Different functional modes of BAR domain proteins in formation and plasticity of mammalian postsynapses

Michael M. Kessels* and Britta Qualmann*

ABSTRACT
A plethora of cell biological processes involve modulations of cellular membranes. By using extended lipid-binding interfaces, some proteins have the power to shape membranes by attaching to them. Among such membrane shapers, the superfamily of Bin–Amphiphysin–Rvs (BAR) domain proteins has recently taken center stage. Extensive structural work on BAR domains has revealed a common curved fold that can serve as an extended membrane-binding interface to modulate membrane topologies and has allowed the grouping of the BAR domain superfamily into subfamilies with structurally slightly distinct BAR domain subtypes (N-BAR, BAR, F-BAR and I-BAR). Most BAR superfamily members are expressed in the mammalian nervous system. Neurons are elaborately shaped and highly compartmentalized cells. Therefore, analyses of synapse formation and of postsynaptic reorganization processes (synaptic plasticity) – a basis for learning and memory formation – has unveiled important physiological functions of BAR domain superfamily members. These recent advances, furthermore, have revealed that the functions of BAR domain proteins include different aspects. These functions are influenced by the often complex domain organization of BAR domain proteins. In this Commentary, we review these recent insights and propose to classify BAR domain protein functions into (1) membrane shaping, (2) physical integration, (3) action through signaling components, and (4) suppression of other BAR domain functions.

KEY WORDS: BAR domain proteins, Dendritic spine shape, Glutamate receptor, Postsynaptic plasticity, Synapse formation

Introduction
Shaping the topology of locally defined membrane areas is crucial for many processes in life because such mechanisms do not only underlie membrane trafficking processes but also define the specialized morphologies of many cells in our body. Modulations of membrane topologies furthermore give rise to segregated cellular subcompartments and establish microdomains that are instrumental for spatially defined assemblies of cellular machineries. Neurons are characterized by their highly elaborate morphology, effective membrane trafficking pathways and a highly organized compartmentalization, which, for example, manifests itself in specialized synaptic compartments. The complex functions of the brain rely on all of these properties. Thus, studying each of them provides important insights into how the brain works and into neurological diseases. Brain research integrates functional studies at the molecular level, direct visual analyses of neuronal function, physiological studies of communication in neuronal networks and circuits, and studies at the macroscopic and tissue architecture level during development, maturation and degeneration, as well as behavioral and psychological studies with model organisms and man.

The development of neurons involves a variety of locally defined decisions and their physical execution against the odds of entropy and counteracting membrane and environmental forces. The developmental steps in neuromorphogenesis include axon and dendrite formation and branching, the generation of presynaptic sites of neurotransmitter release, the establishment of dendritic spines as postsynaptic specializations for reception and integration of neurotransmitter signals, their maturation and their plastic modulation and adaptation – a basis of learning and memory formation. Studies on the formation and plastic rearrangement of postsynapses of excitatory neurons (Fig. 1) highlight important cellular aspects that are derailed in a variety of human diseases, such as intellectual disability, degenerative diseases, schizophrenia and autism, which all have been found to be associated with defects in dendritic spine formation and organization (Fiala et al., 2002; Kulkarni and Firestein, 2012). In this Commentary, we will focus on discussing the functions of membrane-shaping proteins of the BAR domain superfamily in formation and plastic rearrangement of postsynapses.

Two aspects of cell biology that are of utmost importance in synapse and spine formation and postsynaptic plasticity are: (1) induction, elaboration, shaping and morphological remodulation of the synaptic compartment, and (2) locally defined assembly and reorganization of signal-receiving machinery. Among the latter, the internalization of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors during desensitization [long-term depression (LTD); Box 1] and their insertion into the plasma membrane during sensitization [long-term potentiation (LTP); Box 1] take center stage (Fortin et al., 2012). BAR domain superfamily proteins appear to be involved in both of these synaptic processes.

BAR domain proteins have been suggested to impose their curved shapes onto membranes (Peter et al., 2004) (see Box 2). BAR domains form anti-parallel dimers composed of a central six-helix bundle formed by three relatively long helices of each monomer and two protruding arms. BAR domains come in structurally slightly different subtypes and can also exist in combinations with further domains (Fig. 2). The functions of BAR domains are reflected in their ability to self-associate and bend membranes, which leads to the establishment of distinct membrane microdomains that display either convex or concave curvatures and that might furthermore serve as organizational protein platforms to which further cellular components can be recruited. This interconnected, organizational aspect of BAR domain superfamily proteins is mostly brought about by combining protein–protein interaction modules with BAR domains (Fig. 2). In addition, the functions of many BAR domain superfamily members reflect the action of domains affecting signaling components (Fig. 2), such as GTPase-activating protein (GAP) domains that function in...
in vivo (Fig. 3) and use them to provide a more integrated view on the shapes the human brain (Cao et al., 2007; Charrier et al., 2012).

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In this Commentary, we introduce these four functional aspects (Fig. 3) and use them to provide a more integrated view on the in vivo functions of BAR domain proteins.

Working to shape membranes

Most BAR domain proteins have the ability to shape liposomes and many have also been reported to overcome the membrane tensions of living cells and to form tubules or membrane protrusions upon their overexpression (Frost et al., 2009; Qualmann et al., 2011; Mim and Unger, 2012; Suetsugu et al., 2014). Recently, an increasing number of loss-of-function studies has addressed whether these observations have any cell biological or physiological importance. These analyses indeed frequently reveal phenotypes in endocytosis and/or neuronal morphology establishment – processes that involve extensive and spatially restricted changes in membrane topology (Qualmann et al., 2011; Suetsugu et al., 2014) – thereby suggesting that BAR domain proteins are indeed crucial for shaping membranes in vivo (Fig. 3A).

In vitro reconstitutions and, to some degree, also overexpression studies have shown two distinct behaviors of BAR domain proteins. Many are able to tubulate liposomes and/or cellular membranes, that is, promote the formation of convex (positive) curvatures upon membrane association. A few BAR domain proteins, however, show the opposite behavior, that is, they promote the formation of concave lipid surfaces (negative curvatures) (Box 2) and are therefore called inverse (I-)BAR domain proteins (Fig. 2; Qualmann et al., 2011). Of the I-BAR proteins, to our knowledge, only insulin receptor tyrosine kinase substrate p53 (IRSp53, also known as BAIAP2) has been characterized in neurons and shown to be of importance for synapse formation and function (Choi et al., 2005; Kim et al., 2009; see below), although the explicit involvement of membrane-shaping functions of IRSp53 still remains to be addressed.

Besides the I-BAR proteins, other members of the BAR domain superfamily have also been found to act as inverse membrane shapers in vitro and in overexpression studies in different cells. These proteins, which structurally belong to the so-called F-BAR family (Fig. 2), are termed inverse F-BAR (iF-BAR) domain proteins. They currently include the Slit-Robo GTGase-activating proteins 1–4 (srGAP1–srGAP4; srGap4 is also known as ARHGAP4) and localize along negatively curved membranes (Guerrier et al., 2009). This called for functional analyses that vigorously address the putative membrane-shaping functions of these BAR domain proteins in vivo. Overexpression of srGAP3 (also called WRP), as well as its isolated iF-BAR domain, but not of a lipid-binding deficient srGAP3 mutant, increases the number of dendritic filopodia in neurons (Carlson et al., 2011). Consistent with this, srGAP3 loss-of-function in early development manifests as a reduction of dendritic filopodia and spine density, and also leads to impairments in learning and memory, as assessed by multiple

Box 1. A brief overview of synaptic plasticity

Synaptic plasticity refers to the activity-dependent modification of the strength or efficacy of synaptic transmission at pre-existing synapses. The underlying mechanisms include both presynaptic alterations, which typically change the amount of neurotransmitter released from the presynaptic active zones into the synaptic cleft, as well as postsynaptic mechanisms that change the efficiency of the postsynaptic cell to respond to the neurotransmitter signal.

A presynaptic vesicle filled with neurotransmitter

AMPA-type glutamate receptor subunits GluR1,2,3

NMDA-type glutamate receptor subunits 1,2

Other receptors

Proximal scaffold protein (e.g. PSD-95)

Peripheral scaffold proteins (e.g. ProSAP/Shanks)

Actin filament

Fig. 1. The postsynaptic compartment and its structural adaptation during synaptic plasticity processes. The majority of glutamatergic excitatory postsynapses are located in F-actin-rich spines that emanate from the dendritic arbor of neurons. Postsynapses are a signal-receiving and signal-processing subcompartment of neuronal cells. The levels of AMPA-type glutamate receptors in the plasma membrane are modulated during synaptic plasticity processes, such as LTD and LTP. In these states, the adapted response to a previously sensed input is either decreased (LTD) or increased (LTP) by the functional and structural remodeling of postsynapses – a cellular correlate of learning processes.

GTPase cycles (see Box 3). Finally, the functions of BAR domain proteins can be suppressed by tight association with another BAR domain protein, usually a superfamily member with a relatively simple domain organization. Recent work suggests that suppression of the function of BAR domain proteins by BAR–BAR heterooligomerization is an important molecular mechanisms that shapes the human brain (Cao et al., 2007; Charrier et al., 2012).

The underlying mechanisms include both presynaptic alterations, which typically change the amount of neurotransmitter released from the presynaptic active zones into the synaptic cleft, as well as postsynaptic mechanisms that change the efficiency of the postsynaptic cell to respond to the neurotransmitter signal. Synaptic plasticity is commonly accepted to represent a major basis of learning and memory. Hereby, long-term potentiation (LTP) refers to a persistent increase in synaptic strength following high-frequency stimulation. LTP-inducing stimuli increase the amount of functional AMPA-type glutamate receptors in the postsynaptic membrane, mainly through receptor exocytosis that leads to a reinforcement of synaptic transmission. Furthermore, LTP-induction causes the formation of new synapses and the modulation of the morphology of existing synapses, particularly the increase of the head size of dendritic spines, the small dendritic protrusions that harbor the postsynaptic compartment of most excitatory synapses. In contrast, long-term depression (LTD) is an activity-dependent reduction in the efficacy of synapses that mainly results from a decrease in postsynaptic AMPA-type glutamate receptor content in the postsynaptic plasma membrane. This involves receptor internalization through enhanced endocytosis but also structural changes that result in a reduced number of and smaller postsynapses. Synaptic plasticity thus involves both structural and functional changes at synapses.

Table 1. Key constituents of postsynaptic and presynaptic compartments

Key constituents

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Box 2. Different shapes of BAR domain superfamily proteins fit different membrane curvatures

BAR domain superfamily proteins (purple) are located in the cytoplasm and promote positive, convex curvatures of the membrane, such as invaginations from the plasma membrane or protrusions from endomembranes (left panel).

In contrast, proteins of the I-BAR subfamily and iF-BAR proteins (brown) promote negative, concave curvatures, such as those found inside of outward-pointing plasma membrane protrusions (right panel).

Note that most membrane structures of the cell include areas with distinct curvatures, that is, they can accommodate membrane-binding proteins with different curvatures, such as those shown.

Aldrich syndrome protein (N-WASP) –

tests. Importantly, the impaired filopodia formation upon srGAP3 knockout cannot only be rescued by the full-length protein but also by its isolated iF-BAR domain (Carlson et al., 2011).

srGAP1 appears to be functionally different because its iF-BAR domain is not sufficient to induce filopodia formation, but rather suppresses the formation of these protrusions, whereas the isolated iF-BAR domain of srGAP2, similar to that of srGAP3, induced neuronal filopodia in cortical neurons (Coutinho-Budd et al., 2012). Strikingly, loss-of-function of srGAP2 does not impair spine formation but results in increased spine density; however, spine maturation is delayed (Charrier et al., 2012). This phenotype is mirrored by overexpression of srGAP2C, a truncated form of srGAP2 that only encodes an iF-BAR domain, whereas overexpression of srGAP2, which in addition to the iF-BAR domain also comprises a Rhogap and an SH3 domain, leads to opposite spine morphology effects compared to the loss-of-function phenotypes (Charrier et al., 2012).

BAR domain proteins that exhibit shapes that might support convex membrane curvatures, have been implicated in several functions that involve modulations of the membrane topology and that are directly related to synapse formation and postsynaptic plasticity. In line with the data for BAR domain proteins in non-neuronal cells (Qualmann et al., 2011), endophilin (Chowdhury et al., 2006), PICK1 (Xia et al., 1999, 2000), rapostlin (Wakita et al., 2011) and syndapin I (Pérez-Otaño et al., 2006; Anggono et al., 2013) have been implicated in the endocytic uptake of different postsynaptic receptors, such as transferrin, AMPA or N-methyl-D-aspartate (NMDA) receptors. However, to our knowledge, only in the case of PICK1 has BAR-domain-mediated lipid binding explicitly been demonstrated to be required for regulating neurotransmitter receptor surface expression and clustering (Jin et al., 2006).

An important challenge to understand BAR domain protein functions is to distinguish the functions of their cytosolic pool, which might either exist as an unbound fraction or interact with other structural components (such as cytoskeletal elements), from those of their membrane-associated pool, and to correlate this membrane-associated pool with membrane topology and/or further organizational parameters in a quantitative manner. Our recent quantitative analysis of the localization of the F-BAR protein syndapin I in dendritic spines, which used a combination of membrane-freeze fracturing and immunogold labeling, has provided one possible answer to this (Schneider et al., 2014). Freeze fracturing and sample preparation restricts the observation specifically to membrane-associated proteins. A perpendicular view onto such samples using transmission electron microscopy visualizes topologies of large fields of membranes and immunolabeled proteins at ultrastructural resolutions (Schneider et al., 2014). Interestingly, the membrane-associated fraction of syndapin I strongly accumulates in dendritic spines and is particularly pronounced in membrane areas representing the heads of spines that harbor synaptic machinery (stubby and mushroom-type spines). In contrast, it is rarely found inside the cylindrical-shaped necks of spines, whose strongly negatively curved membranes would be more suited to fit I-BAR proteins rather than the curvature of F-BAR proteins, such as syndapin I (Schneider et al., 2014). Nevertheless, it is intriguing that membrane nanotopologies in the proximity of the endogenous syndapin I in neurons are typically relatively flat. These in vivo findings are in line with in vitro experiments with the F-BAR domain protein formin-binding protein 17 (FBP17, also known as FNBP1 and rapostlin) and surface-attached lipids, which suggest that, despite their curved shape, F-BAR domains can also associate with flat membrane topologies (Frost et al., 2008). Thus, high-resolution localization studies of other endogenous BAR domain proteins with regard to the local membrane topologies will be important for gaining a deeper understanding of the physiological functions of BAR domain proteins.

The power of physical integration

Several BAR domain proteins with curvatures that would fit with positively curved membranes, and therefore cannot line and stabilize protrusions through lattice formation at the internal cylindrical membrane surfaces of filopodia and spines, have nevertheless been shown to shape spines. In particular, this includes the syndapins (syndapin I, II and III, also known as PACSIN1–PAC3 and the CIP4/Toca subfamily [CIP4 and Toca (also known as TRIP10 and FNBP1L, respectively), and FBP17] of F-BAR domain proteins (Fig. 2). For instance, knockdown of FBP17 leads to reduced spine densities (Wakita et al., 2011). Similarly, knockout of syndapin I leads to a reduction of dendritic spine density, which we have shown is caused by post synaptic loss of syndapin I (Schneider et al., 2014) and does not result from neurotransmission defects that are caused by the presynaptic membrane trafficking impairments that are also seen upon syndapin I knockout (Koch et al., 2011). Interestingly, rescue experiments have shown that the functions of syndapin I in both dendritic spine and synapse formation, as well as in postsynaptic function, are dependent on F-BAR-domain-mediated membrane binding and on SH3 domain interactions (Schneider et al., 2014). This suggests that there is an additional functional aspect in the synaptic functions of these proteins that goes beyond BAR-domain-mediated membrane association (Fig. 3).

Among the SH3-domain-mediated interactions of syndapins and of CIP4/Toca subfamily members are for instance those with dynamin, a crucial factor for receptor-mediated endocytosis (Qualmann et al., 1999; Qualmann and Kelly, 2000; Kamioka et al., 2004; Fricke et al., 2009; Bu et al., 2009; Wu et al., 2010) and the neural Wiskott–Aldrich syndrome protein (N-WASP) (Qualmann et al., 1999; Qualmann and Kelly, 2000; Ho et al., 2004; Kakimoto et al., 2006; Leibfried et al., 2008; Takano et al.,
known as Shank2) in the heads of dendritic spines (Schneider et al., 2014) and have been linked to autism spectrum disorders (Jiang and Ehlers, 2013). Syndapin I loss-of-function leads to a spatial misorganization of ProSAP1 (also known as Shank1), with PSD-95 (Soltau et al., 2004), one of the SH3-domain-interacting binding partners, such as ProSAP3 (positioned even further away from the plasma membrane) (Valtschanoff and Weinberg, 2001; Burette et al., 2014). Although these IRSp53-mediated connections from the plasma membrane) (Valtschanoff and Weinberg, 2001; Burette et al., 2014) postsynaptic receptors) and ProSAP3 (positioned even further away from the plasma membrane), IRSp53 has on average been detected between PSD-95 (positioned beneath the postsynaptic receptors) and ProSAP3 (positioned even further away from the plasma membrane) (Valtschanoff and Weinberg, 2001; Burette et al., 2014). Although these IRSp53-mediated connections between major factors in postsynapse formation and organization are reflected in decreased spine size and density upon acute IRSp53 loss-of-function (Choi et al., 2005), both the density and the ultrastructure of dendritic spines were unchanged in IRSp53-knockout mice (Kim et al., 2009). However, these mice have defects in spatial learning and novel object recognition suggesting that despite putative compensatory mechanisms that ensure synapse formation, proper brain function might nevertheless require the presence of the integrative component IRSp53 in postsynapses.
Whether and to what extent the synaptic functions of IRSp53 indeed involve any membrane shaping or at least binding of the I-BAR domain to membranes has to our knowledge not been addressed so far. A recent quantitative ultrastructural study revealed that endogenous IRSp53 shows no enrichment at the plasma membrane of dendrites, not even at highly curved plasma membrane areas, such as dendritic branches and the base and neck of spines. Instead, the immunolabeling density of IRSp53 was maximal at the cytoplasmic face of the PSD (Burette et al., 2014). These data suggest that the synaptic functions of IRSp53 might not center on membrane-shaping functions that are represented by its classical I-BAR domain but that IRSp53 might rather act as an integrative scaffolding factor in the F-actin-rich PSD (Fig. 3).

Additional BAR domain proteins with established roles in organizing postsynapses include PICK1, a relatively small protein that consists only of a C-terminal BAR domain and a PDZ domain (Fig. 2). PICK1 associates with the AMPA receptor subunit GluA2 (also known as GluR2 and GRIA2) and is involved in AMPA receptor clustering and GluA2 endocytosis (Rocca et al., 2008) – a function that might also involve syndapin I (Anggono et al., 2013). Additionally, PICK1 was reported to interact with F-actin and the Arp2/3 complex and to act as a suppressor of WASP- and WAVE-mediated Arp2/3 complex activation (Rocca et al., 2008). In line with this, PICK1 knockdown does not phenocopy the Arp2/3 complex loss-of-function, but instead leads to an increase in spine size (Nakamura et al., 2011). The inhibitory effect of PICK1 on Arp2/3 complex-mediated actin nucleation can be released by the interaction of the small GTPase Arf1 with PICK1, and is considered an important mechanism in PICK1-mediated endocytosis of AMPA.

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Box 3. The GTPase cycle

Regulatory GTPases function as molecular switches cycling between GDP-bound ‘OFF’ and GTP-bound ‘ON’-states. Flipping the switch in a directed mode from the active, GTP-bound form of the GTPase to the inactive, GDP-bound form is achieved by hydrolysis of the bound GTP through the intrinsic GTPase activity – an irreversible reaction that forces the cycle to run only in one direction (see box figure). GTP hydrolysis performed by the GTPases is intrinsically very slow but can be accelerated by orders of magnitude by GTPase-activating proteins (GAPs), which are specific for the respective GTP-binding proteins.

Exchange of the bound GDP with GTP to reactivate the GTPase is facilitated by guanine-nucleotide-exchange factors (GEFs), which increase the dissociation rate of nucleotides by several orders of magnitude. In their GTP-bound stage, GTPases can interact with effectors, which are defined as those proteins that only bind tightly to the ON-state. Thereby, only the active state of the GTPase can transduce a signal.

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receptors (Rocca et al., 2008, 2013). Very recently, detailed biochemical and biophysical studies by another laboratory, however, have questioned the basic findings underlying this model. In this study, PICK1 was found to neither bind to the Arp2/3 complex nor inhibit its ability to nucleate actin. Furthermore, the reported interaction between PICK1 and F-actin in vitro was found to be merely caused by unpecific, probably electrostatic, interactions (Madasu et al., 2015). Electrostatic interactions with F-actin have also been observed in recent in vitro reconstitution studies using actin and the F-BAR protein syndapin II (Kostan et al., 2014). It will be interesting to clarify whether BAR domain proteins use their curved, positively charged BAR domain interfaces for direct binding of actin or actin-related molecules such as those incorporated in the Arp2/3 complex, or whether their crosstalk with actin-filament-promoting factors under physiological conditions instead requires interactions between other domains, such as the SH3-domain-mediated interactions of syndapin or CIP4/Toca subfamily members with activators of the Arp2/3 complex.

Although the integration of the functions of different binding partners appears to be an important aspect of BAR domain function in synapse formation and plasticity, recent observations suggest that there is a second level of physical integration. Anti-syndapin I immunolabeling of freeze-fractured membranes of neurons unveiled that syndapin I is not homogenously distributed, but that it forms nanoclusters at the membrane of dendritic spines (Schneider et al., 2014). Such BAR domain protein nanoclusters would be well suited for integrating the functions of syndapin-I-interacting factors (Fig. 3B). By establishing such nanodomains, which might also be formed by other BAR domain proteins, given that, for example PICK1 binds to and clusters AMPA receptors (Xia et al., 1999), BAR domain proteins might be able to physically interconnect and to spatially organize their different interaction partners and thereby assemble the large cellular machines (Fig. 3B) underlying the formation and the plastic reorganization of synapses.

Control by signaling
Functional analyses of synapse formation and modulations have revealed that the gain- and the loss-of-function phenotypes of a diverse group of BAR domain superfamily members reflect the modulation of the activity of signaling components (Fig. 3C). These include BAR domain proteins with GAP domains (Fig. 2).

RNA interference (RNAi) and antisense experiments have suggested that loss-of-function of the Rho-GAP oligophrenin1 (Fig. 2) is associated with decreased spine length. This effect is mediated through hyperactivation of RhoA signaling, as the cellular effects of oligophrenin1 knockdown could be completely suppressed by inhibiting Rho-associated protein kinase (ROCK) with Y-27632 (Govek et al., 2004). Oligophrenin1 has been associated with X-linked intellectual disability (Billauret et al., 1998), and oligophrenin1-knockout mice show behavioral, social and cognitive phenotypes that resemble that of human patients (Khelfaoui et al., 2007). However, in contrast to the reported RNAi phenotypes, the spines at basal dendrites of CA1 pyramidal neurons display no impairments in length and their abundance is also unchanged (Khelfaoui et al., 2007).

Further loss-of-function experiments have suggested that postsynaptic oligophrenin1 has a crucial role in the activity-dependent maturation and plasticity of glutamatergic synapses by selectively enhancing AMPA-receptor-mediated synaptic transmission (Khelfaoui et al., 2009). For instance, examinations of endogenous AMPA receptor subunits revealed that specifically the endocytosis of GluA1 (also known as GluR2 and GRIA1) subunits, which are part of activity-dependently recycling GluR1–GluR2 complexes, but not endocytosis of GluA2 subunits, which are additionally present in constitutively recycling GluR2–GluR3 AMPA receptors, was affected by oligophrenin1-knockout (Khelfaoui et al., 2009). Defects in NMDA-dependent LTD induced by low-frequency stimulation, which have been observed in oligophrenin1-knockout mice, confirmed that specifically the activity-dependent receptor internalization was affected (Khelfaoui et al., 2009). Interestingly, similar to the suppression of spine organizational defects that are induced by oligophrenin1 loss-of-function (Govek et al., 2004), the LTD effects mediated by loss of oligophrenin1 could also be fully overcome with the inhibition of ROCK with Y-27632 (Khelfaoui et al., 2009). Because any presynaptic defects could also be alleviated with the administration of the PKA and RhoA–ROCK pathway inhibitor Fasudil (Khelfaoui et al., 2013), it appears that in all cases studied thus far, the observed oligophrenin1-knockout phenotypes in fact reflect a misregulation of signaling pathways rather than functions of oligophrenin1 as a membrane-shaping factor (Fig. 3C).

In fact, in vitro experiments with recombinant BAR and BAR-PH domains have demonstrated that the oligophrenin1 BAR domain does not bind to brain liposomes and, moreover, that even the combination of its BAR domain with the membrane-binding PH domain fails to result in effective liposome tubulation (Peter et al., 2004). Consistent with this, overexpression of the oligophrenin1 BAR-PH domain fails to elicit membrane tubulation in COS-7 cells (Peter et al., 2004). Furthermore, full-length oligophrenin1 might have an even lower ability to shape membranes due to autoinhibitory interactions (Fauchereau et al., 2003).

Another group of BAR-domain-containing proteins with signaling roles are the ASAP and ACAP/centaurin β subfamilies, which contain an Arf GAP domain (Fig. 2). Overexpression of ASAP1 in dissociated neurons results in decreased spine density and this effect can be partially rescued by overexpression of ARf4 or constitutive active ARf4 (Jain et al., 2012). It therefore appears that, despite the presence of a BAR- and PH-domain, ASAP1 largely exerts its functions in neuronal morphogenesis and spine formation by modulating GTPase activities through its GAP domain. Therefore, ASAP1 appears to functionally act in a mode similar to oligophrenin1 (Fig. 3C).

Other BAR-domain-containing proteins that contain a GAP domain are the members of the RICH-like subfamily (SH3BP1, RICH1 and RICH2; RICH1 and RICH2 are also known as ARHGAP17 and ARHGAP44, respectively), as well as the sRGA subfamily (Fig. 2). Members of both subfamilies show GAP activity towards Rac1 (Cicchetti et al., 1995; Endris et al., 2002; Raynaud et al., 2014). Similar to RhoA, Rac1 is also known as an important regulator of the actin cytoskeleton. Therefore, RacGAPs are also expected to modulate neuronal morphogenesis and synaptic functions. Indeed, through a reduction of Rac1 activity, the Rac1 GAP SH3BP1 was shown to mediate a morphological collapse of developing neurons (Tata et al., 2014).

Furthermore, RICH2 overexpression increased the size and decreased the density of dendritic spines, whereas RICH2 knockdown decreased both spine size and density – an effect that was phenocopied by Rac1 inhibition. The observation that treatment with a Rac inhibitor rescued the phenotype of RICH2 knockdown strongly suggests that these functions of RICH2 are exerted through its GAP activity (Raynaud et al., 2014) (Fig. 3C).
Another example for the modulation of Rac1 activity by BAR-domain proteins is the function of Arfaptin, although it does not contain a GAP domain (Fig. 2). Arfaptin interacts with Rac1, as well as with the GTPases Arf and Arl (D’Souza-Schorey et al., 1997; Lu et al., 2001). Structural analyses have shown that the membrane-binding interface of the Arfaptin BAR domain is blocked upon Rac1 binding. Interestingly, two molecules of Arl were able to compete with Rac1 in a reaction that leaves the concave membrane interface open for membrane association (Nakamura et al., 2012). However, similar to the inability of the BAR domains of the Arf-interacting proteins oligophrenin and centaurin β2 (also known as ACAP2), the arfaptin BAR domain alone did not bind to membranes and the performance of the arfaptin BAR domain also appears to be promoted by neighboring protein domains. Arfaptin has been implicated in synapse formation and modulation, as it was shown to physically associate with components of the dynactin complex, which has been linked to neurodegenerative diseases, and to mediate the membrane association of the dynactin complex in motor neurons (Nakamura et al., 2012; Chang et al., 2013).

Similar to the GAP-domain-containing BAR-domain proteins discussed above, APPL1 also appears to largely execute its functions in synapse formation and modulation through crosstalk with signaling components. APPL1 and its relative APPL2 are BAR-PH domain proteins (Fig. 2) and associate with the serine/threonine kinase Akt, which acts downstream of phosphoinositide 3-kinase (PI3K). APPL1 also contains a PDZ-interacting motif and thereby forms complexes with NMDA receptors through the postsynaptic scaffold PSD95 (also known as DLG4). By connecting synaptic NMDA receptors with PI3K/Akt signaling, APPL1 is crucial for the formation of synapses and synaptic plasticity (Cao et al., 2007; Wang et al., 2012). In line with the observation that Akt knockdown and overexpression of dominant-negative Akt lead to dramatic defects in spine and synapse formation, APPL1 RNAi also resulted in a significant decrease in the number of both spines and synapses. Consistent with this, mutational analyses have suggested that the functions of APPL1 in spine and synapse formation are dependent on its interaction with Akt (Majumdar et al., 2011).

Taken together, these data highlight that besides their BAR domains and classical protein–protein interaction modules, crosstalk with signaling components is also an important aspect of BAR domain protein function in synapse formation and plasticity.

**Acting as suppressive partners**

An additional mode of BAR domain protein function in dendritic spine and synapse formation has recently emerged and might be of far-reaching importance, namely, the suppression of the activity of other BAR domain proteins though complex formation (Fig. 3D). For instance, the small Arfaptin-related protein islet cell autoantigen of 69 kDa (ICA69, also known as ICA1) (Fig. 2) has been shown to associate with the BAR domain protein PICK1 and to inhibit its functions in AMPA receptor trafficking and synaptic plasticity (Cao et al., 2007). More than 75% of all ICA69 and PICK1 molecules have been reported to associate with each other in the brain; however, these inhibitory complexes only occurred in the cell body and dendrites (Cao et al., 2007; Wang et al., 2013). ICA69-mediated inhibition of PICK1 thus might be an important mechanism to restrict PICK1 function to synapses.

Mechanistically, ICA69 appears to form heteromeric ICA69–PICK1 complexes, which prevent the formation of homomeric PICK1 complexes. It is currently unclear whether ICA69–PICK binding reflects the formation of heterodimer BAR domain proteins or the heterooligomerization of homomorphic BAR dimers. As dimers of BAR domain proteins are usually relatively stable and, moreover, the C-terminus of ICA69 might also be involved in the interaction (Cao et al., 2007), heterooligomerization and disruption of putative PICK1 oligomeric structures appears more likely.

Apart from ICA69–PICK1 heterooligomerization, oligomers formed from almost identical BAR domain proteins also have the potential to modulate their function. For instance, a truncated form of srGAP2 that only comprises the iF–BAR domain of srGAP2 (srGAP2C) has been found to naturally occur in humans and might restrict srGAP2 function (Charrier et al., 2012) (Fig. 3D). srGAP2C, which is thought to have arisen from human-specific gene duplication, is expressed in the developing and adult human brain and associates with srGAP2 resulting in the inhibition of its function. This mechanism leads to neoteny during spine maturation and to increased densities of longer spines (Charrier et al., 2012). Because these features are specific for the human brain, this suggests that the inhibition of srGAP2 function by its human-specific paralog srGAP2C has contributed to the evolution of the human brain.

**Conclusions and perspectives**

Members of the BAR domain superfamily have recently emerged as being involved in receptor endocytosis and recycling and in the structural (re)organization of synapses, as well as in the functional integration of these processes. It is increasingly becoming clear that they accomplish these tasks by four different abilities (Fig. 3). First, they shape membranes directly by imposing their curved shapes onto membranes. Second, they integrate the membrane binding and protein interactions that they undergo. Thereby, they act as important membrane-anchoring and/or spatial targeting devices for the cellular machineries that they integrate. This mode of BAR domain function is further promoted by their ability to form larger macromolecular complexes and nanodomains that might organize and control the physical integration of different functional aspects in cellular processes. Third, BAR domain proteins that contain specific additional functional domains are able to crosstalk with signaling pathways; here, they can either act as ‘on’ and ‘off’ switches or they act through their protein–protein interaction domains. The combination of such domains with BAR domains might enable them to act as scaffolds to facilitate the formation of signaling platforms. Finally, BAR domain interactions are important mechanisms for restricting and fine-tuning BAR domain protein functions (Fig. 3).

As recent work suggests that all four of these functional aspects of BAR domain proteins are important molecular mechanisms for shaping and fulfilling the functions of the brain, further research on BAR domain proteins will advance our understanding of these intriguing and versatile spatial and functional organizers. We anticipate that unveiling the exact physiological functions of these proteins by gene knockout studies and addressing the membrane-associated subpool of endogenous BAR domain proteins in vivo by innovative techniques, such as immunolabeling of freeze-fractured membranes, will continue to reveal fundamental cell biological principles underlying synapse formation and synaptic reorganization processes—a fascinating field of cell biology that represents the basis for memory, learning and all higher brain functions.

**Competing interests**

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COMMENTARY


