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Mechanisms of Membrane Deformation by Lipid-Binding Domains

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Abstract

Among an increasing number of lipid-binding domains, a group that not only binds to membrane lipids but also changes the shape of the membrane has been found. These domains are characterized by their strong ability to transform globular liposomes as well as flat plasma membranes into elongated membrane tubules both *in vitro* and *in vivo*. Biochemical studies on the structures of these proteins have revealed the importance of the amphipathic helix, which potentially intercalates into the lipid bilayer to induce and/or sense membrane curvature. Among such membrane-deforming domains, BAR and F-BAR/EFC domains form crescent-shaped dimers, suggesting a preference for a curved membrane, which is important for curvature sensing. Bioinformatics in combination with structural analyses has been identifying an increasing number of novel families of lipid-binding domains. This review attempts to summarize the evidence obtained by recent studies in order to gain general insights into the roles of membrane-deforming domains in a variety of biological events.

Abbreviations

FCH, Fer and CIP4 Homology; BAR, Bin1 Amphiphysin Rvs161/167p; F-BAR, FCH and BAR; EFC, Extended FCH; I-BAR, Inverse BAR; IMD, IRSp53 and MIM; ENTH, Epsin N-terminal Homology, ALPS motif, ArfGAP1 lipid packing sensor motif

1. Introduction

Cellular membranes have unique shapes that are defined by various factors including different lipid compositions between leaflets, cross-linked transmembrane protein, pulling or protruding force by the cytoskeleton (and associated motors), and scaffolding effects by cytosolic coat proteins [1, 2]. Intracellular membrane traffic is initiated by vesicle formation in which dynamic shape change of the membrane takes place. Namely, it is a sequential process of "budding," where the flat membrane is deformed to an invaginated dome; "vesicle growth," where the invaginated membrane becomes a globular vesicle by narrowing its neck; and "fission," where mechanical forces by associated protein(s) pinch off the membrane neck to complete vesicle formation. Recent findings have revealed an increasing number of cytosolic proteins that actively control the shape of the membrane at every stage of this process. A notable feature of such proteins is their intrinsic ability to transform globular liposomes into long and narrow membrane tubules [3-6].

Two types of proteins induce membrane tubulation. One is mediated by the overall protein structure as exemplified by dynamin, a membrane-associated GTPase essential for the tubulation and fission steps of endocytosis [7-9]. The other is achieved solely by part of a protein, structurally defined as a "domain." A growing number of "membrane-tubulating domains" shared by various proteins implicated in membrane trafficking have been emerging.

In this review, we start by describing the BAR domain, the first of the membrane-tubulating domains reported. Recent findings have further extended this particular domain family by including structurally identified new members such as

Arfaptin, sorting nexins, and FCH-domain-containing F-BAR/EFC domains. We then discuss the role of ENTH domains, another membrane-deforming domain family involved in the early onset of endocytosis. Finally, we describe the new concept for membrane deformation or curvature sensing by ALPS motifs or tandem C2 domains. Structural studies on these domain families have started to dissect the mechanism by which an interaction with lipid molecules in the bilayer eventually bends the membrane. We aim to present an emerging picture of the cellular membrane whose shape is dynamically controlled by membrane-deforming domains in a wide range of cellular events such as endocytosis, exocytosis, and cytoskeletal regulation.

2. BAR domain

2.1. BAR domain is a membrane-tubulating module

The BAR domain was originally found as an N-terminal region highly conserved in mammalian and yeast amphiphysins, and is thus named after these proteins (Bin1, amphiphysin, and Rvs161/167p). In yeast, *RVS161* and *RVS167* genes are essential for cell viability and BAR domains are required to suppress the mutant phenotype [10]. Initially, the biochemical role of the BAR domain was thought to be a heterodimerizaton or homodimerization interface of amphiphysin or endophilin [11-13]. Consistently, the secondary structure of this domain was predicted to be highly α-helical and to even form a coiled-coil structure. Despite these findings, together with the fact that amphiphysin and endophilin are involved in clathrin-dependent endocytosis by forming a complex with dynamin or synaptojanin [14-16], the exact role of the BAR domain was unclear.

The understanding of the BAR domain as a membrane-bending module comes from the context of its binding partner—dynamin. Dynamin has a strong ability to deform liposomes into membrane tubules, *in vitro*, on which it polymerizes with a characteristic striation pattern [5, 6]. When pre-incubated with amphiphysin, the dynamin–amphiphysin complex shows a wider striation, indicating a hybrid coat on the tubule generated by both proteins [4]. Surprisingly, amphiphysin by itself has the ability to induce membrane tubulation with a diameter of similar range to that of dynamin-induced tubules [4]. Deletion experiments have identified the N-terminal 284 amino acids to be the region sufficient for this tubulation activity, corresponding to the BAR domain of amphiphysin.

Subsequently, similar membrane-tubulating activity was found in another major dynamin-binding protein—endophilin [3]. A careful search of the amphiphysin and endophilin sequences identified a highly homologous region of 30 amino acids at the N-termini of both proteins. Helical wheel modeling of this region revealed an amphipathic property, in which hydrophobic and hydrophilic residues align on the opposite sides of the helix, respectively [3]. One of the hydrophobic amino acids in this helix, Phe10, was shown to be essential for tubulation when a point mutant of this residue, F10E, lost its activity [3]. This hydrophobicity in the amphipathic helix is believed to be important for membrane curvature formation by penetrating into the lipid bilayer, expanding the surface area of the cytosolic leaflet. This eventually led to an interpretation of the mechanism by which the epsin ENTH domain deforms the plasma membrane at the onset of clathrin-dependent endocytosis [17], as discussed later.

An immunofluorescent study of the muscle-specific isoform of amphiphysin2 (M-amph2) revealed a T-tubule structure in skeletal muscle [18]. In Drosophila, amphiphysin was also shown to localize at the T-tubule, and the disruption of *amph* gene resulted in an abnormal structure and function of the T-tubule/sarcoplasmic reticulum system [19]. Consistently, overexpression of M-amph2 in CHO cells resulted in a robust membrane tubulation invaginated from the plasma membrane [20], indicating its direct role in the formation and maintenance of the T-tubule network.

2.2. Structure of BAR domain—a banana-shaped dimer

The crystal structure of the BAR domain of Drosophila amphiphysin (dAmph) as well as mammalian endophilin has been solved [21-24]. It consists of three long helices with no strands, forming a dimer at a certain angle with each other. This angled association

and the kinked shape of helices 2 and 3 make the dimer resemble a banana. Electrostatic surface modeling revealed that positively charged amino acids, such as K137 and R140 as well as K161 and K163, were located at the concave surface of the dAmph BAR dimer. Point mutants of these positive residues resulted in a loss of membrane binding as well as tubulation activity. These findings indicate that the concave surface of the banana attaches onto the membrane and also suggest that the banana-like structure is suitable for interaction with the curved membrane. The diameter of the best-fitted membrane tubules or vesicles was calculated to be 22 nm [21], consistent with that of the dynamin-induced tubular membrane neck [25]. A curvature-dependent membrane binding was shown for the amphiphysin BAR domain, which preferably co-sedimented with 50-nm-sized liposomes but not with larger ones [21].

2.3. Growing family of the BAR domain

The preceding study helped highlight the structural neighbors of the dAmph BAR domain [21]. The most striking similarity was found in the Arfaptin structure, an Arf- and Rac-binding protein previously implicated in Golgi functions and actin cytoskeleton regulation [26, 27]. Intensive database searches of distantly related sequences unveiled vast numbers of proteins of this family [21, 28], including centaurins (Arf GAPs), oligophrenins (Rho GAPs), and sorting nexins (PX domain-containing proteins) (Fig. 1). Similar to the dAmph BAR domain, Arfaptin bound and even tubulated liposomes when incubated at higher concentrations [21]. Newly found BAR domains of centaurin, oligophrenin, and sorting nexin also preferred high curvature in membrane binding [21, 29, 30], indicating that curvature sensing is an evolutionarily conserved property of the BAR domain.

3. F-BAR/EFC domain

3.1. F-BAR/EFC domain, a novel BAR-related domain

A two-hybrid screen identified a protein that binds to the active form of Cdc42, a Rho subfamily of small GTPase [31]. This protein, named CIP4 (Cdc42-interacting protein 4), had an N-terminal region highly conserved in several other proteins such as tyrosine kinase Fes and Fer, SH3-containing protein syndapin/PACSIN, and Rho GTPase-activating protein (GAP) WRP/srGAPs [32-38]; thus, it was named the FCH domain (for FER–CIP4 homology) [31] (Fig. 2). Fer tyrosine kinase is known to participate in the regulation of cell–cell adhesion through interaction with p120-catenin [39, 40]. Syndapin1 was initially identified as a dynamin-binding protein [35] and was found to be involved in endocytosis [41]. It also binds to N-WASP, an activator of the Arp2/3 complex, to coordinate the actin functions in endocytosis [42]. WRP was identified as a binding protein for WAVE1, another Arp2/3 activator family member related to N-WASP [33], and srGAP1 is a Cdc42 GAP involved in the slit-robo pathway, which controls neuronal migration [36]. The FCH domain is also found in yeast proteins such as Cyk2p/Hof1p, Bzz1p, and Rgd1-2p in budding yeast, and Cdc15p, Imp2p, YB65 (pombe Bzz1p), and Rga7-9p in fission yeast (Fig. 2) [43-49]. Most of these are functionally linked to actin polymerization and cytokinesis. All these findings strongly support the view that FCH proteins are involved in the cellular events dependent on the actin cytoskeleton and its regulatory machinery.

3.2. Membrane-tubulating activity of F-BAR/EFC domain

When overexpressed in the cell, some of the FCH-containing proteins induced, and

localized at, a filamentous structure seemingly reminiscent of the microtubule [50-52]. Dual-color immunofluorescence studies clearly demonstrated that this structure did not overlap any cytoskeleton (microtubules, F-actin, or intermediate filament). Interestingly, it was connected to the outer space and thus represented an invaginated plasma membrane [53], similar to the membrane tubules induced by the overexpression of M-amph2 [20]. The membrane-tubulating activity of FBP17 was directly demonstrated by incubating its recombinant protein with liposomes [54]. A secondary structure prediction anticipated a larger structure composed of three long α-helices, covering the FCH domain up to the following coiled-coil region, which is always found at the C-terminal side of almost all FCH domains. This means that the conventionally defined FCH domain is only part of a larger domain—the F-BAR/EFC domain (F-BAR for FCH and BAR, and EFC for extended FCH) [54, 55]. Many of the F-BAR/EFC-domain-containing proteins also have at their C-termini SH3 domains that bind to dynamin. FBP17 and CIP4 not only induced robust membrane tubulation when overexpressed, but also recruited dynamin onto these tubules [25, 53, 54]. Treatment with LatrunculinB, a blocking agent of actin polymerization, enhanced the growth of membrane tubules [54]. This effect was found to be reversible as wash out of the drug promoted fission of tubules into small vesicles [54]. These results suggest a close interplay between the F-BAR/EFC-induced membrane tubulation and actin cytoskeleton by which dynamin–FBP17/CIP4 complexes could utilize the actin cytoskeleton for fission at the vesicular neck in endocytosis.

3.3. Structure of the F-BAR/EFC domain

The crystal structure of the F-BAR/EFC domains from FBP17, CIP4, and FCHo2

comprises six-helix bundles, which also form banana-shaped dimers as formed by BAR domains [56, 57]. They also have conserved basic patches on their concave surfaces to interact with acidic lipids. A marked difference from the BAR domain structure is that the curvature is much shallower, making the F-BAR/EFC structure fit the membrane surface with diameter of ~60 nm. When viewed from the convex face, the dimer forms a tilde (~) shape, which is suitable for lateral interactions [57]. This lateral interaction involves several hydrophobic and charge interactions between monomers, believed to "coat" the surface of the membrane tubule and provide it with some rigidity [58, 59]. Another feature of the F-BAR/EFC structure is the "tip-to-tip" interaction, which is believed to be important for the formation of filamentous structures surrounding the membrane tubule [54, 56]. However, this mode of interaction seems to be canceled out when narrower tubules are formed [58], indicating a regulatory mechanism of tubule diameter by controlling the tip-to-tip interactions.

4. ENTH domain

4.1. ENTH domain in clathrin-dependent endocytosis and actin function

A 94 kDa protein was identified as a major binding partner of the EH domain of Eps15, an important regulatory protein in endocytosis [60]. This protein, named epsin (after Eps15-interacting protein), had NPF motifs at its C-terminal region, consistent with its strong binding affinity for EH domains [60, 61]. In addition, epsin also contained DPW motifs at its central region that bind to the appendage domain of α-adaptin, a subunit of the AP-2 complex [60, 61], as well as clathrin-binding motifs that bind to the terminal domain of clathrin heavy chain [62, 63], indicating its function as an adaptor in the formation of endocytic machinery complex. Besides these short peptide motifs at the

center of the C-terminal region, the N-terminal ~180 amino acids were anticipated to form a globular structure evolutionarily conserved from yeast to mammal [60, 64]—the ENTH domain (for epsin N-terminal homology). The ENTH domain is mainly found in epsin family members such as mammalian epsin1-3, epsinR, and budding yeast proteins Ent1-5p.

Yeast genetics has indicated an essential role for the ENTH domain of Ent1p and Ent2p, two of at least five epsin homologs in budding yeast [65]. Double deletion of both *ENT1* and *ENT2* genes resulted in a lethal phenotype, concomitant with an aberrant actin structure and growth arrest. Exogenous expression of either gene in this double-mutant cell suppressed these phenotypes, although ENTH-domain-deleted forms could not. Furthermore, an error-prone PCR approach, which introduces random point mutations in the *ENT1* and *ENT2* genes, identified eight of nine mutations in the ENTH domain that failed to reverse the *ent1/2* double-deletion phenotype [65], indicating an essential role of this domain in epsin function.

4.2. ENTH domain binds phosphoinositides

At the molecular level, an explanation for the role of the ENTH domain was provided by the finding that the epsin 1 ENTH domain interacts with $PI(4,5)P_2$, a phosphoinositide mainly localized at the plasma membrane [66]. Overexpression of epsin 1 K76A, which lost the $PI(4,5)P_2$-binding affinity of ENTH, as well as that of epsin 1 ΔENTH, in which the entire ENTH domain was deleted, efficiently blocked EGF internalization [66, 67]. This finding indicated an essential role of the ENTH–$PI(4,5)P_2$ interaction in endocytosis. It also provided important information about the molecular mechanism of endocytosis that occurs at the plasma membrane,

where $PI(4,5)P_2$ acts as a key regulator in recruiting endocytic machinery under the control of phosphoinositide-metabolizing enzymes such as PIP 5-kinases (PIP5K Iγ) and $PI(4,5)P_2$ 5-phosphatases (synaptojanin) [68-70].

EpsinR (also called enthoprotin or Clint) is a unique member of the epsin family in that it only shares with other epsin members an ENTH domain and clathrin-binding motifs but not DPW or NPF motifs [71-74]. Instead, it binds through its DFxDW/F motifs to the γ-ear domain of AP-1, an adaptor complex implicated in vesicular trafficking from the Golgi membrane. Knockdown experiments demonstrated that epsinR is required for retrograde transport of Shiga toxin, TGN38/46, and mannose 6-phosphate receptor from the early and/or recycling endosomes to the trans-Golgi network [75]. The ENTH domain of epsinR was also found to bind phosphoinositides, with the highest affinity for PI4P [73, 74]. This preference seems consistent with the established function of PI4P on the Golgi membrane, where it recruits effector proteins such as AP-1 and FAPP1 PH domain for the budding of cargo-containing vesicles [76, 77].

Different specificities in phosphoinositide binding were reported for the ENTH domains of yeast epsin Ent1-5p. The Ent1p ENTH domain shows a broad specificity for most of the phosphoinositides and other acidic phospholipids such as phosphatidic acid or phosphatidylserine [78]. On the contrary, the ENTH domain of Ent3p, as well as Ent5p, binds almost exclusively to $PI(3,5)P_2$ [79-81]; this interaction is thought to mediate protein transport into the multivesicular body (MVB) but not endocytosis at the plasma membrane. The study by Wendland *et al.* [65] demonstrated that although *ENT1* and *ENT2* genes are mutually redundant, the *ent1*/*ent2* double mutant showed a lethal phenotype, indicating their distinct roles from those of other *ENT* genes such as *ENT3*

or *ENT5*. This distinction may, at least in part, be explained by the difference in phosphoinositide-binding specificities of ENTH domains between Ent1/2p and Ent3/5p.

4.3. Structure of ENTH domain and membrane deformation

Both the crystal and NMR structures of the epsin ENTH domain were solved [66, 82, 83] and revealed to consist only of α-helices. The N-terminal 14 residues were not ordered in both studies, indicating their labile structural nature in solution. Comparisons of chemical shift values in the absence or the presence of Ins(1,4,5)P_3, the head group of PI(4,5)P_2, manifested a large structural change at the N-terminal region upon phosphoinositide binding [66]. In fact, deletion of N-terminal 18 residues, as well as alanine substitution of Arg8 in this region, abolished phosphoinositide binding [66]. These results clearly indicated the key role of the N-terminal region in lipid binding, which was subsequently supported by the crystal structure of the Ins(1,4,5)P_3-bound state of the epsin ENTH domain [17]. The results revealed that the N-terminal region actually forms an extra α-helix (helix 0), forming a concave surface where positively charged residues such as R63 and H73 are coordinated with the phosphates of Ins(1,4,5)P_3. More interestingly, helix 0 also retained an amphipathic property, as had been proposed for BAR domains [3]. Consistently, the epsin ENTH domain can also change the shape of liposomes into tubules [17], in a PI(4,5)P_2-dependent manner on the liposome. A hydrophobic residue, Leu6, is not only located at the putative membrane-embedded side of the helix but was also shown to be necessary for tubulation. The tubulating activity of ENTH is believed to reflect its ability to deform a flat plasma membrane into an invaginated membrane at the onset of endocytosis, as observed for invaginated clathrin-coated pits reconstituted on a lipid monolayer *in vitro*

[17]. This idea was supported by the consideration that in clathrin-dependent endocytosis, the rigidity of the clathrin triskelion is not sufficiently high to bend the flat membrane and thus needs an additional force (by the epsin ENTH domain) for membrane deformation [84, 85].

5. Two faces of amphipathic helix

5.1. Amphipathic helix as a curvature driver

As has been discussed for both the BAR and ENTH domains, the key structural factor for membrane deformation is the presence of the amphipathic helix (Fig. 3A). An amphipathic helix is formed in the epsin ENTH domain structure only when bound to Ins(1,4,5)P_3 [17]. On study found that the N-terminal region of the dAmph BAR domain was not ordered in the crystal structure [21], probably because the protein was not in association with its ligand, phospholipids. However, circular dichroism spectra indicated that there is significant increase in α-helicity in the BAR structures of both dAmph and rat endophilin [21, 23]. Furthermore, specific spin labeling and subsequent electron paramagnetic resonance spectroscopy revealed that the N-terminal region indeed becomes ordered upon lipid binding [23].

Further, the amphipathic helix is also utilized by small GTPases involved in other membrane-deforming processes. Arf1 is a small GTPase that controls the budding and uncoating cycle of the COPI coat at the Golgi membrane. In its GDP-bound state, N-terminal 17 amino acids apply a helical conformation at the central core of the protein, and then become exposed upon GTP binding [86-88]. The same structural transition was observed for Sar1, which controls the COPII coat assembly [89]. Interestingly, N-terminal helices of both GTPases retain amphipathic properties and were also shown

to be necessary for membrane binding. In fact, Arf1 as well as Sar1 can tubulate liposomes *in vitro*, and the hydrophobic residues in the N-terminal helices were important [90-93].

5.2. Amphipathic helix as a curvature sensor—ALPS motifs

The banana-shaped dimer is not the sole structure that could be a curvature sensor. ArfGAP1, which negatively regulates Arf GTPase and is thus involved in coatomer disassembly after the formation of the cargo-containing vesicle, is activated by diacylglycerol. Interestingly, the lipid with unsaturated carboxyl chains was most efficient in activating ArfGAP1 [94]. In theory, unsaturated lipids are less packed and therefore provide a larger space in the bilayer into which membrane-binding proteins can penetrate. This is highly reminiscent of the situation at the surface of the highly curved membrane, which resulted in smaller vesicles. Indeed, ArfGAP1 activation was shown to depend on liposome size; that is, the degree of membrane curvature [95]. The minimal region responsible for this curvature dependency was identified to be the central 40 amino acids with amphipathic property when modeled as a helix, called the ALPS motif for ArfGAP1 lipid-packing sensor [96]. Circular dichroism spectra showed that the ALPS motif actually forms a helical structure only when bound to the membrane [96]. These findings collectively indicate that the amphipathic helix could be another mechanism of curvature sensing (Fig. 3B).

In a database search utilizing an algorithm that specifically picks up amphipathic peptide sequences, Drin *et al.* [97] found a large number of potential ALPS motifs. Among such candidates, including the sterol transporter Kes1p and nucleoporin Nup13, the team further demonstrated a membrane-tethering ability of golgin GMAP-210 in a

manner dependent on vesicular size [98].

6. Curvature toward the extracellular space

The topology of membrane bending is also an interesting question to be explored. In the cell, there could be two types of membrane deformations: invagination and evagination. Most of the membrane-tubulating domains identified so far concern membrane invagination, where positive curvature is generated on the side protein attachments, exemplified by endocytosis from the plasma membrane or vesicular budding from the organellar membrane. By contrast, evagination is a process in which positive curvature is formed on the opposite side of the cytosol, which eventually pushes the membrane toward the outside of the cell or the inner space of organelles. This “reverse deformation” is typically observed in several important cellular events including cell movement, which is accompanied by “pushing” of the plasma membrane.

6.1. I-BAR domain in membrane evagination

A branch of the BAR domain family is characterized by a structure that is not a banana-like dimer rather straight and more like a zeppelin. This subfamily, called the I-BAR domain for inverse BAR or IMD after the names of representative proteins such as IRSp53 and MIM [99, 100], induces robust plasma membrane protrusion when overexpressed in various cell types [101, 102]. More interestingly, the I-BAR domain is able to reproduce such membrane protrusion when incubated with liposomes *in vitro*, resulting in the formation of a tubular membrane elongating toward the inner space of globular liposomes [103]. This may be mediated by the highly conserved positively charged residues located at the extreme ends of the I-BAR structure that simultaneously interacts with Rac GTPase and the membrane [102]. The combination of this type of

membrane-deforming and actin-polymerizing activities seems to be important for the pushing force of the plasma membrane during cell migration.

6.2. Snf7, a new player for membrane evagination through circular array of filamentous structures

Another group for membrane evagination, which is more vesicular, has been observed in viral budding or MVB formation. Although studies had revealed the involvement of several molecules such as ESCRT proteins, little was known about the mechanism of the membrane evagination process because of the lack of information on their membrane interaction properties. Recently, a study utilizing electron microscopy has revealed a novel mode of membrane evagination by one of the components of the ESCRT-III complex—hSnf7 [104]. Also called CHMP4, hSnf7 is localized both at endosomes and the plasma membrane when overexpressed. Visualizing the inner surface of the plasma membrane of the expressing cell by “deep etch” electron microscopy, it was found that a filamentous structure aligns in a circular pattern. More interestingly, when co-expressed with a mutant form of Vps4, an ATPase involved in disassembly of the ESCRT-III complex, hSnf7 filaments form thicker bundles and apparently push the plasma membrane outward, resulting in a tubular protrusion of the plasma membrane [104]. This finding is quite important because it has provided the first evidence for membrane protrusion toward the outer space of the cytosol, simultaneously corresponding to the inner space of organelles, which may drive the formation of inner vesicles of MVB.

7. Tandem C2 domains in membrane bending and fusion

Membrane fusion takes place during a variety of physiological events such as synaptic transmission, myotube differentiation, and viral entry. It is believed to occur through the state of “hemifusion” in which each outer leaflet of opposing lipid bilayer is intermixed. To achieve hemifusion, the high-energy barrier must be overcome by membrane-bending forces, which may pull lipid molecules out of the opposing outer leaflet. Such activity was reported for synaptotagmin, a transmembrane protein localized at the synaptic vesicle with tandem C2 domains, a Ca^{2+}-dependent lipid-binding module. When the tandem C2 domains of synaptotagmin I was incubated with liposomes in the presence of Ca^{2+}, membrane tubules with very small diameter (~17 nm) were induced[105]. This activity was not observed if the two C2 domains were structurally separated, indicating that changes in their steric state upon Ca^{2+} binding is important. Considering the large number of proteins containing tandem or multiple C2-domain-containing proteins, this mode of membrane deformation will potentially be found in wider range of physiological events.

8. Conclusion

Recent findings about novel interactions between proteins and lipids have revealed a more dynamic picture of cellular membrane based on the powerful tubulating and/or deforming activity inherent to a special group of lipid-binding domain. The domain species described in this review would be just a fraction of a more diverse family of domains that control and/or recognize the shape of the membrane. More careful reexamination of already known lipid-binding domains, as well as extensive search for novel domains, seems necessary to precisely understand the mechanism of membrane dynamics in the cell. This new paradigm proposed for protein–lipid interactions is clearly at the dawn of an explosive growth in cell biology. To obtain a sound basis for this new concept, a much comprehensive approach will be necessary.

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Figure Legends

Figure 1 BAR domain family

Schematic presentation of BAR domain family proteins. SH3, src homology 3; PH, pleckstrin homology; PX, phox homology; PTB, phosphotyrosine binding; GAP, GTPase activating protein, GEF, guanine nucleotide exchange factor; PDZ, PSD-95/Dlg/ZO homology; Ank, ankyrin repeat. Sc, yeast Saccharomyces cerevisiae.

Figure 2 F-BAR/EFC domain family

Schematic presentation of BAR domain family proteins. HR1, Rho effector, TyrK; tyrosine kinase. See Fig. 1 for the definition of the other domains. Sp, yeast Schizosaccharomyces pombe.

Figure 3 Mechanisms of curvature-generation and -sensing

(A) Amphipathic helix in a protein (depicted as a "wedge"-like structure) can penetrate into the lipid bilayer, increasing the surface area of the inner leaflet (upper side of the bilayer) thus inducing curvature. (B) Different membrane curvatures could be sensed by amphipathic helices (upper) or banana-shaped dimers (lower). The difference of the sensed curvature would be attributed to the volumes of the helices that could penetrate into the bilayer, or the depths of the concave surface of banana that interacts with the membrane.

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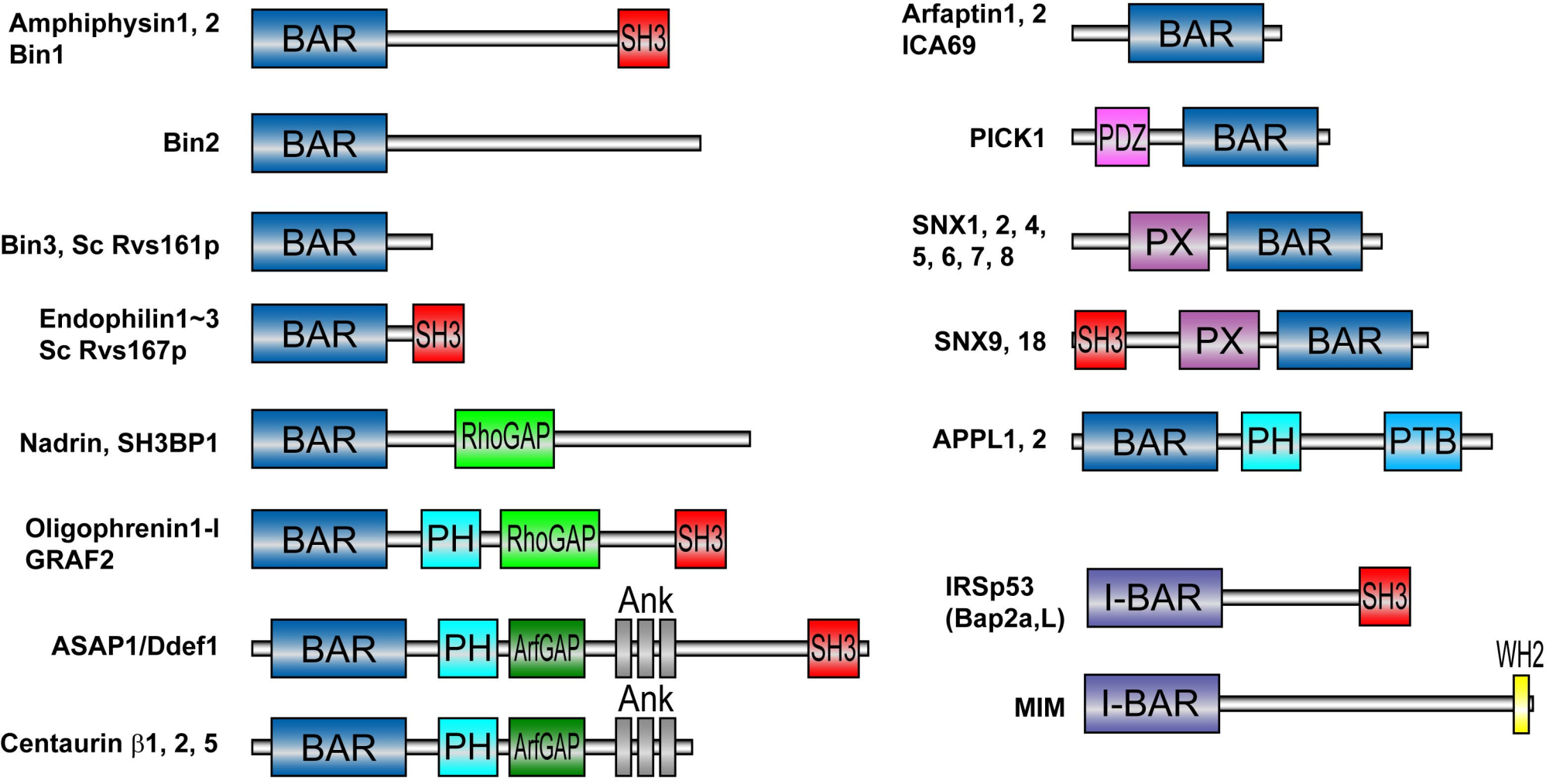
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BAR
SH3
Bin2
BAR
Bin3, Sc Rvs161p
BAR
Endophilin1~3
Sc Rvs167p
BAR
SH3
Nadrin, SH3BP1
BAR
RhoGAP
Oligophrenin1-I
GRAF2
BAR
PH
RhoGAP
SH3
ASAP1/Ddef1
BAR
PH
ArfGAP
Ank
SH3
Centaurin β1, 2, 5
BAR
PH
ArfGAP
Ank
Arfaptin1, 2
ICA69
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PICK1
PDZ
BAR
SNX1, 2, 4,
5, 6, 7, 8
PX
BAR
SNX9, 18
SH3
PX
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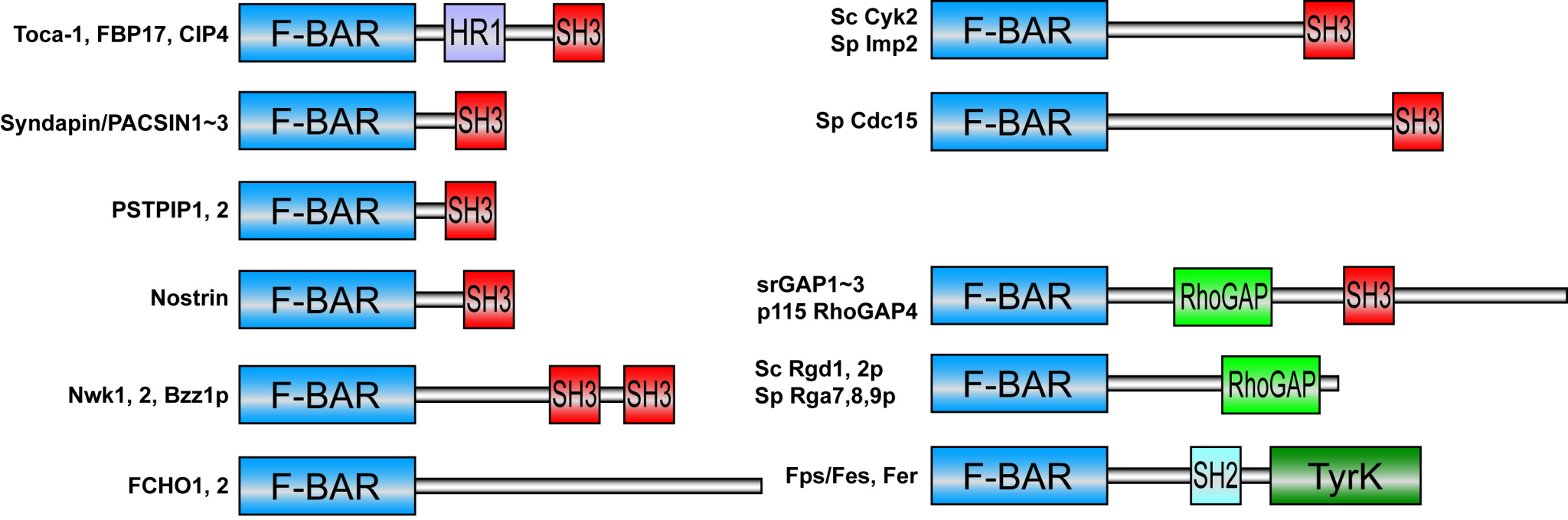
PH
PTB
IRSp53
(Bap2a,L)
I-BAR
SH3
MIM
I-BAR
WH2

Fig. 1


Toca-1, FBP17, CIP4
F-BAR
HR1
SH3
Syndapin/PACSIN1~3
F-BAR
SH3
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F-BAR
SH3
Nostrin
F-BAR
SH3
Nwk1, 2, Bzz1p
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SH3
SH3
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F-BAR
Sc Cyk2
Sp Imp2
F-BAR
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TyrK

Fig. 2

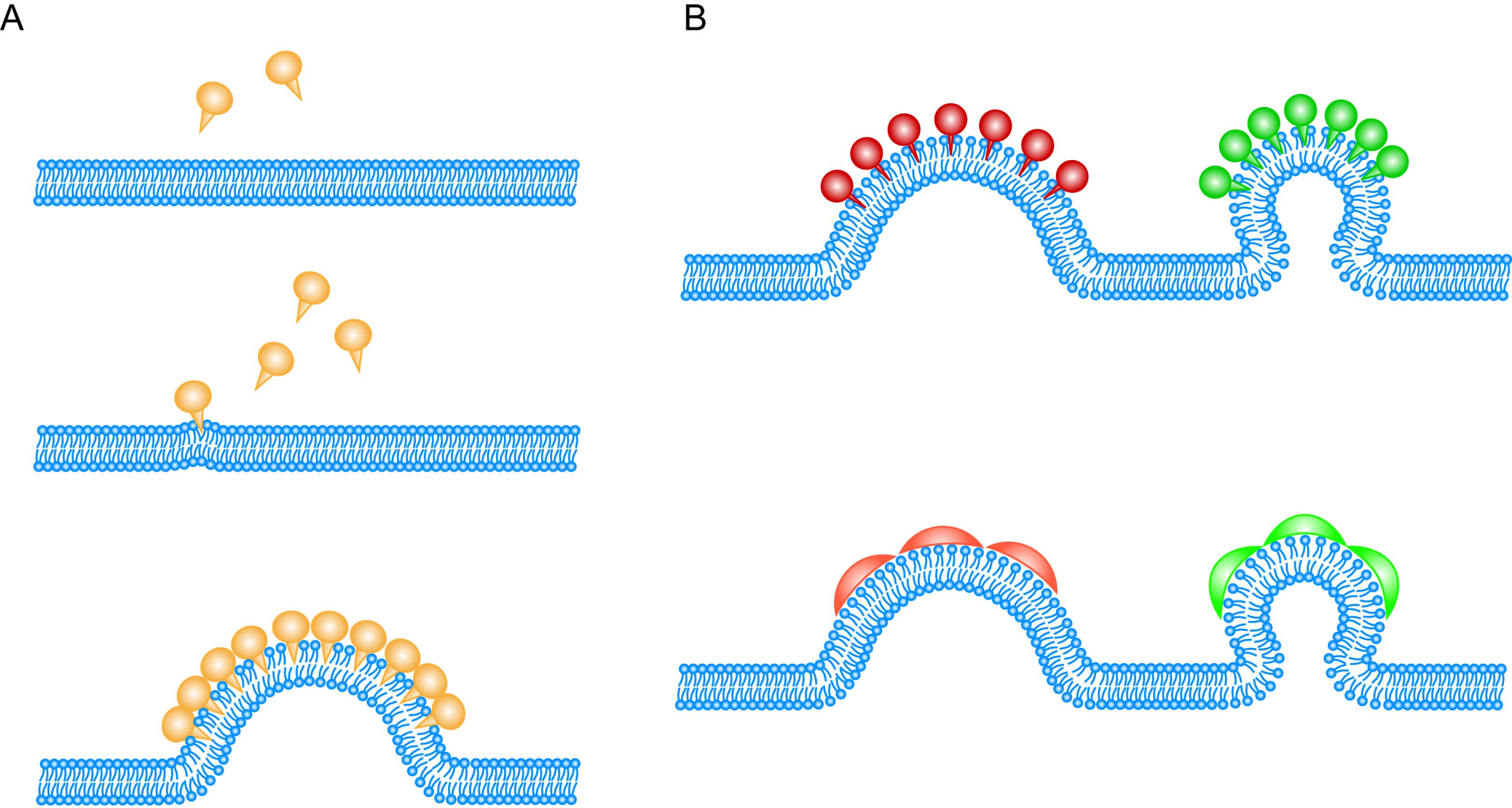

Fig. 3