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Mijo Simunovic, Emma Evergren, Andrew Callan-Jones, Patricia Bassereau. Curving the cells inside and out: Roles of BAR-domain proteins in intracellular trafficking, shaping organelles, and cancer. Annual Review of Cell and Developmental Biology, 2019, 10.1146/annurev-cellbio-100617-060558 . hal-02390315

HAL Id: hal-02390315
https://hal.sorbonne-universite.fr/hal-02390315

Submitted on 3 Dec 2019

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Curving the cells inside and out: Roles of BAR-domain proteins in intracellular trafficking, shaping organelles, and cancer

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Shortened running title: Membrane shaping mechanisms by BAR proteins

Abstract

A great number of cellular processes rely on precise and timely deformation of the cell membrane. While many proteins participate in membrane reshaping and scission, usually in highly specialized ways, Bin/amphiphysin/Rvs (BAR) domain proteins dominate as they not only participate in almost every aspect of cell survival and function, but they are also highly versatile membrane remodelers. Subtle changes in the shape and size of the BAR domain can have great impact on the way they interact with the membrane. Furthermore, the activity of BAR proteins can be tuned by external physical parameters and so they behave differently depending on protein surface density, membrane tension or membrane shape. They can form three-dimensional structures that mold the membrane and alter the underlying liquid properties, even promoting scission under various circumstances. As such, they have found numerous roles within the cell. Endocytosis is among the most highly studied processes where BAR proteins take important roles. However, over the years, a more complete picture has emerged in which BAR proteins are tied to almost all intracellular compartments, from endosomal sorting to tubular networks in endoplasmic reticulum and T-tubules, for instance. They also have a role in autophagy and their activity has been linked with cancer. Here, we briefly review the history of BAR protein discovery, we discuss mechanisms by which they induce curvature, and attempt to settle important controversies in the field. Finally, we review BAR proteins in the context of a cell, highlighting their emerging roles in endosomal sorting and intracellular trafficking, shaping organelles, autophagy and cancer.

Keywords

BAR-domain proteins; curvature; scaffold; amphipatic helices; scission; membrane traffic

A brief history of BAR proteins

Lipid membranes are involved in almost all aspects of cell survival and function. They serve as a physical barrier between the cell and its environment and they compartmentalize the organelles. Membranes also house numerous receptors, ion channels, and many other biological components, and they transmit the mechanical and biochemical cues from the environment. However, they are much more than a passive interface. Membranes are highly dynamic, having the capacity to rapidly and precisely change shape so to facilitate the trafficking of cargo, cell division, motility, and many other processes. The key physical property which allows lipid membranes such flexibility is that they are large yet very thin materials. At the microscopic scale, they retain much of the molecular behavior, including diffusivity, while at the macroscopic scale, they behave like highly elastic solids. Indeed, theoretical calculations and various *in vitro* experiments demonstrated that lipid membranes can adopt a plethora of different shapes depending on lipid composition, phase separation, particle binding, crowding, etc. (Bassereau et al 2014, Lipowsky 2013). In biology, however, timing and spatial localization are crucial. Therefore in cells, proteins take on the role of modulating membrane shape, achieved in a highly orchestrated fashion so to rapidly and precisely bend or cut the membrane.

Proteins belonging to a family of Bin/amphiphysin/Rvs (BAR) domain proteins are perhaps the largest group of membrane-curving proteins in the cell. Some of its most notable members, amphiphysin and endophilin, have originally been found as proteins extremely enriched in the mammalian brain, especially at the nerve terminals (Giachino et al 1997, Lichte et al 1992). There, they where found to bind the membrane during synap-

tic transmission (De Camilli et al 1993). Soon after the discovery, their sequence was likened to Bin1, a splice variant of amphiphysin 2 that interacts with myc and inhibits its oncogenic activity (Sakamuro et al 1996), and also to yeast proteins Rvs161 (Crouzet et al 1991) and Rvs167 (Bauer et al 1993), whose mutation leads to alterations in cell morphology and the cytoskeleton. Over the years, their role in membrane-remodeling phenomena has become more and more prominent, especially in clathrin-mediated endocytosis, a process by which cells internalize nutrients, signaling proteins, and other cargo. Already in the early studies it was shown that amphiphysin, for instance, contains binding sites for the canonical endocytic proteins dynamin and the clathrin adaptor protein AP2, which is why it was thought to be the key recruiter of the clathrin machinery to the endocytic site (David et al 1996). Interestingly, in a different context, an alternative splice variant of amphiphysin 2 was discovered, which does not bind AP2, and was found enriched in the highly reticulated membranous structures of skeletal muscle called the T-tubules (Butler et al 1997). Around the same time, another group of proteins with sequence similarities participating in membrane-remodeling activities were found; namely, CIP4, a target of the Rho protein Cdc42, along with tyrosine kinases FER and Fes/Fps (Aspenström 1997). The commonality between these and many other homologous proteins is that they help regulate the membrane-dependent cytoskeleton rearrangement and, as was later identified, contain a BAR domain.

Although unknown at the time, the early works began to indicate that BAR proteins are not just connectors for various cytoskeleton and membrane effectors, but are likely more direct modulators of membrane shape. Indeed, when purified amphiphysin was

mixed with synthetic lipid vesicles for the first time, the protein transformed the membrane into tubules (Takei et al 1999) (see microgram in Figure 1, *top example*). Although it was shown to interact with dynamin, amphiphysin alone was sufficient to considerably deform the membrane. Importantly, the membrane-binding and deforming activity was attributed to the ~300-residue-long N-terminal domain of the protein, which is in fact the conserved BAR domain. Endophilin and various CIP4 and FER-related proteins displayed the same tubule-inducing effect *in vitro* (Farsad et al 2001, Itoh et al 2005) (see micrograms in Figure 1).

It was not until the discovery of the atomic structure of a BAR domains that the molecular basis of why they cause curvature was finally elucidated. The first X-ray structure of a BAR domain was that of arfaptin, a protein that mediates the crosstalk between the Rho and Arf GTPases in cytoskeletal-induced membrane ruffling (Tarricone et al 2001). Several years later, amphiphysin's BAR domain was resolved (Peter et al 2004) showing that it is very similar to arfaptin's. Namely, dimerized BAR domains form into a curved bundle of helices with the shape reminiscent of a crescent moon (Figure 1), where the concave surface contains positively charged residues, providing the intuitive understanding of why BARs induce curvature (Peter et al 2004). The structure of many other BAR domains was subsequently resolved, showing that the key structural features of the BAR domain are always preserved; however, from one member to another the BAR domain differs in length, charge density, magnitude of curvature, and even sign of curvature (Qualmann et al 2011). Most broadly we divide them into: 1) classical BAR and N-BAR, 2) F-BAR, and 3) I-BAR domains (Figure 1). BARs, N-BARs and F-BARs all induce

positive membrane curvature, i.e., the membrane bends in the direction of the leaflet decorated by the protein, thus forming invaginations, whereas I-BARs cause negative curvature, forming protrusions (Qualmann et al 2011). Furthermore, the radius of tubules generated by BAR proteins is generally correlated to their shape. BAR/N-BAR domains have a more curved structure than the F-BAR domains and hence induce much narrower tubules. For instance, endophilin and amphiphysin, both N-BAR domains, induce tubules of ~10 nm radius when bound to small vesicles at very high concentrations (Mim et al 2012, Peter et al 2004), while F-BAR domains FCHo2 and CIP4 induce much wider tubules, >30 nm in radius (Frost et al 2008, Henne et al 2007) (Figure 1). I-BAR domains resolved so far are similarly curved to F-BARs, however they bind membranes via their convex surface to induce protrusions such as filopodia (Millard et al 2005). One I-BAR member, termed pinkBAR, is almost completely flat and forces membranes into a planar shape (Pykalainen et al 2011) (Figure 1).

In the 25 years of research since the early works that identified BAR proteins, many more are yet to be discovered. The sheer number of BAR domains in a cell begs the question of what mechanisms regulate their precise recruitment at the right place and the right time? What is the extent of BAR interaction with membranes? How diverse are their roles in the cell? In the following sections, we attempt to answer these questions: first, we describe our current understanding of how BAR domains couple with membrane curvature, we discuss the extent to which we can predict their membrane-curving properties based on their shape and structural details, and, finally we review their known roles in important cellular processes hypothesizing potential mechanisms of regulation in the cell.

How do BAR proteins couple to membrane curvature?

Purified BAR proteins with intrinsically more curved BAR domains generate narrower tubules than the ones with flatter BAR domains. One may speculate that the structure of the BAR domain directly correlates with the radius of deformed membranes. While it generally seems true, the intrinsic curvature of the BAR domain cannot be used to predict the precise shape of the membrane. For example, compare the N-BAR proteins in Figure 1. While SNX1 displays a much higher curvature than SNX33 and even amphiphysin, it forms tubules with double the radius. At the same time, SNX33 and amphiphysin form tubules of equal radii, yet display different curvatures in their structures (Figure 1). Recent biophysical measurements, imaging and theoretical predictions showed that 1), the mechanism by which BAR proteins impact curvature is more complex than just imprinting the crescent shape on the surface and 2), how BAR proteins interact with the membrane depends on a number of physical parameters, most importantly protein surface density, but also membrane tension and shape (Simunovic et al 2015).

Mechanisms of curvature generation by BAR proteins

Over the years, the theoretical picture and our understanding of what drives membrane shape has appreciably evolved. That said, most theoretical models today are still based on the relatively simple description developed decades ago (Canham 1970, Helfrich 1973), wherein the bilayer is modeled as a thin elastic sheet whose equilibrium shape is a result of an interplay between bending and stretching deformations. The membrane can be re-

shaped by imposing some kind of asymmetry into the bilayer, either by way of lipid composition, phase separation, local deformations, or active forces (Bassereau et al 2014, Lipowsky 2013). BAR domains causes curvature as a result of multiple different interactions with the membrane (Figure 2).

(1) *Adhesion of BAR domains*. The spontaneous curvature of the membrane can most generally be created by adhesion of objects–proteins, ions, or solid particles to one leaflet (Lipowsky 2013). Although the object does not need to be intrinsically highly curved to impart spontaneous curvature, the curved shape of the BAR domain makes its effect on the membrane much more prominent (Figure 2). Considering that the membrane facing surface of the BAR domain is lined with positively charged residues, the multiple ionic bridges between the single protein and the negatively charged bilayer create a very strong adhesive interface. Indeed, computer simulations have demonstrated that the BAR domain can appreciably locally deform the underlying bilayer (Blood & Voth 2006).

The membrane deformation created by one protein can, in turn, perturb a second, neighboring protein thereby creating indirect protein-protein interactions (Dommersnes & Fournier 1999, Goulian et al 1993, Netz & Pincus 1995). As a result, the membrane itself can mediate the interactions of bound proteins and particles and thus create a feedback loop between protein assembly and curvature generation (Leibler & Andelman 1987). In contrast with a pair of isotropic inclusions or adsorbents (Goulian et al 1993), several theoretical approaches have shown that membrane mediation results in short-range repulsion and long-range attraction between a pair of anisotropic particles, thus providing the

conditions for multi-protein self-assembly (Chou et al 2001, Dommersnes & Fournier 1999, Park & Lubensky 1996, Schweitzer & Kozlov 2015). Membrane-mediated assembly is especially prominent in BAR proteins. The highly anisotropic interactions of BAR domain and its amphipathic helices (AHs) with the bilayer create strong effective attractions between bound N-BAR domains, leading to a variety of large-scale protein organization coupled to membrane deformation, as revealed by computer simulation (Noguchi 2014, Noguchi 2016, Ramakrishnan et al 2013, Simunovic et al 2013a, Simunovic et al 2017b, Simunovic et al 2013b, Simunovic & Voth 2015).

(2) *Scaffolding of BAR proteins around membrane tubes*. Cellular trafficking events, such as endocytosis, generally involve tubular membrane structures or buds connected by a tubular neck. Some endocytic proteins polymerize to form 3D structures on such membranes and thereby act as a mold on the underlying bilayer. Dynamin, for example, polymerizes into a spiral at the neck of the endocytic bud (McMahon & Boucrot 2011). BAR proteins, at sufficiently high surface densities, self-assemble to form a scaffold which shapes the membrane into very stable tubules (Simunovic et al 2016a, Sorre et al 2012). We refer to a scaffold as a large-scale, many-protein structure with mechanical properties that are distinct from those of the underlying membrane. As such, when BAR proteins scaffold a membrane tube, the tube radius is modified and becomes roughly independent of membrane tension (Simunovic et al 2016a) (Figure 3A). Note that this definition is different from that of others, which use the term scaffold to refer to a single BAR protein.

From the structural point of view, electron microscopy experiments show BAR scaffolds as tightly packed proteins stacked both side-by-side and tip-by-tip, completely coating the tubules, e.g., in the case of N-BAR domain of endophilin (Mim et al 2012). However, recent fluorescence microscopy and computer simulation studies showed that a stable scaffold forms at densities much lower than close packing (Simunovic et al 2016a, Sorre et al 2012) (Figure 3B, C). These lower density scaffolds, where BARs cover ~40% of the membrane tubule, are stabilized by strong membrane-mediated interactions among proteins (see above), and are likely the molecular structure seen in various endocytosis processes (Sundborger et al 2011, Takei et al 1999). Interestingly, a low-density helical scaffold as predicted from measurements and computer simulations (Simunovic et al 2016a) was recently seen by electron microscopy and high-speed atomic force microscopy of tubes scaffolded by an amphiphysin-dynamin complex (Takeda et al 2018). The scaffolds are remarkably similar having equal surface density as measured with confocal microscopy and even displaying the same helical pitch of ~20 nm predicted in simulations (see Figure 3C for the molecular structure of an N-BAR scaffold). With BAR proteins such as endophilin and centaurin, low density scaffolds pin the radius of the underlying membrane substrate to a value independent of membrane tension, whereas the radius of the tube bound by a non-scaffolding protein, epsin, is not significantly affected (Simunovic et al 2016a) (Figure 3).

The remarkable advantage of BAR proteins over other membrane-curving proteins is that slight changes in their structure—the curvature and surface charge of the BAR domain, number of AHs, etc.—can result in scaffolds of different radii. Therefore,

we speculate that the sheer variety of BAR proteins is part of the cell's tools to precisely time and target trafficking events via coupling to membrane shape and composition.

(3) *Shallow insertion*. AHs shallowly insert into the bilayer, pushing lipids apart thus inducing local curvature. Many BAR proteins, if not most, contain one or more AHs in their structure, which amplify their curvature-generating capacity (Peter et al 2004, Qualmann et al 2011). It has recently been suggested that other domains in BAR proteins can participate in similar ways in curvature induction. Namely, the pleckstrin homology (PH) domain of β1 centaurin (also known as ACAP1) contains a short amphipathic moiety that can wedge into the bilayer (Pang et al 2014). The wedging mechanism in general is relatively simply explained as expanding one leaflet of the bilayer, while contracting the other, thus inducing curvature (Figure 2, *center*). According to theoretical studies, the magnitude of bilayer deformation–specifically, bending–depends on the depth of insertion in a non-monotonic way: at first increasing with depth, then decreasing and finally switching signs once reaching the bottom leaflet (Campelo et al 2008, Zemel et al 2008). In the physics literature, insertion depth defines spontaneous curvature of the membrane induced by the protein.

(4) *Crowding*. Many bound particles clustered near the membrane can speculatively generate curvature so to maximize the distance between particles (Figure 2, *bottom*). Such entropy-driven mechanism has been demonstrated computationally showing that grafted polymers may induce curvature (Bickel et al 2001, Breidenich et al 2000) and later in experiments where tethered DNA or proteins on the membrane generated tubules

(Nikolov et al 2007, Stachowiak et al 2012). Proteins with the BAR domain contain a number of other regions, which contain disordered sub-regions that could contribute to crowding-generated membrane curvature (Busch et al 2015).

Sensing *versus* inducing membrane curvature

Targeting and recruitment to tubes or tubular necks. In cell membranes, highly curved transport intermediates are produced by external force coming from actin polymerization or molecular motors, or by polymerization of spherical cages made of coat proteins. BAR proteins are, most of the time, recruited to these membrane structures. Hence, it is important to understand how BAR proteins interact with already curved membranes. Various *in vitro* assays have been designed to measure the curvature sensitivity of BAR proteins, typically by measuring protein fluorescence on tubular or spherical membranes as a function of membrane radius (Simunovic et al 2016b). Usually these assays are performed at low (<5%) surface fraction of proteins, where enrichment of the protein of a factor of 10–100 on highly curved membranes compared to flat or very low-curved membranes is found (Baumgart et al 2011). At these low densities, BAR proteins locally deform the membrane (Simunovic et al 2013b), but do not induce membrane tubules (Shi & Baumgart 2015). All tested BAR proteins so far have shown to be sorted on membrane tubules; namely, amphiphysin (Heinrich et al 2010, Sorre et al 2012), endophilin (Simunovic et al 2016a, Zhu et al 2012), BIN1 (Wu et al 2014), ArfGAP1 (Ambroggio et al 2010) (all N-BARs); β2 centaurin (Simunovic et al 2016a) (classical BAR domain); syndapin 1 (Ramesh et al 2013) (F-BAR); and IRSp53 (Prevost et al 2015) (I-BAR). Presumably, this curvature sensitivity is the cell's mechanism to recruit BAR proteins and

thus other proteins they bind to membrane trafficking structures, in a curvature-dependent manner, and so important for downstream scission. For a more detailed understanding of the physics underlying curvature sensing by BAR domains, we refer the reader to previous reviews on this topic (Baumgart et al 2011, Callan-Jones & Bassereau 2013, Simunovic et al 2016b).

Curvature sensing: spheres versus tubes. Broadly speaking, curvature sensitivity is a consequence (1) of the curved backbone of the protein or its 3D polymeric assembly whose redistribution to curved membranes maximizes the contact with the bilayer, and (2), of the more efficient insertion of AHs or other wedging domains, if present, into lipid packing defects, which are more abundant on curved membranes (Cui et al 2011, Drin & Antonny 2010). However, recent works showed that BAR domains sense tubular membranes differently from spherical ones. Namely, while different BAR proteins have a different propensity for tubules based on their radius or sign of curvature, on highly curved spherical vesicles, surprisingly, they are equally sorted regardless of the sign (convex *vs*. concave) or the magnitude of the curvature of the BAR domain (Bhatia et al 2009). Their enrichment on spherical vesicles depends solely on the presence of AHs. Interestingly, epsin, which interacts with membrane only through its AH, is a curvature sensor both on tubes (Capraro et al 2010) and spherical vesicles (Madsen et al 2010).

There is, in fact, a structural difference at the molecular level in the way BAR proteins interact with spherical as opposed to tubular membranes. On tubes, proteins are able to collectively tilt their long axis to match their curvature to that of the tube

(Mesarec et al 2017) thereby maintaining close contact; thus, the backbone and amphipathic helices contribute to curvature sensing, but the helices are not indispensable. In contrast, on spheres such tilting is not possible (without deforming the membrane), the backbone is not in contact, and as a result only the helices participate in curvature sensing. Indeed, a different contact distance between the BAR domain and AHs from the bilayer surface was observed when measuring BARs coating tubules *vs*. spheres (Ambroso et al 2014, Isas et al 2015). These observations hint at the way in which, at higher densities, BAR proteins deform membranes: by forming tubes as opposed to spheres, proteins are able to maximize adhesion and avoid unfavorable packing defects.

Tubulation. At high enough bound surface density, the spontaneous curvature produced by the bound nanoparticles, proteins, or ions results, quite dramatically, in spontaneous tubulation of membrane vesicles (Lipowsky 2013, Saric & Cacciuto 2012). In fact, as mentioned in the Introduction, the structure and membrane-deforming capacity of BAR proteins was initially discovered through tubulation studies (Lee et al 2002, Peter et al 2004, Takei et al 1999). Quantitative tubulation assays have also been used in establishing the importance of membrane tension in modulating BAR-induced deformation by controlling the threshold bound density for tubulation (Shi & Baumgart 2015).

BAR protein-induced membrane deformation can occur in more subtle ways, but with potentially significant biological consequences. BAR proteins are also able to modify the curvature of pre-formed membrane tubes, as shown by *in vitro* studies: continuously increasing the bound surface fraction results in a departure from the radius of a bare

membrane tube. Moreover, with increasing density, BAR proteins are able to stabilize tubes: at a given tension, the force needed to hold the tube decreases with density, reminiscent of their tubulation capacity (Prevost et al 2015, Simunovic et al 2016a, Sorre et al 2012). *In vitro* studies have also shown the tendency of BAR proteins on pre-formed tubes to form scaffolds—imposing a radius independently of the membrane tension—at sufficiently high density, as discussed above (Simunovic et al 2016a, Sorre et al 2012). We point out that the scaffold radius as measured from *in vitro*, pre-formed tubes generally agrees with measurements by electron microscopy upon spontaneous tubulation. These measurements relate to the structure formed by a protein assembly, and its radius does not necessarily correspond to the measured inverse curvature of a single BAR domain from X-ray crystallography.

Controversies: case closed!

The role of AHs. An apparent conflict in the study of BAR proteins has been the importance of the role of AHs in curvature sensing and generation. The debate over AHs has been due to the aforementioned opposing results obtained on spherical and tubular membranes (Bhatia et al 2009, Chen et al 2016, Prevost et al 2015, Simunovic et al 2016a). Precise measurements of BAR proteins binding to the membrane at the atomic level, where it was seen that the BAR domain backbone binds much tighter to tubular than to spherical membranes, help understand the discrepancy (Ambroso et al 2014, Isas et al 2015). The current proposal for the role of AHs in BAR proteins is to increase their membrane affinity through insertion into lipid packing defects; however, since they most-

ly bind *in vivo* to tubes or tubular necks, curvature sensing and generation is essentially effectuated through the curved backbone (Chen et al 2016).

Sensing vs. inducing membrane curvature: two consequences of the same mechanism?
Second, curvature sensing and induction are often discussed in opposition, but are in fact results of the same underlying physics: for energetic reasons, an asymmetric protein–whether an insertion into a bilayer or a curved adsorbent–prefers to bind to a locally curved membrane. The key determinant of whether a protein predominantly senses or generates curvature is the bound density on the membrane (Simunovic et al 2015). *In vivo*, both protein expression level and membrane affinity must be fine-tuned to direct a BAR protein towards a sensing *vs*. induction mode. For instance, muscular amphiphysin 2 forms T-tubules in myotubes. It also tubulates membranes when expressed exogenously, but this tubulation capacity is lost upon mutation that affects binding affinity (Lee et al 2002). To our knowledge, BAR proteins, such as amphiphysin 1, are expressed at levels below the threshold for tubulation, and therefore most likely operate as a curvature sensors (Peter et al 2004).

Membrane scission

Recent studies have revealed a direct role played by the N-BAR protein endophilin in scission during clathrin-independent endocytosis (Boucrot et al 2015, Renard et al 2015). An earlier, *in vitro* study demonstrated the ability of AHs to cut small (~200 nm), spherical vesicles when wedged into the membrane outer layer. This effect is antagonized by the BAR backbone which has an affinity for tubules. Interestingly, vesicle-bound en-

dophilin, which has the dimer backbone and four AHs, produces a mixture of tubules and vesicular fragments (Boucrot et al 2012). In contrast, recent work with static pre-formed tubes found that endophilin was not able to drive membrane scission. Remarkably, when these tubes were then extended, scission did indeed occur (Renard et al 2015, Simunovic et al 2017a). These two studies point to a fundamentally different mechanism by which BAR proteins scission spherical buds and tubular extensions.

Scission of spherical buds by AH insertion. When AHs insert into small spherical vesicles they favor positive Gaussian membrane curvature (i.e., a spherical, as opposed to saddle-shaped, region). The effect on magnitude of curvature depends on the insertion depth: a as the insertion gets closer to the bilayer mid-plane, the effect becomes weaker (Campelo et al 2008, Zemel et al 2008). Thus, shallow AH insertion favors vesiculation (Boucrot et al 2012). In contrast, BAR proteins bind closely on a tube, and the AH insertion is deeper, which opposes vesiculation (Ambroso et al 2014). It could be advantageous for cells to produce spherical, rather than tubular, endocytic buds, since these are necessarily attached to the donor membrane by a saddle-shaped neck, which is destabilized by AH insertion. A tubular extension is not susceptible to scission by the aforementioned mechanism, and other strategies for tube scission are required.

Scission of tubes through BAR scaffold friction. Tubular transport intermediates have been identified as alternative to spherical vesicles, in different coat-independent endocytic pathways. Paradoxically, the BAR domain scaffold stabilizes static membrane tubes, but there is incontrovertible evidence that at the same time these structures con-

tribute to scission (Renard et al 2015). We demonstrated recently that when scaffolded tubes are dynamically extended from a vesicle, these tubes undergo scission through membrane lysis. Scission occurs because the scaffold creates a frictional barrier for lipid flow; tube elongation thus builds up tension along the tube until lysis, in a process that we dubbed Friction-Driven Scission (Simunovic et al 2017a). In cells, the pulling force is provided by dynein motors, and potentially by actin polymerization.

Emerging roles of BAR proteins in the cell

Intracellular organelles are functional units within the cell that undergo a constant flux of membrane fission and fusion. In order for this to occur, membrane trafficking, tubulation and vesiculation are key mechanisms, making curvature-generating proteins key players in a range of cellular compartments and functions. Important roles of BAR, N-BAR, and F-BAR proteins in clathrin-mediated and clathrin-independent endocytosis has been recognized and much effort has been devoted to understanding their recruitment order in the formation of the endocytic site. However, BAR proteins also associate with a variety of intracellular membrane compartments (Figure 4), and evidence is emerging that members of the family not only mediate endocytosis but are also regulating organelle shape, biogenesis, and cell signaling.

Endosomal trafficking. Activated receptors at the plasma membrane initiate intracellular signaling and, at the same time, adaptors for internalization are recruited, endocytic vesicles containing the receptor complex bud off and are then trafficked to early endosomes.

Receptors may continue to signal in this vesicular compartment allowing the signaling to be spatially controlled. Endosomes are important signaling platforms that regulate signaling both temporally and spatially, and consequently regulate fundamental processes in cells such as proliferation, migration and survival (Di Fiore & von Zastrow 2014).

From the early endosomes internalized receptors can be either recycled back to the plasma membrane or targeted for degradation via the endo-lysosomal pathway. A subset of signaling endosomes are marked by the BAR domain-containing protein APPL1. It is an adaptor protein that associates with activated receptors via its phosphotyrosine-binding (PTB) domain (Diggins & Webb 2017). Recent evidence shows that APPL1 is localized to an early endocytic tubo-vesicular membrane compartment that potentially can spatially compartmentalize receptor signaling independently of Rab5-positive early endosomes (Kalaidzidis et al 2015). It is likely that the APPL1 BAR-PH domain can mediate both tubulation and fission of this organelle in accordance with observations by live cell imaging (Kalaidzidis et al 2015). The BAR domain of APPL1 homodimerizes and forms a crescent shaped membrane binding surface that has a specific affinity for phosphoinositides marking early endosomes containing PI(3)P and PI(3,5)P_2 (Chial et al 2008). Investigating this novel role for APPL1 in reshaping early endosomes may be a significant step towards understanding how receptor signaling is regulated by membrane trafficking both in space and time. This is particularly important in cancer cells where receptor signaling is deregulated resulting in uncontrolled proliferation, migration and survival (Di Fiore & von Zastrow 2014, Mellman & Yarden 2013).

Endosomal sorting and endoplasmic reticulum. Interactions between the endoplasmic reticulum (ER) and endosomes have recently emerged as an important regulator of endosome positioning and may also be involved in positioning of other organelles as its network stretches throughout the cell (Neefjes et al 2017). Endosomes act as a network hub for intracellular membrane trafficking as they receive cargo from the plasma membrane and the biosynthetic pathway/trans-Golgi network (TGN), sort and destine for either degradation in the lysosome, retrograde pathways or recycling of cargo to the plasma membrane (Burd & Cullen 2014). Tubulation of endosomes play a key role in sorting and trafficking of cargo and therefore indirectly also for signaling. We predict that more BAR proteins will emerge as regulators of this cellular mechanism. To date research has shown that interactions of the ER with endosome tubules determines the site of fission by the retromer complex and the BAR domain-containing SNX proteins (Dong et al 2016). The retromer complex is responsible for retrograde transport of proteins from the endosome to the TGN and is composed of a complex responsible for cargo selection (VPS26-VPS35-VPS29) and membrane tubulating proteins including BAR proteins SNX1/2 and SNX5/6 (Burd & Cullen 2014). Recently a novel mechanism involving the F-BAR domain-containing protein, pacsin 2, was shown to promote endosome tubulation and vesicle transport of cargo to the TGN (Billcliff et al 2016). Depletion of pacsin 2 leads to a reduction in length of tubules, and an inhibition of the retrograde transport of the receptor to the TGN. This mechanism was shown to be dependent on its partners IPI27A and OCRL (Billcliff et al 2016). OCRL is an inositol polyphosphate 5-phosphatase that is mutated in Lowe Syndrome (De Matteis et al 2017), a severe developmental disorder characterized by renal failure, cataracts and cognitive impairment.

Another mechanism for regulating cell signaling is controlling the trafficking of receptors from the ER to the cell surface. For example the BAR domain of PICK1 is important in regulation of the exit of the AMPA receptor subunit GluR2 from the ER (Lu et al 2014). This mechanism has important implications in neurons in particular for protection from ischemia (Lu et al 2014) and could emerge to be a mechanism that regulates other types of cell surface receptors.

Autophagy and cancer. Autophagy is an intracellular trafficking pathway that is used for bulk degradation of obsolete organelles, pathogens and misfolded proteins. The autophagy process begins with the formation of an autophagosome that encases the cargo, which then fuses with the lysosome leading to degradation. The formation of the autophagosome is regulated by a number of autophagy-related proteins (Atg), wherein Atg20 and Atg24, for instance, contain a BAR domain (Inoue & Klionsky 2010, Zhao et al 2016), and, in addition, the N-BAR protein endophilin B1 (Bif-1) is required for the process (Takahashi et al 2007). The exact molecular mechanism is not known, but it has been proposed to involve curvature induction and membrane deformation leading to maturation of the autophagosome (Takahashi et al 2007).

Remodeling of the endo-lysosomal membrane trafficking pathway is a crucial regulator of cellular signaling pathways and has been linked to mechanisms of cancer development (Mellman & Yarden 2013, Schmid 2017). It controls cell behaviors such as proliferation, apoptosis and survival. Evidence is emerging demonstrating that a large

number of proteins in the BAR family are altered at the expression level in a variety of cancer types and contribute to disease mechanisms (Baldassarre et al 2015, Huang et al 2017b, Johnson et al 2015, Ogi et al 2013, Pinheiro et al 2001, Wang et al 2017). In some cases, BAR proteins can even serve as robust prognostic markers for development of metastatic disease (Dai et al 2017, Hu et al 2016, Huang et al 2017a). Therefore, further research around the function and molecular mechanisms of BAR proteins relevant to cancer cell biology is warranted. Furthermore, cell type specific functions of BAR proteins are also an area that requires more work.

Members of the BAR protein family mediate remodeling of membranes in endocytic intermediates at the plasma membrane as well as various intracellular organelles. These functions are important for regulation of intracellular signaling pathways. Deregulation of expression patterns of BAR proteins in different cancer types urges future functional investigations of this protein family to elucidate novel signaling and trafficking pathways that potentially can be used to identify novel drug targets.

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Figure captions

Figure 1: Structural and curvature-inducing diversity of BAR domains. Shown are X-ray crystal structures of various BAR proteins with each monomer color-coded. Additional domains, such as the amphipathic helices, PH, SH3, PX, etc. are not shown. For each BAR domain, corresponding micrograms of tubulated liposomes by the same protein and the average diameter of induced tubules are shown. Proteins: amphiphysin 1 (PDB ID: 1URU; microgram and measurement taken from (Takei et al 1999); scale bar, 500 nm;), SNX33 and SNX1 (PDB IDs: 4AKV and 4FZ7, respectively; micrograms and measurements taken from (van Weering et al 2012); scale bars, 200 nm), CIP4 (PDB ID: 2EFK; microgram and measurement from (Frost et al 2008); scale bar, ~45 nm), MIM (PDB ID: 2D1L; microgram and measurement from (Mattila et al 2007); scale bar, 100 nm), pinkBAR (PDB ID: 3OK8; microgram taken from (Pykalainen et al 2011); scale bar, 10 μm).

Figure 2: Illustration of mechanisms of membrane deformation by BAR proteins.

Figure 3: BAR domain backbone forms a scaffold on tubular membranes. (A) BAR domain proteins at high density stabilize a tubular membrane whose radius does not change despite change in membrane tension (black plot: control in the absence of proteins). BAR domain is crucial for the formation of the scaffold and does not depend on the presence of amphipathic helices (compare N-BAR protein endophilin A1 and classical BAR protein β2 centaurin). Correspondingly, epsin, a protein without a BAR back-

bone but with an amphipathic helix, affects tube radius, but it does not stabilize it with respect to membrane tension. (B) A confocal slice of a tubular membrane coated with a scaffold of endophilin A2, showing a strong enrichment of the protein on the tube. The tube is pulled out of a giant vesicle (left) and held with optical tweezers (right). Based on work from (Simunovic et al 2016a). Scale bar, ~2 μm. (C) The structure of an N-BAR scaffold at the molecular resolution. Shown is a snapshot of self-assembled N-BAR domains decorating a thin lipid nanotube in a coarse-grained computer simulation from (Simunovic et al 2016a). Scale bar, 20 nm.

Figure 4: BAR proteins in the cell. A not-to-scale cartoon of a cell highlighting some known BAR-domain-containing proteins participating in various cellular phenomena.

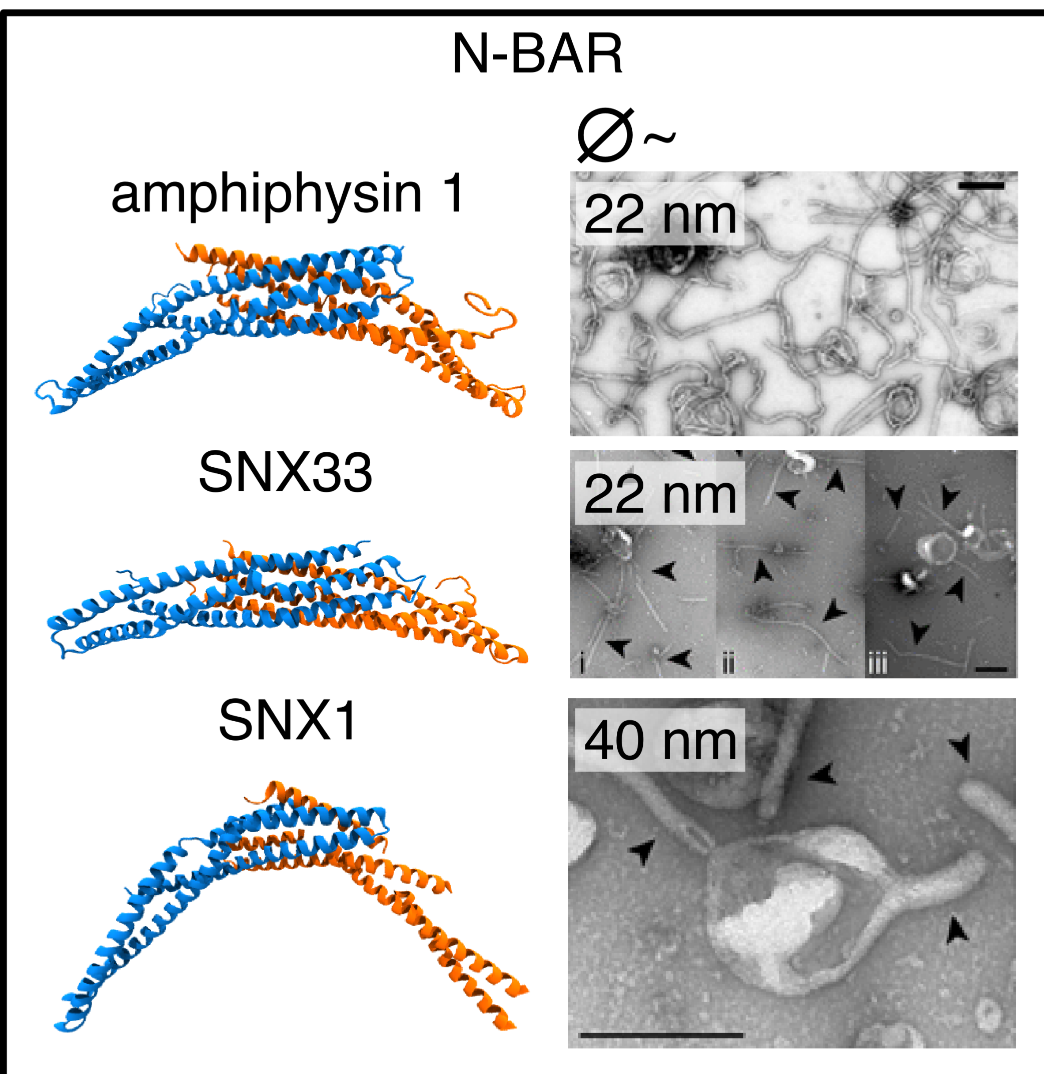


Figure 1

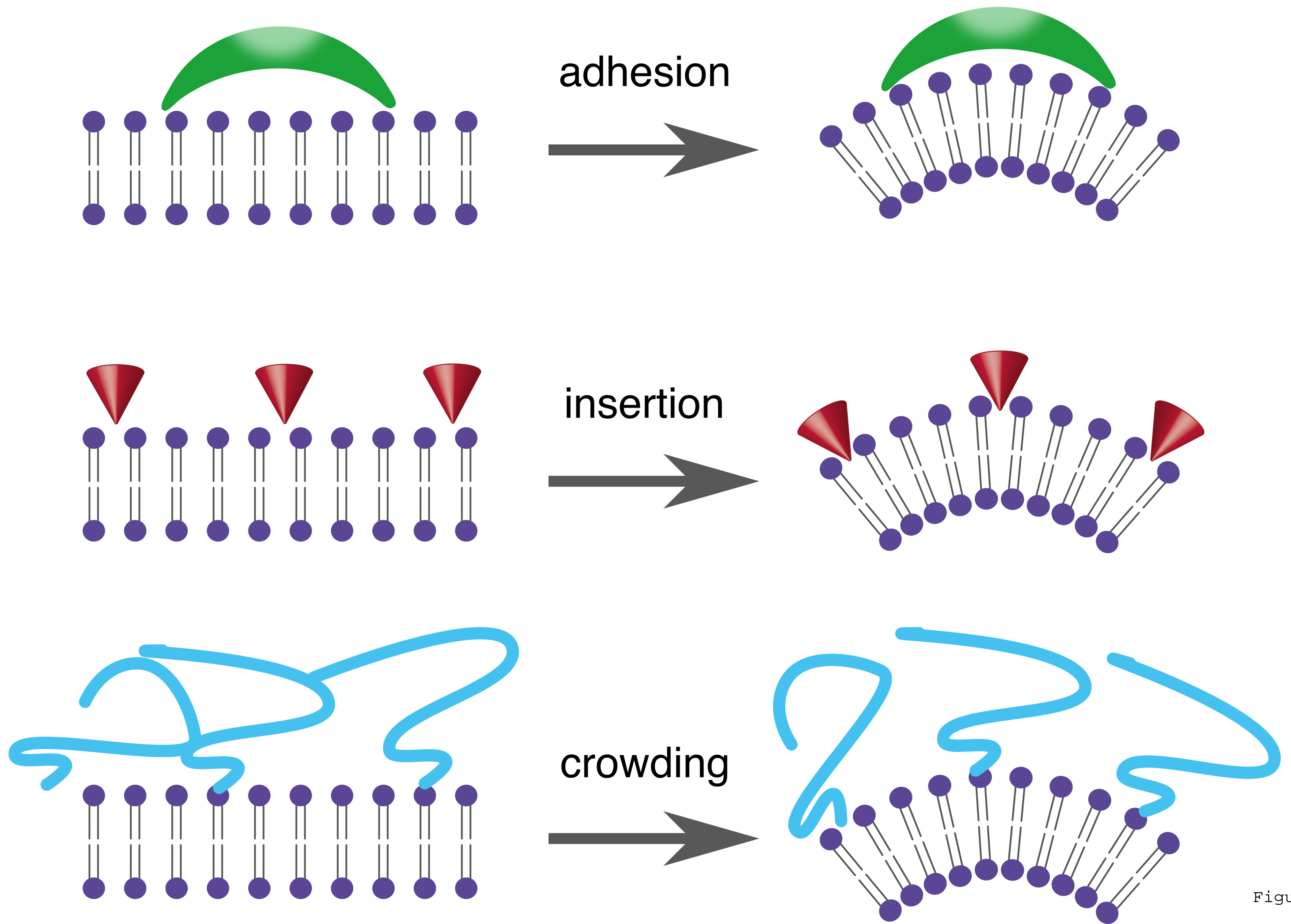


Figure 2

A

endophilin A2 β2 centaurin epsin

r (nm)

100
75
50
25
0

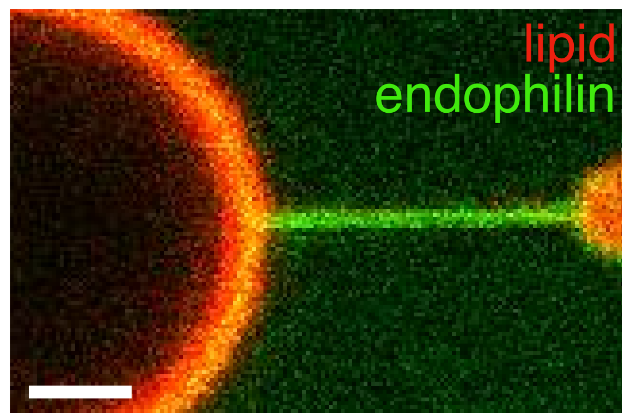
0 0.1 0.2 0.3

100
75
50
25
0

0 0.1 0.2 0.3

σ (mN m^{-1})

B



C

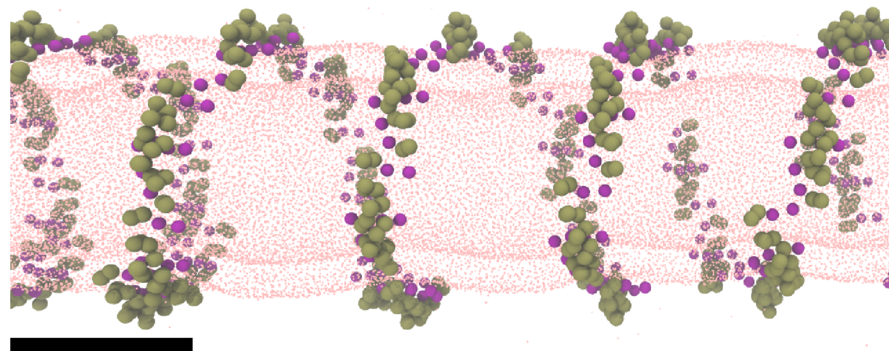


Figure 3

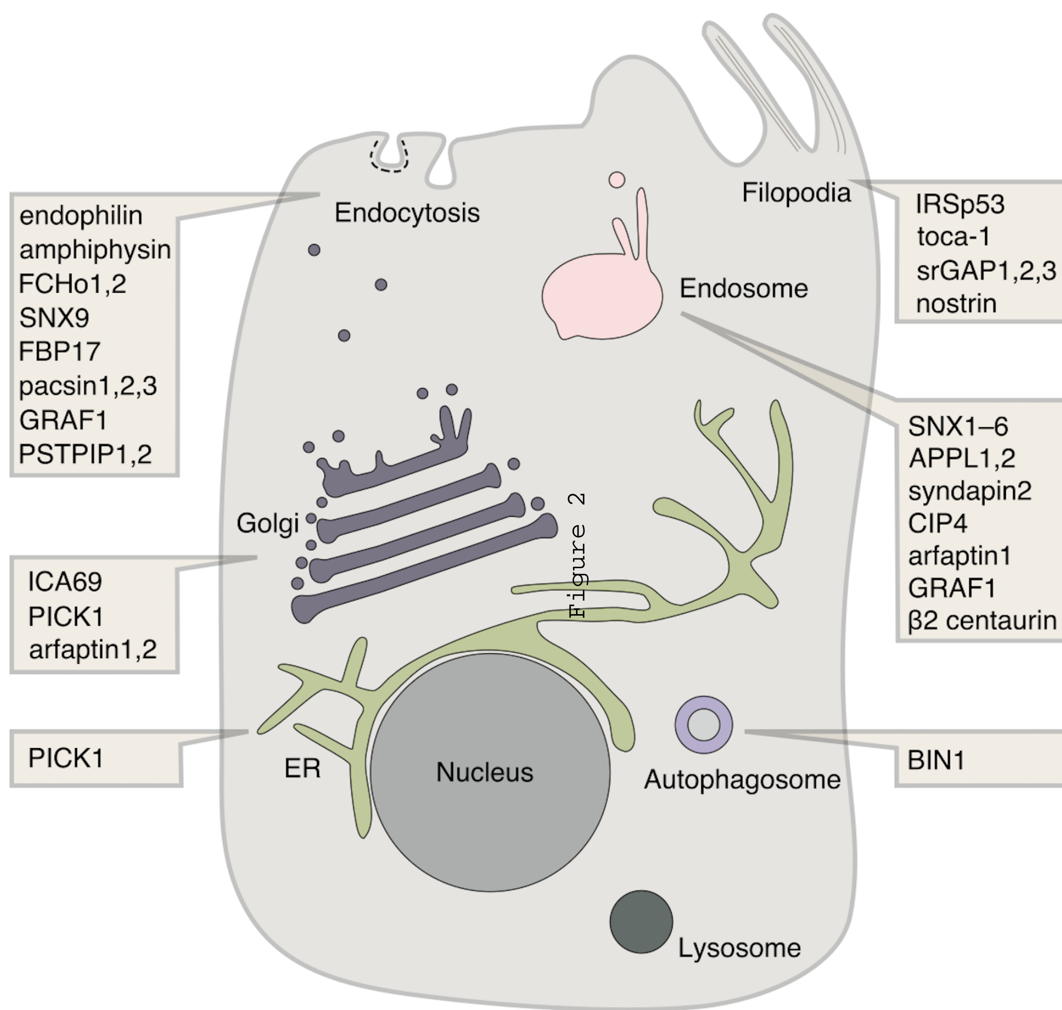


Figure 4