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Review

Membrane-associated cargo recycling by tubule-based endosomal sorting

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ABSTRACT

The endosome system is a collection of organelles that sort membrane-associated proteins and lipids for lysosomal degradation or recycling back to their target organelle. Recycling cargo is captured in a network of membrane tubules emanating from endosomes where tubular carriers pinch off. These tubules are formed and stabilized through the scaffolding properties of cytosolic Bin/Amphiphysin/Rvs (BAR) proteins that comprise phosphoinositide-detecting moieties, recruiting these proteins to specific endosomal membrane areas. These include the protein family of sorting nexins that remodel endosome membrane into tubules by an evolutionary conserved mechanism of dimerization, local membrane curvature detection/induction and oligomerization. How the formation of such a tubular membrane carrier is coordinated with cargo capture is largely unknown. The tubular structure of the membrane carriers could sequester membrane-bound cargo through an iterative mechanism of geometric sorting. Furthermore, the recent identification of cargo adaptors for the endosome protein sorting complex retromer has expanded the sorting signals that retrieve specific sets of cargo away from lysosomal degradation through distinct membrane trafficking pathways.

Contents

Abbreviations: BAR, Bin/Amphiphysin/Rvs; ERC, endosome recycling compartment; FERM, 4.1/ezrin/radixin/moesin; GPCR, G protein coupled receptor; PDZ, PSD-95/Dlg1/Zo-1; PX, phox homology; SNX, sorting nexin; TEN, tubular endosomal network; TfR, transferrin receptor; TGN, *trans*-Golgi network; WASH, Wiskott–Aldrich syndrome protein and SCAR homologue.

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1. Introduction

Endosomes are pleomorphic organelles that sort membrane-associated proteins and lipids into several different membrane trafficking routes. Early endosomes are the main entry point for proteins and lipids, here a major decision is made: are these components to be recycled or are they to be disassembled by degradation in the lysosome. If cargo is to be degraded, it is enriched in specific subdomains where the endosome membrane is remodelled to form intra-luminal vesicles. During the lifetime of the endosome, more intra-luminal vesicles accumulate inside the organelle forming the late endosome (also morphologically termed the multi-vesicular body). In parallel, degradative proteases are delivered to the endosome lumen by membrane trafficking and the pH of the internal milieu is continuously acidified for protease activation. This entire process is termed early-to-late endosome maturation [1–3]. The late endosome can fuse with lysosomes to form a hybrid organelle where the luminal content, including those molecules enriched in intra-luminal vesicles, is degraded. In parallel to maturation of the endosome, sorting events retrieve cargoes away from this degradative route for recycling back to the plasma membrane or to the biosynthetic pathway at the level of the *trans*-Golgi network (TGN). This is achieved by concentrating specific cargo in a network of tubular endosomal sub-domains that regulate membrane traffic: the tubular endosomal network (TEN).

The TEN was first observed by electron microscopy 30 years ago as a network of "interconnected vacuoles and cisternae" filled with electron-dense labelling of different recycling receptors [4–6]. The TEN is highly dynamic, new sorting tubules are continuously formed. One endosome can give rise to several molecularly distinct sorting tubules, in which selected cargo molecules are concentrated. We are just starting to understand the mechanisms involved in remodelling of the endosome membrane to form tubular transport carriers and how this process is coordinated with selective recycling cargo capture.

2. Tubule-based membrane carriers

Tubular-shaped membrane compartments are a common feature of the TEN. Their high surface-to-volume ratio allows the sorting and transport of mainly membrane-bound components without much exchange of luminal content between the endosome and the target-organelle [7,8]. The tubular shape of a lipid bilayer in an aqueous environment is not energetically optimal and requires factors that model and stabilize this configuration. Membrane remodelling can be achieved by different mechanisms including: changing the relative lipid composition across the phospholipid bilayer; altering the trans-membrane protein composition; association of scaffolding proteins on the membrane surface; shallow insertion of hydrophobic protein domains into the phospholipid layer; and, application of mechanical force by linking the membrane to the cytoskeletal motor proteins (reviewed in [9,10]). Combinations of these mechanisms are being employed to generate endosomal sorting tubules associated with the TEN and the tubular membrane carriers that recycle selected cargo.

2.1. SNX-BAR proteins – an evolutionary conserved protein family regulating endosome membrane remodelling

A variety of proteins can make and stabilize membrane tubules, of which many contain a Bin/Amphiphysin/Rvs (BAR) domain [11]. The BAR domain is a banana-shaped protein-dimerization domain that contains positively charged residues at its concave side that associate with membranes through electrostatic interactions (Fig. 1A and B) [12]. Given the arced structure of the BAR domain, these proteins preferably associate with similarly curved membrane structures, thereby functioning as membrane curvature sensors (Fig. 1C′). In addition, BAR domains can oligomerize to form a stabile helical array that induces and stabilizes curved membrane structures such as tubules and vesicles (Fig. 1C″ and D) [13]. Some BAR proteins are recruited to specific membrane subdomains by additional lipid binding moieties that recognize phosphoinositides as organelle identity markers [14]. These include sorting nexins (SNXs) containing a BAR domain (SNX-BAR) that are effectively recruited to the TEN by co-incidence detection of phosphoinositides through their phox homology (PX) domain and membrane curvature through their BAR domain [15,16].

SNX-BAR proteins are evolutionary conserved in most eukaryotes. The *Trypanosoma burcei* genome contains only one putative BAR gene that encodes for a SNX-BAR protein homologous to VPS5 [17]. VPS5 was originally identified in *Saccharomyces cerevisae* as part of retromer, a protein sorting complex that regulates endosome-to-TGN membrane traffic [18]. Retromer comprises a subcomplex of VPS26–VPS29–VPS35 that is proposed to regulate cargo selection, and a subcomplex of SNX-BARs that remodels the endosomal membrane into tubular membrane carriers (for retromer reviews see [19,20]). In yeast, the SNX-BAR subcomplex comprises a heterodimer of VPS5 with VPS17 [18]. The SNX-BAR heterodimer is evolutionary conserved to mammals and has undergone gene duplication, as VPS5 is represented by SNX1 and SNX2 and VPS17-homologues are recognized in SNX5, SNX6 and SNX32 [17,21]. Less complex eukaryotes, like as Excavata and Archaeolastida, lack a VPS17 homologue, indicating that the earliest form of retromer comprises a homodimer of VPS5-homologues [17]. The overall structure of retromer shares similarities with the ancestral coat-complex protocoatomer (also found in coat complexes such as clathrin, COPI and COPII), suggesting that retromer can function as a coat for endosomal recycling transport [22,23].

Many, but not all, of the 12 different mammalian SNX-BAR proteins will induce extensive endosome tubulation when overexpressed in cells [15,21,24–26], and can reshape liposomes into tubular structures *in vitro* [15,24,25,27]. The overall pattern of homo-/hetero-dimerization of SNX-BAR pairs overlaps with the ability of remodelling membrane *in vitro* (Fig. 2), indicating that BAR-dimerization is crucial for membrane remodelling [27]. However, forming a BAR dimer is not sufficient to remodel the lipid bilayer, three different interactions are required for the formation of membrane tubules by SNX-BARs [27].

First, SNX-BAR proteins form specific BAR dimers (Figs. 1B and 2), which is partly achieved by complementary positioning of charged residues within the hydrophobic BAR dimerization interface [27,28]. The SNX-BAR proteins of the retromer complex form predominantly heterodimers, where SNX1 or SNX2 form dimers with SNX5 or SNX6/SNX32, with limited capacity to also from homodimers [21,29]. Potentially, SNX1 and SNX2 have retained some homodimerization capacity as an ancient feature of the VPS5-homodimers found in early eukaryotes. Similarly, SNX4 can form homodimers and heterodimers with SNX7 and SNX30, while SNX7 and SNX30 do not form homodimers [27,30]. SNX4 functions therefore as a central "hub" for SNX4/SNX7/SNX30-dependent membrane remodelling (Fig. 2). SNX8 and the subfamily of SNX-BAR proteins containing a SH3 domain (SNX9, SNX18 and SNX33) all form predominantly homodimers [25,27,28,31], although heterodimerization of SNX9 with SNX18 or SNX33 has also been reported [32,33].

Second, amphipathic helixes (AHs) are inserted into the lipid bilayer (Fig. 1C′ and C″). Many BAR-proteins contain an N-terminal AH and are classified as N-BARs [11,34]. The AH of these proteins is essential for membrane remodelling [34] and curvature sensing [35] (more discussion in Section 2.3). All SNX-BAR proteins contain a AH sequence that is essential for membrane remodelling *in vitro*,

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