Current Biology, Vol. 14, R250–R252, March 23, 2004, ©2004 Elsevier Ltd. All rights reserved. DOI 10.1016/j.cub.2004.02.060

Membrane Curvature: How BAR Domains Bend Bilayers

Dispatch

Joshua Zimmerberg[1] and Stuart McLaughlin[2]

An important new structure suggests the BAR domain is a membrane-binding module that can both produce and sense membrane curvature. BAR resembles a banana that binds membranes electrostatically through its positively charged, concave surface.

There have been a number of reports that adding certain proteins to phospholipid bilayer membranes results in their tubulation or vesiculation, but it is not clear how these proteins work. One hypothesis is that hydrophobic groups on a protein insert into one leaflet of a bilayer, increasing either the spontaneous curvature or the lateral area of this monolayer [1,2]. An alternative hypothesis is that when certain membrane-binding proteins, such as the vesicle coat proteins clathrin or COPI and COPII, polymerize into a coat or a cage, the oligomerization bends the membrane into an endocytic vesicle [2,3]. Peter *et al.* [4] recently reported the X-ray crystallographic structure of the BAR domain of *Drosophila* amphiphysin and proposed a third mechanism for protein-induced membrane curvature: electrostatic attraction between the protein and lipids plasters the membrane to the concave surface of the BAR domain, apparently in the absence of any substantial hydrophobic insertion into the membrane.

Elegant work from the De Camilli [5] laboratory previously established that addition of amphiphysin forms tubules from larger liposomes *in vitro*, and that overexpression of amphiphysin 2 in cells leads to internal membrane tubulation [6]. The newly recognized BAR (Bin/Amphiphysin/Rvs) domain is present in a number of proteins — amphiphysins, endophilin, arfaptins, nadrins, beta-centaurins and oligophrenins — some of which are critical for the recycling of synaptic vesicles and T-tubule formation in muscle [4]. The structure of the BAR domain, an elongated 'banana-shaped' dimer, has sufficient rigidity, curvature, and charge to immediately suggest its function.

Figure 1A, taken from Peter *et al.* [4], shows the structure of the BAR domain. The authors describe each monomer as "a coiled-coil of three long kinked α-helices, forming a six-helix bundle around the dimer interface" and note "the curvature of the dimer is partly due to the way the monomers intersect and partly due to the kinks in helices 2 and 3". The hydrophobic residues are largely placed at the interface between the monomers, raising the question: how does BAR bind to bilayers? The authors propose the answer is simple electrostatics. Figure 1B illustrates the electrostatic profile predicted by application of the Poisson–Boltzmann equation to a model of the BAR domain: the blue areas indicate regions of positive electrostatic potential. Note the flexible loop between helices 2 and 3 found at the extreme ends of the dimer is strongly basic, and that the concave surface of the dimer has several patches of positively charged residues. Thus Peter *et al.* [4] suggest that "this is the surface that interacts with phospholipid membranes"; it would fit a curved membrane with an outer radius of 11 nm.

Many studies have shown that clusters of basic residues on proteins can help anchor proteins to the acidic lipids on the cytoplasmic leaflet of the plasma membrane, which typically contains 15–30% monovalent acidic phospholipids, mainly phosphatidylserine, and about 1% multivalent phosphatidyinositol bisphosphate (PIP_2). These negatively charged lipids produce a negative electrostatic potential that attracts counterions, such as K^+, from the cytoplasm. As first recognized by Helmholtz in the 19th century, this produces a 'diffuse double layer' or ion atmosphere which extends a few Debye lengths — a few nanometers under physiological conditions — away from the surface [7,8]. The negative surface potential, which is about –30 mV for a membrane with 20% phosphatidylserine [8], also attracts clusters of basic residues on proteins.

This effect is illustrated by the carboxy-terminal basic cluster on K-Ras, consisting of seven contiguous lysine residues, or the amino-terminal basic cluster on Src, with net charge +5, which help anchor these proteins to the plasma membrane by simple electrostatic attraction to the acidic lipids. These basic clusters do not, however, provide quite enough electrostatic energy to anchor the protein tightly to the membrane; K-Ras4B requires an adjacent, covalently attached farnesyl group and Src an adjacent, covalently attached myristoyl group, which act as hydrophobic anchors in concert with the electrostatic interactions [9].

The electrostatic binding energies of these basic clusters can be predicted theoretically by applying the non-linear Poisson–Boltzmann equation to atomic level models of bilayers and proteins/peptides [9]. As predicted theoretically and shown experimentally by a number of different laboratories using simple model peptides and phospholipid vesicles, the binding energy increases linearly with the mole fraction — surface density — of acidic lipids in the bilayer and the number of basic residues in the cluster. An increase in salt concentration screens the charges and decreases the binding.

The proposal by Peter *et al.* [4] that BAR domains use simple electrostatics to bind membranes thus has both biological precedents — for example, Src, K-Ras4B, MARCKS, HIV-Gag and AKAP79 — and good theoretical and experimental support from model

[1]Laboratory of Cellular and Molecular Biophysics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892, USA.
[2]Department of Physiology and Biophysics, Health Science Center, State University of New York at Stony Brook, Stony Brook, New York 11794, USA.

membrane studies with simple peptides. For example, peptides with three, five and seven basic residues — Lys_3, Lys_5 and Lys_7 — bind to vesicles containing 30% phosphatidylserine with energies of 3, 5 and 7 kcal mol^{-1}, respectively [9]. If the membrane contains only half as much phosphatidylserine, then ten rather than five basic residues are required to produce a binding energy of 5 kcal mol^{-1}.

The hypothesis that the ability of *Drosophilia* amphiphysin to tubulate liposomes *in vitro* depends on simple electrostatic interaction of the BAR domain with acidic lipids is strongly supported by mutagenesis experiments. Peter *et al.* [4] mutated pairs of positively charged residues — lysine and arginine — to negatively charged glutamate in both the disordered loop and the concave face, that is, the ends and inner surface of the banana. As expected, the mutations reduced the binding to liposomes and inhibited tubulation of liposomes. Similar mutations in mammalian amphiphysins were also effective. Earlier work by Takei *et al.* [5] showed that amphiphysin-induced tubulation of vesicles formed from mixtures of the zwitterionic lipid phosphatidylcholine and the acidic lipid phosphatidylserine increased in parallel with the mole fraction of phosphatidylserine, which is also consonant with simple electrostatic interactions.

BAR will induce curvature only if the electrostatic binding energy is greater than the energy required to bend the membrane. We can crudely estimate these bending energies if we assume a standard bilayer bending modulus (k_b) of ~20 k_BT [10, 11] where k_B is Boltzmann's constant, and T the temperature in degrees Kelvin. If we assume at half-maximal packing each BAR domain occupies about one-fourth of a cylinder of length (L) = 5 nm and radius (R) 11 nm (Figure 1), then the bending energy for a spontaneously flat membrane to become such a cylinder is given by $E_b = \pi k_b L/R = 28\ k_BT$ [10,11]. Then each BAR domain needs 7 k_BT, or about 5 kcal mol^{-1}, to bend a membrane into a tube of diameter 22 nm. From model membrane studies with peptides, we expect at least 5–10 basic residues will be required to produce an electrostatic interaction that is strong enough to bend the bilayer, which agrees qualitatively with the reported structure and mutation results [4].

If a biological membrane has more bending rigidity than the nominal 20 k_BT [10], or if a protein's BAR domain has fewer basic residues in the concave surface, then the BAR domain might not be able to bend the membrane. The electrostatic binding mechanism described above would, however, explain a new putative function for BAR, that of a curvature sensor [4]. It takes about as much work to bind to a membrane as to deform it into a tube of 11 nm, so membrane affinity should be much tighter for membranes that have geometric curvature approaching that of the BAR domain. In fact, Bigay *et al.* [12] have proposed that ArfGAP may act as a curvature sensor in COPI-mediated vesicle coat assembly, as they found that the curvature of the liposomes used in their assay strongly affected ArfGAP binding.

Peter *et al.* [4] note that none of the BAR domains they tested exhibits strong lipid specificity, as expected

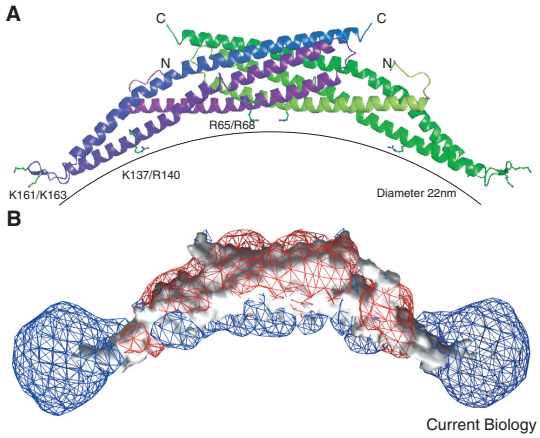

Figure 1. Structure of BAR domain of *Drosophila* amphiphysin [4].

BAR is a homodimer of α-helical bundles with extensive overlap; the two subunits are colored green and purple. The ends and concave surface have a number of basic residues, some of which are shown here. (A) Ribbon rendering. (B) Electrostatic equipotential surfaces in 0.1 M KCl (red = $-k_BT/e$ = –25 mV; blue = $+k_BT/e$) calculated from the Poisson–Boltzmann equation and displayed using GRASP. Figure 1B generously provided by Diana Murray.

for simple electrostatic interactions. A neighboring PH or PX domain might, however, confer such specificity, and might also be required to help anchor the BAR domain to membranes. Several proteins with BAR domains do have adjacent PH, PX or basic domains that should provide additional lipid interactions. Peters *et al.* [4] suggest that the PH or PX domains (reviewed in [13]) and BAR domains could act together, with the former targeting the protein and the latter detecting membrane curvature. This suggestion is supported by their experiments showing that a protein construct with a 'BAR plus PH' domain localizes to membrane tubules, but that constructs with a point mutation in either domain are distributed throughout the cytoplasm [4].

There is a straightforward biophysical basis for this phenomenon: spatial juxtaposition of two membrane-binding sites produces a synergism or apparent cooperativity in their binding (see references in [14]). For example, the membrane binding energies add — or the binding constants multiply — for the two moieties that anchor Src to membranes, a myristate and adjacent cluster of basic residues [9]. Peter *et al.* [4] thus suggest the membrane curvature-sensing BAR domain could act together with another membrane-binding domain, a PH domain for example, as a coincidence counter, sensing both membrane curvature and the presence of specific lipids.

Protein kinase C (PKC) is a well established example of a coincidence counter, or logical AND gate: it requires both the lipid diacylglycerol (DAG) and Ca^{2+} for effective activation. Binding of DAG to PKC's C1 domain, and phosphatidylserine to its Ca^{2+}-activated C2 domain, are required to produce effective translocation of PKC to the plasma membrane. This leads to concomitant activation of the kinase, as membrane binding of C1 and C2 domains removes the amino-terminal

pseudosubstrate domain from PKC's active site [15]. N-WASP is another example: it has two autoinhibitory regions, a basic (B) and a GTPase-binding domain (GBD). Binding of PIP_2 to the B domain and activated Cdc42 to the GBD activate N-WASP, as explored in detail in an interesting recent report by Lim and colleagues [16]. The proposal by Peter *et al.* [4] on coincidence counting is thus very reasonable.

Of course much more work is needed to determine if the electrostatic membrane-bending mechanism proposed by Peter *et al.* [4] for the BAR domain explains the physiological role of the amphiphysins and other BAR-containing proteins. For example, mutations of the basic residues on the concave surface of BAR that prevent tubulation of phospholipid vesicles should have profound physiological effects; these mutations in mammalian amphiphysin 2 or *Drosophila* amphiphysin should affect T-tubule formation [6,17]. Furthermore, the relationship between the highly curved surface of BAR, with a fixed radius of curvature $r = 11$ nm, and the tubules it produces from liposomes or in cells, $r > 11$ nm and highly variable, is not clear. The tubules made by amphiphysin seem to have even larger mean curvatures. Finally, the role of the amino-terminal helix, which is important but not essential for tubulation by amphiphysin [4,18], remains to be determined.

References

1. Sheetz, M.P., and Singer, S.J. (1974). Biological membranes as bilayer couples. A molecular mechanism of drug-erythrocyte interactions. Proc. Natl. Acad. Sci. USA *71*, 4457-4461.
2. Huttner, W.B., and Zimmerberg, J. (2001). Implications of lipid microdomains for membrane curvature, budding and fission. Curr. Opin. Cell. Biol. *13*, 478-484.
3. Lippincott-Schwartz, J., and Liu, W. (2003). Coat control by curvature. Nature *426*, 507-508.
4. Peter, B.J., Kent, H.M., Mills, I.G., Vallis, Y., Butler, P.J.G., Evans, P.R., and McMahon, H.T. (2003). BAR domains as sensors of membrane curvature: the amphiphysin BAR structure. Science *303*, 495-499.
5. Takei, K., Slepnev, V.I., Haucke, V., and De Camilli, P. (1999). Functional partnership between amphiphysin and dynamin in clathrin-mediated endocytosis. Nat. Cell Biol. *1*, 33-39.
6. Lee, E., Marcucci, M., Daniell, L., Pypaert, M., Weisz, O.A., Ochoa, G.C., Farsad, K., Wenk, M.R., and De Camilli, P. (2002). Amphiphysin 2 (Bin1) and T-tubule biogenesis in muscle. Science *297*, 1193-1196.
7. Dill, K.A., and Bromberg, S. (2003). Molecular Driving Forces. (Garland Science), Chapters 20-23.
8. McLaughlin, S. (1989). The electrostatic properties of membranes. Annu. Rev. Biophys. Biophys. Chem. *18*, 113-136.
9. Murray, D., Arbuzova, A., Honig, B., and McLaughlin, S. (2002). The role of electrostatic and nonpolar interactions in the association of peripheral proteins with membranes. Curr. Top. Memb. *52*, 277-307.
10. Boal, D. (2002). Mechanics of the Cell. (Cambridge University Press), pp 156-159.
11. Chernomordik, L.V., and Kozlov, M.M. (2003). Protein-lipid interplay in fusion and fission of biological membranes. Annu. Rev. Biochem. *72*, 175-207.
12. Bigay, J., Gounon, P., Robineau, S., and Antonny, B. (2003). Lipid packing sensed by ArfGAP1 couples COPI coat disassembly to membrane bilayer curvature. Nature *426*, 563-566.
13. DiNitto, J.P., Cronin, T.C., and Lambright, D.G. (2003). Membrane recognition and targeting by lipid-binding domains. Sci STKE. re16.
14. McLaughlin, S., Wang, J., Gambhir, A., and Murray, D. (2002). PIP2 and proteins: interactions, organization and information flow. Annu. Rev. Biophys. Biomol. Struct. *31*, 151-175.
15. Hurley, J.H., and Misra, S. (2000). Signaling and subcellular targeting by membrane lipids. Annu Rev. Biophys. Biomol. Struct. *29,* 49-79.
16. Dueber, J.E., Yeh, B.J., Chak, K., and Lim, W.A. (2003). Reprogramming control of an allosteric signaling switch through modular recombination. Science *301*, 1904-1908.
17. Razzaq A, Robinson I.M., McMahon H.T., Skepper J.N., Su Y., Zelhof A.C., Jackson A.P., Gay N.J., and O'Kane C.J. (2001). Amphiphysin is necessary for organization of the excitation-contraction coupling machinery of muscles, but not for synaptic vesicle endocytosis in Drosophila. Genes Dev. *15*, 2967-2979.
18. Farsad, K., Ringstad, N., Takei, K., Floyd, S.R., Rose, K. and De Camilli, P. (2001). Generation of high curvature membranes mediated by direct endophilin bilayer interactions. J. Cell Biol. *155*, 193-200.