynamin



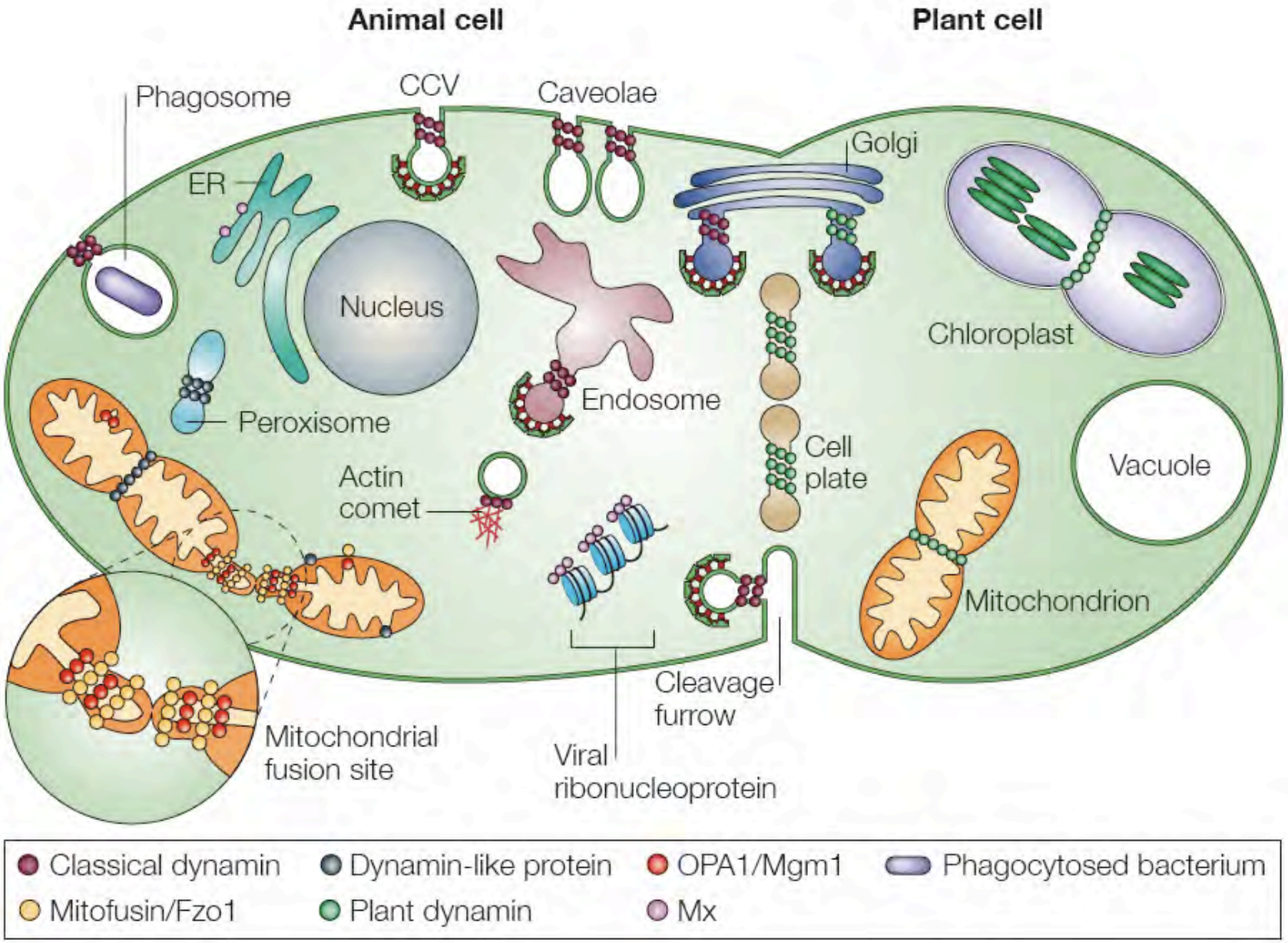
Ferguson & De Camilli (2012)

Oligomerization of dynamin into helical arrays around membrane



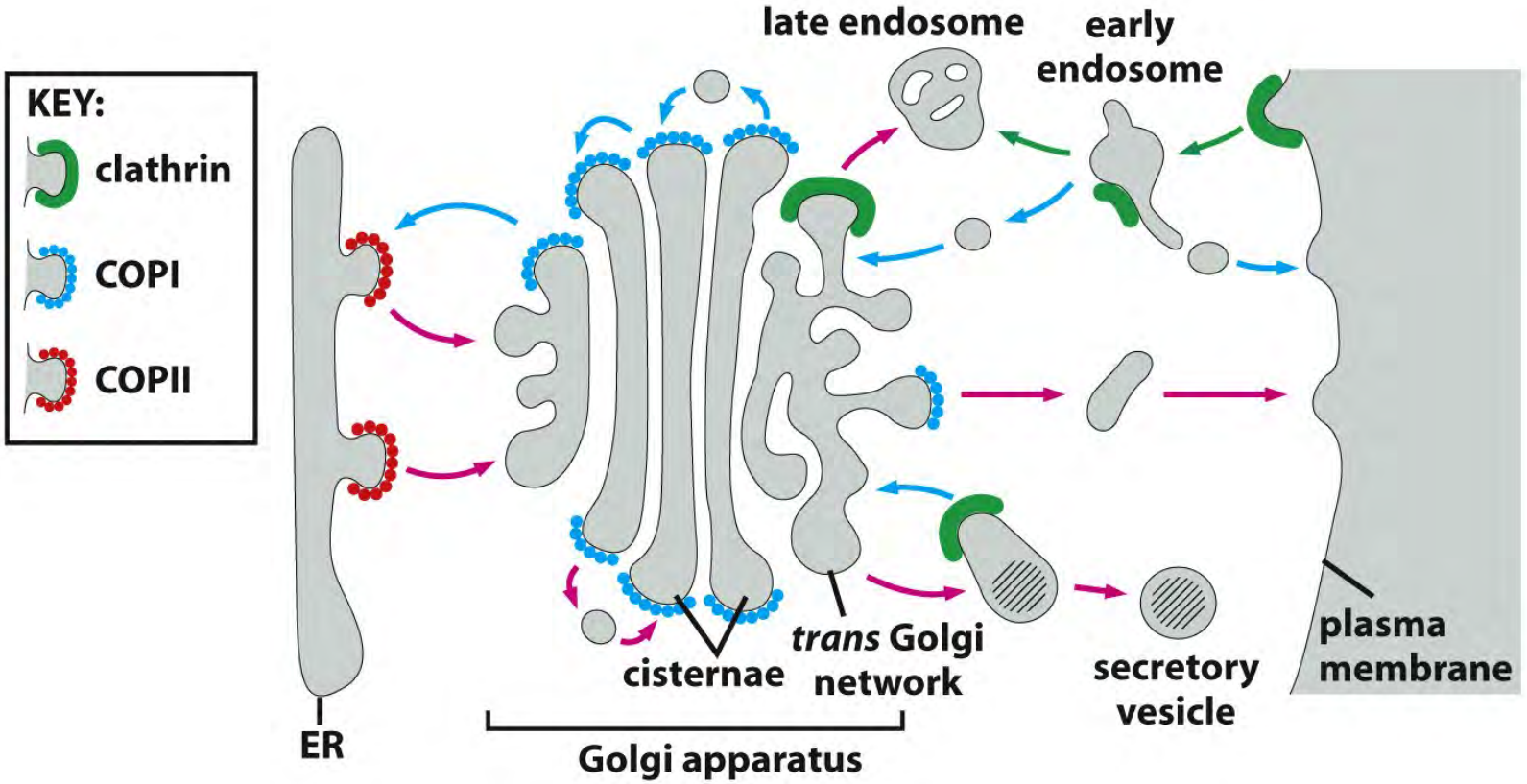
Ferguson & De Camilli (2012)

Dynamin family members remodel diverse cellular membranes



Praefcke and McMahon, 2004

In addition to clathrin, 2 other types of coated vesicles transport cargo from organelle to organelle



Clathrin: endocytosis and trans-Golgi ⇒ late endosomes [ARF, ADP-ribosylation factor]

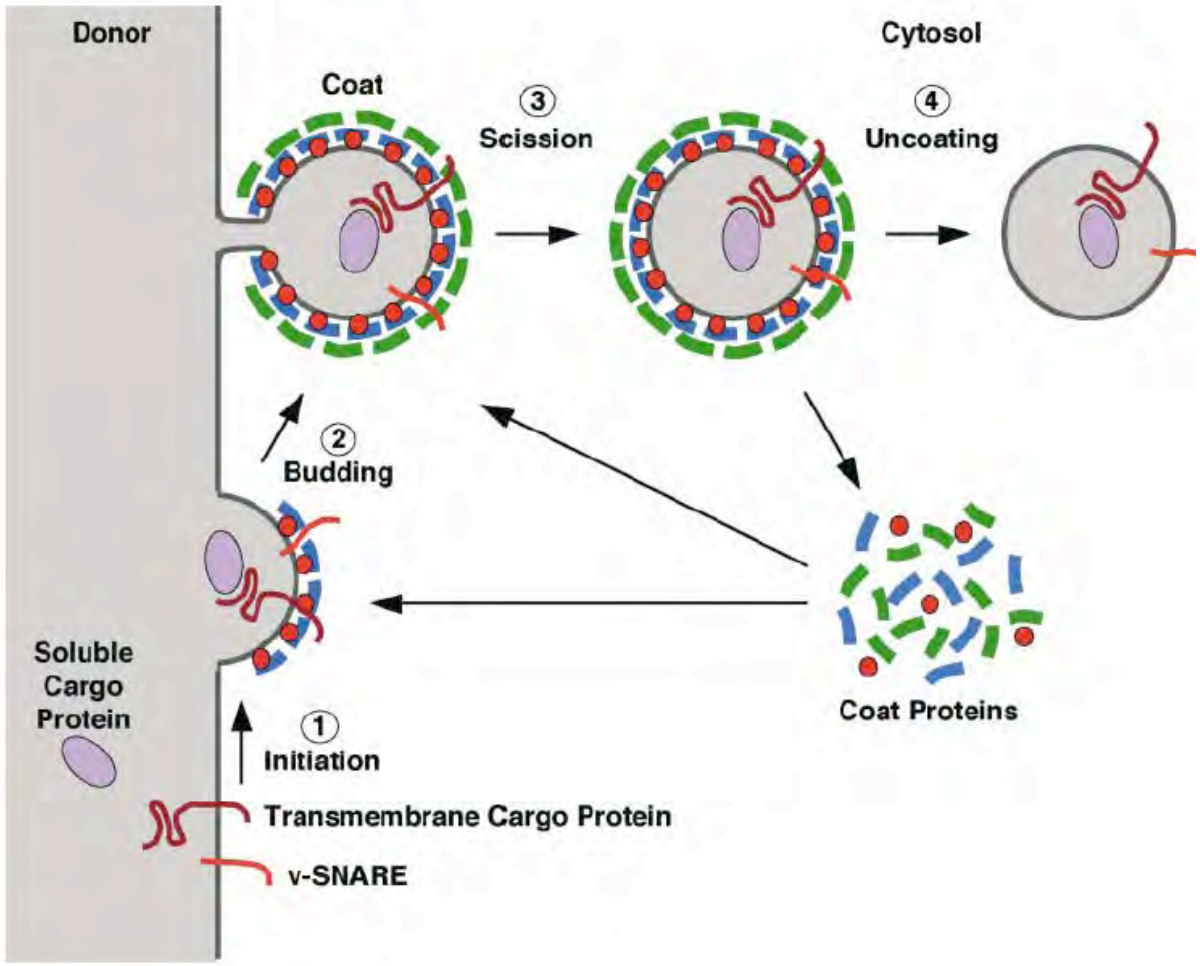
COPII: rough ER ⇒ Golgi (Sar1)

COPI: retrograde Golgi and Golgi ⇒ ER (ARF)

Coat protein polymerization produces high membrane curvature

Figure 13-5 *Molecular Biology of the Cell* (© Garland Science 2008)

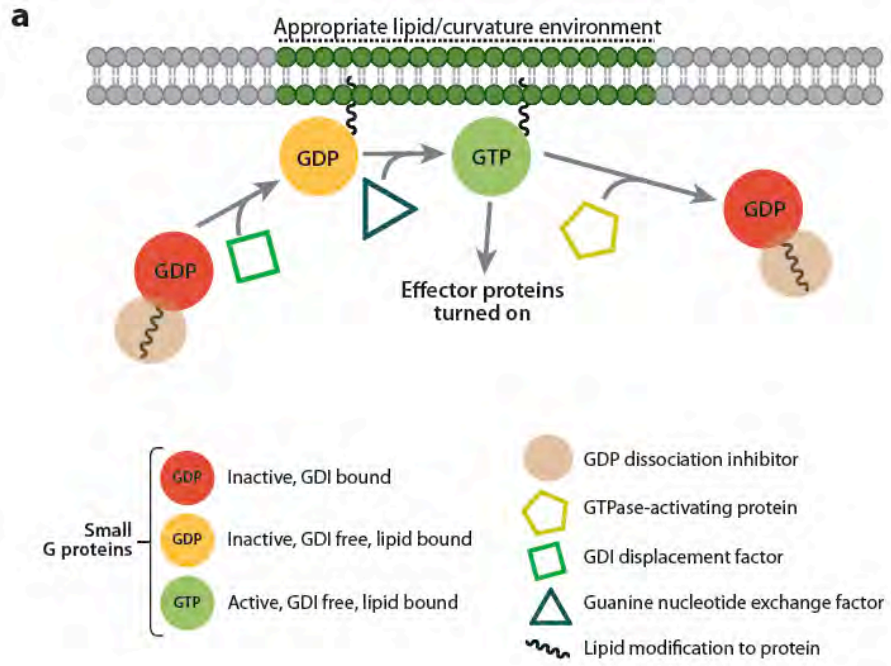
Coat proteins are central in vesicle budding



- Coat proteins promote formation of vesicle.
- Coat is transient.
- What controls the formation and dissociation of the coat?

Bonifacino and Glick (2004) Cell

Small G proteins can regulate timing of molecular interactions



G proteins appear to regulate membrane curvature.

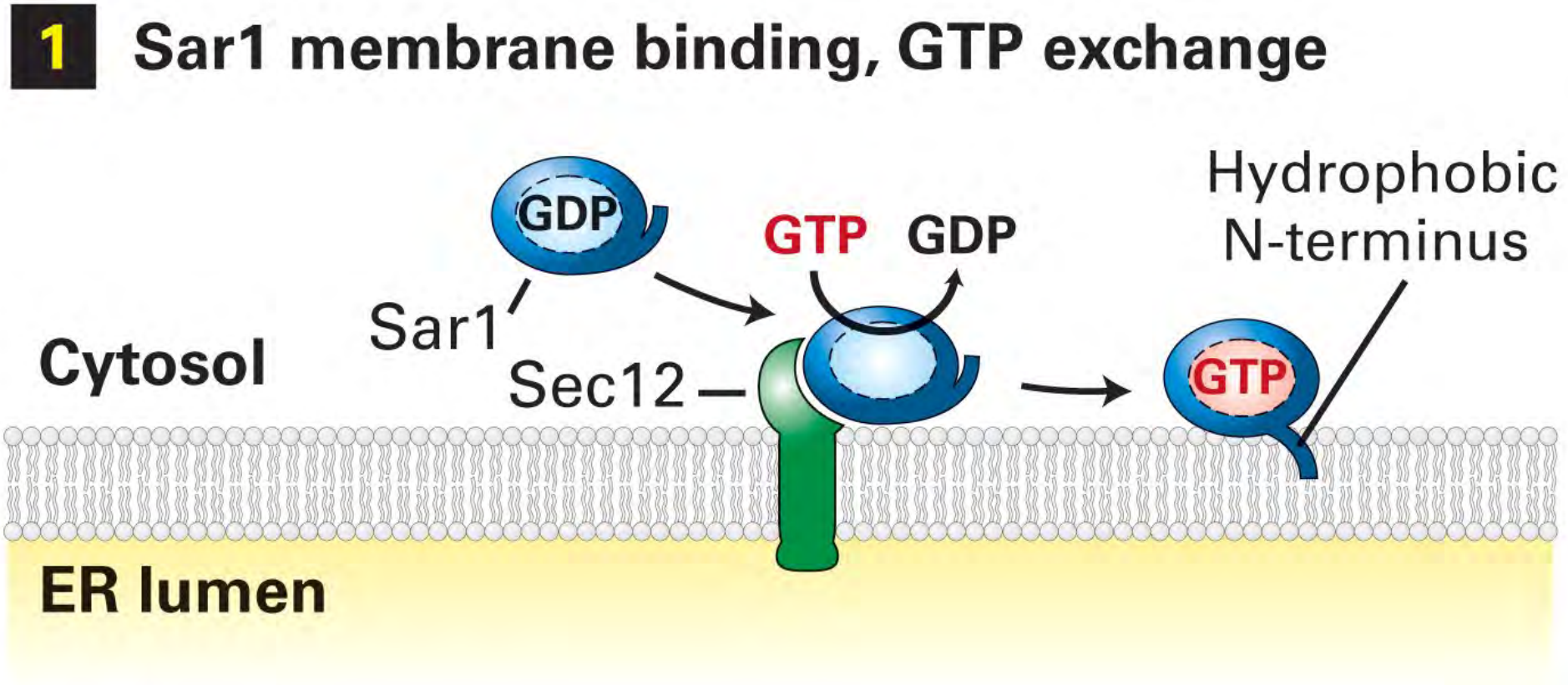
•direct affects on membrane curvature.

•recruitment of coat proteins.

•Example: Sar1 is involved in formation of COPII vesicles.

Doberty &McMahon (2009) Ann Rev Biochem

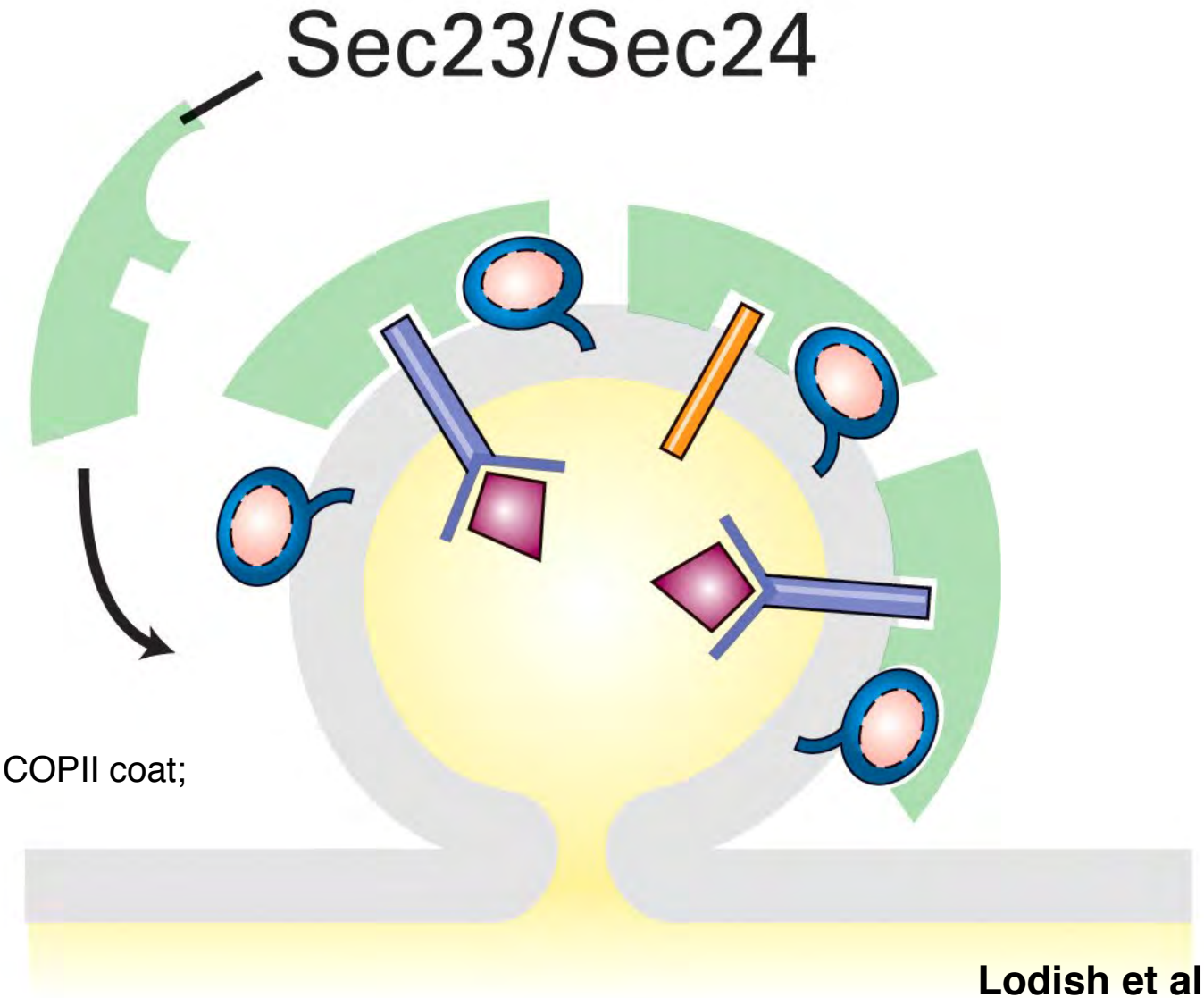
COPII coat formation and dissociation coupled to nucleotide-bound state of Sar1



- Sec12 is integral ER protein that functions as an nucleotide exchange factor for Sar1
- Recruits Sar1 to ER and exchanges GDP for GTP; GTP conformation allows hydrophobic N-terminus to insert into membrane

Lodish et al, 2004

2 COPII coat assembly

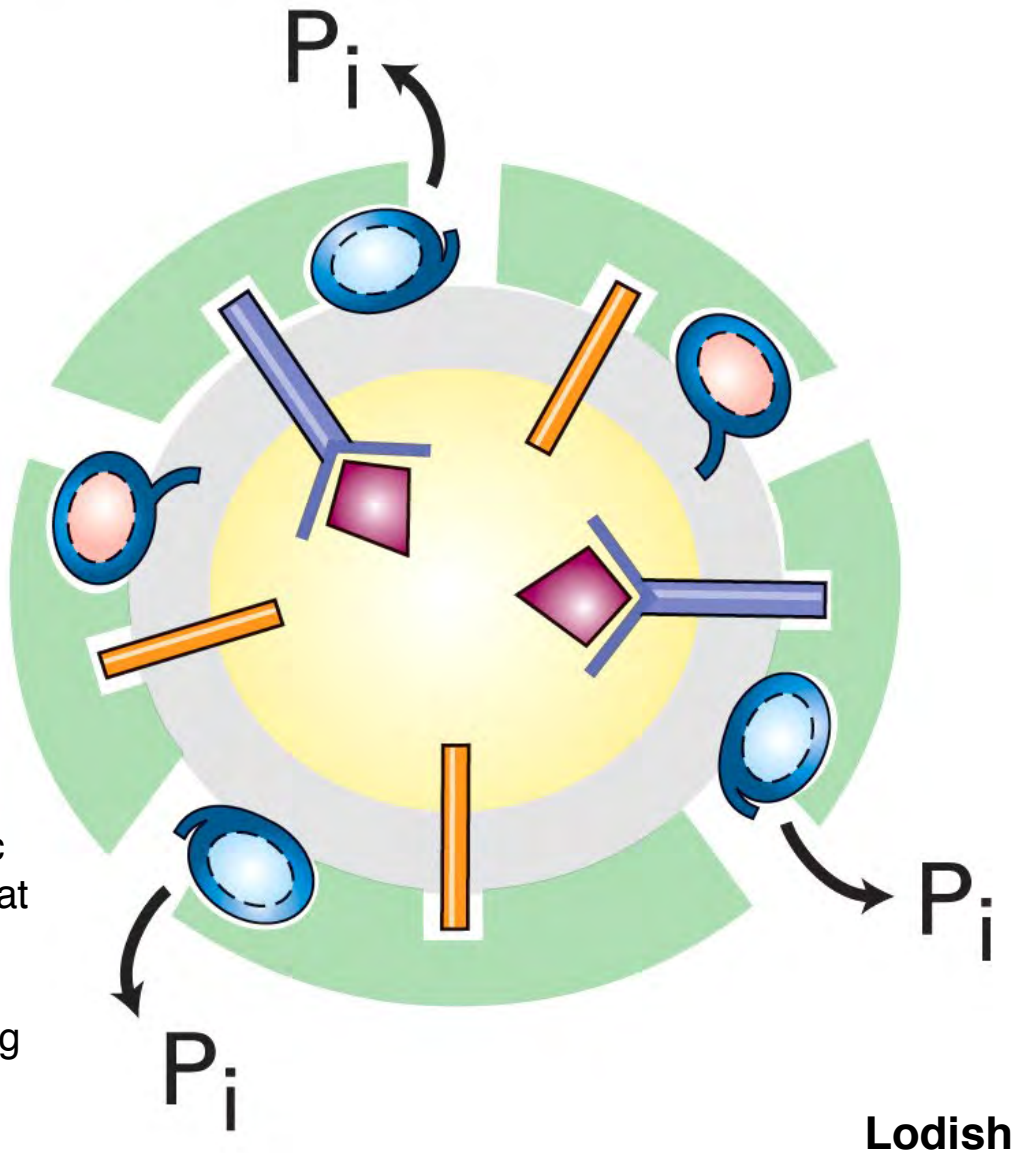


- Sar1-GTP recruits COPII coat;

Coat recruits cargo

Lodish et al, 2004

3 GTP hydrolysis

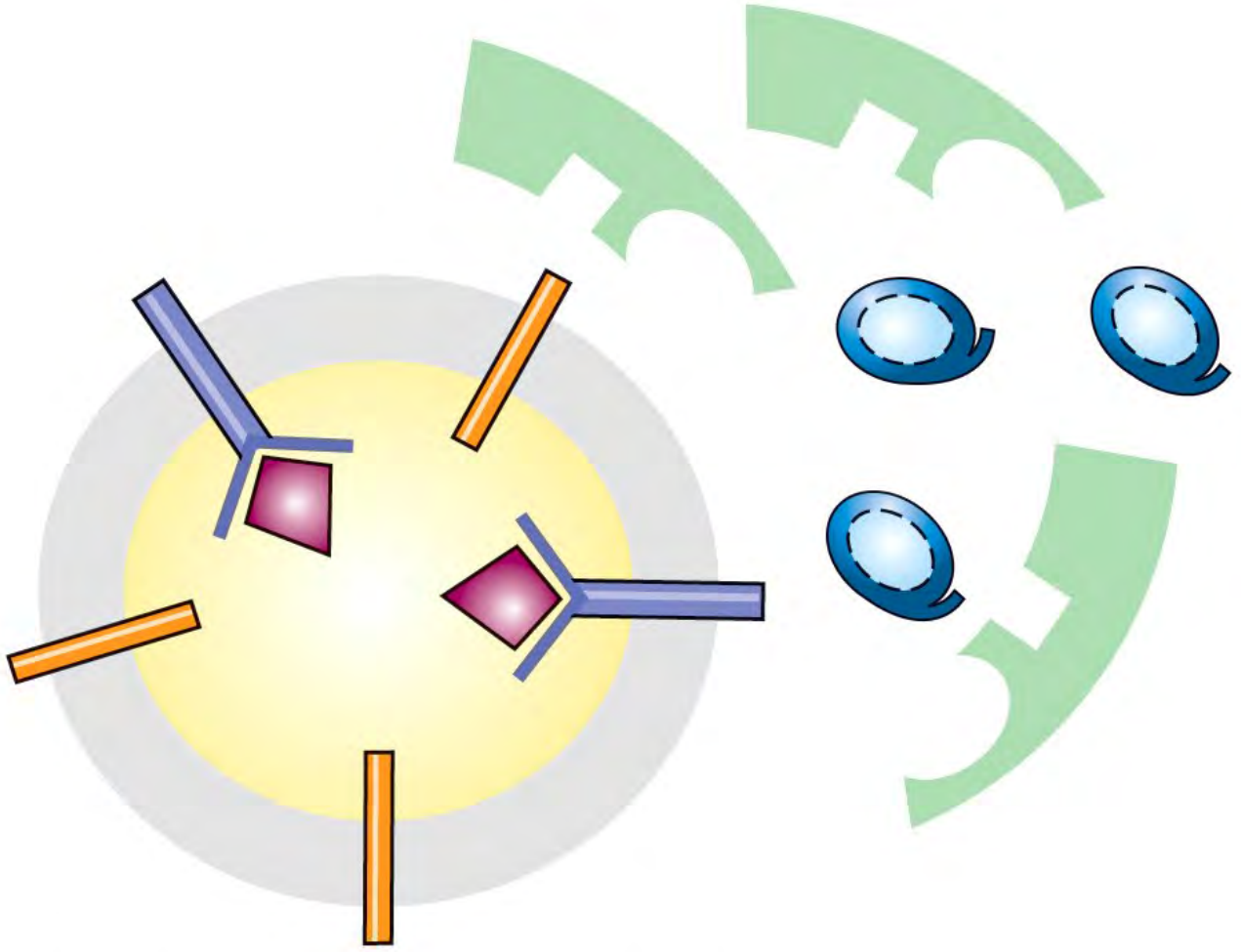


- Sar1 hydrolysis (promoted by Sec 23 coat subunit) disassembles coat

Summary: Through Sar1, GTP binding recruits vesicle coat, and GTP hydrolysis dissociates coat. The GTPase cycle controls the timing.

Lodish et al, 2004

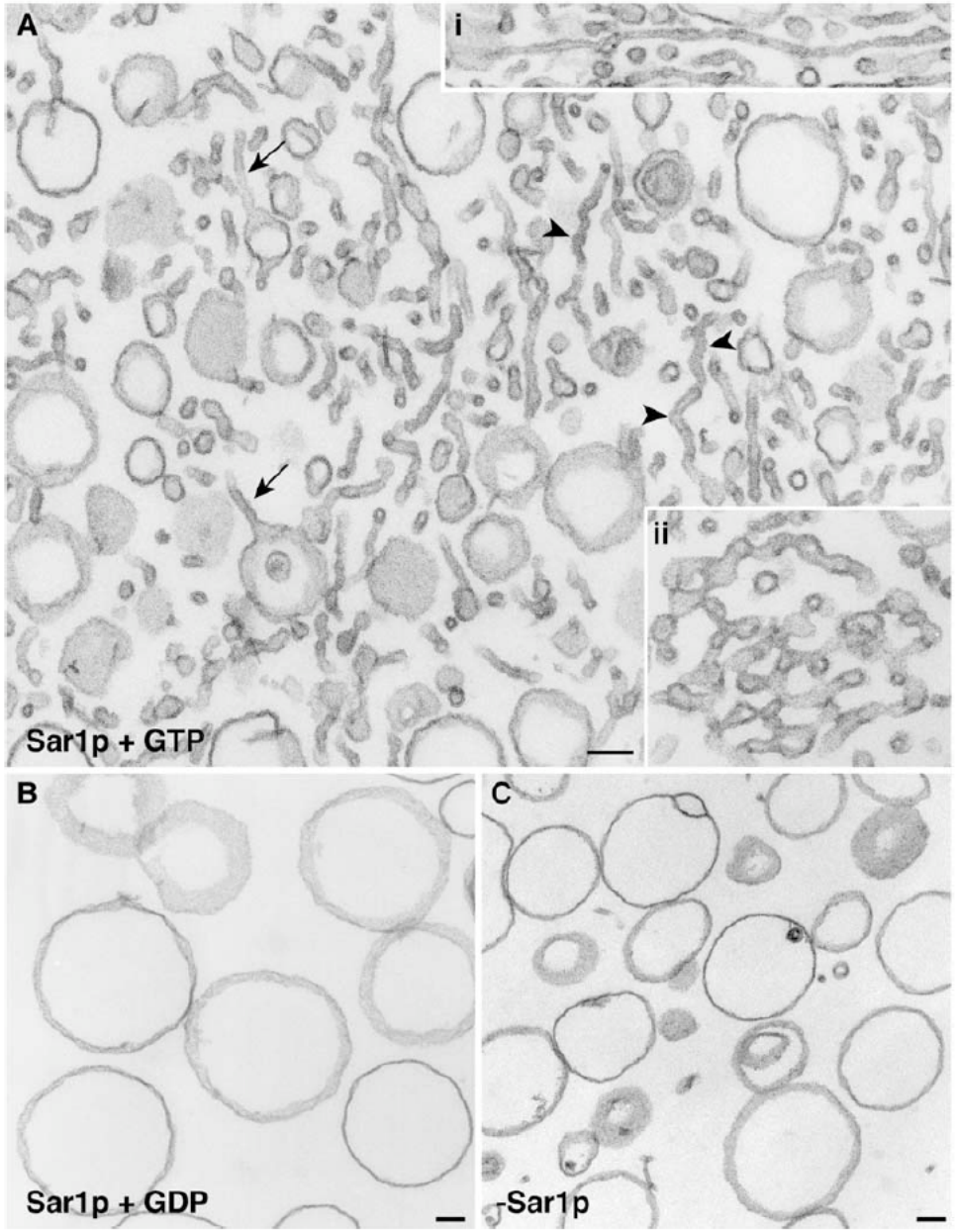
4 Coat disassembly



Uncoated vesicle

Lodish et al, 2004

The Sar1 GTPase can tubulate synthetic liposomes



A)
• Sar1 (alone) tubulates membranes in presence of GTP.

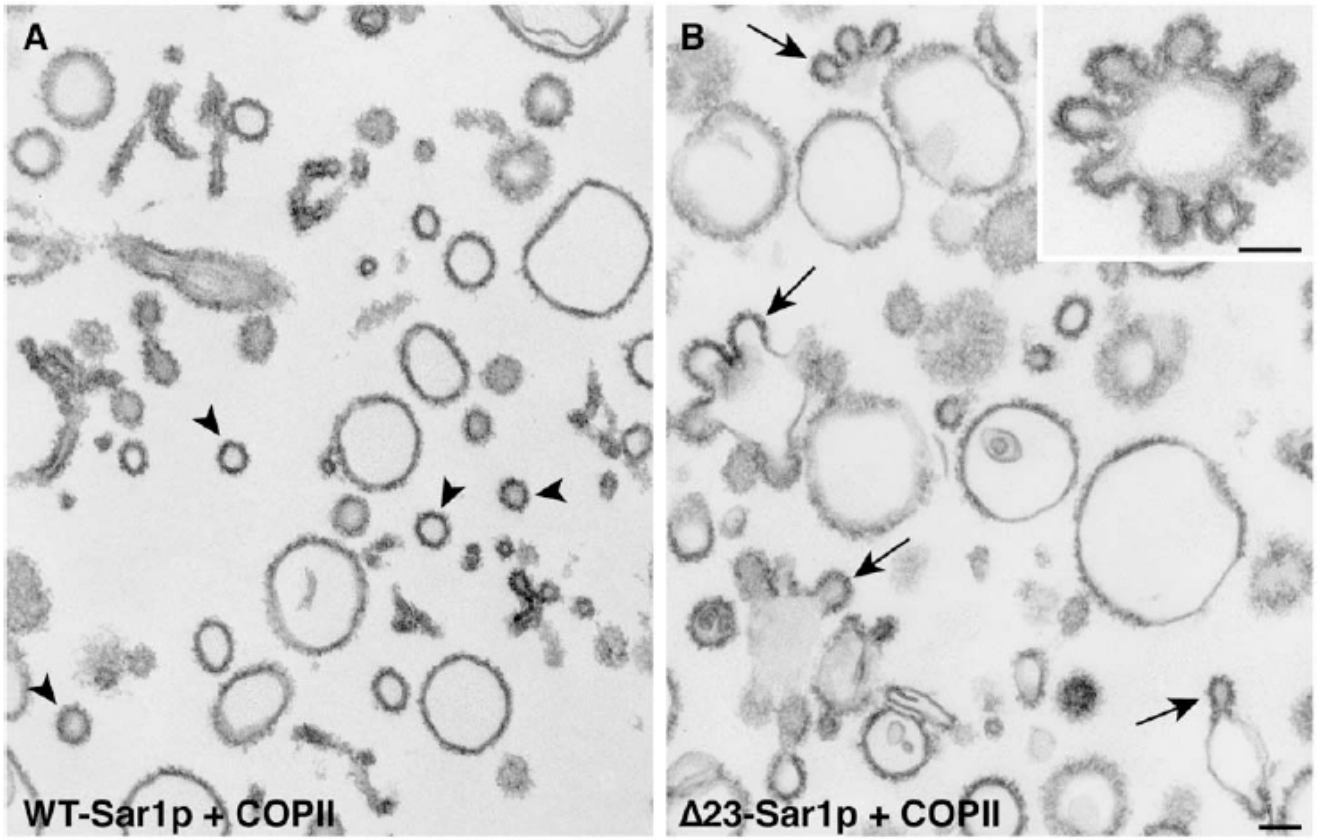
• Average diameter ~26 nm; length up to 1 μm long.

• COPII vesicle ~60-90 nm.

B) GDP
C) no Sar1

Lee et al. (2005) Cell

The Sar1 and COPII coat proteins cooperate



• Wt Sar1 reactions contain more vesicles, and tubules.

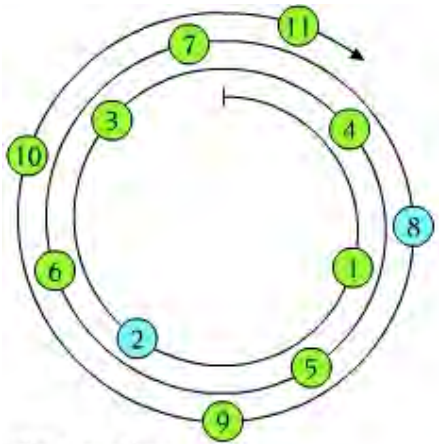
• Δ23-Sar1 reactions contain buds. (this Sar1 mutant previously shown to be unable to tubulate membranes)

• suggests that fission is facilitated by Sar1.

COPII consists of Sec23/24 and Sec 13/31

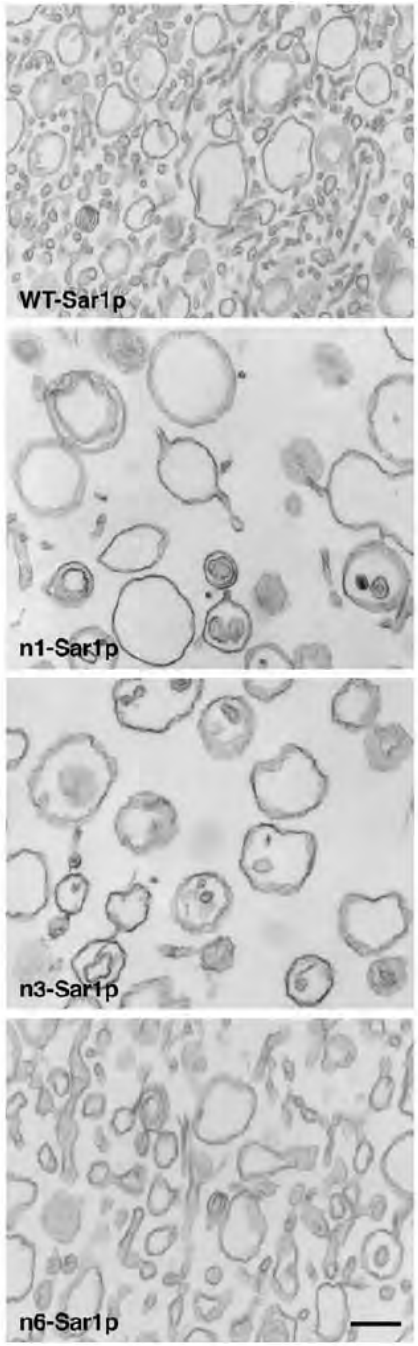
Lee et al. (2005) Cell

N-terminal amphipathic helix important for tubulation



Helical wheel representation; 100˚ per residue

Sar1 mutants



- N-terminal helix of Sar1 is amphipathic.
- Mutation of bulky hydrophobic residues reduces tubulation activity.
- Model: helix insertion into outer leaflet causes membrane deformation.

N6 mutant (RD to AA) has intermediate phenotype

Lee et al. (2005) Cell