F-BAR domain proteins
Families and function

Sohail Ahmed,†,* Wenyu Bu, Raphael Tze Chuen Lee, Sebastian Maurer-Stroh†,3 and Wah Ing Goh†

1Neural Stem Cell Laboratory; Institute of Medical Biology; Immunos, Singapore; 2Bioinformatics Institute; Singapore; 3School of Biological Sciences (SBS); Nanyang Technological University (NTU); Singapore

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Abbreviations: BAR, Bin-Amphiphysin-Rvs; F-BAR, Fes/CIP4 homology-BAR; I-BAR, inverse-BAR; PX, phosphoinositide binding motif; CC, coiled coil; PCH, POMBe Cdc15 homology; EFC, extended FCH; RhoGAPs, Rho GTPase activating proteins; Fps, Fujinami poultry sarcoma; Fes, feline sarcoma; WRP, WAVE associated Rac GTPase activating protein; Syndapins, synaptic dynamin-associated proteins; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate

The F-BAR domain is emerging as an important player in membrane remodeling pathways. F-BAR domain proteins couple membrane remodeling with actin dynamics associated with endocytic pathways and filopodium formation. Here, we provide a comprehensive analysis of F-BAR domain proteins in terms of their evolutionary relationships and protein function. F-BAR domain containing proteins can be categorized into five subfamilies based on their phylogeny which is consistent with the additional protein domains they possess, for example, RhoGAP domains, Cdc42 binding sites, SH3 domains and tyrosine kinase domains. We derive a protein-protein interaction network suggesting that dynamin1/2, N-WASP, Huntingtin, intersectin and Cdc42 are central nodes influencing F-BAR domain protein function.

Introduction

All eukaryotic cells are surrounded by a plasma membrane, and they also contain multiple membrane-based organelles and structures inside cells. Thus membrane remodeling is likely to be important for most cellular activities and development. Recently, the Bin-Amphiphysin-Rvs (BAR) domain superfamily of proteins has been found to play a major role in remodeling cellular membranes linked with organelle biogenesis, membrane trafficking, cell division, cell morphology and cell migration.1 The BAR domain superfamily of proteins is evolutionarily conserved with representative members present from yeast to man.

BAR Domains

Currently there are three distinct families of BAR domain proteins: classical BAR, F-BAR (FCH-BAR e.g., Fes/CIP4 homology BAR e.g., Toca-1) and I-BAR (inverse-BAR e.g., IRSp53).

Structural studies have revealed common elements of the various BAR domains which include dimerisation modules that possess curvature and positively charged surfaces.1 Two basic residues in the BAR domain interact with the negatively charged phospholipids in the membrane. BAR domain dimers form a banana-shaped structure, with each dimer possessing a distinct curvature. BAR domains can deform membranes in vitro2 but may also detect curvature in membranes. The classical BAR domain family includes N-BAR and other additional lipid-binding domains. The N-BAR domain is distinguished from BAR domains by an amphipathic helix in front of the classical BAR domain, which enhances its ability to bind liposomes with no effect on tubulation.2 BAR domains are sometimes coupled with phosphoinositide binding motifs, phox (PX) or PH domains, which enable these BAR proteins to associate specifically with certain types of membranes.3

The I-BAR domain is “cigar shaped” and exists as a six-helix bundle dimer. Positively charged amino acids are located at the end of the I-BAR domain4-6 and are the binding sites for the membrane and actin. The I-BAR domain binds to phosphatidylinositol-rich membranes and has membrane tubulation activity.4-6 Saarikangas et al.8 showed that clustering of PIP2 occurred after I-BAR binding. The membrane was deformed by electrostatic interactions and the I-BAR domain was still dynamically associated with the inner side of the deformed membrane tubules.8 I-BAR domains bind to lipid bilayers through a convex surface and promote the formation of outward protrusions of membranes.8 The I-BAR domain containing protein IRSp53 induces filopodia by coupling membrane protrusion and actin dynamics.9

The fission yeast ‘Pombe Cdc15 homology’ (PCH) family proteins are adaptor proteins involved in cytokinesis and actin dynamics.10 PCH family members are expressed in diverse eukaryotic species with limited sequence similarity, but they do have similar domain structures—an N-terminal Fes/CIP4 homology (FCH) domain, followed by a coiled coil (CC) region and one or more SH3 domains at the C-terminal.10,11 The F-BAR domain, also known as the extended FCH domain (EFC) in PCH family proteins includes the FCH domain and...
CC region and is only weakly similar to the BAR domain. By sequence alignment, structural, biochemical and cell biological studies, it has been shown that the EFC is actually an extended BAR domain, and that F-BAR domains share similar properties with BAR domains. The F-BAR domain plays a role in dimerization and membrane phospholipid binding. Three pairs of basic residues are conserved in the F-BAR domain, which bind lipids and mediate membrane tubulation activity. The F-BAR domain binds specifically to certain kinds of lipids. This is in contrast to the BAR domain, which does not have such lipid preferences. The F-BAR domain has high affinity for PIP₂, moderate affinity for PIP₃ and phosphatidylserine, and does not bind lysophosphatidic acid, lysophosphocholine or sphingosine-1-phosphate. The F-BAR domain tubulates the membrane in vitro and in vivo. The F-BAR domain induces a gently curved helical-bundle dimer with a length ~200 Å fitting into a ~600 Å tubulated membrane. End to end oligomerisation of the F-BAR domain of formin binding protein 17 (FBP17) and thyroid hormone receptor interactor 10 (TRIP10) form filaments that can induce cell membrane invagination.

F-BAR Domain Protein Families

To provide a comprehensive overview of the repertoire of F-BAR domain proteins and to study their evolutionary relationship, we first had to create a proper F-BAR domain alignment since the existing FCH domain model in PFAM covered only the N-terminal part (about one third) of the F-BAR globular domain, as seen in the superimposition of crystal structures of three different F-BAR proteins. Using this structural alignment as seed, we created a new alignment of the whole F-BAR domain (see Suppl. Material for details) and used a derived hidden Markov model to identify a total of 1287 F-BAR domain-containing proteins in the UniRef100 database. Finally, focusing on nine representative species, we carried out a phylogenetic analysis of 305 F-BAR domain proteins (Fig. 1). The phylogenetic subgrouping in the resulting tree clusters proteins with similar domain composition and architecture together which indicates robustness of the analysis as the tree structure itself is based solely on the sequence similarity distances within the F-BAR domain regions. From this analysis, five subfamilies of F-BAR domain proteins can be distinguished. Subfamily 1, the Toca family (FBP17, TRIP10 or CIP4, and Toca-1 or FBPI). Both family 1 and 4 are involved in endocytosis. The Toca family are characterized by the presence of a Cdc42 binding site (homology region 1 (HR1) domain) which is absent in the Pacsin family. Subfamily 2 is the Fps/Fes and Fer subfamily of non-receptor protein tyrosine kinases, including Fps (Fujinami poultry sarcoma) and Fer (feline sarcoma), which are oncogenes identified from transforming retroviruses that encode non-receptor tyrosine kinases, and FCHSD1/2. Subfamily 3 contains the Rho GTPase activating protein (RhoGAP) domain, which regulates the functions of these Rho GTPases and includes SLIT-ROBO Rho GTPase activating protein (SrGAP) 1–3. Subfamily 4 includes syndapins/pacsins and members of this subfamily have a domain structure and function similar to those in Subfamily 5 consisting of growth arrest-specific protein (GAS7), proline-serine-threonine phosphatase-interacting protein 1 (PSTPIP1), cell division cycle 15 (CDC15), FCH domain only 2 (FCHO2) and Nostrin.

F-BAR Domain Protein Function

srGAP1-3. srGAP1-3 contain an F-BAR domain at the N-terminal, a RhoGAP domain in the middle and an SH3 domain at the C-terminal. WAVE associated Rac GTPase activating protein (WRP) or SrGAP1 was first identified by Soderling et al. as a scaffolding protein that inhibited Rac function with a potential link to mental retardation. Guerrier et al. showed that srGAP2-induced filopodium formation was dependent on its F-BAR domain and that this activity was associated with neurite outgrowth and branching. Furthermore, srGAP2 inhibited neuronal migration. Thus srGAP2 contributes to neuronal morphogenesis. These data suggest that there are functional similarities between the F-BAR domain and I-BAR domain in that both can generate membrane protrusions. Thus F-BAR domains have diverse structure and membrane remodeling activities.

Fes/Fer. The Fps/Fes and Fer proteins are a distinct family of non-receptor tyrosine kinases with prominent roles in inflammation and immunity (reviewed in ref. 11). Fer localises to microtubule ends and can phosphorylate the adhesion molecule platelet/endothelial cell adhesion molecule 1 (PECAM-1). Fer has also been linked to actin dynamics through cortactin and the phospholipase D-phosphatidic acid (PLD-PA) pathway. Interestingly, Fer is implicated in growth cone collapse downstream of semaphorin 3A in dorsal root ganglion neurons and in dorsal closure in Drosophila by influencing the stability of adherens junctions.

Syndapins/Pacsins. Syndapins (synaptic dynamin-associated proteins), also known as pacsins, are a group of F-BAR domain proteins that have been studied in detail. Syndapins have three isoforms and they are expressed in a tissue-specific manner and have been implicated in the regulation of the endocytic events. Syndapins contain an N-terminal F-BAR domain and a C-terminal SH3 domain. There are no Cdc42 binding sites in syndapins. Overexpression of the SH3 domain of syndapins inhibits receptor-mediated endocytosis, while overexpression of the full-length protein generates microspikes and lamellipodia-like structures. It would be interesting to examine the dynamics of the microspikes generated by syndapins to see if these structures are filopodia, as has been found to be the case for IRSp53. Syndapins can oligomerise through their N-terminal CC region. This oligomerisation is thought to increase the concentration of the SH3 domain for the recruitment of N-WASP and dynamin. Syndapins bind to synaptojanin and sos, which are important proteins in endocytosis. Other functions of syndapins include regulation of the membrane trafficking events at the trans-Golgi network and endosomal recycling. Recently, the function of syndapin in the formation of the postsynaptic membrane system in Drosophila has been documented. The structural study by Wang et al. revealed that the specific characteristics of the F-BAR domain of syndapins contributed to its ability to generate small tubules and tubule constriction, in contrast to the wide tubules generated...
CIP4 (Toca-1-3) contain an F-BAR domain at the N-terminal, an HR1 domain (Cdc42 binding site) in the middle and a C-terminal SH3 domain. Toca-1 was identified as an essential component of Cdc42-mediated actin polymerisation and it directly or indirectly activates N-WASP. Using an in vitro system, by other F-BAR domains. In additional, full length syndapin lacks membrane tubulation activity and thus probably exists in an autoinhibited state.

Toca-1 (FBP1), -2 (FBP17) and -3 (TRIP10/CIP4). A subset of F-BAR domain proteins including Toca-1, FBP17, TRIP10/CIP4 (Toca-1-3) contain an F-BAR domain at the N-terminal, an HR1 domain (Cdc42 binding site) in the middle and a C-terminal SH3 domain. Toca-1 was identified as an essential component of Cdc42-mediated actin polymerisation and it directly or indirectly activates N-WASP. Using an in vitro system,
The F-BAR domain proteins couple membrane remodeling and actin dynamics associated with endocytic pathways and filopodium formation. Further analysis of the F-BAR domain subfamilies and their interacting partners is likely to give us mechanistic insight into membrane trafficking and morphology pathways.

**Note**

Supplementary material can be found at: www.landesbioscience.com/supplement/AhmedCIB3-2-Sup.pdf
www.landesbioscience.com/supplement/AhmedCIB3-2-Sup.xls
Figure 2. An interaction network between several F-BAR domain-containing proteins generated using Ingenuity Pathway Analysis (Ingenuity Systems, www.ingenuity.com). Genes or gene products are represented as nodes, and the biological relationship between two nodes is represented as a line. All lines are supported by at least one reference from the literature, from a textbook, or from canonical information stored in the Ingenuity Pathways Knowledge Base. Human, mouse and rat orthologues of a gene are stored as separate objects in the Ingenuity Pathways Knowledge Base, but are represented as a single node in the network. The F-BAR domain-containing proteins are colored according to their categorization into one of five subfamilies. Subfamilies (colour): 1, pink; 2, olive; 3, red; 4, blue; 5, green, see text for details. Network Generation. A data set containing identifiers for the F-BAR domain-containing proteins was uploaded into Ingenuity Pathway Analysis (Ingenuity Systems, www.ingenuity.com). The identifiers were mapped to their corresponding gene objects in the Ingenuity Pathways Knowledge Base. Networks of these genes were then algorithmically generated based on their connectivity. All five generated networks were merged and the resulting network was grown to form a larger network, using the all molecules option. Molecules that do not directly interact with F-BAR domain-containing proteins were removed. From the 20 F-BAR domain-containing genes that were found in the merged networks, PSTPIP2, FCHO2, FCHO1 and ARHGAP4 were removed as they were linked to the rest of the network by only a single line via genes that are non-specifically connected to several hundreds of other genes.

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