Focus Review

Let's go bananas: revisiting the endocytic BAR code

Britta Qualmann*, Dennis Koch* and Michael Manfred Kessels*

Institute for Biochemistry I, University Hospital Jena—Friedrich Schiller University Jena, Jena, Germany

Against the odds of membrane resistance, members of the BIN/Amphiphysin/Rvs (BAR) domain superfamily shape membranes and their activity is indispensable for a plethora of life functions. While crystal structures of different BAR dimers advanced our understanding of membrane shaping by scaffolding and hydrophobic insertion mechanisms considerably, especially life-imaging techniques and loss-of-function studies of clathrin-mediated endocytosis with its gradually increasing curvature show that the initial idea that solely BAR domain curvatures determine their functions is oversimplified. Diagonal placing, lateral lipid-binding modes, additional lipid-binding modules, tilde shapes and formation of macromolecular lattices with different modes of organisation and arrangement increase versatility. A picture emerges, in which BAR domain proteins create macromolecular platforms, that recruit and connect different binding partners and ensure the connection and coordination of the different events during the endocytic process, such as membrane invagination, coat formation, actin nucleation, vesicle size control, fission, detachment and uncoating, in time and space, and may thereby offer mechanistic explanations for how coordination, directionality and effectiveness of a complex process with several steps and key players can be achieved.

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Cellular membranes—between relaxation and defined topology

The lipid bilayers of a cell represent natural barriers and thereby bring about the required compartmentalization of life functions. Well-controlled transport processes across

membranes and vesicular transport ensure crosstalk and material exchange among the different compartments and with the extracellular space. The functions of the plasma membrane hereby involve the generation of functional microdomains, which can often also be defined structurally. Structural membrane inhomogeneities are brought about (i) by lipid components (e.g., phosphoinositides and lysophosphatidic acid) that are inverted conical lipids and induce positive curvatures (i.e., convex surfaces), (ii) by integral proteins and (iii) by proteins associating transiently with the membrane surface and manifest in different sorts of cellular protrusions and invaginations.

Shaping membranes with BAR domains

The local shape of a membrane depends on which components are present and on how they are spatially distributed. *Vice versa*, a given membrane will resist bending because alteration of its topology will create tension in the lipid bilayer and/or requires spatial redistribution of topology-influencing membrane components (Figure 1). Despite these obstacles, proteins evolved that are able to overcome predefined membrane topologies and bending resistances and have the power to shape membranes. Two major mechanistic principles of membrane bending and curvature sensing were suggested, the hydrophobic insertion mechanism and scaffolding mechanisms (McMahon and Gallop, 2005; Zimmerberg and Kozlov, 2006; Figure 1).

The BIN/Amphiphysin/Rvs (BAR) domain protein superfamily of membrane shapers allows for highlighting both mechanisms, the conceptual requirements and the functional importance of membrane shaping. The BAR domain was first recognized as conserved domain in BIN1, amphiphysins and the yeast proteins Rvs167p and Rvs161p (David *et al*, 1994). The crystal structure of the amphiphysin BAR domain a decade later then revealed that BAR domains are dimers (Peter *et al*, 2004). The core of these dimers (also called BAR modules) is a 6-helix bundle formed by three, relatively long helices of each monomer (helix 1–3). From the central helix bundle, two arms protrude (Figure 2).

Since overall the shape of BAR modules is curved and amphiphysin was known to tubulate liposomes (Takei *et al*, 1999), Peter *et al* (2004) suggested that a variety of proteins that are structurally related to amphiphysins may also sense and/or induce membrane curvatures upon recruitment from the cytosol to the cytosolic membrane surface. A number of crystal structures, lipid-binding studies and liposome tubulation assays since then confirmed this hypothesis and expanded the superfamily, which according to (weak) sequence homologies and structural similarity today contains classical BAR, N-BAR, BAR-PH, PX-BAR, F-BAR and I-BAR proteins (Frost *et al*, 2009; Suetsugu *et al*, 2010; Figure 2).

*Corresponding author. B Qualmann or D Koch or MM Kessels, Institute for Biochemistry I, University Hospital Jena—Friedrich Schiller University Jena, Nonnenplan 2-4, Jena, Thuringen D-07743, Germany. Tel.: +49 3641 9396 300; Fax: +49 3641 9396 302; E-mail: britta.qualmann@mti.uni-jena.de or dennis.koch@mti.uni-jena.de or michael.kessels@mti.uni-jena.de

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Figure 1 Mechanisms of membrane shaping by BAR domains. (A) Scaffolding mechanism promoted by binding to inverted cone-shaped negatively charged lipids (red). (B, C) Hydrophobic insertion mechanisms embedding protein parts into the lipid layer.

Scaffolding mechanism

Common to the scaffolding mechanisms is the binding of a hydrophilic protein domain with an intrinsically curved shape to the membrane surface forcing it to adopt a similar shape. In curvature sensing, already existing lipid topologies are preferentially interacting with protein scaffolds of suitable curvature. Both processes are not mutually exclusive but may also occur simultaneously (Figure 1).

The scaffolding mechanism relies on several prerequisites:

First, a defined membrane-binding interface is needed. As shown by a plethora of mutational studies (Peter *et al*, 2004; Gallop *et al*, 2006; Masuda *et al*, 2006; Suetsugu *et al*, 2006; Henne *et al*, 2007; Mattila *et al*, 2007; Pylypenko *et al*, 2007; Shimada *et al*, 2007, 2010; Frost *et al*, 2008; Reider *et al*, 2009; Saarikangas *et al*, 2009; Wang *et al*, 2009; Rao *et al*, 2010), BAR superfamily proteins bind to membranes exclusively by the so-called N-surface, which is composed of residues of helix 1 not oriented towards the helix bundle (Millard *et al*, 2005) and which is complemented by lipid-binding residues of the flanking arms. Thereby, the lipid-binding surface correlates well with the concave surface of the banana-shaped BAR modules (Figure 2). The positively charged nature of the membrane-interacting residues fits very well with the negatively charged lipids, such as phosphoinositides and phosphatidylserine, that BAR domains select for binding (e.g., see Peter *et al*, 2004; Itoh *et al*, 2005; Mattila *et al*, 2007 and Dharmalingam *et al*, 2009; Figure 1).

The second requirement is that the lipid-binding surface has intrinsic curvature. BAR superfamily proteins have a variety of very different curvatures, which first was suggested

to be specific for the subfamilies (Figure 2). One extreme is sharp convex curvatures, such as those observed in the crystal structures of arfaptin (classical BAR; Tarricone *et al*, 2001), APPL (PH-BAR; Li *et al*, 2007; Zhu *et al*, 2007) and endophilin A1 (N-BAR; Weissenhorn, 2005; Gallop *et al*, 2006; Masuda *et al*, 2006). The curvatures of these molecules correspond to circles with diameters of only ~ 15 – 17 nm (Figure 2). Also, the crystal structures of amphiphysins have strongly curved lipid-binding surfaces (Peter *et al*, 2004; Casal *et al*, 2006; Figure 2). Structurally, the overall shape of BAR modules is mainly determined by the angle of dimerization. In strongly curved structures, the angle of helix 2 interception is $\sim 30^\circ$, whereas for F-BAR proteins it is only $\sim 10^\circ$ (Henne *et al*, 2007; Shimada *et al*, 2007; Wang *et al*, 2009).

Also, helix kinks contribute to the shape of BAR modules (reviewed in Masuda and Mochizuki, 2010; Figure 2). One extreme case is the so-called I-BAR (inverse BAR) family with its cigar-shaped dimers. Their overall straight, cigar-like shapes provide slightly concave surfaces promoting negative curvatures, such as those found inside of plasma membrane protrusions (Zhao *et al*, 2011).

The third prerequisite for curvature induction by scaffolding is a rigid surface. Mutational analyses of endophilin confirmed the general requirement of rigidity for scaffolding (Masuda *et al*, 2006). It seems that the stiffness of BAR modules is brought about by the central, tightly packed six-helix bundle. Crosslink experiments with endogenous proteins in brain extracts (Ringstad *et al*, 2001; Kessels and Qualmann, 2006) suggest that also *in vivo*, dimers are very stable and represent the prevalent state of BAR superfamily proteins. Also, the crystal structures showing many hydrophobic surface residues being buried upon dimerization suggest that BAR modules are very stable (Masuda and Mochizuki, 2010). Furthermore, successful reconstructions of solution structures of BAR domains (Wang *et al*, 2008, 2009) as well as visualizations of lipid-bound F-BAR dimers by cryo-EM (Frost *et al*, 2008) suggest that BAR modules have a defined, invariant shape.

The fourth prerequisite is that the membrane-binding interface is large enough to impose a defined topology on a significant area of membrane. The lipid-binding surfaces of BAR domains are only ~ 16 – 23 nm in length and ribbonary (Figure 2). Clusters of positive residues may explain how BAR modules can attach their entire N-surface to the membrane but calculations suggest that the energy releases of membrane binding do not provide the energy required for deforming a significant portion of the bilayer. This demands cooperation among many BAR modules (Ayton *et al*, 2007). For endophilin, syndapins, SNXs, and APPL, self-associations were directly shown biochemically (Ringstad *et al*, 2001; Kessels and Qualmann, 2006; Shin *et al*, 2007; Chial *et al*, 2008; Dislich *et al*, 2010). The observation of coats of BAR superfamily proteins around lipids in *in vitro* experiments (for review, see Gallop and McMahon, 2005 and Itoh and Takenawa, 2009), crystal structures and cryo-EM analyses also suggest that BAR modules self-assemble into larger complexes and that, in these lattices, individual members may be in contact in an organized manner (Shimada *et al*, 2007; Frost *et al*, 2008; Wang *et al*, 2009; Henne *et al*, 2010). Interestingly, tubule formation of CIP4 and self-association of syndapin I was based on similar acidic residues just

Figure 2 BAR modules adopt folds with different degrees of curvature. BAR modules (one monomer in yellow and the other in blue) from different subfamilies viewed from the side (left) and from top (right). Additional domains (PH and PX) are in grey. PDB codes are given in brackets. Note that FCHo and syndapin have tilde shapes. Arrowheads mark positions within the BAR modules for hydrophobic insertion into membranes.

C-terminal of the F-BAR domain (Kessels and Qualmann, 2006; Frost *et al*, 2008) that were suggested to form lateral interactions in CIP4 crystal structures (Frost *et al*, 2008). In general, lattice formation adds macromolecular aspects to BAR domain function.

Hydrophobic insertion mechanism

Partial embedding of hydrophobic or amphipathic protein domains into the membrane matrix was shown by biochemical and spectroscopical analyses for endophilin and amphiphysin (Farsad *et al*, 2001; Gallop *et al*, 2006; Henne *et al*, 2007). It is a powerful mechanism for strong hydrophobic interactions and for bending membranes and/or for recognizing sharply bent membranes, which easily allow for such insertions at sites of positive curvature (Figure 1). The presence of N-terminal amphipathic helices distinguishes the N-BAR subfamily composed of amphiphysins/BINs and endophilins and nadrins from the rest of the BAR superfamily (Figures 1 and 2).

Intriguingly, Bhatia *et al* (2009) demonstrated that the amphipathic α -helix of endophilin is required for sensing a given membrane curvature, whereas the positively charged, curved N-surface of the BAR domain was unable to do so. Similarly, elegant genetic analyses in *D. melanogaster* questioned the general importance of the BAR domain core for endophilin function *in vivo*, as mutations in the central amphipathic helix located in the BAR domain did not affect larval viability or synaptic function. In contrast, a mutation in the membrane-inserted helix 0, that is, outside of the BAR domain core, lacked membrane binding and tubulation activity *in vitro* and was unable to rescue the impaired synaptic function in endophilin mutants (Jung *et al*, 2010). Also in worms, deleting the membrane-inserting helix 0 failed to restore functions in endophilin (*unc-57*) mutants, while mutations in helix 1 of the BAR domain core at least partially rescued (Bai *et al*, 2010).

In addition to N-BAR proteins at least one member of the F-BAR family uses hydrophobic insertion mechanisms, syndapin I dimers contain two loops of hydrophobic residues, which protrude from the lipid-binding surface and seem to be inserted into the membrane like wedges (Wang *et al*, 2009; Rao *et al*, 2010). This is a mechanism to promote membrane bending and/or sensing of bent membranes.

Testing the BAR domain hypothesis *in vivo* using the defined stages of endocytic vesicle formation

Intriguingly, many cellular processes do not seem to involve single members of the BAR domain superfamily but diverse, however, distinct sets of BAR domain-containing proteins from different subfamilies. It, therefore, has been suggested that classical BAR, BAR-PH, PX-BAR, N-BAR, I-BAR and F-BAR domains induce and/or prefer different membrane curvatures (BAR domain hypothesis) and that this predetermines their temporal and spatial involvement in cellular membrane shaping processes. These hypotheses were based on the shape of BAR modules seen in crystal structures and of the membrane tubulation reactions observed in *in vitro* reconstitutions with liposomes and proteins.

A particularly well-defined membrane shaping process is clathrin-mediated endocytosis proceeding through sequential,

well-defined stages with different membrane curvatures (Figure 3). Nucleation of adaptors and clathrin at the plasma membrane is followed by the invagination of the plasma membrane. When the deformation has led to a deeply invaginated clathrin-coated pit with an appropriately narrow neck, scission mediated by the large GTPase dynamin releases a clathrin-coated vesicle, which then is rapidly uncoated aided by synaptojanin, GAK/auxilin and Hsc70 and then undergoes further endosomal trafficking (reviewed in Doherty and McMahon, 2009 and Schmid and Frolov, 2011; Figure 3).

Over several decades, biochemical and structural approaches have revealed the protein machineries for endocytic vesicle formation and their properties. Advanced live-imaging techniques now allow for investigation of individual endocytic events, characterization of their heterogeneity and examination of the temporal and spatial organization of the endocytic machinery by determining the order of recruitment and release of fluorescently tagged individual components, by correlating them with distinct stages (i.e. membrane curvatures) and by determining whether the composition of a forming clathrin-coated pit affects its properties (reviewed in Merrifield, 2004; Rappoport, 2008 and Kirchhausen, 2009).

Some studies applying life-cell total internal reflection (TIRF) microscopy, a technique that allows to visualize fluorescent molecules selectively within, at or just adjacent to the plasma membrane, have correlated the recruitment of a few BAR superfamily proteins to the disappearance or increased mobility of clathrin-coated pits. Using this approach as an indicator for endocytosis, endophilin A2 (Perera *et al*, 2006), APPL1 (Erdmann *et al*, 2007; Zoncu *et al*, 2009), FBP17 (Shimada *et al*, 2007) and SNX9 (Soulet *et al*, 2005) were assigned to be recruited to late stages of clathrin-mediated endocytosis. For the endosomal BAR-PH protein APPL1, time-lapse TIRF microscopy demonstrated the appearance of GFP-APPL1 at a subset of clathrin-coated pits right after the disappearance of the clathrin signal (Zoncu *et al*, 2009).

Recent studies have highlighted that an even closer look both at the temporal dynamics and at the apparent heterogeneity of forming clathrin-coated structures at the plasma membrane is necessary. In addition to rather spot-like clathrin-coated pits, larger so-called clathrin-coated patches or plaques were described at the coverslip-attached surface in certain cell types (Gaidarov *et al*, 1999; Ehrlich *et al*, 2004; Keyel *et al*, 2004; Saffarian *et al*, 2009; Batchelder and Yarar, 2010; Figure 3). Furthermore, clathrin-coated pit lifetime distribution analyses suggested two short-lived abortive populations and a longer lived productive population (Loerke *et al*, 2009). Recently, Taylor *et al* (2011) assessed the accuracy of clathrin disappearance as reference point for internalization in TIRF microscopy and showed that the error is as large as the time course of clathrin-coated structure invagination and vesicle formation. For precise timing of endocytic protein recruitments relative to scission, the authors thus monitored the accessibility of pH-sensitive fluorescent cargo of clathrin-mediated endocytosis to rhythmically imposed changes in extracellular pH to detect single scission events by TIRF microscopy (Merrifield *et al*, 2005; Taylor *et al*, 2011).

The process of formation of a clathrin-coated vesicle is characterized by a continuous increase in membrane

Figure 3 Vesicle formation correlates with increased curvature. The formation of a clathrin-coated vesicle from pre-existing clathrin-coated plaques (A) and from a clathrin-coated pit (B) is marked by an increase of membrane curvature and a drop of the corresponding circle diameters (black circles in (A) and (B)).

curvature (Figure 3) and according to the BAR domain hypothesis should, therefore, offer a direct correlation of the recruitment pattern of the differentially curved BAR superfamily proteins and the increasing curvature during vesicle formation. This predictability offered a unique possibility to experimentally test the BAR domain hypothesis. Taylor *et al* (2011) analysed the recruitment patterns of 34 endocytic proteins including 10 proteins of the BAR domain superfamily. Interestingly, in these examinations, the same set of proteins was recruited in the same order to scission events at diverse dynamic classes of clathrin-coated structures (defined by size, lifetime, terminal versus non-terminal events).

Detailed analyses of the kinetics of appearance and disappearance of the BAR superfamily proteins revealed that only some of these proteins showed recruitment dynamics correlating with the curvature of their BAR domains (Figure 4). The FCHO F-BAR domain has a very shallow curvature (Henne *et al*, 2007; Figure 2). In line with this shape, FCHO proteins were suggested to have a role in clathrin-coated pit nucleation and in the induction of curvature early in the invagination of the coated membrane based on biochemical examinations and loss-of-function analyses in cell culture experiments (Henne *et al*, 2007, 2010). Consistently, Taylor *et al* (2011) observed that FCHO1 and FCHO2 were indeed recruited very early to clathrin-coated structures with very similar kinetics and scaling relationships between both clathrin-coated structure size and lifetime and the relative amount of protein recruited when compared with clathrin, but particularly when compared with the clathrin adaptor AP2 (Taylor *et al*, 2011; Figure 4B).

In contrast, the F-BAR protein syndapin II and the N-BAR proteins endophilin A2, BIN1 and amphiphysinI showed a burst of recruitment right before scission. This time of

appearance reflects the highest degree of membrane curvature occurring during clathrin-coated vesicle formation—the deeply invaginated clathrin-coated pit and particularly the narrow vesicle neck (Figure 4). Syndapins, endophilins and amphiphysins indeed have a more sharply curved lipid-binding N-surface than the very shallow F-BAR proteins of the FCHO family with a circle diameter of ~200 nm (Peter *et al*, 2004; Casal *et al*, 2006; Gallop *et al*, 2006; Henne *et al*, 2007; Wang *et al*, 2009). Upon amphipathic helix insertion, endophilins have the sharpest curvature of all three (Gallop *et al*, 2006). Amphiphysins have slightly shallower curvatures corresponding to a circle diameter of ~22 nm (Peter *et al*, 2004; Casal *et al*, 2006). Syndapins, finally, have even shallower curvatures with N-surfaces corresponding to roughly 50 nm circle diameter (Wang *et al*, 2009). The observation that members of these F-BAR and N-BAR subfamily members with their different, distinct shapes nevertheless appear together and also show a synchronous disappearance after scission when the highly curved membrane neck collapses (Taylor *et al*, 2011) thus was unexpected. Interestingly, their dynamic appearance and disappearance parallels the temporal signatures of two of the main binding partners of syndapins, amphiphysins and endophilins, dynamin and N-WASP (David *et al*, 1996; Ringstad *et al*, 1997; Qualmann and Kelly, 2000; Kessels and Qualmann, 2002; Otsuki *et al*, 2003; Yamada *et al*, 2009; Figure 4A–C).

Also, the recruitment of the very sharply curved BAR-PH protein APPL1 only after the scission event cannot simply be based on the geometry of the BAR domain (Li *et al*, 2007), but may rather coincide with the pattern of recruitment of interaction partners of APPL1 including the endosomal component Rab5 (Miaczynska *et al*, 2004).

Strikingly, the post-scission recruitment of the PX-BAR protein SNX9 and the complex, biphasic recruitments of the

Figure 4 Changes of membrane topology during clathrin-mediated endocytosis. (A–C) Detailed view of recruitment traces of BAR superfamily proteins (A), F-BAR proteins (B) and dynamin, F-actin (lifeact) and N-WASP (C). At time $t = 0$, fission of a clathrin-coated vesicle was detected. Images modified from Taylor *et al* (2011). (D) Colour-coded changes in membrane topology at distinct sites of vesicle formation. (E) Localization of BAR superfamily proteins to different sites of membrane curvature during clathrin-mediated endocytosis. For simplicity, clathrin is omitted. F-BAR domains with very shallow curvature (yellow), shallow curvature (orange) and strongly curved BAR modules are in purple. White bars mark defined positions on the molecules to visualize rotations. (F) Electron micrograph of a stimulated lamprey synapse injected with anti-endophilin antibodies (reprinted from Ringstad *et al*, 1999 with permission from Elsevier) displays an unusual accumulation of clathrin-coated pits that were arrested at different stages of vesicle formation, that is, with different curvatures (arrows). Bar, 100 nm. Endo2, endophilin A2; Amph1, amphiphysin I; Synd2, syndapin II; Dyn1, dynamin I.

F-BAR proteins FBP17 and CIP4 with phases of recruitment both before and after scission (Taylor *et al*, 2011) cannot be explained solely on the basis of structural information. Based on the curvatures deduced from the crystal structures of the F-BAR domains of FBP17 and CIP4 (Shimada *et al*, 2007) and of the PX-BAR domain of SNX9 (Pylypenko *et al*, 2007), with curvatures ranging from ~40 to 60 nm, a recruitment pattern during the formation of clathrin-coated vesicles rather similar to that of syndapin II would have been expected. An alternative explanation, that the kinetics of binding partners dictate the kinetics of FBP17 and SNX9, also is inconsistent with the experimental data. Clear temporal discrepancies were observed between SNX9 and CIP4/FBP17/Toca on one side and their binding partners dynamin and N-WASP (Lundmark and Carlsson, 2003; Ho *et al*, 2004; Kamioka *et al*, 2004; Tsujita *et al*, 2006; Yarar *et al*, 2007; Hartig *et al*, 2009) on the other side. The kinetics were opposed (Taylor *et al*, 2011; Figure 4A–C).

This indicates that the original BAR domain hypothesis is oversimplified.

Versatility of BAR domain interactions with membranes

Solving the first structures of different versions of BAR domains served the recognition and the establishment of different subfamilies within the BAR domain superfamily. Structural analyses have also contributed significantly to our understanding of the general properties of BAR domains and furthermore revealed specialized features of individual members of the superfamily. Some of these observations also give hints towards why the direct correlation of N-surface curvature with stages of endocytic vesicle formation reflecting distinct membrane curvatures (Figure 3) is too simplistic and therefore, in several cases, did not lead to the expected results. Multiple modes of membrane interaction, geometric considerations and structural diversity of especially the F-BAR domain may explain some of the observations made in time-resolved localization studies of endocytosis.

Frost *et al* (2008) observed by cryo-EM that the CIP4 F-BAR domain has at least two modes of lipid binding. Besides binding to curved surfaces using its N-surface, CIP4 F-BAR dimers were able to attach laterally to flat membranes in *in vitro* reconstitutions, when the stiffness of the membrane was artificially raised by strong cooling. It has yet to be shown whether also other F-BAR or maybe even other BAR domain proteins have several modes of binding and how such observations relate to cellular processes *in vivo*. Another indication that F-BAR proteins may have different membrane-binding modes stems from the observation that, *in vitro*, syndapins and FCHo have the potential to induce a broad range of tubule diameters, which are by far shallower than their N-surface (Wang *et al*, 2009; Henne *et al*, 2010).

The situation is additionally complicated by the fact that the F-BAR protein subfamily possesses an extreme diversity of shapes. Whereas the N-surface of F-BAR domains is relatively shallow, and the F-BAR domain of srGAP even has been shown to induce negative curvatures (Guerrier *et al*, 2009), some F-BAR domains exhibit sharp helix kinks in the arms, which in the case of FCHo and syndapins result in tilde shapes when viewed from top (Figure 2). In syndapin

I and syndapin II, with two angles of 61°, the kinks are extreme (Wang *et al*, 2009) and would offer curvatures completely different from that of the N-surface. Lateral binding to flat membranes, in contrast, will be impaired by tilde structures, as geometrical considerations would only allow for few lipid contact points.

Intriguingly, tilde shapes can also be used for establishing a tight clamp around extremely curved lipid surfaces. For this, the N-surface has to be placed diagonal onto the axis of the cylinder instead of perpendicular (Figure 4E). This way, syndapins can be fit onto tubules of a diameter of as little as ~15 nm. This situation fits very well with the fission reaction in vesicle formation processes. These geometrical considerations would give an explanation for the fact that syndapins just appear right before and disappear right after fission (Taylor *et al*, 2011).

Another unique feature of the curved and tilde-shaped F-BAR protein syndapin is that such geometry allows synchronous binding to two different curvatures, a key function at the transition from the constricted neck to the vesicle formed (Figure 4D). Association at such zones requires a diagonal placement onto the lipid tubule (Figure 4E) and a slight rotation of the axis of the syndapin dimer, that is, again, some degree of lateral binding versatility (Figure 4E, white indicator bar).

These geometrical considerations and the finding that there seems to be some rotation flexibility in how at least F-BAR domains are placed onto membranes may explain some of the findings in time-resolved localization studies of endocytic vesicle formation. They also highlight why molecules that apparently have the same curvature of the N-surface behave so differently and why the members of the F-BAR subfamily although all providing shallow N-surfaces seem to prefer and/or promote different membrane curvatures during vesicle formation.

Testing the BAR domain hypothesis *in vivo* by loss-of-function paradigms

As described above, TIRF analyses revealed only some correlations between recruitment of a BAR superfamily protein and the curvature of its BAR domain. Also, such analyses leave entirely open the question whether transient appearances of BAR superfamily proteins at some sites of endocytosis reflect a crucial role of these proteins in vesicle formation. Therefore, a closer look at the loss-of-function analyses of BAR superfamily proteins involved in clathrin-mediated endocytosis conducted thus far is consequential.

I-BAR family

During formation of clathrin-coated pits, flat membranes need to be converted into positively curved structures, although also small areas of negative curvature occur at the basis of the forming invagination (Figure 4D). Based on the structure–function relationships *in vitro*, I-BAR proteins generate negative curvature (Zhao *et al*, 2011), suggesting that they are not involved in clathrin-mediated endocytosis or may even have inhibitory effects. Quinones *et al* (2010) tested this hypothesis and found that silencing the vertebrate I-BAR protein MIM in mouse embryonic fibroblasts led to increased clathrin-mediated endocytosis. Also, the *Drosophila* orthologue DMIM had an inhibitory effect on endocytosis that was

accompanied with border cell migration defects of *dmim* mutants, suggesting conservation of MIM function among species. Interestingly, the antagonistic mechanism of MIM on endocytosis seemed to be based on the competition between distinct BAR superfamily proteins for common endocytic effector proteins (Quinones *et al*, 2010).

In contrast to I-BAR proteins, almost all other BAR domain proteins induce positive curvature *in vitro* (Frost *et al*, 2009). These proteins should thus induce or stabilize the progressively increasing positive curvature of the membrane during vesicle formation (Figure 4). In general, the N-surface of classical BAR, BAR-PH, PX-BAR and especially N-BAR domains turned out to be much stronger curved than that of F-BAR domains (Figure 2). These observations suggested a hierarchical order during progression of endocytosis with F-BAR proteins functioning at earlier stages, that is, at sites of low curvature and wide tubular diameters. Other BAR superfamily proteins would participate at later stages, that is, at sites of high curvature and thin tubular diameters, such as those found at the constricted neck of a forming vesicle (Figures 3 and 4D).

Endocytic proteins—FCHo and N-BAR family

Overexpression of the shallow F-BAR protein FCHo led to increased nucleation of clathrin-coated pits, whereas silencing FCHo strongly reduced clathrin-coated pit formation and blocked clathrin-mediated endocytosis. FCHo even preceded the recruitment of AP2/clathrin (Henne *et al*, 2010). Thus, it seems to function as a nucleator for clathrin-coated pit formation during very early phases of membrane invagination. These phases correspond to very shallow curvatures. FCHo appearance, structure and function are thus fully consistent with the BAR domain hypothesis.

At the first look, the same is true for the N-BAR proteins endophilin and amphiphysin. Gene knockout (KO) of endophilin A in worm and fly as well as attacking endophilin A by antibody or peptide introduction in the lamprey all revealed a conserved function of endophilin A in late stages of clathrin-mediated endocytosis, as increased levels of deeply invaginated clathrin-coated pits devoid of dynamin rings were observed. Consequently, in neurons, the replenishment of synaptic vesicles was disturbed and the synaptic vesicle pool was strongly depleted (Ringstad *et al*, 1999; Gad *et al*, 2000; Verstreken *et al*, 2002; Schuske *et al*, 2003). These data suggest that endophilin A acts upstream of dynamin recruitment, a stage during clathrin-mediated endocytosis with high curvature and small radius at the neck of the clathrin-coated pit and is in line with the proposed function of N-BAR proteins and their highly curved structure. These studies furthermore show a functional relationship between endophilin A and synaptojanin. Recent *in vitro* studies suggest that interaction of synaptojanin with SH3 domain-containing curvature sensors, such as endophilin, recruits synaptojanin and controls its enzymatic activity thereby leading to a preferential dephosphorylation of PI(4,5)P₂ at curved membranes (Chang-Ileto *et al*, 2011). Consistently, in *Drosophila*, single mutants of *endoA* and *synj* phenocopy each other both on the ultrastructural and the electrophysiological level and synaptojanin was mislocalized and destabilized in *endoA* mutants (Verstreken *et al*, 2003). *Drosophila endoA* null mutants are sluggish and die as second or early third instar larvae (Verstreken *et al*, 2002).

Worm endophilin A null mutants (*unc-57*) are viable but show an uncoordinated phenotype and thereby phenocopy synaptojanin/*unc-26* mutants (Schuske *et al*, 2003). Recently, data by Bai *et al* (2010) in *C. elegans* questioned the SH3 domain-mediated role of endophilin in synaptojanin recruitment, as endophilin Δ SH3 (i.e., of an N-BAR domain-containing mutant lacking synaptojanin binding) expression rescued *unc-57* mutants. It remains possible, however, that this apparent contradiction does not reflect species differences but the fact that the importance of the endophilin/synaptojanin interaction is activity dependent (Mani *et al*, 2007).

Attacking the N-BAR protein amphiphysin in the lamprey also led to severe depletions of the synaptic vesicle pool. The number of clathrin-coated pits was drastically increased and most of them had a small homogenous size with a narrow neck, suggesting an arrest or strong kinetic delay of endocytosis at a late stage preceding fission (Shupliakov *et al*, 1997; Gad *et al*, 2000). Since no spirals around the neck were observed, these data suggested that, in line with the predictions from its highly curved N-BAR domain structure, also amphiphysin mainly acts upstream of dynamin at the vesicle scission step.

Thus far, the loss-of-function analyses described strongly support the concept that BAR superfamily proteins become functionally important at stages of clathrin-mediated endocytosis, in which membrane curvature exactly corresponds to the curvature of their BAR modules. There are, however, several findings that dismiss this simple correlation. Thorough analysis of endophilin-deficiency phenotypes in worm, fly and lamprey additionally revealed accumulations of shallow-coated pits without constricted necks (Figure 4F). These data emphasize a role for this N-BAR protein in early stages of clathrin-mediated endocytosis with low membrane curvature (Ringstad *et al*, 1999; Verstreken *et al*, 2002; Schuske *et al*, 2003; Andersson *et al*, 2010) and thereby challenge the BAR domain hypothesis.

Furthermore, Mettlen *et al* (2009) reported that clathrin-coated pit dynamics in endophilin A2 knockdown cells had no effect on the relative proportions of abortive and productive clathrin-coated pits, but selectively decreased the lifetime of late abortive events. Therefore, the authors concluded that the N-BAR domain of endophilin may allow for sensing critical early stages in clathrin-coated pit maturation that generate curvature and feed this information into a so-called 'endocytic checkpoint' (Mettlen *et al*, 2009). In physiology, suppressed endophilin A expression, as seen in various tumours, for example breast carcinoma (Sinha *et al*, 2008), apparently has consequences for downregulation of EGF receptors or other growth factor receptors and may thereby promote tumour development or malignancy (Petrelli *et al*, 2002; Soubeyran *et al*, 2002).

Further support for a more complex view of N-BAR proteins functioning at multiple stages corresponding to different degrees of membrane curvature comes from the analysis of amphiphysin I KO mice, which due to an induced instability of amphiphysin II forming heterodimers with amphiphysin I are deficient for both amphiphysin isoforms in the brain. These mice displayed learning deficits in the Morris water maze task and suffered from tonic-clonic seizures accompanied with reduced survival (Di Paolo *et al*, 2002). This argues for some imbalances between excitation and inhibition. However, there is no evidence that the human *amphiphysin*

gene loci are associated with idiopathic generalized epilepsies (Turnbull *et al*, 2005).

At the synapse level, amphiphysin I KO mice showed synaptic vesicle repriming defects as well as a reduced functional synaptic vesicle pool but the total size of the synaptic vesicle pool was unaffected (Di Paolo *et al*, 2002).

Biochemical analyses revealed reduced lipid associations of the late clathrin-mediated endocytosis component synaptojanin, and of the early clathrin-mediated endocytosis components AP2 and clathrin, but not of dynamin in brain extracts from amphiphysin I KO mice (Di Paolo *et al*, 2002). These data were striking because amphiphysins were thought to recruit and activate dynamin at the neck of a forming vesicle based on the observations that amphiphysins associate with lipids (Takei *et al*, 1999), that they directly associate with dynamin (David *et al*, 1996) and that they have the ability to stimulate dynamin's GTPase activity in the presence of lipids (Yoshida *et al*, 2004). Subsequently, Lundmark and Carlsson (2004) revealed that dynamin recruitment to membranes in non-neuronal cells relies on SNX9 and our recent experimentation show that, in the murine brain, the F-BAR protein syndapin I is a crucial dynamin recruiter (Koch *et al*, unpublished).

The observed role of amphiphysin I in AP2 and clathrin recruitment, that is, in early stages of clathrin-mediated endocytosis, emphasized the importance of amphiphysin's AP2/clathrin interaction interface, the CLAP domain (McMahon *et al*, 1997; Ramjaun *et al*, 1997; Slepnev *et al*, 2000). In line, microinjection of GST-amphiphysin CLAP or of anti-amphiphysin CLAP antibodies in the lamprey axons blocked early stages of clathrin-mediated endocytosis (Evergren *et al*, 2004). The importance of the CLAP domain for the endocytic functions of amphiphysins is also supported by the analysis of amphiphysin II KO mice and *Drosophila* amphiphysin mutants. Loss of both of these muscle-enriched amphiphysins lacking a CLAP domain led to muscle phenotypes and embryonic lethality but not to endocytosis defects (Razaq *et al*, 2001; Muller *et al*, 2003). Strikingly, *Drosophila* amphiphysin did not only fail to bind clathrin, but also did not bind dynamin (Razaq *et al*, 2001).

Taken together, the modular domain organization of amphiphysins with their membrane- and protein-interacting domains is essential for their overall function and for participation at distinct stages during clathrin-mediated endocytosis. This conclusion also applies to F-BAR proteins. Elegant rescue experiments of the FCHO RNAi phenotype on clathrin-coated pit formation with FCHO harbouring mutations in the AP2- μ homology domain (μ HD) that abolished binding of eps15 and intersectin or with FCHO mutants with a defective F-BAR domain clearly demonstrated that both the F-BAR domain and its μ HD were essential for its clathrin nucleation ability (Henne *et al*, 2010). These data exemplify that the modular domain organization of a BAR superfamily protein needs to be entirely intact to ensure the particular function of the protein at a distinct step during clathrin-mediated endocytosis.

BAR proteins with additional lipid-binding modules

Modular organization and complex formation with several endocytic proteins can also be observed for oligophrenin-1, which has a BAR domain fused with a PH domain (BAR-PH

protein) and an Rho-GTPase-activating (RhoGAP) domain. Loss of oligophrenin-1 has been associated with X-linked mental retardation (Billuart *et al*, 1998) and gene KO in mice results in impaired spatial memory and dendritic spine immaturity (Khelifaoui *et al*, 2007). On the molecular level, postsynaptic oligophrenin-1 has a critical role in the activity-dependent maturation and plasticity of excitatory synapses through its RhoGAP activity and AMPA receptor binding (Nadif Kasri *et al*, 2009) and is important for presynaptic compensatory endocytosis (Nakano-Kobayashi *et al*, 2009). Interestingly, rescue experiments showed that proper oligophrenin-1 function required both an interaction with endophilin A1 and the RhoGAP domain (Nakano-Kobayashi *et al*, 2009). Thus, as for amphiphysin and FCHO, a modular organization and additional protein interactions are crucial for the function of the BAR-PH protein oligophrenin-1 in synaptic endocytic vesicle formation.

In PICK1, an inhibitor of the Arp2/3 complex actin nucleation machinery (Rocca *et al*, 2008), modularity in membrane interaction manifests in an additional use of a PSD-95/Dlg/ZO1 (PDZ) domain as a lipid-binding module (Pan *et al*, 2007). PICK1 was found to interact with postsynaptic AMPA receptors (Xia *et al*, 1999). A lack of cerebellar LTD in PICK1 KO mice suggested some role in AMPA receptor endocytosis. Interestingly, both the PDZ and the BAR domains were required for LTD (Steinberg *et al*, 2006). Recent studies, however, showed that PICK1 functions in NMDA receptor-induced intracellular retention of AMPA receptors rather than in AMPA receptor endocytosis (Rocca *et al*, 2008; Citri *et al*, 2010). Disruption of PICK1 interactions with metabotropic glutamate receptors is correlated with absence epilepsy and mutations in the *PICK1* gene have been linked to schizophrenia (Dev and Henley, 2006; Bertaso *et al*, 2008).

A combination of BAR domains with an additional membrane-binding module, in this case a phosphoinositide-binding phox homology (PX) domain, is realized in certain members of the sorting nexin (SNX) family. The SNX family is a large family with 33 members in mammals and seems to have roles in different membrane transport processes (van Weering *et al*, 2010). It is important to note that, whereas 12 members of the family seem to have PX-BAR domains, only the 3 members, which additionally harbour an Src homology 3 (SH3) domain, are implicated in endocytosis. These proteins (SNX9, 18 and 33; previously annotated as SNX30) constitute the so-called SNX9 subfamily (Lundmark and Carlsson, 2009).

KO studies on SNX9 family members are not yet available but SNX9 knockdown led to a diminished transferrin internalization in HeLa cells (Soulet *et al*, 2005). In the presynaptic terminal, SNX9 knockdown significantly slowed synaptic vesicle endocytosis (Shin *et al*, 2007). SNX33 (Heiseke *et al*, 2008; Schöbel *et al*, 2008) and SNX18 (Park *et al*, 2010) seem to function in clathrin-mediated endocytosis similar to SNX9, but there are also findings suggesting that SNX18 has a more unique role in endosomal trafficking (Håberg *et al*, 2008).

At nerve terminals, primarily the interaction of SNX9 with dynamin I was required for endocytosis (Shin *et al*, 2007). In line with this observation, SNX9 contributes to the membrane recruitment of dynamin II in non-neuronal cells and stimulates dynamin's GTPase activity when bound to the membrane (Lundmark and Carlsson, 2004; Soulet *et al*,

2005). Mechanistically, SNX9 is able to stimulate dynamin's GTPase activity when bound to the membrane (Soulet *et al*, 2005), and thereby shows some functional similarity to amphiphysin (Yoshida *et al*, 2004). These data suggest that SNX9 is important for proper clathrin-mediated endocytosis progression during the fission step where it recruits and subsequently activates dynamin during vesicle release. However, this conclusion does not fit with the observations that the SNX9 recruitment to clathrin-coated pits during clathrin-mediated endocytosis peaked after the scission of a clathrin-coated pit and thus significantly later than dynamin (Taylor *et al*, 2011; Figure 4A–C).

F-BAR proteins of the CIP4/GBP17/Toca-1 and the syndapin family

The F-BAR family forms the largest subfamily of all BAR superfamily proteins and with the exception of PSTPIP2 and FCHO all members additionally contain SH3 domains. Especially the F-BAR proteins of the CIP4/GBP17/Toca-1 and syndapin (also called PACSIN) family resemble the endocytic N-BAR proteins amphiphysin and endophilin because with dynamin, synaptojanin and N-WASP they also share major SH3 domain interaction partners. Consistently, functional analyses on the cellular level suggested that these proteins have roles in endocytosis (Qualmann and Kelly, 2000; Chang *et al*, 2002; Kamioka *et al*, 2004; Braun *et al*, 2005).

Some genetic data, however, appear contradictory at a first glance. In *D. melanogaster*, loss-of-function analyses of syndapin did not reveal any endocytic roles, syndapin was found to be completely dispensable for synaptic vesicle endocytosis (Kumar *et al*, 2009a). This is in line with its localization, fly syndapin was found on the postsynaptic, that is, muscular side of neuromuscular junctions, in which it participates in the biogenesis of the subsynaptic reticulum (Kumar *et al*, 2009b). Somewhat similar, also amphiphysins apparently lack endocytic functions in lower organisms, such as worms (Pant *et al*, 2009) and flies (Razzaq *et al*, 2001). Interestingly, also fly amphiphysin is expressed at the muscular side of neuromuscular junctions (Razzaq *et al*, 2001). The BAR superfamily has greatly been expanded during evolution to higher eukaryotes. Therefore, it seems that endocytic functions of individual subfamily members evolved later. Along these lines, *C. elegans* endophilin A (*unc-57*) mutants can be rescued with mammalian endophilin A but not with B (Bai *et al*, 2010).

Syndapins seem to work in clathrin-mediated endocytosis in close conjunction with their direct binding partners dynamin and the Arp2/3 complex activator N-WASP (Qualmann *et al*, 1999; Modregger *et al*, 2000; Qualmann and Kelly, 2000; Braun *et al*, 2005) and are even able to physically link dynamin and N-WASP *in vivo* (Kessels and Qualmann, 2006). Syndapins integrate N-WASP into clathrin-mediated endocytosis (Kessels and Qualmann, 2002). Consistently, the cytoskeletal component N-WASP has not only been shown to be recruited to sites of endocytic vesicle formation (Merrifield *et al*, 2004) but to have an important role in clathrin-mediated endocytosis by acute impairments of N-WASP functions (Kessels and Qualmann, 2002) and by analyses of N-WASP-deficient fibroblasts (Benesch *et al*, 2005). Further analyses showed that syndapin is able to recruit N-WASP to the membrane and to control N-WASP/Arp2/3 complex-mediated actin nucleation by N-WASP auto-

inhibition release (Dharmalingam *et al*, 2009). In the F-BAR family, N-WASP autoinhibition release was also observed for Toca-1 (Ho *et al*, 2004) and GBP17 (Takano *et al*, 2008).

Besides linking actin nucleation to endocytosis, the endocytic function of syndapins seems to centre on dynamin. Syndapin I is predominantly expressed in neurons and has thus been suggested to have an important role in compensatory, synaptic clathrin-mediated endocytosis (Qualmann *et al*, 1999). In presynaptic terminals, synaptic vesicle endocytosis is regulated by phosphorylation and syndapin I has been identified as the phosphosensor of dynamin I (Tan *et al*, 2003; Anggono *et al*, 2006), a function that appears to be of special importance during elevated synaptic activity (Clayton *et al*, 2009). Consistently, in lamprey giant axons injected with antibodies directed against either syndapin full length or syndapin's SH3 domain, membrane trafficking defects were selectively obvious after stimulation. The synaptic vesicle numbers were decreased and a massive increase of membranous cisternae still connected to the plasma membrane and enriched with clathrin-coated pits were observed (Andersson *et al*, 2008). Interestingly, synaptopHluorin measurements in neurons showed that also dynamin I is selectively required for endocytic uptake after stimulation (Ferguson *et al*, 2007). Syndapin I KO mice partially phenocopy dynamin I KO mice, as exemplified by loss of synaptic vesicle size control. Syndapin I turned out to be important for efficient membrane recruitment of all three dynamin isoforms (Koch *et al*, unpublished). This intimate interplay fits well with the coincidence detection of both proteins in TIRF analyses (Taylor *et al*, 2011) and represents another example highlighting the importance of BAR superfamily protein-mediated recruitment of binding partners to distinct membrane areas.

The endocytic defects of syndapin I KO mice in the brain as well as those of a naturally occurring mouse mutant with a mutation in the first alternatively spliced region of dynamin Ia (fitful mice) lead to seizures with tonic-clonic convulsions (Boumil *et al*, 2010; Koch *et al*, unpublished). Interestingly, the human gene locus 6p21.3, which includes the *syndapin* I gene, is associated with idiopathic generalized epilepsies (Turnbull *et al*, 2005).

In contrast, CIP4 KO mice did not show any neurological phenotypes. This probably reflects the very low abundance of CIP4 in the brain (Aspenström, 1997). Some role of CIP4 in endocytosis was shown by the finding that CIP4 KO mice display slightly increased early glucose uptake after glucose challenge. This phenotype was a result of elevated plasma membrane levels of the glucose transporter GLUT4 due to reduced and delayed rates of clathrin-mediated endocytosis (Feng *et al*, 2010). Similar results for CIP4-mediated GLUT4 trafficking were observed in cell culture experiments after CIP4 knockdown (Hartig *et al*, 2009). Analysing transferrin uptake, single, double or triple knockdowns in cultured cells showed that also the other two members of the mammalian CIP4/GBP17/Toca-1 family are involved in clathrin-mediated endocytosis (Itoh *et al*, 2005; Tsujita *et al*, 2006).

Analyses in flies and worm revealed additional physiological processes relying on CIP4/GBP17/Toca-1 family members and provided further insights into the molecular mechanisms these proteins employ during endocytosis. Targeted deletion of *Drosophila* CIP4, the only member of the CIP4/GBP17/Toca-1 family in flies, resulted in defective wing hair development, a process that is dependent on

endocytic trafficking (Fricke *et al*, 2009). Knockdown of CIP4 in flies revealed that also regulated E-cadherin endocytosis in epithelial cells is dependent on CIP4 and that CIP4 functions through dynamin and WASP (Leibfried *et al*, 2008). Analyses of the *C. elegans* mutants of the two CIP4/GBP17/Toca-1 orthologues showed diminished clathrin-mediated endocytosis-dependent yolk uptake into oocytes. The endocytic phenotype was accompanied by reduced egg production and defects in embryonic morphogenesis manifesting in some embryonic lethality (Giuliani *et al*, 2009). Otherwise, animals behaved normal, that is, there were no signs for a block of neuronal transmission. In line with N-WASP being an important interaction partner of CIP4/GBP17/Toca proteins, the defect in clathrin-mediated yolk uptake was mediated through WASP and WAVE, another Arp2/3 complex activator (Giuliani *et al*, 2009).

Mechanistic insights into the function of CIP4/GBP17/Toca-1 family members were also provided by a cell-free system suitable for high-resolution imaging of formed endocytic intermediates (Wu *et al*, 2010). This work allowed for a significantly increased resolution in z-direction when compared with TIRF microscopy. GBP17 was localized to the shaft of tubulated plasma membrane generated by arresting dynamin's GTPase cycle with GTP γ S (Wu *et al*, 2010) leading to extended tubular, endocytic structures that are not commonly observed in unmanipulated cells, probably because they are strongly antagonized by membrane tension (Zimmerberg and Kozlov, 2006). Strikingly, GBP17 was found on wider portions of the tubules with diameters of 50–120 nm, that is, diameters that correlate well with the sizes of tubules induced by GBP17 overexpression and those seen in *in vitro* reconstitutions, respectively (Itoh *et al*, 2005; Tsujita *et al*, 2006). GBP17 was axially segregated from the narrower neck of the tubules linking them to the clathrin-coated bud, which were coated with clathrin/dynamin and endophilin (Wu *et al*, 2010). These narrower diameters are consistent with ~35 nm tubules observed upon liposome tubulations with dynamin or endophilin (Takei *et al*, 1995; Ringstad *et al*, 1999).

Taken together, the functional data on BAR superfamily proteins suggest that the vesicle recycling machinery uses the functional diversity and the versatility of membrane binding and shaping offered by the BAR superfamily and the different complexes these proteins form with further components to provide means to efficiently retrieve membrane material.

Conclusions

The structures of single BAR dimers are informative and have advanced our understanding of membrane shaping by scaffolding and hydrophobic insertion mechanisms considerably. Cytosolic components capable of shaping membranes by transient membrane binding emphasize an intimate interplay between membrane compartments and the underlying cytosol. In this respect, membrane shaping by BAR domain proteins represents an excellent example for the compartmentalization of life functions.

In vivo studies clearly show that the initial idea that the differentially curved N-surface of BAR superfamily proteins determines their functions is oversimplified. Diagonal placing of BAR domain structures on tubular membranes, lateral

lipid-binding modes, combinations with further lipid-binding modules integrating their own distinct properties, additional lateral kinks giving rise to tilde-shaped curved structures and presumably also the formation of macromolecular lattices, which are likely to be the actual actors in membrane topology modulations, and their putative versatility in organization and arrangement make the picture much more complex than first thought.

Future work will therefore have to concentrate on revealing the exact cellular and physiological functions of BAR superfamily proteins by acute and constitutive loss-of-function paradigms in different organisms. These efforts will certainly also allow to better understand the diseases associated with altered functions of a variety of BAR superfamily proteins.

Additionally, in order to reveal the molecular functions of BAR superfamily proteins and their mechanistic details, it will be of utmost importance to understand the different types of lattices formed and how they can be rearranged dynamically to empower BAR superfamily proteins to shape membranes. Besides the distinct intrinsic properties of BAR domains, these architectures are likely to be strongly influenced by interactions with lipid components in the membrane, by molecular mechanisms that regulate BAR domain function (Roberts-Galbraith and Gould, 2010) and by the interaction partners of BAR superfamily proteins. Certain protein associations, such as Rac binding of arfapin (Tarricone *et al*, 2001), have been reported to interfere with the lipid binding of BAR domains. Also oligomerization and scaffolding of BAR superfamily protein interaction partners contribute to the functions of macromolecular assemblies. Dynamin, a prominent interaction partner of several endocytic BAR superfamily proteins, self-associates and forms spiral structures itself (Schmid and Frolov, 2011) and will thereby influence the geometry of BAR domain protein lattices. Other binding partners of BAR superfamily proteins may contribute to lattice geometry by offering multiple binding interfaces for BAR proteins, such as recently shown for the syndapin interaction partner Cobl (Schwintzer *et al*, 2011). *Vice versa*, BAR domain lattice geometries themselves will have profound impact on the arrangement—and presumably function—of binding partners that are recruited by the BAR domain proteins to membranes.

Thus, BAR superfamily proteins create macromolecular platforms, which have been shown to recruit and physically and functionally connect different binding partners and can thereby link and coordinate different processes that are of importance during endocytic vesicle formation, such as membrane invagination, coat formation, actin nucleation, vesicle size control, fission, detachment and uncoating, in time and space. A BAR domain-determined sequential recruitment and function of endocytic proteins may offer mechanistic explanations for the cell biological problem how a complex process with several steps and key players can be coordinated and given directionality and effectiveness.

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Conflict of interest

The authors declare that they have no conflict of interest.

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