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Control of Rho GTPase function by BAR-domains

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Cytoskeletal dynamics are key to the establishment of cell polarity and the consequent coordination of protrusion and contraction that drives cell migration. During these events, the actin and microtubule cytoskeleton act in concert with the cellular machinery that controls endo-and exocytosis, thus regulating polarized traffic of membranes and membrane-associated proteins. Small GTPases of the Rho family orchestrate cytoskeletal dynamics. Rho GTPase signaling is tightly regulated and mislocalization or constitutive activation may lead to, for example, morphogenetic abnormalities, tumor cell metastasis or apoptosis. There is increasing evidence that traffic to and from the plasma membrane constitutes an important mechanism controlling Rho GTPase activation and signaling. This brief overview discusses a group of proteins that function at the interface between membrane dynamics and RhoGTPase signaling. These proteins all share a so-called BAR domain, which is a lipid and protein binding region that also harbors membrane deforming activity. In the past 15 years, a growing number of BAR domain proteins have been identified and found to regulate Rho GTPase signaling. The studies discussed here define several modes of RhoGTPase regulation through BAR-domain containing proteins, identifying the BAR domain as an important regulatory unit bridging membrane traffic and cytoskeletal dynamics.

Introduction

Rho GTPases constitute a distinct subfamily within the superfamily of Ras-related small GTPases and are involved in the regulation of cell polarity and motility through their effects on the actin cytoskeleton, membrane traffic and cell adhesion.[1,2] RhoGTPases act as molecular switches, cycling between an inactive GDP-bound state and an active GTP-bound state. This transition is regulated by guanine-nucleotide-exchange factors (GEFs) that promote the exchange of GDP for GTP[3] and by GTPase activating proteins (GAPs) that stimulate the low intrinsic GTPase activity.[4] While activated Rho GTPases generally are localized at the plasma membrane, inactive Rho GTPases, with some exceptions, e.g., RhoB, associate with a cytosolic chaperone Rho guanine nucleotide dissociation inhibitor (RhoGDI).[5]

Increasing evidence indicates that traffic to and from the plasma membrane is an important event controlling Rho GTPase signaling. For example, active Rac1 resides in cholesterol-enriched membrane domains[6] and cell detachment can trigger internalization of these domains resulting in the inactivation of Rac1. Thus, internalization plays a key role in the regulation of Rac1 activity. In line with this, it was shown that the large GTPase Dynamin, which is involved in endocytosis, plays an indispensable role in Rac1 traffic. Dynamin inhibition results in an increase in Rac1 activity.[7] This is accompanied by a relocation of active Rac1 to aberrant dorsal ruffles which results in inhibition of cell spreading and lamellipodia formation.[7] Conversely, Rho GTPases control endocytosis and membrane dynamics. For example, Cdc42 regulates the uptake of GPI-anchored proteins and bacterial toxins via the CLIC/GEEC pathway which functions independently from clathrin or Caveolin-mediated internalization.[8] Furthermore, constitutively active Rac1 and RhoA can inhibit clathrin-mediated endocytosis.[9,10] Thus, membrane traffic and its regulation are tightly linked to RhoGTPase activation and signaling.

In a recent study, we showed that the adaptor protein PACSIN2 regulates the activity of Rac1. PACSIN2 is an F-BAR and SH3-domain-containing protein which is involved in membrane dynamics such as tubulation and internalization. Our findings suggest that PACSIN2 controls cell spreading and migration by targeting Rac1 to intracellular compartments for GAP-mediated inactivation.[11] PACSIN2 is part of the BAR-domain family of proteins that are important regulators of membrane dynamics. Currently, this family comprises proteins encoding one of six classes of BAR-domains: the archetypical BAR domain, or N-BAR, BAR-PH, PX-BAR, F-BAR and I-BAR domains.[12] BAR-domain proteins are capable of sensing membrane curvature and, by binding as banana-shaped dimers to phospholipids (the specificity of lipid binding depends on the type of BAR protein), they can further promote curvature, which eventually leads either to membrane invagination or protrusion depending on the type of BAR domain.[13] As most BAR domain proteins can form dimers and contain one or more protein-binding scaffolding/adaptor domains, they link membrane dynamics to signaling proteins that control actin dynamics. As a result, many BAR-domain containing proteins are potentially important regulators of Rho GTPase-dependent signaling.

Table 1. BAR-Domain-containing proteins lacking a RhoGAP/GEF domain that regulate Rho GTPases

Name	Regulates/Target	BAR Type	Accession #	References
PACSIN2	Rac1	F-BAR	Q9UNF0	11
CIP4	Cdc42	F-BAR	Q15642	14, 16
Toca-1	Cdc42	F-BAR	Q5T0N5	15, 17
Nwk	Cdc42	F-BAR	Q9VSU8	20
IRSp53	Cdc42, Rac1	I-BAR	Q9UQB8	21–24
MIM (B)	Rac1, not Cdc42	I-BAR	O43312	25, 27
Abba-1	Rac 1	I-BAR	Q765P7	26, 28

This table shows BAR-domain-containing proteins involved in regulation of Rho GTPases. GTPase specificity, the type of BAR domain and the Uniprot KB accession number are indicated.

Here, we discuss the role of BAR-domain proteins in the regulation of Rho GTPases. So far, two classes of BAR-domain proteins have been characterized that affect Rho GTPase function: proteins harboring a BAR domain that regulate Rho GTPase function (**Table 1**) and proteins that, in addition to their BAR domain, encode a RhoGAP/GEF domain and regulate Rho GTPase activity (**Table 2**).

Regulation of RhoGTPase Function by BAR Domain Proteins that Lack a RhoGAP/GEF Domain

Over the past 15 years, several BAR-domain-containing proteins have been described that regulate the function of RhoGTPases (**Table 1**). These proteins are all structurally related and encode, next to the common BAR domain, one or more adaptor- or scaffolding domains (**Fig. 1**). Recently, we have shown that the F-BAR domain protein PACSIN2 specifically interacts, through its SH3 domain, with the small GTPase Rac1. Via its F-BAR domain, PACSIN2 can bind to and induce invagination of the plasma membrane. We found that in HeLa cells, loss of PACSIN2 expression increases Rac1GTP levels and, as a consequence, promotes spreading and migration of cells. The effect of PACSIN2 on Rac1 activity depends on their association as well as on membrane binding, since a PACSIN2 BAR-domain mutant, deficient in membrane tubulation, fails to inactivate Rac1. Furthermore, we showed that inactivation of Rac1 by PACSIN2 is prevented when dynamin is inhibited. Our

Table 2. BAR-domain-containing proteins that harbor a RhoGAP or RhoGEF domain

Name	Synonym	Regulates/Target	BAR Type	GEF/GAP	Accession #	References
srGAP1	ARHGAP13	Cdc42, RhoA, not Rac1	F-BAR	GAP	Q7Z6B7	33
srGAP2/FNBP2	ARHGAP34	Rac1, not RhoA, not Cdc42	F-BAR	GAP	O75044	31
srGAP3/WRP	ARHGAP14	Rac1, not RhoA, not Cdc42	F-BAR	GAP	O43295	30, 34
srGAP4/p115	ARHGAP4	RhoA, not Cdc42, not Rac1	F-BAR	GAP	Q86UY3	32, 35
RICH1/Nadrin	ARHGAP17	Cdc42, Rac1, RhoA	BAR	GAP	Q68EM7	37–39
RICH2	ARHGAP44	Rac	BAR	GAP	Q17R89	41
Oligophrenin-1	ARHGAP41	Cdc42, Rac1, Rhoa	BAR	GAP	O60890	46
GRAF1	ARHGAP26	Cdc42, RhoA	BAR	GAP	Q9UNA1	43, 45
GRAF2/PSGAP	ARHGAP10	Cdc42, RhoA	BAR	GAP	A1A4S6	49
GRAF3	ARHGAP42	unknown	BAR	GAP	A6NI28	
GMIP	ARHGAP46	RhoA	BAR	GAP	Q9P107	50, 53
SH3BP1	ARHGAP43	Rac1/2, RhoG, Cdc42, not Rho	BAR	GAP	Q9Y3L3	51, 54
Tuba/DNMBP	ARHGEF36	Cdc42, not Rac1, RhoA	BAR	GEF	Q6XZF7	55–57

This table shows BAR-domain-containing proteins, harboring a GAP/GEF domain, involved in regulation of Rho GTPases. GTPase specificity, the type of BAR domain, presence of GAP/GEF domain, ARHGAP synonym and the Uniprot KB accession number are indicated.

data, therefore, suggest a model in which PACSIN2, in conjunction with dynamin, promotes internalization of Rac1GTP, subsequently targeting it to intracellular sites for GAP-mediated inactivation.[11]

Another family of F-BAR domain-containing proteins that controls RhoGTPase function is the CIP4 family, consisting of CIP4 and Toca-1. Both CIP4 and Toca-1 interact with the small GTPase Cdc42 in fibroblasts,[14,15] regulating Cdc42-dependent actin reorganization.[15,16] Activated Cdc42 interacts with Toca-1 and the N-WASP-WIP (WASP-interacting protein) complex which leads to activation of N-WASP and Arp2/3-mediated actin polymerization.[15] Similar to Toca-1, CIP4 is an effector of activated Cdc42.[14] In addition, CIP4 promotes formation of invadopodia in breast cancer cells through the activation of N-WASP.[16] Both CIP4 and Toca-1 localize to membranes via their F-BAR domains where they act as scaffolding proteins for N-WASP and Cdc42. Whether the F-BAR domain is dispensable for this function remains to be established. However, it is worth mentioning that binding of Cdc42 and N-WASP to Toca-1 regulates its tubulating capacity which depends on its F-BAR domain as an F-BAR domain mutant failed to induce tubulation even in presence of activated Cdc42 and N-WASP.[17] Interestingly, a third family member, FBP17 (forming-binding protein 17), is involved in actin reorganization as well. Similar to CIP4 and Toca-1, FBP17 localizes to sites of membrane curvature via its F-BAR domain and targets the N-WASP-WIP complex to the membrane, stimulating Arp2/3-dependent actin polymerization.[18] However, unlike Toca-1 and CIP4, FBP17 does not interact with Cdc42,[19] leaving its mode of regulation to be established.

Another F-BAR domain protein that acts in conjunction with Cdc42 is Nwk (Nervous Wreck). Nwk is present at the Drosophila larval neuromuscular junction. The mammalian genome encodes two Nwk homologs but these have not been characterized yet.[20] Drosophila Nwk interacts with various endocytic proteins via its SH3 domain and promotes, together with Cdc42, WASP-mediated actin polymerization, which is important in the regulation of synaptic morphology.[20] The exact role of the F-BAR domain and whether Nwk physically interacts with Cdc42, similar to CIP4 and Toca-1, remains to be established.

In addition to the proteins discussed above, one other family of BAR domain-containing proteins has been described to control RhoGTPase function. This family consists of IRSp53, MIM(B) and Abba. They all share an N-terminal IMD domain which is also known as I-BAR domain. IRSp53 is an effector of both Rac1 and Cdc42 and binds to active Rac1 via the I-BAR domain and to active

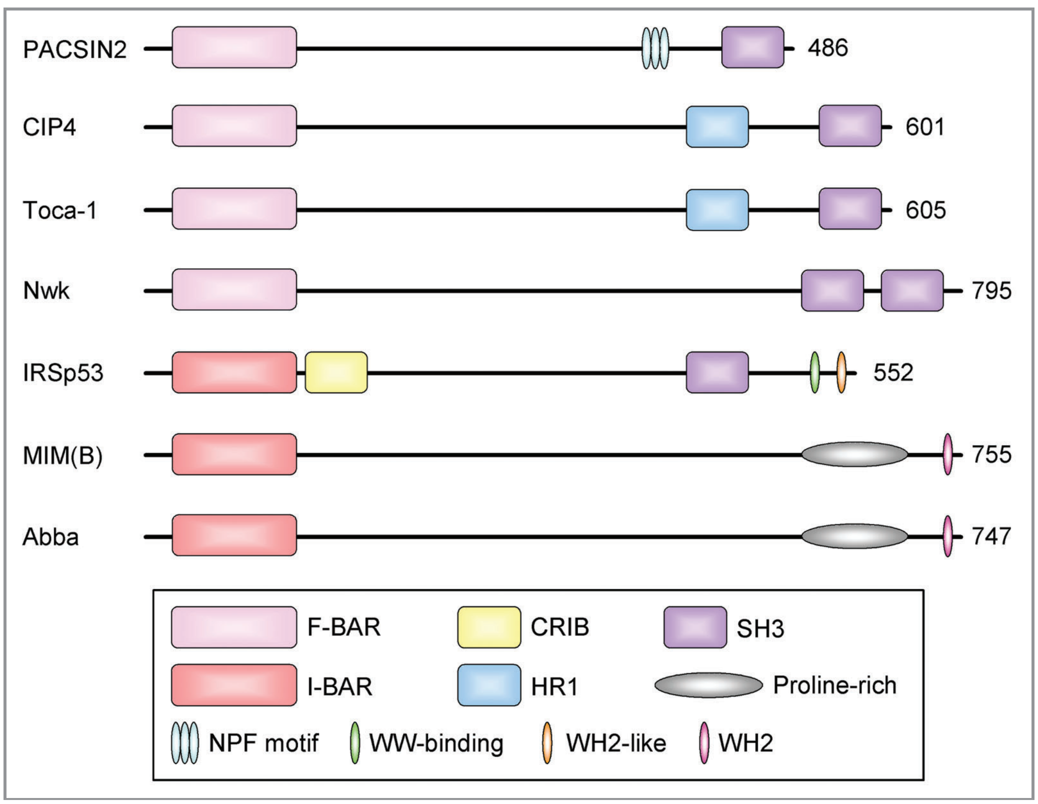

Figure 1. BAR-Domain proteins lacking a RhoGAP/GEF domain that regulate Rho GTPase function. Several BAR-domain-containing proteins have been shown to regulate Rho GTPase function. These proteins encode, in addition to their common BAR domain, one or more adaptor or scaffolding domains. Abbreviations for domains are as follows: CRIB, Cdc42/Rac1 interactive binding domain; F-BAR, Fes/CIP4 homology Bin/Amphiphysin/Rvs; I-BAR, inverted-Bin/Amphiphysin/Rvs; HR1, homology region 1 (Cdc42-binding domain); SH3, Src homology 3; WH2 (like), Wiskott-Aldrich homology 2 (like). Numbers indicate the number of amino acids. Drawings are not to scale.

Cdc42 via its CRIB domain.[21,22] IRSp53 mediates the interaction between Rac1 and WAVE2 (via its SH3 domain) which is important because WAVE proteins, unlike WASP, lack a GTPase binding domain (GBD). IRSp53 thus couples Rac1 to WAVE2 resulting in proper actin polymerization and formation of lamellipodia.[22,23] In addition to its function in Rac1-dependent actin dynamics, IRSp53 also acts as a Cdc42 effector stimulating the formation of filopodia by coupling membrane protrusion (mediated by the I-BAR domain) with actin dynamics through SH3-domain mediated interactions with proteins such as N-WASP.[21,24] Thus, whereas the IRSp53 I-BAR domain is involved in both Rac1 binding and formation of protrusions (by creating outward curvature), for Cdc4, the I-BAR domain mainly functions to create outward curvature. Via its CRIB domain, IRSp53 targets activated Cdc42 to these sites.

Unlike IRSp53, which mediates signals from both Rac1 and Cdc42, Abba and MIM(B) interact with Rac1 but not with Cdc42. Whereas Abba associates to GTP-bound Rac1, MIM(B) binds Rac1 in a nucleotide-independent fashion.[25,26] MIM (B) binds and bundles actin filaments and induces membrane protrusions through the interaction with and activation of Rac1 (both processes mediated via the IMD/I-BAR domain). Moreover, MIM(B) acts as a scaffold protein to recruit Rac1 effectors that drive actin assembly.[25,27] Abba regulates plasma-membrane- and actin dynamics as well and interacts with Rac1 via its IMD/I-BAR domain, similar to MIM(B).[28] Abba localizes with active Rac1 in membrane ruffles and was shown to bind to both wild-type and constitutively active Rac1.[26] PDGF treatment enhanced the Abba-Rac1 interaction and an Abba mutant, deficient in Rac1 binding, prevented Rac1 activation and induction of membrane ruffling by PDGF.[26] These results reveal an important role for Abba in Rac1 signaling downstream of the PDGF receptor.

Thus, it is clear that BAR-domain proteins play key roles in regulating RhoGTPases and that the BAR domain itself is important for this function. Although BAR-domain proteins have similar structures, the mechanisms by which they regulate GTPases differ. Whereas some are targeted, via their BAR domain, to specific sites to control GTPase traffic (e.g., PACSIN2), or act in concert with GTPases to ensure efficient activation of downstream signaling (e.g., Toca-1), others form a physical link via their BAR domain between GTPases and their upstream activators (e.g., Abba) or downstream effectors (e.g., IRSp53). Moreover, some of the BAR-domain proteins (e.g., Toca-1) act either as positive regulators or signal transducers, whereas others (e.g., PACSIN2) serve to downregulate GTPase output.

Regulation of RhoGTPase Function and Activation by BAR Domain-Containing GAPs or GEFs

A large number of RhoGEF and RhoGAP proteins have been identified so far.[3,29] More recently, several of these GAP/GEF proteins were shown to contain a BAR domain as well (**Table 2**) and to have important functions in controlling the activity and consequently the function of RhoGTPases. Similar to the BAR-domain proteins described in the previous section, these BAR-GAP/GEF proteins are structurally similar in that they all harbor a BAR domain, a GAP/GEF domain, and one or more scaffolding domains/regions (**Fig. 2**).

The Slit-Robo (sr)GAPs are critical for neuronal migration because of their inactivation of RhoGTPases. Four different family members (srGAP1-4) have been characterized.[30-33] Slit proteins are secreted, cell or extracellular matrix-associated proteins that guide neuronal migration through binding to the transmembrane Robo receptors. Slit proteins increase the interaction between Robo1 and srGAP1 which results in the activation of srGAP1 and consequent inactivation of GTPases.[33] Whereas srGAP1 regulates Cdc42, both srGAP2 and srGAP3 mediate their function through inactivation of Rac1. The srGAP2 F-BAR domain promotes formation of filopodia-like membrane protrusions and neurite outgrowth in cortical neurons.[31] Thus, the F-BAR and RhoGAP domain of srGAP2 cooperate to regulate neuronal cell migration. The third family member, srGAP3/WRP, is part of the WAVE1 complex.[34] WAVE1 induces actin polymerization downstream of activated Rac1.[35] As part of the WAVE1 complex, srGAP3/WRP functions as a signal-termination factor for Rac1 through its Rac1-GAP activity.[34] Furthermore, srGAP3/WRP regulates spine development through F-BAR domain-dependent formation of dendritic filopodia, and loss of srGAP3/WRP results in impaired long-term memory in mice.[30] Finally, the less-well characterized srGAP family member srGAP4/p115, is predominantly expressed in hematopoietic cells and was shown to stimulate the intrinsic GTPase activity of RhoA and to inhibit stress-fiber formation.[32] Furthermore, srGAP4/p115 associates to MEKK1, thereby reducing MEKK1-induced signaling to the transcription factor AP-1.[36] However, additional studies are necessary to understand the biological function of srGAP4/p115 and the role of the F-BAR domain in this process.

Similar to srGAPs, RICH1 (also called Nadrin), is a BAR domain-containing protein which also posseses a RhoGAP domain. RICH1 shows GAP activity toward Cdc42, Rac1, and RhoA.[37,38] 3T3 fibroblasts expressing full-length RICH1 or its isolated GAP domain were unable to form membrane ruffles after PDGF stimulation.[38] Furthermore, RICH1 localizes to tight- and adherens junctions in epithelial cells, mediated through its interaction with the adaptor protein Amot. RICH1 associates via its BAR domain with the ACCH domain of Amot.[39,40] This interaction inhibits RICH1 function preventing RICH1 from properly downregulating activated Cdc42. In addition, Amot induces relocalization of the polarity proteins Pals1 and Par-3. Thus, RICH1 in conjunction with Amot maintains the integrity of tight junctions through the regulation of Cdc42 activity and trafficking of polarity proteins.[39] As an additional member of this family RICH2, a RacGAP, was identified as a regulator of the actin cytoskeleton in epithelial cells. RICH2 and Ezrin interact with the integral membrane protein CD317 linking it to the actin cytoskeleton at the apical surface of polarized epithelial cells.[41] As RICH2 inhibits formation of Rac1-induced

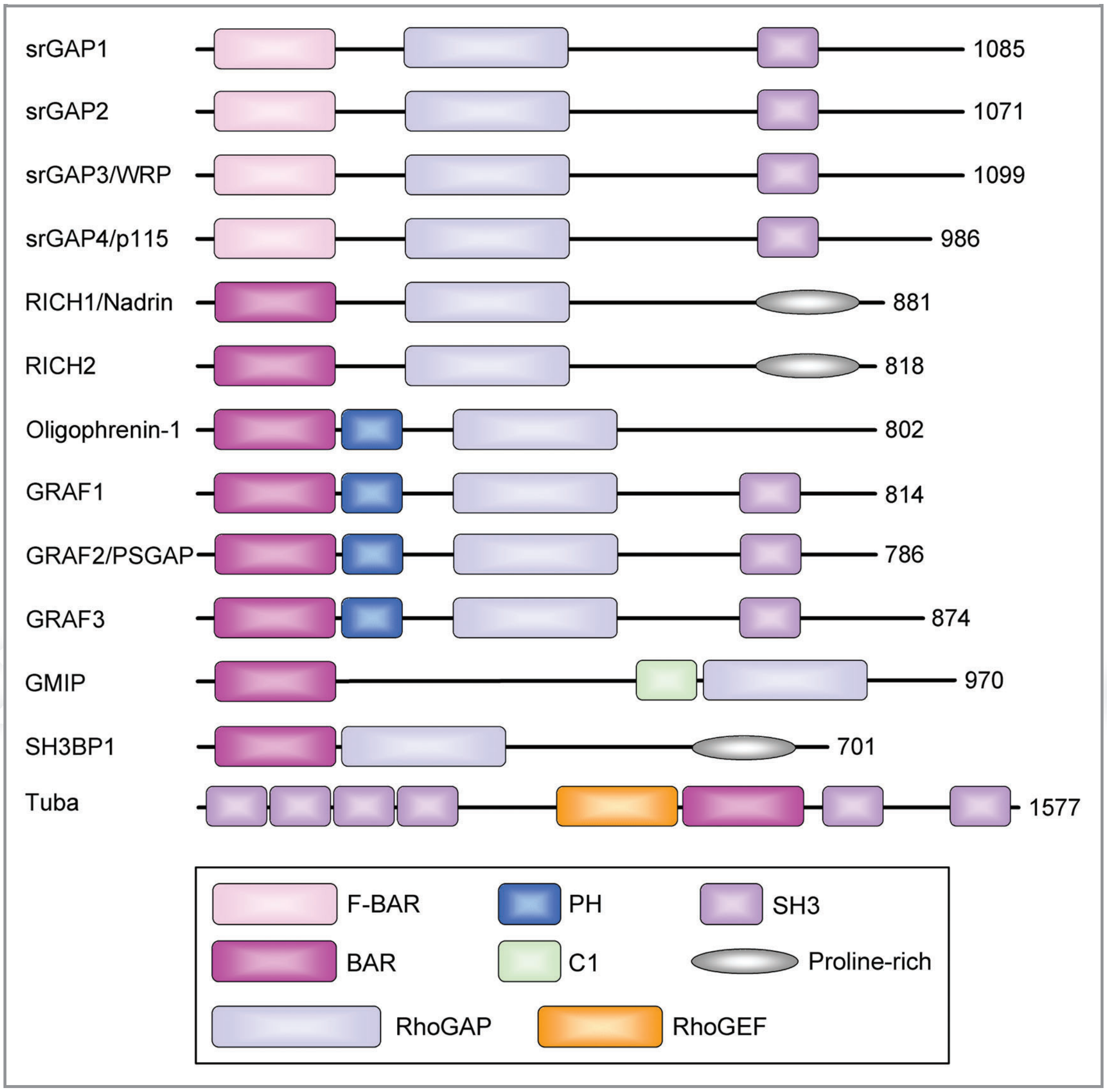

Figure 2. BAR-Domain-containing RhoGAP/GEF proteins. Several BAR-domain proteins have been characterized that harbor, in addition to their BAR domain, a RhoGAP/GEF domain also. In addition, they encode of one or more scaffolding or adaptor domains. Abbreviations for domains are as follows: BAR, Bin/Amphiphysin/Rvs; C1, cysteine-rich phorbol ester binding; F-BAR, Fes/CIP4 homology Bin/Amphiphysin/Rvs; PH, pleckstrin homology; RhoGAP, Rho GTPase activating protein; RhoGEF, Rho guanine-nucleotide-exchange factors; SH3, Src homology 3. Numbers indicate the number of amino acids. Drawings are not to scale.

membrane ruffles,[38] its presence in this complex possibly ensures proper regulation of actin cytoskeleton remodeling at the apical side of polarized epithelial cells. The exact role of the BAR domain is not known in this process although it could well be that RICH2 is targeted to the membrane (where it interacts with CD317) via the lipid-binding properties of the BAR domain.

In addition to the srGAP family and the RICH family, one more family of BAR-domain containing RhoGAP proteins is expressed in mammalian cells. The GRAF (GTPase regulator associated with focal adhesion kinase-1) family consists of four members, GRAF 1-3 and Oligophrenin-1. GRAF proteins play a role in the clathrin-independent endocytosis pathway CLIC/GEEC.[42] GRAF1 exhibits GAP activity toward RhoA and Cdc42 and binds to Focal Adhesion Kinase (FAK) via its SH3 domain.[43] Moreover, GRAF1 regulates the uptake of, for example, GPI-anchored proteins and bacterial toxins via the CLIC/GEEC pathway and internalization via this pathway was shown to be dependent on Cdc42 activation.[8,44] Through its BAR domain, GRAF1 localizes to tubular and vesicular membranes that define the CLIC/GEEC pathway. Here, GRAF1 regulates internalization of cargo by regulating the activity of Cdc42 via its GAP domain. Depletion of GRAF1, leading to impaired CLIC/GEEC function, reduces cell spreading and migration[45] indicating the importance of well-coordinated membrane dynamics and protein traffic in the control of cell shape and motility.

A close relative of GRAF1, Oligophrenin-1, stimulates GTP hydrolysis of Cdc42, Rac1, and RhoA.[46] Through the regulation of GTPase activity and the interaction with endophilin A1, Oligophrenin-1 controls synaptic vesicle endocytosis.[47]

Oligophrenin-1 was also shown to be involved in cognitive impairment.[46] As malfunctions in synaptic vesicle recycling are linked to cognitive defects[48] it could well be that Oligophrenin-1-associated cognitive impairment is caused by a defect in synaptic vesicle traffic due to improper Oligophrenin-1 signaling. A third GRAF family member, GRAF2, also known as PSGAP, has been shown to interact with PYK2 which is structurally related to FAK. PYK2 binds to the GRAF2 SH3 domain thereby inhibiting its RhoGAP function. This results in activation of Cdc42 and cytoskeletal reorganization.[49] The exact role of the GRAF2 BAR domain needs further investigation, but it could well be involved in targeting of GRAF2 to sites where GTPase regulation is required.

Finally, two more BAR-RhoGAP proteins have been characterized so far, GMIP and SH3BP1.[50,51] GMIP associates with the Ras-related protein Gem which is involved in regulating voltage-gated Ca^{2+} channels and cytoskeletal reorganization (Aresta et al., 2002; Beguin et al., 2001).[50,52] Gem, which binds Ezrin at the plasma membrane, downregulates RhoA-dependent stress fibers via its interaction with GMIP which exhibits GAP activity toward RhoA but not Cdc42 and Rac1.[50,53] The exact role of the GMIP BAR domain remains unclear. However, it was shown that the GMIP-Gem interaction is mediated via the GMIP N-terminal part which harbors the BAR domain.[50] Similar to IRSp53,[22] GMIP possibly uses its BAR domain for protein-protein interactions.

SH3BP1 exhibits GAP activity toward the Rac family GTPases and was shown to inhibit PDGF-induced membrane ruffling.[51] Furthermore, it was shown that SH3BP1 binds Exo84 and Sec8, both exocyst components, in a BAR domain-dependent fashion.[54] Together with the exocyst, SH3BP1 is targeted to the leading edge of polarized, motile cells. Here it mediates cell migration by regulating the activity of Rac1. Loss of SH3BP1 causes formation of disorganized instable protrusions.[54] Thus at the leading edge, in concert with GEF-mediated activation of Rac1, SH3BP1 ensures proper Rac1 inactivation to mediate efficient cell migration.

Whereas several RhoGAP proteins encode BAR domains, only one BAR-RhoGEF protein, called Tuba, has been described so far. Tuba has four N-terminal SH3 domains, a central DH domain followed by a BAR domain and two C-terminal SH3 domains (**Fig. 2**). Tuba was shown to exhibit GEF activity toward Cdc42 but not Rac1 and RhoA.[55,56] As Tuba can bind both Dynamin and actin-regulatory proteins such as N-WASP and WAVE1,[56] Tuba was proposed to be an important link between endocytosis, actin dynamics, and GTPase signaling.[56] Furthermore, Tuba activates Cdc42 and subsequently atypical PKC, thereby regulating polarized spindle orientation in epithelial cells.[57] To be functional, RhoGEF proteins generally need a DH-PH motif. The DH domain forms the catalytic core while the PH domain can be involved in plasma membrane targeting and in protein-protein interactions.[3] In general, DH domains without adjacent PH domains are less active than those that are flanked by a PH domain.[3] Intriguingly, it was shown that the Tuba DH domain showed little activity compared with the DH-BAR fragment.[56] This suggests that the BAR domain of Tuba acts as a substitute for a PH domain.

It is clear that BAR-GAP/GEF proteins are important regulators of GTPase activation and consequent signaling. In general, the BAR domain is important for the targeting to membranes and to sites of actin dynamics where they can induce membrane curvature. In addition, the BAR domain can mediate protein-protein interactions. Thus, the BAR domain and GAP/GEF domain cooperate to regulate processes dependent on membrane traffic and actin remodeling including cell spreading, cell polarization and motility. It is perhaps not coincidental that apparently more RhoGAPs than RhoGEFs encode BAR domains. GTPase activation is generally associated with the translocation to the plasma membrane. Although it is not as firmly established that turning off GTPase signaling requires the reverse process, e.g., GTPase internalization, there is accumulating support for this notion, based on previous studies showing that e.g., dynamin, caveolin-1 and PACSIN2 are all required for proper Rac1 inactivation. The fact that also many RhoGAPs encode BAR domains therefore suggest a functional link between membrane traffic and termination of GTPase signaling.

Concluding Remarks

Over the past 15 years, a series of BAR domain-containing proteins have been characterized that are linked to Rho GTPase signaling pathways. The BAR domain itself, through its capacity to bind lipids as well as proteins, plays an important role in the regulation of Rho GTPase activity and output. BAR domains play important roles in the targeting of proteins to specific regions within the plasma membrane where actin remodeling is necessary (e.g., for formation of protrusions or stimulating endocytosis). At these sites, BAR-domain proteins can control Rho GTPase activity, either by regulating the activation status of Rho GTPases, as some of these proteins harbor a RhoGAP/GEF domain, or by linking Rho GTPases to their upstream activators (e.g., growth factor signaling) or to their downstream effectors (e.g., the actin machinery proteins such as WASP proteins and the Arp2/3 complex). Strikingly, the Rho GTPase-regulating BAR domain proteins identified so far all harbor BAR, F-BAR, or I-BAR domains but not any of the other types of BAR domain. In conclusion, BAR-domain proteins are emerging as an important group of RhoGTPase regulators. As this field is relatively young and many previously identified proteins are now found to also include a BAR domain, it is very likely that in the near future more BAR-domain proteins that regulate Rho GTPase signaling will be identified. The challenge then lies in defining their contribution to the promotion or inhibition of localized GTPase signaling.

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Abbreviations

ACCH, Amot coiled-coil homology; AP-1, activator protein 1; BAR, Bin/Amphiphysin/Rvs; C1, cysteine-rich phorbol ester binding domain 1; CIP4, Cdc42-interacting protein 4; CLIC/GEEC, clathrin independent carriers/GPI-enriched endocytic compartments; CRIB, Cdc42/ Rac1 interactive binding domain; DH, Dbl homology; FAK, focal adhesion kinase; F-BAR, Fes/CIP4 homology Bin/ Amphiphysin/Rvs; FBP17, formin binding protein 17; GAP, GTPase activating protein; GDI, guanine nucleotide dissociation inhibitor; GEF, guaninenucleotide- exchange factor; GRAF, GTPase regulator associated with focal adhesion kinase-1; HR1, homology region 1; I-BAR, inverted-Bin/Amphiphysin/Rvs; IRSp53, insulin receptor substrate p53; MEKK1, mammalian MAP/ERK kinase kinase 1; MIM, missing in metastasis; Nwk, nervous wreck; PACSIN2, protein kinase C and casein kinase 2 substrate in neurons 2; PDGF, platelet derived growth factor; PH, pleckstrin homology; PSGAP, PH and SH3 domain-containing RhoGAP protein; PYK2, protein tyrosine kinase 2; SH3, Src homology 3; SH3BP1, SH3-domain binding protein 1; srGAP, slit-robo GAP; Toca-1, transducer of Cdc42-dependent actin assembly-1; WASP, Wiskott-Aldrich syndrome protein; WH2, Wiskott-Aldrich homology 2; WIP, WASP interacting protein; WRP, WAVE-associated Rac GAP

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