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Membrane curvature and mechanisms of dynamic cell membrane remodelling

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Membrane curvature is no longer seen as a passive consequence of cellular activity but an active means to create membrane domains and to organize centres for membrane trafficking. Curvature can be dynamically modulated by changes in lipid composition, the oligomerization of curvature scaffolding proteins and the reversible insertion of protein regions that act like wedges in membranes. There is an interplay between curvature-generating and curvature-sensing proteins during vesicle budding. This is seen during vesicle budding and in the formation of microenvironments. On a larger scale, membrane curvature is a prime player in growth, division and movement.

Cellular membranes change conformation in striking ways during such processes as movement, division, the extension of neuronal arbors and vesicle trafficking. Vesicle budding and fusion occur with flux constantly maintaining the communication between membrane-bound compartments. In other cases, membrane curvatures are stabilized and are more permanent, for example in microvilli or the dendritic tree. In Fig. 1a we highlight the areas of the cell where there are regions of high membrane curvature.

Dynamic membrane remodelling is achieved by the interplay between lipids and proteins, and in this review we discuss the mechanisms that are used by the cell to generate, sense and stabilize local regions of membrane curvature. Areas of high membrane curvature frequently exist for only limited periods of time, and this is achieved primarily by using surrounding proteins to change the morphology. Thus in the formation of highly curved vesicles, the curvature is induced by the effects of membrane-associated proteins, the 'coat proteins'. The curvature is readily reversible when the coats dissociate, leaving the vesicle more fusogenic (as their curvature is not stabilized) and the coat proteins can now be reused in a further round of vesicle formation (giving an efficiency to protein usage).

Recent studies have shown how the highly dynamic changes in membrane curvature that accompany vesicle trafficking are brought about, and we discuss this emerging field. The topology of a budding vesicle has different degrees of positive and negative curvature (Fig. 2). There are key roles for the insertion of amphipathic helices in generating curvature and for BAR domains in sensing and stabilizing curvature. We introduce the ideas of local curvature generation, and how this is transmitted and maintained over a wider area by stabilizing domains and coat proteins.

We go on to address how membrane subdomains with a given curvature may have precise biological properties. They may lead to spatially regulated clustering of downstream interaction partners, or to the colocalization of transiently interacting proteins on the basis of curvature. Curvature modules within proteins are conjugated with other protein motifs and domains, and from these collaborative activities we can suggest some new ideas for how membrane curvature can be generated by multiple mechanisms and integrated into cell biology. But we start by considering the properties of membranes and how the lipid and protein components can influence bilayer topology.

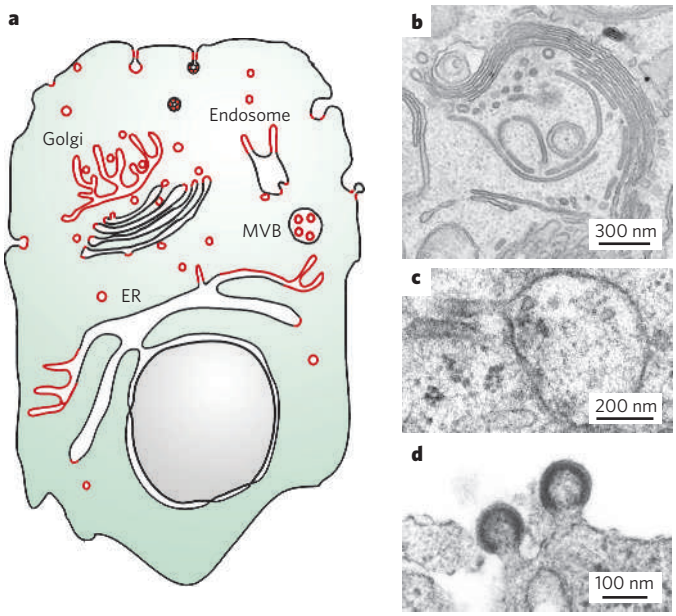

Figure 1 | Local differences in membrane curvature are hallmarks of cellular membranes. Many of the fine details of high local membrane curvature can be seen from the diagram (**a**) and the sample electron micrographs: **b**, fenestrations in the Golgi (from C. Hopkins and J. Burden, Imperial College London); **c**, tubule on endosomes (from P. Luzio and N. Bright, University of Utah); and **d**, HIV-1 viral budding (from W. Sundquist and U. von Schwedler, University of Utah). All of these can be described as local areas of positive or negative curvature (areas of high positive membrane curvature in a cell highlighted in red). Although it is fascinating to wonder how different membrane morphologies are adapted to the functions of different organelles, we concentrate here on how dynamic changes in morphology are generated. MVB, multi-vesicular body; ER, endoplasmic reticulum.

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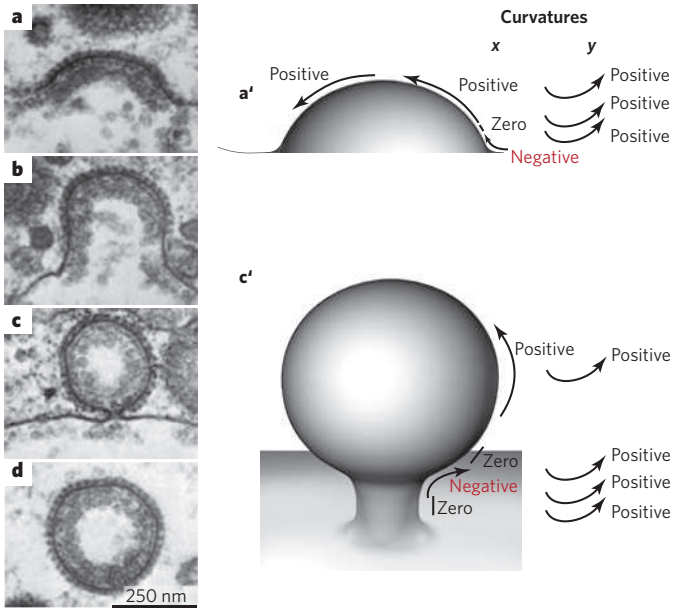


Figure 2 | Clathrin-coated vesicle budding where yolk protein is being incorporated into vesicles in oocytes. (From ref. 88; reproduced with permission from the The Company of Biologists.) The different stages (**a**–**d**) show progression of invagination and the corresponding membrane curvatures (**a**′, **c**′). Given that the membrane surface is two-dimensional we need to describe curvature in perpendicular directions. A sphere is positive in both directions, and the curvature of a tubule is positive in one direction and zero in a perpendicular direction. The curvature of the initial stage of vesicle budding is positive in both directions (**a**′). The curvature of a late-stage budding vesicle is more complex (**c**′). There is bidirectional positive curvature around the body of the vesicle, negative plus positive curvature (in perpendicular directions) at the neck and interface with the parent membrane, and positive plus zero curvature at the neck of a deeply invaginated vesicle. These types of curvature are constantly being formed and dissolved by the interplay between lipids and proteins.

The lipid component of membranes

The bilayer is a permeability barrier that separates the cell from its exterior and organelles from the cytoplasm. This allows a complex range of reactions both within these compartments and on the membrane surfaces. To communicate between the compartments, vesicles and tubules bud from donor compartments and fuse with others[1–4]. We may well wonder how these intermediates are formed, as such extreme deformation is unlikely to form spontaneously. The lipids in cell membranes are in a disordered liquid state[5], meaning that they are free to diffuse and mix in the plane of the bilayer leaflets, although the process may be more complicated than simple brownian diffusion[6]. Lipid mixtures *in vitro* do not readily reconstitute the local variations in curvature of organelle membranes. However, high mole fractions of some lipids are capable of deforming liposomes into tubules[7], and curvature-gymnastics are seen in giant liposomes of relatively simple lipid compositions, where different lipids segregate according to their chemical properties and lead to the formation of buds and domains on the liposome[8,9]. Such behaviours seem very 'cell-like' and clearly the lipid component of the membrane is capable of achieving distinct topologies, although the scale of these deformations is much larger than those discussed here. Moreover, the much more complex lipid mixtures present in a biological membrane, the significant protein component and the control that is needed over membrane dynamics mean that proteins have a crucial function in generation of cell-membrane morphology.

Membrane topology

A large portion of this review concerns vesicle trafficking, and so we describe the membrane curvatures that form a budding vesicle. We use 'positive' to indicate regions of membrane that curve inwards towards the cytoplasm. By this definition, the early stages of vesicle budding (shallow pits) have positive curvature and viruses budding out of the cell have negative curvature (see Figs 1 and 2). We will first consider the early stages of budding. The curvature of the dome (Fig. 2a,a′) can be described as having positive curvature in two directions. This matures into a deeply invaginated vesicle, which is ready to bud off (Fig. 2c,c′). At this stage there are more components of curvature present than there are in a simple dome. At the transition between the dome of the vesicle and the neck there is both positive and negative curvature, in perpendicular directions. There is positive curvature because the neck is still round (a transverse section would give a circle), but there is also negative curvature because there is formation of a concave surface (a longitudinal section of the budding vesicle shows an omega shape). The neck itself, present to a greater or lesser degree, is shaped like a cylinder; there is still positive curvature in one direction (it is still round) but zero curvature in the other (the sides approximate a straight line).

Five ways to bend a membrane

There are several mechanisms that could generate positive or negative curvature. The following five divisions (see Fig. 3) are used for simplicity, and we do not expect these processes to work in isolation.

Changes in lipid composition

At the very least, lipids have a permissive role in membrane curvature. The chemical properties of different lipid acyl chains or headgroups can favour different membrane curvatures: for example, lysophosphatidic acid (LPA) and phosphatidic acid (PA), which are interconverted by lysophosphatidic acid acyl transferase and phospholipase A_2 activity respectively[10–12], favour opposite curvatures. In addition, flippases (which transfer lipids from one leaflet to the other) give rise to membrane asymmetry[13,14], and enzymes that change lipid headgroup size will influence the area occupied by the lipids[15] and thus affect membrane curvature. Some of these changes may well be localized by limited diffusion barriers (for example the presence of transmembrane proteins or the knitting together of proteins by cytoskeletal or scaffolding attachments), and thus they may assist or antagonize changes in topology.

Lipid headgroups are the attachment sites for peripheral membrane proteins and therefore aid the recruitment of proteins necessary to generate curvature. Phosphoinositides (PtdIns) are particularly important as their headgroups are easily modified (see the review by Behnia and Munro in this issue, p. 597). For example, the presence of PtdIns(4,5)P_2 in the plasma membrane is essential for the budding of clathrin-coated vesicles, largely because the budding machinery binds to PtdIns(4,5)P_2 (refs 16–20). Similarly, in the invagination of vesicles into late endosomes there is a requirement for PtdIns-3-OH kinase[21], with which Hrs and other FYVE domain proteins interact[22], and for PtdIns(3)P-5-OH kinase[23].

As well as assisting or antagonizing curvature, lipids may also respond to curvature by concentrating in domains of curvature that they prefer. This is seen in the tubule-pulling experiment of the Goud laboratory[24] where lipids segregate into the tubules.

Inherent in the small size (and high curvature) of transport intermediates is an imbalance in the number of lipids in the inner and outer leaflets of the bilayer. In a liposome with an outer diameter of 50 nm with a membrane thickness of 5 nm, there is 56% more lipid in the outer leaflet than in the inner. So when a vesicle fuses, the imbalance in the outer and inner leaflet lipids has to be accommodated or there has to be a compensatory change. For example, the generation of negative curvature at the neck of the vesicle will, at least in part, relax the positive curvature of the dome.

When making dynamic or reversible changes in membrane curvature (as in making a transport vesicle or tubule) it may well be advantageous to avoid giving long-term stability to the high membrane curvatures as these trafficking intermediates will need to fuse with

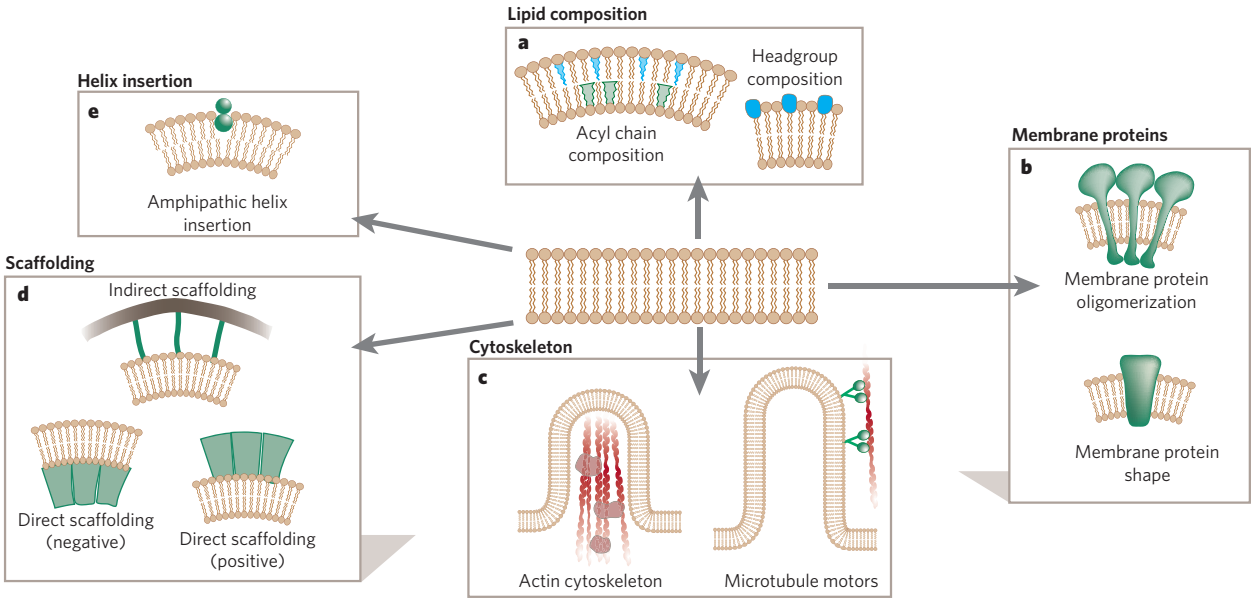


Figure 3 | Mechanisms of membrane deformation. The phospholipid bilayer can be deformed causing positive or negative membrane curvature. There are five main categories: **a**, changes in lipid composition; **b**, influence of integral membrane proteins that have intrinsic curvature or have curvature on oligomerization; **c**, changes in cytoskeletal polymerization and pulling of tubules by motor proteins; **d**, direct and indirect scaffolding of the bilayer; **e**, active amphipathic helix insertion into one leaflet of the bilayer.

recipient membranes, and this process may be aided by the instability and tension inherent in the high curvature. In such cases, peripheral protein association would be the primary driver of curvature, although timed headgroup turnover (as in the hydrolysis of PtdIns(4,5)P_2 by synaptojanin[25]) could also participate.

Influence of integral membrane proteins

Transmembrane proteins with a conical shape will naturally prefer curvatures that mould around their shapes. This shape is seen for the transmembrane domain of the nicotinic acetylcholine receptor, which has been observed at the tops of membrane folds at the neuromuscular junction[26,27] and is also seen in the structure of the voltage-dependent K^+-channel[28]. Acetylcholine receptors and many other transmembrane receptors and channels can be clustered by attachment proteins[29,30], leading to a greater effect on local curvature. If the membrane-spanning domain itself is not funnel shaped then curvature could theoretically still be caused by the overall conformation of clustered proteins or a conformational change, perhaps in response to ligand binding. Given that the structures of so few transmembrane proteins are known, the contribution of intrinsic shape to membrane curvature localization is a virgin field. It would be interesting if receptors destined for endocytosis were to partition and concentrate into regions of high positive curvature (leading to the exclusion of receptors not to be trafficked) or even aid the induction of curvature by lowering the energetic requirements. Indeed, progression of coated pits into vesicles occurs in tandem with cargo loading[31]. The role of curvature in defining membrane domains and in ion channel activity and receptor activation remains largely unexplored and has potential for new insights[32–34].

Cytoskeletal proteins and microtubule motor activity

Cytoskeletal assembly and disassembly is intimately linked with membrane-shape changes of the plasma membrane and of organelles[35,36]. Branching, bundling and treadmilling of actin filaments are involved in the generation and remodelling of many areas of high membrane curvature, including filopodia, pseudopodia, phagocytic cups and axonal growth cones. The ability of the cytoskeleton to influence membrane-shape changes is affected by membrane tension[37], and decreases in tension can help the generation of local curvature (for example, membrane trafficking events[13,38–40]). The cytoskeleton has a large role in maintaining membrane tension, and conversely actin rearrangements are responsive to changes in tension[41]. Therefore, we would envisage constant interplay between the responsive and propulsive power of the cytoskeleton and all the other factors that influence membrane tension and curvature, including trafficking and cell–cell adhesion.

Bursts of actin polymerization have been implicated in many endocytic invagination events[42–46]. Because actin polymerization has a force-generating role during motility and phagocytosis, it is tempting to assume that the reason for it here is the same, in aiding fission[43], but this is not yet clear.

In vivo imaging of cells shows that many tubules and vesicles move along microtubule tracks[47]. *In vitro* it can be demonstrated that kinesin motors attached to Golgi membranes can pull out tubules, and this can be achieved from liposomes with a modest number of motors[24]. Thus it is very likely that motors are at least partly responsible for fenestrated or tubulated organelle morphology (for example, the ER, Golgi and endosome) and the generation of some transport intermediates[48,49]. Given the evidence in favour of microtubules in vesicle generation, it is also possible that actin has a similar role with transport of vesicles mediated by myosins[50].

It is not surprising that cytoskeletal changes influence membrane remodelling in cell motility[51] and in tubule and vesicle carrier formation[52,53], but the cytoskeleton also has another function in directing the location of fusing and endocytosing vesicles and in localizing receptors and signalling complexes[54]. Also, many BAR-domain proteins have links by way of signalling proteins to the actin- and microtubule-polymerization machinery (for example tuba, β-centaurins and nadrins[55]; see also http://www.endocytosis.org/BARdomains/BARs.html). Much future interest will certainly lie in this interface between the cytoskeleton and the proteins that sense or drive curvature.

Scaffolding by peripheral membrane proteins

This can take different forms. Here we consider several families of proteins that deform a membrane by bracing it like a scaffold.

Proteins of the dynamin family bind to inositol lipids and form helical oligomers, constraining the membrane topology into a tubular shape[56,57]. They have an important role in the constriction of organelles during their division, in forming the necks of invaginating vesicles and

promoting their scission from the parent membrane. In plants they generate tubules during cell-wall formation[58]. This family of proteins uses GTP hydrolysis to effect membrane fission[57,59]. An analogy for the role of these proteins is an exoskeleton, supporting and sculpting the membrane from the outside. This can also be achieved by an endoskeleton, as in viral budding[60].

Coat proteins such as clathrin, COPI and COPII can also be considered as exoskeletons that influence membrane bending by polymerizing into curved structures, but these coat proteins do not have direct membrane associations and so are likely to act in conjunction with other proteins (see below)[61,62]. Caveoli are flask-shaped membrane invaginations where caveolin oligomerizes to form the coat[63]. Unlike COP and clathrin-coated vesicles, caveolin is membrane-associated and this could aid in membrane bending by insertion.

BAR domains are modules that sense membrane curvature (see Box 1). This ability to bind preferentially to curved membranes can be deduced from the concave shape of the membrane-binding region. The sensing is shown by its tighter binding to liposomes whose curvature is closer to the intrinsic curvature of the BAR[55]. The energetics of BAR-domain binding to membranes for amphiphysin also leads to the conclusion that the binding energy is close to that required to bend the membrane[64].

BAR domains are formed by dimerization, which is probably enhanced by membrane binding, and therefore the other constituent domains of the protein are presented as pairs. This could, for example, lead to the co-recruitment of two binding partners or a change in selection of a monomeric for dimeric/multimeric partners and thus generate a unique downstream signal based on the initial curvature-sensitive binding event.

BAR domains are also frequently found in combination with N-terminal amphipathic helices (Box 2). They are then called N-BAR domains (see below). This is an interesting combination and can be seen in amphiphysin, endophilin, BRAP and nadrin. All these N-BAR domains lead to membrane tubulation *in vitro*[55,65–68]. In *Drosophila* the N-BAR protein amphiphysin is involved in T-tubule formation in flight muscles and in its absence the T-tubule network is disrupted, preventing flight. In the synapse, amphiphysin is proposed to form or stabilize a very different tubule structure, that of the neck of clathrin-coated vesicles. The degree of positive curvature of the neck is close to that of the BAR, and thus this protein is suited for the recruitment of its binding partner, dynamin, to its correct location[65,69]. Dynamin may also aid in neck formation as it polymerizes into tubules of the same diameter (see exoskeleton discussion above). BAR domains and homologous domains are found in many trafficking proteins and their role in curvature sensing and stabilization will need much more study. We have recently shown that the BAR-domain protein sorting nexin-1 is involved in tubule extension from endosomes[70]. This protein seems to coat the tubule extensions that are involved in trafficking mannose-6-phosphate receptors to the *trans*-Golgi network (TGN).

Active helix insertion into membranes

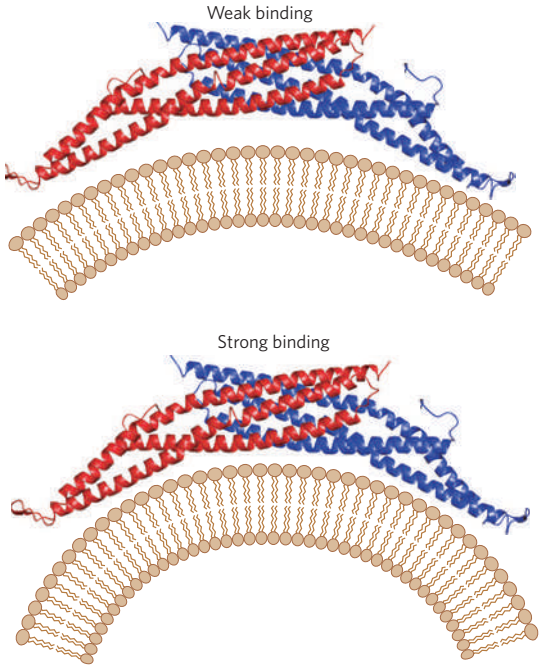
Amphipathic helices inserted into the bilayer result in increased positive membrane curvature (Box 2). In the case of epsin this helix folds and inserts on PtdIns(4,5)P_2 binding. Epsin in turn binds to clathrin and promotes its polymerization into a cage-like structure, and this stabilizes the change in local curvature. Amphiphysin, endophilins, BRAPs and nadrins all have BAR domains with an amphipathic helix at the N terminus (N-BAR domains). These should work in a similar manner[68] to cause local membrane curvature and in these cases we would predict curvature stabilization by the banana-shaped BAR domain instead of by clathrin or another coat protein. Arf and Arl proteins also have N-terminal amphipathic helices that are extended in response to GTP binding, and Arfs are involved in COP1 vesicle budding and in recruitment of GGA and AP1 complexes to membranes[71–73]. By analogy with epsins, these Arfs and Arls are predicted to function in curvature generation alongside stabilization by coat proteins. Sar1 (another small GTPase with an N-terminal amphipathic helix) is likely to function in a similar manner for COPII-coated vesicle budding. The COPII coat structure has already been shown to have a surface that will follow the curved membrane and thus stabilize the curvature[74].

Box 1 | BAR domains and stabilization of membrane curvature

BAR domains are banana-shaped lipid-binding domains found in a wide variety of proteins, which bind to membranes through their concave surface[56] (see also http://www.endocytosis.org/BARdomains/BARs.html). They are dimers, and given that the dimer interface and the membrane-binding region overlap, membrane binding may stabilize the dimer formation[89]. If dimerization is more effective on membrane binding than in the cytosol then multimeric effectors will be better recruited to a membrane-bound protein. For example, dynamin (which is a dimer) binding to amphiphysin will clearly be of much higher avidity when amphiphysin is a dimer.

The BAR interaction with membranes is largely electrostatic and binds to negatively charged membranes. A high concentration of lysine and arginine residues between helices 2 and 3 in some BARs help to give some PtdIns(4,5)P_2 preference over PtdSer (see also the review by McLaughlin and Murray in this issue, p. 605). Other BAR proteins contain specific membrane-targeting PH or PX domains to locate them to specific compartments[56]. BAR domains bind more readily to highly curved liposomes (see Box 1 Fig. 1)[56]. Thus the domain on its own is a sensor of high positive curvature. We should also consider that given a high concentration of a curvature sensor it is clearly possible that a sensor will become an inducer.

An additional feature of some BAR domains is the presence of an N-terminal amphipathic helix (an N-BAR domain). As discussed in Box 2, this amphipathic helix will lead to membrane bending. Thus it is interesting to find these two curvature modules side by side in many proteins.



Box 1 Figure 1 | The amphiphysin BAR domain in association with low-curvature and high-curvature membranes. The BAR domain binds better to the more highly curved membranes because there is more opportunity for electrostatic interactions across the complete membrane-binding surface of the BAR.

Coupling curvature to function

The examples below illustrate the involvement of lipids and proteins in the formation of positive and negative membrane curvature. We concentrate on the making of transport vesicles where membrane curvature is mediated by the collaboration of different mechanisms at different stages of budding events. The lessons can be extended to the

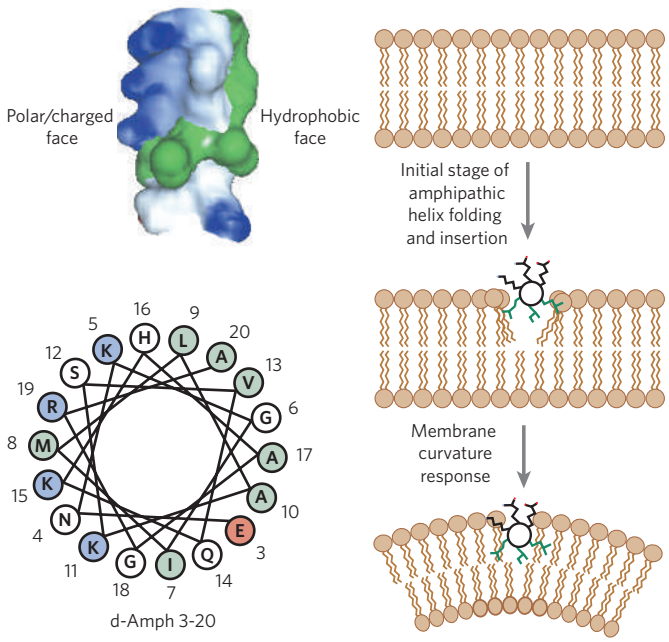
Box 2 | Amphipathic helices and membrane curvature

Amphipathic helices are stretches of α-helix, one side of which is polar (charged) and the other hydrophobic. These helices are frequently unstructured until they insert into membranes, when the helices are predicted to sit flat on the membrane surface with the hydrophobic residues dipping into the hydrophobic phase of the membrane[17,55]. The result will be a displacement of lipid headgroups and a reorientation of acyl chains, giving an orientation more favourable to higher curvature. The fact that this mechanism of curvature can be reproduced on a lipid monolayer[18] shows that this is not just a generation of bilayer asymmetry, nor simply headgroup displacement, but that it is primarily the reordering of the lipids in an individual leaflet with a tighter bend. We can model how the helix looks by taking an ideal α-helix and modelling the sequence of interest. In the figure we illustrate this for the initial residues of *Drosophila* amphiphysin, which *in vivo* is involved in stabilization of T-tubule formation in muscles[67]. This model is an oversimplification as there is sometimes a kink in the helix and the nature of the polar face may give different properties to these helices (see work on synuclein and on ArfGAP1 and synthetic peptides[78,89,90]). We have previously shown that the N-terminal residues of amphiphysin adopt a helical conformation on membrane binding and it is clear that from residue 9 forward there is a strong hydrophobic face and a polar face. Another way to visualize the amphipathic nature of a stretch of amino acids is to use an axial projection of the helix (a helical wheel, see http://www.site.uottawa.ca/~turcotte/resources/HelixWheel/).

Amphipathic helices are found on trafficking proteins as diverse as small G proteins, epsins and proteins containing BAR domains. These helices are all predicted to fold on membrane binding. In the case of epsin[1] this folding was partly induced by the interaction of polar residues with PtdIns(4,5)P_2 in the membrane, and thus this protein folds the helix around the headgroup, engulfing it in a pocket[17].

The most important feature of the amphipathic helix for this review is its effect on membrane curvature. Given the asymmetric insertion (see figure) it acts like a wedge inserted into one leaflet of the membrane. All the amphipathic helices we have studied effect membrane curvature given a high local concentration. Thus it makes sense that epsins bind and promote clathrin polymerization, concentrating the curvature into a local membrane area.

A second feature of an amphipathic helix insertion is that hydrophobic interactions are relatively short-range but strong, so release from the membrane is slow. We have shown this for the displacement of epsin from membranes once its helix is inserted[91]. Both features probably work together to generate local curvature.



We have argued that amphipathic helices will promote an increase in membrane curvature when folded and inserted between the lipid headgroups, but it is also entirely possible that some amphipathic helices will insert only in response to high curvature, and thus even a humble helix may act like a curvature sensor. This is likely to be the case for ArfGAP1, which promotes Arf1 GTP hydrolysis during COPI vesicle budding, thus coupling vesicle budding to the initial stages of the uncoating reaction[78].

Colour coding: green is hydrophobic, white is polar, blue is positive charge, red is negative charge. The back face of the helix is frequently positively charged, probably in part because of the proximity of the (negatively charged) membrane.

formation of tubule carriers, viral budding and the generation of non-trafficking curvatures.

Creating transport carriers

The study of clathrin-coated pit invagination revealed that a number of proteins work together to promote membrane bending and nascent vesicle formation. It has previously been widely believed that coat protein polymerization drives curvature formation[75], but it is now recognized that this is a process driven by direct membrane–protein interactions. Epsins can generate membrane domes *in vitro* by insertion of an amphipathic helix on PtdIns(4,5)P_2 binding and on polymerization of clathrin (see helix insertion above). Clathrin performs an important function by concentrating epsins and by forming a scaffold around the curvature. Clathrin cannot extend around the negative curvature of the junction between the neck and vesicle body and so other proteins are needed. Amphiphysin with its N-BAR domain also has a role in assisting or in generating curvature[55]. The unidirectional curvature and limited depth of the domain may well be suited to the transition region between the vesicle body and neck (see Fig. 2). It may therefore ultimately prefer to locate or aid formation of the neck and thus recruit dynamin to the correct region. Finally the protein dynamin polymerizes on membrane binding and forms an exoskeleton around the vesicle neck. Upon GTP hydrolysis by dynamin, it may undergo a lengthwise extension[58] and/or shrinkage in width (constriction)[59], resulting in vesicle scission. A further factor leading to scission may be the need to relax the mechanical stress in the membrane at the neck[76]. Thus all these proteins (and lipids) participate in the generation of curvatures seen in nascent vesicle formation.

This proposal that amphipathic helices of epsins and amphiphysin helps drive membrane curvature was a new departure from the thought that the clathrin coat alone would force curvature on the membrane. This same traditional thread flows through the COPI- and COPII-coated vesicle field where coat polymerization alone was thought to define the vesicle curvature[74]. For COPI vesicle budding, recent studies have highlighted the presence of a GAP for Arf1 in the coat whose activity is sensitive to curvature[77,78] and clearly these same coats have an Arf1 with its amphipathic helix[79]. Thus it is tempting to speculate that Arf1 and the coat proteins together generate the bud curvature. The evidence suggests that ArfGAP1 is activated by bud formation and so Arf1 GTP is hydrolysed, thus aiding the beginning stages of uncoating, even before the vesicle is detached.

Budding with the opposite topology

Multivesicular bodies (MVBs) are late endosomes with internal vesicles that sort membrane proteins destined for degradation into these vesicles. From the viewpoint of the cytoplasm these vesicles have the opposite curvature to clathrin and COP-coated vesicles. Despite a great deal of work in this area the mechanisms of bending with a negative curvature remain elusive. Budding of vesicles from the limiting membrane of the late endosome into the multivesicular body and viral budding at the cell surface (or indeed from other organelles) are thought to involve similar molecular components. The lipid 2,2′-LBPA is enriched on the internal membranes of late endosomes[80] and favours budding into this compartment[81]. Proteins involved in sorting of cargo into the yeast vacuole were isolated as Vps mutants (vacuolar protein sorting)[82]. The class E mutants have an enlarged late endoso-

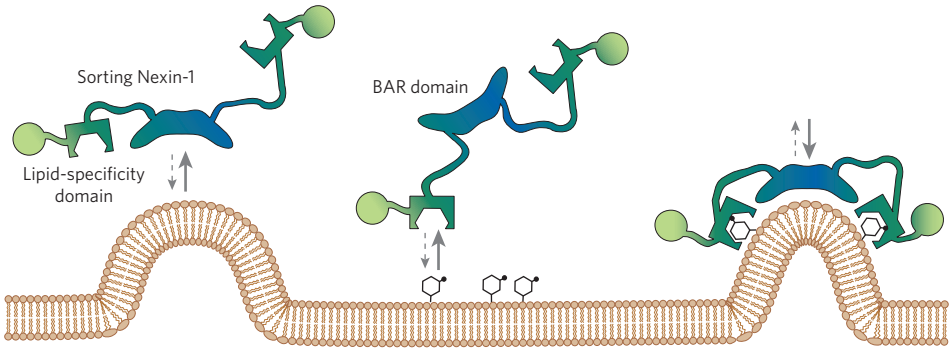


Figure 4 | Coincidence detection. In sorting nexin-1 there is a dimerized BAR domain (blue), which recognizes membrane curvature, and an additional lipid specificity domain (dark green, PX domain). Binding of both domains — that is, coincidence detection of both membrane composition and curvature — is required for recruitment of the protein and for stabilization of membrane curvature. Hexagons represent phosphatidylinositol phosphate headgroups to which PX domains bind.

mal compartment, presumably because of an inability to invaginate the limiting membrane to form the MVB. These proteins can be sorted into complexes and associated proteins that together dynamically interact with endosomal membranes. They help sort cargo and may well have an active role in the exvagination process. Alix/Bro1, a class E Vps mutant involved in MVB formation[83], binds to LBPA-containing liposomes and regulates the formation of internal vesicles[83]. The structure of a Bro1 domain has an interesting boomerang shape[84] (somewhat like the BAR domain) and could potentially function in negative curvature generation, but there is as yet little evidence for this. RNA interference of LIP5 and CHMP4 inhibit MVB formation and also the budding of HIV-1 viral particles[85]. Given the number of coiled-coil proteins involved in MVB formation, it would be interesting if there were a inverse BAR domain, an 'I-BAR'.

Future perspectives

Coincidence detection and membrane microenvironments

A cell has many curved membranes (Fig. 1) and so additional mechanisms of selection must be used if the cell is to sense and respond selectively to membrane curvature. The coincident detection of a number of inputs is a common theme in biology that minimizes noise and gives highly selective responses. BAR-domain proteins give us an example of coincidence detection between lipid curvature and composition. We have already noted the presence of PH domain or PX domains alongside BAR domains in the same proteins[55]. Point mutants show that the PH domains, the PX domains or the BAR domains alone are insufficient for membrane targeting but that these domains work together (Fig. 4)[55,70]. This gives rise to a precise localization of the protein in question. Given the range of domains found in proteins with BAR modules it is likely that this coincidence detection will work for many different levels. Again we can use an example from proteins containing BAR domains. Some of those so far identified have GAP and GEF activities. By analogy to the above examples with PX or PH domains, it can be predicted that these may be curvature sensitive. What seemed remarkable about the PH-BAR and PX-BAR examples is that the proteins do not target visibly in the absence of either domain. The BAR domain lipid-binding mutant was not expected to disrupt dimerization of the BAR, but surprisingly the supposedly dimeric protein (thus two PH domains) does not localize to membranes. This may hint at the importance of membrane binding for stable dimer formation, or it may simply mean that the BAR and the two PH domains are all required for membrane binding.

The selective binding of proteins depending on curvature and the partitioning of lipids favouring that curvature into these regions gives the exciting possibility of a local microenvironment on the membrane. It could, for example, favour the segregation of transmembrane proteins for incorporation into vesicles or tubules or the preferential localization of ion channels in protrusions. It would be interesting to know whether the transport tubules extending from endosomes could concentrate cargo by curvature preference. Similarly, the localization of GAP or GEF activities according to curvature could lead to tight regulation of small G-protein GTP/GDP status and therefore (for instance) selective actin polymerization or signalling pathway activation at these domains. This is like an ecological niche, where curvature defines a protein–lipid microenvironment in which specific interactions are more likely to occur. This could be a dynamic environment where the domain is transient and only forms in response to a range of coincident stimuli.

The interplay between lipids and proteins is key to how cells control membrane shape. This ability of proteins to alter membrane curvature directly is an emerging field of study and the above discussions readily illustrate the importance of multiple mechanisms to obtain effective membrane curvature changes. As in clathrin-coated vesicle formation there is a network of interactions[86] and interlinking pathways that must be considered before we will have understood how cells generate, control and respond to curvature domains and dynamics. ■

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