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# The proposed functions of membrane curvatures mediated by the BAR domain superfamily proteins

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**The plasma membrane, the outermost surface of eukaryotic cells, contains various substructures, such as protrusions or invaginations, which are associated with diverse functions, including endocytosis and cell migration. These structures of the plasma membrane can be considered as tubules or inverted tubules (protrusions) of the membrane. There are six modes of membrane curvature at the plasma membrane, which are classified by the positive or negative curvature and the location of the curvature (tip, neck or shaft of the tubules). The BAR domain superfamily proteins have structurally determined positive and negative curvatures of membrane contact at their BAR, F-BAR and I-BAR domains, which generate and maintain such curved membranes by binding to the membrane. Importantly, the SH3 domains of the BAR domain superfamily proteins bind to the actin regulatory WASP/WAVE proteins, and the BAR/F-BAR/I-BAR domain-SH3 unit could orient the actin filaments towards the membrane for each subcellular structure. These membrane tubulations are also considered to function in membrane fusion and fission.**

*Keywords*: actin cytoskeleton/BAR domain/EFC domain/F-BAR domain/I-BAR domain/IMD domain/membrane curvature/membrane fission/membrane fusion/plasma membrane.

*Abbreviations*: Arp2/3, actin-related protein 2/3; BAR, Bin-Amphiphysin-Rvs167; EFC, extended Fes-CIP4 homology; FCH-BAR, F-BAR, the same domain as EFC; I-BAR, inverse-BAR; IMD, IRSp53-MIM homology domain; N-WASP, neural WASP; WASP, Wiskott–Aldrich syndrome protein; WAVE, WASP family Verprolin-homologous protein.

The plasma membrane contains various membrane microstructures, ranging in size from ~10 nm to few microns, which are supported by the cytoskeleton and/or membrane binding proteins. These fine structures of the plasma membrane include invaginations, such as caveolae and clathrin-coated pits, as well as protrusions, such as filopodia and lamellipodia. Each of these fine micro-membrane structures has its specific morphological characteristics, which have been mainly defined by extensive electron micrographic studies. In most cases, actin filaments accompany these structures. However, it has been unclear how these characteristic structures are formed by the dynamic collaboration between the membrane binding proteins, the actin cytoskeleton and the plasma membrane.

Recently, increasing numbers of membrane-deforming proteins connecting the actin cytoskeleton and the plasma membrane have been found. These proteins belong to the BAR domain superfamily, and contain the Bin-Amphiphysin-Rvs167 (BAR), extended Fes-CIP4 homology (EFC)/FCH-BAR (F-BAR) and IRSp53-MIM homology domain (IMD)/inverse-BAR (I-BAR) domains, which have structurally determined membrane contact surfaces that are considered to function in membrane curvature formation, recognition and maintenance. BAR domain superfamily proteins deform membranes to a geometry that corresponds to the structures of the membrane-binding surface of the proteins, and/or bind to the membranes that fit their structures, and thus function to generate specific membrane geometries (*1*–*3*). These BAR domain superfamily proteins not only deform membranes but also have several other functional domains. For example, the SH3 domains also bind to N-WASP/WAVE proteins, regulator of actin cytoskeleton and dynamin, a molecule that pinches the membrane into vesicles, as described in several reports (Fig. 1) (*1*, *2*, *4*, *5*).

If we consider the morphogenesis of these fine structures of the plasma membrane, the entire cellular or plasma membrane is a relatively flat structure, and therefore, the plasma membrane is considered to be flat, as compared to the fine structures at the membrane. The structures are broadly classified into two kinds of tubulation, corresponding to membrane invaginations and inverted invaginations or protrusions (Fig. 2). There are six curvatures, which are classified by the positive or negative curvature and the position of the membrane tubule on the flat membrane, such as the neck, the tip and the shaft. Therefore, the modes of membrane curvature are classified into six subcategories, although the sizes and the detailed shapes are diverse. Among the six modes of membrane curvature, three have been assigned to the BAR domain proteins (Fig. 3A and B).

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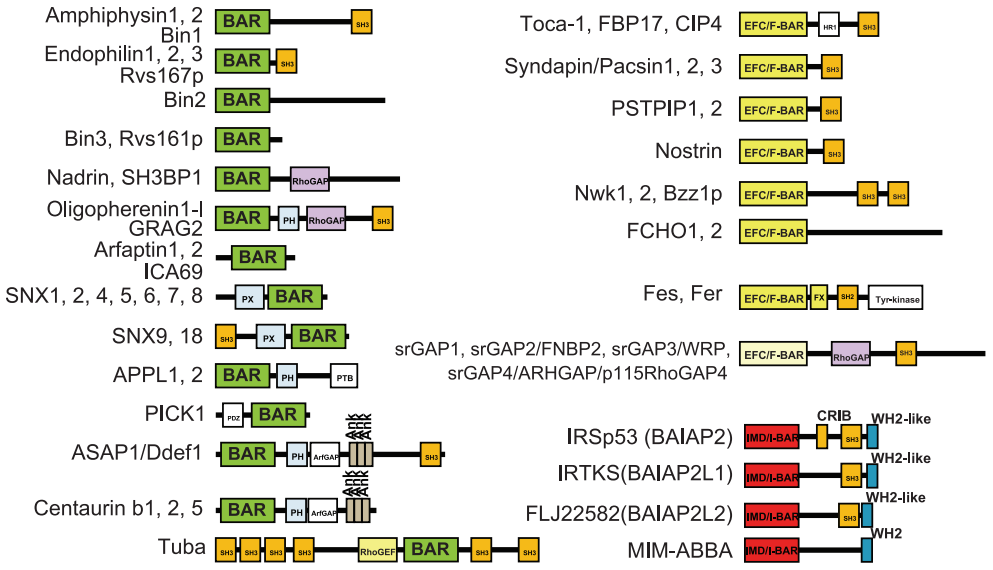


**Fig. 1 Diagram of the domain structures of BAR-, EFC/F-BAR- and IMD/I-BAR -domain-containing proteins.** BAR: Bin Amphiphysin Rvs domain, SH3: Src homology 3 domain, RhoGAP: Rho GTPase activating protein domain, PX: phox homology domain, PH: pleckstrin homology domain, ArfGAP: Arf Rho GTPase activating protein domain, PTB: phospho-tyrosine binding domain, PDZ : Psd-95, Dlg and ZO1 domain, RhoGEF: Rho guanine-nucleotide exchange factors domain, Ank: Ankyrin domain, IMD: IRSp53-MIM homology domain, I-BAR: inverse BAR domain, WH2: wasp homology 2 (verproline homology) domain, EFC: extended FCH domain, F-BAR: FCH-BAR domain, Tyr-kinase: tyrosine kinase domain, ENTH: Epsin N-terminal homology domain, I/LWEQ: actin filament binding domain, HR1: protein kinase C-related kinase homology region 1 domain, FX; F–BAR extension domain, CRIB: Cdc42-Rac interactive binding region, SH2: Src homology 2 domain. The diagram was modified from previous reports (*1*, *65*).

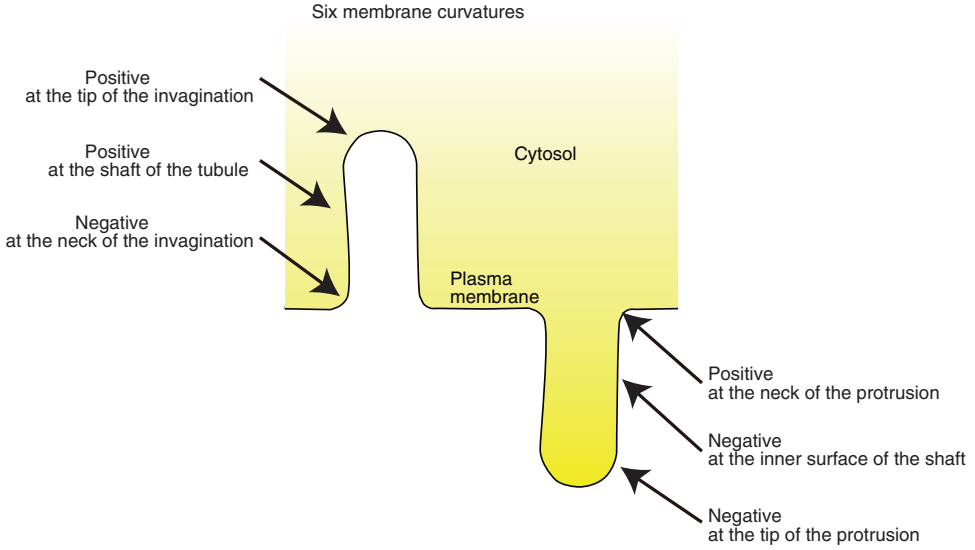


**Fig. 2 Six modes of membrane curvature for protrusion and invagination.** Since the entire cell has a relatively large plasma membrane, as compared to those of the subcellular structures, the subcellular structures are considered to be generated on a flat plasma membrane. The curvature of the membrane can be either positive or negative. Thus, all of the structures of the plasma membrane could be regarded as tubules, with a great variety of sizes and shapes. There are six curvatures, which are classified by the positive or negative curvature and the position in the membrane tubule on the flat membrane, such as the neck, the tip and the shaft.

## BAR and F-BAR domains and invagination structures at the plasma membrane

### *Clathrin-coated pits*

The clathrin-coated pit is one of the most well-defined invaginations of the plasma membrane. Clathrin-coated pits are characterized by an invagination surrounded by a dense lattice of clathrin, and are ~100–200 nm in diameter (*3*, *6*, *7*). Clathrin-mediated endocytosis plays an important role in receptor internalization, synaptic vesicle recycling and somatic nutrient uptake. The formation of clathrin-coated vesicles involves three steps (*8*, *9*). First, the clathrin coat assembles on the flat membrane with other proteins, such as AP-2, and captures the cargo to form a hemispherical clathrin-coated pit. Second, the clathrin-coated pit slowly invaginates, and the actin polymerizes. Third, scission proteins are recruited to the neck of the invaginated pit, for the separation of



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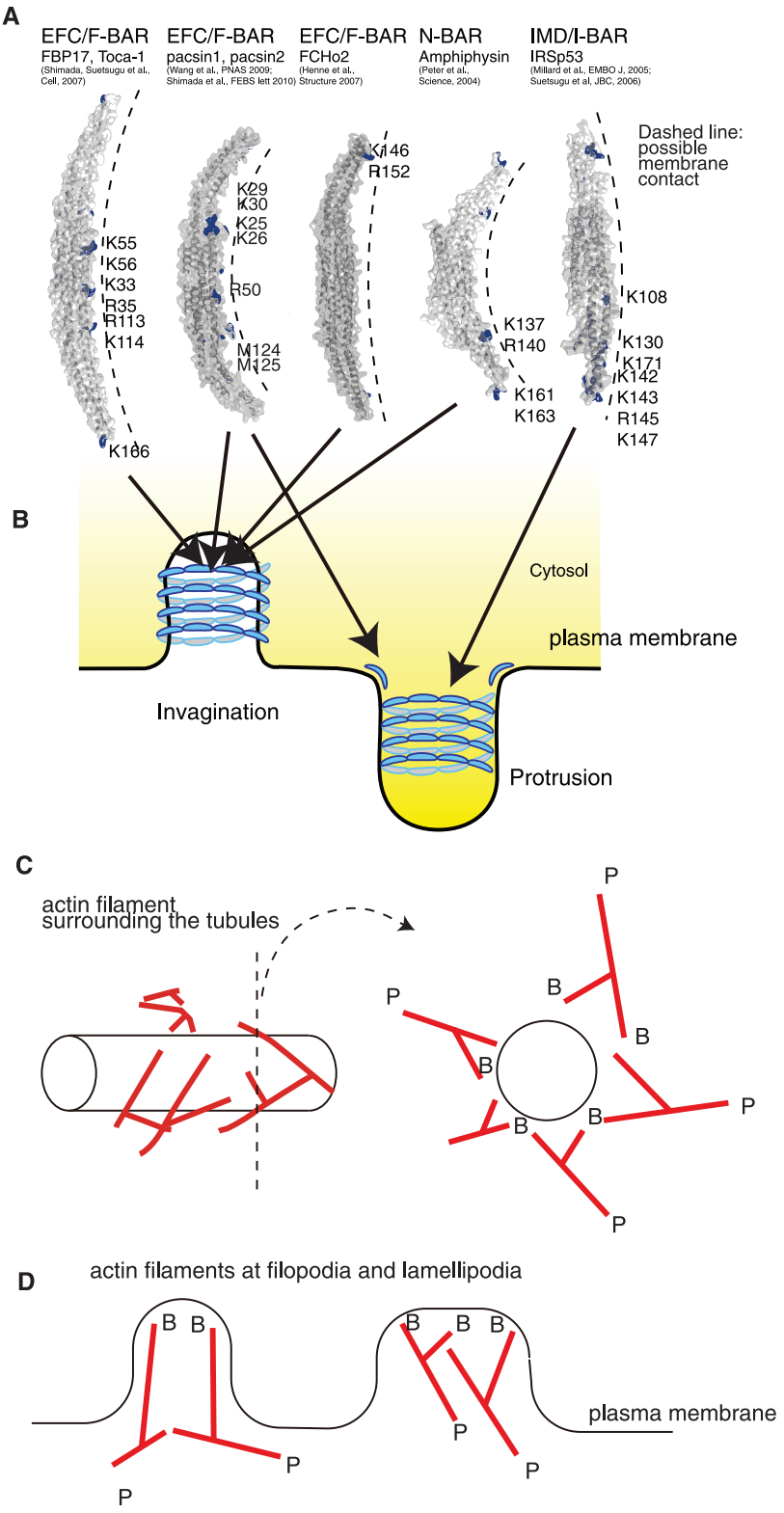


**Fig. 3 Various membrane curvatures and the BAR domain proteins.** (A) The structures of FBP17, pacsin2, FCHo2, Drosophila amphiphysin and IRSp53. The amino acids responsible for lipid binding are coloured blue. Dashed lines indicate putative membrane contacts, as determined by amino-acid substitution studies. (B) Possible mechanism of membrane deformation, based on the results of the EFC/F-BAR study (*22*), in which the spiral formed by the EFC/F-BAR protein is observed on the surface of the tubulated liposome. The EFC/F-BAR domain displays homo-interactions between the dimers: end-to end and lateral. These homo-interactions may facilitate the sensing of the curvature of the membrane and the membrane deformation by the EFC/F-BAR domain. However, these putative mechanisms of BAR-domain- and IMD-induced membrane deformation have not been confirmed. The domains associated with the tubulated membrane are in the cytosol. The membrane protrusions or invaginations are hypothesized to be generated by the spiral formation by the domains (*66*). The positive curvatures at the neck of the protrusion may also be formed by the EFC/F-BAR domain (*40*). (C) The actin filaments induced by FBP17 around the FBP17-induced tubules are illustrated (*30*). (D) Actin filaments for protrusions are illustrated. B and P indicate the barbed end (fast growing end) and the pointed end (slow growing end), respectively.

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the newly formed clathrin-coated vesicles from the plasma membrane.

Various proteins involved in clathrin-mediated endocytosis have been described. Epsins bind to AP-2 and clathrin, and participate in the initial clathrin assembly and clathrin-coat formation steps (*10*), while dynamins play a role in the scission step (*11*). Epsins also have membrane deforming ability through the insertion of an α-helix into the membrane, which induces positive curvature (*1*, *3*). In clathrin-coated pits, the actin filaments induced by the N-WASP activation of Arp2/3 is required for second invagination and actin polymerization step as well as the scission of the clathrin-coated pit from the plasma membrane, although epsins do not bind to either N-WASP or dynamin.

The BAR and EFC/F-BAR-domain proteins, such as amphiphysin, endophilin, SNX-9, FBP17 and Toca-1, have an SH3 domain that can bind to N-WASP, dynamin and/or other proteins, and function in actin polymerization and scission step. Each curvature of membrane contact surface suggests the sequential recruitment of each domain. However, the sequential order of membrane tubulation mediated by each of the BAR domain superfamily protein and actin polymerization or dynamin-mediated fission has not been clarified experimentally. Each domain protein clearly induces membrane tubulation *in vitro*; however, it is still unclear whether each domain actually generates membrane tubulation, or just senses the membrane curvature. Here, we describe the characters of each BAR domain proteins that are reported in clathrin-mediated endocytosis.

### BAR proteins that function with dynamin and N-WASP

All of the domains within the BAR domain superfamily domain form homodimers. The structure of the BAR domain from amphiphysin was the first to be solved, and the protein was demonstrated to be important for membrane deformation. The BAR domain forms a crescent-shaped dimer, with a positively charged concave surface (Fig. 3A) (*12*). The positively charged surface of the domain binds to the negatively charged inner surface of the plasma membrane, mostly through phosphatidylserine, an abundant lipid in the plasma membrane. Furthermore, the BAR domains from endophilin and amphiphysin contain hydrophobic amino acids on the concave surface and dimer ends, respectively (*12–14*). The hydrophobic amino acids on the concave surface or dimer ends are inserted into the membrane, thereby strengthening the interaction between the membrane and the BAR domain. In amphiphysin, N-terminal amphipathic helix precedes the BAR domain; therefore, this type of BAR domain sometimes is referred as N-BAR domain.

The BAR domain of amphiphysin deforms the membrane into narrow tubular invaginations in cells, and into tubules *in vitro* in a reconstitution assay with protein-free liposomes and the BAR domain protein alone. Therefore, these BAR domains from amphiphysin and endophilin are considered to function at the shaft of the tubules (Figs 2 and 3A and B). These tubules are thought to correspond to the location of amphiphysin function at the late stage of endocytic vesicle formation.

The BAR domain of sorting nexin, SNX9, is closely connected to the PX domain, and the BAR and PX domain units of SNX9 have broad phosphoinositide specificity. The BAR-PX unit of SNX9 also deforms membranes into tubules, and SNX9 functions in late stages of clathrin-mediated endocytosis with dynamin and endosomal trafficking (*15–17*).

The above-mentioned BAR domains, such as in the amphiphysin, endophilin and SNX9 proteins, have SH3 domains that bind to dynamin and N-WASP, providing the connection between the membrane shape and the actin polymerization machinery (*17–19*). Amphiphysin induces N-WASP-dependent actin polymerization that is dependent on liposomes, as demonstrated using a cytosolic fraction from amphiphysin knockout mice (*20*).

Endophilins with BAR domain have been proposed to have a lysophosphatidic acid acyl transferase activity that could modify the shape of the liposome by changing the lipid acyl chains, *i.e.* LPA, an inverted-cone-shaped lipid, to phosphatidic acid, a cone-shaped lipid. This mechanism was proposed to generate negative curvature at the neck of the invagination. However, this enzymatic activity was found to be a contaminant of the endophilin purification, and the membrane deformation ability resides in the BAR domain at the N-terminus of endophilin (*21*).

### EFC/F-BAR proteins that function with dynamin and N-WASP

The structure of the EFC or F-BAR domain from CIP4 and FBP17 was the first to be solved among the EFC/F-BAR domains (Figs 1 and 3A) (*22*). The EFC/F-BAR domain of Toca-1 is almost identical to those of CIP4 and FBP17 (*22–24*). The EFC/F-BAR domain forms a crescent-shaped dimer, in which the concave surface binds to the membrane for tubulation *in vitro* (*22*). As for the BAR domain, the overexpression of the EFC/F-BAR domain protein fragment alone induces tubular membrane invaginations in cells. In addition, the EFC/F-BAR domain protein fragment induces the tubulation of protein-free liposomes. Therefore, the EFC/F-BAR domain of CIP4 or FBP17 appears to function at the shaft of the tubule (Fig. 3A and B) Overexpression of full-length FBP17 also induces tubulation. The curvature generated by the EFC/F-BAR domains of CIP4 and FBP17 was much larger than that induced by the BAR domains from amphiphysin and endophilin, and appeared to correspond to the curvature of the initial stages of clathrin-coated pits. FBP17 and CIP4 function in the endocytosis of clathrin-coated pits, presumably by recruiting dynamin and actin polymerization machinery to the membrane curvature (*22–27*). Interestingly, the tubulation induced by the overexpression is antagonized by the co-expression of dynamin and N-WASP, suggesting that the tubules are first

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generated, then fission occurs by N-WASP and dynamin. However, this sequential order was not confirmed experimentally. CIP4 may also be involved in endosomal trafficking (*28*). In a reconstitution assay, FBP17 and Toca-1 were shown to induce N-WASP and Arp2/3 complex-mediated actin polymerization dependent on membrane curvature (*29*, *30*).

These EFC/F-BAR domains bind to phosphatidylserine and PI(4,5)$P_2$, and deform artificial liposomes and cell membranes into tubules. The binding of the EFC/F-BAR domains to phosphatidylserine was confirmed by many laboratories, and is supported by the crystal structure (*22–24*, *31*, *32*). The binding of the EFC/F-BAR domain to PI(4,5)$P_2$ is reasonable, based on its strong negative charge and its involvement in endocytosis, because many proteins involved in endocytosis bind to PI(4,5)$P_2$ (24).

Pacsin/Syndapin forms one branch of the F-BAR domain protein family (*2*, *23*, *33–35*). Initial studies of syndapin II/pacsin2 revealed that the overexpression of the SH3 domain inhibits the endocytosis of transferrin (*36*, *37*). This inhibition was apparently a consequence of titrating out dynamin or N-WASP from the invagination structures, because the C-terminal SH3 domain of pacsin2/Syndapin II binds to N-WASP and dynamin (*38*). The structures of the EFC/F-BAR domains of human pacsin1/Syndapin I, pacsin2/Syndapin II and Drosophila pacsin/Syndapin were reported (*32*, *39*, *40*). The crystal structure of the pacsin F-BAR domain revealed a shallower concave surface, and the lateral surface is curved into a tilde-like shape. Consistent with the diameters of the curvatures of the concave surfaces, the membrane tubules induced by the pacsin F-BAR domain are narrower than those induced by the F-BAR domains of FBP17, CIP4, and Toca-1 (Fig. 3A) (*32*). The narrower diameter of the tubules induced by pacsin2 suggests that it is recruited to the clathrin-coated pit at the late stage of clathrin coated vesicle fission, where narrower membrane tubules connect the plasma membrane to the coated vesicles (Fig. 3A). However, pacsin was reportedly not colocalized with clathrin (*41*). Therefore, the F-BAR domain of pacsin2 forms a clearly distinct subfamily in the F-BAR family, and pacsin2 appears to function in the biogenesis of different cellular organelles than those involving FBP17, CIP4 or Toca-1. The F-BAR domains of pacsin1 and pacsin2 have a hydrophobic insertion loop, as also found in the endophilin BAR domain. The tubules with pacsin2 localization appeared after the disruption of actin filaments, suggesting the role of the actin cytoskeleton in tubule formation (*23*). The SH3 domain of pacsin/Syndapin also binds to N-WASP and dynamin (*36*).

### *BAR and F-BAR proteins that function with other binding partners*

PICK1 is unique BAR-containing protein that contains a PDZ domain, but lacks an SH3 domain. Interestingly, the BAR domain of PICK1 reportedly interacts with the Arp2/3 complex to suppress the nucleation of actin filaments, thereby inhibiting the endocytosis of neurotransmitter receptors (*42*, *43*). However, the functional mechanism of PICK1 in endocytosis is still unclear.

FCHo1 is reportedly present at clathrin-coated pits (*44*, *45*). However, the location of FCHo2 function in cells is still unclear. Interestingly, FCHo1 and FCHo2 have a cargo-binding μ homology domain that binds to Eps15, a cargo adaptor protein (*45*, *46*). The yeast homologue of FCHo1, Syp1, reportedly inhibited actin polymerization induced by Las17p, a yeast homologue of WASP, in uncharacterized manner *(47)*. The F-BAR domain of FCHo2 also forms a crescent-shaped dimer, but the curvature of its membrane binding concave surface is larger than that of the EFC/F-BAR domains of CIP4 and FBP17 (*22*, *31*). In contrast to the EFC/F-BAR domains of CIP4 and Toca-1, the lateral surface of the F-BAR domain of FCHo2 has a tilde-like shape (*31*). The curvature of the membrane contacts of FCHO2 among the FCHo2 (Fig. 3A), FBP17/CIP4, and amphiphysin/endophilin is the largest, suggesting that the FCHos are recruited earlier to the clathrin-coated pits than the other BAR and F-BAR proteins.

### *BAR and F-BAR proteins may function in caveolae and other membrane invaginations*

Caveolae are also well-known structures at the plasma membrane, and are localized in the vicinity of the actin filaments that run beneath it. Nostrin, an EFC/F-BAR protein, is reportedly localized at caveolae (*48*), and the SH3 domain of Nostrin binds to N-WASP and dynamin (*49*). The treatment of cells with an actin depolymerizing drug resulted in the mislocalization of caveolae (*50*). Dynamin is also localized at the neck of caveolae (*51*, *52*). A small percentage of caveolae are engaged in endocytosis, such as of SV40, in an actin- and dynamin-dependent manner (*53*, *54*).

There are several less characterized invaginations in cells, such as those of the CLIC-GEEC pathway, muscle T-tubules and others (*3*). The CLIC-GEEC pathway has deeper invaginations, in which the function of the BAR domain protein GRAF1 has been indicated (*55*). T-tubules are essential for calcium signalling in muscles, and the involvement of caveolin-3 and the BAR domain protein amphiphysin was demonstrated (*56–58*).

## The IMD/I-BAR domain is involved in negative membrane curvature at the shaft and/or tip of the protrusions

There are several types of protrusive structures in cells. Most of these structures contain actin filaments, presumably for the stability and mechanical strength required to exert the force needed for cell motility or the uptake of extracellular materials. The best-characterized cellular protrusions are filopodia and lamellipodia (*2*, *59–61*).

Filopodia are spike- or needle-like cellular protrusions containing bundled actin filaments. Lamellipodia are relatively flat cellular structures that protrude in the direction of cell movement. Branched actin filaments fill the inside of the lamellipodia. Protrusive

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structures driven by actin polymerization are also observed in phagocytosis. During phagocytosis, the protruding lamellipodia-like structure surrounds the material that is incorporated into the cell.

For a long time, filopodia and lamellipodia were considered to be generated solely as a consequence of actin polymerization. WAVE2-mediated activation of the Arp2/3 complex is essential for the formation of branched actin filaments at lamellipodia (*2*). The actin bundle formation at filopodia appears to be generated by several pathways, which are classified as Arp2/3 dependent and Arp2/3 independent (*59*). In the Arp2/3-dependent mechanisms, the branched filament is bundled by additional factors. In the Arp2/3 independent mechanisms, the unbranched filaments are bundled. These mechanisms appear to be sufficient for the generation of protrusions. Therefore, the discovery of membrane protrusions lacking actin filaments was surprising, and their existence *in vivo* is still in question.

The structures of the IRSp53-MIM homology domain (IMD)/inverse-BAR (I-BAR) domain from IRSp53 and MIM were clarified. The IMD/I-BAR domain (hereafter, we refer to this as I-BAR) binds to the membrane through its convex surface, and is considered to bind to the negatively curved membrane at the shaft or tip of protrusions. The I-BAR domain, with the inverted geometry of the membrane-binding surface, is involved in the plasma membrane protrusions of filopodia and lamellipodia (*62–65*). The binding of I-BAR to the membrane on the inner surface of the tubules was confirmed by cryo-electron microscopy (*66*). Most of the interaction occurred through phosphatidylserine, but a preference for $PI(4,5)P_2$ and $PI(3,4,5)P_3$ was observed for the I-BAR of IRSp53 (*63*, *66*). In addition to its induction of a negatively curved membrane structure, the I-BAR from MIM also has a helix for insertion into the membrane (*66*).

Some I-BARs reportedly interact with Rac, a small GTPase. The IMD of IRSp53 binds to the active form of Rac, whereas the I-BAR of MIM binds to the inactive form (*63*, *67*, *68*). These differences in the affinities to the small GTPases may modulate the membrane binding of the IMDs.

The overexpression of the I-BAR fragment alone clearly induced highly dynamic membrane protrusions that persisted even in the presence of an actin polymerization inhibitor (*63*, *64*, *69*, *70*). When the full-length proteins were overexpressed, the induced protrusions contained actin filaments, presumably because the SH3 domain recruits proteins that bundle and/or induce the formation of actin filaments. However, several regions without actin filaments were also observed in these protrusions (*63*, *71*). The I-BAR domain fragment is localized throughout the protrusions, suggesting its role at the shaft and the tip of the protrusive tubules.

Interestingly, the SH3 domain of IRSp53 binds to the Arp2/3 activator, WAVE2, which plays essential roles in lamellipodium formation, and also to N-WASP, which is considered to function in filopodium formation and endocytosis (*67*, *72*, *73*). MIM has the Verproline/WH2 domain, and directly binds to actin (*74*, *75*). The IMD itself has been proposed to have an actin-filament bundling activity, although this function is still controversial (*65*). IMD binding to the actin filament has been confirmed by several laboratories (*62–64*). The IRSp53 SH3 domain reportedly binds to dynamin (*73*), but the significance of dynamin in membrane protrusions is still unclear. IRSp53 also has a PDZ binding motif, and it binds to several proteins with the PDZ domain, which may be important for the assembly of some cellular structures (*76–79*).

IRSp53 is involved in both filopodium and lamellipodium formation, as suggested from the localization and the binding of WAVE2 and N-WASP, VASP and Mena (*67*, *73*, *80*, *81*). An analysis with N-WASP knockout cells indicated that the IRSp53-mediated formation of filopodium-like protrusions requires N-WASP, but its Arp2/3 complex activating ability was not involved in the protrusion formation (*73*). The siRNA mediated knockdown of IRSp53 also revealed its role in lamellipodia formation (*72*). The localization of IRSp53 at the lamellipodium may be modulated by the phosphorylation-dependent binding of 14-3-3 at the SH3 domain (*82*).

The membrane protrusions with cylinder-like shapes, which are induced by the IMD, may be extended by the dendritic Arp2/3-mediated branched actin filaments to form lamellipodia (Fig. 3D). However, it is quite difficult to observe these transient structures in cells. The lamellipodia-like structures induced by WAVE2 and IRSp53 are involved in phagocytosis (*83*, *84*).

## The F-BAR domain members form cellular protrusions by a variety of mechanisms

Four proteins, named slit-robo GAP (srGAP) 1–4, contain a GAP domain for small GTPases, an EFC/F-BAR domain and an SH3 domain (*23*, *24*). The specificity of the GAP activity on small GTPases differs among the srGAP1–4 proteins. The SH3 domain often binds to WASP/WAVE proteins. srGAP1 binds to WASP, and inactivates Cdc42 (*85*). srGAP2 binds to N-WASP and inactivates Rac (*86*, *87*). srGAP3/WRP binds to WAVE1, and inactivates Rac (*88*).

Recently, the F-BAR domain of srGAP2 was reported to induce membrane invagination *in vitro*, with the same geometry as that found in membrane protrusions, such as filopodia (*87*). The overexpression of the F-BAR domain-containing fragment of srGAP2 induced filopodia-like protrusions without actin filament localization in a similar manner as overexpression of IMD/I-BAR domain of IRSp53. Therefore, the F-BAR domain of srGAP2 may function as an I-BAR domain; however, its membrane-binding mechanism is still unclear, because the structure of the F-BAR domain of srGAP2 has not been solved yet. Although the role of the SH3 and GAP domains should be investigated, srGAP2 is involved in neuronal migration through its membrane deforming ability (*87*).

Interestingly, a homologue of FBP17, Toca-1, is apparently a negative regulator of neurite extension (*89*).

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The EFC/F-BAR protein, FBP17, was shown to be involved in both phagocytosis and the formation of podosomes, invasive structures that degrade the extracellular matrix (*90*). However, the role of EFC/F-BAR domain for these structures has been unclear.

The Fes and Fer proteins have the EFC/F-BAR domain, and also contain the conserved kinase domain found among the Src family kinases. Recently, the region adjacent to the Fes or Fer F-BAR domain was shown to bind to phosphaditic acid (PA), and was named the F-BAR extension (FX) domain. The F-BAR and FX units function as a membrane binding module with a preference for PA. The F-BAR-FX unit is essential for the membrane-dependent activation of the Fer kinase activity, which is involved in lamellipodia formation and cell migration (*91*). Since PA has the smallest head group among the phospholipids, the preferential binding to PA may induce the negative membrane curvature for lamellipodia membrane protrusions, by decreasing the volume of the lipid head group at the inner leaflet of the membrane.

Some BAR domain proteins are also involved in protrusion formation. The Tuba protein is localized at synapses and dorsal ruffles (*92*, *93*), and contains the BAR domain, the SH3 domain and the Cdc42-specific GEF domain.

Overexpression of the pacsin2 EFC/F-BAR domain resulted in tubular localization inside cells and deformed liposomes into tubules in vitro (*40*, *94*). We found that overexpression of the pacsin2 EFC/F-BAR domain induced cellular microspikes, with the pacsin2 EFC/F-BAR domain concentrated at the neck. The hydrophobic loops and the basic amino-acid residues on the concave surface of the pacsin2 EFC/F-BAR domain are essential for both the microspike formation and tubulation. Since the curvature of the neck of the microspike and that of the tubulation share a similar geometry, the pacsin2 EFC/F-BAR domain is considered to facilitate both microspike formation and tubulation. Pacsins/Syndapins function in the morphogenesis of neurons and in zebrafish notochord development, presumably through endocytosis and/or protrusive structure formation (*39*, *94*).

PSTPIPs are F-BAR-containing proteins with an SH3 domain that associates with N-WASP. PSTPIP1 is localized at the cleavage furrow. PSTPIP2 has an actin filament bundling activity that induces filopodia (*95*) (reviewed in Aspenstrom *et al.*, 2006) (*96*), although the functions of PSTPIPs in the membrane context are still unclear.

## Membrane fusion mediated by high membrane curvature

Membrane fusion occurs in various aspects of cell morphological changes. It is very important to note that the fusion intermediate is the tubular membrane structure (Fig. 4). Therefore, membrane tubules could be formed by the fusion of vesicles with the flat membrane. Tubular membranes could be either vesiculated or flattened, depending on the context of the associated proteins (*97*). Interestingly, invagination of the plasma membrane has been observed in exocytosis (*98*).

The BAR or F-BAR domain protein fragment induces membrane tubules that appear to have a larger surface area than the original spherical liposomes. The induction of tubulation or a highly curved membrane promotes membrane fusion, as assessed by a FRET-based membrane fusion assay (*13*). The induction of high membrane curvature apparently promotes membrane fusion by exposing the hydrophobic, non-polar interior of the lipid bi-layer, which will contact the other curved membrane that eventually fuses into one liposome. The limited contact area of liposomes upon tubulation also will help to fuse the membrane. The homophilic interactions of F-BARs, such as those observed for FBP17 (*22*), may synergistically assist in membrane fusion.

The synaptotagmin C2 domain deforms membrane into tubules, which reportedly play an essential role in the membrane fusion of synaptic vesicles (*99*). The tubulation at the flat membrane by the synaptotagmin C2 domain is essential for SNARE-mediated vesicle fusion to flat giant liposomes (*100*). Importantly, the tubulation ability of the C2 domain could be substituted by the tubulation by the BAR domain, indicating that the tubulation is a critical and important step in membrane fusion (*100*).

## Spatially ordered actin filament formation on the membrane by the BAR domain superfamily proteins

The structure of the BAR domain superfamily protein determines the diameter of the tubulated membranes or liposomes. The BAR domain superfamily members have certain preferences for the membrane curvatures that they deform into tubules at least *in vitro*. The EFC/F-BAR domain of FBP17 prefers liposomes with a large diameter of more than ~500 nm, rather than small liposomes. In contrast, the BAR domain of amphiphysin has a preference for smaller liposomes (*22*).

Consistently, the induction of actin polymerization by FBP17 or Toca-1 is dependent on the size of the liposomes added to the actin polymerization assay system (*29*). Although the membrane curvature dependence of actin polymerization has only been examined for the FBP17 and Toca-1 proteins, the curvature-dependent actin polymerization was not achieved by the EFC/F-BAR domain alone. The acidic amino acid residues adjacent to the SH3 domain are required for rapid actin polymerization by FBP17 or Toca-1. These acidic amino acids are conserved among FBP17, CIP4, and Toca1, but not among pacsin/syndapin. The acidic amino acid residues reportedly influence the position of the following SH3 domain relative to the membrane by electrostatic repulsion (*29*). The binding of N-WASP to the SH3 domain of FBP17 and Toca-1 was controlled by this electrostatic repulsion. The direct membrane binding of N-WASP through its basic region was required for the membrane curvature-dependent actin

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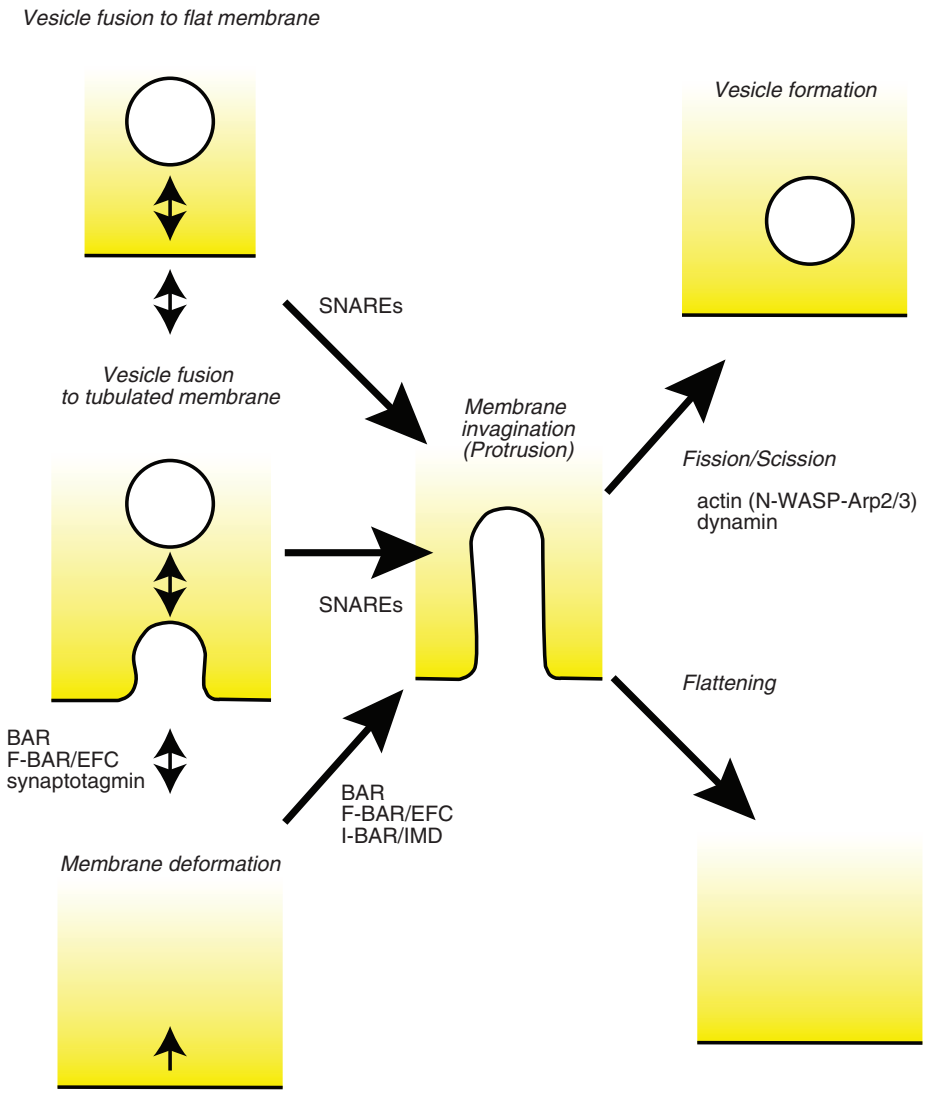


**Fig. 4 Tubular membrane formation by membrane deformation or membrane fusion, with subsequent flattening or vesiculation.** Tubular membrane structures are considered to participate in various aspects of cell morphological changes. The tubular membrane may be the intermediate state of a membrane that is either pinching off or fusing. The tubulated membrane could eventually become flat, either by the fission/scission step most commonly mediated by dynamin and actin polymerization, or simply by retraction into the flat membrane. The representative proteins for each step are also indicated.

polymerization (*29*). Therefore, the curvature-dependent actin polymerization requires the association of both the EFC/F-BAR proteins and the N-WASP–WIP complex with the membrane, in a spatially ordered manner. Cryo-electron microscopy revealed the existence of membrane surfaces between the spirals of the EFC/F-BAR domain (*101*), where the binding proteins may contact the membrane.

The orientation of the actin filament induced by the EFC/F-BAR protein FBP17 was examined (*30*). The orientation was uniform, and the barbed end faced towards the tubules. It is interesting that in both protrusions and invaginations, the barbed ends always appear to face the membrane (Fig. 3C and D). The BAR domain superfamily proteins fit the various membrane curvatures (Fig. 3A and B), and activate WASP/WAVE-Arp2/3 mediated actin polymerization. Therefore, the BAR domain superfamily proteins could control the direction of the actin filaments made by the WASP/WAVE family proteins and the Arp2/3 complex for various sub-cellular structures.

## Correspondence of the BAR domain superfamily proteins and membrane curvature, and implications in diseases

In summary, the BAR domain superfamily proteins appear to utilize three binding modes for membrane curvature formation: the concave surface of the protein binding to the positive curvature of the membrane at the liposome tubule/cellular invagination (amphiphysin, endophilin BARs and FBP17, CIP4, pacsin, FCHO2 EFC/F-BARs), the convex surface of the protein binding to the negative curvature of the membrane at the liposome invagination/cellular protrusion (IRSp53 and MIM IMD/I-BAR) and the concave



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surface of the protein binding to the positive curvature of the membrane at the neck of the liposome invagination/cellular protrusion (pacsin EFC/F-BAR) (Fig. 3A). There may be unknown protein-membrane interactions involved in the negative curvature at the neck of the cellular invaginations and at the tip of cellular protrusions, and at the positive curvature of the tip of tubulated cellular invaginations.

There are several cases of BAR domain involvement in human disease. The best characterized is the case of PSTPIP1, which is mutated in auto-inflammatory disease (PAPA syndrome), and where mutations (E250Q and A230T mutations) were found in the F-BAR domain, although the effect of these mutations has not been clarified (*102*). The srGAP3 gene is deleted in a severe type of mental retardation (*103*). Furthermore, the SH3 domains of CIP4 and pacsin1 bind to huntingtin, a protein responsible for Huntington's disease (*104*, *105*). FBP17 is a fusion partner with the mixed lineage leukemia (MLL) gene, where FBP17, including the partial EFC/F-BAR domain, is fused at the C-terminus of MLL (*106*). In addition, CIP4 is implicated in renal carcinoma, where the mutation causes the expression of a truncated fragment with the EFC/F-BAR domain and lacking the SH3 and HR1 regions (*107*). Fes is a well-known viral oncogene (*108*). Pacsin1 and CIP4 bind to the huntingtin protein through their SH3 domains (*104*, *105*). The connection to diseases will clarify the roles of the cellular membrane structures for various cellular functions.

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**Conflict of interest**
None declared.

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