Current Biology 19, 95–107, January 27, 2009 ©2009 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2008.12.029

Article

Molecular Mechanisms of Membrane Deformation by I-BAR Domain Proteins

Juha Saarikangas,[1,5] Hongxia Zhao,[1,5] Anette Pykäläinen,[1] Pasi Laurinmäki,[2] Pieta K. Mattila,[1,6] Paavo K.J. Kinnunen,[3] Sarah J. Butcher,[2] and Pekka Lappalainen[1,4,*]
[1]Program in Cell and Molecular Biology
[2]Program in Structural Biology and Biophysics
Institute of Biotechnology
P.O. Box 56
[3]Helsinki Biophysics & Biomembrane Group
Medical Biochemistry
Institute of Biomedicine
P.O. Box 63
[4]Neuroscience Center
P.O. Box 56
University of Helsinki
FIN-00014 Helsinki
Finland

Summary

Background: Generation of membrane curvature is critical for the formation of plasma membrane protrusions and invaginations and for shaping intracellular organelles. Among the central regulators of membrane dynamics are the BAR superfamily domains, which deform membranes into tubular structures. In contrast to the relatively well characterized BAR and F-BAR domains that promote the formation of plasma membrane invaginations, I-BAR domains induce plasma membrane protrusions through a poorly understood mechanism.
Results: We show that I-BAR domains induce strong PI(4,5)P_2 clustering upon membrane binding, bend the membrane through electrostatic interactions, and remain dynamically associated with the inner leaflet of membrane tubules. Thus, I-BAR domains induce the formation of dynamic membrane protrusions to the opposite direction than do BAR and F-BAR domains. Strikingly, comparison of different I-BAR domains revealed that they deform PI(4,5)P_2-rich membranes through distinct mechanisms. IRSp53 and IRTKS I-BARs bind membranes mainly through electrostatic interactions, whereas MIM and ABBA I-BARs additionally insert an amphipathic helix into the membrane bilayer, resulting in larger tubule diameter in vitro and more efficient filopodia formation in vivo. Furthermore, FRAP analysis revealed that whereas the mammalian I-BAR domains display dynamic association with filopodia, the *C. elegans* I-BAR domain forms relatively stable structures inside the plasma membrane protrusions.
Conclusions: These data define I-BAR domain as a functional member of the BAR domain superfamily and unravel the mechanisms by which I-BAR domains deform membranes to induce filopodia in cells. Furthermore, our work reveals unexpected divergence in the mechanisms by which evolutionarily distinct groups of I-BAR domains interact with PI(4,5)P_2-rich membranes.

Introduction

In addition to the well-established role of the cytoskeleton in producing forces to generate plasma membrane protrusions and invaginations, many membrane-associated proteins have also been shown to directly sculpt biological membranes. These proteins generate membrane curvature through insertion of hydrophobic or amphiphatic motifs into the membrane to induce bilayer asymmetry and through the formation of membrane-bound protein scaffolds with intrinsic curvature [1–8].

The BAR (Bin, Amphiphysin, Rvs) domain superfamily of proteins are central regulators of membrane remodeling in all eukaryotes. Mutations in genes encoding BAR domain proteins have been linked to many diseases [9–11], and inactivation of these proteins in cells and animals is often characterized by severe phenotypes resulting from altered membrane dynamics [12–14]. Based on structural features and phylogenetic relationships, the BAR domains can be divided into distinct subfamilies [15].

The canonical BAR domain is a dimeric module, where three kinked antiparallel α helices of each monomer form a banana-shaped dimeric 6-helix bundle [16]. BAR domains interact with cellular membranes through their concave surface, which typically contains charged amino acids [16]. A subset of BAR domains (N-BARs) also contain an N-terminal amphiphatic helix that folds upon membrane interaction and penetrates into the bilayer [16–18]. In a number of proteins, the BAR domain is also functionally linked to other membrane-binding motifs such as PH or PX domains [19–21]. Thus, although the curved shape of BAR domains appears to be critical for membrane tubulation, in many cases the membrane curvature-sensing/generation activity is enhanced by additional lipid-binding motifs.

F-BAR domain was originally identified as a FER-CIP4 homology (FCH) domain in the N-terminal region of many actin-regulating proteins. Subsequent studies revealed overall sequence homology between FCH and BAR domains and demonstrated that F-BAR (FCH and BAR) domains tubulate membranes in vitro and in vivo like BAR domains [13, 22]. The structure of F-BAR domain differs from the canonical BAR domain by containing five α helices per monomer. Importantly, being more elongated and gently curved, F-BAR domains induce thicker membrane tubules in comparison to BAR domains [23–25]. A recent cryo-EM study demonstrated that F-BAR domains self-assemble into a helical coat around the membrane tubules, providing evidence that these domains use a combination of scaffolding and cooperative assembly to induce membrane curvature [26].

The I-BAR domain, which is also known as IM (IRSp53/MIM homology) domain, was first identified as an F-actin crosslinking domain at the N-terminal region of mammalian IRSp53 and missing-in-metastasis (MIM) proteins [27]. However, subsequent studies suggested that I-BAR/IM domains do not significantly crosslink actin filaments under physiological

*Correspondence: pekka.lappalainen@helsinki.fi
[5]These authors contributed equally to this work
[6]Present address: Cancer Research UK London Research Institute, 44 Lincoln's Inn Fields, London WC2A 3PX, UK

conditions and revealed that the domain displays structural homology to BAR domains [28–30]. I-BAR/IM monomer consists of three α helices that dimerize into an antiparallel structure, which resembles a zeppelin or inverse BAR (I-BAR) domain shape. Biochemical studies demonstrated that I-BAR domains of MIM and IRSp53 directly bind and deform membranes into tubules in vitro [30, 31]. However, in contrast to the concave-shaped lipid-binding interface of BAR and F-BAR domains, the positively charged lipid-binding surface of I-BAR domains displays a convex geometry [30, 31]. This provided a possible structural explanation for why I-BAR domains induce membrane protrusions rather than invaginations when expressed in cells [27, 32, 33]. However, direct evidence for this "inverse mechanism" has not been demonstrated. Furthermore, possible differences in the membrane deformation properties within the I-BAR domain family (there are five I-BAR domains proteins in mammals, two in *Drosophila*, and one in *C. elegans*) have not been examined [34].

Here, we demonstrate that I-BAR domains bind to the inner leaflet of membrane tubules, thereby inducing the formation of dynamic membrane tubules in the opposite orientation to that of BAR and F-BAR domains. Furthermore, by comparing the membrane interactions of different vertebrate I-BAR domains and their *C. elegans* homolog, we reveal that different I-BAR domains utilize partially distinct mechanisms to deform membranes. These results provide important new mechanistic insights into the role of I-BAR proteins in the formation of plasma membrane protrusions such as filopodia.

Results

I-BAR Domains Bind to the Inner Leaflet of Membrane Tubules and Thereby Deform Membranes in the Opposite Direction to that of BAR Domains

To examine the directionality and dynamics of I-BAR domain-induced membrane tubules in vitro, we monitored their effects on giant unilamellar vesicles (GUVs). Because GUVs are relatively large ($\sim$5–500 μm) and thus have similar tension properties to that of cellular membranes, they are considered good models to monitor protein-induced membrane deformation in vitro [35]. Within 20–200 s after addition of MIM I-BAR, dynamic membrane tubules that invaginated toward the interior of GUVs appeared. At longer incubation times, these multiple invaginations led to the shrinkage of the GUVs (Figure 1A and data not shown). In contrast, the N-BAR domain induced long outward protrusions at the surface of the GUV that in many cases resulted in the breakdown of the vesicle within $\sim$100–200 s after addition of the protein (Figure 1B).

To reveal whether I-BAR domains indeed bind to the inner leaflet of the membrane tubules, as expected from the orientation of the I-BAR domain-induced membrane tubules on GUVs, we incubated I-BAR domains with multilamellar vesicles (MLVs) that were subsequently visualized by cryo transmission electron microscopy (cryo-EM). The morphology of the membrane structures in the electron micrographs fell under two distinct classes: tubular structures and spherical vesicles (Figures 1C–1F). Comparison of different I-BAR domains revealed that the tubules induced by the mammalian MIM and IRSp53 I-BARs were less uniform than the ones induced by the *C. elegans* I-BAR. MIM and IRSp53 I-BAR-induced membrane tubules typically contained perpendicularly oriented striations at the inner leaflet, indicating that these domains indeed associate with the inner leaflet of the membrane tubules (Figures 1C and 1D). Similar striations were not observed in MLVs incubated with the *C. elegans* I-BAR domain, although the inner leaflet of the membrane tubules induced by the *C. elegans* I-BAR appeared thicker and more electron dense in the images. To detect the location of the *C. elegans* I-BAR domain in the membrane tubules, electron-density profiles of the membrane with and without I-BAR domains were compared. For this purpose, perpendicular density profiles of 20 randomly picked tubule sections and spherical vesicles were calculated. Density profiles demonstrated that in the *C. elegans* I-BAR-induced membrane tubules, additional electron density (Figure 1E inset, area shaded by red lines) was always detected at the inner face of the inner membrane leaflet, whereas no additional density was detected at the inner leaflet of vesicles exhibiting spherical morphology (Figures 1E and 1F). Together, these data demonstrate that I-BAR domains deform membranes by binding to the inner leaflet of the membrane tubules.

I-BAR Domains Cluster PI(4,5)P_2 upon Membrane Binding

Previous studies established that I-BAR domains interact with phospholipid-rich membranes through positively charged patches located at the distal ends of the I-BAR domain [30, 31, 36]. The possible effects of I-BAR domains on PI(4,5)P_2 were first examined by microscopy of GUVs containing NBD-labeled phosphatidylcholine (PC) and bodipy-TMR-labeled PI(4,5)P_2. As a control, we used BSA, which had no visible effects on the morphology of the GUVs or their lipid distribution. Interestingly, in addition to the membrane invaginations and protrusions described in the previous paragraph above, I-BAR and amphiphysin N-BAR domains induced visible clustering of PI(4,5)P_2 on GUVs. The PI(4,5)P_2 clusters were stable and rarely dissociated once formed. Importantly, fluorescently labeled PC did not typically cocluster with PI(4,5)P_2, demonstrating that the bright PI(4,5)P_2 spots on GUVs are not a result of general membrane clustering/ruffling at certain foci (Figure 2A). Importantly, PI(4,5)P_2 clustering was typically associated with the formation of membrane tubules (Figure 2B).

To quantify PI(4,5)P_2-clustering activities of different I-BARs, the self quenching of Bodipy-TMR-PI(4,5)P_2 resulting from clustering upon addition of protein was monitored by measuring fluorescence intensity. All the I-BAR domains tested and the amphiphysin N-BAR domain resulted in self quenching of the fluorescent probe molecules (Figure 2B). The results were plotted (equations are given in the Supplemental Experimental Procedures available online) to obtain values for PI(4,5)P_2 clustering of each protein at different concentrations. These data suggest that the IRSp53 I-BAR domain induced the strongest clustering of PI(4,5)P_2, whereas the MIM and ABBA I-BARs were $\sim$15% less efficient in clustering PI(4,5)P_2. Also, the amphiphysin N-BAR domain clustered PI(4,5)P_2, but significantly less efficiently than I-BARs. Furthermore, these experiments revealed that the positively charged "lipid-binding interface" of the I-BAR domains is essential for PI(4,5)P_2 clustering, because neutralization of positively charged residues at these regions correlated with a decrease in the capacity of the I-BAR domain to cluster PI(4,5)P_2 (Figure 2C; Figure S1). We also tested whether I-BAR domains are capable of clustering another negatively charged lipid, phosphatidylserine (PS). Importantly, the I-BAR domains induced only very weak clustering of PS, whereas the amphiphysin BAR domain clustered PS nearly as efficiently as PI(4,5)P_2 (Figure 2E).