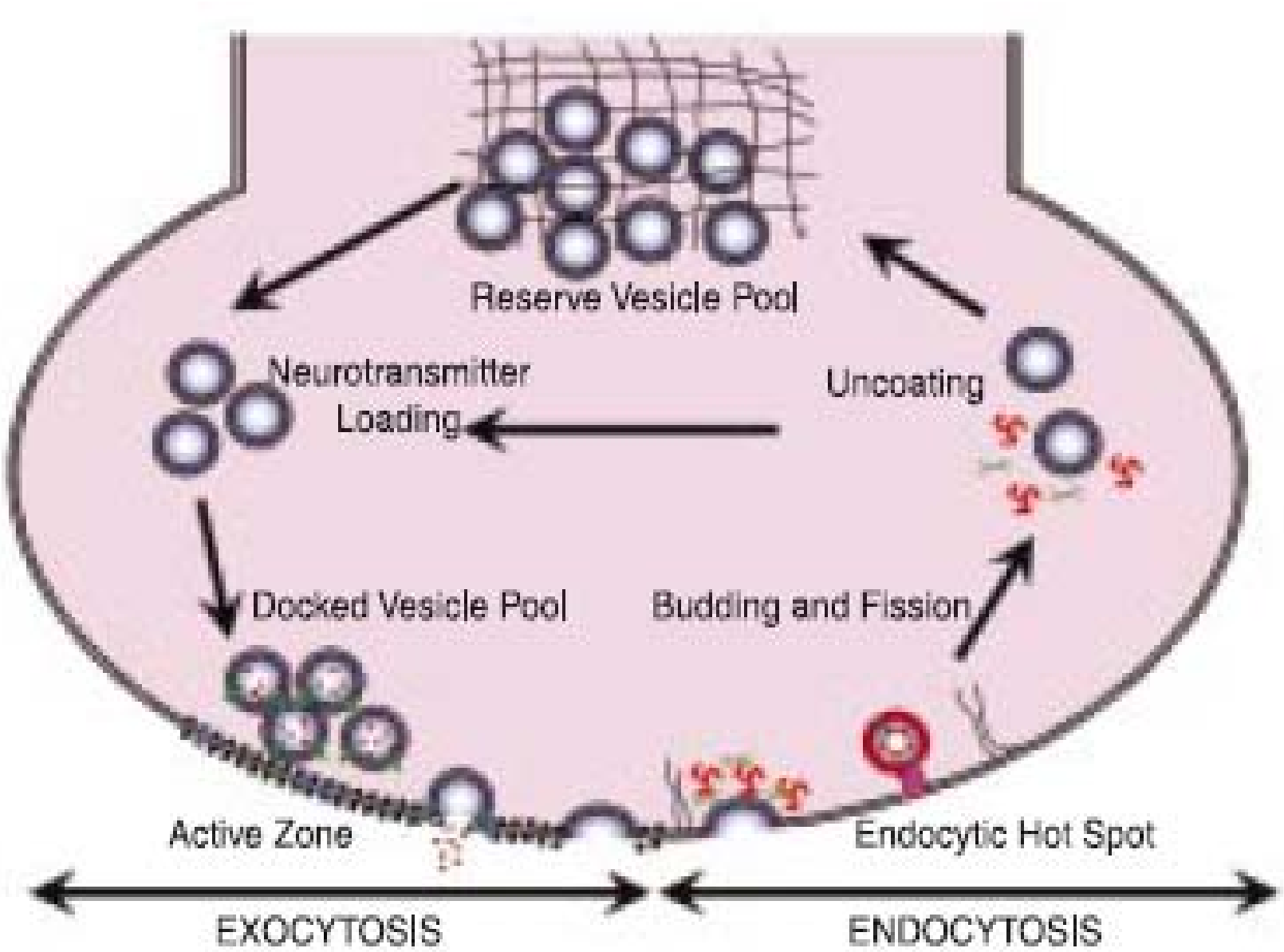
The Synaptic Vesicle Cycle



The Eukaryotic Commandments: T. Cavalier-Smith

(i)origin of the endomembrane system (ER, Golgi and lysosomes) and coated-vesicle budding and fusion, including endocytosis and exocytosis;

(ii) origin of the cytoskeleton, centrioles, cilia and associated molecular motors;

(iii) origin of the nucleus, nuclear pore complex and trans-envelope protein and RNA transport;

(iv) origin of linear chromosomes with plural replicons, centromeres and telomeres;

(v) origin of novel cell-cycle controls and mitotic segregation;

(vi) origin of sex (syngamy, nuclear fusion and meiosis);

(vii) origin of peroxisomes; (ix) origin of mitochondria;

(viii) novel patterns of rRNAprocessing using small nucleolar-ribonucleoproteins (snoRNPs); (x) origin of spliceosomal introns.

EM of Chemical synapse

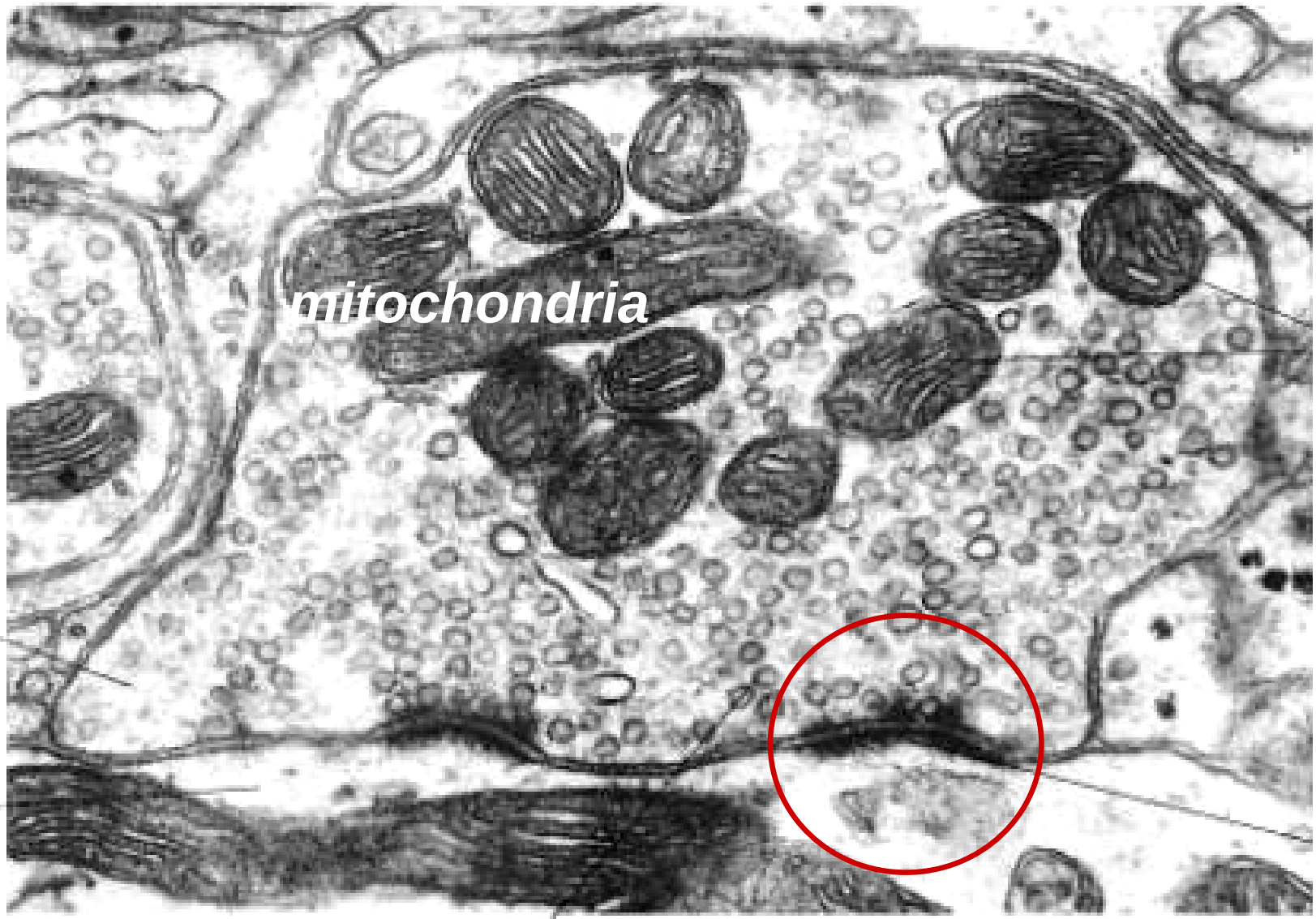


Figure 5.3, Bear, 2001

T. Cavalier-Smith
International Journal of Systematic and Evolutionary Microbiology (2002), 52, 297–354

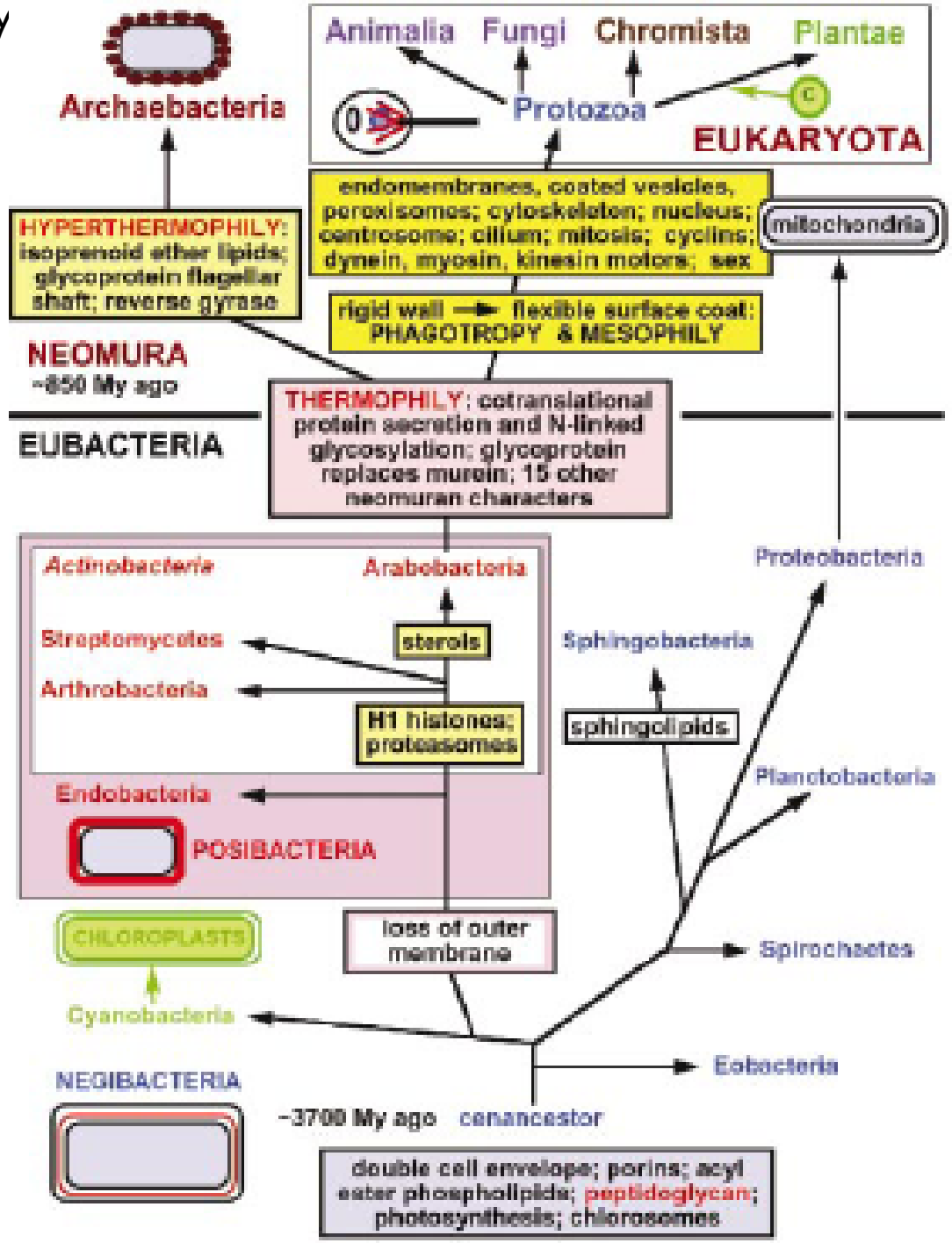


Fig. 1. The bacterial origins of eukaryotes as a two-stage process.

I phase:
The ancestors of eukaryotes, the stem neomura, are shared with archaebacteria and evolved during the neomuran revolution, in which ***N-linked glycoproteins*** *replaced* murein peptidoglycan and 18 other suites of characters changed radically through adaptation of an ancestral actinobacterium to thermophily, as discussed in detail by Cavalier-Smith (2002a).

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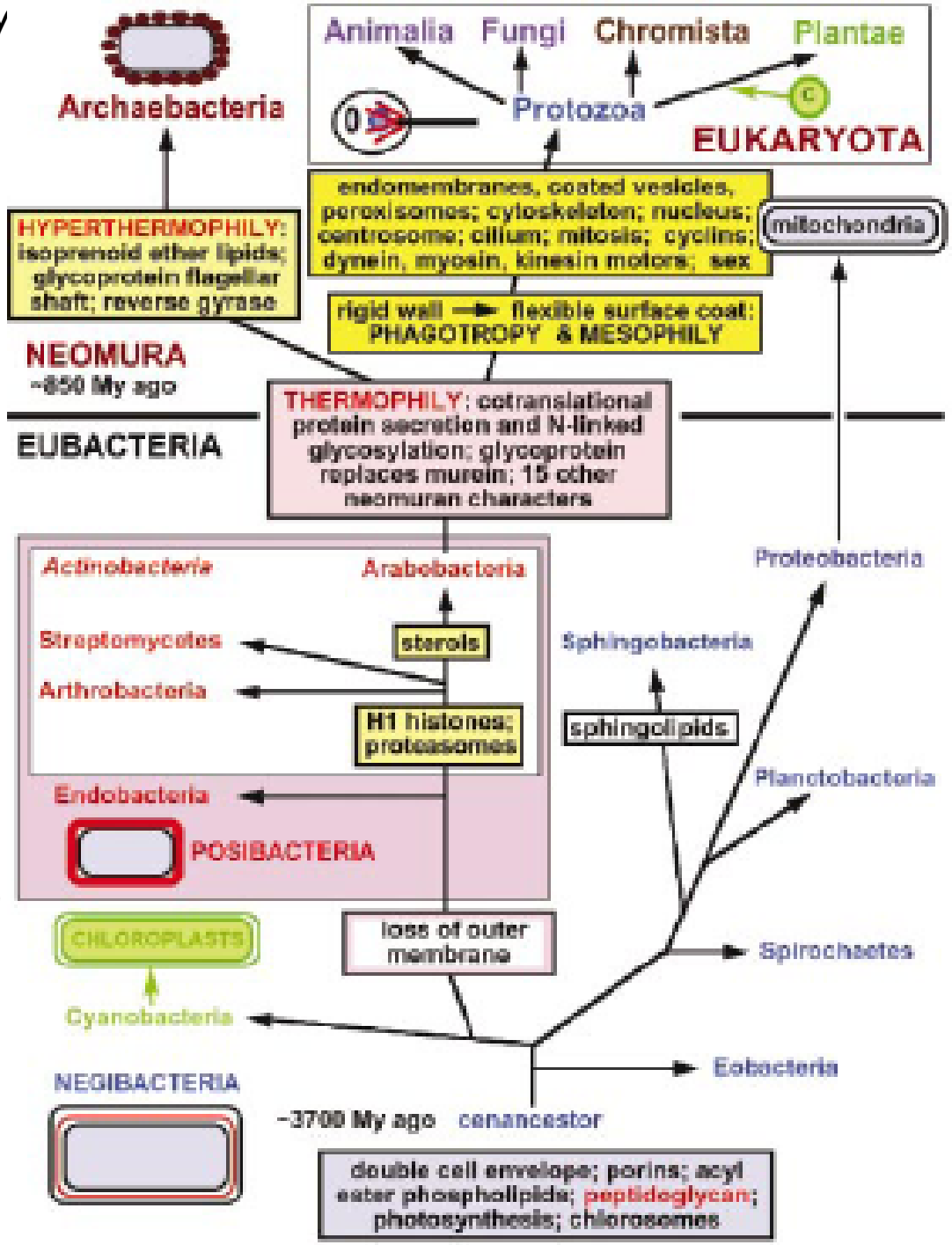
Fig. 1. The bacterial origins of eukaryotes as a two-stage process.

II phase: In the next phase, archaebacteria and eukaryotes diverged dramatically. Archaebacteria retained the wall and therefore their general bacterial cell and genetic organization, but became adapted to even hotter and more acidic environments by substituting prenyl ether lipids for the ancestral acyl esters and making new acid-resistant flagellar shafts (Cavalier-Smith, 2002a).

At the same time, eukaryotes converted the glycoprotein wall into a **flexible surface coat** and **evolved rudimentary phagotrophy** for the first time in the history of life. This triggered a massive reorganization of their cell and chromosomal structure and enabled an *a-proteobacterium to be enslaved and converted into a protomitochondrion to form the first aerobic* eukaryote and protozoan, around 850 My ago.

Substantially later, a cyanobacterium (green) was enslaved by the common ancestor of the plant kingdom to form the first chloroplast (*C*).



T. Martin Embley & William Martin
Eukaryotic evolution, changes and challenges
Nature 2006,

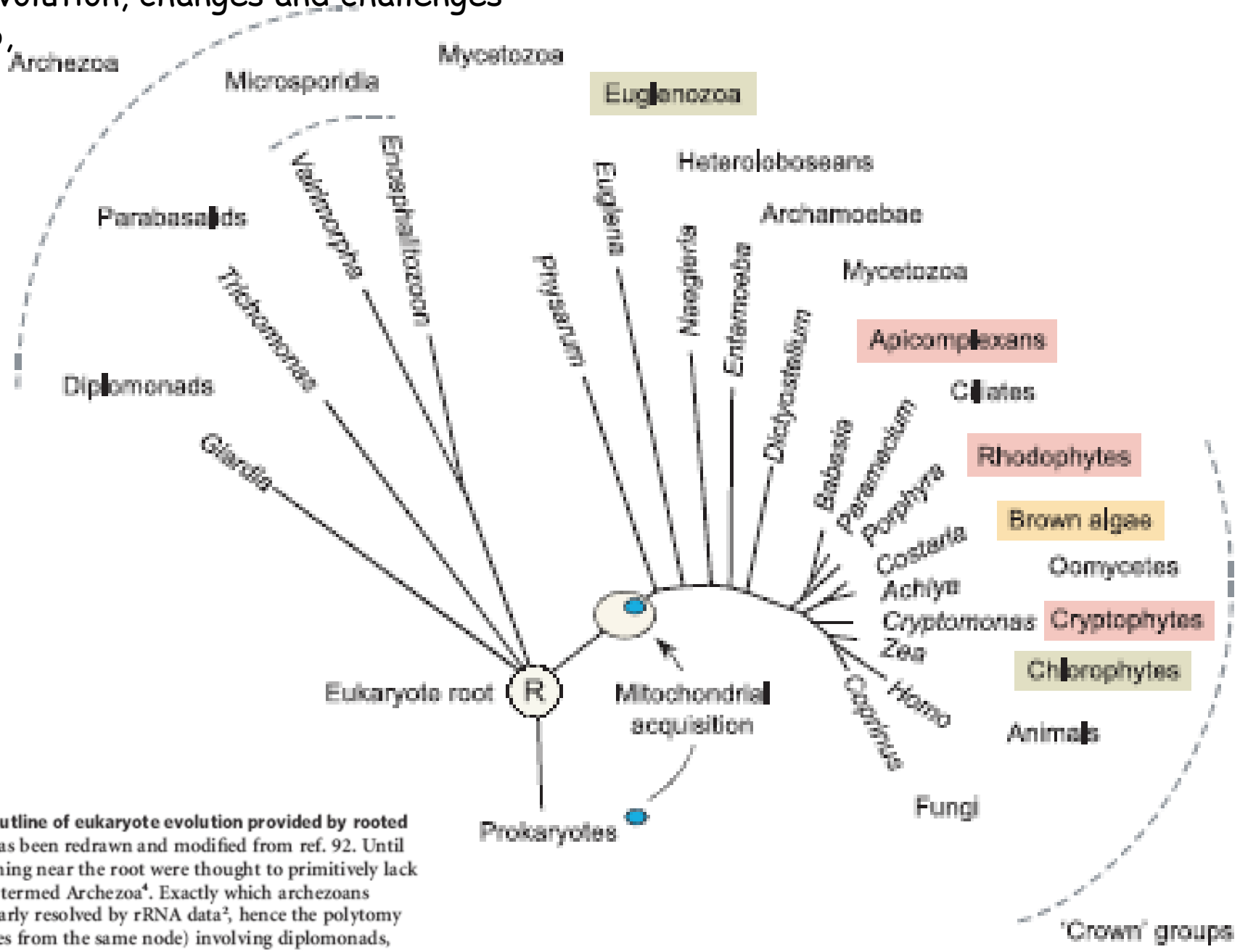


Figure 1 | The general outline of eukaryote evolution provided by rooted rRNA trees. The tree has been redrawn and modified from ref. 92. Until recently, lineages branching near the root were thought to primitively lack mitochondria and were termed Archezoa[4]. Exactly which archezoans branched first is not clearly resolved by rRNA data[2], hence the polytomy (more than two branches from the same node) involving diplomonads, parabasalids and microsporidia at the root. Plastid-bearing lineages are indicated in colours approximating their respective pigmentation. Lineages furthest away from the root, including those with multicellularity, were thought to be the latest-branching forms and were sometimes misleadingly (see ref. 60) called the 'crown' groups.

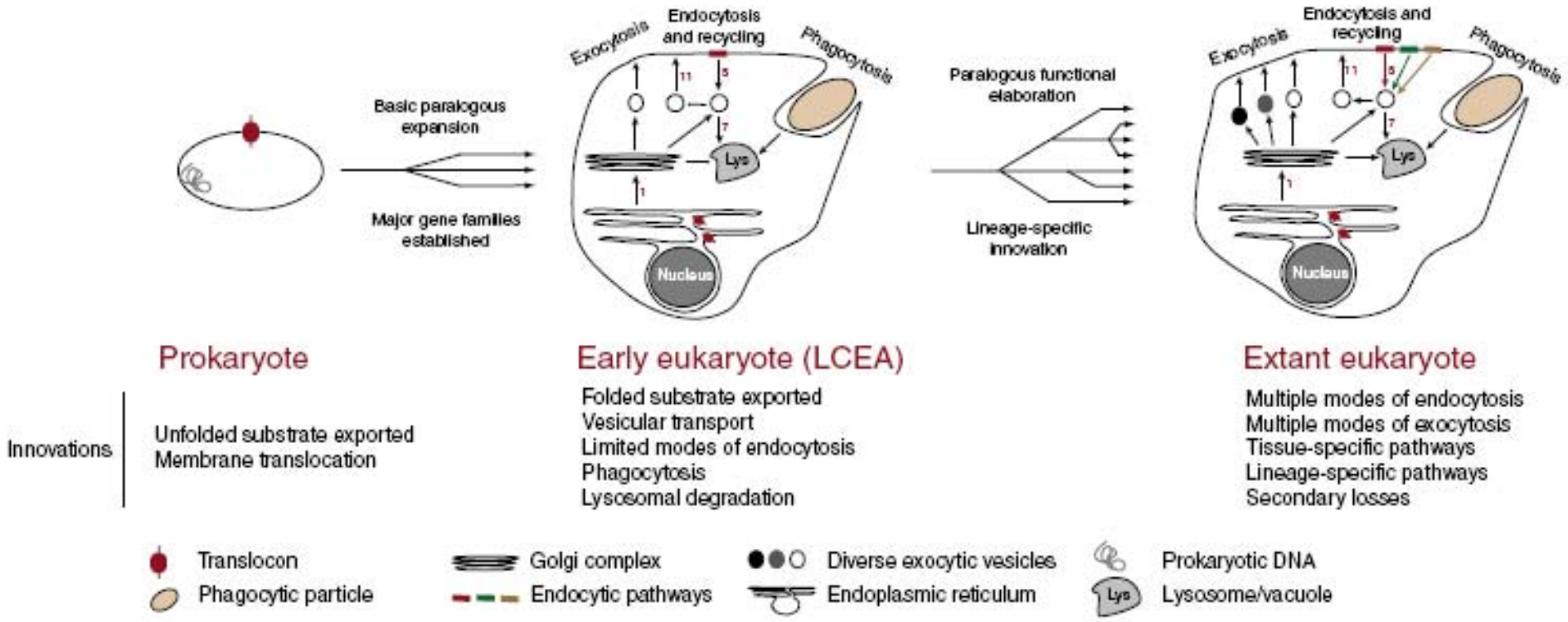
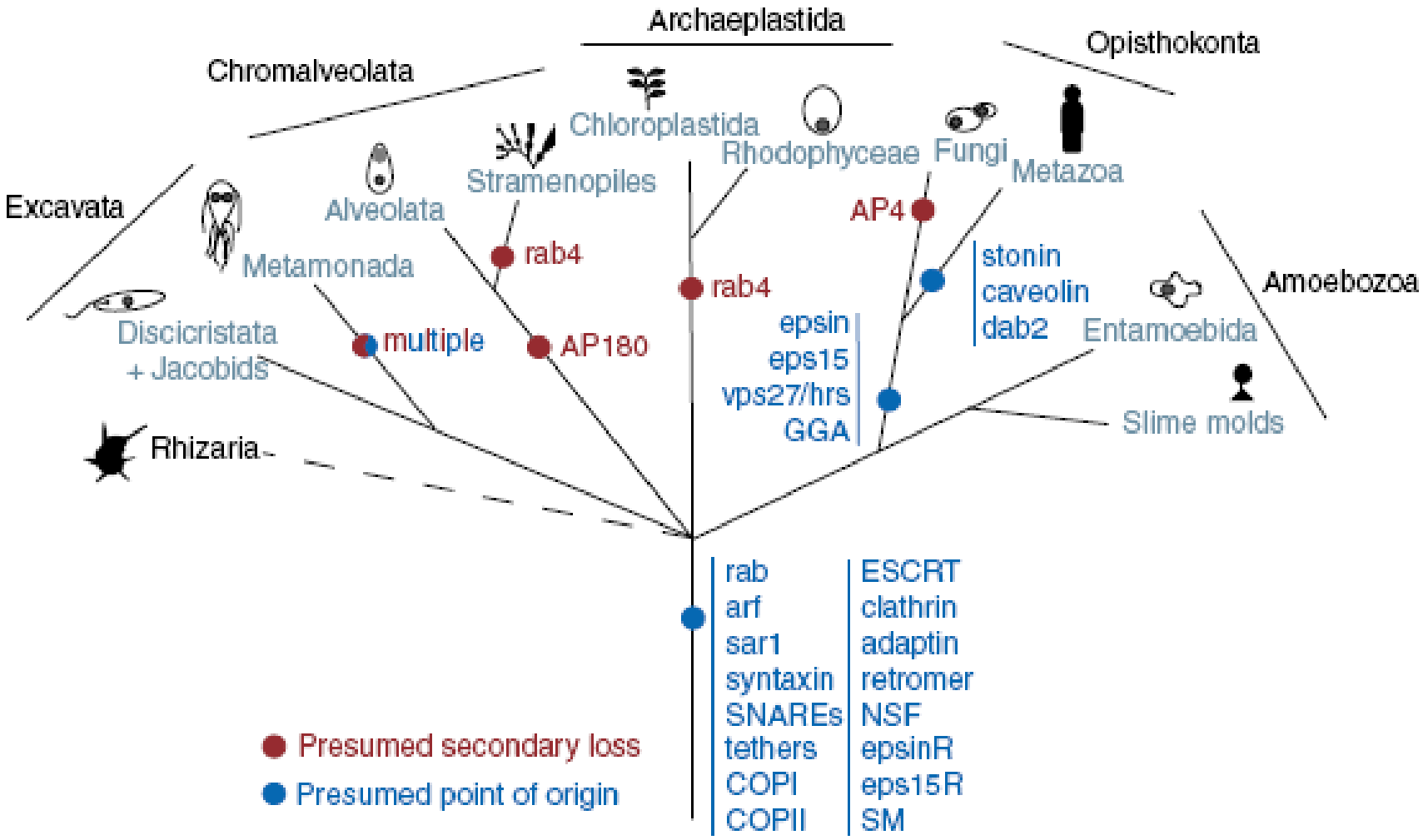


Fig. 3. Major transitions in evolution of the endomembrane system. In prokaryotes, secretion is a comparatively simple matter of translocation of polypeptides across the plasma membrane. Although there are several distinct mechanisms for achieving this, all appear to require an unfolded substrate for translocation. The type I, SRP/SecY-mediated, pathway is homologous to the co-translational ER import pathways of eukaryotes. In the hypothesized LCEA, comparative genomic evidence suggests that the major structures and pathways constituting the endomembrane system were already present, including the ER, Golgi complex and the main features of the endocytosis and recycling systems. The paralogous relationship of the families of SNAREs, Rabs, SM proteins and GTPases, as well as the homology of many coat components to each other and also to components of the nuclear pore complex, provide a potential mechanism for how this system arose. Compartmentalization and gene family expansion led to establishment of multiple protein systems capable of deforming membranes (i.e. transport steps). Elaboration of this basic pattern has been a major driving force for subsequent diversification of the endomembrane system, giving rise to the array of systems present in extant taxa. Subsequent evolution yielded multiple modes of endocytosis, specialized exocytic pathways and increased complexity of post-Golgi pathways. The red ovals indicate a trans-membrane translocation system. Lys, lysosome or vacuole; LCEA, last common eukaryotic ancestor. Small numbers in red indicate an associated pathway-specific Rab protein. The grey structure in the prokaryote indicates the non-compartmentalized genome.

Dacks and Field, J. Cell Science 2009

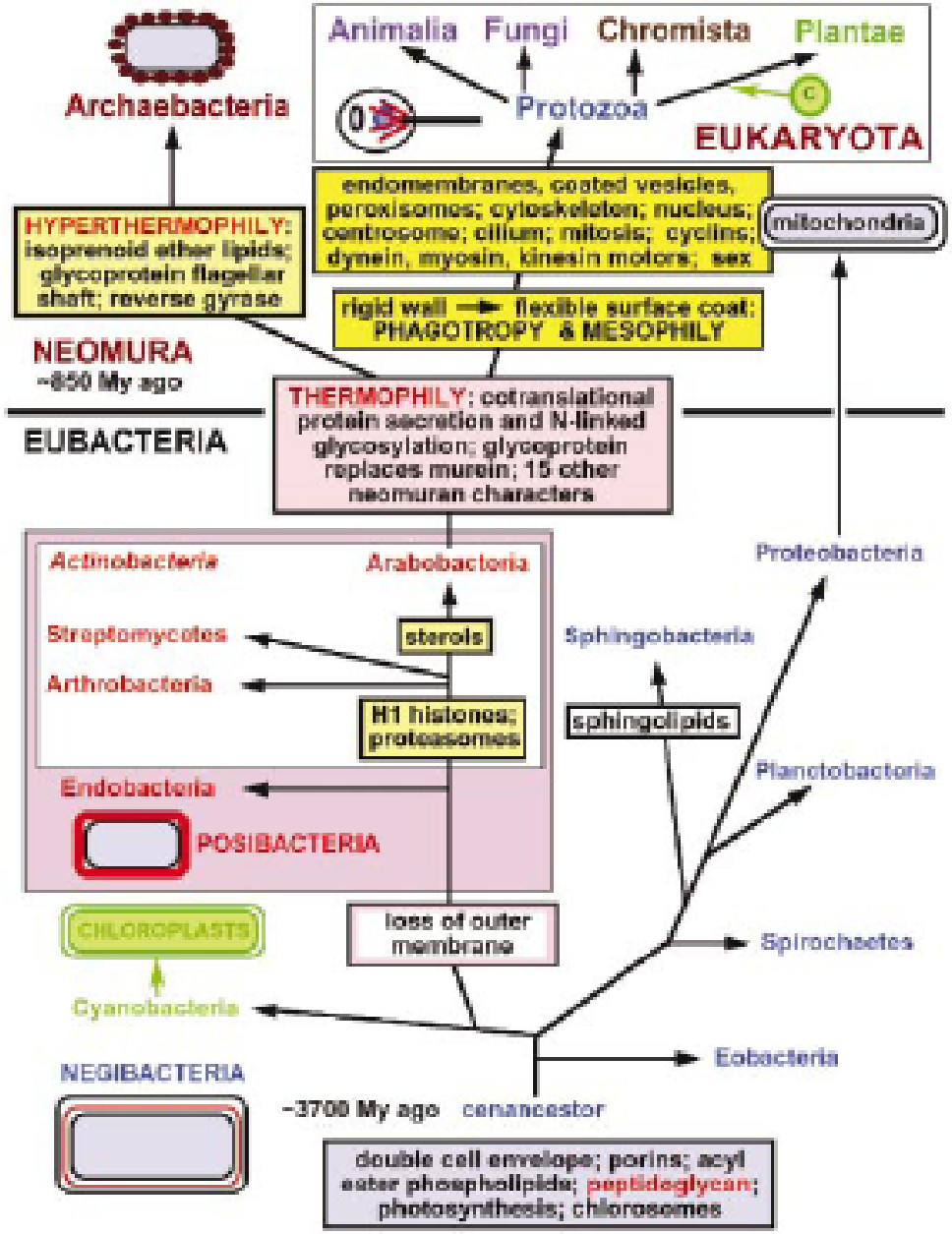
Dacks and Field, J. Cell Science 2009

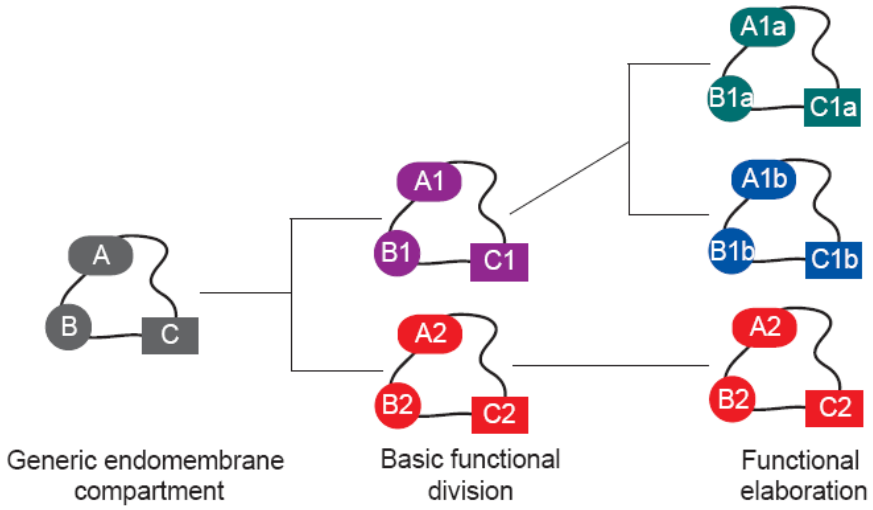


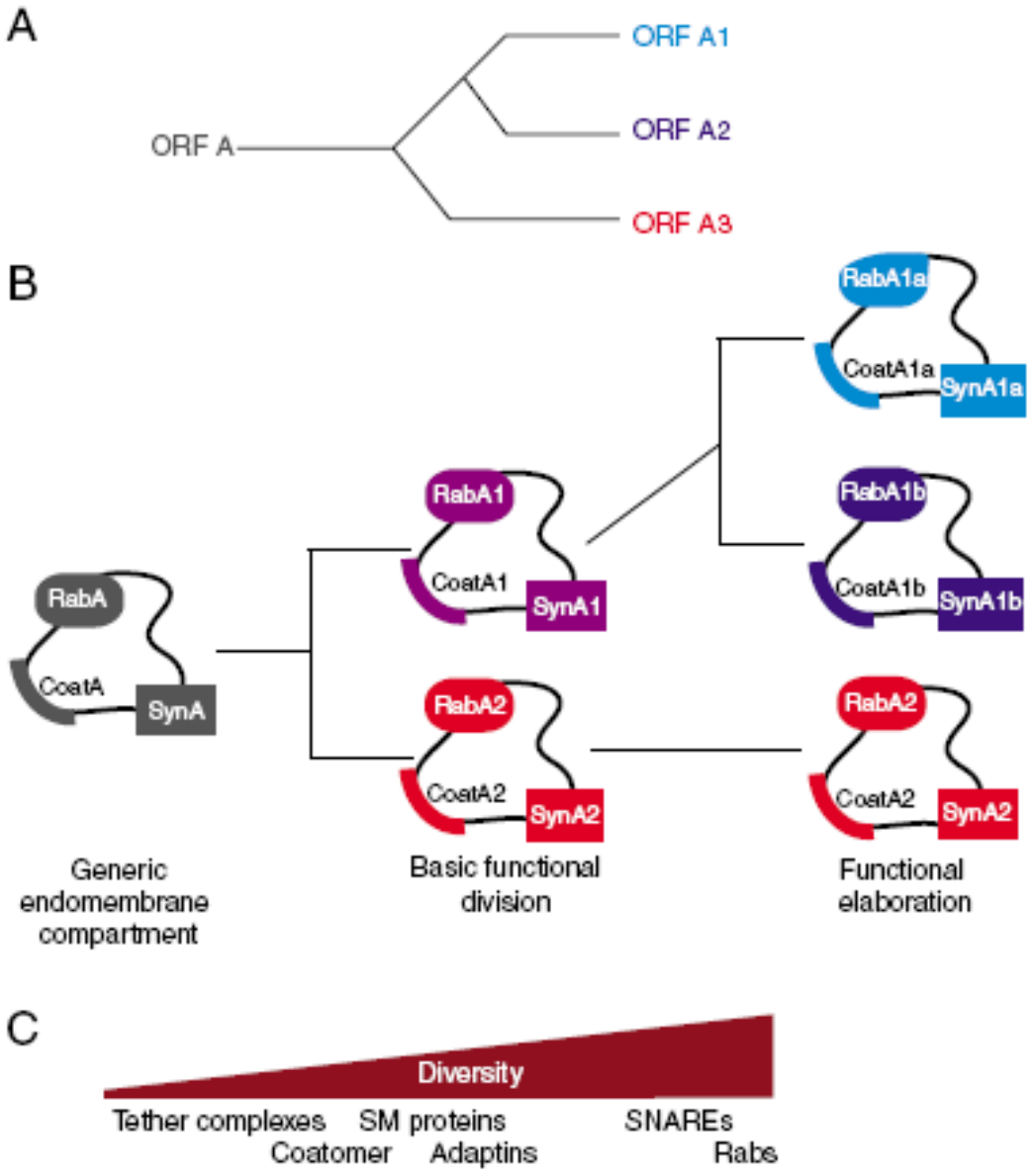
T. Cavalier-Smith
International Journal of Systematic and Evolutionary Microbiology (2002), 52, 297–354

Molecular Clocks

'temporal pattern of molecular change is very different in different categories of molecules, which show the classical phenomenon of mosaic evolution: different molecules alter their rates of evolution to greatly differing degrees in the same lineage The hundreds of molecules that were specifcally involved in the drastic changes that created the ancestral neomuran (e.g. rRNA, protein secretion molecules, vacuolar ATPase) underwent temporarily vastly accelerated evolution (quantum evolution) during those innovations in the stem neomuran, but thousands of other genes, notably most metabolic enzymes, were more clock-like.'







Dacks and Field, J. Cell Science 2009

Mayor and Pagano , Nature Reviews MCB 2007

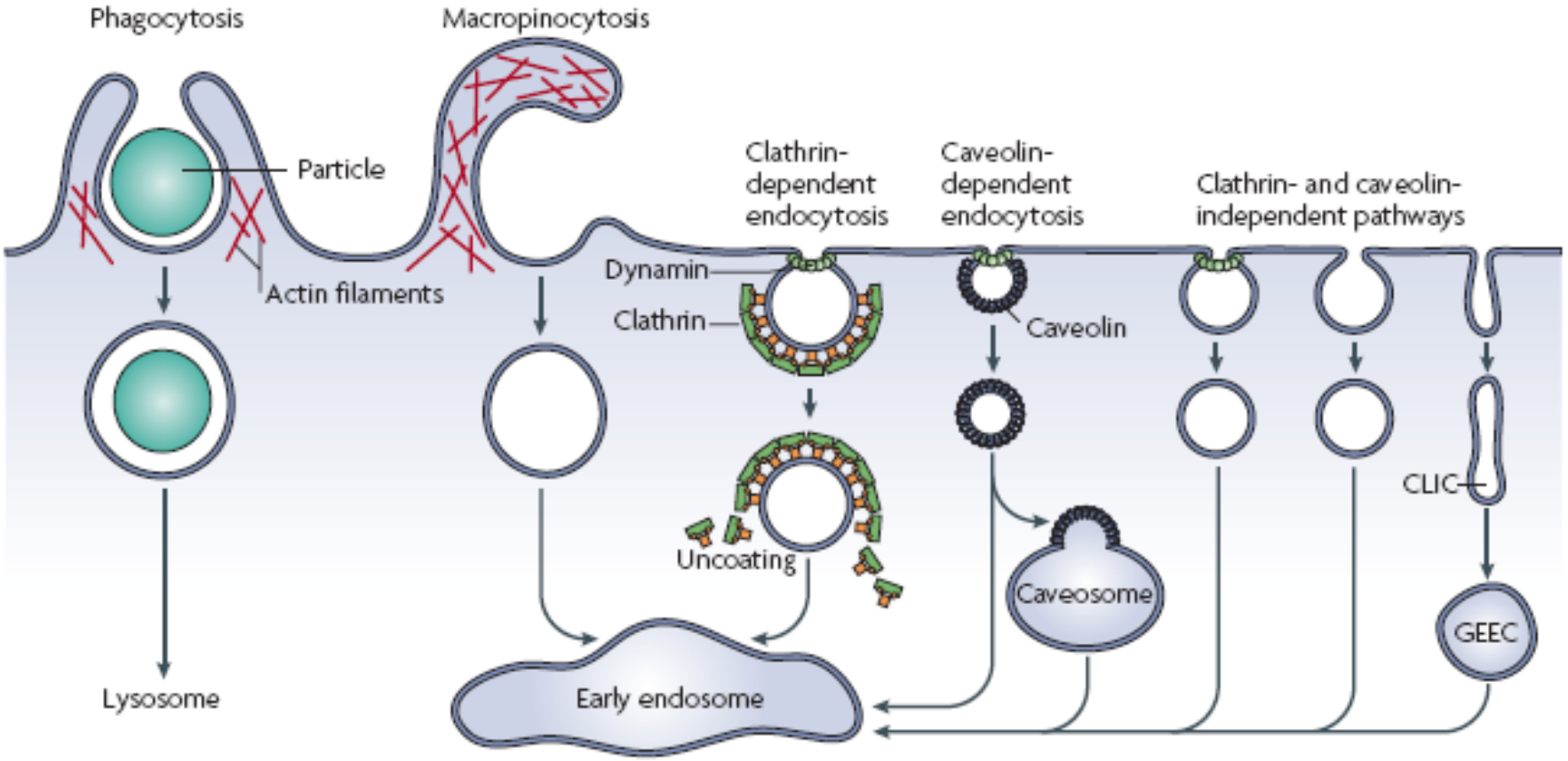
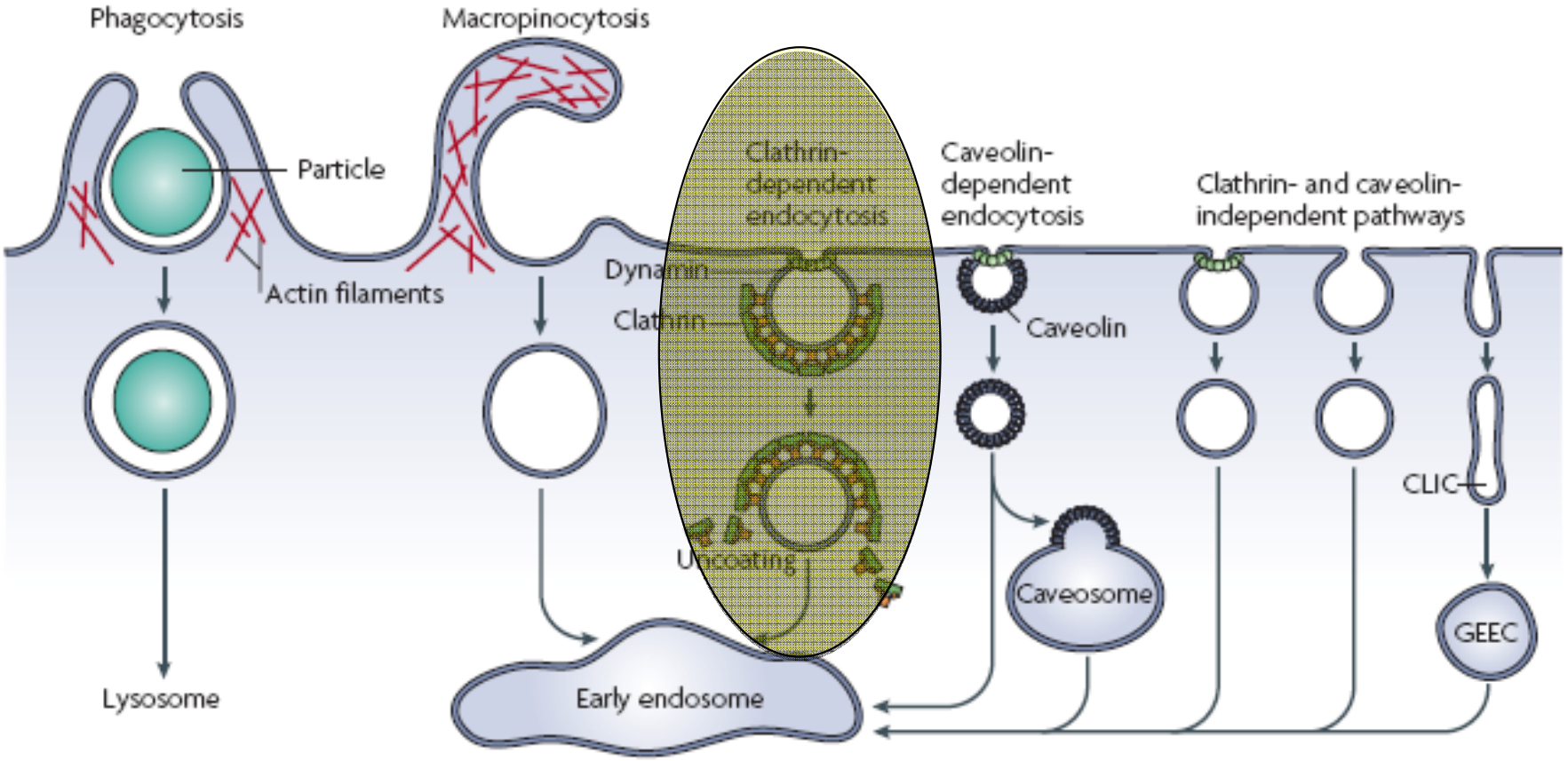


Figure 1 | **Pathways of entry into cells.** Large particles can be taken up by phagocytosis, whereas fluid uptake occurs by macropinocytosis. Both processes appear to be triggered by and are dependent on actin-mediated remodelling of the plasma membrane at a large scale. Compared with the other endocytic pathways, the size of the vesicles formed by phagocytosis and macropinocytosis is much larger. Numerous cargoes (TABLE 1) can be endocytosed by mechanisms that are independent of the coat protein clathrin and the fission GTPase, dynamin. This Review focuses on the clathrin-independent pathways, some of which are also dynamin independent (FIGS 2,3). Most internalized cargoes are delivered to the early endosome via vesicular (clathrin- or caveolin-coated vesicles) or tubular intermediates (known as clathrin- and dynamin-independent carriers (CLICs)) that are derived from the plasma membrane. Some pathways may first traffic to intermediate compartments, such as the caveosome or glycosyl phosphatidylinositol-anchored protein enriched early endosomal compartments (GEEC), *en route* to the early endosome.

Mayor and Pagano , Nature Reviews MCB 2007



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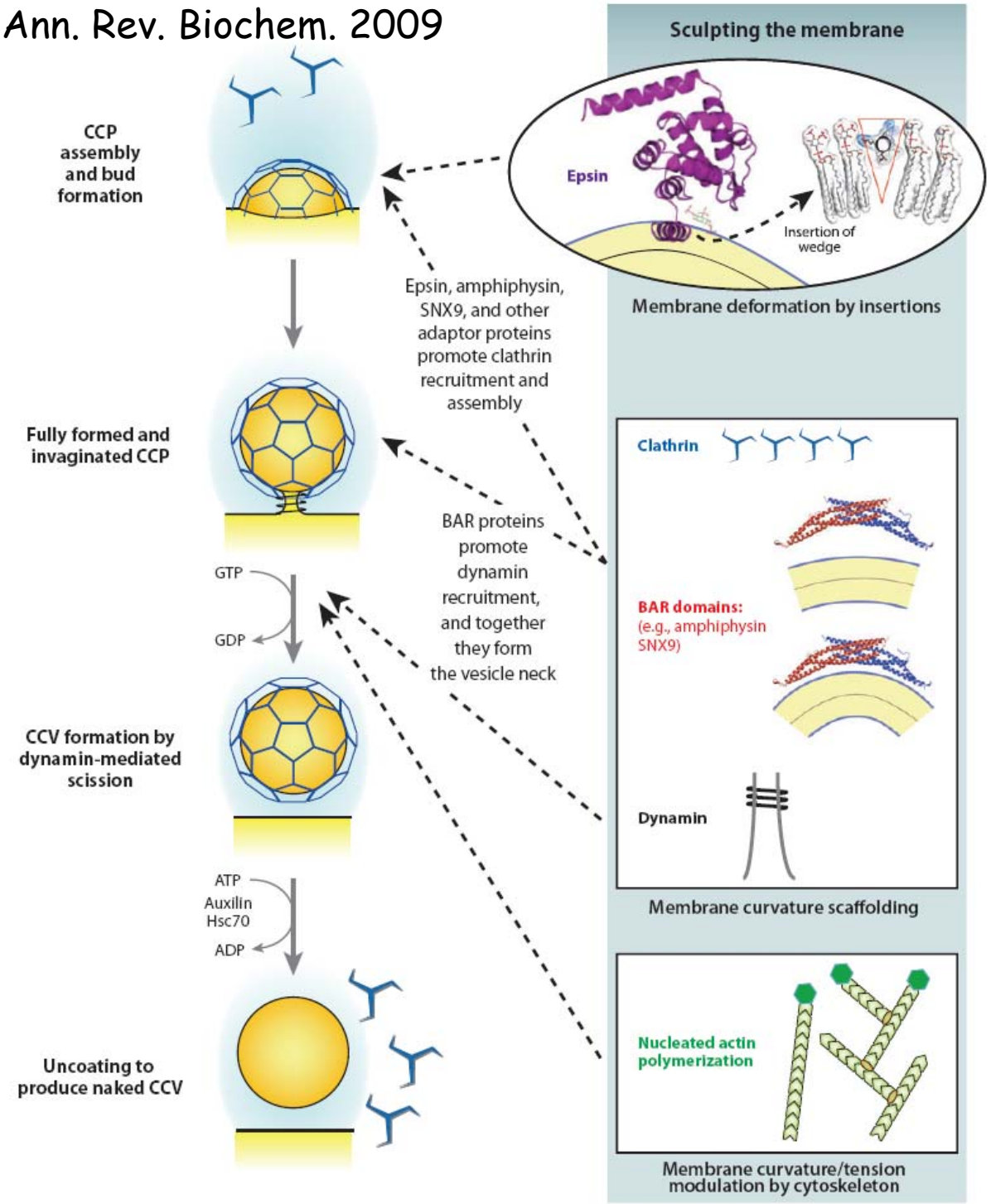
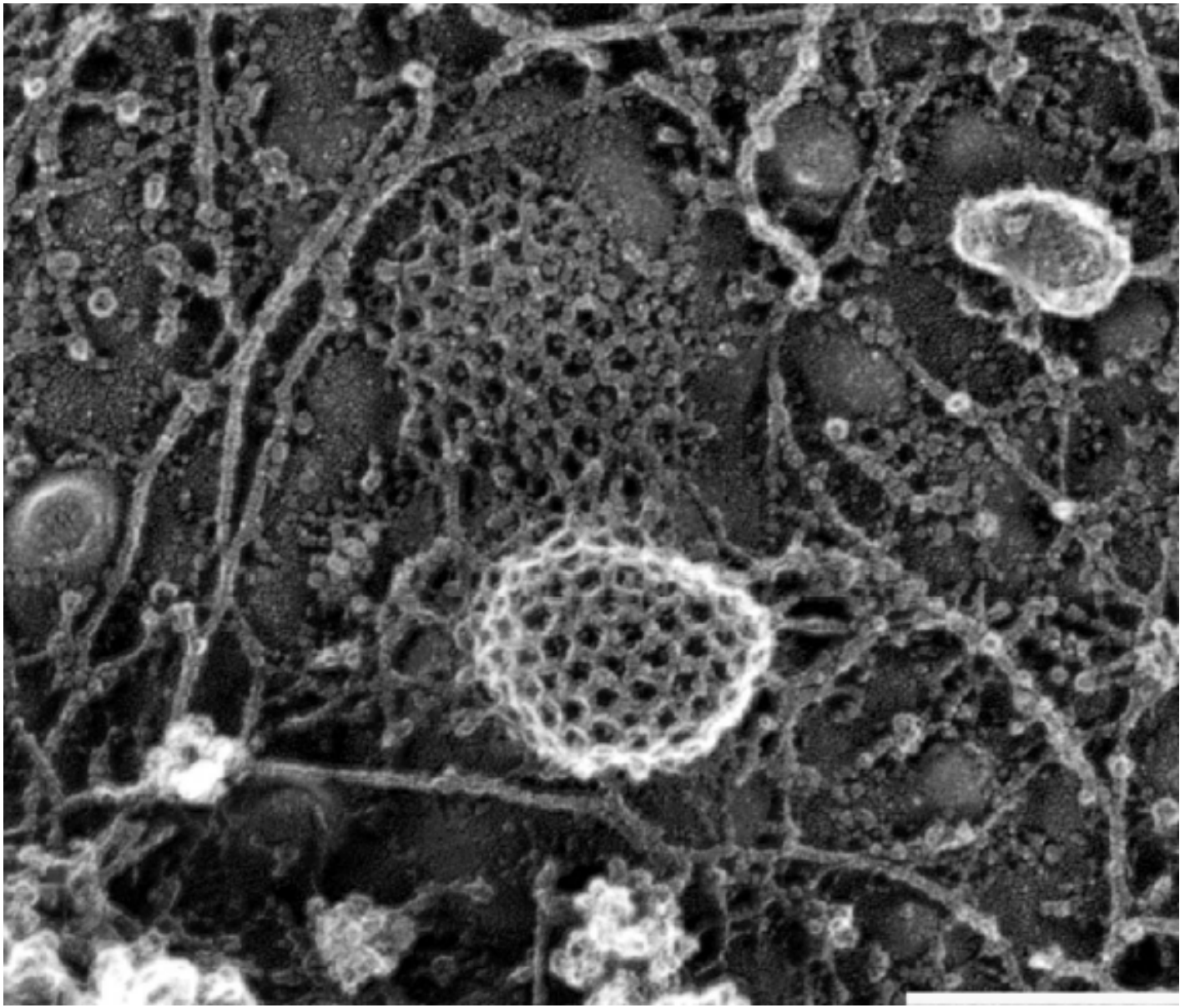
Doherty ·McMahon Ann. Rev. Biochem. 2009



Clathrin-dependent endocytosis



Morone and Kusumi

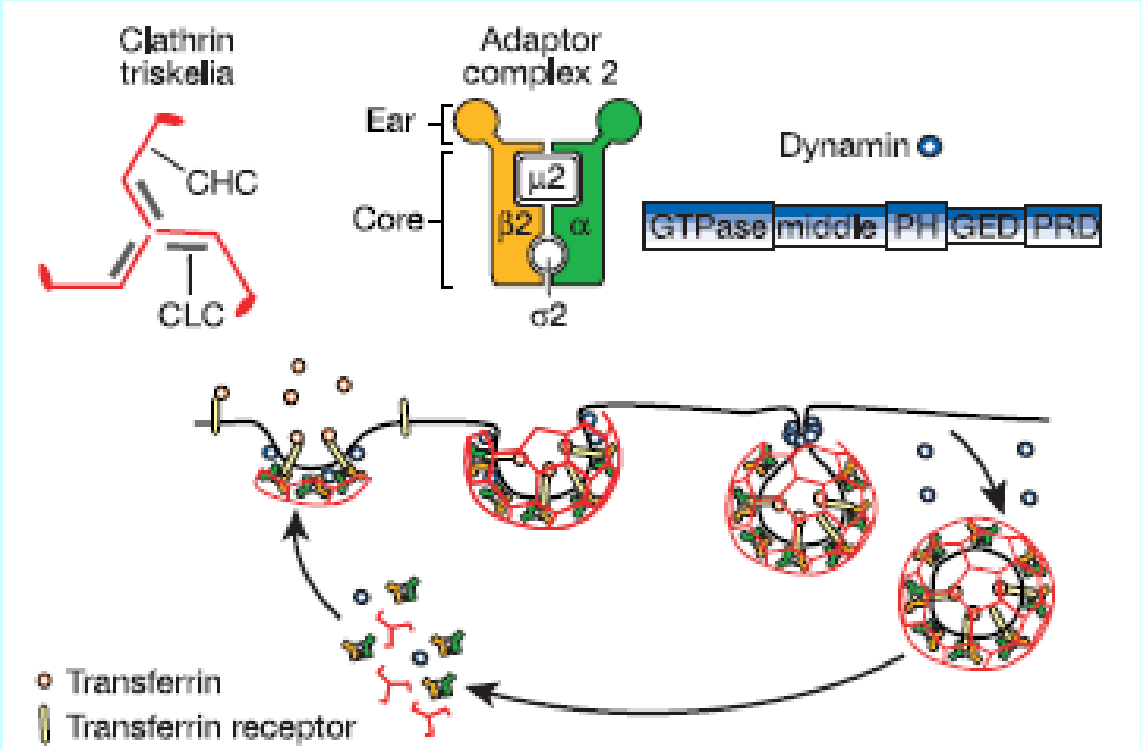
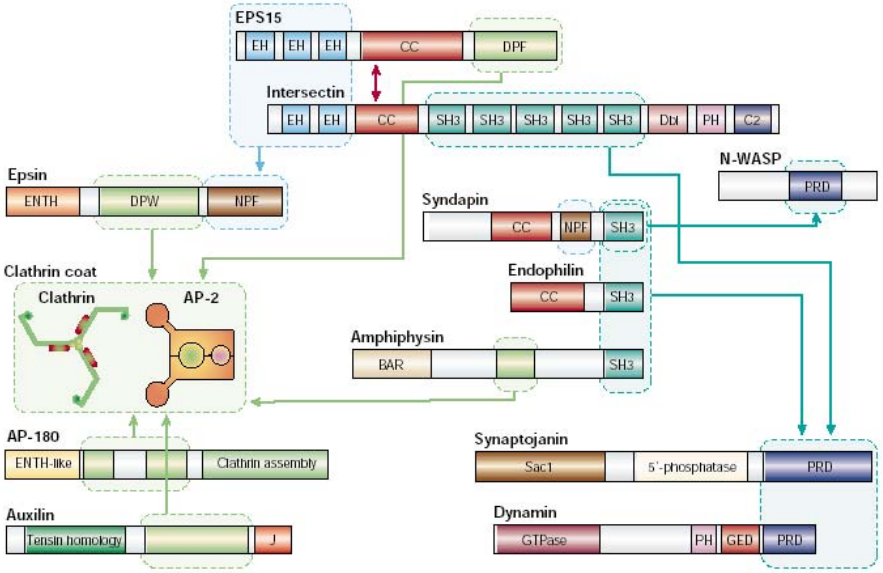


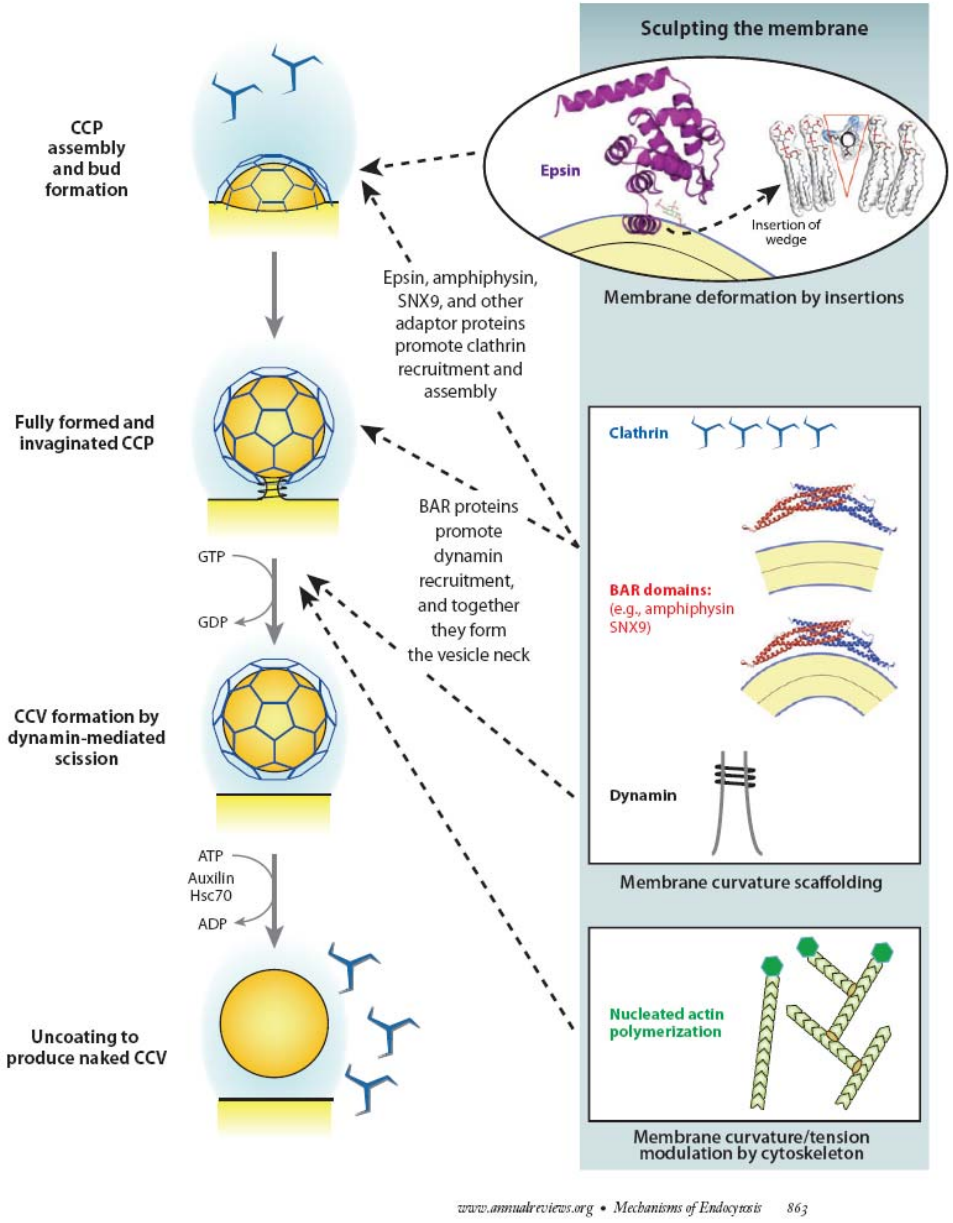
Figure 3 Core components of the machinery driving clathrin-mediated endocytosis. Clathrin triskelions, composed of three clathrin heavy chains (CHC) and three tightly associated light chains (CLC), assemble into a polygonal lattice, which helps to deform the overlying plasma membrane into a coated pit. Heterotetrameric AP2 complexes are targeted to the plasma membrane by the α-adaptin subunits, where they mediate clathrin assembly through the β2-subunit, and interact directly with sorting motifs on cargo molecules through their μ2 subunits. Dynamin is a multidomain GTPase that is recruited to the necks of coated pits, where it can assemble into a spiral or 'collar' to mediate or monitor membrane fission and the release of CCVs (see text for details). A subsequent uncoating reaction recycles the coat constituents for reuse.

Connor and Schmid, 2003

Doherty ·McMahon Ann. Rev. Biochem. 2009



Slepnev VI, De Camilli P, Nat Rev Neurosci. 2



Connor and Schmid, 2003

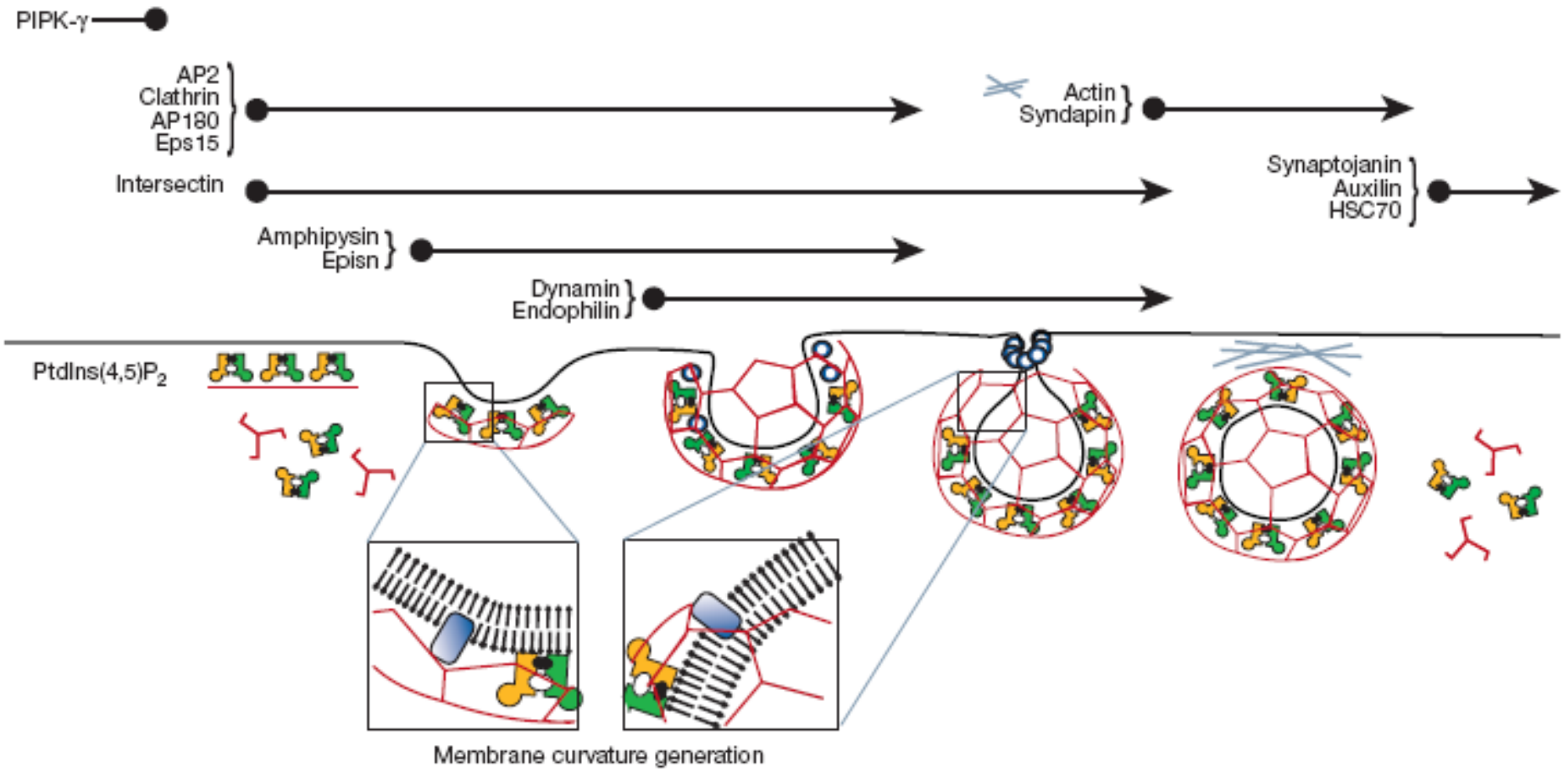
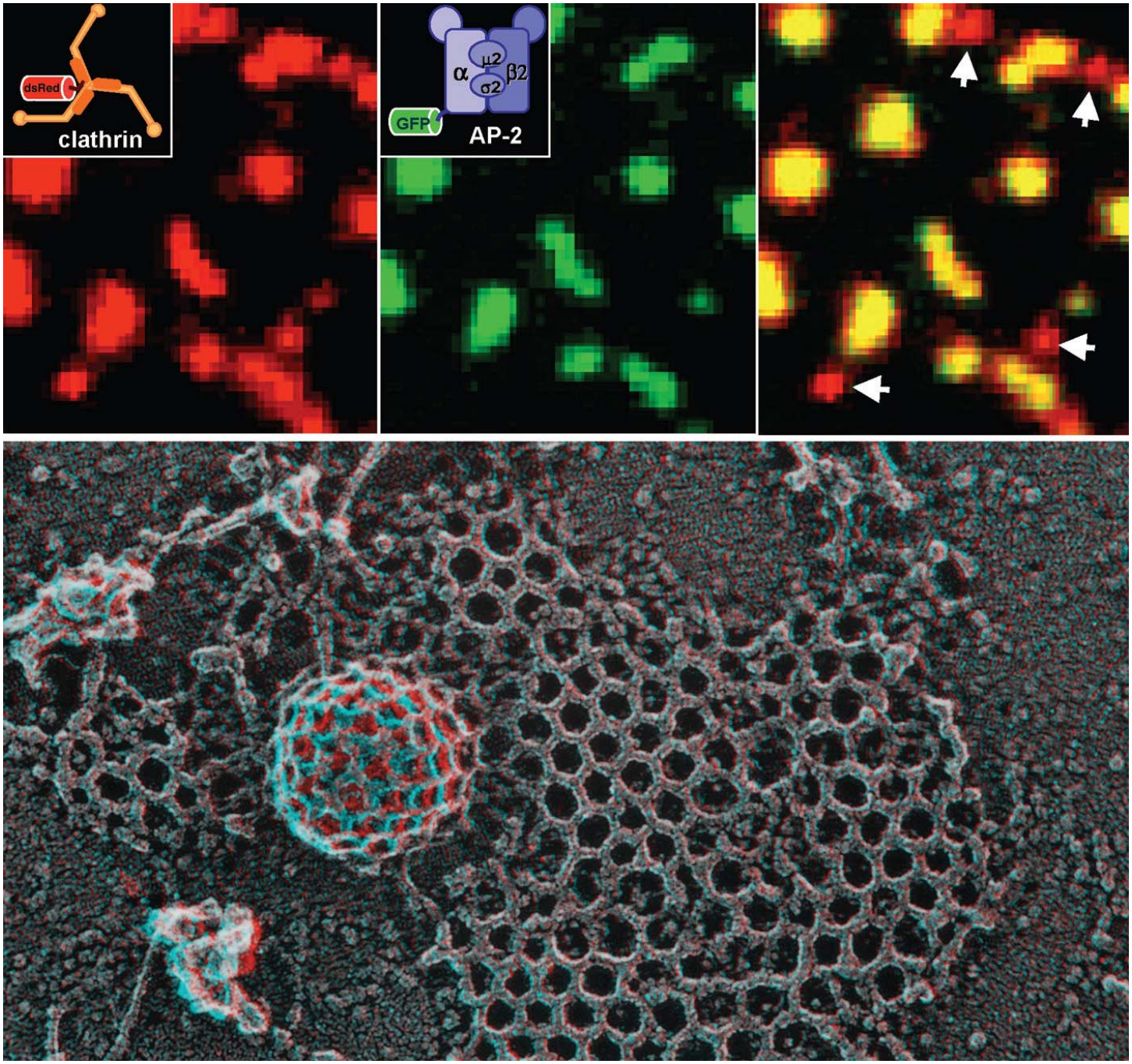
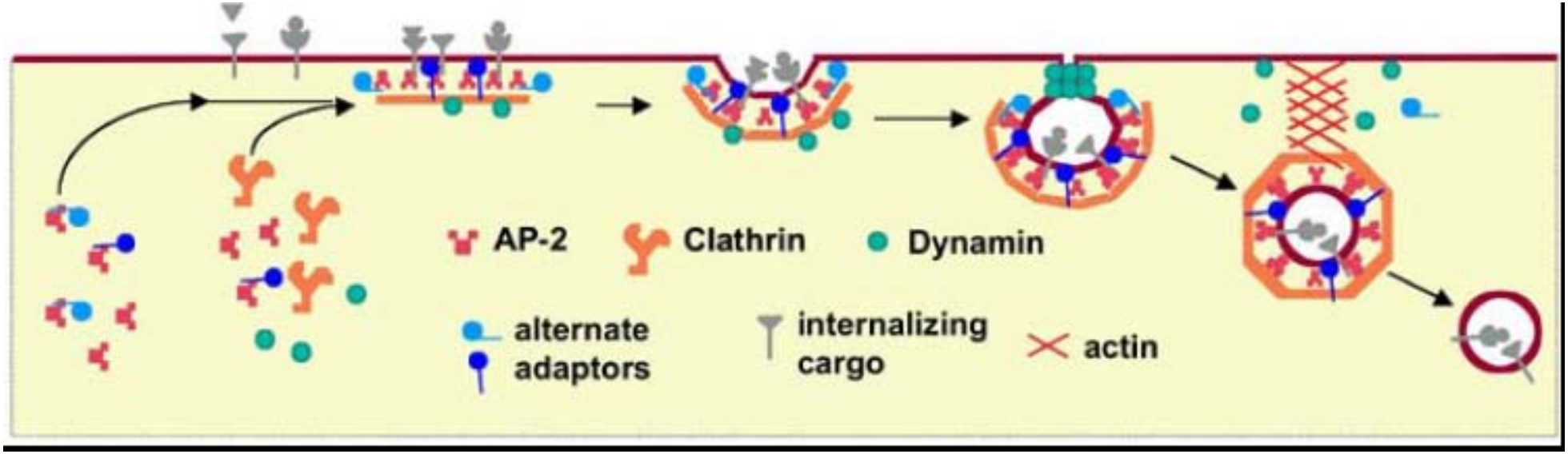


Figure 4 Clathrin-mediated endocytosis is accompanied by the temporally and spatially regulated interactions of multiple factors. The time lines shown are approximate and serve to illustrate the temporal relationships between the dynamic interactions governing CME. Neither the exact hierarchy of these protein interactions nor their exact role in CME is understood. Several accessory proteins involved in endocytosis, including amphiphysin, endophilin, epsin and dynamin, can induce membrane curvature by asymmetrically inserting a portion of their mass into the outer lipid monolayer (see insets), and might function both early and late in CCV formation. PIPK-γ, phosphatidylinositol-4-phosphate 5-kinase gamma; HSC70, heat shock cognate 70.

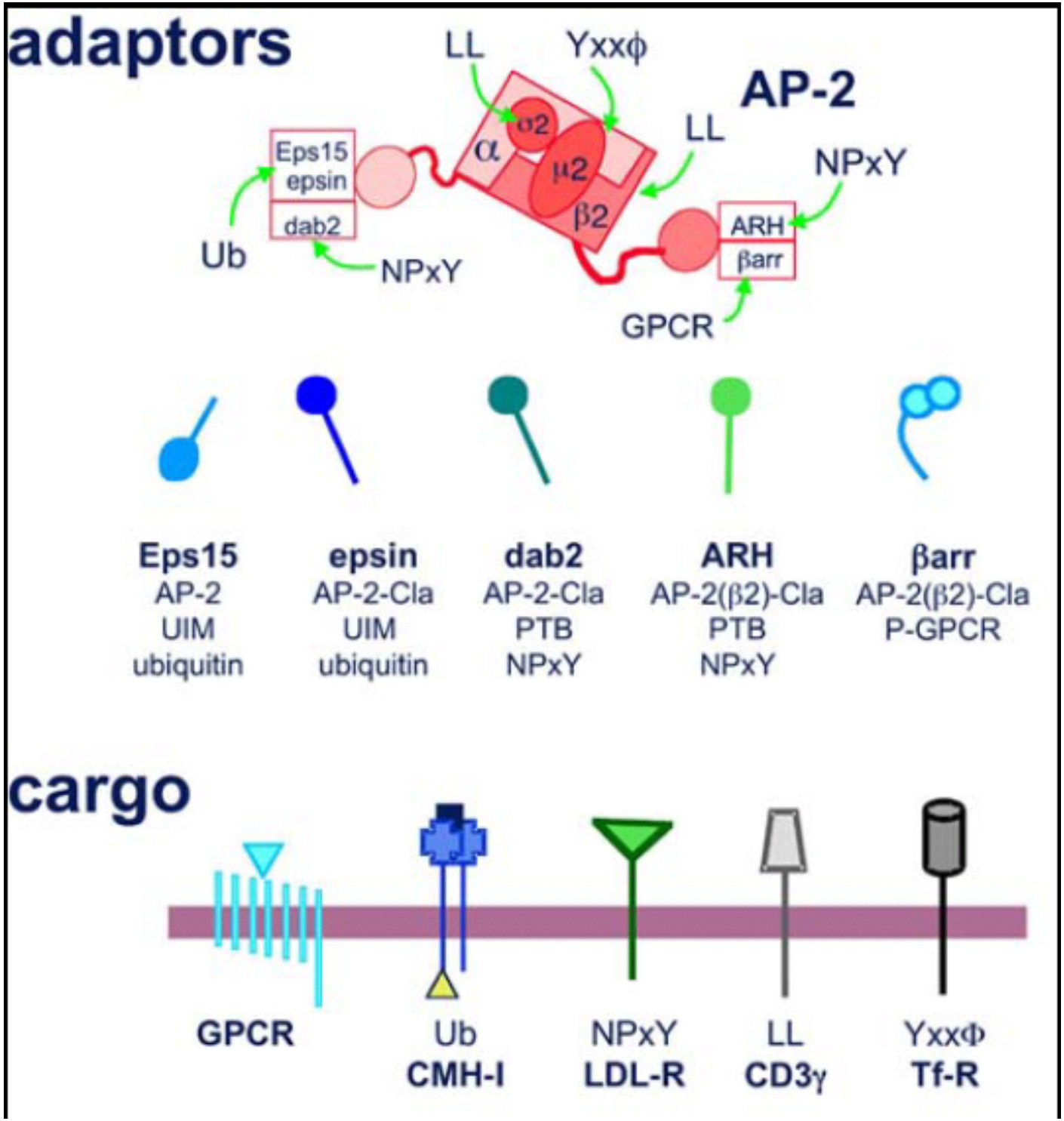
Cargo Recognition



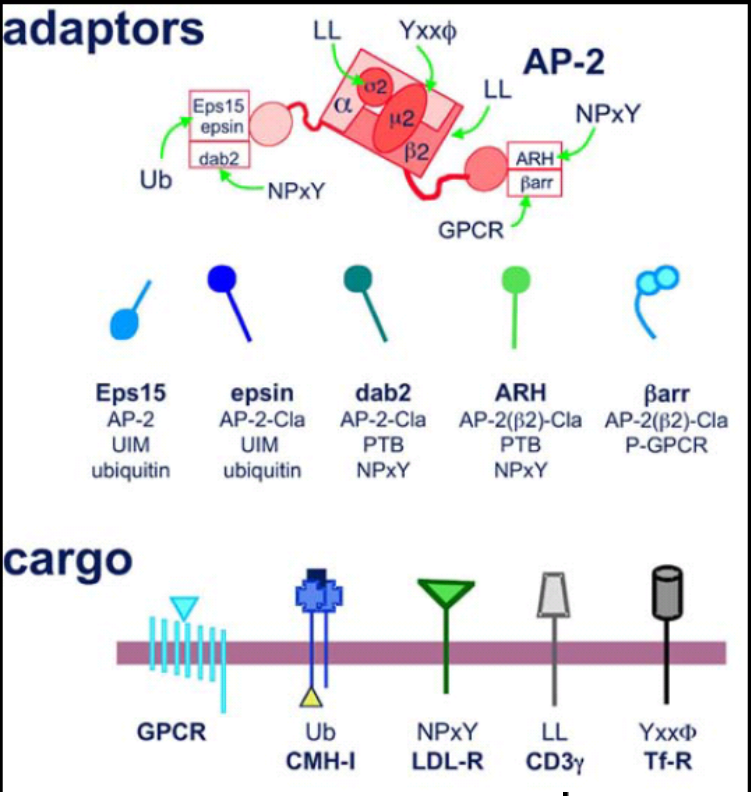
Cargo Recognition

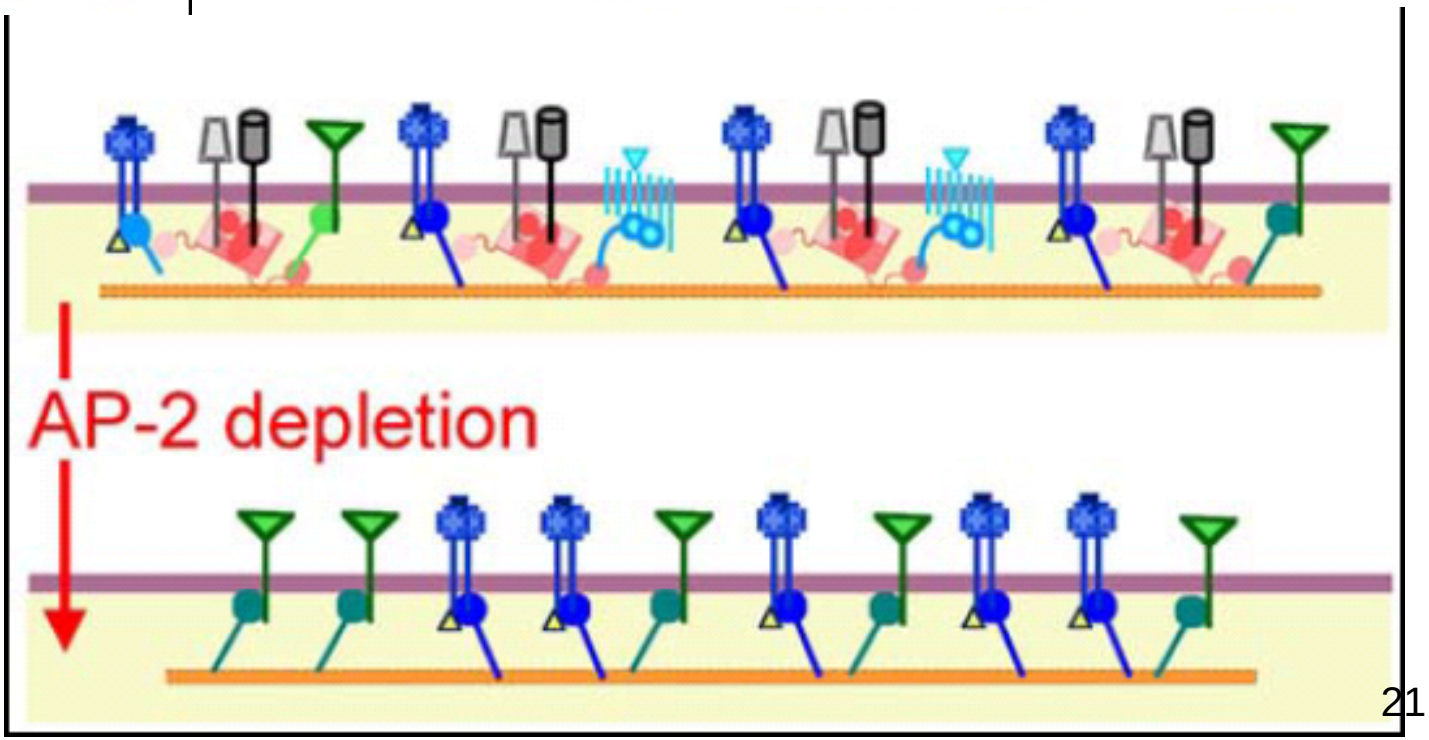


Benmerah and Lamaze, Traffic 2007



Benmerah and Lamaze, Traffic 2007





Benmerah and Lamaze, Traffic 2007

Cargo Recognition

(A) Cross-section of a clathrin-coated vesicle showing the major interactions involved in cargo sorting. The atomic structures correspond to the N-terminal domain of clathrin (red) making contact with the clathrin box (black) at the flexible β-hinge (green) between the C-terminal ear and N-terminal trunk (gray) of β-adaptin. The AP-core contains σ2 (yellow), μ2 (orange), and the N-terminal head of α-adaptin (dark blue). The α-ear is depicted in the back of the core (light blue). The positions of ears and hinges are not known with certainty.
(B) Bottom view of the AP-2 core seen from the plane of the membrane.
(C) Several side views of the core in the inactive or closed conformation, rotated with respect to an axis perpendicular to the membrane (yellow). The orientation of the core is as proposed by Collins et al. (2002); it locates the binding site in α-adaptin (red) for the phosphate groups of the membrane-bound phosphoinositides (light red) on a position close to the inner leaflet of the membrane (yellow).
(D) The YppØ sorting motif (red) within different locations in the cytosolic tail of transmembrane cargo proteins (gray) can make contact with the C-terminal domain of μ2; depicted are possible different active or open conformations, from partial (left) to total (right) extension. When totally extended, a region of μ2 would also interact with a second membrane-bound phosphoinositide.

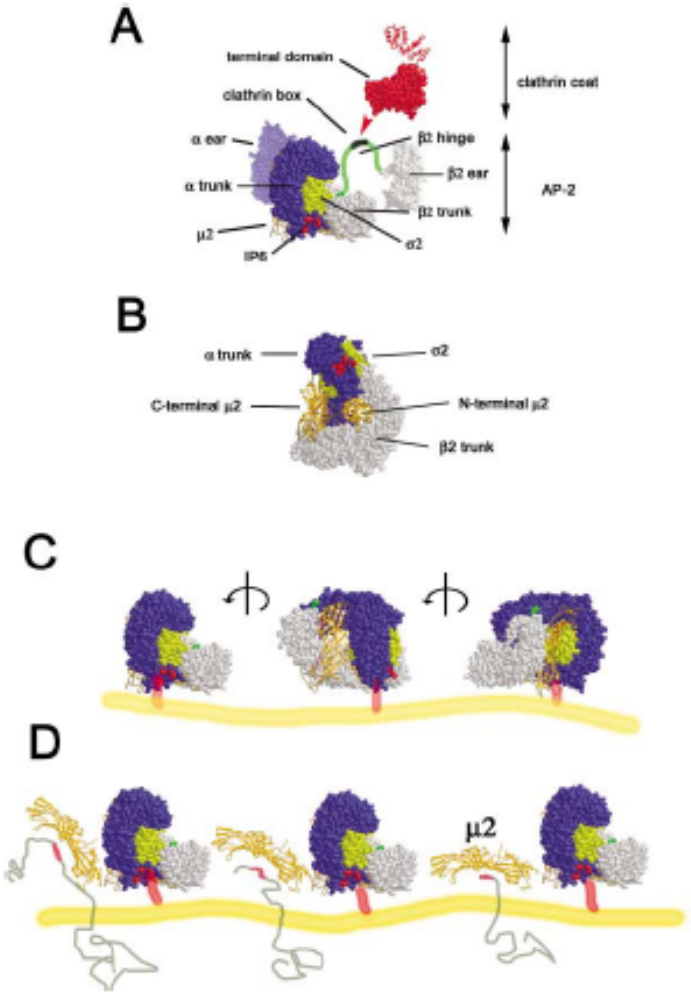
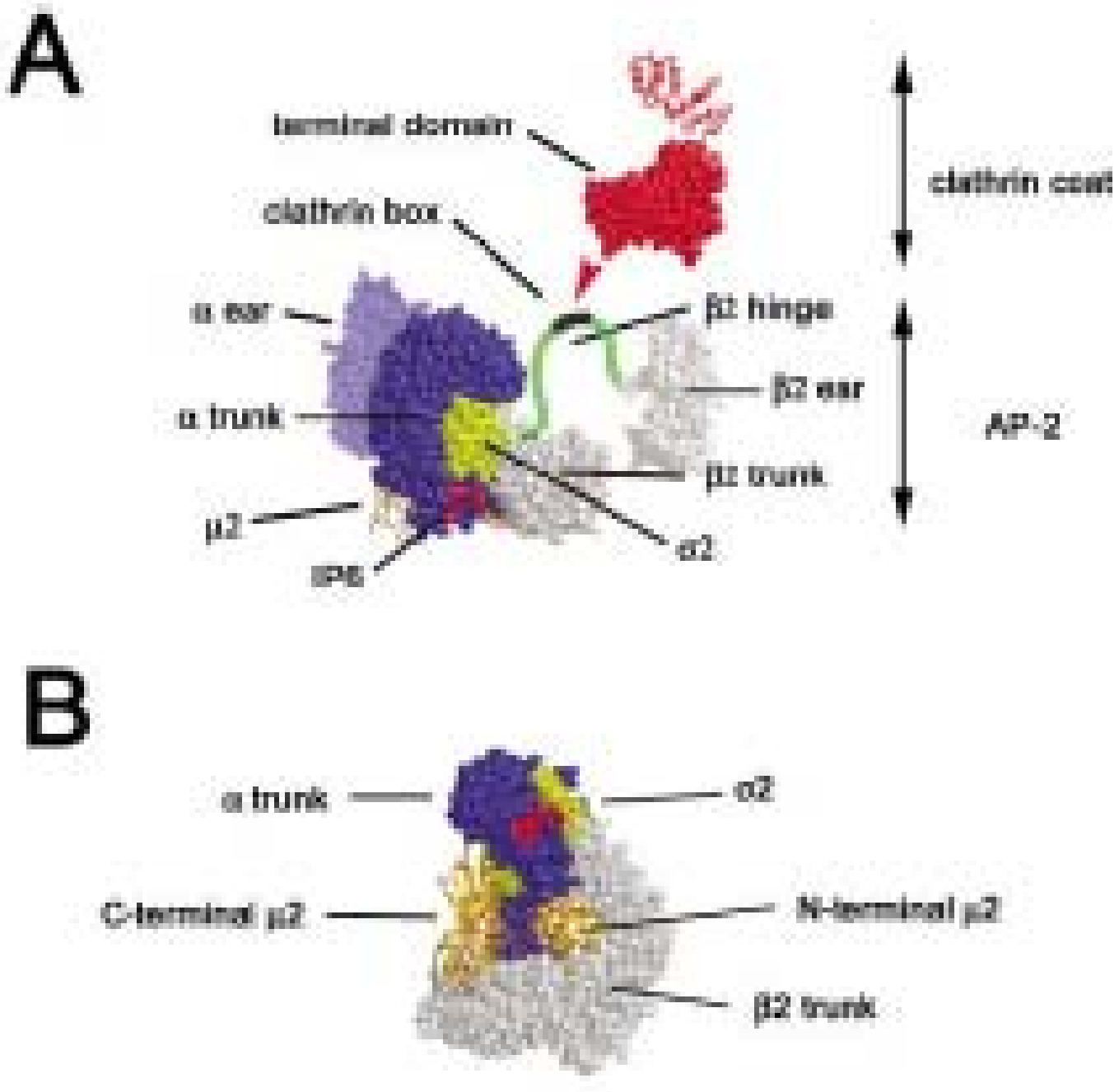


Figure 1. Coat Formation and Cargo Recognition

Owens et al, Cell 2004


A
terminal domain
clathrin box
α ear
α trunk
μ2
σ2
β2 hinge
β2 ear
β2 trunk
clathrin coat
AP-2
B
α trunk
σ2
C-terminal μ2
N-terminal μ2
β2 trunk

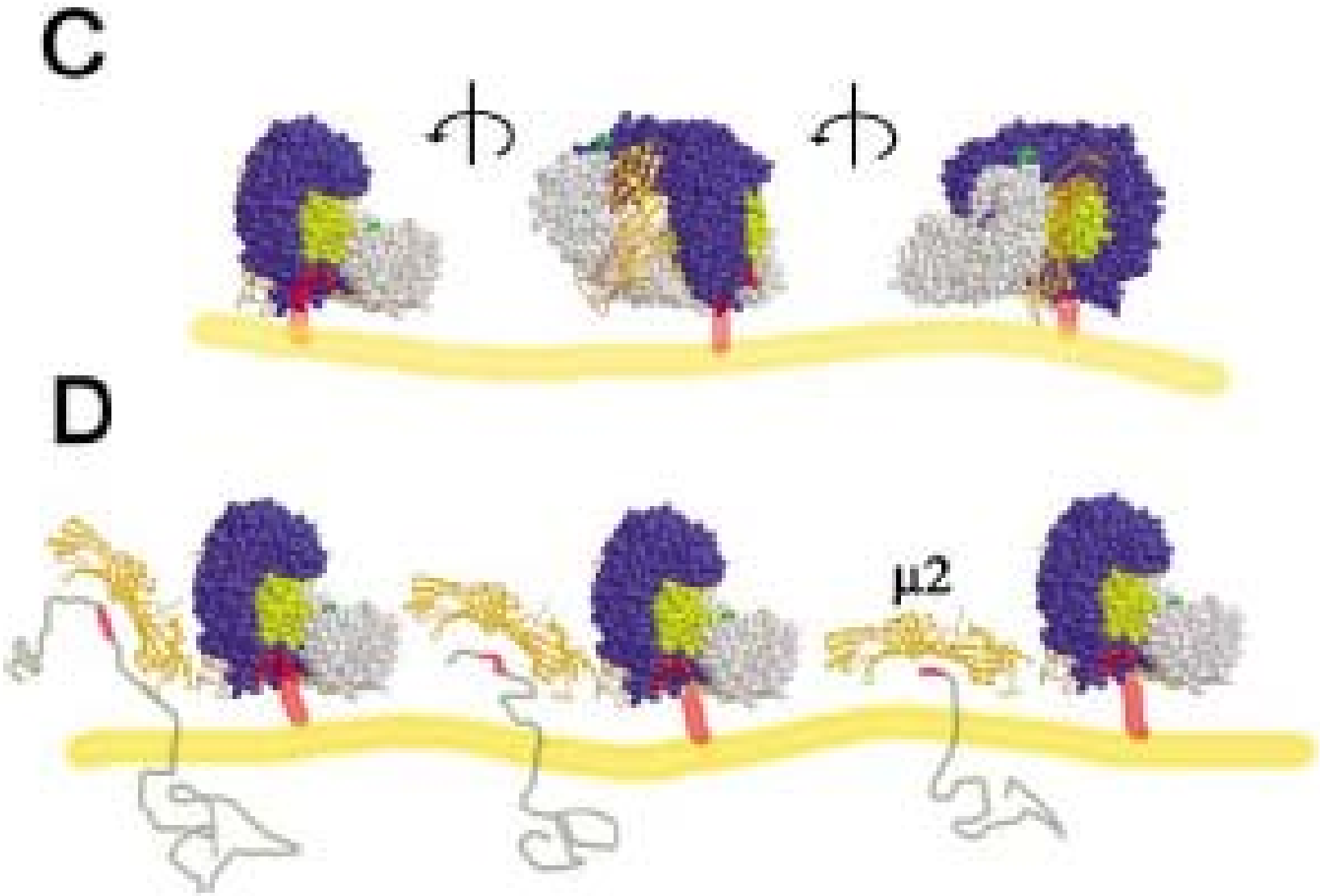


Figure 1. Coat Formation and Cargo Recognition

Capturing Cargo

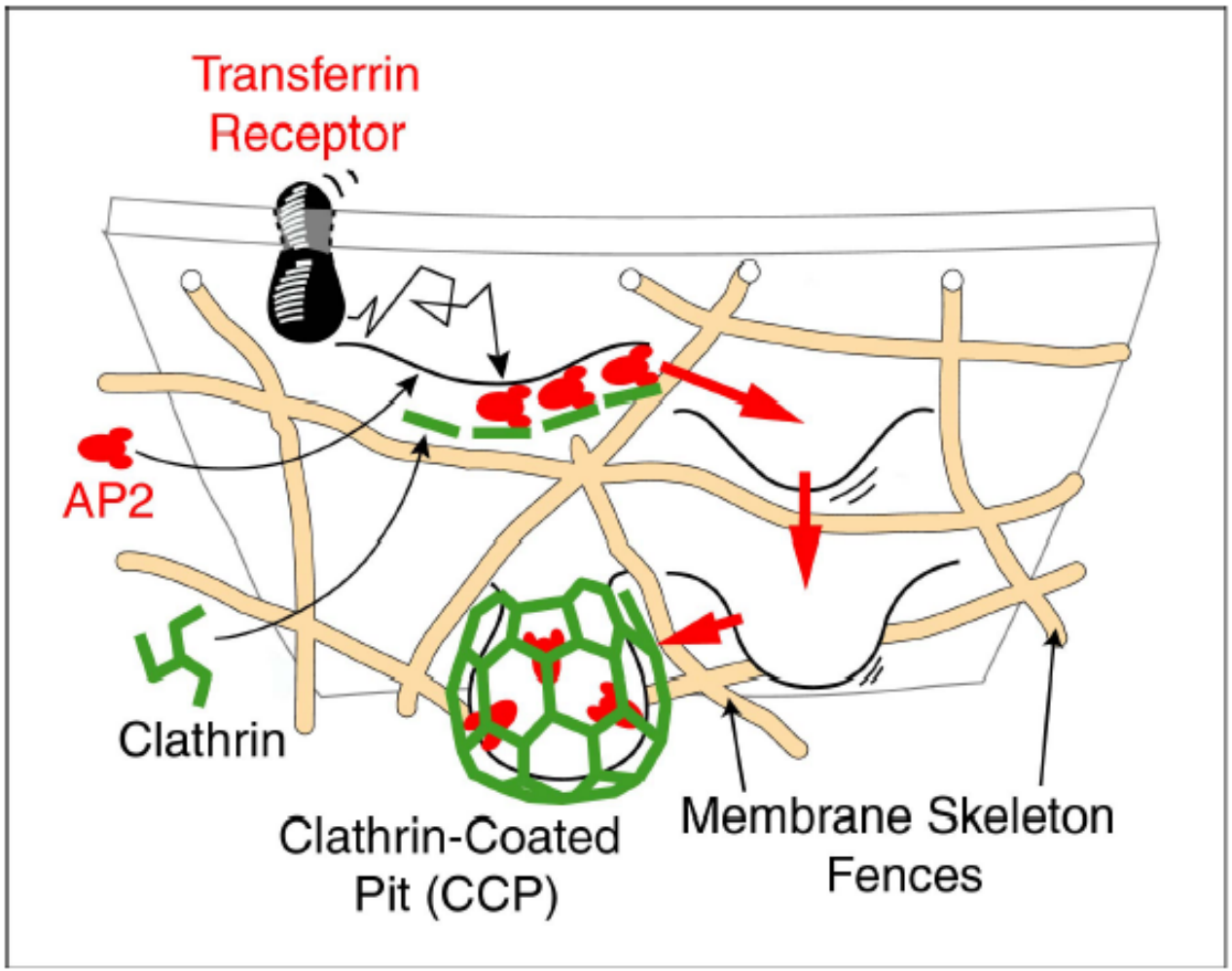
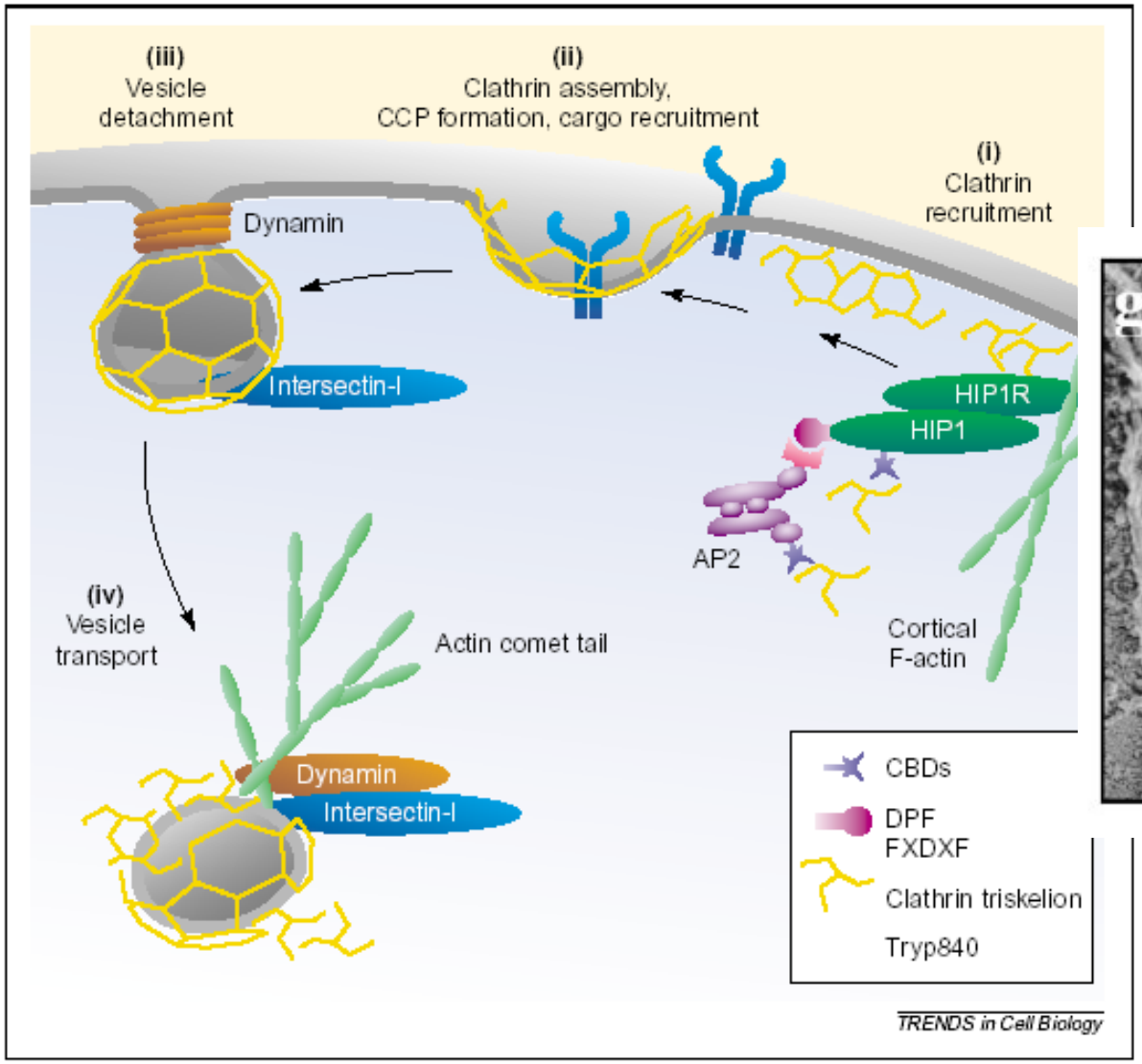
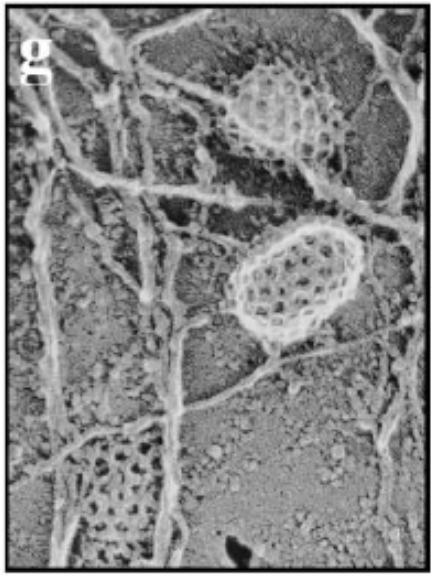


Figure 33. Clathrin-coated pits (CCPs) tend to form repeatedly at certain locations in the membrane. The transmembrane protein transferrin receptor and the cytoplasmic adaptor protein AP2 were visualized at the single molecule level to elucidate the dynamics of CCP formation.

Fujiwara and Kusumi : unpublished

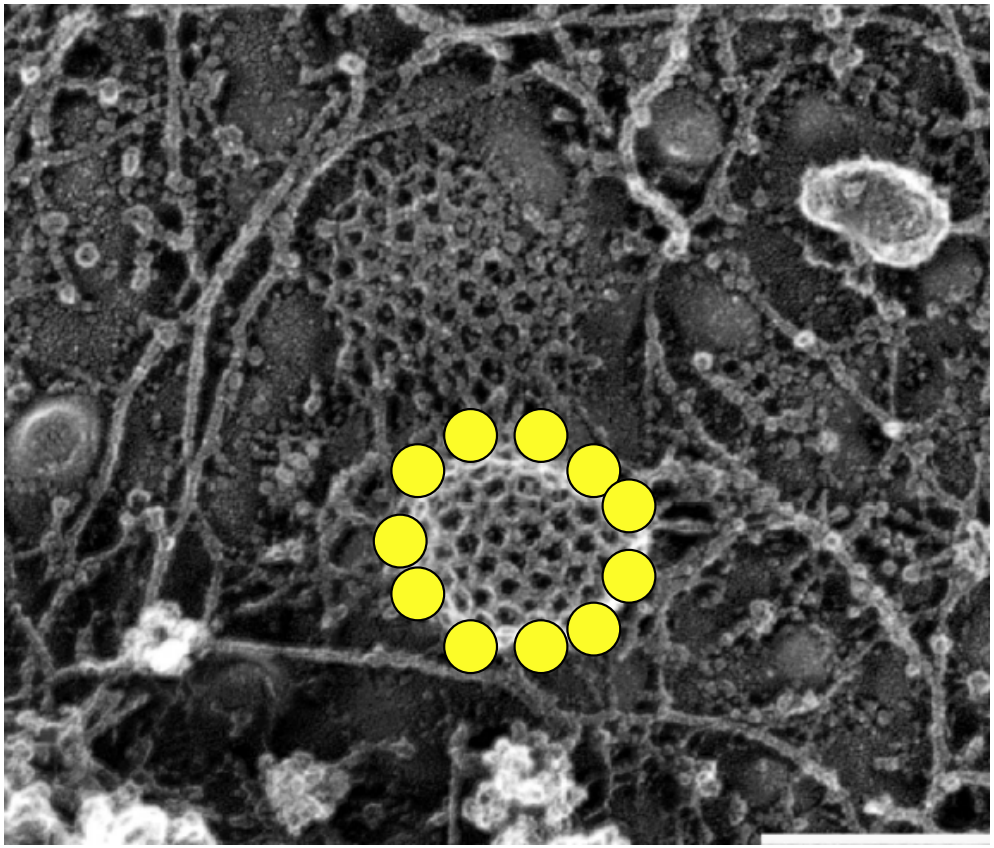
Construction of a clathrin pit



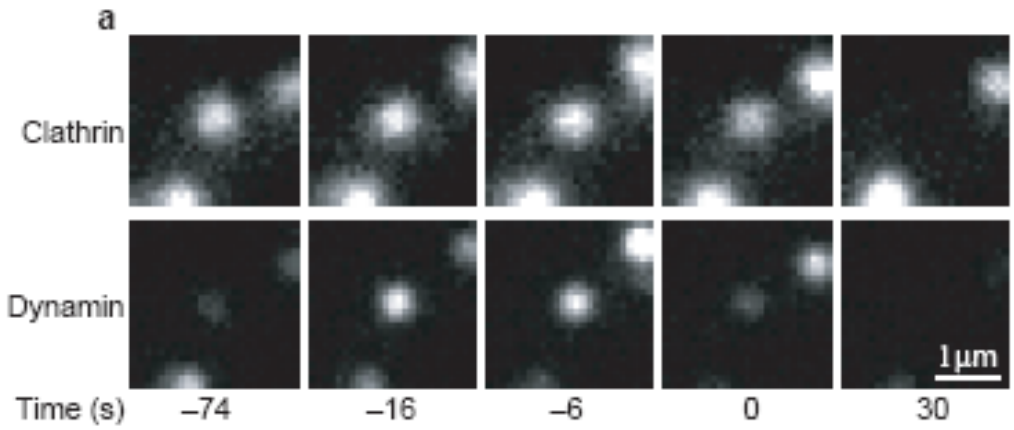


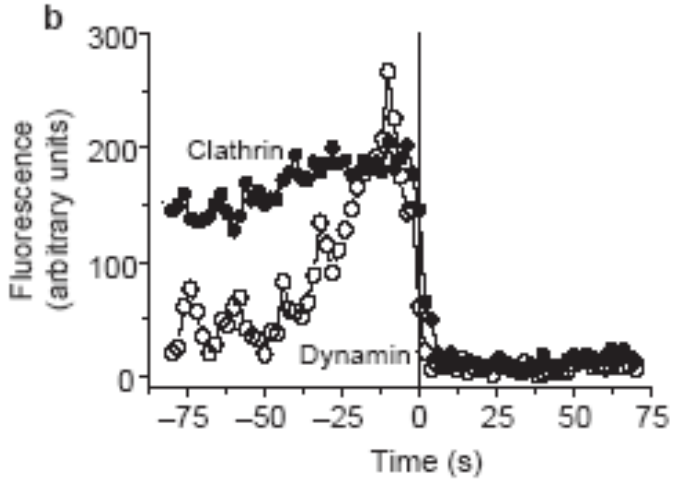
Scission Mechanisms:

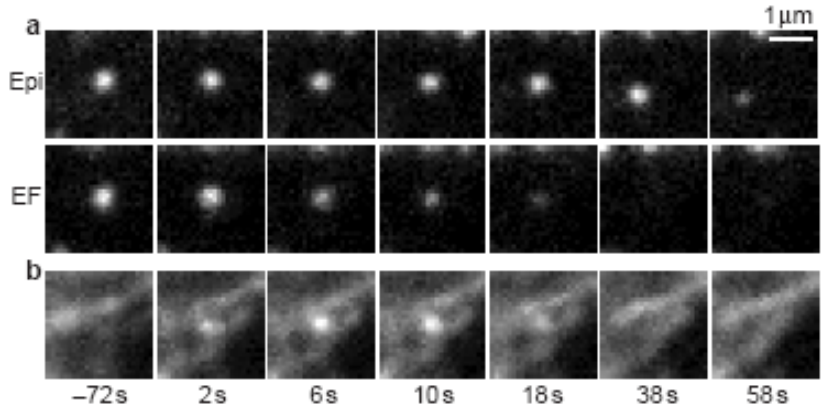
Dynamin-dependent endocytosis

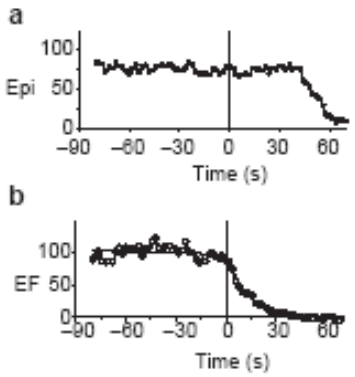


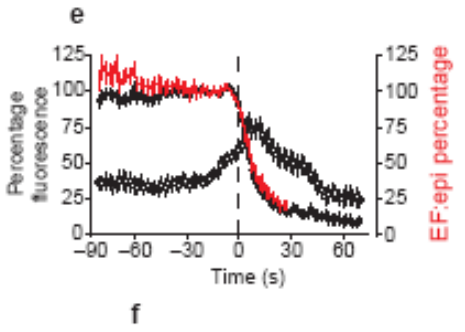
Merrifield et al. Nature Cell Biol 2001,











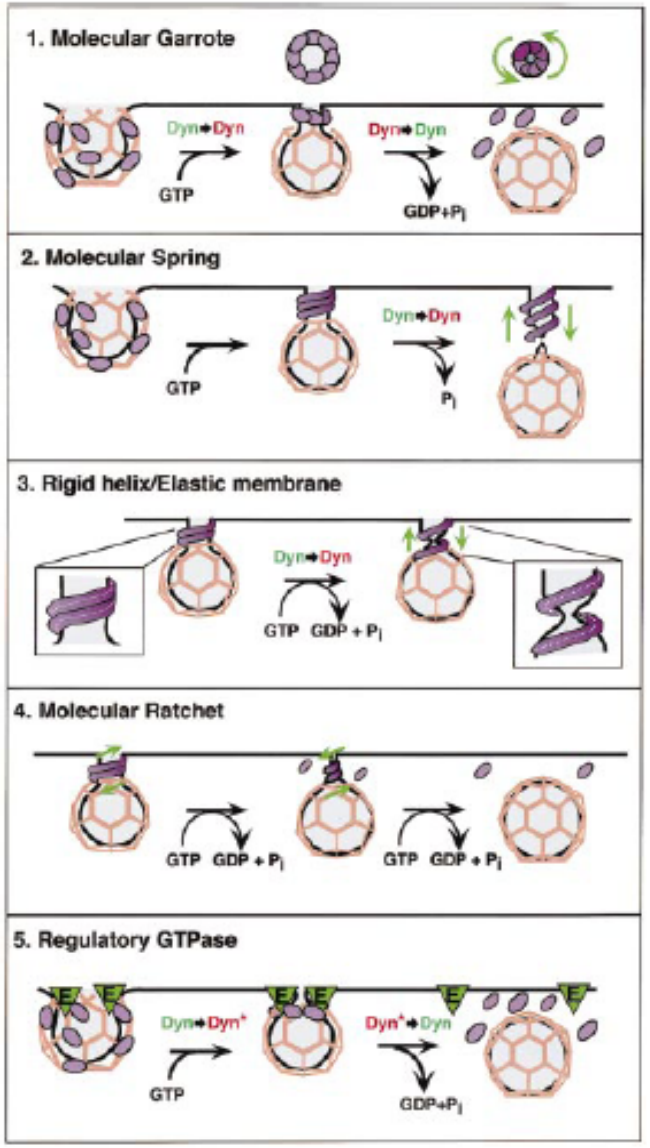
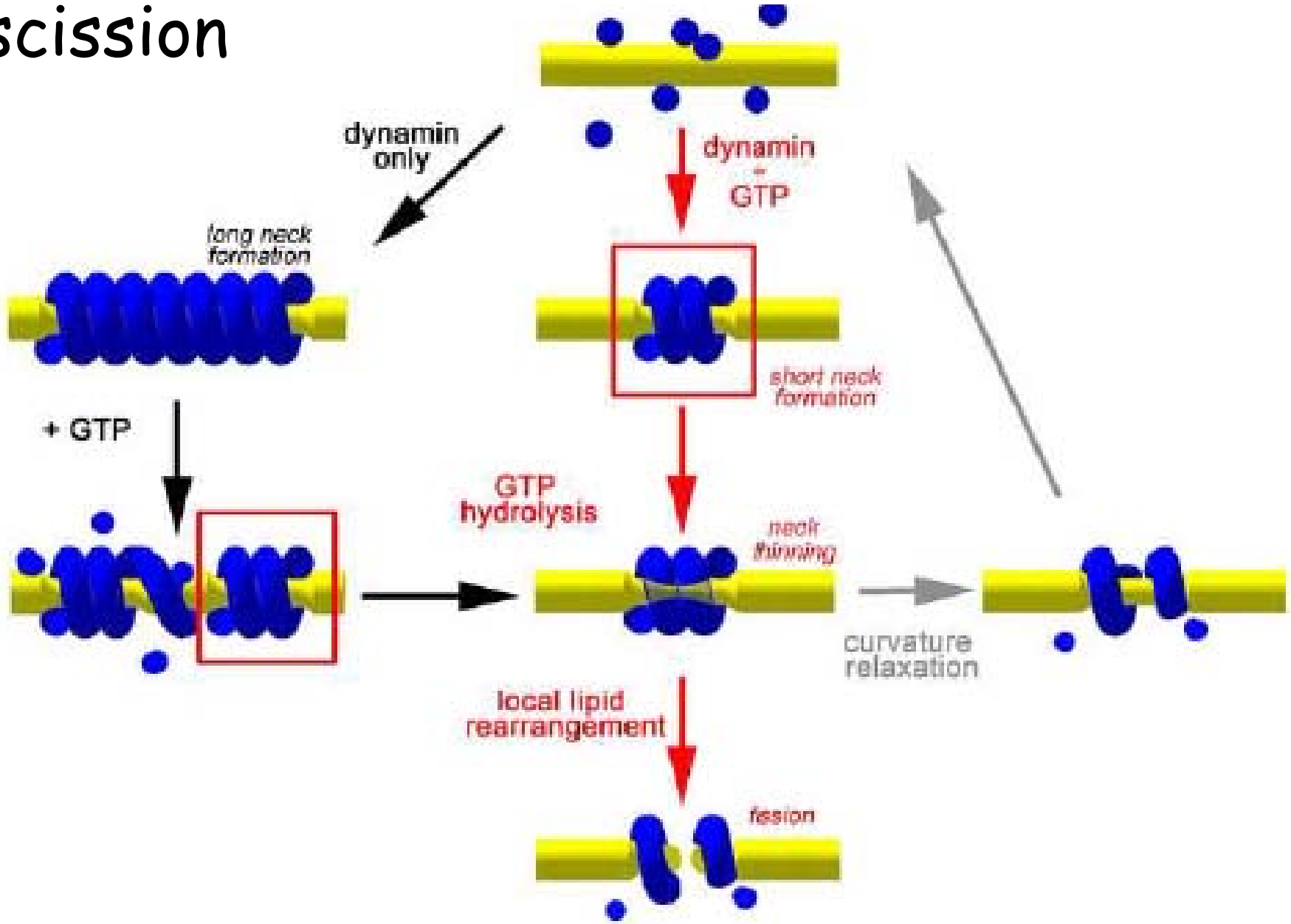


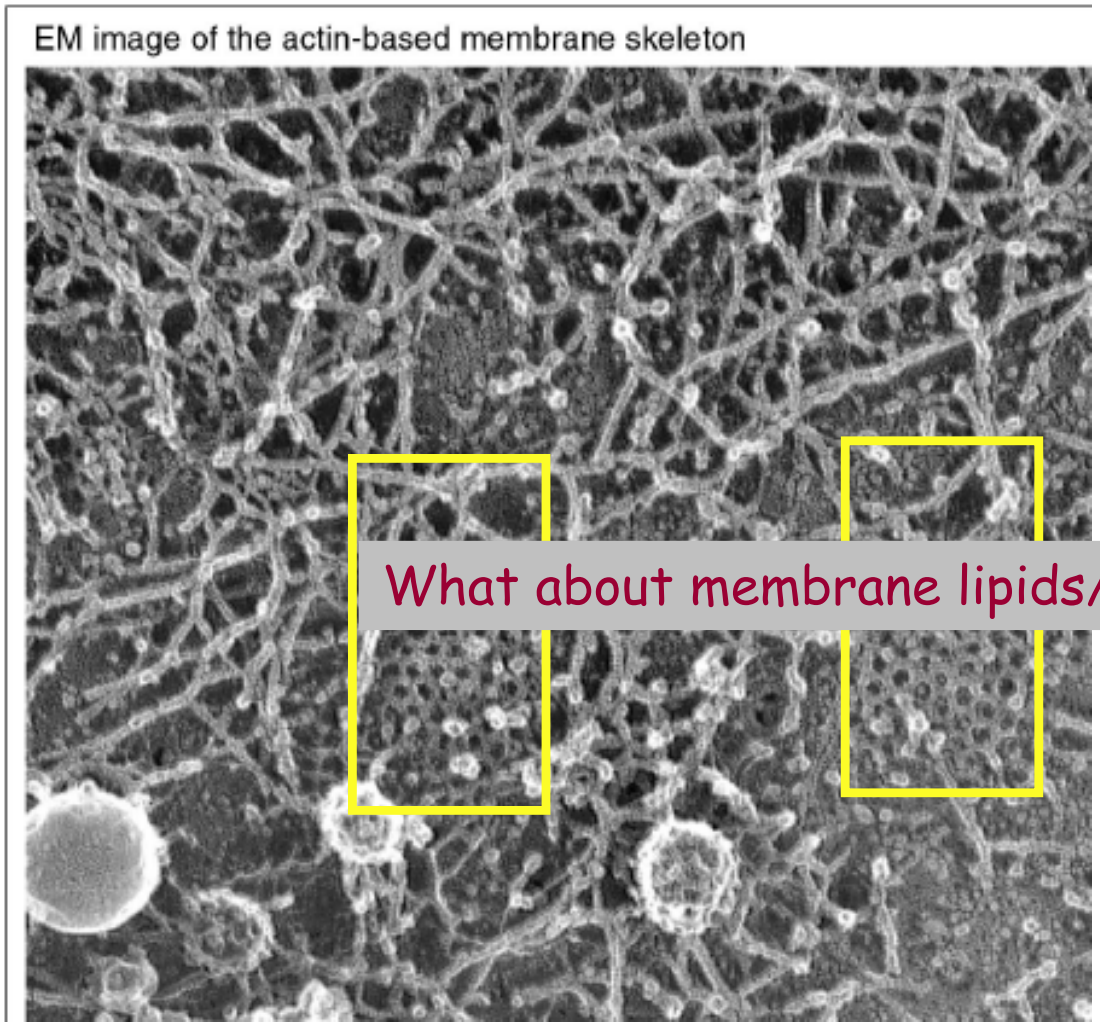
Figure 3: Current models for dynamin function in endocytic coated vesicle formation. Stages at which dynamin binds and hydrolyzes GTP are indicated. A color change reflects a conformation change of dynamin. Green arrows indicate motion accompanying conformational changes of dynamin. In model 3, the inset shows an enlarged view of the dynamin helix. The dotted line indicates the line of attachment to underlying lipids required by the model. In model 5, 'E' designates an unknown effector molecule. See text for details.

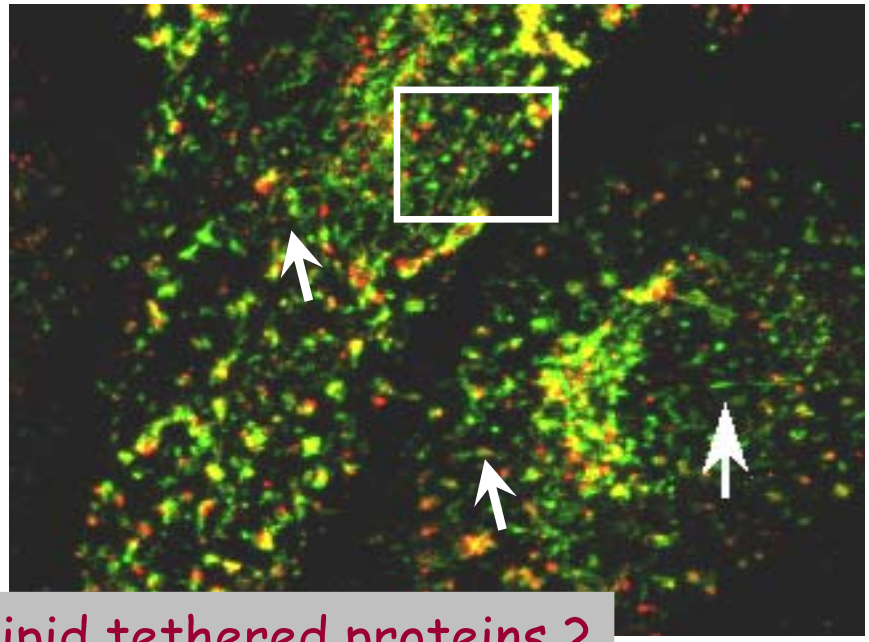
New Model for Dynamin scission



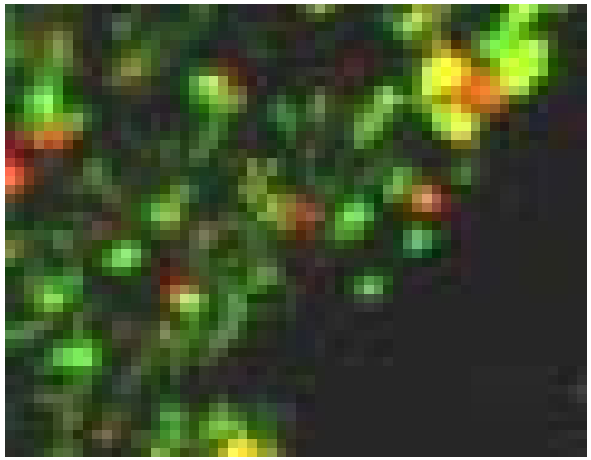
Pucadyil and Schmid, + Bashikirov et al. Zimmerberg and Frolov Cell 2009

Clathrin-dependent endocytosis

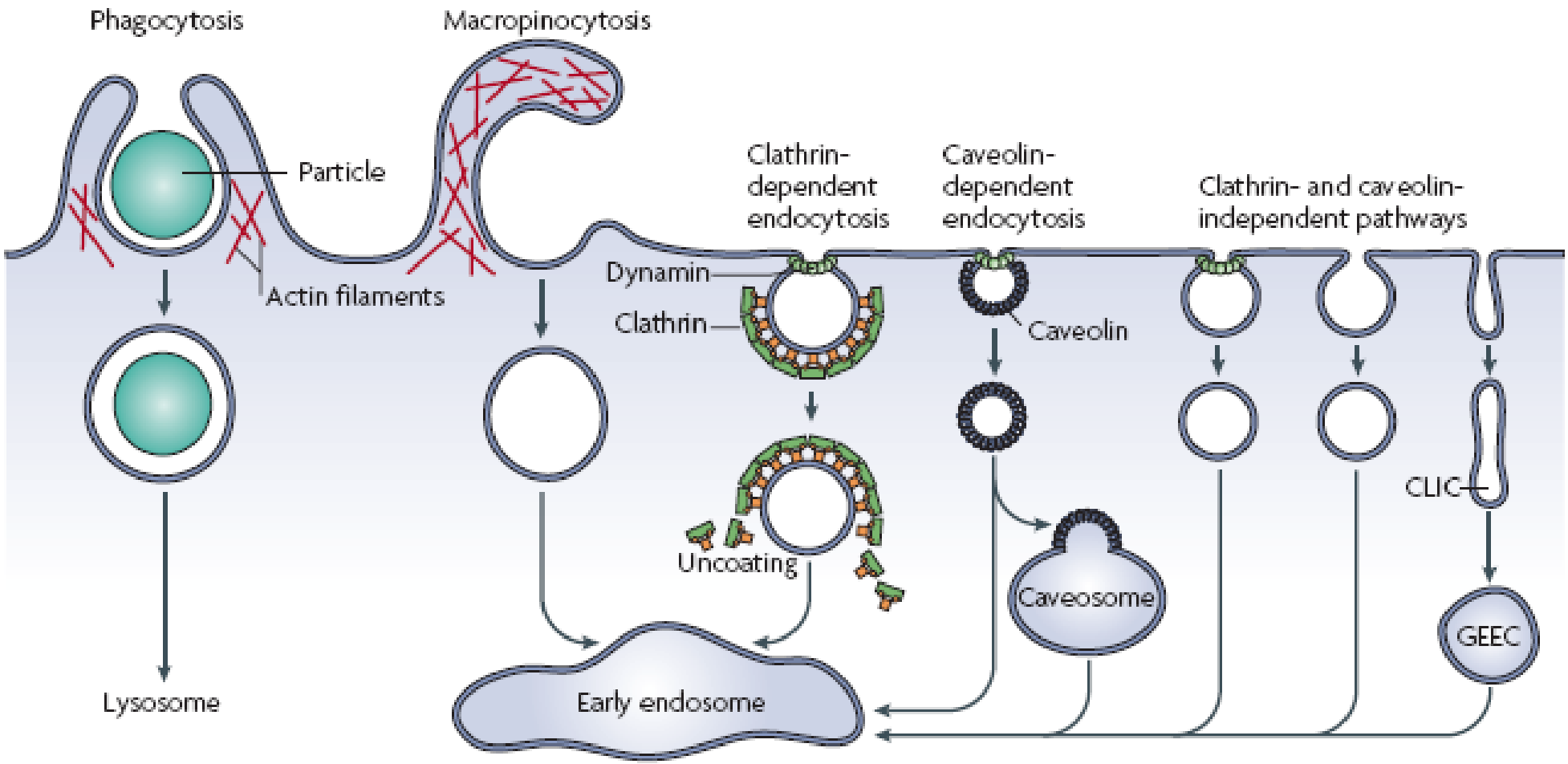




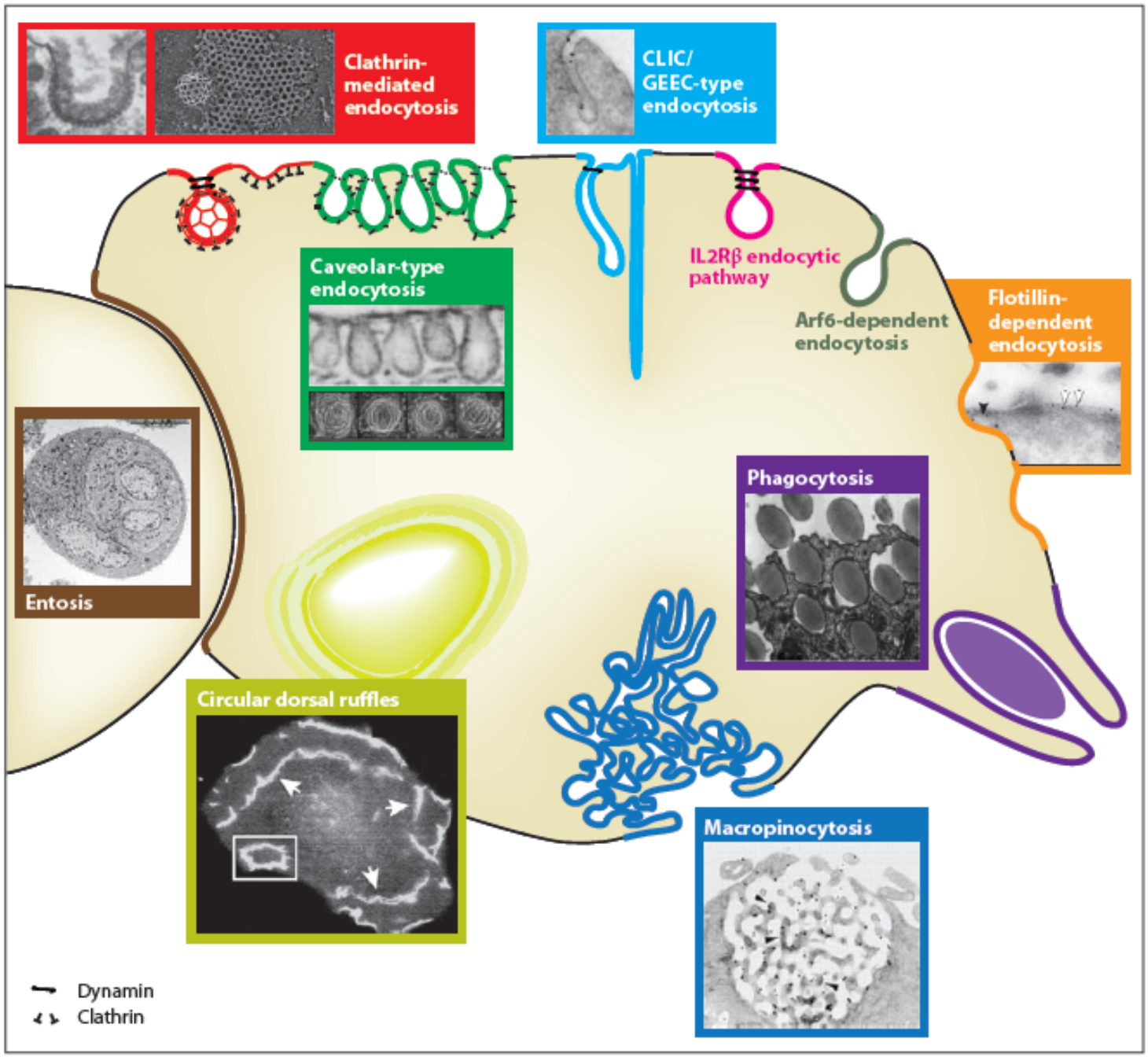
What about membrane lipids/ lipid tethered proteins ?



Morone & Kusumi, *Membrane Organizer Project;* pers. Comm.



Mayor and Pagano , Nature Reviews MCB 2007



Doherty ·McMahon Ann. Rev. Biochem. 2009

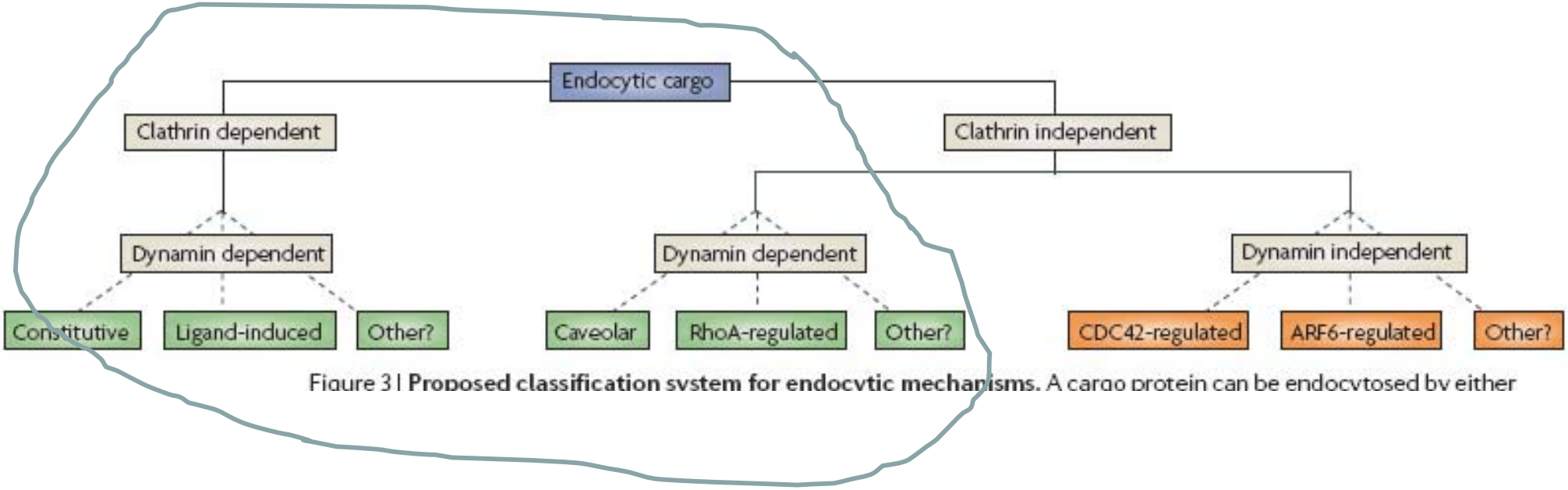
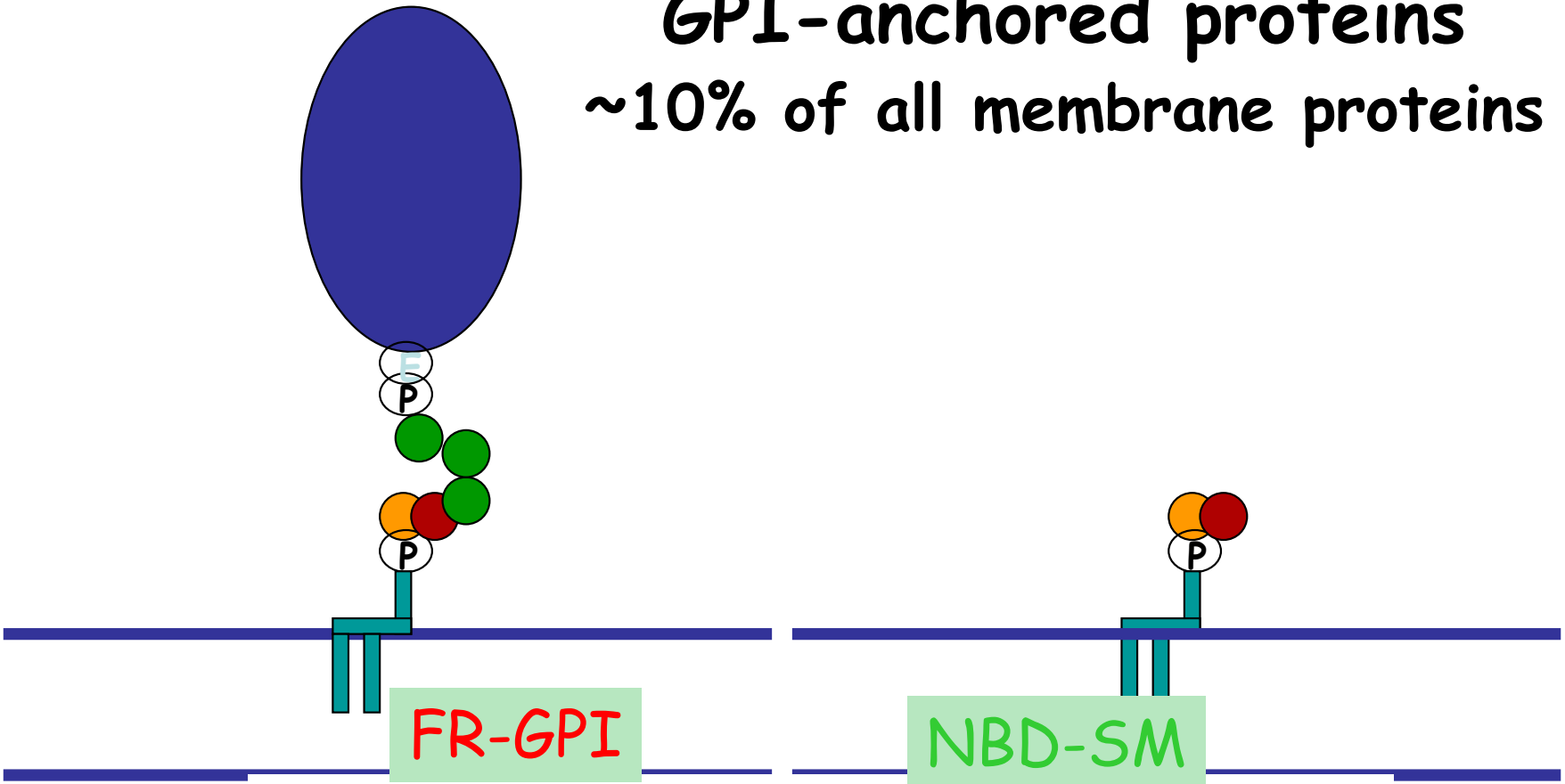


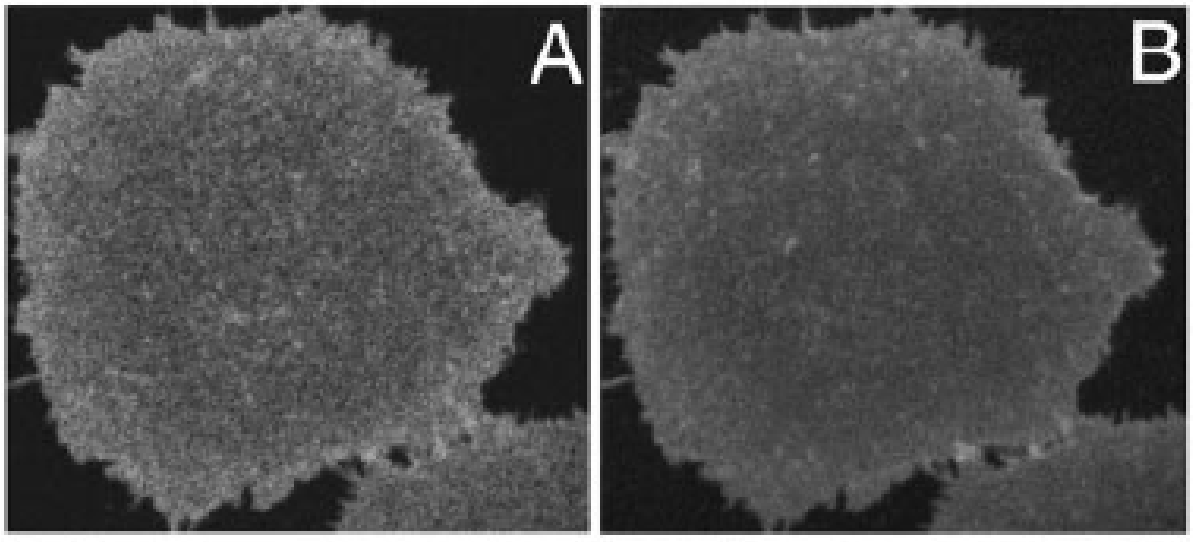
Figure 3 | **Proposed classification system for endocytic mechanisms.** A cargo protein can be endocytosed by either

Mayor and Pagano , Nature Reviews MCB 2007

GPI-anchored proteins

~10% of all membrane proteins

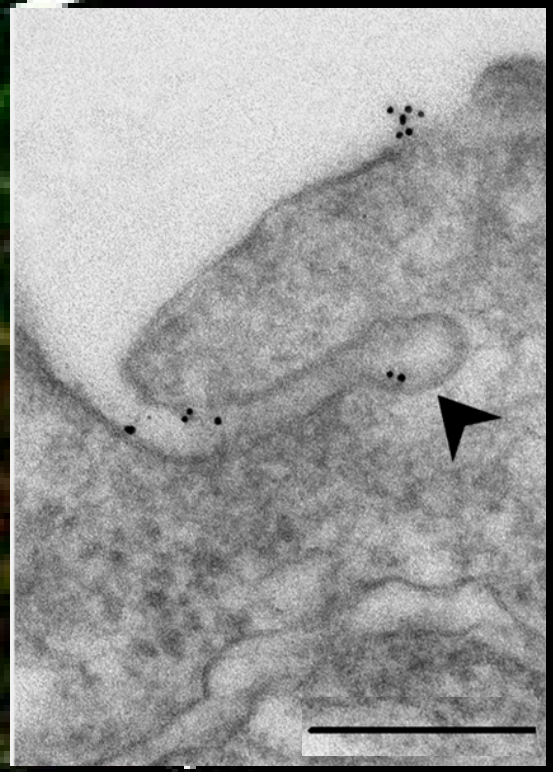




GPI-anchored proteins are selectively endocytosed into distinct endosomal compartments called 'GEECs'

FR-GPI

NBD-SM



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Sabharanjak et al. Dev. Cell, 2002