

example, mutations similar to *bbs1* (*jhu598*) that specifically affect ciliary entry of the BBSome and IFT tip turnaround, but have functional BBSome proteins at the ciliary base, will probably cause a milder phenotype than mutations that disrupt BBSome function at the ciliary base and tip.

It will be interesting to test this model in other ciliated organisms where endogenous proteins can be analysed more readily than in *C. elegans*, and to assess the behaviour of

further IFT components, such as other IFT-A and dynein 2 subunits, in the *dyf-2* and *bbs1* mutants.

COMPETING FINANCIAL INTERESTS

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Bending membranes

Tom Kirchhausen

It is widely assumed that peripheral membrane proteins induce intracellular membrane curvature by the asymmetric insertion of a protein segment into the lipid bilayer, or by imposing shape by adhesion of a curved protein domain to the membrane surface. Two papers now provide convincing evidence challenging these views. The first shows that specific assembly of a clathrin protein scaffold, coupled to the membrane, seems to be the most prevalent mechanism for bending a lipid bilayer in a cell. The second reports that membrane crowding, driven by protein–protein interactions, can also drive membrane bending, even in the absence of any protein insertion into the bilayer.

Bending a planar lipid bilayer requires work. Calculations based on the classic Helfrich model suggest that it costs about 200–250 kcal per mole of vesicle to create a closed spherical bilayer from an initially flat one¹. Certain lipids can generate spontaneous membrane curvature when asymmetrically inserted into the lipid bilayer² (Fig. 1a). Lipids with a large area ratio of polar head groups to acyl chains create positive curvature; lipids with the opposite ratio create negative curvature (as does the insertion of cholesterol). This source of membrane bending is clearly insufficient, however, to explain the remarkable spatial and temporal heterogeneity and specificity of shapes and curvatures observed in the internal membranes of cells. Coordinated assembly of a protein scaffold can provide the driving force to induce membrane curvature if the components of the scaffold are anchored firmly to the lipid bilayer^{3,4} (Fig. 1b). Budding of some enveloped viruses proceeds in this way and, in principle, a similar process can induce the membrane deformation required

for the budding of vesicular and tubular carriers of intracellular cargo. This mechanism has a role in the formation of most intracellular vesicular carriers, particularly those associated with clathrin or the COPI or COPII coat protein complexes. The discovery of membrane-associated proteins with curved, lipid-facing surfaces (for example, those with so-called BAR (Bin–Amphiphysin–Rvs) protein domains), and of others that appear to bind with the hydrophobic side of an amphipathic helix inserted into the head-group–hydrocarbon interface (for example, the epsin N-terminal homology (ENTH) domain), has given rise to the proposal that these proteins also have a role in driving membrane curvature⁵ (Fig. 1c,d), as well as — or even instead of — the assembly of the coats⁶. In the case of clathrin-coated pits, for example, it has been argued that the lattice of clathrin triskelions that surrounds the pit simply follows the curvature induced by recruitment of epsin⁶ and the F-BAR-containing proteins FCHO1 and FCHO2 (ref. 7).

Two important papers, one by Dannhauser and Ungewickell⁸ and the other by Stachowiak *et al.*⁹, provide convincing, straightforward and elegant evidence addressing two fundamental questions. First, is clathrin sufficient to drive

membrane curvature? Second, are specific membrane–protein interactions required to deform the underlying membrane, driven either by the shape of the protein in contact with the membrane or by protein insertion?

Dannhauser and Ungewickell present clear *in vitro* evidence supporting the hypothesis that clathrin can drive membrane deformation without the need for other, potentially membrane-deforming proteins⁸. Their work shows that induction of membrane curvature mediated by proteins such as epsin is dispensable for the creation of deeply invaginated, clathrin-coated pits on liposomes and, by extension, at the plasma membrane. Their experimental system allowed precise control of the molecular components present, enabling them to recapitulate scission mediated by dynamin and GTP, as well as uncoating mediated by auxilin, Hsc70 and ATP. These authors recruited clathrin to the liposomes by using a truncated form of epsin, still capable of interacting with the N-terminal domain of clathrin but lacking the N-terminal ENTH domain, hence unable to interact directly with a lipid bilayer. The authors replaced the ENTH domain with a short flexible peptide linker, tagged at its N-terminus with 6 histidines; this tag binds tightly to Ni-NTA, covalently attached to the

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headgroup of a lipid introduced in small molar fraction into the bilayer. Incubation of a mixture containing clathrin, the truncated epsin and large liposomes containing the Ni-NTA lipid led to the formation of clathrin coats clearly associated to membrane invaginations or buds with sizes similar to those observed *in vivo*. The membrane necks of these buds were sufficiently narrow to support recruitment of dynamin, which, upon activation with GTP, promoted tubule scission and release of coated vesicles. Finally, the clathrin coat surrounding these coated vesicles could be released by the ATP-dependent uncoating reaction mediated by Hsc70 and auxilin. These observations are important, not only because they are the first experiments in which clathrin-coated vesicles have been formed from liposomes using such a minimal set of proteins, but also, and more crucially to the bending question, because vesicle formation occurred perfectly well in the absence of membrane-curvature-inducing domains.

The paper by Stachowiak *et al.* is likewise a significant advance, as it shows that membrane crowding, driven by protein–protein interactions, is sufficient to drive membrane bending, even in the absence of any protein insertion into the lipid bilayer⁹ (Fig. 1e). The authors show that high local protein concentration (crowding) can drive membrane curvature and tubulation, regardless of how the proteins are recruited to the membrane. They used giant unilamellar vesicles (GUVs) containing phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P₂) as the membrane substrate, and showed that tubulation could be achieved with either the ENTH domain of epsin or the ANTH domain of AP180 (another protein found in endocytic clathrin-coated pits and vesicles). The ANTH domain resembles the ENTH domain, but lacks the amphipathic helix. Using fluorescence-lifetime Förster resonance energy transfer (FRET), they found that tubules formed efficiently when ~20% of the membrane was covered by the epsin ENTH domain; at this level, only 2% of the PtdIns(4,5)P₂-rich area would have been occupied by the amphipathic helix, equivalent to 1 epsin per 100 lipid molecules, well below the 10–25% coverage predicted to generate high curvature by hydrophobic insertion. An even more remarkable result was the observation that an ENTH domain with its putative membrane insertion helix replaced by a 6-histidine tag also drove tubulation of GUVs containing Ni-NTA lipids and lacking PtdIns(4,5)P₂ when coverage

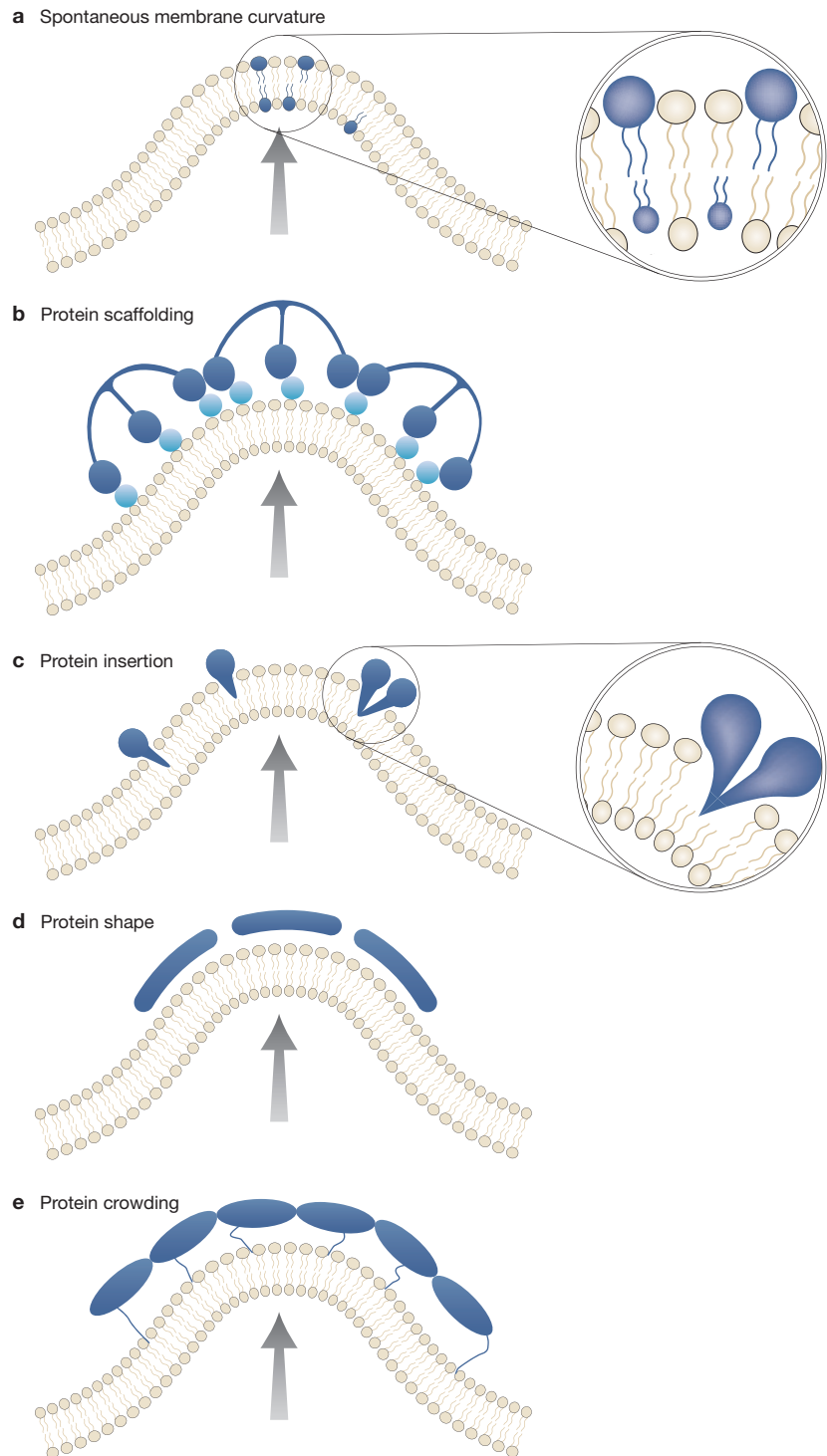


Figure 1 Mechanisms to generate membrane curvature. **(a)** Local spontaneous membrane curvature generated by lipids. Lipids with a large area ratio of polar head groups to acyl chains create positive curvature; lipids with the opposite ratio create negative curvature (as does the insertion of cholesterol). **(b)** Intracellular membrane curvature generated by coordinated assembly of a protein scaffold. Assembly of protein scaffolds can generate membrane curvature if their components are firmly anchored to the lipid bilayer by direct contacts or through adaptor proteins. This is the most common mechanism involved in the generation of intracellular carriers associated with clathrin or the COPI and COPII coat protein complexes. **(c)** Membrane curvature generated by high local surface density of hydrophobic or amphipathic protein domains asymmetrically inserted into the lipid bilayer. **(d)** Membrane curvature generated by large amounts of curvedly shaped protein domains adhered to the lipid bilayer. **(e)** Membrane curvature generated by protein crowding. Energy for membrane deformation provided by high local surface density of proteins even if they are nonspecifically associated to the membrane.

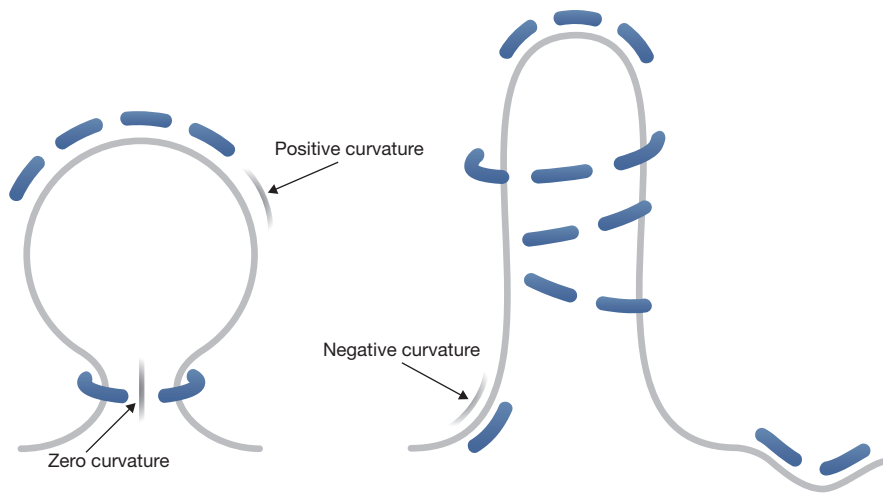


Figure 2 Membrane curvature sensors. These are proteins whose binding affinity for membranes depends on the extent of membrane curvature. Curvature sensing typically relies on insertion of amphipathic domains into the lipid bilayer, or on the interaction of a curved protein domain with the surface of the membrane.

reached the same 20% threshold. Tubulation was also obtained using 6-histidine-tagged green fluorescence protein (GFP), even though this protein is not known to bind membranes or to have any specific membrane-bending function. What appears to be happening is that the membrane bends to dilute the concentrated protein layer, even though the protein has only a peripheral tether linking it to the lipid headgroups.

How do these new data bear on the view that peripheral membrane proteins can generate functionally relevant membrane curvature — either by hydrophobic or amphipathic protein domains that penetrate the lipid bilayer matrix, or by intrinsically curved, hydrophilic protein domains (or assemblies) that adhere to the lipid bilayer surface and impress their shapes on the membrane? The results that led to this view came primarily from *in vitro* observations of membrane deformation driven by excess protein. However, the concentrations of membrane-bound protein in those experiments far exceeded the concentrations of the same or similar proteins in cells. In effect, concentrating the proteins in the laboratory generated the energy required for lipid bilayer deformation. There is no evidence for any comparable concentrating mechanism in cells. As Stachowiak *et al.*⁹ show, high local surface density of even proteins nonspecifically associated with the membrane (crowding) can induce

membrane deformation, but such crowding has yet to be demonstrated *in vivo* under normal conditions of protein expression.

Experiments in cells that sought to identify the role of the F-BAR proteins FCHo1 and FCHo2 in clathrin-coated pit formation led to the proposal that these proteins initiate coated pit formation by inducing membrane curvature⁷. Subsequently, more sensitive live cell imaging experiments instead indicated that these proteins arrive after initiation of coat assembly, and that they stabilize the growing clathrin lattice to prevent its dissociation¹⁰. Developmental studies also show a regulatory rather than an initiating role for these F-BAR proteins¹¹. As the work of Dannhauser and Ungewickell⁸ illustrates, assembly of curved coats with a defined shape, along with a set of connections between the coat assembly units and the membrane, provides a more logical explanation of how bending and curvature are introduced than does an extrapolation of the *in vitro* properties of curvature-preferring molecules. The energy comes from the protein–protein interactions in the coat, and the unidirectionality of the process, from the splitting of ATP by the Hsc70 uncoating enzyme¹². Proteins such as those with BAR or ENTH domains, or other proteins that insert into the membrane as hairpins (for example, the ER-associated reticulons¹³), can clearly act as membrane curvature

sensors, marking the locations of deformed membrane (Fig. 2). All the curvature-sensor proteins have properties consistent with this role^{14,15}. In some cases, membrane localization activates enzymatic activities supported by other domains of the same protein; in others, the sensors in turn localize other proteins to particular curved membrane domains. The sensor proteins will, of course, also stabilize the curvature of the membrane with which they associate.

The papers by Dannhauser and Ungewickell⁸ and by Stachowiak and colleagues⁹ raise the bar for convincing explanations of how membrane deformations are generated in cells. Although their work comes from *in vitro* analyses of relatively artificial systems, they clearly show that any physical model for membrane bending must be evaluated for its relevance at the protein concentrations and membrane compositions occurring naturally in cells. Perhaps the simplest general model, consistent with present data, is that specific protein assembly, coupled to the membrane, is the most prevalent mechanism for bending a lipid bilayer in a cell. The polymerizing protein can be a coat (clathrin, COPI or COPII) or a cytoskeletal element (actin or tubulin). Curvature-sensing proteins can then accumulate to define the subsequent function and fate of the bent membrane.

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