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REVIEW

Membrane shaping by the Bin/amphiphysin/Rvs (BAR) domain protein superfamily

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Abstract BAR domain superfamily proteins have emerged as central regulators of dynamic membrane remodeling, thereby playing important roles in a wide variety of cellular processes, such as organelle biogenesis, cell division, cell migration, secretion, and endocytosis. Here, we review the mechanistic and structural basis for the membrane curvature-sensing and deforming properties of BAR domain superfamily proteins. Moreover, we summarize the present state of knowledge with respect to their regulation by autoinhibitory mechanisms or posttranslational modifications, and their interactions with other proteins, in particular with GTPases, and with membrane lipids. We postulate that BAR superfamily proteins act as membrane-deforming scaffolds that spatiotemporally orchestrate membrane remodeling.

Keywords BAR domain · Membrane remodeling · Membrane deformation · Curvature-sensing · Endocytosis · Synaptic vesicle recycling · Protrusion formation · Neuromorphogenesis · GTPases · Autoinhibition

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Structure of Bin/amphiphysin/Rvs (BAR) domains

Eukaryotic cells are characterized by a diverse array of membraneous structures including vesicles, tubules, and pleiomorphic vacuoles that enable cellular processes such as organelle biogenesis, cell division, cell signaling and migration, secretion, and endocytosis. In many cases, dynamic membrane remodeling is accomplished by the reversible assembly of membrane-sculpting or deforming proteins [1], most notably by members of the BAR domain superfamily. Members of this protein superfamily are involved in membrane remodeling in various cellular pathways ranging from endocytic vesicle and T-tubule formation to cell migration and neuromorphogenesis [2–7]. These proteins contain BAR/N-BAR, EFC/F-BAR and IMD/I-BAR domains (Fig. 1).

BAR/N-BAR domain

The BAR domain was originally identified as an evolutionary conserved region shared by the yeast proteins Rvs161, Rvs167 and the metazoan amphiphysins (the splice variants of which are also called Bin1) [8–11]. The crystal structure of the N-terminal domain of Arfaptin first elucidated how these domains might be able to associate with curved membrane domains [12]. BAR domains are dimerized via α-helical coiled-coils, and the dimerization module forms a positively charged surface that associates with the negatively charged inner surface of cellular membranes, mostly through interaction with negatively charged phospholipids [12–14]. BAR domain superfamily proteins deform membranes to a geometry that corresponds to the structures of the membrane-binding surface of the protein (a crescent- or banana-shaped dimer) (Fig. 1a–e, j). In vitro, BAR domains are able to bind to negatively

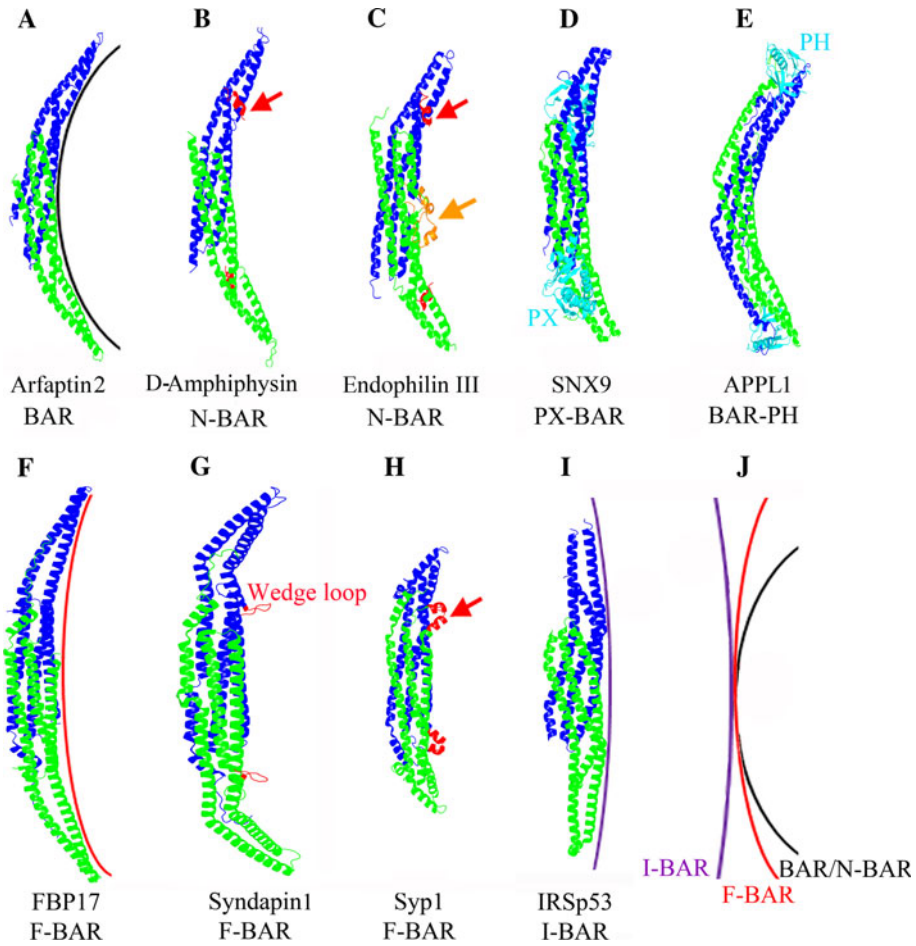

Fig. 1 Architecture of BAR domains and their role in membrane deformation. **a–e** Crystal structures of BAR/N-BAR domains: the arfaptin BAR domain (1I49) (**a**), the *Drosophila melanogaster* amphiphysin BAR domain (D-Amph) (1URU) (**b**), the endophilin III BAR domain (2Z0V) (**c**), the PX-BAR domain from SNX 9 (3DYT) (**d**), and the BAR-PH domain from APPL1 (2Q12) (**e**). **f–h** Structural insights into F-BAR domain architecture: the F-BAR domains of FBP17 (2EFL) (**f**), syndapin 1 (2X3X) (**g**), and yeast Syp1 (3G9G) (**h**). **i** Crystal structure of the IRSp53 I-BAR domain (1WDZ). **j** Membrane deformation by BAR/N-BAR, F-BAR, and I-BAR domains. BAR and F-BAR domains induce positive membrane curvature, whereas I-BAR domains generate negative membrane curvature. Amphipathic α-helices are indicated by *red arrows* in (**b**), (**c**) and (**h**). The additional amphipathic α-helix within the endophilin BAR domain is indicated by an *orange arrow* in (**c**). Crystal structures were generated with Pymol

charged phospholipids and to induce tubulation of liposomes [15]. Recent structural studies have revealed that BAR domains are frequently found in conjunction with a second membrane binding sequence such as an amphipathic α-helix (termed N-BAR domain), a PH domain (pleckstrin homology domain), or a PX domain (Phox homology domain) (Fig. 1b–e). Amphipathic α-helices are found in the endophilin and amphiphysin subfamilies, which are involved in endocytosis and the activity-dependent recycling of synaptic vesicle membranes [16–21]. Such amphipathic helices are often unstructured until they insert in an asymmetric fashion into one leaflet of the membrane. They are predicted to sit flat on the membrane surface with their hydrophobic residues dipping into the hydrophobic phase of the membrane [13, 14, 22, 23]. Mutational inactivation of this hydrophobic face will eliminate liposome binding activity and BAR domain-induced membrane tubulation in vitro [20, 21]. Biophysical and computational studies indicate that such amphipathic helices in conjunction with the concave nature of the BAR domain fold are important determinants of the ability of BAR domain proteins to sense and induce membrane curvature [13, 17, 18]. Hence, the N-BAR module serves a dual role in regulating membrane curvature: via their N-terminal amphipathic helices N-BAR domain proteins sense curved membrane domains by probing the surface for lipid packing defects; such curved microdomains then become stabilized by the lateral association of banana-shaped BAR domain assemblies [16, 17, 24, 25]. A variation of this theme is found in endophilin N-BAR, which contains an additional helix inserted within the BAR domain conserved among endophilin subfamily proteins

[20, 21, 26]. This additional amphipathic helix is located in the center of the banana-shaped BAR domain and inserts into the lipid bilayer in an asymmetric fashion, similar to the N-terminal amphipathic helices found in amphiphysins [18, 20, 21, 26].

BAR domains on their own indiscriminately bind to negatively charged phospholipids. However, the presence of a neighboring PH or PX domain can confer lipid specificity, as PH or PX domains associate with phosphoinositides, including PtdIns(3)P, PtdIns(4,5)P2 or PtdIns(3,4,5)P3 [27–29], and can thus assist targeting of BAR domain-containing proteins to distinct membrane (sub)compartments. For example, the BAR-PX domain of Sorting Nexin 1 (SNX1), a critical component of the retromer complex [30, 31], has been shown to target SNX1 to a highly curved PtdIns (3)P-containing tubular microdomain on early endosomes [31], a function required for retrograde sorting of a variety of cargo ranging from mannose 6-phosphate receptors to Wnt signaling components [32, 33].

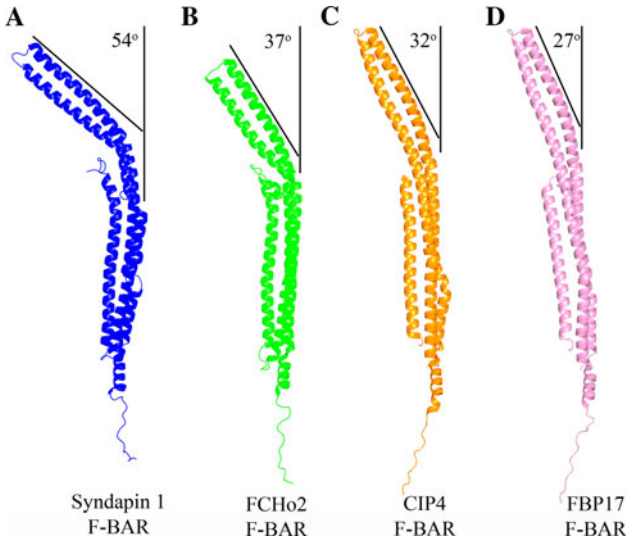

Fig. 2 Comparison of F-BAR domains. Monomer structures of the F-BAR domains from syndapin 1, FCHo2 (2V0O), CIP4 (2EFK), and FBP17 are shown. The degree of lateral bending was quantified by determining the angle between the long axis of the central 3-helix bundle (*concave surface*) and the long axis of the lateral helical region

F-BAR domain

F-BAR proteins were formerly referred to as Pombe *Cdc15 homology* (PCH) proteins. Owing to primary sequence similarity, a domain with a distant relation to BAR domains was identified within them [34]. The archetypal feature of this protein family is their *Fer/CIP4 homology* (FCH) domain [35], which constitutes a functional unit with a neighboring coiled-coil region, together forming the F-BAR domain [34]. F-BAR domain-containing proteins often contain various combinations of SH3 domains, SH2 domains, tyrosine kinase domains, and GAP domains at their C-terminal part [33, 36]. Similar to BAR/N-BAR domains, F-BAR domains form a dimeric positively charged membrane-binding module that associates with negatively charged phospholipids. F-BAR domains usually display a more extended shape with a much shallower curvature than canonical BAR/N-BAR domains (Fig. 1f–h, j). These structural features explain why membrane tubules induced by F-BAR domains are typically of a larger diameter compared to those generated by more strongly curved BAR domain proteins [37, 38]. Similar to the N-BAR domain, an additional wedge loop or amphipathic α-helix is found in several F-BAR domains so far investigated (Fig. 1g, h). For example, X-ray crystallographic studies show that the F-BAR domain of syndapin 1 (also named as PACSIN) dimerizes into an elongated "S" shape with a wedge loop consisting of ^{119}HKQIMGGF^{126} with hydrophobic residues (I122–M123) at the tip in each monomer. These residues are required for the membrane-deforming activity of syndapin [39, 40]. Replacing the hydrophobic amino acids I122 and M123 within the wedge loop by hydrophilic residues (I122T/M123Q) completely eliminated tubule formation in cells, although this mutation did not alter membrane phospholipid binding [39]. In the crystal structure of Syp 1 F-BAR, a distinctive amphipathic α-helix, similar to the amphipathic helix contained within the N-BAR domain of endophilin, protrudes from the membrane interaction face (Fig. 1h), suggesting a role in inducing and/or sensing membrane curvature. Comparison with known crystal structures of other F-BAR domains reveals differences with respect to the lateral curvature relative to the central dimerization region (Fig. 2). Specifically, this region in syndapin F-BAR domain is bent away from its central body at a ~54° angle, which generates a pronounced twisted S-shape in the dimeric molecule (Fig. 2). These differences in lateral curvature together with a distinctive amphipathic helix or wedge loop may allow F-BAR domains to interact and stabilize membranes with a broad range of curvatures.

I-BAR domain

The I-BAR domain (inverse BAR) was first identified in IRSp53 based on sequence homology as an F-actin crosslinking domain at the N-terminal region of mammalian IRSp53 and MIM (missing-in-metastasis) proteins [41]. The IRSp53 protein family comprises IRTKS (insulin receptor tyrosine kinase substrate; also known as BAIAP2L1), MIM/ABBA (missing in metastasis/actin-bundling protein with BAIAP2 homology), and FLJ22582 (BAIAP2L2) [42]. Crystal structure analysis of the I-BAR domain of IRSp53 shows strong structural similarity to the

BAR domain family. One monomer consists of three α helices that dimerize into an antiparallel structure, which resembles a distinct, rather flat, cigar-shaped curvature [43]. The positively charged lipid-binding surface of I-BAR domains displays a convex geometry, thus recognizing negative membrane curvature (Fig. 1i, j) [43–45]. Like BAR/F-BAR domains, the I-BAR domains of MIM and IRSp53 can also directly bind and deform membranes into tubules in vitro [43–45]. However, I-BAR modules stabilize membrane tubules that penetrate into liposomes when bound to the membrane [45]. Strikingly, different I-BAR domains deform PI(4,5)P2-rich membranes through distinct mechanisms. The I-BAR domains of IRSp53 and IRTKS bind membranes mainly through electrostatic interactions, whereas the I-BAR domains of MIM and ABBA insert an additional amphipathic helix into the membrane bilayer, resulting in a larger tubule diameter in vitro and more efficient filopodia formation in vivo [44].

Oligomerization of BAR domains

Oligomerization around the curved membrane is essential for BAR domain function. In vivo, overexpression of BAR/F-BAR domains or BAR/F-BAR domain proteins generates membrane tubules in cells. However, different BAR domain proteins segregate within distinct areas of these tubules that correspond to their preferred curvature [15, 46]. In vitro, liposomal assays have been used to study the membrane tubulating activity of BAR domains. Previous studies have shown that BAR domain-containing proteins, such as amphiphysin, endophilin, syndapin, FBP17 and CIP4, are able to bind lipids and induce tubulation of liposomes [15, 21, 47–49]. A recent cryo-EM analysis of the CIP4 F-BAR domain illustrated how the BAR domain associates with membranes to form cylindrical tubules [49]. To form membrane tubules, F-BAR domain self-assemble into a helical oligomeric coat. Besides the tip-to-tip interactions found in the crystal structure of FBP17 F-BAR [37], the broad contacts between laterally-adjacent dimers are also required for the oligomerization of the F-BAR domain. Additionally, reorientation of the lateral interaction surface crucially triggers the oligomerization of F-BAR domain and the propagation of membrane bending. By fitting the crystal structure of the CIP4 F-BAR domain into cryo-electron microscopic (EM) reconstructions of membrane tubules, a cluster of positive residues (R/K) on the concave surface of the F-BAR module was identified that is required for membrane binding and for enabling rigid F-BAR dimers to deform the membrane [49]. This mechanism was also confirmed by a recent cryo-EM analysis of endophilin A1 N-BAR domain assemblies on liposomes [50].

BAR domain function

BAR domain superfamily proteins have been identified as central regulators of membrane remodeling, including the formation of plasma membrane protrusions and invaginations as well as formation of vesicular or tubular transport carriers (Fig. 3a). As examples of BAR domain protein function, we focus here on clathrin-mediated endocytosis and the formation of filopodial membrane protrusions.

BAR domain function in clathrin-mediated endocytosis

Clathrin-mediated endocytosis is the major pathway for the uptake of nutrients and signaling molecules in higher eukaryotic cells and for the recycling or degradation of transmembrane receptors including the activity-induced reformation of synaptic vesicles at nerve terminals in neurons [51–54]. A clathrin coat is assembled on the cytoplasmic face of the plasma membrane, which progressively matures into a clathrin-coated pit (CCP). Late-stage CCPs constrict at the neck region and finally pinch off from the membrane in a reaction driven by the oligomeric GTPase dynamin. Formation of endocytic vesicles involves differentially curved membrane intermediates at each stage of the pathway, thereby necessitating tight control of membrane remodeling by BAR domain proteins including the N-BAR proteins endophilin and amphiphysin, the BAR-PX domain protein SNX9, and the F-BAR domain proteins FCHo and syndapin. Many of these factors, in addition to their BAR/F-BAR domains, also contain a Src homology 3 domain (SH3) domain that allows them to associate with other endocytic and cytoskeletal proteins, such as the GTPase dynamin (mediating vesicle fission), or N-WASP (an initiator for actin polymerization) (Fig. 3a) [6, 15, 55–59].

During clathrin-mediated endocytosis, F-BAR domain proteins typically arrive early to the site of endocytosis, and may be involved in the nucleation of CCPs [60–63]. The F-BAR-containing proteins FCHo arrive early at sites of endocytosis and specifically bind to PtdIns(4,5)P2-enriched membranes and sculpt the initial vesicle bud site by their F-BAR-encoded membrane-bending activity [61]. Recent studies suggest that another F-BAR domain protein FBP17 has a dual role in shaping and stabilizing membrane curvature via its F-BAR domain and in recruiting machinery for actin polymerization [58]. The latter may provide the force to push vesicle buds away from the plasma membrane and/or to narrow down the neck region at the stage of coat propagation. Blocking clathrin assembly prevents deep membrane invaginations even in the presence of abundant membrane-tubulating proteins in the cytosol, thereby inhibiting CCP maturation at an early stage [58].

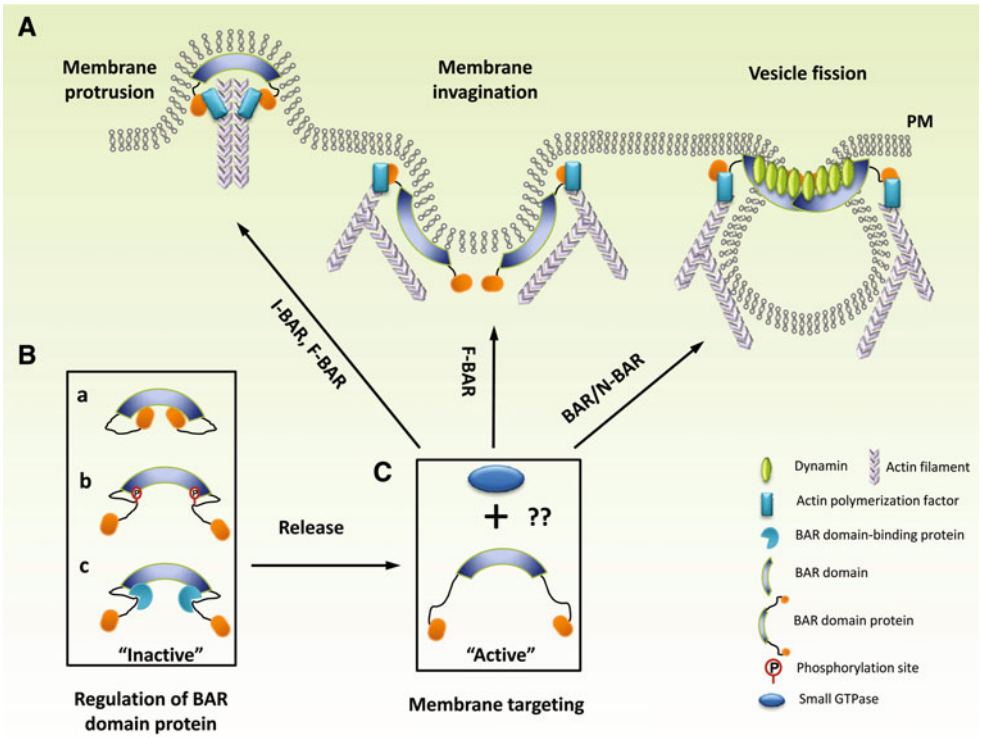

Fig. 3 Biological functions and regulation of BAR domain superfamily proteins. **a** BAR domain proteins function in the generation of membrane protrusions and membrane invaginations, as well as in vesicle fission. **b** Regulation of BAR domain proteins. Mechanisms include autoinhibition (*a*), phosphorylation/dephosphorylation (*b*), and protein–protein interactions (*c*). The latter may keep BAR domain proteins in an "inactive" state. **c** Membrane targeting of BAR domain proteins. Interactions with GTPases aid targeting of some BAR domain proteins to specific membrane sites. See text for further details

Deep clathrin-coated membrane invaginations eventually undergo dynamin-mediated fission (Fig. 3a). Dynamin is a multidomain protein containing a GTPase domain that binds and hydrolyzes GTP, a middle domain, a PH domain for interactions with lipid membranes, a GTPase effector domain (GED) and a proline-rich domain (PRD) at the carboxyl terminal end, which facilitates dynamin recruitment by SH3 domain proteins such as SNX9, amphiphysin, endophilin, and intersectin [64–66]. Oligomeric dynamin recruited to the "neck region" of late stage CCPs hydrolyzes GTP, resulting in a conformational change within the protein that causes vesicle scission [65–68]. Dynamins function together with BAR-SH3 domain proteins, such as amphiphysin, endophilin and SNX9 in bud neck constriction and membrane fission. Consistent with this, dynamin preferentially associates with narrow tubules of a diameter similar to that generated by the N-BAR domains of amphiphysin and endophilin in vitro [47, 48, 58]. In spite of the similar morphological appearance of endophilin- and amphiphysin-coated tubules, both proteins show opposite effects with respect to the regulation of dynamin-mediated membrane fission. Amphiphysin upon co-assembly with dynamin into ring-like structures enhances the fragmentation of liposomal tubules in the presence of GTP [48], whereas endophilin inhibits dynamin-GTP-dependent vesiculation of lipid tubules [47]. Similar effects to those seen for endophilin have been reported for SNX9, which is recruited transiently to CCPs together with a burst of dynamin during late stages of vesicle formation [69]. Although the molecular mechanistic basis for the differential behavior of BAR domain proteins remains unknown, one might speculate that endophilin and SNX9 are stabilized at CCPs by the presence of the amphipathic helix inserted into the BAR domain of endophilin or the PX domain of SNX9, respectively. Hence, structural variations with respect to the interaction of BAR domain proteins with membranes may at least in part underlie their functional diversity at different stages of vesicle formation.

A common, yet poorly understood, feature of many BAR domain proteins in endocytosis is their functional and/or physical association with the machinery for actin polymerization. As shown for FBP17 and other BAR domain proteins, the concomitant presence of SH3 domains allows for recruitment of actin nucleation promoting factors including N-WASP and ARP2/3 to sites of endocytosis. Local actin polymerization may serve to push vesicle buds off from the plasma membrane, facilitate dynamin-mediated fission, and/or propel primary endocytic vesicles away from the cell surface. Clearly, further studies are needed to fully explore the underlying molecular mechanisms.

Filopodial membrane protrusions

Filopodia are often found embedded in or protruding from the lamellipodial actin network [70, 71] and play an important role in neurite outgrowth, wound healing and cell migration, and function as precursors for dendritic spines in

neurons [72]. A prominent feature of I-BAR domains is their ability to induce filopodia formation (Fig. 3a). A well-studied example is the I-BAR domain protein IRSp53. This protein features an autoinhibited conformation in its "inactive" state [4]. The interaction of IRSp53 with the small GTPase Cdc42 in its GTP-bound state releases IRSp53 from autoinhibition and recruits it to the plasma membrane, where IPSp53 via its I-BAR domain induces negative membrane curvature. Induction of membrane protrusions also involves SH3 domain-mediated recruitment of the IPSp53 specific binding partners mDia (a downstream effector of the small GTPase Rho implicated in stress fiber formation and cytokinesis [73]) and Mena (a member of Ena/WASP family protein involved in actin cytoskeleton for adhesion and cell migration [74]), which synergistically promote filopodia formation [4]. Surprisingly, in addition to I-BAR domains, negative membrane curvature is also promoted by a subset of F-BAR domain-containing factors including syndapins and srGAP2. Overexpression of the F-BAR domain of syndapin in addition to the generation of positively curved membrane tubules causes the formation of membrane protrusions or filopodial extensions [39, 75]. A similar phenomenon was observed in the case of the F-BAR domain-containing protein srGAP2. Membrane protrusions formed by srGAP2 negatively regulate neuronal migration and induce neurite outgrowth and branching [76]. More recent studies have also shown that the F-BAR domain of SRGP-1 induces negative membrane curvature, thereby facilitating cell–cell adhesion during *C. elegans* morphogenesis [77]. The underlying mechanism for the peculiar activity of F-BAR domain to induce negative membrane curvature remains uncertain.

Filopodia formation by I-BAR domain-containing proteins depends on the inherent negative curvature of the I-BAR domain itself and on phospholipid-binding residues on the convex side of the I-BAR homodimers. Initial I-BAR-mediated membrane deformation may then be followed by actin polymerization into the space generated, i.e. by the interaction between the SH3 domain of IRSp53 and actin polymerization factors [3, 44, 45, 72]. However, work on SRGP-1 suggests that the formation of filopodia driven by its F-BAR domain occurs independently from actin polymerization [77]. In contrast, the F-BAR family protein PSTPIP 2 induces filopodia by directly associating with F-actin [78]. Hence, more than one mechanism may underlie BAR domain protein-mediated filopodia formation.

Regulation of BAR domain protein function

Since BAR domain proteins are involved in dynamic membrane remodeling by binding and bending cell membranes and by re-organizing the cytoskeleton, their function must be tightly regulated. These mechanisms of regulation include autoinhibition, phosphorylation/dephosphorylation, and interactions with other proteins (Fig. 3b).

Autoinhibition

Multidomain proteins are frequently observed to form autoinhibitory conformations that arise from intramolecular interactions [79–82]. This is exemplified by the recently determined crystal structure of syndapin 1. Autoinhibition is achieved by the association of its F-BAR and SH3 domains, thereby blocking the membrane-tubulating activity of the F-BAR domain in living cells [39, 83]. Release from the clamped conformation is driven by association of syndapin's SH3 domain with the PRD of dynamin 1 unlocking its potent membrane-bending activity. A similar autoinhibitory mechanism has been proposed for srGAP2 and SRGP-1 [76, 77]. A molecularly distinct autoinhibitory mechanism involving the BAR and GAP domains has recently been proposed for members of the GRAF family (GTPase regulator associated with focal adhesion kinase) and the Arf GTPase-activating protein ASAP [84, 85].

Phosphorylation/dephosphorylation

Phosphoregulation of BAR domain proteins has shown demonstrated early on for the F-BAR domain protein Cdc15, a protein with a C-terminal SH3 domain that plays important roles in a variety of cellular processes including endocytosis, cytokinesis, cell motility, and neuritogenesis [86–88]. In *S. pombe*, Cdc15 is an early-acting component in the formation of the contractile ring during cytokinesis. This function involves nucleation of F-actin filament assembly [88] via interactions of Cdc15 with formin and the type I myosin Myo1. Furthermore, Cdc15 stabilizes the contractile ring by associating with the C2-domain protein Fic1 and with paxillin homolog Pxl1 via its C-terminal SH3 domain [89]. Recent studies show that the biological function of Cdc15 in cytokinesis is tightly regulated by phosphorylation/dephosphorylation [90]. Multi-site phosphorylation of Cdc15 within the linker region connecting the F-BAR and the SH3 domains generates a closed inactive conformation (Fig. 3). This modification abolishes Cdc15 assembly at the division site in interphase cells and blocks complex formation with its above-mentioned binding partners. Dephosphorylation of Cdc15 releases this intramolecular block, thereby generating an open active conformation, in which the F-BAR domain engages the membrane and Cdc15 is able to interact with its binding partners [90]. A phosphoregulatory mechanism also controls the activity of Hof 1, the homolog of PSTPIP1 in

mammals [91, 92], and Cdc15 in fission yeast [93], which is released from autoinhibition by Dbf2 kinase-mediated phosphorylation [94].

Regulation by protein interactions

As mentioned above, phosphorylation of Hof1 by the protein kinase Dbf2 activates its function. However, activity of Hof1 in addition is controlled by its association with other proteins. In budding yeast, Hof1 plays an essential role in the regulation of cytokinesis during which an actomyosin ring contracts to trigger cleavage furrow formation and membrane ingression [95]. Hof1 accumulates at the site of cell division once cells enter a new cell cycle [96], but its function is blocked by its direct binding to septins [94]. During cytokinesis, phosphorylation of Hof1 by Dbf2 relocates the protein from septins to the actomyosin ring.

BAR domain proteins have also been shown to be regulated by interaction with small GTPases. As association with small GTPases contributes to BAR domain targeting to membranes, these mechanism are discussed in further detail below.

Membrane targeting of BAR domain proteins

From the above, it is clear that BAR domain protein function needs to be tightly controlled. This includes the correct targeting of BAR domain proteins to organelles or organellar membrane subdomains. How is this achieved? While BAR domain proteins directly bind to membrane phospholipids, these interactions on their own are often insufficient to confer specific association with membranes. Several studies link membrane targeting of BAR domain proteins to signaling pathways involving small GTPases (Fig. 3c) [4, 6, 12, 97–99]. Small GTPases cycle between an active GTP-bound and an inactive GDP-bound state. The GTP/GDP cycle functions as a molecular switch through which effector function including membrane trafficking, actin dynamics, or cell signaling cascades are regulated [100–104]. Examples include regulation of filopodia formation by Cdc42 [4] and Rac-dependent formation of lamellipodial protrusions at the cell periphery [105]. Furthermore, subsets of small GTPases have been demonstrated to bind to and deform membranes via insertion of amphipathic helices or hydrophobic lipid moieties (i.e., myristoyl, prenyl, farnesyl, or palmitoyl groups) [106–110].

The BAR domain protein Arfaptin was originally identified as a binding partner of the Arf family of small GTPases. Recent work has shown that Arl1 associates with the BAR domain of Arfaptin in a GTP-dependent manner [97]. Overexpression of Arfaptin induces formation of Golgi-derived membrane tubules, while exogenous expression of Arl1 alone neither generates membrane tubules nor enhances Arfaptin-dependent tubulation activity. However, depletion of Arl1 abolishes the association of Arfaptin with the Golgi complex, a defect that can be cured by re-expression of exogenous Arl1. Thus, Arl1 is required for targeting Arfaptins to Golgi membranes. Given that Arl1 itself associates with membranes and generates membrane curvature, the BAR domain of Arfaptin may recognize lipid packing defects induced by Arl1-GTP, thereby facilitating the local production of highly curved membranes. Similar principles may target BAR domain proteins with GAP activity such as srGAP, GRAF, and ASAP to their correct subcellular location, although the precise molecular determinants involved may differ from those used by Arl1–Arfaptin. Indeed, biochemical analyses indicate that the early endosomal protein APPL associates with Rab5 via its BAR and PH domains, and that these interactions are critical for APPL-mediated regulation of cell proliferation [98, 99] and for its targeting to endosomal membranes [98]. Consistent with these data, overexpression of inactive GDP-bound Rab35S34N causes the redistribution of endogenous APPL endosomes to the cytoplasm [98]. Collectively, these results support the hypothesis that membrane targeting of APPL depends on the small GTPase Rab5.

Other small GTPases besides Arl1 and Rab5 have also been linked to BAR domain protein association with membranes. For example, Cdc42 has been shown to regulate membrane association of I-BAR and F-BAR domain proteins [4, 6, 111]. Cdc42 directly associates with the I-BAR domain protein IRSp53 via a partial Cdc42/Rac interactive binding region (CRIB) motif in a GTP-dependent manner. Activation of Cdc42 by expressing the DH/PH domain of FGD1, a Cdc42-specific guanine nucleotide exchange factor, dramatically increases filopodia formation, an effect blocked by an inhibitory fragment derived from IRSp53 [4]. Similarly, active Cdc42 has been observed to directly interact with the F-BAR domain proteins Toca-1 and CIP4 [6, 35, 111]. In these cases, association with Cdc42 does not involve a CRIB motif but rather occurs via a protein kinase C-related kinase homology region (HR1) domain. Disruption of complex formation between Cdc42 and Toca-1 impairs the ability of Cdc42 to facilitate N-WASP-WIP-mediated actin polymerization [6]. Activated GTP-bound Cdc42 (Q61L) significantly enhances the tubulating ability of CIP4, while the same mutant inhibits FBP17-induced membrane tubulation [112]. Cdc42 possibly also associates with other F-BAR domain proteins such as syndapin. Expression of mutant Cdc42 abrogates the ability of overexpressed syndapin to control neuromorphogenesis as evidenced by a

reduced number of dendrites and branching points [2]. Whether these effects reflect a direct interaction between Cdc42 and syndapin remains to be determined. Most recently, it has been shown that the Cdc42 relative Rac1 interacts with syndapin 2 and regulates cell spreading and migration [113].

Conclusions and perspectives

It is clear that the BAR domain superfamily proteins play important roles in a wide variety of cellular processes by regulating dynamic membrane remodeling, including the formation of membrane vesicles or tubules, membrane protrusions, and invaginations as well as membrane fission. While the molecular basis for these various activities has become clear through combined structural and cell biological studies, much remains to be learnt about the physiological roles of BAR domain proteins at the organismic level. Moreover, we have only just begun to get a glimpse onto the modes and mechanisms of regulating BAR domain protein activity and function, i.e., during stimulus-induced signaling cascades. Lastly, given that the human proteome contains more than 60 members of the BAR domain protein superfamily, it is likely that many of their cell physiological functions have not been explored. All of these questions deserve attention and will need to be addressed in future studies.

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