Research News

Membrane curvature: a case of endofeelin'…

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Endophilin A1, a cytoplasmic protein essential for the budding and fission of synaptic vesicles from presynaptic plasma membranes, is implicated in the generation of membrane curvature. Endophilin A1 exhibits intrinsic lysophosphatidic acid acyl transferase activity, reflecting its interaction with both the hydrophobic portion and the headgroup of acidic phospholipids. A recent study demonstrates that endophilin A1 binds to liposomes and alone is sufficient to deform them into narrow tubules, thus generating positive bilayer curvature. The recently identified endophilins B, which associate with membranes of the early secretory pathway, also bind to acidic phospholipids and tubulate liposomes, as do the endophilin-interacting proteins amphiphysin and dynamin. Thus, a novel concept for tubulo-vesicular membrane dynamics emerges in which a team of proteins distinct from, but often operating in concert with, the 'classical' coat proteins is pivotal in the generation of membrane curvature.

Membrane dynamics between subcellular compartments of eukaryotic cells involve changes in membrane shape. For membrane traffic to occur, the two major types of membrane structures formed are vesicles and tubules, and these are characterized by a distinct geometry. Vesicles ideally are spherical objects (Fig. 1a, c), whereas tubules, which may or may not pinch off from the donor membrane, are for the most part cylindrical (Fig. 1b, c). Distinct geometries imply differences in membrane curvature. Spherical vesicles are characterized by positive bilayer curvature [1] in all three dimensions (Fig. 1; green), whereas the curvature of the cylindrical portion of a membrane tubule is, in principle, two-dimensional (Fig. 1; blue).

The changes in membrane shape that occur in the formation of vesicles and tubules vary, depending on the shape of the donor membrane. In the case of intracellular compartments, membrane traffic typically originates from cisternae and tubules, whereas in the case of the plasmalemma, the donor membrane often has an essentially planar shape. In the case of such a donor membrane, the transition zone to the forming vesicle or tubule is characterized by a saddle-like shape (Fig. 1a, b; red). The latter combines elements of both positive and negative curvature (depending on whether the membrane surface is being followed in parallel, or perpendicular, to the plane of the donor membrane).

The shape of a biological membrane reflects the shape of its principal constituents – that is, membrane lipids and integral membrane proteins, as well as their interaction with each other and with peripherally associated proteins (including glycoproteins and proteoglycans), the cytoskeleton and the extracellular matrix. Three principal types of interaction exist: (i) protein–protein, (ii) lipid–lipid, and (iii) protein–lipid. The classical paradigm of a membrane shape change – that is, vesicle formation mediated by a cytoplasmic coat, has traditionally focused on the first type of interaction, that between the coat proteins themselves and between coat components and the cytoplasmic domains of integral membrane proteins [2–5]. Lipid rafts [6,7] have been the most frequently studied example of the second type of interaction, but their implications for changes in membrane curvature are only just beginning to be addressed [8]. Here, we concentrate on protein–lipid interactions that result in changes of membrane shape. Specifically, we discuss proteins that operate, in concert with the clathrin coat, in synaptic vesicle budding and fission from the plasma membrane – dynamin, amphiphysin and, in particular, endophilin.

Following the seminal observations that the GTPase dynamin can polymerize into rings [9] and assemble as such on tubules generated from presynaptic membranes by brain cytosol in the presence of GTPγS [10], it was shown that dynamin alone is sufficient to change the shape of liposomes, causing either tubulation [11] or vesiculation [12], depending on the lipid composition. Subsequently, it was shown that amphiphysin, a protein binding to the clathrin coat as well as to dynamin [13,14], also is alone sufficient to tubulate liposomes and enhances the liposome vesiculation by dynamin [15]. De Camilli and colleagues now report [16] that a third protein, endophilin [14,17], which interacts with both dynamin [18] and amphiphysin [19], and which has been implicated in membrane curvature [17], also is able to tubulate liposomes.

Endophilin and membrane curvature

The endophilins are a family of evolutionarily conserved proteins that can associate with the cytoplasmic surface of membranes. They comprise two subgroups – endophilins A and endophilins B – of which the neuron-specific endophilin A1 of mammals has been studied the most extensively [17]. Work using cell-free systems [20,21], microinjection into the lamprey giant synapse [22,23] and *Drosophila* mutants [24,25] has provided compelling evidence for an essential role of endophilin A in the endocytosis of the synaptic vesicle membrane from the presynaptic plasmalemma. Endophilin A can engage in both protein–protein and protein–lipid interactions, and each property is conserved in evolution [17]. Through its C-terminal Src-homology 3 (SH3) domain, endophilin A1 can interact with the proline-rich domains (PRDs) of amphiphysin [19] and dynamin [18], as well as synaptojanin [18], a phosphatidylinositol 5′-phosphatase implicated in synaptic vesicle uncoating [26].

Evidence that endophilin A1 is able to interact directly with lipids originated from the observation that it can bind to lysophosphatidic acid (LPA) and fatty acyl-coenzyme A (CoA) and condense them to phosphatidic acid (PA) [21]. Given that lipid shape affects membrane curvature [1] and that LPA and PA have distinct shapes, it was hypothesized that endophilin A1 might alter the curvature of the cytoplasmic membrane leaflet, promoting either negative [21] or positive [17] curvature, depending on whether the lipids bound are originally cytosolic or

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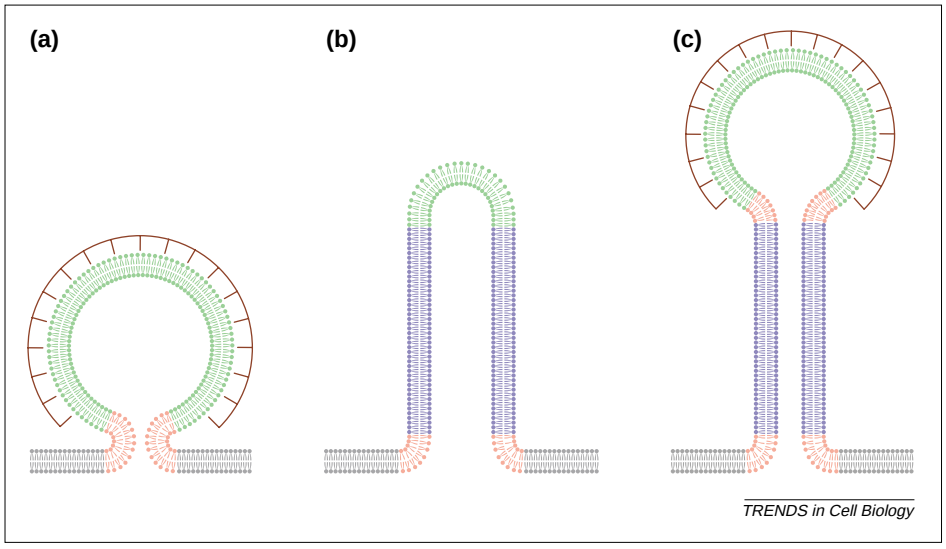

Fig. 1. Membrane vesiculation and tubulation. (a) Vesicle formation from a planar donor membrane; (b) tubule formation; (c) vesicle formation from a membrane tubule. Blue, cylindrically shaped membrane with two-dimensional curvature – likely site of action of tubulating proteins such as dynamin, amphiphysin and endophilin. Green, spherically shaped membrane with positive bilayer curvature in all three dimensions; inducers of positive curvature such as amphiphysin and endophilin might also operate here. Red, saddle-shaped membrane with mixed, positive and negative, curvature – proposed site of changes in lipid shape resulting from lysophosphatidic acid acyl transferase (LPA-AT) activity of endophilin. Brown, protein coat.

already embedded in the membrane. Condensation of membrane-embedded LPA and cytosolic fatty acyl-CoA to PA would promote negative curvature, in particular when unsaturated fatty acyl-CoA such as arachidonoyl-CoA is used [21] (Fig. 1; red). By contrast, negative curvature would be reduced if endophilin A1 were to convert membrane-embedded unsaturated fatty acyl-CoA to PA by addition of LPA from a cytosolic pool. If endophilin A1 utilizes both LPA and fatty acyl-CoA from cytosolic pools and mediates insertion of the resulting PA into the cytoplasmic membrane leaflet, this would generate positive curvature [17,27] (Fig. 1; blue and green).

While morphological data consistent with the proposed role of endophilin A1 in changing membrane curvature were reported previously [22], direct evidence has now been provided in a recent, important report [16]. Using liposomes prepared from either brain lipid extract or synthetic lipids, endophilin A1 was found not only to bind to liposomes, as anticipated from the previous data [21], but, interestingly, to deform them into tubules with outer diameters ranging between 20–100 nm [16]. Endophilin A1 is apparently assembled in rings around the tubules, consistent with it generating positive bilayer curvature (Fig. 1; blue).

Like its intrinsic LPA acyl transferase (LPA-AT) activity [21], the liposome-tubulating activity of endophilin A1 resides in its N-terminal domain [16]. Deletion of the first 33 amino acid residues of endophilin A1 abolishes, and an endophilin A1 fragment consisting of the N-terminal 125 amino acid residues is sufficient for, liposome binding and tubulation. Amino acid residues 7–24 of endophilin A1 are predicted to yield an amphipathic helix, of which the hydrophilic face contains several basic residues likely to interact with acidic phospholipids. Remarkably, mutation of a single phenylalanine residue of the hydrophobic face of the putative helix to a hydrophilic amino acid residue abolishes both liposome binding and tubulation. This suggests that endophilin A1 generates membrane curvature through the interaction of crucial hydrophobic residues with the bilayer, perhaps promoted by, or in concert with, the binding to acidic phospholipids (notably PA, see below).

Interestingly, the N-terminal domain of amphiphysin, which, as in the case of endophilin A1, mediates liposome tubulation [15,16], contains a stretch of sequence that is homologous to the corresponding domain of endophilin and is also predicted to yield an amphipathic helix [16]. Hence, endophilin A1 and amphiphysin exhibit the same type of protein–protein and protein–lipid interactions:

- binding to the PRD of dynamin through their C-terminal SH3 domains; and
- the tubulation of liposomes through their N-terminal putative amphipathic helices.

Reflecting these common features, both amphiphysin [15,16] and endophilin A1 [16] co-oligomerize with dynamin rings on liposome tubules. However, endophilin A1 differs from amphiphysin in that it inhibits liposome vesiculation by dynamin.

Role of PA and the LPA-AT activity of endophilin

Membrane fission is one, although not necessarily the only, step in synaptic vesicle endocytosis that has been proposed to be promoted by the intrinsic LPA-AT activity of endophilin A1 [21]. This proposal was also supported by the demonstration that a distinct protein also exhibiting intrinsic LPA-AT activity, BARS, induces fission of Golgi tubules [28]. Although liposome tubulation is presumably more related to membrane shape changes occurring prior to fission – that is, during vesicle budding and constriction of the neck of the nascent vesicle (see below), than to the fission process itself, the question arises whether the LPA-AT activity of endophilin A1 has a role in liposome tubulation. Farsad *et al.* [16] report that endophilin A1 is able to generate tubules from liposomes made using a defined mixture of synthetic phospholipids lacking the substrates for its intrinsic LPA-AT activity – LPA and fatty acyl-CoA. This shows that liposome tubulation mediated by endophilin A1 does not require ongoing LPA-AT activity. The efficiency of tubulation mediated by endophilin A1 increased when liposomes made from a brain lipid extract were used [16], but it remains to be determined whether this reflects the presence of the substrates for endophilin LPA-AT activity, its product PA or other lipids promoting liposome tubulation. These observations have implications for the role of the LPA-AT activity of endophilin A1 in the budding of the synaptic vesicle membrane, of which we find the following the most likely.

First, consistent with observations made on the formation of synaptic-like microvesicles in perforated PC12 cells [21], the LPA-AT activity of endophilin A1 might have a more crucial role in the case of budding of the synaptic vesicle

membrane from the presynaptic plasma membrane than in the case of tubulation of liposomes. Aside from the fact that biological membranes, in contrast to liposomes, contain integral and peripheral proteins, the lipid composition of presynaptic membranes and synaptic vesicles is clearly different from that of the phospholipid liposomes shown to undergo endophilin A1-mediated tubulation without acyl transfer to LPA [16]. For example, presynaptic membranes and synaptic vesicles are characterized by a high content of cholesterol, a key component of lipid microdomains [6,7] that constitute an important parameter for membrane curvature changes, budding and fission [8].

Second, the LPA-AT activity of endophilin A1 might not be essential as such for membrane curvature changes occurring during synaptic vesicle budding, but might have a modulatory role, for example by increasing the speed at which such changes take place.

Third, as proposed previously [17], the intrinsic LPA-AT activity of endophilin A1 might be a means of putting the protein into a PA-bound state. It is conceivable that the ability of endophilin A1 to tubulate liposomes and hence to generate positive curvature reflects its binding to acidic phospholipids, in particular PA. The LPA-AT activity of endophilin A1 shows a high preference for LPA as compared with other lyso-phospholipids [21], indicating that endophilin A1 specifically binds to the LPA/PA headgroup. In light of these three lines of consideration, it would be interesting to know whether the presence of PA in cholesterol-containing liposomes increases the rates of tubulation by endophilin A1.

Significance of liposome tubulation for the site of action of endophilin A1 *in vivo*

What might be the significance of the ability of endophilin A1 to tubulate liposomes with regard to its site of action in the cascade of steps in synaptic vesicle budding and fission from the plasma membrane? Morphological analyses dissecting the various steps of synaptic vesicle budding and fission have been carried out on the lamprey giant synapse microinjected with either antibodies against the SH3 domain of endophilin A [22] or the SH3 domain itself [23] and on the neuromuscular junction of *Drosophila* mutants with drastically reduced endophilin A levels [24]. These analyses indicate that endophilin A operates at multiple steps in synaptic vesicle budding and fission, being required for:

- the early stages of synaptic vesicle budding, i.e. the formation of shallow pits up to the hemi-spherical stage [24];
- the late stages of budding, i.e. the formation of deeply invaginated, constricted pits [22]; and
- fission [23].

Consistent with these observations, the interaction of the N-terminal domain of endophilin A1 with lipids, which leads to liposome tubulation [16] – that is, the generation of positive bilayer curvature – may well promote vesicle budding (Fig. 1; green). Additional possible roles of endophilin-mediated membrane tubulation (Fig. 1; blue) include, obviously, the constriction of the neck of the budding vesicle. In this context, it is interesting to note that the endophilin-induced tubules had outer diameters of 100 down to 20 nm [16], which is a range including the diameter of shallow pits and constricted vesicle necks.

Endophilin – a bifunctional molecule

In contrast to the N-terminal domain of endophilin A1, which interacts with lipids, its C-terminal SH3 domain interacts with other proteins, notably dynamin [18]. The latter interaction is required for endophilin A1 to stimulate synaptic vesicle endocytosis in a cell-free system [21] and, specifically, has been shown to be of crucial importance for fission [23]. In this context, roles upstream and downstream to the action of dynamin, which are not mutually exclusive, have been discussed for this interaction of endophilin A. On the one hand, endophilin A might be part of a dynamin-controlled effector system for fission, with the endophilin SH3 domain being essential for endophilin A to be recruited to the neck of the nascent vesicle [21,23]. On the other hand, endophilin A might be required for the proper recruitment of dynamin to exert its mechanical role in fission of the vesicle neck. The available morphological evidence, showing (i) the localization of endophilin A1 on dynamin-coated tubules generated from synaptic membranes [22] and (ii) the reduced occurrence of such tubules upon depletion of endophilin A1 [22], is consistent with either role.

Beyond synaptic vesicle endocytosis

Synaptic vesicle endocytosis is not the only membrane shape change originating from the plasmalemma for which the lipid binding and tubulating activity of endophilin–amphiphysin–dynamin complexes is likely to be of crucial importance, nor is the role of these proteins confined to the plasma membrane. Endophilin A and dynamin each constitute protein families that include not only neuron-specific isoforms such as endophilin A1 and dynamin 1 but also members such as endophilin A2 and dynamin 2 whose ubiquitous expression is inconsistent with a role solely in synaptic vesicle endocytosis [18]. Thus, dynamin 2 has been reported to have a role in the formation and/or maintenance of tubular invaginations of the plasma membrane at podosomes [29], and endophilin A2, which binds to dynamin 2 [18], is colocalized with dynamin 2 at podosomes [29].

Moreover, a second group of proteins with significant sequence homology to, and a similar domain organization as, the endophilins A, referred to as endophilins B [17], has recently been characterized. Like the endophilins A, endophilins B are conserved in evolution, bind to lipids, tubulate liposomes and exhibit LPA-AT activity [16,17; J. Modregger, A.A. Schmidt, B. Ritter, W.B. Huttner and M. Plomann, in prep.]. However, in contrast to the endophilins A, endophilins B do not appear to operate in endocytosis and are not localized to the plasma membrane and endosomes but, rather, to the endoplasmic reticulum and Golgi complex, suggestive of a role in the early secretory pathway ([16]; J. Modregger, A.A. Schmidt, B. Ritter, W.B. Huttner and M. Plomann, in prep.).

Finally, another protein, referred to as RICH (RhoGAP interacting with CIP4 homologs), which catalyzes GTP hydrolysis on the small GTP-binding proteins Cdc42 and Rac1 and binds to CIP4 (Cdc42-interacting protein 4), was found to also contain an N-terminal domain with homology to that of the endophilins [30]. This suggests that such domains might have evolved as functional modules in a variety of protein families.

Concluding remarks

Dissecting the mechanism of synaptic vesicle endocytosis has led to a conceptual

advance as to how the changes in membrane shape that occur during tubulo-vesicular membrane dynamics come about. As exemplified by the endophilins, the generation of membrane curvature involves proteins that operate in concert with 'classical' coat components and that (i) bind to certain lipids, (ii) modify their shape, and (iii) deform the bilayer. It does not take too much foresight to predict that the role of such proteins in membrane dynamics will turn out to be broader than is presently known, and that the characterization of the lipid microdomains to which they bind will be rate-limiting for understanding the details of their function.

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