Published in final edited form as:

Curr Opin Cell Biol. 2010 August ; 22(4): 430–436. doi:10.1016/j.ceb.2010.05.002.

Interplay of proteins and lipids in generating membrane curvature

Todd R Graham¹ and Michael M Kozlov²

¹Department of Biological Sciences, Vanderbilt University, Nashville, TN 37235 USA

²Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, 69978 Tel Aviv, Israel

Abstract

The majority of intracellular membranes have strongly bent shapes with radii of curvature ranging from 20 – 50 nm. Many different proteins provide the substantial energy needed to generate and sustain this curvature. One of the most effective mechanisms of curvature creation is based on asymmetry of membrane monolayers. Proteins generate this asymmetry by flipping phospholipid across the membrane, modifying lipid molecules or embedding their hydrophobic domains into the membrane matrix. We review the physical principles of these mechanisms of membrane bending and highlight the action of specific proteins driving vesicle-mediated transport. A model of clathrin-mediated vesicle budding from the *trans*-Golgi network is described to illustrate the interplay and mutual reinforcement of different mechanisms for generating membrane curvature.

Introduction

Cell membranes adopt a broad diversity of shapes corresponding to the specific functions of the membrane compartments [1]. A particularly active membrane-shaping process in eukaryotic cells is the formation of vesicles and tubules that mediate protein transport in the secretory and endocytic pathways. These transport intermediates are typically small, tightly curved structures with radii of only a few tens of nanometers [2,3]. Generation of membrane curvature for vesicle budding was previously thought to be driven by assembly of coat proteins onto membranes, but is now appreciated to be a more complex process involving multiple transport factors and distinct mechanisms [2]. Here we review recent advances in this area emphasizing proteins that work to create membrane asymmetry to drive curvature.

Membrane curvature

A membrane shape is characterized and quantified by curvature of the membrane surface. Curvature is a local geometrical characteristic which, in general, varies from point to point along the membrane plane, but whose average value can be used to describe the shape as a whole. If one considers a small fragment of membrane surface, its shape is described by two circular arcs which lie on the fragment face and are oriented in specific mutually perpendicular directions called the principal directions (see e.g. [4]). The two arcs are characterized by their radii R_1 and R_2 and the reciprocals of these radii, $C_1 = 1/R_1$ and $C_2 =$

© 2010 Elsevier Ltd. All rights reserved.

Corresponding authors: Graham, Todd R (tr.graham@Vanderbilt.Edu), Kozlov, Michael M (michk@post.tau.ac.il).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

$1/R_2$ are called the principal curvatures. Conventionally, curvature of an intracellular membrane is defined as positive if it describes membrane bulging into the cytosol and negative for bulging in the opposite direction. Curvature is considered to be small if the radii R_1 and R_2 are much larger than the membrane thickness, $h \approx 4nm$. An example is plasma membrane for which $h/R \approx 10^{-4}$ or less. In contrast, vesicular and tubular intracellular transport intermediates have $h/R \approx 10^{-1}$ so that their curvatures are considered to be large.

To generate membrane curvature a cell has to perform some work. The reason for that is an intrinsic tendency of a lipid bilayer to adopt and keep a flat shape. In a self-assembling lipid bilayer the two monolayers will tend, energetically, to have the same lipid composition, as favored by the entropy of lipid distribution in the membrane. The resulting bilayer will be completely symmetric with respect to its mid plane, and, hence, there will be no preferential direction for its curving. As a result, the bilayer will acquire neither positive nor negative curvature and adopt a flat shape. Moreover, this bilayer will oppose curving by external forces with a resistance quantified by a bending modulus [5,6]. The value of the latter of about $\approx 10^{-19}$ Joule sets the scale of energies needed for bilayer bending.

What are the specific mechanisms and the related molecular machineries a cell can use to produce and maintain the required curvatures of its membranes? Since the lipid bilayer matrix of any membrane tends to be flat, membrane bending must be performed by membrane associated proteins. To generate membrane curvature, proteins can either make the lipid bilayer asymmetric with respect to its mid plane, or apply forces or mechanical constraints (scaffolds) to the membrane surface forcing the membrane to bend. While these two strategies have been discussed in several recent reviews (see e.g. [2,3,7,8]), we will focus here on the former since evidence is accumulating that membrane asymmetry is a ubiquitous physical reason for membrane curvature.

Generation of the membrane asymmetry can be done in two ways. The first is by modifying the lipid compositions of the membrane monolayers in such a way that the monolayers become different in terms of the either the total amounts of the lipid molecules, or concentrations of diverse lipid species, or both. The second is by direct insertion of protein domains into the membrane monolayers changing the structure of one of them with respect to the other. Within the first strategy the proteins act as “white collar workers” leaving the direct job of membrane bending to lipids themselves, while the second strategy assumes that proteins bend the membrane by “their own hands”, i.e. serve as “blue collar workers”.

White collar workers

These are flippases mediating directional transfer of lipid molecules between the two membrane monolayers, and lipid modifying enzymes that inter-convert lipid species.

Phospholipid flippases

By translocating phospholipid molecules unidirectionally across the membrane, flippases can drive curvature by generating a difference in the number of lipid molecules between the two membrane monolayers and, hence, a difference in the monolayer areas. The membrane bulges in the direction of the monolayer with the larger area. This principle of curvature generation is referred to as the bilayer couple mechanism [9,10]. This mechanism is most effective if the flippases generating the difference in the monolayer areas are distributed around a relatively small membrane compartment. In contrast, if a flippase acts only within a limited area of a larger surface, such as the plasma membrane, its effect will be averaged over the entire surface and the net influence on membrane curvature should be expected, in most cases, to be relatively small.

Type IV P-type ATPases (P4-ATPases)—*P4-ATPases* translocate phospholipid directionally from the exofacial leaflet to the cytosolic leaflet and are linked to changes in membrane curvature and transport vesicle budding [11,12]. There are five P4-ATPase genes in budding yeast that are collectively essential for viability and all five are required for various vesicular transport steps in the secretory and endocytic pathways (reviewed in [13]). Critical roles for P4-ATPases in vesicular transport have also been found recently in *Arabidopsis*, *C. elegans* and cultured mammalian cells [14–16]. Uncertainty as to whether the P4-ATPases are flippases or not has been significantly alleviated by the recent reconstitution of phospholipid flippase activity with two different purified P4-ATPases [17,18].

Lipid modifying enzymes

The chemical nature of lipids determines how they pack side-by-side in a monolayer and thereby influences the preferable monolayer shape. The typical examples are lysolipids (e.g. lysophosphatidylcholine (LPC)) building positively curved monolayers; phosphatidylcholine (PC) forming nearly flat monolayers; and dioleoylphosphatidylethanolamine (DOPE) or diacylglycerol (DAG) self-assembling into monolayers of negative curvature (see for review [3,19]). Each individual lipid can be characterized quantitatively by the curvature of the monolayer it forms spontaneously. This curvature is referred to as the spontaneous curvature of this lipid. Importantly, the spontaneous curvature of a monolayer formed by a lipid mixture is equal, within a good approximation, to a weighted average of the spontaneous curvatures of the constituent lipids [20,21]. The spontaneous curvature of a bilayer is equal to the difference between the spontaneous curvatures of its outer and inner monolayers. Hence, an asymmetric change to the composition of one monolayer by enzymes can induce membrane curvature and examples from the literature are described below.

Lysophospholipid acyltransferases (LPAT) and phospholipase A (PLA)—PLA hydrolyses the acyl chain in the sn-1 (PLA₁) or sn-2 (PLA₂) position of a phospholipid to produce a lysophospholipid with positive spontaneous curvature, while LPAT catalyzes transfer of a fatty acid from Acyl-CoA to an acceptor lysophospholipid, thus restoring the diacylated phospholipid and eliminating the positive spontaneous curvature. The microtubule-independent formation of membrane tubules from the Golgi and endosomes requires the activity of a PLA₂. Antagonists of PLA₂ prevent formation of tubules from the Golgi and endosomes and also perturb protein trafficking through these organelles [22]. PLA₂ activity that is unopposed by LPAT, through application of specific LPAT inhibitors, leads to enhanced tubulation of the Golgi [23]. Important recent advances in this area are the identification of Golgi-associated LPAT (lysophosphatidic acid acyltransferases 3) and PLAs (cPLA₂ and iPLA₁) that control the degree of tubulation and rate of protein trafficking through the Golgi complex [24–26].

Sphingomyelinase—Sphingomyelin has zero spontaneous curvature, but cleavage of this lipid by sphingomyelinase releases the phosphocholine headgroup and produces the lipid ceramide with negative spontaneous curvature. This mechanism appears to be used by cells to drive formation of exosomes [27], which are vesicles that bud into the lumen of the endosome and are ultimately exocytosed. Exosome membranes are enriched for ceramide, suggesting the involvement of a sphingomyelinase in their formation. This prediction was supported experimentally through the use of sphingomyelinase inhibitors and the observation that RNAi knockdown of neutral sphingomyelinase 2 abrogated the sorting of an exosome marker to the endosomal lumen and its release from the cells [27]. Thus, it appears that sphingomyelin conversion to ceramide on the cytosolic leaflet of the endosome induces budding of vesicles into the luminal space.

Blue collar workers

These proteins generate asymmetry of the membrane bilayer by inserting their hydrophobic or amphipathic domains into the bilayer matrix on one membrane side. Similarly to the “white collar workers”, the hydrophobic insertions can modify the membrane curvature in two complementary ways: they can generate a local curvature, or act according to the bilayer couple mechanism by expanding the total area of one membrane monolayer with respect to the other. Which of these two effects prevails depends, mainly, on the depth to which a hydrophobic insertion penetrates the lipid matrix. Protein domains inserting shallowly into one lipid monolayer generate, primarily, a local spontaneous curvature; insertions spanning completely one of the monolayers contribute, mainly, to the bilayer couple mechanism of membrane bending; the membrane bending effect of protein domains spanning the whole bilayer can be related only to the domain shape and is expected to be relatively weak. Here we consider the shallow protein insertions, which appear to be the most powerful drivers of membrane curvature.

To this class belong the small amphipathic helices of N-BAR domains of various proteins [28,29], of epsin ENTH domains [30], and of small G-proteins such as Arf1 and Sar1 (see below). These helices embed into one of the membrane monolayers to about 30-40% of the monolayer thickness and, therefore, expand, mainly, the region of the lipid polar heads of this monolayer, while the region of the hydrocarbon chains remains undisturbed. This leads to a strong asymmetry on the monolayer structure and, hence, to generation of a positive membrane curvature. In spite of their small size (in the range of few nanometers), these amphipathic helices are able to generate very large positive curvatures. The radius of curvature R characterizing a local deformation of the monolayer produced by one inserted amphipathic helix is close to the lipid monolayer thickness $h/R \approx 0.75$ which is three times larger than that of the non-bilayer lipid LPC [31]. The results of theoretical analysis of the membrane curvature generation by shallow insertion are presented in Fig.1.

Synaptotagmin-1 and Doc2b

To the class of shallow insertions belong also small hydrophobic regions of C2 domains of synaptotagmin-1 and Doc2b, which, upon Ca^{2+} binding, get embedded into the lipid monolayer matrix up to about a third of its thickness [32-35]. These little hydrophobic regions act according to the same principle as the amphipathic helices – they splay the lipid polar heads. According to theoretical estimations and experimental results [34,36], the C2 domains are equally or even more effective curvature generators as the amphipathic helices. Generation of a high but localized membrane curvature has been suggested as the essence of the action of synaptotagmin-1 and Doc2b in membrane fusion [32,34-36].

Dynamin

The class of shallow insertions must include also the hydrophobic loops of PH domains of proteins of the dynamin family ([37] and references therein). Although dynamins have been commonly believed to bend membranes by attaching the lipid bilayer surface to the curved positively charged faces of their oligomers, evidence is accumulating for a crucial role of the PH hydrophobic insertions in membrane deformation leading to membrane fission [37].

Sar1p and Arf

As described above, the N-terminal amphipathic α -helices of Sar1 and Arf can induce membrane curvature as they are inserted into the cytosolic leaflet of the bilayer. The conformational change driving this event is induced by GTP binding, which displaces the N-terminal domain from the “body” of these small GTP-binding proteins and presents the amphipathic helix to the membrane [38]. Indeed, GTP-bound Sar1p and Arf effectively

tubulate liposomes while the GDP-bound forms do not [39-41]. Work on Sar1p provided strong evidence for the importance of this membrane-insertion mechanism in vesicle budding [41]. Mutations were identified that uncouple the membrane-bending and coat-recruitment activities of Sar1. These Sar1p amphipathic helix mutants specifically compromised for membrane-bending were markedly deficient in supporting COPII vesicle budding *in vitro* and COPII-dependent protein transport *in vivo* [41]. While the scaffolding property of the COPII coat can deform liposomes in the absence of Sar1p [41], it appears this membrane bending mechanism alone provides insufficient energy to drive vesicle budding *in vivo*.

Similar studies with Arf and its role in budding COPI-coated vesicles are leading to the same conclusions but with a few interesting new twists [39,42]. The ability of Arf-GTP to tubulate membranes appears to be strongly dependent on its ability to dimerize on the membrane surface. An Arf1 mutation (Y35A) that prevents dimerization without disrupting COPI interaction, the GTPase cycle or membrane association, also prevents liposome tubulation. Importantly, Arf1-Y35A cannot support COPI vesicle budding *in vitro* or Arf function *in vivo* [39]. This effect should be due to a larger membrane affinity of the Arf dimers as compared to monomers and, possibly so some structural features of the dimers. Another important observation on the Arf membrane tubulating activity is how membrane curvature influences nucleotide exchange on Arf. Presenting Arf with a curved membrane significantly enhances GTP binding, presumably because the amphipathic helix can more easily penetrate the membrane if the headgroups are splayed and this transition stabilizes the GTP-binding conformation [42]. This observation implies a mechanism for cooperativity of membrane binding and insertion of Arf-GTP dimers leading to local amplification or propagation of membrane curvature.

Epsin, endophilin and amphiphysin

The discovery that the N-terminal amphipathic helices of the endophilin and amphiphysin N-BAR domains, and of the Epsin 1 ENTH domain could potentially tubulate liposomes ignited interest in the membrane insertion mechanism for bending membranes [30,43-45]. The function of these multidomain proteins in endocytosis and regulation of actin dynamics has been extensively reviewed [2,28,46,47].

Dp1/Yop1

The hydrophobic hairpins of reticulons and DP1/Yop1p, the proteins shaping the smooth ER, may belong to the same class of shallow insertions. These hairpins are long enough to span completely one membrane monolayer and penetrate, partially, the second one. At the same time, they could be located in only one monolayer lying parallel to its surface at a small depth under the lipid-water interface.

Concerted action of different mechanisms of curvature generation in clathrin-mediated vesicle budding from the TGN

An important goal is to better define the extent to which different mechanisms contribute to membrane curvature for individual vesicle budding events and how different mechanisms are integrated and controlled. An interesting example for how different mechanisms may be integrated has emerged from studies of the P4-ATPase Drs2p in budding yeast [48-50]. Drs2p localizes to the TGN and its flippase activity is required for AP-1/clathrin-dependent protein trafficking between the TGN and early endosomes [49]. Drs2p also binds directly to the Arf guanine nucleotide exchange factors (GEFs) Gea1p and Gea2p [51]. However, Drs2p is not needed to recruit these ArfGEFs to the Golgi membranes. In fact ArfGEF, Arf, AP-1 and clathrin all associate with the TGN membrane in *drs2* cells [49,51], even though

AP-1/clathrin function is abrogated and the *drs2* mutant TGN membranes are notable for the lack of curvature (tubules) or budding profiles [48,49]. This was a surprising observation because it implies that Arf and the clathrin coat are insufficient to drive membrane curvature at the TGN in the absence of Drs2p flippase activity. The recent discovery that binding of ArfGEF and PtdIns(4)P to the Drs2 C-terminal tail activates flippase activity [50] suggests a mechanism for the integration of different membrane curvature drivers. In the model shown in Fig.2, PtdIns(4)P and ArfGEF are proposed to integrate the function of membrane curvature drivers by activating Drs2p flippase activity and Arf membrane insertion, as well as recruiting AP1/clathrin to stabilize the curvature.

Conclusions

There are several different strategies by which proteins produce membrane asymmetry followed by generation of membrane curvature. The most powerful curvature drivers appear to be small hydrophobic and amphipathic protein domains shallowly inserting into one of the membrane leaflets. At the same time, the difference in the monolayer lipid compositions produced by flippases and the lipid modifying enzymes appears to be indispensable for such processes as vesicle budding from TGN. Hence, the effectiveness and robustness of the intracellular generation of membrane curvature must result from a concerted action of several protein-based mechanisms.

Acknowledgments

This work was supported by NIH GM062367 to T.R.G. A Financial support for MMK from the Israel Science Foundation (ISF) and the Marie Curie Network "Virus Entry" is gratefully acknowledged.

References and recommended reading

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

* of particular interest

** of outstanding interest

- Shibata Y, Hu J, Kozlov MM, Rapoport TA. Mechanisms shaping the membranes of cellular organelles. *Annu Rev Cell Dev Biol.* 2009; 25:329–354. [PubMed: 19575675]
- McMahon HT, Gallop JL. Membrane curvature and mechanisms of dynamic cell membrane remodelling. *Nature.* 2005; 438:590–596. [PubMed: 16319878]
- Zimmerberg J, Kozlov MM. How proteins produce cellular membrane curvature. *Nat Rev Mol Cell Biol.* 2006; 7:9–19. [PubMed: 16365634]
- Spivak, M. A comprehensive introduction to differential geometry. Vol. I. Waltham, Mass.: Brandeis University; 1970.
- Helfrich W. Elastic properties of lipid bilayers: Theory and possible experiments. *Z Naturforsch.* 1973; 28c:693–703.
- Helfrich, W. Elasticity and thermal undulations of fluid films of amphiphiles. In: Charvolin, J.; Joanny, JF.; Zinn-Justin, J., editors. *Les Houches, 1988 - Liquids and interfaces.* Vol. 48. Elsevier; 1990. p. 212-237.
- Campelo F, Fabrikant G, McMahon HT, Kozlov MM. Modeling membrane shaping by proteins: Focus on EHD2 and N-BAR domains. *FEBS Lett.* 2009
- Kozlov MM. Biophysics: Joint effort bends membrane. *Nature.* 2010; 463:439–440. [PubMed: 20110984]
- Devaux PF. Is lipid translocation involved during endo- and exocytosis? *Biochimie.* 2000; 82:497–509. [PubMed: 10865135]

10. Sheetz MP, Singer SJ. Biological membranes as bilayer couples. A molecular mechanism of drug-erythrocyte interactions. *Proc Natl Acad Sci U S A*. 1974; 71:4457–4461. [PubMed: 4530994]
11. Daleke DL. Phospholipid flippases. *J Biol Chem*. 2007; 282:821–825. [PubMed: 17130120]
12. Devaux PF, Herrmann A, Ohlwein N, Kozlov MM. How lipid flippases can modulate membrane structure. *Biochim Biophys Acta*. 2008; 1778:1591–1600. [PubMed: 18439418]
13. Muthusamy BP, Natarajan P, Zhou X, Graham TR. Linking phospholipid flippases to vesicle-mediated protein transport. *Biochim Biophys Acta*. 2009; 1791:612–619. [PubMed: 19286470]
- *14. Poulsen LR, Lopez-Marques RL, McDowell SC, Okkeri J, Licht D, Schulz A, Pomorski T, Harper JF, Palmgren MG. The Arabidopsis P4-ATPase ALA3 localizes to the Golgi and requires a beta-subunit to function in lipid translocation and secretory vesicle formation. *Plant Cell*. 2008; 20:658–676. This paper provides the first evidence outside of the yeast system for a P4-ATPase requirement in exocytic vesicle production. [PubMed: 18344284]
15. Ruaud AF, Nilsson L, Richard F, Larsen MK, Bessereau JL, Tuck S. The *C. elegans* P4-ATPase TAT-1 regulates lysosome biogenesis and endocytosis. *Traffic*. 2009; 10:88–100. [PubMed: 18939953]
- *16. Xu P, Okkeri J, Hanisch S, Hu RY, Xu Q, Pomorski TG, Ding XY. Identification of a novel mouse P4-ATPase family member highly expressed during spermatogenesis. *J Cell Sci*. 2009; 122:2866–2876. This paper provided the first evidence that a P4-ATPase influences the structure and function of the mammalian Golgi. [PubMed: 19657017]
- **17. Coleman JA, Kwok MC, Molday RS. Localization, purification, and functional reconstitution of the P4-ATPase Atp8a2, a phosphatidylserine flippase in photoreceptor disc membranes. *J Biol Chem*. 2009; 284:32670–32679. The Coleman and Zhou [18] papers provided direct biochemical evidence that P4-ATPases are phospholipid flippases. [PubMed: 19778899]
- **18. Zhou X, Graham TR. Reconstitution of phospholipid translocase activity with purified Drs2p, a type-IV P-type ATPase from budding yeast. *Proc Natl Acad Sci U S A*. 2009; 106:16586–16591. See comment with reference 17. [PubMed: 19805341]
19. Chernomordik LV, Kozlov MM. Protein-lipid interplay in fusion and fission of biological membranes. *Annu Rev Biochem*. 2003; 72:175–207. [PubMed: 14527322]
20. Kozlov M, Helfrich W. Effects of a cosurfactant on the stretching and bending elasticities of a surfactant monolayer. *Langmuir*. 1992; 8:2792–2797.
21. Leikin S, Kozlov MM, Fuller NL, Rand RP. Measured effects of diacylglycerol on structural and elastic properties of phospholipid membranes. *Biophys J*. 1996; 71:2623–2632. [PubMed: 8913600]
22. Brown WJ, Chambers K, Doody A. Phospholipase A2 (PLA2) enzymes in membrane trafficking: mediators of membrane shape and function. *Traffic*. 2003; 4:214–221. [PubMed: 12694560]
23. Drecktrah D, Chambers K, Racoosin EL, Cluett EB, Gucwa A, Jackson B, Brown WJ. Inhibition of a Golgi complex lysophospholipid acyltransferase induces membrane tubule formation and retrograde trafficking. *Mol Biol Cell*. 2003; 14:3459–3469. [PubMed: 12925777]
- *24. Morikawa RK, Aoki J, Kano F, Murata M, Yamamoto A, Tsujimoto M, Arai H. Intracellular phospholipase A1gamma (iPLA1gamma) is a novel factor involved in coat protein complex I- and Rab6-independent retrograde transport between the endoplasmic reticulum and the Golgi complex. *J Biol Chem*. 2009; 284:26620–26630. The Morikawa and San Pietro [25] papers identify PLAs associated with tubule formation and protein trafficking in the Golgi system, providing confirmation for prior inhibitor studies (see ref 22). [PubMed: 19632984]
- *25. San Pietro E, Capestrano M, Polishchuk EV, DiPentima A, Trucco A, Zizza P, Mariggio S, Pulvirenti T, Sallese M, Tete S, et al. Group IV phospholipase A(2)alpha controls the formation of inter-cisternal continuities involved in intra-Golgi transport. *PLoS Biol*. 2009; 7:e1000194. See comment to reference 24. [PubMed: 19753100]
- **26. Schmidt JA, Brown WJ. Lysophosphatidic acid acyltransferase 3 regulates Golgi complex structure and function. *J Cell Biol*. 2009; 186:211–218. This paper describes the specific LPAT that regulates tubule formation from the Golgi. [PubMed: 19635840]
- **27. Trajkovic K, Hsu C, Chiantia S, Rajendran L, Wenzel D, Wieland F, Schwille P, Brugger B, Simons M. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science*. 2008; 319:1244–1247. This study implies that the asymmetric formation of ceramide on

- the cytosolic leaflet of endosomes induces inward budding of vesicles into the lumen. [PubMed: 18309083]
28. Frost A, Unger VM, De Camilli P. The BAR domain superfamily: membrane-molding macromolecules. *Cell*. 2009; 137:191–196. [PubMed: 19379681]
 29. Gallop JL, Jao CC, Kent HM, Butler PJ, Evans PR, Langen R, McMahon HT. Mechanism of endophilin N-BAR domain-mediated membrane curvature. *Embo J*. 2006; 25:2898–2910. [PubMed: 16763559]
 30. 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. [PubMed: 12353027]
 - **31. Campelo F, McMahon HT, Kozlov MM. The hydrophobic insertion mechanism of membrane curvature generation by proteins. *Biophys J*. 2008; 95:2325–2339. This paper presents the first quantitative analysis of the hydrophobic insertion mechanism of membrane shaping. [PubMed: 18515373]
 - **32. Groffen AJ, Martens S, Arazola RD, Cornelisse LN, Lozovaya N, de Jong AP, Goriounova NA, Habets RL, Takai Y, Borst JG, et al. Doc2b Is a High-Affinity Ca²⁺ Sensor for Spontaneous Neurotransmitter Release. *Science*. 2010 This paper is the first demonstration of the role of membrane insertion of Doc2b C2 domain in membrane remodeling.
 33. Martens S. Role of C2 domain proteins during synaptic vesicle exocytosis. *Biochem Soc Trans*. 2010; 38:213–216. [PubMed: 20074062]
 - **34. Martens S, Kozlov MM, McMahon HT. How synaptotagmin promotes membrane fusion. *Science*. 2007; 316:1205–1208. This paper is the first demonstration of the role of membrane insertion of synaptotagmin C2 domain in membrane remodeling. [PubMed: 17478680]
 35. Martens S, McMahon HT. Mechanisms of membrane fusion: disparate players and common principles. *Nat Rev Mol Cell Biol*. 2008; 9:543–556. [PubMed: 18496517]
 36. McMahon HT, Kozlov MM, Martens S. Membrane Curvature in Synaptic Vesicle Fusion and Beyond. *Cell*. 2010; 140:601. [PubMed: 20211126]
 - **37. Ramachandran R, Pucadyil TJ, Liu YW, Acharya S, Leonard M, Lukiyanchuk V, Schmid SL. Membrane insertion of the pleckstrin homology domain variable loop 1 is critical for dynamin-catalyzed vesicle scission. *Mol Biol Cell*. 2009; 20:4630–4639. This paper demonstrates for the first time the crucial role for membrane remodeling of insertion of the dynamin PH domain into the membrane matrix. [PubMed: 19776347]
 38. 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. [PubMed: 9109679]
 - **39. Beck R, Sun Z, Adolf F, Rutz C, Bassler J, Wild K, Sinning I, Hurt E, Brugger B, Bethune J, et al. Membrane curvature induced by Arf1-GTP is essential for vesicle formation. *Proc Natl Acad Sci U S A*. 2008; 105:11731–11736. This paper, along with [40 and 42] demonstrate the critical function of the Arf1 N-terminal amphipathic helix in COPI vesicle formation. It also provides evidence that Arf1 dimerization on the membrane is important for membrane deformation. [PubMed: 18689681]
 - **40. Krauss M, Jia JY, Roux A, Beck R, Wieland FT, De Camilli P, Haucke V. Arf1-GTP-induced tubule formation suggests a function of Arf family proteins in curvature acquisition at sites of vesicle budding. *J Biol Chem*. 2008; 283:27717–27723. See [39]. [PubMed: 18693248]
 - **41. Lee MC, Orci L, Hamamoto S, Futai E, Ravazzola M, Schekman R. Sar1p N-terminal helix initiates membrane curvature and completes the fission of a COPII vesicle. *Cell*. 2005; 122:605–617. Outstanding paper demonstrating the importance of the Sar1p N-terminal amphipathic helix insertion to the formation of COPII vesicles in vitro and in vivo. [PubMed: 16122427]
 - **42. Lundmark R, Doherty GJ, Vallis Y, Peter BJ, McMahon HT. Arf family GTP loading is activated by, and generates, positive membrane curvature. *Biochem J*. 2008; 414:189–194. See comment for [39]. In addition, evidence is presented for curvature sensing by Arf and stimulation of nucleotide exchange by curved membranes. [PubMed: 18597672]
 43. 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. [PubMed: 11604418]

44. Peter BJ, Kent HM, Mills IG, Vallis Y, Butler PJ, Evans PR, McMahon HT. BAR domains as sensors of membrane curvature: the amphiphysin BAR structure. *Science*. 2004; 303:495–499. [PubMed: 14645856]
45. 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. [PubMed: 10559861]
46. Itoh T, De Camilli P. BAR, F-BAR (EFC) and ENTH/ANTH domains in the regulation of membrane- cytosol interfaces and membrane curvature. *Biochim Biophys Acta*. 2006; 1761:897–912. [PubMed: 16938488]
47. Maldonado-Baez L, Wendland B. Endocytic adaptors: recruiters, coordinators and regulators. *Trends Cell Biol*. 2006; 16:505–513. [PubMed: 16935508]
48. Chen CY, Ingram MF, Rosal PH, Graham TR. Role for Drs2p, a P-type ATPase and potential aminophospholipid translocase, in yeast late Golgi function. *J Cell Biol*. 1999; 147:1223–1236. [PubMed: 10601336]
- *49. Liu K, Surendhran K, Nothwehr SF, Graham TR. P4-ATPase requirement for AP-1/clathrin function in protein transport from the trans-Golgi network and early endosomes. *Mol Biol Cell*. 2008; 19:3526–3535. This paper provides evidence that Drs2p flippase activity is required for AP1/clathrin function by a mechanism that is independent of coat recruitment to the TGN. [PubMed: 18508916]
- **50. Natarajan P, Liu K, Patil DV, Sciorra VA, Jackson CL, Graham TR. Regulation of a Golgi flippase by phosphoinositides and an ArfGEF. *Nat Cell Biol*. 2009; 11:1421–1426. The activation of Drs2p flippase activity by PtdIns(4)P and ArfGEF suggested a mechanism for integrating different drivers of membrane curvature at a nascent bud site (see Fig. 2). [PubMed: 19898464]
51. Chantalat S, Park SK, Hua Z, Liu K, Gobin R, Peyroche A, Rambourg A, Graham TR, Jackson CL. The Arf activator Gea2p and the P-type ATPase Drs2p interact at the Golgi in *Saccharomyces cerevisiae*. *J Cell Sci*. 2004; 117:711–722. [PubMed: 14734650]

Fig.1.

The hydrophobic insertion mechanism of curvature generation.

Left panel: local membrane deformation generated by insertion of an amphoteric helix. As a specific example the H₀ helix of the epsin1 ENTH domain [30] is shown. The membrane shape has been computed based on an elastic model of the bilayer interior [31] the color code describing distribution of the elastic stresses within the membrane.

Right panel: the effective spontaneous curvature generated by of an amphoteric helix. The color code describes the elastic stress distribution throughout the lipid bilayer [31].

Fig. 2.

Potential mechanism for integrating different drivers of membrane curvature during the budding of AP1/clathrin-coated vesicles from the TGN.

Drs2p is a phospholipid flippase that plays a role in budding AP1/clathrin clathrin-coated vesicles from the yeast TGN. Recent studies indicate that the flippase activity of Drs2p is synergistically activated by binding of the Drs2p C-terminal tail to ArfGEF (Gea2p) and PtdIns(4)P synthesized by Pik1p [50]. ATP-dependent translocation of the preferred Drs2p transport substrate, phosphatidylserine (PtdSer, blue lipid), across the bilayer will increase the cytosolic leaflet surface area while decreasing that of the lumenal leaflet. This monolayer area difference along with the intrinsic positive curvature of PtdSer will induce bending of the membrane towards the cytosol. Although Drs2p flippase activity will broadly impart curvature to the membrane, the Drs2p-bound ArfGEF should enhance this effect locally by stimulating nucleotide exchange on Arf. Insertion of the myristoylated amphipathic helix of Arf-GTP into the membrane would increase local curvature at the nascent bud site. The stimulatory influence of membrane curvature on Arf nucleotide exchange should increase Arf-GTP occupancy and facilitate dimerization to further drive membrane deformation. Arf-GTP and PtdIns(4)P also recruits AP1 and clathrin onto the membrane. The growing clathrin lattice provides a scaffold to stabilize the curvature, and also maintains a high local concentration of ArfGTP/AP1 by preventing their lateral diffusion. AP-1 and clathrin can also recruit EpsinR (Ent3p, Ent5p in budding yeast), with its ENTH domain providing another potential driver of membrane curvature (not shown).