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Review

SNX–BAR proteins in phosphoinositide-mediated, tubular-based endosomal sorting

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ABSTRACT

The endocytic network is morphologically characterized by a wide variety of membrane bound compartments that are able to undergo dynamic re-modeling through tubular and vesicular structures. The precise molecular mechanisms governing such re-modeling, and the events that co-ordinated this with the major role of endosomes, cargo sorting, remain unclear. That said, recent work on a protein family of sorting nexins (SNX) - especially a subfamily of SNX that contain a BAR domain (SNX-BARs) – has begun to shed some much needed light on these issues and in particular the process of tubular-based endosomal sorting. SNX-BARs are evolutionary conserved in endosomal protein complexes such as retromer, where they co-ordinate membrane deformation with cargo selection. Furthermore a central theme emerges of SNX-BARs linking the forming membrane carrier to cytoskeletal elements for transport through motor proteins such as dynein. By studying these SNX-BARs, we are gaining an increasingly detailed appreciation of the mechanistic basis of endosomal sorting and how this highly dynamic process functions in health and disease.

Contents

1. Introduction

The BAR (*B*in/Amphiphysin/*R*vs) domain is a protein dimerization motif made up of three α-helices that dimerize to form a rigid banana-shaped structure [1,2], of which the concave surface contains a number of basic residues that allow association with the phospholipid bilayer through electrostatic interactions [3].

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Given the rigid nature of the BAR domain, it has been argued that these electrostatic interactions are optimally configured to associate with curved rather than flat membranes [4]. In this way, BAR domains can sense membrane curvature. In addition, BAR domains are also able to drive membrane deformation by forming higher ordered helical arrays, thereby stabilizing the formation of high curvature membrane tubules and vesicles [5].

BAR proteins are encoded in most, if not all eukaryotic genomes [6]. Many of these proteins are evolutionary conserved from budding yeast to human, spanning a wide variety of cellular functions including cell division, apoptosis, phagocytosis, endocytosis, exocytosis, cytoskeleton dynamics and transcriptional regulation (reviewed recently by Ref. [7]). Some BAR proteins contain additional membrane-binding modules to control their membrane association. One such module is the phosphoinositide-binding phox homology (PX) domain [8]. In mammalian cells, there are 12 proteins that contain both PX and BAR domains [9–11]. These PX–BAR or SNX–BAR (for sorting nexin-BAR, see Section 2) proteins appear to play essential roles in the regulation of tubular-based sorting events within the endocytic network [11].

2. The SNX–BAR family

2.1. SNX–BARs — a sub-family of sorting nexins

SNX–BARs are a subset of a larger protein family called the sorting nexins (SNXs): these proteins are grouped solely on the basis that they all contain a SNX–PX domain [10–12]. So far, 33 different SNXs have been annotated in the mammalian genome, of which 12 variants contain a C-terminal BAR domain (SNX1, SNX2, SNX4, SNX5, SNX6, SNX7, SNX8, SNX9, SNX18, SNX30, SNX32 and SNX33, see Fig. 1A and Ref. [11]). For those SNX–BARs that have been studied, they are localized to tubular and vesicular membrane profiles throughout the endocytic network and have been described to be involved in clathrin-dependent and -independent endocytosis as well as an increasing array of endosomal sorting events [11].

2.2. Co-incidence detection in membrane targeting of SNX–BARs

SNX–BARs are peripheral membrane proteins that cycle through a dynamic cytosol-to-membrane equilibrium that is governed by the kinetics of membrane association versus disassociation. For membrane association SNX–BARs combine at least two membrane-binding properties. The first is the binding of the PX domain to specific phosphoinositides that form part of the 'identity code' of different endocytic compartments [13]. PX domains display a common structure of four α-helixes and three β-strands, folded into a "baseball-glove" that binds the phosphoinositides in the pocket formed by β1, β2, α2 and their linking loops [14]. Subtle variations in the residues lining the pocket leads to specific phosphoinositide binding, with the insertion of a hydrophobic loop into the membrane serving to further enhance membrane association [15]. Thus, the PX domain of SNX9 associates with phosphatidylinositol 4,5-bisphosphate (PI(4,5)P_2), thereby aiding the targeting of this SNX–BAR to PI(4,5)P_2-enriched regions of forming endocytic pits [16–19]. In contrast, the PX domain of SNX1 associates with the early and late endosomal phosphoinositides, phosphatidylinositol 3-monophosphate (PI(3)P) and phosphatidylinositol 3,5-bisphosphate (PI(3,5)P_2) respectively, aiding the targeting of this protein to maturing early endosomes (Fig. 4 and Refs. [20,21]).

The second membrane-binding activity of SNX–BARs stems from the ability of their BAR domains to sense membrane curvature [2]. Mutagenic studies targeting key residues in the electrostatic association of the SNX1 BAR domain to curved membranes has revealed that, even though the PX domain of SNX1 retains the ability to bind PI(3)P (and PI(3,5)P_2), the mutant protein displays a cytosolic localization [12]. Similarly, a SNX1 PX domain mutant that lacks the ability to bind these phosphoinositides, while retaining a wild-type BAR domain, is also entirely cytosolic [12]. Together these data establish that optimal targeting of SNX1 to the endosomal membrane requires simultaneous binding to phosphoinositides and an ability to sense membrane curvature. A similar dual requirement has also been documented for SNX4 and SNX9 [16,22]. The crystal structure of SNX9 has revealed that the PX domain is situated at the lateral side of the BAR dimer through a flexible linker such that the phosphoinositide-binding pocket lies in the same orientation as the basic concave side of the BAR domain (Fig. 2 and Ref. [16]). On basis of these findings, it has been suggested that SNX–BARs utilize a process of 'co-incidence detention' to target to sub-domains of the endocytic network that are composed of specific phosphoinositides and an appropriate degree of membrane curvature (Fig. 3 and Ref. [23]).

2.3. SNX–BARs induce membrane deformation leading to membrane tubulation

Like other BAR domain-containing proteins, the dose-dependent addition of recombinant SNX–BARs to artificial liposomes leads to the deformation of vesicles into high curvature membrane tubules *in vitro* [12,16,24]. The BAR domains of SNX–BARs share little sequence homology with other BAR domains, but their structure and degree of curvature is related to the "classical" BAR/N-BARs (for N-terminal amphipathic helix-BARs) such as endophilin and amphiphysin [2,9,16]. For N-BARs, the amphipathic helix is a flexible structure that forms in the aqueous-lipid interface: hydrophobic residues are clustered at one side of the helix that embeds into the region of fatty acyl chains of the phospho-bilayer while the hydrophilic residues of the helix face the aqueous surface at the level of the polar head groups [2,25]. The insertion of the helix in the bilayer functions as a wedge, pushing the lipids aside in one layer of the membrane. The discrepancy in tension between the layers in the bilayer leads to 'local' curvature that is further stabilized by the BAR domain (Fig. 3 and Ref. [26]). Several SNX–BARs are now predicted to contain similar amphipathic helixes (e.g. SNX1, SNX2, SNX4 and SNX9) [16,27]. Mutations in the predicted amphipathic helix of SNX9, results in reduced membrane affinity and reduced tubulation capacity of lipid vesicles *in vitro* [16]. A similar type of analysis for the other SNX–BARs should shed more light on the role of these predicted helices in membrane tubulation.

How is 'local' membrane deformation translated into a 'global' deformation that drives tubule formation? Work on a different class of BAR proteins (so-called F-BARs) has revealed that the BAR dimer can coat membrane tubules in a highly ordered fashion, by which the tips of the BAR dimer interact to form a spiral coat while lateral contacts regulate the angle of the BAR dimer relative to the tubule axis (Fig. 2B and Ref. [5]). For SNX9, the only SNX–BAR so far crystallized with a complete PX–BAR unit, tip-to-tip interactions are observed in the crystals consistent with the observations from F-BARs [5,17]. The lateral interactions described in F-BAR sheets are, however, unlikely to play a role in SNX–BAR oligomerization as the PX domain is located at this site of the BAR dimer (see Fig. 2B and Ref. [16]). Whether other contacts allow for lateral interactions between the PX domain and adjacent PX–BAR unit remains an important unanswered question. Equally important is the issue of how many distinct tubular profiles are driven by SNX–BARs. If homodimeric interactions prevailed within this family one would predict that each SNX–BAR would coat a