# Mechanisms of membrane deformation

Khashayar Farsad and Pietro De Camilli*

Membrane traffic requires the generation of high-curvature lipid-bound transport carriers represented by tubules and vesicles. The mechanisms through which membranes are deformed has gained much recent attention. A major advance has been the demonstration that direct interactions between cytosolic proteins and lipid bilayers are important in the acquisition of membrane curvature. Rather than being driven only by the formation of membrane-associated structural scaffolds, membrane deformation requires physical perturbation of the lipid bilayer. A variety of proteins have been identified that directly bind and deform membranes. An emerging theme in this process is the importance of amphipathic peptides that partially penetrate the lipid bilayer.

**Addresses**
Department of Cell Biology, Howard Hughes Medical Institute, Boyer Center for Molecular Medicine, Yale University School of Medicine, 295 Congress Avenue, New Haven, CT 06510, USA
*Correspondence: Pietro De Camilli
e-mail: pietro.decamilli@yale.edu

**Current Opinion in Cell Biology** 2003, **15**:372–381

This review comes from a themed issue on
Membranes and organelles
Edited by Alice Dautry-Varsat and Alberto Luini

0955-0674/$ – see front matter


**DOI 10.1016/S0955-0674(03)00073-5**

**Abbreviations**

| | |
|---|---|
| **COP** | coatomer complex |
| **LPAAT** | lysophosphatidic acid acyltransferase |
| **MVB** | multivesicular body |
| **PI(4,5)P2** | phosphatidylinositol 4,5-bisphosphate |

## Introduction

Cellular compartmentalisation requires membrane-bound structures. Traffic between membranous organelles occurs via tubular and vesicular membrane carriers that bud and fuse, effectively maintaining the compartmentalised state while allowing for dynamic flux. Over the past few years, we have garnered greater understanding of the molecular processes by which the trafficking organelles — tubules and vesicles — form and behave. Generation of these structures can be driven by a cooperation of mechanisms both extrinsic and intrinsic to the membrane. Mechanical forces applied to the membrane by the cytoskeleton can induce membrane tubule formation. Proteinaceous coats selectively associated with the surface of membrane buds are key mediators of vesicle formation in the endocytic and secretory pathways. Accessory factors to the main constituents of coat proteins have also recently been found to be an integral part of both vesicle formation and cargo selection within the bud. Proteins that can deform the membrane into tubules have been identified and characterised. In addition, lipid components of the membrane, either directly or via interaction with proteins, have been suggested to facilitate the structural changes necessary to deform membranes.

In this review, we will primarily focus on recently described mechanisms for membrane deformation that have expanded our understanding of this process.

## Extrinsic forces on the membrane

Cytoskeletal elements have long been known to play some role in membrane traffic, not only by forming the structural scaffold and network over which membrane traffic flows, but also by directly deforming membranes (Figure 1) [1–3]. A characteristic property of membrane bilayers is that the application of an external focal force results in bilayer tubule formation, rather than a broad 'tenting' of the membrane. Many intracellular membrane tubules are generated in this fashion [4•,5–8]. For example, microtubule motors can pull a developing membrane tubule along a preformed microtubule track *in vitro* [1,4•,5]. Microtubule-dependent mechanisms, possibly in cooperation with other cytosolic factors [9], have also been shown to play a role *in vitro* and *in vivo* for the tubular dynamics of the ER [1,2,10,11], as well as for the Golgi and endosome tubulation events following treatment with the fungal metabolite brefeldin A (BFA) [12].

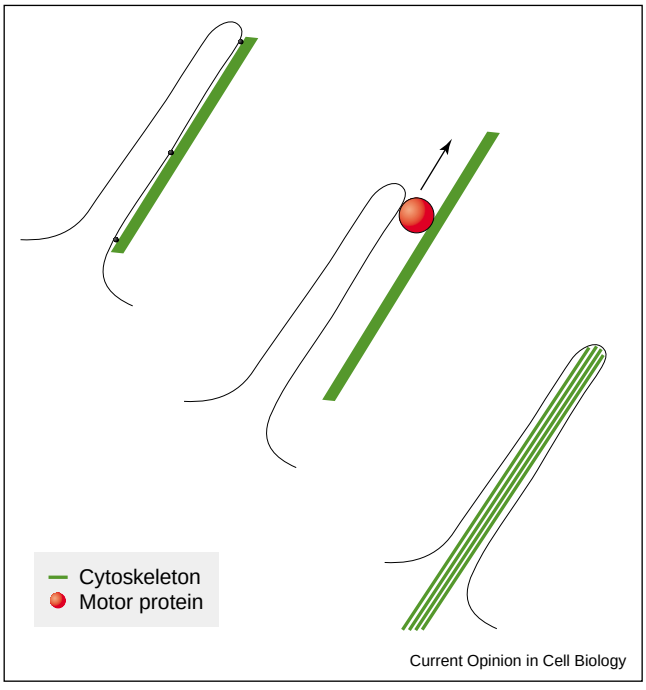
Other cytoskeletal elements, such as actin filaments and membrane-tethered myosin motors, may similarly participate in membrane deformation [13–15,16•,17]. One obvious example of actin-dependent membrane deformation is the formation of cell-surface tubular microvilli, formed by the extension of actin filaments against the plasma membrane. Thus, the cytoskeleton might affect membrane traffic by both structural and dynamic forces acting on the membrane.

## Intrinsic forces on the membrane

### Protein-mediated effects

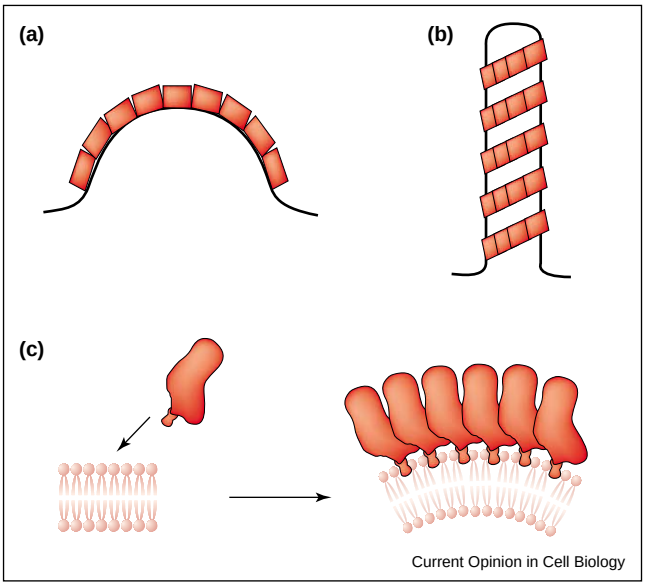
Over the past few years, emerging data have implicated cytosolic proteins in bilayer deformation upon recruitment to the membrane. Oligomerisation of these proteins into a coat scaffold on the membrane has traditionally been thought to promote budding by imposing curvature on the membrane [18–20] (Figure 2a). This view, first developed for the clathrin coat, was then extended to other protein coats observed on vesicles, such as COPI

**Figure 1**




Cytoskeletal mechanisms for membrane deformation. Cytoskeletal elements may have multiple roles in membrane deformation. Above left: cytoskeleton-dependent formation and maintenance of tubular organelle structures; middle: formation of membrane tubules pulled by a cytoskeletal motor protein; below right: external cytoskeletal forces abutting the membrane and causing deformation.

**Figure 2**




Two modes of protein-mediated membrane deformation. **(a,b)** Proteins that polymerise with an intrinsic curvature (forming either spheres or spirals) could potentially drive bilayer curvature and influence membrane shape. **(c)** Membrane deformation according to the bilayer-couple hypothesis. By penetration of an amphipathic helix into the interfacial section of the bilayer, proteins could drive membrane deformation owing to bilayer surface-area discrepancy.

[20] and COPII [21], and has since been supported by data revealing an intrinsic curvature in the structure of coat-protein scaffolds [22,23•]. Importantly, the observation that coat assembly, bilayer invagination, and, in some cases, even fission could occur on protein-free liposomes demonstrated that no integral membrane proteins were required for this process [24–29].

As with the cytosolic proteins which form the coat on a developing bud, cytosolic proteins have also been found to drive bilayer tubulation following recruitment to the membrane (Figure 2b). The first evidence that cytosolic proteins play a physiological role in the generation of membrane tubules came from studies of dynamin, a GTPase critically implicated in the fission reaction of clathrin-coated vesicles and other membrane trafficking events [30–32]. Purified dynamin has the property to self-assemble into rings and spirals, both in solution and at the narrow tubular stalks of endocytic vesicles [33,34]. Both *in vitro* and *in vivo*, dynamin can deform lipid bilayers into narrow tubules coated by dynamin spirals [25,35,36]. A predominant theory emerged: coat proteins were involved in budding from the donor membrane, and dynamin rings were involved in forming the tubular neck of the clathrin-coated bud [34]. Upon GTP hydrolysis, constriction of the dynamin ring would mediate fission [31,36,37•] (but see [38,39] for alternative interpretations).

The identification and characterisation of proteins associated with clathrin and dynamin, in conjunction with recent theoretical considerations of membrane biophysics, have since expanded and revised this view. With respect to clathrin, for example, to drive membrane curvature effectively, the rigidity of the coat protein polymer has to supercede the resistance of mechanically bending the membrane, described as the membrane-bending elastic modulus [40•]. This notion has recently been challenged for clathrin, because of estimations that the rigidity of clathrin triskelia is similar to the membrane-bending elastic modulus [40•]. If true, clathrin could at best serve to maintain an already curved membrane, thereby preventing its collapse back into an effectively planar form [40•]. Thus, mechanisms in addition to coat-protein lattice formation that may help in deforming the bilayer are likely to come into play.

With respect to dynamin and tubulation, the identification of other endocytic proteins that bind and tubulate lipid bilayers has expanded the repertoire of proteins involved in this process. Amphiphysin and endophilin, two major interactors of dynamin, were found to robustly deform liposomes into narrow membrane tubules [41,42•]. Epsin, an interactor of clathrin and of the clathrin

adaptor AP-2 [43–45], was also shown to induce membrane tubulation [46•]. There is currently no evidence that these proteins self-assemble into rings, like dynamin, in the absence of a lipid bilayer. The property of these proteins to deform membranes is likely to reflect a unique interaction with the membrane bilayer (see below), because not all proteins that bind lipid bilayers are able to induce deformation.

There is evidence for a role for these endocytic proteins in the early stages of clathrin-mediated budding, before the generation of a tubular neck, suggesting that the physiological role for these proteins might not be restricted to the formation of tubular membranes. For example, dynamin can be found on the dome of clathrin-coated buds, and antibody disruption of dynamin function at the lamprey synapse leads to markedly impaired clathrin-coated bud formation [47]. Furthermore, impairment of endophilin function at the lamprey synapse by antibody injection or by genetic disruption (in *Drosophila*) results in synaptic vesicle depletion and the accumulation of shallow clathrin-coated pits [48,49,50•,51•].

Amphiphysin also binds clathrin and AP-2 in addition to dynamin [52,53], and clathrin-coated buds generated in the presence of amphiphysin have a more homogenous, smaller size than buds generated in the presence of clathrin-coat fractions alone (K Farsad *et al.*, unpublished data). Finally, epsin is able to recruit clathrin onto a lipid monolayer and induces 'puckered' clathrin-coated structures [46•]. Thus, via their ability to deform planar membranes, these proteins might assist clathrin in early stages of bud formation.

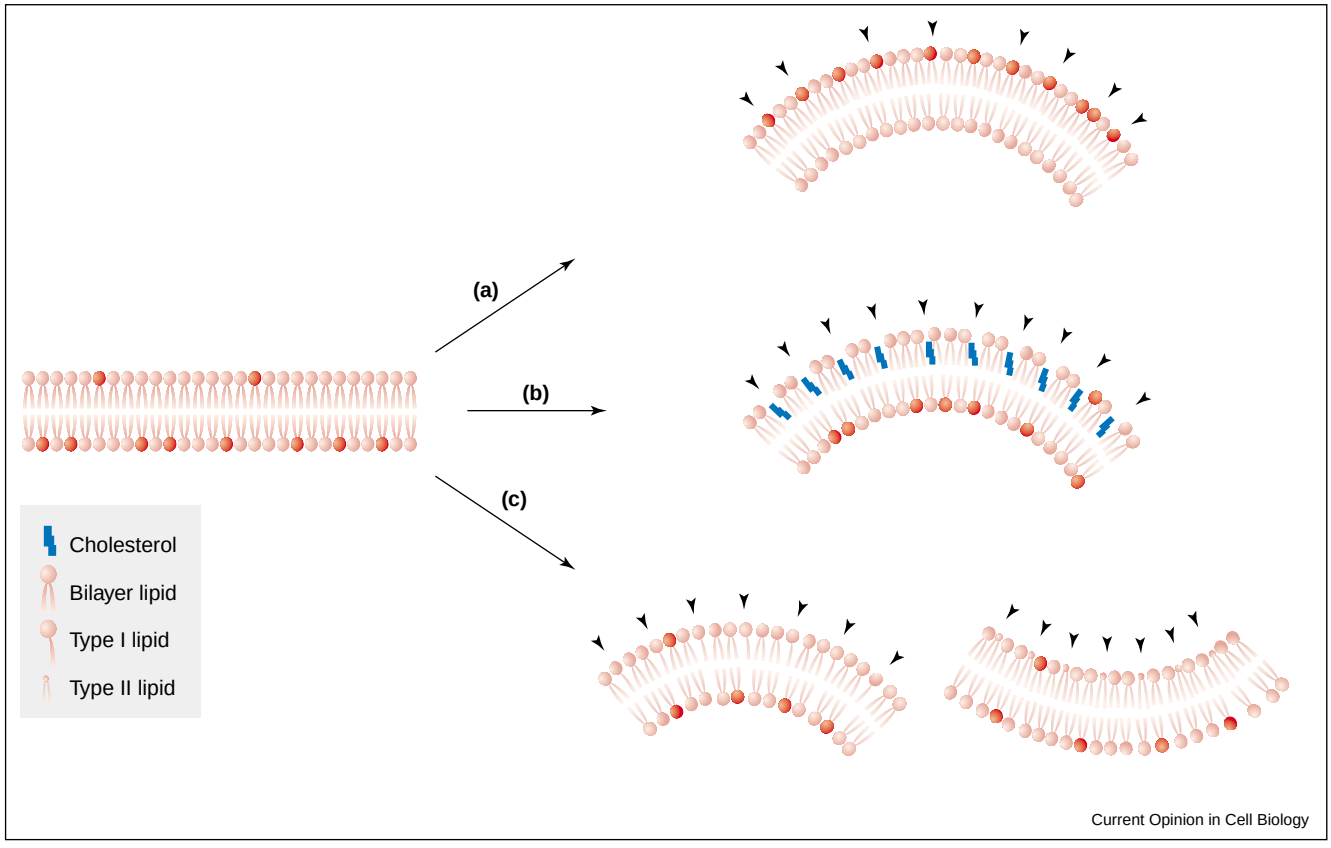
Although the above considerations apply to clathrin coats, it is still unknown whether similar mechanisms (i.e. cooperation of scaffold proteins and membrane-deforming proteins) might also function in coats comprising the COP proteins. In addition, caveolin polymerisation and membrane interactions are thought to play a role in caveolar budding [54–57]. As a final note, one possibility is that various properties of integral membrane proteins might also contribute to membrane deformation. For example, this has been proposed for peripherin, a transmembrane protein concentrated at areas of high curvature in the outer segment discs of retinal photoreceptors, and which induces flattened microsomal vesicles when expressed *in vitro* [58–60].

### Lipid-mediated effects

The role of lipid-specific dynamics in enabling or generating membrane curvature has also been an area of provocative research [61–63]. For example, selective transfer of lipids between bilayer leaflets has been proposed as a means by which surface area asymmetries could influence budding and endocytosis (see below and Figure 3a) [62,64]. In addition, certain lipid species are postulated to favour bilayer curvature owing to their physico-chemical properties, their relative geometries, or both [65,66]. Cholesterol, for example, is required for the generation of high-curvature clathrin-coated buds *in vivo* — cholesterol-depleting compounds prevent maturation of a bud past a shallow level of curvature [67,68]. One possible function of cholesterol is selectively to intercalate into the budding-side leaflet of the bilayer to enable bud formation without producing unfavourable hydrophobic–hydrophilic interactions as the bilayer is distorted (Figure 3b). Two proteins enriched in endocytic vesicle carriers, synaptophysin and caveolin, bind cholesterol [69,70]. These proteins could function to concentrate this lipid selectively in the budding portion of the bilayer, thus allowing more favourable phase interactions as the nascent bud forms [69,70]. In this way, the influence of cholesterol on membrane structure would encompass two non-exclusive possibilities. First, selective enrichment of cholesterol into one leaflet of the membrane might alter the relative bilayer surface areas to favour budding. Second, through differential partitioning, cholesterol might minimise the energy needed for budding by both decreasing local membrane stiffness and by preserving hydrophobic and van der Waals forces between the leaflets as the bilayer deforms [71,72,73•].

In addition, enzymatic alteration of lipids has been suggested to facilitate membrane deformation by generating particular lipid geometries (Figure 3c). Formation of type I lipids (i.e. those that tend to form micelles in aqueous solutions based on the geometry of their head groups and hydrocarbon tails), such as lysophosphatidylcholine, could favour positive curvature by adopting a wedge-like geometry, and formation of type II lipids (i.e. those which tend to form inverted hexagonal structures in aqueous solutions based on the geometry of their head groups and hydrocarbon tails), such as phosphatidylethanolamine, might favour negative curvature by effectively creating the reverse geometry [65,66]. For example, sphingomyelinase, an enzyme that cleaves phosphorylcholine from sphingomyelin to generate the type II lipid ceramide promotes the formation of bilayer invaginations independently from a protein-mediated effect [74]. Furthermore, phospholipase A2 activity was shown to be required for the 60–80 nm Golgi tubules formed upon treatment with BFA, as well as for the formation of tubular endosomal recycling organelles [75,76•]. Although the effect of phospholipase A2 on membrane deformation is not well understood mechanistically, one suggestion is that formation of type I lysolipids in the membrane might have a role. Putative roles for phospholipase C (PLC) and phospholipase D in influencing membrane structure through lipid modification have also been described [77–79]. Of note, lipid metabolism could alter bilayer structure not only directly, by affecting lipid geometries, but also indirectly, via the regulated recruitment of membrane-deforming proteins [46•,80].

**Figure 3**




Lipid-driven membrane deformation. **(a)** Transfer of lipids to one leaflet could promote deformation by creating surface-area discrepancy between the leaflets. **(b)** Selective accumulation of cholesterol could decrease membrane rigidity, create bilayer surface-area discrepancy, and facilitate budding. **(c)** Type I and type II lipids, based on their relative geometries, have been postulated to play a role in membrane deformation. Black arrowheads indicate the transferred lipids in (a), cholesterol molecules in (b), and the various type I or type II lipid species in (c).

Endophilin has been reported to have lysophosphatidic acid acyltransferase (LPAAT) activity, mediating the transfer of a fatty acyl-CoA to lysophosphatidic acid, a type I lipid [81,82]. Similarly, BARS (brefeldin A ADP-ribosylated substrate), a protein involved in Golgi tubulation and fission, was also reported to have LPAAT activity [83]. It remains unclear what role LPAAT activity plays in induction of membrane curvature, especially since endophilin has been shown directly to deform lipid bilayers independently from this enzymatic activity [42•]. However, although the importance of the LPAAT activity of these proteins is currently undefined, the ability of these proteins to bind certain lipids might prove important for their biological function.

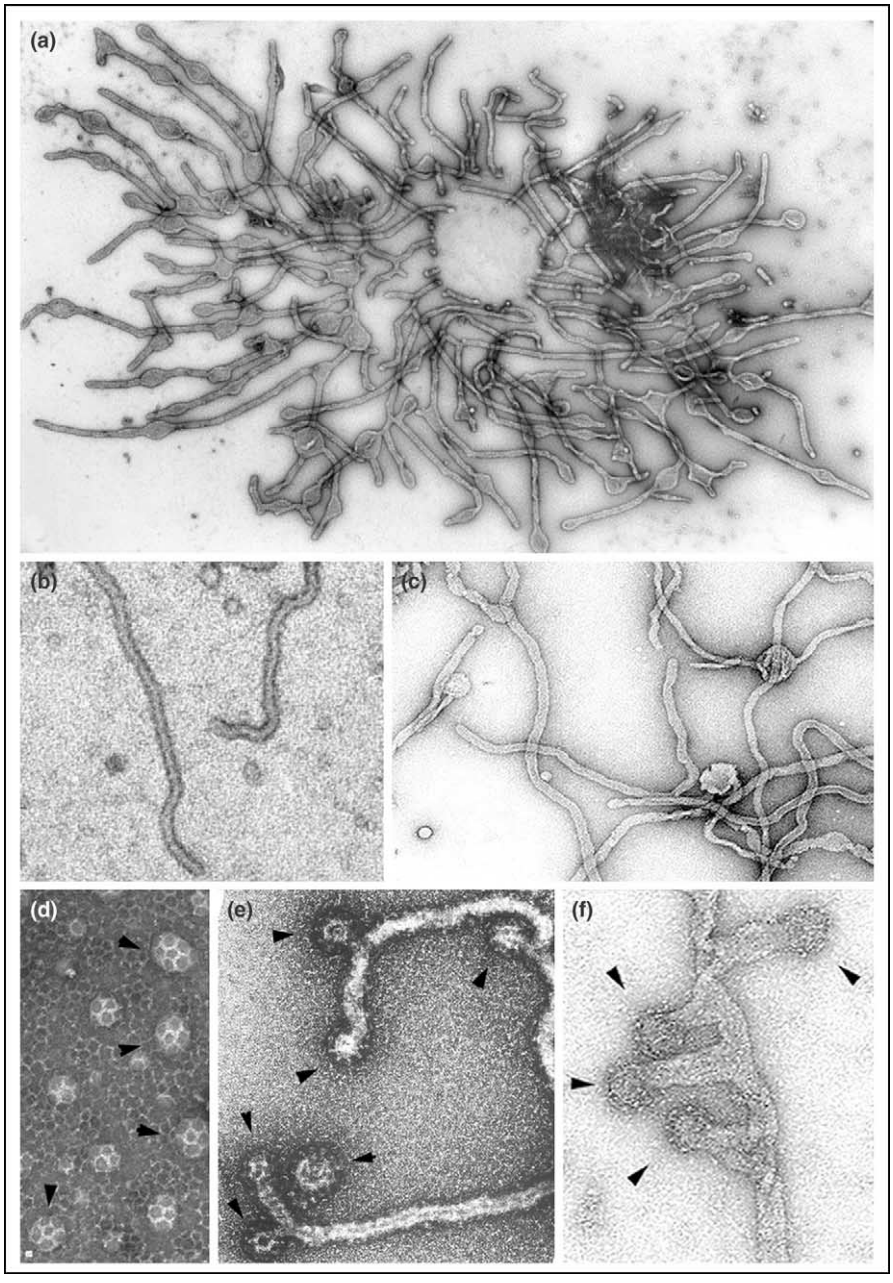
## Amphipathic peptides and the bilayer-couple hypothesis

The bilayer-couple hypothesis, initially popularised by Sheetz and Singer in 1974, postulates that the two halves of a closed lipid bilayer, by virtue of asymmetries between the bilayer leaflets, could have differential responses to various perturbations [84,85]. Thus, a relative increase in surface area of one leaflet of a closed bilayer, as discussed above, is predicted to increase the spontaneous curvature of the bilayer. To minimise its energy state and maintain hydrophobic and van der Waals interactions between the leaflets, an unopposed bilayer will conform to its spontaneous curvature [86]. Specifically, the leaflet to which additional surface area is added will be the side to which the bilayer will deform in compensation. Sheetz and Singer observed that compounds with amphipathic qualities, presumably by intercalating into a particular leaflet of the membrane bilayer, were able to deform erythrocyte membranes according to the predictions of the bilayer-couple theory [84,85].

The bilayer-couple theory could explain the mechanism through which certain proteins affect morphological changes in planar membranes. By physically penetrating into one face of the bilayer, amphipathic peptides could cause membrane deformation (Figure 2c). The amino termini of the tubulogenic proteins endophilin and

amphiphysin contain an amino acid stretch predicted to form an amphipathic helix necessary for lipid bilayer tubulation [42•]. The epsin amino-terminal homology (ENTH) domain of epsin also forms an amphipathic helix on binding phosphatidylinositol 4,5-bisphosphate (PI[4,5]P2), and this induced helix is necessary for bilayer tubulation [46•]. In addition, the ARF-family GTPases, involved in recruitment of coat proteins for vesicular trafficking along the secretory and endocytic pathways, have an amino-terminal amphipathic helix critical for membrane binding [87–90,91•], which potentially could play a role in budding. An intriguing possibility is that all

**Figure 4**




Tubulation and clathrin-mediated budding. Tubular bilayer deformation caused by incubation of **(a)** amphiphysin with liposomes, **(b)** epsin with liposomes, and **(c)** endophilin with liposomes. **(d)** On lipid monolayers doped with PI(4,5)P2, epsin stimulates clathrin recruitment and the formation of monolayer 'puckers', as demonstrated by clathrin pentagonal and hexagonal polymeric structures. **(e)** Clathrin-coated buds are seen associated with amphiphysin tubules. **(f)** When incubated with purified clathrin-coat proteins, endophilin-mediated membrane tubules are often capped with clathrin-coated buds, despite no biochemical binding between endophilin and clathrin-coat proteins. The image shown in (a) is courtesy of Kohji Takei; (b) and (d) are reproduced with permission from *Nature* (http://www.nature.com/) [46•]; (e) is reproduced from the *Nature Cell Biology* [41] by copyright permission of the Rockefeller Press; and (c ) and (f) are reproduced, with permission, from *The Journal of Cell Biology* [42•].

of these proteins share a common mechanism for enabling membrane deformation through the interactions of an amphipathic peptide with the lipid bilayer. Identifying the mechanisms for how these protein–bilayer interactions are regulated will be paramount to understanding the dynamics of this process.

Recent biophysical studies using the prototypical amphipathic helical peptide melittin have shown that an amphipathic helix oriented parallel to a lipid bilayer surface would be ideally poised to reside at an interfacial location between the head groups and the hydrophobic core [92•,93]. The steep gradient of polarity between the head groups and the hydrocarbon tails within a lipid monolayer is estimated to be on the order of the diameter of an α helix, rendering an amphipathic helix an appropriate structural solution to a protein binding the monolayer in this fashion [94]. Thus, perhaps many of these proteins generating membrane deformation function by partially inserting an amphipathic helix into the bilayer in such a way as to create changes in membrane structure. A striking illustration of the power of an amphipathic peptide in affecting such a process was shown in the ability of a designed amphipathic 18-mer peptide to form extensive tubules 40–50 nm in diameter and up to 600 nm in length from liposomes comprising various lipid combinations [95•].

Penetration into the lipid bilayer seems necessary for this process, since proteins which bind superficially to the bilayer without penetration, such as the pleckstrin homology domain of PLCδ or the ANTH domain of AP180 (similar to the epsin ENTH domain, but without the ligand-induced amphipathic helix), do not cause deformation [42•,46•,96,97]. Another likely part of this process is clustering of these proteins such that sufficient concentrations are achieved to enable a significant membrane-deforming effect. Proteins such as endophilin and amphiphysin might cluster by polymerisation, whereas proteins such as epsin might cluster owing to the presence of localised interacting proteins. *In vitro*, and with overexpression, clustering might be a product of high protein concentrations, whereas the situation *in vivo* is probably more subtle and regulated. It remains to be seen whether different mechanisms for clustering have different effects on membrane structure.

Studies with melittin have shown that the monomeric amphipathic helix had only modest effects on bilayer structure at lower concentrations, causing only slight increases in area per lipid. By contrast, melittin monomers cysteine-linked to create dimers affected a significant change in bilayer structure and perturbation at the same monomer/lipid concentration used for monomeric melittin [92•]. Thus, the self-association of an amphipathic helix is thought to have a qualitatively different effect, compared with non-associating monomers, on the structural perturbation of a lipid bilayer [92•]. As such, it is possible that biological membrane dynamics use the effects of both monomeric and polymeric proteins to create variations on bilayer perturbation and deformation. The ability of these proteins to concentrate in the membrane, either alone or in various combinations, might affect the degree to which membrane structure is perturbed. Indeed, at high concentrations, both monomeric and dimeric melittin significantly perturb bilayer structure, and are ultimately membrane-lytic [92•].

Membrane-deforming proteins probably work in conjunction with clathrin-coat proteins to promote budding. *In vitro* data with epsin, amphiphysin and endophilin illustrate this point (Figure 4). As mentioned above, co-incubation of epsin with clathrin induced puckered clathrin polymers on a lipid monolayer [46•] (Figure 4d). Clathrin-coat proteins incubated with amphiphysin resulted in coated buds associated with amphiphysin tubules [41] (Figure 4e). Both epsin and amphiphysin interact biochemically with clathrin, and as such, it makes sense that these proteins could couple clathrin bud formation with other membrane-deforming activities. Interestingly, when incubated with coat proteins, many endophilin-generated tubules were also capped by clathrin-coated buds, despite the fact that endophilin has no known binding properties to clathrin-coat proteins [42•] (Figure 4f). This suggests that endophilin tubules might serve as structural, rather than biochemical, substrates for clathrin coats under conditions that promote budding [42•]. Thus, proteins that alone are able to drive membrane curvature might facilitate clathrin-mediated bud formation by altering bilayer structure to favour this process.

## Conclusions and future directions

Our understanding of the mechanisms generating membrane deformation will no doubt increase our awareness of how this process affects various aspects of cell biology. Roles for membrane budding and tubulation have been described in both immunity and disease. For example, 'reverse' budding — budding away from the cytosol — is a mechanism for the formation of the multivesicular bodies (MVBs) in the late endosomal pathway [98–100]. Recent work in this field has demonstrated a role for three protein complexes, ESCRT (endosomal sorting complex required for transport) I, II and III [101•–103•], in addition to the monoubiquitin pathway [104,105] and phosphoinositide metabolism [106,107], in the generation of the lumenal vesicles of the MVBs. Budding into the MVBs is an efficient way to target membrane proteins/receptors to lysosomes for degradation [100]. In addition, the lumenal vesicles of MVBs are also involved in the immune response [100,108–111]. Loading of the major histocompatibility complex (MHC) class II molecules with antigen involves MVB lumenal vesicles [100]. MVB lumenal vesicles are also secreted as immunomudulatory organelles termed exosomes. Exosomes contain MHC class

II molecules and T cell co-stimulatory factors, and as such are potent immune stimulators and potential anti-tumour agents [100,108–111]. Furthermore, in enveloped viruses such as HIV-1, viral budding from the plasma membrane has apparently usurped the MVB machinery in an analogous process of budding away from the cytosol into the extracellular space [100,104,112,113]. How this budding process occurs is likely to represent a new mechanism in membrane deformation.

The parasite *Toxoplasma gondii* represents a new example of the role for tubulating proteins in disease. Once the parasite enters the cell into the parasitic vacuole, parasite secreted proteins play a role in the generation of a 60–90 nm tubular network emanating into the vacuole from the vacuolar membrane [114•]. The parasite secretes a protein, Gra2, which is involved in the formation of these tubules, and impaired tubule formation results in diminished parasite virulence [114•]. Gra2 contains two amphipathic α-helical regions that are critical for tubulation [114•]. Thus, *T. gondii* uses a secreted tubulogenic protein, with requisite amphipathic helices, in its infectious biology.

Membrane-deforming proteins involved in diverse cellular processes other than intracellular membrane traffic have also been described. A member of the amphiphysin protein family has been localised to the muscle T-tubule system, where its membrane-deforming properties are likely to play a role in the biology of these structures [115•]. Furthermore, dynamin and endophilin isoforms have been localised to the tubular plasma membrane invaginations often observed at podosomes, dynamic actin–membrane structures involved in motility and adhesion [116].

The number of factors known to be involved in generating membrane curvature has increased and has underscored our appreciation of the complexity of the process. Many issues remain to be resolved, and it is likely that the process is driven by a cooperation of both proteins and lipids. A major contribution to bilayer deformation is from the reversible recruitment of cytosolic proteins, which have the advantage of being recycled, to the membrane. Ultimately, more membrane-deforming factors will be identified, and we will probably find that nature has created more than one solution to the same problem.

## References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest


1. Dabora SL, Sheetz MP: **The microtubule-dependent formation of a tubulovesicular network with characteristics of the ER from cultured cell extracts**. *Cell* 1988, **54**:27-35.

2. Vale RD, Hotani H: **Formation of membrane networks *in vitro* by kinesin-driven microtubule movement**. *J Cell Biol* 1988, **107**:2233-2241.

3. Lippincott-Schwartz J, Roberts TH, Hirschberg K: **Secretory protein trafficking and organelle dynamics in living cells**. *Annu Rev Cell Dev Biol* 2000, **16**:557-589.

4. • Roux A, Cappello G, Cartaud J, Prost J, Goud B, Bassereau P: **A minimal system allowing tubulation with molecular motors pulling on giant liposomes**. *Proc Natl Acad Sci USA* 2002, **99**:5394-5399.
The authors have recreated a tubular network using only kinesin attached to giant unilammelar liposomes via polysterene beads, microtubules and ATP. This minimal machinery was sufficient to generate membrane tubules using both synthetic liposomes and Golgi-derived membranes.

5. Robertson AM, Allan VJ: **Brefeldin A-dependent membrane tubule formation reconstituted *in vitro* is driven by a cell cycle-regulated microtubule motor**. *Mol Biol Cell* 2000, **11**:941-955.

6. Allan VJ, Schroer TA: **Membrane motors**. *Curr Opin Cell Biol* 1999, **11**:476-482.

7. Hirokawa N: **Kinesin and dynein superfamily proteins and the mechanism of organelle transport**. *Science* 1998, **279**:519-526.

8. Tooze J, Hollinshead M: **In AtT20 and HeLa cells brefeldin A induces the fusion of tubular endosomes and changes their distribution and some of their endocytic properties**. *J Cell Biol* 1992, **118**:813-830.

9. Dreier L, Rapoport TA: ***In vitro* formation of the endoplasmic reticulum occurs independently of microtubules by a controlled fusion reaction**. *J Cell Biol* 2000, **148**:883-898.

10. Klopfenstein DR, Kappeler F, Hauri HP: **A novel direct interaction of endoplasmic reticulum with microtubules**. *EMBO J* 1998, **17**:6168-6177.

11. Waterman-Storer CM, Salmon ED: **Endoplasmic reticulum membrane tubules are distributed by microtubules in living cells using three distinct mechanisms**. *Curr Biol* 1998, **8**:798-806.

12. Lippincott-Schwartz J, Yuan L, Tipper C, Amherdt M, Orci L, Klausner RD: **Brefeldin A's effects on endosomes, lysosomes, and the TGN suggest a general mechanism for regulating organelle structure and membrane traffic**. *Cell* 1991, **67**:601-616.

13. Schafer DA: **Coupling actin dynamics and membrane dynamics during endocytosis**. *Curr Opin Cell Biol* 2002, **14**:76-81.

14. Hasson T, Mooseker MS: **Molecular motors, membrane movements and physiology: emerging roles for myosins**. *Curr Opin Cell Biol* 1995, **7**:587-594.

15. Geli MI, Riezman H: **Role of type I myosins in receptor-mediated endocytosis in yeast**. *Science* 1996, **272**:533-535.

16. • Buss F, Arden SD, Lindsay M, Luzio JP, Kendrick-Jones J: **Myosin VI isoform localized to clathrin-coated vesicles with a role in clathrin-mediated endocytosis**. *EMBO J* 2001, **20**:3676-3684.
An isoform of myosin VI, one of only two known minus-end-directed actin motors, is found to co-localise to clathrin-coated pits/vesicles in cells. Myosin VI is found in a complex with clathrin and AP2 and is enriched in purified clathrin-coated vesicles. Binding to clathrin-coat proteins is mediated by the myosin VI carboxy-terminal tail, and overexpression of this domain inhibits transferrin uptake in cultured fibroblasts. This is the first time an actin motor has been shown to be directly associated with clathrin-mediated endocytic dynamics.

17. Morris SM, Arden SD, Roberts RC, Kendrick-Jones J, Cooper JA, Luzio JP, Buss F: **Myosin VI binds to and localises with Dab2, potentially linking receptor-mediated endocytosis and the actin cytoskeleton**. *Traffic* 2002, **3**:331-341.

18. Kadota K, Kadota T: **Isolation of coated vesicles, plain synaptic vesicles, and flocculent material from a crude synaptosome fraction of guinea pig whole brain**. *J Cell Biol* 1973, **58**:135-151.

19. Heuser JE, Keen J: **Deep-etch visualization of proteins involved in clathrin assembly**. *J Cell Biol* 1988, **107**:877-886.

20. Rothman JE: **Mechanisms of intracellular protein transport**. *Nature* 1994, **372**:55-63.

21. Kuehn MJ, Schekman R: **COPII and secretory cargo capture into transport vesicles**. *Curr Opin Cell Biol* 1997, **9**:477-483.

22. Musacchio A, Smith CJ, Roseman AM, Harrison SC, Kirchhausen T, Pearse BM: **Functional organization of clathrin in coats: combining electron cryomicroscopy and X-ray crystallography**. *Mol Cell* 1999, **3**:761-770.

23. • Bi X, Corpina RA, Goldberg J: **Structure of the Sec23/24-Sar1 pre-budding complex of the COPII vesicle coat**. *Nature* 2002, **419**:271-277.
The Sec23–Sec24–Sar-1 pre-budding complex of COPII coats was crystallised and demonstrated to show a concave, bow-tie conformation with positively charged residues poised to interact with acidic phospholipids of the membrane bilayer. The structure of the complex, with its intrinsic curvature, is suggestive of a role for this complex in generating or stabilising membrane deformation.

24. Matsuoka K, Orci L, Amherdt M, Bednarek SY, Hamamoto S, Schekman R, Yeung T: **COPII-coated vesicle formation reconstituted with purified coat proteins and chemically defined liposomes**. *Cell* 1998, **93**:263-275.

25. Takei K, Haucke V, Slepnev V, Farsad K, Salazar M, Chen H, De Camilli P: **Generation of coated intermediates of clathrin-mediated endocytosis on protein-free liposomes**. *Cell* 1998, **94**:131-141.

26. Spang A, Matsuoka K, Hamamoto S, Schekman R, Orci L: **Coatomer, Arf1p, and nucleotide are required to bud coat protein complex I-coated vesicles from large synthetic liposomes**. *Proc Natl Acad Sci USA* 1998, **95**:11199-11204.

27. Bremser M, Nickel W, Schweikert M, Ravazzola M, Amherdt M, Hughes CA, Sollner TH, Rothman JE, Wieland FT: **Coupling of coat assembly and vesicle budding to packaging of putative cargo receptors**. *Cell* 1999, **96**:495-506.

28. Zhu Y, Drake MT, Kornfeld S: **ADP-ribosylation factor 1 dependent clathrin-coat assembly on synthetic liposomes**. *Proc Natl Acad Sci USA* 1999, **96**:5013-5018.

29. Drake MT, Zhu Y, Kornfeld S: **The assembly of AP-3 adaptor complex-containing clathrin-coated vesicles on synthetic liposomes**. *Mol Biol Cell* 2000, **11**:3723-3736.

30. Damke H, Baba T, Warnock DE, Schmid SL: **Induction of mutant dynamin specifically blocks endocytic coated vesicle formation**. *J Cell Biol* 1994, **127**:915-934.

31. Hinshaw JE: **Dynamin and its role in membrane fission**. *Annu Rev Cell Dev Biol* 2000, **16**:483-519.

32. McNiven MA, Cao H, Pitts KR, Yoon Y: **The dynamin family of mechanoenzymes: pinching in new places**. *Trends Biochem Sci* 2000, **25**:115-120.

33. Hinshaw JE, Schmid SL: **Dynamin self-assembles into rings suggesting a mechanism for coated vesicle budding**. *Nature* 1995, **374**:190-192.

34. Takei K, McPherson PS, Schmid SL, De Camilli P: **Tubular membrane invaginations coated by dynamin rings are induced by GTP-gamma S in nerve terminals**. *Nature* 1995, **374**:186-190.

35. Sweitzer SM, Hinshaw JE: **Dynamin undergoes a GTP-dependent conformational change causing vesiculation**. *Cell* 1998, **93**:1021-1029.

36. Marks B, Stowell MH, Vallis Y, Mills IG, Gibson A, Hopkins CR, McMahon HT: **GTPase activity of dynamin and resulting conformation change are essential for endocytosis**. *Nature* 2001, **410**:231-235.

37. • Zhang P, Hinshaw JE: **Three-dimensional reconstruction of dynamin in the constricted state**. *Nat Cell Biol* 2001, **3**:922-926.
A three-dimensional structure of dynamin minus the carboxy-terminal proline-rich domain is resolved to 20 Å from cryoelectron micrographs of polymerised tubular dynamin in the constricted state. The study determines the relative positions of the major dynamin domains in relation to the lipid bilayer, and suggests a conformation suited for a constrictase able to impart force on the underlying lipid bilayer.

38. Sever S, Muhlberg AB, Schmid SL: **Impairment of dynamin's GAP domain stimulates receptor-mediated endocytosis**. *Nature* 1999, **398**:481-486.

39. Stowell MH, Marks B, Wigge P, McMahon HT: **Nucleotide-dependent conformational changes in dynamin: evidence for a mechanochemical molecular spring**. *Nat Cell Biol* 1999, **1**:27-32.

40. • Nossal R: **Energetics of clathrin basket assembly**. *Traffic* 2001, **2**:138-147.
Using a thermodynamic model with parameters accounting for clathrin triskelion rigidity, intrinsic triskelion curvature, and changes in free energy upon triskelia interactions, the author provides a formula that factors these elements in the determination of clathrin cage size distributions. The paper suggests that triskelion rigidity *per se* is not sufficient to overcome the bending resistance of the plasma membrane.

41. Takei K, Slepnev VI, Haucke V, De Camilli P: **Functional partnership between amphiphysin and dynamin in clathrin-mediated endocytosis**. *Nat Cell Biol* 1999, **1**:33-39.

42. • Farsad K, Ringstad N, Takei K, Floyd SR, Rose K, De Camilli P: **Generation of high curvature membranes mediated by direct endophilin bilayer interactions**. *J Cell Biol* 2001, **155**:193-200.
Using an *in vitro* assay with purified proteins and liposomes, a direct bilayer tubulating activity for endophilin was found which was independent of a reported lysophosphatidic acid acyltransferase activity. When co-incubated with clathrin coat proteins, endophilin-mediated membrane tubules were often capped with a clathrin-coated bud, demonstrating a favourable association between these two structures, despite the lack of physical binding between endophilin and the clathrin coat.

43. Chen H, Fre S, Slepnev VI, Capua MR, Takei K, Butler MH, Di Fiore PP, De Camilli P: **Epsin is an EH-domain-binding protein implicated in clathrin-mediated endocytosis**. *Nature* 1998, **394**:793-797.

44. Rosenthal JA, Chen H, Slepnev VI, Pellegrini L, Salcini AE, Di Fiore PP, De Camilli P: **The epsins define a family of proteins that interact with components of the clathrin coat and contain a new protein module**. *J Biol Chem* 1999, **274**:33959-33965.

45. Traub LM, Downs MA, Westrich JL, Fremont DH: **Crystal structure of the alpha appendage of AP-2 reveals a recruitment platform for clathrin-coat assembly**. *Proc Natl Acad Sci USA* 1999, **96**:8907-8912.

46. • Ford MG, Mills IG, Peter BJ, Vallis Y, Praefcke GJ, Evans PR, McMahon HT: **Curvature of clathrin-coated pits driven by epsin**. *Nature* 2002, **419**:361-366.
The crystal structure of epsin's ENTH domain is resolved in the presence of its ligand, and an amphipathic helix is induced which is not present in the absence of ligand. Hydrophobic residues in the amphipathic helix are critical for tubular membrane deformation. The authors conclude that membrane insertion by the amphipathic helix *per se* is sufficient for epsin to facilitate clathrin-coated budding events on lipid monolayers.

47. Gad H, Bloom O, Löw P, De Camilli P, Slepnev V, Shupliakov O, Brodin L: **Dynamin plays a role in early stages of clathrin-mediated synaptic vesicle endocytosis**. *Mol Biol Cell* 2000, **11(Suppl)**:218a.

48. Ringstad N, Gad H, Low P, Di Paolo G, Brodin L, Shupliakov O, De Camilli P: **Endophilin/SH3p4 is required for the transition from early to late stages in clathrin-mediated synaptic vesicle endocytosis**. *Neuron* 1999, **24**:143-154.

49. Gad H, Ringstad N, Low P, Kjaerulff O, Gustafsson J, Wenk M, Di Paolo G, Nemoto Y, Crun J, Ellisman MH *et al.*: **Fission and uncoating of synaptic clathrin-coated vesicles are perturbed by disruption of interactions with the SH3 domain of endophilin**. *Neuron* 2000, **27**:301-312.

50. • Guichet A, Wucherpfennig T, Dudu V, Etter S, Wilsch-Brauniger M, Hellwig A, Gonzalez-Gaitan M, Huttner WB, Schmidt AA: **Essential role of endophilin A in synaptic vesicle budding at the *Drosophila* neuromuscular junction**. *EMBO J* 2002, **21**:1661-1672.
P-element disruption of the *endophilin* gene in *Drosophila* results in death at the third instar larval stage of development. There is a marked affect on the morphology of synapses, with a substantial depletion of synaptic vesicles and, in occasional synapses, an arrest of clathrin-mediated synaptic vesicle recycling at shallow stages of bud formation. These results support the data seen earlier with acute disruption of endophilin function in a living synaptic preparation (see [47,48]), and suggest a critical role for endophilin in synaptic vesicle recycling, potentially through its role in membrane deformation.

51. • Verstreken P, Kjaerulff O, Lloyd TE, Atkinson R, Zhou Y, Meinertzhagen IA, Bellen HJ: **Endophilin mutations block clathrin-mediated endocytosis but not neurotransmitter release**. *Cell* 2002, **109**:101-112.

The authors show that endophilin *Drosophila* mutants are depleted in synaptic vesicles and have an expansion of the presynaptic membrane. The mutants have dramatically impaired endocytosis, but are able to sustain 15–20% of normal neurotransmission, suggesting a possible alternate pathway for vesicle recycling dynamics.

52. Slepnev VI, Ochoa GC, Butler MH, De Camilli P: **Tandem arrangement of the clathrin and AP-2 binding domains in amphiphysin 1, and disruption of clathrin coat function by amphiphysin fragments comprising these sites**. *J Biol Chem* 2000, **275**:17583-17589.

53. Brett TJ, Traub LM, Fremont DH: **Accessory protein recruitment motifs in clathrin-mediated endocytosis**. *Structure* 2002, **10**:797-809.

54. Rothberg KG, Heuser JE, Donzell WC, Ying YS, Glenney JR, Anderson RG: **Caveolin, a protein component of caveolae membrane coats**. *Cell* 1992, **68**:673-682.

55. Fra AM, Williamson E, Simons K, Parton RG: ***De novo* formation of caveolae in lymphocytes by expression of VIP21-caveolin**. *Proc Natl Acad Sci USA* 1995, **92**:8655-8659.

56. Drab M, Verkade P, Elger M, Kasper M, Lohn M, Lauterbach B, Menne J, Lindschau C, Mende F, Luft FC *et al.*: **Loss of caveolae, vascular dysfunction, and pulmonary defects in caveolin-1 gene-disrupted mice**. *Science* 2001, **293**:2449-2452.

57. Fernandez I, Ying Y, Albanesi J, Anderson RG: **Mechanism of caveolin filament assembly**. *Proc Natl Acad Sci USA* 2002, **99**:11193-11198.

58. Molday RS, Hicks D, Molday L: **Peripherin. A rim-specific membrane protein of rod outer segment discs**. *Invest Ophthalmol Vis Sci* 1987, **28**:50-61.

59. Arikawa K, Molday LL, Molday RS, Williams DS: **Localization of peripherin/RDS in the disk membranes of cone and rod photoreceptors: relationship to disk membrane morphogenesis and retinal degeneration**. *J Cell Biol* 1992, **116**:659-667.

60. Wrigley JD, Ahmed T, Nevett CL, Findlay JB: **Peripherin/RDS influences membrane vesicle morphology. Implications for retinopathies**. *J Biol Chem* 2000, **275**:13191-13194.

61. Evans EA: **Bending resistance and chemically induced moments in membrane bilayers**. *Biophys J* 1974, **14**:923-931.

62. Farge E, Devaux PF: **Shape changes of giant liposomes induced by an asymmetric transmembrane distribution of phospholipids**. *Biophys J* 1992, **61**:347-357.

63. Lipowsky R: **The conformation of membranes**. *Nature* 1991, **349**:475-481.

64. Farge E, Ojcius DM, Subtil A, Dautry-Varsat A: **Enhancement of endocytosis due to aminophospholipid transport across the plasma membrane of living cells**. *Am J Physiol* 1999, **276**:C725-733.

65. Chernomordik L: **Non-bilayer lipids and biological fusion intermediates**. *Chem Phys Lipids* 1996, **81**:203-213.

66. Burger KN: **Greasing membrane fusion and fission machineries**. *Traffic* 2000, **1**:605-613.

67. Subtil A, Gaidarov I, Kobylarz K, Lampson MA, Keen JH, McGraw TE: **Acute cholesterol depletion inhibits clathrin-coated pit budding**. *Proc Natl Acad Sci USA* 1999, **96**:6775-6780.

68. Rodal SK, Skretting G, Garred O, Vilhardt F, van Deurs B, Sandvig K: **Extraction of cholesterol with methyl-beta-cyclodextrin perturbs formation of clathrin-coated endocytic vesicles**. *Mol Biol Cell* 1999, **10**:961-974.

69. Thiele C, Hannah MJ, Fahrenholz F, Huttner WB: **Cholesterol binds to synaptophysin and is required for biogenesis of synaptic vesicles**. *Nat Cell Biol* 2000, **2**:42-49.

70. Murata M, Peranen J, Schreiner R, Wieland F, Kurzchalia TV, Simons K: **VIP21/caveolin is a cholesterol-binding protein**. *Proc Natl Acad Sci USA* 1995, **92**:10339-10343.

71. Brown MF, Thurmond RL, Dodd SW, Otten D, Beyer K: **Elastic deformation of membrane bilayers probed by deuterium NMR relaxation**. *J Am Chem Soc* 2002, **124**:8471-8484.

72. Brown MF, Thurmond RL, Dodd SW, Otten D, Beyer K: **Composite membrane deformation on the mesoscopic length scale**. *Phys Rev E Stat Nonlin Soft Matter Phys* 2001, **64**:010901.

73. • Baba T, Rauch C, Xue M, Terada N, Fujii Y, Ueda H, Takayama I, Ohno S, Farge E, Sato SB: **Clathrin-dependent and clathrin-independent endocytosis are differentially sensitive to insertion of poly(ethylene glycol)-derivatized cholesterol in the plasma membrane**. *Traffic* 2001, **2**:501-512.

Insertion of polyethylene glycol (PEG)-cholesterol into the outer leaflet of cultured cells progressively inhibited clathrin-dependent and clathrin-independent endocytosis, but at different concentrations. Concentrations of PEG-cholesterol corresponding to >10% membrane surface area inhibited pinocytosis and caveolar endocytosis, but double that concentration was required to inhibit clathrin-mediated endocytosis. The authors reason that the higher rigidity of clathrin triskelia relative to that of lipid bilayers causes this difference. This study highlights the effects of differential bilayer surface area asymmetry on the tendency to bud, suggesting that endocytosis is sensitive to some degree of relative leaflet surface area.

74. Holopainen JM, Angelova MI, Kinnunen PK: **Vectorial budding of vesicles by asymmetrical enzymatic formation of ceramide in giant liposomes**. *Biophys J* 2000, **78**:830-838.

75. de Figueiredo P, Drecktrah D, Katzenellenbogen JA, Strang M, Brown WJ: **Evidence that phospholipase A2 activity is required for Golgi complex and trans Golgi network membrane tubulation**. *Proc Natl Acad Sci USA* 1998, **95**:8642-8647.

76. • de Figueiredo P, Doody A, Polizotto RS, Drecktrah D, Wood S, Banta M, Strang MS, Brown WJ: **Inhibition of transferrin recycling and endosome tubulation by phospholipase A2 antagonists**. *J Biol Chem* 2001, **276**:47361-47370.

The authors show that, in addition to tubulation from the Golgi apparatus, phospholipase A2 activity plays a role in endosomal recycling events. Inhibitors of phospholipase A2 block endosomal recycling of transferrin receptor with accumulations in centrally located endosomes. These inhibitors also prevent *in vitro* endosome tubule formation, and the authors conclude that endosomal tubulation, like Golgi tubulation, is dependent on phospholipase A2 activity.

77. Ktistakis NT, Brown HA, Waters MG, Sternweis PC, Roth MG: **Evidence that phospholipase D mediates ADP ribosylation factor-dependent formation golgi coated vesicles**. *J Cell Biol* 1996, **134**:295-306.

78. Liscovitch M: **Phospholipase D: role in signal transduction and membrane traffic**. *J Lipid Mediat Cell Signal* 1996, **14**:215-221.

79. Basanez G, Ruiz-Arguello MB, Alonso A, Goni FM, Karlsson G, Edwards K: **Morphological changes induced by phospholipase C and by sphingomyelinase on large unilamellar vesicles: a cryo-transmission electron microscopy study of liposome fusion**. *Biophys J* 1997, **72**:2630-2637.

80. Burger KN, Demel RA, Schmid SL, de Kruijff B: **Dynamin is membrane-active: lipid insertion is induced by phosphoinositides and phosphatidic acid**. *Biochemistry* 2000, **39**:12485-12493.

81. Schmidt A, Wolde M, Thiele C, Fest W, Kratzin H, Podtelejnikov AV, Witke W, Huttner WB, Soling HD: **Endophilin I mediates synaptic vesicle formation by transfer of arachidonate to lysophosphatidic acid**. *Nature* 1999, **401**:133-141.

82. Modregger J, Schmidt AA, Ritter B, Huttner WB, Plomann M: **Characterization of endophilin B1b, a brain-specific membrane-associated lysophosphatidic acid acyltransferase with properties distinct from endophilin A1**. *J Biol Chem* 2003, **278**:4160-4167.

83. Weigert R, Silletta MG, Spano S, Turacchio G, Cericola C, Colanzi A, Senatore S, Mancini R, Polishchuk EV, Salmona M *et al.*: **CtBP/BARS induces fission of Golgi membranes by acylating lysophosphatidic acid**. *Nature* 1999, **402**:429-433.

84. Sheetz MP, Singer SJ: **Biological membranes as bilayer couples. A molecular mechanism of drug–erythrocyte interactions**. *Proc Natl Acad Sci USA* 1974, **71**:4457-4461.

85. Sheetz MP, Painter RG, Singer SJ: **Biological membranes as bilayer couples. III. Compensatory shape changes induced in membranes**. *J Cell Biol* 1976, **70**:193-203.

86. Nossal R, Zimmerberg J: **Endocytosis: curvature to the ENTH degree**. *Curr Biol* 2002, **12**:R770-R772.

87. Amor JC, Harrison DH, Kahn RA, Ringe D: **Structure of the human ADP-ribosylation factor 1 complexed with GDP**. *Nature* 1994, **372**:704-708.

88. Antonny B, Beraud-Dufour S, Chardin P, Chabre M: **N-terminal hydrophobic residues of the G-protein ADP-ribosylation factor-1 insert into membrane phospholipids upon GDP to GTP exchange**. *Biochemistry* 1997, **36**:4675-4684.

89. Goldberg J: **Structural basis for activation of ARF GTPase: mechanisms of guanine nucleotide exchange and GTP-myristoyl switching**. *Cell* 1998, **95**:237-248.

90. Pasqualato S, Menetrey J, Franco M, Cherfils J: **The structural GDP/GTP cycle of human Arf6**. *EMBO Rep* 2001, **2**:234-238.

91. • Pasqualato S, Renault L, Cherfils J: **Arf, Arl, Arp and Sar proteins: a family of GTP-binding proteins with a structural device for 'front-back' communication**. *EMBO Rep* 2002, **3**:1035-1041.
This review presents data comparing the Arf family proteins and suggests a new way of classifying these proteins according to their structural switch regions, which are involved in nucleotide binding. An amino-terminal amphipathic helix is shown to protect the nucleotide-binding site in the inactive protein, which then opens upon interaction of this helix with membranes, demonstrating a critical role for membrane binding by this amphipathic helix for Arf activation.

92. • Hristova K, Dempsey CE, White SH: **Structure, location, and lipid perturbations of melittin at the membrane interface**. *Biophys J* 2001, **80**:801-811.
Using novel analytical methods, X-ray diffraction results of a prototypical amphipathic helical peptide, melittin, in association with a lipid bilayer, demonstrates that the helix is oriented parallel to the bilayer, and resides at the interfacial position between the polar head groups and the hydrophobic core. Despite its location in the bilayer, monomeric melittin had only a modest effect on bilayer structure. By contrast, dimeric melittin formed by a cysteine linkage had a much more profound effect on bilayer structure at the same monomer:lipid concentrations as monomeric melittin. This underscores the fact that monomeric amphipathic helices have qualitative and quantitative differences with respect to their effects on a lipid bilayer compared with amphipathic helices in multimeric forms.

93. Lam YH, Wassall SR, Morton CJ, Smith R, Separovic F: **Solid-state NMR structure determination of melittin in a lipid environment**. *Biophys J* 2001, **81**:2752-2761.

94. White SH, Ladokhin AS, Jayasinghe S, Hristova K: **How membranes shape protein structure**. *J Biol Chem* 2001, **276**:32395-32398.

95. • Lee S, Furuya T, Kiyota T, Takami N, Murata K, Niidome Y, Bredesen DE, Ellerby HM, Sugihara G: ***De novo*-designed peptide transforms Golgi-specific lipids into Golgi-like nanotubules**. *J Biol Chem* 2001, **276**:41224-41228.
The authors show that a *de novo* designed 18-mer amphipathic peptide alone is able to create robust tubular networks on the order of 40–50 nm in diameter and up to 600 nm in length. The dynamics of the process were influenced by the length and hydrophobicity of the helix, as well as by the lipid composition of the bilayer. This study highlights the profound structural effects of an amphipathic peptide on a lipid bilayer.

96. Hurley JH, Misra S: **Signaling and subcellular targeting by membrane-binding domains**. *Annu Rev Biophys Biomol Struct* 2000, **29**:49-79.

97. Ford MG, Pearse BM, Higgins MK, Vallis Y, Owen DJ, Gibson A, Hopkins CR, Evans PR, McMahon HT: **Simultaneous binding of PtdIns(4,5)P2 and clathrin by AP180 in the nucleation of clathrin lattices on membranes**. *Science* 2001, **291**:1051-1055.

98. Piper RC, Luzio JP: **Late endosomes: sorting and partitioning in multivesicular bodies**. *Traffic* 2001, **2**:612-621.

99. Stahl PD, Barbieri MA: **Multivesicular bodies and multivesicular endosomes: the 'ins and outs' of endosomal traffic**. *Sci STKE* 2002, **2002**:PE32.

100. Katzmann DJ, Odorizzi G, Emr SD: **Receptor downregulation and multivesicular-body sorting**. *Nat Rev Mol Cell Biol* 2002, **3**:893-905.

101. • Katzmann DJ, Babst M, Emr SD: **Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I**. *Cell* 2001, **106**:145-155.
With Babst *et al.* (2002) [102•,103•], the ESCRT sorting complex mediating sorting into multivesicular bodies is identified. This represents a potentially new machinery for budding away from the cytoplasm, which further data has shown is used by enveloped viruses for budding from the cell.

102. • Babst M, Katzmann DJ, Snyder WB, Wendland B, Emr SD: **Endosome-associated complex, ESCRT-II, recruits transport machinery for protein sorting at the multivesicular body**. *Dev Cell* 2002, **3**:283-289.
See annotation Katzmann *et al.* (2001) [101•].

103. • Babst M, Katzmann DJ, Estepa-Sabal EJ, Meerloo T, Emr SD: **ESCRT-III: an endosome-associated heterooligomeric protein complex required for MVB sorting**. *Dev Cell* 2002, **3**:271-282.
See annotation Katzmann *et al.* (2001) [101•].

104. Hicke L: **A new ticket for entry into budding vesicles-ubiquitin**. *Cell* 2001, **106**:527-530.

105. Dupre S, Volland C, Haguenauer-Tsapis R: **Membrane transport: ubiquitylation in endosomal sorting**. *Curr Biol* 2001, **11**:R932-934.

106. Odorizzi G, Babst M, Emr SD: **Fab1p PtdIns(3)P 5-kinase function essential for protein sorting in the multivesicular body**. *Cell* 1998, **95**:847-858.

107. Odorizzi G, Babst M, Emr SD: **Phosphoinositide signaling and the regulation of membrane trafficking in yeast**. *Trends Biochem Sci* 2000, **25**:229-235.

108. Denzer K, Kleijmeer MJ, Heijnen HF, Stoorvogel W, Geuze HJ: **Exosome: from internal vesicle of the multivesicular body to intercellular signaling device**. *J Cell Sci* 2000, **113**:3365-3374.

109. Schartz NE, Chaput N, Andre F, Zitvogel L: **From the antigen-presenting cell to the antigen-presenting vesicle: the exosomes**. *Curr Opin Mol Ther* 2002, **4**:372-381.

110. Thery C, Zitvogel L, Amigorena S: **Exosomes: composition, biogenesis and function**. *Nat Rev Immunol* 2002, **2**:569-579.

111. Wolfers J, Lozier A, Raposo G, Regnault A, Thery C, Masurier C, Flament C, Pouzieux S, Faure F, Tursz T *et al.*: **Tumor-derived exosomes are a source of shared tumor rejection antigens for CTL cross-priming**. *Nat Med* 2001, **7**:297-303.

112. Pornillos O, Garrus JE, Sundquist WI: **Mechanisms of enveloped RNA virus budding**. *Trends Cell Biol* 2002, **12**:569-579.

113. Garrus JE, von Schwedler UK, Pornillos OW, Morham SG, Zavitz KH, Wang HE, Wettstein DA, Stray KM, Cote M, Rich RL *et al.*: **Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding**. *Cell* 2001, **107**:55-65.

114. • Mercier C, Dubremetz JF, Rauscher B, Lecordier L, Sibley LD, Cesbron-Delauw MF: ***Toxoplasma parasitophorous* vacuole induced by parasite proteins**. *Mol Biol Cell* 2002, **13**:2397-2409.
The authors show that secreted proteins from the *Toxoplasma* parasite are critical for the formation of the tubular network seen in the parasitophorous vacuole. This network is postulated to play a role in nutrient delivery to the parasite, and mutations that prevent the tubular network render the parasite less virile. Interestingly, the gra2 parasite secreted protein responsible for tubulation contains two critical amphipathic helices that are required for this process.

115. • Lee E, Marcucci M, Daniell L, Pypaert M, Weisz OA, Ochoa GC, Farsad K, Wenk MR, De Camilli P: **Amphiphysin 2 (Bin1) and T-tubule biogenesis in muscle**. *Science* 2002, **297**:1193-1196.
An amphiphysin 2 isoform present in high abundance in muscle cells has been shown to localise to the phosphatidylinositol 3,4-bisphosphate-rich T-tubule system involved in excitation–contraction coupling, and demonstrates evolving roles for membrane-deforming proteins in diverse processes within the cell.

116. Ochoa GC, Slepnev VI, Neff L, Ringstad N, Takei K, Daniell L, Kim W, Cao H, McNiven M, Baron R *et al.*: **A functional link between dynamin and the actin cytoskeleton at podosomes**. *J Cell Biol* 2000, **150**:377-389.