CELL BIOLOGY

BAR Domains Go on a Bender

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One of the basic needs of any growing cell is the ability to remodel the lipid membranes that both surround the cell and make up its intracellular organelles. At any point in time, a multitude of tiny bubbles of membrane known as vesicles are busy acting as couriers, picking up and delivering lipids and proteins with exquisite specificity. Although there is still much to be learned about how the many distinct flavors of vesicle are generated, one of the central themes that has emerged is that vesicle formation requires an interplay between the membrane lipids themselves and the cytosolic proteins that coat and deform the membrane. Of particular interest is how these coat proteins and additional effector molecules cooperate in a temporal and spatial manner to initiate membrane curvature, sculpt a vesicle of defined size, and pinch off the new bud. On page 495 of this issue, Peter *et al*. (*1*) describe the structure of a conserved protein domain that seems ideally suited to the task of sensing, and perhaps even generating, the membrane-bending events required during vesicle formation.

The BAR domain—named for the Bin-Amphiphysin-Rvs proteins in which it was first identified—is found in proteins implicated in vesicle generation and membrane remodeling in mammals, *Drosophila*, and yeast (*2*). The founder of the BAR protein family, amphiphysin I, is enriched at synaptic nerve terminals in the brain where it helps to coordinate vesicle budding from the plasma membrane, a process known as endocytosis. It does this through interactions with a variety of other molecules, including the clathrin coat proteins, the AP2 complex whose job is to populate the vesicle with cargo proteins, and the guanosine triphosphatase (GTPase) dynamin, which is recruited to the constricted "neck" of an almost-budded vesicle to drive fission from the donor membrane (*3*, *4*). Amphiphysin thus seems well placed to couple the recruitment of cargo and coat proteins to regions of membrane curvature. But how is membrane bending initiated, and how is progress during budding monitored?

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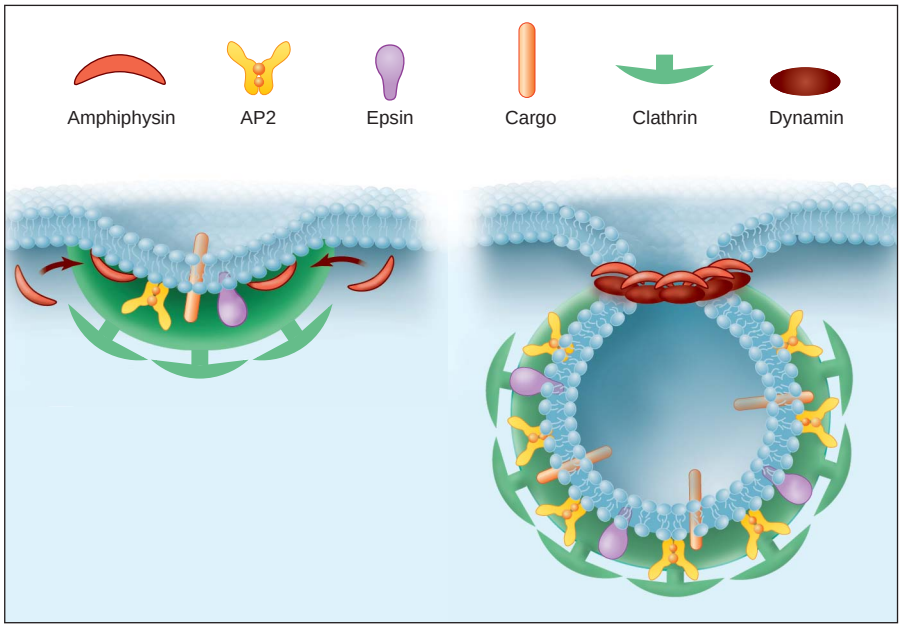
If you were to design a protein domain for detecting or imposing membrane curvature, you would likely come up with something that closely resembles the structure of the *Drosophila* BAR domain now solved by Peter *et al*. It is, simply, a curve itself, although the crescent-shaped structure is only revealed upon dimerization of two slightly kinked monomers, which suggests that the domain functions only as a dimer. Intuition would probably tell you that the concave surface of the crescent would face the membrane. Satisfyingly, Peter *et al*. show that mutation of positively charged amino acid residues in the concave part of the BAR domain abolishes the ability of the isolated domain to bind to negatively charged membranes and to induce them to form tubes. The story gets even more interesting as the authors identify previously unsuspected BAR domains in a variety of proteins such as nadrins, oligophrenins, centaurins, and sorting nexins. These proteins all participate in some way in membrane-remodeling events, and they present the BAR domain in the context of other lipid-sensing or effector domains. Thus, BAR-containing proteins are able to integrate multiple signals from their protein partners and from specific lipid species (such as phosphatidylinositol 4,5-bisphosphate) with regions of membrane curvature.

At the most basic level, the BAR domain senses rather than imposes membrane curvature, and Peter *et al*. identify both "strong" BAR domains (which can do both) and "weak" BAR domains (which prefer their membranes already bent). In general, the strong BAR domains possess an amino-terminal amphipathic α helix that enhances their ability to deform membranes into tubes. This additional helix is reminiscent of a similar structure in the protein epsin, which, like amphiphysin, is important for endocytosis. Although epsin does not contain the crescent-shaped BAR domain, it can still deform membranes by virtue of this α helix, which is thought to induce spontaneous curvature of the lipid bilayer by partially penetrating into one leaflet of the membrane (*5*, *6*). Weak BAR domains are able to generate membrane curvature at high concentrations, presumably as a result of their intrinsic shape. The use of a concave membrane-binding sur-



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Raising the BAR. BAR domains may sense and impose curvature on membranes. During endocytosis, strong BAR domains, such as those in the amphiphysin and endophilin proteins, may cooperate with proteins such as epsin to promote membrane deformation. Other regions of amphiphysin interact with AP2 and clathrin coat proteins, potentially coupling cargo and coat recruitment to regions of membrane curvature. Late during vesicle budding, the amphiphysin BAR domain may recognize the sharp curvature of the vesicle neck, directing assembly of dynamin to the vesicle neck before membrane fission when the vesicle breaks free from the membrane.

face to promote curvature may turn out to be a widespread feature found in other vesicle budding events. The Sec23-24p complex, which coats COPII vesicles, has a curved underbelly that may stimulate deformation of the membrane during budding from the endoplasmic reticulum (*7*).

Given the variety of proteins with BAR domains and the versatility of the crescent structure, it is not hard to imagine that roles for BAR domains will be discovered for all stages of vesicle biogenesis, from the initial generation of curvature to vesicle fission, and possibly even after vesicle release. In the case of the amphiphysin BAR domain, it seems plausible that it may operate at multiple stages of clathrin-mediated endocytosis (see the figure). Stimulation of membrane invagination may proceed in cooperation with other accessory factors such as epsin and endophilin, all of which possess the potent amphipathic α helix that could induce spontaneous curvature (*5*, *8*). Through additional domains on amphiphysin that bind to the AP2 complex and clathrin itself, clathrin recruitment may also participate in membrane bending or act to stabilize curvature. Late in the budding process, amphiphysin has been shown to recruit dynamin to the vesicle neck where it forms a ring-like collar, finalizing the fission process (*9*). It is here that the amphiphysin BAR domain may really pay its dues, delaying dynamin recruitment until the sharp, positive curvature of a vesicle neck is presented. Peter *et al*. estimate that the natural shape of the BAR domain would best suit a curved membrane ~22 nm in diameter, closer perhaps to the narrow neck of an almost budded vesicle than to the 50- to 100-nm diameter of the clathrin-coated vesicles themselves.

There are other scenarios where the detection of sharp membrane curvature would be required, although BAR domains have yet to be directly implicated. The first is during a specialized form of vesicle fusion at nerve terminals known as "kiss-and-run" exocytosis, where vesicles never completely fuse with the plasma membrane but form only a transient pore through which neurotransmitter is released (*10*). This process, which may require dynamin action, is in some ways the converse of the final step of vesicle fission and could also make use of BAR domain proteins to sense and regulate the fusion pore aperture. A second intriguing scenario has recently been uncovered by Bigay *et al*. (*11*) for COPI vesicles, which traffic from the Golgi to the endoplasmic reticulum and between Golgi cisternae. Bigay and co-workers observed that the activity of ArfGAP1, a catalytic partner for the small G protein Arf1, can be stimulated by more than two orders of magnitude as the membrane curvature of artificial liposomes approaches that found on a budded COPI vesicle. The ability of ArfGAP1 to respond to membrane curvature thus provides an elegant mechanism to prevent premature vesicle coat disassembly. It is not clear whether ArfGAP1 has a BAR domain (*12*); perhaps there are yet more ways to recognize curvature. But for proteins such as nadrins, centaurins, and oligophrenins, the presence of a BAR domain in conjunction with Arf and Rho effector domains suggests that there are more bends in the road in store for the beautifully simple curve of the BAR.

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BIOCHEMISTRY

Mimicking Posttranslational Modifications of Proteins

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One of the surprises of the complete first draft of the human genome was that only about 30,000 genes were found—only about two times as many as in the fly or worm. However, human complexity is not simply a result of the direct protein products of genes. A higher order of complexity can be added after a gene has been translated into the corresponding protein. Recent advances in mimicking these modifications are helping to elucidate the role of the modifications and hold great promise for future pharmaceuticals.

Genomes of more complex species typically contain more enzymes and proteins involved in posttranslational modifications of proteins than do the genomes of simpler organisms. The most widespread and complex form of posttranslational modification, glycosylation, requires about 1% of genes (*1*), yet is typically absent in simple prokaryotic organisms such as bacteria.

Posttranslational modifications range from the widespread (such as glycosylation, phosphorylation, ubiquitination, and methylation) to the obscure (such as glutathionylation, hydroxylation, sulfation, transglutamination, and epimerization). Their effects often fundamentally alter protein function. For example, posttranslational modification of proline residues in the transcription factor HIFα (the α subunit of the hypoxia-inducible factor) is a key oxygen-sensing mechanism within cells (*2*), and phosphorylation cascades are a central part of intracellular signaling (*3*).

Why are proteins altered after translation, away from the typically tight control of the gene expression process? One reason may be that it is far easier to create a spectrum of slightly different proteins by taking one basic protein scaffold and fine-tuning or even entirely switching its properties, than to build each protein from scratch just to find that it may not be needed at a given time. Posttranslational modifications create a dynamic combinatorial library of properties that can rapidly respond to systemic stimuli such as oxygen levels or hormonal concentrations. This flexibility may, however, also be turned against us. A recent study found that HIV uses a dynamically changing "shield" of posttranslational glycosylation to evade our immune systems (*4*).

The dynamic complexity of posttranslational modification is often difficult to elucidate in the laboratory. Working out the role of each (sometimes very minor but important) protein component requires abundant sources and extensive purification. Furthermore, to continue to precisely exploit the power of proteins in therapeutics requires the creation of pure protein drugs (most today are sold as mixtures).

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